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SURVIVAL AND GROWTH OF TRIPLOID *CRASSOSTREA VIRGINICA* (GMELIN, 1791) AND *C. ARIAKENSIS* (FUJITA, 1913) IN BOTTOM ENVIRONMENTS OF CHESAPEAKE BAY: IMPLICATIONS FOR AN INTRODUCTION

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ABSTRACT Survival and growth of triploid *Crassostrea virginica* and triploid *C. ariakensis* were investigated at four sites surrounding Chesapeake Bay, United States, that varied in salinity, tidal regime, water depth, predation intensity and disease pressure. Four experimental treatments were established at each site: *C. virginica*; *C. ariakensis*; 50:50 of *C. virginica*: *C. ariakensis*; and shell only. Oysters were deployed at mean shell heights of 12.80 mm and 13.85 mm (*C. virginica* and *C. ariakensis*, respectively), at an overall density of 347.5 oysters m^{-2}. Oyster survival and growth varied significantly with site and species. Survival was significantly higher in *C. virginica* than *C. ariakensis* at the intertidal site, and significantly higher in *C. ariakensis* than *C. virginica* at the highest salinity, subtidal site. Survival did not differ significantly between species at the mid and low salinity, subtidal sites. For both species, survival differed significantly between sites, with lowest survival in both species occurring at the intertidal site. Among the subtidal sites, *C. virginica* survival varied inversely with salinity, whereas *C. ariakensis* had the lowest survival at the mid salinity site. Eight months after deployment *C. ariakensis* were significantly larger than *C. virginica* at all sites. This difference generally persisted throughout the experiment, though the size differences between oyster species at the lowest salinity site were small (< 10%). Shell heights within single-species treatments differed significantly between sites; highest growth rates were observed at the high salinity, subtidal site, whereas lowest growth rates were observed at the high salinity, intertidal site. At low and mid salinity subtidal sites, *C. ariakensis* shell heights were significantly greater in the single-species treatment compared with the mixed-species treatment. *Perkinsus marinus* infections occurred in both species at all sites, with prevalences varying between sites. In *C. virginica*, moderate and high intensity infections were only common at the two higher salinity sites, whereas infections in *C. ariakensis* were generally low to rare. *Haplosporidium nelsoni* infections in *C. virginica* were only observed at the two higher salinity sites and prevalences were generally low. Two out of 53 *C. ariakensis* tested at the high salinity, subtidal site had rare *H. nelsoni* infections. *Bonamia* spp. infections were never observed. Our study supports previous laboratory findings and observations from its native range that *C. ariakensis* survives poorly in intertidal habitats. In subtidal habitats, however, *C. ariakensis* displayed broad environmental tolerances, often exceeding native oyster survival and growth rates. Post-introduction *C. ariakensis* populations would be shaped by the survival and growth patterns described here, but also by their reproductive success, larval survival, predator-prey interactions and prevailing disease dynamics.

KEY WORDS: *Crassostrea ariakensis*, growth, non-native oysters, species introduction, survival

INTRODUCTION

Populations of the native eastern oyster, *Crassostrea virginica* along much of the mid-Atlantic coast of the United States have declined during the last century because of a combination of over-harvesting (Gross & Smyth 1946), habitat degradation (Rothschild et al. 1994), reduced water quality (Seliger et al. 1985), disease (Ford & Tripp 1996, Lenihan et al. 1999) and the interactions among these factors (Lenihan & Peterson 1998). Newell (1988) estimated that the extant population of *C. virginica* in Chesapeake Bay was approximately 1% of the biomass present a century earlier. While many factors have contributed to this decline, epizootics of two oyster diseases, *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX), have played a major role over the past several decades. A declining oyster population and the consequent collapse of the fishery have prompted the consideration of intentionally introducing a non-native oyster species to the region (National Research Council 2004). Mann et al. (1991) and Gottlieb and Schweighofer (1996) have argued that introducing a non-native oyster species with greater resistance to disease would restore both the ecosystem services once provided by *C. virginica* and the commercial oyster fishery.

While there is a substantial historical precedent for introducing a non-native oyster species to replace a declining native oyster species (see Mann 1979, Mann 1983, Chew 1990, Ruesink et al. 2005 for reviews), such introductions into marine and estuarine ecosystems raise serious concerns (Cohen & Carlton 1998, Pew Oceans Commission 2003). Naturalization of the Pacific oyster, *Crassostrea gigas* in northwest Europe is a classic example of the unanticipated spread of a non-native species

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beyond its intended range. *C. gigas* was intentionally introduced to the coastal waters of several European countries during the 1960s and 1970s (e.g., Walne & Helm 1979, Grizel & Héral 1991, Drinkwaard 1999), primarily for aquaculture purposes. Low seawater temperatures were expected to prevent reproduction, thereby restricting its distribution to the introduction sites (Drinkwaard 1999). Wild *C. gigas* populations, however, have spread rapidly through much of northwest Europe, from southern Portugal to northern Germany (Wehrmann et al. 2000, Cardoso et al. 2007), in some cases impacting native benthic communities (e.g., Reise 1998, Reise et al. 2006). The uncontrolled expansion of *C. gigas* in this region is testimony to the need for caution when considering a deliberate non-native introduction (see McKindsey et al. 2007 for review). Given the widely reported ecological and economic costs of non-native species (e.g., Pimentel et al. 2000), deliberate introductions warrant very careful consideration (see Mack et al. 2000, Sala et al. 2000, Lodge et al. 2006).

An initial study by Calvo et al. (1999) examined the potential for introducing *C. gigas* to the region because of its known disease resistance and long history as an aquaculture species. These authors observed that *C. gigas* had comparable or lower growth rates in mid and low salinity waters and was more susceptible to infestation by spionid polychaetes (*Polydora* spp.) than *C. virginica*. In a later study of the Suminoe oyster, *Crassostrea ariakensis*, Calvo et al. (2001) reported low disease susceptibility, limited *Polydora* spp. infestations, and high survival and growth rates over a range of salinities, prompting the current consideration of its introducing to Chesapeake Bay (National Research Council 2004).

The initial study by Calvo et al. (2001) and subsequent field studies (Grabowski et al. 2004, Hudson et al. 2005, Paynter et al. 2008) comparing the growth of triploid *C. ariakensis* and *C. virginica* used cultchless, triploid *C. ariakensis* grown in predator-exclusion cages suspended above the bottom. Two of these studies (Calvo et al. 2001, Grabowski et al. 2004) compared triploid *C. ariakensis* to diploid *C. virginica* and thus must be interpreted with caution because triploidy enhances growth rates in oysters (Allen & Downing 1986). In addition, the suspension of oysters above the bottom in predator-exclusion cages limits their exposure to the stresses commonly encountered in benthic environments, including predation, siltation, food limitation and inter- and intraspecific competition. When Grabowski et al. (2004) evaluated the impacts of suspension above the bottom on *C. ariakensis, C. gigas* and *C. virginica* they found that while all three species exhibited higher survival and growth rates when suspended above the bottom, the magnitude of this difference was greater for triploid *C. ariakensis* than for either triploid *C. gigas* or diploid *C. virginica*. Finally, all prior studies have stocked predator exclusion cages with a single species of oyster, precluding evaluation of the effects of interspecific competition between *C. ariakensis* and *C. virginica*.

In an effort to provide a more realistic assessment of the potential performance of *C. ariakensis* if introduced to the Chesapeake Bay region, we compared the survival and growth of triploid *C. virginica* and triploid *C. ariakensis* in both intertidal and subtidal "natural" bottom habitats, across a range of salinities. Oysters of the two species were set on shell and grown side-by-side in single-species and mixed-species treatments. Our objective was to assess the capacity of *C. ariakensis* to survive and grow in bottom reef environments, and to tolerate the interspecific competition it would face if introduced to Chesapeake Bay and coastal environments along the mid-Atlantic seaboard.

MATERIALS AND METHODS

Production of Triploid Oysters

To reduce the risk of an unintentional introduction of *C. ariakensis* and to ensure that results were not confounded by differences in ploidy, all oysters used in these experiments were triploids produced by the Aquaculture Genetics and Breeding Technology Center (ABC) at the Virginia Institute of Marine Science (VIMS) in Gloucester Point, VA. Triploid *C. ariakensis* were produced in a quarantined hatchery in accordance with protocols established by the International Council for the Exploration of the Seas (ICES 1988). For both species, triploids were produced by crossing tetraploid males with diploid females, as described in Guo et al. (1996). Oysters were spawned on 16 June 2005 and larvae were set on aged oyster shell in the first week of July 2005. To facilitate species identification in mixed-species treatments, *C. virginica* were set on left valves and *C. ariakensis* were set on right valves. Prior to deployment, the ploidy status of 3000 randomly selected *C. ariakensis* was assessed using flow cytometry, as described in Guo & Allen (1994). Analyses revealed the presence of a single diploid oyster, satisfying the criteria set by the United Sates Army Corps of Engineers permit for deployment (<0.1% diploid oysters).

After setting, juvenile oysters (spat) were held for approximately three months in land-based flow-through tanks at ambient temperature and salinity at the VIMS Eastern Shore Laboratory (ESL) in Wachapreague, VA and the University of Maryland Center for Environmental Science's (UMCES) Horn Point Laboratory (HPL) in Cambridge, MD. During this holding period, excess spat were removed from shells to achieve target densities of 5–13 spat per shell. Oyster shells were then randomly assigned either to an experimental treatment or to be recounted and measured for final estimates of deployment sizes and densities.

Study Sites

Four field sites were selected to encompass a range of tidal environments, predicted salinities, predicted disease pressures and predicted relative predator abundances (Table 1, Fig. 1). The high salinity Machipongo River location was an intertidal site chosen to reflect the distribution of native oysters in the salt marsh lagoon system on the eastern side of the Delmarva Peninsula. The three sites within Chesapeake Bay were all subtidal, and included one high salinity site (York River, VA), one mid salinity site (Patuxent River, MD) and one low salinity site (Severn River, MD). The York River site was shallower (1–2 m) than the two Maryland sites (3–4 m). At each site a YSI 600 XLM meter measured temperature and salinity at 15-min intervals throughout the course of the 23 mo experiment.

Experimental Design

The experiment used a 4 × 4 block design with one replicate of each treatment in each of two blocks at each of four sites. The

TABLE 1.

Field site characteristics and predicted disease pressures and relative predator abundances.

Site	Tidal Regime	Depth (m)	Salinity (psu) (Avg.; Range)	Predicted Disease Pressure[a]	Predicted Relative Predator Abundance[b]
Machipongo	Intertidal	0–2	High (25.8; 3–34)	High Dermo High MSX	Highest
York	Subtidal	1–2	High (16.5; 8–22)	High Dermo High MSX	High
Patuxent	Subtidal	3–4	Mid (11.6; 8–16)	Low Dermo No MSX	Moderate
Severn	Subtidal	3–4	Low (9.6; 3–14)	No Dermo No MSX	Low

[a]Supporting citation for *a priori* prediction of disease patterns across sites: Calvo et al. (1999).
[b]Supporting citation for *a priori* prediction of predation patterns across sites: White & Wilson (1996).

blocking factor, proximity to shore, was chosen to account for differences in elevation of the substratum between blocks at the intertidal site and was expected to have minimal influence at subtidal locations. The four experimental treatments within each block consisted of two single-species treatments (*C. virginica* only and *C. ariakensis* only), one mixed-species treatment (50:50 *C. virginica*: *C. ariakensis*), and a control treatment (oyster shell only). The shell only treatment served as a control for the presence of live oysters for investigations of differences in macrofaunal diversity and abundance between treatments to be reported elsewhere (Harwell et al. in prep). The other treatments were used to examine the effects of three factors and their interactions—site (four total), species (*C. ariakensis* or *C. virginica*; nested within block) and single/mixed (single- or mixed-species treatment, used to investigate the effects of the presence or absence of interspecific competition, nested within species)—on survival and growth.

Regrettably the process of producing triploids from diploid-tetraploid crosses results in a small proportion of diploid offspring. For this reason, treatment plots at each site were arranged to maximize the distance between treatments containing *C. ariakensis*, thereby reducing the likelihood of successful fertilization between diploids across plots during the course of the experiment. To further increase biosecurity, the eight experimental treatment plots at each site were deployed within large galvanized steel-framed cages (3.05 m × 3.05 m × 0.61 m high; one replicate cage per treatment per block at each site).

The cage design was a compromise between biosecurity concerns and the desire to allow predation, facilitate adequate water flow and reduce fouling. The design was intended to allow predation by small benthic organisms such as xanthid and portunid crabs, and gastropods, while preventing the disturbance or loss of oysters caused by large predators (e.g., cownose rays, *Rhinoptera bonasus*), human activities (e.g., illegal fishing) and storms. To this end, the sides of cages and the access doors on the tops of cages were covered with 5-cm diagonal mesh galvanized chain-link fencing. A gap slightly larger than 5 cm remained between two hinged access doors on the top of the cage, however, potentially allowing access to larger predators capable of swimming. Cage bottoms were lined with plastic mesh (6.4 mm diameter Vexar). Each cage contained a 5 × 5 array of plastic trays (58.4 cm × 58.4 cm × 7.4 cm deep, with 0.64 cm diameter holes). Each tray was lined with 2-mm fiberglass window screen and filled with enough oyster shell to completely cover the bottom of the tray.

Oysters were deployed at all four sites between October 27 and November 1, 2005 by haphazardly placing shells set with experimental oysters within each of the 25 trays in their previously-assigned experimental treatment plots. The target density of oysters for all treatments was 400 oysters m^{-2} (= 136 oysters $tray^{-1}$). Realized initial densities differed slightly across sites and between treatments (Virginia sites: *C. virginica* = 358.1 oysters m^{-2}, *C. ariakensis* = 325.9 oysters m^{-2}, mixed-species treatments = 342.0 oysters m^{-2}; Maryland sites: all treatments = 353.1 oysters m^{-2}). Mean shell heights of *C. virginica* and *C. ariakensis* at deployment were 12.80 mm (n = 1362, SD = 5.68) and 13.85 mm (n = 1272, SD = 5.45), respectively.

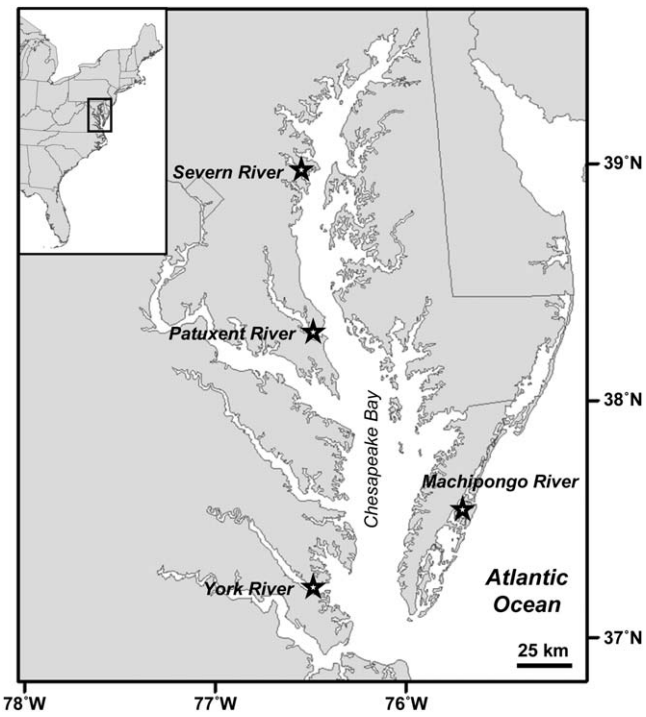

Figure 1. Study site locations in Chesapeake Bay and on the Atlantic coast of Virginia.

Sampling

Sampling occurred one month post-deployment and again in spring, summer and fall of each year (Table 2). Trays within each cage were selected for sampling using a risk-averse sampling design that maximized the distance between unsampled trays remaining in the cage, thereby reducing the likelihood of successful fertilization across trays between any rare diploids that might have been present. During each sampling period, three trays were removed from each cage at each site and replaced with trays filled with clean shell. Prior to its removal from the cage, each tray was capped to prevent the loss of oysters or associated macrofaunal organisms. All 24 trays (3 trays cage^{-1} $\times$ 4 treatments $\times$ 2 blocks) from a site were sampled on a single day and transported to the laboratory for processing.

Once returned to the laboratory, each tray was photographed intact. Valves originally set with experimental oysters were then removed and photographed individually. Care was taken to distinguish between experimental oysters and naturally-recruiting oysters. Naturally-recruiting oysters were easily identified by size because the first episode of natural recruitment occurred in summer 2006, eight months after deployment of experimental oysters. Once photographed, experimental oysters were identified to species, counted, and shell heights measured to the nearest millimeter using a vernier caliper. After all oysters were removed, the remaining material in each tray was washed over a 1-mm mesh sieve and all retained material fixed with 10% neutral buffered formalin for later analyses of macrofaunal abundance and diversity, to be reported elsewhere (Harwell et al. in prep.). Tissue ash-free dry weights were determined for five oysters (of each species in the case of the mixed-species treatment) from each replicate tray by drying all soft tissues at 40°C to a constant weight (1–7 days depending on oyster size) and combustion at 500°C for 5 h. Length-weight regressions were used to estimate total biomass within each treatment.

Disease Diagnostics

Prior to deployment, 25 oysters of each species from each holding facility (UMCES HPL and VIMS ESL) were tested for disease. *Perkinsus marinus* (the causative agent of Dermo disease) infections were assayed using Ray's fluid thioglycollate medium (RFTM, Ray 1952). Infections by *Haplosporidium nelsoni* (the causative agent of MSX disease), *Bonamia* spp. and other metazoan parasites were assessed by standard histological techniques (Burreson et al. 1988). The presence of *H. nelsoni* detected by histology was verified by *in situ* DNA hybridization (ISH) techniques. Disease assays were subsequently conducted in the summer (July) and fall (September to October) of both 2006 and 2007. In summer samplings, where sufficient survival permitted, five oysters were selected from each tray sampled (up to 30 oysters species^{-1} treatment^{-1}); in fall samplings, 10 oysters of each species were selected from each tray (up to 60 oysters species^{-1} treatment^{-1}) to permit a more thorough investigation of *P. marinus* transmission dynamics.

All disease and histology analyses were conducted by the VIMS Shellfish Pathology Laboratory. Following methods from Ray (1954), Mackin (1962), and Burreson et al. (1988), infection intensities were scored as:

$$\text{Weighted prevalence} = ((n_1 * 5) + (n_2 * 3) + (n_3 * 1) + (n_4 * 0.5))/N$$

where n_i = number of oysters rated as (i), i = infection intensity (1 = high, 2 = moderate, 3 = light, and 4 = rare), and N = total number of oysters of each species tested from each site during each sampling period.

Ploidy Testing

Evidence exists that a small percentage of triploid oysters are capable of reverting to a mosaic state (i.e., combination of original triploid cells and subsequently reverted diploid cells), and that this process may be progressive over time (Zhou 2002). We therefore conducted ploidy testing of *C. ariakensis* from each site in each year using methods described in Guo et al. (1996). Briefly, gill tissue samples were collected from each oyster (target $n = 100$ *C. ariakensis* per site), DNA was stained with 10 μg mL^{-1} DAPI-10% DMSO, and DNA contents were analyzed using a PARTEC Cell Cycle Analyzer with UV light excitation. Output histograms of relative DNA content were

TABLE 2.

Deployment phase and sampling event dates for each of the four field sites.

Task	Machipongo	York	Patuxent	Severn
Deployment dates				
Steel cages	26 Sept 2005	29 Sept 2005	30 Sept 2005	12 Oct 2005
Plastic trays	6 Oct 2005	10 Oct 2005	30 Sept 2005	12 Oct 2005
Spat-on-shell	31 Oct 2005	1 Nov 2005	28 Oct 2005	27 Oct 2005
Sampling dates				
Winter 2005	28 Nov 2005	29 Nov 2005	13 Dec 2005	7 Dec 2005
Spring 2006	5 Apr 2006	10 Apr 2006	24 Apr 2006	17 Apr 2006
Summer 2006	5 July 2006	24 July 2006	10 July 2006	17 July 2006
Fall 2006	17 Oct 2006	4 Oct 2006	10 Oct 2006	24 Oct 2006
Spring 2007	18 Apr 2007	n/a	10 Apr 2007	2 & 26 Apr. 2007[a]
Summer 2007	30 July 2007*	n/a	17 July 2007	24 July 2007*
Fall 2007	10 Sept 2007*	n/a	19 Sept 2007	7 Sept 2007*

[a]Incorrect sampling of one tray in one of the *C. ariakensis* only cages on April 2nd 2007 required a return visit to the Severn to sample the correct tray.

* Indicates a reduced number of cages sampled because of reasons outlined in Reductions in experimental design.

used to confirm oyster species identifications and to categorize individual oysters as diploids, triploids or mosaics.

Statistical Analyses

Percent survival was calculated on a per tray basis using counts of live oysters present at each sampling date. Survival data from July 2006 (the last date for which all treatments at all sites were present) met the assumptions of normality (Shapiro-Wilk, $P = 0.058$) and homoscedasticity ($F_{calc} < F_{max}$) and were analyzed using a four-way, fixed-factor ANOVA model with site, block (nested within site), species (nested within block) and single/mixed (nested within species) as factors. The effect of single/mixed treatment was not significant and was therefore removed from the model. We observed a significant site x species interaction ($F = 7.64$, $P = 0.0002$) and a significant block effect ($F = 4.07$, $P = 0.0097$), so we next ran series of two-way ANOVAs, first by site with species and block as main effects, and second by species with site and block as main effects. In cases where block was nonsignificant, we ran reduced one-way ANOVA models with either species or site as the single factor.

Oyster shell height data from July 2006 were initially analyzed using a partially nested, five-way ANOVA with site, block (nested within site), species (nested within block), single/mixed (nested within species), and tray (nested within each species x single/mixed treatment combination) as factors and individual oysters within trays as replicates. Tray was found to be nonsignificant and was removed from the model. Significant interactions were found both for site x species ($F = 137.88$, $P < 0.0001$) and site x single/mixed ($F = 6.50$, $P = 0.0002$), requiring

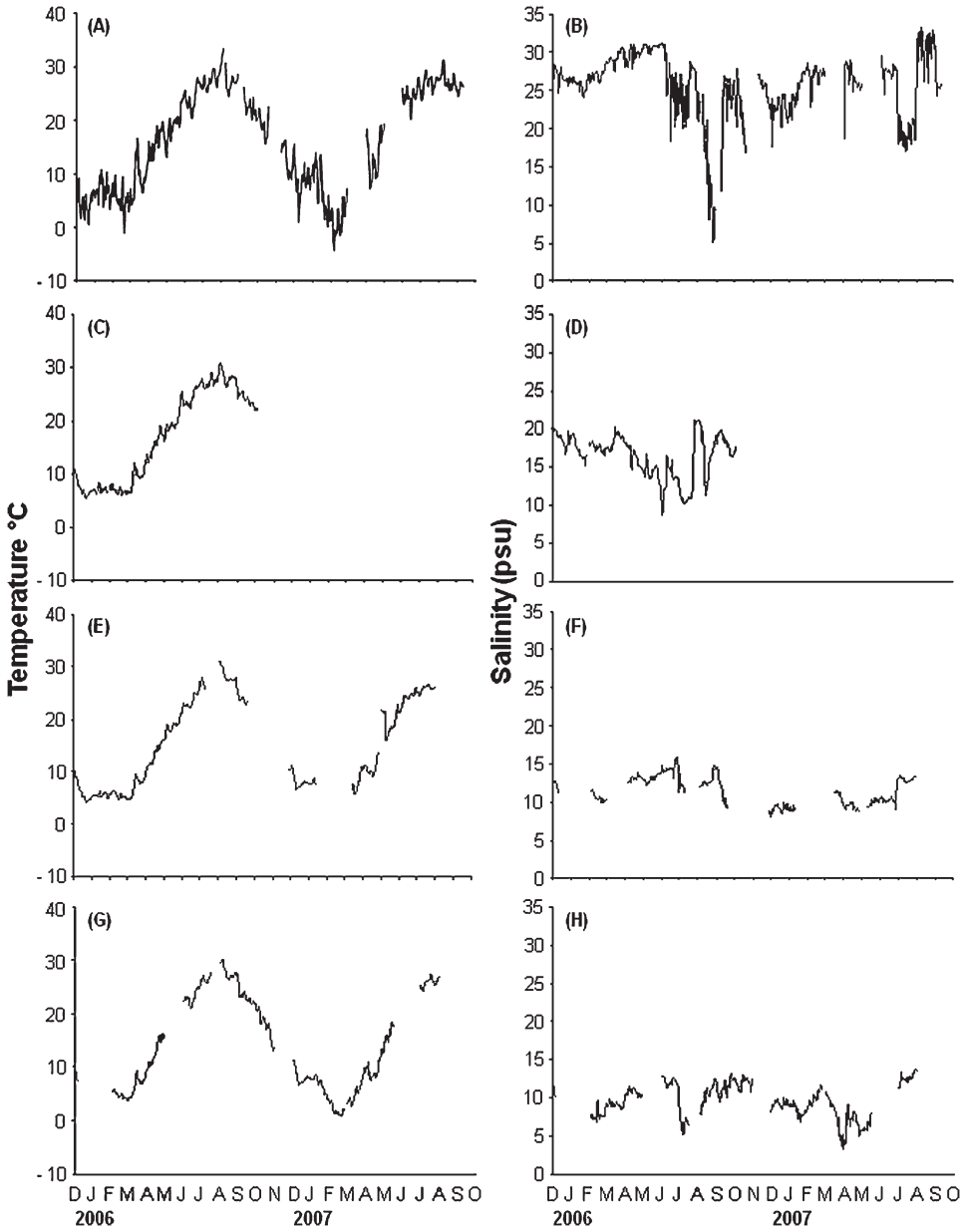

Figure 2. Temperature (left panels) and salinity (right panels) data at 15-min intervals at each of the 4 sites, presented as mean daily values for the Machipongo (A, B), York (C, D), Patuxent (E, F) and Severn (G, H) Rivers from 12/1/05 onwards.

separate testing for the effects of site, species and single/mixed as main effects within appropriate subsets of the data. Each of these subsets was tested for the assumptions of normality and homoscedasticity. In some cases the assumption of normality was not met (Shapiro-Wilk, $P < 0.05$) and we performed a Box-Cox procedure to determine the transformation most closely resembling a normal distribution (Sokal & Rohlf 1981). Having observed a significant block effect in the reduced four-way ANOVA model ($F = 11.16$, $P < 0.0001$), we conducted a series of two-way, fixed-factor ANOVAs for each main effect (site, species or single/mixed) with cage as a blocking factor. In two-way ANOVA models for particular site x species x single/mixed treatment combinations, where block effects were nonsignificant, block was removed from the model and a one-way ANOVA model was conducted. Multiple comparisons for significant effects of site were conducted using Scheffé's test.

Shell height and biomass data for individual oysters were transformed using $\log_{10}$ and $\log_{10} + 1$, respectively, to generate linear regression equations describing oyster growth form by site, species and treatment (i.e., single/mixed). The slopes of these regression equations were compared by generating 95% confidence intervals (C.I.s) and significant differences inferred between regressions when the 95% C.I. for one slope did not overlap the other slope estimate.

RESULTS

Reductions in Experimental Design

During the course of these experiments, three modifications were made to the original experimental design. First, the experiment at the York River was terminated early (Oct 2006) as a result of Tropical Storm Ernesto and a subsequent nor'easter storm in the lower Chesapeake Bay. Although no oysters were released from the cages, there was considerable redistribution of oysters between trays within cages. This limited the value of future data collected from the site and raised enough concerns about biosecurity to warrant early termination of this site. Second, at the Machipongo River site, one block of cages was located at approximately MLLW and the second block at approximately 0.25 m above MLLW. In both Oct 2006 and April 2007 samples, no *C. ariakensis* survived in either the single- or mixed-species cages from the block situated above MLLW. We terminated collection of *C. ariakensis* data from this block, but continued to gather *C. virginica* data from both single/mixed treatments in both blocks through the end of the experiment (Sept 2007). Third, in June to July 2007 illegal fishing activity damaged one replicate cage of both single-species treatments (*C. virginica* only and *C. ariakensis* only) at the Severn River, partly revealed by observations made during July 2007 sampling dives. Extensive search and recovery efforts were made to remove all oysters within and around the damaged cages. While we continued to sample the remaining cages at the Severn site through Sept 2007, data on survival, growth and oyster biomass m^{-2} are presented only up until Apr 2007. Disease data from the Severn site for July 2007 and Sept 2007 were gathered from animals in the remaining single- and mixed-species treatment cages. Table 2 provides a complete list of the deployment and sampling dates for each site.

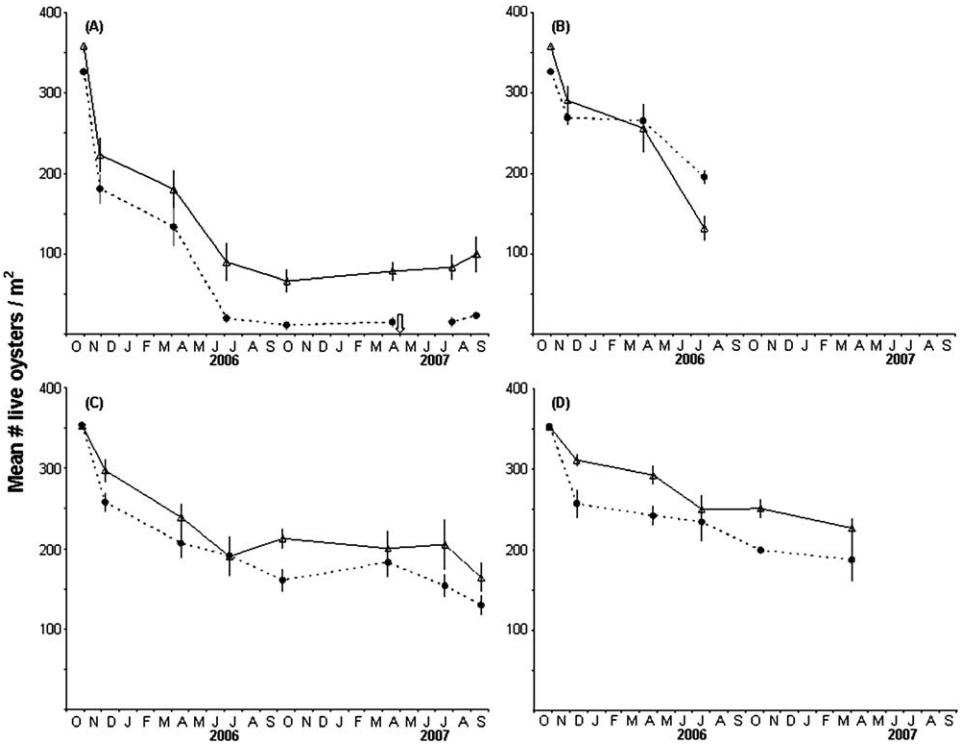

Figure 3. Survival of *C. virginica* (open triangles, solid lines) and *C. ariakensis* (closed circles, broken lines) at each study site: (A) Machipongo, (B) York, (C) Patuxent and (D) Severn. Data are expressed as mean numbers of live oysters m^{-2} (± 1 S.E.) for the single-species treatment only. At the Machipongo, *C. ariakensis* data from July 2007 onwards are from one block only (arrow indicates termination of *C. ariakensis* data collection from the second block).

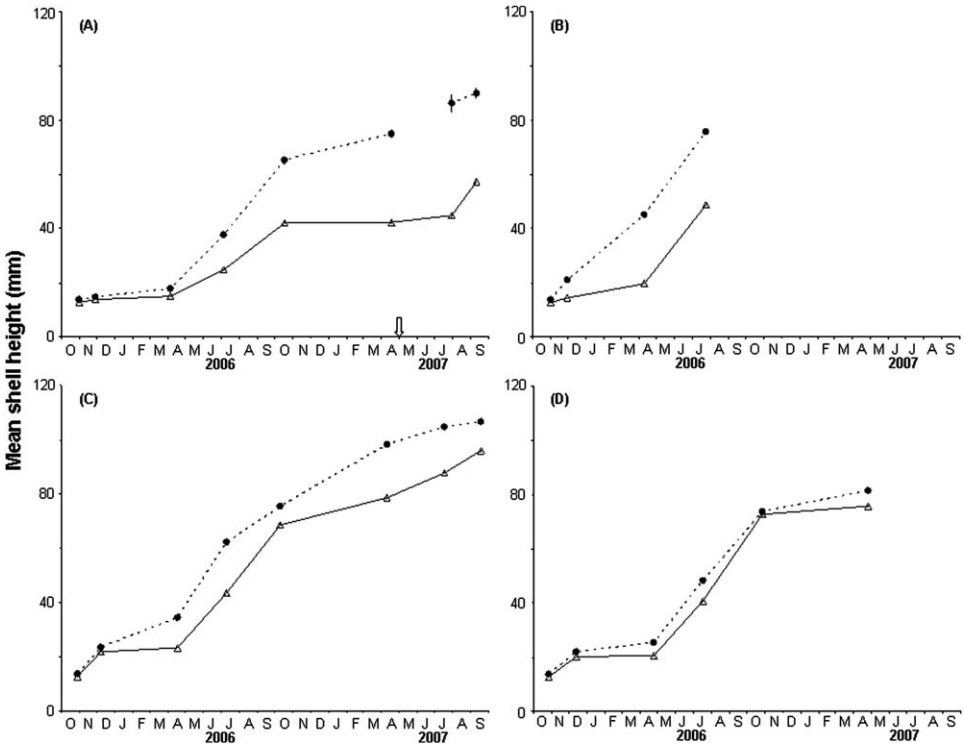

Figure 4. Growth of *C. virginica* (open triangles, solid lines) and *C. ariakensis* (closed circles, broken lines) at each study site: (A) Machipongo, (B) York, (C) Patuxent and (D) Severn. Data are expressed as mean shell height (mm) (± 1 S.E.) for the single- and mixed-species treatments combined. At the Machipongo, *C. ariakensis* data from July 2007 onwards are from one block only (arrow indicates termination of *C. ariakensis* data collection from the second block).

Temperature and Salinity

The intertidal Machipongo River site exhibited greater variability in temperature and salinity than the three subtidal sites (Fig. 2) because it was alternately exposed to seawater and air temperatures within each tidal cycle. Both the lowest (–8.8°C on Feb 6, 2007) and highest (38.0°C on June 21, 2006) individual 15-min interval temperature records observed during this experiment occurred at the Machipongo River site during low tide (not shown by plotting daily averages in Fig. 2A, chosen for figure clarity). Shortly after deployment (Dec 2005 to Feb 2006) and again in the second winter (Dec 2006 to Feb 2007), oysters at this site experienced subzero temperatures. At the three subtidal sites, recorded seasonal temperature profiles were similar across sites (Figs. 2 C, E, & G), showed less short-term temporal variability than the Machipongo River site, and never fell below zero. Salinity was much less variable at the subtidal sites than at the Machipongo River site (Fig. 2), reflecting the close proximity of the intertidal site to the shoreline and its direct exposure to freshwater run-off during rain events. In Aug 2006 several rain events at the Machipongo River site caused the salinity to fall from 24.5 psu on 8 Aug 2006–5.3 psu on 26 Aug. 2006. In general, however, draught conditions prevailed for much of the two-year study period, and salinities at the Patuxent and Severn River sites were elevated compared with long-term averages (Chesapeake Bay Program monitoring data, see http://www.chesapeakebay.net/monitoring.aspx?menuitem=19916).

Survival and Growth

Survival and growth of *C. virginica* and *C. ariakensis* varied across sites (Figs. 3–6, Tables 3–7). During the first month after deployment mortality rates were high at all sites, with a consistent trend of lower survival of *C. ariakensis* (Fig. 3). The lowest survival was observed at the Machipongo River site, where, during the first month, *C. virginica* and *C. ariakensis* suffered 37% and 43% mortality, respectively. Initial growth rates during this first month were similar for both species at the Machipongo, Patuxent, and Severn Rivers; however, at the York River, *C. ariakensis* exhibited higher growth rates than *C. virginica* (Fig. 4).

Over the first winter little mortality was observed at the York and Severn River sites, while both oyster species at the Machipongo and Patuxent River sites experienced 20% to 24% mortality (Fig. 3). Growth rates of *C. ariakensis* during this period were greater than those of *C. virginica* at the York and Patuxent River sites and comparable at the other two sites (Fig. 4).

Between April and July 2006 high mortality was observed for both species at the high salinity sites. At the Machipongo River site, *C. ariakensis* experienced almost complete mortality, while *C. virginica* had over 50% mortality during this period (Fig. 3A), whereas at the York River site (Fig. 3B), mortalities were 26.52% and 48.47% for *C. ariakensis* and *C. virginica*, respectively, over these 3 mo. Growth rates during this period were similar between species with the exception of the Machipongo River site, where *C. ariakensis* grew more rapidly than the native species (Fig. 4A). In general, survival and growth patterns across sites and species were established by the July

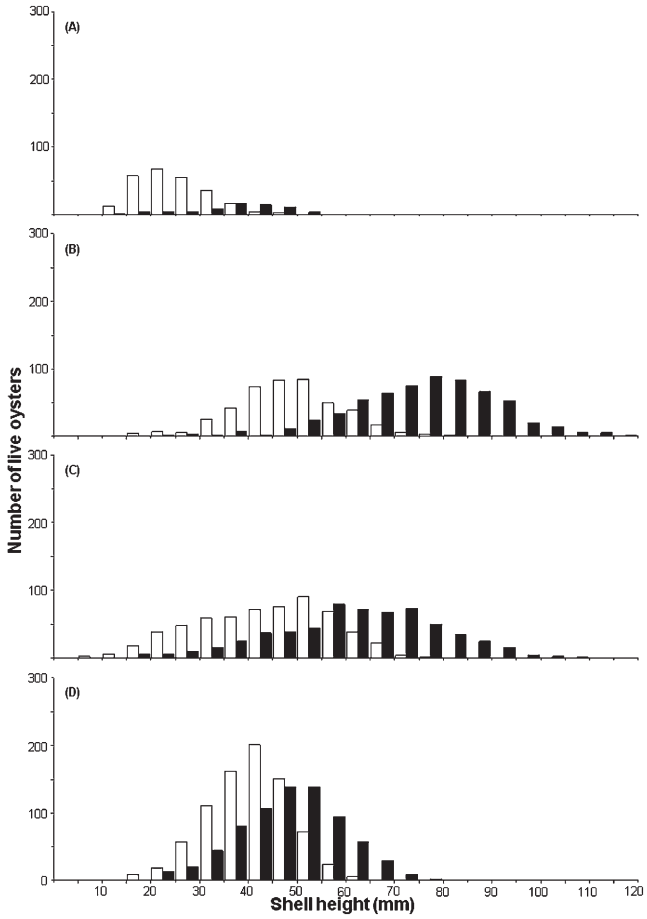

Figure 5. Size distribution of live oysters by site in July 2006. The total number of live *C. virginica* (white bars) and *C. ariakensis* (black bars) within each shell height bin (5-mm) are shown for each site: (A) Machipongo, (B) York, (C) Patuxent and (D) Severn.

2006 sampling date, the last sampling period for which all treatment replicates were intact across all sites.

Significant effects of species on survival were observed at the Machipongo and York River (Table 3). At the Machipongo River, *C. virginica* had higher survival than *C. ariakensis*, presumably because of its greater tolerance of aerial exposure. At the York River, *C. ariakensis* had higher survival rates than *C. virginica*, potentially caused by a partial predation refuge conferred by size. Survival did not differ between the two species at the Patuxent and Severn River sites (Table 3).

Survival of both *C. virginica* and *C. ariakensis* varied significantly across sites in July 2006 (Table 4, Figure 3). For *C. virginica*, mean survival was highest at the Severn River, intermediate at the Patuxent and York Rivers, and lowest at the Machipongo River. *C. ariakensis* survival did not differ significantly between the Severn, York, and Patuxent River sites but was significantly lower at the Machipongo River (Table 4).

In July 2006, significant differences in shell height were observed between species, sites, and single/mixed treatments (Tables 5–7, Figs. 4 and 5). At all sites and for single- and mixed-species treatments, *C. ariakensis* had greater shell heights than *C. virginica* ($P < 0.0001$ in all cases), though these differences were small (<10 mm) at the Severn River (Table 5). For both species, and in both single/mixed treatments, there was a

TABLE 3.

Effect of Species by Site on oyster percent survival from one-way ANOVA.

	Mean % Survival		Effect of Species by Site	
Site	*C. virginica*	*C. ariakensis*	F-value	p-value
Machipongo	21.29	6.38	21.35	<0.0001
York	42.04	62.32	7.99	0.0098
Patuxent	55.99	55.44	0.01	0.9190
Severn	77.94	68.18	2.89	0.1034

Note: Significant block effects occurred for the Machipongo ($F = 16.00$, $P = 0.0006$) and Patuxent ($F = 7.06$, $P = 0.0147$) only, requiring two-way ANOVA models. For the York and Severn block effects were nonsignificant and are not shown.

significant effect of site on shell height (Table 6, $P < 0.0001$ in all cases). For both oyster species, in the single-species treatments, all sites differed significantly from one another, with the York > Patuxent > Severn > Machipongo ($P \leq 0.05$, Scheffé's multiple comparisons tests). Site differences in the mixed-species treatments were more complicated and varied between oyster species; however, the order of ascending mean shell heights mirrored that of the single-species treatments described above. The effect of single/mixed was more variable than site as a main effect, with differences observed in *C. ariakensis* shell heights in only two cases (Table 7). At both the Patuxent and Severn River sites, *C. ariakensis* was larger in the single-species treatment than in the mixed-species treatment.

We observed significant block effects on shell height in several instances (Machipongo: species as main effect in mixed-species treatment, and single/mixed as main effect in *C. ariakensis*; York: species as main effect in mixed-species treatment; Patuxent: species as main effect in single-species treatment, and single/mixed as main effect in *C. ariakensis*; Severn: species as main effect in single-species treatment, and single/mixed as main effect in *C. virginica*; site as main effect for both species and both single/mixed treatments), indicating some effect of the position of the cage within our block design on oyster growth. At the subtidal sites, observations made during sampling events suggest that these effects may have been the result of differential bedload transport of sediments into cages.

Shell height to biomass relationships were computed for each combination of site, species, and treatment by regressing $\log_{10}$ (shell height) *versus* $\log_{10} + 1$ (ash-free dry weight) (Table 8). The resulting regression equations differ between treatments (single/mixed) in only two site x species combinations: (1) at the Patuxent River site for *C. virginica*, where the slope for single-species treatment (slope = 3.210, SE = 0.054) was significantly greater than that for the mixed-species treatment (slope = 3.068, SE = 0.057); and (2) at the Machipongo River site for *C. ariakensis*, where again the slope for the single-species treatment (slope = 3.268, SE = 0.082) was significantly greater than that for the mixed-species treatment (slope = 3.008, SE = 0.066). There was also a consistent pattern of steeper slopes (i.e., more biomass per unit shell height) for *C. ariakensis* versus *C. virginica* across all sites (Table 8).

Shell height to biomass regressions were used to estimate oyster biomass within each site, species and single/mixed treatment combination throughout the study. Reported as

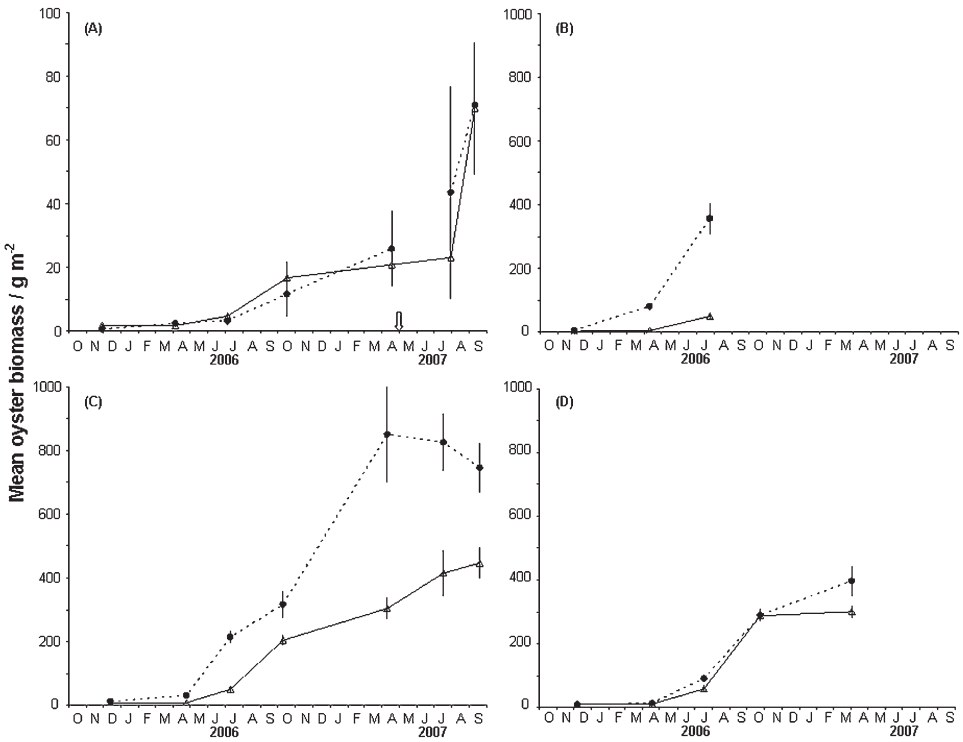

Figure 6. Mean oyster biomass per unit area (g m^{-2}) for the single species cages of *C. virginica* (open triangle, solid lines) and *C. ariakensis* (closed circles, broken lines) at each study site: (A) Machipongo, (B) York, (C) Patuxent and (D) Severn. Error bars are ±1 SE. At the Machipongo, *C. ariakensis* data from July 2007 onwards are from one block only (arrow indicates termination of *C. ariakensis* data collection from the second block). Note that the y-axis scale for the Machipongo River is an order of magnitude lower.

grams ash-free dry weight m^{-2}, these data show the combined effects of survival and growth on oyster biomass at each site for each of the single-species treatments (Fig. 6). Final biomass per unit area was greater for *C. ariakensis* than for *C. virginica* at the three subtidal sites and comparable between the two species in the lower intertidal block at the Machipongo (Fig. 6).

Oyster Disease Status

Few *P. marinus* infections were observed in the July 2006 samples, but by Oct 2006 infections were observed in both *C. virginica* and *C. ariakensis* at all sites (Table 9). The prevalence and intensity of *P. marinus* infections in *C. virginica* varied with salinity throughout the study, as expected, with values generally increasing with salinity. *C. ariakensis* infections also followed this pattern, with the exception that the Patuxent River site had the lowest infection levels in 2007. In *C. ariakensis* most infections were light or rare with a few cases exhibiting moderate prevalence (York River in July 2006 and Patuxent River in Oct 2006). No high intensity *P. marinus* infections were ever observed in *C. ariakensis*. We had not expected to observe *P. marinus* infections at the Severn River; however, infections were observed at low prevalence (20%) and light and rare intensities in both species in Sept 2007. These infection levels are not expected to have caused mortality.

The few cases of *H. nelsoni* infections detected in *C. virginica* and *C. ariakensis* occurred at the two highest salinity sites (Table 10). *H. nelsoni* infections of *C. virginica* were found in July and Oct 2006 samples from the York River and in July and Sept 2007 samples from the Machipongo River. *H. nelsoni* infections of *C. ariakensis*, initially recorded from histological examinations and subsequently confirmed by *in situ* hybridization (ISH) techniques, were found in two oysters from the York River in Oct 2006.

No infections by *Bonamia* spp. were observed in any of the histological tissue samples examined for either oyster species.

Post-Deployment Ploidy Testing of *C. ariakensis*

C. ariakensis were collected for ploidy testing beginning on 10 May 2006 with the collection of 101 samples from the York River site only. All 101 animals tested were triploid with no mosaic or diploid tissue. After the July 2006 sampling, we tested (where available) at least 100 animals from each site for ploidy ($n = 49$, 105, 113, and 100 for the Machipongo, York, Patuxent, and Severn, respectively). Of the total 367 oysters tested, all were triploid. In April 2007, we again conducted ploidy testing ($n = 61$, 113, and 117 for the Machipongo, Patuxent and Severn, respectively; no samples were collected from the terminated York site). Of the 291 animals tested, 290 were identified as triploid *C. ariakensis*. A single animal from the Severn River was triploid but yielded a DNA content indicative of *C. virginica*.

After damage to one of the *C. ariakensis* cages at the Severn River site in June to July 2007, we collected samples for additional ploidy analyses from 99 individuals on July 26, 2007. All oysters were triploid *C. ariakensis*. Thus, of the 858 *C. ariakensis* analyzed for ploidy throughout the course of this experiment, no diploid or genetically mosaic *C. ariakensis* were observed. All remaining *C. ariakensis* were removed from the field at the termination of the experiment in Sept 2007 and we

TABLE 4.

Effect of Site by Species on oyster percent survival from one-way ANOVA. Values with equivalent letters were not different ($P > 0.05$) by Scheffé's multiple comparisons tests*.

	Mean % Survival				Effect of Site by Species	
Species	Machipongo	York	Patuxent	Severn	F-value	*p*-value
C. virginica	21.29[a]	42.04[b]	55.99[b]	77.94[c]	31.17	<0.0001
C. ariakensis	6.38[a]	62.32[b]	55.44[b]	68.18[b]	48.55	<0.0001

*For significant differences between sites ($P < 0.05$), compare letter values within species only.

have no reason to suspect that this research resulted in an unintentional introduction.

DISCUSSION

Survival and growth of *C. virginica* and *C. ariakensis* varied significantly across bottom environments of Chesapeake Bay and adjacent coastal waters. Survival of the two species was comparable at the low and mid salinity sites. *C. ariakensis* had higher survival than the native oyster species at the high salinity subtidal site, whereas at the intertidal high salinity site the native oyster, *C. virginica*, had higher survival than the non-native species. The growth rate of *C. ariakensis* exceeded that of *C. virginica* at all sites; however, this difference was small at the lowest salinity site. Combined survival and growth resulted in comparable production over the 23 mo duration of the study by the two species in the lower intertidal block only at the Machipongo; the higher intertidal block had no surviving *C. ariakensis*. Higher production was observed for *C. ariakensis* than *C. virginica* at all of the subtidal sites. Growth rates of both species were high in subtidal environments, with mean shell heights exceeding 75 mm for each at the two subtidal sites (Patuxent and Severn) by April 2007.

Our data are the first collected on survival and growth rates of *C. ariakensis* in bottom habitats within the Chesapeake Bay region. Direct comparisons between these rates and those observed from studies in off-bottom cages (Calvo et al. 2001, Grabowski et al. 2004, Hudson et al. 2005, Paynter et al. 2008) should be made with caution because other factors (e.g., initial size and density of oysters) varied between studies. There is ample evidence from studies with *C. virginica* that both survival and growth rates of oysters elevated above the bottom (either suspended in the water column or on high relief reefs) exceed those of oysters in low relief bottom habitats (e.g., Lenihan 1999, Lenihan et al. 1996, Lenihan & Peterson 1998, Bartol et al. 1999, Moroney & Walker 1999, Saoud et al. 2000). Thus, we suggest that the rates reported here are more appropriate for predicting survival and growth rates of *C. ariakensis* in bottom habitats than those previously reported.

Our experiments, however, have several significant limitations with regard to estimating natural mortality rates. First, the size of the oysters at the time of deployment (ca. 13-mm shell height) likely limited the effects of small predators such xanthid crabs (e.g., *Panopeus herbstii*, *Eurypanopeus depressus*, and *Rhithropanopeus harrisii*) and the polyclad flatworm, *Stylochus ellipticus* (Newell et al. 2000). Juvenile *C. ariakensis* are more susceptible to predation by xanthid crabs than *C. virginica* (Newell et al. 2007), thus rates of early post-settlement mortality would likely have been higher for *C. ariakensis* had we used smaller oysters. Second, the cages themselves likely reduced predation rates by limiting the access of predators such as large blue crabs, *Callinectes sapidus* (although some were found inside the cages) and cownose rays, *Rhinoptera bonasus*. Because *C. ariakensis* shells are structurally weaker and thus more susceptible to crushing by predators than *C. virginica* shells (Bishop & Peterson 2006, Newell et al. 2007), relative mortality rates for adult *C. ariakensis* would also likely be higher under natural conditions. Lastly, none of our experimental sites were located in areas expected to experience episodes of low dissolved oxygen (DO). Evidence exists that *C. ariakensis* has a lower tolerance of low DO than *C. virginica* (Harlan & Paynter unpublished data).

C. ariakensis exhibited little tolerance for intertidal exposure. At the intertidal Machipongo site, mortality was 43% by the end of the first month. By the end of the first year, all of the *C. ariakensis* in the higher block of cages (approximately 0.25 m above MLLW) were dead and <5% persisted in the lower block (approximately MLLW). This pattern is consistent with previous

TABLE 5.

Sample size (n), mean and standard deviation (SD) of oyster shell height in July 2006 for species by site and single- *versus* mixed-species treatments. F- and *P* values are for the effect of single *versus* mixed treatment from one-way and two-way ANOVAs (when block effects [not shown] are included).

		Shell Height (mm)							
		C. virginica			*C. ariakensis*			Effects of Species	
Site	Single/Mixed	n	Mean	SD	n	Mean	SD	F-value	*p*-value
Machipongo	Single	184	25.05	7.10	41	36.89	8.49	86.56	<0.0001
	Mixed	64	23.98	6.11	22	39.07	11.06	64.99	<0.0001
York	Single	270	48.17	10.26	399	75.06	15.04	659.61	<0.0001
	Mixed	173	49.59	11.85	216	76.55	14.08	412.23	<0.0001
Patuxent	Single	389	44.11	13.31	391	64.04	16.14	366.05	<0.0001
	Mixed	210	42.82	14.23	205	58.93	16.00	114.48	<0.0001
Severn	Single	512	40.82	8.35	479	49.02	10.67	183.45	<0.0001
	Mixed	307	40.58	8.22	253	47.02	10.13	68.89	<0.0001

TABLE 6.

Mean oyster shell height in July 2006 for each site by species and single- versus mixed-species treatments. n and SD associated with each mean can be found in Tables 4 A and C. F- and *P* values are for the effect of single *versus* mixed treatment from one-way and two-way ANOVAs (when block effects [not shown] are included).

		Shell Height (mm)				Effects of Site	
Species	Single/Mixed	Machipongo	York	Patuxent	Severn	F-value	*p*-value
C. virginica	Single species	25.05[d]	48.17[a]	44.11[b]	40.82[c]	216.63	<0.0001
	Mixed species	23.98[c]	49.59[a]	42.82[b]	40.58[b]	90.84	<0.0001
C. ariakensis	Single species	36.89[d]	75.06[a]	64.04[b]	49.02[c]	326.13	<0.0001
	Mixed species	39.07[c]	76.55[a]	58.93[b]	47.02[c]	208.11	<0.0001

*Within single rows, means with different letters are significantly different from one another ($P < 0.05$).

experiments in quarantine (Kingsley-Smith & Luckenbach 2008) and with observations of *C. ariakensis* in its native range (Luckenbach et al. 2005, Wang et al. 2008, Yoon et al. 2008), where it appears to be limited to subtidal and low intertidal locations. The experimental conditions at this site may have resulted in harsher physical conditions than on a natural intertidal oyster reef, where reef interstices provide some buffering from temperature extremes and desiccation. Nevertheless, the observed pattern is consistent with what we know about each oyster species.

Given differing tolerances to intertidal exposure, spatial segregation could develop between the species in high salinity environments, with *C. virginica* occupying the high and mid intertidal zones and *C. ariakensis* dominating the low intertidal and subtidal zones. In high salinity regions of the Gulf of Mexico and lower Middle and South Atlantic coasts of the United States, *Crassostrea virginica* occurs predominantly in the intertidal zone (Coen et al. 1999, Coen & Grizzle 2007), where the lower limit of its distribution is determined by predation and competition (Galtsoff & Luce 1930, Chestnut & Fahy 1952, Dame 1979, Ortega 1981, O'Beirn et al. 1996) and the upper limit by physiological tolerance of exposure (Nichy & Menzel 1967, Michener & Kenny 1991, Roegner & Mann 1995, Shumway 1996). Provided *C. ariakensis* can survive ambient predation and competition, it has the potential to establish populations in subtidal high salinity areas. A similar niche separation driven by aerial exposure tolerance has been described for interactions between native (*Saccostrea glomerata*) and non-native (*C. gigas*) oysters on the east coast of Australia (Krassoi et al. 2008). At mid and low intertidal elevations, *S. glomerata* is rapidly overgrown by *C. gigas*. In the high intertidal, *C. gigas* suffers high mortality and *S. glomerata* appears to be unaffected by the presence of the non-native species. Other examples of spatial separation between native and introduced oyster species have been documented by Walne & Helm (1979) and Andrews (1980).

Our experimental design allowed us to investigate some aspects of competition between the two species. The mixed-species treatment was established by placing equal numbers of shells set with *C. virginica* and shells set with *C. ariakensis* into each tray at an overall oyster density equal to that of the single-species treatments. Although our design did not permit us to evaluate early interspecific competition for space on individual shells, both species grew quickly (especially at subtidal sites) and soon occupied much of the space within each tray. Direct contact between clumps of *C. virginica* and *C. ariakensis* became common. Under these conditions, the two species were expected to compete primarily for food. While we do not expect food to be broadly limiting in the eutrophic Chesapeake Bay, localized food depletion can occur in the immediate vicinity of the oysters (Harsh & Luckenbach 1999). Our experiments found evidence for negative impacts of interspecific competition

TABLE 7.

Sample size (n), mean and standard deviation (SD) of oyster shell height in July 2006 for single and mixed species treatments by site and species. F- and *P* values are for the effect of single *versus* mixed treatment from one-way and two-way ANOVAs (when block effects [not shown] are included).

		Shell Height (mm)							
		Single Species			Mixed Species			Effects of Single/Mixed	
Site	Species	n	Mean	SD	n	Mean	SD	F-value	*p*-value
Machipongo	*C. virginica*	184	25.05	7.10	64	23.98	6.11	0.86	0.3541
	C. ariakensis	41	36.89	8.49	22	39.07	11.06	0.47	0.4960
York	*C. virginica*	270	48.17	10.26	173	49.59	11.85	1.79	0.1822
	C. ariakensis	399	75.06	15.04	216	76.55	14.08	1.30	0.2548
Patuxent	*C. virginica*	389	44.11	13.31	210	42.82	14.23	1.08	0.2990
	C. ariakensis	391	64.04	16.14	205	58.93	16.00	14.78	0.0001
Severn	*C. virginica*	512	40.82	8.35	307	40.58	8.22	0.15	0.6941
	C. ariakensis	479	49.02	10.67	253	47.02	10.13	6.20	0.0130

TABLE 8.

Oyster growth form investigated from $\log_{10}$ shell height in mm (x) to $\log_{10}$ + 1 oyster biomass ash-free dry weight in grams (y) linear regression equations by site, species and treatment (single/mixed). Significant differences between treatments were determined from nonoverlapping 95% C.I.s of slopes (ns = slopes not significantly different from one another).

Site	Species	Single/Mixed	Equation	R^2 value	Slope Comparison Results
Machipongo	*C. virginica*	Single	$y = 3.062x - 2.636$	0.838	ns
		Mixed	$y = 2.892x - 2.427$	0.897	
	C. ariakensis	Single	$y = 3.268x - 2.945$	0.941	Single > Mixed
		Mixed	$y = 3.008x - 2.469$	0.951	
York	*C. virginica*	Single	$y = 3.459x - 3.276$	0.883	ns
		Mixed	$y = 3.326x - 3.083$	0.852	
	C. ariakensis	Single	$y = 3.793x - 3.948$	0.897	ns
		Mixed	$y = 3.767x - 3.865$	0.884	
Patuxent	*C. virginica*	Single	$y = 3.210x - 2.831$	0.945	Single > Mixed
		Mixed	$y = 3.068x - 2.602$	0.934	
	C. ariakensis	Single	$y = 3.348x - 3.103$	0.903	ns
		Mixed	$y = 3.329x - 3.080$	0.911	
Severn	*C. virginica*	Single	$y = 2.843x - 2.288$	0.923	ns
		Mixed	$y = 2.773x - 2.161$	0.926	
	C. ariakensis	Single	$y = 3.272x - 2.992$	0.898	ns
		Mixed	$y = 3.326x - 3.073$	0.910	

on *C. ariakensis* at the low and mid salinity subtidal sites (Severn and Patuxent, respectively; Table 7). At both sites, the mean shell height of *C. ariakensis* was greater in the single-species treatment than in the mixed-species treatment. We found no evidence for reduced growth rates of *C. virginica* as a result of interspecific competition. The patterns we observed imply that interspecific competition with *C. virginica* reduced the growth rate of *C. ariakensis* more than competition with conspecifics. The mechanism by which this occurs, however, remains unclear.

Our analyses frequently revealed significant block effects on oyster growth. These effects are explicable at the Machipongo River, where the blocks differed in tidal elevation, and possibly at the York River, where one block was located closer to the shoreline. Block effects are less readily explained at the sites in Maryland, where the arrangement of cages into blocks did not follow an obvious environmental gradient. It is possible that these effects are related to differences in bedload sediment transport and/or microhabitat conditions that influenced oyster physiology and growth. Importantly, these block effects did not alter the patterns we observed across sites and between species.

Disease did not play a major role in the survival patterns observed in this study. *P. marinus* infections were observed in both oyster species at all sites during the course of the study. Patterns of infection in *C. virginica* and *C. ariakensis* were similar to those observed in previous studies (Paynter et al.

TABLE 9.

Prevalence and intensity of *Perkinsus marinus* (Dermo) infections (n = number of oysters tested).

		C. virginica							*C. ariakensis*						
			%	Intensity level (%)						%	Intensity level (%)				
Sample date	Site	n	infected	High	Moderate	Light	Rare	WP	n	infected	High	Moderate	Light	Rare	WP
July 2006	Machipongo	76	0.00	0.00	0.00	0.00	0.00	0.00	49	4.08	0.00	0.00	4.08	0.00	0.04
	York	60	5.00	0.00	0.00	0.00	5.00	0.03	60	10.00	0.00	1.67	0.00	8.33	0.09
	Patuxent	60	0.00	0.00	0.00	0.00	0.00	0.00	55	0.00	0.00	0.00	0.00	0.00	0.00
	Severn	60	5.00	0.00	0.00	0.00	5.00	0.03	60	3.33	0.00	0.00	0.00	3.33	0.02
Oct 2006	Machipongo	104	63.46	6.73	17.31	34.62	4.81	1.23	37	43.24	0.00	0.00	35.14	8.11	0.39
	York	85	41.18	2.35	10.59	14.12	14.12	0.65	97	14.43	0.00	0.00	7.22	7.22	0.11
	Patuxent	120	35.83	0.00	5.00	22.50	8.33	0.42	119	38.66	0.00	2.52	25.21	10.92	0.38
	Severn	120	5.83	0.00	0.00	3.33	2.50	0.05	119	19.33	0.00	0.00	9.24	10.08	0.14
July 2007	Machipongo	45	86.67	22.22	37.78	17.78	8.89	2.47	24	50.00	0.00	0.00	45.83	4.17	0.48
	Patuxent	60	3.33	0.00	0.00	1.67	1.67	0.03	45	0.00	0.00	0.00	0.00	0.00	0.00
	Severn	44	18.18	0.00	0.00	2.27	15.91	0.10	45	6.67	0.00	0.00	0.00	6.67	0.03
Sept 2007	Machipongo	72	90.28	11.11	45.83	27.78	5.56	2.24	13	46.15	0.00	0.00	46.15	0.00	0.46
	Patuxent	116	28.45	0.00	9.48	12.07	6.90	0.44	118	16.10	0.00	0.00	7.63	8.47	0.12
	Severn	50	20.00	0.00	0.00	16.00	4.00	0.18	50	20.00	0.00	0.00	8.00	12.00	0.14

TABLE 10.

Prevalence and intensity of *Haplosporidium nelsoni* (MSX) infections (n = number of oysters tested).

		C. virginica							*C. ariakensis*						
				Intensity level (%)							Intensity level (%)				
Sample date	Site	n	% infected	High	Moderate	Light	Rare	WP	n	% infected	High	Moderate	Light	Rare	WP
July 2006	Machipongo	56	0.00	0.00	0.00	0.00	0.00	0.00	39	0.00	0.00	0.00	0.00	0.00	0.00
	York	59	27.12	1.69	1.69	15.25	8.47	0.33	60	0.00	0.00	0.00	0.00	0.00	0.00
	Patuxent	60	0.00	0.00	0.00	0.00	0.00	0.00	55	0.00	0.00	0.00	0.00	0.00	0.00
	Severn	60	0.00	0.00	0.00	0.00	0.00	0.00	58	0.00	0.00	0.00	0.00	0.00	0.00
Oct 2006	Machipongo	54	0.00	0.00	0.00	0.00	0.00	0.00	27	0.00	0.00	0.00	0.00	0.00	0.00
	York	51	13.73	5.88	5.88	0.00	1.96	0.48	53	3.77	0.00	0.00	0.00	3.77	0.02
	Patuxent	60	0.00	0.00	0.00	0.00	0.00	0.00	60	0.00	0.00	0.00	0.00	0.00	0.00
	Severn	60	0.00	0.00	0.00	0.00	0.00	0.00	60	0.00	0.00	0.00	0.00	0.00	0.00
July 2007	Machipongo	45	4.44	0.00	0.00	2.22	2.22	0.03	24	0.00	0.00	0.00	0.00	0.00	0.00
	Patuxent	60	0.00	0.00	0.00	0.00	0.00	0.00	45	0.00	0.00	0.00	0.00	0.00	0.00
	Severn	44	0.00	0.00	0.00	0.00	0.00	0.00	45	0.00	0.00	0.00	0.00	0.00	0.00
Sept 2007	Machipongo	67	1.49	0.00	1.49	0.00	0.00	0.04	13	0.00	0.00	0.00	0.00	0.00	0.00
	Patuxent	60	0.00	0.00	0.00	0.00	0.00	0.00	60	0.00	0.00	0.00	0.00	0.00	0.00
	Severn	50	0.00	0.00	0.00	0.00	0.00	0.00	50	0.00	0.00	0.00	0.00	0.00	0.00

2008). Prevalences of *C. virginica* with moderate to high *P. marinus* intensity infections only exceeded 15% at the Machipongo River site, where in July and September 2007 weighted prevalences reached values (2.0–2.5) associated with disease-induced mortality events in the field (Audemard et al. 2006, Paynter et al. 2008). All *P. marinus* infections in *C. virginica* at the Severn River site, and most of *P. marinus* infections at the Patuxent River, were scored as light or rare. Although there was no indication of high mortalities associated with these infections, it is likely that had the experiment run longer *C. virginica* at the Machipongo and York River sites would have experienced some mortality associated with Dermo disease (Audemard et al. 2006). *P. marinus* infections were observed in *C. ariakensis* at all sites, but only during Oct 2006 at the Patuxent River, when 3 of 119 animals tested exhibited moderate intensity infections, did the intensity levels exceed light or rare. Moss et al. (2006) have demonstrated, however, that under laboratory conditions, *P. marinus* is capable of causing lethal intensity infections in *C. ariakensis*.

H. nelsoni infections were only observed in *C. virginica* at the Machipongo and York River sites, where prevalences and intensities remained generally low. At the final sampling of the York River in Oct 2006, 12% of *C. virginica* sampled had high or moderate *H. nelsoni* infections. If the experiment had continued longer at this site, some mortality from MSX disease would likely have occurred.

The observation of *H. nelsoni* infections in *C. ariakensis* tissue samples collected from the field is, to the best of our knowledge, novel. Infection intensity was scored as rare in both of the oysters in which the parasite was found, and there was no sign of advanced MSX disease. Despite the lack of previous reports of *H. nelsoni* infections in *C. ariakensis*, it is not particularly surprising to find infections with low prevalence and intensity. The York River site, adjacent to the VIMS campus, has been used for numerous oyster field studies for at least the past 50 years, and is known to be an area with high MSX disease pressure (e.g., Calvo et al. 1999). Moreover, the presence of low level, nonpathogenic *H. nelsoni* infections in the closely-related Pacific oyster, *C. gigas*, has been previously reported (Burreson et al. 2000). An alternative interpretation of this novel finding is sample contamination. The histological identifications of the parasite as *H. nelsoni* were confirmed by *in situ* DNA hybridization; however, no genetic confirmations of the oyster species were conducted. We have no reason to suspect, however, that the oysters in question were not *C. ariakensis*. Each oyster species was set onto a different valve (right valves only in the case of *C. ariakensis*) and we are proficient at distinguishing morphologically between adults of the two species. Nevertheless, it would be prudent to await further findings of *H. nelsoni* infections in *C. ariakensis* before declaring this species susceptible to the MSX parasite.

Histological examinations of tissue samples from 786 *C. virginica* and 649 *C. ariakensis* (see Tables 9 and 10 for temporal and spatial distribution) did not reveal the presence of any *Bonamia* spp. in oysters from this study. Burreson et al. (2004) have reported that *Bonamia* sp. was partly responsible for high mortalities of triploid *C. ariakensis* in high salinity areas of Bogue Sound, NC and Audemard et al. (2008) have suggested that some risk exists for the spread of this parasite to Virginia waters with salinity >20 psu. The lack of *Bonamia* spp. in samples from our experiment is consistent with the results of other oyster disease screening from Virginia to date.

If introduced to Chesapeake Bay or surrounding waters, the invasive potential of *C. ariakensis* will depend on several factors. Some of these have been clarified by this research. Growth of *C. ariakensis* in shallow subtidal bottom habitats comparable to those studied here exceeds that of *C. virginica*, although the differences are small in low salinity environments. Survival of the non-native species is low in the intertidal zone, and its ability to persist in high salinity environments, where native oysters are largely restricted to the intertidal, will depend in part on its ability to overcome high predation rates in the subtidal. It remains unclear whether the ability of *C. ariakensis* to achieve a size refuge from predation *via* a high growth rate is sufficient to offset its increased susceptibility because of its low shell strength. This did seem to be the case at the York River

site, although early postsettlement mortality and overall predator abundances were influenced by our experimental design. It is clear from this study that, if introduced to the region, the ability of *C. ariakensis* to become established and the outcome of its interactions with native oysters will vary significantly with location.

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