

2008

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### Recommended Citation

Kingsley-Smith, P.R. and Luckenbach, M.W., "Post-settlement survival and growth of the Suminoe oyster, *Crassostrea ariskensis*, exposed to simulated emersion regimes" (2008). *VIMS Articles*. 1371.  
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## POST-SETTLEMENT SURVIVAL AND GROWTH OF THE SUMINOE OYSTER, *CRASSOSTREA ARIAKENSIS*, EXPOSED TO SIMULATED EMERSION REGIMES

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**ABSTRACT** In high salinity habitats along the Middle and South Atlantic coasts of the United States the Eastern oyster, *Crassostrea virginica* occupies an intertidal refuge from predation, facilitated by its tolerance of aerial exposure and associated desiccation and temperature stress. Observations of the Suminoe oyster, *C. ariakensis* in its native environments in Asia reveal that this species is most commonly found subtidally or in the very low intertidal zone, suggesting that it may be less tolerant of aerial exposure. With serious consideration being given to introducing *C. ariakensis* to the mid-Atlantic region, it is important to understand the ability of this non-native species to invade and become established in the intertidal zone. We conducted experiments in an outdoor quarantined facility to compare the tolerances of *C. virginica* and *C. ariakensis* to varying levels of aerial exposure. Diploid *C. virginica* and *C. ariakensis* were set on 10 cm × 10 cm PVC tiles, held in a flow-through quarantine system exposed to ambient weather conditions, and subjected for eight weeks to four simulated tidal emersion regimes—(1) high intertidal (3.5 h emersion), (2) mid intertidal (2 h emersion), (3) low intertidal (1 h emersion), and (4) subtidal (constant immersion)—and four exposure orientations—(1,2) vertical north- and south-facing, and (3,4) horizontal up- and down-facing. Complete mortality of both species occurred in the high intertidal treatment by the end of week 1. No *C. ariakensis* had survived in the mid intertidal treatment by week 2 and very few remained alive in the low intertidal treatment. By the end of week 5, only 1.25% of the *C. ariakensis* had survived in the low intertidal treatment, whereas survival of *C. ariakensis* in the subtidal treatment was 36.88%. Significantly, *C. virginica* survival was 80.63% in the subtidal treatment and 67.50% and 28.13% on the vertically-oriented tiles (north- and south-facing treatments combined) in the low intertidal and mid intertidal treatments, respectively. Growth rates of *C. virginica* across tidal treatments were greatest in the subtidal treatment and *C. ariakensis* grew faster in the subtidal treatment than *C. virginica*. These results indicate that even with modest aerial exposure, under climatic conditions characteristic of summers in the mid-Atlantic region of the United States, *C. ariakensis* would suffer high rates of early post-settlement mortality, effectively restricting this non-native oyster species to subtidal environments if introduced to the region.

**KEY WORDS:** *Crassostrea ariakensis*, exotic species, intertidal exposure, Suminoe oyster

### INTRODUCTION

Patterns of intertidal zonation among sessile marine invertebrates have classically been attributed to post-settlement mortality resulting from biological interactions and physical stress, with the lower limits of a species distribution typically set by competition and predation, and the upper limits set by the physiological stress of aerial exposure (Stephenson & Stephenson 1949, Lewis 1961, Connell 1972, Paine 1974). Numerous later studies have clearly demonstrated that combinations of pre- and post-settlement processes affect the vertical distributions of intertidal marine invertebrates (e.g., Grosberg 1982, Underwood & Denley 1984, Gaines & Roughgarden 1985, Underwood & Fairweather 1989, Michener & Kenny 1991, Roughgarden et al. 1991). Nevertheless, physiological tolerances of thermal stress and desiccation remain critical factors in the ability of a species to exploit intertidal habitats.

The vertical distribution of the Eastern oyster, *Crassostrea virginica* (Gmelin 1791) along the United States Atlantic and Gulf of Mexico coasts varies from exclusively subtidal in the northern extent of its range, to largely intertidal along the southern Atlantic coast, with mixed subtidal and intertidal distribution in the lower mid-Atlantic and Gulf of Mexico (Coen et al. 1999, Kennedy & Sanford 1999, Coen & Grizzle 2007 and references therein). In general, populations along the South Atlantic coast from northeastern Florida to southern North Carolina (approximately 29°N to 34°N latitude) are restricted to the intertidal zone (Coen & Grizzle 2007), a situation

that we note also prevails in high salinity environments along the outer coast of Virginia (approximately 37°N to 38°N latitude) (pers. obs.). The restriction of *C. virginica* to intertidal habitats within this region has frequently been attributed to the effects of predation and competition in subtidal habitats (Galtsoff & Luce 1930, McDougall 1942, Chestnut & Fahy 1952, Dame 1979, Ortega 1981, O'Beirn et al. 1995, O'Beirn et al. 1996), though Giotta (1999) also highlighted the roles of sedimentation and flow reduction. Larval behavior and settlement patterns may also contribute to this distribution (Michener & Kenny 1991). Experiments conducted in Chesapeake Bay, USA reveal that *C. virginica* exhibits higher settlement rates subtidally (Roegner & Mann 1990, Bartol & Mann 1997), but that higher early post-settlement survival rates in the mid- and low- intertidal zones (Roegner & Mann 1995, Bartol et al. 1999) modify the vertical distribution of this species. Exposure stress has been shown to set the upper limit of horizontal distribution for several oyster species (e.g., Nichey & Menzel 1967, Potter & Hill 1982, Michener & Kenny 1991, Krasso et al. 2008) and, as with several rocky intertidal organisms (e.g., Connell 1972, Newell 1979, Davenport & Davenport 2005), thermal and desiccation stress have been implicated. Ice scour and winter air temperatures are presumed to preclude the survival of *C. virginica* in the intertidal zone in northern parts of its distribution, whereas at more southerly latitudes high temperature stress, particularly among newly-settled oysters, has been suggested as an important factor in setting upper exposure limits (Roegner & Mann 1995, Michener & Kenny 1991).

The consideration currently being given to an intentional introduction of *C. ariakensis* (Fujita, 1913) to the Middle

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Atlantic coast of the United States highlights the need to predict where this species might survive and grow in the region, if introduced. One component of this is the tolerance of this species to intertidal exposure. In the Ariake Bay, on the Japanese island of Kyushu, the Kumamoto oyster, *C. sikamea* is the dominant oyster occupying the high intertidal zone. The mid-intertidal region is dominated by the Pacific oyster, *C. gigas*, whereas the Suminoe oyster, *C. ariakensis* occurs predominantly in the lower regions of the intertidal and subtidally (e.g., Hedgecock et al. 1999, Luckenbach et al. 2005). Whereas earlier reports of the distribution of *C. ariakensis* from other areas of its native range suffer from taxonomic ambiguities (see Wang et al. 2008), more recent studies in China (Wang et al. 2008) and Korea (Yoon et al. 2008) also indicate that this species is restricted to subtidal and very low intertidal habitats. The absence of *C. ariakensis* in its native range from the mid and upper regions of the intertidal zone may be explained by one, or some combination of the following hypotheses: (i) behavioral responses of *C. ariakensis* larvae are such that they do not settle high in the intertidal; (ii) *C. ariakensis* larvae settle high in the intertidal, but do not survive there because of a physiological intolerance to prolonged periods of exposure; (iii) post-settlement *C. ariakensis* are able to survive in these regions, but are out-competed or overgrown by the superior performances of other oyster species, such as *C. sikamea* and *C. gigas*.

In this study we evaluate hypothesis (ii) above, while conducting a direct comparison of the early post-settlement survival and growth of *C. virginica* and *C. ariakensis* over a range of simulated tidal emersion regimes, each incorporating varying degrees of shading. Post-settlement survival and growth of *C. ariakensis* in both intertidal and shallow subtidal habitats will determine the potential niche overlap and resource competition with other sessile species, including the native oyster, *C. virginica* and also the extent to which *C. ariakensis* may present a significant biofouling issue if introduced to waters of the Chesapeake Bay and Middle Atlantic coast.

## MATERIALS AND METHODS

### Brood Stock Conditioning and Larval Rearing

Brood stock conditioning, spawning and larval rearing of local diploid *C. virginica*, and diploid *C. ariakensis* were conducted in quarantined facilities at the Castagna Shellfish Research Hatchery at the Virginia Institute of Marine Science's Eastern Shore Laboratory, Wachapreague, VA, USA. Wild native *C. virginica* ( $n = 69$ ) were collected from intertidal habitats in the vicinity of the laboratory (37°36'N, 75°41'W) and conditioning began during the week of March 14, 2005 and continued for 9 wk. *C. ariakensis* brood stocks used in this study were derived from stocks accidentally imported to the US West coast from Japan among shipments of *C. gigas* (Breese & Malouf 1977) and subsequently reared through numerous generations in hatcheries. Conditioning of *C. ariakensis* ( $n = 10$ ) began during the week of March 21, 2005 and continued for 8 wk.

*Crassostrea virginica* and *C. ariakensis* were thermally-induced to spawn on May 17 and 19, 2005 respectively. Eggs released from individual females of each species were combined and fertilized with pooled sperm from multiple males. The offspring were reared in batch culture at 30 psu and 25°C and

fed on multispecies diets of cultured *Isochrysis galbana*, *Chaetoceros neogracile*, *Pavlova pinguis*, and *Tetraselmis striata*.

### Settlement and Density Manipulations

Experiments were conducted using oysters set on replicate 10 cm × 10 cm × 0.64 cm thick white "seaboard" PVC tiles (Norva Plastics Inc., Norfolk, VA). Prior to setting the oysters these tiles were pre-soaked in flowing seawater for three days to allow for the development of natural biofilms that are known to stimulate the settlement of *Crassostrea* spp. (Tamburri et al. 2008). Tiles were then suspended in 200 L plastic containers of 30 psu seawater at 25°C and competent pediveliger larvae of each species were added to separate containers (*C. virginica*: approx.  $1.9 \times 10^6$  larvae, 79% with eye spots; *C. ariakensis*: approx.  $2.1 \times 10^6$  larvae, 36% with eye spots). Seawater was aerated vigorously, oyster larvae were fed daily on a mixed algal diet, and water changes were conducted on alternate days.

Observations made 48 h later indicated that adequate setting had occurred in the *C. virginica* tanks, such that the container was drained, refilled with filtered water, but larvae were not fed. At this time, *C. ariakensis* larvae showed lower setting densities than *C. virginica* larvae, and the *C. ariakensis* pediveliger larvae were returned to the container, fed and allowed a further 24 h to enhance settlement. All tiles were then examined using a binocular dissecting scope. Tiles with <10 attached and metamorphosed oysters on at least one side were rejected. On all other tiles, 10 metamorphosed oysters were identified on one side, marked (circled with pencil) and all remaining oysters removed by scraping with a razor blade, yielding an initial oyster density of 10 oysters per tile (= 1,000 oysters m<sup>-2</sup>). A total of 128 tiles were thus manipulated for each oyster species and maintained in filtered seawater prior to their transportation to an outdoor quarantined experimental facility (described below).

### Experimental Design

Both the duration of aerial exposure and the orientation of the substrate relative to the sun may affect the degree of desiccation experienced by oysters. We investigated each of these factors in a replicated, full factorial design. Four simulated tidal regimes (1) high intertidal (3.5 h emersion every 12.5 h), (2) mid intertidal (2 h emersion every 12.5 h), (3) low intertidal (1 h emersion every 12.5 h), and (4) subtidal (constant immersion) were established in separate fiberglass seawater tables (2.44 m long × 66 cm wide × 15 cm deep). All four seawater tables were fitted with a 5 cm diameter drain-pipe and received continuous flow-through seawater. Secondary drains with independent solenoid valves were fitted to seawater tables (1) to (3). Electronic timers were set to switch on and off, thereby opening and closing the solenoid valves and, at appropriate times, approximating the natural ebb and flood of the tide. Two complete emersion/immersion cycles for simulated tidal regimes (1) to (3) above advanced the tidal cycle by 1 h each day. The midpoints of the simulated low tides were synchronized across all tidal treatments.

Four orientation treatments (1) up (horizontal, face up), (2) down (horizontal, face down), (3) north (vertical, north-facing), and (4) south (vertical, south-facing) were established by fixing the tiles in the specified orientation on a PVC rack using stainless steel nuts and bolts. Seawater tables were oriented in

an east-west direction and tiles for both of the vertical treatments (north and south) were held parallel to the current flow and the long axis of the seawater table. Each rack held one replicate tile of each of the four orientation treatments. Tiles and racks were set up in a manner that established eight replicates of each orientation for each species within each of the tidal exposure regimes, with the location of the orientation treatments varying along the axis of each seawater table in a repeated Latin square design (see Fig. 1). Thus, the full factorial design contained two species (*C. virginica* and *C. ariakensis*)  $\times$  four exposure treatments (high, mid and low intertidal, and subtidal)  $\times$  four orientations (up, down, north, and south)  $\times$  eight replicates for a total of 256 tiles, each with 10 oysters at the initiation of the experiment.

Manipulated tiles, secured in their appropriate racks, were transported from the hatchery to the outdoor quarantined facility in a covered trailer in four empty, uncovered fiberglass seawater tables. Oysters were aerielly exposed for <45 min in transit from the hatchery to the quarantined facility located approximately 10 km away. The quarantine facility was located in a clearing approximately 200 m from a high salinity (>30 psu) tidal creek from which seawater was pumped to the tables. Effluent from the tables was captured as described below in *Quarantine Measures*. Deployment of the oysters into the experimental seawater tables occurred at 2,300 h on June 9, 2005 and oysters experienced the midpoint of their first low tide exposure period the following morning at 0501 h, simulating the natural tide. The experiment was exposed to ambient conditions of sunlight, air, seawater temperature and precipitation throughout the experiment.

Flow rates through the experimental seawater tables were maintained at 8 L min<sup>-1</sup> to ensure that oysters were not food limited. (This flow rate was based on a filtration rate, F (liters h<sup>-1</sup>) for *C. virginica* of  $F = 6.79W^{0.73}$ , where W = g tissue ash-free dry weight, Riisgård 1988). The seawater system was checked daily to ensure proper functioning. 50  $\mu$ m-pore bag filters were placed over the intakes to the seawater tables to prevent the settlement of invertebrate larvae on experimental tiles. Bag filters were rinsed and replaced at least daily because of high rates of sedimentation, particularly after strong wind events. Twice-weekly the tiles were gently rinsed with freshwater during the simulated low tide to prevent smothering-induced

mortality. The temperature in each seawater table was continuously monitored at hourly intervals using submersible *iBCod* data loggers obtained from Alpha Mach Inc., beginning at 0000 h on June 11, 2005. (Note: Data from loggers were unavailable on June 9 and 10, 2005, when a min-max alcohol thermometer was used to measure air temperature adjacent to the high intertidal treatment.)

Tiles were photographed after initial density manipulations and at weekly intervals after deployment for a period of 8 weeks. Tiles were removed from each species pair of racks in turn and individually photographed using a digital camera mounted 45 cm above an illuminated photographic platform. Digital images were later analyzed using Image Pro Plus version 6.0 software and used to determine weekly oyster survival and growth. Survival was determined by counting the total number of oysters on each tile image; growth was determined from oyster size, measured as shell height (SH) to the nearest 0.01 mm, of individual oysters from each tile image in a repeated measures design.

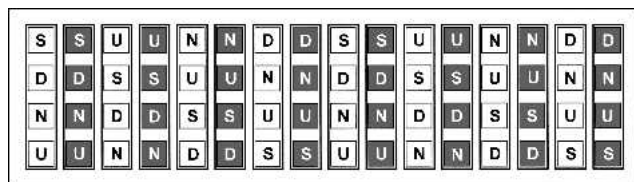
#### Quarantine Measures

All effluent waters from the quarantine hatchery in which brood stock conditioning, spawning, and larval rearing were conducted was treated with 5% sodium hypochlorite for a minimum of 24 h and then dechlorinated with sodium thio-sulphate before being released into the adjacent tidal creek. Effluent waters from the seawater tables in which the experiments were conducted passed to a series of closed ponds, located at the outdoor quarantined facility, permitting their containment and slow percolation through a sandy soil, ensuring that no viable gametes, larvae, or juvenile oysters were lost to the surrounding waters.

#### Statistical Analyses

The experiment ran for 8 wks with weekly estimates of survival and growth for all treatment replicates. Initial inspection of the data revealed that, beginning in week 6 and continuing through week 8, surviving oysters of both species occupied most of the space on the tiles, especially in the subtidal treatment, and an increasing number of oysters were found to have slipped off the tiles. Thus, we restricted all of our subsequent analyses to the first five weeks of the experiment. The experiment was designed to evaluate oyster survival in a replicated four-way ANOVA model with time, species, tidal exposure, and orientation as main effects. Untransformed and transformed (square-root, logarithmic, and arcsine) data, however, failed to meet assumptions of both normality and homoscedasticity. Furthermore, significant ( $P < 0.05$ ) two- and three-way interactions occurred under all multiway ANOVAs, resulting in part from the complete or near complete mortality of oysters in some treatment combinations. The data were thus partitioned into a series of one-way ANOVAs and tested against the assumptions of equal variances ( $F_{\max}$  test) and normality (Shapiro-Wilk test). Once again, the assumptions of ANOVA were not met with transformed or untransformed data for the majority of one-way ANOVA comparisons.

The significance of each main effect (time, species, tidal exposure and orientation) on oyster survival was therefore evaluated with a one-way nonparametric Kruskal-Wallis test at each level of each other variable where sufficient oysters



**Figure 1.** An illustration of the full factorial, repeated Latin square design of the 10 cm  $\times$  10 cm  $\times$  0.64-cm thick PVC tiles, as arranged in each of the four *Tidal Exposure* treatments (high, mid, and low intertidal, subtidal) established in independent seawater tables. Tiles set with *C. virginica* are shown in white; tiles set with *C. ariakensis* are shown in grey. Blocks of four tiles shown vertically represent individual PVC racks used to hold set of four tiles, containing each of four orientations (S = south-facing, D = down-facing, N = north-facing and U = up-facing). The sequence of orientations progresses by one orientation after each species pair of racks down the entire length of each seawater table. Seawater was supplied to the tables from the left relative to this illustration.

survived. Where significant main effects were observed ( $P < 0.05$ ) with the Kruskal-Wallis test, a nonparametric *a posteriori* multiple pairwise comparison was conducted using a series of Mann-Whitney *U*-tests, with an experiment-wise  $\alpha$ -level of 0.05. Mann-Whitney *U*-tests were not conducted for significant species effects, as only two levels were present.

Treatment effects on growth were assessed by analyzing shell heights at week 5 for those treatment levels with oysters surviving on two or more tiles. This constraint left the following valid hypotheses related to oyster shell height:

**Exposure  $H_0$ :**

Mid = Low = Subtidal (*C. virginica* only; all orientations);

**Orientation  $H_0$ :**

Up = South = North = Down (Subtidal only; spp. separately);

**Species  $H_0$ :**

*C. virginica* = *C. ariakensis* (Subtidal only; all orientations).

For each hypothesis, the subsets of data were tested against the assumptions of normality and homoscedasticity, and if satisfied, one-way ANOVAs were conducted for tests with more than two levels. In the case of *Species* as a main effect (only two levels), a *t*-test was performed using the Cochran & Cox (1950) approximation of *P* values for unequal variances. *A posteriori* multiple comparisons were conducted using Tukey's test.

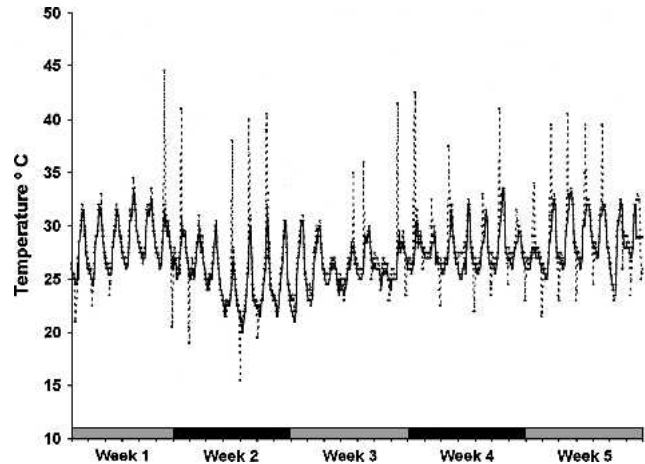
## RESULTS

### Temperature

Minimum and maximum air temperatures for June 9–10, 2005, recorded using an alcohol thermometer, prior to the deployment of the *iBCod* data loggers, were 17.8°C and 32.2°C, respectively. Temperature records from midnight June 11, 2005 (~2 days after deployment) through the end of week 5 (July 14, 2005), obtained from the *iBCod* data loggers, varied between treatments (see Fig. 2; for clarity we show only subtidal and mid intertidal temperature data). The temperature record in the subtidal treatment is continuous seawater temperature, whereas the mid intertidal temperature record incorporates seawater temperatures and air temperatures during simulated low tides. When all four seawater tables were experiencing flow-through temperatures across seawater tables tracked each other closely; however, when the simulated tide ebbed out, temperatures in the intertidal treatments deviated greatly from the subtidal treatment, with air temperatures exceeding those of seawater during the day and being generally lower at night. Temperature in the mid intertidal treatment ranged from 15.5°C to 44.5°C, whereas temperature in the subtidal treatment ranged from 20.0°C to 33.5°C.

### Survival

During the first week of the experiment, *C. ariakensis* experienced high mortality in all intertidal treatments, while high mortality of *C. virginica* also occurred in the high intertidal treatment (see Fig. 3). Significant effects of *Time*, *Species*, *Tidal Exposure* and *Orientation* on survival were observed at various levels within each of the other main effects (see Tables 1–3, Fig. 3).



**Figure 2.** Comparison of temperatures in the mid intertidal (dotted line) and subtidal (solid line) treatments. The mid intertidal data incorporate seawater and air temperatures, whereas the subtidal data are a constant record of seawater temperature. Temperature data collected at hourly intervals using Alpha Mach Inc. *iBCod* submersible data loggers are shown here for the period June 11 to July 14, 2005 inclusive.

### Time Effects

Time had a significant effect on survival (Kruskal-Wallis test,  $P < 0.001$ ) only in the case of *C. virginica* on up-facing tiles in the mid intertidal and low intertidal treatments, where survival in both these tidal treatments was significantly higher in weeks 1 and 2 than in weeks 3, 4 and 5 (Mann-Whitney *U*-test,  $P < 0.05$ ). This indicates that significant mortality occurred between weeks 2 and 3 in these treatments. Otherwise, the general lack of significant time effects reflects the fact that the majority of mortality in both species occurred during the first week of the study; thereafter little change occurred in the number of oysters surviving.

### Species Effects

Survival did not differ significantly between oyster species in the high intertidal treatment as a result of the early complete mortality of both species in this treatment (see Fig. 2). Survival did, however, differ significantly between species ( $P < 0.05$ ) in the mid intertidal, low intertidal and subtidal treatments, with the exceptions of the mid intertidal, down-facing tiles in weeks 1–5, the mid intertidal, up-facing tiles in week 5 only, and the low intertidal, up-facing tiles in weeks 3–5 (see Table 1). These exceptions occurred in situations where both species experienced low survival (see Orientation Effects later). In all cases in which a significant species difference was found, survival was greater for *C. virginica* than for *C. ariakensis* (see Table 1).

### Tidal Exposure Effects

Tidal exposure treatment had a significant effect on survival in all weeks and for all orientations in both oyster species (see Table 2, Fig. 2). The rank ordering of survival generally followed a pattern of decreasing survival with increasing tidal exposure; however, the significance of the differences between tidal exposure treatments varied across weeks, orientations and species (see Table 2).

For *C. ariakensis*, in all weeks and in all orientations, survival was significantly higher in the subtidal treatment than

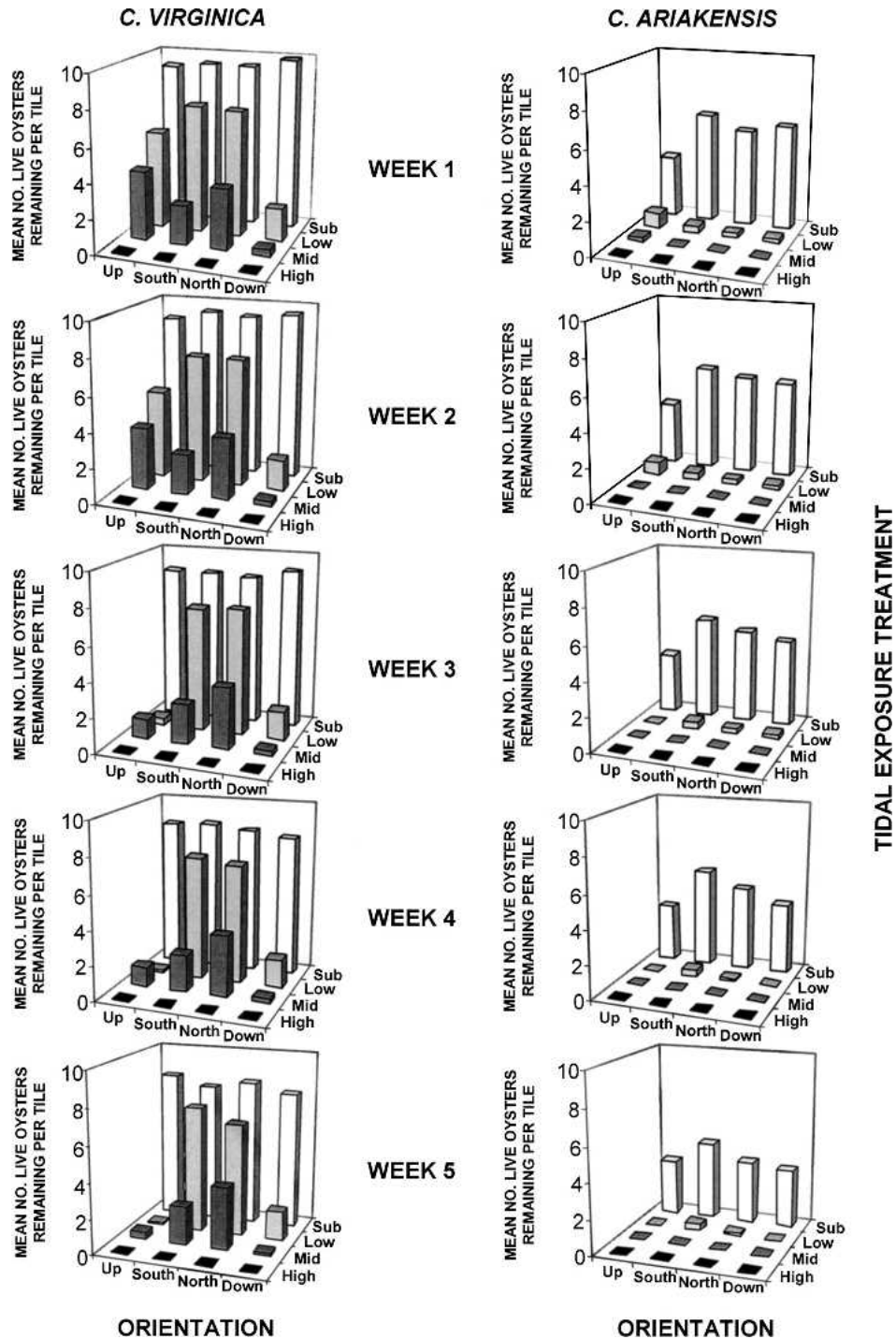


Figure 3. Comparative survival of *C. virginica* and *C. ariakensis* at week 5. Bars represent the mean number of live spat present across eight replicate tiles across tidal emersion levels and orientations. Initial number of oysters per tile = 10. Error bars are not shown for clarity.

in the three intertidal treatments, which did not differ significantly from one another. *Crassostrea ariakensis* survived very poorly in all three simulated intertidal treatments, with the majority of mortality occurring in week 1.

Patterns of survival in *C. virginica* in relation to tidal exposure were more complex. The high intertidal treatment always had significantly lower survival rates than the subtidal treatment, but differences between these treatments and the

other intertidal treatments varied both with orientation and time (see Table 2).

**Orientation Effects**

Significant effects of orientation on survival were not observed for *C. ariakensis*, which only survived well in the subtidal treatment (see Table 3, Fig. 2). Significant orientation effects on survival were also not found for *C. virginica* in the

TABLE 1.

Summary of Species Effects on survival by *Tidal Exposure, Orientation and Time (Week)*. Values under *Species* indicate either non-significant (NS) or significant differences in survival between species (Mann-Whitney *U*-test, experiment-wise  $\alpha < 0.05$ ). Weeks with equivalent results are collapsed for brevity.

| <i>Tidal Exposure</i> | <i>Orientation</i> | <i>Time (Week)</i> | <i>Species</i>            |
|-----------------------|--------------------|--------------------|---------------------------|
| High intertidal       | Up                 | 1-5                | NS                        |
| High intertidal       | South              | 1-5                | NS                        |
| High intertidal       | North              | 1-5                | NS                        |
| High intertidal       | Down               | 1-5                | NS                        |
| Mid intertidal        | Up                 | 1-4                | <i>C.v.</i> > <i>C.a.</i> |
| Mid intertidal        | Up                 | 5                  | NS                        |
| Mid intertidal        | South              | 1-5                | <i>C.v.</i> > <i>C.a.</i> |
| Mid intertidal        | North              | 1-5                | <i>C.v.</i> > <i>C.a.</i> |
| Mid intertidal        | Down               | 1-5                | NS                        |
| Low intertidal        | Up                 | 1-2                | <i>C.v.</i> > <i>C.a.</i> |
| Low intertidal        | Up                 | 3-5                | NS                        |
| Low intertidal        | South              | 1-5                | <i>C.v.</i> > <i>C.a.</i> |
| Low intertidal        | North              | 1-5                | <i>C.v.</i> > <i>C.a.</i> |
| Low intertidal        | Down               | 1-5                | <i>C.v.</i> > <i>C.a.</i> |
| Subtidal              | Up                 | 1-5                | <i>C.v.</i> > <i>C.a.</i> |
| Subtidal              | South              | 1-5                | <i>C.v.</i> > <i>C.a.</i> |
| Subtidal              | North              | 1-5                | <i>C.v.</i> > <i>C.a.</i> |
| Subtidal              | Down               | 1-5                | <i>C.v.</i> > <i>C.a.</i> |

high intertidal treatment, because of the complete mortality of this species in this treatment during week 1, nor in the subtidal treatment where survival was uniformly high. Orientation, however, did influence the survival of *C. virginica* in the mid

intertidal and low intertidal treatments, where poor survival was observed, in the down-facing tiles beginning in week 1 and in the up-facing tiles by week 3 (see Table 3, Fig. 2).

**Growth**

Despite the high mortality rates in several of the treatments, we were able to test a subset of growth hypotheses related to the effects of Tidal Exposure, Orientation, and Species (see earlier and Table 4). Because the initial sizes of oyster in the experiment were both small (<250  $\mu$ m SH) and comparable between species, we use oyster size at week 5 of the experiment, measured on July 14, 2005, as our metric of growth.

**Tidal Exposure Effects on Size of *C. virginica***

Mean size of *C. virginica* in the mid intertidal, low intertidal and subtidal treatments at week 5 ranged from 20.37 SH mm to 22.88 SH mm. The data satisfied the assumptions of normality (Shapiro-Wilk,  $P = 0.2779$ ) and homoscedasticity ( $F_{calc} = 1.57$ ;  $F_{max} = 3.8$ ,  $P < 0.05$ ) and one-way ANOVA revealed a significant effect of tidal exposure on oyster growth, with oysters in the mid intertidal treatment found to be significantly smaller than those in the subtidal treatment (see Table 4).

**Orientation Effects on Size in the Subtidal Treatment**

A two-way ANOVA for the effects of species and orientation on size in the subtidal treatment failed the assumption of normality (Shapiro-Wilk,  $P = 0.0078$ ) yet found significant effects of species, orientation and their interaction ( $P < 0.0001$ , in all cases). Separate one-way ANOVAs were subsequently performed for each oyster species to investigate the effect of orientation on

TABLE 2.

Effects of *Tidal Exposure* level on oyster survival by *Species, Orientation and Time (= Week)*. Kruskal-Wallis tests identified significant effects of *Tidal Exposure* on oyster survival at all combination of these variables. Rank orders of survival by *Tidal Exposure* level within all treatment combinations are shown in ascending order left to right, with different superscripts indicating significant differences in mean oyster survival (Mann-Whitney *U*-test, experiment-wise  $\alpha < 0.05$ ).

| <i>C. virginica</i> |             |   | <i>C. ariakensis</i> |             |  |
|---------------------|-------------|---|----------------------|-------------|--|
| <i>Orientation</i>  | <i>Time</i> | <i>Tidal Exposure Effects</i>   | <i>Orientation</i>   | <i>Time</i> | <i>Tidal Exposure Effects</i>  |
| Up                  | 1           | High <sup>a</sup> Mid <sup>b</sup> Low <sup>b</sup> Sub <sup>c</sup>  | Up                   | 1           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 2           | High <sup>a</sup> Mid <sup>b</sup> Low <sup>b</sup> Sub <sup>c</sup>  |                      | 2           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 3           | High <sup>a</sup> Low <sup>a</sup> Mid <sup>a</sup> Sub <sup>b</sup>  |                      | 3           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 4           | High <sup>a</sup> Low <sup>a</sup> Mid <sup>a</sup> Sub <sup>b</sup>  |                      | 4           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 5           | High <sup>a</sup> Low <sup>a</sup> Mid <sup>a</sup> Sub <sup>b</sup>  |                      | 5           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
| South               | 1           | High <sup>a</sup> Mid <sup>b</sup> Low <sup>c</sup> Sub <sup>c</sup>  | South                | 1           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 2           | High <sup>a</sup> Mid <sup>b</sup> Low <sup>c</sup> Sub <sup>c</sup>  |                      | 2           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 3           | High <sup>a</sup> Mid <sup>b</sup> Low <sup>c</sup> Sub <sup>c</sup>  |                      | 3           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 4           | High <sup>a</sup> Mid <sup>b</sup> Low <sup>c</sup> Sub <sup>c</sup>  |                      | 4           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 5           | High <sup>a</sup> Mid <sup>b</sup> Low <sup>c</sup> Sub <sup>c</sup>  |                      | 5           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
| North               | 1           | High <sup>a</sup> Mid <sup>b</sup> Low <sup>c</sup> Sub <sup>c</sup>  | North                | 1           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 2           | High <sup>a</sup> Mid <sup>b</sup> Low <sup>c</sup> Sub <sup>c</sup>  |                      | 2           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 3           | High <sup>a</sup> Mid <sup>b</sup> Low <sup>c</sup> Sub <sup>c</sup>  |                      | 3           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 4           | High <sup>a</sup> Mid <sup>b</sup> Low <sup>bc</sup> Sub <sup>c</sup> |                      | 4           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 5           | High <sup>a</sup> Mid <sup>b</sup> Low <sup>bc</sup> Sub <sup>c</sup> |                      | 5           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
| Down                | 1           | High <sup>a</sup> Mid <sup>ab</sup> Low <sup>b</sup> Sub <sup>c</sup> | Down                 | 1           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 2           | High <sup>a</sup> Mid <sup>ab</sup> Low <sup>b</sup> Sub <sup>c</sup> |                      | 2           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 3           | High <sup>a</sup> Mid <sup>ab</sup> Low <sup>b</sup> Sub <sup>c</sup> |                      | 3           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 4           | High <sup>a</sup> Mid <sup>ab</sup> Low <sup>b</sup> Sub <sup>c</sup> |                      | 4           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |
|                     | 5           | High <sup>a</sup> Mid <sup>ab</sup> Low <sup>b</sup> Sub <sup>c</sup> |                      | 5           | High <sup>a</sup> Mid <sup>a</sup> Low <sup>a</sup> Sub <sup>b</sup> |

TABLE 3.

Effects of *Orientation* on the survival of *C. virginica* within each *Tidal Exposure* treatment and *Time (Week)*. NS indicates nonsignificant differences in survival across orientations (Kruskal-Wallis test,  $P > 0.05$ ). Where significant orientation effects were observed rank order of survival by orientation within all treatment combinations are shown in ascending order left to right, with different superscripts indicating significant differences in mean oyster survival (Mann-Whitney *U*-test, experiment-wise  $\alpha < 0.05$ ). No significant orientation effects were observed for *C. ariakensis*.

| <i>Tidal Exposure</i> | <i>Time (Week)</i> | <i>Orientation Effects</i>   |
|-----------------------|--------------------|--|
| High intertidal       | 1                  | NS   |
|                       | 2                  | NS   |
|                       | 3                  | NS   |
|                       | 4                  | NS   |
|                       | 5                  | NS   |
| Mid intertidal        | 1                  | Down <sup>a</sup> South <sup>b</sup> North <sup>b</sup> Up <sup>b</sup>  |
|                       | 2                  | Down <sup>a</sup> South <sup>b</sup> North <sup>b</sup> Up <sup>b</sup>  |
|                       | 3                  | Down <sup>a</sup> Up <sup>ab</sup> South <sup>b</sup> North <sup>b</sup> |
|                       | 4                  | Down <sup>a</sup> Up <sup>ab</sup> South <sup>b</sup> North <sup>b</sup> |
|                       | 5                  | Down <sup>a</sup> Up <sup>a</sup> South <sup>b</sup> North <sup>b</sup>  |
| Low intertidal        | 1                  | Down <sup>a</sup> North <sup>b</sup> South <sup>b</sup> Up <sup>b</sup>  |
|                       | 2                  | Down <sup>a</sup> South <sup>b</sup> North <sup>b</sup> Up <sup>b</sup>  |
|                       | 3                  | Up <sup>a</sup> Down <sup>a</sup> South <sup>b</sup> North <sup>b</sup>  |
|                       | 4                  | Up <sup>a</sup> Down <sup>a</sup> North <sup>b</sup> South <sup>b</sup>  |
|                       | 5                  | Up <sup>a</sup> Down <sup>a</sup> North <sup>b</sup> South <sup>b</sup>  |
| Subtidal              | 1                  | NS   |
|                       | 2                  | NS   |
|                       | 3                  | NS   |
|                       | 4                  | NS   |
|                       | 5                  | NS   |

size in the subtidal treatment only. For *C. virginica*, a one-way ANOVA satisfied the assumptions of normality and homoscedasticity and found a significant effect of orientation on size, with oysters on down-facing tiles found to be significantly larger than those on south-facing tiles (see Table 4). Likewise, the assumptions of ANOVA were satisfied for the *C. ariakensis*

data and a significant effect of orientation on growth was observed, with higher growth rates on north- and down-facing tiles compared with up- and south-facing tiles (see Table 4).

Species Effects on Growth in Subtidal Treatments

This subset of data satisfied the assumption of normality (Shapiro-Wilk,  $P = 0.3603$ ), but failed that of homoscedasticity ( $F_{calc} = 18.77$ ;  $F_{max} = 2.15$ ,  $P > 0.05$ ), attributable to the greater variance in size for *C. ariakensis* at week 5 compared with *C. virginica* ( $\sigma^2 = 95.17$  for *C. ariakensis* cf.  $\sigma^2 = 5.07$  for *C. virginica*). We thus applied a *t*-test with the Cochran & Cox (1950) approximation for unequal variances (see Table 4) and found that by week 5 *C. ariakensis* were significantly larger than *C. virginica* in the subtidal treatment (mean size = 27.54 mm SH and 22.88 mm SH for *C. ariakensis* and *C. virginica*, respectively).

DISCUSSION

Our results support the hypothesis that the survival of *C. ariakensis* in the intertidal zone is limited by physiological stress caused by aerial exposure. The native oyster species, *C. virginica*, had higher survival than the non-native species, *C. ariakensis*, across most treatments. Only in the cases of the high intertidal exposure level, up-facing tiles in low and mid intertidal levels and (inexplicably) the down-facing tiles in the mid intertidal level, where *C. virginica* also had very low survival, was its survival not greater than that of *C. ariakensis*. The high intertidal exposure level (3.5 h exposure at each low tide) in our study clearly exceeded the physiological tolerances of newly-settled oysters of both species. Based upon our field observations, we had expected that *C. virginica* would have exhibited modest survival at this simulated high intertidal exposure level on the tiles with orientations sheltered from direct sunlight (down- and north-facing); however, the temperature extremes in our experimental set-up may have exceeded those of certain field locations at which we have observed oysters tolerating similar durations of exposure. An important limitation of our experimental design is that the duration of tidal emersion remained fixed within each tidal exposure treatment. In a natural setting, tidal range varies

TABLE 4.

Effects of *Tidal Exposure*, *Orientation* and *Species* on oyster size (SH = shell height in mm at week 5) for subsets of data from treatments with sufficient survival. Mean sizes are given for each treatment level and different superscripts indicate significant differences (Tukey's test,  $\alpha = 0.05$ ).

| Hypothesis  | Test (ANOVA or <i>t</i> -test)                        | Mean Size (SH mm) Multiple Comparisons |                             |                                |                            |
|---|---|--|-----------------------------|--------------------------------|----------------------------|
| <i>Tidal Exposure</i>                                       |   |  |                             |                                |                            |
| (C. virginica only; all orientations)                       |   |  |                             |                                |                            |
| H <sub>0</sub> : Mid = Low = Subtidal                       | F = 5.89, df = 2, P = 0.004                           | Mid<br>20.37 <sup>a</sup>              | Low<br>21.41 <sup>ab</sup>  | Subtidal<br>22.88 <sup>b</sup> |                            |
| <i>Orientation</i>  |   |  |                             |                                |                            |
| (Subtidal only; week 5 only)                                |   |  |                             |                                |                            |
| H <sub>0</sub> : Up = South = North = Down                  |   |  |                             |                                |                            |
| <i>C. virginica</i> :                                       | F = 3.31, df = 3, P = 0.035                           | South<br>21.66 <sup>a</sup>            | Up<br>22.01 <sup>ab</sup>   | North<br>23.33 <sup>ab</sup>   | Down<br>24.52 <sup>b</sup> |
| <i>C. ariakensis</i> :                                      | F = 29.01, df = 3, P < 0.001                          | Up<br>16.48 <sup>a</sup>               | South<br>23.31 <sup>a</sup> | North<br>31.85 <sup>b</sup>    | Down<br>39.14 <sup>c</sup> |
| <i>Species</i>  |   |  |                             |                                |                            |
| (Subtidal only; all orientations)                           |   |  |                             |                                |                            |
| H <sub>0</sub> : <i>C. virginica</i> = <i>C. ariakensis</i> | t = -2.51, df = 30, P = 0.018<br>(Cochran & Cox 1950) | <i>C.v.</i><br>22.88                   |                             | <i>C.a.</i><br>27.54           |                            |



with the neap-spring cycle and meteorological conditions, allowing for more variations in emersion times. This natural variation was not incorporated into our experimental apparatus.

Near complete mortality of *C. ariakensis* was observed across all intertidal treatments in this study, while *C. virginica* survived significantly better than the non-native species in the majority of mid and low intertidal treatment combinations. Even in the low intertidal treatment, in which oysters experienced only 1 h of aerial exposure twice daily, most *C. ariakensis* died within the first week of the study. (Only four *C. ariakensis* from a total of three tiles persisted in the low intertidal exposure treatment to the end of week 5, i.e., July 14, 2005). During the first week of the study air temperatures, recorded at the height of the experiment tiles during simulated low tides, deviated above and below water temperatures on several occasions, most notably reaching 44.5°C during one daytime low tide in the mid intertidal treatment (see Fig. 2), and 47.5°C in the high intertidal treatment. Temperatures recorded in the seawater tables during simulated low tides at times were higher than ambient air temperatures, reflecting the radiant heating of the table surfaces. Similar solar heating of rock surfaces has been shown to affect barnacle survival distribution (Bertness 1989, Wethey 2002). Furthermore, *in situ* measurements of body temperature in barnacles (Bertness 1989) and mussels (Helmuth et al. 2006) have been observed to exceed ambient air temperatures. Several temperate intertidal invertebrates have been shown to have thermal tolerances exceeding 40°C (e.g., littorinid gastropods: McMahon 2001, *Mytilus californianus*: Helmuth et al. 2006), and numerous studies (reviewed in Shumway 1996) have shown that adult *C. virginica* have wide temperature tolerances, including surviving air temperatures between 44°C to 49°C during low tide (Galtsoff 1964). The small size of oyster spat during the first week of this study likely reduced their tolerance of high temperatures.

We are not able to determine from our study if the observed mortalities were a direct consequence of thermal intolerance, desiccation stress, or a combination of these factors. Our observations are, however, consistent with those from the native range of *C. ariakensis* where it is apparently restricted to subtidal and low intertidal habitats in Korea (Yoon et al. 2008), China (Wang et al. 2008, Luckenbach pers. obs.), and Japan (Luckenbach et al. 2005). Further, these observations are consistent with data currently being gathered from a field study incorporating sites in Chesapeake Bay and on Virginia's Atlantic coast, USA using sterile *C. ariakensis* (Kingsley-Smith et al. unpubl. data).

Survival patterns for both species across tidal exposure levels and substrate orientations established within the first week of the study were generally maintained throughout the five week period. Similar results were reported by Roegner & Mann (1995) in their investigations of the effects of tidal zonation and season on the early recruitment and growth of *C. virginica* in Chesapeake Bay in which these authors determined the relative contributions of larval settlement and early post-settlement mortality to the vertical distribution of oysters. High mortality rates were observed within one week of settlement at all tidal heights, followed by a sharp decline in mortality rates two weeks post-settlement, such that mortality in the first week strongly influenced later abundance patterns. In their field study *C. virginica* did not recruit to the upper intertidal zone and survival above the MLW was severely reduced when air temperatures exceeded 30°C during the spring and summer months.

The growth rate of *C. virginica* in our study varied with the duration of tidal emersion, with oysters in the mid intertidal exposure treatment showing significantly slower growth, inferred from a smaller size at week 5, than those continually submerged (see Table 4). Roegner & Mann (1995) reported that growth rates of *C. virginica* exposed >25% of the time were markedly slower than those of oysters immersed for longer periods. Similarly, Mackin (1946) found growth rates of intertidal *C. virginica* to decrease with increasing exposure time. In our study we were unable to compare growth rates of *C. ariakensis* across tidal exposure levels due to the low survival of this species in all intertidal treatments. The highest growth rates for either oyster species in our study were observed for *C. ariakensis* growing in the subtidal treatment, especially on the more sheltered down- and north-facing tiles. Bishop & Peterson (2006a), investigating the relationship between duration of aerial exposure and growth rates for triploid *C. ariakensis* found that immersion time, and thus available feeding time, provided a good predictor of growth rate during early-to-mid winter. This relationship, however, was seasonally dependent and immersion time failed to predict differences in growth rates between oysters growing at different tidal elevations at other times, because of the indirect effects of competition with other suspension feeders in subtidal treatments.

The outcomes of competitive interactions can be greatly affected by conditions of the physical environment that create trade-offs between competitive ability and tolerance of abiotic stress. Krassoi et al. (2008) demonstrated an example of such a trade-off on the east coast of Australia for interactions between the native Sydney rock oyster, *Saccostrea glomerata* and the Pacific oyster, *C. gigas*, introduced to Australasia for aquaculture (Dinamani 1991). At mid and low intertidal elevations, *C. gigas* rapidly overgrew the native oyster, *S. glomerata*, while at high intertidal elevations, where *C. gigas* suffered very high mortality (~80%), *S. glomerata* was unaffected by the presence of the invader. There are clear parallels here with the potential interactions between *C. virginica* and *C. ariakensis*, at least at subtidal and lower intertidal elevations.

The poor survival of *C. ariakensis* in intertidal habitats has implications for its potential to (1) become established in high salinity environments, (2) interact with native oyster populations, and (3) pose a potential fouling nuisance. In high salinity environments of the Gulf of Mexico and Middle and South Atlantic coasts, high abundances of *C. virginica* are generally only observed in the intertidal zone where this oyster species achieves a partial refuge from predation and competition (Galtsoff & Luce 1930, McDougall 1942, Mackin 1946, Chestnut & Fahy 1952, Ortega 1981). If introduced to the region, poor survival by *C. ariakensis* in the intertidal should lead to spatial segregation between the native and non-native oyster species, at least in high salinity environments, reducing the likelihood of localized competition for food and space. Furthermore, *C. ariakensis* shells require less force to be crushed than those of *C. virginica*, making them more susceptible to predation by portunid (*Callinectes sapidus*) (Bishop & Peterson 2006b) and xanthid crabs (Newell et al. 2007). Bishop & Peterson (2006b) suggest that this increased susceptibility to predation might prevent *C. ariakensis* from reaching population densities sufficient to support a commercial fishery. This may be especially true in high salinity environments that exhibit higher predation pressure. The inability of *C. ariakensis* to use the

intertidal refugia exploited by the native oyster, *C. virginica* may ultimately affect its ability to successfully establish populations in high salinity environments. Poor survival of *C. ariakensis* in the intertidal zone will also limit its potential to become an intertidal fouling nuisance on shoreline stabilization and mooring structures.

#### ACKNOWLEDGMENTS

The authors thank Stephanie Bonniwell and Lynn Walker for their assistance through hatchery support and tile manipula-

tion. Reade Bonniwell provided invaluable expertise in the installation of the simulated tidal emersion systems through his plumbing and electrical wizardry. Al Curry and Raija Bushnell provided additional oversight at the quarantine facility and helped with the day-to-day maintenance of the experiment and photographing of tiles. The authors also thank PG Ross for his statistical consultations in the analyses for this manuscript. This work was supported by a grant from the NOAA Chesapeake Bay Office (NA04NMF4570429). This is contribution number 2918 from the Virginia Institute of Marine Science.

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