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

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EXECUTIVE SUMMARY

In response to Virginia General Assembly's House Joint Resolution No. 450, VIMS initiated intensive field investigations with non-native oysters, *Crassostrea gigas*, during 1997. In May 1997, 600 juvenile triploid *C. gigas* (mean size = 19.2 mm, age = 10 mo.) and 600 juvenile triploid *Crassostrea virginica* (mean size = 31.7 mm, age = 11 mo.), were deployed at each of three replicate low salinity (<15ppt), medium salinity (15ppt-25ppt), and high salinity (>25ppt) sites in Chesapeake Bay and the Atlantic Coast of Virginia. In addition to triploid oysters, two groups of selected stocks of diploid *C. virginica* which had previously demonstrated either good growth or disease tolerance were deployed and evaluated at a subset of the sites. All oysters were maintained in floating mesh cages until May 1998, when the experiment was terminated to avoid risks of reproduction in triploid *C. gigas* during an additional summer in the field.

The study demonstrated that the comparative performance of *C. virginica* and *C. gigas* in Chesapeake Bay and the Atlantic Coast of Virginia varied with salinity regime. For the most part, selected stocks of diploids did not perform better than triploid *C. virginica*; therefore, the following comparisons refer to triploids. At low salinity sites in Chesapeake Bay, average cumulative mortality for *C. virginica* (10%) was significantly lower than that for *C. gigas* (63%). Growing at 0.10 mm/d, *C. virginica* reached a final average shell height of 67.8 mm, while *C. gigas,* with a significantly lower growth rate (0.06 mm/d), attained a final average size of 41.1 mm. During the 1-year deployment, 14% of the *C. virginica* and 0% of the *C. gigas* at low salinity sites reached market size (3 inches or 76.2 mm). However, *C. virginica* was more susceptible than *C. gigas* to *Perkinsus marinus* infections. Average *P. marinus* prevalence at low salinity sites peaked during fall at 44% for *C. virginica* and 3% for *C. gigas.*

At medium salinity sites, survival and growth rate for *C. virginica* were not different from that observed for *C. gigas*. However, *C. gigas* was less susceptible than *C. virginica* to *P. marinus* infections. Both species experienced moderately high cumulative mortality at the medium salinity sites–35% for *C. virginica* and 53% for *C. gigas* –but considerable variation among sites was observed. With a final average size of 74.1 mm, 41% of the *C. virginica* reached market size at medium salinity sites, while final average size and percent reaching market size for *C. gigas* was respectively 65.1 mm and 11%. Average *P. marinus* prevalence at medium salinity sites peaked during fall at 72% for *C. virginica* and 26% for *C. gigas*.

At high salinity sites, mean cumulative mortality was similarly low (<11%) for both species, while growth rate of *C. virginica* was significantly lower than that of *C. gigas*, and prevalence and intensity of *P. marinus* infections were higher for *C. virginica* than for *C. gigas*. With a mean growth rate of 0.24 mm/d, *C. gigas* grew nearly twice as fast as *C. virginica* at 0.13 mm/d. At the end of the experiment, in May 1998, average shell height of *C. gigas* was 108.1 mm and 100% had attained market size, while for *C. virginica* average shell height was 78.4 mm and 52% had attained market size. Average *P. marinus* prevalence at high salinity sites peaked during fall at 77% for *C. virginica* and 3% for *C. gigas*.

Infestations by the mud-worm *Polydora* spp. were higher for *C. gigas* than for *C. virginica* at low and medium salinity sites in October 1997, but similar for both species for other times and locations. Condition index was higher for *C. virginica* than for *C. gigas* at low salinity in May 1998, but similar for both species for other times and locations. All *C. gigas* were removed from the experiment by May 1998, after one year of deployment. *Crassostrea virginica* remaining at medium and high salinity sites until October 1998 had high prevalence and intensity of *P. marinus* infections and suffered high mortality (>67%).

In conclusion, *C. virginica* clearly outperformed *C. gigas* in low salinity sites (< 15 ppt) and *C. gigas* outperformed *C. virginica* at high salinity sites (>25 ppt). Performance was similar for the two species at medium salinity sites (15-25ppt) during the course of the study from May 1997 to May 1998.

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INTRODUCTION

As native oyster, *Crassostrea virginica*, stocks declined in Chesapeake Bay, due in part to protozoan parasites (Burreson and Ragone Calvo 1996), the shellfish industry in the region has been increasingly interested in the potential of non-native oyster species to restore the fishery. By 1995, when the annual oyster harvest in Virginia had declined to 2% of its level thirty years before, the Virginia General Assembly directed the Virginia Institute of Marine Science (VIMS) to conduct investigations to determine the suitability of selected non-indigenous oyster species for cultivation in Virginia. After conducting a thorough review of the existing literature, two candidate species within the genus *Crassostrea*, the Pacific oyster, *Crassostrea gigas,* and the Suminoe oyster, *Crassostrea ariakensis* (=*rivularis*), were selected based upon current knowledge and inferences regarding ecological requirements and disease tolerance (VIMS 1996). Prior studies at VIMS had indicated that *C. gigas* was more resistant to protozoan pathogens than the native oyster, at least under some environmental conditions. In laboratory disease challenge experiments with *Perkinsus marinus* (the organism which causes Dermo disease), *C. gigas* exhibited lower disease prevalence and intensity and had lower mortality than *C. virginica* (Meyers et al. 1991, Barber and Mann 1994). A field challenge experiment conducted in the York River using sterile oysters also indicated that *C. gigas* had reduced susceptibility to *P. marinus* and *Haplosporidium nelsoni* (the causative agent of MSX disease) compared to the native oyster (Burreson et al. 1994). In this field study, which lasted only five months, *C. gigas* had comparable shell growth rates to the native oysters, but became heavily infested by the mud worm *Polydora websteri*, resulting in poor meat quality. However, these studies were limited in duration and spatial extent, and it was clear that more extensive field experiments were necessary to better evaluate the performance of *C. gigas* within a broader range of salinity and other environmental conditions. No growth or disease challenge studies are available for *C. ariakensis* in the region; however, for locations on the West Coast of the US, Langdon and Robinson (1991) reported growth rates similar to that of *C. gigas*.

Responsible decisions regarding the potential introduction of an exotic species into a new area outside its native range require a considerable body of knowledge. In addition to understanding disease susceptibility and growth rates across a range of environmental conditions, it is important to have some knowledge of the reproductive capabilities of the exotic species and its ecological interactions with other species in the new environment. Towards this end VIMS has initiated a broad-based research effort with both of the candidate non-indigenous oyster

species, which includes evaluating environmental influences on reproduction and larval survival, predator-prey interactions with a major predator in the system (the blue crab *Callinectes sapidus*), and direct competition with the native oyster. In this report we detail one portion of this work—field experiments conducted with *C. gigas* over a range of habitats in Chesapeake Bay and the Atlantic coast of Virginia. These experiments were designed to (1) test the hypothesis that comparative performance of *C. gigas* and *C. virginica* would vary with salinity, (2) compare disease susceptibility in the same two species across salinity regimes, and (3) compare infestations by shell-boring organisms (e.g., mud worms and boring sponges). To ensure that these experiments resulted in neither the unintended reproduction of *C. gigas* nor the introduction of potential exotic pathogens, we used sterile triploid oysters produced from progeny of quarantined brood stocks, in accordance with protocols developed by the International Council for the Exploration of the Seas (ICES).

Background on Crassostrea gigas

Crassostrea gigas is the primary oyster species supporting shellfish industries around the globe. It has been estimated that 80% of the world oyster production is derived from *C. gigas* (Chew 1990). Experience with the transfer of *C. gigas* beyond its native range in the Indo-Pacific coast of Asia, particularly in Japan, has been considered both successful and problematic. For example, transfer of *C. gigas* to the Pacific Northwest region of the US has restored the shellfish industry, which used to rely on the native oyster *Ostrea lurida* (Chew 1990). Transfer of *C. gigas* to France has rehabilitated the industry by substituting for *Crassostrea angulata*, which was decimated by a viral disease (Grizel and Héral 1991). Transfer of exotic oysters, however, carries the risk of parallel transfer of pests and disease agents, and undesired competition of exotic species with their native counterparts. For instance, spread of the viral disease affecting *C. angulata* in France has been correlated with the introduction of *C. gigas*, which was conducted in bulk and without proper measures for disease prevention (Andrews 1980, Grizel and Héral 1991). Following transplantation into southeastern Australia, *C. gigas* successfully reproduced and displaced the native oyster, *Saccostrea commercialis*, from some of its habitat (Chew 1990).

Objectives

As a crucial component of non-native oyster research at VIMS, the present study was directed at examining the potential for growing *C. gigas* in Virginia. The study was not intended to serve as the sole basis for evaluating potential introductions of non-native oysters in Virginia,

but rather to support the decision making process involving the Virginia Marine Resources Commission and other state and regional bodies.

Specific objectives were to evaluate and compare the performance (survival, growth, and disease status) of *C. gigas* and *C. virginica*, within a range of salinity regimes in Chesapeake Bay and Atlantic Coast waters of Virginia.

METHODS

Study sites

Nine 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 triplicate locations within low salinity (>15 ppt), medium salinity (15-25 ppt), and high salinity (>25 ppt) areas (Fig 1). Low and medium salinity sites were established near the margins of rivers (Corrotoman, Great Wicomico, Coan, and York); or in shallow creeks surrounded by marshes (Woodas Creek, a tributary to the East River, and Nandua Creek). 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 various sites for intervals ranging from weekly to monthly.

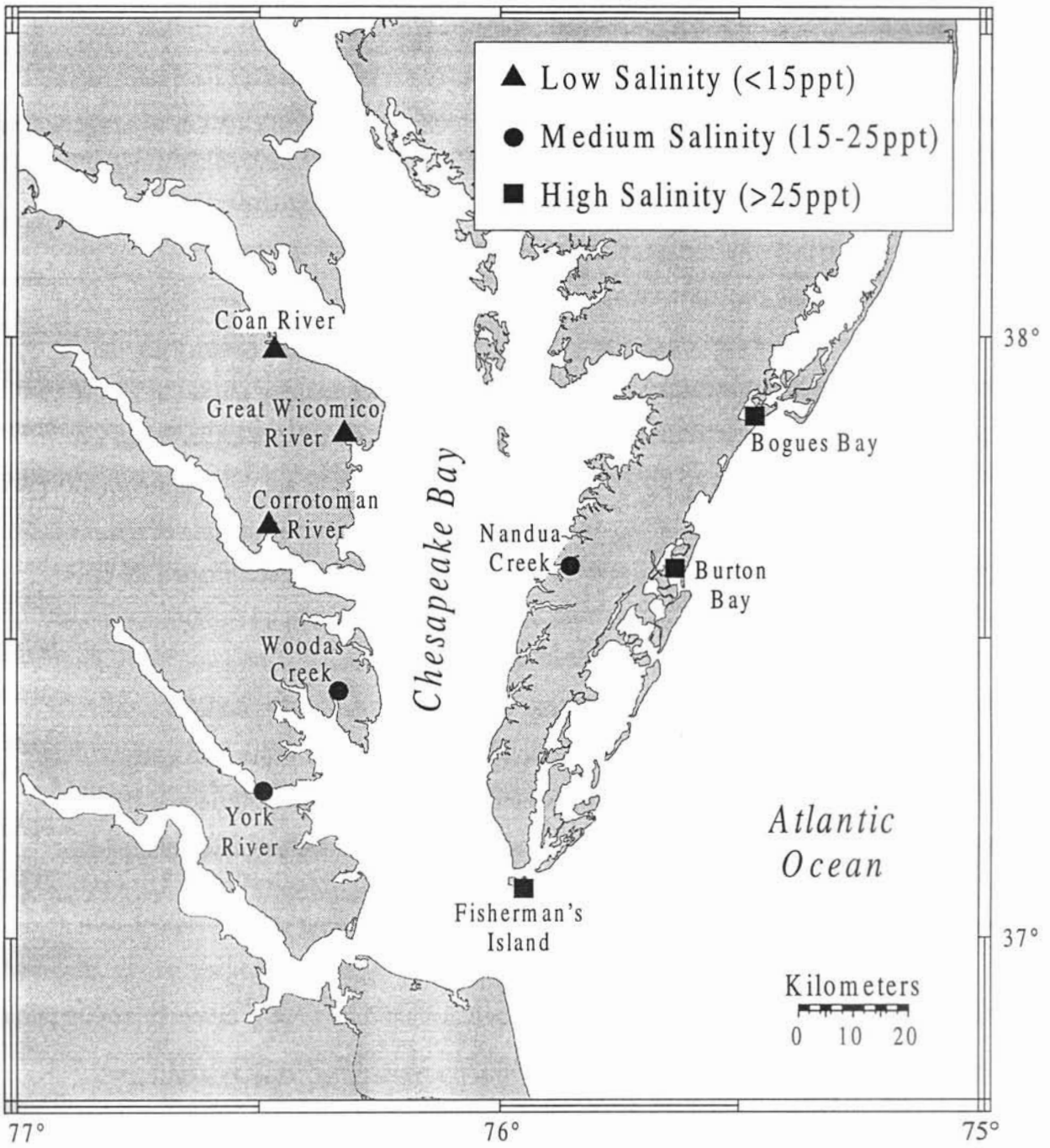

Fig. 1. Location of study sites in Chesapeake Bay and the Atlantic Coast of Virginia.

Oyster groups

Triploid *C. gigas* (3CG) and triploid *C. virginica* (3CV) were produced for this study by Haskin Shellfish Research Laboratory (HSRL) during June-July 1996 (Table 1). Brood stock for 3CG was Miyagi strain *C. gigas* originating from the Pacific Northwest Coast of the USA and maintained in quarantine at HSRL for several generations. Triploid *C. gigas* were produced by mating tetraploid and diploid parent stocks, an approach which results in complete triploidy of progeny (Guo et al. 1996). Brood stock for 3CV was a Delaware Bay strain naturally selected against *P. marinus* and *H. nelsoni* in Delaware Bay. Triploidy in *C. virginica* was chemically induced by treatment of fertilized eggs with cytochalasin-B using the methods described by Allen and colleagues (Downing and Allen 1987, Allen et al. 1989). Additionally, two *C. virginica* diploid stocks were produced for comparisons with the triploid oysters. One stock (2CVa) was produced at HSRL from brood stock originating from Delaware Bay strains with previously demonstrated resistance to *P. marinus* and *H. nelsoni* in Chesapeake Bay (Ragone Calvo et al. 1997). A second diploid *C. virginica* group (2CVb) was produced at VIMS by crossing stocks from Chincoteague Bay and the Lynnhaven River which had previously shown good performance in high salinity sites in Virginia. *Crassostrea virginica* groups selected for the present study represented the best available native stocks in terms of disease resistance and growth within Chesapeake Bay and the Atlantic Coast of Virginia. *Crassostrea gigas* stocks, which had been domesticated and raised in quarantine in Delaware Bay water for several generations, represented the best available pre-selected stocks for environments in Virginia.

Table 1. Oyster groups used in the present study.

Species	Group code	Hatchery	Date spawned	Size in May 97*
C. gigas	3CG	HSRL	16 July 96	19.2mm
C. virginica	3CV	HSRL	11 June 96	31.7mm
C. virginica	2CVa	HSRL-VIMS	11 July 96	20.5mm
C. virginica	2CVb	VIMS	19 June 96	43.2mm

Key to group codes: 2 = diploid, 3 = triploid, CG = *C. gigas*, CV = *C. virginica*
* = Mean shell height at the time of deployment.

Experimental design

Until field deployment in May 1997, juvenile 3CG were maintained first in flow-through tanks with ambient Delaware Bay water and quarantined effluents at HSRL Cape Shore, NJ, and then with York River ambient water and quarantined effluents at VIMS Gloucester Point, VA. Juvenile 3CV and 2CVa groups were also maintained first at HSRL Cape Shore, NJ, and then at

Gloucester Point, VA in flow-through tanks without quarantined effluents. Diploid Chincoteague/Lynnhaven oysters were maintained in field nursery at the Eastern Shore of Chesapeake Bay, VA.

Between 28 April and 16 May 1997, oysters were dispensed into triplicate 3.2 mm mesh bags and placed within individual floating trays at selected sites as described below. There were 200 oysters per bag and 600 oysters per floating tray. 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 were cleaned of fouling organisms at least once a month during regular site visits and more often if necessary. All sites were visited monthly (± 10 days). As oysters grew they were transferred from 3.2 mm mesh bags to 9.5 mm mesh bags in July 1997. In March 1998, when 3CG at high salinity sites approached space limitation within bags, all oyster groups at high salinity sites were split by placing half of the oysters into new bags. Oysters in the new bags were placed in a float adjacent to the original one.

A full factorial design, with three replicate sites within each of the three salinity regimes, was employed to examine the effects of triploid *C. virginica* and *C. gigas* (species) and salinity regime on final cumulative mortality, final condition index, and weighted prevalence of *Polydora* spp. A nested design, with sites nested within salinity regime and individual oysters as replicates, was employed to examine the effects of salinity regime on the growth rates of each species. Differences in mean final cumulative mortality, mean overall growth rate, mean final condition index, and mean weighted prevalence of *Polydora* spp., between species within salinity regime, between salinity regimes within species, and between times where appropriate, were further examined by Newman-Keuls test (Zar 1974). Data were examined for compliance with ANOVA assumptions using Bartlett chi-square test for homogeneity of variance and plots of means vs. standard deviations, and arcsine transformations were used where appropriate (Zar 1974).

Because of insufficient number of *C. virginica* available from the diploid stocks selected, 2CVa oysters were deployed at single sites (Coan River, York River, and Burton Bay) within salinity regimes, and 2CVb oysters were only deployed at a single high salinity site (Burton Bay). Therefore, comparisons involving diploid stocks were restricted to specific sites.

Mortality, growth, and condition

All live and dead oysters within each float were counted monthly to determine survival. Monthly mortality for each oyster group 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 of each oyster group was calculated as the sum of interval mortality (Barber 1994, Krebs 1972).

To follow growth, 100 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 and February 1998. Overall growth rate was calculated as initial size minus final size divided by total time elapsed in days. To provide a measure of production potential, the proportion of individually labeled oysters that attained Virginia legal market size for wild stocks (3 in = 76.2 mm), within each salinity regime, was calculated at the end of the experiment.

Whole weight, shell weight, and tissue wet and dry weights were measured on the same oysters (n=25) collected for disease diagnoses in October 1997 and May 1998. Following Lawrence and Scott (1982), condition index (CI) was calculated, by the formula:

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

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 (n = 25) was taken to assess the disease status of oyster groups prior to deployment on 25 March 1997 for 3CV, 3CG, and 2CVa, and on 22 April for 2CVb. Subsequent disease samples (n=25) were collected, depending on group and site, during 30 June-7 July 1997, 29 September-8 October 1997, and 15 April-18 May 1998. *Perkinsus marinus* was diagnosed using Ray's fluid Thioglycollate medium (RFTM) assays (Ray 1952) on combined mantle, gill, and rectum tissue. *Haplosporidium nelsoni* 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). Histological sections were also used

to document the presence of other parasites and to examine development of oyster gonads. All disease and histology work was performed by 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 disease diagnoses in October 1997 and May 1998. 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:

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

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

Reproductive status and ploidy

A baseline sample (n =125 larvae) for 3CV and (n=35 juveniles) for 3CG was taken to confirm ploidy status prior to deployment. To follow ploidy status during field deployment, 3CG samples (n = 35 oysters for each site) were collected, depending on site, during 2-10 June 1997, 30 June-9 July 1997, 28 July-5 August 1997, and 4-7 May 1998. Only *C. gigas* was examined for ploidy status during field deployment but *C. virginica* was collected at the same time that ploidy samples were taken, to standardize the number of oysters removed from trays by sampling. Ploidy status was determined by flow cytometry of gill biopsies from individually labeled oysters. When gill tissues 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 determination was made at HSRL and the VIMS Aquaculture Genetics and Breeding Technology Center.

RESULTS

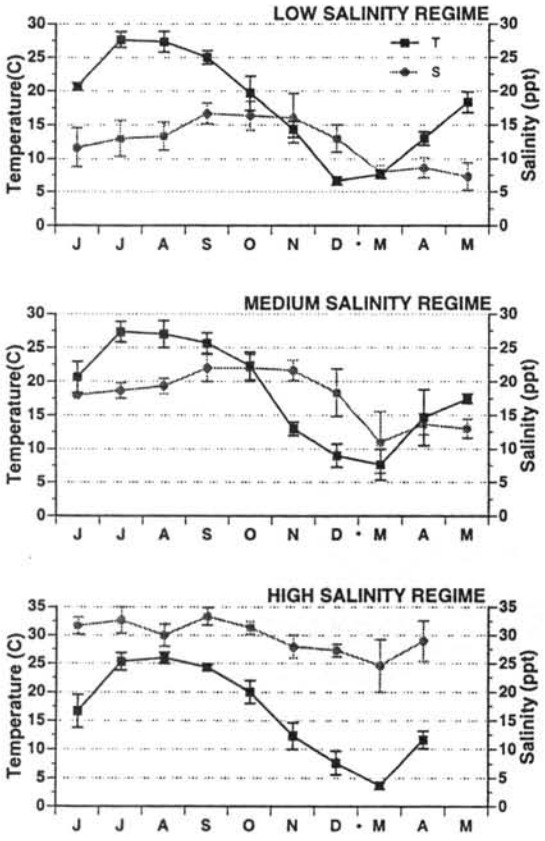

Fig. 2. Mean monthly (±SD) temperature and salinity of 3 sites within low, medium, and high salinity regimes, using stem thermometer and refractometer. * = Break in monthly sampling.

Environmental parameters

Salinity was within the range established for low, medium, and high salinity sites for most of the monthly measures (Fig. 2). Low salinity sites experienced relatively high mean salinity (>15 ppt) during September, October, and November because of drought conditions during the summer and relatively low mean salinity (<10 ppt) during March, April, and May because of high rainfall during the winter. The Coan River site experienced extreme low salinity with mean daily values of 3 ppt during April and May (Appendix I). Medium salinity sites experienced relatively low salinity (<15 ppt) during March, April, and May (Fig. 2).

Temperature followed similar seasonal trends at all sites with a maximum of 27-29 °C in July and a minimum of 3-6 °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).

Turbidity, measured in Nephelometric Turbidity Units (NTU), was highest at the medium salinity Nandua Creek site and Woodas Creek site. Maximum daily mean turbidity at Nandua Creek and Woodas Creek was respectively 436 NTU and 149 NTU, while maximum daily mean values at other sites was < 38 NTU (Appendix I).

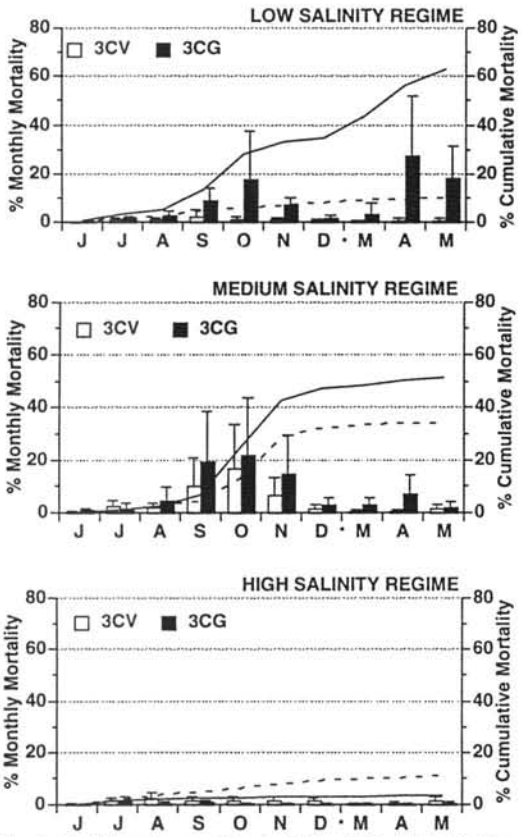

Fig. 3. Monthly and cumulative mortality of triploid *C. virginica* (3CV) and triploid *C. gigas* (3CG) from June 1997 trough May 1998. Bars = Mean (+SD) monthly mortality of 3 sites within low, medium, and high salinity. Dashed lines = Mean cumulative mortality of 3CV. Black lines = Mean cumulative mortality of 3CG. * = Break in monthly sampling.

Mortality

At low salinity, mortality of 3CV was very low (<3%) at all times while mortality of 3CG reached 18% in October 1997 and peaked at 28% in April 1998. At medium salinity, monthly mortality was highest for both groups in the fall with a maximum of 17% for 3CV and 22% for 3CG in October 1997. At high salinity, monthly mortality was very low (<3%) for both oyster groups at all times. Within salinity regimes, the pattern of mortality was similar among sites except for the Nandua Creek site that exhibited extremely high mortality (Appendix IIIa-c).

When *C. gigas* was removed from the study in May 1998, cumulative mortality of 3CV vs. 3CG at low, medium and high salinity sites, respectively, was 10% vs. 63%, 35% vs. 53%, and 11% vs. 4% (Fig. 3). Salinity regime, oyster species, and their interaction had significant effects on cumulative mortality (Table 2A), with 3CV surviving better at low salinity sites, and both groups having similar survival at medium and high salinity sites (Fig. 3 and Table 2B).

After *C. gigas* was removed from the study, cumulative mortality of *C. virginica* continued to increase during July and October 1998. By October 1998, mean (n = 2 sites) cumulative mortality of 3CV for low, medium, and high salinity sites was respectively 40%, 80%, and 67%. By May 1998, the diploid stocks (2CVa and 2CVb) both had greater cumulative mortality than 3CV, but in most cases exhibited the same patterns of survival relative to *C. gigas* as 3CV (Appendix IIIa-c). However, by October 1998, 2CVa had considerably higher survival than any other *C. virginica* stock deployed at Burton Bay (Appendix IIIc).

Table 2. Effects of salinity regime and oyster species on final cumulative mortality.

A. Two-way ANOVA

Effect	df	MS	F	p
Salinity	2	0.347	8.052	0.006
Species	1	0.216	5.008	0.044
Salinity*Species	2	0.212	4.918	0.027
Error	12	0.043		

B. Multiple comparisons (Newman-Keuls test) of mean cumulative mortality between triploid *C. virginica* and triploid *C. gigas* within salinity regimes, and between salinity regimes within triploid *C. virginica* and triploid *C. gigas*.

Comparison		Significance ($\alpha<0.05$)
Within	Between	
Low salinity	*C. virginica and C. gigas*	S
Medium salinity	*C. virginica and C. gigas*	NS
High salinity	*C. virginica and C. gigas*	NS
C. virginica	Low salinity vs. medium salinity	NS
C. virginica	Low salinity vs. high salinity	NS
C. virginica	Medium salinity vs. high salinity	NS
C. gigas	Low salinity vs. medium salinity	NS
C. gigas	Low salinity vs. high salinity	S
C. gigas	Medium salinity vs. high salinity	S

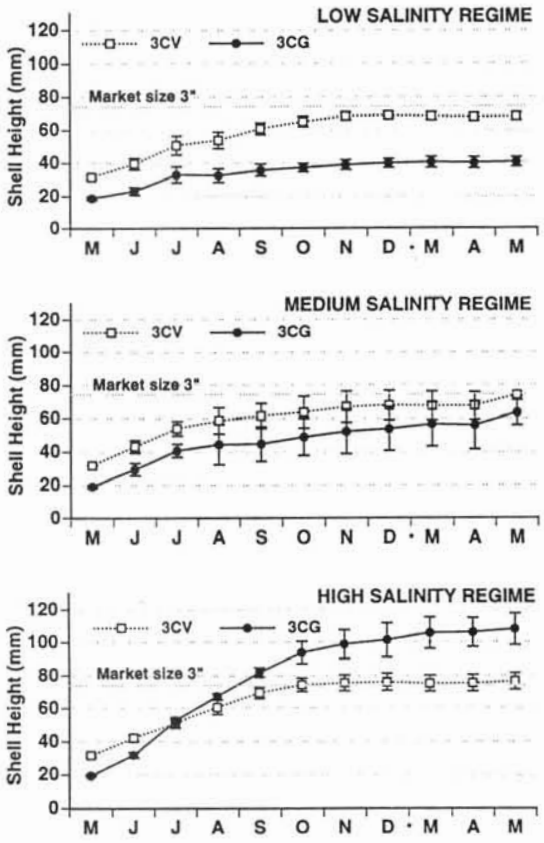

Fig. 4. Monthly shell height of triploid C. virginica (3CV) and triploid C. gigas (3CG) from May 1997 to May 1998. Mean (±SD) of 3 sites within salinity regime. * = Break in monthly sampling.

Growth

Before field deployment in April 1997, mean size of 3CV and 3CG was, respectively, 31.7 mm and 19.2 mm; subsequent growth varied with salinity regime. At low salinity, 3CV increased its initial size advantage over 3CG resulting in a mean shell height of 67.8 mm for 3CV and 41.1 mm for 3CG in May 1998. At medium salinity, the initial size differential between species was maintained throughout the study yielding a final mean shell height of 74.1 mm for 3CV and 65.1 mm for 3CG. At high salinity, the initially smaller 3CG had reached the same size as 3CV 3 mo. after deployment by July 1997. *Crassostrea gigas* continued to grow throughout the fall and winter months, while *C. virginica* stopped growing after October 1997 (Fig 4). A similar pattern was observed in the proportion of each species reaching legal market size (76.2 mm), with the exception that at the medium salinity sites *C. virginica* out performed *C. gigas* (Table 3).

Salinity regime, site within salinity regime, oyster species, and their interactions had significant effects on mean growth rate (Table 4A). At low salinity, mean overall growth rate of 3CV (0.10 mm/d) was significantly greater than that of 3CG (0.06 mm/d) (Table 4B), with most of the growth in *C. virginica* occurring between July and October (Fig. 4). At the medium salinity sites, mean overall growth rate for both oyster groups (0.10 mm/d) was not significantly different and the monthly pattern of growth was similar for both species. At high salinity, mean overall growth rate of 3CV (0.13 mm/d) was significantly lower and nearly half than that of 3CG (0.24 mm/d). The diploid *C. virginica* performed comparable to or worse than the triploids of the same species with respect to the numbers reaching market size (Table 3) and growth rate (Appendix III a-c).

Table 3. Percent market size (>76.2 mm) oysters in May 1998, based on the legal size for wild harvested oysters in Virginia. X = Group not deployed at that salinity regime. In parenthesis, number of market size oysters/total number of live oysters.

	Oyster group			
Salinity regime	3CV	3CG	2Cva	2CVb
Low	14% (38/268)	0% (0/69)	0% (0/84)	X
Medium	41% (65/159)	11% (10/91)	2% (1/55)	X
High	52% (131/252)	100% (260/261)	3% (2/77)	60% (48/80)

Table 4. Effects of salinity regime, site nested within salinity regime, and oyster group on growth rate.

A. Three-way ANOVA

Effect	df	MS	F	p
Salinity	2	0.016	1390.037	<0.0005
Site	6	0.000	47.502	<0.0005
Group	1	0.000	42.463	<0.0005
Salinity*Site	2	0.009	810.027	<0.0005
Site*Group	6	0.000	8.009	<0.0005
Error	1122	0.000		

B. Multiple comparisons (Newman-Keuls test) of mean growth rate between triploid *C. virginica* and triploid *C. gigas* within salinity regimes, and between salinity regimes within triploid *C. virginica* and triploid *C. gigas*.

Comparison		Significance (α<0.05)
Within	Between	
Low salinity	*C. virginica and C. gigas*	S
Medium salinity	*C. virginica and C. gigas*	NS
High salinity	*C. virginica and C. gigas*	S
C. virginica	Low salinity vs. medium salinity	NS
C. virginica	Low salinity vs. high salinity	S
C. virginica	Medium salinity vs. high salinity	S
C. gigas	Low salinity vs. medium salinity	S
C. gigas	Low salinity vs. high salinity	S
C. gigas	Medium salinity vs. high salinity	S

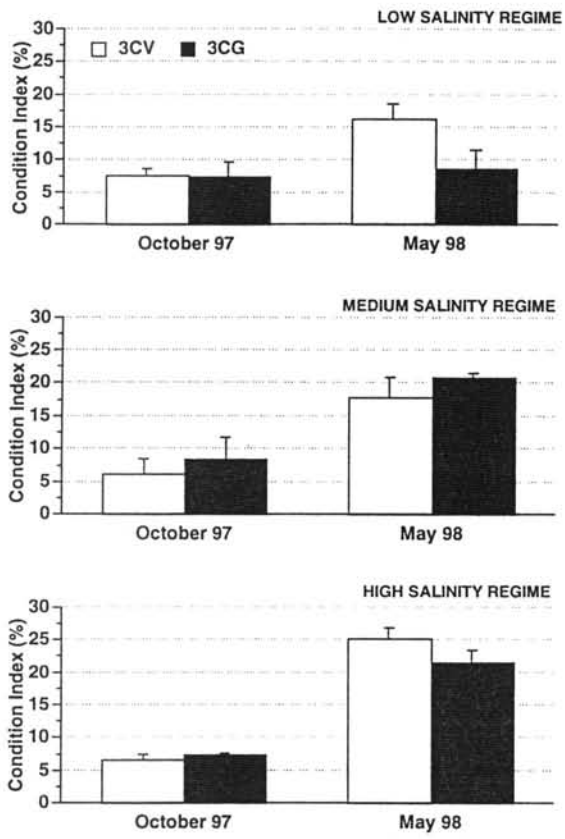

Fig. 5. Condition index in triploid *C. virginica* (3CV) and triploid *C. gigas* (3CG). Mean (+SD) of 3 sites within low, medium, and high salinity regime.

Condition index

Salinity regime, time, and the interactions of salinity and species and salinity and time had significant effects on final oyster condition (Table 5A). For October 1997, there were no significant differences in condition index between species within any salinity, or between salinities within a species. For May 1998, at low salinity, mean condition index of 3CV (16.2%) was significantly higher than of 3CG (8.7%); and at other salinities no significant differences were detected between species. For May 1998 within either species, condition index significantly increased with salinity, except for *C. gigas* between medium and high salinity. For both species within any salinity, except for *C. gigas* within low salinity, condition index increased with time (Table 5B).

Table 5. Effects of salinity regime, oyster species, and time on condition index.

A. Three-way ANOVA

Effect	df	MS	F	p
Salinity	2	82.662	18.840	<0.0005
Species	1	6.806	1.551	0.226
Time	1	1022.249	232.990	<0.0005
Salinity*Species	2	29.052	6.621	0.006
Salinity*Time	2	100.039	22.800	<0.0005
Species*Time	1	25.236	5.751	0.254
Salinity*Species*Time	2	10.863	2.476	0.107
Error	22	4.387		

Condition index for diploid groups in May 1998, particularly for 2CVa, was higher than that for 3CV. Among sites within salinity regimes, oysters at Nandua Creek and Woodas Creek had lower condition index than that at the York River in October 1997 (Appendix II). No results were available for Nandua Creek in May 1998 because at that site oysters did not survive beyond April 1998.

Relative to whole oyster weight, shells of *C. virginica* were heavier than shells of *C. gigas* (Appendix II). For all samples combined, the percentage of shell weight relative to whole weight was 66% in 3CV and 57% in 3CG. Proportional shell weight remained fairly constant for 3CV at low, medium, or high salinity, between October 1997 and May 1998, while it decreased in 3CG at low and medium salinity and increased in 3CG at high salinity (Appendix II).

B. Multiple comparisons (Newman-Keuls test) of condition index between triploid *C. virginica* and triploid *C. gigas* within salinity regimes, and between salinity regimes within triploid *C. virginica* and triploid *C. gigas*.

Comparisons		Significance ($\alpha < 0.05$)
For October 97, within	Between	
Low salinity	*C. virginica and C. gigas*	NS
Medium salinity	*C. virginica and C. gigas*	NS
High salinity	*C. virginica and C. gigas*	NS
C. virginica	low salinity vs. medium salinity	NS
C. virginica	low salinity vs. high salinity	NS
C. virginica	medium salinity vs. high salinity	NS
C. gigas	low salinity vs. medium salinity	NS
C. gigas	low salinity vs. high salinity	NS
C. gigas	medium salinity vs. high salinity	NS
For May 98, within	Between	
Low salinity	*C. virginica and C. gigas*	S
Medium salinity	*C. virginica and C. gigas*	NS
High salinity	*C. virginica and C. gigas*	NS
C. virginica	low salinity vs. medium salinity	NS
C. virginica	low salinity vs. high salinity	S
C. virginica	medium salinity vs. high salinity	S
C. gigas	low salinity vs. medium salinity	S
C. gigas	low salinity vs. high salinity	S
C. gigas	medium salinity vs. high salinity	NS
For *C. virginica* within	Between	
Low salinity	October 97 vs. May 98	S
Medium salinity	October 97 vs. May 98	S
High salinity	October 97 vs. May 98	S
For *C. gigas* within	Between	
Low salinity	October 97 vs. May 98	NS
Medium salinity	October 97 vs. May 98	S
High salinity	October 97 vs. May 98	S

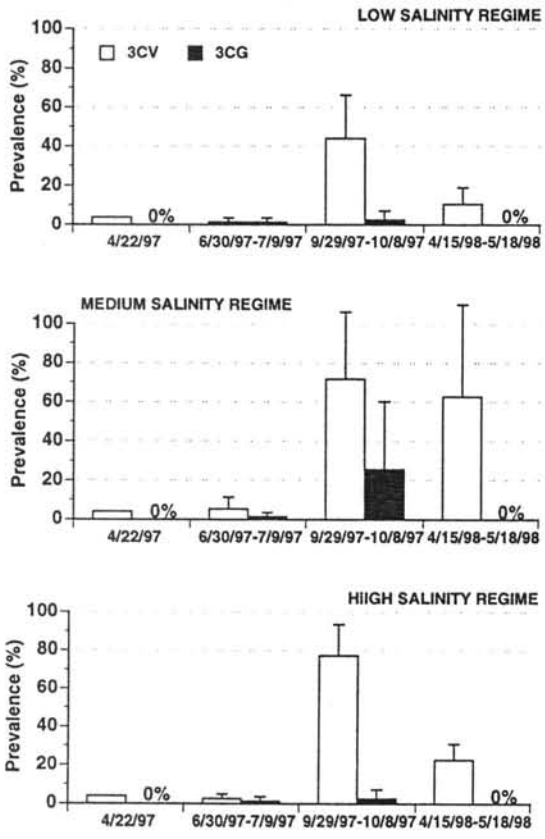

Fig. 6. Prevalence of *Perkinsus marinus* in triploid *C. virginica* (3CV) and triploid *C. gigas* (3CG) from April 1997 through May 1998. Mean (+SD) of 3 sites within low, medium, and high salinity regime.

Disease

Perkinsus marinus infections in 3CV were low during the first spring and summer of deployment and peaked in fall with higher prevalences observed at higher salinities (Fig. 6). Prevalence of *P. marinus* infections in *C. gigas* was generally low at most times and sites (Fig. 6, Appendix V); however, relatively high prevalence in Nandua Creek resulted in a mean prevalence of 26% in *C. gigas* at medium salinity sites during the fall. Prevalence of *P. marinus* in 3CV at the medium salinity Nandua Creek and Woodas Creek sites remained high (>80%) in spring 1998 when mean prevalence at low and high salinity sites subsided below 23%. *Crassostrea virginica* had a higher proportion of moderate and high intensity infections by *P. marinus* than *C. gigas* (Appendix V).

Prevalence of *P. marinus* in diploid groups, particularly 2CVa, was lower than that in 3CV but higher than in *C. gigas* (Appendix V). *Haplosporidium nelsoni* was present at low prevalence in 3CV at medium and high salinity, but was absent in *C. gigas* (Appendix V). At low salinity *H. nelsoni* was not detected in any of the samples. Triploid *C. virginica* at the York River in July experienced the highest MSX prevalence (16%) for oysters at medium salinity (Appendix V). The diploid group (2CVb) at Burton Bay experienced the highest MSX prevalence (46% in September 97 and 50% in May 98) recorded during the study (Appendix V).

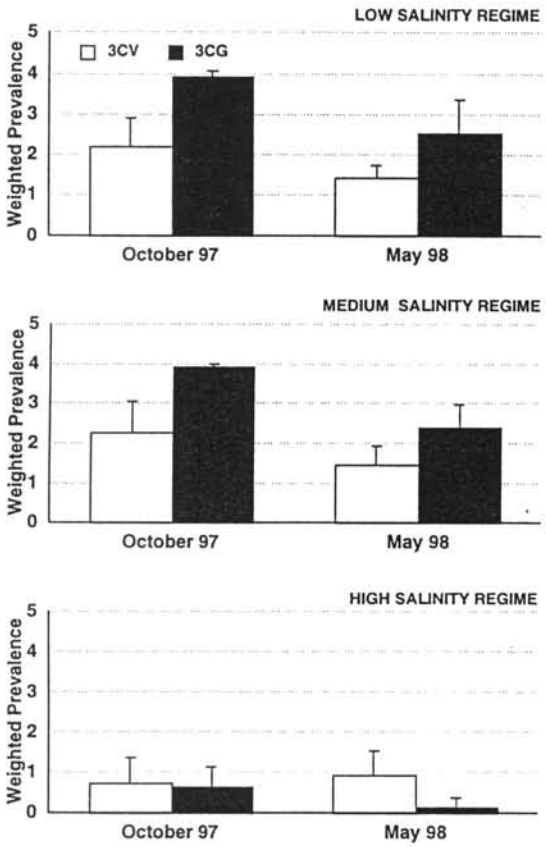

Fig. 7. Intensity of *Polydora* infestations in triploid *C. virginica* (3CV) and triploid *C. gigas* (3CG). Mean (+SD) of 3 sites within low, medium, and high salinity regime.

Polydora

At low and medium salinity sites, mean prevalence was high (>95%) for 3CV and 3CG regardless of time. At high salinity sites, however, while mean prevalence for 3CV remained at 64%, it decreased for *C. gigas* from 52% in October 1997 to 12% in May 1998. Differences in weighted prevalence (Equation 2) between oyster species were more pronounced than differences in prevalence. Triploid *C. virginica* had lower weighted prevalence than *C. gigas* at medium and low salinity sites in October and similar levels of *Polydora* spp. infestation at all other times and locations (Fig. 7). Salinity, oyster species, time, and the interaction of salinity and oyster species had significant effects on mean weighted prevalence (Table 6A).

Table 6. Effects of salinity regime, oyster species, and time on *Polydora* spp. weighted prevalence.

A. Three-way ANOVA

Effect	df	MS	F	p
Salinity	2	14.085	49.296	<0.0005
Species	1	4.814	16.851	<0.0005
Time	1	5.586	19.550	<0.0005
Salinity*Species	2	3.204	11.215	<0.0005
Salinity*Time	2	0.881	3.084	0.065
Species*Time	1	0.910	3.185	0.088
Salinity*Species*Time	2	0.003	0.013	0.986
Error	22	0.286		

B. Multiple comparisons (Newman-Keuls test) of *Polydora* spp. weighted prevalence between triploid *C. virginica* and triploid *C. gigas* within salinity regimes, and between salinity regimes within triploid *C. virginica* and triploid *C. gigas*.

Comparisons		Significance ($\alpha < 0.05$)
For October 97, within	Between	
Low salinity	*C. virginica and C. gigas*	S
Medium salinity	*C. virginica and C. gigas*	S
High salinity	*C. virginica and C. gigas*	NS
C. virginica	low salinity vs. medium salinity	NS
C. virginica	low salinity vs. high salinity	S
C. virginica	medium salinity vs. high salinity	S
C. gigas	low salinity vs. medium salinity	NS
C. gigas	low salinity vs. high salinity	S
C. gigas	medium salinity vs. high salinity	S
For May 98, within	Between	
Low salinity	*C. virginica and C. gigas*	NS
Medium salinity	*C. virginica and C. gigas*	NS
High salinity	*C. virginica and C. gigas*	NS
C. virginica	low salinity vs. medium salinity	NS
C. virginica	low salinity vs. high salinity	NS
C. virginica	medium salinity vs. high salinity	NS
C. gigas	low salinity vs. medium salinity	NS
C. gigas	low salinity vs. high salinity	S
C. gigas	medium salinity vs. high salinity	S
For *C. virginica* within	Between	
Low salinity	October 97 vs. May 98	NS
Medium salinity	October 97 vs. May 98	NS
High salinity	October 97 vs. May 98	NS
For *C. gigas* within	Between	
Low salinity	October 97 vs. May 98	S
Medium salinity	October 97 vs. May 98	S
High salinity	October 97 vs. May 98	NS

For 3CV, within any salinity, mean weighted prevalence was not significantly different between October and May, while for 3CG at low and medium salinity mean weighted prevalence significantly decreased from October to May. Within 3CG, at high salinity, mean weighted prevalence was not significantly different between October and May (Table 6B). Diploid *C. virginica* had infestations by *Polydora* spp. of similar intensity than those of triploid oysters of the same species (Appendix II).

Ploidy

Baseline samples confirmed 100% triploidy among naturally induced triploid *C. gigas* and revealed 85% triploidy among chemically induced triploid *C. virginica*. The proportion of *C. gigas* gill samples in which combinations of diploid and triploid cells (mosaics) were detected by flow-cytometry varied with time and salinity (Table 7). The proportion of mosaics, pooled for all salinity regimes, increased from 0.0% in June 1997 to 6.1% in April 1998, and then decreased to 3.6% in May 1998. The proportion of mosaics, pooled for all times within low, medium, and high salinity, was respectively, 4.0%, 2.5%, and 1.8%. For all samples collected during the study combined, regardless of salinity, the overall proportion of mosaics was 2.7%.

Table 7. Percent genetic mosaics among *C. gigas* by salinity regime and date. In parenthesis number of mosaics/number of oysters examined.

Date/Salinity	Low	Medium	High	Row total
2-10 June 97	0.0% (0/105)	0.0% (0/105)	0.0% (0/105)	0.0% (0/315)
30 June - 9 July 97	0.0% (0/105)	2.8% (3/105)	0.0% (0/105)	0.9% (3/315)
28 July - 5 August 97	4.7% (5/105)	0.9% (1/105)	0.0% (0/105)	1.9% (6/315)
6-15 April 98	5.0% (3/60)	8.3% (8/96)	4.8% (5/105)	6.1% (16/261)
4-7 May 98	6.1% (20/325)	1.7% (4/233)	2.5% (9/358)	3.6% (33/916)
Column total	4.0% (28/700)	2.5% (16/644)	1.8% (14/778)	2.7% (58/2122)

Among a group of 23 oysters with mosaic gill cells examined, for samples collected from June 1997 through April 1998, 5 were females, 15 were males, and 3 were not identified as to sex. Among oysters with mosaic gill cells there was only one male individual, in Bogues Bay samples collected 14 April 1998, in which haploid gonad cells were detected. Detection of an individual *C. gigas* with haploid gametes triggered immediate action to avoid potential reproduction of experimental oysters. VIMS Director for Research and Advisory Services decided to terminate the experiment and by 6 May 1998 all *C. gigas* were removed from the water and maintained in quarantine conditions at VIMS.

DISCUSSION

This study demonstrated that the comparative performance of *C. virginica* and *C. gigas* in Chesapeake Bay and the Atlantic Coast of Virginia varied with salinity regime. At low salinity, survival, growth rate, final condition index, and resistance to infestations by *Polydora* spp. were greater for *C. virginica* than for *C. gigas*. However, *C. virginica* was more susceptible than *C. gigas* to *P. marinus* infections. High mortality (63%) and poor growth (0.06 mm/d) observed for *C. gigas* at low salinity sites were not surprising considering the previously reported optimal salinity of 35‰ for growth in this species (Mann et al. 1991). High mortality of *C. gigas* at the low salinity Coan River site in April (56%) can probably be attributed to a prolonged period of extreme low mean daily salinity (3‰ for 1 month). Most of the growth for *C. virginica* and *C. gigas* occurred in the spring subsequent to deployment.

At low and medium salinity, shells of *C. gigas* with severe *Polydora* spp. infestations were very fragile and often disintegrated during monthly inspections of labeled individuals for growth. The decrease in the severity of *Polydora* spp. infestations between October 1997 and May 1998, primarily for medium and high salinity sites, can be attributed to oyster shell repair. In May 1998 nacar shell deposits were often observed to cover blisters. Comparing shell weight for oysters of similar size, Barber (Barber and Mann 1994) found that shell weight was significantly greater for similar sized *C. virginica* than *C. gigas*. Similarly, in the present study, *C. virginica* had heavier shells proportional to whole oyster weight relative to *C. gigas*. It is likely that the relatively thinner shells in *C. gigas* make it more susceptible to heavy *Polydora* spp. infestations.

At medium salinity sites, mean cumulative mortality, growth rate, and final condition index of *C. virginica* were not different than that of *C. gigas*. *Crassostrea gigas* was more susceptible to infestations by *Polydora* spp. and less susceptible to *P. marinus* than *C. virginica* in this salinity regime. Both *C. virginica* and *C. gigas* experienced a high variability in mortality and growth rate due to extremely poor performance at Nandua Creek, relative to the other two medium salinity sites. High mortality and poor condition of *C. virginica* and *C. gigas* at Nandua Creek can be attributed to prevalent and severe *P. marinus* infections. Among medium salinity sites, for both *C. virginica* and *C. gigas*, *P. marinus* infections were most prevalent and severe at the Nandua Creek site, and to a lesser extent at the Woodas Creek site, relative to the York River site. Apparently, sites that were fairly enclosed, with silty bottoms and high turbidity, as the Nandua Creek and Woodas Creek sites, were conducive to higher *P. marinus* infections. We

speculate that high density of other oyster lots present in the immediate vicinity of the experimental oysters, coupled with relatively poor water exchange, resulted in high disease pressure and environmental stress at those sites. Despite higher salinity being more favorable for the development of lethal *P. marinus* infections, triploid oysters at high salinity sites suffered only minor mortality (<12%) compared to that at the medium salinity Nandua Creek site (>67%). To some extent, overall cooler temperature at high salinity sites may have contributed to prevent disease-induced mortality comparable to that at medium salinity sites.

As documented in prior studies comparing growth of diploid *C. gigas* and diploid *C. virginica* in quarantined systems at the York River (Barber and Mann 1994), most of the growth for both species at medium salinity occurred in the spring and fall. However, there were important differences between the two studies. Barber and Mann (1994) reported greater growth rates for *C. gigas* than *C. virginica* at the York River site, while this study did not find significant differences in growth of the two species at the site. This incongruity may arise from different environmental conditions at the site between years or from differences in the timing of spawns and handling of oysters between the studies.

At high salinity sites, growth rate of *C. gigas* was higher than that of *C. virginica*, while there was no difference in survival and final condition between oyster species, except for higher condition index in diploid *C. virginica* than in *C. gigas* at Burton Bay in May 1998. Infestations by *Polydora* spp. were very light for both species, while prevalence and intensity of *P. marinus* infections were higher for *C. virginica* than for *C. gigas*. During the 1 year deployment at high salinity, mean cumulative mortality was similarly low (<11%) for both species. With a mean growth rate of 0.24 mm/d, *C. gigas* at high salinity sites grew nearly twice as fast as triploid *C. virginica*. By November 1997, six months after deployment, 95% of *C. gigas* attained 76.2 mm, the legal market size for wild caught oysters in Virginia. By comparison, despite their larger initial size, only 47% of triploid *C. virginica* reached the same size in that period. Most of the growth for *C. virginica* and *C. gigas* occurred in the spring and continued into the fall. While *C. virginica* stopped growing in October, *C. gigas* continued to grow during the winter months.

In summary, during the course of the study *C. gigas* performed no better than *C. virginica* at low and medium salinities in Chesapeake Bay. In contrast, performance of *C. gigas* at high salinity in the Atlantic Coast of Virginia was clearly superior to that of *C. virginica*. However, considering the large variability in performance between the two oyster species among medium salinity sites and given the wide temporal salinity fluctuations in Chesapeake Bay, caution should be exercised in extrapolating performance of *C. gigas* at these sites over longer periods of

time. For *C. virginica* remaining at medium and high salinity sites after *C. gigas* was removed in May, a second summer of disease exposure was devastating. At medium salinity for triploid *C. virginica*, prevalence of *P. marinus* was 100% and cumulative mortality reached 66% - 94% in October 1998. Therefore, it is likely that at medium salinity *C. gigas* would have out performed *C. virginica* if they remained in the water for at least a second summer.

The results of this study are not, however, sufficient for concluding that *C. gigas* is or is not an appropriate species for introduction or use in these environments. A prerequisite for responsible utilization of an exotic species in fisheries enhancement is an understanding of the associated environmental risks. Use of reproductively capable diploid *C. gigas* would likely result in its introduction into some regions within the waters of Virginia and neighboring states. An important determinant of the extent to which this species might spread if introduced is the interactive effects of temperature and salinity on reproduction and larval development. Experiments to address this issue are currently underway at VIMS, but final results are not yet available. Further, interactions with other species—such as competitive interactions with *C. virginica* and predator-prey interactions with dominate predators in the system—may be important in determining the spread and impact of introducing this species. Experiments addressing these issues have recently been initiated at VIMS. Ultimately, any decision to attempt to establish an exotic oyster species or to permit its use in aquaculture within Virginia is a management issue which must weigh the risks and benefits to society. While this work has demonstrated some benefits with respect to rapid growth and disease tolerance by *Crassostrea gigas* in high salinity environments, at the present time we do not believe that sufficient information is available to evaluate the risks associated with such an introduction.

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APPENDICES

I. Daily temperature, salinity, and turbidity, by site and weekly-monthly interval.

II. Biomass, condition index, and weighted prevalence of *Polydora* spp., for *C. virginica* and *C. gigas* by site and date
   A. October 1997
   B. May 1998

III. Cumulative mortality of *C. virginica* and *C. gigas* by site.
   A. Low salinity sites
   B. Medium salinity sites
   C. high salinity sites

IV. Monthly shell height of *C. virginica* and *C. gigas* by site.
   A. Low salinity sites
   B. Medium salinity sites
   C. high salinity sites

V. Prevalence and intensity *P. marinus* and *H. nelsoni* by site and date.
   A. Baseline sample
   B. Low salinity sites
   C. Medium salinity sites
   D. High salinity sites

Appendix I. Mean (SD) daily temperature, salinity, and turbidity. Statistics of hourly measures recorded with Hydrolab Minisonde© during deployment at various sites.
- = No record available.

Salinity regime	Site	Date interval	Temperature (°C)	Salinity (ppt)	Turbidity (NTU)
Low	CORV	09/04/97-09/16/97	25.2 (0.8)	9.8 (3.2)	31.2 (67.9)
	GWRV	08/04/97-08/18/97	28.5 (1.1)	9.4 (1.6)	15.0 (22.9)
		04/07/98-05/04/98	16.9 (1.4)	8.3 (0.4)	111.7 (73.6)
	CNRV	08/04/97-08/20/98	28.2 (1.1)	11.1 (0.7)	12.8 (41.4)
		12/09/97-12/26/97	6.0 (0.6)	7.2 (0.9)	4.2 (4.4)
		04/07/98-05/04/98	17.0 (1.6)	3.3 (0.7)	23.2 (21.3)
Medium	NACK	06/23/97-06/30/97	29.6 (1.4)	-	345.2 (216.3)
		07/03/97-07/14/97	28.6 (1.3)	-	436.4 (305.5)
		09/29/97-10/22/97	19.9 (3.0)	20.8 (0.6)	19.0 (26.5)
	WOCK	06/11/97-06/24/97	27.3 (2.6)	13.7 (2.7)	133.2 (192.9)
		07/01/97-07/08/97	29.2 (1.5)	17.3 (0.5)	148.8 (241.2)
		09/29/97-10/13/97	21.8 (1.5)	18.3 (1.9)	36.4 (79.0)
High	BOBY	10/30/97-11/30/97	10.6 (3.0)	24.5 (1.9)	24.6 (24.8)
		01/20/98-03/06/98	7.3 (1.6)	23.5 (2.4)	38.6 (52.8)
	BUBY	10/31/97-11/30/97	10.7 (3.0)	26.3 (4.9)	12.6 (26.4)
	FIIS	01/20/98-03/06/98	6.7 (1.7)	24.0 (2.2)	10.8 (29.9)

Site codes: CORV = Corrotoman River, GWRV = Great Wicomico River, CNRV = Coan River, NACK = Nandua Creek, WOCK = Woodas Creek, BOBY = Bogues Bay, BUBY = Burton Bay, FIIS = Fisherman's Island.

Appendix II. Mean (SD) biomass and condition index (CI), and weighted prevalence of *Polydora* spp., for *C. virginica* and *C. gigas* by site in (A) October 1997 and (B) May 1998.

A. October 1997

Salinity regime	Site	Group	N	Whole wt. (g)	Shell wt. (g)	Wet wt. (g)	Dry wt. (g)	CI (%)	Polydora
Low	CORV	3CV	25	30.9 (7.9)	20.7 (5.3)	4.6 (1.3)	0.7 (0.2)	6.9 (1.2)	2.2
		3CG	23	10.3 (2.2)	6.6 (1.3)	1.2 (0.3)	0.2 (0.1)	5.5 (1.3)	4.0
	GWRV	3CV	25	22.0 (5.7)	12.9 (3.2)	3.8 (1.1)	0.7 (0.2)	7.5 (0.9)	2.9
		3CG	25	5.3 (1.1)	2.6 (0.6)	0.9 (0.2)	0.2 (0.0)	6.6 92.3)	4.0
	CNRV	3CV	25	30.2 (9.0)	18.2 (4.9)	5.4 (1.9)	1.1 (0.4)	8.8 (1.3)	1.5
		3CG	23	7.6 (1.4)	4.3 (1.0)	1.6 (0.40	0.3 (0.1)	10.0 (2.3)	3.8
		2CVa	25	13.0 (3.7)	8.5 (2.5)	2.3 (0.8)	0.5 (0.2)	10.1 (1.9)	1.4
Medium	NACK	3CV	22	14.3 (4.3)	9.6 (2.8)	1.5 (0.5)	0.2 (0.1)	3.9 (1.5)	2.5
		3CG	17	5.8 (1.4)	3.9 (0.9)	0.6 (0.3)	0.1 (0.1)	5.8 (2.8)	4.0
	YKRV	3CV	25	33.8 (8.4)	23.0 (5.4)	5.2 (2.0)	0.9 (0.4)	8.4 (2.1)	1.4
		3CG	25	32.0 (6.3)	20.1 (3.9)	7.3 (1.9)	1.4 (0.4)	12.2 (2.0)	3.9
		2CVa	25	14.2 (4.6)	10.0 (3.3)	2.4 (1.0)	0.4 (0.2)	10.3 (1.7)	2.0
	WOCK	3CV	25	30.3 (7.9)	20.1 (5.0)	4.1 (1.5)	0.7 (0.2)	6.3 (1.8)	2.9
		3CG	24	14.7 (3.5)	9.0 (2.0)	2.5 (0.8)	0.4 (0.1)	7.3 (1.8)	3.9
High	BOBY	3CV	24	41.6 (14.6)	29.4 (9.7)	5.3 (2.2)	0.8 (0.3)	6.9 (1.9)	1.0
		3CG	25	73.0 (21.4)	42.7 (12.3)	10.7 (3.1)	2.0 (0.7)	7.0 (2.6)	0.7
	BUBY	3CV	25	36.3 (13.3)	24.5 (8.6)	4.2 (1.8)	0.7 (0.3)	5.7 (1.2)	0.0
		3CG	25	60.7 (14.0)	33.4 (7.2)	10.9 (3.5)	2.0 (0.7)	7.4 (1.8)	0.1
		2CVa	25	23.7 (7.1)	16.9 (5.3)	2.6 (0.8)	0.5 (0.2)	6.8 (1.0)	0.0
		2CVb	24	40.7 (13.2)	27.7 (9.3)	4.6 (1.7)	0.8 (0.3)	6.0 (1.7)	0.1
	FIIS	3CV	25	44.6 (12.7)	27.8 (8.7)	5.6 (1.8)	1.1 (0.4)	7.4 (4.5)	1.2
		3CG	25	83.1 (23.5)	46.8 (12.2)	13.5 (4.2)	2.8 (0.9)	7.6 (2.1)	1.1

Site codes: CORV = Corrotoman River, GWRV = Great Wicomico River, CNRV = Coan River, NACK = Nandua Creek, WOCK = Woodas Creek, BOBY = Bogues Bay, BUBY = Burton Bay, FIIS = Fisherman's Island. Group codes: 3CV = triploid *C. gigas*, 3CV = triploid *C. virginica*, 2CVa = diploid Delaware Bay *C. virginica*, 2CVb = diploid Chincoteague/Lynnhaven *C. virginica*.

Appendix II. Mean (SD) biomass and condition index (CI), and weighted prevalence of *Polydora* spp., for *C. virginica* and *C. gigas* by site.

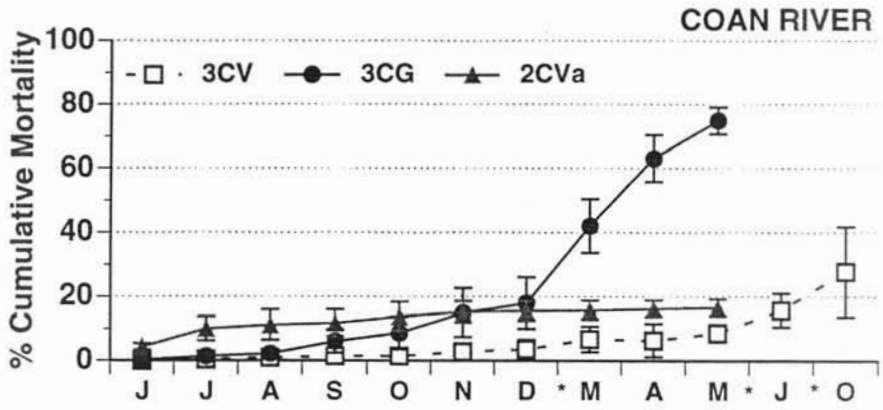
B. May 1998

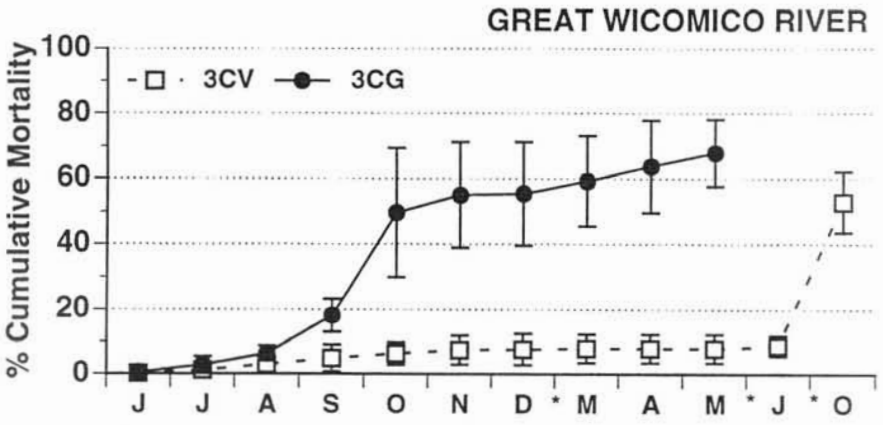
Salinity regime	Site	Group	N	Whole wt. (g)	Shell wt. (g)	Wet wt. (g)	Dry wt. (g)		
			25	36.2 (10.5)	21.8 (6.6)	5.9 92.0)	1.9 (0.3)	13.6 (2.5)	1.8
		3CG	25	13.1 (2.9)	5.5 (1.4)	2.4 (0.8)	0.5 (0.2)	7.1 (1.5)	2.9
	GWRV	3CV	25	32.2 (8.9)	20.2 (5.4)	6.3 (2.1)	2.1 (0.5)	18.2 (3.4)	1.3
		3CG	25	6.8 (1.7)	3.2 (0.8)	1.9 (0.6)	0.4 (0.2)	12.0 (1.6)	1.6
	CNRV	3CV	25	42.2 (9.9)	27.1 (6.4)	7.6 (2.1)	2.5 (0.5)	16.7 (2.9)	1.2
		3CG	25	8.0 (1.2)	3.8 (0.6)	1.5 (0.3)	0.3 (0.1)	7.0 (1.0)	3.1
		2CVa	25	16.9 (3.4)	11.2 (2.3)	2.8 (0.8)	1.6 (0.2)	28.7 (3.6)	1.2
Medium	YKRV	3CV	25	46.3 (12.5)	32.0 (8.7)	8.2 (3.2)	2.8 (0.9)	20.0 (3.3)	1.1
		3CG	25	52.0 (10.2)	30.1 (5.7)	13.5 (1.3)	4.7 (1.1)	21.4 (2.2)	2.8
		2CVa	25	33.5 (11.9)	23.4 (8.2)	5.8 (2.4)	2.2 (0.5)	23.5 (4.8)	1.0
	WOCK	3CV	24	40.6 (10.6)	26.0 (6.8)	6.8 (1.9)	2.2 (0.4)	15.6 (2.8)	1.8
		3CG	24	20.9 (7.7)	9.5 (3.6)	5.5 (2.3)	2.1 (0.5)	20.5 (6.0)	2.0
High	BOBY	3CV	25	51.2 (13.0)	36.7 (10.0)	5.7 (2.0)	3.7 (0.5)	26.4 (4.8)	1.5
		3CG	25	142.1 (24.9)	93.5 (16.8)	28.8 (5.7)	10.5 (1.7)	22.6 (4.8)	0.4
	BUBY	3CV	25	49.0 (12.8)	33.5 (9.2)	4.4 (1.6)	3.4 (0.4)	23.2 (5.1)	0.3
		3CG	25	103.8 (24.2)	69.8 (15.7)	18.3 (5.0)	7.4 (1.5)	22.6 (4.0)	0.0
		2CVa	24	32.3 (10.1)	23.5 (7.5)	4.4 (1.6)	3.2 (0.3)	40.2 (13.6)	0.2
		2CVb	23	41.5 (14.1)	29.4 (10.7)	4.2 (1.9)	3.2 (0.4)	28.3 (6.5)	0.1
	FIIS	3CV	23	55.1 (15.7)	38.0 (10.7)	7.6 (2.5)	4.1 (0.6)	25.7 (6.2)	1.0
		3CG	25	165.3 (25.6)	103.0 (15.0)	32.7 (7.4)	12.0 (2.2)	19.4 (2.4)	0.0

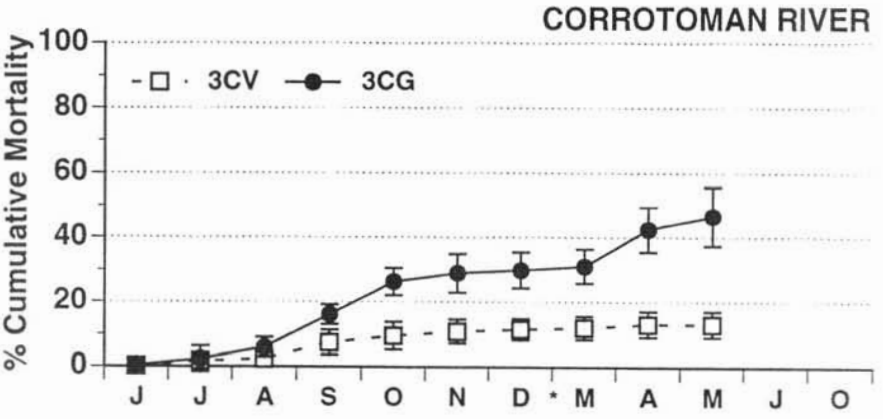
Site codes: CORV = Corrotoman River, GWRV = Great Wicomico River, CNRV = Coan River, WOCK = Woodas Creek, BOBY = Bogues Bay, BUBY = Burton Bay, FIIS = Fisherman's Island. Group codes: 3CV = triploid *C. gigas*, 3CV = triploid *C. virginica*, 2CVa = diploid Delaware Bay *C. virginica*, 2CVb = diploid Chincoteague/Lynnhaven *C. virginica*.

Appendix III. Cumulative mortality of *C. virginica* and *C. gigas* by site.

A. Low salinity sites. Mean (±SD) of 3 bags, starting with 200 oysters each, from May 1997 to October 1998. 3CV = triploid *C. virginica*, 3CG = triploid *C. gigas*, 2CVa = diploid Delaware Bay *C. virginica*. * = Break in monthly sampling.

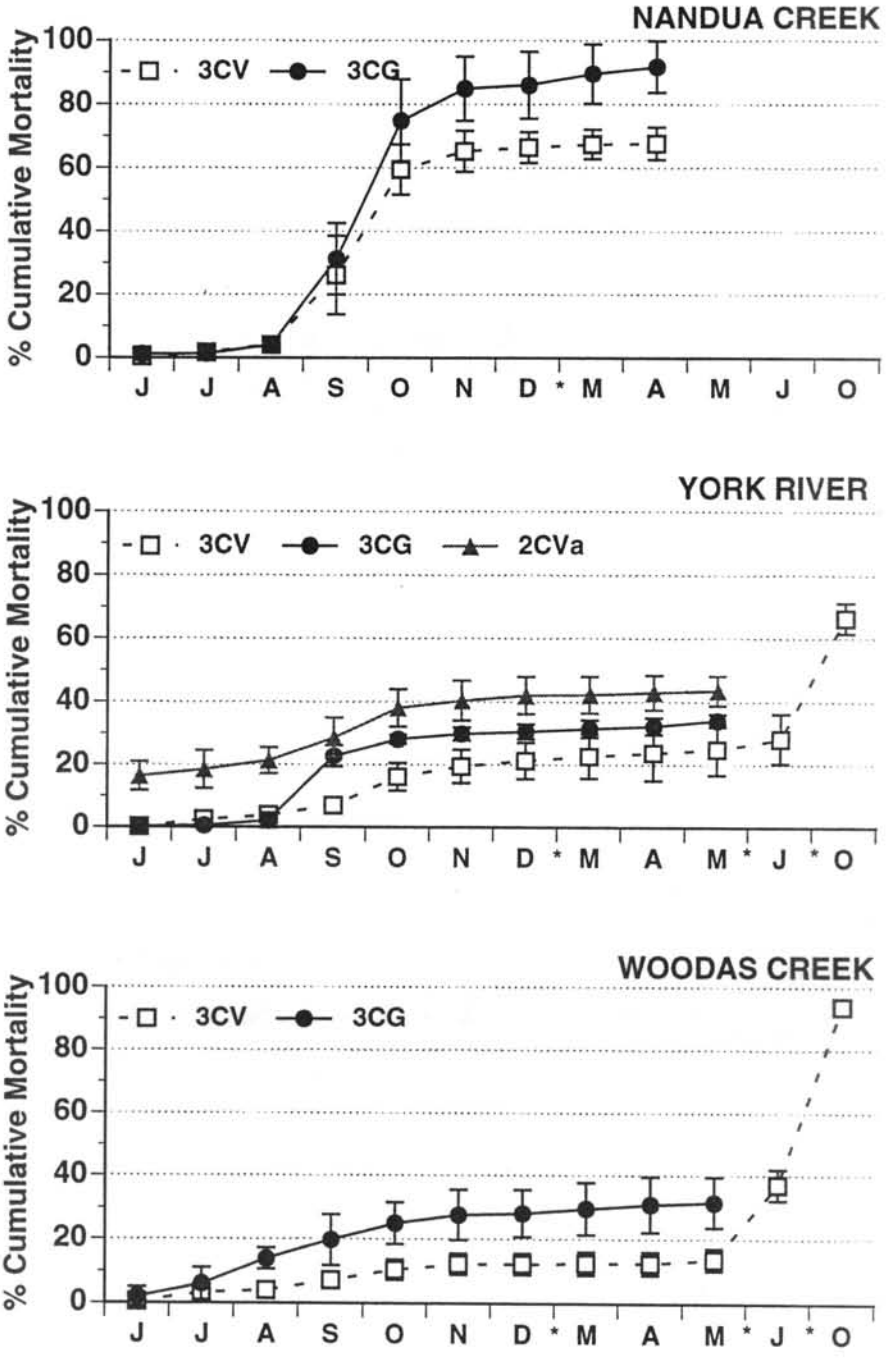






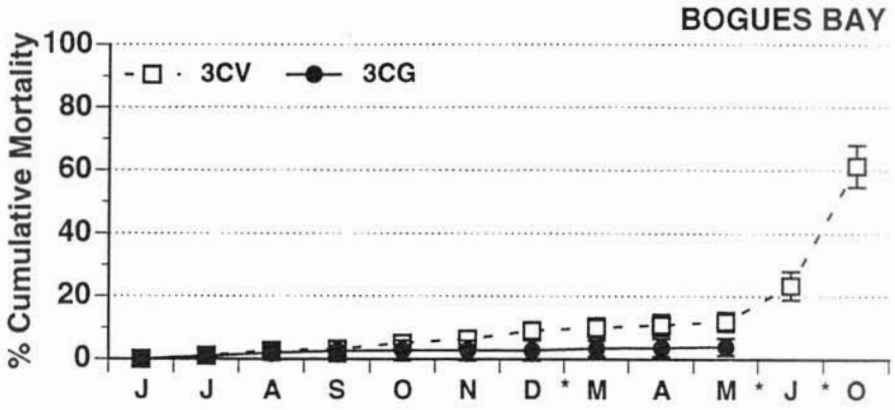
Appendix III. Cumulative mortality of *C. virginica* and *C. gigas* by site.

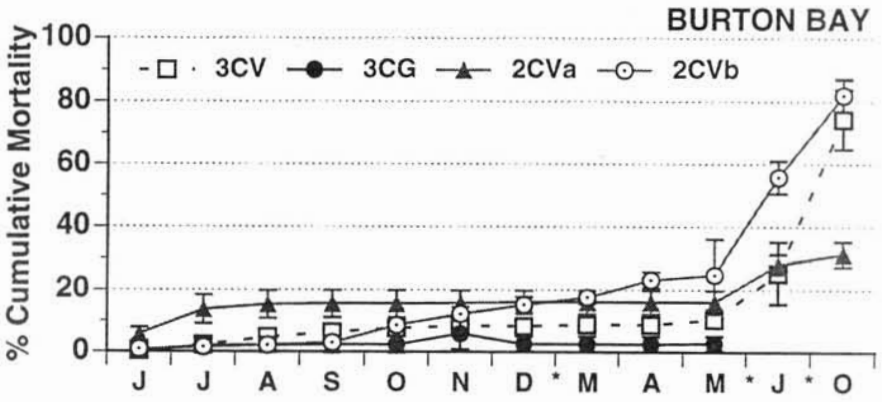
B. Medium salinity sites. Mean (±SD) of 3 bags, starting with 200 oysters each, from May 1997 to October 1998. 3CV = triploid *C. virginica*, 3CG = triploid *C. gigas*, 2CVa = diploid Delaware Bay *C. virginica*. * = Break in monthly sampling.

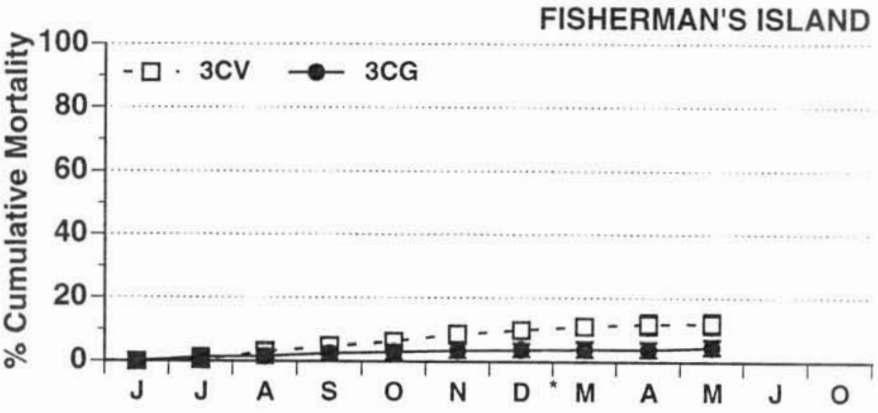


Appendix III. Cumulative mortality of *C. virginica* and *C. gigas* by site.

C. High salinity sites. Mean (±SD) of 3 bags, starting with 200 oysters each, from May 1997 to October 1998. 3CV = triploid *C. virginica*, 3CG = triploid *C. gigas*, 2CVa = diploid Delaware Bay *C. virginica*, 2CVb = diploid Chincoteague/Lynnhaven *C. virginica* . * = Break in monthly sampling.

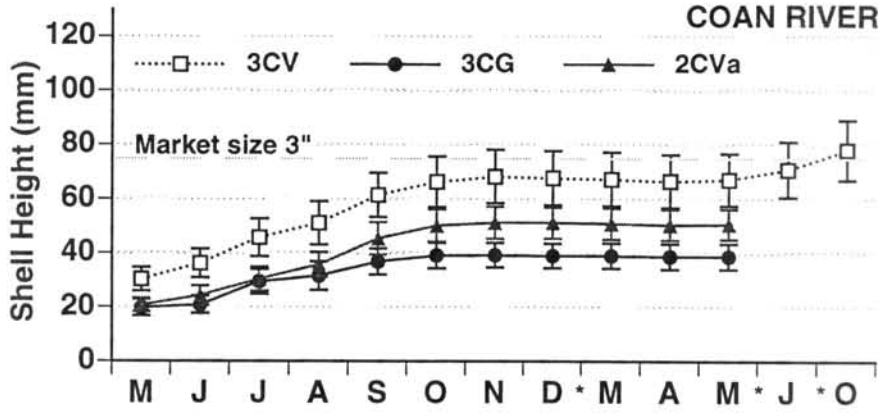


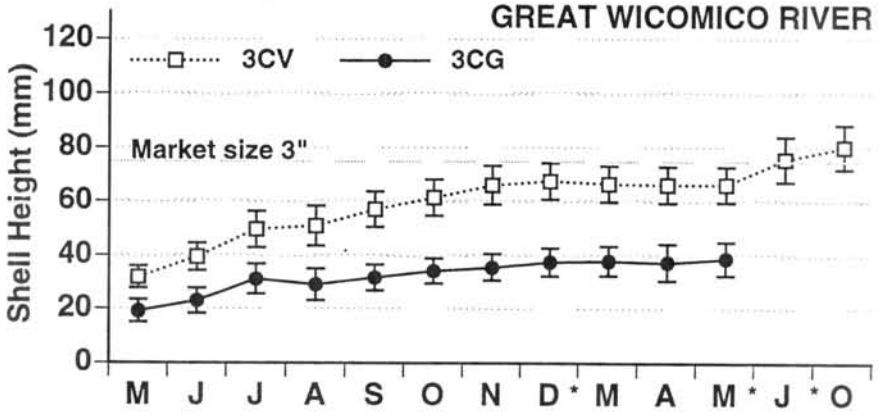


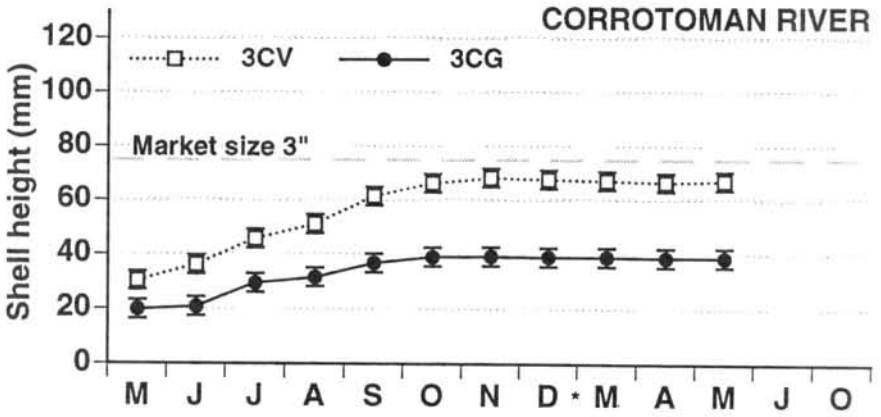


Appendix IV. Monthly shell height of *C. virginica* and *C. gigas* by site.

A. Low salinity sites. Mean (±SD) of 27-100 individual oysters from May 1997 to October 1998. 3CV = triploid *C. virginica*, 3CG = triploid *C. gigas*, 2CVa = diploid Delaware Bay *C. virginica*. * = Break in monthly sampling.

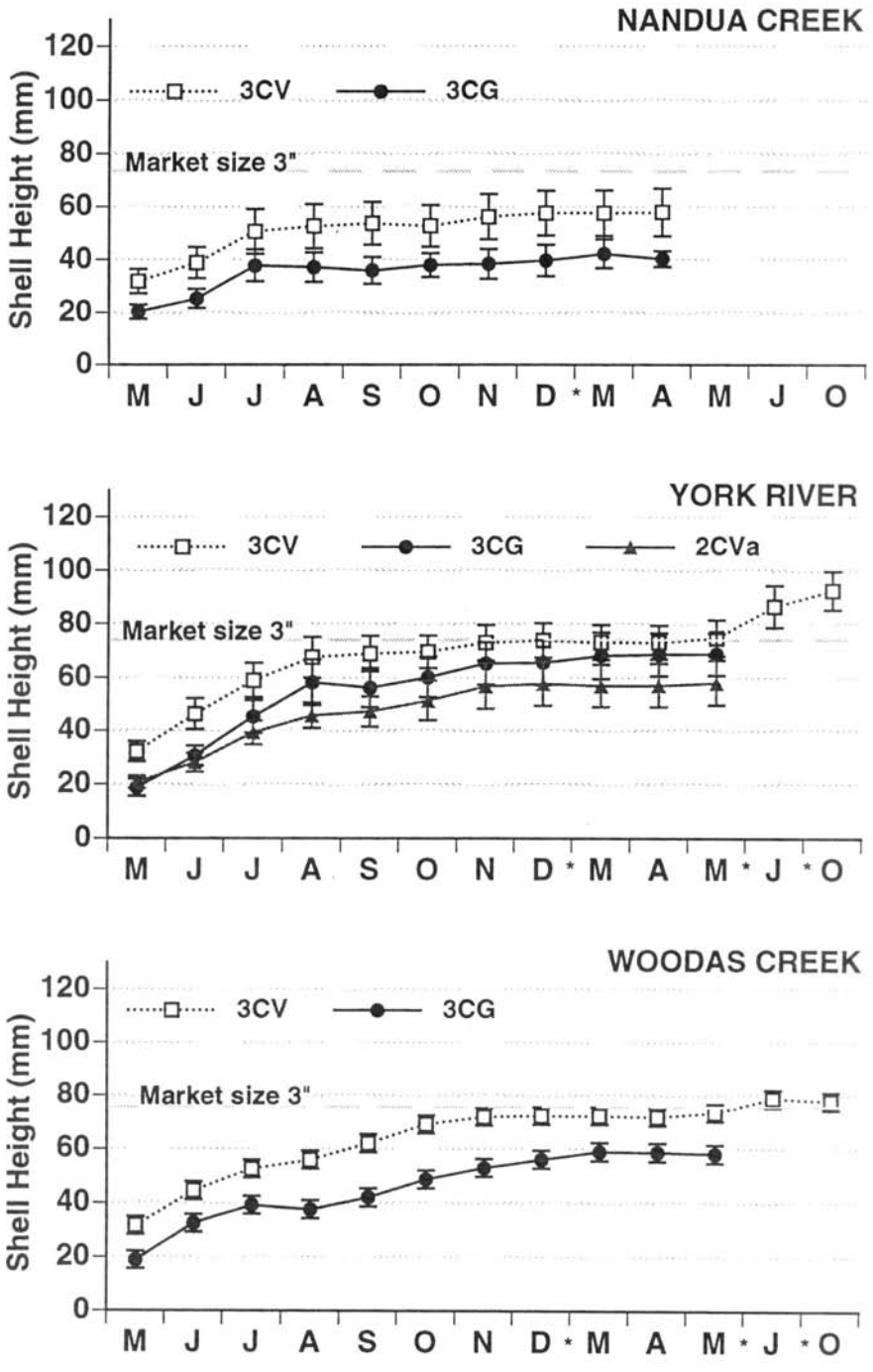






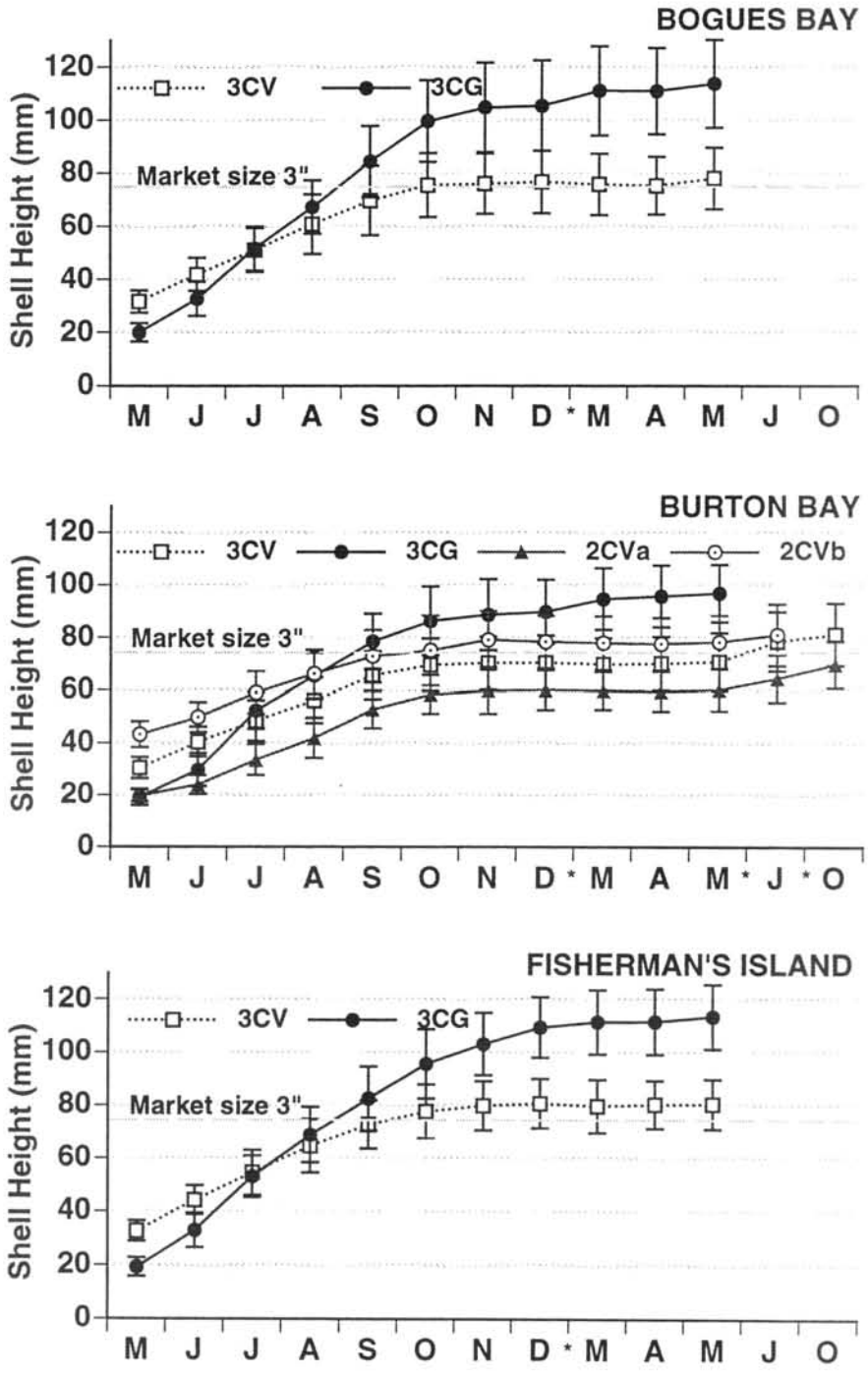
Appendix IV. Monthly shell height of *C. virginica* and *C. gigas* .

B. Medium salinity sites. Mean (±SD) of 3-100 individual oysters from May 1997 to October 1998. 3CV = triploid *C. virginica*, 3CG = triploid *C. gigas*, 2CVa = diploid Delaware Bay *C. virginica*. * = Break in monthly sampling.



Appendix IV. Monthly shell height of *C. virginica* and *C. gigas* by site.

C. High salinity sites. Mean (±SD) of 18-100 individual oysters from May 1997 to May 1998. 3CV = triploid *C. virginica*, 3CG = triploid *C. gigas*, 2CVa = diploid Delaware Bay *C. virginica*, 2CVb = diploid Chincoteague/Lynnhaven *C. virginica* . * = Break in monthly sampling.



Appendix V. Prevalence and intensity of *P. marinus* and *H. nelsoni* in (A) Baseline samples, (B) Low salinity sites, (C) Medium salinity sites, and (D) High salinity sites.

A. Baseline samples.

Site	Date	Group	*P. marinus*				*H. nelsoni*			
			%Prevalence	L*	M	H	%Prevalence	L*	M	H
YKRV	25 Mar 97	2CVb	0% (0/26)	0	0	0	0% (0/25)	0	0	0
	22 May 97	3CV	4% (1/25)	1	0	0	4% (1/25)	0	0	1
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
		2CVa	0% (0/26)	0	0	0	0% (0/25)	0	0	0

*L, M, H = Number of oysters with respectively light, moderate, and heavy infections. Site code: YKRV = York River. Group codes: 3CV = triploid *C. gigas*, 3CV = triploid *C. virginica*, 2CVa = diploid Delaware Bay *C. virginica*, 2CVb = diploid Chincoteague/Lynnhaven *C. virginica*.

Appendix V. Prevalence and intensity of *P. marinus* and *H. nelsoni.*

B. Low salinity sites.

Site	Date	Group	*P. marinus*				*H. nelsoni*			
			%Prevalence	L*	M	H	%Prevalence	L*	M	H
CORV	8 Jul 97	3CV	0% (0/25)	0	0	0	0% (0/25)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
	8 Oct 97	3CV	24% (6/25)	6	0	0	0% (0/25)	0	0	0
		3CG	8% (2/25)	2	0	0	0% (0/25)	0	0	0
	8 May 98	3CV	20% (5/25)	5	0	0	0% (0/25)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
GWRV	7 Jul 97	3CV	4% (1/25)	1	0	0	0% (0/24)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
	3 Oct 97	3CV	68% (17/25)	15	2	0	0% (0/25)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
	4 May 98	3CV	4% (1/25)	1	0	0	0% (0/25)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/24)	0	0	0
	29 Sep 98	3CV	100% (25/25)	13	7	5	0% (0/25)	0	0	0
CNRV	7 Jul 97	3CV	0% (0/25)	0	0	0	0% (0/25)	0	0	0
		3CG	4% (1/25)	1	0	0	0% (0/25)	0	0	0
		2CVa	0% (0/25)	0	0	0	0% (0/24)	0	0	0
	3 Oct 97	3CV	40% (10/25)	9	0	1	0% (0/25)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/23)	0	0	0
		2CVa	0% (0/24)	0	0	0	0% (0/25)	0	0	0
	4 May 98	3CV	8% (2/25)	2	0	0	0% (0/25)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
		2Cva	0% (0/25)	0	0	0	0% (0/25)	0	0	0
	29 Sep 98	3CV	52% (13/25)	13	0	0	0% 90/25)	0	0	0

*L, M, H = Number of oysters with respectively light, moderate, and heavy infections. Site codes: CORV = Corrotoman River, GWRV = Great Wicomico River, CNRV = Coan River. Group codes: 3CV = triploid *C. gigas*, 3CV = triploid *C. virginica*, 2CVa = diploid Delaware Bay *C. virginica.*

Appendix V. Prevalence and intensity of *P. marinus* and *H. nelsoni.*

C. Medium salinity sites.

Site	Date	Group	*P. marinus*				*H. nelsoni*			
			%Prevalence	L*	M	H	%Prevalence	L*	M	H
NACK	30 Jun 97	3CV	12% (3/25)	3	0	0	0% (0/25)	0	0	0
		3CG	4% (1/24)	1	0	0	0% (0/25)	0	0	0
	29 Sep 97	3CV	95% (21/22)	12	3	6	0% (0/25)	0	0	0
		3CG	65% (11/17)	9	0	2	0% (0/25)	0	0	0
	May 15 98	3CV	88% (22/25)	20	2	0	0% (0/25)	0	0	0
		3CG	0% (0/27)	0	0	0	0% (0/17)	0	0	0
YKRV	9 Jul 97	3CV	0% (0/25)	0	0	0	16% (4/25)	0	0	4
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
		2CVa	0% (0/25)	0	0	0	0% (0/25)	0	0	0
	7 Oct 97	3CV	32% (8/25)	8	0	0	0% (0/25)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
		2CVa	12% (3/25)	2	1	0	0% (0/25)	0	0	0
	5 May 98	3CV	8% (2/24)	2	0	0	0% (0/24)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
		2CVa	0% (0/25)	0	0	0	4% (1/25)	1	0	0
	28 Sep 98	3CV	100% (25/25)	13	7	5	4% (1/25)	1	0	0
WOCK	8 Jul 97	3CV	4% (1/25)	1	0	0	0% (0/24)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
	8 Oct 97	3CV	88% (22/25)	18	1	3	0% (0/25)	0	0	0
		3CG	12% (3/25)	3	0	0	0% (0/25)	0	0	0
	5 May 98	3CV	92% (22/24)	17	3	2	0% (0/24)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
	30 Sep 98	3CV	100% (15/15)	4	5	6	0% (0/15)	0	0	0

*L, M, H = Number of oysters with respectively light, moderate, and heavy infections. Site codes: YKRV = York River, NACK = Nandua Creek, WOCK = Woodas Creek. Group codes: 3CV = triploid *C. gigas*, 3CV = triploid *C. virginica*, 2CVa = diploid Delaware Bay *C. virginica.*

Appendix V. Prevalence and intensity of *P. marinus* and *H. nelsoni.*

D. High salinity sites

Site	Date	Group	*P. marinus*				*H. nelsoni*			
			%Prevalence	L*	M	H	%Prevalence	L*	M	H
BOBY	1 Jul 97	3CV	0% (0/25)	0	0	0	4% (1/25)	0	1	0
		3CG	4% (1/25)	1	0	0	0% (0/25)	0	0	0
	29 Sep 97	3CV	60% (15/25)	13	1	1	0% (0/25)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/20)	0	0	0
	4 May 98	3CV	16% (4/25)	4	0	0	0% (0/25)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
	13 Oct 98	3CV	96% (24/25)	18	3	3	0% (0/25)	0	0	0
BUBY	1 Jul 97	3CV	4% (1/25)	1	0	0	4% (1/25)	0	0	1
		3CG	0% (0/20)	0	0	0	0% (0/20)	0	0	0
		2CVa	0% (0/25)	0	0	0	0% (0/21)	0	0	0
		2CVb	4% (1/25)	0	1	0	4% (1/25)	0	0	1
	30 Sep	3CV	80% (20/25)	17	2	1	0% (0/20)	0	0	0
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
		2Cva	32% (8/25)	8	0	0	0% (0/20)	0	0	0
		2CVb	60% (15/25)	13	0	2	46% (11/24)	5	1	5
	5 May 98	3CV	20% (5/25)	4	0	1	4% (1/24)	1	0	0
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0
		2CVb	6% (1/17)	1	0	0	50% (6/12)	2	0	4
	7 May 98	2CVa	0% (0/25)	0	0	0	4% (1/25)	1	0	0
	7 Oct 98	3CV	92% (23/25)	16	2	5	0% (0/25)	0	0	0
		2CVa	84% (21/25)	19	2	0	0% (0/25)	0	0	0
		2CVb	80% (20/25)	15	1	4	8% (2/25)	2	0	0
FIIS	30 Jun 97	3CV	4% (1/25)	1	0	0	0% (0/25)	0	0	0
		3CG	0% (0/21)	0	0	0	0% (0/25)	0	0	0
	30 Sep 97	3CV	92% (23/25)	14	4	9	0% (0/25)	0	0	0
		3CG	8% (2/25)	2	0	0	0% (0/25)	0	0	0
	6 May 98	3CV	32% (8/25)	8	0	0	8% (2/25)	0	0	2
		3CG	0% (0/25)	0	0	0	0% (0/25)	0	0	0

*L, M, H = Number of oysters with respectively light, moderate, and heavy infections. Site codes: BOBY = Bogues Bay, BUBY = Burton Bay, FIIS = Fisherman's Island. Group codes: 3CV = triploid *C. gigas*, 3CV = triploid *C. virginica*, 2CVa = diploid Delaware Bay *C. virginica*, 2CVb = diploid Chincoteague/Lynnhaven *C. virginica.*

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