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Assessment of Off-Bottom Oyster (Crassostrea virginica) Aquaculture Techniques on Biofouling in the Northern Gulf of Mexico

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ASSESSMENT OF OFF-BOTTOM OYSTER (CRASSOSTREA VIRGINICA) AQUACULTURE TECHNIQUES ON BIOFOULING IN THE NORTHERN GULF OF MEXICO

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Renewable Natural Resources

by
Ellis Leroy Chapman Junior
B. S., Christopher Newport University, 2015
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Abstract

Although off-bottom oyster aquaculture is a rising industry in the northern Gulf of Mexico, it is susceptible to biofouling, the accumulation of organisms on industry surfaces. Biofouling creates problems for commercial growers by increasing the costs of labor associated with biofouling management. The most used off-bottom production techniques involve aerial exposure. *OysterGro*™ 6-slot off-bottom oyster cages were used in this project to test aerial exposure frequency, antifouling coatings and bag position on mortality, growth rates, shell ratios and condition indices on four sites in the northern Gulf of Mexico. Aerial exposure of floating cages was performed in increments of once a week, once every two weeks, and once every three weeks, with and without antifouling coatings. The results of the experiment suggest that site location makes the largest differences in production; also weekly aerial exposure and bag position inside cages impact production. These effects contributed to differences in size, shape, biofouling accumulation, quality and survival. The use of antifouling coatings on pontoons and bags may have reduced wet bag weights in Florida, but otherwise did not impact production in this project.

Chapter 1. Introduction

Off-bottom oyster production is expanding in the northern Gulf of Mexico This production method has the potential to produce high-quantities of high-quality oysters which can sell for more premium values (Walton *et. al*, 2012). Off-bottom culture results in faster growth of oysters, increased survival from predation, improved fan and cup ratios, and improved product consistency (Walton *et.al*, 2013). One of the major problems with off-bottom production is the costs of labor associated with biofouling control (Adams *et. al*, 2011) which reduces grower profits. Biofouling is defined here as the accumulation of all macro-organisms, micro-organisms, biofilms, sediment, organic material, and inorganic material on hard industry surfaces (Bixler & Bhushan, 2012).

Adams *et. al* (2011) state that biofouling maintenance is the number one expense for off-bottom production accounting for almost 15% of all costs of production. A New Zealand study estimated NZ-$64.3 million dollars (US-$41.1 million) over a 24-year period would be required for biofouling management at a green-lipped mussel farm (Soliman & Inglis, 2018). Fouling organisms such as barnacles, mussels and oysters create numerous maintenance issues for off-bottom culture. These organisms may have pointed edges that can warp and damage equipment; such as cages and ropes, by cutting rope or plastic via mechanical chaffing (Soliman & Inglis, 2016 & Cavour *et. al*, 2003). Sharp-edged fouling species may make the equipment dangerous to use for the handlers. Organisms may also attach themselves directly to oysters, creating spatial problems by limiting cage space for harvestable livestock (Fitridge *et. al*, 2012). Organisms, such as macroalgae and tunicates, may clog the mesh of containers and have negative effects such as reduction of food availability. Restriction of water flow can create hypoxic conditions in the containers themselves, and build-up of nitrogenous waste inside containers can increase mortality (Collin *et. al*, 2010). Overcrowding has been known to reduce growth, increase mortality, reduce meat weight and negatively affect shell shape of cultured oysters (Lacoste *et. al*, 2014). Fouling organisms can cause direct physical constraints upon the livestock which manipulate shape and meat weight (LaCoste *et. al*, 2014).

The shift to off-bottom production has created the need for distinct management strategies not associated with on-bottom production. Biofouling does not occur uniformly throughout the water column and the most affected areas are in the pelagic, photic, and intertidal zones (Claereboudt *et. al*, 1994). These represent the primary production area in which off-bottom production is based. Current oyster production incorporates biofouling reduction through techniques via aerial drying (Comeau, 2013 and Lacoste *et. al*, 2015) and biofouling resistant chemical coatings (Dunham *et. al*, 2012 and Braithewaite *et. al*, 2007). Aerial drying targets fouling organisms by exposing them to air and direct sunlight, which makes them vulnerable to desiccation (South *et. al*, 2017). Coatings on equipment reduce biofouling in multiple ways. Coatings may act as biocides and kill setting organisms that adhere to cage surfaces (Edwards *et. al*, 2015 & Dunham *et. al*, 2012) or deter organisms from attaching by chemically changing the surface, which reduces biofouling settling-success. Generally, cages and bags are extruded polyethylene plastic mesh which is hydrophobic. A change of cage and bag surfaces to a hydrophilic state makes it difficult for setting organisms to attach (Ashraf *et. al*, 2017 & Zheng *et. al*, 2012).

Aerial drying of floating cages is one of the most common techniques in off-bottom production and there is evidence that suggests this technique will reduce biofouling accumulation

(Mallet *et. al*, 2009, & Comeau, 2013 and Walton *et. al*, 2013). Aerial drying is so effective at reducing biofouling that the culture technique was specifically designed for aerial exposure. Evidence of the efficacy of this technique was first observed in in colder latitudes than the Gulf region (Carver *et. al* 2009), specifically Australia, New Zealand and then to the Northwest and Northeast USA coastlines, but some southern growers have started adopting this technique (Walton *et. al,* 2013, La Peyre *et. al,* 2017). Increased temperatures of the Gulf allow for faster growth of livestock but also substantially increase biofouling rates compared to colder environments.

Site selection is a key aspect to consider when establishing aquaculture farms because all locations will have distinct management requirements (Leonhardt *et. al*, 2017 & Stelzenmuller *et. al*, 2017). Temperature and salinity affect oyster growth more than any other abiotic factors and certain locations may also have riverine influences, extreme tidal ranges, currents, and salinity variations that can alter oyster growth (Leonhardt *et. al*, 2017 & Miller *et. al*, 2017). This study was part of a collaboration through the Southeast Regional Aquaculture Center (SRAC) to assess biofouling control via aerial drying for both the southeast US Atlantic and northern Gulf of Mexico coasts. The project was designed to include two teams (one team in the SE Atlantic and one team in the Northern Gulf) to deploy floating cages and assess aerial drying of these cages through weekly (flipped once a week), biweekly (flipped once every two weeks) and triweekly (flipped once every three weeks) increments, with and without antifouling coatings. The Atlantic team had test sites in North Carolina, South Carolina and Georgia. The Gulf team had test sites in Alabama, Louisiana, Florida and Mississippi.

Chapter 2. Effects of flipping frequency and antifouling coatings on biofouling in off-bottom oyster (*Crassostrea virginica*) aquaculture production in the northern Gulf of Mexico

Materials and Methods

Sites

The Gulf team sites were located in Grand Isle, LA, Biloxi, MS, Fort Morgan, AL, and Cedar Key, FL.

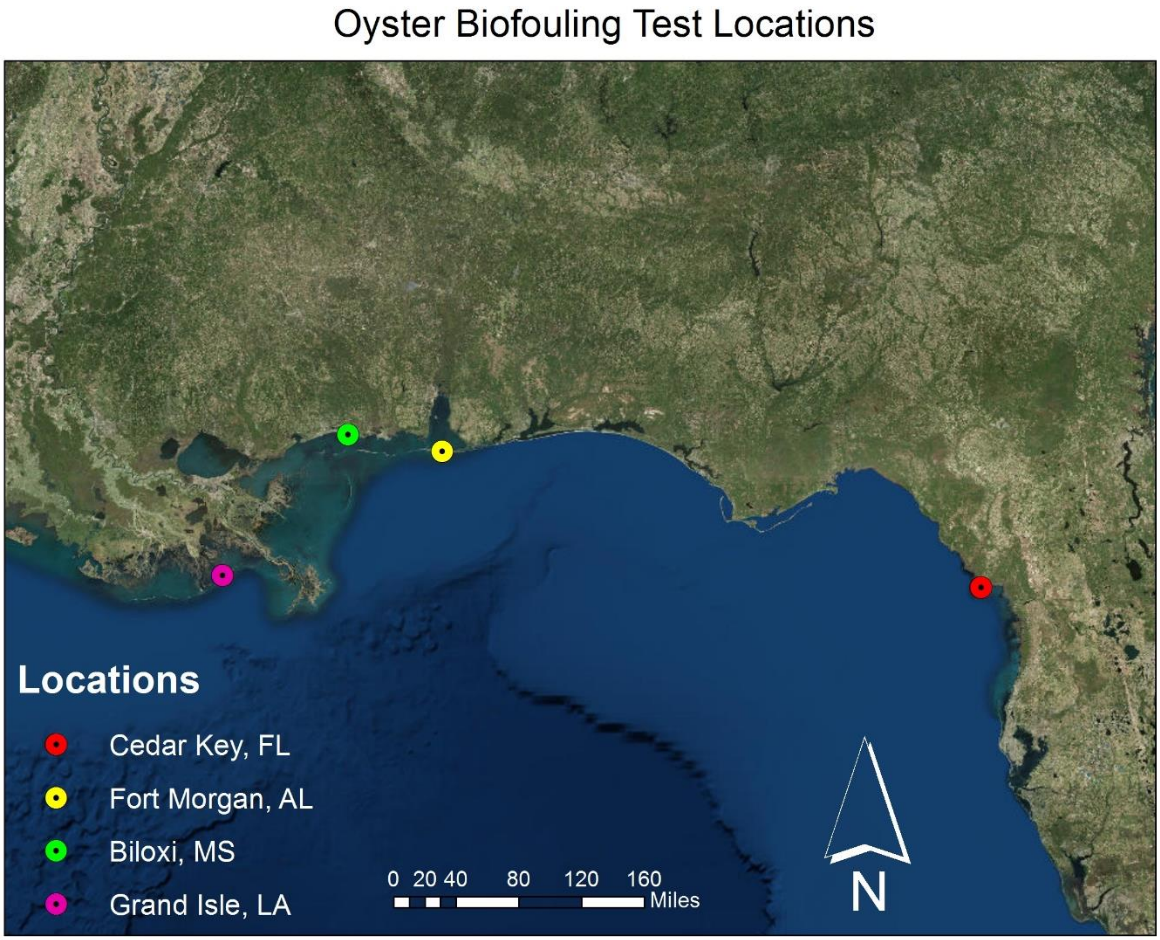

Figure 1. Gulf of Mexico testing sites. Source: ESRI Online®.

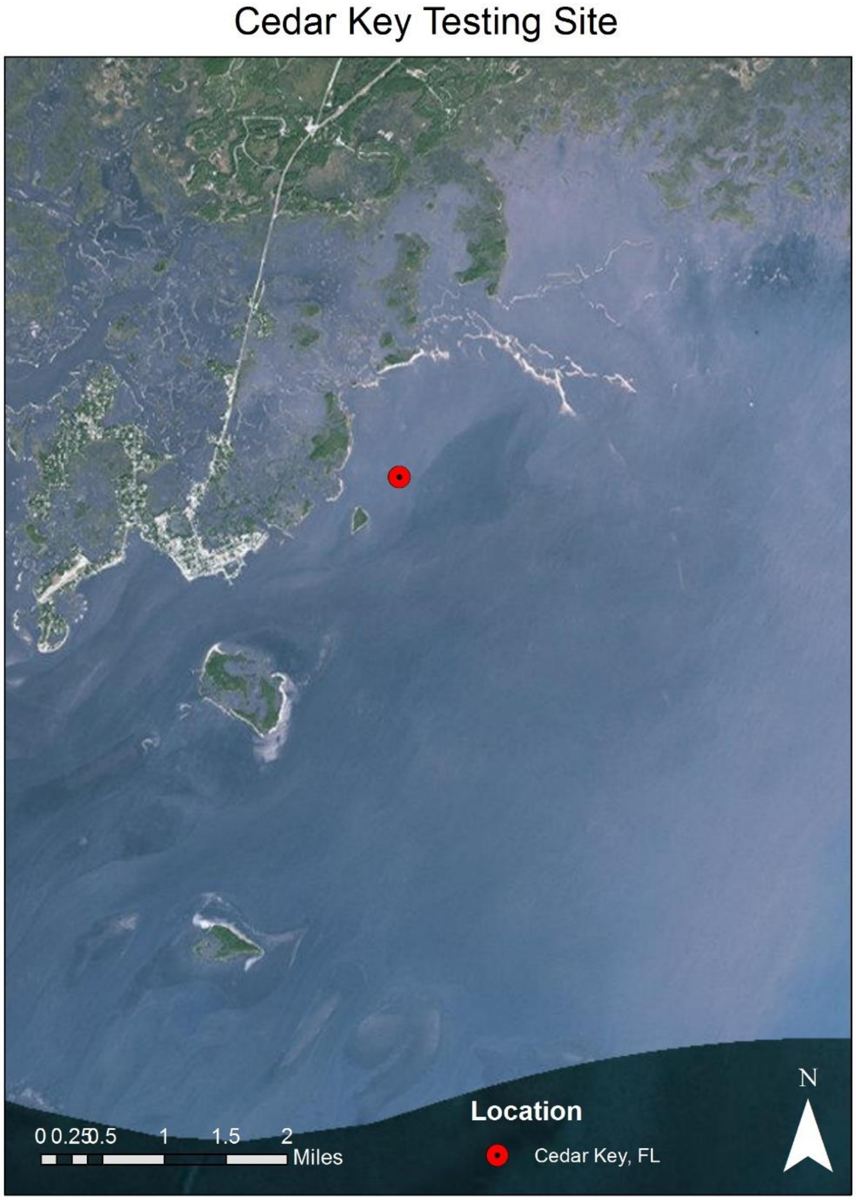

Figure 2. Satellite image of Cedar Key, FL testing site. Source: ESRI Online®.

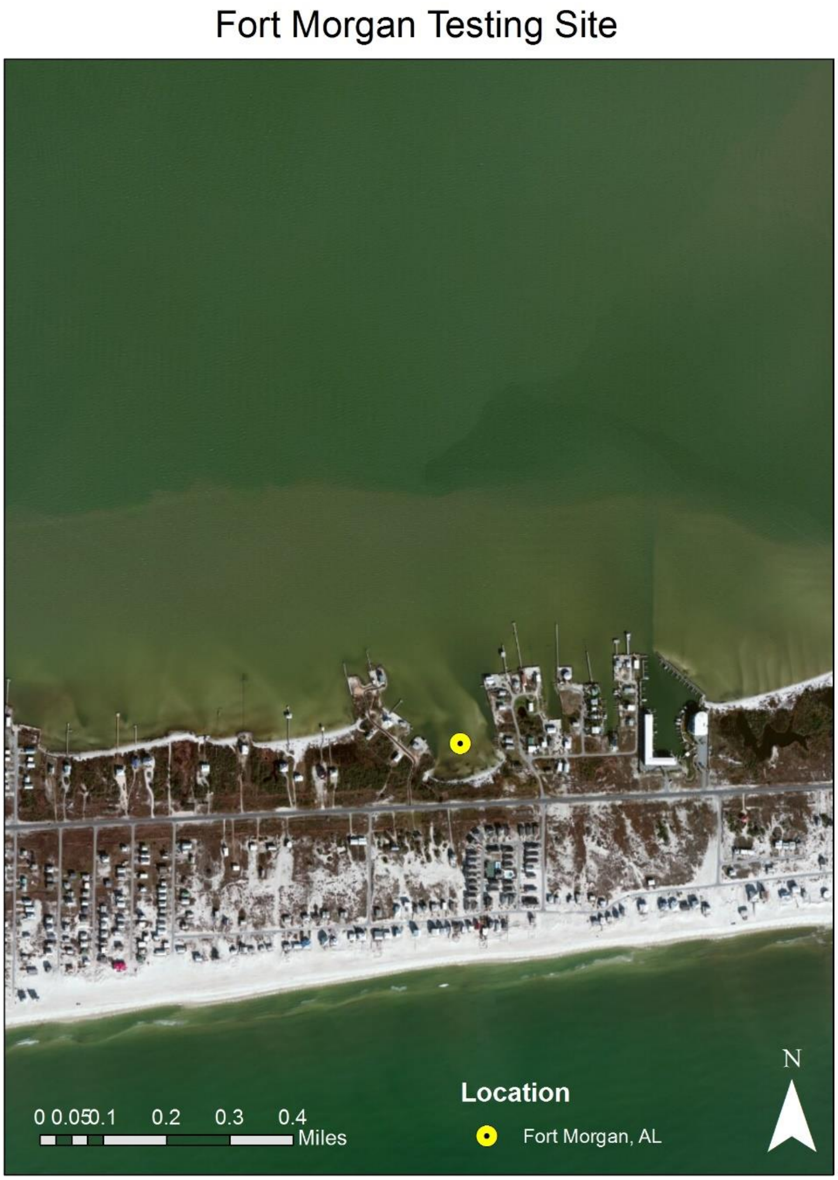

Figure 3. Satellite image of Fort Morgan, AL testing site. Source: ESRI Online®.

Figure 4. Satellite image of Biloxi, MS testing site. Source: ESRI Online®.

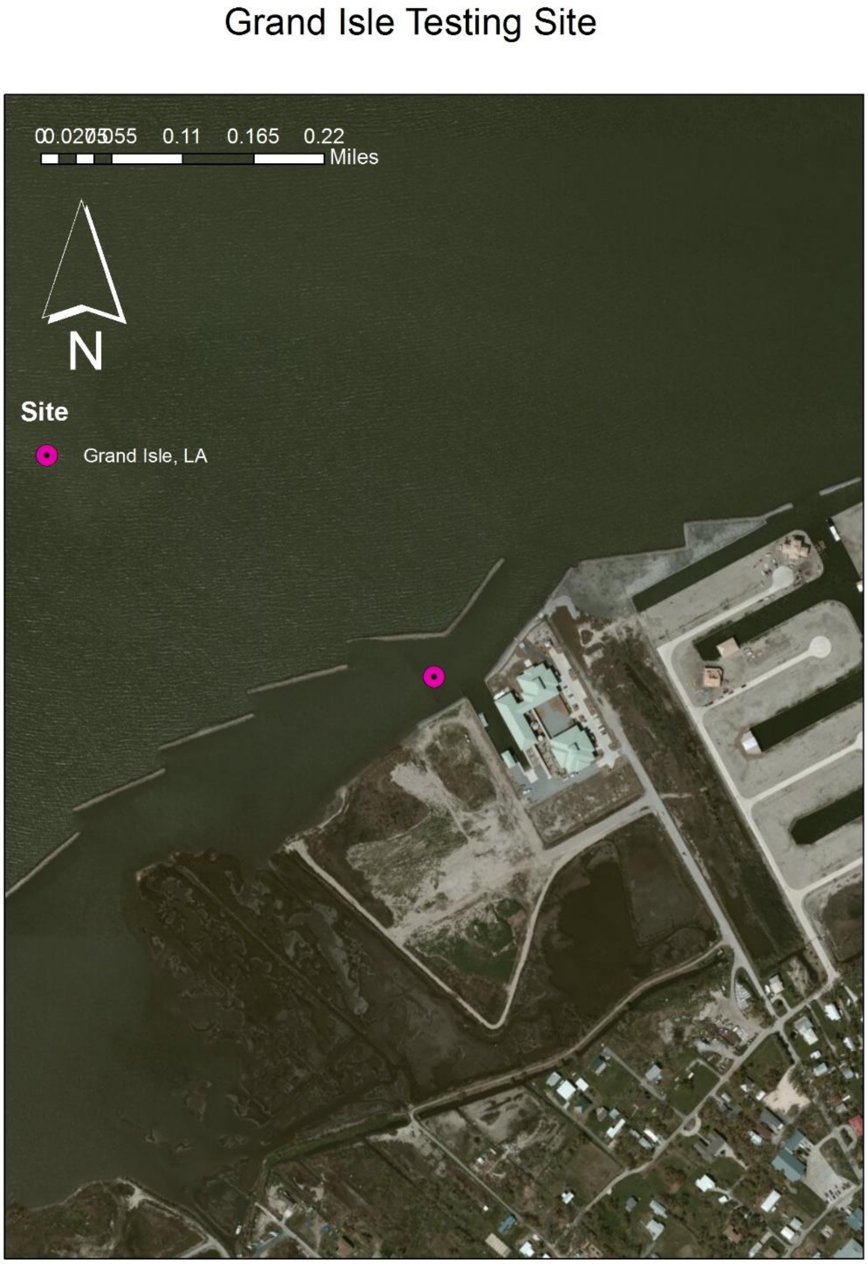

Figure 5. Satellite image of Grand Isle, LA testing site. Source: ESRI Online®.

The Florida site (29.14434 N, -83.00606 W) was chosen by Co-PI, Leslie Sturmer of the George C. Kirkpatrick Laboratory in Cedar Key, FL, who also operated the site. This site was open to the Gulf of Mexico on the southern and eastern sides which made it very exposed to open water and storms. The bottom was mud and sand based and the average depth was 120-150 cm with a tidal range of 75-105 cm. The salinity here remained above 18 ppt and the site was not prone to hypohaline events.

The Fort Morgan, AL site (30.2329586 N, -87.9797712 W) was located at Navy Cove Oyster Company, a commercial collaborator in the study. The site was on the southern shore of Mobile Bay. A large fetch to the north made this site prone to storms and strong winds in the fall and winter months. The site had a sandy bottom and the depth averaged ~120 cm with a ~30 cm tidal range. This site was prone to hypohaline events from the river discharges of the Mobile and Tensaw Rivers into the northern portion of Mobile Bay.

The Biloxi, MS site (30.3655297 N, -88.8392207 W) was located 500 m south of Deer Island and 500 meters north of Katrina Key. This site was southerly exposed to open water and was prone to storms during the spring and summer months. The site was selected by the Mississippi Department of Marine Resources (MDMR). This location was also prone to hypohaline events from the Biloxi and Tchoutacabouffa Rivers discharge into Biloxi Bay. The site had a sandy bottom at an average depth of ~120 cm with a 30-45 cm tidal range.

The Grand Isle, LA site (29.2389165 N, -90.00028173 W) was located on the northern side of Grand Isle near the shore of Barataria Bay. The site was designed for horizontal flow across the cages in shallow waters between shoreline and artificial riprap rock structures. The site was also protected from a wide fetch over the Barataria Bay by the riprap. As a result, there appeared to be much less wind and wave action impacting the growing cages. This site was chosen for the Louisiana Sea Grant Oyster Research and Demonstration Farm. The site had a muddy bottom and the average depth was ~120 cm with a ~30 cm tidal range. This site was also particularly vulnerable to frequent prolonged periods of hypohaline events due to its proximity to the Mississippi River delta.

Culture Equipment

More research is required to characterize off-bottom biofouling control methods in the northern Gulf of Mexico. This study investigated the effectiveness of three aerial drying regimes (by varying frequency) and antifouling coatings as potential solutions.

The OysterGro^{TM} six-slot floating cage system (Figure 6) is a metal wire cage that can store individual Velar mesh bags of various size. These cages were donated by Bouctouche Bay Industries from New Brunswick, Canada. There were 12 cages at each site over 4 sites yielding 48 cages overall.

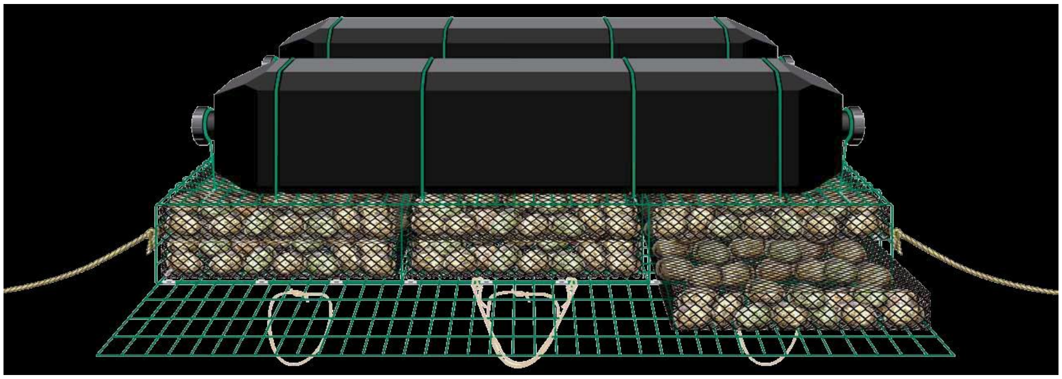

Figure 6. The OysterGro™ floating cage. Each cage consists of an outer housing made of 12-gauge vinyl-coated wire mesh which houses six Velar® bags. Each cage measures 1.524 m (60” in) long, 0.914 m (36” in) wide, and 0.152 m (6”) deep. Source: www.oystergro.com

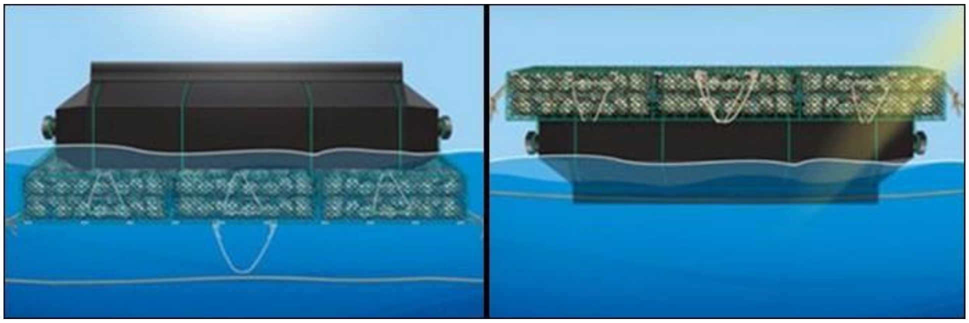

Figure 7. Growing position (left) and drying position (right) of the cage. Source: oystergro.com

The floating cage system was designed to flip the cages and pontoons while deployed in the water. Floating cages operate in two positions: the growing position and the drying position (Figure 7). Once deployed, all pontoons were in the growing position until scheduled aerial drying, which was achieved by flipping the cage 180 degrees in the water. This left the bags and oysters in the air, putting fouling organisms at risk of desiccation, starvation and suffocation (Carver *et. al*, 2009). Comeau (2013) observed that oysters can survive out of water for up to twenty-four hours without adverse side effects. One advantage of this method is that the grower can decide when and for how long to the cages are flipped and exposed to the air. A disadvantage is that this requires intensive labor.

Netminder^{TM} is a commercially available brand of paint marketed as a deterrent for marine fouling organisms on submerged painted surfaces. The company selling this product worked in collaboration with the project and provided the chemical antifouling agent free-of-charge. The coating was a water, hydrogen peroxide and silicon based solution that was applied via the instructions from Netminder^{TM}. Hydrogen peroxide quickly dissolve so longevity is a question to the use of these coatings. The coatings were applied before deployment and allowed time to dry before put into use in the field.

Pre-Deployment

Activities at each site's activities were supervised by the designated local specialists. The Michael C. Voisin Oyster Hatchery/Sea Grant Oyster Research Laboratory on Grand Isle, LA supplied the seed for the experiment. Seed for the project came from this one source to ensure consistency among all Gulf States. Triploid oyster seed, from crossing tetraploid males with diploid females, were used for the experiment because of known superior growth rates, disease resistance and neutral effects on genetic variability on wild populations due to sterility (Allen, 1992 & Supan, 2001). Dr. Ryan Carnegie's laboratory from the Virginia Institute of Marine Science tested for the presence of diseases (*Perkinsus marinus & Haplosporidium nelson*) before the seed was deployed to individual states as per state regulations for interstate shipping of shellfish. After disease screening, Florida and Louisiana seed were grown at the Voisin hatchery until reaching a size that would be retained in 12 mm grow-out bags (Sept. 2017). During August 2017, seed was sent to Dauphin Island, AL to condition until field deployment at the Auburn University Shellfish Laboratory at the Dauphin Island Sea Lab in Dauphin Island, AL. Both states were conditioned at Dauphin Island. Approximately 43,200 individual seed were used for the entire project split equally over four states resulting with roughly 10,800 seed per site (Table 1).

Table 2.1. Experimental design for floating cages of each site.

Total Cages	Cages with Pontoons Coated	Bags per Cage	Bags Coated per Cage	Oysters per Bag	Total Oysters in Cages per Site
12	6	6	3	150	10800

Pontoon-cage interfaces can become heavily fouled, but the tops of the pontoon are rarely affected by fouling, so the coating was applied on the bottoms of the pontoons only. The caging was not coated because fouling is less problematic and due to difficulty applying paint onto caging. Bags were coated and loaded into cages to disperse the variance inside the cages between coated and non-coated bags. Coating was applied per instructions from Netminder™. (Figure 8).

Figure 8. Design of oyster growing bags with and without antifouling fouling coatings inside growing cages.

At each site there were twelve sets pontoons in total (Figure 9). Four sets of pontoons were flipped weekly (once every week), four sets of pontoons were flipped biweekly (once every two weeks), and four sets of pontoons were flipped triweekly (once every three weeks). A synchronized schedule was given to extension agents for uniformity of flipping schedules across all sites.

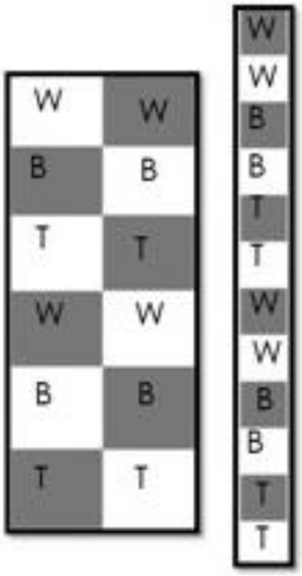

Figure 9. Pontoon arrangement at each site. The Louisiana site had the arrangement on the left. Mississippi, Florida and Alabama had the arrangement on the right. “W”, “B” and “T” respectively refer to weekly flipping regime, biweekly flipping regime and triweekly flipping regime. Shaded-background areas indicate coated pontoons and white-backgrounds indicate non-coated pontoons.

Sampling Methods

Throughout the study, quarterly samplings were conducted (December 2017, March 2018 & June 2018) with ten oysters selected haphazardly from each bag. These oysters were measured for shell length, shell height and shell width (Galtsoff *et. al*, 1964, Figure 10) which were used to calculate fan and cup ratios. Fan ratio is the shell height divided by the shell length and cup ratio is the shell width divided by the shell length. Photographs of the bags were used for visual determination of percent fouling. Photographic assessment was based upon a picture of each treatment bag and estimated “percent fouling” of a specific point in the middle of the narrow side of the bag. This area was bordered by a ruler marking 6 inches (15.24 cm) across and a notecard labelling the sample. Initially percent fouling was to be measured on pontoons and bags using ImageJ® software, but due to poor and inconsistent backgrounds in photographs, using the software was unreliable and visual assessment of the middle 15 pores, 3x5, in the bag pictures was used (Figure 11).

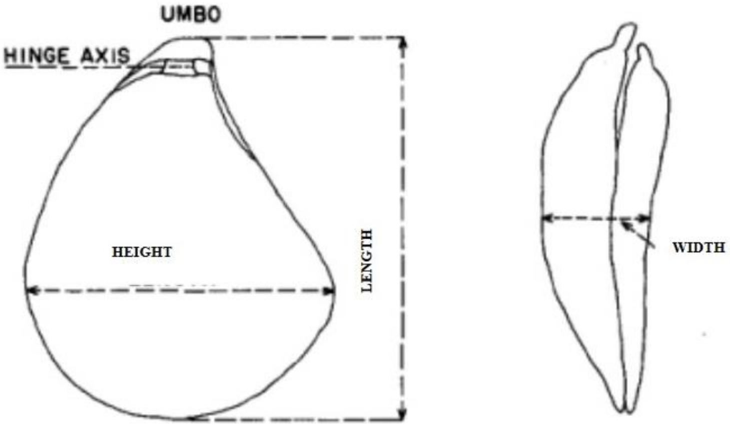

Figure 10. Shell dimensions as described by Galtsoff, 1964.

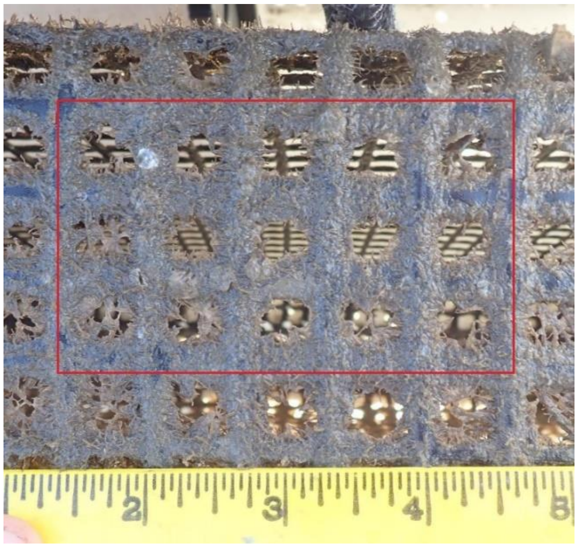

Figure 11. Reference bag describing the visual quantification of percent. This would be ranked as 0.00 percent fouling. Source: Shannon Kirk, University of Georgia.

Bag Position Sampling Considerations

The same dataset from the experiment was used in this analysis and used the same experimental design. For this study, the application of antifouling coatings was considered negligible and it is assumed that coatings do not impact production. All four sites were assessed randomly and independently. Each cage has 6 slots and each specific bag-slot was labelled to relate to Figure 12. It was observed in the experiment that the differences in the results were associated to two variations upon location. In the first variation, position's 2, 4, and 6 were labelled as "top" because during aerial exposure, these were the sides of the cage exposed to the air. Subsequently, position's 1, 3, and 5 were labelled as "bottom" because they were situated underneath the "top" row during aerial exposure. In the second variation, position's 1, 2, 5 & 6 were labelled as "outside" and position's 3, & 4 were labelled as "inside". This analysis intends to investigate the impacts of flip regime in weekly, biweekly and triweekly increments along with the variability associated to, top/bottom and inside/outside, positional variations.

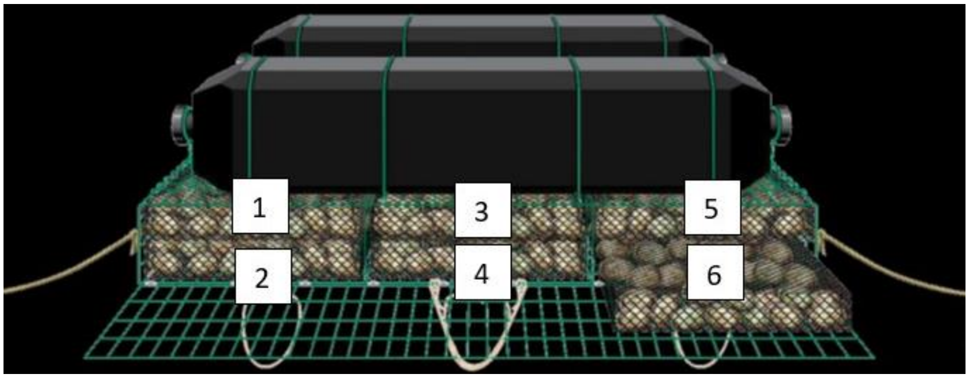

Figure 12. The OysterGro™ floating cage. Each cage consists of an outer housing made of 12-gauge vinyl-coated wire mesh which houses six Velar® bags. Each cage measures 1.524 m (60" in) long, 0.914 m (36" in) wide, and 0.152 m (6") deep. Source: www.oystergro.com

Post-Harvest

The initial plan was to conduct a 12-month analysis and make a final harvest in September 2018. However, the cages (specifically biweekly and triweekly flipped cages) were beginning to sink by April 2018 and it was decided after the second quarterly sampling to use the third quarterly sampling (June 2018) for the final harvest. At final harvest, each bag at each site was collected and oysters sorted for a dead/live count. This allowed for mortality results for each bag after a 9 month grow-out period. Wet bag weights (kg) were measured with a common fish-hook scale and a photograph of each bag was taken for percent fouling assessment. Twenty-five oysters from each bag were put into separate polyethylene freezer bags, placed on ice in a cooler transported back to the laboratory and put in chest freezers for long term storage.

After the samples were frozen, they could then be taken out, rinsed, "patted-dry" and used for further analysis. Of the 25 oysters collected from each of the 288 bags (72 bags per site), all were measured for shell length, shell width, shell height, fan ratio, cup ratio and the whole wet weight at final harvest. Ten individuals, from each twenty-five oyster sample set, were further assessed for volume displacement, weight/volume of fouling on oysters, condition index, and oyster meat quality index (as developed by 2009Australian Seafood Research Convention and modified by the Auburn University Shellfish).

Statistical Analysis

In chapters two and three, the goals are same. The goals are to assess size, shape, biofouling accumulation, quality and survival upon oyster production at four sites in the Gulf of Mexico. The objectives in chapters two and three are similar but not the same.

For chapter two, each response variable was assessed by using a six-factor generalized linear model (logit link-Poisson distribution or logit link-binomial distribution). This was constructed with three main effects being flip regime (FR), bag coating (BC), and pontoon coating (PC). The model used the main effects interacting among tested hypotheses (PROC GLIMMIX, SAS, vers.9.4, SAS Institute Inc., Cary, NC). Post-hoc analyses were conducted on least-squares means with Tukey-adjusted p-values. Generalized linear model assumptions were assessed by goodness-of-fit statistics (lowest AIC and Chi-square/degree of freedom closest to 1). The singular fixed results were used to compare the impacts of just the main factors from site to site. Site was considered a random effect and each data set was assessed independently. The model statement for the chapter two analysis was:

$$y_x = \mu_x = FR_x + BC_x + PC_x$$

In the chapter three assessment for bag position (bag position 1 (BP1) (inside/outside), and position 2 (BP2) (top/bottom)), a general linearized mixed model with a Tukey post-hoc analysis ($\alpha=0.05$) was applied using the same SAS glimmix procedure. The model statement for the chapter three analysis was:

$$y_x = \mu_x = BP1_x + BP2_x$$

The five main oyster attributes assessed were size, shape, biofouling accumulation, quality and survival. While shell length (mm) is a commonly used metric in wild harvest (with 75 mm a typical minimum harvest size in fisheries), size also was quantified by whole wet weight (g) and volume displacement (mL). Shape attributes involved differences in fan and cup ratios at final harvest. These ratios are proportional size indicators, without units. Biofouling accumulation was quantified by percent fouling (ratio), defouled weight (g), defouled volume (mL), and wet bag weights (kg) at harvest. Defouled weight/volume was the weight/volume of the oysters during analysis after having had all biofouling removed. These values reflected the difference in the first measurement (pre-cleaning) and the second measurement (after biofouling removal). These numbers quantified weight and volume of biofouling on the oysters themselves. The wet bag weight (kg) at final harvest was a measure of biofouling on the gear itself.

Quality was quantified by both a qualitative index and condition index. The quality index was adopted from the Auburn University Shellfish Laboratory at the Dauphin Island Sea Laboratory in Dauphin Island, AL. This measurement was based upon three oyster meat criteria (body, mantle & fullness) and each criterion was rated from 0-3 (0=best, 3=worst). The index score was the average sum of the three scores and lower scores indicate higher quality. Condition index is a measurement widely used to describe fecundity and spawn cycles in oysters, but the oysters in this project were triploids, so this number became a “meat-to-shell” ratio that essentially quantified the “meatiness” of the oysters. Survival was directly measured by the portion of animals surviving to harvest in each bag.

Goals and Objectives

Goals

To determine the impacts of flip regime and antifouling coatings evaluate the effects on:

- Size
- Shape
- Biofouling Accumulation
- Quality
- Survival

Objectives

1) To test the effects of flip regime upon oyster production

H_0: $\mu_{weekly} = \mu_{biweekly} = \mu_{triweekly}$
H_A: H_0 false

2) To test the effects of antifouling coatings, on bags and pontoons, upon oyster production

H_0: $\mu_{coating} = \mu_{non\text{-}coating}$
H_A: $\mu_{coating} \neq \mu_{non\text{-}coating}$

Results

Florida Site

Size

Flipping regime had an impact upon size. Weekly flipping restricted shell length (85.18 mm) as compared to biweekly (91.46 mm) and triweekly (91.47 mm) flipping. (F=90.06, P<0.0001, df_{NUM}=2, df_{DEN}=1783). Tukey groupings for flip regime show that weekly flipped oysters were the smallest by weight at harvest (111.40 g), although triweekly flipped oysters (121.94 g) were smaller than biweekly flipped ones (126.60 g) (F=308.76, P<0.0001 df_{NUM} = 2, df_{DEN} = 1783). The results of weekly flipped oysters on volume displacement (64.13 mL) were significantly lower than those of biweekly (81.11 mL) and triweekly (78.67 mL) flipped oysters (F=289.22, P<0.0001, df_{NUM} = 2, df_{DEN} = 707).

For bag coating, Oysters grown in bags with antifouling coatings had significantly smaller shell lengths (88.80 mm) than oysters grown in bags without (89.94 mm) antifouling coatings. Antifouling coatings on bags, although not statistically significant, appeared to reduce the whole wet weight of oysters grown with antifouling coatings (118.72 ± 0.9365 g) compared to those grown without coatings (121.24 ± 0.9497 g). Bag coating had no impact upon volume at this site.

Antifouling coatings on pontoons had no impact on shell length, or volume displacement. Whole wet weight of oysters grown in pontoons with antifouling coatings were significantly smaller (117.85 g) than for oysters growing in pontoons without antifouling coatings (122.11 g) (F=67.55, P<0.0001, df_{NUM} = 1, df_{DEN} = 1783).

Shape

Mean fan ratios at this site were not significantly affected by flipping regime, bag antifouling coatings or pontoon antifouling coatings. Weekly flipped oysters had a greater fan ratio (0.7837 ± 0.03614) than biweekly (0.7416 ± 0.03548) and triweekly (0.7446 ± 0.03529) flipped oysters at final harvest, but it was not significantly greater.

Cup ratios were affected by flipping regime and pontoon antifouling coatings. The cup ratio of weekly flipped oysters (0.3828) was significantly greater than the cup ratios of biweekly (0.3672) and triweekly (0.3622) flipped oysters (Flip Regime; F=29.57, P<0.0001, df_{NUM} = 2, df_{DEN} = 1783). This may or may not be visually evident to a grower or buyer to the extent that "cups appear deeper". Oysters grown in pontoons with antifouling coating exhibited significantly smaller cup ratios (0.3675) than oysters grown in pontoons without (0.3739) coatings (Pontoon Coating; F=7.93, P-0.0049, df_{NUM} = 1783, df_{DEN} = 1), however, this would also be visually difficult for the grower to perceive at final harvest.

Biofouling Accumulation

Flipping regime impacted all four biofouling response measurements at this site (Percent Fouling; F=9.325, P=0.0003, df_{NUM} = 2, df_{DEN} = 56, Defouled Weight; F=79.46, P<0.0001, df_{NUM} = 2, df_{DEN} = 683, Defouled Volume; F=35.68, P<0.0001, df_{NUM} = 2, df_{DEN} = 641, Wet Bag Weight; F=15.80, P<0.0001, df_{NUM} = 2, df_{DEN} = 60). Data indicated weekly flipping reduced the accumulation of biofouling on both, the gear and the oysters themselves. Based on defouled weight and defouled volume, this site had the greatest accumulation of biofouling on the oysters themselves and weekly flipping resulted in a 2-3 fold reduction in biofouling (6.53 g & 5.94 mL) as compared to biweekly (15.51 g & 12..53 mL) and triweekly flipping (15.73 g & 12.34 mL).

Antifouling coatings on pontoons resulted in no significant differences on biofouling. However, the presence or absence of antifouling coatings on bags resulted in significant differences in biofouling. The wet bag weight, and defouled weight of bags and oysters grown in bags treated with antifouling coatings were significantly lower than for oysters and bags without antifouling coatings (Wet Bag Weight; F=21.21, P<0.0001, df_{NUM} = 1, df_{DEN} = 60, Defouled Weight; F=3.63, P=0.0572, df_{NUM} = 1, df_{DEN} = 683). Percent fouling of bags with (0.3132 ± 0.03287) antifouling coatings was 8% lower than the bags without (0.3950 ± 0.03101) antifouling coatings, but this difference was not statistically significant.

Quality

Both quality and quantity of oysters were impacted by flipping regime at this site. The mean quality index of weekly flipped oysters (0.971) indicated significantly higher quality as compared to biweekly (2.561) and triweekly (2.698) flipped oysters (F=54.52, P<0.0001, df_{NUM} = 2, df_{DEN} = 707). Condition index (meat-to-shell ratio) data demonstrated a pattern of increased condition with increased aerial exposure. Weekly flipped oysters had the greatest score (11.36), followed by biweekly flipped oysters (9.45), and triweekly flipped oysters (7.53) (F=135.95, P<0.0001, df_{NUM} = 2, df_{DEN} = 707).

Antifouling coatings on bags and pontoons also affected quality index values. At this site, the oysters growing in bags with antifouling coatings had a significantly lower quality index score (1.86) than oysters grown in bags without antifouling coatings (2.30). (Bag Coating; F=54.52, P<0.0001, df_{NUM} = 2, df_{DEN} = 707) The oysters grown using pontoons with antifouling coatings had a significantly higher quality index score (2.46) than oysters grown in pontoons without antifouling coatings (1.69) (Pontoon Coating; F=26.56, P<0.0001, df_{NUM} = 2, df_{DEN} = 707).

Survival

There were no significant differences in the survival of oysters, at this site, attributable to flip regime, bag coating or pontoon coating. Triweekly flipped oysters had the highest survival and survival without antifouling coatings on bags or pontoons was higher than with antifouling coatings by an insignificant margin.

Table 2.2. Florida oyster response variables separated by weekly, biweekly and triweekly flipping. Presented as (Value ± Standard Error, Tukey Group).

	Flip Regime		
	1	**2**	**3**
Shell Length (mm)	**85.18 ± 0.379, B**	**91.46 ± 0.397, A**	**91.47 ± 0.393, A**
Whole Wet Weight (g)	**111.40 ± 1.073, C**	**126.60 ± 1.218, A**	**121.94 ± 1.169, B**
Volume (mL)	**64.13 ± 1.090, B**	**81.11 ± 1.352, A**	**78.67 ± 1.312, A**
Fan Ratio	0.78 ± 0.036, A	0.74 ± 0.035, A	0.74 ± 0.035, A
Cup Ratio	**0.38 ± 0.002, A**	**0.37 ± 0.002, B**	**0.36 ± 0.002, B**
Percent Fouling (%)	**0.21 ± 0.037, B**	**0.38 ± 0.040, A**	**0.47 ± 0.041, A**
Defouled Weight (g)	**6.53 ± 0.341, B**	**15.51 ± 0.754, A**	**15.73 ± 0.771, A**
Defouled Volume (mL)	**5.94 ± 0.335, B**	**12.53 ± 0.668, A**	**12.33 ± 0.665, A**
Wet Bag Weight (kg)	**2.03 ± 0.091, B**	**2.62 ± 0.091, A**	**2.69 ± 0.091, A**
Quality Index	**0.97 ± 0.079, B**	**2.56 ± 0.162, A**	**2.70 ± 0.170, A**
Condition Index	**11.36 ± 0.168, A**	**9.45 ± 0.169, B**	**7.53 ± 0.168, C**
Survival (ratio)	0.89 ± 0.013, A	0.88 ± 0.013, A	0.90 ± 0.011, A

Table 2.3. Florida oyster response variables separated by oysters grown in bags with antifouling coatings and oysters grown in bags without antifouling coatings. Presented as (Value ± Standard Error, Tukey Group).

	Antifouling Coatings On Bags	
	1 (With Coatings)	**0 (Without)**
Shell Length (mm)	**88.80 ± 0.318, B**	**89.94 ± 0.318, A**
Whole Wet Weight (g)	118.72 ± 0.937, A	121.24 ± 0.950, A
Volume (mL)	73.76 ± 1.017, A	75.52 ± 1.034, A
Fan Ratio	0.76 ± 0.029, A	0.75 ± 0.029, A
Cup Ratio	0.37 ± 0.002, A	0.37 ± 0.002, A
Percent Fouling (%)	0.31 ± 0.033, A	0.40 ± 0.031, A
Defouled Weight (g)	**11.63 ± 0.496, B**	**13.55 ± 0.568, A**
Defouled Volume (mL)	**9.37 ± 0.435, B**	**11.16 ± 0.506, A**
Wet Bag Weight (kg)	**2.20 ± 0.075, B**	**2.69 ± 0.074, A**
Quality Index	**1.86 ± 0.109, B**	**2.30 ± 0.124, A**
Condition Index	9.55 ± 0.138, A	9.34 ± 0.137, A
Survival (ratio)	0.88 ± 0.010, A	0.90 ± 0.010, A

Table 2.4. Florida oyster response variables separated by oysters grown in cages with pontoons with antifouling coatings and oysters grown in cages with pontoons without antifouling coatings. Presented as (Value ± Standard Error, Tukey Group).

	Antifouling Coatings On Pontoons	
	1 (With Coatings)	**0 (Without)**
Shell Length (mm)	89.36 ± 0.323, A	89.38 ± 0.313, A
Whole Wet Weight (g)	**117.85 ± 0.943, B**	**122.11 ± 0.943, A**
Volume (mL)	73.25 ± 1.025, A	76.03 ± 1.027, A
Fan Ratio	0.75 ± 0.029, A	0.76 ± 0.029, A
Cup Ratio	**0.37 ± 0.002, B**	**0.37 ± 0.002, A**
Percent Fouling (%)	0.34 ± 0.031, A	0.37 ± 0.032, A
Defouled Weight (g)	12.73 ± 0.531, A	12.46 ± 0.535, A
Defouled Volume (mL)	10.83 ± 0.500, A	9.70 ± 0.442, A
Wet Bag Weight (kg)	2.46 ± 0.075, A	2.43 ± 0.073, A
Quality Index	**2.46 ± 0.134, A**	**1.69 ± 0.097, B**
Condition Index	9.46 ± 0.140, A	9.43 ± 0.136, A
Survival (ratio)	0.87 ± 0.011, A	0.91 ± 0.009, A

Alabama Site

Size

Oyster sizes were influenced by flip regime. Weekly flipped oysters had a significantly smaller shell length at final harvest than biweekly and triweekly flipped oysters (F=107.65, P<0.0001, $df_{NUM} = 2$, $df_{DEN} = 1787$). Weekly flipped oysters were not fully to harvest size (71.74 mm shell length) as compared to biweekly and triweekly flipped oysters (78.27 mm & 77.80 mm respectively). Oyster size as measured by whole wet weight (g) and volume (mL) indicated similar results. Weekly flipped oysters were lighter and had less volume (71.69 g & 42.98 mL) at harvest than biweekly (86.46 g & 51.36 mL) and triweekly (87.73 g & 54.16 mL) flipped oysters (Weight; F=619.87 , P<0.0001, $df_{NUM} = 2$, $df_{DEN} = 1787$, Volume; F= 173.16, P<0.0001, $df_{NUM} = 2$, $df_{DEN} = 708$).

There were no significant differences in shell length, whole wet weight (g) and volume displacement regarding pontoon and bag antifouling coatings.

Shape

Fan and cup ratios were significantly impacted by flip regime. Oysters flipped more regularly yielded greater fan and cup ratios. Fan ratios (0.8245) of triweekly flipped oysters were significantly smaller than in weekly and biweekly flipped oysters (0.8457 & 0.8447 respectively) (Fan Ratio; $F=12.11$, $P<0.0001$, $df_{NUM} = 2$, $df_{DEN} = 1787$, Cup Ratio; $F= 51.92$, $P<0.0001$, $df_{NUM} = 2$, $df_{DEN} = 1787$).

Oysters grown in pontoons with antifouling coatings had significantly greater fan ratio (0.8445) than did oysters grown in pontoons without antifouling coatings (0.8320) (Pontoon Coating; $F=41.65$, $P<0.0001$, $df_{NUM} = 1$, $df_{DEN} = 1787$).

Biofouling Accumulation

Flipping regime impacted percent fouling, and wet bag weights. Percent fouling measurements were so low that many values were recorded as zero. As a result, some values in Table 2, 3, and 4 were deemed non-estimable (indicated with "*"). For these values, the numbers given are the arithmetic means, not the statistical means also the respective standard deviation is given and not standard error. Weekly flipping resulted in significantly lighter bags (1.24 kg) than biweekly (2.49 kg) and triweekly (2.25 kg) flipped oysters ($F=27.46$, $P<0.0001$, $df_{NUM} = 2$, $df_{DEN} = 60$).

Defouled weight (g) and defouled volume (mL) measurements were similar across treatments except for the defouled weight of oysters grown in pontoons with antifouling coatings. The defouled weight (g) of the oysters grown in pontoons with antifouling coatings (3.80 g) was significantly less than for oysters grown in pontoons without antifouling coatings (5.06 g) ($F=4.33$, $P=0.0380$, $df_{NUM} = 1$, $df_{DEN} = 419$). Antifouling coatings had no significant influences on final wet bag weights (kg).

Quality

The quality index results indicated significantly higher scores for triweekly flipped oysters (0.704) than biweekly flipped oysters (0.476), which in turn were significantly greater than weekly flipped oysters (0.2042) ($F=3023$, $P<0.0001$, $df_{NUM} = 2$, $df_{DEN} = 708$). Condition index values at this site indicated that biweekly flipped oysters had a significantly lower meat-to-shell ratio (9.60) than weekly (11.15) or triweekly (10.47) flipped oysters (Flip Regime; $F=38.80$, $P<0.0001$, $df_{NUM} = 2$, $df_{DEN} = 706$).

The condition index of oysters grown in bags with antifouling coatings (10.11) was significantly less than the condition index of oysters grown in bags without antifouling coatings (10.71) (Bag Coating; $F=17.31$, $P<0.0001$, $df_{NUM} = 2$, $df_{DEN} = 706$).

Antifouling coatings on pontoons had no impact on condition index, nor on the quality index.

Survival

This site had the lowest survival of all sites in the study and it is suspected that this was due to a long-lasting freshet event in Mobile Bay during February and March of 2018. Flip regime impacted oyster survival at this site. Triweekly flipped oysters had significantly higher survival (0.8141) than weekly flipped oysters (0.7215) which in turn exhibited significantly greater survival than the biweekly flipped oysters (0.6076) ($F=15.83$, $P<0.0001$, $df_{NUM} = 2$, $df_{DEN} = 60$). Antifouling coatings on bags and pontoons did not significantly affect survival at this site.

Values indicated with an "*" refer to the arithmetic mean and standard deviation because the statistical mean was non-estimable due to the overwhelming 0.00 scores at this site.

Table 2.5. Alabama oyster response variables separated by weekly, biweekly and triweekly flipping. Presented as (Value ± Standard Error, Tukey Group).

	Flip Regime		
	Weekly	**Biweekly**	**Triweekly**
Shell Length (mm)	**71.73 ± 0.42, B**	**78.26 ± 0.445, A**	**77.79 ± 0.443, A**
Whole Wet Weight (g)	**71.69 ± 0.896, B**	**86.46 ± 1.068, A**	**87.73 ± 1.083, A**
Volume (mL)	**42.98 ± 0.834, B**	**51.36 ± 0.976, A**	**54.16 ± 1.023, A**
Fan Ratio	**0.85 ± 0.003, A**	**0.84 ± 0.003, A**	**0.82 ± 0.003, B**
Cup Ratio	0.45 ± 0.037, A	0.38 ± 0.037, A	0.39 ± 0.037, A
Percent Fouling (%)	0.0083 ± 0.024 *	0.14 ± 0.014 *	0.11 ± 0.0125 *
Defouled Weight (g)	3.79 ± 0.694, A	4.42 ± 0.366, A	5.08 ± 0.461, A
Defouled Volume (mL)	6.84 ± 1.045, A	5.82 ± 0.396, A	6.92 ± 0.511, A
Wet Bag Weight (kg)	**1.24 ± 0.127, B**	**2.49 ± 0.127, A**	**2.25 ± 0.127, A**
Quality Index	**0.20 ± 0.030, C**	**0.48 ± 0.046, B**	**0.704 ± 0.057, A**
Condition Index	**11.15 ± 0.216, A**	**9.60 ± 0.201, B**	**10.47 ± 0.209, A**
Survival (ratio)	**0.72 ± 0.027, B**	**0.61 ± 0.029, C**	**0.81 ± 0.023, A**

Table 2.6. Alabama oyster response variables separated by oysters grown in bags with antifouling coatings and oysters grown in bags without antifouling coatings. Presented as (Value ± Standard Error, Tukey Group).

	Antifouling Coatings On Bags	
	1 (With Coatings)	**0 (Without)**
Shell Length (mm)	75.48 ± 0.354, A	76.39 ± 0.357, A
Whole Wet Weight (g)	81.19 ± 0.824, A	82.74 ± 0.840, A
Volume (mL)	49.32 ± 0.772, A	49.69 ± 0.776, A
Fan Ratio	0.84 ± 0.003, A	0.84 ± 0.003, A
Cup Ratio	0.43 ± 0.030, A	0.38 ± 0.003, A
Percent Fouling (%)	0.11 ± 0.014 *	0.077 ± 0.091 *
Defouled Weight (g)	4.22 ± 0.364, A	4.64 ± 0.485, A
Defouled Volume (mL)	6.82 ± 0.623, A	6.23 ± 0.532, A
Wet Bag Weight (kg)	1.91 ± 0.103, A	2.08 ± 0.103, A
Quality Index	0.48 ± 0.038, A	0.45 ± 0.036, A
Condition Index	**10.11 ± 0.168, B**	**10.71 ± 0.173, A**
Survival (ratio)	0.70 ± 0.022, A	0.73 ± 0.021, A

Table 2.7. Alabama oyster response variables separated by oysters grown in cages with pontoons with antifouling coatings and oysters grown in cages with pontoons without antifouling coatings. Presented as (Value ± Standard Error, Tukey Group).

	Antifouling Coatings On Pontoons	
	1 (With Coatings)	**0 (Without)**
Shell Length (mm)	75.46 ± 0.354, A	76.41 ± 0.357, A
Whole Wet Weight (g)	81.12 ± 0.824, A	82.80 ± 0.840, A
Volume (mL)	49.83 ± 0.778, A	49.17 ± 0.770, A
Fan Ratio	**0.84 ± 0.003, A**	**0.83 ± 0.003, B**
Cup Ratio	0.39 ± 0.003, A	0.43 ± 0.030, A
Percent Fouling (%)	0.065 ± 0.074 *	0.12 ± 0.016 *
Defouled Weight (g)	**3.80 ± 0.311, B**	**5.06 ± 0.520, A**
Defouled Volume (mL)	6.50 ± 0.530, A	6.55 ± 0.625, A
Wet Bag Weight (kg)	2.12 ± 0.103, A	1.86 ± 0.103, A
Quality Index	0.49 ± 0.0382, A	0.43 ± 0.036, A
Condition Index	10.60 ± 0.172, A	10.21 ± 0.168, A
Survival (ratio)	0.74 ± 0.021, A	0.69 ± 0.022, A

Mississippi Site

Size

Size was impacted by flipping regime at this site but not by antifouling coatings on bags or pontoons. Weekly flipped oysters, had smaller shell lengths, lower whole wet weights, and lower volumes (70.66 mm, 65.81 g, & 43.62 mL respectively) than biweekly (81.39 mm, 93.29 g & 63.12 mL, respectively) and triweekly flipped oysters (81.65, 98.27 & 68.42 mL, respectively) (Length; F=302.92, P<0.0001, df_{NUM}=2, df_{DEN}=1738, Weight; F=2291.11 , P<0.0001 ,df_{NUM} = 2, df_{DEN} = 1737, Volume; F=768.02 , P<0.0001 , df_{NUM} = 2, df_{DEN} = 698). Weekly flipped oysters were not fully harvestable size by the final harvest, and biweekly and triweekly flipped oyster average shell lengths were within 1 mm of each other at harvest.

Shape

Fan ratio was not significantly impacted at this site by flip regime, bag coatings or pontoon coatings. In contrast, cup ratio was significantly influenced by all three factors. The cup ratio of weekly flipped oysters (0.4184) was greater than those of biweekly (0.3804) and triweekly flipped oysters (0.3787) (Flip Regime; F=1738, P<0.0001, df_{NUM} = 2, df_{DEN} = 1738).

Oysters grown in bags with antifouling coatings exhibited smaller cup ratios (0.3898) than those grown in bags without antifouling coatings (0.3951) (Bag Coating; F=3.95, P=0.0470, df_{NUM} = 1, df_{DEN} = 1738). Similarly, oysters grown in pontoons with antifouling coatings exhibited smaller cup ratios (0.3884) than those grown in pontoons without antifouling coatings (0.3965) (Pontoon Coating; F=9.10, P-0.0026, df_{NUM} = 1738, df_{DEN} = 1). These cup ratio differences would be difficult to visually distinguish as a grower.

Biofouling Accumulation

Defouled weight and defouled volume were significantly impacted by flip regime at this site. Weekly and biweekly flipping yielded lower defouled weights (1.70 g & 1.27 g, respectively) than did triweekly flipping (3.62 g) (F=11.24, P<0.0001, df_{NUM} = 2, df_{DEN} = 503). Weekly flipping also resulted in lower defouled volume (2.77 mL) than biweekly flipping (2.90 mL) and triweekly flipping (3.67 mL) (F=35.68, P<0.0001, df_{NUM} = 2, df_{DEN} = 641).

This site had the highest values for percent fouling and wet bag weight. However, there were no significant differences in these measurements attributable to flip regime and antifouling coatings on pontoons and bags and there were no significant biofouling accumulation responses associated with the use of antifouling coatings at this site.

Quality

As measured by both the quality Index and condition index, this site had the highest quality meat and highest meat-to-shell ratios. There were no significant differences of the quality Index values associated with flipping regime or antifouling coatings.

Weekly flipping significantly yielded higher condition indices (18.90) than biweekly (12.29) and triweekly flipping (12.67) (Flip Regime; F=34.10, P<0.0001, $df_{NUM} = 2$, $df_{DEN} = 698$). Average condition index of oysters grown in bags with antifouling coatings (16.00) was significantly greater than for those grown in bags without antifouling coatings (13.24) (Bag Coating; F=19.59, P<0.0001, $df_{NUM} = 2$, $df_{DEN} = 698$). In contrast, the condition index of oysters grown in pontoons with antifouling coatings was significantly (12.91) less than that of those grown in pontoons without antifouling coatings (16.32) (Pontoon Coating; F=29.91, P<0.0001, $df_{NUM} = 2$, $df_{DEN} = 698$).

Survival

The only factor affecting survival at this site was flip regime. Triweekly flipped oysters had significantly greater survival (0.9481) than weekly (0.8839) and biweekly flipped oysters (0.9031). Antifouling coatings on bags or pontoons did not impact survival at this site (F=10.85, P<0.0001, $df_{NUM} = 2$, $df_{DEN} = 59$).

Table 2.8. Mississippi oyster response variables separated by weekly, biweekly and triweekly flipping. Presented as (Value ± Standard Error, Tukey Group).

	Flip Regime		
	Weekly	**Biweekly**	**Triweekly**
Shell Length (mm)	**70.66 ± 0.407, B**	**81.39 ± 0.431, A**	**81.65 ± 0.435, A**
Whole Wet Weight (g)	**65.81 ± 0.782, C**	**93.29 ± 1.043, B**	**98.27 ± 1.102, A**
Volume (mL)	**43.62 ± 0.835, C**	**63.12 ± 1.130, B**	**68.42 ± 1.215, A**
Fan Ratio	0.83 ± 0.038, A	0.80 ± 0.004, A	0.81 ± 0.004, A
Cup Ratio	**0.42 ± 0.003, A**	**0.38 ± 0.002, B**	**0.38 ± 0.002, B**
Percent Fouling (%)	0.78 ± 0.023, A	0.75 ± 0.024, A	0.71 ± 0.026, A
Defouled Weight (g)	**1.70 ± 0.174, B**	**1.27 ± 0.134, B**	**3.62 ± 0.233, A**
Defouled Volume (mL)	**2.77 ± 0.203, B**	**2.90 ± 0.274, AB**	**3.67 ± 0.288, A**
Wet Bag Weight (kg)	3.93 ± 0.115, A	4.09 ± 0.115, A	4.11 ± 0.115, A
Quality Index	0.03 ± 0.013, A	0.03 ± 0.035, A	0.05 ± 0.029, A
Condition Index	**18.90 ± 0.722, A**	**12.29 ± 0.411, B**	**12.67 ± 0.430, B**
Survival (ratio)	**0.88 ± 0.013, B**	**0.90 ± 0.012, B**	**0.95 ± 0.009, A**

Table 2.9. Mississippi oyster response variables separated by oysters grown in bags with antifouling coatings and oysters grown in bags without antifouling coatings. Presented as (Value ± Standard Error, Tukey Group).

	Antifouling Coatings On Bags	
	1 (With Coatings)	**0 (Without)**
Shell Length (mm)	78.04 ± 0.344, A	77.76 ± 0.349, A
Whole Wet Weight (g)	85.98 ± 0.800, A	85.60 ± 0.809, A
Volume (mL)	58.76 ± 0.876, A	58.00 ± 0.875, A
Fan Ratio	0.81 ± 0.030, A	0.81 ± 0.031, A
Cup Ratio	**0.39 ± 0.002, B**	**0.40 ± 0.002, A**
Percent Fouling (%)	0.76 ± 0.021, A	0.73 ± 0.019, A
Defouled Weight (g)	1.64 ± 0.133, A	1.97 ± 0.167, A
Defouled Volume (mL)	3.27 ± 0.234, A	2.96 ± 0.183, A
Wet Bag Weight (kg)	4.00 ± 0.094, A	4.09 ± 0.094, A
Quality Index	0.05 ± 0.017, A	0.03 ± 0.030, A
Condition Index	**16.00 ± 0.502, A**	**13.24 ± 0.370, B**
Survival (ratio)	0.91 ± 0.010, A	0.91 ± 0.009, A

Table 2.10. Mississippi oyster response variables separated by oysters grown in cages with pontoons with antifouling coatings and oysters grown in cages with pontoons without antifouling coatings. Presented as (Value ± Standard Error, Tukey Group).

	Antifouling Coatings On Pontoons	
	1 (With Coatings)	**0 (Without)**
Shell Length (mm)	77.84 ± 0.349, A	77.96 ± 0.780, A
Whole Wet Weight (g)	86.70 ± 0.817, A	84.89 ± 0.792, A
Volume (mL)	57.44 ± 0.877, A	59.33 ± 0.883, A
Fan Ratio	0.81 ± 0.003, A	0.81 ± 0.030, A
Cup Ratio	**0.39 ± 0.002, B**	**0.40 ± 0.002, A**
Percent Fouling (%)	0.74 ± 0.020, A	0.76 ± 0.020, A
Defouled Weight (g)	1.83 ± 0.156, A	1.78 ± 0.146, A
Defouled Volume (mL)	3.13 ± 0.223, A	3.09 ± 0.196, A
Wet Bag Weight (kg)	3.95 ± 0.094, A	4.13 ± 0.094, A
Quality Index	0.06 ± 0.014, A	0.01 ± 0.034, A
Condition Index	**12.91 ± 0.360, B**	**16.32 ± 0.509, A**
Survival (ratio)	0.91 ± 0.010, A	0.91 ± 0.009, A

Louisiana Site

Size

Oysters at this site were the largest throughout the project. Half the oysters at this site were of harvestable size by the first quarterly sampling (December 2017), and all oysters at this site were harvestable by the second quarterly sampling (March 2018). Weekly flipping restricted the length, whole wet weight and volume of oysters (97.54 mm, 139.78 g & 90.39 mL, respectively) as compared to biweekly (104.35 mm, 150.67 g & 94.50 mL, respectively) and triweekly (105.38 mm, 150.05 g & 96.05 mL, respectively) flipping (Length; F=108.81, P<0.0001, $df_{NUM} = 2$, $df_{DEN} = 1787$, Weight; F=171.12 , P<0.0001, $df_{NUM} = 2$, $df_{DEN} = 1787$, Volume; F= 22.24, P<0.0001, $df_{NUM} = 2$, $df_{DEN} = 708$). There were no significant differences in the sizes of oysters cultured with or without antifouling coatings, on bags or pontoons.

Shape

Flip regime impacted the cup ratios at this site, but fan ratios were not impacted. Weekly flipped oysters had a deeper cup ratio (0.3435) at final harvest than biweekly (0.3264) and triweekly (0.3221) flipped oysters, which were equivalent (F=28.22, P<0.0001, $df_{NUM} = 2$, $df_{DEN} = 1787$). Antifouling coatings did not impact the fan and cup ratios of these oysters.

Biofouling Accumulation

Of the four measurements describing biofouling accumulation (percent fouling, defouled weight, defouled volume & wet bag weight), only percent fouling exhibited significant differences among the means regarding flip regime. The mean percent fouling of biweekly flipped growing bags (0.3051) was significantly higher than for weekly (0.2134) and triweekly (0.1781) flipped growing bags ($F=7.90$, $P=0.0009$, $df_{NUM} = 2$, $df_{DEN} = 59$). There were no significant differences resulting from antifouling coatings.

Quality

The quality index and condition index were not significantly impacted at this site by flip regime, nor by antifouling coatings. The quality index site average was the second highest among the four sites, suggesting lower quality oysters. Condition index values at this site were the lowest on average throughout the four sites. Oysters at this site were covered in mud inside the bags at final harvest and this may have impacted their quality.

Survival

Survival at this site was the highest throughout the experiment at 96%. There were no differences in survival associated with flip regime or antifouling coatings.

Table 2.11. Louisiana oyster response variables separated by weekly, biweekly and triweekly flipping. Presented as (Value ± Standard Error, Tukey Group).

	Flip Regime		
	Weekly	**Biweekly**	**Triweekly**
Shell Length (mm)	**97.54 ± 0.429, B**	**104.35 ± 0.446, A**	**105.38 ± 0.45, A**
Whole Wet Weight (g)	**139.78 ± 1.162, B**	**150.67 ± 1.246, A**	**151.05 ± 1.248, A**
Volume (mL)	**90.39 ± 1.209, B**	**94.50 ± 1.257, A**	**96.05 ± 1.275, A**
Fan Ratio	0.79 ± 0.004, A	0.74 ± 0.004, A	0.73 ± 0.003, A
Cup Ratio	**0.34 ± 0.002, A**	**0.33 ± 0.002, B**	**0.32 ± 0.002, B**
Percent Fouling (%)	**0.21 ± 0.023, B**	**0.31 ± 0.022, A**	**0.18 ± 0.022, B**
Defouled Weight (g)	2.88 ± 0.322, A	3.11 ± 0.339, A	3.79 ± 0.411, A
Defouled Volume (mL)	2.67 ± 0.329, A	2.52 ± 0.355, A	2.47 ± 0.343, A
Wet Bag Weight (kg)	3.04 ± 0.058, A	3.04 ± 0.058, A	2.86 ± 0.058, A
Quality Index	1.06 ± 0.082, A	1.27 ± 0.093, A	1.35 ± 0.099, A
Condition Index	8.48 ± 0.188, A	8.72 ± 0.191, A	8.80 ± 0.192, A
Survival (ratio)	0.95 ± 0.004, A	0.96 ± 0.004, A	0.97 ± 0.004, A

Table 2.12. Louisiana oyster response variables separated by oysters grown in bags with antifouling coatings and oysters grown in bags without antifouling coatings. Presented as (Value ± Standard Error, Tukey Group).

	Antifouling Coatings On Bags	
	1 (With Coatings)	**0 (Without)**
Shell Length (mm)	102.09 ± 0.359, A	102.75 ± 0.361, A
Whole Wet Weight (g)	148.02 ± 1.000, A	146.32 ± 0.990, A
Volume (mL)	94.03 ± 1.022, A	93.26 ± 1.015, A
Fan Ratio	0.76 ± 0.003, A	0.75 ± 0.003, A
Cup Ratio	0.33 ± 0.002, A	0.33 ± 0.002, A
Percent Fouling (%)	0.24 ± 0.020, A	0.23 ± 0.019, A
Defouled Weight (g)	3.54 ± 0.317, A	2.97 ± 0.267, A
Defouled Volume (mL)	2.81 ± 0.322, A	2.03 ± 0.230, A
Wet Bag Weight (kg)	3.01 ± 0.047, A	2.95 ± 0.047, A
Quality Index	1.16 ± 0.072, A	1.30 ± 0.077, A
Condition Index	8.85 ± 0.157, A	8.48 ± 0.154, A
Survival (ratio)	0.96 ± 0.003, A	0.96 ± 0.000, A

Table 2.13. Louisiana oyster response variables separated by oysters grown in cages with pontoons with antifouling coatings and oysters grown in cages with pontoons without antifouling coatings. Presented as (Value ± Standard Error, Tukey Group).

	Antifouling Coatings On Pontoons	
	1 (With Coatings)	**0 (Without)**
Shell Length (mm)	102.15 ± 0.360, A	102.69 ± 0.360, A
Whole Wet Weight (g)	147.79 ± 1.000, A	146.54 ± 0.991, A
Volume (mL)	94.52 ± 1.027, A	92.77 ± 1.010, A
Fan Ratio	0.76 ± 0.003, A	0.75 ± 0.003, A
Cup Ratio	0.33 ± 0.002, A	0.33 ± 0.002, A
Percent Fouling (%)	0.23 ± 0.020, A	0.23 ± 0.019, A
Defouled Weight (g)	3.26 ± 0.294, A	3.25 ± 0.293, A
Defouled Volume (mL)	2.14 ± 0.246, A	2.68 ± 0.310, A
Wet Bag Weight (kg)	2.96 ± 0.047, A	3.00 ± 0.047, A
Quality Index	**1.11 ± 0.069, B**	**1.35 ± 0.080, A**
Condition Index	8.77 ± 0.157, A	8.56 ± 0.154, A
Survival (ratio)	0.96 ± 0.003, A	0.96 ± 0.004, A

Discussion

Size

Increasing flipping frequency restricted oyster growth rates. Weekly aerial exposure resulted in lower shell length, whole wet weight and volume at all four sites. At final harvest, oysters at every site were of a harvestable size, on average, except for weekly flipped oysters in Alabama and Mississippi. Biweekly and triweekly flipped oysters at these sites were harvestable size. There are multiple mechanisms that could possibly result in reduced growth. First, weekly flipping will limit the exposure time that the livestock were suspended in the water, feeding. Triweekly flipped oysters had two more days out of every twenty-one where they continued to feed and grow while weekly and biweekly flipped oysters were subjected to aerial exposure. The second mechanism that could possibly result in restricted growth is an increase in stressor events. Bodenstein (2019) demonstrated that aerial exposure can increase the likelihood of mortality.

Only at one site (Florida) did antifouling coatings appear to impact the size of oysters at final harvest. Shell lengths of oysters grown in bags with antifouling coatings at the Florida site were one mm less than those of oysters grown in bags without antifouling coatings. Additionally, the whole wet weight of oysters grown in pontoons with antifouling coatings was, on average, five grams less than the whole wet weight of oysters grown in pontoons without antifouling coatings. These differences were statistically significant but to the grower, it would be very difficult to make statements regarding the size of the oysters at final harvest based upon the presence of a coating, especially when results from the other sites in the project strongly suggest that these coatings did not impact size.

Shape

Larger fan and cup ratios can relate to improved shell and growing conditions. Also, these larger ratios are known to relate to an increase in meat tissue, especially increased cup ratios. Deeper cups are known for yielding "meatier" oysters. Increasing aerial exposure (weekly flipping) consistently increased the fan and cup ratios at every site. Fan ratios were significantly different among treatments only at the AL site where there were no differences in cup ratio. Cup ratios were significantly different at the LA, FL and MS sites in regards to flipping regimes. Increasing the fan and cup ratios could increase the overall value of the oyster livestock themselves.

Along with stress, another effect of aerial exposure is the consistent tumbling action caused by localized wave action. Tumbling is another form of stress (Bodenstein, 2019) that is designed to "clean" and "defoul" oysters. As oysters are left exposed in drying positon, mud, sediment, and organic material will be prone to desiccation from solar exposure. Once the inside of the bags have been effectively dried and re-submerged, the dried debris will be washed away under the cage. This in turn opened pore channels that may have been clogged, allowed better hydrodynamic ventilation inside the bag, and increased the growing room of the oyster. Maintaining this regular "self-cleaning" resulted in increased space inside the bag and exposed oysters to increased "tumbling" from wave action. Increased wave-related stress resulted in the oysters increasing their overall fan and cup ratios. If "deeper cupped" or "wider shelled" oysters are considered to be a higher value crop, then weekly aerial exposure is strongly suggested as a management technique.

Antifouling coatings were occasionally associated with significant influences on certain traits, but there was no set trend for fan and cup ratios. At the MS site, oysters grown in pontoons and bags with antifouling coatings had significantly smaller cup ratios than oysters grown without antifouling coatings, while, results at the AL site suggested that antifouling coatings on pontoons can slightly increase the fan ratio. These differences (statistically significant or not) are marginal and would be insufficient for a grower to deem the oysters "better" or "worse" at final harvest.

Biofouling Accumulation

Before considering the effects of flip regime and antifouling coatings, a well-considered biofouling management strategy would assess the location and environmental conditions of any site. This study had four sites in the northern Gulf of Mexico. The Grand Isle, LA site, on the northern side of Grand Isle, is closely protected by an artificial riprap structure that mitigates the majority of wave-action. The AL site in Fort Morgan, AL is exposed (particularly during the fall and winter months) to a large fetch from the north. This causes substantial tumbling and wave-related stress to livestock during these rough winds. During the spring, this site is typically exposed to freshet events as well. The MS site is located on the southern side of Deer Island, MS and is directly exposed to open fetch of the Gulf of Mexico, particularly during the spring and summer months. At final harvest, based on bag weights and percent fouling, this site had the highest levels of gear biofouling. The FL site is similarly, to the MS site, exposed to the Gulf of Mexico from the south and west. As measured by defouled weight and defouled volume, this site had the greatest biofouling accumulation on the oysters themselves. Overall results suggest that the most protected sites actually had the least amount of biofouling (AL and LA) and the most exposed sites had the greatest amount of biofouling (MS and FL).

Flip regime, within this experiment, significantly affected the biofouling accumulation on both gear and livestock. The percent fouling at the AL site was so low that it was deemed "Non-estimable" by the SAS procedure because 0.00 was input repeatedly in the data.

When looking at the influence of flipping regime on defouled weight and defouled volume, AL and LA exhibited no differences; however, observed differences at the FL and MS sites were considered statistically significant. Particularly in FL but also in MS, weekly flipped oysters had significantly less (>50%) defouled weight and defouled volume than biweekly or triweekly flipped oysters. Tumbling action inside of bags at these sites presumably improved fan and cup ratios, while reducing the biofouling accumulation. By increasing spatial allocation, increasing tumbling action, and improving growing conditions, oysters at these more exposed sites seemed to "tumble-off" more biofouling with weekly flipping regimes. If flipping did not occur weekly, then biofouling accumulation was inevitable.

The last measurement of biofouling accumulation was based on wet bag weights. Weekly aerial exposure resulted in lighter wet bag weights at three of the four sites (AL, FL, & MS). The LA site was exceptional in the amount of mud present. The three sites had sandy-based bottoms so sediment was less of an issue. Mud in growing bags at the LA site contributed to higher wet bag weights at final harvest. Wet bag weights at this site were not significantly different due to the high quantity of mud stuck on bags at final harvest.

In Tables 14 & 15, the defouled weight and wet bag weights were used to calculate the total amount of biofouling per cage and differences associated with those values in relation to flipping regime and site. In Table 14, the defouled weight (from results) was used and expressed to estimate total oyster biofouling on harvestable livestock. This was calculated by multiplying the defouled weight by 150 (estimated stocking density) and then was multiplied by the associated survival. Survival was contributed to this value because only oysters being used for consumption at harvest would be pertinent to having defouled weight scraped off. This value does not include the weight of dead shell and biofouling attached to dead shells inside the bags. In Table 15, the wet bag weight (from results) was subtracted by 1.00 kg (approximate weight of growing bags) and then was multiplied by 6 (6 bags per cage) to yield an estimated bag biofouling per cage.

Table 2.14. Total oyster biofouling per cage per state per flipping regime (kg).

Oyster Biofouling per Cage (kg)			
Site	Weekly	Biweekly	Triweekly
Florida	5.24	12.25	12.72
Alabama	2.47	2.42	3.72
Mississippi	1.36	1.40	3.09
Louisiana	2.48	2.70	3.30

Table 2.15. Total bag biofouling per cage per state per flipping regime (kg).

Bag Biofouling per Cage (kg)			
Site	Weekly	Biweekly	Triweekly
Florida	6.18	7.01	10.12
Alabama	1.44	8.94	7.50
Mississippi	17.57	18.50	18.65
Louisiana	12.25	12.26	11.15

Antifouling coatings only appeared to reduce biofouling accumulation at the FL site. At this site, the presence of antifouling coatings on bags significantly decreased oyster defouled weight and defouled volume. This suggests that the presence of the coating inside the bags yielded a direct effect on the surfaces of the oyster's shells, thus resulting an overall reduction in the accumulation of biofouling. Wet bag weight at this site was significantly less with antifouling coating; almost half a kg difference. At the FL site, and only the FL site, the use of the antifouling coatings reduced the accumulation of biofouling. It is also worth noting that shell length was also significantly smaller in bags treated with coating. Pontoon coating did not impact biofouling accumulation during this study at these sites in the Gulf of Mexico.

Quality

Quality index data demonstrated, at three of the four sites, that weekly flipping resulted in lower index scores correlating to higher quality oyster meat. Weekly flipping increased meat quality. In AL, there was a linear progression of the index as flipping regime increased. In FL, weekly flipping resulted in lower scores and biweekly and triweekly flipping resulted in similar higher scores. In LA, though statistically insignificant, the meat quality was still greater in weekly oysters. Only in MS were the results less clear but the MS site had the highest quality oysters and all were comparable.

Condition index results were similar to those of the quality Index. Weekly flipping resulted in significantly greater condition indices than biweekly and triweekly flipping at three of four sites (AL, FL & MS, but not LA). MS oysters, had the greatest condition index along with Auburn Index, which made them the highest quality oysters in the project. The condition index in LA was poorer and this was suspected to be because of the increase of mud and silt at this site that restricted optimal growing conditions. Aerial exposure, at the other three sites, improved growing conditions inside of bags and reduced biofouling on the oysters themselves, making growth, feeding and living conditions more optimal. Understanding that regular aerial exposure increased the quality index and condition index, it can probably be stated that weekly flipping can condition oysters into higher quality categories. Aerial exposure, as a stressor, mimics a low tide event and forces the oyster to close its shell for an extended period of time. This weekly practice increased the oyster's durability during times of stress and actually increased the quality and quantity of the livestock.

Looking at the effects of pontoon coating site-by-site, there are vast disparities. In AL, there were no significant differences based on pontoon coating but there are significant differences related to bag coating. The condition index of oysters in bags with coating was slightly lower than for oysters grown without bag coatings. In LA, the only significant difference was associated to pontoon coating which suggested that pontoon coating improved meat quality, but meat quality was relatively poor at this site compared to others. In FL, results suggest that oysters grown in pontoons with coating yielded lower quality indices. In MS, the results suggest that quality is better without pontoon coating but condition index is better with bag coating.

Differences associated with antifouling coatings on bags and pontoons suggest that the antifouling coatings did not exhibit a specific trend regarding resultant oyster meat quantity and quality. Therefore, it appears, that antifouling coatings did not affect quality or quantity of oysters in this project.

Survival

Site and flip regime influenced survival over the project more than any other factor. The AL site had the lowest survival, which was attributed to a freshet event in February – March 2018, while, the LA site had the greatest survival. Flip regime also impacted survival of oysters. Triweekly flipped oysters consistently had the highest survival at all four sites. Because aerial exposure is a stressor event for oysters, it is theorized that increase in flipping frequency also increase stressor events. An increase in stress-related events can increase mortality.

Antifouling coatings on bags and pontoons resulted in no significant differences in the survival of the oysters in this study. Survival was deemed unaffected by the antifouling coatings used here.

Conclusions

Looking back to the goals and objectives, there may or may not be certain trade-offs when considering size, shape, biofouling accumulation, quality and survival when testing flipping frequency. Increased flipping frequency (weekly versus triweekly) will compromise the size and growth of the oysters and slightly decrease the survival; it will also improve the shell shape and morphometrics, increase condition and quality indices and decrease biofouling accumulation. So, when making management strategies, it may be pertinent to consider these impacts in relation to farm turnover, biofouling management, product quality and product consistency.

Aside from the wet bag weights at the Florida site, there were no significant impacts of antifouling coatings on growing bags or pontoon surfaces in relation to size, shape, biofouling accumulation, quality and survival. Antifouling coatings did not impact production at these sites in the study.

Aside from site, flipping regime and antifouling coatings, there are other variables and sources of error that could have impacted production in this study. Bag position inside cages was observed to affect production. At some sites, outside cages and inside cages yielded different sized oysters and different levels of biofouling on gear and livestock. Stocking densities in this project were approximately 150 oysters per bag, once large enough to be retained in a 12 mm Velar growing bag. If stocking densities were higher or lower, growth rates would be impacted. Additionally, research on adjustable longline system shows there may be disadvantages and advantages to specific densities inside growing bags (Davis, 2013). This experiment utilized the *OysterGro*™ 6-slot growing pontoon, but if biofouling assessments were made using adjustable longline systems, *OysterGro*™ 4-slot growing cages or even 2-slot growing cages, a completely different pattern of biofouling accumulation might be observed.

Chapter 3. Effects of bag position on biofouling for floating cages in off-bottom oyster (*Crassostrea virginica*) aquaculture production in the northern Gulf of Mexico.

Materials and Methods

Data and experimental procedures from Chapman *et. al* (2019) were used in this analysis. There were 12 OysterGro^{TM} 6-slot growing cages deployed at each of four sites (in four states) over a 9-month grow-out period (September 2017 through June 2018). At each site, 4 of the cages were flipped weekly, 4 were flipped biweekly and 4 were flipped triweekly. Also, antifouling coatings were applied on half of all growing pontoons and half of all growing bags at each site. This analysis describes only the impacts of bag positioning because antifouling coatings and flip regime were assessed in chapter two. All four sites were assessed independently.

Each pontoon has 6 slots and bags for each specified slot were uniquely labelled (Figure 12). Bag positions 2, 4, and 6 were labelled as “top” because during aerial exposure, these were the slots of the cage most exposed to the air and sunlight. Similarly, bag positions 1, 3, and 5 were labelled as “bottom” because they were situated underneath the “top” row during aerial exposure. Additionally, bag position’s 1, 2, 5 & 6 were labelled as “outside” while position’s 3, & 4 were labelled as “inside”. This analysis investigated impacts of flipping regime in weekly, biweekly and triweekly increments along with variability in growth, quality traits and biofouling accumulation associated with top/bottom and inside/outside bag positions.

Goals and Objectives

Goals

To determine the impacts of flip regime and antifouling coatings evaluate the effects on:

- Size
- Shape
- Biofouling Accumulation
- Quality
- Survival

Objectives

1) To test the effects of inside and outside bag positioning inside growing pontoons upon oyster production

$$H_0: \left(\frac{\mu1 + \mu2 + \mu5 + \mu6}{4}\right) = \left(\frac{\mu3 + \mu4}{2}\right)$$

$$H_A: \left(\frac{\mu1 + \mu2 + \mu5 + \mu6}{4}\right) \neq \left(\frac{\mu3 + \mu4}{2}\right)$$

2) To test the effects of top and bottom bag positioning inside growing pontoons upon oyster production

$$H_0: \left(\frac{\mu1 + \mu3 + \mu5}{3}\right) = \left(\frac{\mu2 + \mu4 + \mu6}{3}\right)$$

$$H_A: \left(\frac{\mu1 + \mu3 + \mu5}{3}\right) \neq \left(\frac{\mu2 + \mu4 + \mu6}{3}\right)$$

Results

Florida Site

Size

Size at harvest at this site was impacted by inside/outside bag positioning and top/bottom bag positioning. Inside/outside bag positioning affected whole wet weight (F=13.18, df_{Num}=1, df_{Den}=1783, p=0.0003). Inside positioned oysters weighed more than outside positioned oysters (123.5 g vs. 118.4 g respectively). Top/bottom bag positioning significantly affected whole wet weight (F=9.58, df_{Num}=1, df_{Den}=1783, p=0.0020) and volume displacement (F=7.82, df_{Num}=1, df_{Den}=707, p=0.0053). Top positioned oysters had smaller whole wet weights and volume displacements (118.8 g & 73.0 mL respectively) than bottom positioned oysters (123.1 g & 77.3 mL respectively).

Shape

Both fan ratios and cup ratios were not significantly impacted by inside/outside bag positioning or top/bottom positioning at this site.

Biofouling Accumulation

Percent fouling of growing bags at the Florida site was significantly impacted by inside/outside bag positioning (F=5.71, df_{Num}=1, df_{Den}=56, p=0.0203) and top/bottom bag positioning (F=5.47, df_{Num}=1, df_{Den}=56, p=0.0230). Weight difference and volume difference measurements were significantly affected by inside/outside bag positioning (Weight Difference: F=26.13, df_{Num}=1, df_{Den}=697, p<0.0001, Volume Difference: F=13.78, df_{Num}=1, df_{Den}=707, p=0.0002), and top/bottom positioning (Weight Difference: F=15.14, df_{Num}=1, df_{Den}=697, p=0.0001, Volume Difference: F=30.09, df_{Num}=1, df_{Den}=707, p<0.0001).

Inside positioned growing bags had a greater percent fouling (0.3852) than outside positioned growing bags (0.2871). Inside positioned oysters also had greater weight difference and volume difference measurements (14.4 g & 11.7 mL respectively) than outside positioned oysters (11.7 g & 9.5 g). Top positioned growing bags had higher percent fouling (0.3841) than bottom positioned growing bags (0.2881). Weight difference and volume difference of top positioned oysters (14.9 g & 12.4 mL) was greater than in bottom positioned oysters (11.2 g & 8.9 mL).

Quality

The quality index was affected by top/bottom bag positioning (F=21.28, df_{Num}=1, df_{Den}=707, p<0.0001). Top positioned oysters also exhibited higher quality index values (2.52) than bottom positioned oysters (1.71). Condition index was not impacted by bag positioning at this site.

Survival

Both inside/outside bag positioning (F=10.86, df_{Num}=1, df_{Den}=57, p=0.0017) and top/bottom positioning impacted survival (F=11.92, df_{Num}=1, df_{Den}=57, p=0.0011) at this site. Inside positioned oysters (0.8567) had almost 5% lower survival than outside positioned oysters (0.9056), while top positioned oysters (0.9067) had >5% higher survival than inside positioned oysters (0.8556).

Table 3.1. Response variables at final harvest of Florida oysters set to inside and outside bag positions. Expressed as "Value (± Standard Error, Tukey Group)"

	Bag Position	
	Inside	**Outside**
Shell Length (mm)	89.72 (± 0.39, A)	89.21 (± 0.27, A)
Whole Wet Weight (g)	**123.5 (± 1.2, A)**	**118.4 (± 0.8, B)**
Volume Displacement (mL)	76.5 (± 1.3, A)	73.8 (± 0.9, A)
Fan Ratio	0.75 (± 0.035, A)	0.76 (± 0.025, A)
Cup Ratio	0.37 (± 0.001, A)	0.37 (± 0.001, A)
Percent Fouling (%)	**0.39 (± 0.024, A)**	**0.29 (± 0.034, B)**
Weight Difference (g)	**14.4 (± 0.6, A)**	**11.7 (± 0.4, B)**
Volume Difference (mL)	**11.9 (± 0.6, A)**	**9.5 (± 0.3, B)**
Wet Bag Weight (kg)	2.37 (± 0.09, A)	2.49 (± 0.06, A)
Quality Index	2.25 (± 0.15, A)	1.98 (± 0.10, A)
Condition Index	9.17 (± 0.20, A)	9.57 (± 0.14, A)
Survival (ratio)	**0.86 (± 0.013, B)**	**0.91 (± 0.008, A)**

Table 3.2.: Response variables at final harvest of Florida oysters set to top and bottom bag positions. Expressed as "Value (± Standard Error, Tukey Group)"

	Bag Position	
	Top	**Bottom**
Shell Length (mm)	89.41 (± 0.34, A)	89.52 (± 0.34, A)
Whole Wet Weight (g)	**118.8 (± 1.0, B)**	**123.1 (± 1.0, A)**
Volume Displacement (mL)	**73.0 (± 1.1, B)**	**77.3 (± 1.1, A)**
Fan Ratio	0.7521 (± 0.03069, A)	0.7589 (± 0.03083, A)
Cup Ratio	0.3705 (± 0.001691, A)	0.3717 (± 0.001697, A)
Percent Fouling (%)	**0.3841 (± 0.03135, A)**	**0.2881 (± 0.02701, B)**
Weight Difference (g)	**14.9 (± 0.6, A)**	**11.2 (± 0.5, B)**
Volume Difference (mL)	**12.4 (± 0.5, A)**	**8.9 (± 0.4, B)**
Wet Bag Weight (kg)	2.48 (± 0.08, A)	2.38 (± 0.08, A)
Quality Index	**2.52 (± 0.14, A)**	**1.71 (± 0.11, B)**
Condition Index	9.18 (± 0.17, A)	9.56 (± 0.17, A)
Survival (ratio)	**0.9067 (± 0.90092, A)**	**0.8556 (± 0.0120, B)**

Alabama Site

Size

There were significant differences in the size of Alabama oysters attributable to inside/outside bag positioning. Inside/outside bag position statistically significantly affected shell length, whole wet weight, and volume displacement. Inside oysters were larger as measured by shell length (83.02 mm) (F=482.72, df_{Num}=1, df_{Den}=1787, p<0.0001), whole wet weight (100.4 g) (F=549.53, df_{Num}=1, df_{Den}=1787, p<0.0001) and volume displacement (61.0 mL) (F=252.49, df_{Num}=1, df_{Den}=708, p<0.0001) than outside oysters (72.39 mm, 72.7 g & 43.8 mL, respectively).

Whole wet weight was influenced by top/bottom bag positioning. Whole wet weight of top positioned oysters (85.1 g) was significantly less than for bottom positioned oysters (88.0 g) (F=5.84, df_{Num}=1, df_{Den}=1787, p=0.0157). However, shell lengths and volume displacement were not significantly impacted by top/bottom positioning.

Shape

At this site, fan ratios were not impacted by inside/outside bag positioning, or top/bottom positioning. Nonetheless, cup ratio was significantly impacted inside/outside bag positioning (F=103.52, df_{Num}=1, df_{Den}=1787, p<0.0001) and top/bottom bag positioning (F=22.94, df_{Num}=1,

df_{Den}=1787, p<0.0001). Inside positioned oysters had shallower cups (0.3657) than oysters grown in outside positions (0.4266). Additionally, oysters grown in top bag positions had higher cup ratios (0.4105) than those grown in bottom bag positions (0.3818).

Biofouling Accumulation

At this site, many bags were recorded with percent fouling scores equal to 0.00 at harvest, making calculation of percent fouling non-estimable by the SAS software used for analysis. This was the only site where this occurred. As a result, percent fouling for inside positioned oysters, top positioned oysters and bottom positioned oysters were all non-estimable. The results displayed with an "*" after the value are representative of the arithmetic mean, not the statistical mean; also, the respective standard deviation is given and not standard error.

Weight difference and volume difference are related response variables, and both are measures of biofouling on the oysters themselves. At this site, inside/outside bag positioning impacted both values. Effects attributable to inside/outside bag positioning also statistically significantly impacted biofouling on oysters themselves. Inside positioned oysters had greater weight and volume differences (6.0 g & 4.9 mL respectively) than outside positioned oysters (1.1 g & 0.8 mL) (Weight Difference: F=76.18, df_{Num}=1, df_{Den}=708, p<0.0001; Volume Difference: F=36.76, df_{Num}=1, df_{Den}=708, p<0.0001).

Top/bottom positioning statistically significantly affected wet bag weights. Top positioned bags (2.26 kg) weighed 36 percent (0.6 kg) more than bottom positioned bags (1.66 kg) at final harvest (F=22.08, df_{Num}=1, df_{Den}=60, p<0.0001).

Quality

Quality index values and condition index values were affected by inside/outside positioning. Condition index was affected by top/bottom bag positioning, but the quality index was not statistically impacted.

Scores closer to 0.00 indicate higher quality oysters, and inside positioned oysters (0.60) yielded a significantly greater quality index than outside positioned oysters (0.39). (Inside/Outside Position: F=12.73, df_{Num}=1, df_{Den}=708, p<0.0004).

The condition index values of inside positioned oysters (9.68) were significantly lower than those of outside positioned oysters (10.77) (Inside/Outside Position: F=19.06, df_{Num}=1, df_{Den}=706, p<0.0001). The condition index value of top positioned oysters (9.95) was significantly lower than for bottom positioned oysters (10.50) (Top/Bottom Position; F=4.70, df_{Num}=1, df_{Den}=706, p=0.0306).

Survival

Survival at this site was the lowest in the study. This was attributed to a long-lasting freshet event in Mobile Bay, AL between February and March 2018. inside/outside bag positioning, and top/bottom positioning. Inside positioned oyster survival (0.80) was over 10% higher than outside positioned oyster survival (0.67) at this site (Inside/Outside Position: F=35.04, df_{Num}=1, df_{Den}=60, p<0.0001). Top positioned oyster survival (0.76) was almost 5% greater than that of bottom positioned oysters (0.7090) (F=4.96, df_{Num}=1, df_{Den}=60, p=0.0297).

Table 3.3. Response variables at final harvest of Alabama oysters set to inside and outside bag positions. Expressed as “Value (± Standard Error, Tukey Group)”

	Bag Position	
	Inside	**Outside**
Shell Length (mm)	**83.02 (± 0.40, A)**	**72.39 (± 0.26, B)**
Whole Wet Weight (g)	**100.4 (± 1.0, A)**	**72.7 (± 0.6, B)**
Volume Displacement (mL)	**61.0 (± 1.0, A)**	**43.8 (± 0.5, B)**
Fan Ratio	0.83 (± 0.037, A)	0.84 (± 0.026, A)
Cup Ratio	**0.37 (± 0.005, B)**	**0.43 (± 0.004, A)**
Percent Fouling (%)	0.067 ± 0.076 *	0.11 (± 0.013) *
Weight Difference (g)	**6.0 (± 0.5, A)**	**1.1 (± 0.1, B)**
Volume Difference (mL)	**4.9 (± 0.7, A)**	**0.8 (± 0.1, B)**
Wet Bag Weight (kg)	1.85 (± 0.10, A)	2.06 (± 0.07, A)
Quality Index	**0.60 (± 0.05, A)**	**0.39 (± 0.03, B)**
Condition Index	**9.68 (± 0.20, B)**	**10.77 (± 0.15, A)**
Survival (ratio)	**0.80 (± 0.017, A)**	**0.67 (± 0.015, B)**

Table 3.4. Response variables at final harvest of Alabama oysters set to top and bottom bag positions. Expressed as “Value (± Standard Error, Tukey Group)”

	Bag Position	
	Top	**Bottom**
Shell Length (mm)	77.69 (± 0.34, A)	77.72 (± 0.34, A)
Whole Wet Weight (g)	**85.1 (± 0.8, B)**	**88.0 (± 0.8, A)**
Volume Displacement (mL)	52.0 (± 0.8, A)	52.7 (± 0.8, A)
Fan Ratio	0.83 (± 0.032, A)	0.85 (± 0.033, A)
Cup Ratio	**0.41 (± 0.004, A)**	**0.38 (± 0.004, B)**
Percent Fouling (%)	0.094 ± 0.098 *	0.061 ± 0.071 *
Weight Difference (g)	3.2 (± 0.3, A)	3.8 (± 0.4, A)
Volume Difference (mL)	2.5 (± 0.4, A)	3.3 (± 0.5, A)
Wet Bag Weight (kg)	**2.26 (± 0.09, A)**	**1.66 (± 0.09, B)**
Quality Index	0.46 (± 0.04, A)	0.53 (± 0.04, A)
Condition Index	**9.95 (± 0.18, B)**	**10.50 (± 0.18, A)**
Survival (ratio)	**0.76 (± 0.015, A)**	**0.71 (± 0.02, B)**

Mississippi Site

Size

Shell length (F=461.40, df_{Num}=1, df_{Den}=1738, p<0.0001), whole wet weight (F=532.04, df_{Num}=1, df_{Den}=1738, p<0.0001) and volume displacement measurements (F=200.12, df_{Num}=1, df_{Den}=698, p<0.0001) were all impacted by inside/outside bag position. Shell length (Top/Bottom: F=24.69, df_{Num}=1, df_{Den}=1738, p<0.0001) and whole wet weight (Top/Bottom: F=14.01, df_{Num}=1, df_{Den}=1737, p=0.0002) measurements were also influenced by top/bottom position.

Inside positioned oysters had greater shell length, whole wet weight and volume displacement measurements (84.46 mm, 102.6 g & 69.8 mL respectively) than outside positioned oysters (74.52 mm, 77.1 g & 52.6 mL). Top positioned oysters’ shell lengths and whole wet weights (80.63 mm & 87.8 g) were greater than those of bottom positioned oysters (78.34 mm & 91.9 g).

Shape

Fan ratio was not impacted at this site by inside/outside bag position or top/bottom bag position. Cup ratio was not impacted by bag position either.

Biofouling Accumulation

Percent fouling was not influenced by inside/outside bag position or top/bottom bag position. The weight difference measure was significantly affected inside/outside bag position effects (F=26.13, df_{Num}=1, df_{Den}=697, p<0.0001) and top/bottom position effects (F=15.14, df_{Num}=1, df_{Den}=697, p=0.0001). The volume difference measure was affected by inside/outside bag position (F=23.26, df_{Num}=2, df_{Den}=698, p<0.0001) and top/bottom bag position (F=23.74, df_{Num}=1, df_{Den}=698, p<0.0001). Wet bag weight was affected by inside/outside bag position (F=9.24, df_{Num}=1, df_{Den}=60, p<0.0035) and top/bottom bag position (F=20.76, df_{Num}=1, df_{Den}=60, p<0.0001).

Inside positioned oysters also had greater average weight differences (2.7 g) and average volume differences (2.3 mL) than outside positioned oysters (1.3 g & 1.1 mL respectively). Top positioned oysters had smaller average weight differences (1.5 g) and average volume differences (1.1 mL) than bottom positioned oysters (2.5 g & 2.3 mL respectively).

Wet bag weight of inside positioned bags (3.81 kg) was less than that of outside positioned bags (4.16 kg). Top positioned bags (4.25 kg) also weighed more than bottom positioned bags at final harvest (3.72 kg).

Quality

The quality index was not significantly impacted by inside/outside bag position or top/bottom bag position at this site. In contrast, condition index was significantly impacted by inside/outside bag position (F=336.66, df_{Num}=1, df_{Den}=698, p<0.0001) and top/bottom bag position (F=314.36, df_{Num}=1, df_{Den}=698, p<0.0001). Inside positioned oysters (11.40) had lower condition index values than outside positioned oysters (16.71). Top positioned oysters (16.62) had greater condition index values than bottom positioned oysters (11.48).

Survival

Bag position did not impact survival at this site.

Table 3.5. Response variables at final harvest of Mississippi oysters set to inside and outside bag positions. Expressed as “Value (± Standard Error, Tukey Group)”

	Bag Position	
	Inside	**Outside**
Shell Length (mm)	**84.46 (± 0.38, A)**	**74.52 (± 0.26, B)**
Whole Wet Weight (g)	**102.6 (± 1.0, A)**	**77.1 (± 0.5, B)**
Volume Displacement (mL)	**69.8 (± 1.1, A)**	**52.6 (± 0.6, B)**
Fan Ratio	0.82 (± 0.037, A)	0.81 (± 0.027, A)
Cup Ratio	**0.37 (± 0.002, B)**	**0.41 (± 0.006, A)**
Percent Fouling (%)	0.77 (± 0.024, A)	0.74 (± 0.017, A)
Weight Difference (g)	**2.7 (± 0.2, A)**	**1.3 (± 0.1, B)**
Volume Difference (mL)	**2.3 (± 0.2, A)**	**1.1 (± 0.1, B)**
Wet Bag Weight (kg)	**3.81 (± 0.067, B)**	**4.16 (± 0.07, A)**
Quality Index	0.08 (± 0.02, A)	0.01 (± 0.06, A)
Condition Index	**11.40 (± 0.22, B)**	**16.71 (± 0.19, A)**
Survival (ratio)	0.90 (± 0.011, A)	0.92 (± 0.007, A)

Table 3.6. Response variables at final harvest of Mississippi oysters set to top and bottom bag positions. Expressed as "Value (± Standard Error, Tukey Group)"

	Bag Position	
	Top	**Bottom**
Shell Length (mm)	**80.63 (± 0.33, A)**	**78.34 (± 0.33, B)**
Whole Wet Weight (g)	**87.8 (± 0.8, B)**	**91.9 (± 0.8, A)**
Volume Displacement (mL)	60.5 (± 0.9, A)	61.9 (± 0.8, A)
Fan Ratio	0.82 (± 0.032, A)	0.81 (± 0.032, A)
Cup Ratio	**0.40 (± 0.002, A)**	**0.38 (± 0.002, B)**
Percent Fouling (%)	0.72 (± 0.022, A)	0.78 (± 0.019, A)
Weight Difference (g)	**1.5 (± 0.1, B)**	**2.5 (± 0.2, A)**
Volume Difference (mL)	**1.1 (± 0.1, B)**	**2.3 (± 0.2, A)**
Wet Bag Weight (kg)	**4.25 (± 0.08, A)**	**3.72 (± 0.08, B)**
Quality Index	0.04 (± 0.05, A)	0.06 (± 0.02, A)
Condition Index	**16.62 (± 0.22, A)**	**11.48 (± 0.22, B)**
Survival (ratio)	0.92 (± 0.009, A)	0.90 (± 0.010, A)

Louisiana Site

Size

Size, as measured by shell length and whole wet weight, was statistically significantly impacted by inside/outside bag position and top/bottom bag position. Shell length was specifically impacted by top/bottom bag position (F=20.16, df_{Num}=1, df_{Den}=1787, p<0.0297). Whole wet weight was impacted by inside/outside bag position (F=21.89, df_{Num}=2, df_{Den}=1787, p<0.0001). Volume displacement was not impacted by flipping regime.

Top positioned oysters (103.48 mm) exhibited shorter shell lengths than bottom positioned oysters (101.08 mm). Inside positioned oysters' whole wet weights (145.1 g) were less than those of outside positioned oysters (148.2 g).

Shape

Oyster fan ratio was not impacted by inside/outside bag position, or top/bottom bag position at this site. Cup ratios, however, were impacted by top/bottom bag position (F=13.26, df_{Num}=1, df_{Den}=1787, p<0.0003). Top positioned oysters had smaller cup ratios (0.3256) than bottom positioned oysters (0.3350).

Biofouling Accumulation

There were no other significant differences involving biofouling accumulation. Inside/outside bag position and top/bottom bag position did not impact biofouling accumulation at this site.

Quality

There were no statistically significant differences in the quality index or condition index attributable to inside/outside bag position or top/bottom bag position.

Survival

This site had the highest overall survival throughout the project. Survival was not influenced by inside/outside bag position or top/bottom bag position.

Table 3.7. Response variables at final harvest of Louisiana oysters set to inside and outside bag positions. Expressed as “Value (± Standard Error, Tukey Group)”

	Bag Position	
	Inside	**Outside**
Shell Length (mm)	101.86 (± 0.44, A)	102.70 (± 0.31, A)
Whole Wet Weight (g)	**145.1 (± 1.2, B)**	**148.2 (± 0.9, A)**
Volume Displacement (mL)	93.5 (± 1.2, A)	93.7 (± 0.9, A)
Fan Ratio	0.75 (± 0.035, A)	0.76 (± 0.025, A)
Cup Ratio	0.33 (± 0.002, A)	0.33 (± 0.001, A)
Percent Fouling (%)	0.26 (± 0.024, A)	0.22 (± 0.016, A)
Weight Difference (g)	3.7 (± 0.4, A)	3.1 (± 0.2, A)
Volume Difference (mL)	2.3 (± 0.3, A)	2.5 (± 0.2, A)
Wet Bag Weight (kg)	3.04 (± 0.06, A)	2.95 (± 0.04, A)
Quality Index	1.30 (± 0.10, A)	1.19 (± 0.06, A)
Condition Index	8.55 (± 0.19, A)	8.72 (± 0.14, A)
Survival (ratio)	0.96 (± 0.004, A)	0.96 (± 0.003, A)

Table 3.8. Response variables at final harvest of Louisiana oysters set to top and bottom bag positions. Expressed as “Value (± Standard Error, Tukey Group)”

	Bag Position	
	Top	**Bottom**
Shell Length (mm)	**103.48 (± 0.38, A)**	**101.08 (± 0.376, B)**
Whole Wet Weight (g)	145.6 (± 1.0, A)	147.7 (± 1.1, A)
Volume Displacement (mL)	93.9 (± 1.1, A)	93.3 (± 1.1, A)
Fan Ratio	0.75 (± 0.031, A)	0.76 (± 0.031, A)
Cup Ratio	**0.33 (± 0.002, B)**	**0.34 (± 0.002, A)**
Percent Fouling (%)	0.26 (± 0.020, A)	0.21 (± 0.020, A)
Weight Difference (g)	3.5 (± 0.4, A)	3.2 (± 0.3, A)
Volume Difference (mL)	2.3 (± 0.3, A)	2.5 (± 0.3, A)
Wet Bag Weight (kg)	2.96 (± 0.05, A)	3.04 (± 0.05, A)
Quality Index	1.31 (± 0.08, A)	1.18 (± 0.08, A)
Condition Index	8.63 (± 0.16, A)	8.65 (± 0.16, A)
Survival (ratio)	0.96 (± 0.004, A)	0.96 (± 0.004, A)

Discussion

Size

Oyster size at harvest in this study was measured by shell length, whole wet weight and volume displacement. Shell length is a typical measurement of harvest size for growers (>75 mm), however, shell morphometry, biofouling accumulation and meat attributes also go into the overall market value of oysters. Whole wet weight and volume displacement were also used to describe size attributes for a more multidimensional characterization. Size, by itself, did not provide a single best indicator of oyster production and quality.

Throughout this project, oyster growth (and size) was most impacted by individual site characteristics (Chapman *et al.* 2019) and flipping regime, in that order. Bag position also influenced overall oyster size in Alabama, Louisiana, & Mississippi. Variations between the four sites generated significant differences in oyster production variables. The largest oysters based on shell length, whole wet weight and volume displacement, were harvested at the Louisiana site. Seed for the project was produced at the same Louisiana site and there could have been genetic adaptations that provided an advantage over other sites in terms of growth. Additionally, Louisiana oyster seed was being grown in an upweller nearby while seed for other sites was being packaged and shipped. This effectively gave the oysters a 2 to 3 day advantage in growth during post-larval and nursery phases. The Florida site had the second largest oysters in the project, followed by the Mississippi and Alabama sites. Mississippi oysters were only slightly larger than Alabama oysters at harvest. Oysters for these sites were shipped from Louisiana to Dauphin Island, Alabama before stocking to condition the seed to waters closer to their respective grow-out sites.

Flipping regime greatly impacted production at all sites. Weekly flipping resulted in stunted growth while triweekly flipping consistently yielded the largest oysters. Market size (>75 mm) was not achieved by weekly flipped oysters in Alabama or Mississippi, but biweekly and triweekly flipped oysters averaged >75 mm at both sites and all Florida and Louisiana oysters had grown to market size by the final harvest for data collection. The increased aerial exposure increased the amount of time weekly flipped oyster spent out of the water, thus reducing growth rates.

Inside/outside bag positional effects on size (and therefore growth) were evident at the Mississippi and Alabama sites. Inside positioned oysters were larger than outside positioned oysters at these sites. The OysterGro® 6-slot growing pontoon often experienced significant tumbling action due to localized wind-driven wave action. The pontoons oscillate based on wave patterns and the outside positioned oyster bags were most impacted by waves. Inside positioned oysters were at the fulcrum of the wave-induced tumbling action and thus received the least tumbling. Increased wave-induced tumbling on the outside bag slots increased stress on the oysters in these bags and forced the oysters to retract into their shells more often, thus reducing growth.

Top/bottom bag positioning impacted the size of oysters in Alabama, Florida and Mississippi. At these sites, whole wet weight of top positioned oysters was less than for bottom positioned oysters. In Florida, volume displacement of top positioned oysters was also less than for bottom positioned oysters. In Mississippi, average shell length in top positioned oysters was 2 mm greater than in bottom positioned oysters in spite of the higher whole wet weight of bottom positioned oysters. It would appear, except for shell length measurements in Mississippi, that top positioned oysters are generally smaller at harvest than bottom positioned oysters. Increased aerial and solar exposure could increase stress on top-positioned oysters and restrict their growth.

Shape

Site variation was the only factor that affected fan ratios in the project. Alabama oysters had the largest fan ratios followed by Mississippi oysters, while, Louisiana and Florida had lower average fan ratios.

Cup ratios were impacted by site variations, flipping regime, inside/outside bag positional effects and top/bottom bag positional effects. Oysters at the Alabama, Florida, and Mississippi sites had comparable cup ratios between 0.36-0.42 while the Louisiana oysters averaged about 0.33. A reduced cup ratio is also associated with less meat content and the condition index (relative meat-to-shell ratio) values of Louisiana oysters were smaller than for other sites.

Inside/outside bag positional effects significantly impacted cup ratios of oysters grown in Mississippi and Alabama. At both of these sites, outside positioned oysters had significantly greater cup ratios than inside positioned oysters. It would seem, that the increased tumbling and wave action associated with the outside positioned oysters increased pruning and chipping within bags, which resulted in greater cup ratios.

Top/bottom positioning also significantly impacted cup ratios in three of the four sites. The Alabama site is exposed to a very long fetch in the fall/winter months and the Mississippi site is exposed to a longer fetch in the spring/summer months. The Florida site is also exposed to a wide fetch and experienced significant tumbling action. At the Alabama and Mississippi sites, top positioned oysters had greater cup ratios than bottom positioned oysters. Conversely, at the Louisiana site, bottom positioned oysters had greater cup ratios than top positioned oysters. In Alabama and Mississippi, wave action increased surface exposure and increased tumbling and pruning increased top positioned cup ratios. The bottom positioned oysters were “shielded” from exposure by the top positioned bags and oysters. In Louisiana, due to an artificial riprap structure at the location, fetch and overall wave action were significantly diminished. Less tumbling action occurred at this site compared to the other three sites. Mud accumulated inside the bags and by the time of final harvest, it actually began to inhibit aerial and solar exposure.

Biofouling Accumulation

Biofouling accumulation was measured using percent fouling, weight difference, volume difference and wet bag weights. Percent fouling reflected estimated biofouling on the bags, and the weight and volume difference measurements estimated biofouling on the oysters themselves.

Many variables exhibited significant variations between sites. The fall/winter fetch resulted significant tumbling and pruning at the Alabama site, and reduced the fall/winter biofouling accumulation. In the spring, the location of the Alabama site protects the oyster farm minimizing exposure to the open Gulf of Mexico; and thus minimizing biofouling. This probably explains why percent fouling and wet bag weight results were very low at this site.

This phenomenon may also be relevant at the Mississippi site on the southern side of Deer Island, MS. This site was protected from harsh winds and wave action from the north during the fall/winter months, but during spring/summer months, the oysters were directly exposed to the open Gulf of Mexico. The lack of tumbling in the fall/winter months, and the open ocean exposure during spring/summer resulted in significantly higher values for percent fouling. Percent fouling at final harvest and wet bag weights at this site were the highest in the project, on average.

Percent fouling at the Florida site, on average, was the second highest in the study. Wet bag weights in Florida were the second lightest in the project. The southwestern exposure to the Gulf of Mexico left the Florida site particularly vulnerable to biofouling accumulation. Percent fouling on bags was low (relative to other sites) at the Louisiana site but the wet bag weights were the second heaviest in the project. This site was protected from the fetch of the Barataria Bay by an artificial riprap structure directly north of the location. This inhibited open water exposure and reduced fouling on the bags. Wet bag weights were high at this site because of the accumulation of mud and sediment.

Flipping regime also had an impact on biofouling accumulation. Weekly aerial exposure reduced biofouling accumulation as measured by percent fouling, wet bag weights, weight difference and volume difference. Aerial exposure subjects settling organisms, on pontoons, cages, bags and oysters, to desiccation and minimizes biofouling settling success.

In Alabama, weekly flipped oysters had 4 fold less weight difference and volume difference values than biweekly and triweekly oysters. Wet bag weights of weekly flipped bags were 2 fold less than biweekly and triweekly flipped bags. In Louisiana, percent fouling and wet bag weight were not reduced by weekly flipping, but weight and volume difference measures suggested that weekly flipping reduced biofouling on the oysters themselves. The Florida site had the highest biofouling accumulation on oysters. Weekly flipping resulted in a 2 to 3 fold reduction in biofouling accumulation as measured by weight difference, volume difference, and percent fouling. Wet bag weight was also significantly lower with weekly flipping. In Mississippi, biweekly flipping reduced biofouling to a greater extent than weekly and triweekly flipping. Biofouling accumulation at this site was the worst and most excessive at final harvest.

Biofouling accumulation, on oysters as quantified by weight difference and volume difference measurements, was reduced in outside bag positions in Alabama, Florida and Mississippi. Again, pruning and tumbling of oysters may have constantly "knocked off" settling organisms that might have remained attached in inside bag positions. Although the outside positioned oysters themselves exhibited reduced biofouling, in Mississippi the outside positioned bags had increased levels of biofouling. This also occurred in Alabama and Florida, but the results were not statistically significant. Outside positioned bags are exposed to the bulk of the biofouling being dispersed via wave action, while, inside positioned bags are somewhat "shielded" and less available to biofouling organisms. Removing growing bags from cage slots incidentally scrapes off biofouling on some bags and potentially biases results. Inside positioned bags had more biofouling scraped off when being removed from cages than did outside positioned bags.

Top/bottom bag positional effects were observed in Alabama, Florida and Mississippi. Oysters in top positions were less fouled than those in bottom positions. In contrast, bags in top positions were more fouled than the bags in bottom positions. In Alabama, the top position wet bag weights were 0.5 kg greater than for bottom position bags. Though not statistically significant, top positioned weight and volume difference measurements at this site were less than for bottom positioned oysters. In Florida, percent fouling was also greater on top positioned bags than bottom position bags and the wet bag weights, though statistically insignificant, were heavier for top positioned bags. Top positioned oysters were significantly less fouled than bottom positioned oysters based on the weight difference measure. In Mississippi, top positioned bags exhibited increased wet bag weights and decreased weight difference values, reinforcing the trends observed in Alabama and Florida.

Tables 24-25 express biofouling accumulation in terms of total biofouling per cage from the oyster livestock and the growing bags. Table 24 uses the defouled weight (from results) multiplied by 150 (approximate stocking density) multiplied by 6 bags per cage multiplied by the survival (percent from results) of that treatment. Survival was used because only sellable livestock would be pertinent to being defouled. Table 25 used the wet bag weight (from results) minus the weight of a dry bag (approximately 1 kg) multiplied by 6 (bags per cage).

Table 3.9. Total oyster biofouling per cage per site per bag position.

Oyster Biofouling per Cage (kg)				
Site	**Inside**	**Outside**	**Top**	**Bottom**
Florida	3.70	6.36	6.08	4.31
Alabama	1.44	0.44	1.09	1.21
Mississippi	0.73	0.71	0.62	1.02
Louisiana	1.07	1.79	1.51	1.39

Table 3.10. Total oyster biofouling per cage per site per bag position.

Bag Biofouling per Cage (kg)				
Site	**Inside**	**Outside**	**Top**	**Bottom**
Florida	2.74	5.96	4.44	4.14
Alabama	1.70	4.24	3.78	1.98
Mississippi	5.62	12.64	9.75	8.16
Louisiana	4.08	7.80	5.88	6.12

Quality

Although the Auburn index was impacted by site variation and bag position at the Alabama and Florida sites, it was not impacted by these factors in Louisiana or Mississippi. Results did not definitively indicate that bag position increases or decreases meat quality.

Condition index was impacted by site variation and bag position (in Alabama and Mississippi). The Mississippi site had the highest condition index values and Louisiana oysters had the lowest condition index values. Outside positioned oysters, at the Alabama and Mississippi sites, had much higher condition index values than inside positioned oysters. Top positioned oysters also yielded greater condition index scores at the sites.

In positions where the heaviest biofouling occurred and where exposure to wave-action and tumbling was highest, the quality of the oysters was highest. This suggests that increased stress may condition oysters to be meatier and have higher quality meat.

Survival

Site variation and bag position accounted for the largest differences in survival during this project. Inside/outside bag positional effects on survival were present in Alabama and Florida, but no clear trend was apparent. In Alabama, inside positioned oysters had >10% increased survival compared to outside positioned oysters. In Florida, outside positioned oysters had 5% increased survival compared to inside oysters. Top/bottom bag positional effects on survival were also present in Alabama and Florida with a consistent 5% increase in survival for top positioned oysters from both sites. Survival at the Louisiana and Mississippi sites was not impacted by top/bottom position.

Conclusions

The goals in this assessment were to investigate biofouling on the production process of oyster off-bottom farming in terms of size, shape, biofouling accumulation, quality and survival upon two different bag position variations. Differences between the four sites influenced production differently at each site. More exposed (top and outside) bag position slots increased biofouling accumulation while also increasing tumbling on livestock, generating higher quality oysters. The more exposed position slots had a reduction in size but increased survival.

Other variables and sources of error could have impacted production in this study. Higher or lower stocking densities would be expected to impact growth rates. If biofouling assessments are made using adjustable longline systems, *OysterGro*™ 4-slot growing cages or 2-slot growing cages, a completely different pattern of biofouling accumulation could be observed.

Chapter 4. Final Conclusions

Results of this project provide insights for off-bottom oyster aquaculture in the northern Gulf of Mexico. The main questions involved in the development of the study were: 1) Does flipping frequency on the OysterGro™ 6-slot growing pontoons impact production and biofouling accumulation? 2) Does the use of antifouling coatings reduce biofouling accumulation? And 3) though not an initial question, could certain positional effects inside the OysterGro™ 6-slot growing pontoons impact production? Results suggest 1) Yes, biofouling does impact production and can be excessive if not managed, increased aerial exposure (weekly exposure) reduced size, biofouling accumulation and survival (as compared to triweekly exposure) while also decreasing biofouling accumulation on gear and oysters and improving quality of oysters, and increasing relative shell morphometrics. 2) The use of antifouling coatings may have reduced the wet bag weight in one site (Florida), but overall, the use of antifouling coatings had no impact on production and did not reduce biofouling accumulation. And, 3) results of the second assessment demonstrated that bag positioning can greatly impact production and biofouling accumulation. Outside bags experience increased tumbling from wave action, reducing both the size of oysters and biofouling accumulation. Increased stress associated with these positions in combination with weekly flipping yielded oysters with greater cup ratios and the highest condition and meat indices. Inside cages were prone to increased biofouling, poorer condition indices and poorer meat indices, but oysters from these cages were larger and had slightly greater survival. Top/bottom bag position effects demonstrated similar results. Top positioned oysters were more subjected to aerial exposure and tumbling than bottom oysters. The "shielding" by the top row of oysters allowed bottom oysters to become more fouled.

Many factors go into site selection for oyster culture, and one thing that should be assessed is the potential for biofouling accumulation to be managed by location. In this study, there was no quantifiable measure of exposure to a specific site, but one area of further inquiry would be to test exposure to a farm in terms of biofouling accumulation. Biofouling seemed most excessive at sites with greater exposure to open water (Mississippi and Florida). Also, sites that had the most exposure and greatest tumbling action yielded the highest quality oysters. Wave-related stress increased tumbling and yielded greater cup ratios, higher condition indices and superior meat quality indices. These desirable traits came at the cost of slower growth and possible small increases in mortality.

To gain a better grasp of biofouling on off-bottom aquaculture in the northern Gulf of Mexico, more studies will be required for a wider scope of biofouling. Certain drawbacks in this experiment were the use of the OysterGro™ 6-slot growing pontoons. The use of floating bags, adjustable longline systems or even OysterGro™ 2 (4 or 9)-slot growing pontoons could yield altering result than this experiment. One possible study to answer this would be to make this same assessment again but instead of comparing frequency, one could keep the flipping frequencies the same and simply test the assessment based on gear type. It would be difficult to compare ALS systems to OysterGro™ growing pontoons because ALS systems are much more dependent upon tidal regime for aerial exposure rather than manual flipping.

Another limit to this experiment was from the effects of stocking density. Stocking density in this experiment was approximately 150 oysters per bag, but if bags were loaded at greater or lower values, this could alter the biofouling accumulation in the project. An increase of stocking density could have varying effects in terms of biofouling. Starting with higher densities could decrease biofouling but could also stunt livestock growth. Or lower densities could also increase biofouling because of the increase of available bag space.

Lastly, this experiment was limited to four sites in the northern Gulf of Mexico throughout four states. If repeated studies were to progress, then perhaps sites more westward in Texas would be advantageous. At the time of deployment, Texas did not have legislation for off-bottom oyster aquaculture but recent litigation changes have opened Texas to off-bottom oyster aquaculture. Texas has a vast amount of coastline and two distinct subspecies of the Eastern Oysters (*Crassostrea* virginica). Also, Texas coastlines are much higher in salinities than states like Louisiana, Alabama and Mississippi due to the lack of riverine influences on the Texas coastline. Also, increased sites in the Florida eastern panhandle and southern Gulf Florida would also benefit from another biofouling assessment. Cedar Key, FL is south of "the big bend" but much of Florida oyster farming is in Pensacola, Apalachicola and Alligator Harbor, so sites more pertinent to modern industry hotspots would be more ideal to this state. Not only would including more sites be beneficial but changing the sites in Alabama, Mississippi and Louisiana could give a better scope of biofouling in the northern Gulf of Mexico. Navy Cove Oyster Co. (this project's Alabama industry partner) is located on the inside of Mobile Bay which protects it from direct exposure to the Gulf of Mexico but also leaves it prone to hypohyaline events in the spring. Perhaps investigating oyster farms in Grand Bay or other sites may yield differing results than this project. The same is true of sites in Mississippi and Louisiana. Louisiana would particularly benefit due to its extensive coastline and potential for oyster farming in the western part of the state.

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Vita

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