A Comparative Field Study of *Crassostrea ariakensis* and *Crassostrea virginica* in Relation to Salinity in Virginia

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EXECUTIVE SUMMARY

In accordance with the Rational Plan for Testing Application of Non-Native Oyster Species (VIMS 1996) we conducted a field experiment to examine survival, growth and disease susceptibility of *Crassostrea ariakensis* (=*rivularis*) in relation to salinity in Virginia. The performance of triploid *C. ariakensis* in comparison with that of diploid *C. virginica*, (n = 250, age = 2 years, mean shell height = 60-64 mm) was evaluated at replicate sites within low, medium, and high salinity regimes (respectively, < 15‰, 15-25‰, > 25‰) in Chesapeake Bay and the Atlantic Coast. During the course of this study, from June 1998 to September 1999, there was a severe oyster disease epizootic prevailing in Chesapeake Bay. At the end of the study *C. ariakensis* exhibited lower disease prevalence and intensity and superior survival and growth than *C. virginica*. At low salinity sites cumulative mortality in *C. ariakensis* (14%) was significantly lower than that in *C. virginica* (81%). At medium and high salinity sites, cumulative mortality in *C. ariakensis* was less than 16% whereas all *C. virginica* were dead by the end of the experiment. After one year of deployment, mean shell height of *C. ariakensis* at low, moderate, and high salinity sites, was respectively 96 mm, 125 mm, and 140 mm. In comparison, mean shell height of *C. virginica* was respectively 72 mm, 85 mm, and 75 mm. Prevalence and intensity of *Perkinsus marinus* infections were significantly lower in *C. ariakensis* than in *C. virginica*. During the second summer of disease exposure, prevalence in *C. ariakensis* ranged form 0-28% whereas prevalence in *C. virginica* was 100% at all sites. Only light infections were present in *C. ariakensis* whereas heavy infections were found in *C. virginica*. MSX was absent in *C. ariakensis* and present in *C. virginica*. Mud worms 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.

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INTRODUCTION

In contrast with extensive information available for eastern, *Crassostrea virginica,* and Pacific, *Crassostrea gigas,* oysters, reports on Suminoe oysters, *Crassostrea ariakensis* (=*C. rivularis*), are scarce. Suminoe oysters are 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 mostly occurs 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). In the West Coast of USA, where *C. ariakensis* has been introduced with shipments of *C. gigas* and kumamoto oysters 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 condition at various locations along the West Coast.

No studies on the Suminoe oyster are 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 May 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 mid to subtropical 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 than *C. gigas* in Chesapeake Bay. The objectives of the present study were to compare survival, growth, and disease susceptibility of *C. ariakensis* and *C. virginica* in various salinities.

METHODS

Study Sites

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

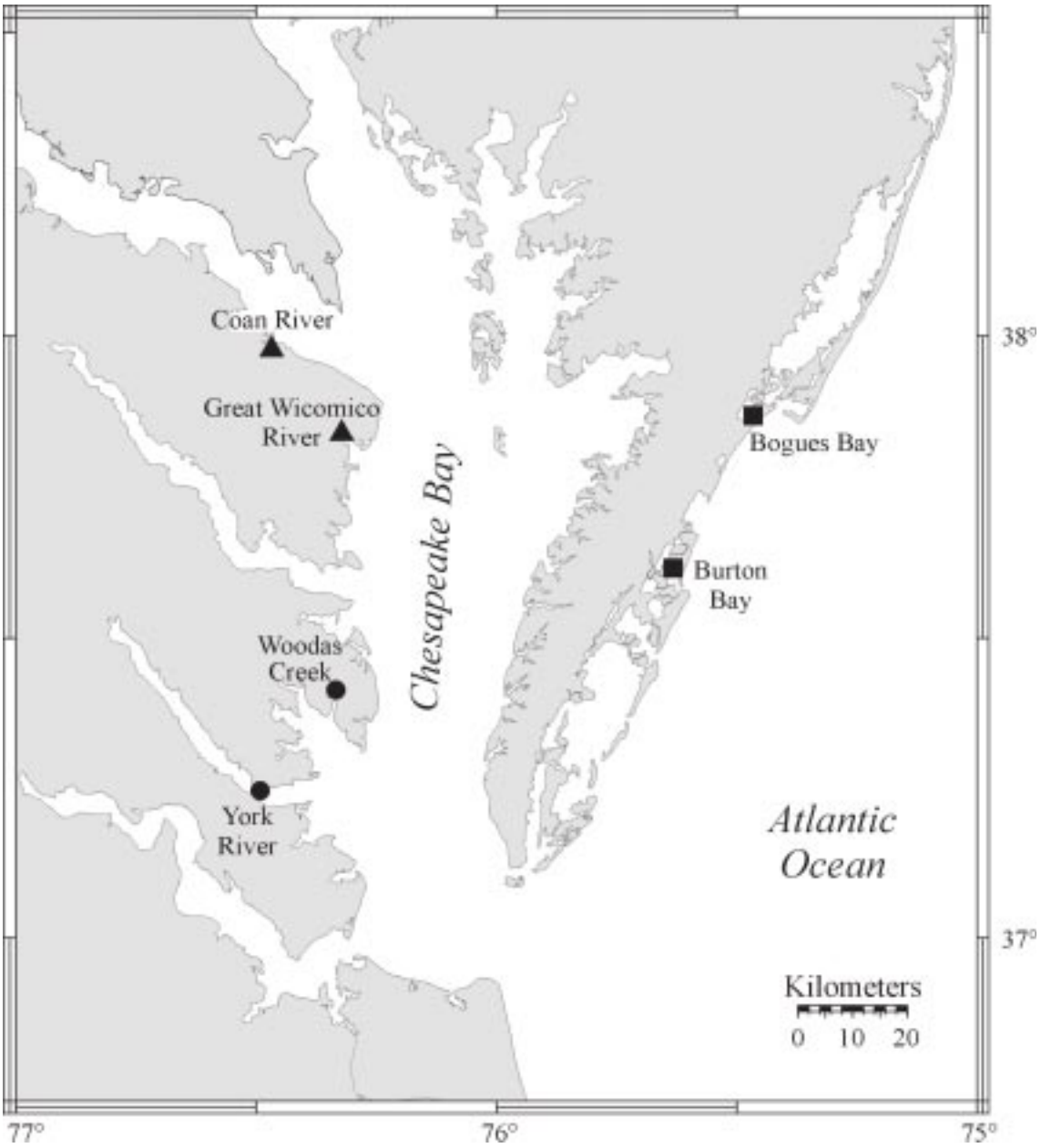

Figure 1. Location of study sites in Chesapeake Bay and the Atlantic Coast of Virginia. Triangles = low salinity (<15‰) sites, circles = medium salinity (15-25‰) sites, squares = high salinity (>25‰) sites.

Minisonde® dataloggers deployed at various sites for weekly to monthly intervals.

Oysters

To ensure that this study resulted in neither the unintended reproduction of a non-native species nor the introduction of potential exotic pathogens, we used individually certified triploid *C. ariakensis* produced and maintained in quarantine first at Haskin Shellfish Research Laboratory, Rutgers University (HSRL) and then at the Virginia Institute of Marine Science's (VIMS) Aquaculture Genetics and Breeding Technology Center. *C. ariakensis* brood stock, originating from an established line at HSRL and derived from sources in 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 below. *C. virginica* brood stock, collected from Mobjack Bay, VA was spawned by a local commercial hatchery in July 1996. Prior to deployment, juvenile diploid *C. virginica* were maintained at the Ware River, VA.

Experimental Design

Between 29 May and 2 June 1998, adult oysters were dispensed into replicate 9.5 mm mesh bags and placed within individual floating trays at the study sites. Each floating tray contained 2 bags with 100 oysters and one bag containing 50 individually labeled oysters, to follow growth, as described below. Floating trays (2.3 m x 0.5 m x 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).

Data were examined for compliance with statistical test assumptions using Bartlett chi-square test for homogeneity of variance and plots of means vs. standard deviations. Arcsine and logarithmic transformations were used when appropriate, and non-parametric tests were employed when necessary (Zar 1974). ANOVAs were used to examine the effects of species and salinity regime on final cumulative mortality, growth rate, *P. marinus* prevalence and weighted prevalence. Differences in mean variables, between species within a salinity regime and between salinity regimes within a species, were further examined by Newman-Keuls tests. Mann-Whitney and Kruskal-Wallis tests were used, respectively, to examine variation in oyster body weights, condition, and *Polydora* spp. by species within a salinity regime, or to examine both the effects of species and salinity on the same variables. Statistical analyses were performed using Statview® and Statistica® softwares.

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 interval, corrected for oysters removed by sampling. Cumulative mortality was calculated as the sum of interval mortality (Barber and Mann 1994, Krebs 1972).

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-May 1999, and divided by the deployment time in days standardized for 30 days.

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

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

Oysters were allowed to air-dry for 15-20 min 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-tared 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.

Diseases and Polydora

A baseline sample of 25 oysters was taken to assess the disease status of each species prior to deployment in spring 1998. Subsequent disease samples for 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 rectum tissue. Infection intensity was rated based on Ray (1954) and Mackin (1962) and for the calculation of weighted prevalence the following numerical values were assigned to intensity categories: (1) light, (3) moderate, and (5) heavy. Weighted prevalence was calculated by the formula:

$$\text{Weighted prevalence} = ((n_1*1) + (n_2*3) + (n_3*5))/N, \quad (2)$$

where n_i = number of cases rated as (i), N = total number of oysters examined in the sample.

Haplosporidium nelsoni was diagnosed using standard paraffin histology procedures with oysters preserved in Davidson's AFA and 6 mm tissue sections stained with Harris' hematoxylin and eosin (Burreson et al. 1988). Infection intensity was rated as light, moderate, and heavy based on Burreson et al. (1988). Histological sections were also used to document the presence of other parasites and to examine development of oyster gonads. All disease and histology analyses 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

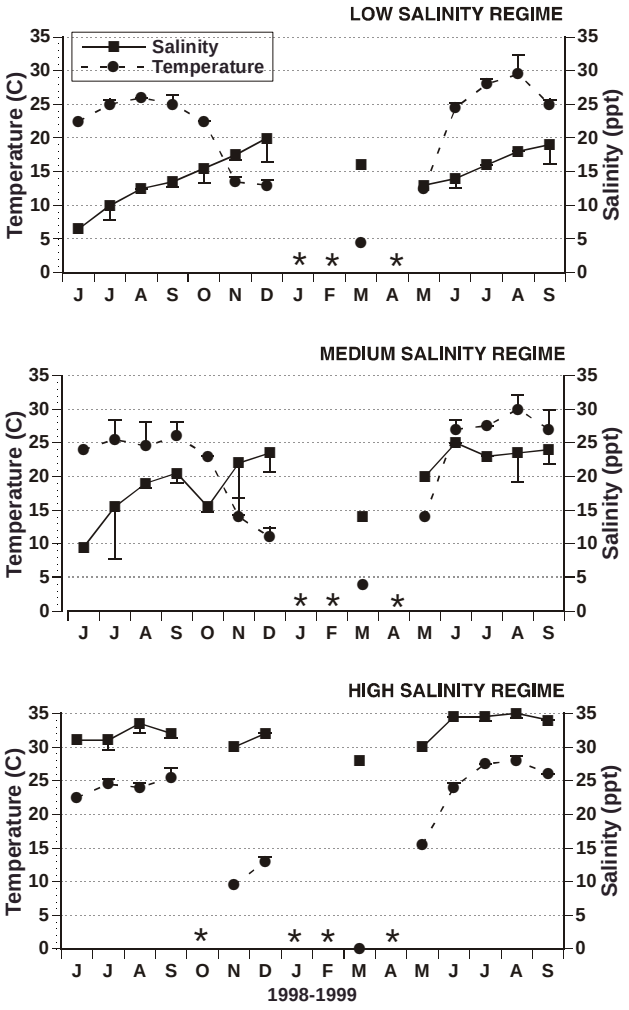

Figure 2. Mean monthly temperature and salinity by salinity regime (N = 2 sites, +/- SD) using stem thermometer and refractometer. * = Break in monthly sampling.

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 by the formula:

$$\text{Weighted prevalence} = ((n_1*1) + (n_2*2) + (n_3*3) + (n_4*4))/N, \quad (3)$$

where n_i = number of cases rated as (i), N = total number of oysters examined in the sample.

Reproductive Status and Ploidy

Baseline samples of *C. ariakensis* were taken to ascertain the extent of triploid individuals in quarantine and to certify triploid individuals to be deployed during the experiment. Over the course of the study 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 was determined by flow cytometry of gill and/or hemolymph biopsies. When gill and/or hemolymph samples were found to contain any diploid cell (a condition termed mosaic), a biopsy of the gonad was examined by flow cytometry, and the remaining gonad tissue was processed by histology. Ploidy assays were conducted at HSRL and the VIMS Aquaculture Genetics and Breeding Technology Center.

RESULTS

Environmental Parameters

Low salinity sites experienced relatively low mean salinity (<10‰) during June-July 1998 because of high rainfall during spring 1998 and relatively high mean salinity (>15‰) during November 1998-March 1999 and in August and September 1999, because of drought conditions starting in fall 1998 and continuing into Spring and Summer 1999. Medium salinity sites experienced relatively low salinity (<15‰) during June 1998 (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 28-32°C in July and a

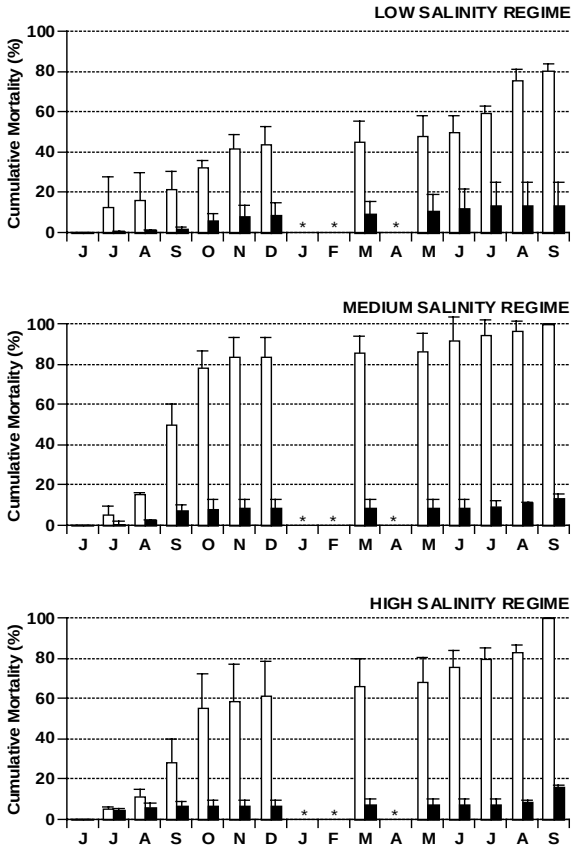

Figure 3. Mean cumulative mortality by salinity regime (N=2 sites, +SD) from June 1998 to September 1999. Open bars=*C. virginica*, solid bars=*C. ariakensis*. *=Break in monthly sampling.

Table 1. Effect of species and salinity regime on mean cumulative mortality.

A. Two-way ANOVA

Effect	df	MS	F	p
Species	1	1.4033	120.400	< 0.0005**
Salinity	2	0.0403	3.715	0.089
Species*Salinity	2	0.0543	4.662	0.060
Error	6	0.0116		

** denotes significance at p = 0.01

minimum of 0-5°C in March. High salinity sites experienced overall cooler temperature with monthly means 2-4°C lower than medium or low salinity sites (Fig. 2).

Mortality

Throughout most of the study and regardless of salinity regime, mortality of *C. virginica* was much higher than that of *C. ariakensis* (Fig. 3). Species had a significant effect on mean cumulative mortality (Table 1). At low salinity sites mean cumulative mortality in *C. ariakensis* (14%) was much lower than that in *C. virginica* (81%). At medium and high salinity sites, mean cumulative mortality in *C. ariakensis* was less than 16% whereas all *C. virginica* were dead by the end of the experiment. 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).

Growth

Growth varied with species and salinity regime (Fig. 4). At the start of the experiment mean shell height was 60 mm in *C. virginica* and 64 mm in *C. ariakensis*. After 1 yr. of deployment, mean shell height of *C. virginica* at low, medium, and high salinity sites was respectively 70 mm, 80 mm, and 73 mm. *C. virginica* stopped growing during the second year and growth at low and high salinity regimes during the first year was minimal. In comparison, mean shell height of *C. ariakensis* at low, moderate, and high salinity sites, was respectively 93 mm, 121 mm, and 137 mm. Most of the growth in *C. ariakensis* occurred during fall 1998 and spring 1999. No growth was observed for either species during July to September 1999.

Species, salinity regime and their interaction had significant effects on mean growth rate (Table 2A). 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 significantly lower than that of *C. ariakensis* (6.2 mm $mo.^{-1}$). For *C. virginica*, growth rate did not significantly differ among salinity regimes. For *C. ariakensis*, growth rate at low salinity was significantly lower than that at medium and high salinity regimes, but growth rate did not significantly differ between medium and high salinity regimes (Table 2B).

Disease

Baseline samples revealed no *P. marinus* and a 4% prevalence of *H. nelsoni* (MSX) in *C. virginica* and 12% prevalence of *P. marinus* and no MSX in *C. ariakensis*. In all subsequent samples prevalence and intensity of *P. marinus* infections were consistently 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 prevalence in *C. ariakensis* ranged 0-28% (Fig. 5). Several heavy infections were found in *C. virginica* whereas only light infections were observed in *C. ariakensis* (Appendix I). During August and October 1998, prevalence and weighted prevalence were significantly higher in *C. virginica* than in *C. ariakensis* (Appendices II and IIIA). In September 1999 when all *C. virginica* at medium and high salinity sites had either died or had been removed by sampling, prevalence and weighted prevalence in *C. ariakensis* were not significantly different among salinity regimes (Appendices IV and V). Maximum prevalence of MSX in *C. virginica* was 25% at the York River site in May 1999. MSX was also present in *C. virginica* at the low salinity Great Wicomico River site in September 1998, and at high salinity sites in October 1998 and May 1999. In general, intensity of infections was light but a few heavy infections were found in medium and high salinity sites. No MSX was found in *C. ariakensis.*

Condition

At low salinity sites, mean condition index in *C. virginica* (3.6%) was not significantly different (Mann-Whitney tests p = 0.121) than that in *C. ariakensis* (6.6%). Similarly, there was no significant (Mann-Whitney tests p = 0.121) difference in body weights between species. 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 (Appendix VI). Within *C. ariakensis*, mean condition index at low, medium and high salinity, respectively, were 6.6%, 5.3% and 9.7% and not significantly different (Kruskal-Wallis test, p = 0.276). Similarly, there were no significant differences (Kruskal-Wallis test, p > 0.102) between mean body weights among salinity regimes.

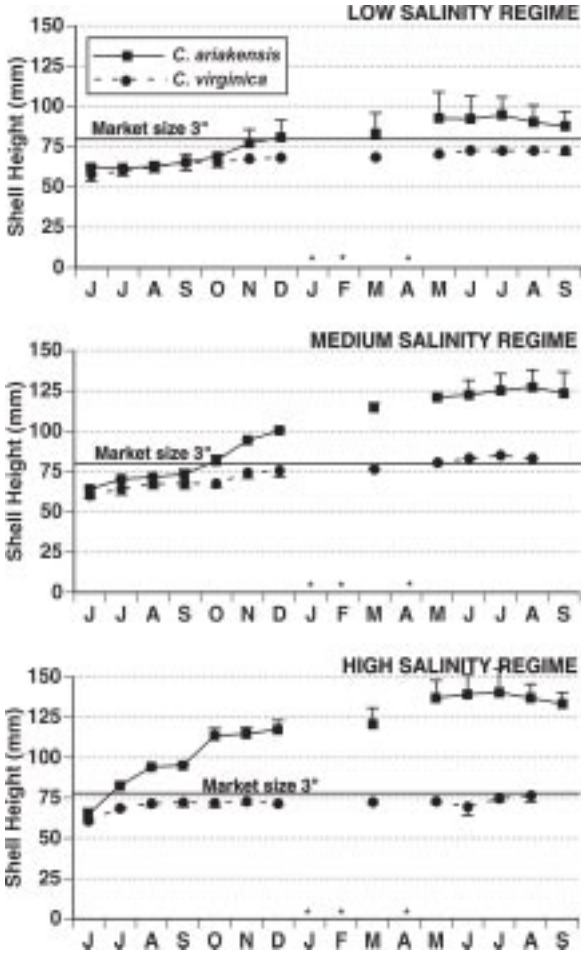

Figure 4. Mean shell height by salinity regime (N=2 sites, +-SD) of 50 individual oysters repeatedly measured from June 1998 to September 1999. Solid lines with squares=*C. ariakensis*, dashed lines with circles=*C.virginica*. *=Break in monthly sampling.

Percent shell 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 was 100% in both species, and weighted prevalence in *C. virginica* (1.1) was not significantly different (Mann-Whitney test p = 0.121) from that in *C. ariakensis* (3.4). 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 (Appendix VII). Within *C. ariakensis*, mean prevalence

Table 2. 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	0.031*
Species*Salinity	2	3.225	6.124	0.035*
Error	6	0.526		

* denotes significance at p = 0.05, ** denotes significance at p = 0.01

B. Multiple comparison (Newman-Keuls test)

Comparison		p
Within	Between	
Low salinity	*C. virginica and C. ariakensis*	0.162
Medium salinity	*C. virginica and C. ariakensis*	0.012*
High salinity	*C. virginica and C. ariakensis*	0.003**
C. virginica	Low salinity vs. medium salinity	0.406
C. virginica	Low salinity vs. high salinity	0.932
C. virginica	Medium salinity vs. high salinity	0.613
C. ariakensis	Low salinity vs. medium salinity	0.008**
C. ariakensis	Low salinity vs. high salinity	0.007**
C. ariakensis	Medium salinity vs. high salinity	0.131

* denotes significance at p = 0.05, ** denotes significance at p = 0.01

at low, medium and high salinity, respectively, 100%, 62% and 12% was not significantly different (Kruskal-Wallis test, p = 0.156) among salinity regimes. Similarly, weighted prevalence at low, medium, and high salinity, respectively, 3.4, 2.2 and 1.0 was not significantly different (Kruskal-Wallis test, 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 66 individuals in which combinations of diploid and triploid cells (mosaics) were detected out of 1163 oysters examined (5.7%). The proportion of mosaics ranged from 0% to 16% depending on time and site. For all salinity regimes combined, the proportion of mosaics increased from 0.5% in June 1998 to 7.4% in August 1999. For all times pooled within low, medium, and high salinity regimes, the proportion was respectively, 5.3%, 6.9%, and 4.5% (Table 3). Examination of 39 mosaic individuals revealed that 10 were females, 23 were males, 1 was hermaphroditic, and 5 were undifferentiated.

DISCUSSION

Over the course of the study from June 1998 through September 1999, *C. ariakensis* exhibited higher survival and growth rate, and lower disease susceptibility than *C. virginica*. Drought conditions

and below normal Chesapeake Bay stream flow starting in fall 1998 resulted in increased salinity and severe epizootics of both *H. nelsoni* and *P. marinus* in 1999 (Ragone Calvo & Burreson 1999). Heavy disease pressure prevailing during this study, however, did not affect survival and growth of *C. ariakensis*. For Suminoe oysters deployed at any salinity regime, susceptibility to *P. marinus* was low, no MSX was found, and cumulative mortality was less than 16%. In contrast, Mobjack Bay *C. virginica* employed in this study, which were relevant for the industry because they have been the standard stock for commercial aquaculture in Virginia, experienced high mortality associated with heavy infections. For example, after the first summer of disease exposure, when more than 50% of *C. virginica* in this experiment had died, MSX was present and *P. marinus* was 100% prevalent with severe infections at medium and high salinity sites. A year later when all *C. virginica* at medium and high salinity sites were dead, cumulative mortality at low salinity sites was 81% and *P. marinus* was 100% prevalent with severe infections. Presence of MSX and intensification of *P. marinus* infections 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

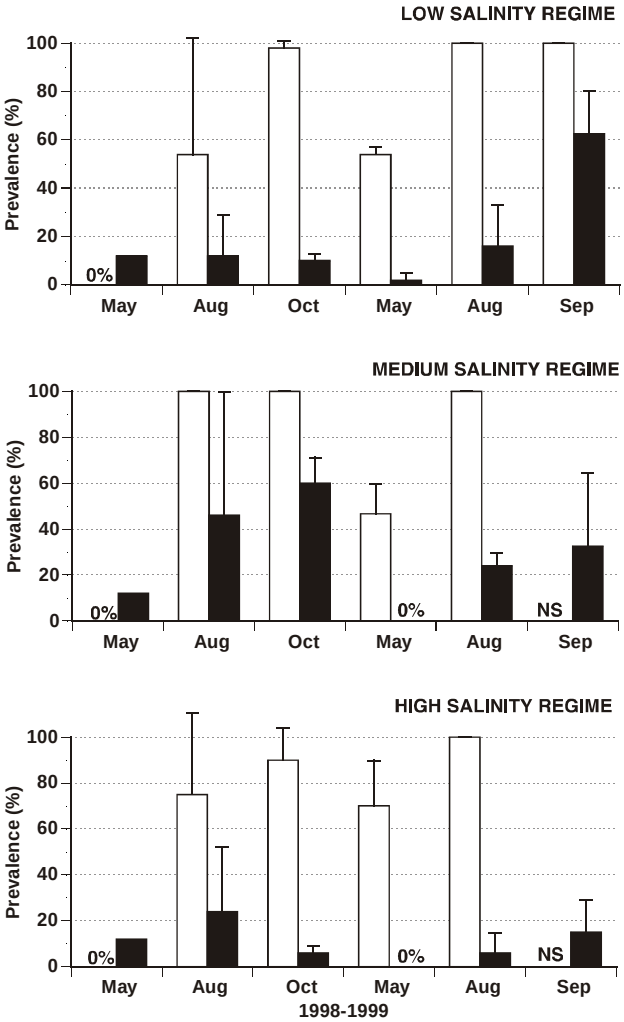

Figure 5. Mean prevalence of *P. marinus* by salinity regime (N = 2 sites, + SD) in samples of 25 oysters. Open bars = *C. virginica*, solid bars = *C ariakensis*. NS = Not sampled.

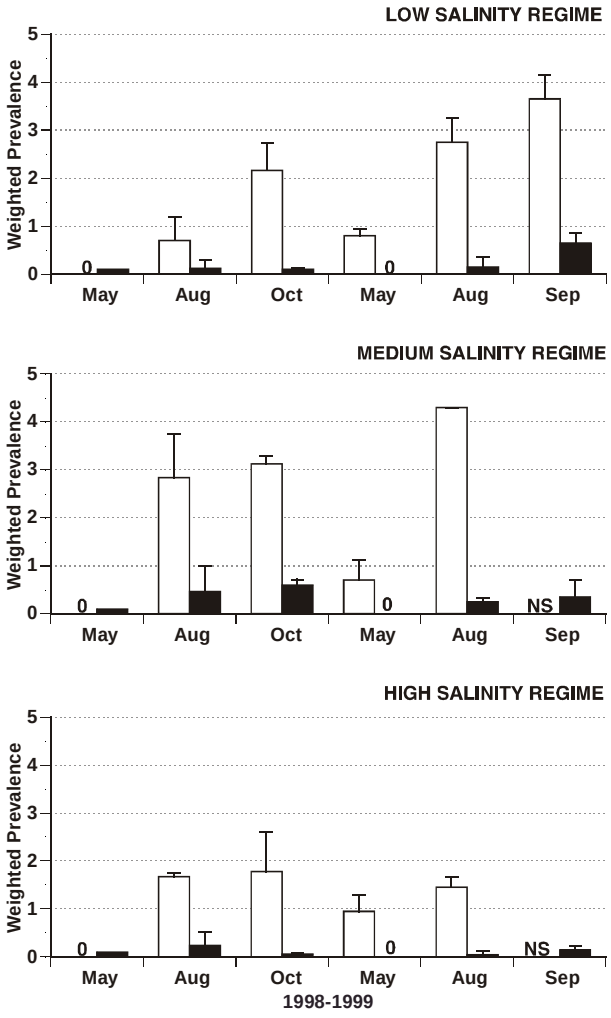

Figure 6. Mean weighted prevalence of *P. marinus* (N = 2 sites, + SD) in samples of 25 oysters. Open bars = *C. virginica*, solid bars = *C. ariakensis*. NS = Not sampled.

Table 3. Percent genetic mosaics by salinity regime and date.

Salinity	1998		1999				
	6/30-7/6	8/3-8//12	5/3-5/6	6/1-6/10	6/30/-7/8	8/6	
Low	1% (1/70)	3% (2/70)	4% (4/70)	7% (5/70)	8% (5/65)	6% (3/50)	5% (20/395)
Medium	0% (0/70)	7% (5/70)	1% (8/70)	8% (6/70)	8% (5/63)	8% (4/48)	7% (28/391)
High	0% (0/70)	3% (3/70)	0% (2/70)	6% (4/70)	2% (1/49)	8% (4/50)	4% (14/379)
	0% (1/210)	5% (10/210)	5% (14/210)	7% (15/210)	6% (11/177)	7% (11/148)	5% (62/1165)

In parenthesis number of mosaics/number of oysters examined

greater than 15‰ during summer and fall is conducive to development of lethal *P. marinus* infections (Burreson and Ragone Calvo 1996). Mud worms were present in both oyster species but infestations did not appear to affect condition or growth of *C. ariakensis*. In Zhanjiang Bay, southern China, mass mortality of *C. ariakensis* has been associated with outbreaks of toxic phytoplankton blooms (Yongjia et al. 1995). However, to the best of our knowledge no parasitic diseases had been reported in Suminoe oysters before this study. More research is needed to examine disease susceptibility and the mechanisms of disease resistance in *C. ariakensis*.

In agreement with the wide salinity tolerance described for *C. ariakensis* in its native range (Guo et al. 1999), Suminoe oysters tested in this study had comparable survival at all salinity regimes and equal growth rate at medium and high salinity regimes. By the end of the experiment, when oysters were 3 years old, mean shell height of *C. ariakensis* at low, medium, and high salinity regimes was respectively 96 mm, 125 mm, 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).

Results of the present investigation suggest that *C. ariakensis* is more adapted to Chesapeake Bay conditions than *C. gigas*. In a study with *C. gigas* at mostly the same low and medium salinity sites used in the present investigation (Calvo et al. 1999), mean cumulative mortality was greater than 50% and growth rate at medium salinity sites was not significantly higher that of *C. virginica*. Both *C. gigas* and *C. ariakensis* had similarly low susceptibility to *P. marinus* infections and no MSX was detected in either oyster species. In high salinity sites at the Atlantic Coast of Virginia, both *C. gigas* and *C. ariakensis* experienced significantly higher growth rate than corresponding *C. virginica* control oysters. Similarly, in a direct comparison of *C. gigas* and *C. ariakensis*, with oysters of the same age in high salinity environments, growth rate was the same for both species at various locations on the West Coast of USA (Langdon & Robinson 1996). For example, juveniles (< 10 mm in shell height) of both non-indigenous oyster species planted on shell strings in July 1990 similarly increased to 90 mm after 1 year of deployment in Yaquina Bay, OR.

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 resulted in higher survival and growth in *C. ariakensis* as compared to *C. virginica*. As previously discussed for *C. gigas* (Calvo et al. 1999), a debate on whether *C. ariakensis* is, or is not, an appropriate species for introduction or 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 following: (a) assess the possibility of introducing pathogens and parasites associated with the species proposed for introduction; (b) assess the potential relationship of the candidate species with other members of the ecosystem; and (c) examine the probable effects including a prediction of the range for the establishment of the species.

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APPENDICES

I. Prevalence and intensity of *P. marinus* in *C. virginica* and *C. ariakensis* by salinity regime, site and date.

II. One-way ANOVA of the effects of species, salinity regime, and time on *P. marinus* prevalence.

III. One-way ANOVA of the effects of species, salinity regime, and time on *P. marinus* weighted prevalence.

IV. One-way ANOVA of the effect of salinity regime on *P. marinus* prevalence in *C. ariakensis.*

V. One-way ANOVA of the effect of salinity regime on *P. marinus* weighted prevalence in *C. ariakensis.*

VI. Mean (SD) biomass and condition index of *C. virginica* and *C. ariakensis* by salinity regime and site in September 1999.

VII. Prevalence and intensity of *Polydora* spp. in *C. virginica* and *C. ariakensis* by salinity regime and site in September 1999.

Appendix I. Prevalence and intensity of *P. marinus* in *C. virginica* and *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*
Low	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
Medium	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
High	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*
Low	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
Medium	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
High	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% (0/20)	0	0	0

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 examined/number of oysters infected. * = Number of oysters with, respectively, light, moderate, and heavy infections. NS = No live oysters remaining for sampling.

Appendix II. Effects of species, salinity regime, and time on *P. marinus* prevalence.

Three-way ANOVA

Effect	df	MS	F	p
Species	1	21480.17	32.669	<0.0005**
Salinity	2	2515.50	3.825	0.052
Time	1	486.17	0.712	0.415
Error	12	657.50		

** denotes significance at p = 0.01

Appendix III. Effects of species, salinity regime, and time on *P. marinus* weighted prevalence.

A. Three-way ANOVA

Effect	df	MS	F	p
Species	1	0.787	91.964	<0.0005**
Salinity	2	0.078	9.112	0.004**
Time	1	0.029	3.427	0.089
Error	12	0.008		

** denotes significance at p = 0.01

B. Multiple comparison (Newman-Keuls test)

Comparison		p
Within	Between	
Low salinity	*C. virginica and C. ariakensis*	0.002**
Medium salinity	*C. virginica and C. ariakensis*	<0.0005**
High salinity	*C. virginica and C. ariakensis*	0.001**
C. virginica	Low salinity vs. medium salinity	0.014*
C. virginica	Low salinity vs. high salinity	0.579
C. virginica	Medium salinity vs. high salinity	0.012*
C. ariakensis	Low salinity vs. medium salinity	0.149
C. ariakensis	Low salinity vs. high salinity	0.852
C. ariakensis	Medium salinity vs. high salinity	0.091

* denotes significance at p = 0.05, ** denotes significance at p = 0.01

Appendix IV. Effect of salinity regime on *P. marinus* prevalence in *C. ariakensis*.

One-way ANOVA

Effect	df	MS	F	p
Salinity	2	1154.167	2.270	0.251
Error	3	508.333		

Appendix V. Effect of salinity regime on *P. marinus* weighted prevalence in *C. ariakensis*.

One-way ANOVA

Effect	df	MS	F	p
Salinity	2	0.011	2.140	0.264
Error	3	0.005		

Appendix VI. Mean (SD) biomass and condition index of *C. virginica* and *C. ariakensis* by salinity regime and site in September 1999.

Salinity	Site	Species	n	Whole wt. (g)	Shell wt. (g)	Wet wt. (g)	Dry wt. (g)	CI (%)
Low	CNRV	Cv	14	70.3 (15.8)	47.8 (14.5)	5.6 (1.6)	1.0 (0.3)	4.5 (1.6)
		Ca	11	83.5 (42.7)	48.2 (27.9)	12.8 (6.0)	2.8 (1.6)	8.2 (2.5)
	GWRV	Cv	5	73.3 (13.3)	42.4 (19.3)	4.6 (2.1)	0.7 (0.4)	2.8 (1.8)
		Ca	20	82.2 (21.1)	50.3 (13.9)	10.1 (3.1)	1.6 (0.7)	5.1 (1.8)
Medium	WOCK	Ca	20	191.7(58.6)	115.3 (35.2)	29.0 (10.3)	5.7 (2.3)	7.4 (1.7)
	YKRV	Ca	20	351.8 (151.4)	211.3 (66.2)	57.3 (17.9)	14.5 (5.0)	12.1 (3.4)
High	BUBY	Ca	20	247.5 (95.4)	161.2 (57.0)	28.9 (10.9)	5.1 (2.4)	6.2 (2.7)
	BOBY	Ca	20	334.1 (75.8)	211.2 (44.4)	33.6 (11.2)	4.6 (1.8)	4.5 (1.9)

Site codes: CNRV = Coan River, GWRV = Great Wicomico River. WOCK = Woodas Creek, YKRV = York River, BUBY = Burton Bay, BOBY = Bogues Bay. Species codes: Cv = *C. virginica*, Ca = *C. ariakensis.*

Appendix VII. Prevalence and intensity of *Polydora* spp. in *C. virginica* and *C. ariakensis* by salinity regime and site in September 1999.

		C. virginica					*C. ariakensis*				
Salinity	Site	Prevalence	I*	II*	III*	IV*	Prevalence	I*	II*	III*	IV*
Low	CNRV	100% (15/15)	11	4	0	0	100%(11/11)	0	2	5	4
	GWRV	100% (5/5)	4	0	1	0	100% (20/20)	0	2	4	14
Medium	WOCK	NS	-	-	-	-	100% (20/20)	0	2	7	11
	YKRV	NS	-	-	-	-	80% (16/20)	15	1	0	0
High	BUBY	NS	-	-	-	-	20% (4/20)	4	0	0	0
	BOBY	NS	-	-	-	-	5% (1/20)	1	0	0	0

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 examined/number of oysters infected. * = Number of oysters with Polydora infestations categorized as (I) Mudblisters affecting less than 25% of the valve; (II) 25%-50% of the valve affected; (III) 50%-75% of the valve affected; (IV) More than 75% of the valve affected. NS = No live oysters remaining for sampling.

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