

**EFFECT OF WAVE ACTION, BIOFOULING CONTROL, AND DENSITY ON THE
PERFORMANCE OF EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*)**

by

John Collins Lewis

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Approved by

William C. Walton, Chair, Associate Professor, School of Fisheries, Aquaculture and Aquatic
Sciences

Todd D. Steury, Associate Professor, School of Forestry and Wildlife Sciences

Matthew W. Gray, Assistant Professor, University of Maryland Center for Environmental
Sciences

Abstract

To better understand the impacts of wave action on oyster performance and its interaction with other farming choices, triploid oysters were deployed utilizing the adjustable longline system at an oyster farm site near Deer Island, Mississippi. A full factorial experiment consisting of two wave action (natural, dampened) x three biofouling control (desiccated, power washed, submerged) x two stocking density (low, high) treatments was deployed at one high energy site. Higher wave action generally increased production quality metrics (cup ratio, condition, cleanliness) and dampened wave action generally increased production quantity metrics (shell height, whole wet weight). To maximize product quantity, submerged, lower stocking density treatments in lower wave environments and power washing, higher stocking density treatments in higher wave sites are recommended. For increased product quality in either a high or low wave energy sites, weekly desiccation and lower stocking densities led to the highest quality. In lower wave sites, the weekly desiccated, higher stocking density treatment seemed to produce the best combination of quantity and quality. In higher wave sites, the submerged, higher stocking density treatment seemed to produce the best combination of quantity and quality.

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CHAPTER ONE:

A BRIEF REVIEW OF EASTERN OYSTER *Crassostrea virginica* AQUACULTURE

PRACTICES ACROSS NORTH AMERICA

Background

The Eastern oyster *Crassostrea virginica* is an economically important bivalve species endemic to North America. Its native range stretches south from the Gulf of St. Lawrence in Canada and covers the entirety of the East Coast of the United States to the tip of Florida, and also spans the entire coast of the Gulf of Mexico bordering the United States (Loosanoff, 1965). In 2019, commercial Eastern oyster harvest accounted for \$186.7 million, with the Gulf of Mexico providing the largest share (\$83.2 million; 44.6%), followed by the Mid-Atlantic region (\$51.0 million; 27.3%), New England (\$43.4 million; 23.3%), and South Atlantic region (\$9.0 million; 4.8%) (NOAA Fisheries Landings Data; see <https://www.fisheries.noaa.gov/foss/f?p=215:200:465814032806::NO>). Despite the magnitude of the harvest by value, there are many areas throughout this native range that have reported marked declines in oyster abundance and landings over the last 70 years. Reviewing available data from the NOAA Fisheries Landings Database, harvest volume decreased from 68.2 million pounds in 1950 to 18.5 million pounds in 2019.

One of the starkest examples of this decline in abundance comes from that of the Chesapeake Bay, where it has been reported that Eastern oyster abundances have declined to <1% of historic levels due to factors such as overharvesting, disease, pollution, and loss of habitat, some of which have continued to plague the ongoing restoration efforts for the species in this area (National Research Council, 2004). In 1950, harvest poundage of oysters in the Chesapeake Bay was reported to be near 30 million pounds yet harvest poundage in 2019 only totaled in at 4.1 million pounds (NOAA Fisheries Landings Data). There is a similar story in Apalachicola Bay, Florida. Historically, Apalachicola oysters have been regarded as some of the best on the market throughout the Gulf of Mexico and oysters harvested from this bay accounted

for 90% of total harvest from Florida in the 1990s (Dugas et al., 1997). More recently, however, there was a marked population decline in 2012 attributed to anthropogenic and environmental factors that created poor conditions for juvenile oyster survival and recruitment, ultimately leading to a downward trend in oyster abundance (Havens et al., 2013). This historic oyster fishery was ultimately shut down by the Florida Fish and Wildlife Conservation Commission in 2020 through the year 2025 in order to allow for habitat restoration and replenishment of the stock. There are numerous examples that highlight similar trends of decline over time in wild oyster harvest in other areas where Eastern oysters were historically harvested due to a wide array of human and environmental causes (Ford, 1997; MacKenzie Jr., 1997; Park et al., 2014).

While many wild stocks of Eastern oysters have experienced decreases in abundance and subsequent harvest declines, oyster aquaculture has grown over this same time period. According to the Food and Agriculture Organization of the United Nations' United States of America Aquaculture Fact Sheet published in 2011, the economic production of the shellfish sector of US aquaculture grew \$210 million between 1998 and 2008, from \$113 million to \$323 million, with Eastern oysters accounting for \$45 million and Pacific oysters *Crassostrea gigas* adding \$33 million to this total (Olin, 2011). In comparison, it is estimated that in 2017, United States oyster aquaculture was valued at \$186.2 million, a \$50.5 million increase since 2012 (valued at \$135.7 million), clearly depicting a positive trend in economic growth since 2008 (National Marine Fisheries Service, 2020).

Expansion into new markets may have allowed for such economic growth in a sector of aquaculture that has been around for nearly a century in the United States (Kennedy, 2004). Due to the wide environmental variation that Eastern oysters can withstand (Loosanoff, 1965) and given the dwindling supply of wild caught oysters available to historic markets (MacKenzie Jr.

and Burrell Jr., 1997), farming of this species began to take root throughout the East Coast of the United States, which continues to be a major producer of farmed oysters (Hudson, 2019; Ozbay et al., 2014). In the Gulf of Mexico, however, this expansion of oyster aquaculture was delayed, possibly due to the retention of harvestable wild oyster populations up until the mid-2000s, when natural disasters and environmental issues began to cause these fisheries to collapse (Havens et al., 2013; Park et al., 2014).

The growth and survival of the Eastern oyster is largely influenced by salinity and temperature, where suitable salinity ranges from 5 to 40 parts per thousand (ppt) and survivable temperature varies between -2° to 36° C throughout their native range (Doiron, 2008; Galtsoff, 1964; Proestou et al., 2016). This made the Eastern oyster a good candidate for aquaculture practices, with early culturing efforts being as simple as collection and transportation of wild oyster seed to areas more suitable for growth or harvest (Wallace, 2001). The oyster aquaculture industry has since evolved with the development of more sophisticated hatchery and nursery practices, allowing industry control over the supply of oyster seed.

Aquaculture Expansion

The state of Virginia has been a leader of the East Coast in oyster aquaculture production for years. In 2018, it was reported that Virginia ranked first in farmed Eastern oyster production on the East Coast, with 32.1 million single market oysters sold for \$13.1 million and oyster aquaculture was titled the most rapidly growing sector of shellfish aquaculture in the state (Hudson, 2019). Oyster farming has also shown considerable growth in Maryland with off-bottom practices comprising a significant portion of the industry as of 2018 (Van Senten et al., 2019). North Carolina has seen more recent growth of oyster aquaculture through an increase in

lease applications, with 58 new bottom leases and 48 new water column leases in 2019, up from zero new leases of either category in 2009 (Lopez, 2020). Trends of growth in oyster aquaculture are also shown in other East Coast states such as New Jersey (Calvo, 2018), Massachusetts (Massachusetts Division of Marine Resources, 2020), and Maine (Cole et al., 2017) to name a few.

Oyster aquaculture expansion in the Gulf of Mexico was delayed compared to the East Coast but is now growing across all five Gulf states. The first off-bottom oyster farms in the Gulf of Mexico were established in Alabama and Louisiana in 2009. In 2016, that number grew to 14 commercial oyster farms operating across 18.1 leased acres in Alabama (Grice and Walton, 2017). As of 2019, there were 21 commercial oyster farms operating across 40 leased acres, which produced 2.4 million single market oysters (Grice and Walton, 2020). Mississippi has seen similar expansion of oyster farms over recent years due to the addition of their Off-bottom Oyster Aquaculture (OBOA) program that started in 2018, which has produced 24 farmers working across 51 leased acres and led to 480,000 farmed oysters sold (Rider, 2020). According to data provided by the University of Florida Institute of Food and Agricultural Sciences (UF IFAS) Florida Aquaculture Surveys website, production of farmed Eastern oysters in Florida rose from 2.9 million to 4.5 million oysters from 2016 to 2019 (see <https://shellfish.ifas.ufl.edu/industry/florida-aquaculture-survey/>). In 2019, Texas passed legislation to legalize oyster farming within state waters which will allow even more expansion of Eastern oyster aquaculture. Currently, off-bottom oyster farming continues to spread to new areas throughout the Gulf of Mexico through education, outreach, and technological advances.

Off-Bottom Oyster Farming

Common aquaculture practices utilized in commercial oyster farms fall into one of two categories: on-bottom and off-bottom. On-bottom oyster aquaculture is a heavily used practice in the mid-Atlantic states (Thomas et al., 2019) and Pacific Northwest (Toba, 2002), as well as the Gulf of Mexico, albeit to a lesser extent (Walton et al., 2013a). On-bottom aquaculture has a long history in the Pacific Northwest that originally relied on the setting of wild oyster seed on artificially placed substrates, but has since evolved with the expansion of hatchery technology (Toba, 2002). Hudson, 2019 mentions that extensive spat-on-shell production is still a viable economic force in Virginia (\$1.4 million farm gate value), with these oyster products mainly supplying the shucked meat market. While still the product of aquaculture practices, this sort of large-volume oyster production has the advantage of reduced labor due to oysters being planted directly on-bottom or in bottom cages (Hudson, 2019; Toba, 2002).

While on-bottom farming is typically less labor intensive, it may not be a practical method at every site due to predation pressures, bottom substrate, and biofouling accumulation (Archer et al., 2014; Thomas et al., 2019; Walton et al., 2013b). This has led the industry to search for new oyster grow-out methods, which has been found in off-bottom farming gear. Off-bottom oyster farming is “the culture of oysters in some type of mesh container (basket, bag, cage, etc.) that is held above the seafloor”, as described by Walton et al., 2012b, or otherwise growing oysters utilizing any method that elevates them within the water column. While off-bottom production methods may vary in gear type, they all provide the added benefit of optional air-drying, which reduces biofouling and can be an extremely important part of the production process (Mallet et al., 2013; Walton et al., 2012b).

Oyster Performance

In the simplest terms, oyster performance in an aquaculture setting can be defined by a few main characteristics: growth and survival. These two metrics are self-explanatory, where good growth paired with high survival leads to high yield, which is typically a universal goal of any farming practice regardless of the species being grown. Aside from these base metrics, there are a few additional performance metrics that are especially applicable to off-bottom oyster aquaculture, including oyster appearance, cleanliness, meat condition, and product consistency, where market demands may place a premium on metrics of quality.

Importance of Appearance

When thinking of high-quality oysters intended for the half-shell market, the food industry quote “*you eat first with your eyes*” comes to mind immediately. The importance of presentation cannot be stressed enough in regards to off-bottom oyster farming, with shell shape being the primary means by which most half-shell products are visually judged (Leavitt et al., 2017; Mizuta and Wikfors, 2018; Sink et al., 2016; Thomas et al., 2019; Walton et al., 2013a). While the ideal shape of an oyster may seem like a subjective topic, there have been successful efforts to quantify aspects of shell shape that the majority of wholesalers and consumers can agree on (Brake et al., 2003; Thomas et al., 2019). Many Eastern oyster studies have adopted or used a variation of the method used in Brake et al., 2003 to determine a threshold that would deem an oyster ‘good’, which was set at >0.25 depth (largest distance from valve to valve) to length (distance from hinge to bill) ratio and >0.63 width (largest distance perpendicular to length) to length ratio. Thomas et al., 2019 used a similar index, but instead used all three measurements, with a ratio of height (in this case, distance from hinge to bill) to length (largest

distance perpendicular to height) to width (largest distance from valve to valve) of 3:2:1. These methods emphasize the importance of cup and fan ratios, where deeper cups paired with wider fans produce the most desirable, highest quality oysters.

Condition Index

A method commonly used that is intended to quantify oyster meat production in comparison with shell cavity volume is condition index. This method consists of weighing intact oysters, then shucking their meats into numbered tins that are placed in a drying oven for 48 hours and allowing the shells to dry at room temperature in matching numbered dishes for 48 hours, subsequently weighing the dry tissue and dry shell and using those numbers to calculate the condition of the oyster's meat (Abbe and Albright, 2003; Abbe and Sanders, 1988; Lawrence and Scott, 1982). This may then be compared to shell shape in order to better quantify the importance of shell shape in producing high quality meats. It is important to note that condition may vary throughout the year depending on environmental stresses, disease impacts, or spawning (Abbe and Sanders, 1988).

Product Consistency

Producing one 'perfect' oyster may be a stroke of luck, but consistency is key when raising oysters for the half-shell market. Oysters grown on bottom are known to exhibit variation in shape, and therefore are typically bound for the shucked oyster market (Cheney, 2010; Walton et al., 2013a). Off-bottom oyster farming, however, has shown to provide reduced variation in oyster growth, leading to a more consistent, uniform product (Cheney, 2010; Thomas et al., 2019; Walton et al., 2012b; Walton, 2014). While off-bottom grow-out systems may reduce

growth (Leavitt et al., 2017), the quality of the product will be improved, therefore driving a higher price-point and aiding in branding efforts (Sink et al., 2016; Walton et al., 2013a). Importantly, off-bottom oyster farming also offers several opportunities for oyster farmers to affect oyster performance. Some of these factors are selected by the farmer (e.g., stocking density, culture method, etc.), but many are environmental factors – which may very well interact with the farmer’s decisions and methods. The main factors affecting performance are reviewed below.

Site Selection

One of the primary factors that affects the performance of off-bottom oyster aquaculture is site selection. With the expansion of oyster aquaculture throughout the Gulf and Atlantic coasts of the United States, farm sites with a wider variety of environmental conditions have been established or are being considered. Universal factors impacting site selection for oyster farming purposes include: 1) classification of the area as an aquaculture use zone in applicable states or site approval via permitting through state and federal agencies; 2) year-round environmental factors such as salinity and other freshwater influence, water temperature, algae production for food supply, pollutant influence, and biofouling prevalence; and 3) physical attributes including average depth, tidal fluctuation, substrate, site accessibility, and site security (Flimlin et al., 2010; Wallace, 2001; Walton et al., 2013a). The order of importance for the factors listed above varies among states and growers. For example, oyster farmers in Florida must select a site that has been predetermined as an aquaculture use zone by the Florida Department of Agriculture and Consumer Services (FDACS) Division of Aquaculture (FDACS Division of Aquaculture, 2020), whereas farmers in North Carolina are allowed to select any area

they believe to be conducive for aquaculture, which must then be certified through several state and federal agencies (Lopez, 2020). In this case, the Florida farmer is given a list of areas for potential leasing and must use the other environmental and physical factors secondarily in selecting a site, where the North Carolina farmer puts these factors into consideration long before applying for a lease in a given area.

Environmental Factors

As mentioned above, salinity and temperature (averages and ranges) are two of the most important factors to consider when selecting a site for oyster farming. There have been numerous studies conducted on the interactive effects of salinity and temperature on the growth and survival of Eastern oysters which have found that extreme conditions of one of the two parameters leads primarily to reduction of growth, and extreme conditions of both ultimately leads to decreased growth and increased mortality (Gunter, 1955; La Peyre et al., 2016; Leonhardt et al., 2017; Lowe et al., 2017). Lowe et al., 2017 observed that temperature extremes less than 10° C and greater than 30° C led to a reduction in growth in Louisiana oysters, and extended periods of low salinities of less than 5 ppt led to growth reduction and increased mortality. Increasing percentage of days at “high salinity” of greater than 15 ppt was also a stressor in this study, with the combination of high temperature and high salinity leading to higher oyster mortality than low salinity and high temperature, although both had a major impact on growth and survival (Lowe et al., 2017).

Salinity

Optimal salinity conditions for Eastern oysters may be region dependent (Kraeuter et al., 2007; La Peyre et al., 2016). For example, near Deer Island, Mississippi, it is reported that optimal salinity for oyster growth sits between 15 and 30 ppt (Rider, 2020) and in North Carolina, oysters are said to thrive at salinities above 20 ppt (Swartzenberg et al., 2005). In contrast, Lowe et al., 2017 described the optimal salinity range for growth in Louisiana oysters as 10.7 to 16.1 ppt, likely due to the long-term influence of freshwater inflow from the Mississippi River. While these ranges are not totally exclusive of one another, temporal salinity fluctuations at a given site should be assessed prior to selection of that site for oyster aquaculture.

Aside from optimal growth, it has been documented that infection intensity of both *Haplosporidium nelsoni* (MSX; where present) and *Perkinsus marinus* (Dermo) parasites is correlated with salinities greater than 15 ppt (Burreson and Ragone Calvo, 1996; Ford and Haskin, 1988). Increased infection rates of either of these parasites results in increased mortality over time (Burreson and Ragone Calvo, 1996; Ford and Haskin, 1982), however there is no evidence to support any widespread presence of *Haplosporidium nelsoni* in the Gulf of Mexico (Ford et al., 2011). Other concerns with salinity come from the influence of predation, where the flatworm *Stylochus frontalis* and Southern and Atlantic oyster drills *Stramonita haemastoma*, *Urosalpinx cinerea* are limited to waters with salinities above 15 ppt (Mississippi Department of Marine Resources, 2010; Swartzenberg et al., 2005).

Not all attributes of varied salinity present negative consequences, however. Many oyster farmers throughout the Gulf and Atlantic Coasts grow their products for a niche market, where oysters are typically sold by count for the half-shell market instead of by weight or volume as is

traditionally done with wild harvested oysters (Walton et al., 2013a). With the traditional wild harvest market, oysters are mainly bound for shucking houses to provide for canned meat products, with emphasis on shucked meat volume (Walton et al., 2013a). The half-shell market tends to value uniqueness of product, with emphasis on oysters grown in a given state, estuary, or tributary (ex. Rappahannock River, Virginia; Wellfleet Bay, Massachusetts; Apalachicola Bay, Florida; etc.), and branding is especially useful for farmers to maximize the marketability of their product (Sink et al., 2016; Wallace, 2001; Walton et al., 2013a). Environmental and physical factors that affect growth and survival of oysters at any given farm site may vary widely between estuaries, and even between tributaries within the same estuary system.

One important- if not the most important- factor emphasized by the half-shell market is taste, which may be heavily dictated by the salinity of the farm site (Sink et al., 2016). Naturally, both raw bars and consumers enjoy having a wide range of taste to choose from, with some patrons preferring sweeter oysters raised in lower salinity waters of estuarine tributaries, and others craving a strong brine flavoring influenced by proximity to the Gulf of Mexico or Atlantic Ocean. This places an emphasis on not only where the oysters were grown, but also when they were harvested, as salinity may vary widely at any given farm site due to patterns of rainfall, tidal fluctuations, and sustained wind direction.

Temperature

A factor that seems to show less variation across regions is water temperature. Sources throughout the Gulf and Atlantic states claim that optimal growing conditions typically fall between 10°C and 30°C (Lowe et al., 2017; Rider, 2020; Swartzenberg et al., 2005). It was observed in Casas et al., 2018 that oysters sourced from New Brunswick, Canada, and Louisiana,

USA showed that clearance rate, or volume of water completely filtered of particles per unit of time (i.e., feeding), was significantly higher at 20°C and 30°C than at 10°C and oysters remained open for a greater percentage of time at 20°C when compared with the other two temperature regimes, regardless of geographic origin.

The Gulf of Mexico typically allows for a year-round growing season for oyster farmers due to increased average water temperatures across the entire year coinciding with the optimal temperature range for oyster growth. This leads to faster production time, with the majority of off-bottom farmed oysters reaching harvestable size (3 inches) in 12 to 15 months from spawn, although a portion may be harvestable by 9 months (Walton et al., 2013a). In comparison, in the Gulf of St. Lawrence, Canada, off-bottom farmed oysters may reach this harvestable size in under four years, with growth taking place from May to September and peak growth in May and June (Doiron, 2008). Off-bottom grow-out periods in the Chesapeake Bay are said to get oysters to this specified market size at 12-14 months, with growth mostly taking place between April and November when water temperatures are well above 10°C (Luckenbach et al., 2008; NOAA, 2016) It should be noted, however, that no matter what state or estuary in which the oysters are farmed, there may be considerable variation in water temperature between systems and across years due to factors such as yearly rainfall and yearly average air temperature (Gillanders and Kingsford, 2002; Vroom et al., 2017).

Hydrodynamics

Another factor affecting the quality of a given grow-out site is flow, specifically freshwater inflow. The freshwater inflow of a site has several attributes that can provide both benefits and detriments under different conditions. Under good, stable conditions, these inflows

are the source of nutrients for phytoplankton production and maintain relatively constant salinity within the system (Campbell and Hall, 2019; Randall and Day, 1987). In unstable conditions freshwater inflow may be too high, resulting in long periods of lowered salinity and subsequent oyster stress or mortality (La Peyre et al., 2016) and increased turbidity, which in turn reduces primary production due to high light attenuation through the water column (Randall and Day, 1987). Low freshwater inflow presents its own set of problems, resulting in lowered production due to nutrient deficiency (Randall and Day, 1987) and increased salinity leading to higher impacts of disease and predation mortality (La Peyre et al., 2016). Aside from freshwater inflows, natural current should also be considered. Higher currents provide the benefits of increased phytoplankton density, allow for mixing of the water column which provides dissolved oxygen as well as consistent temperature and salinities, and carries away waste expelled from oysters (Campbell and Hall, 2019). Just like freshwater inflow, too much or too little current could have negative impacts including increased wear on gear due to strenuous resistance to currents or vertical stratification followed by periods of hypoxic conditions, respectively (Campbell and Hall, 2019).

Off-Bottom Gear Effect on Oyster Performance

The performance of oysters in an aquaculture setting is highly dependent on the gear selected. On-bottom culturing methods may produce high oyster volume, but are consistently outperformed by off-bottom culture in terms of oyster growth, survival, shell shape and appearance, and meat condition (Archer et al., 2014; Thomas et al., 2019; Walton et al., 2013b). While off-bottom culturing methods generally outperform on-bottom methods, there can be differences between these metrics even between off-bottom gear types.

Much research has been done assessing the effect of gear type on oyster performance in an aquaculture setting. Mallet et al., 2013 assessed oyster growth over two years in New Brunswick, Canada between two off-bottom gear types, floating bags versus rope grown oysters (which consists of oysters glued to ropes that run horizontally between a metal structure with PVC floats to keep the structure at the water's surface), and found that across their three sites (inshore, middle, offshore), oysters in the rope grown gear type outperformed the floating bags in both growth and weight gain, no matter the site. Archer et al., 2014 measured the difference between average survival, daily growth rate, and condition index between oysters in floating cages and bottom cages at five sites in Southeastern Massachusetts, and found that the floating cages outperformed the bottom cages in all these categories at most, but not all, of the sites studied. Thomas et al., 2019 compared growth and shape of oysters between floating cages, rack and bag, and bottom cages across five months at two sites in the Maryland portion of the Chesapeake Bay, determining that the floating cage treatment promoted the greatest gain in weight and overall shape in comparison with the other two treatments. Finally, Walton et al., 2013b assessed the effect of gear type (bottom cage, adjustable longline system, floating bag, floating cage) on oyster survival, growth, shape, and condition index at one farm site in Alabama, reporting that survival was significantly better in the off-bottom treatments compared to on-bottom cages due to predation. Additionally, growth was the worst in the bottom cages and comparable between the other off-bottom gear types, oyster shape was not significantly affected by gear type, and condition was highest in the adjustable longline and floating cage systems (Walton et al., 2013b).

Adjustable Longline System

Types of off-bottom gear that are commonly used throughout the United States can be broken down into two categories: suspended and floating gear. Suspended gear is that which is attached to permanently fixed infrastructure, such as lines strung between pilings intentionally set for oyster farming or pre-existing pilings from a dock, for example. A commonly used example of this type of grow-out operation is the adjustable longline system (ALS). This system is comprised of a line that is tensioned between two pilings with supporting structures (2" PVC pipes, for example) placed at even intervals along the line between the two pilings, which provide clips that allow for vertical adjustment of the line, in turn allowing for periodic desiccation of grow-out gear (Davis et al., 2012a). As reviewed in Davis et al., 2012a, aside from the ability to air dry gear, this system allows for natural tumbling of oysters due to wave action, provides hurricane preparedness via the option to lower the line to the lowest vertical setting on the posts while still keeping oysters off the bottom, and allows for easy inventory management. Negative attributes of this gear type involve cost of piling installation and gear and equipment pricing (i.e., PVC structural pipes and clips, longline baskets, longline material), visible structures above the water line such as pilings and structural pipes, and limitation to shallow areas for proper operation of this equipment (Davis et al., 2012a). Areas with major tidal swings may also be less ideal for this fixed gear type, as severe low tides may affect site accessibility and expose oysters to air-drying more frequently than desired, and severe high tides may affect the workability of the gear and reduce the ability to air dry when desired.

Floating Bag System

In contrast, floating gear comes in all manners of choices for equipment. The options for floating gear are constantly expanding, with companies new and old making variations to make their product unique and answer the demands of the industry, but the majority of floating gear is comprised of either floating bags or floating cages (see Walton et al., 2013b for comparisons between off-bottom and on-bottom gear types). Floating bags consist of exactly what they sound like: a mesh bag made of plastic with either foam floats or plastic floats filled with air attached on one side, which are strung in succession along a main line that is anchored into the substrate at either end (Walton et al., 2012a). This system provides the benefits of being highly customizable, works in a wide range of depths, easy gear desiccation by simply flipping the bag so the floats are facing down, and allows for natural tumbling, but depending on the floats used, oysters may stay submerged even when the bag is flipped to the air-dry position, negating the positive effects of air drying on the oysters themselves, and a hurricane plan will need to be created at a site-to-site basis (Walton et al., 2012a).

Floating Cage System

Finally, there is the floating cage grow-out system. While there are many manufacturers that produce floating cages, the general structure is the same: a rigid metal frame with a closure on one side that provides access to compartments which offer space for two, four, or six mesh bags to be inserted, topped with two large, air-filled plastic pontoons (Davis et al., 2012b). Similar to the floating bag system, these cages are tethered to a main line that is anchored at either end and cages can be flipped up, allowing the pontoons to hold the cage out of the water, but in this case both the bags and oysters are exposed to air-drying. Other benefits to utilizing the

floating cage system is it is effective in any depth of water and a variety of sites (so long as anchors can be successfully installed into the substrate), cages are easily movable once in the water due to the flotation provided by the pontoons, and the pontoons may be filled with water in the event of a hurricane which, if done properly, allows the pontoons to hold the cage and oysters inside off the bottom while anchoring the cage in place, reducing the chance of losing gear or oysters in severe weather (Davis et al., 2012b). Floating cages do have cons, however, which include increased labor to effectively desiccate gear compared to the other off-bottom gear types, decreased capacity per acre due to needed spacing of cages both on a mainline and between mainlines to eliminate tangling, and most commonly, pontoon failure due to a puncture or leaky end caps, resulting in a half or fully sunken cage that requires additional labor and expenses to fix (Davis et al., 2012b).

Ploidy

Another factor affecting the growth of Eastern oysters comes in the form of genetic material, most notably in the number of sets of chromosomes the animal possesses. In the wild, oysters obtain one set of chromosomes each from the haploid sperm and egg at the time of fertilization, resulting in a diploid organism. Polyploid organisms are those that contain more than two sets of chromosomes, which was successfully induced in Eastern oysters via the treatment of fertilized eggs with cytochalasin B, hereafter referred to as CB (Stanley et al., 1981). Further exploration into this novel topic was done showing that triploid oysters grew faster than their diploid counterparts (Stanley et al., 1984), which provided the framework for continued research into the topic of producing polyploid oysters (Barber and Mann, 1991; Guo et al., 1996, 1994). Some of the main disadvantages to inducing triploidy in oysters using the above

method is that CB is highly toxic, which poses health risks to the person administering the drug and raises concerns over treating oysters intended for food production with it, and it is also isn't 100% effective in inducing triploidy (Guo et al., 1994).

These issues led to the search for a better method of producing triploids, which was found by Guo et al., 1996. While Guo et al., 1996 evaluated Pacific oysters, the process works the same for Eastern oysters (Guo et al., 2002). It was shown that 100% triploid offspring can be produced by mating tetraploid oysters with diploid oysters, effectively reducing the concerns for efficiency and reducing the need for handling of CB in the hatchery (Guo et al., 1996). It should be noted, however, that to produce viable tetraploids, CB is administered after fertilization between triploid eggs and diploid sperm, effectively creating a tetraploid oyster that may be later used to create triploids as described above (Guo and Allen, 1994).

Growth and Survival of Triploid Oysters

The main benefit provided by triploid oysters is growth, which has been shown to be significantly greater in triploids when compared to diploid counterparts in both shell height and overall weight (Barber and Mann, 1991; Stanley et al., 1984; Walton et al., 2013b). This faster growth has been mostly attributed to the inhibition of gametogenesis caused by the triploidy condition, which allows the oyster to exert that energy surplus as overall growth (Barber and Mann, 1991; Guo et al., 1996; Mizuta and Wikfors, 2018). Naturally, increased growth rate has major implications for aquaculture practices, allowing farmers to get more product to the market in a shorter period of time. While triploid oysters may not possess any significant disease resistance or immunity (Barber and Mann, 1991; Degremont et al., 2012), their increased growth

may allow them to reach harvestable size before disease can affect their growth or survival (Barber and Mann, 1991).

Comparisons of survival between diploid and triploid oysters, however, are much less clear. There are conflicting studies that report differences in survival between the two ploidies, such as Degremont et al., 2012 and Wadsworth et al., 2019. Degremont et al., 2012 compared the growth, yield, and survival between diploids and triploids in fixed bags attached to rebar racks across three sites in the Chesapeake Bay, reporting that across all sites triploids had 34% less cumulative mortality in comparison to diploids while still outperforming the diploids in shell height, weight, and yield. In contrast, Wadsworth et al., 2019 compared the growth, condition index, and mortality between diploids and triploids using the adjustable longline system across four sites in Alabama and reported that triploids exhibited significantly higher mortality across all four sites while still outperforming diploids in terms of growth and condition. Interestingly, environmental data from this study showed that triploids were more sensitive to extended low salinity (less than 5 ppt) at one site, but the cause of increased mortality at the other sites was considered to be unknown (Wadsworth et al., 2019). This alludes to mortality being site specific, with lower, but still significant, differences in mortality between diploids and triploids at the Grand Bay site (Wadsworth et al., 2019) where ploidy was shown to have no effect on survival in a previous study (Walton et al., 2013b). An important thing to note in Walton et al., 2013b is the performance of triploid oysters was modified by gear, where triploids grown in bottom cages produced poor results while those grown using the adjustable longline system excelled, furthering the importance of gear type on selection of not only farm site but ploidy. Due to unknown causes of mortality events in areas such as the northern Gulf of Mexico, however, it is recommended that farmers entertain the idea of using both ploidies in their farming operations,

hedging their production against mass mortality events seen in triploid oysters (Wadsworth et al., 2019).

Selective Breeding

Aside from ploidy, selective breeding has been utilized in oyster hatcheries for multiple purposes including disease resistance and accelerated growth (Allen et al., 1993; Proestou et al., 2016; Ragone Calvo et al., 2003). The theory behind this relaxed method of genetic selection is fairly simple, where individuals that exhibit faster growth rates or those that survive a disease outbreak are spawned together, ideally passing down those targeted genes and creating genetically superior offspring (Allen et al., 1993). The simplicity of this method allows for any hatchery to implement selective breeding, especially for increased growth selection as no determination of disease presence must be implemented. This in turn gives farmers broader access to superior genetic lines throughout the aquaculture industry. Both Allen et al., 1993 and Proestou et al., 2016 touch on the fact that even with genetically superior lines, the perceived outcome of either accelerated growth or disease resistance is the product of interaction between oyster genetics and their environment, where the oyster in question may have stellar genetics but be placed in a lesser quality environment, leading to poor performance. Factors affecting the expression of superior genes in selectively bred oysters may be environmental, such as salinity and temperature, or products of the farming environment, including stocking density and biofouling (Proestou et al., 2016). Therefore, farmers should take into account not only the brood line of seed oysters they are purchasing for grow-out but also evaluate the quality of their site and growing practices to ensure those oysters can reach their full performance potential.

Stocking Density

The effect of density on wild Eastern oyster growth has been extensively documented, with crowding causing oysters to grow long yet narrow (Galtsoff, 1964). This idea isn't only reserved for wild oysters, however. In off-bottom oyster aquaculture, stocking density has been shown to have an impact on the growth and condition of both Eastern and Pacific oysters (Chávez-Villalba et al., 2010; Davis, 2013; Gamble, 2016; Marshall and Dunham, 2013). In Pacific oysters, as density is increased there is a reduction in shell shape and cup depth (Marshall and Dunham, 2013), as well as condition, ultimately resulting in a less desirable product (Chávez-Villalba et al., 2010). For Eastern oysters, Davis, 2013 observed that oysters grown at higher stocking density using the adjustable longline system had greater shell height by the end of the experiment, but this came at the cost of reduced cup and fan ratios as well as reduced condition, which again make this an inferior product when compared to oysters grown at lower stocking densities. Gamble, 2016 observed a significant impact of high oyster density grown in floating cages on decreased cup depth and decreased condition, specifically in triploid oysters. These results fortify the importance of stocking density in regard to producing the highest quality product possible, furthering the delicate balance between production volume and product quality.

The ideal stocking density in off-bottom oyster farming is that which allows for space within the container for oysters to be naturally tumbled and obtain the high quality shape and condition that the half-shell oyster market demands (Mizuta and Wikfors, 2018). This differs across the common gear types due to the variety of sizes offered for oyster farming equipment. An effective grow-out stocking density of 150 oysters per bag is recommended for the floating cage system, with each cage holding two, four, or six bags and runs typically containing ten cages (Davis et al., 2012b). Similarly, the floating bag system also supports a grow-out stocking

density of 150 oysters per bag, with a run being constituted of 200 bags (Walton et al., 2012a). Grow-out density for the adjustable longline system is recommended as half of the other two, with 75-100 oysters per basket, 96 to 108 baskets per line, and two lines per run (Davis et al., 2012a; Glen Chaplin, pers. comm.). There is no evidence to support density having any impact on biofouling accumulation in off-bottom oyster farming (Davis, 2013; Gamble, 2016; Marshall and Dunham, 2013).

Impact of Biofouling

Unwanted organisms that congregate on oysters and farming gear are termed biofouling, and are a considerable headache for oyster farmers to manage, imposing additional labor cost and time investment (Adams et al., 2011; Doiron, 2008; Flimlin et al., 2010; Swartzenberg et al., 2005; Walton et al., 2013a). Examples of biofouling experienced in oyster farming include barnacle strikes, oyster overset, mussels, various species of algae (especially if raised in the upper portions of the water column), sea squirts, and fouling mosses (Doiron, 2008; Swartzenberg et al., 2005; Walton et al., 2013a). Aside from added labor, biofouling may also reduce water flow or increase competition for food resources within the grow-out container effectively reducing the amount of food available to the oysters inside, in turn decreasing overall growth and in extreme cases leading to mortality (Flimlin et al., 2010; Swartzenberg et al., 2005). Fouling that accumulates on the oysters themselves must be dealt with as well, and if improperly managed there could be serious impacts to the profitability of a farming operation, be it through further increased labor and time costs or through a buyer's reluctance to buy "dirty" or "unsightly" oysters, especially in the half-shell market (Adams et al., 2011; Swartzenberg et al., 2005; Walton et al., 2013a).

Not all aspects of biofouling are a negative, however. Dealteris et al., 2004 observed that given the additional surface area provided by submerged aquaculture gear for biofouling to accumulate, there was greater abundance of organisms and species richness sampled on or around the submerged aquaculture gear in comparison with native eelgrass *Zostera marina* habitat, with many of the organisms using the aquaculture gear like nursery habitat, as refuge from predation and a source of food. Small American lobsters *Homarus americanus* were often found within oyster gear and larger predatory fish were observed frequenting the study area including recreationally and commercially important species for Rhode Island, USA, such as striped bass *Morone saxatilis*, American shad *Alosa sapidissima*, and winter flounder *Pleuronectes americanus* (Dealteris et al., 2004). Similarly, it was observed in Louisiana and Alabama, USA, that off-bottom oyster farming gear provided valuable habitat for juvenile blue crabs *Callinectes sapidus*, with increased abundance and survival when compared to unvegetated bottom (Stewart, 2015). In addition to fouling organisms such as barnacles and sea squirts, O'Beirn et al., 2004 observed 45 taxa associated with Taylor floats containing oysters near Chincoteague Island, Virginia, USA, including several species of crabs, mollusks, and finfish.

Fouling Management

Fouling control is a universal issue for oyster farmers in the U.S. and often influences the selection of farm equipment used (Adams et al., 2011; Walton et al., 2013a). The most common method employed by oyster farmers is desiccation, or air-drying the grow-out gear and oysters alike for a prolonged period of time, which allows the oysters to close up tight and remain alive while the fouling organisms dry out and die (Flimlin et al., 2010; Swartzenberg et al., 2005; Walton et al., 2013a). While duration of air-drying may be site specific, it is recommended that

oyster farmers air dry their equipment once a week for a period of 24 hours to control fouling during the peak growing season, with exceptions during periods of extreme heat (air temperature of 35°C or higher) when desiccation should be limited to overnight, and frequency may be reduced during the winter months when fouling organisms grow slower (Walton, 2014). Once dried, the oysters and gear should be reintroduced to the water so oysters may resume feeding. Fouling that is managed by air drying farming equipment may act as a food source for other species of fish and crabs, further maximizing the environmental use of farm gear by other organisms (Flimlin et al., 2010). It is important to note, however, that frequent desiccation may have a negative effect on oyster growth, with more frequent air drying efforts leading to less time the oyster gets to feed and/or increased disturbance that may chip away new growth, ultimately slowing overall growth in comparison with those air dried less frequently (Gamble, 2016).

One of the other main risks associated with air drying of oysters is the impact of elevated temperatures on *Vibrio* levels inside the oyster, which is shown to significantly elevate the density of these bacteria (Grodeska et al., 2019; Kinsey et al., 2015). The two naturally occurring species of greatest concern for public health associated with eating raw oysters are *Vibrio vulnificus* and *Vibrio parahaemolyticus*, with the former being the cause of 95% of seafood related deaths in the United States, boasting a mortality rate of 50%, and the latter causing temporary and non-fatal gastroenteritis (Blackwell and Oliver, 2008; Oliver, 2013). Luckily for the oyster industry, *Vibrio* levels typically return to ambient conditions seven days post-resubmersion and oysters are then safe to harvest for consumption (Grodeska et al., 2019; Kinsey et al., 2015). Public health regarding shellfish products is regulated by agencies such as the National Shellfish Sanitation Program (NSSP) which is governed by the U.S. Food and Drug Administration (FDA), and shellfish sanitation offices under supervision of state public health

departments (see <https://www.fda.gov/food/federalstate-food-programs/national-shellfish-sanitation-program-nssp> for more information).

Other methods of controlling biofouling include power washing, tumbling, dipping the oysters themselves in either brine or freshwater baths, or manual removal of fouling by brushing or scraping (Flimlin et al., 2010; Hood et al., 2020; Mizuta and Wikfors, 2018). Power washing of equipment is typically done post-harvest or after transferring oysters to a clean container, as to clean the dirtied container for future use. Gear containing oysters may also be cleaned this way, however the long-term effects of this rough handling are not clear, where fouling on the oysters themselves may also be removed but damage to the shell may occur. Brine or freshwater dips are effective in killing fouling organisms without adverse effects to the oyster provided they aren't left for extended periods of time (Flimlin et al., 2010; Mizuta and Wikfors, 2018). Manual removal via scraping or brushing may be feasible for a very small farm dealing limited quantities of product, but with increases in operation size, this method likely becomes ineffective in both time and labor costs.

Tumbling

Tumbling may be the most dynamic of all the biofouling controls listed here, as it can be broken down into two categories and provide numerous services at once for oyster farms. Mechanical tumbling consists of placing oysters in a rotating cylindrical tube set at a shallow angle which allows oysters to 'tumble' from the opening to the exit. This tube typically has nozzles that spray freshwater onto oysters as they advance through the tube and holes cut out throughout the tube in various sizes. This provides the services of: 1) Cleaning of biofouling off oysters through collision with the tube and other oysters aided by freshwater spray; 2) size

sorting of oysters via various-sized holes that are cut out of the tube; 3) chipping away new growth which leads to deeper-cupped, higher-quality oysters (Mizuta and Wikfors, 2018; Ring, 2012). While these services are highly useful in oyster farming, there are also drawbacks to mechanical grading. Oysters must be removed from the water and taken to the area that the tumbler is located, which is typically on land or on large, sturdy platforms since tumblers are bulky machines that require electricity to function, and then restocked post-tumble, which may be labor intensive depending on the location of the tumbler in relation to the farm (Mizuta and Wikfors, 2018).

The other category of tumbling could be deemed natural tumbling, where oysters are placed within grow-out systems that intentionally allow for movement through wave energy or tidal fluctuations which allows the environment to do the labor of tumbling for the farmer (Leavitt et al., 2017; Mizuta and Wikfors, 2018). As a result, oysters collide with one another throughout the grow-out period, which provides similar services of biofouling control and deepened cup as compared to the mechanical tumbling (Leavitt et al., 2017; Mizuta and Wikfors, 2018). As is mentioned in Leavitt et al., 2017, increased tumbling does reduce growth but the amount of natural tumbling experienced by the oysters may be highly variable for different gear types across different sites depending on tidal fluctuations and wave energy, in turn emphasizing that selection of these grow-out methods and subsequent product quality is likely very site specific (Flimlin et al., 2010; Gamble, 2016). These systems of natural tumbling are likely to still be paired with air drying, as the gear itself will still accumulate biofouling if left submerged.

Wave Action

One major factor that may have a substantial impact on oyster performance yet is lesser studied is wave action. Across three sites, Mallet et al., 2013 observed that rope grown oysters exhibited relatively similar growth, where the floating bags produced “widely different growth performance”, specifically in the offshore zones where the rope grown oysters performed best. The authors point to this difference in growth likely being from the “avoidance of shell erosion associated with wave action”, since the rope grown oysters are fixed in place and not colliding with one another, and are also assumed to have the same access to food resources (Mallet et al., 2013). Biofouling was eliminated as a potential impacting factor on growth as it was noted in previous trials that oysters in cleaned bags grew the same as those in fouled bags in similar areas to those studied here, and density was held constant in this study, further pointing to wave action being the main driving factor in suppressing oyster growth in floating bags (Mallet et al., 2013). For oyster farmers in Canada, the growing season is already short (Doiron, 2008), so any further reduction in growth could potentially cause some farming operations to shut down due to loss of revenue, as is mentioned in the conclusion of Mallet et al., 2013.

Though tested in a different fashion, Thomas et al., 2019 came to a similar conclusion about wave action and gear type, except its effect on oyster growth has different implications for oyster farmers in the Chesapeake Bay. The effect of a five month ‘finishing period’ was tested in this study, with the results indicating that the oysters in the floating cage system achieved significantly higher weight and the most optimal shell shape in comparison to the other gear types, which were both attributed to the increased tumbling of oysters provided by the floating cages (Thomas et al., 2019). In this case, however, there is the possibility of increased primary production in the upper portions of the water column that could be contributing to the

performance boost of floating cage raised oysters, which has been claimed to be another benefit to off-bottom oyster culture (Paynter and Dimichele, 1990; Walton et al., 2012b). If so, this is a very important detail for oyster farmers, as increased food availability and subsequent increased growth could offset the potentially harmful impacts of increased wave action, resulting in an oyster that continues to effectively grow while being groomed into the ideal shape for the half-shell market.

Conclusion

While these studies, along with a handful of others (i.e. Brake et al., 2003; Davis, 2013; Gamble, 2016; Mizuta and Wikfors, 2018; Swartzenberg et al., 2005) mention wave action as a proposed reason for differences in off-bottom oyster performance, few studies have set out with the specific goal of determining the impact of wave action in these settings. In comparison, other culturing practice factors such as grow-out gear type, stocking density, biofouling control, and ploidy have been extensively studied in an off-bottom aquaculture setting resulting in best practice recommendations for these criteria, as presented in the material above. This signifies that more research is needed specifically targeting the effect of wave action and its interaction with other culturing factors on oyster performance in an off-bottom aquaculture setting to provide better management recommendations to current and future oyster farmers throughout the United States.

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CHAPTER TWO:
EFFECT OF WAVE ACTION, BIOFOULING CONTROL, AND DENSITY ON THE
PERFORMANCE OF EASTERN OYSTERS (*Crassostrea virginica*)

1. Introduction

Wild stocks of Eastern oysters *Crassostrea virginica* have shown marked population declines throughout recent history in many areas with historic oyster fisheries, such as the Chesapeake Bay (National Research Council, 2004), Apalachicola Bay (Havens et al., 2013), and several other areas due to a wide range of anthropogenic and environmental causes (i.e. Ford, 1997; MacKenzie Jr., 1997; Park et al., 2014). While wild oyster fisheries have declined over time, the oyster aquaculture sector has grown throughout the United States (National Marine Fisheries Service, 2020; Olin, 2011). The East Coast has long been a producer of farmed oysters, with many states showing continued growth currently (Calvo, 2018; Cole et al., 2017; Hudson, 2019). The Gulf of Mexico, however, has shown delayed expansion of aquaculture efforts, likely due to more recent wild oyster population declines (Havens et al., 2013; Park et al., 2014).

Oyster aquaculture throughout much of the Gulf of Mexico is focused on off-bottom grow-out methods (where oysters are raised in some type of container that is suspended or floating and not in direct contact with the seafloor). On-bottom oyster production (where oysters are grown directly on the seafloor) faces numerous hurdles along the Gulf Coast, including high predation pressure, sedimentation, and biofouling accumulation (Archer et al., 2014; Thomas et al., 2019; Walton et al., 2013b). Off-bottom oyster production methods have the added benefit of increased oyster performance which encompasses a variety of criteria, including growth, survival, shell shape, oyster cleanliness, and meat condition (Archer et al., 2014; Thomas et al., 2019; Walton et al., 2013b). These are all important production factors for off-bottom oyster farmers due to the destination of their end product, which is primarily the premium half-shell oyster market (Brake et al., 2003; Walton et al., 2013a).

Within off-bottom oyster production, careful site selection is key to maximizing oyster performance (Wallace, 2001). Environmental factors such as salinity and temperature have been shown to have interactive effects on oyster performance, with extreme combinations leading to major impacts on oyster growth and survival (La Peyre et al., 2016; Lowe et al., 2017). Flow at a given site is also important, as it may impact both salinity and temperature depending on weather patterns (particularly flood or drought conditions), phytoplankton abundance for oyster nutrition via nutrient transportation, and water column mixing that promotes necessary concentrations of dissolved oxygen (Campbell and Hall, 2019; La Peyre et al., 2016; Randall and Day, 1987). Once a site is selected, farmers then make choices throughout the production cycle that have substantial impacts on their final product. One major decision is gear selection, where different types of off-bottom grow-out gear have been shown to have significant impacts on oyster growth, shell shape, and meat condition (Mallet et al., 2013; Thomas et al., 2019; Walton et al., 2013b).

In addition to gear selection, factors such as ploidy and seed source have been studied extensively, where triploid oysters exhibit significantly greater growth in shell height as well as overall weight (Barber and Mann, 1991; Stanley et al., 1984; Walton et al., 2013b), and selective breeding can produce seed oysters with higher disease resistance and increased growth rates (Allen et al., 1993; Proestou et al., 2016). Stocking density has been shown to have a significant impact on shell shape and meat condition in both Pacific oysters *Crassostrea gigas* (Chávez-Villalba et al., 2010; Marshall and Dunham, 2013) and Eastern oysters (Davis, 2013; Gamble, 2016). In terms of overall cleanliness, biofouling control must be implemented to reduce the accumulation of unwanted organisms on both oysters and gear, which if left unchecked can cause additional labor costs, reduced water flow throughout the grow-out container (in turn

reducing growth), and reduced end-product value (Adams et al., 2011; Flimlin et al., 2010; Walton et al., 2013a).

While all these subjects have been extensively studied in an off-bottom oyster aquaculture setting, wave action is a topic that is often overlooked. Though mentioned as a possible influence on oyster performance in off-bottom oyster aquaculture in a handful of studies (Brake et al., 2003; Davis, 2013; Gamble, 2016; Mallet et al., 2013; Thomas et al., 2019), the explicit impact that wave action has on oyster performance has not been experimentally tested. The purpose of this study was to determine the impact wave action has on oyster growth, survival, and condition when paired with distinct density and biofouling control regime treatments at one high-energy site while holding gear type and ploidy constant. Treatments were selected to best replicate common grow-out practices and biofouling control strategies utilized by oyster farmers in the northern Gulf of Mexico. We hypothesized that oyster performance would be positively impacted by wave action manipulation and negatively impacted by increased density and lack of biofouling control.

2. Methods

2.1 Oysters

Oysters used for this study were spawned at the Auburn University Shellfish Laboratory (AUSL) in Dauphin Island, Alabama on April 29, 2020. Single-seed triploid oysters were selected for this study due to their common use in off-bottom oyster farming throughout the Gulf of Mexico. Triploid oysters were produced via mating of Louisiana Sea Grant GNL tetraploid sperm with eggs from a Gulf-wide mixed diploid line maintained by AUSL. Oysters were set on 200–500-micron micro-cultch and raised in a land-based nursery system prior to deployment in

1.5mm Intermas oyster flat bags (available from Ketcham Supply Co. Inc., New Bedford, Massachusetts, USA) in OysterGro® floating cages (OysterGro Aquafarming Systems, New Brunswick, Canada) at the Auburn University Research Farm located in Sandy Bay, Alabama. Once large enough, oysters used for this study were transferred to 6mm BST™ baskets (BST Oyster Supplies, Cowell, South Australia, Australia) and placed on an adjustable longline system at this same site before being collected and redistributed into treatment baskets for the initial deployment at the Mississippi Department of Marine Resources Commercial Aquaculture Park near Deer Island, Mississippi.

2.2 Site Selection

This study took place at one farm site provided by Mississippi Department of Marine Resources (MS DMR) in Mississippi Sound (30° 22' 5.7066" N, -88° 51' 3.1284" W), near Deer Island, Mississippi (see Fig. 1). Mississippi Sound is a wide, open stretch of water extending from its eastern border between Dauphin Island, Alabama and Cedar Point, Alabama, to its western border from Malheureux Point, Louisiana, to Half Moon Island, Louisiana, to Light House Point, Mississippi, and is bordered along the south by a chain of barrier islands with boundaries drawn between them (Eleuterius, 1978). Given that the nearest barrier island (Ship Island, Mississippi) is between 12.9-16.1 km South of the chosen farm site, this site frequently experiences high wave energy which was a major factor in its selection for this study. The farm site supplied for this experiment was oriented east to west, often experiencing wave action perpendicular to its orientation. Supplemental environmental data were provided by MS DMR via a remote set YSI EXO3 multiparameter sonde equipped with an optical ODO smart sensor and conductivity/temperature smart sensor (YSI, Yellow Springs, Ohio, USA). Readings were

taken every 30 minutes over the course of the day for water temperature, salinity, and dissolved oxygen throughout the experiment aside from times when the data sonde was shut down for maintenance or otherwise not actively recording data.



Figure 1. Map of study site, designated by the star, near Deer Island, MS. Maps obtained from Google Earth™ on February 16, 2021.

2.3 Experimental Design

The adjustable longline system (ALS) was utilized in this experiment, with oysters grown in BST™ Inter-Lock Mesh baskets and lines comprised of 5mm Bayco wire and 10.8mm outside-diameter Dripper tube available from BST™ and SEAPA™ (SEAPA Australia, Edwardstown, South Australia, Australia). The ALS is commonly used in off-bottom oyster farming and is comprised of a line that is tensioned between two pilings with supporting structures (termed ‘riser posts’, 2-inch PVC pipes are typically used) placed at even intervals along the line between the two pilings, which provide clips that allow for vertical adjustment of

the line (Davis et al., 2012a). In this case, the system from piling to piling was 100m long, with riser posts placed approximately 3m inside the starting piling and spaced 2.54m apart for the duration of the run, resulting in a total of 32 ‘bays’, or spaces between riser posts for baskets to be hung. Riser posts had holes drilled every 0.2m (~8 inches) for inserting pins upon which 3-inch PVC sliders with BST™ post riser clips rested, allowing for vertical adjustment of the line. While ALS typically have one line that can be adjusted, in this study to achieve independence among the replicate baskets, two adjustable longlines were installed and set at two static heights along the single run; the lower line was set sub-tidal to mimic the height at which an oyster farmer would typically set their lines for grow-out and the upper line was set at the highest vertical setting allowing each basket to be set at its experimentally assigned treatment without affecting adjacent baskets (Fig. 2). Oysters were assigned to one of twelve unique treatments, with eight replicates per treatment for a total of 96 baskets which were arranged in a completely randomized design along the line. The experimental design was a 3-factor fully crossed design: Wave Action (natural or dampened) x Biofouling Control (weekly, never desiccated, or power washed) x Stocking Density (low or high) for twelve total treatments.

Oysters were first deployed at the experiment site on July 30, 2020 in 6mm BST baskets and were subsequently transferred up to 12mm BST baskets during the first sampling on August 28, 2020. Sampling was conducted every six weeks after the first sampling date with final sampling efforts completed on March 2, 2021, once the majority of oysters reached the harvestable size threshold of 2.5-3 inches (63.5-76.2 mm). Prior to the initial deployment, a pre-sample of 101 oysters was taken consisting of 76 oysters for shell dimension measurements (length, width, height) and 25 oysters for preliminary condition indexing to provide an estimated

starting point for oysters of all treatments. Oysters were deployed at an average shell height of 28.58 (± 0.62 SE) mm.

During the first sample, it was noted that there was basket migration horizontally along the line resulting in equipment damage, so SEAPA™ StormBreaker 11mm clamp bearings were installed in early September 2020 to prevent this migration. Hurricane Zeta made landfall in southeastern Louisiana in late October 2020, resulting in minor basket loss and causing damage to the longline infrastructure that was promptly fixed the following week. In January 2021, it was noted that baskets were being lost after becoming dislodged from the line due to wear at the attachment points, so every remaining basket was secured to the line around the handles by zip ties. Desiccation was not implemented for two weeks in February 2021 due to inclement weather and freezing temperatures as a precaution to prevent undue oyster mortality. Of the 96 12mm baskets deployed on August 28, 2020, the experiment concluded with 71 12mm baskets on March 2, 2021.

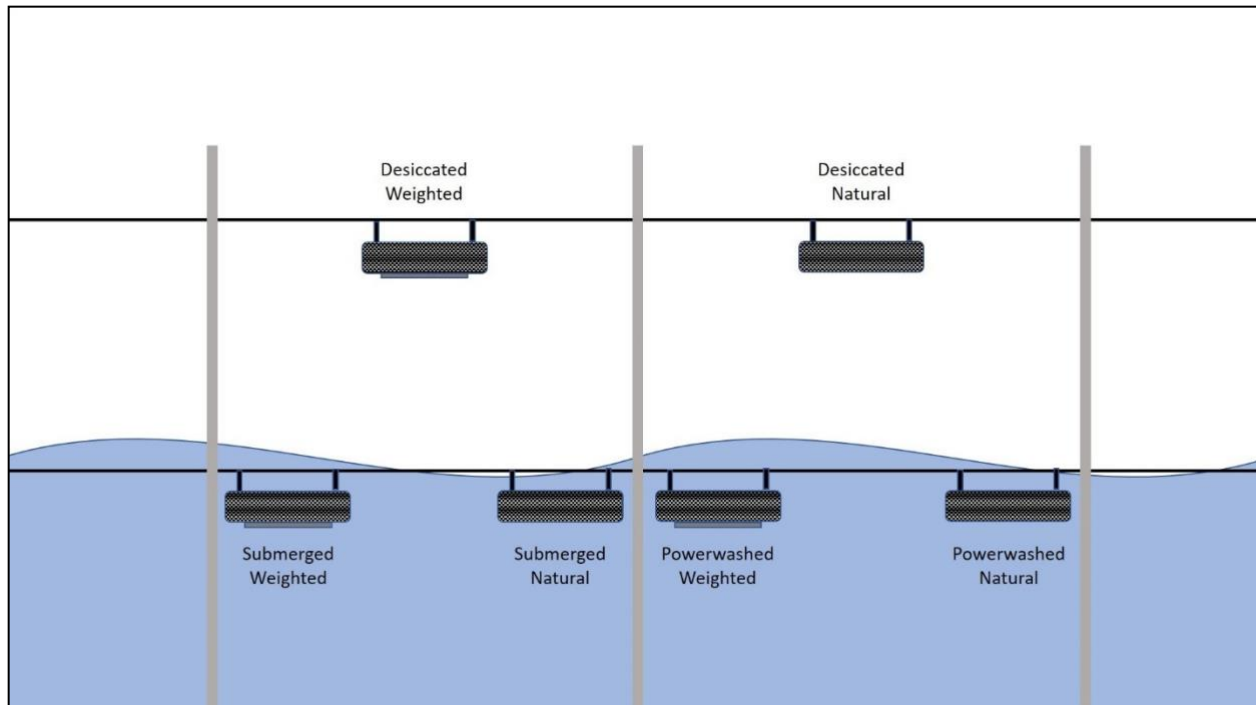


Figure 2. Diagram of experimental longline setup with an illustration of biofouling control and wave action treatments. Each of these was paired with either ‘low’ or ‘high’ density for a total of twelve unique treatments.

2.3.1 Wave Action

There were two wave action treatments within this study. Baskets experienced either natural wave energy, which was observed to be high at this site, or dampened wave energy achieved by modifying BST baskets with weights intended to reduce basket movement. Weights were comprised of approximately 0.61-meter (24 inch) sections of #8-gauge rebar with an average mass of 2.32 (± 0.0017 SE; $n = 10$) kg that were attached to the exterior bottom of the basket with zip ties. The average mass of these sections of rebar was obtained by weighing 10 randomly selected individual weights on a Measuretek™ EHP-B electronic platform scale to the nearest 5 g (Measuretek Scale Co., Ltd, Vancouver, British Columbia, Canada). Qualitatively,

baskets with weights were observed to move substantially less than baskets without weights under a variety of wave conditions and multiple observations. With wave action, baskets without weights were observed to occasionally rotate completely around the line, which was never observed in baskets with weights. TechnoSmArt™ AXY-5 accelerometer sensors were deployed freely inside baskets to quantify differences in basket movement among treatments, with deployments in October 2020 and February 2021 (Technosmart Europe, Rome, Italy).

2.3.2 Biofouling control

Biofouling control was also manipulated in this study. There were three treatments consisting of desiccated weekly (~24-hour duration), never desiccated (hereafter referred to as submerged), or never desiccated but power washed periodically. These treatments represented what a farmer might do to either combat or neglect biofouling. During the initial month prior to the first sample, biofouling control treatments were not implemented due to the small size of the oysters, as is recommended in the industry (Scott Rikard, pers. comm.). Biofouling control treatments were applied once the oysters were stocked into 12mm baskets after one month of deployment. For those baskets that were desiccated weekly, baskets were removed from the lower line and placed on the upper line, allowing for full air-drying of the basket and oysters within, and were placed back onto the lower line the following day. Power washed treatments were cleaned on-site twice during the study, once in October 2020 and once in January 2021 to combat biofouling on equipment and oysters within reflecting the typical schedule used by commercial oyster farmers in the area. Submerged treatments remained underwater throughout the study, aside from during sampling efforts and rare occasions of extreme low tide.

2.3.3 Density

The final variable manipulated in this study was oyster stocking density. For the initial deployment, oysters were stocked by weight into 6mm BST baskets, with the low density treatments receiving half the weight of the high density treatments, at 1.3 kg and 2.6 kg of oysters per basket, respectively. Based off the pre-sample wet weight measurements ($n = 25$), this translates to approximately 669 (± 276 ; 95% CI) and 1,338 (± 551 ; 95% CI) oysters per basket for low and high stocking densities. These weights were obtained using the Measuretek™ EHP-B electronic platform scale. Both density treatments were decreased during the first sampling by hand-counting 50 and 100 oysters from the low and high density treatments respectively to represent densities that may be commonly found in commercial oyster farms. Selection of the two density treatments for this study were set with the recommended stocking density for oyster grow-out in longline baskets in mind. Recommended stocking density is about 75-100 oysters per basket which is represented by the high density variant in this experiment, with half of that representing the low density variant (Davis et al., 2012; Bill Walton, pers. comm.). Due to 6mm basket damage and subsequent oyster loss from 8 of the 12 unique treatment combinations between deployment and the first sample date, individual 12mm BST baskets were restocked with oysters from that same unique treatment in order to repopulate the line to its full extent of 96 baskets (with oysters obtained from the oysters remaining after hand-counting). The only change in stocking density that occurred after this point was the result of oyster mortality within each basket, where dead oysters were discarded from the basket without replacement during sampling.

2.4 Sampling Measurements and Analysis

Non-destructive sampling took place one month after initial deployment of oysters, and every six weeks after this point until the end of the study in March 2021. Non-destructive sampling for each basket consisted of shell dimension measurements on 10 oysters per basket and total live/dead counts for mortality. Destructive samples were taken during the final sampling where individual oysters from each basket ($n = 15$) were collected for biofouling, condition index, and shell shape analysis in the lab. At the time of sampling, final mortality counts were completed and photographs were taken of the baskets to quantify equipment biofouling.

2.4.1 Survival

Between the initial deployment and first sample, survival was not monitored as the stocking densities of these baskets were too high to feasibly count. During the first sample, stocking densities were lowered to a more reasonable number for future survival sampling. After the first sampling period, survival was quantified by performing counts of live and dead oysters in every basket at each sampling period. For empty shells, only left valves with umbos were counted to ensure no overcounting of dead oysters. Dead oysters were discarded after being accounted for during each sampling period. Probability of oyster survival was analyzed during statistical analysis.

2.4.2 Shell Dimensions

Throughout the duration of the study, shell dimensions were recorded to the nearest 0.01 mm using Mitutoyo IP67 electronic calipers (Mitutoyo Corporation, Kanagawa, Japan). During

each non-destructive sampling, oysters ($n = 10$) from each basket were measured for shell height (distance from hinge to bill), length (widest point perpendicular to length measurement), and width (widest point between the two valves) as shown in Fig. 3 and described in Galtsoff, 1964. Shell height was the main metric used to compare overall oyster growth among treatments. All three shell dimension measurements were taken during the condition indexing portion of the final data collection process after the final sample. Measurements taken for shell length and width were used to calculate cup and fan ratios for each individual oyster sampled, with cup ratio being the ratio of shell width to shell height and fan ratio the ratio of shell length to shell height. Prior work (Brake et al., 2003) has documented that cup and fan ratios of at least 0.25 and 0.63 are deemed ‘good’ by buyers and industry members.

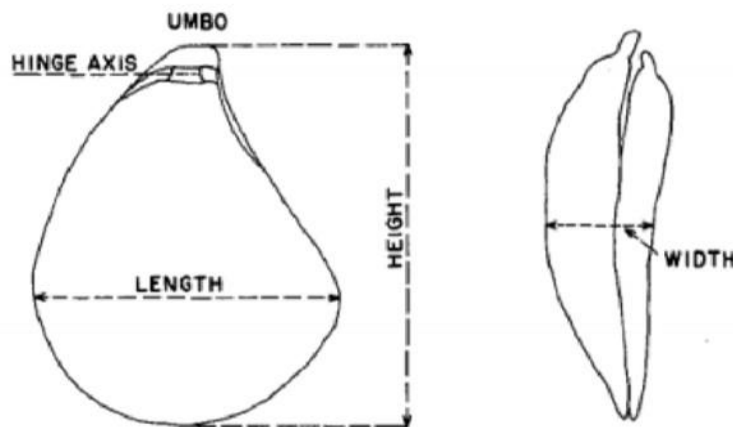


Figure 3. Visual representation of shell dimensions measured for obtaining growth metrics and ratios (Galtsoff 1964).

2.4.3 Condition Index

Condition index was assessed on final oyster samples in the lab. Whole wet weight of each individual oyster was measured to the nearest 0.001 g on a Mettler Toledo AL204 balance

(Mettler-Toledo, LLC, Columbus, Ohio, USA). After weighing, oysters were shucked and all tissues were removed from the shell. Shells were placed in individually labeled petri dishes and allowed to dry for 48 (± 2) hours at room temperature. Oyster tissues were placed in individually labeled VWR™ aluminum boats (VWR International, LLC, Radnor, Pennsylvania, USA) that corresponded to the number given to the petri dish containing the matching shell. Tissues were placed in a Fisher Scientific Isotemp oven (Thermo Fisher Scientific Inc., Pittsburgh, Pennsylvania, USA) set at 80° C for 48 (± 2) hours. Once dried, both the dry tissue and dry shell were weighed on the balance to the nearest 0.001 g. Meat condition indices were calculated using the formula as follows (described in Abbe and Sanders, 1988):

$$\left[\frac{\text{Dry tissue weight (g)}}{\text{Shell cavity volume (ml)}} \right] * 100$$

where shell cavity volume (ml) is equal to the difference between whole wet weight (g) and dry shell weight (g).

2.4.4 Biofouling

Biofouling on two substrates were quantified in this study: oyster fouling and equipment fouling. Quantification of fouling on oysters was performed in the lab after the final sampling, which was done through standardized time of cleaning. Data were collected by recording how long it took to clean each individual oyster from each sample of all biofouling through both brushing and scraping, which provided a comparative representation of how clean or fouled oysters from each treatment were. The average of these times was calculated for each replicate basket, and in turn for each unique treatment group. Equipment fouling was sampled through photography, where a white vinyl background was placed inside the basket and a 5” by 5” white vinyl stencil was attached on the outside of the basket, providing a fixed area with high contrast

to quantify blocked cells. A photograph of each basket was taken on-site during the final sample with an Olympus™ Tough TG-6 waterproof digital camera (OM Digital Solutions Americas, Inc., Center Valley, Pennsylvania, USA) held in a fixed location by a mountable camera stand. Quantification of percent cover was completed later in the lab through visual analysis via counting of “open” or “blocked” cells. “Blocked” cells were defined as those that were >50% blocked. Probability of cell blockage per treatment was analyzed during statistical analysis.

2.4.5 Wave Action

Quantification of wave action was attempted by placing a TechnoSmArt™ accelerometer sensor in one replicate basket of each treatment to compare the amount of jostling inside. Sensors were wrapped in electrical tape to prevent fouling of the connection port and were placed freely within the basket. After collection, data were extracted from each of these sensors in terms of acceleration on a three-dimensional plane and the average magnitude of acceleration was compared among treatments. Due to sensor quantity limitation, this study lacked replication of wave action quantification for each treatment. Inconsistent differences were observed in the analysis of accelerometer data and results were inconclusive.

2.4.6 Statistical Analysis

All statistical analysis was performed in the statistical software program RStudio (R Core Team, 2019). Each response variable aside from basket fouling and survival were modeled using linear mixed-effects models within the *nlme* package (Pinheiro et al., 2019). Basket fouling and survival were analyzed via logistic regression using a generalized linear model from the *stats* package (R Core Team, 2019). In all models, the response variable was modeled via unique

treatments which were comprised of a combination of density, biofouling control, and wave action treatments (where one of the original 12 treatments was lost during the course of the study, leaving 11 unique treatments). Model outputs were analyzed by estimated marginal means (least squares means) using the *emmeans* package (Lenth, 2020). Assumptions of homoscedasticity and normality of residuals were visually tested via graphical outputs of standardized residuals for fitted values and histograms of residuals, respectively, for each response variable. Tukey post hoc pairwise comparisons were performed at a confidence level of 0.95 for all response variables. Graphical outputs were created using the *ggplot2* package (Wickham, 2016) with error bars illustrating upper and lower 95% confidence limits. Due to the loss of one entire treatment by the end of the study, graphical outputs omit the display of that treatment. Environmental data were graphed via *ggplot2* in RStudio, also utilizing the *scales*, *lubridate*, and *dplyr* packages for data and graph manipulation (Grolemund and Wickham, 2011; Wickham, 2016; Wickham et al., 2019; Wickham and Seidel, 2019).

3. Results

For conciseness, treatments will be referred to by their abbreviated ‘treatment’ code hereafter (Table 1). Due to loss of all replicate baskets of one treatment (low oyster density/power washed/natural wave action, or LP-N) during this study, all of the following results omit its inclusion. The number of replicates of each treatment at the end of the experiment that were analyzed are also shown below (Table 1). Confidence was set at 95% ($\alpha = 0.05$) for all analyses.

Table 1. Description of treatment codes with number of replicate baskets present during final sample.

Stocking Density	Biofouling Control	Wave Action	Treatment	Replicates
Low	Desiccated	Natural	LD-N	8
Low	Power washed	Natural	LP-N	0
Low	Submerged	Natural	LS-N	3
High	Desiccated	Natural	HD-N	8
High	Power washed	Natural	HP-N	3
High	Submerged	Natural	HS-N	2
Low	Desiccated	Weighted	LD-W	8
Low	Power washed	Weighted	LP-W	8
Low	Submerged	Weighted	LS-W	7
High	Desiccated	Weighted	HD-W	8
High	Power washed	Weighted	HP-W	8
High	Submerged	Weighted	HS-W	8

Temperature at the experiment site ranged between 6.4° C (February 2021) and 32.7° C (September 2020). Salinity (PSU) ranged between 0 and 29.5. Dissolved oxygen ranged between 46.3% and 168.5% saturation. Daily averages of temperature and salinity (Fig. 4) and dissolved oxygen (Fig. 5) were graphed to illustrate variation over time during the experiment.

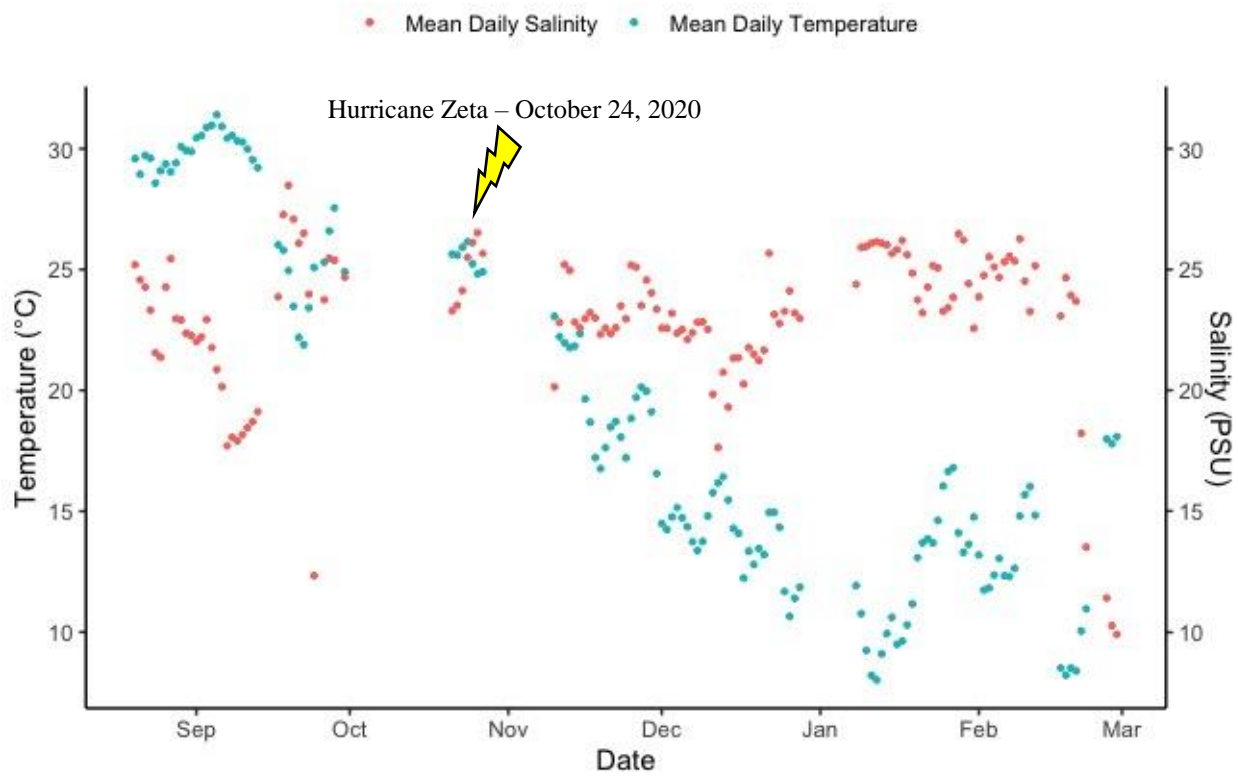


Figure 4. Daily average temperature and salinity readings provided by MS DMR. The lightning bolt signifies the landing of Hurricane Zeta on October 24, 2020.

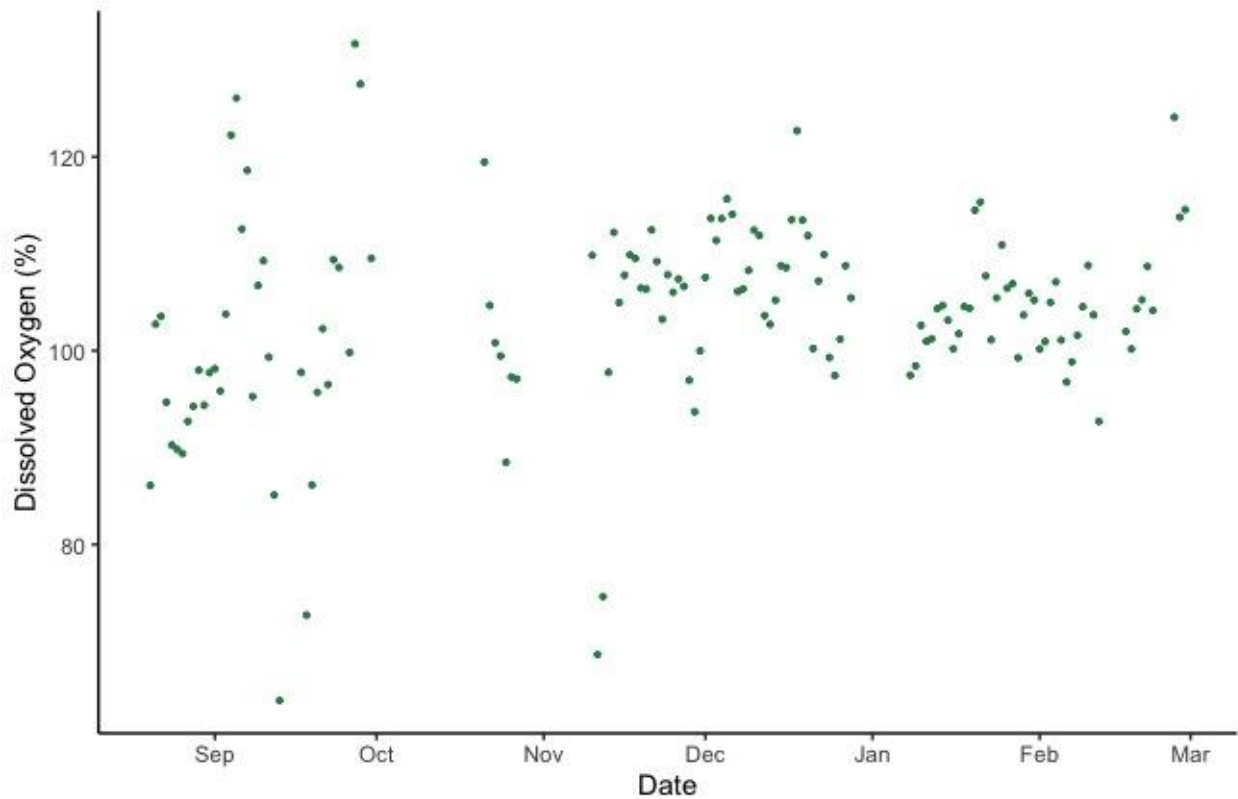


Figure 5. Daily average dissolved oxygen readings provided by MS DMR.

3.1 Survival

Although oyster survival probability was fairly high in this study (≥ 0.867 ; Fig. 6), the two high density power washed treatments, irrespective of wave action, (HP-N and HP-W) had the second and third lowest survival probabilities (0.870; 95% CL: 0.827-0.904 and 0.873; 95% CL: 0.848-0.894, respectively). Submerged treatments represented the lowest (LS-N) and highest (LS-W) survival probabilities seen in this study, however LS-N was not significantly different than any other treatment ($p \geq 0.0544$). This was also the case with HS-N, LD-W, and HD-W ($p \geq 0.1418$). The treatment with the highest survival (LS-W) was only significantly different from three treatments (HP-N, HP-W, HS-W; $p \leq 0.0472$).

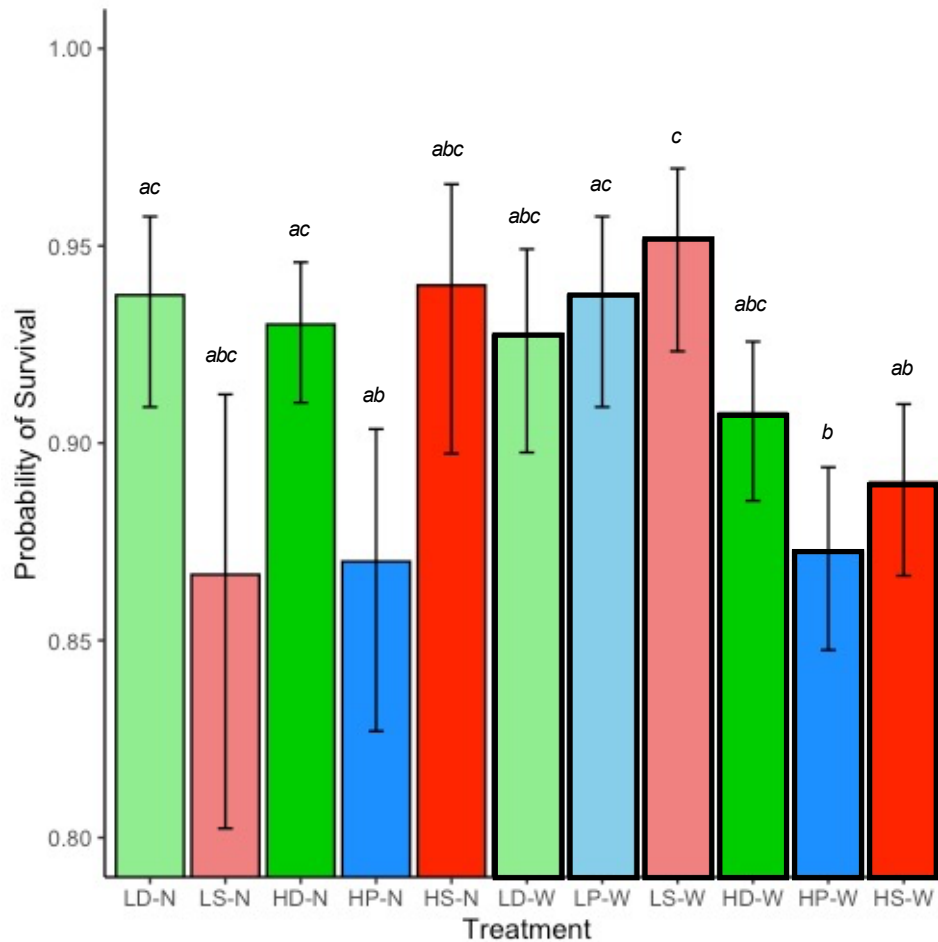


Figure 6. Average survival probability by treatment. Error bars indicate upper and lower 95% confidence limits. Bars that share a letter are not statistically significant ($p > 0.0500$). Bars that have bolded borders indicate those that belong to the weighted wave action treatment and those that share colors illustrate identical density-biofouling control treatment combinations.

3.2 Shell Dimensions

For the primary metric of overall growth, shell height, the largest oysters tended to be in the weighted wave action treatments with some exceptions due to the other applied treatments (Fig. 7). Oysters in HS-W were larger than the oysters in any other treatment ($p \leq 0.0006$),

followed by LS-W ($p \leq 0.0191$). The oysters in HD-W and HP-W did not differ from each other ($p = 0.9674$) but were significantly larger than the remaining treatments ($p \leq 0.0136$). Among the remaining treatments, shell heights overlapped across the two wave action treatments, with LP-W, HP-N, LD-W, HD-N not significantly differing. The significantly smallest treatments were all in the natural wave action treatments (HS-N, LS-N, and LD-N). Shell length and shell width are considered in the calculation of cup and fan (below).

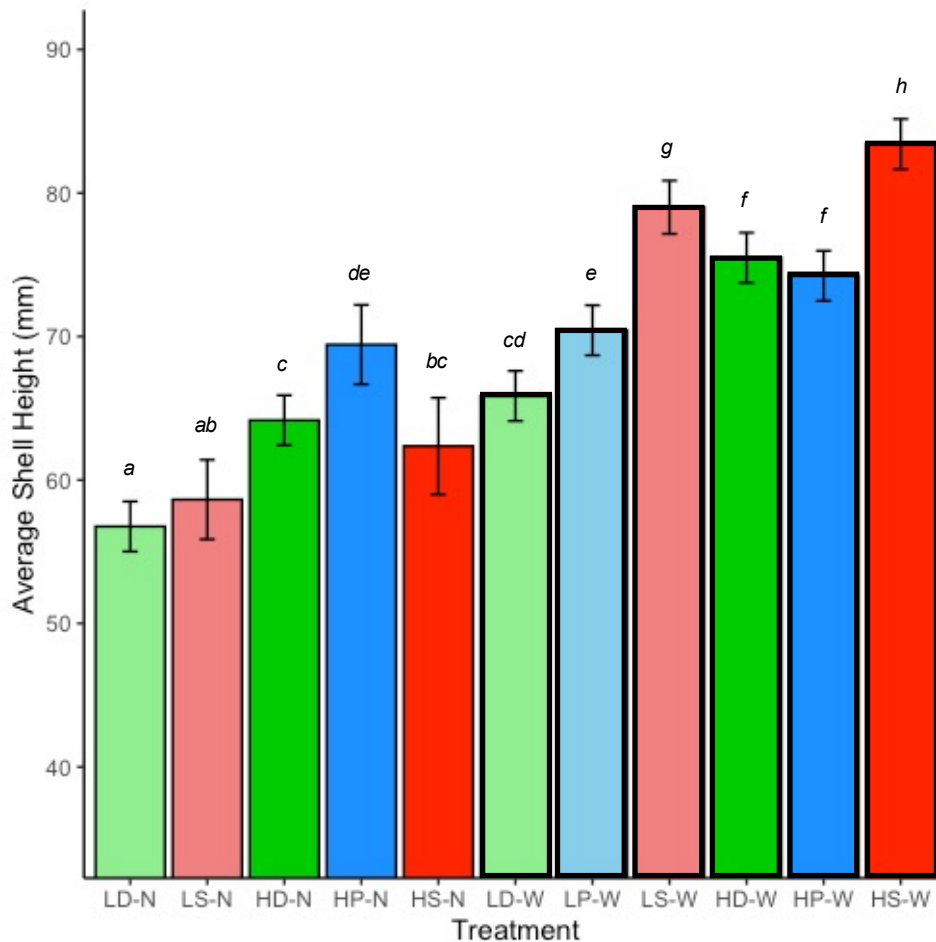


Figure 7. Average shell height (mm) by treatment. Error bars indicate upper and lower 95% confidence limits. Bars that share a letter are not statistically significant ($p > 0.0500$). Bars that have bolded borders indicate those that belong to the weighted wave

action treatment and those that share colors illustrate identical density-biofouling control treatment combinations.

Overall, all treatments tended to have what is considered a ‘good’ cup shape (> 0.25), but there were differences among treatments (Fig. 8). For average cup ratio, the majority of the largest average values fell within the natural wave action treatment regardless of the other treatment combinations, with the exception of LD-W. The two treatments with the highest average cup ratio were LD-N and LS-N, which were not significantly larger than HS-N ($p \geq 0.7449$) but were significantly larger than all remaining treatments ($p \leq 0.0027$). HS-N, LD-W, HD-N, and HP-N showed no significant differences amongst each other ($p \geq 0.5272$). The remaining smallest treatments fell within the weighted wave action treatment, with those in the submerged biofouling control treatments trending lowest in order (LS-W and HS-W in decreasing order). HS-W had the lowest average cup ratio and was significantly lower than all other treatments ($p \leq 0.0209$).

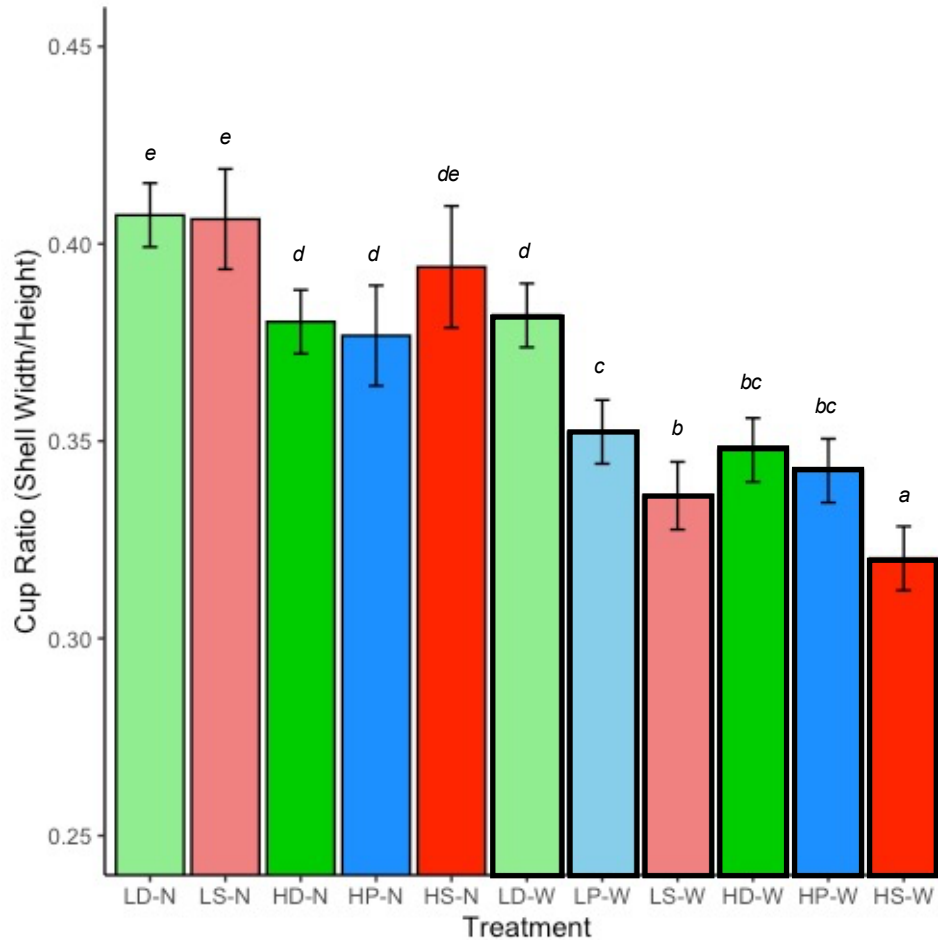


Figure 8. Average cup ratio (shell width/shell height) per treatment. Error bars indicate upper and lower 95% confidence limits. Bars that share a letter are not statistically significant ($p > 0.0500$). Bars that have bolded borders indicate those that belong to the weighted wave action treatment and those that share colors illustrate identical density-biofouling control treatment combinations.

Similar to cup ratio, all treatments had what would be considered ‘good’ fan ratios (> 0.63), but there were fewer clear differences among treatments (Fig. 9). The treatment with the greatest average fan ratio (LS-W) was not significantly larger from the four next highest treatments ($p \geq 0.4468$), and two treatments (HS-N and LS-N) were not significantly different

than any treatment ($p \geq 0.2773$). The two smallest treatments (HD-W and LD-N) were only significantly lower than the greatest three treatments LS-W, HP-N, and LD-W ($p \leq 0.0409$).

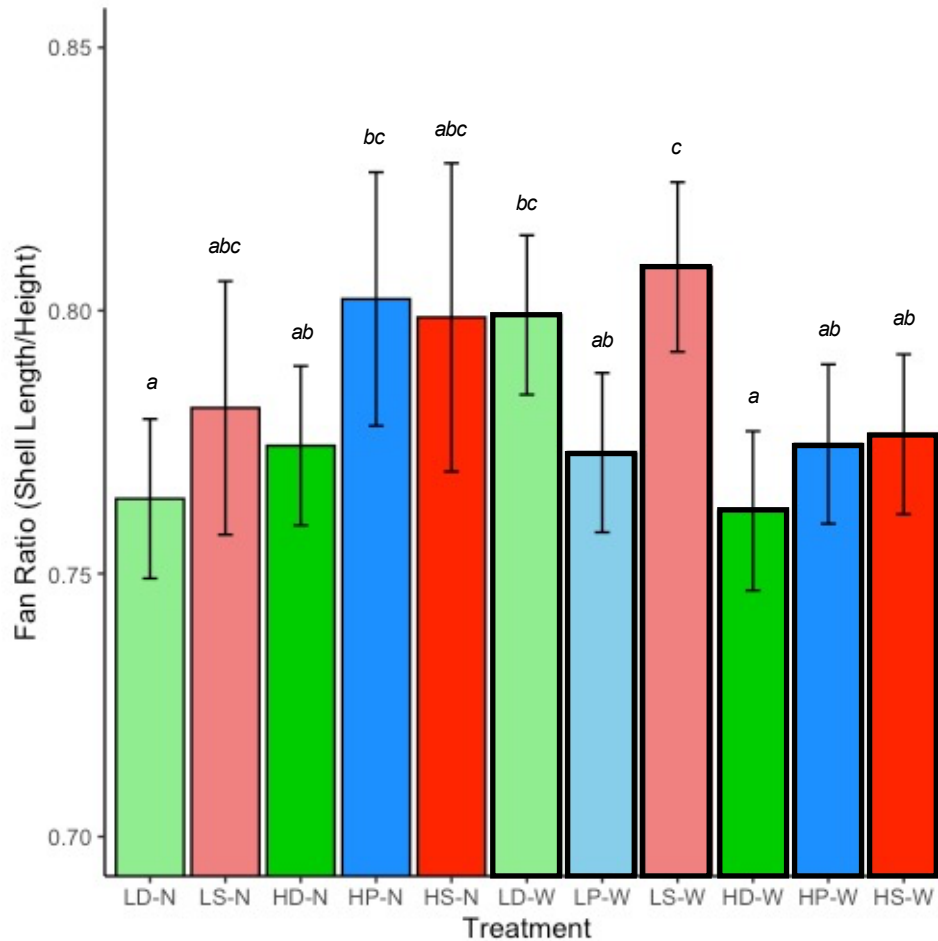


Figure 9. Average fan ratio (shell length/shell height) per treatment. Error bars indicate upper and lower 95% confidence limits. Bars that share a letter are not statistically significant ($p > 0.0500$). Bars that have bolded borders indicate those that belong to the weighted wave action treatment and those that share colors illustrate identical density-biofouling control treatment combinations.

3.3 Condition Index

As an additional metric of production, whole wet weight was analyzed among treatments (Fig. 10). In general, these results follow very closely to those shown for shell height (Fig. 7), with the greatest average wet weight per treatment belonging to the weighted wave action treatments and the two greatest treatments specifically falling within the submerged biofouling control treatments (HS-W and LS-W), which were not significantly different from one another ($p = 0.6317$) but were significantly larger than all other treatments ($p \leq 0.0254$). The third largest treatment on average, HD-W, was the only remaining treatment that was significantly different than all other treatments ($p \leq 0.0265$). The significantly smallest treatments were in the two low stocking density natural wave action treatments (LD-N and LS-N), which were not significantly different from one another ($p = 0.9997$) or from the next highest treatment, HS-N ($p \geq 0.1267$).

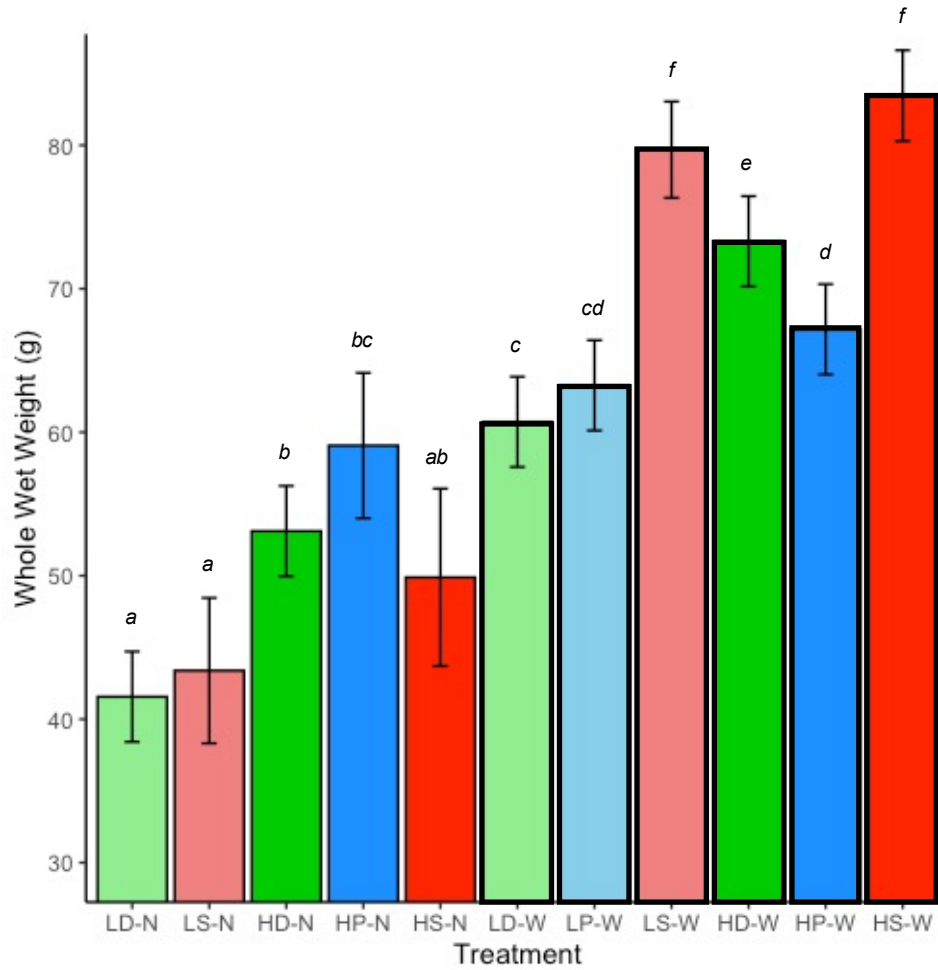


Figure 10. Average whole wet weight (g) per treatment. Error bars indicate upper and lower 95% confidence limits. Bars that share a letter are not statistically significant ($p > 0.0500$). Bars that have bolded borders indicate those that belong to the weighted wave action treatment and those that share colors illustrate identical density-biofouling control treatment combinations.

For a potential measure of product quality as a proxy for ‘shell fullness’, condition index was analyzed among all treatments (Fig. 11). The top four treatments with the highest average condition index (LD-N, LS-N, HS-N, HD-N) were all within the natural wave action treatment

but were not significantly different from one another or the next two highest treatments, LD-W and HD-W ($p \geq 0.0519$). Of these four greatest treatments, the top two were comprised of the low density treatment. Desiccated treatments also trended towards the top half of the rankings, comprising three of the top five condition indices, though there was no statistical significance among them ($p \geq 0.0519$). At the low end of condition index, two of the high density-weighted wave action treatments, HS-W and HP-W, were significantly poorer than all other treatments ($p \leq 0.0002$) but were not significantly different than one another ($p = 0.9999$).

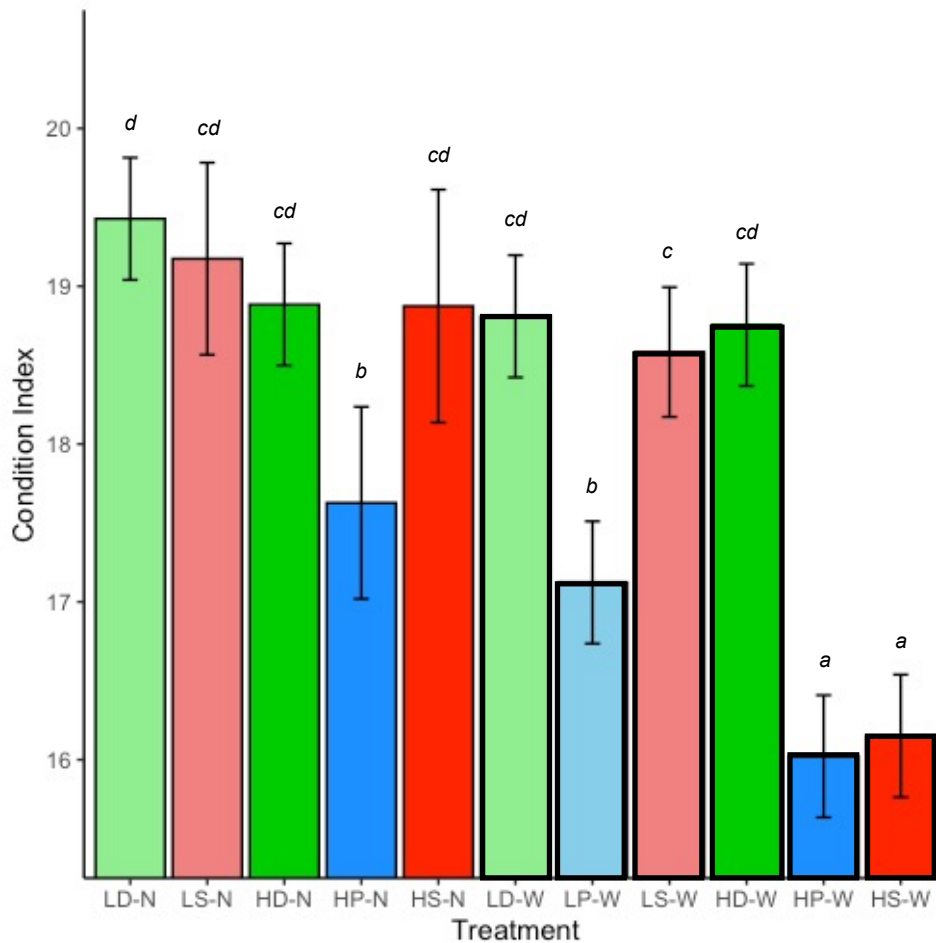


Figure 11. Average condition index per treatment. Error bars indicate upper and lower 95% confidence limits. Bars that share a letter are not statistically significant ($p > 0.0500$). Bars that have bolded borders indicate those that belong to the weighted wave

action treatment and those that share colors illustrate identical density-biofouling control treatment combinations.

3.4 Biofouling

As a measure of fouling on oysters, average cleaning time in seconds for oysters had well-defined differences among treatments (Fig. 12). The greatest average cleaning time was the HS-W treatment, which was significantly greater than all other treatments ($p < 0.0001$), followed by LS-W (which was also significantly greater than all other treatments; $p < 0.0001$), both belonging to the submerged biofouling control and weighted wave action treatments. In descending order, the next three greatest treatments fell within the power washed biofouling control treatments, followed by the remaining two submerged natural wave action treatments (HS-N, LS-N). The four treatment combinations with the shortest cleaning times fell within the desiccated biofouling control treatment, with the lowest two of these falling in the natural wave action treatment (HD-N, LD-N). In general, high stocking density variations of treatments took significantly longer to clean than their low density counterparts when holding biofouling control and wave action constant, with the exception of LS-N and HS-N ($p = 0.9993$) and HD-W and LD-W ($p = 0.6795$).

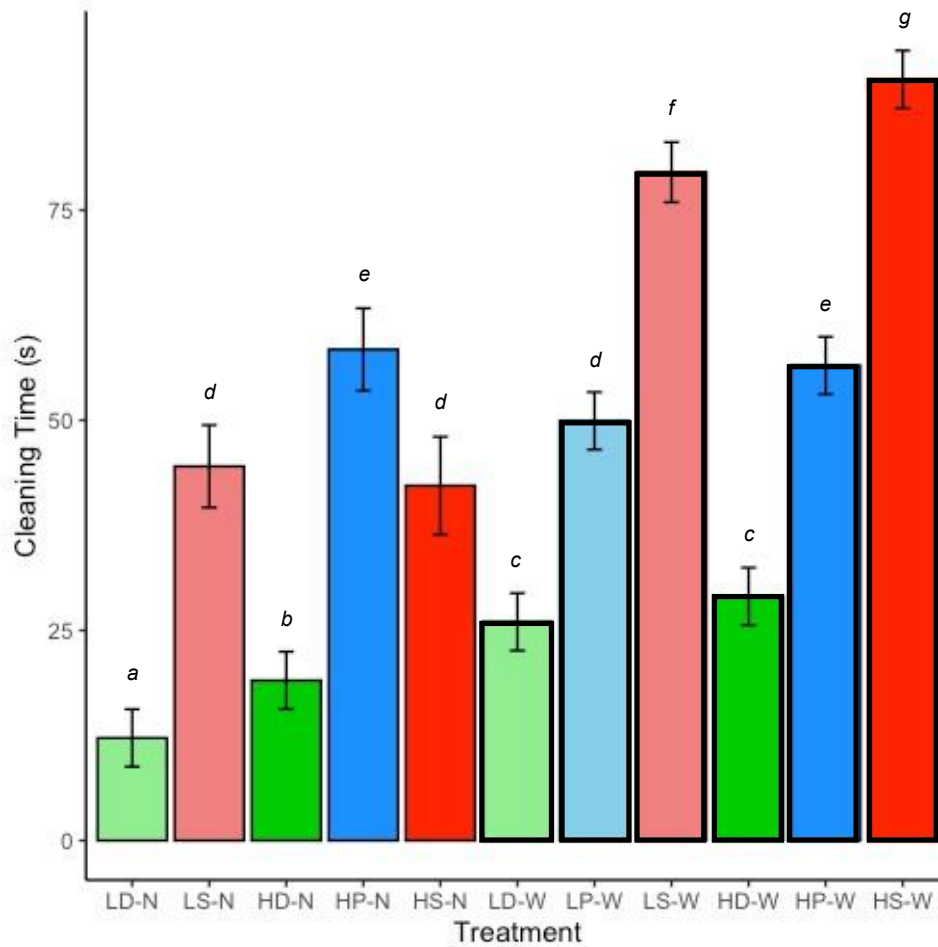


Figure 12. Average cleaning time (in seconds) per oyster for each treatment. Error bars indicate upper and lower 95% confidence limits. Bars that share a letter are not statistically significant ($p > 0.0500$). Bars that have bolded borders indicate those that belong to the weighted wave action treatment and those that share colors illustrate identical density-biofouling control treatment combinations.

As a measure of fouling on the baskets themselves, basket fouling probability was analyzed (Fig. 13). The treatment with the highest degree of basket fouling was HP-W, and three of the top five treatments were comprised of the power washed biofouling control treatments. Four of the top five most fouled baskets were within the high stocking density treatments (HP-

W, HS-N, LP-W, HD-N, HP-N): HP-W was greater than LP-W ($p < 0.0001$) while HS-N was not significantly different than either of these ($p \geq 0.3539$). Moving beyond the top three treatments, the next four most fouled treatments were within the natural wave action treatment; HD-N, HP-N, LD-N, LS-N showed no significant differences amongst each other ($p \geq 0.1460$). Of these, HP-N, LD-N, LS-N also had no significant difference from the next lowest treatment (LD-W; $p \geq 0.4322$). The least fouled baskets were baskets within the weighted wave action treatments. The lowest three of these (HD-W, HS-W, LS-W) were not significantly different ($p \geq 0.2591$).

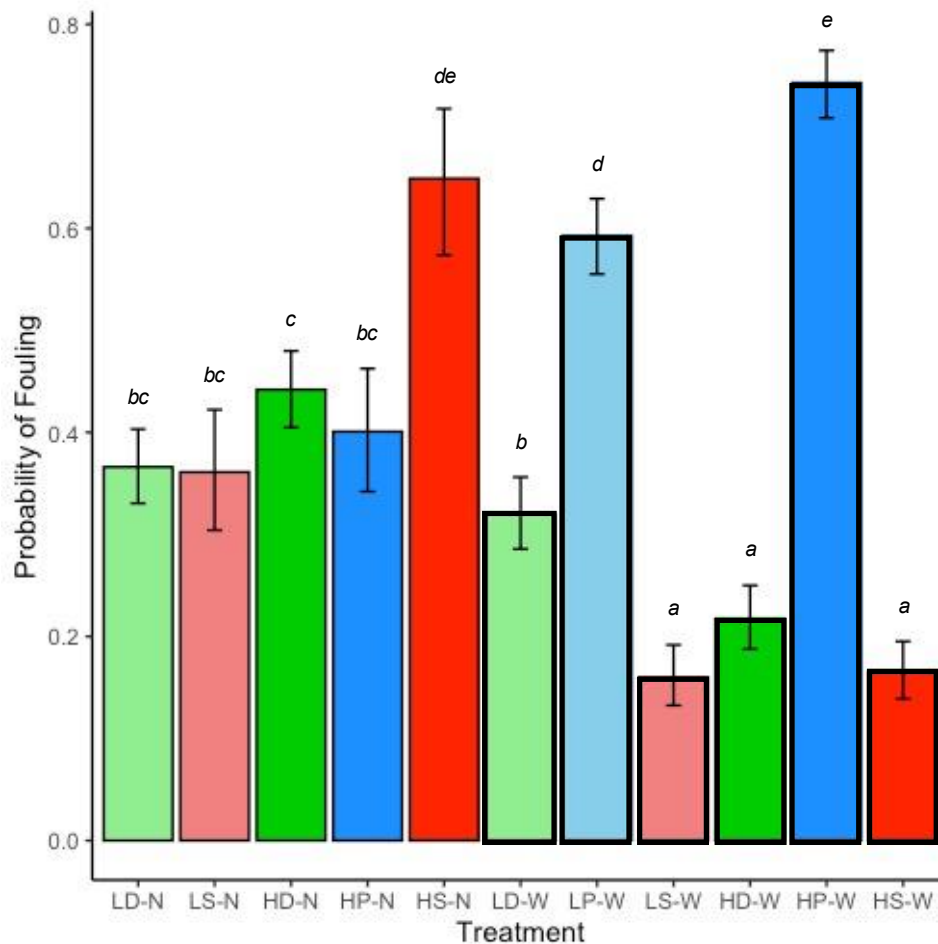


Figure 13. Average probability of basket fouling per treatment. Error bars indicate upper and lower 95% confidence limits. Bars that share a letter are not statistically significant

($p > 0.0500$). Bars that have bolded borders indicate those that belong to the weighted wave action treatment and those that share colors illustrate identical density-biofouling control treatment combinations.

4. Discussion

The results of this study allow a better understanding of how stocking density, biofouling control, and wave action interact to affect different aspects of aquaculture oyster performance in an off-bottom, high energy setting in the northern Gulf of Mexico. Of course, the effects of these factors vary among the different aspects of performance, including measures of production quantity, measures of product quality, and biofouling on the culture gear itself. Oyster production quantity was assessed by a combination of oyster survival, average shell height, and average whole wet weight, which are arguably the most universally important factors in the broad scope of all oyster aquaculture. Second, and most specifically important to oyster farmers who have intent to distribute product to the half-shell market, is oyster quality. This was assessed through average cup ratio, average fan ratio, average condition index and cleaning time, all of which may have an impact on wholesale or consumer willingness to purchase a given oyster product (e.g., Mizuta and Wikfors, 2018). Lastly, basket fouling was assessed as a possible source of additional labor costs and time investment which both cut into the profitability of a farming operation. While it is well known that environmental factors, such as salinity and temperature (e.g., Lowe et al., 2017), food supply (e.g., Campbell and Hall, 2019), and farming practices (e.g., Archer et al., 2014; Thomas et al., 2019; Walton et al., 2013b) can affect aquacultured oyster performance, here we provide one of the first assessments of the effect of wave action combined with common farming practices to determine their impacts on oyster performance.

4.1 Measures of Production Quantity

Oyster survival is an integral factor in oyster aquaculture yield that has a direct impact on the profitability of all oyster farming operations. Within this study, there were no clear differences in survival driven by wave action (Fig. 6). Additionally, there were no signs of mortality due to predation and environmental data provided no indication of extended periods of poor water quality that may have caused mortalities (Fig. 4; Fig. 5). Due to the relatively high survival of oysters among all treatments and considerable variation (Fig. 6), there were no other discernable patterns and very few differences present among treatments. Similar results have been seen in the northern Gulf of Mexico with respect to handling practices and gear type (Ring, 2012), stocking densities and biofouling control (Gamble, 2016), and stocking densities and basket arrangement (Davis, 2013).

Regarding the second component of yield (growth), eight of the eleven treatments had an average shell height greater than 63.5 mm and two of those eight were larger than 76.2 mm. Natural wave action tended to reduce the average shell height (Fig. 7), though it's not clear if this was due to reduced feeding with increased jostling, shell breakage, or a combination of these two factors; oyster movement within baskets affected by waves were expected to experience increased 'pruning' (also referred to as tumbling), or the breaking of new shell growth caused by the collision with the container and other oysters within (Brake et al., 2003; Mallet et al., 2013). Interestingly, within the natural wave action treatments, low stocking density seemed to increase the effect with the two low stocking density treatments having the smallest oysters (and, notably, with a complete loss of the baskets and oysters in the third low density natural wave action treatment). While overstocking of oysters might be expected to reduce growth with increased

competition for food (Capelle et al., 2013; Marshall and Dunham, 2013), here, reduced stocking density appears to interact with reduced wave action to cause a reduction in average shell height. This pattern, however, was not true across all the biofouling control treatments in the natural wave action treatments. Results for whole wet weight closely mirrored those for shell height, with distinct differences between natural and weighted counterparts (Fig. 10). Natural wave action again led to a reduction in whole wet weight and there was a considerable difference between the least and greatest average estimate. Reduced density led to a significant reduction in average wet weight in desiccated treatments for both natural and reduced wave action treatments, but that pattern was not present for power washed or submerged treatments.

If production of harvestable oysters by size is the primary goal of a producer working in a high energy environment, these data suggest stocking baskets at the higher density tested here and power washing as a means of biofouling control (HP-N) is optimal. For producers raising oysters within an environment with reduced wave action, the data suggest that submerged baskets would maximize growth, with an added benefit of stocking at the lower density tested here (LS-W).

4.2 Measures of Production Quality

In contrast to production quantity, wave action tended to increase oyster quality in terms of shell shape, condition index, and oyster shell cleanliness (reduced biofouling). Both cup and fan ratios are used as indicators of quality in oysters destined for the half-shell market (Brake et al., 2003). All averages among treatments for cup and fan ratio were higher than the ‘good’ quality standard described by Brake et al., 2003, which is defined as greater than 0.25 for cup ratio and greater than 0.63 for fan ratio. In fact, of the 1,065 individual oysters processed, only

two oysters had an observed cup ratio slightly lower than 0.25 and only sixteen were below a fan ratio of 0.63. This overall high oyster shell shape quality is likely due to the overarching effects of off-bottom oyster aquaculture, which has been shown to increase the perceived quality of oysters by allowing them to grow deeper cups or wider fans in comparison with on-bottom oysters as previously described (Brake et al., 2003; Leavitt et al., 2017; Mallet et al., 2013; Thomas et al., 2019).

For shell shape, wave action had a clear effect on cup ratio (Fig. 8). All the highest cup ratios were produced in the treatments exposed to natural wave action, with only the low density desiccated treatment from the reduced wave treatments (LD-W) achieving a statistically similar cup ratio. Within the natural wave action treatments, the two low density treatments (LD-N, LS-N) obtained the deepest average cups. This is likely a consequence of the increased pruning that also led to the lower average shell heights in these treatments. Biofouling control provided no discernable pattern while stocking density follows the inverse of the pattern described for shell height, where high density treatments tended to have smaller cups than low density when holding biofouling control and wave action constant. Unlike cup ratio, fan ratio did not appear to have clear, consistent differences among treatments (Fig. 9). This lack of patterns is likely caused by two things: First, there were very few significant differences present and very little absolute difference between the least and greatest ratios; second, the overall fan ratios were very high across all treatments as previously stated.

Condition index is a unit-less quantification of relative meat quality for bivalves (Abbe and Albright, 2003). Although there are no universally set thresholds for what is deemed a 'good' or 'bad' value of condition index, Abbe and Sanders, 1988 stated that values ≥ 10 are generally seen in oysters in good health. Only one oyster processed in this experiment had a

condition index value below 10, with the lowest treatment on average being well above this level of condition. Observed average condition index values in this study (Fig. 11) were also markedly higher than those reported in studies utilizing the same grow-out system (ALS) in similar study areas throughout Mississippi Sound (Wadsworth et al., 2019; Walton et al., 2013b). It is worth noting that triploid oysters have been shown to exhibit a significant reduction in gametogenesis in comparison with diploids (Barber and Mann, 1991), so condition indices reported here should be free from seasonal variation caused by the changes associated with reproduction.

Within this study, results for condition index were complicated, but many of the highest values were observed in the natural wave action treatments while the lowest values were observed in the dampened wave action treatments (Fig. 11). The patterns were seemingly affected by interactions with one or both of the other treatments. For example, power washing appears to have reduced condition index. The overall high levels of condition index observed within this study is again likely due to the overarching effect of off-bottom oyster aquaculture and selection of triploids for use as experimental oysters. Both off-bottom culture and ploidy have been shown to have a positive effect on oyster condition index, which is a common reason farmers make these choices within their operations (Archer et al., 2014; Wadsworth et al., 2019).

Finally, in terms of product quality, the strongest effect on the cleanliness of the oysters produced appears to have been biofouling control, which is not surprising since the explicit intent of this factor is to manage biofouling (Fig. 12). The lowest cleaning times observed were in the four desiccated treatments. Interestingly, there was also a clear effect of wave action, where natural wave action significantly reduced cleaning time relative to the dampened wave action counterpart treatments with the sole exception of the high density, power washed treatments (which did not statistically differ). Also, in a natural wave energy environment, power

washing actually produced more heavily fouled oysters than the submerged treatment, suggesting this is not an effective biofouling control method in this environment (while it did produce cleaner oysters in the wave dampened treatments relative to the submerged treatments). For a producer in a high energy environment, the low stocking density, desiccated treatment produced the cleanest oysters. Within a dampened wave environment, the desiccated treatments (regardless of stocking density) produced the cleanest oysters.

Overall, for a producer in a high wave environment focused on producing high quality product, the low density, desiccated treatment appeared to be the optimal choice, producing oysters with the deepest cups, highest condition index and least amount of fouling on the oysters (albeit it with ‘good’ but relatively reduced fans). The same is true within a low wave environment, where low density-desiccated treatments generally outperformed all others in these metrics.

4.3 Basket Fouling

Percent fouling on baskets gave complicated results (Fig. 13) that are not readily explained by the applied treatments in a predictable way. Within dampened wave action treatments, the power washed treatments were highly fouled while the desiccated and submerged treatments tended to be the least fouled. There were fewer differences within the natural wave action, where only the high density submerged basket stood out as heavily fouled (though all the other treatments were more fouled than the least fouled baskets in the dampened wave action). Equipment fouling has negative impacts for both oysters and farmers in an aquaculture setting. Fouling can block the pores within farming equipment and the organisms can compete for food resources, effectively limiting both flow and food supply which may cause a reduction of

condition or mortality if left untreated (Flimlin et al., 2010; Hood et al., 2020). To reduce these negative impacts, farmers must increase their time and labor to combat fouling which can take many forms including the ones tested here, power washing or desiccating. The results of this study do not provide clear direction of optimal fouling control for equipment. It is worth noting, however, that the method of determination of fouling in this case is very simple and took place as soon as baskets were removed from the water. From qualitative inspection of photographs from all eleven treatments, the desiccated treatments seemed to be mostly comprised of soft, filamentous algae which is easy to remove from equipment. In the photographs, these filamentous algae laid over the holes and caused them to be counted as blocked. In comparison, the submerged baskets were covered in barnacles which allowed less surface area for algae to attach, in turn presenting more open pores in photographs while being harder to remove than algae in reality. Power washed baskets were somewhere between desiccated and submerged in terms of fouling, where many small barnacles were present along with some biofilm.

Conclusion

Oyster farmers face many decisions when operating a commercial aquaculture operation. These decisions are often impacted by and interact with the environment in which the farmer operates. Specifically in this case, we evaluated how wave action impacts the decision of biofouling control and density when paired with a single grow-out system (ALS) and triploid oyster seed.

In areas with reduced wave action, a farmer could expect positive impacts on oyster production quantity with increases in both growth indices (shell height and wet weight) and no discernable negative impact to oyster survival in comparison with natural wave action

treatments. In contrast, oyster farmers in areas of high wave action might expect an increase in production quality measures, including cup ratio, condition index, and oyster cleanliness. Importantly, even within these different wave action environments, a farmer's choices about biofouling control and stocking density can have an impact on the final product. Within a low wave action environment, a farmer attempting to maximize production quantity might simply keep oysters submerged (no biofouling control) at low stocking density (given the significant increase in probability of survival relative to the high density treatment). Within that same lower wave energy environment, however, a farmer intent on maximizing production quality metrics might institute weekly desiccation and lower stocking density as this combination produced oysters with the best combination of deep cups, broad fans, high condition index, and clean shells. While there is no ideal treatment that optimizes production quantity and quality in the lower wave environment, interestingly the weekly desiccated, higher stocking density seems to produce a good combination of product quality and quantity (accepting oysters with relatively smaller fans). Additionally, based on the data, either weekly desiccation or full submersion is recommended in place of power washing to decrease basket fouling in this lower wave environment.

Individuals farming oysters at high wave action sites with intentions of increasing production quantity might opt for power washing and using the higher stocking density. For production quality in a high wave action site, farmers might opt for the same approach used in lower wave environments: weekly desiccation and lower stocking density (though this approach does not produce the best fan ratio of the tested treatments). In this case, however, farmers would enjoy an absolute increase in production quality benefits due to the increased wave action (i.e., oysters with significantly better cup ratios relative to the same treatment in lower wave energy

environments). Interestingly, the submerged, higher stocking density treatment seemed to produce the best compromise between production quantity and quality in the higher wave energy environment. A farmer using this approach would have to accept a substantial increase in biofouling.

These data provide more evidence in support of the importance of site selection in an oyster aquaculture setting, particularly in terms of product quantity versus quality. High wave action sites tended to produce higher quality oysters, especially when paired with low oyster stocking density and weekly desiccation. In comparison, low wave action sites proved to be better for production quantity, where no biofouling control (submerged) is ideal and lower density may be selected for increased survival.

This is not to imply low wave action sites cannot produce a high-quality oyster, however. Based upon our observations, farmers intending to supply oysters to the half-shell market from a low wave action site have the ability to do so if the proper husbandry choices are made, such as proper biofouling control and stocking density. The results presented here provide applicable advice for use in an off-bottom oyster aquaculture setting; however, it is important to note that every choice a farmer makes has a chance to positively (or negatively) impact all other aspects of the oysters' growing environment, ultimately shaping the final product. Additional work utilizing other grow-out gear types such as floating cages or bags, diploid oysters, and different sites should be completed to obtain a better understanding of the impact of wave action on the performance of oysters in an off-bottom oyster aquaculture setting.

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CHAPTER THREE:

RIDING THE WAVE: OYSTER FARMING IN HIGH WAVE ENVIRONMENTS

A Summary for the Oyster Aquaculture Industry

Expansion of Off-Bottom Oyster Aquaculture

Off-bottom oyster aquaculture has experienced growth throughout coastal portions of states along the Gulf Coast over the last decade. Expansion of aquaculture practices in this region has been attributed to the decline of once-abundant wild oyster stocks as well as the development of “niche” half-shell markets throughout the Gulf states. The growth of off-bottom oyster farming in particular is due to its advantages in comparison with on-bottom culture methods, including reduced mortality due to predation and sedimentation, higher end-product value by improved shell shape, appearance, and condition, faster growth, and the ability to control fouling organisms (Walton et al., 2012b). These attributes are typically grouped together within the term oyster “performance”.

Environmental Factors Affecting Performance

In the simplest terms, performance in all sectors of oyster aquaculture refers to yield, which is the combination of survival and growth. Within the lens of off-bottom oyster farming, however, performance can include survival, growth, shell shape and appearance, cleanliness, and meat condition. There are numerous factors that affect oyster performance in an off-bottom aquaculture setting. Environmental factors such as salinity and temperature have an interactive effect on the growth and survival of oysters, where extreme high or low combinations of each factor cause undue stress, ultimately reducing growth and can eventually cause mortality (Lowe et al., 2017). The Gulf of Mexico is often praised by oyster growers for providing good growing conditions almost year-round, allowing farmers to get the majority of oysters to market within 12 months of spawning. Flow at a given site also plays an important role as the food and dissolved

oxygen delivery system to the oysters, where reductions in either of these factors could lead to decreases in growth and increases in mortality (Campbell and Hall, 2019).

Farming Choices

Aside from the environmental factors of a given site, farmers make many choices that also impact oyster performance. Selection of grow-out gear can have a major impact on oyster performance in terms of oyster growth, meat condition, and shell shape. Similarly, selection of diploid or triploid oyster seed can have a substantial impact on performance of an oyster crop, where triploids have been shown to exhibit accelerated growth and higher meat condition in comparison with diploid counterparts (Stanley et al., 1984). Stocking density is yet another choice that farmers must make. Overstocking of grow-out containers may lead to a reduction in both growth rate and quality of oyster shell shape, however understocking can cut the profitability of an operation by limiting production volume. Choice of biofouling control may also affect profitability as controlling biofouling on oysters and gear may be labor intensive, but fouled oysters are less likely to be suitable for the half-shell market. Critically, the outcomes from the choices that farmers make are impacted by the farm's environmental factors. For example, overstocking could be more problematic at higher temperatures.

Wave Action

One environmental factor that is often overlooked yet may play a major role in the performance of oysters in off-bottom aquaculture is wave action. Although it is mentioned as a contributing factor to the growth, shell shape, and consistency of oysters, few studies have assessed the impact of wave action on off-bottom oyster performance. Here, we assessed the

effect wave action has on oyster performance in an off-bottom setting when paired with different stocking densities and biofouling control techniques.

Experimental Design

Triploid oysters spawned at the Auburn University Shellfish Lab were deployed in adjustable longline baskets along a single line at the Mississippi Department of Marine Resources Commercial Aquaculture Park near Deer Island, Mississippi in fall 2020. We assessed every combination of two wave action treatments (natural or dampened) x three biofouling control treatments (~24 hour desiccated, power washed, fully submerged) x two density treatments (low or high) for a total of 12 unique treatment combinations (Fig. 1). Wave energy at this site was high, so natural treatments represent high wave energy. Approximately 5-pound (2.32 kg) rebar weights were added to dampened wave energy treatments with the intent of reducing basket motion to simulate a low wave action site. Densities were set at either 100 oysters per basket ('high' density; represents approximately normal stocking density for longline baskets) or 50 oysters per basket ('low' density). Oysters were sampled every six weeks post-deployment and the experiment concluded when oysters reached a set size threshold of 2.5-3 inches (63.5-76.2 mm). Final sampling and analysis examined oyster survival, shell height, cup and fan ratio, whole wet weight, condition index, oyster cleaning time, and basket fouling.

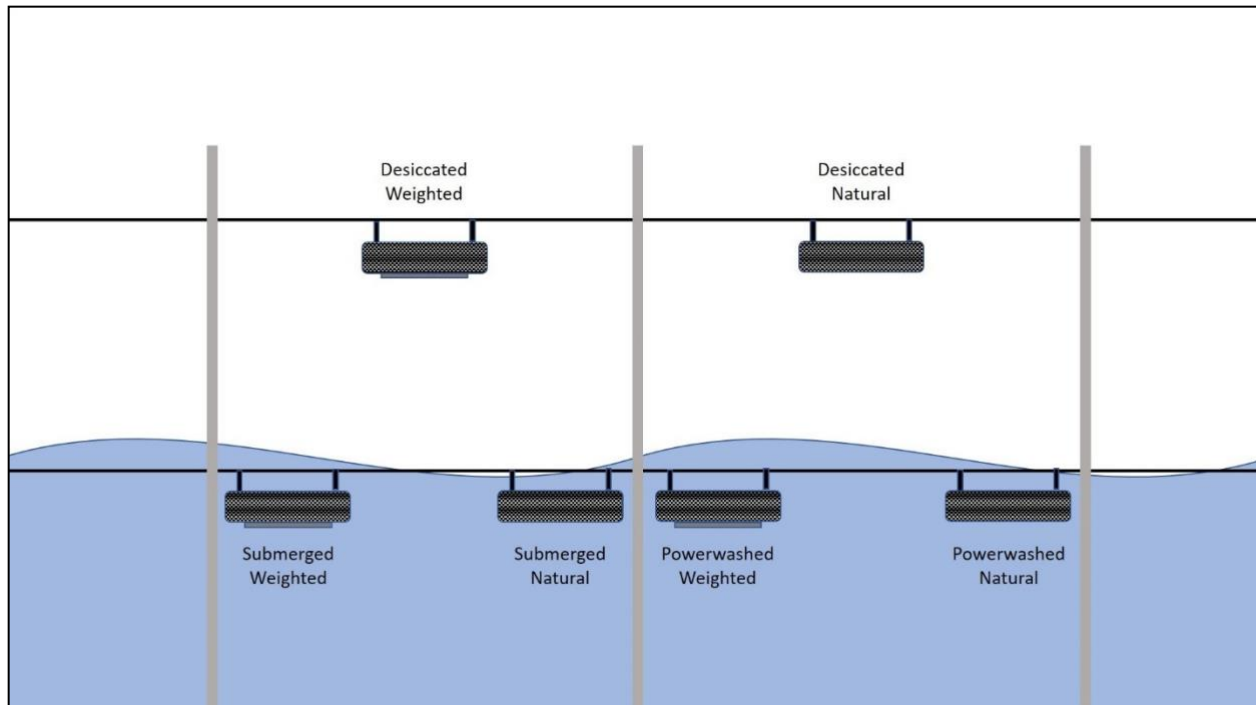


Figure 1. Diagram of experimental longline setup with an illustration of biofouling control and wave action treatments. Each was paired with either ‘low’ or ‘high’ density for a total of twelve unique treatments.

What We Found

Response variables were broken into three categories for easy comparison between treatments: production quantity (survival, shell height, whole wet weight), production quality (cup and fan ratio, condition, cleaning time), and equipment fouling. In general, reduction of wave action led to an increase of metrics within the production quantity (e.g., shell height; Fig. 2) category (aside from survival) but decreased production quality metrics. The opposite was observed for natural wave action treatments (e.g., cup ratio; Fig. 3), where increases in quality metrics and decreases in quantity metrics were shown. Survival throughout this study was generally high regardless of treatment combination.

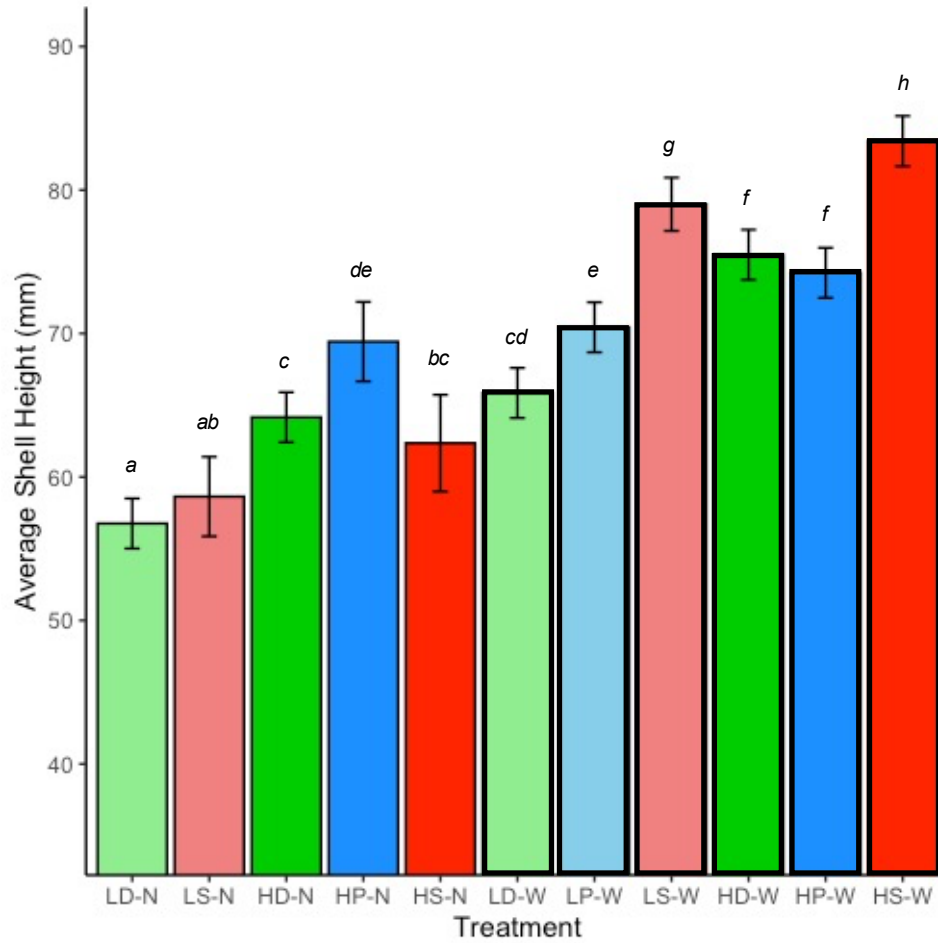


Figure 2. Average shell height (mm) by treatment. Error bars indicate upper and lower 95% confidence limits. Bars that share a letter are not statistically significant ($p > 0.0500$). Bars that have bolded borders indicate those that belong to the weighted wave action treatment and those that share colors illustrate identical density-biofouling control treatment combinations.

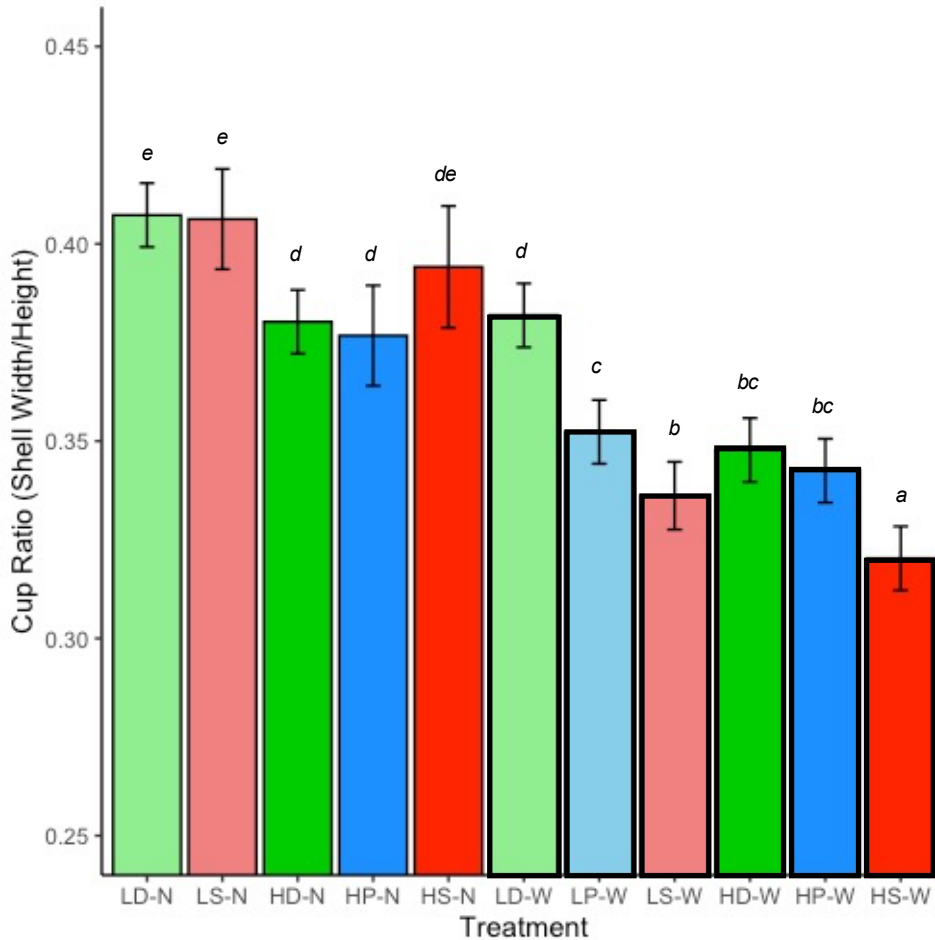


Figure 3. Average cup ratio (shell width/shell height) per treatment. Error bars indicate upper and lower 95% confidence limits. Bars that share a letter are not statistically significant ($p > 0.0500$). Bars that have bolded borders indicate those that belong to the weighted wave action treatment and those that share colors illustrate identical density-biofouling control treatment combinations.

While these were general patterns between the two types of wave action, decisions that farmers make (stocking density and biofouling control) had different outcomes based on wave action. For farmers intending to solely maximize production quantity in a low wave action environment, no biofouling control paired with low density seems to be advantageous. Growers

in the same environment focused on increasing production quality metrics, however, should concentrate on weekly desiccation and reduced stocking density for the best results. For a blend of quantity and quality in a low wave action site, farmers should benefit from combining desiccation with high density. In contrast, producers focused on maximizing production quantity in a high wave action environment may find the best results from power washing and increasing stocking density. Similar to low wave action, farmers focused on production quality should again practice weekly desiccation and maintain low stocking density, however, should expect additional quality metric benefits from higher wave action. Growers looking for a blend of quantity and quality in a high wave action environment should benefit from increased density combined with no biofouling control. Oyster cleanliness, illustrated by cleaning time, was dominated by biofouling control, where weekly desiccation showed a significant decrease in comparison with submersion and power washing, and additional benefits from natural wave action and low density (Fig. 4). In regard to basket fouling in a low wave energy environment, power washed treatments were highly fouled while the desiccated and submerged treatments tended to be the least fouled. Within high wave action, however, only the high density submerged baskets stood out as heavily fouled.

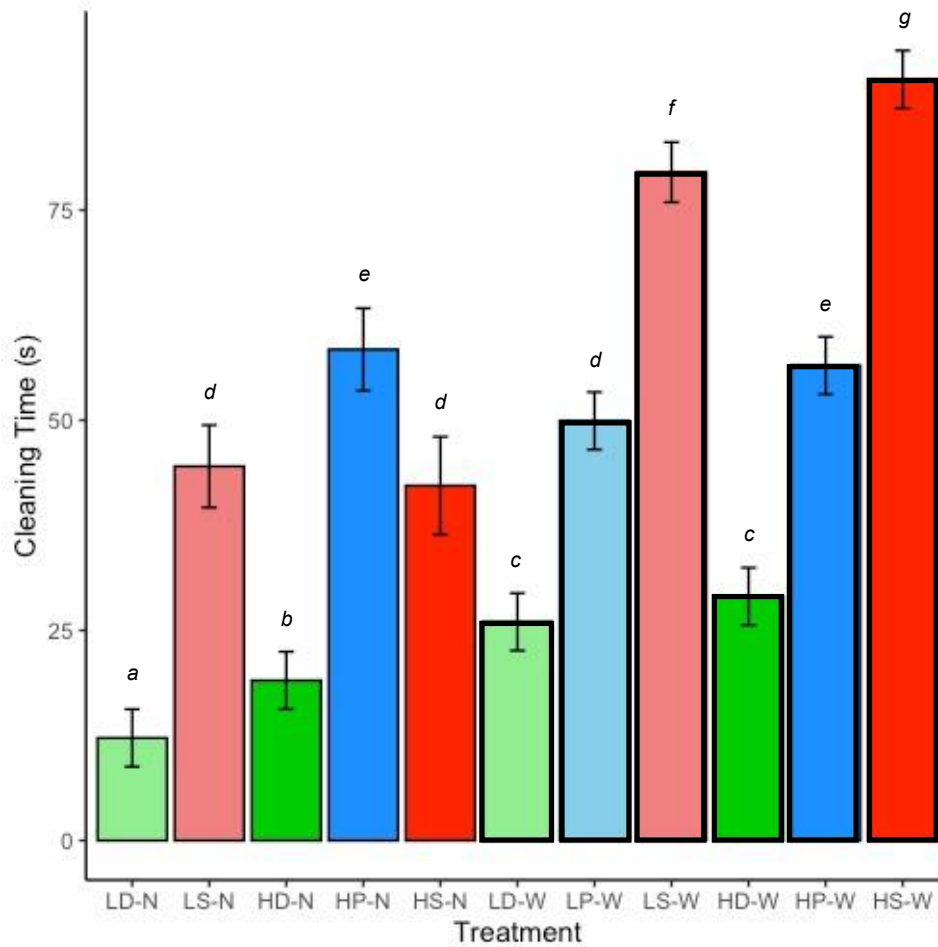


Figure 4. Average cleaning time (in seconds) per oyster for each treatment. Error bars indicate upper and lower 95% confidence limits. Bars that share a letter are not statistically significant ($p > 0.0500$). Bars that have bolded borders indicate those that belong to the weighted wave action treatment and those that share colors illustrate identical density-biofouling control treatment combinations.

Oyster Farming Recommendation

Overall, this study not only supports the importance of site selection for oyster farming but also provides evidence of the interactive impact of other farming practices on the final oyster product. While this study illustrates an increased benefit in production quality for high wave

action sites, it is still possible to produce a high-quality product within a low wave action environment if the proper farming practices are applied. Every grow-out site provides a unique set of environmental and physical characteristics that will shape the outcome of oysters in an off-bottom system, so it is essential for farmers to take in account all farming choices presented here in order to achieve their highest production potential.

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