

ABSTRACT

Title of Thesis: THE EFFECT OF AQUACULTURE GEAR AND TIDAL ZONE ON THE GROWTH AND SHAPE OF THE OYSTER *CRASSOSTREA VIRGINICA* DURING A “FINISHING PERIOD” IN CHESAPEAKE BAY.

Laura Landis Thomas, Master of Science, 2016

Thesis directed by: Assistant Professor, Louis V. Plough, Marine Estuarine Environmental Science
Research Professor, Jeffrey C. Cornwell, Marine Estuarine Environmental Science

This study investigates how gear type and increased wave action as a result of tidal zone location, influenced the shape and weight of *Crassostrea virginica* during a “finishing” period in Chesapeake Bay. For these experiments, oysters were deployed in three different gear treatments in the intertidal zone: bottom cages, OysterGro™ floats, and rack and bag, and a bottom cage deployed in the subtidal zone as a control treatment. Shell length, width, height, total weight and wet meat weight were measured each month from August to December 2015 and an index of shell shape relative to an idealized 3-2-1 ratio of length, width, and height was calculated. OysterGro™ floats produced significant increases in total weight and wet meat weight. Also, OysterGro™ floats showed less deviation from the ideal 3-2-1 ratio, while only one other treatment (intertidal bottom cage) differed from the control (subtidal bottom cage).

**THE EFFECT OF AQUACULTURE GEAR AND TIDAL ZONE ON THE
GROWTH AND SHAPE OF THE OYSTER *CRASSOSTREA VIRGINICA*
DURING A “FINISHING PERIOD” IN CHESAPEAKE BAY.**

By

Laura Landis Thomas

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Master of Science
2016

Advisory Committee:

Assistant Professor, Louis V. Plough, Chair
Research Professor, Jeffrey C. Cornwell, Chair
Associate Professor, Elizabeth W. North
Professor, Standish K. Allen, Jr.

©Copyright by

Laura Landis Thomas

2016

Acknowledgements

I would like to thank my advisors, Dr. Louis Plough and Dr. Jeffrey Cornwell of University of Maryland, Horn Point Laboratory, for their support, expertise, and their willingness to guide me through extraordinary circumstances; without them this would not be possible. I would like to thank the members of my committee for their helpful reviews, suggestions and advice: Dr. Standish K. Allen Jr., Virginia Institute of Marine Science, and Dr. Elizabeth W. North, University of Maryland Horn Point Laboratory. I would like to thank the following organizations and individuals for sharing datasets and materials: Dr. Larry Sanford, University of Maryland Horn Point Lab; Debbie Hinkle, University of Maryland Horn Point Lab; and Nate Geyerhahn, Virginia Institute of Marine Science. I would also like to thank UMCES-HPL for granting me a Graduate Assistantship, and the Izaak Walton League of America: Maryland Mid-Shore Chapter, for their generous scholarship. Additionally, I would like to thank Hoopers Island Oyster Aquaculture Company and True Chesapeake Oyster Co. for sponsoring this study and sharing their invaluable industry knowledge throughout this process. Finally, thank you to my family for supporting my education and allowing me to pursue my goals and love for marine science.

Table of Contents

List of Tables	iv
List of Figures.....	v
Introduction	1
Materials and methods.....	8
Gear types	8
Oyster cohort sources	9
Deployment of gear treatments	10
Sampling procedures	12
Shell shape index analysis	13
Statistical analyses.....	14
Results	15
Length.....	15
Total weight.....	15
Wet meat weight.....	16
3-2-1 Ratio index.....	16
Mortality.....	17
Discussion.....	19
Growth.....	20
3-2-1 Ratio index.....	22
Implications for aquaculture in Maryland	24
Experimental Design/Future Work.....	26
Conclusions	28
References	45

List of Tables

Table 1. Mean initial values for length, width, height (mm), total weight (g), and 3-2-1 ratio of oysters deployed into gear treatments at commencement of study.30

Table 2. Mean final values for length, width, height (mm), total weight (g) and 3-2-1 ratio of oysters sampled from gear treatments at the end of study.30

Table 3. Mean tidal heights at low and high tide levels for sites where gear treatments were deployed. OysterGro™ and subtidal cage were deployed at the far site, rack and bag at the middle site, and intertidal cage at the near site.....31

Table 4. Mean depth below surface (cm) of gear treatments and control deployed at a low tide level.....31

Table 5. Example 3-2-1 deviation calculations on an excel spreadsheet using an adapted Chi-squared test.....32

Table 6. ANOVA and Tukey’s post-hoc results for differences in growth and shape parameters across treatments at the end of the experiment.33

List of Figures

- Figure 1. Schematic illustrating the gear types and details of oyster deployment of across four gear treatments. Three treatments were deployed in the intertidal; bottom cages, rack and bag system, and OysterGro™ floats and one treatment (bottom cage) was deployed in the subtidal as a control. ...34
- Figure 2. OysterGro™ large float system contains six compartments, each of which can hold one Intermas™ Oyster bag (Figure 5). Image source: Oystergro.com35
- Figure 3. Custom manufactured bottom cage with stilts. Numbers indicate sections within top and bottom layers of cage; each section can hold 300 market-sized (~80 mm) oysters.....36
- Figure 4. Location in Chesapeake Bay of Farm A, True Chesapeake Oyster Co., Ridge, MD (red) and Farm B, Hoopers Island Oyster Aquaculture Company, Fishing Creek, MD (blue), where oyster sample populations were obtained from separate shores. Image source: chesapeakebay.net.....37
- Figure 5. Custom manufactured grading tool used to grade oysters to at least 63.5 mm before deployment, to ensure uniformity of initial samples.....38
- Figure 6. Picture of study site from beach at Fishing Creek MD, with farm A treatments deployed center left and farm B treatments deployed center right in the intertidal zone (mean tidal height of 0.89 meters). From the beach, the first set of buoys indicate the intertidal bottom cage, the second set of buoys indicate the rack and bag system, the black floating pontoons indicate the OysterGro™ floats, and finally, a third set of buoys parallel with channel marker indicate the subtidal bottom cages (control).....39
- Figure 7. Intermas™ Oyster bags with 12 mm diamond mesh, initially stocked with 150 73.1 mm oysters.....40
- Figure 8. Box and whisker plots of the final parameter measurements across treatments. For each parameter/treatment combination, the dark bars within boxes represent the median of the data, the boxes on either side of the median represent the lower and upper 25% quartiles, the upper and lower bars extending from the boxes indicate the minimum and maximum values in the data, and the open circles indicate the outliers.....41

Figure 9.	Box plot of 3-2-1 ratio deviation values in August (initial) and December (final) samples across gear treatments. The darker shade bars (or left-most bars of a pair) represent initial values, while the lighter shade bars indicate final values. Error bars indicate standard error of mean (SEM). Significant differences between initial and final values are indicated by p-values.....	42
Figure 10.	Histogram of the initial and final 3-2-1 ratio values observed in the OysterGro™ gear treatment, showing a shift in distribution of the final values towards zero, or lower deviation.	43
Figure 11.	Mean total percent mortality of oysters within each farm replicate, across gear treatments. Farm A (True Chesapeake) displays a trend of approximately 9.83% mean mortality across gear treatments. Farm B (Hoopers Island Oyster Aquaculture Company), displays a trend of approximately 4.45% mean mortality across gear treatments.....	44

Introduction

The eastern oyster, *Crassostrea virginica*, is an autogenic ecosystem engineer that is critically important to estuarine ecosystems such as the Chesapeake Bay (Newell 2004), providing habitat for both epibenthic fishes and invertebrates (e.g. Coen et al., 1999, Peterson et al., 2003, Grabowski et al., 2005, Fulford et al., 2010), increasing water quality through filtration (Coen et al., 2007), aiding chemical processes such as nutrient cycling (e.g. Dames and Libbes 1993; Souchu et al., 2001; Porter et al., 2004, Dumbauld et al., 2009, Kellogg et al., 2013), and stabilizing benthic and intertidal habitats (Grabowski and Peterson 2007). In Chesapeake Bay, *C. virginica* is a keystone species (Mann and Powell 2007) and the health of the oyster populations is critical to the overall health of the Bay and the other fisheries it sustains.

The *C. virginica* fishery in Chesapeake Bay was the largest in the world in the late 1800's (Kennedy and Briesch 1983), but populations have since declined ~99% in Chesapeake Bay (Wilberg et al., 2013) and oyster populations are functionally extinct in many other ecosystems (Beck et al., 2011). The decline in oyster populations is a major cause of concern because of the commercial value of its harvests and the ecosystem services they provide many other species in Chesapeake Bay (e.g. Jackson et al., 2001, Kemp et al., 2005, Lotze et al., 2006, Coen et al., 2007, Grabowski and Peterson 2007). The role *C. virginica* plays in the local economy as a prominent fisheries resource has even been defined as an ecosystem service by Grabowski and Peterson (2007).

Aquaculture provides a conduit to maintain the oyster resource in Chesapeake Bay, while relieving harvest pressure from wild stocks and reducing harmful harvesting practices that are detrimental to reef structures, and in turn the bay ecosystem as a whole.

In the state of Virginia, aquaculture has effectively filled the gap between the demand of the oyster market and the low populations of oysters in Chesapeake Bay (Hudson and Murray 2016), but in Maryland, a number of oppositional forces exist that have hindered the expansion of aquaculture (Wheeler 2015). One of the primary issues is related to leasing regulations and permitting delays, which have presented major hurdles for individuals looking to get into the industry. Despite recent attempts to streamline regulatory requirements and permitting, new businesses applying for a water column or bottom lease often face at least six months and potentially up to multiple years of waiting for government approval from agencies such as the Maryland Department of Natural Resources and the Army Corps of Engineers (Wheeler 2015). Coinciding with governmental delay, NIMBY (“Not In My Back Yard”) opposition is a consistent approval obstacle, as many waterfront property owners oppose the presence of aquaculture gear around their property. Finally, the commercial waterman, though a minority numerically, often provide vociferous opposition to legislation that would expand aquaculture, and have historically had disproportionate political influence over decisions regarding oyster aquaculture (Kennedy and Breisch, 1983). These factors, combined with a strong cultural attachment to the public fishery, have resulted in a relatively slower response and acceptance of aquaculture in Maryland, and consequently, the growth of the industry has been much slower. Despite the slower pace of growth of aquaculture in Maryland, it is clear that current fishing practices are not sustainable (Kennedy and Breisch, 1983, Rothschild et al. 1996, Willberg et al. 2011). Aquaculture represents an important alternative to meet the market demand for oysters and data and studies of gear and regionally specific grow-out methods are needed to accelerate growth

of the industry in Maryland.

Different methods of aquaculture grow-out for the *Crassostrea*, or cupped, genus of oyster are utilized around the world, all of which are influenced by local conditions (salinity, temperature, tidal flux, etc.). Oyster aquaculture gear used by a particular farm is usually a direct reflection of the physical conditions that are encountered at that site (Don Webster, Commercial Aquaculture Regional Specialist, Maryland SeaGrant Extension, personal communication, Paynter et al., 1992). Historically, oysters have been grown on-bottom in both subtidal and intertidal zones, but many industries around the world have implemented off-bottom culture (Chew 1988). While on-bottom culture still remains prevalent in some oyster industries, off-bottom and floating culture in intertidal zones is becoming increasingly popular worldwide, with gear types such as racks, floating cages or bags, and long-line culture being commonly utilized (e.g. Buestel et al., 2009; Nell 1999; Maguire and Nell 2007; Dumbald et al., 2009; Lavoie 2005; Chew 1988; Mallet et al., 2009; 2013; Comeau 2013). Rack-type culture uses galvanized frames to suspend oysters just off bottom in mesh bags, allowing oysters to feed while protecting them from bottom predators such as crabs. This method also allows growers to cultivate in areas that may otherwise have unfit bottom (e.g. Conte et al., 1994). Next, floating cages/bags avoid the bottom all together and utilize wave action to increase access to food and movement, which aids in growth (Conte et al., 1994). One example that originated in Canada is the OysterGro™ float, in which cages are attached to floats that bob at the surface, keeping the cages hanging just below the surface of the water 24 hours per day, facilitating very rapid growth (Archer and Murphy 2014; Comeau 2013; Mallet et al., 2013). Finally, longline culture, which has recently become popular on the west

coast of the United States and both Canadian coasts, suspends oysters in individual plastic mesh cylinders hanging from a single wire with two simple, detachable clips, and is especially useful in areas with extreme tidal flow (e.g. Buestel et al., 2009; Nell 1999; Maguire and Nell 2007; Dumbald et al., 2009; Lavoie 2005; Chew 1988; Mallet et al., 2009; 2013; Comeau 2013).

Many regions with established aquaculture systems, such as Australia (Nell 1999), France (Buestel et al., 2009), Canada (Mallet et al., 2013; Comeau 2013), and the west coast of the United States (Dumbald et al., 2009; Lavoie 2005), grow oysters in intertidal locations. This practice is intended to utilize wave action to help shape the oyster shell and prevent fouling. Additionally, most systems include a “finishing process”, in which oysters are transplanted to an area for the final phase of grow-out to perfect the oyster for market. Notable or popular practices include the relocation of oysters into claires, or warm, algae-rich ponds for the final months of grow-out in France (Buestel et al., 2009), the “hardening” of oysters on the Pacific Coast of the U.S., where oysters are allowed daily periods of exposure to air in the intertidal zone (Helm 2005), and the relocation of oysters from a subtidal to a nutrient-rich intertidal zone, in order to increase oyster exposure to wave action to shape and fatten the oyster (e.g. in Coffin Bay, Australia; Achim Janke, TOPS Oyster, personal communication). Shell shape is a particularly important trait for the half-shell market, and thus is the focus of finishing processes in a number of systems. The deepness of the oyster half-shell cup and associated heavy meat (the two are often correlated), are considered highly desirable traits (e.g. Brake et al., 2003). Shell shape can be described quantitatively by the ratio of height to length (Brake et al., 2003) or even by the ratio of all three dimensions, length to

width to height, in which a 3-2-1 ratio is considered ideal (e.g. Ward et al 2005), Brake et al., 2003 documented the ideal dimensions of height/length of an oyster were 0.32, and width/length was 0.689, which translates to a 3-2-1 ratio. Though, this metric has not yet seen wide quantitative use in the industry.

Oysters subject to more movement and frequent disturbance tend to be more round and have deeper cups (Brake et al., 2003, Carriker 1996; Boulding and Hay 1993; Seed 1968). The movement of oysters during grow-out allows excess shell growth to be chipped off and regrown into a more rounded shape, resulting in an oyster growing more quickly in height than length (Brake et al., 2003), which is referred to as “pruning” (Brake et al., 2003). In addition to benefits for shell shape, recent empirical studies also find that intertidal or higher-energy environments can improve oyster growth rates (e.g. Mallet et al., 2013; Walton et al., 2013; Manley et al., 2009; Comeau 2013; Archer and Murphy 2014; Paynter et al., 1992), and floating gear specifically can improve overall condition index and performance (e.g. Mallet et al., 2013; Walton et al., 2013). Importantly, all of these studies except for Paynter et al. (1992) were carried out in regions outside of the Chesapeake Bay (e.g. the Gulf of Mexico, Canadian Atlantic, and U.S. Atlantic east coast), at locations with varying shoreline types, wave energy, tidal fluxes that could generally be described as more coastal ocean conditions (e.g. Goullion et al., 2010; Ramsey et al., 2005; Manley et al., 2009). Thus, the results of these studies may not be directly transferrable to oyster culture in Chesapeake Bay, which is a more sheltered estuary with an average tidal flux of about one meter maximum (Hagy and Kemp 2012).

Growers in the Chesapeake Bay region are still exploring how to produce a top-grade product for the half-shell market in the most efficient, sustainable, and cost-effective manner. The current standard for growing oysters in Chesapeake Bay, especially Maryland, is “on-bottom”, with 5,700 of 6,000 acres total of oyster leases in Maryland utilizing on-bottom culture, most of which is bottom cage (Don Webster, Commercial Aquaculture Regional Specialist, Maryland SeaGrant Extension, personal communication). On-bottom oyster aquaculture is probably not as effective in general and may be particularly inefficient in Chesapeake Bay due to the potential for heavy siltation (low tidal flux) and mortality from highly prevalent predators such as blue crabs and cow nose rays (Smith and Merriner 1985). However, there is limited empirical data on the relative performance of bottom culture compared with other gear types (e.g. floating cages), which could support recommendations for implementing alternative methods in the region. Paynter et al., (1992) evaluated the performance and economic viability of floating gear treatments in Chesapeake Bay, based on previous knowledge that oysters grown in floating cages had increased growth rates relative to bottom gear (Paynter and DiMichele 1990). The study found that lifting oysters above the sediment, even by a few inches, increased the growth rate by 50 to 100%, and made recommendations that a “finishing process” using such gear treatments should be explored in the region. Some growers in the region are starting to express interest in expanding their practices into off-bottom gear, as well as implementing a “finishing process” relevant to the area (Don Webster, Commercial Aquaculture Regional Specialist, Maryland SeaGrant Extension, personal communication); however, there is uncertainty about how these gear types would actually perform in the Chesapeake Bay

due to a lack of experiments and data, which has resulted in resistance to invest in such gear treatments. Until data on the effects of alternative gear type on oyster production in Chesapeake Bay are available, these gear types may not be implemented locally, potentially limiting the growth of the oyster aquaculture industry

The goal of this research was to examine how alternative gear types and a “finishing process” could improve oyster aquaculture in Chesapeake Bay, compared with traditional bottom cage culture. Specifically, this study sought to test the effect of off-bottom culture (OysterGro™ floats and rack and bag systems; Figure 1), on growth, shape, and weight of an oyster, as well as the effect of culture in an intertidal vs. subtidal zone in the final four months of grow-out. This study addresses the following specific hypotheses: (1) moving oysters from a subtidal bottom cage into an intertidal gear treatment (rack and bag system, OysterGro™ float) for finishing will improve the shape and weight of an oyster, (2) A floating gear type such as the OysterGro™ float will yield higher growth and more optimal shape characteristics than the other gear treatments and (3) deploying the traditional bottom gear in a location with more wave action (intertidal vs. subtidal) will improve growth and shape.

Materials and methods

Gear types

Experimental gear treatments were selected based on their use in the oyster aquaculture industry in Chesapeake Bay (either common or uncommon), and on the expectation that the gear types would be affected differently by wave action, which was predicted to have an effect on shape and weight, as shown in other oyster aquaculture studies conducted in this region and elsewhere (e.g. Paynter et al., 1992; Paynter and DiMichele 1990; Carriker 1996; Boulding and Hay 1993; Seed 1968; Brake et al., 2003). The three gear treatments were OysterGro™ floats, rack and bag systems, and bottom cages, which were deployed in an intertidal location. A control treatment of oysters deployed in subtidal bottom cages was chosen to mimic the grow-out conditions that oysters experienced before the beginning of the experiment, and which is the traditional gear type currently employed in many locations in Maryland (Figure 1). OysterGro™ floats (Figure 1, 2) were chosen because of their lack of prevalence in the local industry but common use elsewhere, such as the west coast of the United States and the Atlantic coast of Canada (Paynter et al., 1992; Mallet et al., 2009; 2013; Comeau 2013). Because they are a surface gear (i.e. they bob at the surface with wave action and tidal fluctuations), OysterGro™ floats facilitate much greater movement of oysters, which may produce a more rounded shape that is desirable for the half-shell market (Brake et al., 2003). Rack and bag systems (Figure 1) were also chosen as a gear type that is uncommon in the local industry (Don Webster, Commercial Aquaculture Regional Specialist, Maryland SeaGrant Extension, personal communication; Helm 2005) but has the potential to improve shape during finishing due to the positioning of oysters off

bottom (0.6 m), which provides greater exposure to wave action and tidal fluctuations (though perhaps not as much as OysterGro™ floats). Finally, bottom cages (intertidal; Figure 1, 3) were chosen because they are the most common gear type used in the local industry (the local ‘control’) and to provide a direct comparison of intertidal vs. subtidal conditions. Bottom cages are stationary and rest about 0.3 meters off of the bottom sediments, receiving little to no wave action in subtidal areas and limited movement of the oysters within the cages.

Oyster cohort sources

Oysters (n = 2,300) that were grown in subtidal bottom cages to a shell height of ~64 mm (just shy of market size) were obtained from two separate farms: True Chesapeake Oyster Co., located on the southern Western Shore of Maryland (henceforth referenced as Farm A; Figure 4) and Hoopers Island Oyster Aquaculture Company, located on the middle Eastern Shore of Maryland (henceforth referenced as Farm B; Figure 4). Oysters were collected from two separate farms on opposite shores of the Chesapeake Bay to reduce the possibility that initial shell shape characteristics or previous growing conditions at a given farm would influence the experimental results. Oysters used were a mix of triploid (three sets of chromosomes) strains (Guo et al., 1996), including Lola, a low salinity Louisiana strain known to be *Perkinsus marinus* (Dermo) and *Haplosporidium nelson* (MSX) resistant, and DEBY, a medium salinity, high performance Delaware Bay strain with some disease resistance (Ragone Calvo et al., 2003). These two strains are commonly used in aquaculture in the region, as they are known to thrive in Chesapeake Bay waters.

To ensure that oysters initially deployed into gear treatments were as uniform as possible, oysters were graded to a shell length of at least 63.5 mm by hand using a custom-made grading tool (Figure 5). At initial deployment, there were no significant differences in width, length, height, or 3-2-1 ratio deviation among treatments (one-way ANOVA, $p > 0.13$; Table 1). For weight, there was a small, but significant initial difference between two of the gear treatments (intertidal cages vs. OysterGro™ floats; 4.55 g mean difference, Tukey HSD $p = 0.011$; Table 1), but no significant differences were found between any other treatments (Tukey HSD $p > 0.13$). Note also that oysters deployed to the OysterGro™ had the lowest initial mean weight of any treatment (Table 1). Thus, any changes observed over the course of the experiment could be attributed to the conditions encountered in the different gear treatments.

Deployment of gear treatments

A set of two replicates per gear treatment were deployed for Farm A and Farm B (4 replicates; Figure 1, 6); however, replicates within farms were combined for measurements, resulting in a total of two *effective* replicates (Farm A combined, Farm B combined) for each experimental gear treatment, including the subtidal control.

OysterGro™ floats (Ketcham Supply), rack and bag systems, and bottom cages were deployed at a uniform mean tidal height of 0.89 meters (Table 3) in the intertidal zone. This site experiences tidal flux of around 1 meter with about 10% exposure time based on manual meter stick measurements and averaged online data from the NOAA National Data Buoy Center, Station BISM2 – 8571421 in Bishops Head, MD, 10 km South East from the project site (data averaged from January 2014- August 2015). Gear treatments were positioned at a very similar mean tidal height so that they were subject to similar

wave action (Table 3; Figure 1, 6). At low tide, the intertidal gear treatments were positioned at an average of 7.83 cm below the surface (Table 4), whereas the subtidal bottom cage (control) was positioned at an average of 25.4 cm (Table 4).

In August 2015, gear treatments utilizing bags (OysterGro™, rack and bag) were stocked with 150 oysters per bag, a density in accordance with the current oyster aquaculture industry standards (Walton et al., 2013; Mallet et al., 2013). InterMas™ Oyster bags with 23mm diamond mesh (Ketcham Supply) were used to hold oysters in the OysterGro™ and rack and bag gear treatments. Bags were open-ended and sealed using a PVC pipe and zip ties (Figure 7). A similar study (Walton et al., 2013) also stocked bags with 150 ~50-60 mm oysters, which they confirmed as a standard stocking density in commercial aquaculture operations within the Gulf of Mexico. Wire-mesh OysterGro™ floating cages contain six individual compartments, each of which can house one individual Vexar™ or InterMas™ bag (45.7 cm × 88.9 cm × 7.6 cm, Ketcham Supply; Figure 2, 7). Though OysterGro™ floats can house six bags per float; only two bags were stocked in the middle sections per float to reduce cost and labor. Four bags for each farm were stocked with 150 oysters per bag, for a total of 600 oysters per farm (total of 1200 oysters across both farms). For the rack and bag treatment, four bags total (2 bags per farm, 150 oysters per bag, 300 oysters per farm, 600 oysters total) were affixed to an 8x4x3' custom-made rebar rack using zip ties. Bottom cages possess two tiers, with each tier being divided into two sections (Figure 3). It is standard practice to stock each section with 300 market sized (76 mm/3 inches) oysters freely in the cage compartment, not within a bag (Johnny Shockley, Hoopers Island Oyster Aquaculture Company and Don Webster, MDDNR, personal communication). A related experiment found that there was

no significant difference in growth and condition index between top and bottom layers of cages (Archer and Murphy 2014), so for this experiment, only the top right section (section 2; Figure 3) of each cage was stocked with 350 oysters, in order to reduce costs and labor. Each bottom cage was deployed in the intertidal zone with the top right section outwards from the shore, to ensure equal wave action was experienced among replicates. The same stocking and deployment methods for intertidal bottom cage were used for the subtidal bottom cage control.

To summarize, there were 350 oysters in the bottom cage treatments, 300 oysters (2 bags) in each OysterGro™ float, and 150 oysters in each bag affixed to the rack. The reason for the different densities within treatments is that the optimal density in each gear type differs due to the size of the gear, and this study aimed at maintaining optimal densities for each gear treatment to accurately compare growth.

Sampling procedures

Oysters were sampled each month from August-December, and measurements of shell length, width, height (mm), and total weight (g) were recorded. Due to differences in the time it took to obtain oysters from the separate farms, Farm A was initially sampled and deployed on 8/5/2015, and Farm B was initially sampled and deployed on 8/20/2015. Farms were then sampled on these dates each month through December 2015, to ensure that they had equal time in the water. Additionally, the study sight was visited each day, and gear exposure and tidal height were recorded.

An initial sampling of 10% of the population (n=232 oysters, 58/gear treatment) was conducted in August 2015 for all traits. Oysters from each gear treatment were collected randomly and measured for length, width, and height in millimeters using a

Neiko 01407A Electronic Digital Caliper, with 0.01mm/0.0005 inch resolution and an accuracy of 0.02mm/0.001 inch. Total weight (g) was measured by placing the entire wet oyster onto an Etekcity 500g Digital Pocket Scale, accuracy (0.001g / 0.001 oz.) up to a weight range of 500g / 17.64 oz. Wet meat weight (g) was obtained by shucking the oyster immediately after the total weight (g) had been taken, and weighing the wet meat on the .001 gram scale. Oysters were sampled and measured for all traits (including wet meat weight) at the August (initial), and December (final) time points for 10% of the animals, and at the October (middle) time point, for 5% of the animals. Intervening time points (September and November) were sampled at 5% of the population (n=96, 24/gear treatment) for shape (length, width, height) and total weight. Mortality was monitored throughout the experiment, and dead animals were removed and recorded at each sampling time point. At the end of the experiment, all remaining live and dead animals were counted, and mortalities were added to the count from previous sampling points. There were only two mortality values recorded for each treatment, so no statistical tests were conducted.

Shell shape index analysis

To characterize 3-dimensional shell shape and changes to shape over time, the ratio, in inches, of the length to width to height of each oyster was calculated and compared to a ratio of 3-2-1, which roughly represents the optimal or most marketable shape sought by growers and chefs for oysters in the half-shell market (e.g. Brake et al., 2003; Ward et al. 2005; Stan Allen and Nate Geyerhahn, Virginia Institute of Marine Science, personal communication). A shell shape index was calculated as the deviation of an individual oyster's length:width:height ratio from the idealized 3-2-1 ratio using

goodness of fit Chi-square tests. A deviation value is produced for each oyster by summing the standardized squared difference between the observed and expected values, the expected values being the proportionate dimensions (3-2-1) for each oyster given their observed values (see Table 5 for example and formulas). A chi-square deviation value of zero for an oyster would indicate perfect fit (no deviation from expected 3-2-1), while a value > 5 would indicate a substantial deviation from the 3-2-1 ratio. To our knowledge, this is one of the first times this calculation has been used to quantitatively assess shell shape or proportionality in oyster aquaculture research, though it is increasingly recognized as a metric for characterizing shell shape.

Statistical analyses

Statistical analyses of differences in mean growth and shape among gear treatments were carried out with one-way analysis of variance (ANOVA) on the final time point (December 2015) and included the two farm replicates, Farm A (n= 1870) and Farm B (n= 2050), which were combined for analyses. Tukey's post-hoc tests were conducted to determine the significance among pairwise comparisons of gear treatments for each parameter, length, total weight, wet meat weight, and 3-2-1 ratio deviation. For the 3-2-1 deviation data, two-tailed t-tests were also used to examine changes in shape that occurred between initial and final samples. All statistical analyses were conducted in R version 3.2.3 (R Core Team 2015).

Results

Measurements taken over the course of the experiment revealed significant changes in total weight, wet meat weight, and shell ratio index across all gear treatments. The OysterGro™ gear treatment yielded the greatest increases in total weight (g) and wet meat weight (g) over time (Table 2). Additionally, the OysterGro™ gear treatment yielded the lowest (closest to ideal) 3-2-1 ratio deviation values. No significant differences in length (mm) were found across treatments, which was unexpected. Results for each growth or shape parameter are described in greater detail below.

Length

The four treatments produced similar increases in mean shell length over the course of the experiment. Final mean length was 90.9 mm (+/- 7.24 mm standard deviation, SD) for OysterGro™ (growth rate of 0.14 mm/day), 89.8 mm (+/- 9.37 mm SD) for rack and bag (growth rate of 0.13 mm./day), 87.9 mm (+/- 8.07 SD) for the intertidal bottom cage (growth rate of 0.12 mm/day), and 85.5 mm (+/- 8.85 SD) for the c subtidal bottom cage control (growth rate of 0.10 mm/day; Table 2, Figure 8). No statistically significant differences in mean length were found among gear treatments (one-way ANOVA, $p > 0.05$; Table 6). Note that each treatment reached market size (length) during the course of the experiment.

Total weight

Mean oyster weight (total) at the end of the experiment varied significantly among gear treatments (one-way ANOVA $p < 0.0001$; Table 6). The highest values were observed in the OysterGro™ treatment (mean of 103 g, +/- 20.6 g SD), followed by the

rack and bag treatment (87.36 g, +/- 11.8 g SD), the control of subtidal bottom cage (81.54 g, +/- 9.9 g SD), and the intertidal bottom cage treatment (80.97 g, +/- 7.7 g std dev; Table 2, Figure 8). Post-hoc testing indicated significant differences between the OysterGro™ and all treatments (Tukey HSD $p < 0.002$), and a significant difference between rack and bag and subtidal cage treatments (Tukey HSD $p < 0.02$), but not among other treatments (Tukey HSD $p > 0.13$). There was no significant difference between the intertidal bottom cage and subtidal bottom cage (control; Tukey HSD $p > 0.88$).

Wet meat weight

Mean oyster wet meat weight at the end of the experiment varied significantly among gear treatments (one-way ANOVA $p < 0.0001$; Table 6). The highest values were observed in the OysterGro™ treatment (mean of 19.3 g, +/- 3.6 g std dev), followed by the intertidal cage treatment (15.4 g, +/- 4.1 g SD), the rack and bag treatment (14.6 g, +/- 4.3 g SD), and the subtidal cage control (14.1 g, +/- 4.0 g SD; Table 2, Figure 8). Post-hoc testing indicated significant differences between the OysterGro™ and all treatments (Tukey HSD $p < 0.0001$; Table 6), but not among other treatments (Tukey HSD $p > 0.19$). There was a strong positive relationship between total and wet meat weight for all oysters sampled (Pearson correlation, $r = 0.98$. $n = 117$).

3-2-1 Ratio index

Gear type had a significant effect on the 3-2-1 ratio deviation values of oysters (one-way ANOVA, $p < 0.04$; Table 6). The OysterGro™ gear treatment yielded the lowest mean 3-2-1 ratio deviation value and the lowest standard deviation values (0.85, +/- 0.87 standard deviation, SD; Table 2) in the December (final) sample, with a significant decrease in the mean 3-2-1 ratio deviation value in the August (initial) sample

(two-tailed T-test $p < 0.02$) The intertidal cage gear treatment yielded the second lowest mean 3-2-1 ratio deviation value (0.94, +/- 1.14 SD; Table 2) in the December (final) sample, with a significant decrease from the August (initial) sample (two-tailed T-test $p < 0.04$; Figure 9). The rack and bag gear treatment yielded the highest mean 3-2-1 ratio deviation value in the December sample (1.56 +/- 3.53 SD; Table 2) and did not significantly change from the initial mean value (two-tailed T-test $p > 0.05$, Figure 9). The control treatment (subtidal cage) yielded the second highest mean 3-2-1 ratio deviation value in the December sample (1.13, +/- 1.14 SD; Table 2), and did not significantly change from mean initial value ($p > 0.05$; Figure 9,10). Post-hoc testing indicated a significant difference in final index values between OysterGro™ and rack and bag gear treatments (Tukey HSD $p < 0.04$; Table 6). Overall, variance in 3-2-1 ratio deviation was high among oysters within all treatments, but was particularly high for the rack and bag treatment (3.53 SD), due to a number of very “ugly” or non-ideal oysters produced, one of which produced a 3-2-1 ratio deviation of 30. Figure 10 illustrates the change (reduction) in distribution of 3-2-1 deviation values from the initial sample to the final sample for the OysterGro™ gear treatment, which yielded the most substantial improvement in 3-2-1 ratio.

Mortality

Mortality did not appear to vary much between treatments (Figure 11), though it did differ between replicates (Farm A vs. Farm B). Farm A had a mean mortality of 9.83% across gear treatments, while Farm B had a mortality of 4.45% across treatments (Figure 11). The elevated mortality in Farm A is possibly due to pre-experiment stress

experienced by the animals during transport to the grow-out site (4 hour drive by automobile). Farm B had essentially no transport time.

Discussion

The goal of this study was to examine the effect of aquaculture gear type and wave action (tidal exposure) on the growth and shape of oysters, and was guided by the hypothesis that gear types with the greatest potential for oyster movement (i.e. the OysterGro™ float) would produce the greatest gains in shape, size, and weight. Overall, OysterGro™ floats produced the greatest increase in growth (total weight and wet meat weight) over the course of the experiment, contrasting sharply with the lower growth and performance of bottom cages, the local industry standard (Table 2, Figure 8). In addition, using our novel 3-2-1 deviation metric, OysterGro™ floats produced the most commercially optimal shape (low mean 3-2-1 deviation), with lower variance compared to all other treatments. Interestingly, the rack and bag treatment, which deploys oysters higher in the water column than bottom cages and was expected to expose oysters to more water movement, only produced modest gains in growth compared with the bottom cage control and yielded significantly deeper, heavier oysters with hard shells with high 3-2-1 ratio deviations. Finally, no significant difference in performance (growth or shape) was observed between the subtidal vs. intertidal bottom cages, which suggests that finishing bottom cage grown oysters in the intertidal may not provide a significant benefit. These results can offer growers in the region much needed information on how different gear treatments and tidal conditions can affect grow-out and finishing of their oysters, and should increase the available information for growers to make informed decisions on gear investment and strategies to remedy problems within current practices and gear types. Below, these results are examined in greater detail and the possible causes of differences in growth and shape among gear types are discussed.

Growth

In this experiment, growth was measured both as the increase in shell length (mm) and weight (g), and the most significant differences in growth among gear treatments were in total and wet meat weight. For shell length (mm), there were subtle differences across treatments at the final sampling period, with OysterGro™ floats possessing the highest mean length and lowest variance and subtidal bottom cage possessing the lowest mean length (Figure 8), but differences were not statistically significant (Table 6). This finding contrasts with prior experimental work, in which significant differences in growth rates (length in mm/day) were found between floating cage gear and gear treatments located lower in the water column or on-bottom (e.g. Archer and Murphy 2014; Comeau 2013; Walton et al., 2013; Paynter and DiMichele 1990). However, a major difference between the current study and these previous grow-out studies is in the duration of the experiment. The previous studies mentioned above tracked oyster growth over many months to years, whereas the current study was only carried out over a 5-month period. Thus, the shorter duration of our study may not have provided adequate time for growth (length) differences to appear. In contrast, another study of gear treatments in the intertidal (Manley et al., 2009) found that the effect of wave action and position in the water column actually had little to no significant effect on oyster growth rate, which concurs with the results of the current study. However, the study by Manley et al., (2009) was conducted during months (October-April) with seasonally low water temperatures that typically inhibit growth in oysters, whereas the current experiment (and others) were carried out at a time of year (August-December) where oysters demonstrated significant and continuous growth. Clearly, duration of the experiment and grow-out season (water

temperature) could impact the observed changes in length. Alternatively, the lack of length difference observed may also reflect trade-offs between better shape (cup) characteristics and length in the higher-movement gear. For example, because oysters were likely being heavily tumbled in the OysterGro™ floats (as intended), leading shell growth may have been chipped more regularly, reducing an increase in shell length while promoting an increase in shell height, and therefore overall weight.

While length did not significantly vary across gear types, weight did, likely due to the oysters rounding, deepening, and hardening instead of increasing in shell length alone. Previous studies have found differences in total weight and meat weight by reporting changes in condition index (actual values divided by expected values x 100, way to measure and compare overall health or performance of an organism) among gear types (Archer and Murphy 2014; Manley et al., 2009; Mallet et al., 2013; Walton et al., 2013). Archer and Murphy (2014) reported a significant increase in condition index for floating cages compared to bottom cages, which agrees with the results from this study. Previous studies have suggested that the benefits of growing oysters higher in the water column likely include increased access to plankton, a greater distance from benthic predators, and warmer water temperatures (Archer and Murphy 2014). The increase in growth and meat weight for the OysterGro™ treatment in the current experiment may have been due to these factors, while the other treatments suffered because they were lower in the water column.

While gear type (e.g. float vs. bottom) did affect growth (weight), no significant effect of tidal location for bottom cage treatments (intertidal vs. subtidal) was detected. This could indicate that for the bottom cage gear type, there is little benefit in moving

oysters from subtidal to intertidal locations, for finishing or during the entire grow-out period. It was expected that increased wave action in the intertidal (compared to subtidal) would result in better growth and production, but there are a few reasons why this might not have occurred. First, actual wave energy or action may not have been different enough between the two treatments to have an observable effect on performance over the course of the experiment. It is also possible that any differences in wave energy had little influence due to the nature of the cage, which is a solid, enclosed structure, designed to protect the oysters within it from outside disturbances like predators and siltation. Waves crashing upon the intertidal could have lost most of their energy when encountering the mesh of the cage itself, leaving little to no wave energy to affect the oysters inside of the cage. Finally, the proximity of the caged gear to the bottom could have inhibited growth in general, despite the more “favorable” location in intertidal zone, because of the potential for increased encounter with predators or fouling organisms and siltation from suspended sediment.

Overall, the superior growth performance of the OysterGro™ treatment suggests that location in the water column may be more important than tidal height. Further, situating gear at the same tidal height may not be as effective in shallow water compared to a body of water with a deeper water column. Aquaculture gear that sits on the surface of a deeper water column appears to have many benefits including optimal exposure to wave action and sufficient distance from bottom sediment and predators.

3-2-1 Ratio index

Our shell shape analyses using the 3-2-1 ratio deviation as a metric of ideal shape, showed that the OysterGro™ float yielded the most commercially optimal shape (lowest

mean 3-2-1 ratio deviation) at the end of the grow-out period (Table 2; Figure 9,10). Variation among oysters was also lowest in the OysterGro™ treatment, indicating that OysterGro™ oysters were also more uniform in shape compared with the other treatments. These results can likely be attributed to the floating nature of the OysterGro™ gear as well as its position high in the water column, where it receives sufficient wave action to naturally “tumble” oysters. It is well known in the local (Chesapeake) industry, and supported through studies (e.g. Brake et al., 2003), that more movement, or tumbling, results in a positive shaping of oysters that are more round or have deeper cups (Wheeler 2013; Don Webster, Commercial Aquaculture Regional Specialist, Maryland SeaGrant Extension, personal communication). Growers can spend tens of thousands of dollars on equipment to shape oysters mechanically (e.g. a single tumbler costs ~ \$30,000 dollars, see: <http://www.hioac.com/equipment-pricing>) while the OysterGro™ float appears to mimic this practice naturally, utilizing wave action to stimulate movement of the oysters and their collision with one another and the cage. Moreover, it appears that improvement in shape for oysters in OysterGro™ floats requires only a short duration during a “finishing” period.

The intertidal cage and rack and bag treatments were also exposed to relatively more wave action than the control (subtidal bottom cage) and thus, were also expected to benefit from increased movement. However, while the intertidal bottom cage treatment did yield the second lowest 3-2-1 deviation at the final sample (Table 2; Figure 9), the rack and bag treatment surprisingly produced oysters with much less ideal shapes than the initial samples (higher 3-2-1 deviation values; Figure 9 and Table 2). Anecdotally, the oysters sampled from the rack and bag treatment were very hard and spherical, which

reflects the high 3-2-1 deviation values and is generally not desirable for the half-shell market. This “reversal” of the 3-2-1 metric in rack and bag oysters would, in general, be regarded as negative, but it may be useful in certain situations. Siting of rack and bag gear may also be important as it is more commonly used in areas with greater tidal flux. Chesapeake Bay has relatively low levels of tidal flux (~1 meter at most; Hagy and Kemp 2012), which might not provide ideal conditions for deploying this gear type. In summary, OysterGro™ appears to be the best gear for finishing oysters to a marketable shape, while rack and bag could be used in limited circumstances to finish brittle, shallow oysters.

Implications for aquaculture in Maryland

The work presented in this thesis provides some of the first data on oyster aquaculture gear performance in Chesapeake Bay, which should be helpful for current and prospective oyster farmers seeking more efficient methods to grow oysters. Growers in the region may be interested in novel or alternative gear types, but have been hesitant to try them due to a lack of Chesapeake-specific data. Some growers may be more concerned with overall meat weight rather than size or shape, but the data presented here allow growers to weigh the costs and benefits of different gear on a variety of traits that might provide higher marketability of their product. Based on the results from this study, a number of rough recommendations can be made, with the important caveat that these data come from only a single study. If farmers want heavier meat (which is generally desirable, e.g. Brake et al. 2003), they should finish in an intertidal zone or in a gear treatment positioned high in the water column (e.g. OysterGro™ floats). Given the relatively limited tidal range in Chesapeake Bay, floating gear might be a better option

for exposing oysters to higher wave energy, and the position of the gear higher in the water column may also limit problems associated with siltation and proximity to predators on the bottom.

This work also sheds light on how gear type or culture technique might impact water quality and nutrient pollution around oyster aquaculture sites. As discussed in the introduction, oysters provide ecosystem services via filtration of the waters they are situated in and through benthic-pelagic coupling, which can remove nitrogen and phosphorus (in the phytoplankton they consume) as pseudofeces deposited to the sediment below (Verway 1952; Newell et al., 2002; Newell 2004; Kellogg et al., 2013). Thus, intensive oyster aquaculture has the potential to assist in management practices to reduce nitrogen and phosphorous inputs into highly eutrophic systems (Newell 2004). However, buildup of pseudofeces (benthic loading) in localized aquaculture areas may cause increased microbial respiration and ultimately the release of Phosphorous back into the water column, resulting in a buildup of H_2S and the creation of a toxic, anoxic surface benthic environment (e.g. Dahlback and Gunnarson 1981; Tenore et al., 1982; Cranford et al., 2003; Newell 2004; Gallardi 2014). I personally have observed a number of oyster farms around the Chesapeake with black, anoxic sediment that reeks of hydrogen sulfide, especially in locations with low flow. However, nutrient loading in and around aquaculture sites may be moderated to some extent by flow conditions and gear type. Increased wave action, flow, and tidal flux can move nutrients and waste products away from farm sites, reducing the potential for benthic loading and local buildup of pseudo feces (Cornwell et al., 2016). Floating gear, such as the OysterGro™ float, may pose less of a risk than bottom culture because in these systems, phytoplankton and excess N and P

are removed by oysters that are higher in the water column (close to the surface), which may allow natural flow conditions to transport pseudo feces out of the area, mitigating benthic loading (Testa et al. 2015). Bottom culture, on the other hand, especially directly on-bottom, may pose a higher risk for benthic overloading because pseudo feces are deposited right on or near the bottom, where flow is often reduced relative to higher in the water column. In future studies, the impact of floating vs. bottom cage gear types on the benthic community and biogeochemistry should be examined, especially if paired with overall performance of gear type on oyster culture.

Experimental Design/Future Work

Despite the overall success of this study and the relatively robust results obtained, there were a few shortcomings associated with the study design that warrant some discussion and can hopefully guide future work in this area. First, there was an error in how data among replicates were recorded. Though there were sufficient replicates for each gear treatment (four), they were not taken advantage of properly, because replicates within separate farms were combined before measurement, resulting in essentially only one replicate per farm, or two replicates per gear treatment total for the study. In future studies, at least three independent replicates of each gear treatment should be included to ensure a robust statistical design and to account for noise or variation due to uncontrolled environmental variability that is inherent in field studies.

Another experimental design issue that could be corrected in future experiments is fouling. Fouling was not controlled in this experiment because an ancillary goal of the study was to test how much fouling would occur on particular gear over the experiment without any intervention. This study indicated that it is not advisable to leave a gear alone

for four months without some sort of standard antifouling procedures, even in cooler waters, as there was moderate fouling of all gear (e.g. presence of sea squirts, boring sponge) in this study. In future studies, control of fouling should be incorporated into the experimental design; however, the fouling experienced in this study may have also been site-specific.

Future experiments of oyster aquaculture gear should also include the collection of data on physical and environmental parameters of the oysters and the environment around the gear. For example, this study did not deploy a wave gauge at the subtidal and intertidal locations (bottom cage treatments) to quantify wave action, thus it is difficult to know how much (or if) the wave energy at these sites actually differed, as was expected. In future studies, data such as these would be helpful in characterizing the physical conditions of gear deployed within the subtidal and intertidal zones, in order to more specifically describe the grow-out sites or to relate performance (growth, shape) to physical (wave energy) conditions. Additionally, measuring turbidity, current/flow, and phytoplankton (chlorophyll distribution as a proxy), would provide important data that might be helpful in explaining performance differences among treatments during the experiment. Finally, incorporating motion-sensing devices into gear or even inside ‘dummy’ oysters could be especially useful in quantifying the movement energy of animals across gear types, shedding light on how exactly the exposure to different levels of wave or tidal energy affect oyster movement.

Finally, It should be noted that the site of the current study would be considered moderate salinity in the Chesapeake Bay (10-20ppt), and experiments at higher salinity sites might result in different growth rates. Higher salinity environments generally favor

increased growth (up to ~35 ppt; Kennedy et al., 1996), but higher salinity environments also tend to have higher disease prevalence (e.g. Dermo, *Perkinsis marinus*), which can cause significant mortality events or reduce growth (Burreson 1991). So, if this study is to be repeated, salinity and possibly even disease status should be monitored so that results can be compared.

Conclusions

The results of this study provide an interesting contrast to previous gear type studies for the aquaculture of eastern oysters and overall, indicate that gear treatments that expose animals to more wave energy and are higher in the water column produce oysters that grow faster and have more marketable shapes. However, the highest performing gear type, OysterGro™ floats, has seen little use in Chesapeake Bay to date, and more research on this gear may be needed in the region before it becomes widely used in the local industry. Differences in the results of the current study compared with previous aquaculture gear studies conducted across a number of locations outside of Chesapeake Bay (many of which differ physically), reinforces the need for more data on grow-out methods in this region. Most oyster industries are highly attuned or adapted to their local conditions, with tradition and on-the-water experience driving the local technology. For the local industry (Chesapeake Bay or other), information on grow-out practices are really only relevant if they relate to the local conditions, which renders complementary studies of gear-use in different areas of the country and world less likely to influence attitudes and implementation of gear type by growers (Don Webster, Commercial Aquaculture Regional Specialist, Maryland SeaGrant Extension, personal communication). Future experiments similar to this study (i.e. in the Chesapeake region)

are encouraged in order to build upon the limited body of knowledge about grow-out methods for oyster aquaculture in Chesapeake Bay, especially in Maryland. Given the major decline in the oyster fishery and the reduction in ecosystem services provided by oysters, there is a great need to invest in and implement novel technologies that can accelerate aquaculture production and the return of the oyster industry to the region.

In summary, this study demonstrates that gear type can have a major effect on the shape and weight of an oyster and that transplanting oysters from a subtidal bottom cage into other gear treatments (e.g. OysterGro™ floats) can be a good option for finishing and possibly for the entire grow out period. This study also marks one of the first explicitly designed gear type experiments performed in Chesapeake Bay, and some of the unique results suggest that gear must be evaluated on a site or regionally specific basis. This study provides the first empirical data on the differences between local (traditional) and emerging gear types, as well as the costs and benefits of transplanting oysters into a different tidal zone in the last four months of growth in Chesapeake Bay. Of course, the data presented here represent a single study that should be repeated before any hard conclusions are drawn about the success of these gear types in Chesapeake Bay. Nevertheless, this information should positively affect the nascent oyster aquaculture industry in Maryland by providing growers with actual data on the effect of different gear, which they can use to make decisions about how to more effectively grow oysters with specific properties of shape and weight.

Tables

Table 1. Mean initial values for length, width, height (mm), total weight (g), and 3-2-1 ratio of oysters deployed into gear treatments at commencement of study.

Gear type	Length (mm)	Width (mm)	Height (mm)	Total Weight (g)	3-2-1 ratio
Subtidal cage (control)	73.37(7.6)	46.82(4.83)	21.66(3.81)	45.37(8.96)	1.31(1.32)
OysterGro™	71.81(6.87)	45.76(4.87)	21.98(3.81)	44.01(9.88)	1.22(1.44)
Rack and Bag	72.7(7.45)	46.34(5.3)	22.60(4.31)	45.87(11.12)	1.38(1.49)
Intertidal Cage	74.14(7.91)	47.09(4.43)	21.73(3.9)	45.58(12.84)	1.29(1.38)

Table 2. Mean final values for length, width, height (mm), total weight (g), and 3-2-1 ratio of oysters sampled from gear treatments at the end of study.

Gear type	Length (mm)	Width (mm)	Height (mm)	Total Weight (g)	3-2-1 ratio
Subtidal cage (control)	85.5(8.85)	58.09(7.96)	29.77(4.18)	81.54(9.9)	1.13(1.14)
OysterGro™	90.9(7.24)	61.06(5.75)	31.55(3.79)	103(20.6)	0.85(0.87)
Rack and Bag	89.8(9.73)	60.22(7.27)	30.83(5.62)	87.36(11.8)	1.56(3.53)
Intertidal Cage	87.9(8.07)	58.59(6.6)	30.32(3.78)	80.97(7.7)	0.94(1.14)

Table 3. Mean tidal heights at low and high tide levels for sites where gear treatments were deployed. OysterGro™ and subtidal cage were deployed at the far site, rack and bag at the middle site, and intertidal cage at the near site.

Site*	Tide level	Tidal height (m)
Far	low	0.762
Middle	low	0.686
Near	low	0.559
Far	high	1.19
Middle	high	1.12
Near	high	1.02

*Far site ~25 yards from coast, middle site ~15 yards from shoreline, near site ~5 yards from shoreline.

Table 4. Mean depth below surface (cm) of gear treatments and control deployed at a low tide level.

Gear	Tide level	Depth below surface (cm)
Subtidal cage (control)	low	25.4
OysterGro™	low	7.62
Rack and bag	low	7.62
Intertidal cage	low	8.26

Table 5. Example 3-2-1 deviation calculations on an excel spreadsheet using an adapted Chi-squared test.

	A	B	C	D	E	F	G	H	I
1	Oyster	Length	Width	Depth	Total (sum)	3-2-1 exp length	3-2-1 exp width	3-2-1 exp depth	Chi-Square $\Sigma[(O-E)^2/E]$
2	1	37	22	7	66	33.00	22.00	11.00	1.94
3	2	34	24	12	70	35.00	23.33	11.67	0.06
4	3	30	15	4	49	24.50	16.33	8.17	3.47
5	4	58	31	20	109	54.50	36.33	18.17	1.19
6	5	69	38	26	133	66.50	44.33	22.17	1.66
7	6	30	20	10	60	30.00	20.00	10.00	0.00
8	7	38	25	15	78	39.00	26.00	13.00	0.37
9	...								
10				Formulas =SUM(C9:E9) =E8*3/6 =E8*2/6 =E8*1/6 =((B8-F8)^2/F.....					
11									
12									
13									=((B8-F8)^2/F8)+((C8-G8)^2/G8)+((D8-H8)^2/H8)

Results for oyster number 7 are highlighted in a gray dashed box and excel formulas are displayed in cells F10:G10 (expected values of each dimension) and F13 (the Chi-square value, moved to fit within figure dimensions).

Table 6. ANOVA and Tukey's post-hoc results for differences in growth and shape parameters across treatments at the end of the experiment.

Trait	Length (mm)	Total weight (g)	Wet meat weight (g)	3-2-1 Ratio
Anova P-Value	0.10	1.15E-10	2.93E-14	0.03
DF	3	3	3	3
F-stat	2.09	16.70	22.73	2.92
n	1520	1526	1114	920
Tukey*	0	3	3	1

*Tally of number of significant pair-wise comparisons (Tukey's) between OysterGro™ and all other treatments.

Figures

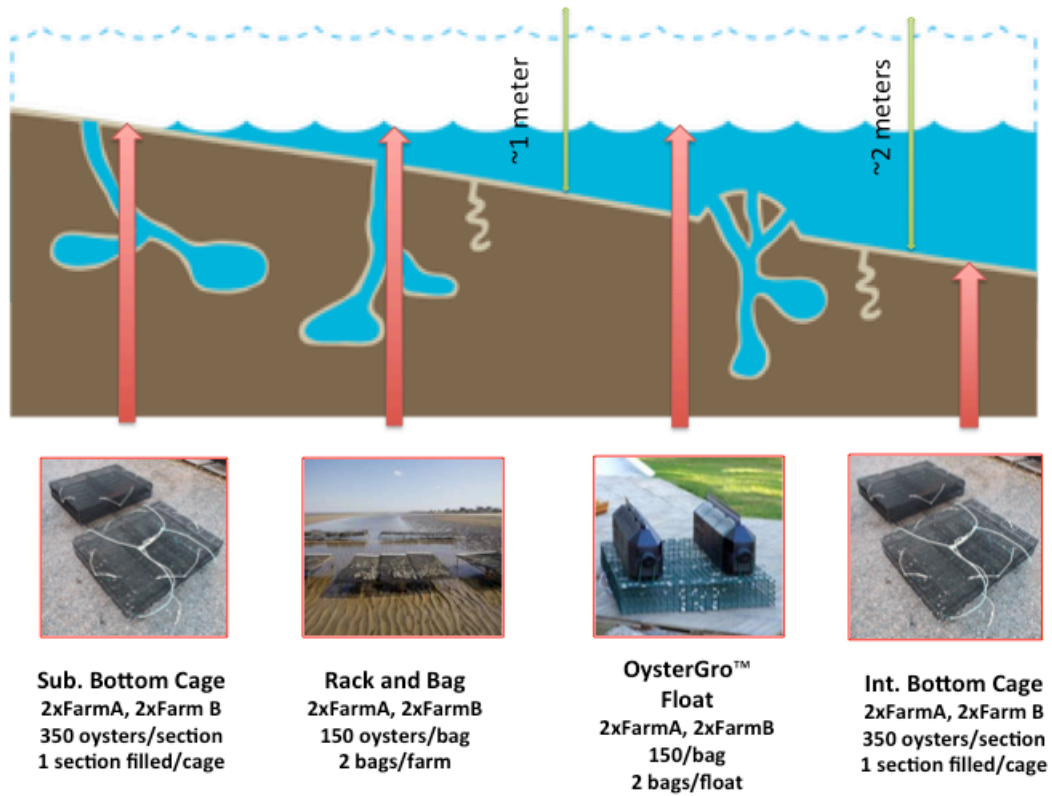


Figure 1. Schematic illustrating the gear types and details of oyster deployment of across four gear treatments. Three treatments were deployed in the intertidal; bottom cages, rack and bag system, and OysterGro™ floats and one treatment (bottom cage) was deployed in the subtidal as a control.

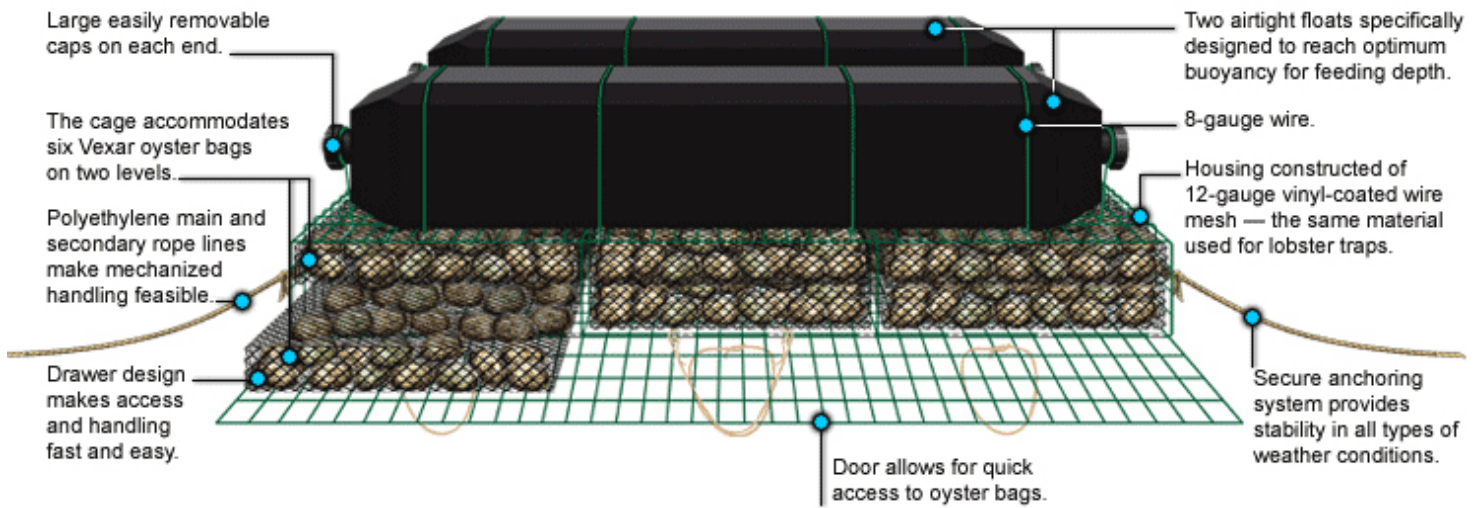


Figure 2. OysterGro™ large float system contains six compartments, each of which can hold one Intermas™ Oyster bag (Figure 5). Image source: Oystergro.com

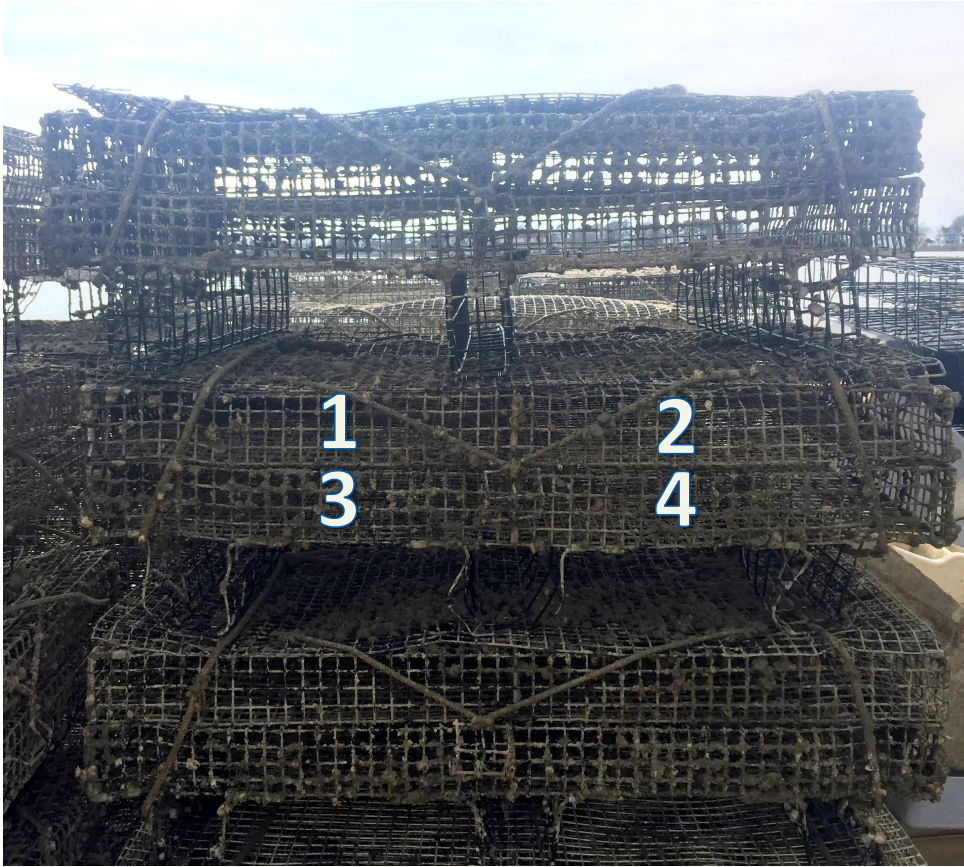


Figure 3. Custom manufactured bottom cage with stilts. Numbers indicate sections within top and bottom layers of cage; each section can hold 300 market-sized (~80 mm) oysters.

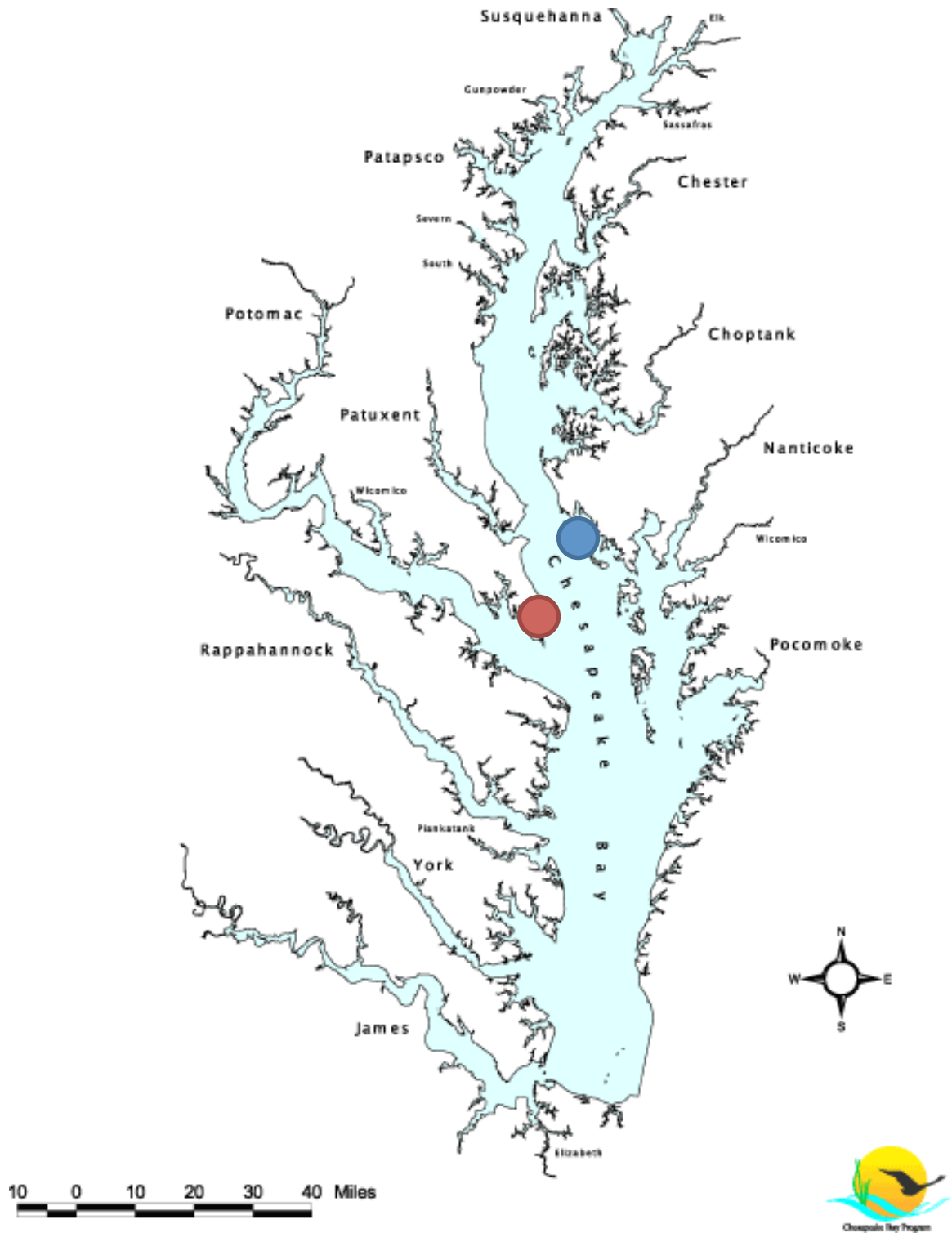


Figure 4. Location in Chesapeake Bay of Farm A, True Chesapeake Oyster Co., Ridge, MD (red) and Farm B, Hoopers Island Oyster Aquaculture Company, Fishing Creek, MD (blue), where oyster sample populations were obtained from separate shores. Image source: chesapeakebay.net



Figure 5. Custom manufactured grading tool used to grade oysters to at least 63.5 mm before deployment, to ensure uniformity of initial samples.



Figure 6. Picture of study site from beach at Fishing Creek MD, with farm A treatments deployed center left and farm B treatments deployed center right in the intertidal zone (mean tidal height of 0.89 meters). From the beach, the first set of buoys indicate the intertidal bottom cage, the second set of buoys indicate the rack and bag system, the black floating pontoons indicate the OysterGro™ floats, and finally, a third set of buoys parallel with channel marker indicate the subtidal bottom cages (control).



Figure 7. Intermas™ Oyster bags with 12 mm diamond mesh, initially stocked with 150 73.1 mm oysters.

Final parameter measurements across gear treatments

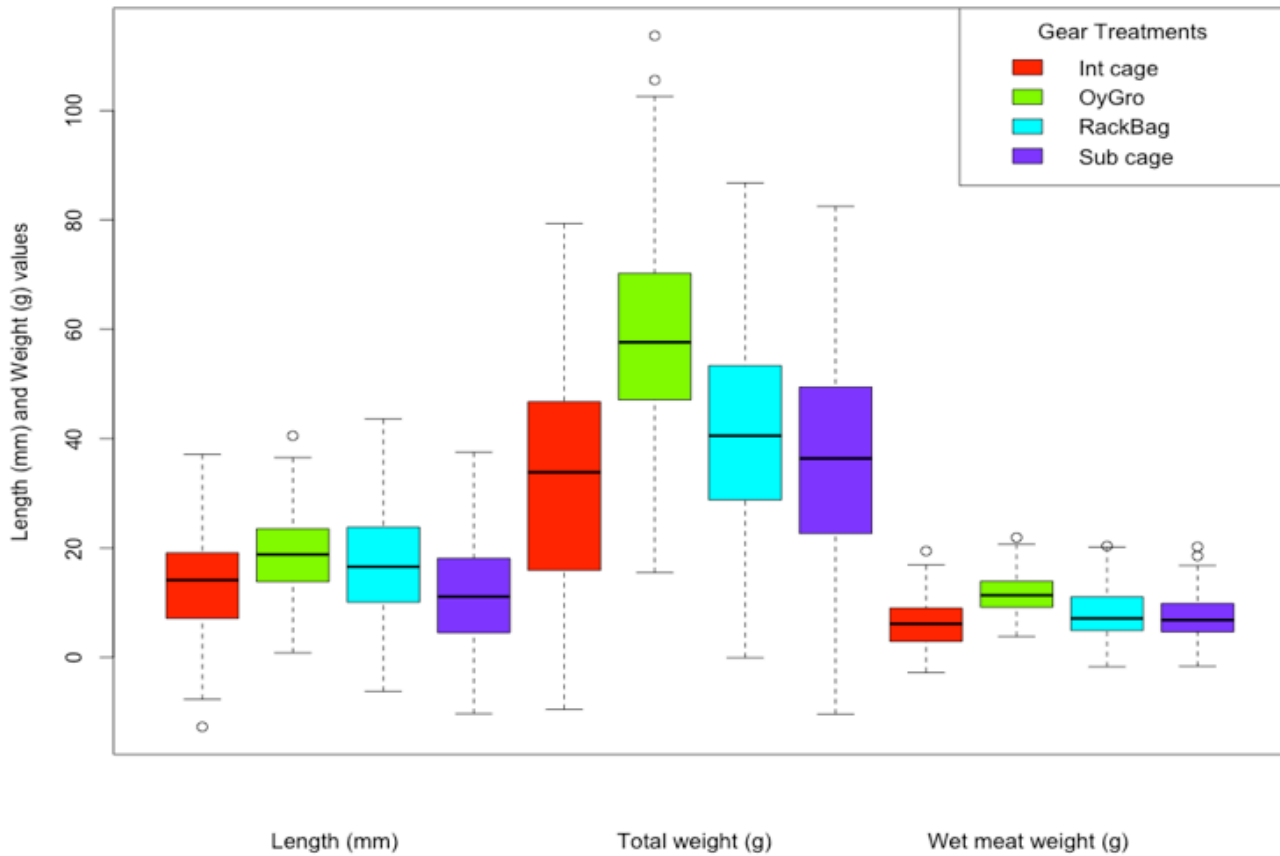


Figure 8. Box and whisker plots of the final parameter measurements across treatments. For each parameter/treatment combination, the dark bars within boxes represent the median of the data, the boxes on either side of the median represent the lower and upper 25% quartiles, the upper and lower bars extending from the boxes indicate the minimum and maximum values in the data, and the open circles indicate outliers.

Final values for 3-2-1 ratios across gear types August (initial) and December (final) samples

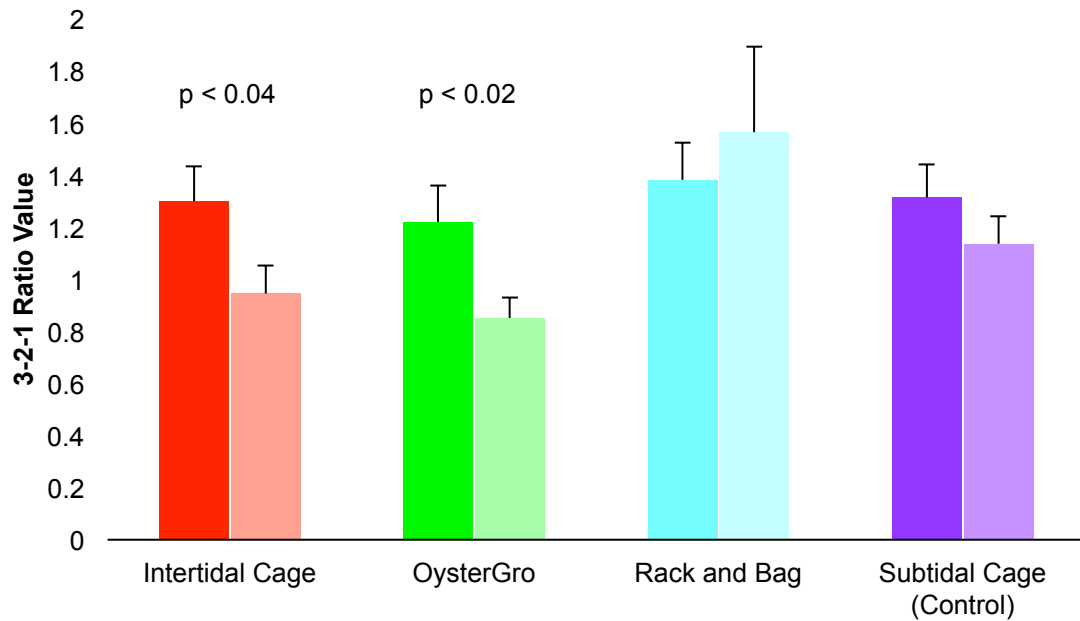


Figure 9. Box plot of 3-2-1 ratio deviation values in August (initial) and December (final) samples across gear treatments. The darker shade bars (or left-most bars of a pair) represent initial values, while the lighter shade bars indicate final values. Error bars indicate standard error of mean (SEM). Significant differences between initial and final values are indicated by p-values.

Frequency of OysterGro™ 3-2-1 ratio values in August (initial) and December (Final) samples

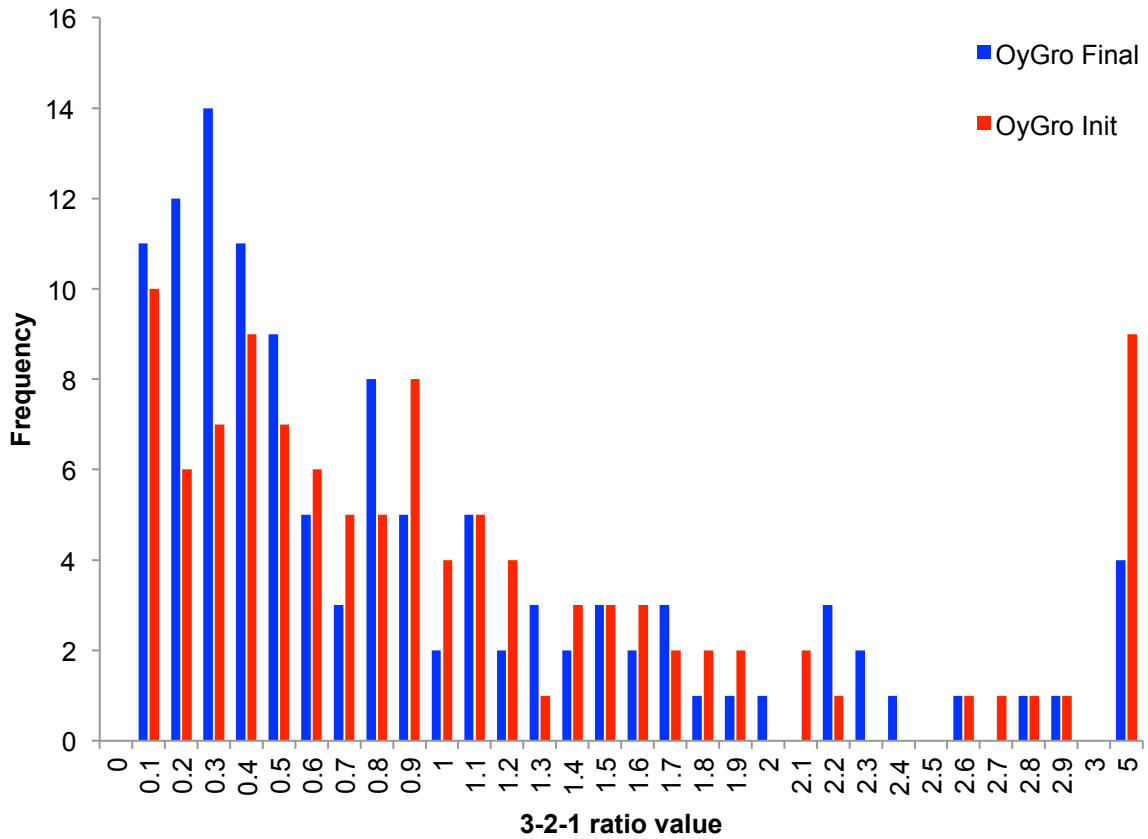


Figure 10. Histogram of the initial and final 3-2-1 ratio values observed in the OysterGro™ gear treatment, showing a shift in distribution of the final values towards zero, or lower deviation.

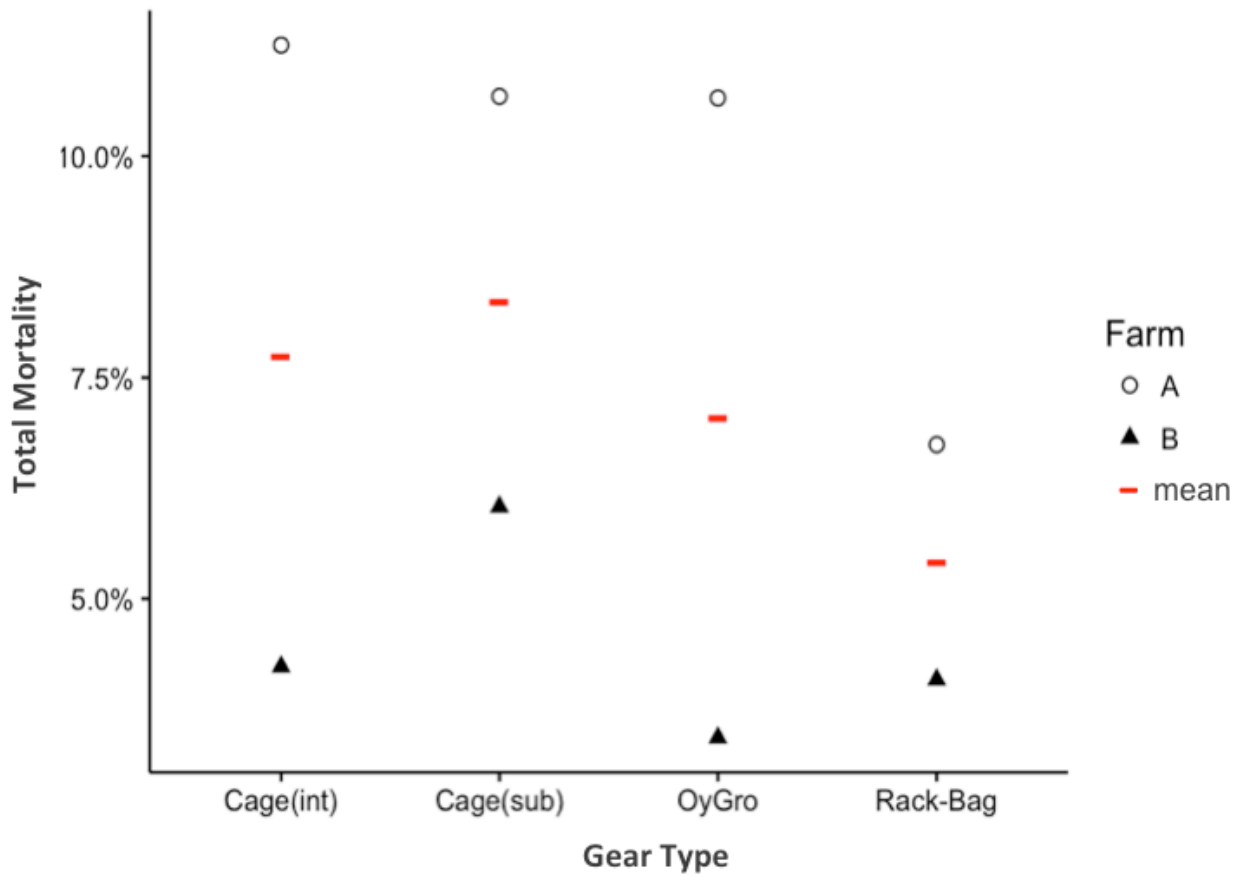


Figure 11. Mean total percent mortality of oysters within each farm replicate, across gear treatments. Farm A (True Chesapeake) displays a trend of approximately 9.83% mean mortality across gear treatments. Farm B (Hoopers Island Oyster Aquaculture Company), displays a trend of approximately 4.45% mean mortality across gear treatments.

References

- Allen, Jr., S.K., Downing, S.L. and Chew, K.K. (1989) Hatchery Manual for Producing Triploid Oysters. University of Washington Press, Seattle, WA, USA.
- Allen, Jr., S.K., Bushek, David (1992) Large-scale production of triploid oysters, *Crassostrea virginica* (Gmelin) using “stripped” gametes. *Aquaculture*. 103: 241-251.
- Archer, A., Reitsma, J., Murphy, D. (2014) A Comparison of Bottom and Floating Gear for growing American Oysters (*Crassostrea virginica*) in Southeastern Massachusetts. Woods Hole Sea Grant and Cape Cod Cooperative Extension.
- Beck, M.W., Brumbaugh, R.D., Airoidi, L., Carranza, A., Coen, L.D., Crawford, C., Defeo, O., Edgar, G.J., Hancock, B., Kay, M.C., Lenihan, H.S., Luckenbach, M.W., Toropova, C.L., Zhang, G., Guo, X. (2011) Oyster reefs at risk and recommendations for conservation, restoration, and management. *Bioscience* 61:107–116.
- Boicourt, William C. (1992) Influences of Circulation Processes on Dissolved Oxygen in the Chesapeake Bay. In: Smith, D., Leffler, M., Mackiernan, G. (eds.) Oxygen dynamics in Chesapeake Bay. A synthesis of results. Maryland Sea Grant, College Park, p. 7-53.
- Bosch, D., Kuminoff, N., Stephenson, K., Miller, A., Pope, J., Harris, A. (2010) Evaluation of Policy Options for Expanding Oyster Aquaculture in Virginia. Department of Agricultural and Applied Economics, Virginia Tech, Blacksburg, Virginia 24061.
- Boulding, E.G., and Hay, T.K. (1993) Quantitative genetics of shell form of an intertidal snail constraints on short-term response to selection. *Evolution*. 47:576-592.
- 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.
- Breitburg, D.C. (1999) Are three-dimensional structure and healthy oyster populations the keys to an ecologically interesting and important fish community? In: Luckenbach, M.W., Mann, R., Wesson, J.A. (Eds.), *Oyster Reef Habitat Restoration: A Synopsis and Synthesis of Approaches*. Virginia Institute of Marine Science Press, Gloucester Point, VA, pp. 239–250.
- Buestel, D., Ropert, M., Prou, J., Gouletquer, P. (2009) History, status, and future of oyster culture in France. *Journal of Shellfish Research*. 28(4) 813-820.

- Burreson, E.M. (1991) Effects of *Perkinsus marinus* Infection in the eastern oyster, *Crassostrea virginica*: I. Susceptibility of native and MSX-resistant stocks. *J. Shellfish Res.* 10(2):417-423.
- Carriker, M.R. (1996) The shell and ligament. In: V.S. Kennedy, R.E.I Newell, and A.F. Eble, editors. The eastern oyster, *Crassostrea virginica*. Pp. 75-168. College Park, MD. Maryland Sea Grant College Publication, pp. 75-168.
- Casas, S., Leonhart, J., Supan, J., La Peyre, J. (2013) Evaluation of four grow-out systems for commercial production of the Eastern Oyster *Crassostrea virginica* in Louisiana estuaries. Poster session presented at the World Aquaculture Society Aquaculture 2013 Meeting, Nashville, Tennessee.
- Chew, K.K. (1988) Oyster aquaculture in the Pacific Northwest. In: Keller, S. (ed.), Bd. 88-4, Proc. 4. Alaska Aquacult. Conf., Alaska Sea Grant Rep. Sitka, 67-75 pp.
- Coen L.D., Luckenbach M.W., Breitburg D.L. (1999) The role of oyster reefs as essential fish habitat: a review of current knowledge and some new perspectives. *Am Fish Soc Symp* 34:303–307
- Coen L.D., Brumbaugh R.D., Bushek D., Grizzle R. and others (2007) Ecosystem services related to oyster restoration. *Mar Ecol Prog Ser* 341:303–307.
- 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.).
- Comeau, L.A. (2013) Suspended versus bottom oysters culture in eastern Canada: comparing stocking densities and clearance rates. *Aquaculture* 410-411:57-65. G.C. Daily (Ed.), *Nature Societal Dependence on Natural Ecosystems*, Island Press, Washington, DC (1997), pp. 275–293.
- Conte, F.S., Harbell S.C. and R.L. RaLonde (1994) Oyster Culture- fundamentals and technology of the West Coast industry. Western Region Aquaculture Center, Seattle, WA.
- Cornwell, J., Rose, J., Kellogg, L., Luckenbach, M., Bricker, S., Paynter, K., Moore, C., Parker, M., Sanford, L., Wolinski, W., Lacatell, A., Fegley, L., Hudson, K. (2016) Panel Recommendations on the Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Determination Decision Framework and Nitrogen and Phosphorous Assimilation in Oyster Tissue Reduction Effectiveness for Oyster Aquaculture Practices. DRAFT for CBP Partnership and Public Review.

- Cranford, P., Dowd, M., Grant, J., Hargrave, B., McGladdery, S. (2003) A scientific review of the potential environmental effects of aquaculture in aquatic systems: Ecosystem level effects of marine bivalve aquaculture. Fisheries and Oceans Canada.
- Dahlback, B. and Gunnarsson, L.A.H. (1981) Sedimentation and sulfate reduction under a mussel culture. *Marine Biology* 63:269-275.
- Dame, R., Libes, S. (1993) Oyster reefs and nutrient retention in tidal creeks. *J Exp Mar Biol Ecol* 171:251–258
- Dumbauld, B.R., Ruesink, J.L., Rumrill, S.S. (2009) The ecological role of bivalve shellfish aquaculture in the estuarine environment: a review with application to oyster and clam culture in West Coast (USA) estuaries. *Aquaculture* 290, 196–223.
- Fulford, R.S., Breitburg, D.L., Luckenbach, M., Newell, R.I.E. (2010) Evaluating ecosystem response to oyster restoration and nutrient load reduction with a multispecies bioenergetics model. *Ecol Appl* 20:915–934
- Gallardi, D. (2014) Effects of Bivalve Aquaculture on the Environment and Their Possible Mitigation: A Review. *Fish Aquac J* 5:105. doi:10.4172/2150-3508.1000105
- Grabowski, J.H., Hughes, A.R., Kimbro, D.L., Dolan, M.A. (2005) How habitat setting influences restored oyster reef communities. *Ecology* 86:1926–1935
- Grabowski, J.H., Peterson, C.H. (2007) Restoring oyster reefs to recover ecosystem services. In: Cuddington K, Byers JE, Wilson WG, Hastings A (eds) *Ecosystem engineers: plants to protists*. Academic Press, Amsterdam p 281–298
- Guo, X., DeBrosse, G.A., Allen, S.K. (1996) All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. *Aquaculture*. 142:149-161.
- Hagy, J. D. and Kemp, W. M. (2012) Estuarine Food Webs, in *Estuarine Ecology, Second Edition* (eds J. W. Day, B. C. Crump, W. M. Kemp and A. Yáñez-Arancibia), John Wiley & Sons, Inc., Hoboken, NJ, USA. doi: 10.1002/9781118412787.ch16
- Hudson, K., and Murray, T.J. (2016) Virginia Shellfish Aquaculture Situation and Outlook Report: Results of the 2015 Virginia Shellfish Aquaculture Crop Reporting Survey. VIMS Marine Resource Report No. 2016-4.

- Kellogg, L.M., Cornwell, J.C., Owens, M.S., Paynter, K.T. (2013) Denitrification and nutrient assimilation on a restored oyster reef. *Mar Ecol Prog Ser* 480:1-19.
- Kemp, W.M., Boynton, W.R., Adolf, J.E., Boesch, D.F. and others (2005) Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Mar Ecol Prog Ser* 303: 1–29.
- Kennedy, V.S., Breisch, L.L. (1983) Sixteen decades of political management of the oyster fishery in Maryland's Chesapeake Bay. *J Environ Manag* 164:153–171.
- Lavoie, R.E. (2005) Oyster Culture in North America: History, Present and Future. Paper read at The 1st International Oyster Symposium. Tokyo, Japan.
- Maguire, G.B. & J.A. Nell. (2007) History, status and future of oyster culture in Australia. *Oyster Res. Inst. News* 19:3–12.
- Malone, T.C. (1992). Effects of water column processes on dissolved oxygen: nutrients, phytoplankton, and zooplankton. In: Smith, D., Leffler, M., Mackiernan, G. (eds.) *Oxygen dynamics in Chesapeake Bay. A synthesis of results*. Maryland Sea Grant, College Park, p. 61-148.
- Mallet, A.L., Carver, C.E.A., Coffen, S.S., Freeman, K.R. (1986) Winter growth of the blue mussel, *Mytilus edulis*: importance of stock and site. *Journal of Experimental Marine Biology and Ecology* 108, 217–228 .
- Mallet, A.L., Carver, C.E.A. (1989) Growth, mortality, and secondary production in natural populations of the blue mussel, *Mytilus edulis*. *Canadian Journal of Aquatic Sciences* 46, 1154–1159.
- 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.
- Manley, J., Power, A., Walker, R. (2009) Effect of Submergence Depth on Eastern Oyster, *Crassostrea virginica* (Gmelin, 1791), Growth, Shell Morphology, Shell Characteristics, *Perkinsus marinus* Infection, and Mortality on Oysters Cultured Intertidally Off-Bottom in Georgia. *Occasional Papers of the University of Georgia Marine Extension Service*. 5: 1-22.
- Mann, Roger; Powell, Eric N. (2007) Why Oyster Restoration Goals in The Chesapeake Bay Are Not and Probably Cannot Be Achieved. *Journal of Shellfish Research* 26(4):905-917.
- Nell, John A. (2001) The History of Oyster Farming in Australia. *Marine Fisheries Review*. 63(3) 14-25.

- Newell, R.I.E. (1988) Ecological changes in Chesapeake Bay: Are they the result of over harvesting of the American oyster (*Crassostrea virginica*)? In: Lynch, M.P., Krome, E.C. (Eds.), *Understanding the Estuary: Advances in Chesapeake Bay Research*. Chesapeake Research Consortium, Solomons, Maryland, pp. 536–546.
- Newell, R.I.E., Cornwell, J.C., Owens, M.S. (2002) Influence of simulated bivalve biodeposition and microphytobenthos on nitrogen dynamics: A laboratory study. *Limnol. Oceanogr.* 47(5): 1367-1379.
- Newell, R.I.E. (2004) Ecosystem Influences of Natural and Cultivated Populations of Suspension-Feeding Bivalve Molluscs: A Review. *Journal of Shellfish Research*. 23:51-61.
- Paynter, K.T. and L. DiMichele (1990) Growth of tray cultured oysters (*Crassostrea virginica* Gmelin) in the Chesapeake Bay. *Aquaculture* 87:289-298.
- Paynter, K.T., Mallonee, M.E., Shriver, S.H. (1992) Cost analysis of floating raft oyster production in Chesapeake Bay. *J. Shellfish Res.* 11(1):163-167.
- Porter, Elka T.; Cornwell, Jeffrey C.; Sanford, Lawrence P. (2004) Effect of oysters *Crassostrea virginica* and bottom shear velocity on benthic-pelagic coupling and estuarine water quality. *MEPS* 271:61-75.
- Quittmeyer, C. L. (1966). A Report on the Chesapeake Bay Fisheries of Maryland. Centreville, Maryland: Seafood Advisory Committee of Wye Institute.
- R Core Team. (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
- Ragone Calvo, L.M., G.W. Calvo and E.M. Burreson (2002) Dual disease resistance in a selectively bred eastern oyster, *Crassostrea virginica*, strain tested in Chesapeake Bay. *Aquaculture* 220:69-87.
- Seed, R. (1968) Factors influencing shell shape in the mussel *Mytilus edulis*. *J. Mar. Biol. Ass. U.K.* 48:561-584.
- Sonier, R., LeBlanc, K., Hardy, M., Ouellette, M., Comeau, L.A., Landry, T. (2011a.) Shellfish monitoring network in Atlantic Canada 1996–2001: temperature, growth, condition and survival. Canadian Data Report of Fisheries and Aquatic Sciences 1234 (x+25 p).
- Sonier, R., LeBlanc, K., Hardy, M., Ouellette, M., Comeau, L.A., Landry, T., (2011b.) Development of a shellfish monitoring network in Atlantic Canada 1996–1997. Canadian Technical Report of Fisheries and Aquatic Sciences 2944 (viii+28 p).

- Souchu, P., Vaquer, A., Collos, Y., Landrein, S., Deslous-Paoli, J.M., Bibent, B. (2001) Influence of shellfish farming activities on the biogeochemical composition of the water column in Thau Lagoon. *Marine Ecology Progress Series* 218, 141–152.
- Smith, J.W. and Merriner J.V. (1985) *Estuaries*. 8: 305. doi:10.2307/1351491
- Stanley, J.G., Allen, Jr., S.K. and Hidu, H. (1981) Polyploidy induced in the American oyster, *Crassostrea virginica*, with cytochalasin B. *Aquaculture*, 23: 1-10.
- Tenore, K., L. Boyer, R. Cal, J. Corral, F. Garcia, M. Gonzalez, E. Gonzales, R. Hanson, J. Iglesias, and M. Krom. (1982) Coastal upwelling in the Rias Bajas, NW Spain: Contrasting the benthic regimes of the Rias de Arosa and Muros. *Journal of Marine Research* 40:701-772.
- Testa J.M., D.C. Brady, J.C. Cornwell, M.S. Owens, L.P. Sanford, C.R. Newell C., S.E. Suttles, and R.I.E. Newell (2015) Modeling the impact of floating oyster aquaculture on sediment-water nutrient and oxygen fluxes. *Aquaculture Environment Interactions*. DOI: 10.3354/aei00151.
- Verwey, J. (1952) On the ecology of distribution of cockle and mussel in the Dutch Waddensea, their role in sedimentation, and their source of food supply. *Arch. Neerl. Zool.* 10:171-239.
- Walton, W.C., Rikard, F.S., Chaplin, G.I., Davis, J.E., Arias, C.R., Supan, J.E. (2013) Effects of ploidy and gear on the performance of cultured oysters, *Crassostrea virginica*: survival, growth, shape, condition index and *Vibrio* abundances. *Aquaculture* 414:260–266.
- Ward, R.D., Thompson, P.A., Appleyard, S.A., Swan, A.A., & Kube, P.D. (2005) Sustainable genetic improvement of Pacific oysters in Tasmania and South Australia. Final Report FRDC Project 2000/2006. Hobart (Tasmania), CSIRO Marine and Atmospheric Research and Fisheries Research and Development Corporation, 193p.
- Wheeler, Timothy B. “Oyster farmers seek quicker approval process”. The Baltimore Sun [Baltimore, Maryland] October 25th, 2015.
- Wilberg, Michael J., Wiedenmann, John R., Robinson, Jason M. (2013) Sustainable exploitation and management of autogenic ecosystem engineers: application to oysters in the Chesapeake Bay. *Ecological Applications*. 23(4) 766-776.