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COMPARATIVE FIELD STUDY OF *CRASSOSTREA GIGAS* (THUNBERG, 1793) AND *CRASSOSTREA VIRGINICA* (GMELIN, 1791) IN RELATION TO SALINITY IN VIRGINIA

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ABSTRACT   To evaluate and compare the performance of triploid juvenile *C. gigas* (mean shell height = 19.2 mm) and triploid juvenile *Crassostrea virginica* (mean shell height = 31.7 mm), 600 oysters of each species were deployed for 1 year in floating mesh cages at three replicate sites within low, medium, and high salinity regimes (respectively, <15‰, 15–25‰, > 25‰) in the Chesapeake Bay and the Atlantic Coast of Virginia. The comparative performance of the two oyster species varied with salinity. At low salinity sites, cumulative mortality of *C. virginica* (10%) was significantly (*P* < .05) lower than that of *C. gigas* (63%), and over-all mean growth rate of *C. virginica* (2.9 mm mo^{-1}) was significantly (*P* < .05) higher than that of *C. gigas* (1.6 mm mo^{-1}). At medium salinity sites, survival and growth rate of *C. virginica* and *C. gigas* were not significantly (*P* > .05) different. 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. At high salinity sites, mean cumulative mortality was similarly low (<11%) for both species; whereas, over-all mean growth rate of *C. gigas* (7.1 mm mo^{-1}) was significantly (*P* < .05) higher than that of *C. virginica* (3.6 mm mo^{-1}). At all sites, *C. gigas* was less susceptible than *C. virginica* to *Perkinsus marinus* infections. Infections by *Haplosporidium nelsoni* were present in *C. virginica* and absent in *C. gigas*. Infestations by mud-worm *Polydora* spp. were more prevalent and severe for *C. gigas* than for *C. virginica* at low and medium salinity sites in October 1997, but similar for both species at other times and locations. Condition index was significantly (*P* < .05) higher for *C. virginica* than for *C. gigas* at low salinity in May 1998, but similar for both species for other times and locations. *Crassostrea virginica* outperformed *C. gigas* in low salinity sites in the Chesapeake Bay, *C. gigas* outperformed *C. virginica* at high salinity sites in the Atlantic Coast, and performance was similar for both species at medium salinity sites in the Chesapeake Bay.

KEY WORDS: *Crassostrea gigas*, triploid, growth, survival, disease susceptibility, Virginia

INTRODUCTION

As native eastern oyster, *Crassostrea virginica* (Gmelin, 1791) stocks have declined throughout much of the mid-Atlantic seaboard of the United States through overharvesting, disease, and water quality deterioration, interest in the potential of non-native oyster species to restore the fishery and ecological functions has grown. This has been particularly apparent in the Chesapeake Bay region, where standing stocks of eastern oysters have been reduced in the last decade to 1% of late nineteenth-century levels (Newell 1988). Given that much of this decline has been caused by devastating Dermo and MSX epizootics resulting from, respectively, the protozoan parasites *Perkinsus marinus* and *Haplosporidium nelsoni* (Burreson and Ragone Calvo 1996), strategies aimed at rehabilitation of stocks largely depend upon the use of disease-resistant oysters. Although development of eastern oyster lines with resistance to MSX has been achieved (Ford and Haskin 1987) and development of lines with resistance to both Dermo and MSX is in progress (Ragone Calvo et al. 1997), applicability of selective breeding programs is mostly limited to aquaculture. Use of disease-resistant eastern oysters for fishery enhancement or ecological restoration is constrained by dilution of their gene pool with that of susceptible oysters in the wild. Furthermore, the gene flow from relatively uninfected and highly susceptible populations in low salinity areas may limit the evolution of resistance in eastern oysters (Gaffney and Bushek 1996).

The Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), has been the species of choice to substitute for depleted local oyster populations decimated by disease and other factors in many countries (Mann et al. 1991, Shatkin et al. 1997). *Crassostrea gigas* is the primary oyster species supporting shellfish industries around the globe, accounting for an estimated 80% of the world oyster production (Chew 1990). Shatkin and collaborators (1997) reviewed the worldwide experience with introductions of *C. gigas* and presented an analysis of economic, legal, and ecological factors relevant for introductions into the Gulf of Maine. 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 United States has restored the shellfish industry that 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). Problems with the transfer of exotic oysters include parallel transfer of pests and disease agents and undesired competition of exotic species with their native counterparts. For example, 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).

During the last decade, the possible introduction of *C. gigas* into the Chesapeake Bay has received considerable attention. Mann and collaborators (1991) developed the rationale and analyzed the risks associated with such an introduction. Gottlieb and Schweighofer (1996) further discussed the potential of *C. gigas* for restoring the Chesapeake Bay ecosystem. In Virginia, a program to

examine the suitability of nonindigenous oyster species to local conditions was established, while efforts to restore native oysters continued (VIMS 1996). Based upon ecological requirements and disease tolerance, two candidate nonindigenous oyster species within the genus *Crassostrea*, *C. gigas* and the Suminoe oyster, *C. ariakensis* (= rivularis) (Fujita, 1913) were initially selected for testing in the Chesapeake Bay (Mann et al. 1991, VIMS 1996). In this paper, we address field studies with *C. gigas*. No growth or disease challenge studies are available for *C. ariakensis* in the region; however, for locations on the West Coast of the United States, Langdon and Robinson (1991) reported growth rates similar to that of *C. gigas*. Studies with *C. ariakensis*, currently underway at Virginia Institute of Marine Science (VIMS), will be the object of a future report.

Both Mann et al. (1991) and Gottlieb and Schweighoffer (1996) have suggested that *C. gigas* has considerable potential for restoration in part of the Chesapeake Bay, but both indicated the need for more research. The need for field studies was particularly emphasized to assess the performance of exotic oysters under local conditions, and because there was no alternative way for challenge against MSX. Prior studies at VIMS 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 *P. marinus*, *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 triploid oysters also indicated that *C. gigas* had reduced susceptibility to *P. marinus* and *H. nelsoni* as compared to the native oyster (Burreson et al. 1994). In this field study, which lasted only 5 months, *C. gigas* had comparable shell growth rates to the native oysters, but became heavily infested by the polychaete *Polydora websteri*, resulting in poor meat quality. However, these studies were limited in duration and spatial extent, and more extensive field experiments were necessary to evaluate the performance of *C. gigas* better within a broader range of salinity and other environmental conditions. The present study was 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).

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‰), medium salinity (15–25‰), and high salinity (>25‰) 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 of 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 characterize environmental variables further, hourly temperature, salinity,
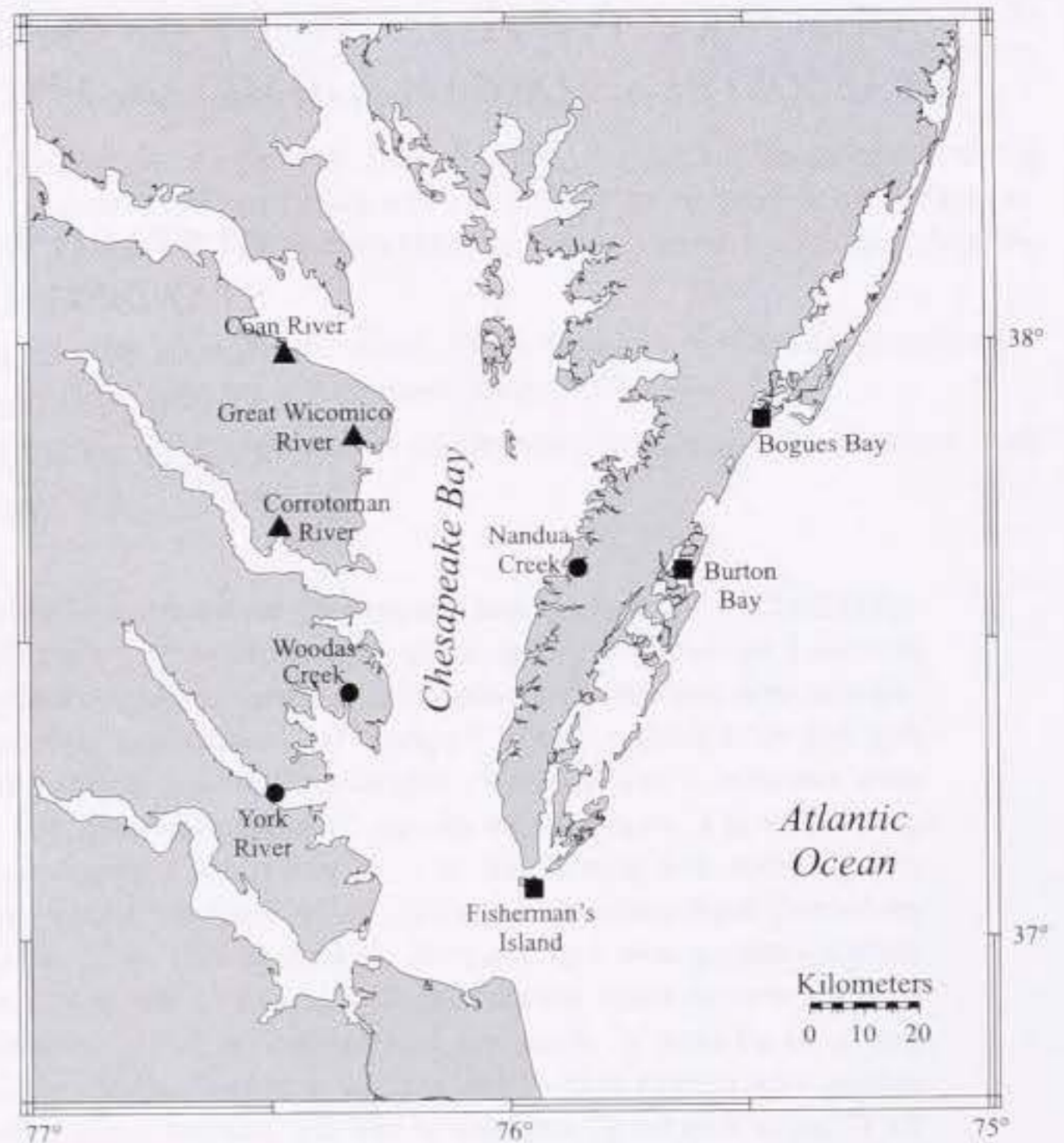

Figure 1. Location of study sites in the Chesapeake Bay and the Atlantic Coast of Virginia. ▲ Low salinity (<15 ppt) sites, ● medium salilnity (15–25 ppt) sites, ■ high salinity (>25 ppt) sites.

and turbidity were measured with Hydrolab-Minisonde® dataloggers deployed at various sites for weekly to monthly intervals.

Oyster Groups

To ensure that this study resulted in neither the unintended reproduction of *C. gigas* nor the introduction of potential exotic pathogens, we used 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). Triploid *C. gigas* (3CG) and triploid *C. virginica* (3CV) were produced for this study by Haskin Shellfish Research Laboratory (HSRL) during June to July 1996 (Table 1). Brood stock for 3CG was Miyagi strain *C. gigas* originating from the Pacific Northwest Coast of the United States and maintained in quarantine at HSRL for several generations. Triploid *C. gigas* were produced by mating tetraploid and diploid parent stocks, an approach that 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

TABLE 1.

Oyster groups used.

Species	Group Code	Hatchery	Date Spawned	Size in May 1997[a]
C. gigas	3CG	HSRL	16 July 96	19.2 mm
C. virginica	3CV	HSRL	11 June 96	31.7 mm

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

TABLE 2.

Percentage market size (>76.2 mm) oysters in May 1998, based on the legal size for wild harvested oysters in Virginia.

Salinity Regime	Oyster Group	
	3CV	3CG
Low	14% (38/268)	0% (0/69)
Medium	41% (65/159)	11% (10/91)
High	52% (131/252)	100% (260/261)

Oyster group codes described in Table 1. In parenthesis, number of market size oysters/total number of live oysters.

cytochalasin-B using the methods described by Downing and Allen (1987) and Allen et al. (1989).

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 were also maintained first at HSRL Cape Shore, NJ, and then at Gloucester Point, VA in flow-through tanks without quarantined effluents. 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 × 0.5 m × 0.3 m) were constructed by fitting wire mesh trays (25-mm square 16-gauge mesh) into floating frames built with 4-inch (10.16 cm) PVC pipe, following the design of Luckenbach and Taylor (1997). Floating trays 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), salinity regime, and time on final cumulative mortality, final condition index, prevalence and weighted prevalence of *P. marinus*, and weighted prevalence of *Polydora* spp.. Differences in mean variables, 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 analysis of variance (ANOVA) assumptions using Bartlett chi-square test for homogeneity of variance and plots of means versus standard deviations. Arcsine and logarithmic transformations were used where appropriate (Zar 1974).

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 sam-

pling. Cumulative mortality of each oyster group was calculated as the sum of interval mortality (Barber and Mann 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. Mean monthly growth rates for individual oysters were calculated as the over-all shell height increment divided by the deployment time in days standardized for 30 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:

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

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 (n = 25) was taken to assess the disease status of oyster groups before deployment in the spring of 1997. Subsequent disease samples (n = 25) were collected, depending upon group and site, during the summer and fall of 1997 and the spring of 1998. *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.

$$\text{Weighted prevalence} = ((n_1 * 1) + (n_2 * 3) + (n_3 * 5))/N, \quad (2)$$
$$\text{where } n_i = \text{number of cases rated as } (i),$$
$$N = \text{total number of oysters examined in the sample.}$$

Haplosporidium nelsoni was diagnosed using standard paraffin histology procedures with oysters preserved in Davidson's AFA and 6-μm 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 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

TABLE 3.

Percentage genetic mosaics among *C. gigas* by salinity regime and date.

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)

In parenthesis number of mosaics/number of oysters examined.

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 following 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

Before deployment, baseline samples of 3CV (n = 125 larvae) and 3CG (n = 35 juveniles) were taken to confirm ploidy status. During deployment, samples of 3CG (n = 35) were collected, depending upon site, at the beginning of the month in June, July, and August 1997 and May 1998. Only *C. gigas* was examined for ploidy during deployment, but an equal number of *C. virginica* were concurrently collected from trays to standardize the number of oysters removed by sampling. Ploidy 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 assays were conducted at HSRL and the VIMS Aquaculture Genetics and Breeding Technology Center.

RESULTS

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 ‰) during September, October, and November because of drought conditions during the summer and relatively low mean salinity

(<10‰) 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‰ during April and May. Medium salinity sites experienced relatively low salinity (<15‰) 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 over-all cooler temperature with monthly means 2–4 °C lower than medium or low salinity sites (Fig. 2).
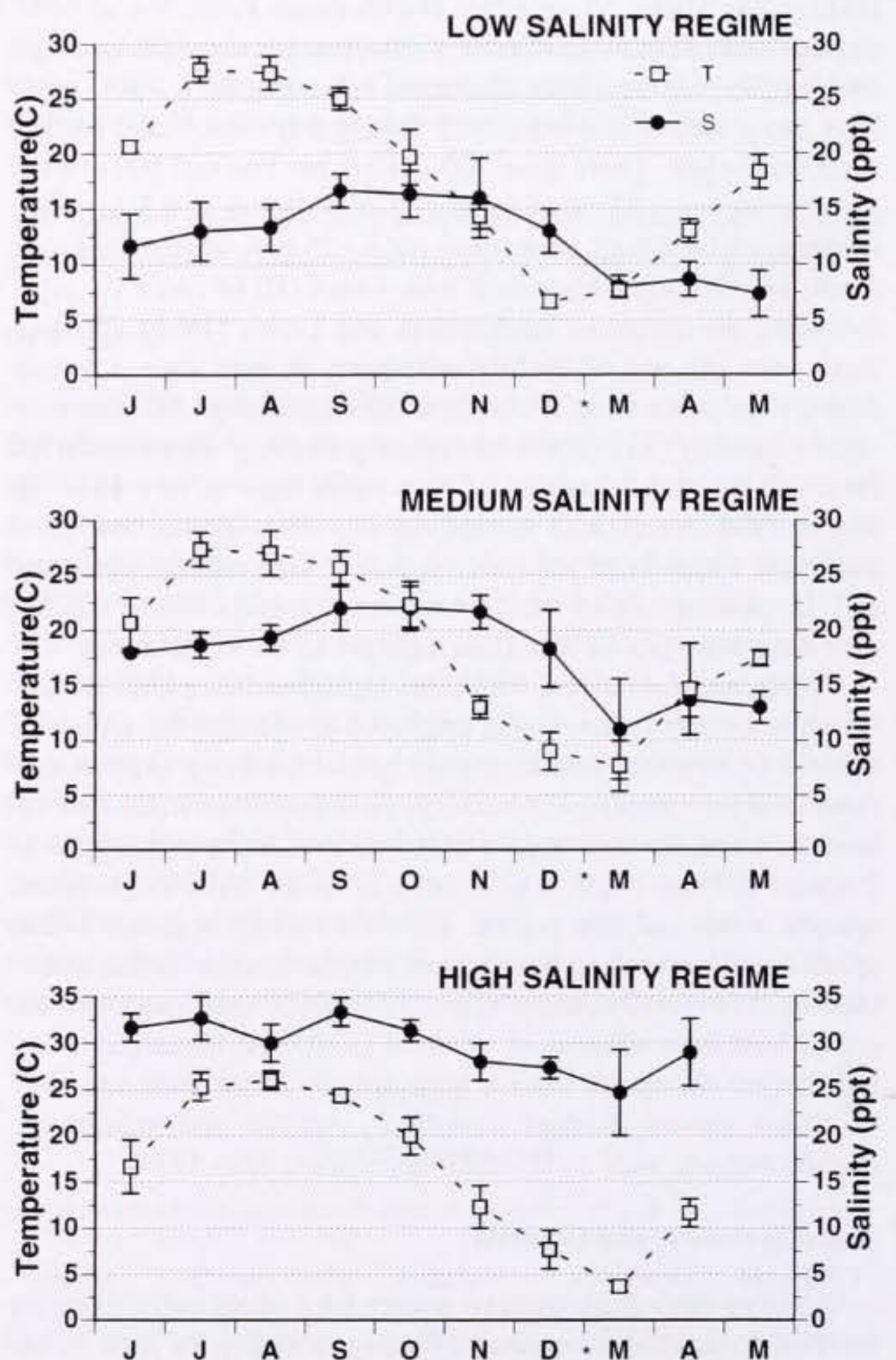

Figure 2. Mean monthly (± SD) temperature and salinity of three sites within low, medium, and high salinity regimes, using stem thermometer. * Break in monthly sampling

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, and maximum daily mean values at other sites was <38 NTU.

Mortality

Species, salinity regime, and their interaction had significant (P < .05) effects on cumulative mortality. At low salinity sites, mean monthly mortality of 3CV was very low (<3%) at all times, and that of 3CG peaked at 28% in April 1998 (Fig. 3). By May 1998, mean cumulative mortality of 3CV (10%) was significantly (P < .05) lower than that of 3CG (63%). At medium salinity sites, mean monthly mortality reached 17% for 3CV and 22% for 3CG in October 1997 (Fig. 3). By May 1998, mean cumulative mortality of 3CV (35%) was not significantly (P > .05) different than that of 3CG (53%). High variability in mortality, for both species, among medium salinity sites was attributable to extremely high mortality at Nandua Creek. At high salinity sites, mean monthly mortality

was very low (<3%) for both species at all times (Fig. 3). In May 1998, mean cumulative mortality of 3CV (11%) was not significantly (P > .05) different from that of 3CG (4%). Within 3CV, there were no significant (P > .05) differences in mean cumulative mortality among salinity regimes. Within *C. gigas*, oysters at low and medium salinity experienced significantly (P < .05) higher mortality than those at high salinity, and no significant (P > .05) difference was detected between oysters at low and medium salinity.

Growth

At the initiation of the experiment, mean size of 3CV and 3CG was, respectively, 31.7 mm and 19.2 mm; subsequent growth varied with salinity regime (Table 2). 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 at the end of the study (Fig. 4). At medium salinity, the 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 (Fig. 4). At high salinity, the initially smaller 3CG reached the same size as 3CV 3 mo after deployment, in July 1997, and continued to grow during fall and winter attaining a final mean shell height of 108.1 mm in
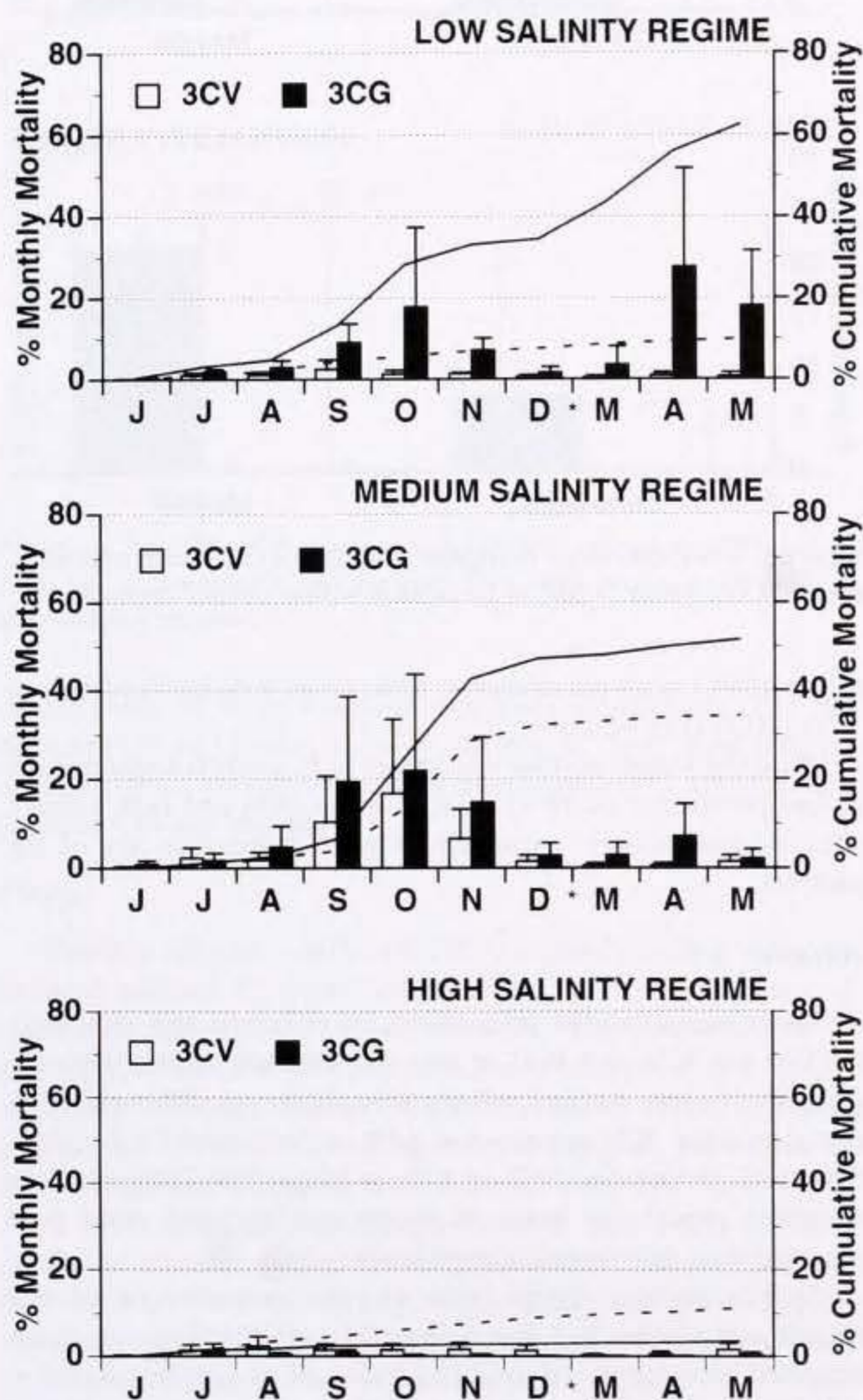

Figure 3. Monthly and cumulative mortality of triploid *C. virginica* (3CV) and triploid *C. gigas* (3CG) from June 1997 through May 1998. Bars = mean (+ SD) monthly mortality of three sites within salinity regimes. Dashed lines = mean cumulative mortality of 3CV. Solid lines = mean cumulative mortality of 3CG. * Break in monthly sampling.
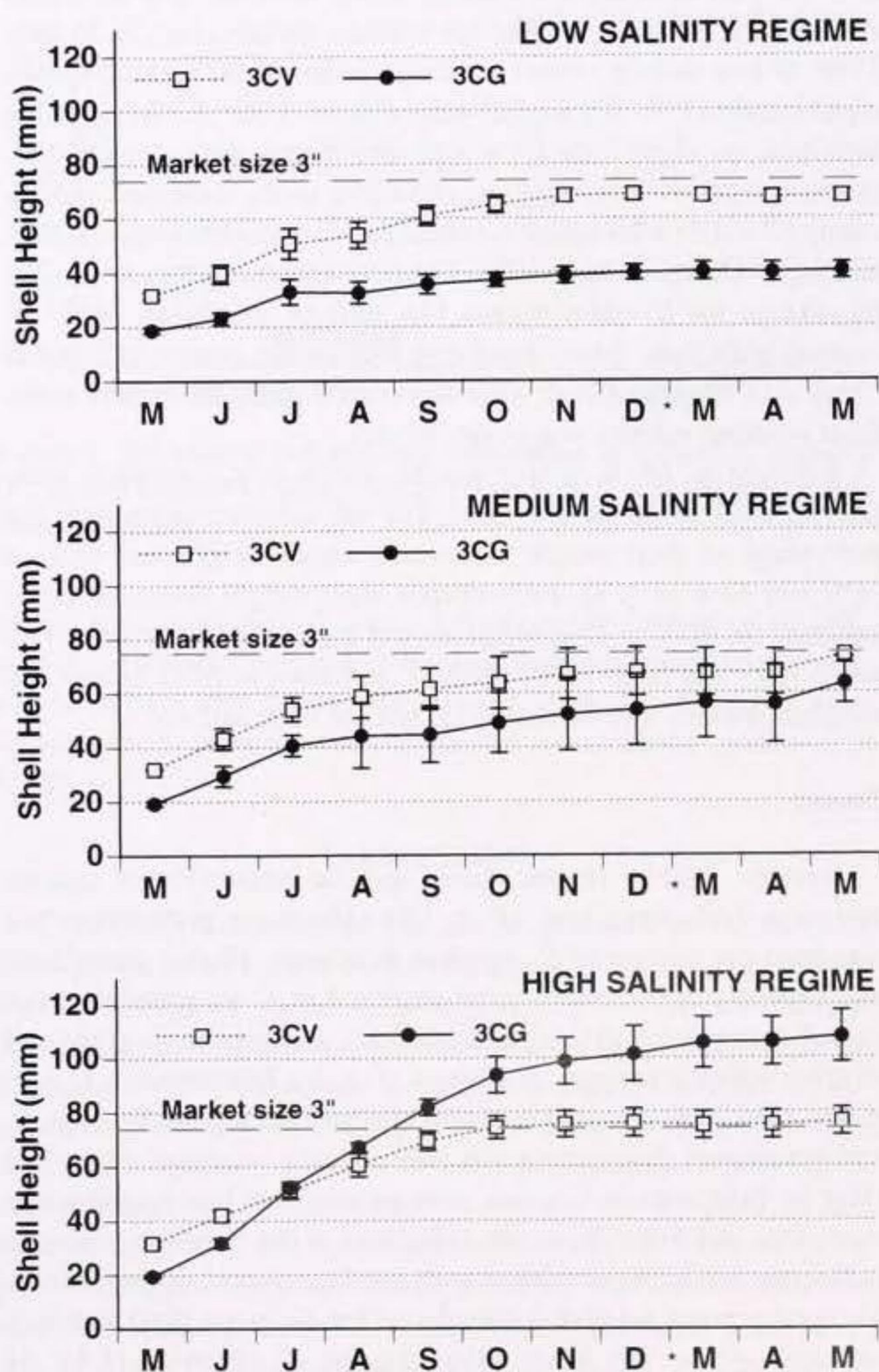
Figure 4. Monthly shell height of triploid *C. virginica* (3CV) and triploid *C. gigas* (3CG) from May 1997 to May 1998. Mean (± SD) of three sites within salinity regimes.

May 1998. By comparison, *C. virginica* stopped growing after October 1997 and reached 78.4 mm in May 1998 (Fig 4). Species, salinity regime, and their interactions had significant ($P < .05$) effects on mean growth rate. At low salinity sites, mean overall growth rate of 3CV (2.9 mm mo^{-1}) was significantly ($P < .05$) greater than that of 3CG (1.6 mm mo^{-1}), with most of the growth in *C. virginica* occurring between July and October (Fig. 4). At medium salinity sites, mean over-all growth rate for both species (3.0 mm mo^{-1}) was not significantly ($P > .05$) different, and the monthly pattern of growth was similar. At high salinity sites, mean over-all growth rate of 3CV (3.6 mm mo^{-1}) was significantly ($P < .05$) lower and nearly half that of 3CG (7.1 mm mo^{-1}). Within 3CV, growth rate did not significantly ($P < .05$) differ between salinity regimes. Within *C. gigas*, growth rate at high salinity was significantly ($P < .05$) higher than that at medium and low salinity regimes, and growth rate did not significantly ($P > .05$) differ between medium and low salinity regimes.

Condition Index

Salinity regime, time, and the interactions of salinity and species and salinity and time had significant ($P < .0005$) effects on final oyster condition. In October 1997, there were no significant ($P > .05$) differences in condition index between species within any salinity, or between salinities within a species (Fig. 5). In May 1998, at low salinity, mean condition index of 3CV (16.2%) was significantly ($P < .05$) higher than that of 3CG (8.7%); at other salinities, no significant ($P > .05$) differences were detected between species. Within species, condition index increased significantly ($P < .05$) with salinity, except for *C. gigas* between medium and high salinity in May 1998. For both species within any salinity, except for *C. gigas* within low salinity, condition index increased with time. Mean condition indices for oysters at Nandua Creek and Woodas Creek were lower than those of oysters at the third medium salinity site (York River).

Relative to whole oyster weight, shells of *C. virginica* were heavier than shells of *C. gigas*. 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, and 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.

Disease

Species, salinity regime, time, and the interaction of species and time had significant ($P < .05$) effects on prevalence and weighted prevalence of *P. marinus* infections. Higher prevalence and intensity of infections were observed in *C. virginica* and occurred at medium salinity during fall as compared to *C. gigas* and to other salinity regimes and times (Fig. 6). Infections in *C. virginica* were low in prevalence and intensity during the first spring and summer of deployment and subsequently increased in the fall (Fig. 6). Infections in *C. gigas* were generally of low magnitude at most sites and times; however, infections at the Nandua Creek site in fall reached 67% prevalence with two heavy intensity infections. Maximum mean weighted prevalence for *C. gigas* (0.4) was significantly ($P < .05$) lower than that for *C. virginica* (1.4). At medium salinity sites, infections remained high in *C. virginica* during spring 1998 (prevalence >62%, weighted prevalence = 0.9), whereas, at low and high salinity sites, infections subsided in
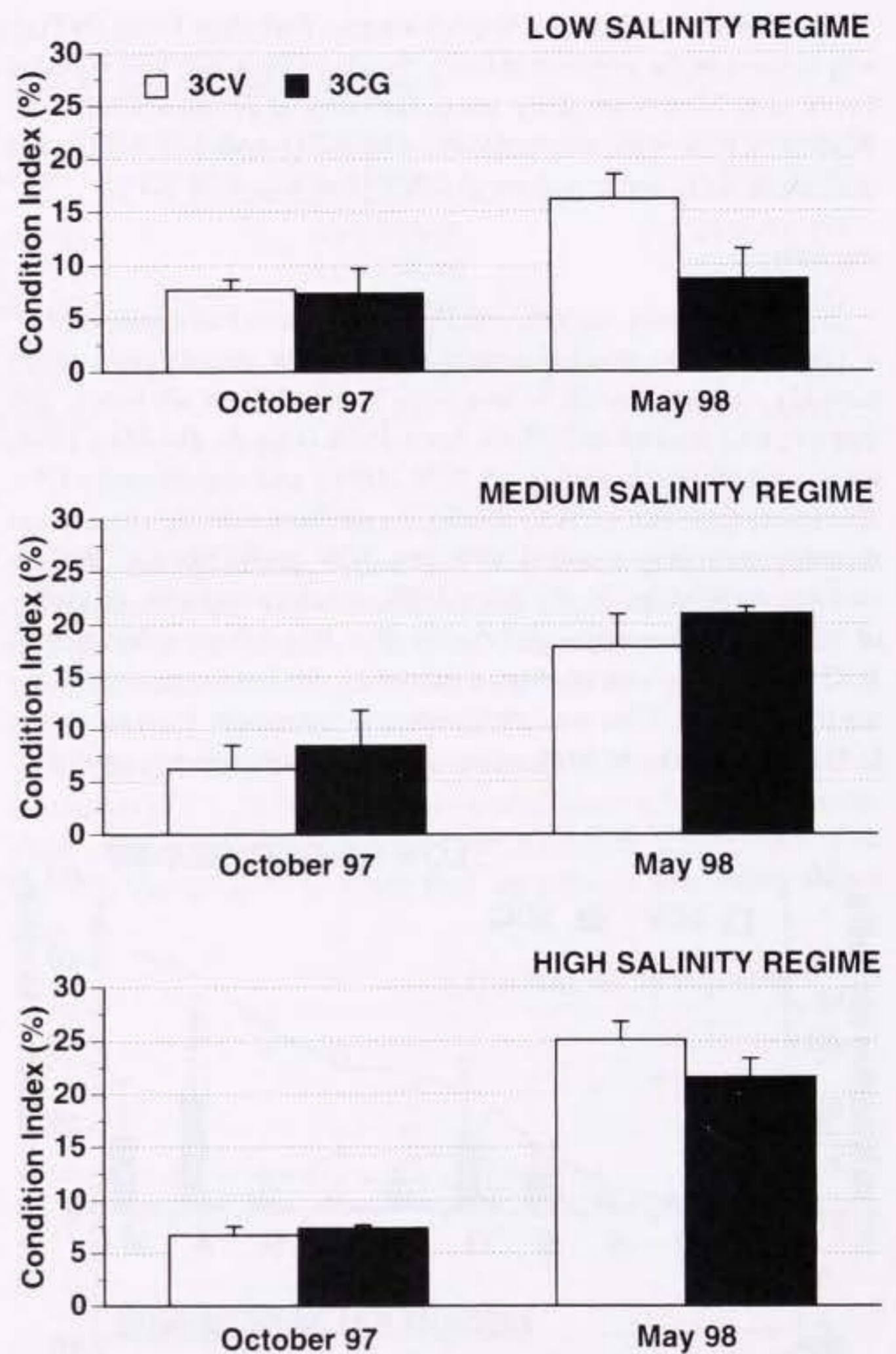

Figure 5. Condition index in triploid *C. virginica* (3CV) and triploid *C. gigas* (3CG). Mean (+ SD) of three sites within salinity regimes.

spring 1998 (mean prevalence < 23%, mean weighted prevalence = 0.1–0.3) (Fig 6).

Haplosporidium nelsoni was absent in *C. gigas* but was present at low prevalence (< 16%) in 3CV at medium and high salinity sites. At low salinity, no infections were detected in any of the samples.

Polydora

Mean prevalence of infestations by *Polydora* spp. was high (>95%) for 3CV and 3CG at low and medium salinity sites regardless of time. At high salinity sites, however, although 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 between oyster species were more pronounced than differences in prevalence.

Species, salinity regime, time, and the interaction of salinity regime and species had significant ($P < .0005$) effects on mean weighted prevalence. Triploid *C. virginica* had significantly ($P < .05$) 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). For 3CV, within any salinity, mean weighted prevalence was not significantly ($P > .05$) different between October and May, whereas, for 3CG at low and
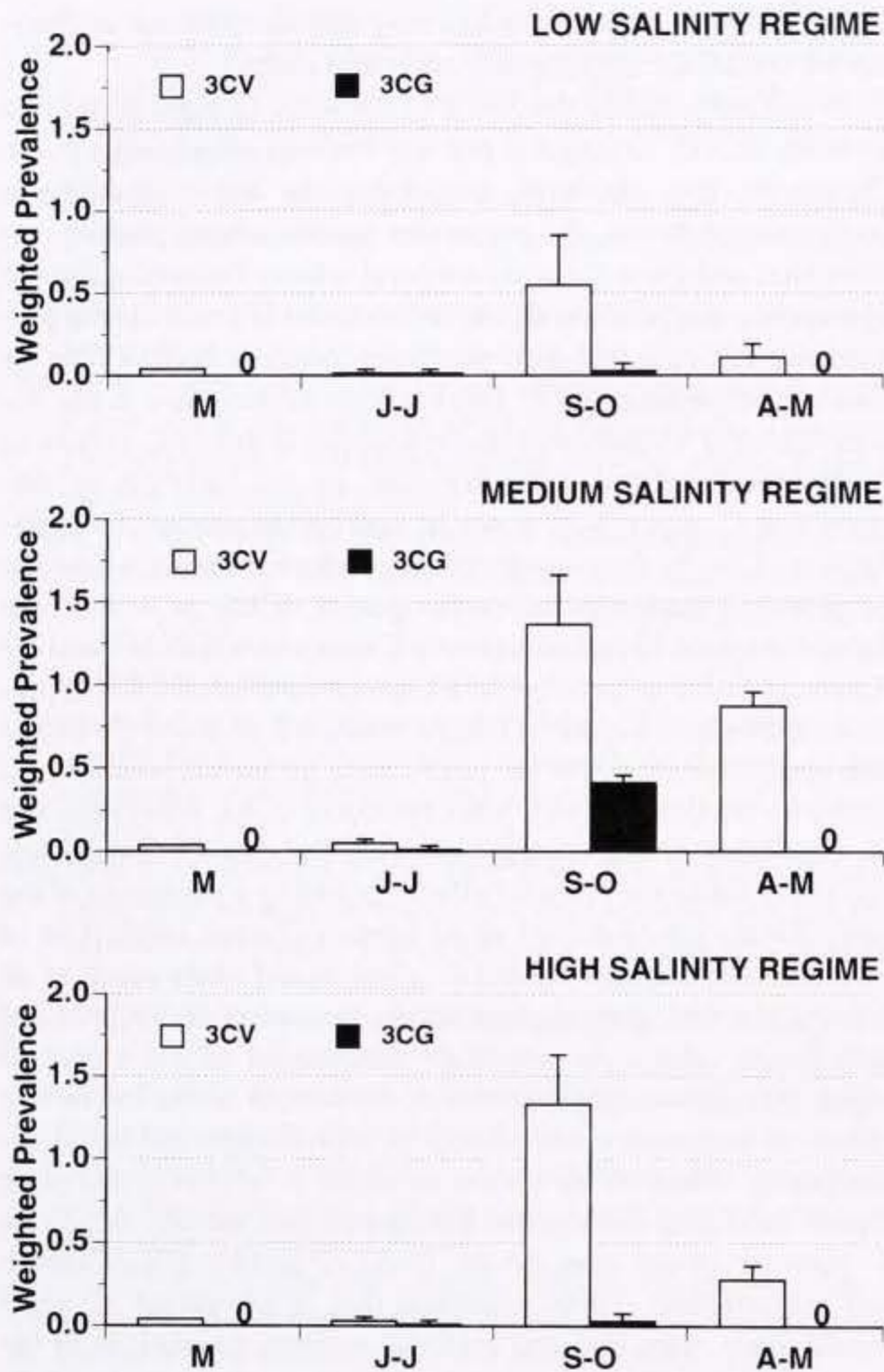
Figure 6. Intensity of *P. marinus* in triploid *C. virginica* (3CV) and *C. gigas* (3CG) from April 1997 through May 1998. Mean (+ SD) of three sites salinity regimes.
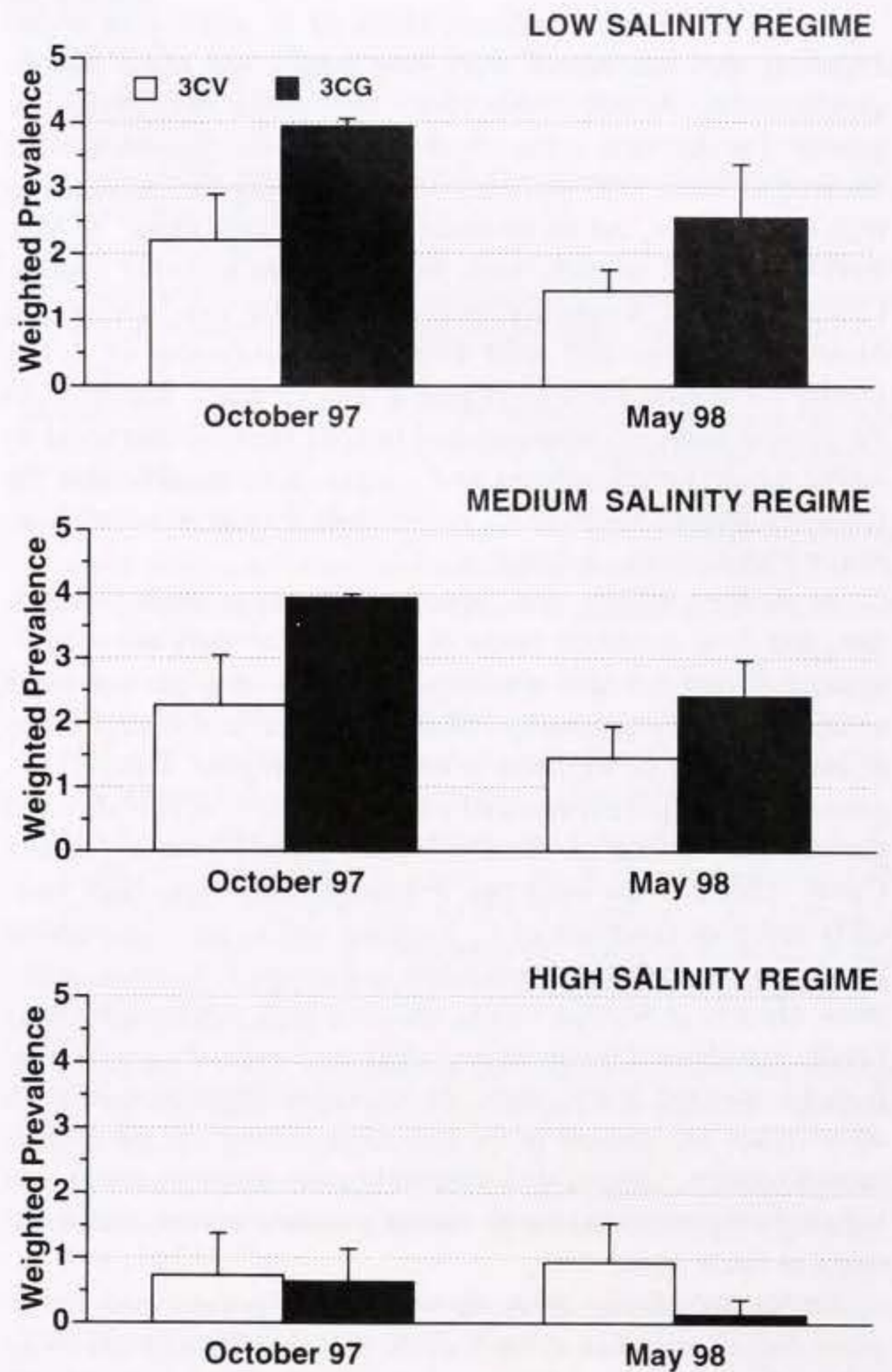

Figure 7. Intensity of *Polydora* spp. infestations in triploid *C. virginica* (3CV) and triploid *C. gigas* (3CG). Mean (+ SD) of three sites within salinity regimes.

medium salinity, mean weighted prevalence significantly ($P < .05$) decreased from October to May. Within 3CG, at high salinity, mean weighted prevalence was not significantly ($P > .05$) different between October and May.

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 3). 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 over-all proportion of mosaics was 2.7%.

Examination of 23 oysters with mosaic gill cells revealed that 5 were females, 15 were males, and 3 were undifferentiated. Among oysters with mosaic gill cells, there was one individual in which haploid cells were detected in a gonad biopsy (a male collected in Bogues Bay on 14 April 1998). Concerns over the po-

tential reproduction of *C. gigas* following the finding of an individual oyster with potentially haploid gametes, resulted in termination of the experiment. 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 the 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 significantly 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 (1.6 mm mo^{-1}) 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 nacre shell deposits were often observed to cover blisters. Comparing shell weight for oysters of similar size, Barber and Mann (1994) found that shell weight was significantly ($P < .05$) 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 possible that the relatively thinner shells of *C. gigas* made 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 significantly 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 because of 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. Oysters at Nandua Creek, and to a large extent at Woodas Creek, experienced the most prevalent and severe *P. marinus* infections recorded in this study. We speculate that high density of other oyster lots present in the immediate vicinity of the experimental oysters, coupled with relatively poor water exchange and high turbidity, resulted in high disease pressure and environmental stress at those sites.

Barber and Mann (1994) reported greater growth rates for *C. gigas* than *C. virginica* at the York River site, although 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. Furthermore, the experiment of Barber and Mann (1994) involved exposing diploid oysters to unfiltered York River water in quarantined tanks, while our study was conducted *in situ* with triploid oysters deployed within mesh cages.

Growth rate of *C. gigas* at high salinity in the present study was higher than that reported in other studies for high salinity environments. In a study of *C. gigas* growth at Seto Inland Sea in southern Japan where temperature ranged from 8–30 °C (Kobayashi et al. 1997), oyster shell height increased from 27.0 to 93.1 mm between May 1990 and January 1991. Studies with *C. gigas* in Canada and Korea reviewed by Kobayashi et al. (1997), reported similar growth rates. By comparison at high salinity sites in the present study, where temperature ranged from 4–27 °C, shell height of *C. gigas* increased from 19.2 to 101.6 mm between May and December 1997. Higher growth rates of *C. gigas* in the present study may be attributed to the use of triploid oysters; whereas, diploid oysters were used in the other studies cited above. In general, because gametogenesis is restricted in triploid oysters, more energy is available for somatic growth. Allen and Downing (1986) and Davis (1989) indicated that increased growth in triploid *C. gigas* mostly occurred during the normal reproductive season. Additional factors that would explain the difference in growth

among *C. gigas* between studies may include different environmental conditions among study areas and times.

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

The results of this study, however, are not sufficient to conclude that *C. gigas* is or is not an appropriate species for introduction or use in these environments. Before reaching a decision concerning introduction of exotic species, ICES, as well as the European Inland Fisheries Advisory Commission (EIFAC) and the American Fisheries Society (AFS), have recommended that appropriate authorities, including fishery managers, examine the candidate species to: (1) assess the justification for the introduction; (2) assess its relationship with other members of the ecosystem and the possibility of introducing associated pathogens and parasites; and (3) examine the probable effects including a prediction of the range for the establishment of the species (Turner 1988). 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. Based on the review by Mann et. al (1991) and other reports indicating that optimal temperature and salinity ranges for *C. gigas* larvae are, respectively, 18–35 °C and 19–35‰, Gottlieb and Schweighofer (1996) postulated that, if introduced, *C. gigas* would likely reproduce and establish resident populations in the lower portion of the Chesapeake Bay. Spreading would likely occur, via larval dispersal, into other areas of the Mid-Atlantic coast of North America. Interactions with other species—such as competitive interactions with *C. virginica* and predator–prey interactions may further influence the possible range extension. Additional investigations into environmental constraints on reproduction, competitive interactions with native species and predator–prey dynamics would enhance our predictive capability to determine the potential range for establishment of *C. gigas* in habitats in the Mid-Atlantic region.

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