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# Effects of ploidy and gear on the performance of cultured oysters, *Crassostrea virginica*: Survival, growth, shape, condition index and *Vibrio* abundances

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ABSTRACT

In 2011 at a commercial oyster farm in Grand Bay, Alabama, we conducted an experimental field test of ploidy (triploids and half-sibling diploids) and gear type: 1) LowPro bottom cages (Chesapeake Bay Oyster Company — CBOC), 2) adjustable long-line baskets (BST, Ltd.), 3) OysterGro floating cages (Ketcham Supply) and 4) floating bags (CBOC). Eastern oysters, *Crassostrea virginica*, were deployed in the four gear types from May 5 to October 11 (166 days), with replicate bags of each ploidy assigned to each gear type ($n \geq 3$). Survival, growth (both shell dimensions and weight measurements, adjusted to account for differences between the ploidies at the onset of the experiment), and oyster shape ('fan' & 'cup' ratios) were quantified at the conclusion of the experiment. Condition indices were determined in both August and October, while abundances of the bacteria, *Vibrio vulnificus* and *V. parahaemolyticus*, were quantified in August and September. Survival was equivalent between ploidies, but differed significantly among gear types with poor survival in the bottom cages (affected by the oyster drill, *Stramonita haemastoma*). In terms of growth, triploids grew better than diploids for almost all metrics. Among gear types, growth was poorest in the bottom cages. For dry tissue weight, there was a significant ploidy by gear interaction; within floating bags, there was no difference between triploids and diploids, but triploids had higher dry tissue weight than the diploids in any other gear type. No differences were observed in fan ratios among treatments, but triploids had significantly higher cup ratios than diploids. For condition index, unexpectedly there was no clear pattern explained by ploidy in the August sample. In October, however, triploid condition index exceeded the diploid condition index. Finally, there was no significant effect of gear or ploidy on the abundances of the two *Vibrio* spp. assessed, but there was a tendency for these abundances to be lower in triploids than diploids. This study adds to the growing body of published evidence of the benefits of genetic triploidy to the Eastern oyster aquaculture industry. We conclude that oyster farmers could expect to benefit from raising triploid oysters, but that the magnitude of these benefits will depend on the type of gear selected.



## 1. Introduction

With the growth of shellfish aquaculture in the United States (USDA, 2006), there has been interest in improving production. This has led to a great deal of research, much of which has emphasized the significance of proper site selection (e.g., Cho et al., 2012; Radiarta et al., 2008; Silva et al., 2011). Practically, though, most current shellfish farmers are not in a position to change sites. For these farmers, tools and techniques that can improve production at any given site are especially valuable.

Genetic improvements (e.g., selection for disease resistance, polyploidy) have been targeted as one means of improving production. In oyster aquaculture, triploids have been found to grow faster than diploid counterparts for both the Pacific oyster, *Crassostrea gigas*, and the Sydney rock oyster, *Saccostrea commercialis* (Allen and Downing, 1986; Garnier-Géré et al., 2002; Hand and Nell, 1999; Hand et al., 2004; Hawkins et al., 2000; Normand et al., 2008). This has led to widespread adoption of triploids by the Pacific oyster aquaculture industry and generated significant interest in triploidy by the Eastern oyster, *Crassostrea virginica*, aquaculture industry. For example, in Virginia, 91% of the oysters planted in 2010 were triploid (Murray and Hudson, 2012). In Massachusetts, over 69% of surveyed shellfish farmers indicated satisfaction with the triploid oysters that they had grown and over 50% were purchasing triploid oyster seed (Walton and Murphy, 2011).

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Despite this interest and widespread use, very few studies have documented experimentally the effect of ploidy on the performance of *C. virginica* that were produced by crossing tetraploids with diploids producing 'genetic triploids' (Dégremont et al., 2012). Harding (2007) found that genetic triploid oysters outgrew a disease-resistant diploid strain in Chesapeake Bay. Dégremont et al. (2012) tested multiple spawns of diploids and genetic triploids in the Virginia portion of Chesapeake Bay, and found that triploids outperformed diploids in all measures of growth. In Massachusetts in an experimental test of half-sibling diploids and triploids, despite significant spatial and temporal variation, triploids had the potential for faster growth, heavier shells and more tissue than the half-sibling diploids, with no instances where diploids performed better (Walton and Murphy, 2011).

In addition to ploidy, the importance of cultivation practices (e.g., Comeau et al., 2011) and culture gear (e.g., Mallet et al., 2013) is widely recognized in oyster aquaculture. Prior studies have focused on cultivation practices such as optimizing stocking densities (e.g. Comeau et al., 2011). In contrast, few formal studies have quantified differences in oyster growth and survival among gear types (but see Coddington-Ring, 2012; Mallet et al., 2013).

Here we test the potentially interactive effects of both ploidy and culture gear on the performance of cultured oysters, *C. virginica*, measured in terms of survival, growth, condition index and abundances of the bacteria, *Vibrio vulnificus* and *V. parahaemolyticus*. To the best of our knowledge, this study is the first experimental test of the performance of triploid oysters in different culture gears.

## 2. Materials and methods

### 2.1. Site description

The study was conducted at a commercial oyster farm in Sandy Bay, near Grand Bay, Alabama (Fig. 1), following the routine practices of this farm (described below). Off-bottom oyster farming is relatively new within the Gulf of Mexico, and oyster farmers are experimenting with different culture methods and gears (Walton et al., 2012). The site is near shore, with mean water depth at low tide varying from 0.75 to 1.25 m at the study location, with minimal tidal variation (~0.5 m). The area consists of firm mud bottom and typically experiences salinities of 15–25 PSU (practical salinity units).

### 2.2. Experimental design and set up

Oysters used in this experiment were spawned at the Auburn University Shellfish Laboratory (Dauphin Island, AL) in May 2010. Both diploids and triploids were spawned from common maternal broodstock. For diploids, two different Cedar Point broodstock lines (CP07B and CP07C) were spawned through thermal shock methods. Eggs from the CP07B line were fertilized with sperm from the CP07C line. For triploids, eggs from the CP07B line were strip spawned and fertilized with sperm from Louisiana Seagrant Program's Grand Isle Bivalve Hatchery tetraploid OBOY oyster line spawned through thermal shock methods. Nine days post fertilization, the larvae were sent to the Aquaculture Genetics and Breeding Technology Center at the Virginia institute of Marine Science for ploidy verification. Resulting "eyed" larvae were set on microcultch to produce single-set spat. Diploid and triploid spat were reared in identical upweller systems. Oysters were moved to the oyster farm location on Sept. 29, 2010 (~20 mm shell height) and held under identical conditions in Australian long-line baskets at the site.

At the onset of the experiment on May 5, 2011, all the oysters (~50–60 mm shell height) of each ploidy were pooled by ploidy. From each of these pooled groups, 50 oysters were haphazardly selected for initial measurements and frozen for subsequent processing. Using Mitutoyo IP67 ABS coolant proof calipers, each oyster's shell height, length and width was measured to the nearest 0.01 mm. Using these measurements, fan ratio (shell length/shell height) and cup ratio (shell width/shell height), were calculated. After measuring, oysters were weighed individually on a Metler Toledo AL204 balance to the nearest 0.001 g as a measure of whole wet weight. Oysters were then dissected and the tissues separated from the shells. Shells were allowed to air dry for 48 h (±2 h) and then weighed as a measure of dry shell weight. Tissues were dried in a Fisher Scientific Isotemp oven at 80 °C for 48 h (±2 h), and then weighed as a measure of dry tissue weight. Using Abbe and Albright's (2003) formula, condition index was calculated as: [(dry tissue weight) / (whole wet weight − dry shell weight)] * 100.

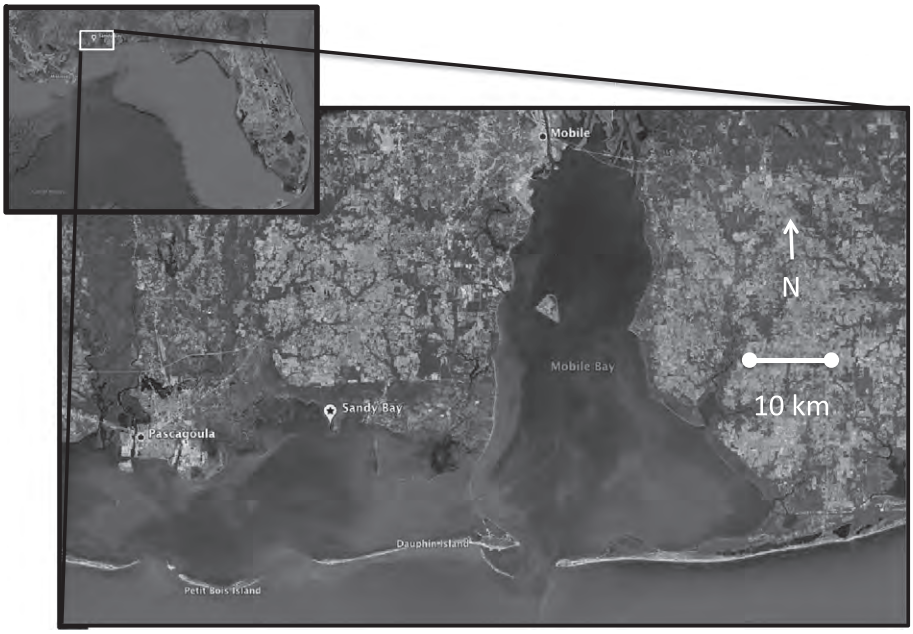


**Fig. 1.** Map (generated with GoogleEarth™) of the study site, a commercial oyster farm in Sandy Bay, near Grand Bay, AL (USA).

The data were compared using a one factor ANOVA (ploidy) to determine if there were significant differences between groups at the onset of the experiment so that these could be taken into account with subsequent measurements.

Diploid and triploid oysters were then selected from the remaining pooled oysters for each ploidy and stocked into one of four types of commercial oyster aquaculture gear (Fig. 2): LowPro™ bottom cages or LP hereafter (Chesapeake Bay Oyster Company), adjustable long-line baskets or ALS hereafter (BST, Ltd.), OysterGro™ floating cages or OG hereafter (Ketcham Supply) and floating bags or FB hereafter (Chesapeake Bay Oyster Company). Each gear type was stocked at 66% of the typical stocking density to avoid crowding. This equated to 150 oysters/bag (of a specific ploidy) for all gear types with the exception of the ALS baskets, which are smaller, which were stocked at 75 oysters/bag. All stocking densities are typical for commercial oyster culture operations within the Gulf of Mexico. Within any gear type, the ploidy treatments were randomly assigned for a total of eight treatments (2 ploidy groups × 4 gear types).

LP cages (n = 3, with 4 bags/cage or 12 bags, 6 for each ploidy) sat on the seafloor, with no handling throughout the experiment other than sample collection. ALS baskets (n = 6, 3 for each ploidy) were clipped to an adjustable long-line that was raised above the high tide line weekly for ~24 h air exposure (as a standard means of controlling fouling). Similarly, the OG cages (n = 2, with 6 bags/cage or 12 bags, 6 for each ploidy) were flipped up onto attached pontoon floats weekly for ~24 h air exposure. Finally, the FB (n = 6, 3 for each ploidy) were flipped weekly to expose the previously downward facing side of the bags to air.

### 2.3. Oyster survival, growth and condition index

Sampling was conducted during the peak of summer to sample condition index and in the fall to sample all response variables when harvest would typically occur (and oysters would typically have ≥75 mm shell height, the typical harvest size which is reached typically within 15–18 months after spawning). On August 2, 2011 (89 days post-deployment), five oysters were haphazardly selected from each bag and analyzed as described above for initial measurements to determine condition index. On October 18, 2011 (166 days post-deployment), all the bags of oysters were retrieved from the site, though one replicate of the triploid FB treatment was missing. Live and dead oysters were counted in each bag and percent survival was calculated. Again, a sample of oysters (n = 5) was collected from each bag, with each oyster measured and weighed as described above for initial measures. To account for any differences between the two ploidy groups at the onset of the experiment, the average values for each response variable within each ploidy were subtracted from each individual measurement to give a change from the onset. For example, the average initial shell height for triploids was subtracted from the individual final shell height for each triploid to yield change in shell height. This adjustment was performed for all response variables, except condition index, fan ratio, cup ratio and measures of *Vibrio* spp. abundance.

### 2.4. Abundance of V. vulnificus and V. parahaemolyticus

On August 2 and September 13, 2011, oysters (n = 12) were collected from each of the eight treatments to sample during high ambient temperatures when Vibrio bacteria were expected to be abundant. Each sample time served as a replicate (n = 2), and samples were processed identically. Oysters were maintained below 10 °C during transport and processed immediately upon arrival to the laboratory (Aquatic Microbiology Laboratory, Auburn University). Oysters were cleaned and shucked using aseptic techniques and homogenized individually with a tissue tearer in alkaline peptone water (APW) to obtain a 1:1 dilution (oysters weight (g):APW volume (ml)). Samples were incubated overnight for 18 h at 35 °C. After incubation, the Qualicon BAX system Vibrio

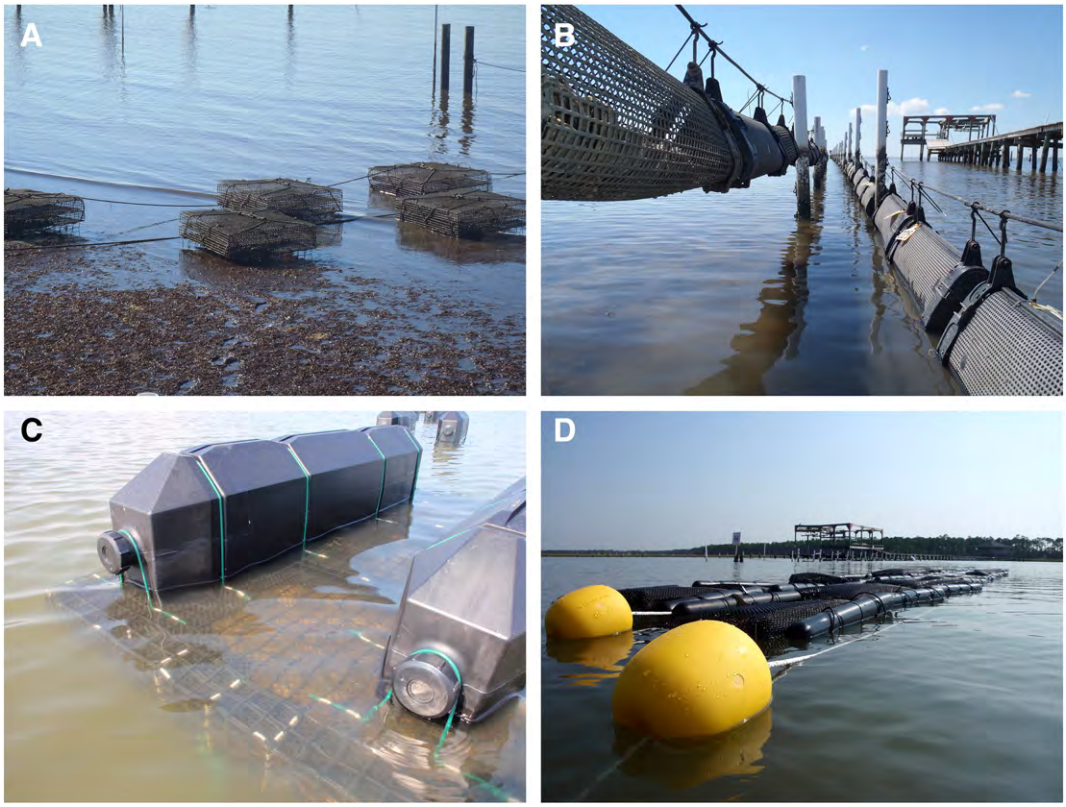


**Fig. 2.** Photographs of the four different gear types used in this study: A. LowPro™ bottom cages = LP (Chesapeake Bay Oyster Company); B. adjustable long-line baskets = ALS (BST, Ltd.); C. OysterGro™ floating cages = OG (Ketcham Supply); and, D. floating bags = FB (Chesapeake Bay Oyster Company).

**Table 1**
Differences between diploid and triploid oysters, *Crassostrea virginica*, at the onset of the field experiment (May 5, 2011). N = 50. Standard deviation is shown in parentheses. Condition index is calculated per Abbe and Albright (2003). Metrics marked with an asterisk (*) significantly differed ($p \leq 0.05$). Percent benefit, or gain, observed in triploids compared to diploids is shown only for significant metrics.

| Metric | Diploid | Triploid | % Benefit |
|---|---|---|---|
| Shell height (mm)* | 53.5 (±7.69) | 59.8 (±6.61) | 11.8 |
| Shell length (mm)* | 38.2 (±4.36) | 41.2 (±4.73) | 7.9 |
| Shell width (mm)* | 14.1 (±1.75) | 16.5 (±1.95) | 17.0 |
| Whole wet weight (g)* | 19.8 (±5.49) | 27.9 (±7.47) | 40.9 |
| Dry shell weight (g)* | 13.4 (±3.66) | 18.8 (±0.73) | 40.3 |
| Dry tissue weight (g)* | 0.66 (±0.215) | 1.12 (±0.403) | 69.7 |
| Condition index* | 10.3 (±1.48) | 12.1 (±1.52) | 17.5 |
| Fan (shell length/shell height) | 0.72 (±0.081) | 0.69 (±0.072) | – |
| Cup (shell width/shell height) | 0.27 (±0.037) | 0.28 (±0.031) | – |

kit was used to simultaneously detect the presence of *V. vulnificus* and *V. parahaemolyticus* by real time PCR. A standard curve with known concentrations of each pathogen was used to correlate the Ct (cycle threshold) values obtained with colony forming unit (CFU)/ml.

### 2.5. Statistical analyses

The study was designed as a two factor (2 ploidy groups × 4 gear types) ANOVA. Assumptions of normality and homogeneity were tested prior to further analysis. Computations were performed using SyStat 13 for Windows, with $p \leq 0.05$ considered significant. Where significant effects were found, a Tukey's post-hoc pairwise comparison was performed to compare all treatment means.

## 3. Results

### 3.1. Initial sizes and weights

At the initiation of the experiment, the triploid oysters were significantly larger than their diploid counterparts in all three shell dimensions (Table 1). Furthermore, triploids had significantly greater whole wet weight, dry shell weight, dry tissue weight and condition index (Table 1). The fan and cup ratios, however, did not significantly differ (Table 1).

### 3.2. Effect of ploidy and gear on oyster survival

There was a significant difference ($p < 0.001$) in survival among gear types over the duration of the experiment (Table 2), but no difference between ploidy or a significant interaction between ploidy and gear ($p \geq 0.74$). Among gears, survival in LP cages was lowest, while survival rates in the OG cages and FB were highest (Table 3).

### 3.3. Effect of ploidy and gear on oyster growth

With few exceptions (Tables 2 & 3), there was a significant effect of both ploidy and gear on most observed measures of oyster growth (measured as change from the average value for each ploidy at the onset of the experiment in May 2011). The only significant interaction between these factors was observed for dry tissue weight, as discussed below.

Triploids grew better than diploids for almost all metrics, though almost all the oysters had reached a typical market size of ≥75 mm shell height. Typical for shell metrics, the change in shell height for triploids was significantly greater than that for diploids (Table 2). Changes in whole wet weight (Table 2) and dry shell weight (Table 2) were also greater for triploids than diploids.

In terms of gear, oysters in LP cages consistently performed relatively poorly (Table 3), while oysters in FB and ALS baskets tended to perform best, followed closely by oysters in OG cages (Table 3). Typical for shell metrics, the change in shell height of oysters grown in FB was significantly greater than that of oysters grown in OG and LP cages, while oysters grown in ALS baskets did not differ from those grown in either FB or OG cages but were greater than those grown in LP cages (Table 3). Changes in whole wet weight (Table 3) and dry shell weight (Table 3) were significantly less in LP cages than any other gear type and changes in whole wet weight of oysters in OG cages were significantly lower than those of oysters grown in FB, with no significant differences among other gear types.

For dry tissue weight (Fig. 3), the results were more complex, but notably diploid oysters grown in LP cages had the significantly lowest change in dry tissue weight, while triploid oysters grown in ALS baskets had the significantly highest dry tissue weight. Additionally, within a gear type, the triploids consistently outperformed their diploid counterparts with the exception of floating bags (Fig. 3) where the difference between ploidy was not significant ($p = 0.14$). Finally, changes in dry tissue weight of triploid oysters grown in LP cages were statistically equivalent to diploids grown in any of the other three gear types (Fig. 3).

Condition index in August revealed a significant interaction between ploidy and gear (Fig. 4). Within any gear type, the only significant difference in condition index between diploids and triploids was observed in LP cages, where triploids had significantly higher condition index (Fig. 4). In October, there were significant effects of both ploidy and gear, but no interaction. During this sampling, triploid oysters had significantly higher condition index than diploid oysters (Table 2). Among gear type (Table 3), the average condition index of oysters grown in ALS baskets was significantly higher than oysters grown in FB and LP

**Table 2**
Effects of ploidy (diploid and triploid) on oyster, *Crassostrea virginica*, survival, growth and condition index during the 2011 field study. Condition index is calculated per Abbe and Albright (2003). Standard deviation is shown in parentheses. Metrics marked with an asterisk (*) significantly differed ($p \leq 0.05$). Percent benefit, or gain, observed in triploids compared to diploids is shown only for significant metrics.

| Metric | Diploid | Triploid | % Benefit |
|---|---|---|---|
| Survival (%) | 72.2 (±3.88) | 69.5 (±5.39) | – |
| Shell height Δ (mm)* | +23.0 (±2.46) | +29.5 (±1.72) | 28.3 |
| Shell length Δ (mm)* | +14.0 (±1.23) | +17.0 (±1.27) | 21.4 |
| Shell width Δ (mm)* | +13.1 (±0.71) | +16.0 (±0.55) | 22.1 |
| Whole wet weight Δ (g)* | +46.2 (±4.06) | +79.8 (±4.75) | 72.7 |
| Dry shell weight Δ (g)* | +32.0 (±2.79) | +59.2 (±3.73) | 85.0 |
| Dry tissue weight Δ (g) | *Significant interaction with gear (see text)* | | |
| August condition index | *Significant interaction with gear (see text)* | | |
| October condition index* | 7.5 (±0.33) | 9.7 (±0.40) | 29.3 |
| Fan [shell length/shell height] | 0.69 (±0.008) | 0.68 (±0.008) | – |
| Cup ([shell width/shell height]* | 0.36 (±0.005) | 0.38 (±0.005) | – |
| *Vibrio vulnificus* abundance log (CFU/g) | 4.5 (±0.47) | 3.4 (±0.36) | – |
| *Vibrio parahaemolyticus* abundance log (CFU/g) | 6.2 (±0.31) | 5.6 (±0.0.10) | – |

**Table 3**
Effects of gear (LowPro™ bottom cages = LP, ALS long-line baskets = ALS, OysterGro™ cages = OG, and floating bags = FB) on oyster, *Crassostrea virginica*, survival, growth and condition index during the 2011 field study. Condition index is calculated per Abbe and Albright (2003). Standard deviation is shown in parentheses. Metrics marked with an asterisk (*) significantly differed ($p \leq 0.05$).

| Metric | LP | ALS | OG | FB |
|---|---|---|---|---|
| Survival (%)* | 49.2 (±4.15)$^{c}$ | 71.0 (±1.69)$^{b}$ | 87.7 (±1.83)$^{a}$ | 82.8 (±1.50)$^{a,b}$ |
| Shell height Δ (mm)* | 17.2 (±1.73)$^{c}$ | 31.7 (±2.86)$^{a,b}$ | 27.2 (±1.65)$^{b}$ | 37.4 (±3.23)$^{a}$ |
| Shell length Δ (mm)* | 10.2 (±0.94)$^{c}$ | 17.3 (±1.59)$^{b}$ | 16.7 (±0.84)$^{b}$ | 22.8 (±1.57)$^{a}$ |
| Shell width Δ (mm)* | 11.7 (±0.73)$^{c}$ | 16.0 (±0.91)$^{a,b}$ | 15.3 (±0.61)$^{b}$ | 17.6 (±0.37)$^{a}$ |
| Whole wet weight Δ (g)* | 42.3 (±4.64)$^{c}$ | 77.3 (±10.68)$^{a,b}$ | 68.2 (±6.37)$^{b}$ | 79.8 (±8.22)$^{a}$ |
| Dry shell weight Δ (g)* | 29.7 (±3.53)$^{b}$ | 55.8 (±8.29)$^{a}$ | 51.0 (±5.15)$^{a}$ | 55.8 (±6.55)$^{a}$ |
| Dry tissue weight Δ (g) | *Significant interaction with ploidy* (*see text*) | | | |
| August condition index | *Significant interaction with ploidy* (*see text*) | | | |
| October condition index* | 7.3 (±0.29)$^{c}$ | 10.5 (±0.65)$^{a}$ | 9.3 (±0.52)$^{a,b}$ | 7.7 (±0.55)$^{b,c}$ |
| Fan [shell length/shell height] | 0.70 (±0.011) | 0.66 (±0.010) | 0.68 (±0.008) | 0.68 (±0.020) |
| Cup [shell width/shell height] | 0.37 (±0.007) | 0.36 (±0.009) | 0.37 (±0.005) | 0.36 (±0.011) |
| *Vibrio vulnificus* abundance log (CFU/g) | 3.5 (±0.75) | 4.5 (±0.64) | 4.1 (±0.43) | 3.8 (±0.83) |
| *Vibrio parahaemolyticus* abundance log (CFU/g) | 6.3 (±0.45) | 5.6 (±0.38) | 6.0 (±0.35) | 5.7 (±0.29) |

cages. Additionally, the average condition index of oysters grown in OG cages was greater than oysters grown in LP cages (with no significant difference from the other gear types).

### 3.4. Effect of ploidy and gear on oyster shape

There were no significant effects of ploidy and gear, or interaction between these factors, on oyster fan ratio (Tables 2 & 3). Average fan ratio values ranged from 0.66 to 0.70. For oyster cup ratio, there was a significant effect of ploidy (Table 2), but no effect of gear or interaction between these factors (Table 3). Triploid oysters were significantly more cupped than diploid oysters (Table 2), with a notable increase from the 0.27–0.28 cup ratio at the start of the experiment in May.

### 3.5. Effect of ploidy and gear on Vibrio abundance

The overall prevalence for *V. parahaemolyticus* and *V. vulnificus* in individual oysters was 100% and 81%, respectively. No clear association was observed between positive oysters and gear or ploidy. When data were analyzed quantitatively (colony forming units of the pathogen/g of oyster meat) and the two sampling times were averaged, there was no significant effect of ploidy or culture gear, nor interaction between these factors, on *V. vulnificus* and *V. parahaemolyticus* abundance (Tables 2 & 3). There was a tendency, however, for *V. vulnificus* and *V. parahaemolyticus* abundance to be lower in triploid oysters (Table 2).

## 4. Discussion

### 4.1. Oyster survival

Overall, survival was good during the experiment (70.9% ± 4.64). In our study, we found no effect of ploidy on survival, in agreement with the other studies with *Saccostrea glomerata* (Hand et al., 2004) and *C. gigas* (Dégremont et al., 2010). In contrast, others have found higher (e.g., Gagnaire et al., 2006) and lower (e.g., Cheney et al., 2000; Goulletquer et al., 1996) survival between triploid and diploid *C. gigas*. In the Eastern oyster, Matthiessen and Davis (1992) and Dégremont et al. (2012) suggest that triploids survived better in areas with disease pathogens. While we did not sample disease, the farm site has been recently documented as having a high body burden of Dermo, *Perkinsus marinus*, in nearby wild oysters (J. LaPeyre, unpub. data). Though all seeds were raised at this site for over 12 mo (including the nursery stage), ploidies did not differ in terms of survival.

There was a significant effect of gear on survival. Survival was poor in the LP bottom cages, which appeared to be largely due to predation by the Southern oyster drill, *Stramonita haemastoma*. This was evidenced by numerous drilled oyster shells in this gear. The intermediate survival in the ALS was not readily explained. Presumably, oysters in the ALS were subjected to exposure to air at frequencies identical to OG cages and FB, and for durations less than or equal to the floating cages (because ALS occasionally experienced shorter durations of exposure due to extreme high tides).

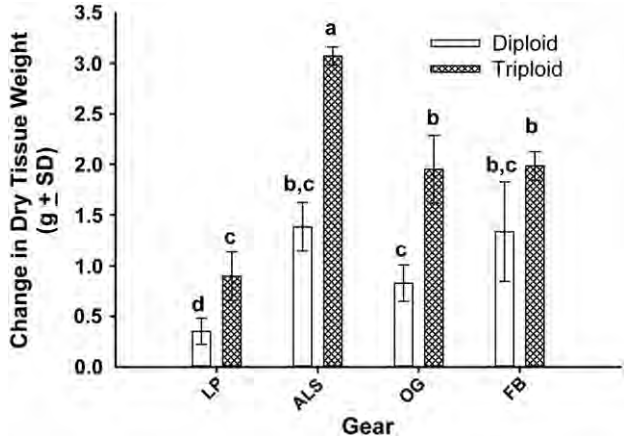


**Fig. 3.** Effect of interaction between ploidy and culture gear on oyster, *Crassostrea virginica*, change in dry tissue weight (g) at a commercial oyster farm in Grand Bay, AL (USA). Oysters were deployed for 166 days from May 5 to October 18, 2011. Error bars represent standard error of the mean. Significant differences among treatments are indicated by different letters. See Fig. 2 for explanation of gear types.

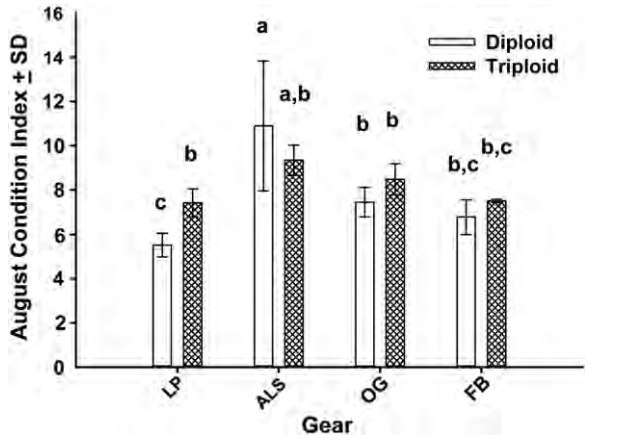


**Fig. 4.** Effect of interaction between ploidy and culture gear on oyster, *Crassostrea virginica*, condition index (per Abbe and Albright, 2003 methodology, measured on August 2, 2011 at a commercial oyster farm in Grand Bay, AL (USA)). Error bars represent standard error of the mean. Significant differences among treatments are indicated by different letters. See Fig. 2 for explanation of gear types.

### 4.2. Oyster growth

Despite a shared spawning date and similar, side-by-side nursery conditions, the triploid seed significantly out-performed half-sibling diploid seed during the nursery phase from spawning in May 2010 to the onset of the field experiment in May 2011 in all measures of growth (Table 1). We emphasize the magnitude of percent gain in weight metrics, as most prior work has focused on shell metrics. These differences suggest an advantage to genetic triploid Eastern oysters even during the first year of production. The mechanism of this advantage deserves further study as presumably the sterility of triploid oysters contributed minimally to this difference with the diploids not expected to be reproductive until perhaps ≥1 yr old (~May 2011).

To account for these differences at the onset of the experiment, in this study we chose to use change in shell and weight metrics, calculated as the difference between final individual measurements and average initial measurements for each ploidy (over a period of 166 d). Using this conservative approach to test for differences between ploidy, we emphasize the significance of the observed differences.

For almost all metrics of growth, differences could be explained by a single factor: ploidy *or* gear. For ploidy, triploids grew better than diploids in regard to shell metrics, whole wet weight and dry shell weight (Table 2). We emphasize the gains observed in measures of weight (Table 2); percent gains for weights were more than double those for shell metrics. Notably, many oyster farmers have noted that fast growth measured as changes in shell height often appears to be correlated with thinner shells (Walton, pers. obs.). Here, however, we document faster growing oysters (triploids) with heavier shells.

For gear, generally growth was poorest in the LP bottom cages, with some relatively minor differences among the remaining three gear types. For dry tissue weight, the effects were more complicated. Within all but the FB, triploids had significantly higher dry tissue weights than their diploid counterparts (with a similar pattern within FB). Importantly, though, the positive effect of triploidy was modified by gear; the poor performance of oysters in LP cages appeared to reduce the dry tissue weight of triploids grown in these cages, while triploids excelled in the ALS.

### 4.3. Oyster condition index

Unexpectedly, despite the expectation that triploids would have a higher summer condition index due to the lack of spawning, we found that within any gear type triploids only had a higher condition index than diploids in the LP cages during the August sampling. Conversely, in October when differences were not expected, triploids had a significantly higher condition index. This perhaps can be attributed to a fall spawning by the diploids, but does not explain the pattern observed in August. It was also interesting that the condition index of oysters in LP cages in October was significantly lower than that of oysters in other gear types, reflecting the pattern observed in growth. Oysters grown in the ALS and the OG cages had the highest condition indices at harvest.

### 4.4. Oyster shape

We found no effect of either treatment upon the fan ratio of the oyster (shell width to shell height, where smaller values are more elongate and values approaching one are more discoid). Overall, the fan ratio was considered to be good. For the cup ratio of the oysters (shell width to shell height, where smaller values are flatter and values approaching one are more deeply cupped), we found that triploids had a significantly higher cup ratio, which is a trait that has been deemed desirable to oyster consumers (Brake et al., 2003). We emphasize, however, that all the treatments had a cup ratio well above the 0.25 threshold value determined by Brake et al. (2003) to be 'good'.

### 4.5. Abundances of *V. vulnificus* and *V. parahaemolyticus*

As expected for this harvest area in summer, abundance of pathogenic vibrios in oyster was high with *V. parahaemolyticus* present in all oysters tested and *V. vulnificus* in more than 80% of samples. Intriguingly, there was a tendency for *V. vulnificus* and *V. parahaemolyticus* abundances to be lower in triploids than in diploids. The variation induced by the low level of replication prevents any clear conclusion but the consistent pattern observed suggests that this potential should be investigated further. While we recognize that the pattern does not suggest that triploids could ever be considered free of risk, we note that any factor that can reduce the risk of illness by these bacteria could be included in a larger risk calculation.

In contrast to the potential role of ploidy, gear type did not appear to affect the abundances of these two bacteria. This is notable due to the concerns raised about the effect of culture methods (which are related to gear type) on human illness.

## 5. Conclusions

In conclusion, this study adds to the growing body of evidence of the benefits of genetic triploidy to the Eastern oyster aquaculture industry. Despite significant effects of gear, there were few interactions observed between ploidy and gear. This suggests that oyster farmers could expect to benefit from raising triploid oysters, but that the magnitude of these benefits will depend on the type of gear selected.

## References


Abbe, G.R., Albright, B.W., 2003. An improvement to the determination of meat condition index for the eastern oysters, *Crassostrea virginica* (Gmelin, 1791). Journal of Shellfish Research 22, 747–752.

Allen Jr., S.K., Downing, S.L., 1986. Performance of triploid Pacific oysters, *Crassostrea gigas* (Thurnberg): I. Survival, growth, glycogen content, and sexual maturation in yearlings. Journal of Experimental Marine Biology and Ecology 102, 197–208.

Brake, J., Evans, F., Langdon, C., 2003. Is beauty in the eye of the beholder? Development of a simple method to describe desirable shell shape for the Pacific oyster industry. Journal of Shellfish Research 22, 767–771.

Cheney, D.P., MacDonald, B.F., Elston, R.A., 2000. Summer mortality of Pacific oysters, *Crassostrea gigas* (Thunberg): initial findings on multiple environmental stressors in Puget Sound, Washington, 1998. Journal of Shellfish Research 19, 353–359.

Cho, Y., Lee, W.-C., Hong, S., Kim, H.-C., Kim, J.B., 2012. GIS-based suitable site selection using habitat suitability index for oyster farms in Geoje-Hansan Bay, Korea. Ocean and Coastal Management 56, 10–16.

Coddington-Ring, C., 2012. Evaluation of a mechanical grader for the improvement of the aquaculture production of the eastern oyster, *Crassostrea virginica*, in the Northern Gulf of Mexico. Auburn University, Auburn, AL (Masters Thesis, 221 pp.).

Comeau, L.A., Arsenault, G., Davidson, J., 2011. Off bottom oyster (*Crassostrea virginica* Gmelin) culture in Prince Edward Island: an evaluation of seed sources and stocking density. Canadian Technical Report of Fisheries and Aquatic Sciences 2976 (viii + 13 pp.).

Dégremont, L., Soletchnik, P., Boudry, P., 2010. Summer mortality of selected juvenile Pacific oyster *Crassostrea gigas* under laboratory conditions and in comparison with field performance. Journal of Shellfish Research 29, 847–856.

Dégremont, L., Garcia, C., Frank-Lawale, A., Allen Jr., S.K., 2012. Triploid oysters in Chesapeake Bay: comparison of diploid and triploid *Crassostrea virginica*. Journal of Shellfish Research 31, 21–31.

Gagnaire, B., Soletchnik, P., Madec, P., Geairon, P., Le Moine, O., Renault, T., 2006. Diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg), reared at two heights above sediment in Marennes-Oleron Basin, France: difference in mortality, sexual maturation and hemocyte parameters. Aquaculture 254, 606–616.

Garnier-Géré, P.H., Naciri-Graven, Y., Bougrier, S., Magoulas, A., Héral, M., Kotoulas, G., Hawkins, A., Gérard, A., 2002. Influences of triploidy, parentage and genetic diversity on growth of the Pacific oyster *Crassostrea gigas* reared in contrasting natural environments. Molecular Ecology 11, 1499–1514.

Goulletquer, P., Joly, J.P., Gerard, A., Le Gagneur, E., Moriceau, J., Peignon, J.M., Heurtebise, S., Phelipot, P., 1996. Performance of triploid Pacific oysters *Crassostrea gigas* (Thunberg) reared in high carrying capacity ecosystem: survival, growth and proximate biochemical composition. Haliotis 25, 1–12.

Hand, R.E., Nell, J.A., 1999. Studies on triploid oysters in Australia. XII. Gonad discolouration and meat condition of diploid and triploid Sydney rock oysters (*Saccostrea commercialis*) in five estuaries in New South Wales, Australia. Aquaculture 171, 181–194.

Hand, R.E., Nell, J.A., Thompson, P.A., 2004. Studies on triploid oysters in Australia. XIII. Performance of diploid and triploid Sydney rock oyster, *Saccostrea glomerata* (Gould, 1850), progeny from a third generation breeding line. Aquaculture 233, 1–4.

Harding, J.M., 2007. Comparison of growth rates between diploid DEBY eastern oysters (*Crassostrea virginica*, Gmelin 1791), triploid eastern oysters, and triploid suminoe oysters (*C. ariakensis*, Fugita 1913). Journal of Shellfish Research 26, 961–972.

Hawkins, A.J.S., Magoulas, A., Héral, M., Bougrier, S., Naciri-Graven, Y., Day, A.J., Kotoulas, G., 2000. Separate effects of triploidy, parentage and genomic diversity upon feeding behaviour, metabolic efficiency and net energy balance in the Pacific oyster *Crassostrea gigas*. Genetical Research 76, 273–284.

Mallet, A., Carver, C.E., Doiron, S., Thériault, M.-H., 2013. Growth performance of eastern oysters *Crassostrea virginica* in Atlantic Canada: effect of the culture gear. Aquaculture 396-399, 1–7.

Matthiessen, G.C., Davis, J.P., 1992. Observations on growth rate and resistance to MSX (*Haplosporidium nelsoni*) among diploid and triploid eastern oysters (*Crassostrea virginica* (Gmelin, 1797)) in New England. Journal of Shellfish Research 11, 449–454.

Murray, T.J., Hudson, K., 2012. Virginia shellfish aquaculture situation and outlook report: results of 2011 Virginia shellfish aquaculture crop reporting survey. Virginia Sea Grant, VSG-12-07, VIMS Marine Resource Report No. 2012-04, Gloucester, VA (20 pp.).

Normand, J., Le Pennec, M., Boudry, P., 2008. Comparative histological study of gametogenesis in diploid and triploid Pacific oysters (*Crassostrea gigas*) reared in an estuarine farming site in France during the 2003 heatwave. Aquaculture 282, 124–129.

Radiarta, I.N., Saitoh, S.I., Miyazono, A., 2008. GIS-based multi-criteria evaluation models for identifying suitable sites for Japanese scallop (*Mizuhopecten yessoensis*) aquaculture in Funka Bay, southwestern Hokkaido, Japan. Aquaculture 284, 127–135.

Silva, C., Ferreira, J.G., Bricker, S.B., DelValls, T.A., Martin-Diaz, M.L., Yáñez, E., 2011. Site selection for shellfish aquaculture by means of GIS and farm-scale models with an emphasis on data-poor environments. Aquaculture 318, 444–457.

US Department of Agriculture (USDA), 2006. Census of Aquaculture (2005). National Agricultural Statistics Service, Washington, DC (116 pp.).

Walton, W.C., Murphy, D.C., 2011. Development of an improved oyster for Massachusetts shellfish farmers: field test of seedless oysters. Final Report to Massachusetts. Department of Agricultural Resources, Boston, MA (45 pp.).

Walton, W.C., Davis, J.E., Chaplin, G.I., Rikard, F.S., Hanson, T.R., Waters, P.J., Swann, D.L., 2012. Off-bottom oyster farming. Timely Information: Agriculture & Natural Resources. Alabama Cooperative Extension System (8 pp.).