Crassostrea Ariakensis In Chesapeake Bay: Growth, Disease And Mortality In Shallow Subtidal Environments

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INTRODUCTION

In recent years, Perkinsus marinus and Haplosporidium nelsoni, two protozoan parasites, have contributed to the dramatic decline in populations of the eastern oyster, Crassostrea virginica (Gmelin, 1791), in Chesapeake Bay and hindered restoration efforts in many areas. Introduction of the Suminoo oyster, Crassostrea ariakensis (Fujita, 1913), has been proposed as a means of rehabilitating the oyster industry and restoring oyster reef habitats in Chesapeake Bay. Previous studies have indicated that C. ariakensis had higher growth rates and lower parasite-related mortality than C. virginica (Calvo et al. 2001). Experiments by Calvo et al. (2001), designed to assess the potential use of triploid C. ariakensis as an aquaculture species, were conducted in floating racks at sites in Virginia and used populations of C. ariakensis and C. virginica that differed in disease history, initial rearing environment, and ploidy. In that study, the C. ariakensis were triploid and deployed directly from the hatchery (i.e., not previously infected by P. marinus), whereas the C. virginica to which they were compared were diploid, older and had acquired P. marinus infections in the previous year. These factors likely contributed to the differences in growth and mortality observed between species in that study because triploid oysters have been shown to grow faster than diploids (Allen & Downing 1986) and that second year infections of P. marinus are typically more virulent than first year infections (Paynter & Burreson 1991).

Our goal was to control for these factors and to assess the performance of C. ariakensis in shallow subtidal habitats, thereby broadening our understanding of the potential utility of C. ariakensis as part of an ecological restoration program aimed at restoring oyster reefs in Chesapeake Bay. We examined growth, disease acquisition, and mortality in side-by-side field grow-out experiments. We used hatchery-produced triploid oysters of both species and deployed them simultaneously in shallow subtidal environments at four sites encompassing an environmental gradient from polyhaline southern to oligohaline northern regions of Chesapeake Bay. We found that after 28 mo, C. ariakensis grew to a larger mean size than C. virginica at all sites. However, the differences in final size were relatively small at the Maryland sites but large at the Virginia site. Because P. marinus prevalences in our experimental triploids were relatively low even after a third summer of exposure at the Virginia site, we compared P. marinus prevalences in diploid C. virginica of similar deployment history at the Virginia site and found much higher prevalences in those oysters.

METHODS

Triploid juvenile C. ariakensis and C. virginica were placed in mesh oyster cages and deployed at three sites in Maryland (Severn, Choptank, and Patuxent Rivers) and one site in Virginia (York River). Long-term data from nearby NOAA Chesapeake Bay Program monitoring buoys indicated that these sites should provide a range of salinity exposures from 5–25. The methods used in our study were modeled after those of Calvo et al. (2001) to assure comparable results and
biosecurity. All deployments were permitted by the Virginia Marine Resource Commission, Maryland Department of Natural Resources and/or Army Corps of Engineers after consultation with the Ad-hoc Exotic Species subcommittee of the Living Resources subcommittee of the Chesapeake Bay Program.

Juvenile *C. virginica* and *C. ariakensis* for these experiments were produced in October, 2003, at the VIMS oyster hatchery by crossing tetraploids of each species produced in 2002 with diploids of the same species (see Guo & Allen 1994). Diploid *C. virginica* came from local Maryland stocks, whereas diploid *C. ariakensis* came from a North American west coast population. Juveniles of both species were held in flow-through tanks at Cherrystone Aquafarms, Cheriton, VA, until deployed in the field in April 2004. At each site, 800 oysters of each species were split between two replicate subtidal cages resulting in four cages (two of each species) at each site. Experimental units consisted of mesh cages (ADPI, Inc) strapped onto underwater racks that held the cages ~0.25 m above the bottom. To prevent reductions in growth rate because of overcrowding, additional cages were periodically added to the experiment and oyster populations split evenly between old and new cages.

Sites were visited monthly with the following exceptions: a single sampling period was used for November/December 2004 and no samples were taken in March 2005. To assess disease acquisition, oysters were sampled at each site monthly from May through October of 2004 and also in November/December of 2004, in January, May, July and October of 2005, and in August of 2006 (York site only). All live oysters, boxes, and gapers were counted to assess mortality and a subset of live measured each month to assess growth. For growth measurements, 30–40 oysters were haphazardly selected *en masse* from each cage and their shell height measured to the nearest mm. During months chosen for disease sampling, 15 of the measured oysters from each of two cages were sacrificed oysters revealed that *C. ariakensis* shells from the York River site appeared to have more natural spat settlement on them than did the native oyster shells. Counts were made of spat on both valves of each species from the York River site. Spat/valve were compared using a simple *t*-test (*α* = 0.05).

**RESULTS**

**Salinity**

Salinity data from NOAA monitoring buoys near each site showed that the Severn River site was characterized by low salinities during the study period (6–12.9) whereas the Choptank and Patuxent River sites had low to moderate salinities (7.9–12.9 and 8.3–15.5, respectively, see Figure 1). The York River site, adjacent to the Virginia Institute of Marine Science (VIMS), was associated with the highest salinity range (16.5–27; see Fig. 1). Although the salinities were more variable at the York River site, no large or unusual shifts in salinity occurred at any of the sites as a result of extended drought or heavy rainfall during the study. Although the Patuxent site reached a high of 15.5 in October 2005, the Maryland salinities remained within a remarkably small range revolving around 10 during most of the study.

**Growth**

As of August 2006, the effects of species, site, and their interaction on August mean shell height were all highly significant. At each site, the mean shell height of *C. ariakensis* was significantly greater than *C. virginica* (Table 1, 1-way ANOVA at each site, *P* < 0.001 for all sites; Fig. 2). Tests of the effects of site within species found that shell heights at the York River site were significantly greater than the other three
sites for both species. Although salinity is unlikely to be the only difference between our sites (see Fig. 1.) and may not be the primary factor driving observed differences in growth, it is clear that *C. ariakensis* growth rates were significantly greater at the Virginia site than at the Maryland sites where *C. ariakensis* reached a mean shell height of nearly 180 mm.

Growth rates of *C. ariakensis* and *C. virginica* within site were nearly identical at all sites for the first four months (Fig. 2). However, during the first fall season (September to December 2004), *C. ariakensis* continued to grow at summertime rates at all sites except the Patuxent River. After the first fall, increases in shell height over time were remarkably similar between both species within site at all Maryland sites (i.e., the trend lines are nearly parallel; Fig. 2a to c). At the York River site in Virginia, growth rates of *C. ariakensis* and *C. virginica* differed for the majority of the study (Fig. 2d). After growing through the first summer and part of fall at rates nearly identical to *C. virginica*, *C. ariakensis* continued to grow until December 2004 when shell height stopped increasing. In contrast, *C. virginica* grew very little between September and December 2004. Shell growth in both species then restarted after March, but *C. ariakensis* growth was more robust. Growth stopped in *C. ariakensis* during the summer months, and restarted in September 2005, whereas *C. virginica* grew at a slower rate throughout the summer and stopped again in October. Growth in *C. ariakensis* continued throughout the winter of 2005/2006 until June 2006, when it again slowed through August 2006. During the same period, *C. virginica* grew very slowly, increasing in shell height only 13 mm between September 2005 and August 2006 compared with the 45 mm increase observed in *C. ariakensis*.

### Disease

Prevalences of *Perkinsus marinus* (Dermo) infections in both species were typical in annual cycle (peaked in October) and in relation to salinity (the highest prevalence in the oysters raised at the highest salinity). First year infections were detected only in native oysters in Virginia (% infected = 26.7, WP = 0.23). Second year infections were detected in both species of oysters at all sites except in *C. ariakensis* at the Severn site (Fig. 3). Although it is difficult to compare disease prevalences statistically (a single sample of 30 oysters is the historic standard for *Perkinsi sia* diagnosis), Figure 3 shows that *P. marinus* prevalences appear to be similar between oyster species raised at the Choptank and Patuxent sites. However, *P. marinus* prevalence in *C. virginica* grown in Virginia was almost 4 times that of *C. ariakensis* held at the same site. Weighted prevalences (Fig. 4) showed similar trends with higher infection levels in *C. virginica* at the Virginia site. However, it should be noted that all WP values were quite low (all but one are less than 0.20) relative to levels observed during disease-induced mortality events (WP ≥ 2.5).

Disease trends in *C. ariakensis* and *C. virginica* at the York River site showed interesting differences (Fig. 4). By the end of the first growing season (October 2004), 26.7% of the native oysters were infected with *P. marinus* but no parasites were detected in the nonnative oysters. By May of the following year, both species had light infections. By October 2005, 66.7% of *C. virginica* were infected as compared with only 16.7% for *C. ariakensis*. Weighted prevalences were also quite different, showing that the native oyster had substantially more intense infections (although, overall, the infections in all oysters remained relatively light). By August, 2006, infection prevalences and weighted prevalences had not changed substantially. However, the diploid *C. virginica* tested that were the same age and held in trays nearby our experimental oysters showed much higher prevalence (93.1%) and weighted prevalence (2.1) than either triploid group.

### Mortality

Patterns of mortality were very similar throughout the study but differed between sites and, at times, between species within
Mean monthly mortality was less than 2% at all sites in both species. However, we believe several events caused “artifactual” mortality during the course of the experiment. First in the fall of 2004, when oyster cages at the Patuxent site had been transferred into a flow through tank because of heavy seas, water flow was cut off for approximately 72 h and the tank fouled. After this exposure, approximately 10% of the C. ariakensis oysters died, whereas less than 2% of the C. virginica died. In February 2005, a freeze event apparently occurred at the Severn and Choptank sites where an extremely low tide exposed several cages to freezing temperatures. In the Choptank, all oysters in two cages, one of C. ariakensis and one of C. virginica were killed. In the Severn, one cage of C. ariakensis experienced over 60% mortality and one of C. virginica over 25%. The C. ariakensis cage was more exposed than the C. virginica cage and likely resulted in higher mortality of that species at that site. Higher box and gaper counts in the two months subsequent to that event were likely related to it.

Analysis of variance of the monthly mortality data, with freezing outliers removed, showed significant site, species, and interaction effects. In general, mortality was lowest at the low salinity Severn site (0.67 ± 0.15 SEM) and highest at the highest salinity York site (1.36 ± 0.16), with the Choptank and Patuxent sites in between. Species-specific mortality rates at each site are shown in Table 2. One-factor ANOVA showed that mean monthly mortality was not different between species at the Severn or Choptank sites. However, mean monthly mortality was significantly higher in C. ariakensis at the Patuxent site but significantly lower at the York site (Table 2).

A few interesting patterns emerged in the monthly mortality graphs. In the York, mortality in both species appeared to be greater in the summer months—especially C. virginica mortalities, which rose over 5% in 2005 & 2006. In the Patuxent, mortalities in both cages of C. ariakensis rose in late summer/fall of 2004 and 2005, whereas C. virginica showed no change. We believe these mortalities were in association with temporary transfer of the cages into flow-through enclosed tanks during bad weather. In the Choptank, oysters in both C. virginica cages showed a spike in mortality over 10% in September 2004 and a smaller increase in July 2005. C. ariakensis showed no such mortality at that site and Perkinsus marinus infections in the native oysters did not appear to be heavy enough to cause such mortalities.

Recruitment onto C. ariakensis versus C. virginica

In summer of 2004, wild C. virginica larvae settled over our study area in the York River but at none of the Maryland sites. Assessment of spat (juvenile oysters) densities conducted in October 2004 found significant differences in the numbers of spat settled on C. ariakensis versus C. virginica (Mann-Whitney Rank Sum, $P < 0.001$). Almost four times as many spat settled on C. ariakensis (4.0 spat/oyster) than on C. virginica (1.1 spat/oyster) held in side-by-side trays.
DISCUSSION

The suitability of C. ariakensis as an ecological substitute for C. virginica in the Chesapeake Bay depends on the physiological ability of the species to survive and flourish in the Chesapeake ecosystem. This project sought to determine the comparative performance of both species in the field one means of gauging that suitability. One of the main goals was to document the mortality caused in both species by P. marinus infections.

Typically at the York river site, P. marinus will induce mass mortalities in oysters in the second and/or third summer season (Burreson 1991, Paynter & Burreson 1991). However, P. marinus infections in the oysters deployed for this project apparently never reached an intensity level high enough to induce or cause mass mortalities in either species. It is possible that triploidy conveyed some physiological advantage to disease, and thus the experimental oysters were not lethally infected within the expected time frame, although Barber & Mann (1991) reported no differences in disease susceptibility between triploid and diploid oysters. Despite a lack of disease-induced mortality, infection levels were indeed lower in C. ariakensis than in the native oyster and one would predict that disease-induced death would come sooner to the natives than C. ariakensis. However, we could not demonstrate that, therefore, one of the key questions as to the value or suitability of C. ariakensis as an ecological substitute for a disease-intolerant native oyster remains unanswered by this study. It should be noted that Moss et al. (2006) have shown that C. ariakensis can acquire lethal infections of P. marinus under laboratory conditions and, thus, may not be entirely physiologically resistant to the parasite.

During the study period, C. ariakensis outgrew native oysters at all sites, however, differences in growth rates between the two species were much greater at the lower salinity Virginia site than at the lower salinity Maryland sites. A review of the literature indicates that C. ariakensis is generally considered a fast growing oyster. We are unaware of other studies that have specifically addressed differences in growth of C. ariakensis as a function of salinity that included sites with low enough salinities to be comparable to those commonly observed on oyster reefs in the Maryland portion of Chesapeake Bay (see Zhou & Allen 2003 for a review). In Japan, the salinity range for C. ariakensis has been reported from 9–30 (Amemiya 1928), whereas in China, multiple reports of optimal ranges cluster around 10–30 (Zhou & Allen 2003). Thus, the three Maryland sites represented suboptimal growth salinities according to the literature, and our results confirm this conclusion. Other studies (Calvo et al. 2001, Grabowski et al. 2004) have shown similar results, although those studies compared diploid native oyster growth to triploid nonnative growth. Triploid oyster growth is
sometimes more rapid than conspecific diploid growth (Allen & Downing 1986, Barber & Mann 1991). Because the present study compares triploids to triploids, it confirms a true species-associated difference in growth rate. Further, the difference in growth rates associated with salinity is remarkable and may indicate that *C. ariakensis* will not grow as much faster than the native oyster in Maryland waters.

In considering whether *C. ariakensis* is a viable replacement for the native species, disease susceptibility may be even more important than growth rate. *P. marinus* infection rates were low at all sites in both species and did not appear to cause disease-related mortalities in either species. However, *P. marinus* infection has been shown to reduce growth rate in *C. virginica* (Andrews 1965, Paynter & Burreson, 1991). The reduced growth rates in both summer seasons in *C. virginica* groups with *P. marinus* infections suggest that parasite infection may have contributed to the differential growth rates between species, causing a reduction in growth rate in *C. virginica*.

Mortality rates were low throughout the study except for low-tide associated freezing events at the Choptank and Severn sites and apparent anoxic stress at the Patuxent site associated with temporary housing in flow-through tanks. Our suspicion that the differential mortality rates observed in the Patuxent in October 2004 may have been caused by differences in hypoxia/anoxia tolerance between the two species has been supported by recent work by Harlan (2007) demonstrating that *C. ariakensis* is less tolerant of oxygen stress than *C. virginica*. She found that most *C. ariakensis* held under anoxic conditions died within a few days, whereas most *C. virginica* survived over two weeks. Given the frequency of anoxic and hypoxic events in Chesapeake Bay (Mackiernan 1987, Smith et al. 1992), evaluation of the utility of *C. ariakensis* as a substitute for the native species will need to carefully weigh any advantages in disease resistance against the disadvantages of higher susceptibility to oxygen stress.

Interestingly, in the York River where the only measurable spatfall occurred, significantly more native oyster spat settled on *C. ariakensis* than *C. virginica* in 2004. Because our analyses did not control for differences in the total surface area available for settlement on the valves of the two species, additional studies will be required to determine whether spat settle at

| River     | Species    | Mean Monthly Mortality (%) | SE  | N  |
|-----------|------------|----------------------------|-----|----|
| Severn    | *C. ariakensis* | 0.59                       | 0.22| 48 |
|           | *C. virginica*  | 0.76                       | 0.22| 48 |
| Choptank  | *C. ariakensis* | 0.40                       | 0.31| 24*|
|           | *C. virginica*  | 1.46                       | 0.32| 24*|
| Patuxent  | *C. ariakensis* | 1.23                       | 0.22| 48 |
|           | *C. virginica*  | 0.37                       | 0.21| 48 |
| York      | *C. ariakensis* | 0.83                       | 0.22| 47 |
|           | *C. virginica*  | 1.88                       | 0.22| 47 |

* Oysters in one cage of *C. ariakensis* and one cage of *C. virginica* were killed by freezing in 2/2005.

** *C. ariakensis* mortality was significantly higher than that of *C. virginica* at the Patuxent site (1-factor ANOVA; *P* < 0.0078).

*** *C. ariakensis* suffered significantly less mortality than *C. virginica* at the York site (1-factor ANOVA; *P* < 0.0024). No significant differences in mortality were detected between species at the Severn or Choptank sites (1-factor ANOVA).

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Figure 5. Monthly mortality of *C. ariakensis* (•) and *C. virginica* (□) at Severn (A), Choptank (B), Patuxent (C), and York (D) sites. Note that the maximum mortality represented on the Y-axis is 30%. Monthly mortality was calculated as (number of boxes + number of gapers)/(number of live + number of boxes + number of gapers) in each of two sampling cages. Boxes and gapers were removed each month. The spikes in mortality in the Severn and Choptank sites were result of a low-tide freeze in one cage of *C. ariakensis* and one cage of *C. virginica* at each site. The two cages frozen at the Choptank site were removed from the study.
higher rates per unit surface area on *Crassostrea ariakensis*. That said, the differences in surface area were probably not large, because the mean shell height of *Crassostrea* at the time of settlement was about 90 mm and that of *C. virginica* was about 80 mm. This could be an important observation. Because larval settlement is a function of both physical and chemical cues and because hybridization between the two species is unproductive (Allen et al. 1993, Gaffney & Allen 1993), increased settlement rates of native oyster larvae on nonnative oysters might lead to complex interactions between populations of the two species.

In summary, *C. ariakensis* grew more rapidly than the native oyster at all sites. In addition, parasitic infections were substantially lower in the nonnative oysters, which will likely lead to lower disease-related mortalities, although that was not quantified by this study. The lack of large differences in growth rates in Maryland suggests that disease tolerance might be the most important quality *C. ariakensis* could bring to the upper, less saline Chesapeake Bay. Because no studies, including this one, have quantified differences in disease-related mortality between triploids of the two species and at least one study has shown that *C. ariakensis* can acquire lethal infections of *P. marinus* (Moss et al. 2006), it is still too soon to draw definitive conclusions regarding the disease tolerance of *C. ariakensis* in the field or its viability as a replacement for the native species.

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