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Lionel Degremont
Virginia Institute of Marine Science

C Garcia

A Frank-Lawale

Standish K. Allen Jr.
Virginia Institute of Marine Science

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TRIPLOID OYSTERS IN THE CHESAPEAKE BAY: COMPARISON OF DIPLOID AND TRIPLOID *CRASSOSTREA VIRGINICA*

LIONEL DÉGREMONT,*[†] CÉLINE GARCIA,[†] ANU FRANK-LAWALE AND STANDISH K. ALLEN, JR.

Virginia Institute of Marine Science, College of William and Mary, PO Box 1346, Gloucester Point, VA 23062

ABSTRACT   Diploid and triploid Eastern oysters, *Crassostrea virginica*, were tested at 3 sites characterized by low or moderate salinity regimes in the Virginia part of the Chesapeake Bay from November 2005 through October 2007. Both diploid and triploid cultures were replicated 3 times by producing separate spawns from different broodstock. Ploidy had a generally consistent effect on the performance of *C. virginica* at the 3 test sites. At the end of the study, in October 2007, and across all sites, triploid oysters had lower cumulative mortality than diploids (–34%), and greater shell height (+25%), whole weight (+88%), and yield (+152%), as well as a higher proportion of market-size oysters (+114%) than diploids. Both diploids and triploids were similarly infected by *Perkinsus marinus* and, to a lesser extent, by *Haplosporidium nelsoni*. In a closer look, growth parameters (shell height growth, whole weight, yield, and percentage of marketable oysters) were always higher in triploids than in diploids regardless of the parental source, strongly supporting the superior advantage of triploids. Similar results were obtained for cumulative mortality, but to a lesser extent as a result of the large variation in mortality for both diploid and triploid cohorts among sites, as well as a significant site-by-cohort interaction. Our report is the first clear illustration of variation for the cumulative mortality exhibited among different spawns in triploids, and comes with the lesson that care must be taken in experiments in which the goal is to test the effect of ploidy on this trait. Our results support the notion that selective breeding programs to reduce mortality, coupled with triploid production to increase growth, can further optimize yield. The best-performing replicate spawn had 80% survival after 2.5 y, and reached an average shell height of 92 mm, weighing 142 g.

KEY WORDS: Crassostrea virginica, diploid, triploid, oyster, mortality, growth, disease, Chesapeake Bay

INTRODUCTION

Eastern oyster populations (*Crassostrea virginica* (Gmelin, 1791)) in the Chesapeake Bay have collapsed as a result of overfishing, loss of habitat, decrease of water quality, and diseases (Vølstad et al. 2008). For the latter, the susceptibility of *C. virginica* to 2 protozoan parasites—*Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX)—leads to episodes of mass mortality, especially in the environmental range of the Virginia portion of the Chesapeake Bay (Ford & Haskin 1982, Andrews 1988, Mann et al. 1991). Genetic improvements, such as selection and polyploidy, have led to new optimism about rebuilding the resource through aquaculture. Development of disease-resistant strains of *C. virginica* shows promising results with the reduction of susceptibility to both diseases (Ragone Calvo et al. 2003, Aquaculture Genetics and Breeding Technology Center 2009). Triploidy also has been popular in contributing to the growth of industry in Virginia, accounting for 91% of oysters planted in 2010 (Murray & Hudson 2011). In other oyster species, triploids grow faster than diploids as a result of their partial sterility, their greater heterozygosity, their different energy allocations for growth and gametogenesis (Allen & Downing 1986, Hand & Nell 1999, Hawkins et al. 2000, Garnier-Géré et al. 2002, Normand et al. 2008), or some combination thereof. Theoretically, if triploid Eastern oysters could reach market size earlier, it would limit disease mortality (Barber & Mann 1991), resulting in increased yields even if oysters had limited disease tolerance. A similar approach was taken successfully with juvenile oyster disease, caused by the bacteria *Roseovarius crassostreae* (Majoy et al.

2007), during which faster growing oysters reached a refuge size that improved overall survival (Barber et al. 1998, Davis & Barber 1999).

Surprisingly, few studies have documented the effect of ploidy on the performance of the Eastern oyster *C. virginica*, especially with oysters produced by crossing tetraploids with diploids. Tetraploid × diploid crosses create all-triploid (so-called genetic) progeny (Guo et al. 1996). Only 1 study has compared genetic triploid with diploid *C. virginica,* and it focused on only 1 parameter (shell height), using 1 cohort of diploids and 1 cohort of triploids (Harding 2007). Performance among cohorts of diploid oysters can vary, however, especially for survival and disease resistance, as shown for *Crassostrea gigas* (Evans & Langdon 2006, Dégremont et al. 2007) and *C. virginica* (Ragone Calvo et al. 2003, Dégremont et al. 2006). That is, it is not the case that experimental spawns from the same broodstock population perform the same because of the nature of "sampling" different parental genes each spawn. Variance among triploid cohorts is likely as well. Testing replicate cohorts, as we report here, provides a measure of the repeatability of the performance of genetic triploid crosses stemming from different parental sources.

Improved performance in triploids could be the result of several factors, none yet documented thoroughly: additive genetic gains may be obtained in triploids resulting from selective breeding programs in the founding diploid populations; physiological gains may be obtained from increased robustness during the usually stressful spawning period in oysters (which also corresponds with disease challenges); and, last, there may be a heterotic effect from the cross between a dissimilar tetraploid male with a diploid female. Hand et al. (2004) concluded that gains obtained through selective breeding for growth could be additive for triploids in *Saccostrea glomerata.* More recently, triploid *C. gigas* produced from crossing diploid females—selected for resistance to summer

mortality with unselected tetraploid males—had greater survival than similar triploids produced from females that were unselected (Boudry et al. 2008, Dégremont et al. 2010). It is reasonable to assume that disease resistance will pass to triploid *C. virginica* as well, because lines with dual resistance are available.

This study involves a design that should better explain the ploidy effects on performance (mortality, growth, yield, proportion of marketable oysters, and disease status) of *C. virginica* by using various diploid and tetraploid broodstocks, comparing 3 cohorts of diploid *C. virginica* with 3 cohorts of triploid *C. virginica* as replicates of each ploidy.

MATERIALS AND METHODS

Biological Material

Three diploid cohorts and 2 triploid cohorts were produced in the Aquaculture Genetics and Breeding Technology Center's (ABC's) hatchery at the Virginia Institute of Marine Science located in Gloucester Point. The third cohort of triploid *C. virginica*, cohort G, was obtained from a local hatchery in northern Virginia to represent a "typical" commercial spawn of triploids. Three disease-resistant strains of diploid *C. virginica* were crossed within strain to produce three representative "cohorts" of diploids for testing against the triploids A, B and C, which were spawned respectively in April 2005, July 2005, and July 2005 (Table 1). Cohort A is the sixth generation of the Delaware Bay line called DBY (*cf.* Ragone-Calvo et al. 2003). Cohort B is the third generation of the DMO line that originated from crossbreeding between the DBY line and a local oyster population from the Chesapeake Bay sampled in the Mobjack Bay. Last, cohort C is the first generation of the DBLA line that was produced from crossbreeding between the DBY line and a Louisiana line, developed and characterized by Louisiana State University (Jerome LaPeyre, Department of Veterinary Sciences) as resistant to *P. marinus*. The line was also highly susceptible to *H. nelsoni*. For triploids, 3 cohorts—named D, E, and G—were produced by crossing tetraploid males with diploids females in April 2005, July 2005, and July 2005, respectively (Table 1). For cohorts D and E, fifth-generation DBY females were used whereas unselected wild females were used to produce cohort G (in the commercial hatchery). Tetraploid oysters used to produce cohort E were the generation G_0 of the tetraploid line originally developed from triploid Louisiana wild oysters and a diploid CROSBreed (XB)

male. Tetraploids used to produce cohort D were from a G_1 cross of the tetraploids noted earlier. Tetraploids used to produce cohort G were from a tetraploid line produced independently by crossing genetic triploids produced from tetraploids E noted earlier with XB, which themselves were out-crossed to the DBY line (thus it is a composite of Louisiana, XB, and DBY germplasm). Thus, each triploid cohort was produced from different tetraploid base populations and different diploid base populations. These crosses were completed fortuitously during the 2005 spawning season with the objective of planting out various diploid and triploid cohorts for comparison. They represent the experience that commercial growers would encounter ordering seed from various hatcheries.

All cohorts were produced following standard hatchery procedures used at the ABC hatchery. Briefly, larvae were grown to eyed pediveliger stage and set using microcultch. One month later, oysters were placed in an inland nursery next to the hatchery, and ploidy was determined by flow cytometry. When juveniles were approximately 10 mm, they were transferred into the York River until the simultaneous deployment of all diploid and triploid cohorts for grow-out evaluation in November 2005.

Field Experiment

Sites

Oysters were deployed at 3 sites in the Chesapeake Bay in November 2005 characterized by 2 salinity regimes and therefore two expected disease pressures. The low-salinity (<15‰) site was located in the Yeocomico River at Kinsale (Fig. 1), where disease pressure is low for *P. marinus* (Dermo) and absent for *H. nelsoni* (MSX). The other 2 sites were medium-salinity sites (15–25‰) located in the York River at Gloucester Point and in Broad Bay, Lynnhaven River, in Virginia Beach (Fig. 1), with high disease pressures for both Dermo and MSX.

Experimental Design

The culture method used was bags fixed on rebar racks. At each site, each cohort was replicated into 3 bags of 150 oysters for survival and yield assessments. A fourth bag was added for sampling for growth and disease evaluation. At each site, oysters were grown in the intertidal zone from March to November, and in deeper water for overwintering in an attempt to avoid freezing-induced mortality. In one case, this was unsuccessful.

TABLE 1.

Pedigree of the diploid (2n) and triploid (3n) cohorts tested.

Ploidy	Cohort Name	Diploid Broodstock*	Tetraploid Broodstock*	Spawn Date	No. of Females†	No. of Males†
2n	A	G_5 DBY		April 2005	10	12
2n	B	G_2 DMO		July 0505	26	18
2n	C	G_0 DBLA		July 2005	39	24
3n	D	G_5 DBY	G_1 Louisiana and XB cross	April 2005	10	6
3n	E	G_5 DBY	G_0 Louisiana and XB cross	July 2005	26	3
3n	G	G_0 wild oysters	G_0 Louisiana, XB and DBY cross	July 2005	3	1

* G indicates generation
† For D, E, and G, the numbers of females are given for the diploid broodstocks, and the numbers of males are given for the tetraploid broodstocks.
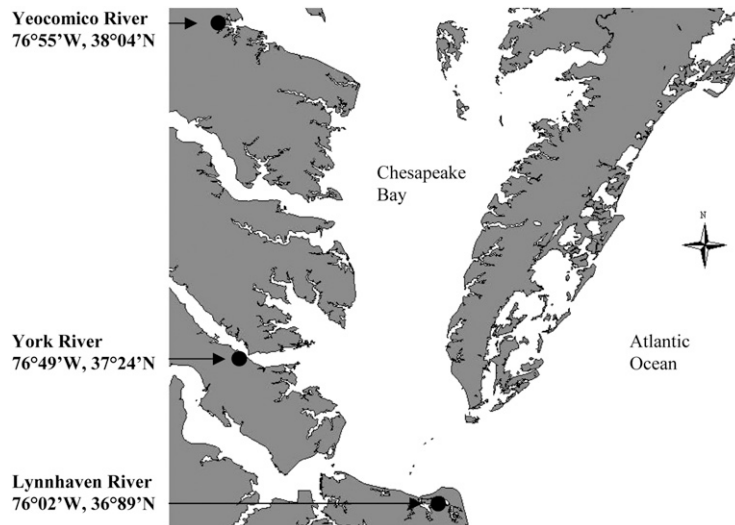DBY, *Andrews* DBY line; XB, *Haskins* CROSBreed line.

Figure 1. Location of study sites in Chesapeake Bay.

Sampling and Data Collection

At deployment, total weight of all oysters in each bag was recorded for yield assessments, and 30 oysters were sampled randomly and measured individually for shell height (in millimeters) and whole weight (in grams). Oysters were monitored from March 2006 to October 2007. Dead and live oysters were counted monthly between May 2006 and September 2006, and also in November 2006. Survival was also recorded in March 2007, June 2007, and October 2007. The cumulative mortality was then determined as the ratio between the number of dead oysters from the beginning to the sampling on the number of live oysters at the beginning. Total weight of live oysters for each of the 3 replicate bags was recorded in November 2006 and October 2007 for the determination of yield. Shell height and whole weight were measured individually from 30 oysters sampled (from the bag dedicated to growth) in November 2006 and from 50 oysters in October 2007. Percentage of oysters that reached market size (76 mm) was also obtained at both dates in fall using the shell height data sets. Last, disease was assessed for all cohorts at all sites in August 2006 at the onset of heavy mortality observed for several cohorts. Fifteen oysters per cohort were screened and *H. nelsoni* infections were diagnosed using stained paraffin sections (Burreson et al. 1988). Parasite *P. marinus* was diagnosed using Ray's fluid thioglycollate medium inoculation of rectal and mantle tissues (Ray 1952). Weighted incidences were then calculated as described in Paynter and Burreson (1991).

Environmental Parameters

Temperature and salinity were recorded monthly using a YSI probe (Yellow Springs Instruments, Yellow Springs, OH), and, in addition, temperature was recorded every 3 h using 2 iBCod temperature loggers (Maxim Integrated Products, Sunnyvale, CA) at each site.

Statistical Analyses

Data Correction

During the overwinter period, all racks were placed in deeper water to protect them from freezing. Unfortunately, this was unsuccessful at the Yeocomico River site. In February 2007, some of the oysters were exposed during a period of exceptionally low tide and low temperatures. Consequently, unusual mortality, ranging from 10–80%, was observed in several bags in March 2007 (5 bags for the diploid cohorts and 2 bags for the triploid cohorts), whereas mortality for the remaining bags was only 1%. As a result, final mortality and associated yield recorded in October 2007 were corrected for these bags. For example, two replicates of 1 cohort had low mortality (0% and 1%) between December 2006 and March 2007, whereas the third had a mortality of 43% as a result of the freezing event. Thus, 56 oysters died of the freezing event. Of the 72 remaining oysters, 6 died from March until September 2007 (8% mortality). So the new number of dead oysters in September 2007 was corrected to 11 ($6 + 56 \times 8\%$), and the final number of alive oysters was 117 ($66 + 56 \times 92\%$), instead of 66. Thus, final mortality for this replicate was corrected from 56% to 22%. A total of 7 bags out of a total of 18 had to be adjusted this way. The same approach was adopted for the final yield determinations.

Dependent Variables and Time Frame

Cumulative mortality, height and weight, yield, and proportion of market-size oysters (>76 mm) were analyzed in November 2006 and October 2007. Disease prevalence for *H. nelsoni* and *P. marinus,* and weighted prevalence for both diseases were analyzed in August 2006, at 1+ y old.

Model Equations

We fitted the following model:

$$Y_{ijkl} = \mu + site_i + ploidy_j + cohort_{k(j)} + \left(site_i \times ploidy_j\right) + \left(site_j \times cohort_{k(j)}\right) + E_{ijkl}$$

where Y_{ijkl} is the dependant variable (mortality, disease prevalence, and weighted incidence), μ is the overall mean, site is the site effect (i is the York River, Lynnhaven River, Yeocomico River), ploidy is the ploidy effect in *C. virginica* (j is diploid, triploid), cohort$_{k(j)}$ is the cohort effect (k is cohorts A, B, C, D, E, and G), site$_i \times$ ploidy$_j$ and site$_i \times$ cohort$_{k(j)}$ are the interactions,

and E_{ijkl} is the residual error. Site, ploidy, and their interactions were defined as fixed effects, whereas cohort and site-by-cohort interaction were random effects. We acknowledge that the ''ploidy'' effect is compounded by a genetic effect, such that triploids and diploids have different genetic backgrounds.

For growth (height and weight) and yield, the models were similar to those described here, except that a covariate was added: time elapsed for height and weight growth (i.e., from the deployment to the sampling) (see Taris et al. (2006) for more details). The covariate for yield was the initial yield (i.e., the total weight of the 150 oysters at the time of deployment).

Statistical Procedures Used

All statistical analyses were performed using SAS software version 9.1. Cumulative mortality, disease prevalence, and market-size oysters were analyzed using the GLIMMIX procedure with a probit transformation and a binomial distribution. The likelihood ratio test—a chi-square distribution and $df = 1$—was used to test the significance of the random effects whereas the significance of fixed effects was based on F tests (Littell et al. 1996). Multiple comparisons were conducted using the least-squares means statement and the SLICE option, which allows testing of the ploidy factor at each site when a significant interaction is found between site and ploidy. Thus, the statistical test is more powerful than rerunning the analysis within site because the degrees of freedom are not reduced (Littell et al. 2002). When a significant interaction between site and cohort was found, the site was dropped from the analyses to test the cohort effect within the site.

Normality and the homogeneity of variance for height, weight, yield, *H. nelsoni* weighted incidence, and *P. marinus* weighted incidence were assessed with the UNIVARIATE procedure using the Shapiro-Wilk statistic, and with the GLM procedure using Levene's test. From the results, the weighted incidence data were transformed using the RANK procedure, and were then analyzed using the MIXED procedure (SAS 1988). Yield was log transformed and analyzed using the MIXED procedure by running an ANCOVA with the initial yield as a covariate. A similar approach was used for the height and weight analyses except that data were not transformed and the covariate was time. Multiple comparisons were conducted using the same approach as described here.

RESULTS

Salinity and Temperature

Average salinity and SD was $10.6 \pm 2.0\%$ at the Yeocomico River site, $16.6 \pm 2.6\%$ at the York River site, and $19.7 \pm 1.7\%$ at the Lynnhaven River site throughout the experiment. Water temperature was quite similar among the 3 sites, ranging from 3°C during the winter to 30°C during the summer (Fig. 2).

Cumulative Mortality

Cumulative mortality, hereafter called mortality, by site and by ploidy recorded in November 2006 and in October 2007 is reported in Table 2. Interaction between site and ploidy was not significant in either year (2006, $P = 0.617$; 2007, $P = 0.897$; Table 3). Within and across sites, mortality was always higher in diploid than in triploid *C. virginica* throughout the duration of our study (Table 2). Mean mortality ($\pm$SD) across all sites was $32 \pm 21\%$ for diploids and $18 \pm 14\%$ for triploids in November 2006, and reached $53 \pm 23\%$ for diploids and $35 \pm 21\%$ for triploids in October 2007 (Table 2). Statistically, however, there was no significant difference in mortality between ploidy in both
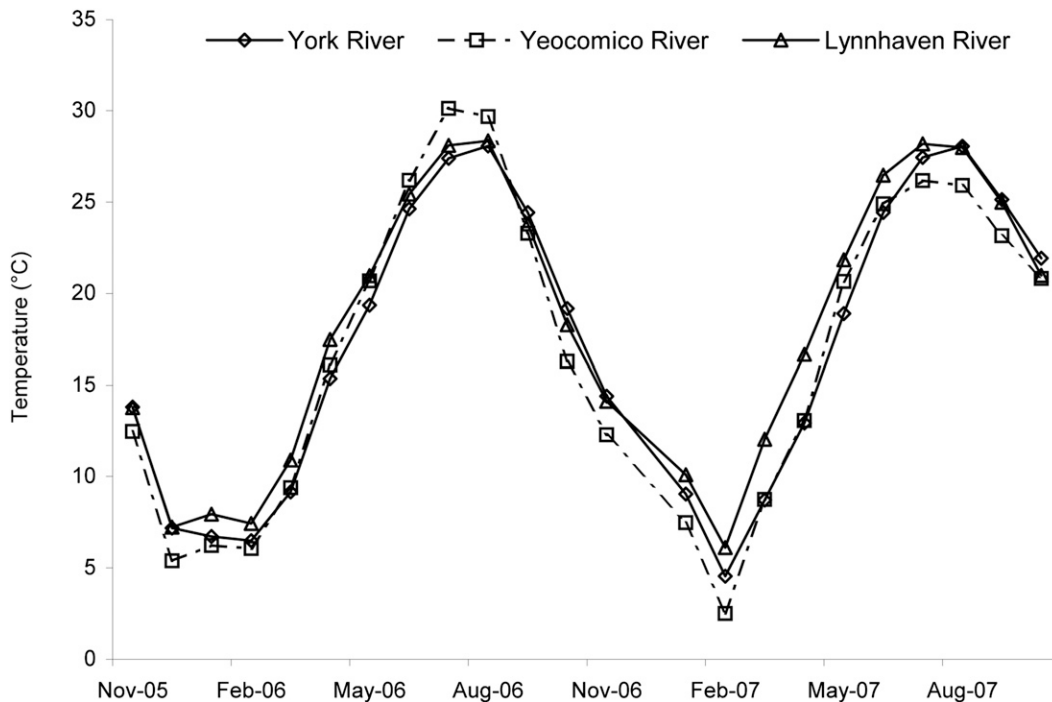

Figure 2. Water temperature at the 3 sites from November 2005 to October 2007.

TABLE 2.

Mean and SD of diploid (2n) and triploid (3n) *C. virginica* for cumulative mortality (as a percentage) and market size (as a percentage) within and among sites in November 2006 and October 2007.

Ploidy	Mortality in November 2006				Mortality in October 2007			
	Lynn R	York R	Yeoc R	All	Lynn R	York R	Yeoc R	All
2n	34 ± 17	48 ± 21	15 ± 10	32 ± 21	57 ± 16	70 ± 16	31 ± 18	53 ± 23
3n	17 ± 11	26 ± 18	9 ± 4	18 ± 14	37 ± 20	50 ± 20	18 ±8	35 ± 21
Both	25 ± 16	37 ± 22	12 ± 8	25 ± 19	47 ± 20	60 ± 20	25 ± 14	44 ± 23

Ploidy	Market size in November 2006				Market size in October 2007			
	Lynn R	York R	Yeoc R	All	Lynn R	York R	Yeoc R	All
2n	20 ± 15	3 ± 3	9 ± 7	11 ± 11	56 ± 9	21 ± 8	51 ± 18	43 ± 20
3n	88 ± 12	62 ± 38	53 ± 7	68 ± 25	98 ± 2	84 ± 7	95 ± 3	92 ± 8
Both	54 ± 39	33 ± 40	31 ± 25	39 ± 35	77 ± 24	53 ± 35	73 ± 27	68 ± 29

Lynn R, Lynnhaven River; York R, York River; Yeoc R, Yeocomico River.

years (2006, $P = 0.220$; 2007, $P = 0.196$), likely because of the high variance in mortality among cohorts (Table 2).

A significant difference in mortality was found among sites in both years (2006, $P = 0.018$; 2007, $P = 0.005$), with lower mortality at the Yeocomico River site, the low-salinity site, whereas mortality was around double that at the York River and Lynnhaven River sites (the medium-salinity sites; Table 2).

Mortality remained very low for all cohorts at all sites from November 2005 to June 2006 (Fig. 3). Mortality occurred between July 2006 and November 2006, and between March 2007 and October 2007 at all 3 sites. At the Yeocomico River site, mortality remained lower than 20% for all cohorts in October 2007, except for the diploid cohort A, which reached 28% in fall 2006 and 50% in fall 2007. At the two other sites, heavy mortality affected diploids of cohort C (57–76%) in November 2006, whereas mortality was very low for the triploid cohort D (6–8%) and intermediate for the other cohorts (15–47%; Fig. 3). In October 2007, similar results were observed with higher mortality that reached 77% in the Lynnhaven River and 90% in the York River for diploid cohort C, whereas mortality of the triploids in cohort D remained low (18–28%; Fig. 3). In both years, there was a significant interaction between site and cohort (2006, $P < 0.001$; 2007, $P < 0.001$), whereas no significant differences in mortality between cohorts were found (Table 3). These results are likely a result of the variation of mortality of the diploid cohorts among salinity regimes, probably attributable to disease. Indeed, when the Yeocomico River site is dropped from the statistical analyses, the interaction between site and cohort was not significant (2006, $P = 0.19$; 2007, $P = 0.58$), and there was a significant difference in mortality between cohorts (2006, $P < 0.001$; 2007, $P < 0.001$). Also, at the Yeocomico River, mortality of cohorts was significantly different in both years (2006, $P < 0.001$; 2007, $P < 0.001$).

Disease

Mean prevalence and weighted incidences of *H. nelsoni* across sites were $18 ± 20$% and $0.1 ± 0.5$, respectively, in diploids, and $7 ± 11$% and $0.6 ± 1.5$ in triploids. Within site, no *H. nelsoni* infection was found among cohorts tested at the low-salinity

Yeocomico River site (Fig. 4). At the medium-salinity sites, only samples from diploid cohorts A and C, and triploid cohort G were infected at the Lynnhaven River site, whereas all cohorts except D were infected at the York River site (Fig. 4). Diploid cohort C had the highest prevalence and weighted incidence at the Lynnhaven River site, with 40% and 1.4, respectively, and at the York River site with 50% and 1.9, respectively. For the mean prevalence of *H. nelsoni*, only the cohort effect was significant ($P = 0.033$; Table 4). For weighted incidence of *H. nelsoni*, only the interaction between site and cohort was significant ($P = 0.034$; Table 5). When the low-salinity site where *H. nelsoni* was absent was dropped from the statistical analyses, however, the interaction was not significant ($P = 0.53$) and the cohort effect became significant ($P = 0.037$).

TABLE 3.

Probit analysis of mortality and market size of oysters in fall 2006 and 2007.

Source	2006			2007		
	df	For Chi-square	P	df	For Chi-square	P
Mortality (%)						
Site	2	6.95	0.018	2	11.35	0.005
Ploidy	1	2.11	0.220	1	2.41	0.196
Cohort (ploidy)	1	*2.31*	0.064	1	*2.20*	0.069
Site × ploidy	2	0.51	0.617	2	0.11	0.897
Site × cohort (ploidy)	1	*229.52*	<0.001	1	*237.78*	<0.001
Market size (%)						
Site	2	5.92	0.026	2	12.92	0.003
Ploidy	1	19.94	0.011	1	106.44	<0.001
Cohort (ploidy)	1	*1.87*	0.171	1	*0.01*	0.920
Site × ploidy	2	1.17	0.359	2	0.17	0.844
Site × cohort (ploidy)	1	*6.90*	0.009	1	*6.86*	0.009

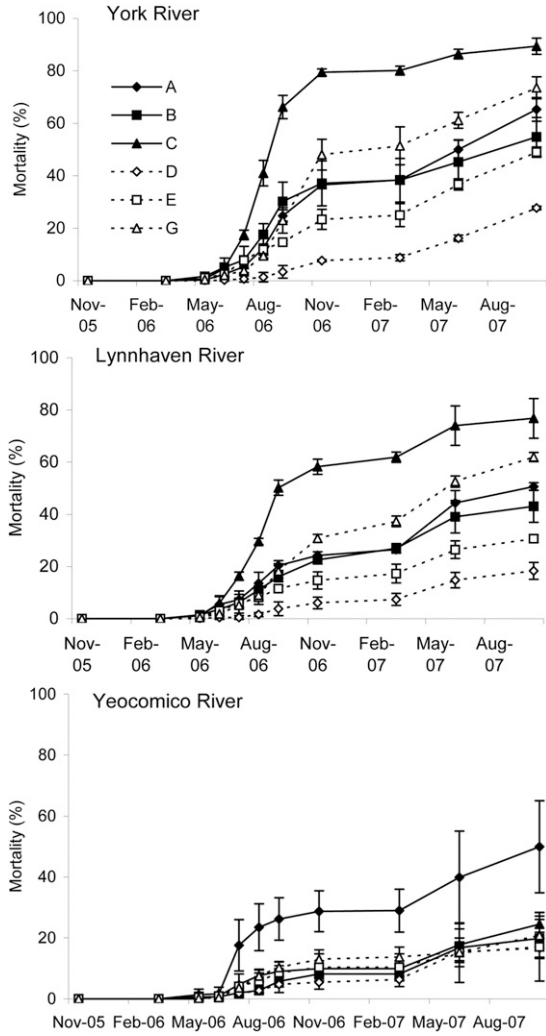Chi-square values are in italics. Parentheses indicate nesting of factors in analyses.

Figure 3. Mean cumulative mortality (percent ± SD) of diploid (solid lines: cohorts A, B, and C) and triploid (dashed lines: cohorts D, E, and G) C. virginica by site from November 2005 to October 2007.
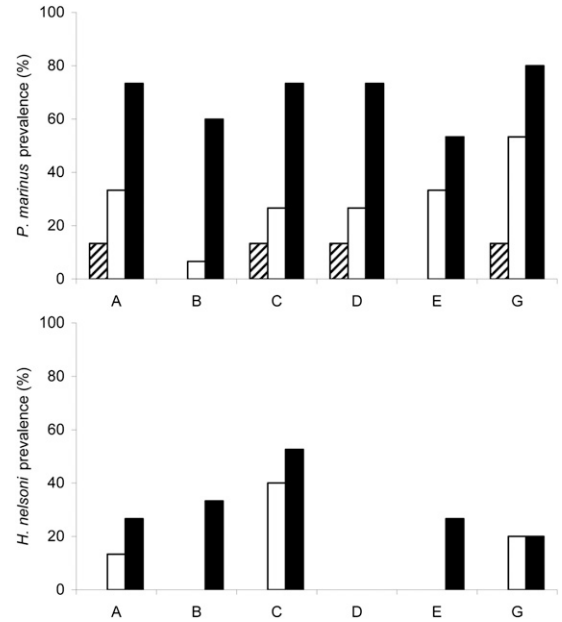

Figure 4. Prevalence of P. marinus (top) and H. nelsoni (bottom) in diploid (cohorts A, B, and C) and triploid (cohorts D, E, and G) C. virginica in the York River (black bars), Lynnhaven River (white bars), and Yeocomico River (dashed bars) in August 2006.

Mean prevalence for *P. marinus* within site and cohort is shown in Figure 4. The mean prevalence among sites was 33 ± 29% in diploids and 39 ± 28% in triploids, and the mean weighted incidences were 0.6 ± 1.2 in diploids and 0.8 ± 1.3 in triploids. Site effect for both prevalence and weighted incidence was significant ($P < 0.001$), as was the cohort effect for weighted incidence ($P = 0.023$; Tables 4 and 5). Prevalence and weighted incidence of *P. marinus* were the highest at the York River site (69 ± 10% and 1.2 ± 1.4, respectively), intermediate at the Lynnhaven River site (30 ± 15% and 0.7 ± 1.3, respectively), and lowest at the Yeocomico River site (9 ± 7% and 0.2 ± 0.6, respectively). Cohort G had the highest weighted incidence (1.1 ± 1.5), and cohorts B and E exhibited the lowest weighted incidence (0.4 ± 0.9).

Growth and Percentage of Market-Size Oysters

The mean weight at deployment was 1.5 ± 0.9 g for diploids and 2.4 ± 1.1 g for triploids, and they grew to 41 ± 15 g and 76 ± 24 g, respectively, by fall 2006, finally reaching 75 ± 26 g and 141 ± 41 g, respectively, in fall 2007 (Table 6). In both years,

interactions between site and ploidy were significant ($P < 0.05$; Table 7). Within sites, triploids always had greater weights than diploids at all sites in both years ($P < 0.01$; Fig. 5). Among sites, mean weight was significantly different in both years, with the greatest weight in the Lynnhaven River, intermediate in the Yeocomico River, and the lowest in the York River ($P < 0.001$; Table 7).

For shell height, diploid *C. virginica* deployed in November 2005 grew from 22–63 mm by fall 2006, and from 22–75 mm by fall 2007. Triploid *C. virginica* grew from 28–82 mm by fall 2006, and from 28–94 mm by fall 2007 (Table 6). There were significant differences in shell height among sites as well as between ploidies in both years ($P < 0.05$; Table 7), but no significant interactions were detected between site and ploidy in either year ($P > 0.05$). Triploids grew faster than diploids; Lynnhaven River supported the highest growth rate, followed by the Yeocomico River and then the York River. There was a significant site-by-cohort interaction in both years (Table 7).

TABLE 4.

Probit analysis of disease prevalence in August 2006.

		P. marinus			*H. nelsoni*	
		For			For	
Source	df	Chi-square	P	df	Chi-square	P
Site	2	34.30	<0.001	2	1.99	0.199
Ploidy	1	0.28	0.624	1	0.00	0.983
Cohort (ploidy)	1	*1.5*	0.221	1	*4.45*	0.033
Site × ploidy	2	0.83	0.469	2	0.03	0.975
Site × cohort (ploidy)	1	*0.00*	0.980	1	*0.86*	0.354

Chi-square values are in italics.

TABLE 5.

Variance analysis of weighted incidence in August 2006.

Source	*P. marinus*				*H. nelsoni*			
	df	MS	F	P	df	MS	F	P
Site	2	141.56	78.17	<0.001	2	34.89	9.90	0.007
Ploidy	1	3.78	0.40	0.560	1	21.33	2.19	0.213
Cohort (ploidy)	4	9.38	5.18	0.023	4	9.74	2.80	0.100
Site × ploidy	2	4.28	2.36	0.156	2	8.00	2.30	0.162
Site × cohort (ploidy)	8	1.81	0.53	0.837	8	3.47	2.12	0.034
Error	252	3.44			252	1.64		

Within site, growth was different among cohorts in all sites both years ($P < 0.05$), except in 2007 in the Lynnhaven River ($P = 0.11$).

Overall, by fall 2006, only 11% of surviving diploid *C. virginica* reached market size (76 mm) compared with 68% of triploids (Table 2). It is also important to note that 90–100% of 2 cohorts of the triploid *C. virginica* (cohorts D and E) had reached market size—notably, at the York River and Lynnhaven River sites. By the end of the experiment, 43% of surviving diploids and 92% of the surviving triploids were market size (Table 2). Significant differences in percentage to market size were detected between ploidies in both years (Table 3). We also observed significant differences in the proportion of marketable oysters among sites in 2006 and 2007, with a higher proportion of oysters reaching market size in the Lynnhaven River, whereas the lowest proportion was seen in the York River (Table 6). There was always a significant difference among cohorts ($P < 0.001$) in the proportion of marketable oysters.

Last, a significant interaction between site and cohort was found for both years and within sites.

Yield

The mean yield (total weight of all live oysters) in 2006 and 2007 within and among sites and ploidies is presented in Table 6. ANCOVAs indicated that initial yield had no effect on final yield, and failed to reveal any significant site-by-ploidy interactions in either year ($P > 0.05$; Table 8). Triploids had a significantly higher yield than diploids in both years, with 14.9 ± 5.1 kg for the former and 5.9 ± 3.0 kg for the latter in 2007 (Table 6). Also, yield was significantly different among sites, with the highest yield observed for the oysters deployed in the Yeocomico River, whereas those deployed in the York River had the lowest yield in 2007 (Table 6). At the end of the experiment, yields ranged from 1.3–10.2 kg in the diploid cohorts, and from 5.7–22.3 kg in the triploid cohorts. There was a significant site-by-cohort interaction in both years, but the effect of cohort was not significant (Table 8) unless site was dropped from the statistical analyses; then, there was always a significant difference in yield among cohorts at each site ($P < 0.05$).

DISCUSSION

Cumulative Mortality

Published results of comparative oyster mortality between diploids and triploids vary. Mortality was found to be similar between the 2 ploidies in *S. glomerata* (Hand et al. 2004) and in *C. gigas* (Dégremont et al. 2010), whereas in an earlier study by Hand et al. (1998b), triploid *S. glomerata* (formerly *Saccostrea commercialis*) had lower mortality than diploid and triploid

TABLE 6.

Mean and SD of yield (in kilograms), shell height (in millimeters), and whole weight (in grams) of diploid (2n) and triploid (3n) *C. virginica* within and among sites in November 2005, November 2006, and October 2007.

Ploidy	Initial Yield in November 2005				Yield in November 2006				Yield in October 2007			
	Lynn R	York R	Yeoc R	All	Lynn R	York R	Yeoc R	All	Lynn R	York R	Yeoc R	All
2n	0.23 ± 0.08	0.25 ± 0.12	0.24 ± 0.08	0.24 ± 0.09	4.5 ± 1.2	2.9 ± 1.2	4.5 ± 1.1	4.1 ± 1.4	5.8 ± 2.0	3.3 ± 1.6	8.6 ± 2.5	5.9 ± 3.0
3n	0.39 ± 0.07	0.41 ± 0.07	0.41 ± 0.08	0.41 ± 0.07	11.8 ± 2.5	8.6 ± 0.5	8.6 ± 0.5	9.6 ± 2.6	16.4 ± 5.5	11.0 ± 4.8	17.4 ± 2.1	14.9 ± 5.1
Both	0.31 ± 0.11	0.33 ± 0.13	0.32 ± 0.12	0.32 ± 0.12	8.2 ± 4.2	6.8 ± 2.0	6.8 ± 2.0	6.9 ± 3.4	11.1 ± 6.8	7.1 ± 5.2	13.0 ± 5.1	10.4 ± 6.2

Ploidy	Initial shell height in November 2005				Shell height in November 2006				Shell height in October 2007			
	Lynn R	York R	Yeoc R	All	Lynn R	York R	Yeoc R	All	Lynn R	York R	Yeoc R	All
2n	21.5 ± 5.4	23.3 ± 5.8	22.3 ± 5.2	22.4 ± 5.5	67 ± 10	59 ± 9	64 ± 8	63 ± 10	79 ± 10	68 ± 8	76 ± 11	75 ± 11
3n	27.5 ± 5.9	28.2 ± 5.2	28.4 ± 5.5	28.0 ± 5.6	89 ± 11	80 ± 15	77 ± 11	82 ± 13	102 ± 13	86 ± 11	94 ± 11	94 ± 13
Both	24.5 ± 6.4	25.8 ± 6.0	25.4 ± 6.2	25.2 ± 6.2	78 ± 5	69 ± 16	71 ± 12	73 ± 15	90 ± 16	77 ± 13	85 ± 14	84 ± 16

Ploidy	Initial whole weight in November 2005				Whole weight in November 2006				Whole weight in October 2007			
	Lynn R	York R	Yeoc R	All	Lynn R	York R	Yeoc R	All	Lynn R	York R	Yeoc R	All
2n	1.4 ± 0.9	1.7 ± 0.9	1.5 ± 0.8	1.5 ± 0.9	47 ± 16	39 ± 15	39 ± 13	41 ± 15	84 ± 29	70 ± 23	71 ± 25	75 ± 26
Both	2.3 ± 1.1	2.6 ± 1.2	2.4 ± 1.0	2.4 ± 1.1	89 ± 20	78 ± 23	62 ± 20	76 ± 24	165 ± 46	130 ± 36	126 ± 30	141 ± 41
All	1.8 ± 1.1	2.2 ± 1.2	1.9 ± 1.0	2.0 ± 1.1	68 ± 28	58 ± 28	50 ± 21	59 ± 26	125 ± 56	100 ± 42	99 ± 39	108 ± 48

Lynn R, Lynnhaven River; York R, York River; Yeoc R, Yeocomico River.

TABLE 7.

Covariance analysis of growth in fall 2006 and 2007 for diploid and triploid *C. virginica*.

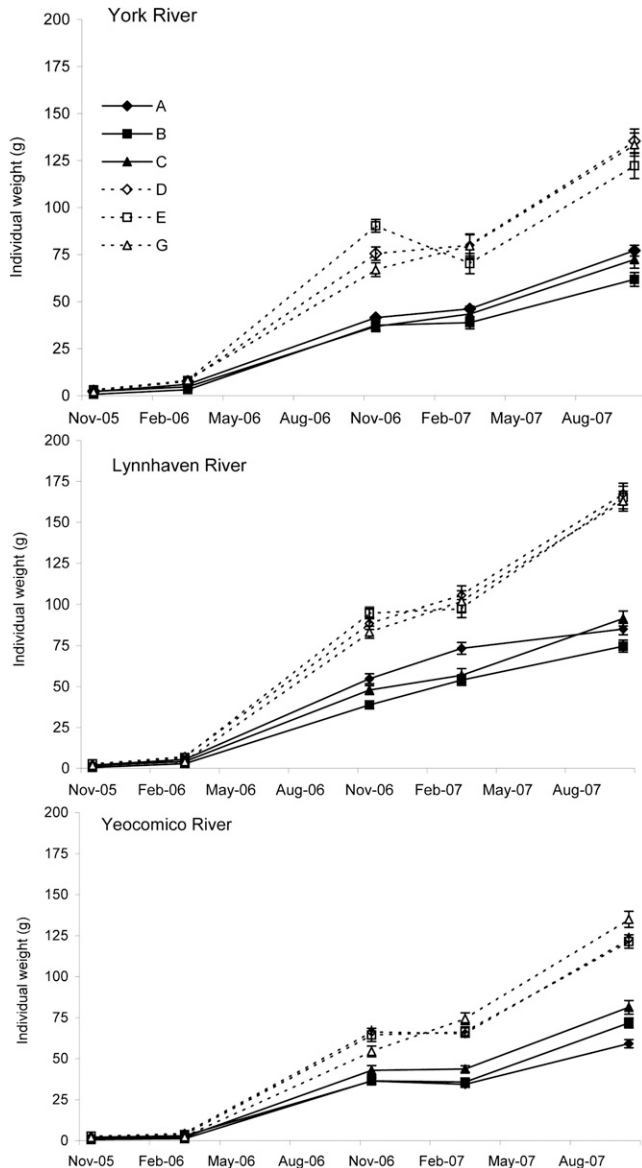| Source	Growth 2006				Growth 2007			
	df	MS	F	P	df	MS	F	P
Whole weight								
Site	2	7.26	20.77	<0.001	2	23.89	24.55	<0.001
Ploidy	1	76.85	59.53	0.002	1	354.36	241.72	<0.001
Cohort (ploidy)	4	1.29	3.69	0.055	4	1.47	1.51	0.288
Site × ploidy	2	2.26	6.47	0.021	2	5.36	5.50	0.031
Site × cohort (ploidy)	8	0.35	2.31	0.019	8	0.97	1.54	0.139
Error	1,044	0.15			1,404	0.63		
Shell height								
Site	2	2.48	7.60	0.014	2	5.92	19.56	<0.001
Ploidy	1	11.11	16.71	0.015	1	15.25	16.73	0.015
Cohort (ploidy)	4	0.66	2.04	0.181	4	0.91	3.01	0.086
Site × ploidy	2	0.68	2.10	0.186	2	0.20	0.68	0.535
Site × cohort (ploidy)	8	0.33	5.68	<0.001	8	0.30	3.94	<0.001
Error	1,044	0.06			1,404	0.08		

C. gigas (Gagnaire et al. 2006); the opposite findings were observed in *C. gigas* by Goulletquer et al. (1996) and Cheney et al. (2000). To our knowledge, comparison of mortality between diploid and triploid *C. virginica* has only been reported twice previously, and both studies used chemically induced triploids. Matthiessen and Davis (1992) found results similar to ours at one of their testing sites in New England where MSX disease was known to be prevalent, whereas mortality was negligible for both diploid and triploid oysters at sites free from disease pathogens. In a challenge experiment using oyster tissue infected with *P. marinus*, however, Meyers et al. (1991) found that there were no differences in mortality between diploid and triploid *C. virginica* because all oysters had died by the end of the trial. It is worth noting, though, that the diploids died at a faster rate and, at the time of their comparisons, disease-resistant lines were not available. In our study, overall mean cumulative mortality was 35% in triploids and 53% in diploids in October 2007 (Table 2), indicating a clear advantage for triploids in survival. The difference was not statistically significant, however, because of the high variation among both diploid and triploid cohorts, especially in the medium-salinity sites (Table 2). Such variation was observed in diploid *C. virginica* (Ragone Calvo et al. 2003), but not before in triploids. Our report is the first clear illustration of this variation exhibited among different spawns, and comes with the lesson that care must be taken in experiments where the goal is to test the effect of ploidy, especially under the following circumstances: (1) the diploid and triploid oysters have a low effective population size (restricted numbers of parents in the spawn), especially if the studied trait is highly heritable (that is to say, it is possible that particular individuals will lead to variation among spawns with few parents sampled within a population (Evans & Langdon 2006, Dégremont et al. 2007)); (2) using diploid and tetraploid oysters derived from different germplasm; and (3) using the same germplasm but not the same parents, such that different sets of parents are used to produce the diploid and triploid oysters, as in the studies by Meyers et al. (1991), Matthiessen and Davis (1992), Cheney et al. (2000), Gagnaire et al. (2006), and the current study. The best method to compare diploids and triploids is to use chemically induced triploids from common females and males, or

eggs from common females crossed with either the diploid or the tetraploid sperm. Even in such cases, comparisons can still break down for the latter if the diploid and tetraploid do not share common founder genotypes. In our case, this was impossible because our tetraploids were derived intentionally from a number of disease-resistant founder sources—DBY, CROSBreed, and Louisiana brood stock; there is no diploid counterpart to this tetraploid. Therefore, we replicated the ploidy factor using several cohorts produced with different spawns to partition the variation (error) in the ploidy factor and thereby increase the precision in the estimate of the difference between the 2 treatments (Hurlbert 1984).

Our study also demonstrates that triploid *C. virginica* exhibit a clear advantage over diploids even when both ploidies are disease resistant, as observed previously in *S. glomerata* for growth (Hand et al. 2004). Thus, it seems the progress in selection for disease-resistant strains is advanced through both the diploid and tetraploid lines (Table 1) and appears to be additive. For example, the lowest cumulative mortality in our study (Fig. 3) was observed for the triploid cohort D, which was produced by crossing a G_1 tetraploid × disease-resistant diploid line; intermediate cumulative mortality was obtained for triploid cohort E, which was made by crossing a G_0 tetraploid × disease resistant diploid line; and, last, the highest cumulative mortality was observed in triploid cohort G, a cross between a G_0 tetraploid × wild oysters. This confirms previous results in *C. gigas* that indicate that summer mortality in juveniles could be reduced significantly using a selective breeding program in both the diploid and tetraploid parents (Boudry et al. 2008, Dégremont et al. 2010). In addition to apparent disease resistance in the triploids, there may be additional effects augmenting the triploid advantage, such as nonadditive gene action, dosage effects, or simple physiological advantage. Further controlled matings are needed to sort out these possible effects on mortality.

Disease

It is important to note that disease sampling/diagnosis was only conducted in August 2006. It would have been interesting to

Figure 5. Growth curves of the individual weight (grams ± SE) of diploid (solid line) and triploid (dashed line) *C. virginica* by site from November 2005 to October 2007.

Source	Yield 2006				Yield 2007			
	df	MS	F	P	df	MS	F	P
Site	2	0.94	3.13	0.099	2	3.01	5.82	0.028
Ploidy	1	1.46	16.29	0.016	1	1.50	8.76	0.042
Cohort (ploidy)	4	0.47	1.90	0.202	4	0.91	2.17	0.162
Site × ploidy	2	0.35	1.14	0.366	2	0.29	0.56	0.590
Site × cohort (ploidy)	8	0.30	50.07	<0.001	8	0.52	23.41	<0.001
Initial yield	1	0.00	0.09	0.760	1	0.05	1.53	0.224
Error	35	0.01			35	0.02		

mortality was observed for any cohort in the Yeocomico River, where *H. nelsoni* was not detected in 2006 (in fact, none has been detected between 2004 and 2007, data not shown). Regarding other cohorts, a significant portion of the mortality may be attributable mostly to *P. marinus* and, to a lesser extent, to *H. nelsoni,* because *P. marinus* prevalence and weighted incidence were greater than those of *H. nelsoni.*

Growth and Percentage of Market-Size Oysters

Our results indicate that weight was twice as high in triploid *C. virginica* as in diploids, and—to a lesser extent—a similar trend was found for shell height, clearly showing another real advantage of triploidy. Triploid cohort D, which was the best cohort for survival, reached an average shell height of 92 mm and weighed 142 g in fall 2007. Growth advantages in oysters resulting from triploidy are common around the world (Nell 2002), and our results are similar to those observed by Hand et al. (1998a) in *S. commercialis,* Hand et al. (2004) in *S. glomerata*, and Goulletquer et al. (1996) and Garnier-Géré et al. (2002) in *C. gigas.* Previous studies in *C. virginica* have compared mostly growth between diploids and chemically induced triploids. Different responses have been reported, however, with either triploids having greater growth than diploids (Stanley et al. 1984, Barber & Mann 1991, Matthiessen & Davis 1992) or diploids and triploids having similar growth, depending on whether triploids were created through meiosis I or meiosis II inhibition (Stanley et al. 1984). To our knowledge, only 1 study has compared genetic triploid *C. virginica,* produced by mating diploids and tetraploids, with diploid *C. virginica.* This study reported that triploid and diploid Eastern oysters attained the same size at 1.2 y and 1.5 y, respectively (Harding 2007), which supports our results. The results of Harding (2007) could be explained by the lack of measurements on smaller oysters combined with large differences in initial size between ploidies, taking into account that the most rapid growth is observed during the first 2 y of life (Kraeuter et al. 2007). Interestingly, cohorts used in her study were the same as those used in ours (derived from the same spawns), with the diploid DBY Eastern oyster referred to here as cohort A and the triploid Eastern oyster referred to here as cohort D. Triploids of cohort D in our study had much greater growth than diploids of cohort A at all sites (Fig. 5), and this was also confirmed by pooling the 3 cohorts within ploidy. Thus, our study is the first to report a greater growth rate for shell height in genetic triploid *C. virginica* than diploid *C. virginica*, which was

gather further data in 2006 and in 2007 to determine more completely the ploidy effect on disease parameters. Nevertheless, some trends could be interpreted from the results in 2006, in relation to the mortality observed the same year. No significant difference was observed between ploidy for all disease variables (Tables 4 and 5), but there was a significant difference for the cohort effect. Perhaps this indicates that the broodstock used as parents, and so selective breeding to improve survival in *C. virginica,* had a greater impact than ploidy on disease. Regarding the particular pathogen involved, diploid cohort C suffered heavy mortality mostly as a result of *H. nelsoni* in the medium-salinity sites. This cohort was produced by crossing the DBLA line, which was a G_0 of DBY, with naive Louisiana broodstock (Table 1). Cohorts A and B, produced from established disease-resistant lines (DBY and DMO), had similar survival at both disease sites. Conversely, no abnormal disease

also confirmed through the whole-weight analyses at all sites and in both years.

Because of the superior performance in triploid oysters, most had reached market size (76 mm in Virginia) by the end of the experiment, whereas only half as many diploids had reached such a size. This agrees with results obtained from *C. virginica* tested in the York River (Barber & Mann 1991), and *C. virginica* at the Fisher Island Sound site investigated by Matthiessen and Davis (1992). Our results confirmed that triploid *C. virginica* take less time than diploids to attain market size, which was recently estimated to be 1.2 y in triploids and 1.5 y in diploids (Harding 2007). Interestingly, 97% of the remaining oysters of cohort E exceeded market size in the Yeocomico and Lynn-haven rivers in November 2006, when they were 15 mo old, and 80% of them had survived. One year later, survival was 20% less (60%). This loss during the second year would have been eliminated if the oysters had been harvested in winter 2006 instead of spring 2007.

Yield

Fast growth combined with low cumulative mortality leads to high yield. On average, among sites, the best triploid cohort (D) yielded 11.4 kg in November 2006 and 18.4 kg in October 2007, whereas the best yield for diploids was 4.4 kg and 7.1 kg, respectively, for cohort B. Furthermore, the lowest yield in triploids was 61% greater than the highest yield in diploids, indicating the superior commercial traits of triploid *C. virginica*. The high variation among cohorts within ploidy, however, and the strong interaction between cohorts and environment, also indicate that specific cohorts might be tailored to specific environments, although the range of salinities in our region of the Chesapeake Bay is greater than the typical range of salinities used for cultivation in other areas along the east coast of the United States.

CONCLUSIONS

Our study clearly demonstrates that triploid *C. virginica* were superior to diploids in growth (height, weight, percentage of market-size oysters) and yield, and to a lesser extent in survival, indicating a significant genetic improvement of the species.

Apparently triploidy, however, does not confer an advantage in terms of disease incidence. Nonetheless, survival was greater in the triploid groups. This finding could potentially uncover the mechanism for disease tolerance in this species. Superior survival in the presence of Dermo and MSX may be the result of the physiological advantages of triploidy derived from sterility, not disease resistance. On the other hand, there is an indication—based on the rank performance among triploids resulting from their genetic foundation from disease-resistant lines (or not)—that some advantages of breeding can also be obtained. Strong variation among cohorts and strong interactions between cohorts and environments, especially between low- and medium-salinity sites, suggest that specific cohorts should be used for each salinity regime. This tailoring of strains to environments has been a hallmark of the ABC breeding program, and it appears that it is warranted for triploids. Particularly intriguing was triploid cohort D, which had a consistently high performance at all sites throughout the duration of the experiment, with the largest yield (18.4 kg) and the greatest survival (80%) after 2.5 y, and reached an average shell height of 92 mm and weighed 142 g. Whether generalist crosses can be produced routinely for greater yields across environments awaits further comparisons using various tetraploid "lines." In general, though, triploid *C. virginica* should be considered to confer a significant added value for aquaculture in this area.

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