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A COMPARATIVE FIELD STUDY OF *CRASSOSTREA ARIAKENSIS* (FUJITA 1913) AND *CRASSOSTREA VIRGINICA* (GMELIN 1791) IN RELATION TO SALINITY IN VIRGINIA

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ABSTRACT We examined survival, growth, and disease susceptibility of triploid *Crassostrea ariakensis* (=*rivularis*) and compared results with that of diploid *Crassostrea virginica*. Two hundred and fifty oysters (age = 2 yr, mean shell height = 60–64 mm) of each species were deployed at duplicate sites, (Chesapeake Bay, and the Atlantic Coast of Virginia) within low, medium, and high salinity regimes respectively (< 15‰, 15–25‰, > 25‰). Over the course of the study, from June 1998 to September 1999, *C. virginica* exhibited low survival, modest growth and high disease susceptibility. In contrast, *C. ariakensis* exhibited high survival, high growth rate, and low disease susceptibility. At low salinity sites, final mean cumulative mortality of *C. virginica* (81%) was significantly higher than that of *C. ariakensis* (14%). At medium and high salinity sites, all *C. virginica* died before the end of the study whereas final mean cumulative mortality in *C. ariakensis* was 13 to 16%. After 1 year of deployment, mean shell height of *C. virginica* at low, moderate, and high salinity sites was respectively 70, 80 and 73 mm. In comparison, mean shell height *of C. ariakensis* was respectively 93, 121 and 137 mm. At low salinity sites, mean growth rate of *C. virginica* was not significantly different from that of *C. ariakensis*. At medium and high salinity sites, mean growth rate of *C. virginica* was significantly lower than that of *C. ariakensis*. Prevalence and intensity of *Perkinsus marinus* infections were significantly higher in *C. virginica* than in *C. ariakensis*. During the second summer of disease exposure, prevalence in *C. virginica* was 100% at all sites whereas in *C. ariakensis* it ranged from 0 to 28%. Heavy intensity of infections were prevalent in *C. virginica* whereas infections in *C. ariakensis* were limited to light intensity. *Haplosporidium nelsoni* (MSX) was present in *C. virginica*, but absent in *C. ariakensis*. Mud worms (*Polydora* spp.) were present in both oyster species, but infestations were low and did not appear to affect condition or growth. In summary, wide salinity tolerance and low disease susceptibility were associated with high survival and growth of *C. ariakensis* in Chesapeake Bay and the Atlantic Coast of Virginia.

KEY WORDS: Chesapeake Bay, aquaculture, species introduction, *Crassostrea ariakensis, C. virginica*

INTRODUCTION

In contrast to extensive information available on the eastern oyster *Crassostrea virginica*, and Pacific oyster *Crassostrea gigas*, reports on the Suminoe oyster *Crassostrea ariakensis* (= *C. rivularis*), are scarce. Suminoe oysters have been reported to be naturally distributed from southern Japan along the south China coast through southeast Asia to the western coast of the Indian subcontinent, but the taxonomy is tenuous in some areas and its actual distribution not clearly known (Carriker & Gaffney 1996).

Larval settlement for *C. ariakensis* is reported to occur primarily in estuarine areas with low salinity, but juvenile and adult oysters grow within a wide range of salinity (Guo et al. 1999, Ahmed et al. 1987, Cai et al. 1992). Cultivation is important in southern China using seed oysters collected from the wild (Guo et al. 1999). On the West Coast of USA, where *C. ariakensis* was introduced with shipments of *C. gigas* and kumamoto oysters (*Crassostrea sikamea*, Anemiya 1928) from southern Japan in the 1970s (Breese and Malouf 1977), its aquaculture potential has been established (Langdon and Robinson 1996). Using field experiments to compare the growth of *C. ariakensis* and *C. gigas*, Langdon and Robinson (1996) found that both species had similar growth and meat condition at various locations along the West Coast.

To the best of our knowledge, no parasitic diseases have been reported in Suminoe oysters within its native range. However, in Zhanjiang Bay, southern China, mass mortality of *C. ariakensis* has been associated with outbreaks of toxic phytoplankton blooms (Yongjia et al. 1995). In Marennes Oleron, France, mortality in association with *Bonamia*-like parasites was observed in quarantined *C. ariakensis* animals exposed to *Bonamia ostreae* endemic waters (Cochennec et al. 1998).

Studies on the potential performance and disease susceptibility of Suminoe oysters are not available for the Atlantic Coast of USA. However, as native eastern oyster stocks collapsed throughout much of the mid-Atlantic seaboard due to over harvesting, disease, and water quality deterioration, interest in the potential use of non-native oyster species has grown. Following a Virginia program to examine the suitability of non-indigenous oyster species to the local environments (VIMS 1996), *C. gigas* was the first species to be evaluated in Chesapeake Bay and the Atlantic Coast of Virginia (Calvo et al. 1999). Over the course of that study, from May 1997 to 1998, *C. gigas* had lower disease susceptibility than *C. virginica*, but survival and growth were equal or superior in native oysters than in *C. gigas* within Chesapeake Bay. Based on its close resemblance to the native oyster and its tolerance of temperate to sub-tropical environments, *C. ariakensis* was the second candidate species selected for testing in Virginia (VIMS 1996). Considering its documented ability to grow in a wide range of salinity, we hypothesized that *C. ariakensis* would perform better relative to *C. virginica* than had *C. gigas* in Chesapeake Bay. The objectives of the present study were to compare survival, growth, disease susceptibility and infestations by shell boring polychaetes in *C. ariakensis* and *C. virginica* deployed over a range of salinity.

MATERIALS AND METHODS

Study Sites

Six sites were selected based several criteria including salinity regime, geographic location, available information on oyster growing conditions and water quality, safety, logistics, and relevance

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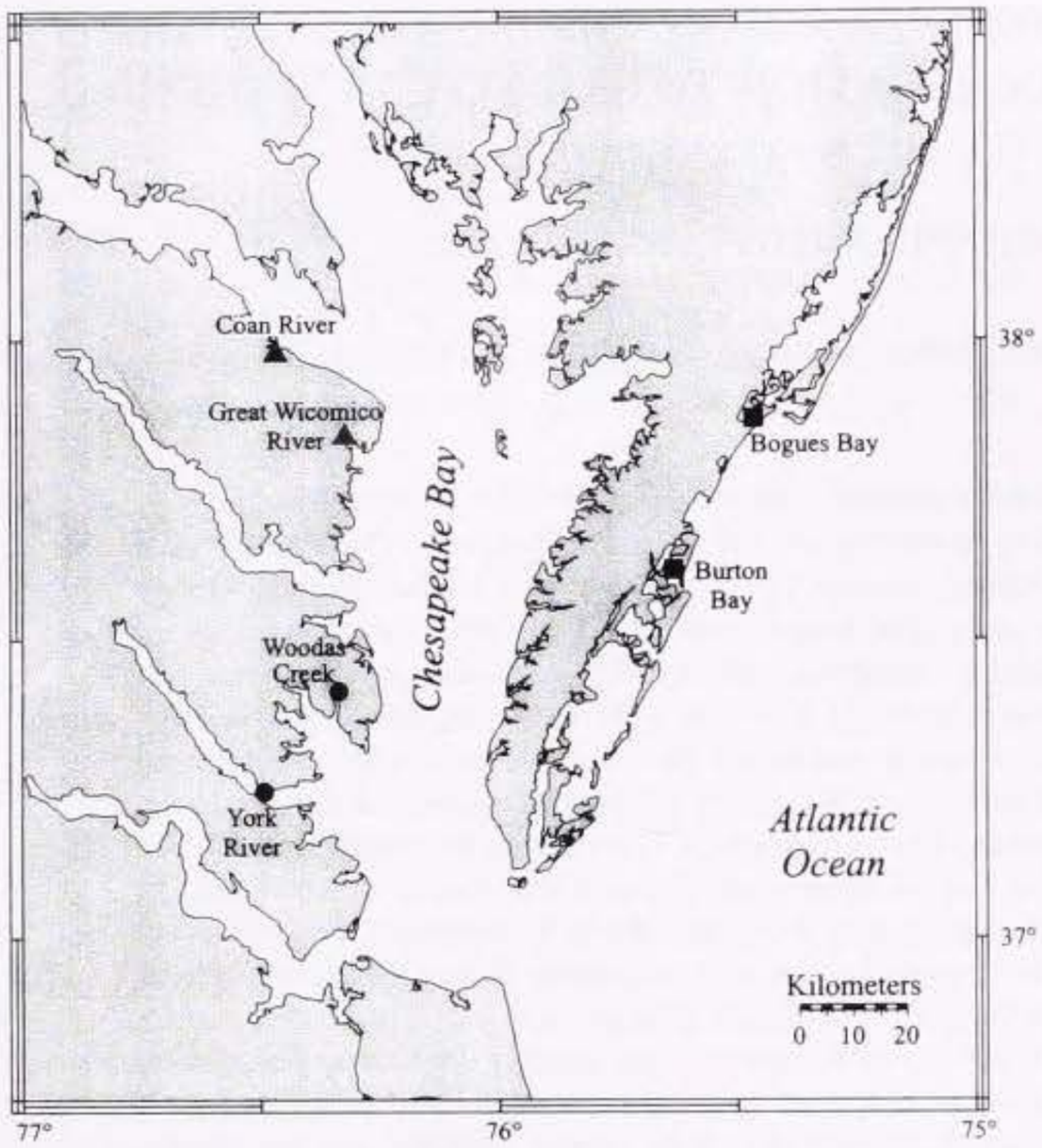

Figure 1. Location of study sites in Chesapeake Bay and the Atlantic Coast of Virginia: ▲ = Low salinity (<15‰) sites, ● = Medium salinity (15–25‰) sites, ■ = High salinity (>25‰) sites.

for the oyster industry. Sites were established at duplicate locations within low salinity (<15‰), medium salinity (15–25‰), and high salinity (>25‰) areas (Fig 1). Low and medium salinity sites were established near the margins of rivers (Coan, Great Wicomico, and York) or in shallow creeks surrounded by marshes (Woodas Creek, a tributary of the East River). High salinity sites were located in well-flushed narrow channels surrounded by marshes and mudflats in the coastal lagoon system of the Atlantic Coast of Virginia.

Temperature and salinity were measured during monthly site visits with a stem thermometer and a refractometer. To further characterize environmental variables, hourly temperature, salinity, and turbidity were measured with Hydrolab-Minisonde® dataloggers deployed at the same sites for weekly to monthly intervals.

Oysters

Individually certified triploid *C. ariakensis* were produced and maintained in quarantine first at the Haskin Shellfish Research Laboratory, Rutgers University (HSRL) and then at the Virginia Institute of Marine Science's (VIMS) Aquaculture Genetics and Breeding Technology Center. *Crassostrea ariakensis* brood stock, originating from an established line maintained in quarantine at HSRL and derived from sources on the West Coast of USA, was spawned in July 1996. Triploidy was induced by treatment of fertilized eggs with cytochalasin-B using the methods described by Downing and Allen (1987) and Allen et al. (1989). Juvenile *C. ariakensis* were transferred to flow-through York River water with quarantined effluents at VIMS, where oysters were maintained until they were individually examined for triploidy, as described later, before field deployment in early 1998. *Crassostrea virginica* brood stock, collected from Mobjack Bay, VA was spawned by a local commercial hatchery (Middle Peninsula Aquaculture) in July 1996. Prior to deployment, diploid *C. virginica* juveniles were maintained by Mobjack Bay Seafood in the Ware River, VA.

Experimental Design

Between May 29 and June 2, 1998, adult oysters were dispensed into replicate 9.5-mm mesh bags and placed within individual floating trays at the study sites. There were two replicate sites within each of three salinity regimes (Fig. 1). Each floating tray contained two bags with 100 oysters and one bag containing 50 individually labeled oysters, to follow growth, as described later. Floating trays (2.3 m × 0.5 m × 0.3 m) were constructed by fitting wire mesh trays (25-mm square 16 gauge mesh) into floating frames built with 4-inch (10.16 cm) PVC pipe, following the design of Luckenbach and Taylor (1997). Floating trays and bags were cleaned of fouling organisms at least once a month during regular site visits and more often if necessary. All sites were visited monthly (± 15 days).

Mortality, Growth and Condition

All live and dead oysters within each float were counted monthly to determine survival. Monthly mortality was calculated as the number of oysters that died during each month interval, divided by the number of live oysters at the beginning of the month, corrected for oysters removed by sampling. Cumulative mortality was calculated as the sum of interval mortality (Barber and Mann 1994, Krebs 1972). Mortality data was examined for normality and homogeneity of variance using plots of means versus standard deviations and Bartlett's chi-square test (Zar 1974). A two-way ANOVA was employed to examine the effects of species and salinity on arcsin-transformed cumulative mortality. Statistical analyses were performed using Statview® and Statistica® software.

To follow growth, 50 oysters within each float were individually labeled and shell height was repeatedly measured to the nearest 0.1 mm using calipers, once monthly except January, February, and April 1999. Monthly growth rates for individual oysters were calculated as the overall shell height increment during the growing period while live oysters of both species were still available at all sites, June 1998 to May 1999, and divided by the deployment time in days standardized to 30 days. When oysters died measurements were taken from the remaining individuals without replacement. Growth rate data was examined for normality and homogeneity of variance using the same tests specified above for mortality data. The effects of species and salinity regime on arcsin-transformed mean growth rate were examined using a two-way ANOVA followed by a Newman-Keuls test.

At the end of the experiment, in September 1999, whole weight, shell weight, tissue wet and dry weights were measured on the same oysters collected for disease diagnoses. Following Lawrence and Scott (1982), Condition Index (CI) was calculated by the formula:

$$CI = \text{tissue dry weight} * 100 / (\text{whole weight} - \text{shell weight}). \quad (1)$$

Oysters were allowed to air-dry for 15 to 20 minutes before weighing and whole oyster weight was recorded to the nearest 0.01g. Oysters were then shucked, shells weighed to the nearest 0.01g, and wet tissues were gently rolled on a paper towel and weighed on pre-tarred vessels to the nearest 0.001g. Wet tissues were dried at 80°C overnight and tissue dry weight was measured the next day

to the nearest 0.001g. Condition index data was examined for normality and homogeneity of variance using the same tests specified above for mortality data. Non-parametric statistics were employed because means and standard deviations were still highly correlated (r = 0.952) after transformation. Mann-Whitney tests were used to examine differences in mean condition index and mean ranked body weights between species. Kruskal-Wallis tests were employed to examine differences in the above parameters among salinity regimes.

Diseases and Polydora

A baseline sample of 25 oysters was taken to assess the disease status of each species prior to deployment in May 1998. Subsequent samples of each species at each site were collected in August and September 1998, and in May, August, and September 1999. *Perkinsus marinus* was diagnosed using Ray's fluid Thioglycollate medium (RFTM) assays (Ray 1952) on combined mantle, gill, and rectal tissue. Infection intensity was rated based on Ray (1954) and Mackin (1962). For the calculation of weighted prevalence infection intensity was ranked, following Paynter and Burreson (1991), as: 0 = negative, 1 = light, 3 = moderate and 5 = heavy. Light-moderate infections were ranked as 1 and moderate-heavy infections were ranked as 3. Weighted prevalence was calculated by the formula:

$$\text{Weighted prevalence} = \Sigma I^{*} n_I / N \qquad (2)$$

Where I = infection intensity rank
n_I = number of oysters within I
N = total number of oysters examined in the sample.

Prevalence and weighted prevalence data were examined for normality and homogeneity of variance using the same tests specified above for mortality data. Two-way ANOVAs followed by Newman-Keuls tests were employed to analyze the effects of species and salinity regime on arcsin-transformed prevalence and untransformed weighted prevalence.

Haplosporidium nelsoni, the causative agent of MSX disease, was diagnosed using standard paraffin histology procedures with oysters preserved in Davidson's AFA and 6 μm tissue sections stained with Harris' hematoxylin and eosin (Burreson et al. 1988). Infection intensity was rated as light, moderate or heavy based on Burreson et al. (1988). Histology sections were also used to document the presence of other parasites and to examine development of oyster gonads. Disease diagnoses and histology were performed by the VIMS Shellfish Pathology Laboratory.

The spionid polychaetes *Polydora websteri* and *P. ligni* are commensal with bivalves, including oysters. These suspension-feeding worms do not feed on the oyster, but the mechanical irritation caused by their presence causes the oyster to lay down additional layers of conchiolin over the worm's tube in what are often termed mud-blisters. At sufficiently high levels of infestation this can severely limit the growth of oysters and reduce their condition index. Examination for mud-blisters associated with *Polydora* spp. was conducted on the same oysters collected for condition and disease diagnoses in September 1999. Worms were not identified to species, but *Polydora websteri* is the most common species affecting oysters in the northeast coast of the United States (Blake and Evans 1972, Wargo and Ford 1993). The internal surface of right valve shells was visually inspected and rated according to the presence and extent of mud-blisters. Examination was restricted to right valves as in Wargo and Ford (1993) who reported that infestations by *Polydora* spp. were equally found in right and left valves. Following the methods of Handley and Bergquist (1997), infestation was rated as: (0) no visible mud-blisters or any evidence of boring by *Polydora* spp.; (1) mud-blisters affecting less than 25% of the valve; (2) 25–50% of the valve affected; (3) 50–75% of the valve affected; or (4) more than 75% of the valve affected. Weighted prevalence was calculated similarly to equation (2) using the five categories above. Prevalence and weighted prevalence data was examined for normality and homogeneity of variance using the same tests specified above for mortality data. Non-parametric tests were employed because zero variances precluded computation of Bartlett's test for determining homogeneity of variance. Mann-Whitney tests were used to examine differences in mean prevalence and mean weighted prevalence between species. Kruskal-Wallis tests were employed to examine differences in the above parameters among salinity regimes.

Reproductive Status and Ploidy

A baseline sample of *C. ariakensis* was collected to ascertain the percentage of triploid individuals in the lot of animals exposed to Cytochalasin-B as described above. Prior to field deployment, all *C. ariakensis* animals were individually certified as triploids following flow-cytometric methods (Allen 1983). Briefly, experimental animals were notched on the dorsal side of the right valve using a Dremel® rotary tool equipped with a fiberglass-cutting wheel. A 1- ml syringe fitted with a 23-gauge needle was inserted into the adductor muscle and 0.05 ml of hemolymph was removed. A 10 μg/ml DAPI-10% DMSO staining solution was added to the hemolymph and the sample was vortexed, aspirated, and filtered through a 23μm Nitex® screen. DNA content of prepared samples was determined on a PARTEC® Cell Cycle Analyzer via ultraviolet light excitation. Histograms of relative DNA content were used to identify diploid cells with modal DNA values 1.5 times lower than that of triploid cells. Individuals with triploid and diploid cells were categorized as mosaics. A 2mm × 2mm piece of gill tissue, as well as a cross-section of gonad tissue were also sampled from mosaic individuals and examined for DNA content as above. The remaining gonad from mosaic individuals was processed by histology.

Over the course of the experiment, samples of *C. ariakensis* (n = 16–35) were collected from each site in July and August 1998 and in May, June, and July 1999. Ploidy assays were conducted at HSRL and the VIMS Aquaculture Genetics and Breeding Technology Center. A two-way ANOVA was employed to examine the effects of salinity and time on mean arcsin-transformed percentage of mosaics. Significant effects were further examined using a Newman-Keuls test.

RESULTS

Environmental Parameters

Means of monthly salinity measures at the two low salinity sites were below 10% only during June and July 1998. Drought conditions prevailed throughout much of the study and salinities above 15% were recorded at the low salinity sites from November 1998 to March 1999. Medium salinity sites experienced relatively low salinity (<15%) during June 1998, but were between 15 to 25% on all other sampling dates (Fig. 2). Salinity fluctuations in

high salinity sites were within the expected range (25–35‰). Temperature followed similar seasonal trends at all sites with a maximum of 28° to 32°C in July and a minimum of 0° to 5°C in March. High salinity sites experienced overall cooler temperature with monthly means 2° to 4°C lower than medium or low salinity sites (Fig. 2). Turbidity was low (<70 Nephelometric turbidity units at all sites and times tested). Dissolved oxygen was relatively low (60% air-saturation) at Woodas Creek in July 1999 compared to all other sites and times measured (68–87% air-saturation).

Mortality

As the experiment progressed, *C. virginica* percent cumulative mortality rapidly increased whereas *C. ariakensis* mortality remained low. The highest increase in mean cumulative mortality, from 5% to 78%, was observed in *C. virginica* at medium salinity between July and October 1998 (Fig. 3). At the end of the experiment, mean cumulative mortality in *C. virginica* (81%–100%) was significantly higher ($p < 0.0005$) than that in *C. ariakensis* (13%–16%). Salinity had a significant ($p = 0.006$) effect on final cumulative mortality. The interactive effect of species and salinity was also significant ($p = 0.011$) and may be attributed to the increase in mortality between low and higher salinities observed for *C. virginica* whereas low mortality was similarly observed for *C. ariakensis* at all salinities (Fig. 4).

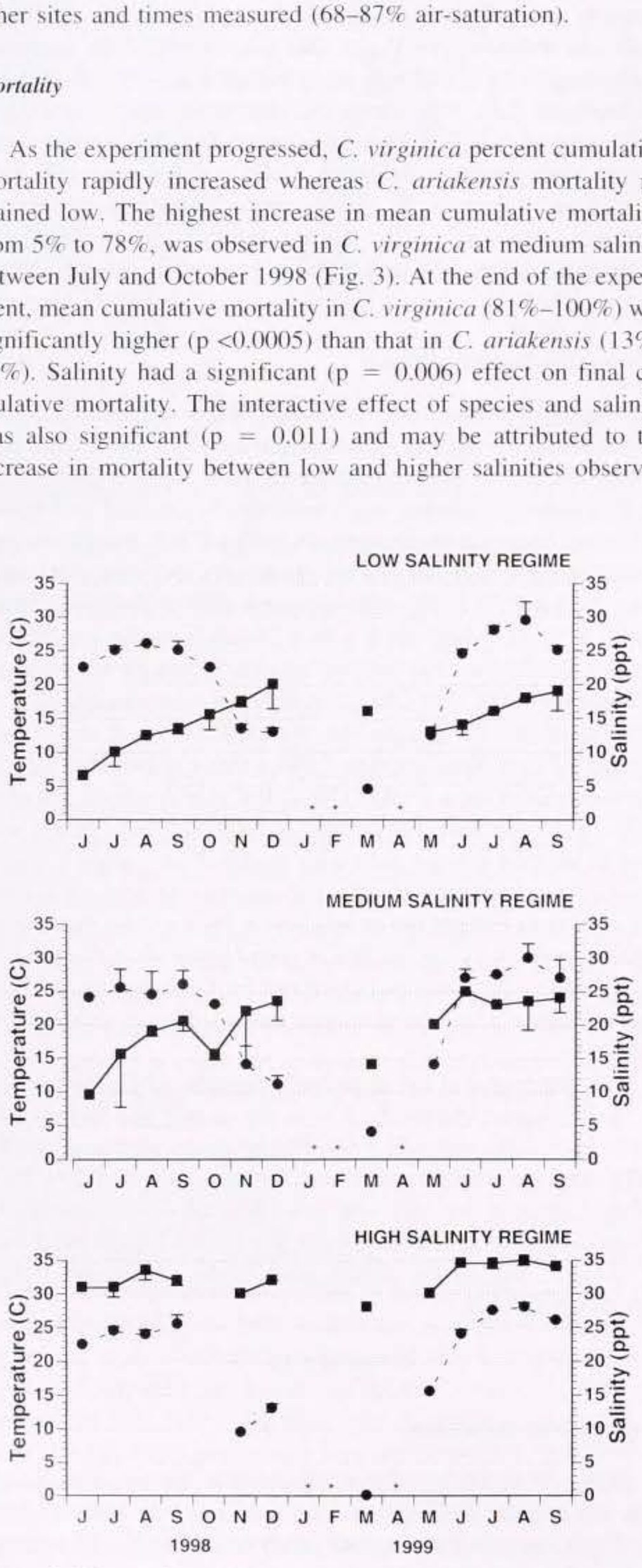

Figure 2. Means of monthly temperature and salinity measurements by salinity regime (N = 2 sites, ± SD), from June 1998 to September 1999. ● = Temperature using stem thermometer. ■ = Salinity using refractometer.

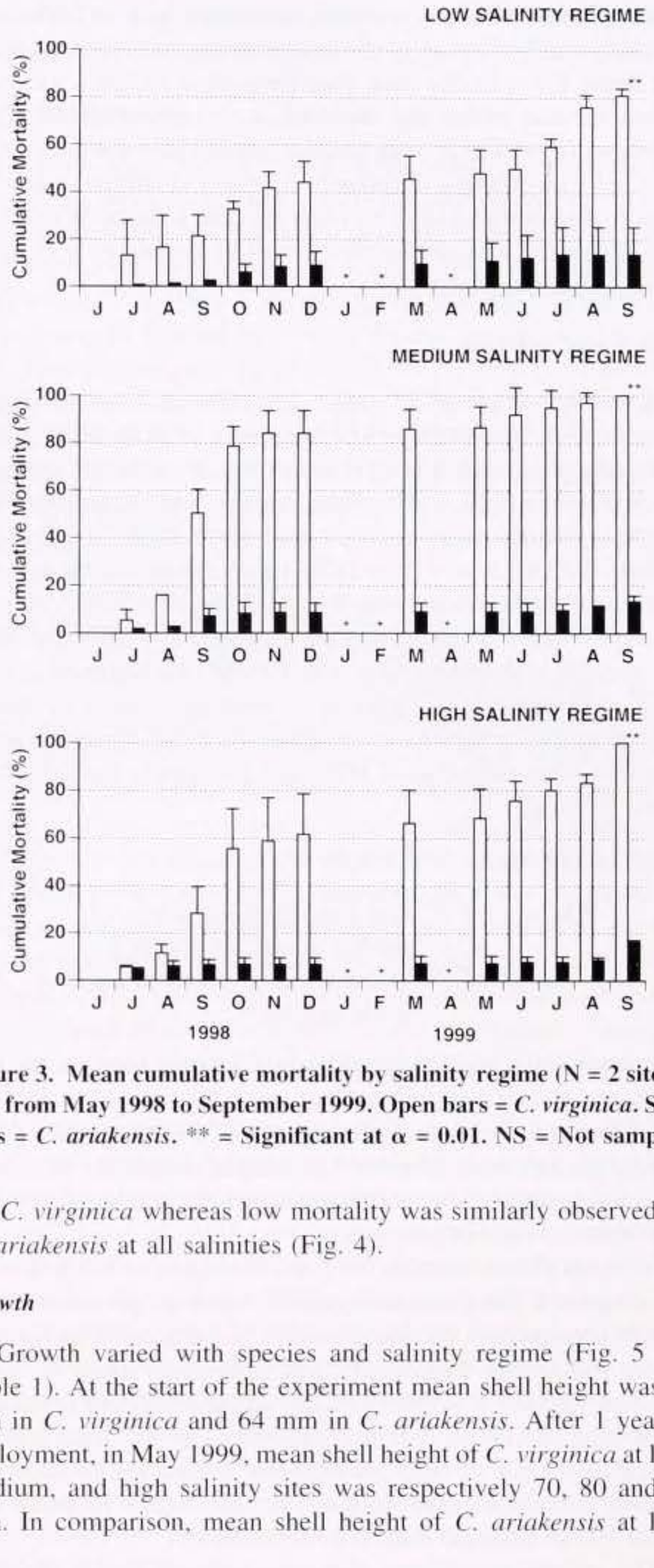

Figure 3. Mean cumulative mortality by salinity regime (N = 2 sites, + SD) from May 1998 to September 1999. Open bars = *C. virginica*. Solid bars = *C. ariakensis*. ** = Significant at $\alpha = 0.01$. NS = Not sampled.

Growth

Growth varied with species and salinity regime (Fig. 5 and Table 1). At the start of the experiment mean shell height was 60 mm in *C. virginica* and 64 mm in *C. ariakensis*. After 1 year of deployment, in May 1999, mean shell height of *C. virginica* at low, medium, and high salinity sites was respectively 70, 80 and 73 mm. In comparison, mean shell height of *C. ariakensis* at low,

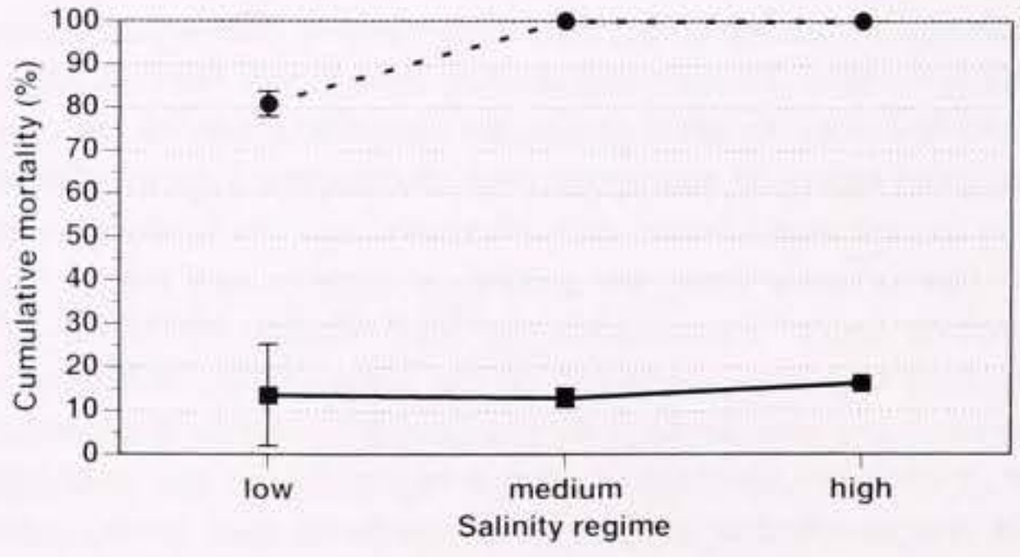

Figure 4. Interaction between oyster species and salinity on final cumulative mortality. Means of 2 sites (± SD). ● = *C. virginica*. ■ = *C. ariakensis*.

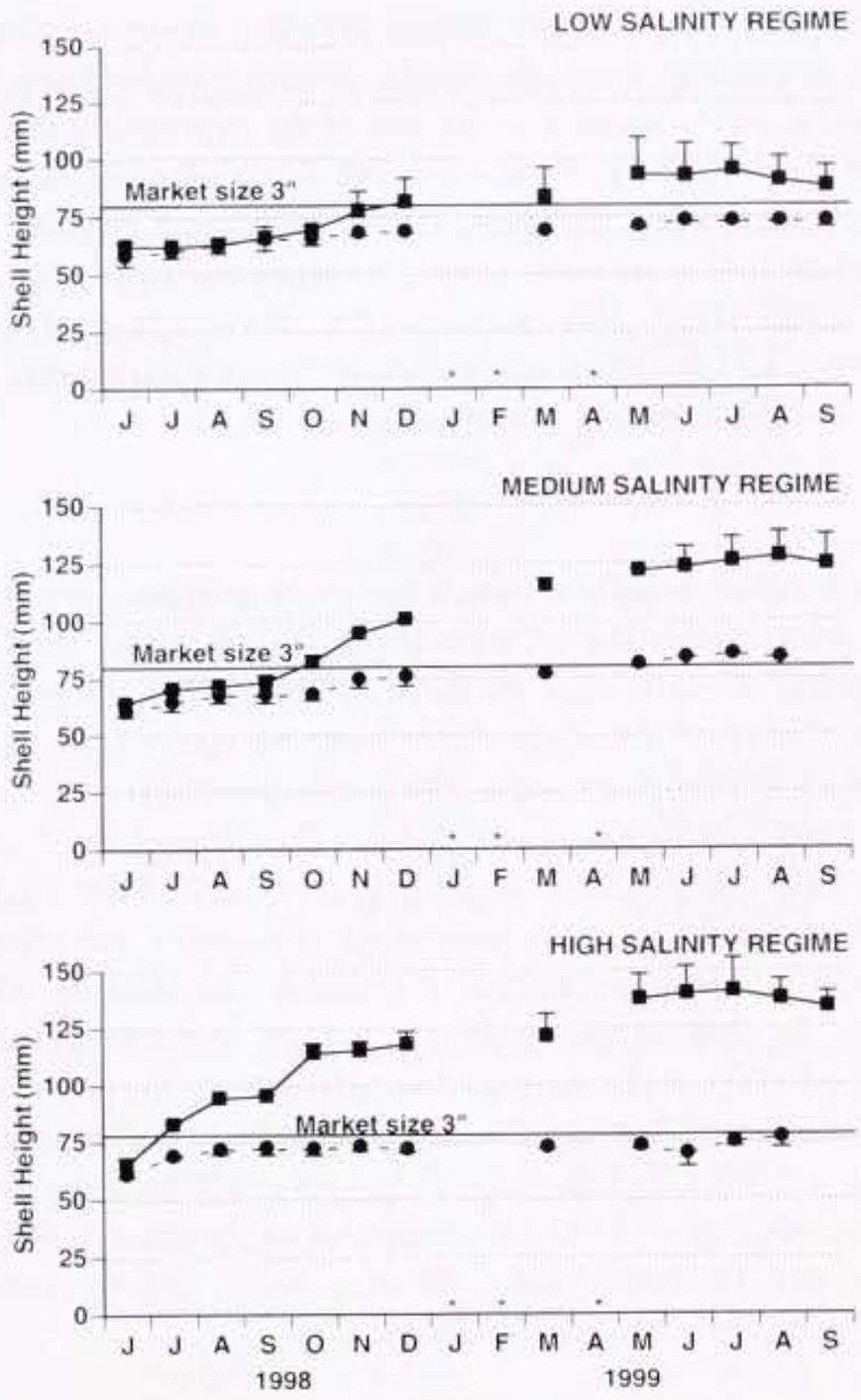

Figure 5. Mean shell height by salinity regime (N = 2 sites, ± SD) of 50 individual oysters repeatedly measured from May 1998 to September 1999. ● 1 = *C. virginica*. ■ = *C. ariakensis*.

TABLE 1.

Effects of species and salinity regime on mean growth rate.

A. Two-way ANOVA

Effect	df	MS	F	p
Species	1	32.293	61.382	<0.0005
Salinity	2	3.441	6.536	0.052
Species*Salinity	2	3.225	6.124	0.076
Error	6	0.526		

B. Multiple comparison (Newman-Keuls test)

Comparison		
Within	**Between**	**p**
Low salinity	*C. virginica* and *C. ariakensis*	0.116
Medium salinity	*C. virginica* and *C. ariakensis*	0.018
High salinity	*C. virginica* and *C. ariakensis*	0.005
C. virginica	Low salinity vs. medium salinity	0.268
C. virginica	Low salinity vs. high salinity	0.931
C. virginica	Medium salinity vs. high salinity	0.440
C. ariakensis	Low salinity vs. medium salinity	0.034
C. ariakensis	Low salinity vs. high salinity	0.018
C. ariakensis	Medium salinity vs. high salinity	0.280

moderate, and high salinity sites, was respectively 93, 121 and 137 mm. Oysters continued to grow until July 1999 when mortality of *C. virginica* approached 100% in all medium and high salinity sites. At that time mean shell height *of C. ariakensis* at low, medium and high salinity sites was respectively, 96, 125 and 140 mm. No growth was observed for either species during July to September 1999. Most of the growth occurred during fall 1998 and spring 1999.

Species and salinity regime had a significant effect on mean growth rate (Table 1A). Similar growth rates were observed for *C. virginica* at all salinities in contrast to increasing growth rates with increasing salinity observed for *C. ariakensis* (Fig 6). At low salinity sites, mean growth rate of *C. virginica* (1.1 mm mo^{-1}) was not significantly different than that of *C. ariakensis* (2.6 mm mo^{-1}). At medium salinity sites, mean growth rate of *C. virginica* (1.7 mm mo^{-1}) was significantly lower than that of *C. ariakensis* (4.9 mm mo^{-1}). At high salinity sites, mean growth rate of *C. virginica* (1.0 mm mo^{-1}) was also significantly lower than that of *C. ariakensis* (6.2 mm mo^{-1}). For *C. virginica*, growth rate did not differ significantly among salinity regimes. For *C. ariakensis*, however, growth rate at low salinity was significantly lower than that at medium and high salinity regimes (Table 1B).

Disease

At the beginning of the study, there was no *P. marinus* and a 4% prevalence of *H. nelsoni* in *C. virginica* and 12% prevalence of *P. marinus* and no *H. nelsoni* in *C. ariakensis* (Fig. 7). In October 1998, prevalence and weighted prevalence of *P. marinus* were significantly higher ($p < 0.0005$) in *C. virginica* than in *C. ariakensis*. In September 1999, when no live *C. virginica* remained at the medium salinity York River site, prevalence in *C. virginica* at all other sites was 100% whereas prevalence in *C. ariakensis* ranged from 0 to 75% and did not differ ($p > 0.250$) among salinity regimes (Fig. 7). Heavy infections were prevalent in *C. virginica* whereas only light infections were observed in *C. ariakensis* (Table 2).

Maximum prevalence of *H. nelsoni* (25%) was observed in *C. virginica* at the York River site in May 1999. *H. nelsoni* was also present in *C. virginica* at the low salinity Great Wicomico River site in September 1998 (4%), and at high salinity sites in October 1998 (4–8%) and May 1999 (4%). Intensity of *H. nelsoni* infections was light except for heavy infections found in oysters sampled from medium (1/132) and high salinity sites (1/157). No *H. nelsoni* was found in *C. ariakensis*. Other parasites observed in histological sections of *C. virginica* were the protozoan *Haplospo-*

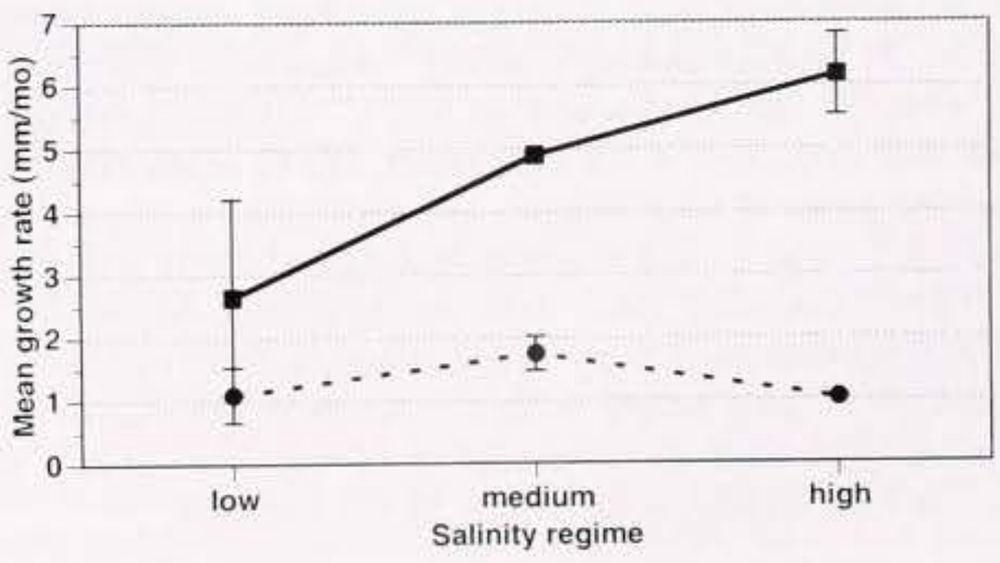

Figure 6. Interaction between oyster species and salinity on mean growth rate. Means of 2 sites (± SD). ● = *C. virginica*. ■ = *C. ariakensis*.

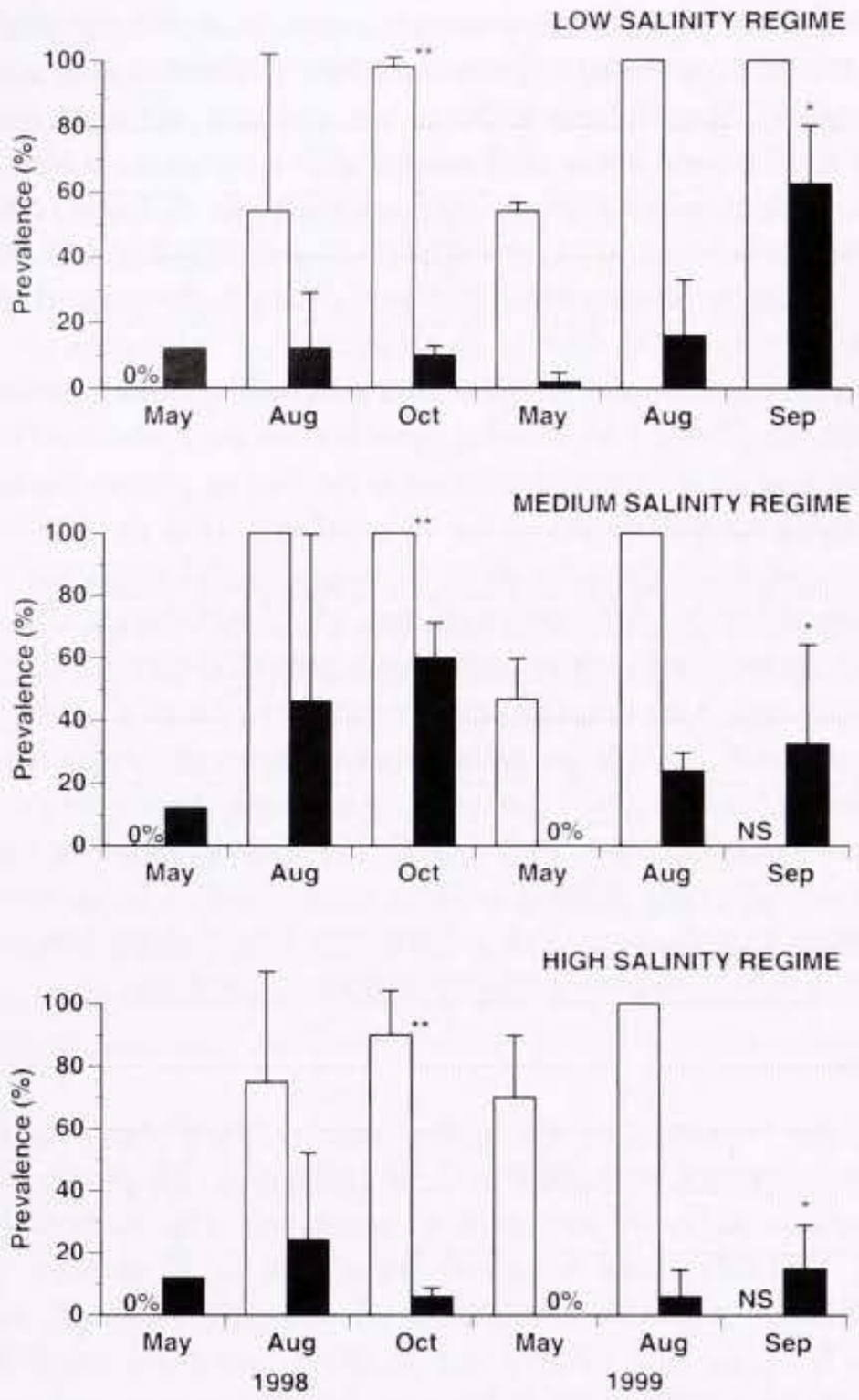

Figure 7. Mean prevalence of *P. marinus* by salinity regime (N = 2 sites, + SD), in samples of 25 oysters, from May 1998 to September 1999. Open bars = *C. virginica*. Solid bars = *C. ariakensis*. ** = Significant at α = 0.01. * = Not significant at α = 0.05. NS = Not sampled because no *C. virginica* remained.

ridium costale (SSO), present at high salinity sites, the trematode *Bucephalus* sp. and a chlamydia-like organism. None of these or other parasites were observed in *C. ariakensis*.

Condition

At low salinity sites, mean condition index in *C. virginica* and *C. ariakensis* were, 3.6 and 6.6, respectively, and means were not significantly different ($p = 0.121$). Similarly, there were no significant differences ($p = 0.121$) in mean body weights between species. At medium and high salinity, comparisons between species were not possible because no live *C. virginica* remained at those sites at the end of the experiment. For *C. ariakensis*, mean condition index at low, medium and high salinity, respectively, were 6.6, 5.3 and 9.7 and means were not significantly different ($p = 0.276$). Similarly, there were no significant differences ($p > 0.102$) between mean body weights among salinity regimes. The percentage of shell weight relative to whole oyster weight in *C. virginica* (62%) was similar to that in *C. ariakensis* at low, medium, or high salinity, respectively, 59, 61 and 65%.

Polydora

At low salinity sites, mean prevalence of *Polydora* spp. was 100% in both oyster species, and there was no significant difference in mean weighted prevalence between oyster species ($p = 0.121$). At medium and high salinity, comparisons between species were not possible because at the end of the experiment there were no live *C. virginica* at those sites. For *C. ariakensis*, there was a trend of decreasing prevalence with increasing salinity. Mean prevalence in *C. ariakensis* at low, medium and high salinity sites were, respectively, 100, 62 and 12%. However, ranked mean prevalence and weighted prevalence were not significantly different ($p = 0.156$) among salinity regimes.

Ploidy

The baseline sample revealed that prior to deployment 94% of the *C. ariakensis* in the lot were triploids. Individual certification assured that triploids were exclusively deployed in the field. During the course of the study, there were 62 individuals in which combinations of diploid and triploid cells (mosaics) were detected out of 1164 oysters examined (5.3%). The proportion of mosaics ranged from 0.0 to 13.8% depending on time and site (Table 3). The effect of time on mean percentage of mosaics was significant ($p = 0.002$). Mean percentage of mosaics increased significantly between the first sampling time and each subsequent sampling time ($p < 0.007$). After the initial sampling time, the percentage of mosaics did not differ significantly among the remaining sampling times ($p > 0.498$). Salinity regime had no effect on mean percentage of mosaics ($p = 0.128$). Examination of 39 mosaic individuals revealed that 10 were females, 23 were males, one was hermaphroditic, and five were undifferentiated.

DISCUSSION

Drought conditions and below normal Chesapeake Bay stream flow starting in fall 1998 resulted in increased salinity and epizootics of both *H. nelsoni* and *P. marinus* during 1999 (Ragone Calvo & Burreson 1999). High disease pressure prevailing in the region was associated with severe infections and high mortality in *C. virginica*, though not in *C. ariakensis*. After the first summer of disease exposure, more than 50% of *C. virginica* had died and prevalence of *P. marinus* at medium and high salinity sites was 100%. A year later when all *C. virginica* at medium and high salinity sites were dead, cumulative mortality at low salinity sites was 81% and prevalence of *P. marinus* was 100% with severe infections present. Maximum prevalence of *H. nelsoni* in *C. virginica* was 25% whereas no *H. nelsoni* was detected in *C. ariakensis*. Presence of *H. nelsoni* and intensification of *P. marinus* infections in *C. virginica* at the low salinity Great Wicomico site was undoubtedly favored by drought conditions resulting in salinity greater than 15% starting in fall 1998 and continuing into spring and summer 1999. Persistence of salinity greater than 15% during summer and fall is conducive to development of lethal *P. marinus* infections (Burreson and Ragone Calvo 1996). In comparison, maximum *P. marinus* prevalence in *C. ariakensis* reached 84%, but infections never exceeded light intensity and mortality remained low (13–16%). With the caveat that this study spanned only 15 months, *C. ariakensis* appears highly tolerant of the dominant parasitic diseases affecting Chesapeake Bay oysters.

A limitation of this study was that conditions were not identical for both species before the beginning of the experiment. Since *C. ariakensis* was quarantined for their first two years in land-based systems with limited inflow of raw York River water, long-term exposure to disease agents may have been reduced in relation to

TABLE 2.

Prevalence and intensity of *P. marinus* in *C. virginica* and in *C. ariakensis* by salinity regime, site and date during 1998 (A) and 1999 (B).

A.

			C. virginica				*C. ariakensis*			
Salinity	**Site**	**Date**	**Prevalence†**	**L***	**M***	**H***	**Prevalence†**	**L***	**M***	**H***
L	CNRV	8/12/98	20% (5/25)	3	2	0	0% (0/25)	0	0	0
		9/30/98	96% (24/25)	18	2	4	12% (3/25)	3	0	0
	GWRV	8/4/98	88% (22/25)	21	0	1	24% (6/25)	6	0	0
		9/30/98	100% (25/25)	12	4	9	28% (7/25)	7	0	0
M	WOCK	8/3/98	100% (25/25)	7	5	13	84% (21/25)	21	0	0
		9/30/98	100% (24/24)	7	7	10	68% (17/25)	17	0	0
	YKRV	8/3/98	100% (25/25)	16	3	6	8% (2/25)	2	0	0
		9/29/98	100% (25/25)	7	11	7	52% (13/25)	13	0	0
H	BUBY	8/6/98	100% (25/25)	20	1	4	44% (11/25)	11	0	0
		10/7/98	80% (20/25)	13	6	1	8% (2/25)	2	0	0
	BOBY	8/6/98	50% (25/50)	19	4	2	4% (1/25)	1	0	0
		10/13/98	100% (25/25)	13	7	5	4% (1/25)	1	0	0

B.

			C. virginica				*C. ariakensis*			
Salinity	**Site**	**Date**	**Prevalence†**	**L***	**M***	**H***	**Prevalence†**	**L***	**M***	**H***
L	CNRV	5/3/99	52% (13/25)	12	0	1	4% (1/25)	1	0	0
		8/2/99	100% (25/25)	10	12	3	4% (1/25)	1	0	0
		9/21/99	100% (14/14)	4	4	6	50% (6/12)	6	0	0
	GWRV	5/3/99	56% (14/25)	11	2	1	0% (0/25)	0	0	0
		8/2/99	100% (24/24)	9	5	10	28% (7/25)	7	0	0
		9/21/99	100% (6/6)	1	1	4	75% (15/20)	15	0	0
M	WOCK	5/5/99	56% (14/25)	11	1	2	0% (0/25)	0	0	0
		8/2/99	100% (3/3)	0	1	2	28% (7/25)	7	0	0
		9/22/99	NS	–	–	–	55% (11/20)	11	0	0
	YKRV	5/4/99	37% (3/8)	3	0	0	0% (0/25)	0	0	0
		8/3/99	NS	–	–	–	19% (4/21)	4	0	0
		9/21/99	NS	–	–	–	10% (2/20)	2	0	0
H	BUBY	5/6/99	84% (21/25)	19	0	2	0% (0/25)	0	0	0
		8/5/99	100% (13/13)	12	0	1	12% (3/25)	3	0	0
		9/2/99	NS	–	–	–	25% (5/20)	5	0	0
	BOBY	5/6/99	56% (14/25)	13	0	1	0% (0/25)	0	0	0
		8/4/99	100% (25/25)	19	4	2	0% (0/25)	0	0	0
		9/21/99	NS	–	–	–	0% (0/25)	0	0	0

Salinity codes: L = low (<15‰), M = medium (15–25‰), H = high (>25‰). Site codes: CNRV = Coan River, GWRV = Great Wicomico River, WOCK = Woodas Creek, YKRV = York River, BUBY = Burton Bay, BOBY = Bogues Bay. † = In parenthesis number of oysters infected/number of oysters examined. * = Number of oysters with, respectively, light, moderate, and heavy infections. NS = No *C. virginica* remaining for sampling.

that of *C. virginica* which was initially maintained in the water at a nearby location. Absence of *P. marinus* in the baseline sample of *C. virginica* collected in May 1998 does not preclude the possibility of sub-clinical infections. Given the low sensitivity of the standard diagnostic assay for detecting *P. marinus* infections during spring (Bushek et al. 1994, Burreson and Ragone Calvo 1996), it is likely that acquisition of infections occurred the prior summer but that overwintering infections were not detected the following spring.

Suminoe oysters tested in this study had comparable survival at all salinity regimes and similar growth rate at medium and high salinity regimes, in agreement with the wide salinity tolerance described for *C. ariakensis* in its native range (Guo et al. 1999). By the end of the experiment, when oysters were three years old, mean shell height of *C. ariakensis* at low, medium, and high salinity regimes was respectively 96, 125 and 140 mm. By comparison, in Zhanjiang Bay (annual salinity range = 7–30‰) average shell height of three-year-old Suminoe oysters is 100 mm (Cai et al. 1992).

In contrast to *C. gigas* in previous studies (e.g., Handley & Berquist 1997, Calvo et al. 1999), *C ariakensis* was not found to be adversely affected by *Polydora* spp. in this study. Mud worms were present in both *C. ariakensis* and *C. virginica*, but infestations were not severe and did not appear to affect growth or survival of either species. However, since the conditions that result in severe infestations are not clearly understood, we cannot dismiss the possibility that *C. ariakensis* might be susceptible to such infestations.

Results of the present investigation suggest that *C. ariakensis* is more adapted to Chesapeake Bay conditions than *C. gigas*. In a

TABLE 3.

Percentage of genetic mosaics by salinity regime, site and time.

		1998		1999					
Salinity	**Site**	**July**	**August**	**May**	**June**	**July**	**August**	**Total by site**	**Total by salinity**
L	CNRV	0.0% (0/35)	2.8% (1/35)	8.6% (3/35)	8.6% (3/35)	9.7% (3/31)	8.0% (2/25)	6.1% (12/196)	5.0% (20/395)
	GWRV	2.8% (1/35)	2.8% (1/35)	2.8% (1/35)	5.7% (2/35)	5.9% (2/34)	4.0% (1/25)	4.0% (8/199)	
M	EARV	0.0% (0/35)	2.8% (1/35)	11.4% (4/35)	8.6% (3/35)	13.8% (4/29)	12.0% (3/25)	7.7% (15/194)	7.2% (28/391)
	YKRV	0.0% (0/35)	11.4% (4/35)	11.4% (4/35)	8.6% (3/35)	2.9% (1/34)	4.3% (1/23)	6.6% (13/197)	
H	BUBY	0.0% (0/35)	8.8% (3/34)	2.8% (1/35)	5.7% (2/35)	0.0% (0/33)	8.0% (2/25)	4.1% (8/197)	3.7% (14/379)
	BOBY	0.0% (0/35)	0.0% (0/35)	2.8% (1/35)	5.7% (2/35)	6.2% (1/16)	8.0% (2/25)	3.3% (6/181)	
Total by month		0.5% (1/210)	4.8% (10/209)	6.7% (14/210)	7.1% (15/210)	6.2% (11/177)	7.4% (11/148)	Overall total = 5.3% (62/1164)	

Salinity codes: L = low (<15‰), M = medium (15–25‰), H = high (>25‰). Site codes: CNRV = Coan River, GWRV = Great Wicomico River, EARV = East River, YKRV = York River, BUBY = Burton Bay, BOBY = Bogues Bay. In parenthesis number of mosaics divided by number of oysters examined.

comparative study with *C. virginica* and *C. gigas* (Calvo et al. 1999), *C. gigas* exhibited high cumulative mortality (63%) at low salinity sites and growth rate was not greater than that of *C. virginica* within Chesapeake Bay. In contrast, *C. ariakensis* tested in this study had less than 16% cumulative mortality and greater growth rate than *C. virginica* within medium salinity sites in Chesapeake Bay. In grow-out trials with *C. gigas* and *C. ariakensis* using various culture methods at high salinity locations on the West Coast of USA, growth rate was the same for both oyster species. For example, *C. gigas* and *C. ariakensis* juveniles (mean shell height = 10 mm) planted directly on the bottom in Puget Sound, Washington, or suspended from floating rafts, in Yaquina Bay, Oregon, increased to 90–100 mm after 1 year of deployment during 1990 to 1991 (Langdon & Robinson 1996). A direct comparison between *C. gigas* and *C. ariakensis* is not available for the East Coast of USA. However, in this study and in Calvo et al. (1999), both species experienced significantly higher growth rate than the corresponding *C. virginica* control oysters at high salinity sites on the Atlantic Coast of Virginia.

The choice of oyster strain must be considered in the interpretation of results. *C. virginica* offspring from wild Mobjack Bay brood stock were employed in this study because they are a standard stock for aquaculture and because they were the only stock available with similar age, size and disease status *as C. ariakensis*. It is likely that a strain of *C. virginica* selected for disease resistance would more favorably compare to *C. ariakensis*. For example, DEBY, a strain of *C. virginica* that has been selectively bred by VIMS against *P. marinus* and *H. nelsoni* for four generations (Ragone Calvo et al. 1997), exhibited similar survival to that of *C. ariakensis* and higher survival, higher growth rate and lower disease susceptibility than that of unselected *C. virginica* from Mobjack Bay deployed at a site in the Great Wicomico River from June 1998 to May 1999 (G. Calvo unpublished data). Similarly, CROSBreed strains (Debrosse and Allen 1996) have been selected for dual resistance to both pathogens in the Mid-Atlantic. Use of disease resistant *C. virginica* strains could provide a more relevant comparison of native and non-native oyster performance for aquaculture.

To some extent, the fact that *C. ariakensis* were triploids may have allowed them to grow faster than diploid *C. virginica*. In general, reduced gametogenesis in triploids corresponds with improved somatic growth (Barber & Mann 1991). However, based on studies with *C. virginica and C. gigas*, ploidy effects alone are unlikely to account for reduced disease susceptibility and increased survival of *C. ariakensis*. Barber and Mann (1991) found that cohorts of diploid and triploid *C. virginica* had the same prevalence (96%) and similar intensity of *P. marinus* after 17 months of deployment at the York River, VA. Meyers et al. (1991) reported equal cumulative mortality for diploid and triploid *C. virginica* (100%) and lower cumulative mortality for diploid (25%) than triploid *C. gigas* (34%), after one year of challenge with *P. marinus*.

In summary, during the course of the study *C. ariakensis* performed better than *C. virginica* in Chesapeake Bay and the Atlantic Coast of Virginia. Wide salinity tolerance combined with low disease susceptibility was associated with high survival and high growth rate in *C. ariakensis*. In contrast, high disease susceptibility was associated with low survival and low growth rate in *C. virginica*.

As previously discussed for *C. gigas* (Calvo et al. 1999), a decision on whether *C. ariakensis* is, or is not, an appropriate species for use in these environments must include other factors beyond the scope of these field investigations. For example, international organizations have recommended that competent local authorities consider the ecological consequences of species introductions by evaluating the following: (1) the possibility that non-indigenous species may carry pests and pathogens into the new environment; (2) the potential relationship of the exotic species with native species; and (3) the potential range for establishment of the exotic species in the new environment. A cautious introduction of triploid *C. ariakensis* for aquaculture purposes following International Council for the Exploration of the Seas guidelines, as it is

currently being considered in Virginia, would minimize risks associated with factors enumerated above. Use of individually certified triploids in a closely monitored research setting allowed us to conduct the present study with minimum risks of unintentionally introducing reproductively capable *C. ariakensis* into the waters of Virginia. At the present time, however, there is no precedent for using triploids to control oyster populations at a commercial scale. In practice, implementation of such a strategy would require significant efforts directed at monitoring the reproductive status of deployed stocks over time, and at designing and enforcing strict quarantine regulations to avoid undesired release of reproductively capable stocks.

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