EFFICACY OF BIOFOULING MITIGATION METHODS FOR FLOATING CAGE PRODUCTION OF SOUTHEASTERN TRIPLOID OYSTERS (*CRASSOSTREA VIRGINICA*)

by

SHANNON E. KIRK

(Under the Direction of Robert Bringolf)

ABSTRACT

Eastern oyster (*Crassostrea virginica*) aquaculture can be a major economic contributor in the Southeastern United States; however, biofouling poses a risk to successful culture and biofouling mitigation methods are needed. Effects of aerial drying frequency and a fouling-release coating on oyster growth, quality, and fouling in floating oyster cages were assessed in NC, SC, and GA. Half of culture bags were treated with a fouling release coating. Drying treatments included 24-hr aerial exposure once every one, two, or three weeks. Oyster shell metrics and bag fouling were documented quarterly. Oysters were harvested in the final two quarters for condition and fouling determination. Generally, drying frequencies and coating had little effect on oyster condition and fouling. However, oysters dried every two or three weeks without coatings showed greater growth compared to oysters dried weekly in coated bags. Additional research is needed to identify optimal biofouling mitigation approaches in the southeastern US.

INDEX WORDS: *Crassostrea virginica*, oyster, aquaculture, biofouling, floating cage, fouling-release coating, aerial drying

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DEDICATION

For my late grandfathers, Paul W. Kirk and Jerry L. Norton, who always inspired me to explore and respect everything aquatic.

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CHAPTER 1

LITERATURE REVIEW

The eastern oyster (*Crassostrea virginica*) is a filter-feeding estuary-dependent bivalve that holds high ecological and economic value. Eastern oysters form reefs that provide ecosystem services ranging from water quality improvement to habitat formation for other reef-dependent species. Eastern oyster shells are comprised primarily of calcium carbonate and consists of two elongate valves; a deeply cupped thicker left (lower) valve and a flattened right (upper) valve. They have a large adductor muscle that creates a pulling force against the ligament attaching the two valves. This, in combination with an inner mantle, creates a watertight joint that allows oysters to withstand tidal air exposure. This joint also prevents entry of water and other potentially damaging organisms.

The eastern oyster is commonly cultured in its native range along the Atlantic coast of the US (Andrews 1990). The southeastern industry was successful in the early 1900s, with Georgia leading with the highest annual landings (Harris 1980). However, a combination of overfishing, disease, and labor shortage resulted in massive population decline and the lowest landings were recorded in Georgia in 1987 (Harris 1980). However, over the past several decades, many southeastern U.S. states have made efforts to restore their oyster industries. For example, notable recoveries in the southeast are occurring in North Carolina and South Carolina; in 2017 North Carolina reported a harvest value of $5.6 million, which is more than double that of 2012 (NCDMF 2018). South Carolina has also embraced the growth in oyster culture, with the South Carolina Sea Grant oyster culture efforts contributing to 14 oyster farming businesses in 2018, making an estimated $2.3 million economic impact (Sea Grant, 2018). Georgia, which holds the record for highest U.S. landings in the early 1900s, opened its first shellfish hatchery in 2015, allowing the state to access similar technological advances used in other areas of the Southeastern U.S. (Harris 1980). This advancement can be attributed to a reemerging demand for high quality single oysters served on the half-shell. While wild reef oysters tend to grow long and skinny in clusters, ideal oysters single oysters have a deep and wide shape (Brake et al. 2003). This more marketable shape can occur with controlled reproduction and early management. By spawning high volumes of oyster larvae and allowing them to settle on microcultch (finely ground oyster shell) in an appropriately sized container, spat develop individually rather than clumped (Callam and Supan 2018). Growing individual oysters reduces the amount of labor needed later to produce a single oyster and encourages shapely growth. These advancements and increased consumer demand have led to the development of oyster hatcheries specializing in single set oysters.

Controlled reproduction of oysters in hatcheries also allows for the manipulation of ploidy, most commonly through production of triploid oysters, which has been used to improve growth in cultured oysters (Stanley et al. 1981; Allen and Downing 1986; Guo et al. 1996; Harding 2007). Triploid (3N) oysters have three sets of chromosomes and are effectively sterile, meaning energy used for spawning and gametic development is redirected for somatic tissue growth, as evident in higher triploid glycogen content during spawning season (Allen and Downing 1986; Barber and Mann 1991; Matthiessen and Davis 1992; Dégremont et al. 2012). Triploid offspring are often produced by crossing male tetraploid oysters with female diploids through natural mating or strip spawning, the latter being considered the most effective (Nell 2002; Stone et al. 2013).

Manipulation of ploidy hosts a wide range of advantages in the proper environment regardless of gear type (Walton et al. 2013). One advantage of triploid oysters is an increased growth rate, which means these oysters tend to reach market size faster than diploids, reducing the amount of time needed for farmers to profit from their efforts (Harding 2007; Stone et al. 2013; Walton et al. 2013; Callam et al. 2016). Increased growth rates are most often reflected in increased wet tissue weight, implying higher meat yields and an overall higher condition oyster (Stone et al. 2013; Callam et al. 2016). However, growth rate can vary depending on multiple environmental and management practices. Use of rigorous husbandry techniques, such as use of a shellfish tumbler, can increase growth performance of diploids to match that of triploids (Stone et al. 2013). Furthermore, in low salinity areas, triploid growth rate advantages are minimal (Callam et al. 2016). Survival rates of triploids can also be reduced to that of diploid strains when oysters become buried in sediment, restricting their ability to filter feed and respire (Colden and Lipcius 2015).

Proper grow-out gear is crucial for growing the highest quality oysters. Bottom-cages, despite common use, have disadvantages related to both mortality and growth rate. Use of this method increases the likelihood that oysters will experience issues like parasitism and predation (Moroney and Walker 1999). Further, in areas with high sediment deposition rates, oysters in bottom cages are exposed to the problem of sediment burial as described above (Moroney and Walker 1999; Colden and Lipcius 2015; Comeau et al. 2017). These effects are especially apparent in instances where the larger left valve is buried and during quiescent stages experienced by oysters in lower-temperature areas, when oysters narrowly open their valves to flush out waste (Comeau et al. 2017). Use of bottom cages or table structures can also drastically increase sediment deposition, increasing the impact on surrounding benthic habitat and overall environmental footprint (Mallet et al. 2006).

Oyster farmers are gravitating towards suspended cage culture methods as more research emerges supporting claims of rapid growth and reduced mortality attributed to the benefits of suspension in plankton-rich surface waters (Adams et al. 1991; Moroney and Walker 1999; Manley et al. 2009; Walton et al. 2013; Thomas et al. 2019). The South Carolina oyster aquaculture industry is embracing this method, with a 25% increase in suspended culture observed in 2016, 10 suspended culture lease permit applications submitted in 2017, and 14 farms operating in 2018 (Davis 2016, Davis 2017, Sea Grant 2018). These cages also make oysters more accessible to growers, decreasing the amount of time and energy spent on maintenance such as biofouling mitigation (Williamson et al. 2015). However, suspended oysters may be more prone to biofouling, an issue that can negatively affect growth, condition, and survival.

Suitable shellfish aquaculture conditions are congruent with many other invertebrate species and the benefits bivalves gain from being suspended in plankton-rich surface waters are mirrored by many fouling organisms (Carman et al. 2010). Biofouling is the settlement of unwanted organisms on culture gear or the oysters themselves and is caused by an initial settlement of dissolved organic material that allows for colonization of bacteria and algae (Callow and Callow 2002). These organisms form a biofilm or “slime” that encourages larger organisms to colonize gear surfaces (Callow and Callow 2002; Willemsen 2005). Fouling communities vary both spatially and temporally, but most consist of suspension-feeding organisms (Fitridge et al. 2012). In terms of shellfish culture specifically, fouling results in unwanted invertebrate communities forming on gear and the cultured oysters themselves, and can cost oyster industries up to $300 million in damages or approximately 5-10% of production costs (Willemsen 2005; Fitridge et al. 2012). While there is evidence that this decreased flow can encourage food production by trapping plankton in the euphotic zone, the benefit is situation specific and is often outweighed by other (negative) impacts (Ross et al. 2002). Fouling organisms cause physical damage to gear, interfere with the mechanical function of bivalve shells, and compete with cultured bivalves for such as food and oxygen (Fitridge et al. 2012). If not addressed early on, these communities can grow to reduce flow to cultured oysters, increasing mortality and reducing commercial quality (Adams et al. 1991; Moroney and Walker 1999; Fitridge et al. 2012). Higher incidences of fouling can be associated with decreased shell height and dry tissue mass that may negate growth advantages of longer feeding times, increasing time to harvest (Bishop and Peterson 2006; Sievers et al. 2017). Biofouling can also weigh down cages, putting them at risk for sinking or loss during storms (Sala and Lucchetti 2008; Fitridge et al. 2012).

Macrofouling communities can consist of “hard” or “soft” fouling organisms and the former is more problematic. “Soft” fouling refers to algae and soft-body invertebrates, such as sponges, tunicates, and hydroids (Callow and Callow 2002). These organisms are easier to remove and typically are not destructive when proper mitigation strategies are used. For example, tunicates, or more specifically ascidians, can quickly adhere to untreated aquaculture gear, as suitable shellfish conditions are similar to their preferred habitat and gear can provide hard substrates needed for settlement (Carman et al. 2010). However, these organisms are easily removed through physical mitigation methods such as air-drying and freshwater sprays (Carman et al. 2010; Sievers et al. 2017). In contrast, "hard" fouling describes attachment of invertebrates such as barnacles, mussels, and wild oysters (Callow and Callow 2002). Hard fouling colonies are considered more detrimental to shellfish culture because hard fouling is not as easily mitigated through physical methods. Hard fouling organisms are more tolerant to air-drying in later life stages and high adhesion strength. For example, larval barnacles searching for hard substrates leave adhesive trails behind that can induce settlement of other organisms (Callow and Callow 2002). Fouling organisms can also have toxic effects through production of antipredation or antifoulant metabolites (Willemsen 2005). The use of artificial substrates for bivalve culture can also increase the prevalence of invasive fouling organisms, as it weakens the competitive advantage of native species (Tyrrell and Byers 2007).

A variety of manual biofouling mitigation techniques have been used to reduce the attachment of fouling organisms while maximizing caged oyster growth and each has advantages and disadvantages. Oysters placed off-bottom intertidally during fouling recruitment periods and on-bottom sub tidally during warmer months can maximize growth with reduced fouling attachment (Adams et al. 1991; Moroney and Walker 1999). However, this method is labor-intensive and may not be available to farmers working on small leases. Another commonly used practice to reduce biofouling is aerial drying for 24 hours. Aerial drying is commonly used with suspended oyster gear and creates an artificial extended low tide. Oysters have a large adductor muscle that creates a pulling force against the ligament attaching the left and right valves which creates a watertight joint, allowing older seed to withstand air exposure

(Harris 1980). Cultured seed can survive drying mitigation methods while smaller organisms get stressed and die off, reducing fouling accumulation levels when drying is performed even once during a growing season (Mallet et al. 2009). However, this method is not effective for reducing fouling by wild oysters and barnacles unless done during initial wild settlement (Adams et al. 1991). Aerial drying may also have negative effects on cultured oysters in terms of growth, shell shape, and mortality if not performed properly. For example, drying too frequently decreases the amount of time oysters can feed and could thereby reduce growth rates especially in colder months when biofouling isn't as prevalent (Bishop and Peterson 2006).

Chemical options for biofouling control have also been assessed as an alternative to manual methods. Dipping oysters in low concentrations of acetic acid or lime can greatly reduce fouling while maintaining oyster survival rates over 80% and having little effect on oyster growth (Rolheiser et al. 2012). Anti-fouling coatings can also be applied to culture gear to reduce attachment and preemptively mitigate fouling. Copper oxide based biocidal coatings are widely used in aquaculture, but this has resulted in elevated levels of copper in water and sediment surrounding the culture site (Willemsen 2005). For this reason, the interest in use of fouling-release coatings is increasing in popularity because they are available in peroxide-based, biodegradable formulas. Fouling-release coatings lack heavy metals and can be applied to submerged gear to discourage settling and minimize the strength of fouling organism attachments (Callow and Callow 2002). Netminder®, a water-based silicone barrier coating, has been assessed with lantern nets in scallop culture. It has been suggested to reduce fouling on culture gear, but may increase fouling on bivalves themselves as a result of organisms bypassing hard cage substrates (Tettelbach et al. 2014).

Floating cages are known to simplify biofouling mitigation methods and are ecologically advantageous. These cages make oysters more accessible to growers, decreasing the amount of time and energy spent on maintenance such as aerial drying regimes (Williamson et al. 2015). Floating cages also provide year-round habitat for other estuarine species by providing protection, forage, and habitat for obligate reef residents in as little as a month after deployment (Dealteris et al. 2004; Marenghi and Ozbay 2010). Floating cage management does not adversely impact sediment biochemistry and benthic communities by means of nutrient deposition in areas with adequate intertidal flow (Mallet et al. 2009). Also, floating cage culture does not increase bacterial abundance despite grower concerns (Walton et al. 2013).

The goal of this project was to inform methods for floating cage oyster culture for the Southeastern U.S. Specific objectives were 1) to gain better understanding of the efficacy of aerial drying and fouling-release coating treatment methods for reducing biofouling and 2) assess effects of the anti-fouling treatments on oyster growth and quality. To address the first objective, we also aimed to determine what combination of treatments affected hard, soft, and total fouling accumulation on oysters and grow-out gear throughout seasonal growth periods. To address our second objective, we aimed to determine what combination of treatments affected oyster growth, shell shape, weight, and condition throughout seasonal growth periods. Findings from this project can also inform assessment of the economic effect of each methodology on production cost. In combination with subsequent extension work, our findings can be used to enhance oyster grower knowledge and improve methodologies for producing high quality oysters in multiple regions of the southeastern U.S. Atlantic coast.

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CHAPTER 2

GROWTH

Introduction

Over the past several decades, many southeastern US states have made efforts to restore their eastern oyster (*Crassostrea virginica*) industries. For example, notable recoveries in the southeast are occurring in North Carolina and South Carolina; in 2017 North Carolina reported a harvest value of $5.6 million, which is more than double that of 2012 (NCDMF 2018). South Carolina has also embraced growth in oyster culture, with the South Carolina Sea Grant Consortium oyster culture efforts contributing to 14 oyster farming businesses in 2018, making an estimated $2.3 million economic impact (Sea Grant, 2018). Georgia, which holds the record for highest US landings in the early 1900s, opened its first shellfish hatchery in 2015, allowing the state to access similar technological advances used in other areas of the Southeastern US (Harris 1980). This advancement can be largely attributed to a reemerging demand for high quality single oysters served on the half-shell. While wild reef oysters tend to grow long and skinny in clusters, ideal single oysters have a deeper and wider shape (Brake et al. 2003). This more marketable shape can occur with controlled reproduction and early management. By spawning high volumes of oyster larvae and allowing them to settle on microcultch (ground oyster shell) in a controlled environment, spat develop individually rather than clumped (Callam and Supan 2018). This culture method reduces the amount of labor needed later to produce a single oyster and creates optimal conditions for desirable shell shape growth. These advancements and increased consumer demand have led to the development of oyster hatcheries specializing in single set oysters.

Controlled reproduction of oysters in hatcheries also allows for the manipulation of ploidy, most commonly through the spawning of triploid oysters, which has been used to improve growth in cultured oysters (Stanley et al. 1981; Allen and Downing 1986; Guo et al. 1996; Harding 2007). Triploid oysters have three sets of chromosomes and are effectively sterile, meaning energy used for spawning and gametic development is redirected for somatic tissue growth as evident in higher glycogen content in triploids during spawning season (Allen and Downing 1986; Barber and Mann 1991; Matthiessen and Davis 1992; Dégremont et al. 2012). Manipulation of ploidy hosts a wide range of advantages in the proper environment regardless of gear type (Walton et al. 2013). One advantage is an increased growth rate, which means these oysters tend to reach market size faster than diploids, reducing the amount of time needed for farmers to profit from their efforts and the time culture operations are at risk from dangerous storms or other environmental stressors (Harding 2007; Stone et al. 2013; Walton et al. 2013; Callam et al. 2016). Increased growth is most often reflected in increased wet tissue weight, implying higher meat yields and an overall higher body condition (Stone et al. 2013; Callam et al. 2016). However, growth rate can vary depending on multiple environmental and management practices. Use of rigorous husbandry techniques, such as using a shellfish tumbler, can increase growth performance of diploids to match that of triploids (Stone et al. 2013). Furthermore, triploid growth rate advantages are minimal in low salinity areas and survival rates of triploids can be reduced to that of diploid strains when oysters become buried in sediment, restricting their ability to filter feed and respire (Colden and Lipcius 2015; Callam et al. 2016).

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Oyster farmers are gravitating towards suspended cage culture methods as more research emerges supporting claims of rapid growth and reduced mortality attributed to the benefits of suspension in plankton-rich surface waters (Adams et al. 1991; Moroney and Walker 1999; Manley et al. 2009; Walton et al. 2013; Thomas et al. 2019). These cages also make oysters more accessible to growers, decreasing the amount of time and energy spent on maintenance such as biofouling mitigation (Williamson et al. 2015). However, suspended oysters may be more prone to biofouling, an issue that can negatively affect growth, condition, and survival.

Suitable shellfish aquaculture conditions are congruent with many other invertebrate species and the benefits bivalves gain from being suspended in plankton-rich surface waters are mirrored by many other invertebrates, increasing the potential for biofouling (Carman et al. 2010). Biofouling is the settlement of unwanted organisms on culture gear or the oysters themselves and is caused by an initial settlement of dissolved organic material that allows for colonization of bacteria and algae (Callow and Callow 2002). In terms of shellfish culture specifically, fouling results in unwanted invertebrate communities forming on gear and the cultured oysters themselves, and can cost oyster industries up to $300 million in damages or approximately 5-10% of production costs (Willemsen 2005; Fitridge et al. 2012). Fouling organisms can cause physical damage to gear, interfere with the mechanical function of bivalve shells, and compete with cultured bivalves for resources such as food and oxygen (Fitridge et al. 2012). Higher incidences of fouling can be associated with decreased shell height and dry tissue mass that may negate growth advantages of longer feeding times (Bishop and Peterson 2006). Biofouling can also weigh down suspended culture gear, putting it at risk for sinking or loss, particularly during storms (Sala and Lucchetti 2008; Fitridge et al. 2012). Biofouling mitigation is an important part of culturing high-quality oysters, as it may reduce both the negative biological and economical effects of high fouling occurrences. Biofouling accumulation may be mitigated by use of proper drying regimes and fouling-release agents (Callow and Callow 2002; Mallet et al. 2009). However, mitigation is used to control invertebrates including other oysters, meaning certain methods may affect cultured oyster growth, shape and quality.

The objective of this project was to gain a better understanding of the efficacy of aerial drying and fouling-release coating treatment methods on oyster growth and quality in three southeastern US states (Georgia, South Carolina, and North Carolina). We aimed to determine what combination of factors affected oyster growth, shell shape, weight, and condition throughout seasonal growth periods. These findings can allow us to evaluate the economic effect of each methodology on production cost. In combination with subsequent extension work, the findings can also be used to enhance oyster grower knowledge and improve methodologies for producing high quality oysters in multiple regions of the southeastern US Atlantic coast.

Methods

Study area

This study occurred at three sites on the southeastern US Atlantic coastline; Georgia (GA), North Carolina (NC), and South Carolina (SC) (Figure 1-1A). The GA study site was in the protected Halfmoon River inshore artificial reef boundary in Wassaw sound, which opens directly to the Atlantic Ocean (Figure 1-1B). It resided at the confluence of the Bull and Wilmington rivers in waters approved for shellfish harvest and was adjacent to a commercial clam lease. While the suitability of this location for oyster aquaculture was previously unknown, we were limited in site selection to areas in which research permitting had been acquired. Salinity values and water temperatures were measured by a HOBO U24-002-C data logger that was attached with zip ties to a randomly selected cage. Data were recorded every 30 minutes from October 13, 2017 until May 18, 2018, but barnacle growth over the logger negated the accuracy of April and May data and those months were removed from analysis. Loggers were not removed during drying treatments, so all datapoints with salinities lower than 3 psu were removed from analysis. The SC site was on an oyster lease owned by Lady's Island Oyster Company off the Coosaw River near Brown's Island (Figure 1-1C). SC water quality data for 2018 were obtained by SC Department of Natural Resources at shellfish monitoring site 14-13. The North Carolina site was in Core Sound near Cedar Island on a shellfish lease owned by Carolina Mariculture Company (Figure 1-1D). No water quality data could be obtained from NC. All sites varied in tidal range, salinity, and wave action to allow for observations of treatment effects across different environmental parameters.

Experimental design

OysterGro® floating cage systems were acquired from BBI group (Bouctouche, New Brunswick, Canada), and 12 were managed at each site (one site per state). The cages were 1.52 m long, 0.91 m wide, and 0.15 m deep and each held 6 grow-out bags. A total of 72 Vexar (12 mm) grow-out bags were used per site. Half of the bags for each cage were treated with the fouling-release coating Netminder® (Gladwyne, Pennsylvania, U.S). The coating was applied using paint rollers with the assistance of a company representative on August 24, 2017. Bag coating treatments were considered coated (with Netminder®) or uncoated (no Netminder®). All 12 cages per site were randomly anchored on a single line with anchors between each three cages. Cages were approximately 1.8 meters apart and the total length of the line was approximately 38 meters. Cages were deployed in GA and NC on October 13, 2017 and in SC on October 15, 2017. The three drying treatments were weekly (once every week), biweekly (once every two weeks), or triweekly (once every three weeks). Biweekly drying was considered the control treatment, as most of the growers we collaborated with implemented this regime.

Drying treatments were assigned to the first six cages on the anchor line with the pattern two weekly, two biweekly, and two triweekly and this pattern was repeated for the remaining six cages. This non-random pattern was required to make cage management easier for growers, who were managing multiple commercial cages as well as our research cages for the full growth period. Following a Latin Squares Design, three randomly selected bags of each coating treatment were placed within each cage to account for error associated with bag placement. Bag orientations within the cages were not changed throughout the experiment.

Spawning/stocking

Triploid oysters were spawned June of 2017 at Lady's Island Oyster Hatchery (Seabrook, SC, US) with a SC tetraploid x SC diploid cross through strip spawning. When the oysters reached an average height of 25mm, 10,800 were haphazardly selected for each site and stocked in mesh bags for deployment at weight estimated densities of 150/bag. Bags were randomly selected and transported to each site manager in early October 2017. GA oysters were held in a floating upweller system (FLUPSY) for one week prior to deployment. SC and NC oysters were deployed directly to their field locations. To determine starting measurements, 100 randomly selected oysters were measured on October 11, 2017 in GA using General 6" dial calipers for shell height (SH), length (SL), and width (SW) to nearest 0.1 mm using methods described by Galtsoff (1964). These measurements were used for SC and NC starting measurements as oysters were deployed less than a week after measurement. Each previously stocked mesh bag was randomly assigned a Vexar bags and oysters were transferred to their assigned Vexar bag before being deployed in cages. Oyster stocking and bag deployment occurred within the same week that cages were anchored. Oysters grew in the same cage and bag for the entire growth period. Stocking densities were determined in October 2018 by accounting for mortalities, live oysters, and numbers of harvested oysters in each remaining bag (n = 14). Only 12 bags from SC and 2 bags in GA were available for stocking density counts because SC oysters were replaced by smaller seed in all but one bag per cage after June in compliance with the grower's preferences, NC oysters were lost to Hurricane Florence prior to October, and the majority of GA bags were too fouled to determine which mortalities were from the original stock. Because bags were randomly distributed, we used this count to apply to all bags in the experiment.

Quarterly shell metrics

Oyster growth was monitored at each site during quarterly sampling trips, which occurred in December of 2017, and March, June, and October of 2018. All three sites were sampled within a two-week period for each quarter. During December and March sampling, a subsample of 10 oysters per bag were haphazardly selected and shell metrics (SH, SL, SW) were measured with the methods described previously (Galtsoff 1964). These measurements allowed for calculation of cup (SW:SH) and fan (SL:SH) ratios, which are indicators of oyster quality. Oysters were returned to their bags and cages after measurement. During June sampling, 25 oysters were randomly selected from each bag and 10 randomly selected oysters from those 25 were frozen and retained for determination of condition. Shell metrics were measured later during condition procedures and before shucking. Mortalities were counted and retained for later measurement. All GA oyster bags remained stocked in their cages until Oct sampling while SC oysters were reduced to one bag of original oysters per cage, removing the bag coating treatment. Oyster measurements and condition analyses were available from all bags at each site in December, March, and June (n = 72). Like June, October sampling consisted of harvesting and freezing oysters. Four GA bags were lost during Hurricane Michael prior to sampling, reducing the number of bags to 68. In GA, 5 oysters per bag were processed (n = 68 bags) and in SC we processed 10 per bag (n = 12 bags). Again, shell metrics were measured later during condition determination and before shucking. NC oysters were lost during Hurricane Florence and were not sampled for October. All oysters were kept frozen for a minimum of 1 week before condition analysis began.

Condition

All June oyster weights were measured to the nearest 0.01 g with a Sartorious CP124S balance scale and in October with a Denver Instrument XE-410D scale. Different scales were used because intense fouling increased weights above the threshold of the Sartorious CP124S. Frozen oysters were cleaned with running cold water and a wire brush, dried with a paper towel, and weighed to obtain wet weight (WW). Oysters that gaped before or after cleaning were noted and removed from wet weight, wet tissue weight, and condition analyses. Oysters were measured for shell metrics after cleaning and before being shucked. Each oyster was assigned a plastic weigh boat and a standardized 0.9 g aluminum tin. Soft tissue was removed from shells with a shucking knife and placed in its assigned aluminum tin. The inside of both valves was scraped with a stainless-steel razor to remove any remaining soft tissues, which was added to its respective tin. Soft tissues and tins were weighed, and the tin weight subtracted to determine wet tissue weight (WT). Both valves were patted dry with a paper towel to remove excess liquid before being placed exterior side down in its assigned plastic weight boat. Valves were weighed to determine wet shell weight (WS). Scales were tared with plastic weigh boats prior to placement of the shells and no subtraction of plastic boat weights was needed. Tins with wet tissues were placed on aluminum trays and dried for 48 ± 1 hours at 80^{o}C. Shells were air-dried for 48 hours in accordance to condition index procedures (Abbe and Albright 2003). When removed from the oven, tins and dried soft tissues were left to cool at room temperature for ~5 minutes. Soft tissues were weighed with their tins and the tin weight subtracted from the total weight to determine dry tissue weight (DT). Shells were transferred to an already tared plastic weigh boat, ensuring all pieces of broken valves were transferred as well, and weighed to determine dry shell weight (DS). Scales were tared between each measurement for quality control. Condition index (CI) was calculated according to Abbe and Albright's (2003) formula below:

$$[(DT) / (WW - DS)] * 100$$

Growth and mortality

Oyster growth was monitored using the SH data recorded during each quarterly sampling trip by subtracting mean SH values for each bag from the previous value for the same bag. Mortality was monitored during June and October sampling. Oysters found gaped or with separated valves were considered dead and were bagged to bring back for further investigation. Only left valves of collected mortalities were counted and SH measured to avoid double counts. These counts allowed us to estimate occurrences of mortality events. Mortalities with SH < 40 mm were used to determine survival rates that exclude initial die offs that occurred before deployment. June mortalities were summed and subtracted from the mean stocking number to determine survival, divided by the mean stocking number, and multiplied by 100 to determine survival percentage per bag. Small (<40 mm) mortalities were also summed, subtracted from total mortalities, divided by the mean stocking number, and multiplied by 100 to determine total grow-out survival percentages per bag. Both survival percentages per bag were used to determine survival rates with and without initial die-offs. Mean survival rates for each drying treatment and bag x drying treatment were calculated by taking the mean survival rates per bag for each treatment. October mortalities were added to mortalities of their corresponding bags and the previously described methods repeated to calculate October survival rates.

Data analysis

Data were organized using Excel®2016. All data were analyzed using RStudio (RStudio Team 2003). Vexar bags within cages represented our experimental units as these bags were the lowest level of randomly assigned treatment and each oyster sampled was considered a subsample. Therefore, mean oyster measurements, growth rates, weights, and CI were calculated per bag.

Shell metrics, growth rates, CI, and weights were analyzed using a two-factor (aerial drying treatment x bag coating treatment) split-plot ANOVA to determine treatment interactions and to account for error among cages. Comparisons were made within states (GA, SC, NC) with data analyzed quarterly to determine seasonal effects of treatments. No comparisons were made among states because of site variation. Treatment comparisons were made with all states combined for December, March, and June. Data for combined comparisons were blocked by location. No combined comparisons were made for October because of the loss of NC oysters and the reduction in numbers of SC bags. Differences among drying treatments and among main effects of drying treatments with bag coating treatments were determined using Tukey's HSD tests. Residual distributions were checked using Shapiro-Wilks tests and if residuals were not normally distributed, appropriate transformations were used to normalize the data. December GA, October GA, and March NC cup ratios were normalized with LOG transformations. March SC SL, December NC SL, and October SC SW were normalized with SIN transformations. October GA WW and October SC WT were also normalized using SIN transformations.

If normalization was not achieved through transformation, we evaluated main effects with non-parametric Kruskal-Wallis tests and Dunn's tests for differences. December GA fan ratios, and June GA SW could not be normalized with transformations and thus were assessed with Kruskal-Wallis and Dunn's tests. For combined states' (GA, SC, NC) data, December SL and June SL, SW, CI, and WW could not be normalized with transformations and thus were assessed with Kruskal-Wallis and Dunn's tests.

One factor ANOVA and Tukey's HSD tests were used for SC Oct analysis because the bag coating treatment was no longer testable with the bag reduction. Oysters that gaped during or before cleaning were removed from weight and CI analyses. All quarterly time points were analyzed individually and $\alpha = 0.05$ for all tests. All results are presented as mean ± SE unless otherwise noted. All survival percentages were analyzed using Kruskal-Wallis and Dunn's tests.

Results

Study area

Water temperature at the GA site ranged from 9-28^{o}C with a mean of 17 ± 0^{o}C from October through December 2017. Salinity ranged from 23-29 psu with a mean of 27 ± 0 psu during the same period. From January through March 2018, water temperatures varied from 2-22^{o}C with a mean of 13 ± 0^{o}C. Salinity ranged from 13-28 psu with a mean of 24 ± 0 psu. Water temperature at the SC site ranged from 9-13^{o}C with a mean of 11 ± 1^{o}C from January through March 2018. Salinity ranged from 27-28 psu with a mean of 27 ± 0 psu. SC water temperatures ranged from 21-31^{o}C with a mean of 27 ± 2^{o}C from April to October 2018. Salinity ranged from 20-28 psu with a mean of 26 ± 1 psu. No temperature or salinity data were available for the NC site.

Spawning/stocking

Oysters were stocked at a mean SH of 30.9 ± 0.4 mm (n = 100). Twelve bags in SC and two bags in GA were counted in Oct for stocking densities and the mean number of oysters stocked per bag was 137 ± 1 (n = 14). Not all bags from all sites were measured because NC bags were lost during Hurricane Florence, only one SC bag per cage remained with the originally stocked oysters, and most GA bags were too fouled to distinguish between originally stocked oyster mortalities and fouling oyster mortalities.

Quarterly shell metrics

Georgia

In December, SH differed among drying treatments, and weekly drying (40.2 ± 0.4 mm) was lower (N = 72, $F_{2, 69}$ = 22.13, p < 0.001) than biweekly drying (44.9 ± 0.6 mm) and triweekly drying (45.6 ± 0.5 mm) (Figure 1-2A). Bag coatings also produced different SH in December, with uncoated bags (44.6 ± 0.6 mm) having significantly larger (N = 72, $F_{1, 70}$ = 29.0, p < 0.001) SH than coated bags (42.6 ± 0.6 mm). December fan ratio in weekly drying treatments (0.75 ± 0.0) were significantly lower (χ^2 (2, N = 72) = 17.04, p < 0.001) than biweekly (0.78 ± 0.0) while neither varied from triweekly (0.76 ± 0.0) (Figure 1.3A). However, there were no fan ratio differences between bag coating treatments among drying treatments (Figure 1.3A). There was no difference among cup ratios for drying treatments or bag coating treatments in December (Figure 1.4A).

March SH differed among drying treatments; weekly drying (44.0 ± 0.5 mm) was significantly lower (N = 72, $F_{2, 69}$ = 16.10, p = 0.001) than biweekly (50.0 ± 0.7 mm) and triweekly (50.7 ± 0.6 mm) (Figure 1.2A). There were no differences among bag coatings for March SH (Figure 1.2A). March fan ratios differed among drying treatments only, as biweekly drying (0.77 ± 0.0) and triweekly drying (0.77 ± 0.0) were significantly greater (N = 72, $F_{2, 69}$ = 13.25, p = 0.002) than weekly drying (0.74 ± 0.0) (Figure 1.3A). There were no differences among drying treatments or between bag coating treatments for cup ratios (Figure 1.4B).

June SHs within weekly drying treatments (59.0 ± 0.9 mm) were significantly lower (N = 72, $F_{2, 69}$ = 5.35, p = 0.030) than triweekly (67.7 ± 1.3 mm), but neither varied from biweekly (66.9 ± 0.8 mm) (Figure 1.2A). June cup ratios also varied by drying treatment, as biweekly (0.40

± 0.0) and triweekly (0.40 ± 0.0) were significantly lower (N = 72, $F_{2, 69}$ = 10.83, p = 0.004) than weekly drying (0.43 ± 0.0). June SW for weekly drying (25.3 ± 0.4 mm) was significantly lower ($\chi^2(2, N = 72) = 13.56$, p = 0.001) than biweekly (26.4 ± 0.5 mm) and triweekly (26.9 ± 0.3 mm). There was no difference among fan ratios for drying treatments (Figure 1.3A). No June measurements showed differences between bag coatings and there were no interactions among drying and bag coating treatments. Cup ratios for weekly drying (0.43 ± 0.0) were higher (N = 72, $F_{2, 69}$ = 10.83, p = 0.004) than biweekly (0.40 ± 0.0) and triweekly (0.40 ± 0.0) (Figure 1.4A).

October SH showed no differences among drying treatments, but coated bags (85.9 ± 1.5 mm) were significantly lower (N = 68, $F_{2, 65}$ = 5.71, p = 0.041) than uncoated bags (88.6 ± 1.4 mm) (Figure 1.2A). However, when whole effects of drying and bag coating treatments were assessed, there was no difference among any combination (Figure 1.2A). October fan ratios showed no differences among drying or between bag coating treatments (Figure 1.3A). Similarly, October cup ratios showed no differences among drying treatments or between bag coating treatments (Figure 1.4A).

South Carolina

There was no difference among drying or bag coating treatments for SH in December in SC (Figure 1.2B) and fan ratios did not differ among drying treatments (Figure 1.3B). Bag coating treatments produced different fan ratios, and uncoated bags (0.69 ± 0.005) were significantly higher (N = 72, $F_{2, 36}$ = 12.59, p = 0.006) than coated (0.67 ± 0.0). However, there were no difference between bag coating treatments within drying treatments (Figure 1.3B). December drying treatment cup ratios were not different, but cup ratios varied by bag coating treatments and uncoated bag cup ratios (0.30 ± 0.0) were significantly greater ($N = 72$, $F_{1, 70} = 16.89$, $p = 0.003$) than coated bags (0.29 ± 0.0) (Figure 1.4B).

In March, SH differed within drying treatments; weekly drying (59.7 ± 0.7 mm) and biweekly (62.5 ± 0.5 mm) treatments were significantly lower ($N = 72$, $F_{2, 69} = 13.74$, $p = 0.002$) than triweekly drying (65.9 ± 0.8 mm) (Figure 1.2B). Bag coating also had an effect on SH, and uncoated bags (63.8 ± 0.8 mm) were significantly higher ($N = 72$, $F_{1, 70} = 12.75$, $p = 0.006$) than coated bags (61.6 ± 0.6 mm). However, there were no differences among bag coatings within drying treatments (Figure 1.2B). For March SL, weekly drying (43.2 ± 0.4 mm) was significantly lower ($N = 72$, $F_{2, 69} = 7.77$, $p = 0.011$) than biweekly drying (45.0 ± 0.4 mm) with neither varying from triweekly (47.9 ± 0.5). Neither fan ratios nor cup ratios varied among drying treatments or between bag coatings (Figures 1.3B, 1.4B).

In June, SH differed among drying treatments again, and weekly drying (74.5 ± 0.8 mm) was significantly lower ($N = 72$, $F_{2, 69} = 18.34$, $p = 0.001$) than biweekly (79.8 ± 0.6 mm) and triweekly (82.6 ± 0.7 mm) (Figure 1-2B). Fan ratios in weekly drying treatments (0.80 ± 0.0) were significantly higher ($N = 72$, $F_{2, 69} = 9.45$, $p = 0.006$) than triweekly (0.76 ± 0.0), but neither significantly differed from biweekly (0.78 ± 0.0) (Figure 1.3B). Cup ratios in June also differed by drying treatment, as weekly (0.35 ± 0.0) was lower ($N = 72$, $F_{2, 69} = 7.42$, $p = 0.013$) than biweekly (0.34 ± 0.0) and triweekly treatments (0.33 ± 0.0) (Figure 1-4B).

In October, there were no differences among drying treatments for SH (Figure 1.2B). However, October SW for weekly drying (59.2 ± 0.8 mm) was significantly lower ($N = 12$, $F_{2, 9} = 5.56$, $p = 0.027$) than biweekly (62.5 ± 0.8 mm) while neither differed from triweekly (62.2 ± 1.0

mm). October fan ratios also varied, as weekly (0.65 ± 0.0) was significantly lower (*N* = 12, $F_{2, 9}$ = 7.72, *p* = 0.011) than both biweekly (0.69 ± 0.0) and triweekly (0.69 ± 0.0) (Figure 1.3B). October cup ratios for weekly drying (0.34 ± 0.0) was significantly lower (*N* = 12, $F_{2, 9}$ = 4.97, *p* = 0.035) than triweekly (0.36 ± 0.0), but neither differed from biweekly (62.5 ± 0.0) (Figure 1-4B). No bag treatments were assessed because only one bag per cage remained after June harvest.

North Carolina

In NC, there were differences among drying treatments for SH in December, as weekly drying (49.8 ± 0.6 mm) was significantly lower (*N* = 72, $F_{2, 69}$ = 4.82, *p* = 0.038) than biweekly (52.2 ± 0.6 mm) and triweekly (52.3 ± 0.6 mm). However, Tukey's HSD results showed no difference among drying treatments for SH in December as well as no differences between bag coating treatments (Figure 1.2C). There were also no differences among drying treatments for fan ratios (Figure 1.3C). However, there was a difference between bag coating treatments for fan ratios; fan ratios in coated bags (0.72 ± 0.0) were significantly higher (*N* = 72, $F_{1, 70}$ = 14.19, *p* = 0.004) than uncoated bags. There were no differences between bag coating treatments within drying treatments for fan ratios (Figure 1.3C). There were also no differences among drying treatments or bag coating treatments for cup ratios in December (Figure 1.4C).

In March, SH varied among drying treatments, as weekly drying (59.0 ± 0.7 mm) was significantly lower (*N* = 72, $F_{2, 69}$ = 9.18, *p* = 0.007) than biweekly (62.4 ± 0.68 mm) and triweekly drying (61.8 ± 0.6 mm) (Figure 1.2C). For March SL, there were no differences (*N* = 72, $F_{2, 35}$ = 3.32, *p* = 0.083) among weekly drying (44.5 ± 0.5 mm), biweekly drying (46.1 ± 0.4 mm), and triweekly drying (46.1 ± 0.5). However, bag coating treatments varied and coated bags (46.2 ±

0.4 mm) were higher (N = 72, $F_{1,35}$ = 5.83, p = 0.039) than uncoated bags (44.8 ± 0.4 mm). There were no differences among drying treatments or bag coating treatments for March fan ratios (Figure 1.3C). March cup ratios differed among drying treatments, as weekly drying (0.29 ± 0.0) was significantly higher (N = 72, $F_{2, 69}$ = 5.28, p = 0.031) than biweekly (0.28 ± 0.0), but neither differing from triweekly (0.29 ± 0.0) (Figure 1.4C).

June drying treatments differed in SH, and weekly drying (74.0 ± 0.8 mm) was significantly lower (N = 72, $F_{2, 69}$ = 6.08, p = 0.021) than triweekly drying (78.8 ± 0.7 mm) but neither varied from biweekly (76.1 ± 0.8 mm) (Figure 1.2C). However, there were no differences among bag coating treatments for June SH (Figure 1.2C). There were also no differences among any treatment for cup ratio (Figure 1.4C). There were no other differences among drying treatments or bag coatings for fan ratio (Figure 1.3C). However, there was one significant interaction among drying and bag coating treatments for SL (N = 72, $F_{2, 69}$ = 6.02, p = 0.022). No NC shell metrics were analyzed in October because of hurricane losses.

<u>All states</u>

With all states' (GA, SC, NC) data combined, there were significant differences among drying treatments for all quarterly metrics in December. December SH differed by drying treatment only, as weekly (46.6 ± 0.6 mm) was significantly lower (N = 216, $F_{2, 213}$ = 21.33, p < 0.001) than biweekly (49.4 ± 0.5 mm) and triweekly (50.0 ± 0.5 mm) (Figure 1.2D). For December SL, there was a significant difference among drying treatments as weekly drying (32.7 ± 0.3 mm) was significantly less (χ^2(2, N = 216) = 51.59, p < 0.001) than biweekly (35.3 ± 0.2 mm) and triweekly (35.9 ± 0.3 mm). However, there was no SL difference (χ^2(1, N = 72) =

1.104, $p < 0.293$) between uncoated bags (34.8 ± 0.2 mm) and coated bags (34.4 ±0.3 mm). For December SW, biweekly drying (15.0 ± 0.2 mm) and triweekly drying (15.2 ± 0.1 mm) were significantly higher (N = 216, $F_{2, 213} = 8.00$, p = 0.002) than weekly drying (14.4 ± 0.2 mm). Fan ratios differed among drying treatments, and weekly (0.71 ± 0.0) was significantly smaller (N = 216, $F_{2, 71} = 3.98$, $p = 0.029$) than triweekly (0.73 ± 0.0), but neither treatments differed from biweekly (0.72 ± 0.0) (Figure 1.3D). There were no differences among drying nor bag coating treatments for cup ratios (Figure 1.4D).

March SH showed differences among drying treatments only, as weekly drying (54.2 ± 0.9 mm) was significantly lower (N = 216, $F_{2, 213} = 28.57$, $p < 0.001$) than the other biweekly drying (58.3 ± 0.8) and triweekly drying (59.5 ± 0.9) (Figure 1.2D). Drying treatments also varied for SL, as weekly drying (40.1 ± 0.7 mm) was significantly lower (N = 216, $F_{2, 213} = 24.51$, $p <$ 0.001) than biweekly (43.2 ± 0.5 mm) and triweekly (44.2 ± 0.6 mm). March SW for weekly drying (14.3 ± 0.2 mm) was also significantly lower (N = 216, $F_{2, 213} = 12.19$, $p < 0.001$) than biweekly (18.0 ± 0.1 mm) and triweekly (18.4 ± 0.1 mm). There were no significant differences among drying treatments for fan ratios, but fan ratios for coated bags (0.75 ± 0.0) were significantly higher (N = 216, $F_{1, 107} = 4.42$, $p = 0.043$) than uncoated (0.74 ± 0.0), p = 0.043) (Figure 1.3D). March cup ratio varied among drying treatments, as weekly drying (0.32 ± 0.0) was significantly higher (N = 216, $F_{2, 213} = 5.32$, $p = 0.010$) than biweekly (0.32 ± 0.0) and triweekly (0.32 ± 0.0), although differences were beyond reportable significant digits. Cup ratios also differed between bag coatings, and uncoated bags (0.32 ± 0.0) were higher (N = 216, $F_{1, 107}$ = 4.42, $p = 0.043$) than coated bags (0.32 ± 0.0), but differences again were beyond reportable significant digits (Figure 1.4D).

June SH again varied among drying treatments only, as weekly drying (69.2 ± 1.0 mm) was significantly lower (N = 216, $F_{2, 213}$ = 19.75, p < 0.001) than biweekly drying (74.3 ± 0.8 mm) and triweekly drying (76.4 ± 0.9 mm) (Figure 1.2D). There were SL differences among drying treatments as well, and weekly (55.7 ± 0.7 mm) was significantly lower (χ^2(2, N = 216) = 27.10, p < 0.001) than biweekly and triweekly (59.7 ± 0.4 mm). There were no differences (χ^2(1, N = 216) = 0.05, p < 0.815) between uncoated bags (58.1 ± 0.5 mm) and coated bags (58.1 ± 0.5 mm). For SW in June, weekly drying (25.2 ± 0.2 mm) was significantly lower (χ^2(2, N = 216) = 24.51, p < 0.001) than biweekly (25.9 ± 0.2 mm) and biweekly was significantly lower than triweekly (26.5 ± 0.2 mm). June fan ratios for weekly drying (0.81 ± 0.0) was significantly higher (N = 216, $F_{2, 213}$ = 5.10, p = 0.012) than triweekly (0.79 ± 0.0) but neither varied from biweekly (0.80 ± 0.0) (Figure 1.3D). Cup ratios differed by drying treatment, as weekly drying (0.37 ± 0.0) was significantly higher (N = 216, $F_{2, 213}$ = 15.77, p < 0.001) than biweekly (0.35 ± 0.0) and triweekly (0.35 ± 0.0) (Figure 1.4D). Bag coating treatments also showed different cup ratios in June, and coated bags (0.36 ± 0.0) were higher (N = 216, $F_{1, 107}$ = 5.16, p = 0.030) than uncoated (0.36 ± 0.0), although these differences were beyond reportable significant digits. Reductions in stocks in SC and hurricane losses in NC meant that states could not be combined for the final sampling period. As a result, no combined state shell metrics were analyzed for October.

Condition

<u>Georgia</u>

In June, CI did not differ among drying treatments or bag coating treatments (Figure 1.5A). There were differences among drying treatments for WW, as weekly drying (39.69 ± 1.50

g) was significantly lower (*N* = 72, $F_{2, 69}$ = 6.18, p = 0.021) than triweekly drying (57.86 ± 1.65 g) while neither varied from biweekly drying (53.81 ± 2.36 g). WW for coated bags (50.29 ± 2.01 g) and uncoated bags (50.61 ± 2.01 g) did not differ significantly (*N* = 72, $F_{2, 69}$ = 0.14, p = 0.716). There were no WT differences among drying treatments or bag coating treatments (Figure 1.6A).

October condition was similar, as CI did not differ among drying treatments or bag coatings (Figure 1.5A). October WW did not significantly differ among drying treatments (*N* = 66, $F_{2, 63}$ = 3.03, *p* = 0.099), although weekly treatment (90.46 ± 4.41 g) trended lower than biweekly (104.08 ± 4.80 g) and triweekly (111.80 ± 2.95 g). WW in October for coated (99.34 ± 3.71 g) and uncoated bags (103.8 ± 3.85 g) also did not differ significantly (*N* = 66, $F_{2, 63}$ = 0.26, *p* = 0.620). WT weights did not differ among bag coating treatments or drying treatment (Figure 1.6A).

South Carolina

In June, CI showed no difference among any treatments (Figure 1.5B). There was a difference among drying treatments for WW, as weekly drying (57.45 ± 1.13 g) weighed significantly less (*N* = 69, $F_{2, 66}$ = 14.96, *p* = 0.001) than biweekly (65.41 ± 1.23 g) and triweekly (70.31 ± 0.85 g). However, coated bags (64.21 ± 1.18 g) and uncoated bags (64.34 ± 1.36 g) did not differ significantly in June (*N* = 69, $F_{2, 66}$ = 0.00, *p* = 0.992). There was also a difference among drying treatments only for WT, with weekly drying (11.42 ± 0.28 g) weighing significantly less (*N* = 69, $F_{2, 66}$ = 9.37, *p* = 0.006) than triweekly (14.34 ± 0.25 g), but neither differing from biweekly (13.26 ± 0.41 g) (Figure 1.6B).

In October, CI did not differ among any treatments (Figure 1.5B). October WW also showed no differences among any treatments. WT did not differ among any treatment (Figure 6B).

North Carolina

In June, CI did not differ among drying treatments in NC (Figure 1.5C). In contrast drying treatments differed in WW with biweekly drying (57.09 ± 0.94 g) and triweekly drying (59.46 ± 1.13 g) weighing significantly more ($N = 59$, $F_{2, 56} = 13.34$, $p = 0.002$) than weekly drying (53.27 ± 0.82 g). Drying treatments also differed in terms of WT following the same pattern as weekly drying (12.85 ± 0.36 g) weighed significantly less ($N = 59$, $F_{2, 56} = 9.91$, $p = 0.005$) than biweekly (14.23 ± 0.39 g) and triweekly (14.69 ± 0.47 g) (Figure 1.6C). There were no differences between bag coating treatments for CI (Figure 1.5C) or WT (Figure 1.6C). Condition was not analyzed for NC in October because no oysters were sampled.

All states

With all states' (GA, SC, NC) data combined in June, there were no differences among drying treatments or bag coating treatments for CI (Figure 1.5D). Drying treatments produced different results for June WW across all drying treatments, with weekly drying (50.00 ± 1.16 g) weighing significantly less ($\chi^2(2, N = 200) = 56.64$, $p < 0.001$) than biweekly (58.70 ± 1.17 g) and triweekly (62.66 ± 1.01 g) weighing significantly more than both weekly and biweekly. However, June WW for coated (56.97 ± 1.03 g) and uncoated bags (57.04 ± 1.09 g) did not differ significantly (χ^2(2, N = 200) = 0.00, p = 0.989). WT differed by drying treatment only, with weekly drying (10.80 ± 0.29 g) weighing less than biweekly (12.63 ± 0.33 g) and triweekly (13.35 ± 0.29 g) (Figure 1.6D). Reductions in stocks in SC and hurricane losses in NC meant that states could not be combined for the final sampling period. As a result, no combined state condition data were analyzed for October.

Growth and mortality

Georgia

In GA, drying treatments produced different SH growths from deployment until December, as weekly drying (9.3 ± 0.4 mm) increased significantly less (N = 72, $F_{2, 69}$ = 22.13, p < 0.001) than biweekly (14.0 ± 0.6 mm) and triweekly drying (15.1 ± 0.6 mm). Bag coating treatments also showed differences in growth, and coated bags (11.6 ± 0.6 mm) increased significantly less (N = 72, $F_{1, 70}$ = 29.07, p < 0.001) than uncoated bags (13.7 ± 0.6 mm). However, there was only a difference between bag coatings within the biweekly drying treatment, with biweekly coated bags (12.7 ± 0.9 mm) showing less growth than biweekly uncoated bags (15.3 ± 0.7 mm).

There were no differences in SH growth among drying treatments from December to March, with differences among weekly drying (3.8 ± 0.5 mm), biweekly drying (5.0 ± 0.6 mm), and triweekly drying (5.1 ± 0.6 mm) being insignificant (N = 72, $F_{2, 69}$ = 1.86, p = 0.211). However, there were differences between bag coating treatments, and coated bags (5.6 ± 0.4 mm) increased significantly more (N = 72, $F_{1, 70}$ = 45.22, p < 0.001) than uncoated bags (3.6 ± 0.5 mm).

Drying treatments again showed no differences in growth from March to June, with differences among weekly drying (15.0 ± 0.6 mm), biweekly drying (16.9 ± 0.8 mm), and triweekly drying (17.3 ± 0.5 mm) being insignificant (N = 72, $F_{2, 69}$ = 0.73, p = 0.508). Bag coating treatments also didn't vary, with differences between coated bags (15.9 ± 0.6 mm) and uncoated bags (16.8 ± 0.6 mm) being insignificant (N = 72, $F_{1, 70}$ = 1.55, p = 0.245). Grow-out survival rates (excluding small mortalities) were >95% for all drying and bag coating treatments in June.

From June to October, SH growth was similar across drying treatments, with weekly drying (24.4 ± 1.2 mm), biweekly drying (22.0 ± 0.9 mm), and triweekly drying (21.6 ± 1.3 mm) differing insignificantly (N = 68, $F_{2, 65}$ = 0.85, p = 0.461). There were no differences between bag coating treatments either, with coated bags (21.9 ± 0.9 mm) differing insignificantly (N = 68, $F_{1, 66}$ = 3.15, p = 0.110) from uncoated bags (23.5 ± 1.0 mm). Only two October GA bags, both from the triweekly drying treatment, were assessed for mortality, eliminating the possibility to accurately compare treatments. Estimated triweekly drying grow-out survival was 89%.

<u>South Carolina</u>

From deployment until December, SH growth in SC was lowest for weekly drying (19.0 ± 0.6 mm), but it did not differ significantly (N = 72, $F_{2, 69}$ = 3.03, p = 0.084) from biweekly drying (20.3 ± 0.6 mm) or triweekly drying (21.1 ± 0.8 mm). Differences in SH growth between coated bags (20.8 ± 0.5 mm) and uncoated bags (19.5 ± 0.6 mm) were also insignificant (N = 72, $F_{1, 70}$ = 3.37, p = 0.100).

SH growth from December to March again showed no differences (N = 72, $F_{2, 69}$ = 4.27, p = 0.050) among weekly drying (9.8 ± 1.0 mm), biweekly drying (11.3 ± 0.9 mm), and triweekly drying (13.9 ± 1.1 mm). There was a difference in SH growth between bag coating treatments, with uncoated bags (13.4 ± 0.9 mm) increasing significantly more (N = 72, $F_{1, 70}$ = 12.33, p = 0.007) than coated bags (9.9 ± 0.7 mm).

Differences in SH growth among drying treatments diminished from March until June and there were no significant differences (N = 72, $F_{2, 69}$ = 1.113, p = 0.370) among weekly drying (14.8 ± 0.9 mm), biweekly drying (17.3 ± 0.9 mm), and triweekly drying (16.8 ± 0.9 mm). Grow-out survival rates (excluding small mortalities) were >95% for all treatments in June.

From June to October, growth was only assessed for drying treatments. There were no differences (N = 12, $F_{2, 9}$ = 2.77, p = 0.115) in SH growth among weekly drying (16.8 ± 2.5 mm), biweekly drying (10.9 ± 2.7 mm), and triweekly drying (10.3 ± 0.6 mm) from June to October. Grow-out survival rates in October were above 90% for all drying and bag coating treatments.

North Carolina

From deployment until December, SH growth in NC for weekly drying (18.9 ± 0.6 mm), biweekly drying (21.3 ± 0.6 mm), and triweekly drying (21.4 ± 0.4) differed significantly (N = 72, $F_{2, 69}$ = 4.81, p = 0.038). However, Tukey' HSD test results show no differences among drying treatments. Deployment to December SH growth in coated (20.3 ± 0.5 mm) and uncoated bags (20.8 ± 0.6 mm) did not differ significantly (N = 72, $F_{1, 70}$ = 0.85, p = 0.381).

From December until March, SH growth did not vary among drying treatments, as weekly drying (9.2 ± 0.7 mm), biweekly drying (10.2 ± 0.8 mm), and triweekly (9.5 ± 0.7 mm) drying resulted in insignificant differences (N = 72, $F_{2, 69}$ = 0.61, p = 0.565). Coated bags (10.3 ± 0.6 mm) and uncoated bags (8.9 ± 0.6 mm) also showed no significant differences (N = 72, $F_{1, 70}$ = 3.36, p = 0.100).

Trends from the previous growth periods in NC repeated for SH growth from March to June; weekly drying (15.0 ± 0.9 mm), biweekly drying (13.7 ± 1.1 mm), and triweekly drying (17.0 ± 0.7 mm) did not vary significantly (N = 72, $F_{2, 69}$ = 2.60, p = 0.128). Bag coating treatments also did not vary, with coated bag (14.9 ± 0.8 mm) SH growth being similar (N = 72, $F_{1, 70}$ = 0.43, p = 0.528) to uncoated bags (15.6 ± 0.7 mm). Survival was high in NC in June and total survival for weekly drying (99.9 ± 0.0%), biweekly drying (99.9 ± 0.1%), and triweekly drying (100.0 ± 0.0%) were not different ($\chi^2(2, N = 72)$ = 0.48, p = 0.787). Total survival for coated bags (99.9 ± 0.1%) and uncoated bags (100.0 ± 0.0%) did not differ significantly ($\chi^2(1, N = 72)$ = 1.07, p = 0.300). Grow-out survival (with no small mortalities) was >99% among all drying treatments in June. Hurricane losses in NC meant that growth and survival could not be analyzed for October.

<u>All states</u>

With all states (GA, SC, NC) combined, SH growth rates from deployment until December showed no significant differences among weekly drying (15.7 ± 0.6 mm), biweekly drying (18.5 ± 0.5 mm), and triweekly drying treatments (19.1 ± 0.5 mm). SH growth comparisons between coated bags (17.6 ± 0.4 mm) and uncoated bags (18.0 ± 0.4 mm) also showed no significant differences (N = 216, $F_{1, 214}$ = 1.08, p = 0.307).

From December to March, trends of little variation among treatments remained the same for SH growth as weekly drying (7.6 ± 0.5 mm), biweekly drying (8.9 ± 0.6 mm), and triweekly drying (9.5 ± 0.6 mm) did not differ significantly (N = 216, $F_{2, 213}$ = 0.97, p = 0.389). Again, coated bags (8.6 ± 0.5 mm) and uncoated bags (8.7 ± 0.5 mm) did not significantly differ (N = 216, $F_{1, 214}$ = 0.01, p = 0.940).

For March to June, SH growth trends repeated, with weekly drying (14.9 ± 0.5 mm), biweekly drying (16.0 ± 0.6 mm), and triweekly drying (16.9 ± 0.4 mm) showing no differences (N = 216, $F_{2, 213}$ = 2.10, p = 0.138). Again, coated bags (16.0 ± 0.4 mm) and uncoated bags (15.9 ± 0.4 mm) did not differ significantly (N = 216, $F_{1, 214}$ = 0.02, p = 0.903). Total survival percentages for weekly (96.6 ± 0.9%), biweekly (97.7 ± 0.4%), and triweekly drying (95.4 ± 0.9%) treatments did not differ significantly (χ^2(2, N = 216) = 0.49, p = 0.781). Total mortality for coated bags (96.3 ± 0.7%) and uncoated bags (96.9 ± 0.6%) also did not differ significantly (χ^2(1, N = 216) = 0.12, p = 0.729). Grow-out survival (excluding small mortalities) was similar, with weekly (99.0 ± 0.1%), biweekly (98.4 ± 0.2%), and triweekly drying (98.5 ± 0.2%) differences being insignificant (χ^2(2, N = 216) = 2.35, p = 0.310). Survival also did not differ by bag coatings, with grow-out survival percentages from coated bags (98.6 ± 0.2%) being statistically similar (χ^2(1, N = 216) < 0.01, p = 0.982) to uncoated bags (98.7 ± 0.2%). Reductions in stocks in SC and hurricane losses in NC meant that combined states' growth could not be analyzed for October.

Discussion

Efficacy of aerial drying and fouling-release coating treatment methods on oyster growth metrics varied by state; however, some consistent trends were evident. Higher drying frequencies seemed to have a negative relationship with SH during earlier growth periods. For example, December, March, and June had lower SH in weekly drying treatments in GA. Weekly drying oysters also grew at slower rates in December. In SC, triweekly drying oysters had higher SH and faster growth rates in March. Weekly drying SH was also lower than biweekly and triweekly drying in NC in March. With all state's data combined, SH was consistently lower in weekly drying treatments across all sampling periods. This may be attributed to oysters having short feeding times due to more frequent drying, as oysters with longer feeding times may show faster growth in colder months when fouling isn't as prevalent (Bishop and Peterson 2006). However, it appears that growth in weekly drying treatments were redirected towards other metrics. GA, SC, and NC weekly drying oysters had higher cup ratios in June with SC weekly drying also having higher fan ratios. Higher cup and fan ratios may mean that the differences could be caused by additional handling, which can break off new growth and encourage shell thickening (Stone et al. 2013). These higher cup ratios may not be a negative result as some growers find that high cup ratios imply high oyster quality (Brake et al. 2003). It is likely that the collisions among oysters within the bags and the additional tumbling from weekly bag flipping may have chipped oysters more frequently, ultimately influencing shell shape to be more desirable, which is congruent with other work evaluating suspended oysters (Manley et al. 2009; Mallet et al. 2013; Thomas et al. 2019).

Shell metric trends appeared to change as oysters continued to grow beyond the desirable harvest size of 76 mm. While GA biweekly and triweekly drying oysters trended to have higher SH in October, the differences among drying treatments were not evident after October. GA oysters also showed no difference among drying treatments when looking at cup and fan ratio. This may be because intense fouling caused cages to flip back over, altering drying frequencies and increasing fouling. The GA site was also adjacent to the sound, so wave action was likely greater due to direct energy input from the ocean. SC oyster shell metric trends also changed in October and SH no longer varied by drying treatment. However, October SC cup and fan ratios in biweekly and triweekly drying treatments were significantly higher than weekly drying ratios, which was a more drastic change than in GA. SC ratio trends may have been more noticeable because cage weight was reduced when many bags were restocked, meaning cages did not flip back over during drying treatments. Changes in cup and fan ratio trends may be a result of increased fouling presence or simply a change in morphological growth of oysters at a certain size.

Bag coating treatment had little effect on shell metrics, especially towards the end of the growth period. However, coated bags had a negative relationship with SH early in the first quarterly period. In GA, oysters grown in uncoated bags had higher SH in December and greater growth in December and March. Uncoated bag SH and growth was higher in SC in March as well. Coating treatments also seemed to affect cup and fan ratios, albeit in different ways depending on the location. SC fan ratios in uncoated bags were higher in December, but NC fan ratios in uncoated bags were lower. These effects may be influenced by sloughing of the coating, which occurred within the first 6 months and may have made the coating available for consumption by the cultured oysters. However, this result contrasts observations of coating effects on SH of other bivalves (Tettelbach et al. 2014). Without histological evidence and more extensive research, we cannot conclude that the coating had a direct negative effect on shell metrics. Uncoated bags also had higher SH in GA in October, long after the coating disappeared.

Also, differences between bag coatings were not evident when all states' data were combined, as differences were minor or non-existent for shell metrics among all sampling periods. This leads us to believe that there may be another factor influencing differences among bags.

Treatment effects on oyster quality varied among states, but there were identifiable similarities. Bag coating had no effect on any condition parameter among any state. There were no differences among treatments for CI in any state. However, when all states' data were combined, weekly drying oysters had higher CI. While CI is important, most growers tend to prefer oysters with higher WW and WT as this is what consumers can observe. All states and combined data demonstrated a negative relationship between high drying frequency and WW in June, with biweekly and triweekly drying providing greater weights than weekly drying. However, this effect disappeared by October, with no difference among treatments in GA and SC. WT was also affected by drying treatments, with SC, NC, and combined data demonstrating higher weights for biweekly and triweekly drying treatments in June. While GA had no differences among drying treatments or bag coatings for WT in June or October, biweekly and triweekly drying oysters had higher WW in June. SC oysters had higher WT and WW for biweekly and triweekly drying oysters in June, but those differences disappeared by October. High WW and WT may increase the marketability of an oyster. These observations suggest that reducing drying regimes may provide benefits early on, but not later in summer months when fouling is more prevalent. For a more complete assessment of drying treatments, effects of fouling should be considered as well.

In summary, initial differences in growth parameters among treatments were apparent early but subsided by the time oysters reached harvest size across all states. There were no interactions between drying and bag coating treatments, indicating one does not influence the effects of the other. Bag coating treatments had minimal effect on oyster growth and condition towards the end of the grow-out period while drying treatment effects were more substantial. Overall, biweekly and triweekly drying treatment oysters grown in uncoated bags had slightly better performance than weekly drying or coated bag oysters. By limiting handling of oysters, decreasing numbers of trips for management, and avoiding additional costs of a fouling-release coating, growers may be able to see higher economic returns upon harvest by employing biweekly or triweekly. In contrast, increased handling may result in shell thickening and higher cup ratios, increasing oyster aesthetics and marketability (Brake et al. 2003; Manley et al. 2009; Mallet et al. 2013; Stone et al. 2013; Thomas et al. 2019). As all treatments and sites produced high survival, the tradeoff between fast growth and oyster shell metrics is ultimately up to the grower. Each state showed varying performance that can be attributed to a site effect, which can significantly alter culture success, demonstrating the importance of determination of management techniques based on location and other environmental parameters (Mallet et al. 2009). To determine whether the methods tested in the present study would make an economic difference for growers, effects of the treatments on fouling must be evaluated as well. However, this study provides critical preliminary information for floating oyster cage management options that could improve oyster culture in the southeastern (US) Atlantic states.

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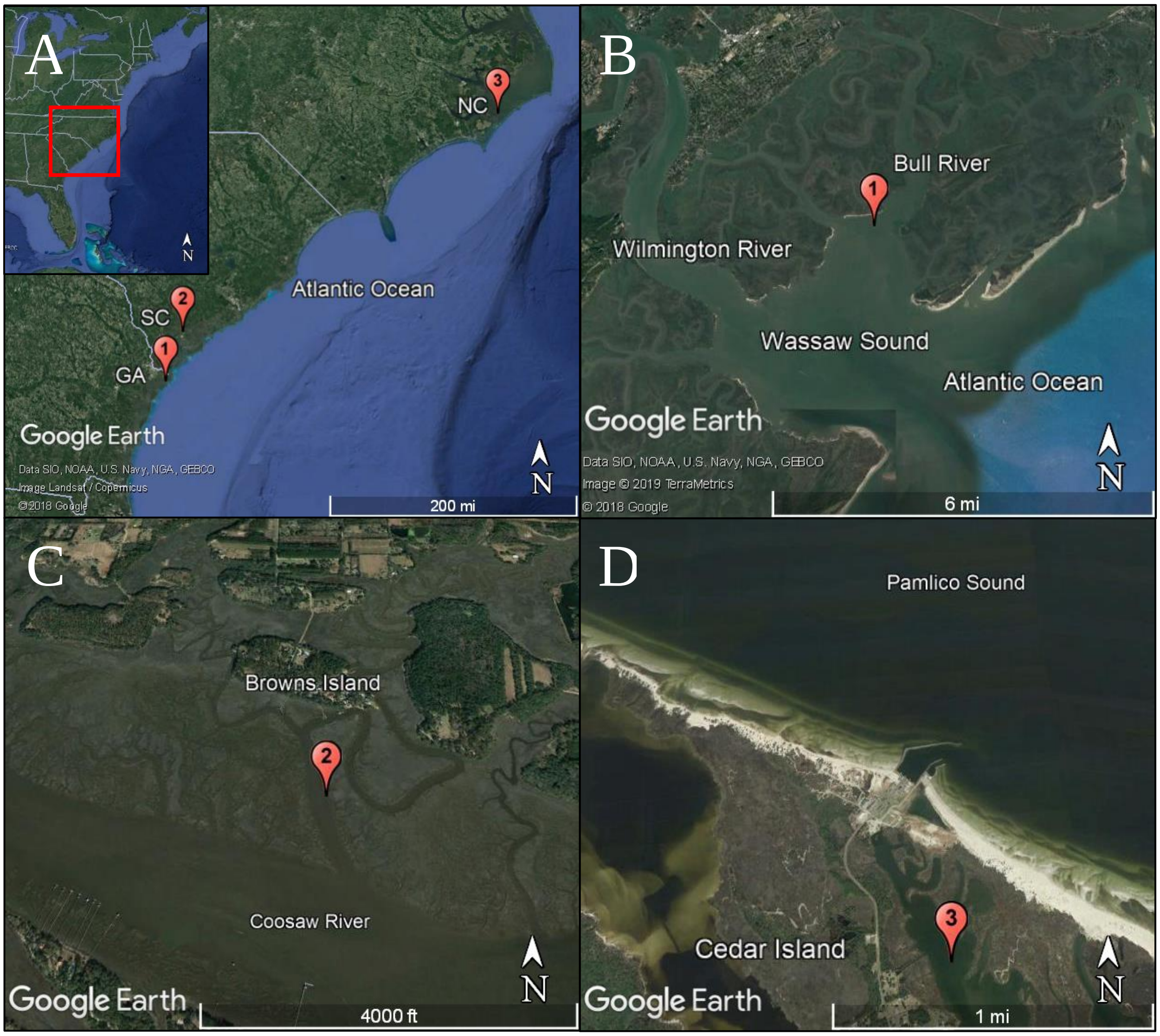

Figure 1.1: Oyster floating cage locations (A) in Georgia (B), South Carolina (C), and North Carolina (D). Pin 1 represents Georgia location and can be viewed closer in map B. Pin 2 represents South Carolina location can be viewed closer in map C. Pin 3 represent North Carolina location and can be viewed closer in map D. Images obtained through Google Earth.

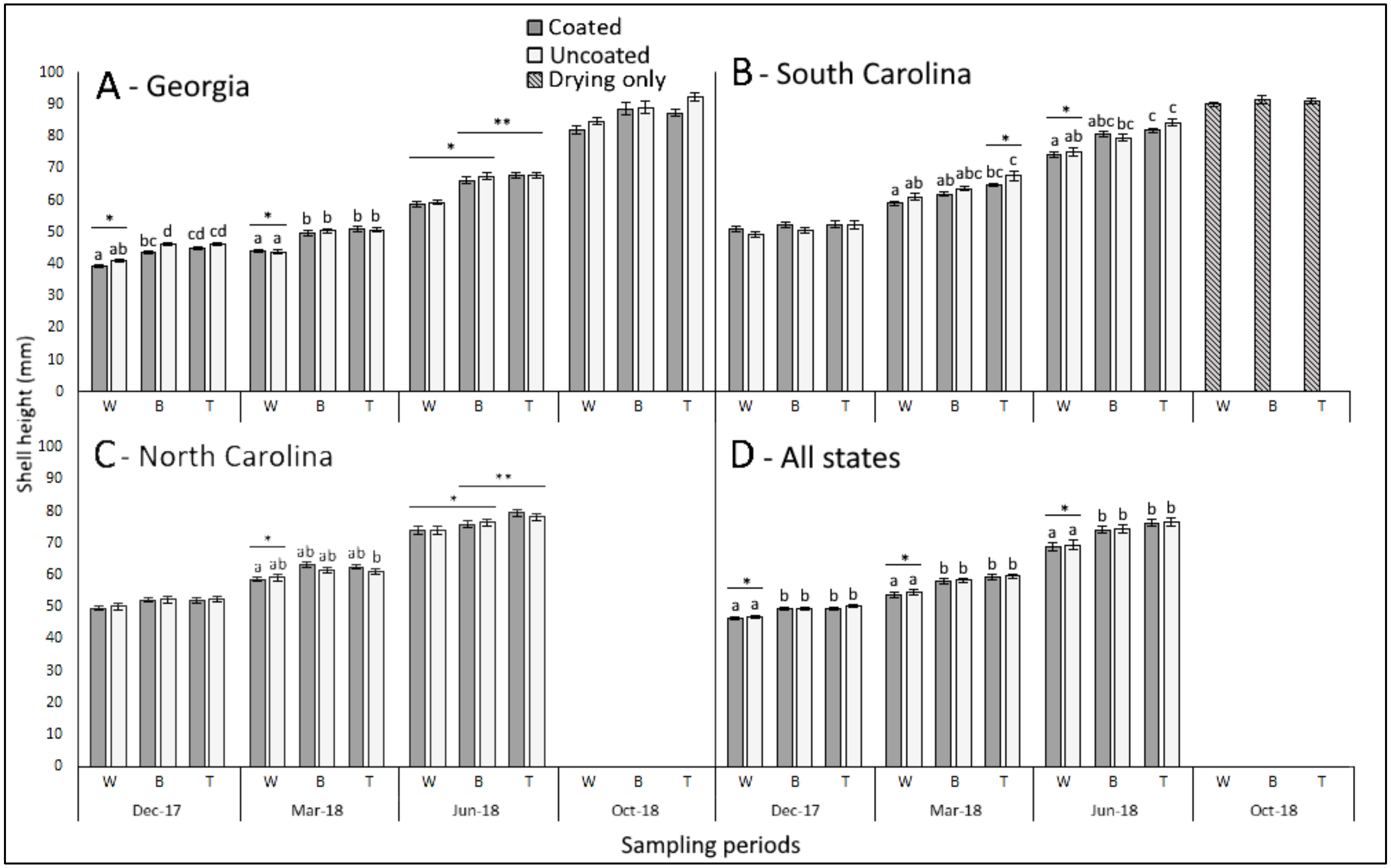

Figure 1.2: Oyster shell height means (± standard error) for oysters grown in floating cages in Georgia (A), South Carolina (B), North Carolina (C), and all states' data combined (D) under different drying (W = weekly, B = biweekly, T = Triweekly) and bag coating treatments (coated, uncoated) over four sampling periods. Initial oyster stocking occurred in October 2017. Error bars are standard error. Darker bars represent coated bags, lighter bars represent uncoated bags, and hatched bars represent bags where bag coating was not investigated. North Carolina bags were lost in the October season and no data can be presented. Figure 1.2D only shows December-June seasons because of changes in management practices and the loss of North Carolina oysters prior to October. Lowercase letters denote significant differences (p<0.05) among drying and bag treatments as main effects. Bars with asterisks represent differences among drying treatments only.

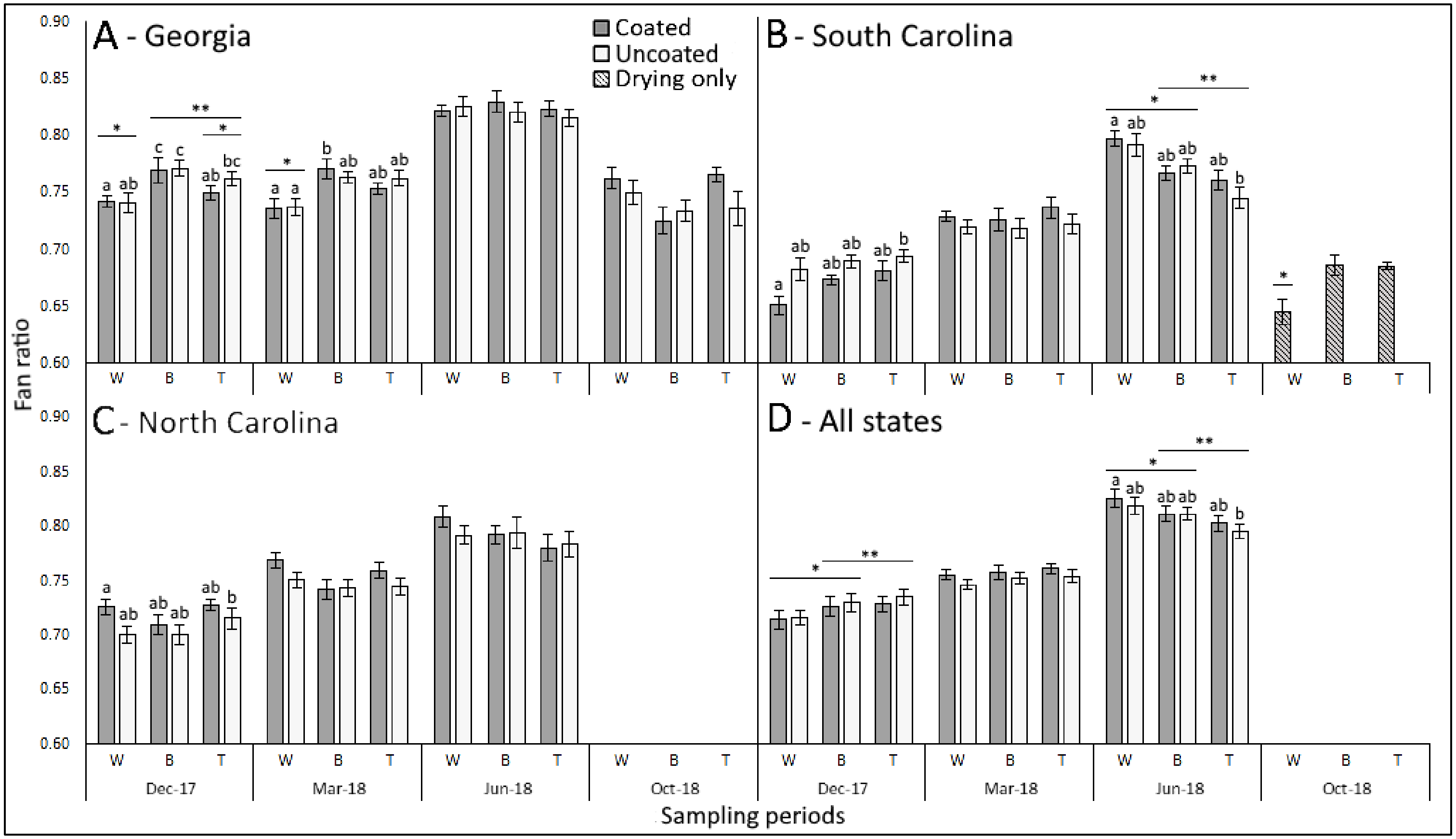

Figure 1.3: Fan ratio (shell width/shell height) means (± standard error) for oysters grown in floating cages in Georgia (A), South Carolina (B), North Carolina (C), and all states' data combined (D) under different drying (W = weekly, B = biweekly, T = Triweekly) and bag coating treatments (coated, uncoated) over four sampling periods. Initial oyster stocking occurred in October 2017. Error bars are standard error. Darker bars represent coated bags, lighter bars represent uncoated bags, and hatched bars represent bags where bag coating was not investigated. North Carolina bags were lost in the Oct season and no data can be presented. Figure 1.3D only shows December-June seasons because of changes in management practices and the loss of North Carolina oysters prior to October. Lowercase letters denote significant differences ($p<0.05$) among drying and bag treatments as main effects. Bars with asterisks represent differences among drying treatments only.

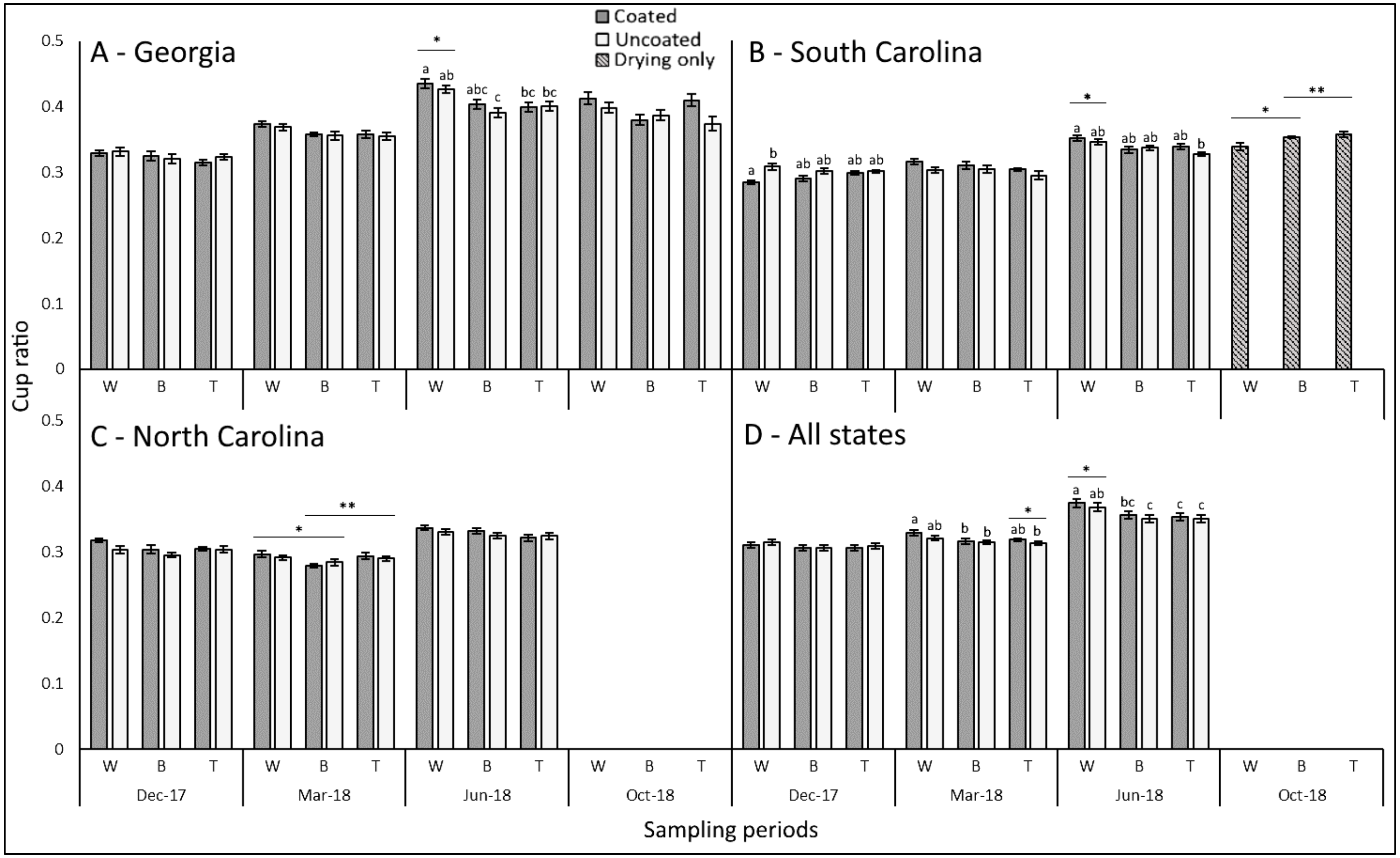

Figure 1.4: Cup ratio (shell width/shell height) means (± standard error) for oysters grown in floating cages in Georgia (A), South Carolina (B), North Carolina (C), and all states data combined (D) under different drying (W = weekly, B = biweekly, T = triweekly) and bag coating (coated, uncoated) treatments over four sampling periods. Initial oyster stocking occurred in October 2017. Error bars are standard error. Darker bars represent coated bags, lighter bars represent uncoated bags, and hatched bars represent bags where bag coating was not investigated. Figure 1.4D show December-June seasons because of changes in management practices and the loss of NC oysters prior to October. Lowercase letters denote significant differences ($p<0.05$) among drying and bag treatments as main effects. Bars with asterisks represent differences among drying treatments only.

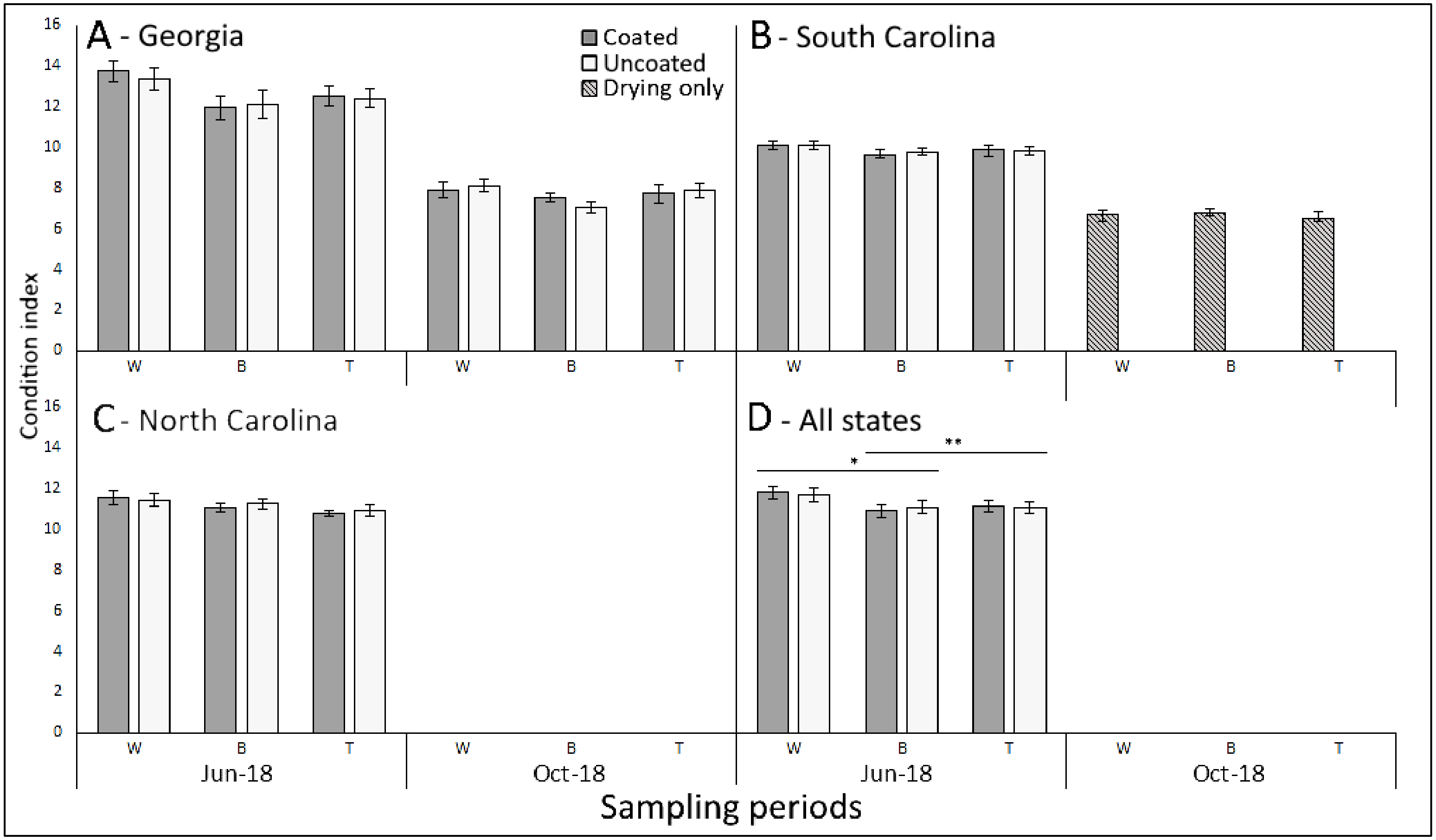

Figure 1.5: Condition indices means (± standard error) for oysters grown in floating cages in Georgia (A), South Carolina (B) North Carolina (C), and all states' data combined (D) under different drying (W = weekly, B = biweekly, T = triweekly) and bag coating (coated, uncoated) treatments over two sampling periods. Initial oyster stocking occurred in October 2017. Error bars are standard error. Darker bars represent coated bags, lighter bars represent uncoated bags, and hatched bars represent bags where bag coating was not investigated. Figure 1.5D shows June season only because of changes in management practices and the loss of NC oysters prior to October. Lowercase letters denote significant differences ($p<0.05$) among drying and bag treatments as whole effects. Bars with asterisks represent differences among drying treatments only.

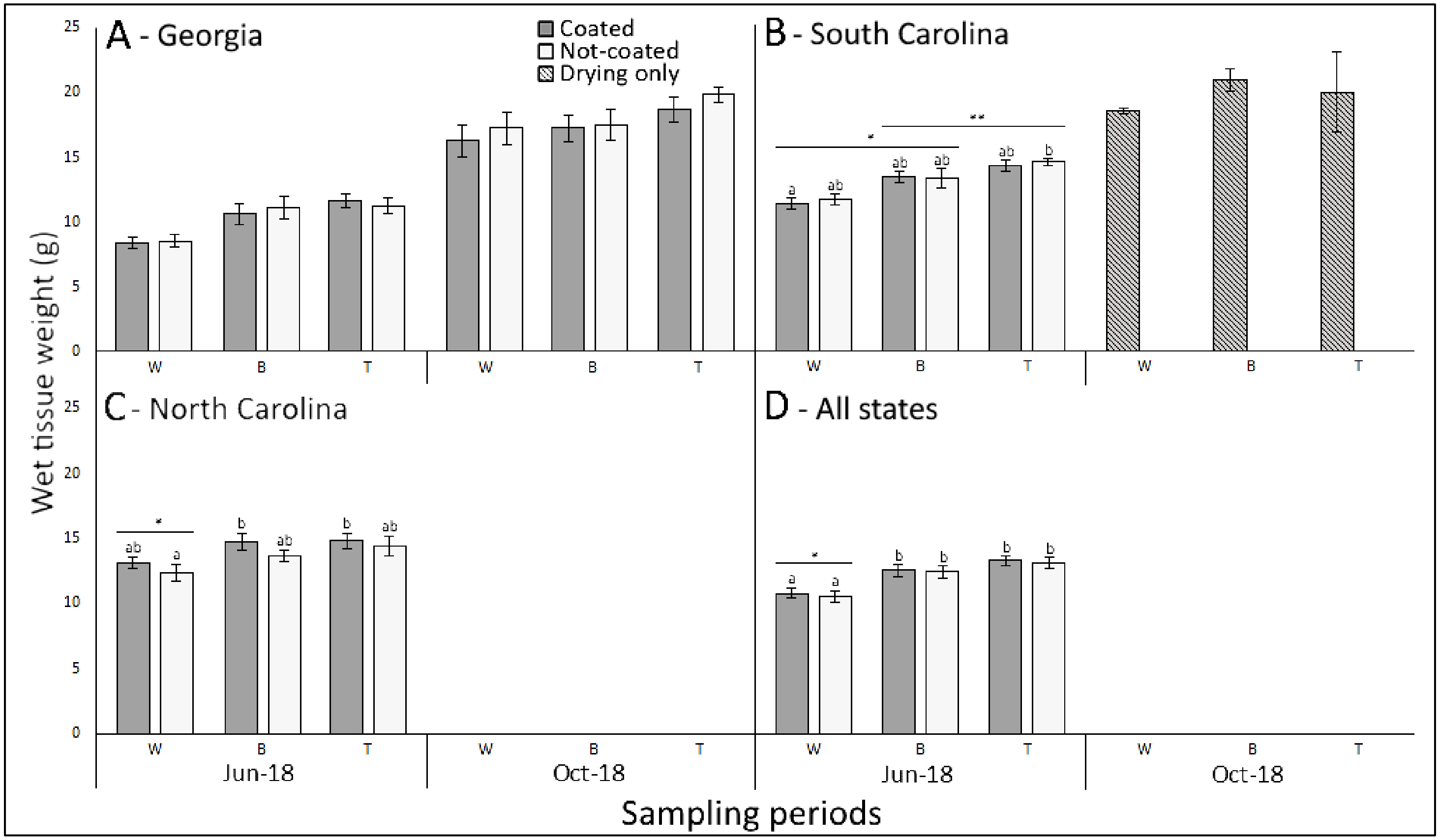

Figure 1.6: Wet tissue weight (g) means (± standard error) for oysters grown in floating cages in Georgia (A), South Carolina (B), North Carolina (C), and all states' data combined (D) under different drying (W = weekly, B = biweekly, T = triweekly) and bag coating (coated, uncoated) treatments over two sampling periods. Initial oyster stocking occurred in October 2017. Error bars are standard error. Darker bars represent coated bags, lighter bars represent uncoated bags, and hatched bars represent bags where bag coating was not investigated. Figure 1.6D shows June season only because of changes in management practices and the loss of NC oysters prior to October. Lowercase letters denote significant differences ($p<0.05$) among drying and bag treatments as main effects. Bars with asterisks represent differences among drying treatments only.

CHAPTER 3

FOULING

Introduction

Over the past several decades, many southeastern U.S. states have made efforts to restore their eastern oyster (*Crassostrea virginica*) industries. For example, notable recoveries in the southeast are occurring in North Carolina and South Carolina; in 2017 North Carolina reported a harvest value of $5.6 million, which is more than double that of 2012 (NCDMF 2018). South Carolina has also embraced growth in oyster culture, with the South Carolina Sea Grant oyster culture efforts contributing to 14 oyster farming businesses in 2018, making an estimated $2.3 million economic impact (Sea Grant, 2018). Georgia, which holds the record for highest U.S. landings in the early 1900s, opened its first shellfish hatchery in 2015, allowing the state to access similar technological advances used in other areas of the Southeastern U.S. (Harris, 1980). This advancement can be attributed to a reemerging demand for high quality single oysters served on the half-shell. While wild reef oysters tend to grow long and skinny in clusters, ideal single oysters have a deeper and wider shape (Brake et al. 2003). This more marketable shape can occur with controlled reproduction and early management. By spawning high volumes of oyster larvae and allowing them to settle on microcultch (finely ground oyster shell material) in an appropriately sized container, spat develop individually rather than clumped (Callam and Supan, 2018). Growing oysters independently reduces the amount of labor needed later to produce a single oyster and encourages shapely growth. These advancements and increased consumer demand have led to the development of oyster hatcheries specializing in single set oysters.

Proper grow-out gear and culture techniques are crucial for growing the highest quality oysters. Bottom-cages, despite common use, have disadvantages related to both mortality and growth rate. Use of this method increases the likelihood that oysters will experience issues like parasitism and predation (Moroney and Walker, 1999). Further, in areas with high sediment deposition rates, oysters in bottom cages are exposed to the problem of sediment burial as described above (Colden and Lipcius, 2015; Comeau et al., 2017; Moroney and Walker, 1999). These effects are especially apparent in instances where the larger left valve is buried and during quiescent stages experienced by oysters in lower-temperature areas, when oysters narrowly open their valves to flush out waste (Comeau et al., 2017). Use of bottom cages or table structures can also drastically increase sediment deposition, increasing the impact on surrounding benthic habitat and overall environmental footprint (Mallet et al., 2006).

Oyster farmers are gravitating towards suspended cage culture methods as more research emerges supporting claims of rapid growth and reduced mortality attributed to the benefits of suspension in plankton-rich surface waters (Adams et al., 1991; Manley et al., 2009; Moroney and Walker, 1999; Thomas et al., 2019; Walton et al., 2013). The South Carolina oyster aquaculture industry is embracing this method, with a 25% increase in suspended culture observed in 2016, 10 suspended culture lease permit applications submitted in 2017, and 14 farms operating in 2018 (Davis 2016, Davis 2017, Sea Grant 2018). These cages also make oysters more accessible to growers, decreasing the amount of time and energy spent on maintenance such as biofouling mitigation (Williamson et al., 2015). However, suspended oysters may be more prone to biofouling, an issue that can negatively affect growth, condition, and survival.

Suitable shellfish aquaculture conditions are congruent with many other invertebrate species and the benefits bivalves gain from being suspended in plankton-rich surface waters are mirrored by many fouling organisms, increasing the prevalence of biofouling (Carman et al., 2010). Macrofouling communities can consist of “hard” or “soft” fouling organisms and the former is more problematic. “Soft” fouling organisms are easier to remove and typically are not destructive when proper mitigation strategies are used. For example, tunicates, or more specifically ascidians, can quickly adhere to untreated aquaculture gear as suitable shellfish culture conditions are similar to their preferred habitat and gear can provide hard substrates needed for settlement (Carman et al., 2010). However, these organisms are easily removed through physical mitigation methods such as air-drying and freshwater sprays (Carman et al., 2010; Sievers et al., 2017). In contrast, “hard” fouling organisms, such as that by wild-type oysters and barnacles, are considered more detrimental to shellfish culture because hard fouling is not as easily mitigated through physical methods. The use of artificial substrates for bivalve culture can also increase the prevalence of invasive fouling organisms, as it weakens the competitive advantage of native species (Tyrrell and Byers, 2007).

In terms of shellfish culture specifically, biofouling results in unwanted invertebrate communities forming on gear and the cultured oysters themselves, and can cost oyster industries up to $300 million in damages or approximately 5-10% of production costs (Fitridge et al., 2012; Willemsen, 2005). Fouling organisms cause physical damage to gear, interfere with the mechanical function of bivalve shells, and compete with cultured bivalves for resources such as food and oxygen (Fitridge et al., 2012). If not addressed early on, these communities can grow to reduce flow to cultured oysters, increasing mortality and reducing commercial quality (Adams et al., 1991; Fitridge et al., 2012; Moroney and Walker, 1999). Higher incidences of fouling can be associated with decreased shell height, lower decreased growth rates, and lower dry tissue mass that may negate growth advantages provided by longer feeding times and increasing time to harvest (Bishop and Peterson, 2006; Sievers et al., 2017). Biofouling can also weigh down suspended culture gear, putting them at risk for sinking or loss, particularly during storms (Fitridge et al., 2012; Sala and Lucchetti, 2008).

A variety of culture techniques have been used to reduce the attachment of fouling organisms while maximizing caged oyster growth and each technique has advantages and disadvantages. A commonly used practice to reduce biofouling is aerial drying for 24 hours. Aerial drying is used with suspended oyster gear and creates an artificial extended low tide. Cultured oyster seed can survive drying mitigation methods while other organisms get stressed and die off, reducing fouling accumulation when this is performed even once during a drying season (Mallet et al., 2009). However, this method is not effective for reducing fouling by wild oysters and barnacles unless done during initial wild settlement (Adams et al., 1991). Aerial drying may also have negative effects on cultured oysters in terms of growth, shell shape, and mortality if not performed properly. For example, drying too frequently decreases the amount of time oysters can feed and could thereby reducing growth rates, especially in colder months when biofouling isn’t as prevalent (Bishop and Peterson, 2006).

Chemical options for biofouling control have also been assessed. Copper oxide based biocidal coatings are widely used in aquaculture, but this has resulted in elevated levels of copper in water and sediment surrounding the culture site (Willemsen, 2005). For this reason, the use of fouling-release coatings is increasing in popularity because they are available in peroxide-based, biodegradable formulas. Netminder®, a water-based silicone barrier coating, has been applied and assessed on lantern nets in scallop culture. The fouling-release coating has been suggested to reduce fouling on culture gear, but may increase fouling on bivalves themselves as a result of organisms bypassing hard cage substrates (Tettelbach et al., 2014).

The objective of this project is to gain a better understanding of the efficacy of aerial drying in combination with fouling-release coating treatment methods for reducing biofouling in three southeastern United States (Georgia, South Carolina, and North Carolina). We aimed to determine what combination of factors affected hard, soft, and total fouling accumulation on oysters and grow-out gear throughout seasonal growth periods. These objectives, in combination with subsequent extension work, can be used to enhance oyster grower knowledge and improve methodologies for producing high quality oysters in multiple regions of the southeastern US Atlantic coast.

Methods

Study area

This study occurred in three states; Georgia (GA), North Carolina (NC), and South Carolina (SC) (Figure 2.1A). The GA study site was in the protected Halfmoon River inshore artificial reef boundary in Wassaw sound, which opens directly to the Atlantic Ocean (Figure 2.1B). It resided at the confluence of the Bull and Wilmington rivers in waters approved for shellfish harvest and was adjacent to a commercial clam lease. While the suitability of this location for oyster aquaculture was previously unknown, we were limited in site selection to areas in which research permitting had been acquired. Salinity values and water temperatures were measured by a HOBO U24-002-C data logger that was attached with zip ties to a randomly selected cage. Data were recorded every 30 minutes from October 13, 2017 until May 18, 2018, but barnacle growth over the logger negated the accuracy of April and May data and those months were removed from analysis. Loggers were not removed during drying treatments, so all datapoints with salinities lower than 3 psu were removed from analysis. The SC site was on an oyster lease owned by Lady's Island Oyster Company off the Coosaw River near Brown's Island (Figure 2.1C). SC water quality data for 2018 were obtained by SC DNR at shellfish monitoring site 14-13. The North Carolina site was in Core Sound near Cedar Island on a shellfish lease owned by Carolina Mariculture Company (Figure 2.1D). All sites varied in tidal range, salinity, and wave action to allow for observations of treatment effects across different environmental parameters.

Experimental design

OysterGro® floating cage systems were acquired from BBI group (Bouctouche, New Brunswick, Canada), and 12 cages were managed at each site (one site per state). The cages were 1.52 m long, 0.91 m wide, and 0.15 m deep and each held 6 grow-out bags. A total of 72

Vexar (12 mm) grow-out bags were used per site. Half of the bags for each cage were treated with the fouling-release coating Netminder® (Gladwyne, Pennsylvania, U.S). The coating was applied using paint rollers with the assistance of a company representative on August 24, 2017. Bag coating treatments were considered coated (coated with Netminder®) or uncoated (not coated with Netminder®). All 12 cages per location were anchored on a single line with anchors between each three cages. Cages were approximately 1.8 meters apart and the total length of the line was approximately 38 meters. Cages were deployed in GA and NC on October 13, 2017 and in SC on October 15, 2017. The three drying treatments were weekly (once every week), biweekly (once every two weeks), or triweekly (once every three weeks). Biweekly drying was considered the control treatment, as most of the growers we collaborated with implemented this regime. Drying treatments were assigned to the first six cages on the anchor line with the pattern two weekly, two biweekly, and two triweekly. This pattern was repeated for the remaining six. This non-random pattern was required to make cage management easier for growers, who were managing multiple commercial cages as well as our research cages for the full growth period. Following a Latin Squares design, three randomly selected bags of each coating treatment were placed within each cage to account for error associated with bag placement. Bag orientations within the cages were not changed throughout the experiment.

Spawning/stocking

Triploid oysters were spawned June of 2017 at Lady's Island Oyster Hatchery (Seabrook, SC, U.S.) using a SC tetraploid x SC diploid cross through strip spawning. When the oysters reached an average height of 25mm, 10,800 were haphazardly selected for each site and stocked in mesh bags for deployment at weight estimated densities of 150/bag. Bags were randomly selected and transported to each site manager in early October 2017. Actual stocking densities were determined in October by accounting for mortalities, live oysters, and numbers of harvested oysters in each remaining bag (n = 14). Only 12 bags in SC and 2 bags in GA were available for stocking density counts because oysters were removed from all but one SC bag per cage and replaced with younger oysters after June sampling, NC oysters were lost to Hurricane Florence, and the majority of GA bags were too fouled to determine which mortalities were from the original stock. Because bags were randomly distributed, we used this count to estimate stocking density for all bags in the experiment. GA oysters were held in a floating upweller system (FLUPSY) next to the University of Georgia's Marine Extension Services building on Skidaway Island for one week prior to deployment. SC and NC oysters were deployed directly to their field locations. To determine starting measurements, 100 randomly selected oysters were measured on October 11, 2017 in GA using General 6" dial calipers for shell height (SH), length (SL), and width (SW) to nearest 0.1 mm using methods described by Galtsoff (1964). These measurements were used for SC and NC starting measurements as oysters were deployed less than a week after measurement. Each previously stocked mesh bag was randomly assigned a Vexar bag and oysters were transferred to that assigned Vexar bags before being deployed. Oyster stocking and bag deployment occurred within the same week that cages were anchored. Oysters grew in the same cage and bag for the entire growth period.

Bag fouling

Sampling periods occurred in December of 2017, and March, June, and October of 2018. Fouling accumulation was monitored at each site through photographic documentation of bags during each sampling period. Bags within and across all locations were photographed within a two-week period. During each sampling period, the side of each Vexar bag was photographed and a 16 cm ruler was included for scale. Each photograph was visually analyzed to quantify fouling as follows: three consecutive rows of five Vexar mesh holes, or a surface area of ~40 cm^2, were assigned percent coverage values for a total of 15 observations per picture. If there was no fouling obstruction of the hole, it was assigned a value of zero (0%) and if it was completely obstructed, it was be assigned a value of one (100%). Intermediate values were estimated to the nearest 0.1 (10%). If the mesh was entirely obstructed by fouling and mesh holes could not be identified, the bag was automatically assigned a value of one (100%). Percent fouling coverage for each bag was determined by taking the mean obstruction value of all 15 observations. Time and resource constraints resulted in only one researcher performing coverage analysis. Percent fouling coverage analysis was available from all bags at each site in December, March, and June (n = 72). After the June sampling period, oysters were removed from all but one SC bag per cage and replaced with younger oysters in compliance with the grower's preferences. Despite the change in stocks, SC bags remained in their original orientation through October to observe summer fouling. In GA, four bags were lost during hurricane Michael prior to October sampling, reducing the number of bags to 68. All bags and cages in NC were lost during hurricane Florence and thus not sampled in October. At the end of the experimental growth period, all remaining bags were emptied and weighed immediately using a fish scale to determine the overall wet weight of bag fouling and bag sides were photographed again. All bags were constructed using the same specifications, were randomly distributed between coating treatments, and no coating remained on the bags by October, allowing us to compare fouling without subtracting original bag weights. Growth was also monitored by during each sampling period as reported in chapter 2.

Oyster fouling

During June sampling, 10 randomly selected oysters from each bag in each state were harvested and frozen for later determination of fouling intensity (n = 72). In October, oysters were harvested and frozen, but in GA, 5 oysters per bag (n = 68) were processed and 10 oysters per bag (n = 12) were processed in SC. All June oyster weights were measured with a Sartorious CP124S balance scale and October weights with a Denver Instrument XE-410D scale to the nearest 0.01 g. Different scales were used because the intense fouling increased weights above the threshold of the Sartorious CP124S. Frozen oysters were rinsed gently with cold running water to remove excess sediment without removing loosely attached fouling, and then patted dry with a paper towel. Oysters were weighed to determine fouled wet weight (FWW). Hard fouling was scraped off and weighed to determine hard fouling weight (HFW). Soft fouling was then removed using running cold water and a wire brush over a sieve to catch any remaining hard fouling or new growth that broke off. Oysters were dried with a paper towel and weighed to obtain clean wet weight (CWW). Additional hard fouling that came off during the cleaning process was weighed and added to HFW and new growth was added to CWW. Oysters that gaped before or after cleaning were noted and removed from all analysis that required CWW. HFW and CWW were subtracted from FWW to determine soft fouling weight (SFW). All fouling weights were divided by CFW to determine total, hard, and soft fouling ratios. Common fouling organisms were also identified. Spat, barnacle, ascidian (*Mogula sp.*), mussel, and bryozoan (*Membranipora sp.*) presence was recorded as present (1) and absent (0).

Data analysis

Data were organized with Excel®2016. All data were analyzed with RStudio (RStudio Team 2003). Vexar bags within cages were treated as experimental units because these bags were the smallest unit to which treatments were randomized. Each oyster sampled within a bag was considered a subsample of the bag. Therefore, mean hard, soft, and total fouling ratios were calculated per bag. Mean percent presence of spat, barnacle, ascidian, and bryozoan were also calculated per bag by summing presence and dividing by number of sampled individuals.

Residual distributions were first checked using Shapiro-Wilks tests. If residuals were not normally distributed, appropriate transformations were used to normalize the data. GA and SC bag weights were normalized using a LOG transformation. All June GA fouling ratios were LOG transformed. June SC soft fouling ratios were log transformed. June NC total fouling ratios and soft fouling ratios were LOG transformed. All states (GA, SC, NC) June total fouling ratios and soft fouling ratios were LOG transformed. If transformation was unable to normalized data, we combined drying treatments and bag coating treatments to look at main effects using Kruskal-Wallis tests and Dunn's tests for differences. Percent fouling coverage of bags and fouling presence percentages could not be normalized with transformations and thus were assessed with Kruskal-Wallis and Dunn's tests. June SC hard and total fouling ratios also could not be normalized with transformations and were assessed with Kruskal-Wallis and Dunn's tests. June NC hard fouling ratios and combined states (GA, SC, NC) hard fouling ratios also could not be normalized and were assessed using Kruskal-Wallis and Dunn's tests. All bag weights, percent presence of fouling organisms on oysters, as well as hard, soft, and total oyster fouling ratios that had normal residual distribution before or after transformations were analyzed with a two-factor (aerial drying treatment x bag coating treatment) split-plot ANOVA to determine treatment interactions and to account for error among cages. Differences among drying treatments and among main effects of drying treatments with bag coating treatments were determined using Tukey's HSD tests.

All sampling periods were assessed separately to determine seasonal effects of treatments. Comparisons were made within states (GA, SC, NC) and with all states' data combined. No comparisons were made among states because of site variation. Treatment comparisons were made with all states' data combined for December, March, and June only. Data for combined comparisons were blocked by location. No combined comparisons were made for October because of the loss of NC oysters and the reduction in numbers of SC bags. One factor ANOVAs and Tukey's HSD tests were used for SC October analysis because stock reductions eliminated our ability to assess bag coating treatments. All results are presented as mean ± SE unless otherwise noted and $\alpha = 0.05$ for all tests.

Results

Study area

Water temperature at the GA site ranged from 9-28^{o}C with a mean of 17 ± 0^{o}C from October through December 2017. Salinity ranged from 23-29 psu with a mean of 27 ± 0 psu during the same period. From January through March 2018, water temperatures ranged from 2-22^{o}C with an average of 13 ± 0^{o}C. Salinity ranged from 13-28 psu with a mean of 24 ± 0 psu. Water temperature at the SC site ranged from 9-13^{o}C with a mean of 11 ± 1^{o}C from January through March 2018. Salinity ranged from 27-28 psu with a mean of 27 ± 0 psu. SC water temperatures ranged from 21-31^{o}C with a mean of 27 ± 2^{o}C from April to October 2018. Salinity ranged from 20-28 psu with a mean of 26 ± 1 psu. No temperature or salinity data were available for the NC site.

Spawning/stocking

Oysters were stocked at a mean SH of 30.9 ± 0.4 mm (n = 100). Not all bags were available for stocking density counts in October. NC bags were lost during Hurricane Florence, only one SC bag per cage remained with the originally stocked oysters, and most GA bags were too fouled to distinguish between originally stocked oyster mortalities and fouling oyster mortalities. Therefore, initial stocking densities were estimated based on the 12 SC bags and 2 GA bags that remained viable in October. We determined that 137 ± 1 oysters were stocked per bag. Mean oyster shell height in SC and NC reached a legal harvest size of ≥ 76.0 mm by the June sampling period, with SC averaging 79.0 ± 0.4 mm and NC averaging 76.3 ± 0.3 mm. GA oysters did not reach the 76 mm threshold by June, when shell height averaged 64.5 ± 0.3 mm. However, GA oysters were at the legal harvest size by October with an average shell height of 87.2 ± 0.7 mm (see chapter 2 for growth results).

Bag fouling

Georgia

Bag fouling percent coverage trends varied among sampling periods for GA and differences mainly resided among drying treatments (Figure 2.2A). In December, bag fouling coverage in GA among weekly drying (1.5 ± 0.3%), biweekly drying (18.3 ± 3.1%), and triweekly drying (7.5 ± 0.8%) all differed significantly (χ^2 (2, N = 72) = 42.69, p < 0.001), with the lowest fouling coverage on bags dried weekly. Coated bags (9.5 ± 2.0%) and uncoated bags (8.6 ± 1.8%) did not differ significantly (χ^2 (1, N = 72) < 0.01, p = 0.978) in fouling coverage in December. By March, GA bag fouling coverage did not differ significantly (χ^2 (2, N = 72) = 1.91, p = 0.384) among weekly drying (27.4 ± 6.1%), biweekly drying (11.3 ± 1.6%), and triweekly drying (20.6 ± 1.6%). Coated bags (19.5 ± 3.8%) and uncoated bags (20.0 ± 3.6%) again did not differ significantly (χ^2 (1, N = 72) = 0.39, p = 0.534). In June, fouling coverage in GA was significantly different (χ^2 (2, N = 72) = 31.77, p < 0.001) among weekly drying (20.0 ± 4.6%), biweekly drying (35.4 ± 5.2%), and triweekly drying (66.3 ± 4.9%) with the lowest coverage on weekly bags. Again, coated bags (43.3 ± 5.1%) and uncoated bags (37.9 ± 5.1%) did not differ significantly (χ^2 (1, N = 72) = 0.78, p = 0.377).

Bag fouling coverage was greatest in October in GA, but weekly drying (35.0 ± 3.4%), biweekly drying (40.7 ± 4.7%), and triweekly drying (36.6 ± 4.7%) did not differ significantly (χ^2 (2, N = 68) = 0.37, p = 0.830). There was no difference (χ^2 (1, N = 68) = 2.69, p = 0.101) between coated bags (41.8 ± 3.6%) and uncoated bags (33.4 ± 3.2%). While GA triweekly bags (4.2 ± 0.5 kg) trended higher in weight, the difference was not significantly (N = 69, $F_{2, 66}$ = 1.76, p = 0.226) from weekly (3.0 ± 0.2 kg) or biweekly drying (4.0 ± 0.3 kg) (Figure 2.3A). GA coated bags (3.8 ± 0.2 kg) and uncoated bags (3.5 ± 0.3 kg) also showed no significant differences (N = 69, $F_{1, 67}$ = 1.57, p = 0.241) (Figure 2.3A).

<u>South Carolina</u>

In SC, bag fouling coverage trends varied among sampling periods and only differed among drying treatments (Figure 2.2B). Drying treatments in SC varied in December, with significantly lower (χ^2 (2, N = 72) = 47.64, p < 0.001) fouling coverage percentages on weekly (0.2 ± 0.2%) and biweekly bags (0.9 ± 0.4%) compared to triweekly drying (7.1 ± 0.9%). Coated bags (2.4 ± 0.7%) and uncoated bags (3.1 ± 0.7%) did not differ significantly (χ^2 (1, N = 72) = 0.26, p = 0.612) in fouling coverage in December. March fouling coverage in SC trends were like December, as weekly (0.2 ± 0.1%) and biweekly drying (0.5 ± 0.2%) was again significantly lower (χ^2 (2, N = 72) = 49.32, p < 0.001) than triweekly drying (19.0 ± 4.8%). Fouling coverage on coated bags (4.9 ± 2.1%) and uncoated bags (8.4 ± 3.2%) again did not differ significantly (χ^2 (1, N = 72) < 0.01, p = 0.990) in March. Bag fouling in SC was highest in June, but weekly drying (82.0 ± 4.3%), biweekly drying (69.8 ± 6.3%), and triweekly drying (67.0 ± 6.2%) did not differ significantly (χ^2 (2, N = 72) = 2.90, p = 0.235). For bag coating in June, there was no significant difference (χ^2 (1, N = 72) = 0.02, p = 0.875) between coated (71.9 ± 5.0%) and uncoated bags (73.9 ± 4.5%).

Percent fouling coverage in SC in October trended higher in triweekly drying treatments triweekly (49.8 ± 5.2%), did not differ (χ^2 (2, N = 72) = 5.89, p = 0.053) among weekly (33.5 ± 4.1%) and biweekly (36.9 ± 4.3%) treatments. There were no significant differences (χ^2 (1, N = 72) = 0.35, p = 0.554) between coated bags (38.7 ± 4.0%) and uncoated bags (41.4 ± 3.7%) in October. Final bag weights in SC were lowest for biweekly drying (2.2 ± 0.1 kg), but did not differ significantly (N = 72, $F_{2, 69}$ = 2.50, p = 0.137) from weekly (2.5 ± 0.1 kg) and triweekly drying (2.7 ± 0.1 kg) (Figure 2.3B). There were also no significant differences (N = 72, $F_{1, 70}$ = 2.34, p = 0.160) between coated bags (2.4 ± 0.1 kg) and uncoated bags (2.5 ± 0.1 kg) for October bag weights.

North Carolina

In NC, bag fouling coverage differed by both drying treatment and bag coating treatment (Figure 2.2C). In December, percent fouling coverage in NC on weekly drying bags (0.5 ± 0.2%) was significantly lower (χ^2 (2, N = 72) = 27.92, p < 0.001) than biweekly (5.0 ± 0.9%) and triweekly drying (4.0 ± 0.7%). Bag coating treatments also differed in December, and fouling coverage on uncoated bags (4.4 ± 0.7%) was significantly higher (χ^2 (1, N = 72) = 5.47, p = 0.019) than on coated bags (1.9 ± 0.4%). By March, drying treatment effects subsided in NC, with weekly drying (27.8 ± 6.4%), biweekly drying (19.1 ± 4.5%), and triweekly drying (20.8 ±

4.0%) resulting in similar fouling coverage (χ^2 (2, N = 72) = 0.24, p = 0.888). Coated bags (18.2 ± 3.9%) and uncoated (26.2 ± 4.1%) also did not differ significantly (χ^2 (1, N = 72) = 1.53, p = 0.216) in March. Bag fouling coverage decreased in NC by June, although biweekly (6.2 ± 0.3%) and triweekly drying (6.2 ± 0.4%) showed significantly lower fouling coverage (χ^2 (2, N = 72) = 11.96, p = 0.003) than weekly drying (8.7 ± 0.9%). October fouling coverage did not vary among bag coatings, with coated (6.7 ± 0.3%) and uncoated bags (7.2 ± 0.6%) showing no significant differences (χ^2 (2, N = 72) = 0.39, p = 0.534).

<u>All states</u>

With all states combined (GA, SC, NC), fouling coverage only varied among drying treatments, and differences were not apparent in each season (Figure 2.2D). For all states' data in December, percent fouling coverage on weekly drying bags (0.7 ± 0.1%) was significantly lower (χ^2 (2, N = 216) = 74.36, p < 0.001) than biweekly (8.1 ± 1.4%) and triweekly drying (6.2 ± 0.5%). Fouling coverage on coated bags (4.6 ± 0.8%) and uncoated bags (5.3 ± 0.7%) did not differ significantly (χ^2 (1, N = 216) = 2.84, p = 0.092) in December. By March with all states' data combined, fouling coverage for weekly (18.2 ± 3.3%) and biweekly drying (10.4 ± 1.8%) was significantly lower (χ^2 (2, N = 216) = 15.00, p = 0.001) than triweekly drying (20.1 ± 2.5%). Coated bags (14.1 ± 2.0%) and uncoated bags (18.4 ± 2.2%) again did not vary (χ^2 (1, N = 216) = 1.51, p = 0.219) for fouling coverage in March. All states' data in June showed that bag fouling coverage trended lower with weekly (36.9 ± 4.4%) and biweekly drying (37.1 ± 4.1%) when compared to triweekly drying (46.5 ± 4.3%), but no treatments differed significantly (χ^2 (2, N =

216) = 1.64, p = 0.439). June percent fouling coverage on coated bags (41.0 ± 3.5%) and uncoated bags (39.4 ± 3.4%) again did not differ significantly (χ^2 (1, N = 216) = 0.20, p = 0.651).

Oyster fouling

<u>Georgia</u>

Ratios of hard, soft, and total fouling were small in June (<0.05) and there were no differences among any fouling treatments (Table 2.1). Total fouling ratios for weekly drying (0.02 ± 0.00), biweekly drying (0.01 ± 0.00), and triweekly drying treatments (0.01 ± 0.00) did not differ in June (N = 72, $F_{2, 69}$ = 1.11, p = 0.371). Coated bags (0.01 ± 0.00) and uncoated bags (0.01 ± 0.00) also did not differ significantly (N = 72, $F_{1, 70}$ = 0.26, p = 0.620) for total fouling ratios in June. GA hard fouling ratios in June also showed little variation, with weekly drying (0.01 ± 0.00), biweekly drying (0.01 ± 0.00), and triweekly drying (0.01 ± 0.00) not differing significantly (N = 69, $F_{2, 66}$ = 1.04, p = 0.394). Hard fouling ratios in coated bags (0.01 ± 0.00) and uncoated bags (0.01 ± 0.00) did not differ (N = 69, $F_{1, 67}$ = 0.16, p = 0.697) in June. GA soft fouling ratios in June followed the same trend, as weekly drying (0.01 ± 0.00), biweekly drying (0.01 ± 0.00), and triweekly drying (0.01 ± 0.00) were statistically similar (N = 69, $F_{2, 66}$ = 1.52, p = 0.270). Coated bags (0.01 ± 0.00) and uncoated bags (0.01 ± 0.00) also did not vary significantly (N = 69, $F_{1, 67}$ = 0.87, p = 0.376) for soft fouling ratio in June.

Wild oyster spat, barnacles, and bryozoans were all present on the cultured GA oysters in June. There were no differences in percent occurrence for spat or barnacles among drying treatments, but bryozoan occurrence differed (Table 2.2). Spat percent occurrence among weekly drying (86.7 ± 4.8%), biweekly drying (85.7 ± 3.8), and triweekly drying (84.1 ± 5.3%) did not differ significantly (χ^2 (2, N = 69) = 1.71, p = 0.426). Spat occurrence trended lower in uncoated bags (82.1 ± 4.4%) but did not differ significantly (χ^2 (1, N = 69) = 0.89, p = 0.344) from coated bags (88.9 ± 2.9%). Barnacle occurrence did not differ significantly (χ^2 (2, N = 69) = 2.22, p = 0.329) among weekly (47.5 ± 4.3%), biweekly (38.3 ± 4.9%), or triweekly drying treatments (42.3 ± 7.1%). Uncoated bags (45.6 ± 4.8%) and coated bags (40.0 ± 4.1%) did not vary (χ^2 (1, N = 69) = 0.72, p = 0.398) for barnacle occurrence in June. June bryozoan percent occurrence varied among drying treatments, as presence in triweekly drying treatments (27.5 ± 7.5%) was significantly higher (χ^2 (2, N = 69) = 7.63, p = 0.022) than weekly drying (5.0 ± 2.3%), but neither differed from biweekly drying (16.7 ± 7.6%). There was also a significant difference in June bryozoan presence among bag coating treatments, and occurrence in coated bags (8.0± 3.0%) was lower (χ^2 (1, N = 69) = 4.16, p = 0.041) than uncoated bags (23.6 ± 6.5%).

Fouling in GA increased by October, but there were still no differences among any treatments for any fouling ratios (Table 2.1). Total fouling ratios trended lower in weekly (0.68 ± 0.08) and triweekly drying (0.62 ± 0.10), but neither differed significantly (N = 66, $F_{2, 63}$ = 0.39, p = 0.686) from biweekly drying (0.79 ± 0.12). While October total fouling ratios trended lower in uncoated bags (0.62 ± 0.08), it did not differ significantly (N = 66, $F_{1, 64}$ = 3.30, p = 0.103) from coated bags (0.79 ± 0.08). October hard fouling ratios trended lower in weekly (0.67 ± 0.08) and triweekly drying (0.61 ± 0.10), but again did not differ significantly (N = 66, $F_{2, 63}$ = 0.40, p = 0.683) from biweekly drying (0.77 ± 0.11). October hard fouling ratios for uncoated bags (0.61 ± 0.08) trended lower but did not differ significantly (N = 66, $F_{1, 64}$ = 3.30, p = 0.103) from coated bags (0.77 ± 0.08). October soft fouling ratios did not vary significantly (N = 66, $F_{2, 63}$ = 1.19, p =

0.347) among weekly (0.02 ± 0.00), biweekly (0.02 ± 0.00), and triweekly drying (0.02 ± 0.00). Soft fouling ratio for uncoated bags (0.02 ± 0.00) trended lower, but did not differ significantly (N = 66, $F_{1, 64}$ = 3.25, p = 0.105) from coated bags (0.02 ± 0.00) in October.

Wild oyster spat, barnacles, ascidians, mussels, and bryozoans were all present in GA in October (Table 2.2). Spat occurrence did not differ significantly (χ^2 (2, N = 68) = 4.13, p = 0.13) among weekly drying (96.7 ± 1.6%), biweekly drying (100.0 ± 0.0%), and triweekly drying (98.0 ± 1.4%). October spat occurrences did not vary among bag coatings, as uncoated (98.2 ± 1.0%) and coated bags (98.2 ± 1.0%) did not differ (χ^2 (1, N = 68) = 0.00, p = 1.000). Barnacle occurrence mirrored spat occurrence in October, as weekly (96.7 ± 1.6%), biweekly (100.0 ± 0.0%), and triweekly drying (98.0 ± 1.4%) were similar (χ^2 (2, N = 68) = 4.13, p = 0.127). Barnacle occurrence for coated bags (98.2 ± 1.0%) and uncoated bags (98.2 ± 1.0%) did not differ significantly (χ^2 (1, N = 68) = 0.00, p = 1.000) in October. Ascidian occurrence varied by drying treatment, as biweekly (2.5 ± 1.8%) and triweekly drying (0.0 ± 0.0%) percent occurrence was lower (χ^2 (2, N = 68) = 12.88, p = 0.002) than weekly drying (13.3 ± 4.1%). Occurrence of ascidians on coated bags (8.2 ± 2.9%) and uncoated bags (2.9 ± 1.7%) in October did not differ significantly (χ^2 (1, N = 68) = 2.65, p = 0.104). October mussel percent occurrence among weekly drying (53.3 ± 6.0%), biweekly drying (54.2 ± 5.0%), and triweekly drying (51.0 ± 6.1%) did not differ significantly (χ^2 (2, N = 68) = 0.14, p = 0.930). Coated bags (56.5 ± 4.4%) and uncoated bags (49.4 ± 4.7%) did not vary significantly (χ^2 (1, N = 68) = 1.38, p = 0.240) for October mussel occurrence percentages. Bryozoan percent occurrence in October was like June, as weekly drying (1.7 ± 1.7%) was significantly lower (χ^2 (2, N = 68) = 8.18, p = 0.017) than triweekly drying (15.0 ± 5.6%), but neither varied from biweekly drying (6.7 ± 3.1%). However, there were no differences (χ^2 (1, N = 68) = 0.33, p = 0.566) between coated bags (7.1 ± 3.4%) and uncoated bags (7.6 ± 2.7%) for mussel occurrence in October.

South Carolina

Fouling in SC in June was minimal (≤0.01) and fouling ratios did not vary among coating and drying treatments (Table 2.3). June total fouling ratio among weekly (0.01 ± 0.00), biweekly (0.02 ± 0.00), and triweekly drying (0.01 ± 0.00) did not differ significantly (χ^2 (2, N = 69) = 1.31, p = 0.520). Bag coating total fouling ratios in June for coated bags (0.01 ± 0.00) and uncoated bags (0.01 ± 0.00) did not vary (χ^2 (1, N = 69) = 0.08, p = 0.783). June hard fouling ratios were not different (χ^2 (2, N = 69) = 4.66, p = 0.097) among weekly (0.00 ± 0.00), biweekly (0.00 ± 0.00), and triweekly drying (0.00 ± 0.00). There were no significant differences (χ^2 (1, N = 69) = 0.07, p = 0.789) between coated (0.00 ± 0.00) and uncoated bags (0.00 ± 0.00) for hard fouling ratios in June. June soft fouling ratios in weekly drying (0.01 ± 0.00), biweekly drying (0.01 ± 0.00), and triweekly drying treatments (0.01 ± 0.00) did not differ significantly (N = 69, $F_{2, 66}$ = 0.06, p = 0.939). Soft fouling ratios in June did not differ significantly between coated (0.01 ± 0.00) and uncoated bags (0.01 ± 0.00).

Wild oyster spat, barnacles, ascidians, and bryozoans were present on cultured SC oysters in June (Table 2.4). Spat percent presence did not differ significantly (χ^2 (2, N = 72) = 0.18, p = 0.913) among weekly drying (2.9 ± 1.8%), biweekly drying (1.7 ± 1.0%), and triweekly drying (1.7 ± 0.8%). Spat presence for coated bags (2.5 ± 0.8%) and uncoated bags (1.7 ± 1.2%) also did not differ significantly (χ^2 (1, N = 72) = 2.46, p = 0.117). However, there was a difference among drying treatments for barnacle percent presence, as weekly (2.1 ± 1.0%) and biweekly drying (2.1 ± 0.8%) presence was significantly lower (χ^2 (2, N = 72) = 8.76, p = 0.013) than triweekly drying (7.5 ± 1.0%). June barnacle percent presence did not differ significantly (χ^2 (1, N = 72) = 0.52, p = 0.471) between coated (3.3 ± 1.1%) and uncoated bags (4.4 ± 1.2%). Ascidian percent presence in June also did not vary significantly (χ^2 (2, N = 72) = 0.44, p = 0.804) among weekly (64.2 ± 4.3%), biweekly (64.6 ± 5.5%), and triweekly drying (66.7 ± 5.5%). June ascidian presence for coated bags (64.7 ± 4.4%) did not differ significantly (χ^2 (1, N = 72) < 0.01, p = 0.964) from uncoated bags (65.6 ± 4.0%). Bryozoan percent presence in June did not vary among drying treatments, as weekly (1.6 ± 1.3%), biweekly (0.4 ± 0.4%), and triweekly drying (0.8 ± 0.8%) were similar (χ^2 (2, N = 71) = 0.51, p = 0.775). Bryozoan presence for coated bags (0.8 ± 0.6%) did not differ significantly (χ^2 (1, N = 71) < 0.01, p = 0.966) from uncoated bags (1.1 ± 0.9%) in June.

We only assessed drying treatments for SC in October and there was little variation in fouling ratios among drying treatments (Table 2.3). October total fouling ratios trended lower for weekly (0.10 ± 0.03) and biweekly drying (0.10 ± 0.02) but did not differ significantly (N = 12, $F_{2, 9}$ = 0.15, p = 0.864) from triweekly drying (0.12 ± 0.02). October hard fouling ratios also trended lower for weekly (0.08 ± 0.02) and biweekly drying (0.09 ± 0.02), but neither differed significantly (N = 12, $F_{2, 9}$ = 0.14, p = 0.870) from triweekly drying (0.10 ± 0.02). October soft fouling ratios also did not vary among drying treatments, as weekly drying (0.02 ± 0.00), biweekly drying (0.02 ± 0.00), and triweekly drying (0.02 ± 0.00) did not differ significantly (N = 12, $F_{2, 9}$ = 0.22, p = 0.805).

Wild oyster spat, barnacles, ascidians, bryozoans, and mussels were all present on SC oysters in October and only drying treatment effects were assessed (Table 2.4). October spat percent presence for weekly drying (97.5 ± 2.5%), biweekly drying (100 ± 0.0%), and triweekly drying (100 ± 0.0%) did not differ significantly (χ^2 (2, *N* = 12) = 2.00, *p* = 0.368). October barnacle percent presence also did not vary by drying treatment, as weekly drying (97.5 ± 2.5%), biweekly drying (100 ± 0.0%), and triweekly drying (100 ± 0.0%) were similar (χ^2 (2, *N* = 12) = 2.00, *p* = 0.368). October ascidian presence trended lower for biweekly (25.0 ± 5.0%) and triweekly drying (22.5 ± 10.3%) but neither differed significantly (χ^2 (2, *N* = 12) = 2.61, *p* = 0.272) from weekly drying (65.0 ± 19.4%). October percent bryozoan presence was lowest for biweekly drying (0.0 ± 0.0%) but did vary significantly (χ^2 (2, *N* = 12) = 1.10, *p* = 0.577) from weekly drying (2.5 ± 2.5%) or triweekly drying (2.5 ± 2.5%). Mussels were only present in one weekly drying treatment oyster bag (5.0 ± 5.0%) in October and presence did not differ significantly (χ^2 (2, *N* = 12) = 2.00, *p* = 0.368) from biweekly (0.0 ± 0.0%) or triweekly (0.0 ± 0.0%).

<u>North Carolina</u>

Fouling in NC in June was minimal (≤0.02) and fouling ratios only differed among drying treatments (Table 2.5). June total fouling ratios did not differ significantly (*N* = 59, $F_{2, 56}$ = 1.72, *p* = 0.234) among weekly drying (0.02 ± 0.00), biweekly drying (0.01 ± 0.00), and triweekly drying (0.02 ± 0.00). Total fouling ratios in June in coated bags (0.02 ± 0.00) and uncoated bags (0.01 ± 0.00) also did not vary significantly (*N* = 59, $F_{1, 57}$ = 0.77, *p* = 0.403). However, June hard fouling ratios differed, as triweekly drying (0.00 ± 0.00) differed significantly (χ^2 (2, *N* = 59) = 6.52, *p* =

0.038) from biweekly (0.00 ± 0.00) but neither varied from weekly (0.00 ± 0.00), although the differences were beyond reportable significant digits. Hard fouling ratios for coated bags (0.00 ± 0.00) and uncoated bags (0.00 ± 0.00) did not differ significantly (χ^2 (2, N = 59) = 0.27, p = 0.606) in June. June soft fouling ratios for weekly drying (0.01 ± 0.00), biweekly drying (0.01 ± 0.00), and triweekly drying (0.01 ± 0.00) did not vary significantly (N = 59, $F_{2, 56}$ = 0.30, p = 0.748). June soft fouling ratios for coated bags (0.01 ± 0.00) uncoated bag (0.01 ± 0.00) also did not differ significantly (N = 59, $F_{1, 57}$ = 1.21, p = 0.299).

In June, fouling presence on cultured oysters consisted of wild oyster spat, barnacles, and bryozoans only (Table 2.6). There were no differences among drying treatments for spat percent presence, as weekly drying (0.4 ± 0.4%), biweekly drying (0.4 ± 0.4%), and triweekly drying (0.8 ± 0.6%) differed insignificantly (χ^2 (2, N = 72) = 0.52, p = 0.770). Spat percent presence for coated bags (0.6 ± 0.4%) did not vary significantly (χ^2 (1, N = 72) = 0, p = 1) from uncoated bags (0.6 ± 0.4%). Barnacle percent presence trended lower in June for weekly drying (52.9 ± 4.8%) and biweekly drying (50.4 ± 5.6%), but neither differed significantly (χ^2 (2, N = 72) = 0.37, p = 0.833) from triweekly drying (55.4 ± 4.5%). Coated bags (53.9 ± 4.6%) and uncoated bags (51.9 ± 3.4%) barnacle percent presence was also similar (χ^2 (2, N = 72) = 0.12, p = 0.733) in June. There was variation among drying treatments for June bryozoan percent presence, as weekly (6.2 ± 3.2%) was significantly lower (χ^2 (2, N = 57) = 7.08, p = 0.029) than biweekly drying (16.3 ± 4.4%) but neither differed from triweekly (14.0 ± 4.4%). June bryozoan percent presence in coated bags (9.3 ± 2.6%) and uncoated bags (14.0 ± 3.6%) did not vary significantly (χ^2 (1, N = 57) = 0.769, p = 0.380).

All states

With all states' (GA, SC, NC) data combined, June fouling was low (≤0.02) overall (Table 2.7). Total fouling ratios in June did not vary among drying treatments, with no significant differences (N = 200, $F_{2,\,197}$ = 0.31, p = 0.735) among weekly drying (0.01 ± 0.00), biweekly drying (0.01 ± 0.00), and triweekly drying (0.01 ± 0.00). June total fouling ratios in coated bags (0.01 ± 0.00) did not differ significantly (N = 200, $F_{1,\,198}$ = 0.66, p = 0.421) from uncoated bags (0.01 ± 0.00). June hard fouling ratios did not vary (χ^2 (2, N = 200) = 2.88, p = 0.237) among weekly (0.00 ± 0.00), biweekly (0.00 ± 0.00), and triweekly drying (0.00 ± 0.00). Coated bags (0.0 ± 0.00) and uncoated bags (0.00 ± 0.00) were also similar (χ^2 (1, N = 200) = 0.46, p = 0.497) in June for hard fouling ratios. June soft fouling ratios did not differ significantly (N = 200, $F_{2,\,197}$ = 0.19, p = 0.828) among weekly (0.01 ± 0.00), biweekly (0.01 ± 0.00), and triweekly drying (0.01 ± 0.00). June soft fouling ratios for coated bags (0.01 ± 0.00) and uncoated bags (0.01 ± 0.00) did not differ significantly (N = 200, $F_{2,\,197}$ = 0.81, p = 0.373).

Wild oyster spat, barnacles, ascidians, and bryozoans were all present among all states (GA, SC, NC) in June (Table 2.8). June percent presence of spat did not differ significantly (χ^2 (2, N = 213) = 0.24, p = 0.888) among weekly drying (30.0 ± 5.0%), biweekly drying (28.5 ± 4.9%), and triweekly drying (27.3 ± 4.9%). Spat percent presence among coated bags (30.1 ± 4.1%) and uncoated bags (27.1 ± 4.0%) also did not vary significantly (χ^2 (1, N = 213) = 0.52, p = 0.469) in June. Barnacle percent presence in June among weekly (34.2 ± 3.4%), biweekly (30.1 ± 3.5%), and triweekly drying (34.9 ± 3.7%) did not differ significantly (χ^2 (2, N = 213) = 1.11, p = 0.574).

Coated bags (32.3 ± 2.9%) and uncoated bags (33.8 ± 2.8%) were also similar (χ^2 (1, N = 213) = 0.15, p = 0.703) for barnacle percent presence in June. Much like spat and barnacle presence, ascidian percent presence in June did not differ significantly (χ^2 (2, N = 216) < 0.01, p = 0.998) among weekly (21.4 ± 3.9%), biweekly (21.5 ± 4.0%), and triweekly drying (22.2 ± 4.2%). June ascidian percent presence in coated bag (21.6 ± 3.3%) and uncoated bag (21.9 ± 3.3%) were statistically similar (χ^2 (1, N = 216) < 0.01, p = 0.992). Bryozoan percent presence among weekly drying (4.0 ± 1.4%), biweekly drying (8.2 ± 2.1%), and triweekly drying (10.0 ± 2.4%) did not vary significantly (χ^2 (2, N = 154) = 5.31, p = 0.070) in June. Bryozoan percent occurrence in June for coated bags (5.1 ± 1.2%) and uncoated bags (9.4 ± 2.0%) were not significantly different (χ^2 (1, N = 154) = 1.90, p = 0.168).

Discussion

Fouling on oysters appeared to be unaffected by both drying regimes and bag coatings. This was unexpected, as tidal aerial exposure has been shown to decrease fouling coverage on oysters (Bishop and Peterson, 2006). Increased fouling on cultured shellfish within bags coated with fouling-release agents has also been observed, as organisms settle on the next hard uncoated substrate they encounter (Sievers et al., 2017; Tettelbach et al., 2014). While there were small but significant differences among drying treatments in hard fouling ratios for SC and NC oysters in June, there were no differences among any treatments for total or soft fouling ratios among any states in June or October.

Biofouling accumulation trends varied among sampling seasons. Overall, there seemed to be a negative relationship between drying frequency and fouling accumulation in December. Triweekly drying treatments had the highest fouling percent coverage in SC and weekly drying bags had significantly lower percent fouling coverage for GA, NC, and combined data. In contrast, biweekly drying in GA had higher fouling coverage compared to weekly and triweekly bags. However, fouling coverage in all drying treatments in December was low (<20%) and oysters were not yet at harvest size. The negative trend between drying frequency was not as evident in March. While SC and combined data showed that triweekly bags had higher percent coverage, GA and NC showed no difference.

Although trends differed over time and across states, bag coating treatments seemed to have little to no effect throughout. All evidence suggests that there may be a benefit to drying oysters more frequently as it may decrease fouling. However, the lack of significant differences among all treatments for bag weight shows that both drying frequency and coating have little effect on fouling accumulation after a summer season. Most oysters reached harvest size in June before peak summer fouling, when growers would have the opportunity to clean gear before restocking. While GA still showed a clear negative trend between drying frequency and fouling accumulation (weekly drying oysters had lower fouling coverage than biweekly and triweekly drying had the highest coverage) it was the only state that was not at harvest size by June. In direct contrast to December trends, NC had higher coverage in weekly drying treatments, although fouling was still below 20% coverage in June. There was no distinction among drying treatments in SC in June, although assessments may not be accurate because most bags had mud clogging the mesh, making it hard to distinguish between fouling accumulation and sediment caking. There were also no significant differences among treatments with all states' data combined, although triweekly coverage was slightly higher. In October, GA and SC bags showed different results. While GA bags showed no differences among drying treatments, SC bags showed that the negative relationship between drying frequency and fouling accumulation may still be evident, as weekly drying showed lower fouling coverage than triweekly drying.

Presence of fouling organisms followed different trends compared to the other fouling quantifying metrics. GA had the highest fouling occurrence in June, and wild oyster spat was the most frequently present. This is not unexpected, as oysters grown off-bottom in GA are known to have higher occurrence of spat fouling (Adams et al., 1991; Moroney and Walker, 1999; O'Beirn et al., 1996). However, some October trends went against our previous observations of a negative correlation between drying frequency and fouling occurrence. While bryozoans occurred more frequently in triweekly treatments in October, both wild spat and barnacles occurred more frequently in biweekly treatments compared to weekly and triweekly treatments. Also, ascidians showed higher occurrences in weekly treatments in October while mussel occurrence did not seem to be influenced by any treatment. SC fouling occurrence was more dominated by ascidians than other organisms in June, which is likely explained by the ascidians' nature towards settling on available substrate and a lack of hard fouling (Carman et al., 2010). However, there were no differences among any treatments in ascidian occurrence. Barnacles were the only organism that appeared to be affected by treatment in SC, with biweekly and weekly drying oysters showing lower occurrences than triweekly drying oysters. October fouling occurrences in SC shifted towards higher barnacle and spat presence, but there were no differences among any treatment for any fouling type. NC oysters, which had the highest barnacle occurrence compared to any other fouling type, showed no difference among treatments for spat, barnacles, grapes, or mussels. However, bryozoan occurrence was lower in the weekly drying treatment compared to biweekly and triweekly drying, implying it may have been influenced by drying frequency. With all states data combined, there were no differences among any treatments for presence except for bryozoans, which occurred less frequently in the weekly drying treatment. While this difference is notable, bryozoans aren't typically considered a problem fouling organism and focus should remain on the results of little to no differences among any treatment for spat, barnacle, ascidian, and mussel occurrences.

While our findings suggest that aerial drying frequency and bag coating treatments provide little difference among fouling coverage, ratios, and occurrences, there are many factors that could have altered our findings. Differences in October bag coverage trends and the lack of difference among bag weights in GA and SC may be explained by the overall success of the flipping regimes. With SC having reduced stocks, drying regimes continued without any issue while GA oysters grew heavier than the cages could handle, causing them to flip back over before the full 24-hour drying time was complete, thus decreasing the efficacy of the drying regimes. While fouling ratios may have showed little to no treatment effects, the length of time between sampling season may have resulted in us overlooking differences. By the October sampling period, GA oysters were so significantly fouled that stocked bags were full, and oysters needed to be broken apart in the bags prior to harvest. Freezing oysters prior to fouling analysis may have also affected our results. Ascidians either fell off the oysters as they were removed or lost water while traveling back to the freezers, decreasing both their mass and occurrence. If fouling was assessed biweekly or monthly and with live organisms, differing trends may have been easier to identify.

Summary

Overall, the chosen drying frequency and bag coating treatments appeared to have little effect on fouling, implying that drying less frequently or using alternate mitigation methods may be more beneficial for growers. While fouling through December, March, and June was minimal, there were still instances of early set hard fouling. Other mitigation methods may be more successful at reducing fouling coverage of cultured oysters and gear. Mitigation approaches such as more frequent flipping regimes during the summer, pressure washing during barnacle and oyster spawning seasons, or sinking oyster cages to the bottom during recruitment periods (Adams et al., 1991; Carman et al., 2010; Moroney and Walker, 1999) may prove to be more successful than the methods we tested in the present study. Site dynamics such as wave action, environmental parameters, and ecology of the region can greatly influence the efficacy of fouling mitigation treatments and should be considered when deciding management strategies (Mallet et al., 2009). With additional research regarding growth or condition of cultured oysters, we may find that these treatments show advantages and disadvantages in ways other than fouling mitigation. However, this study provides crucial information for floating oyster cage management strategies that could improve oyster culture in the southeastern (US) Atlantic states.

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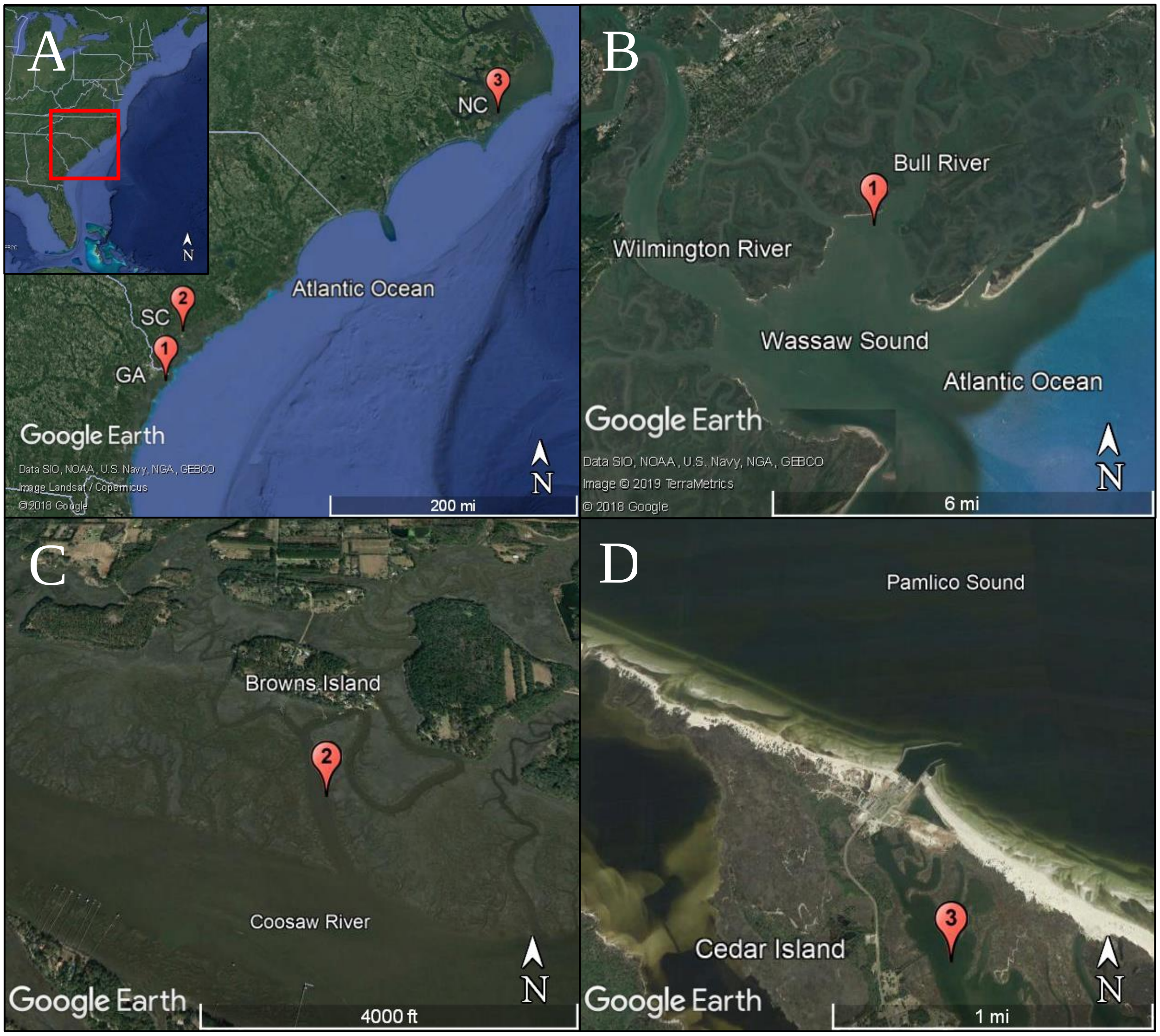

Figure 2.1: Oyster floating cage study locations (A) in Georgia (B), South Carolina (C), and North Carolina (D). Pin 1 represents Georgia location and can be viewed closer in map B. Pin 2 represents South Carolina location can be viewed closer in map C. Pin 3 represent North Carolina location and can be viewed closer in map D. Images obtained through Google Earth.

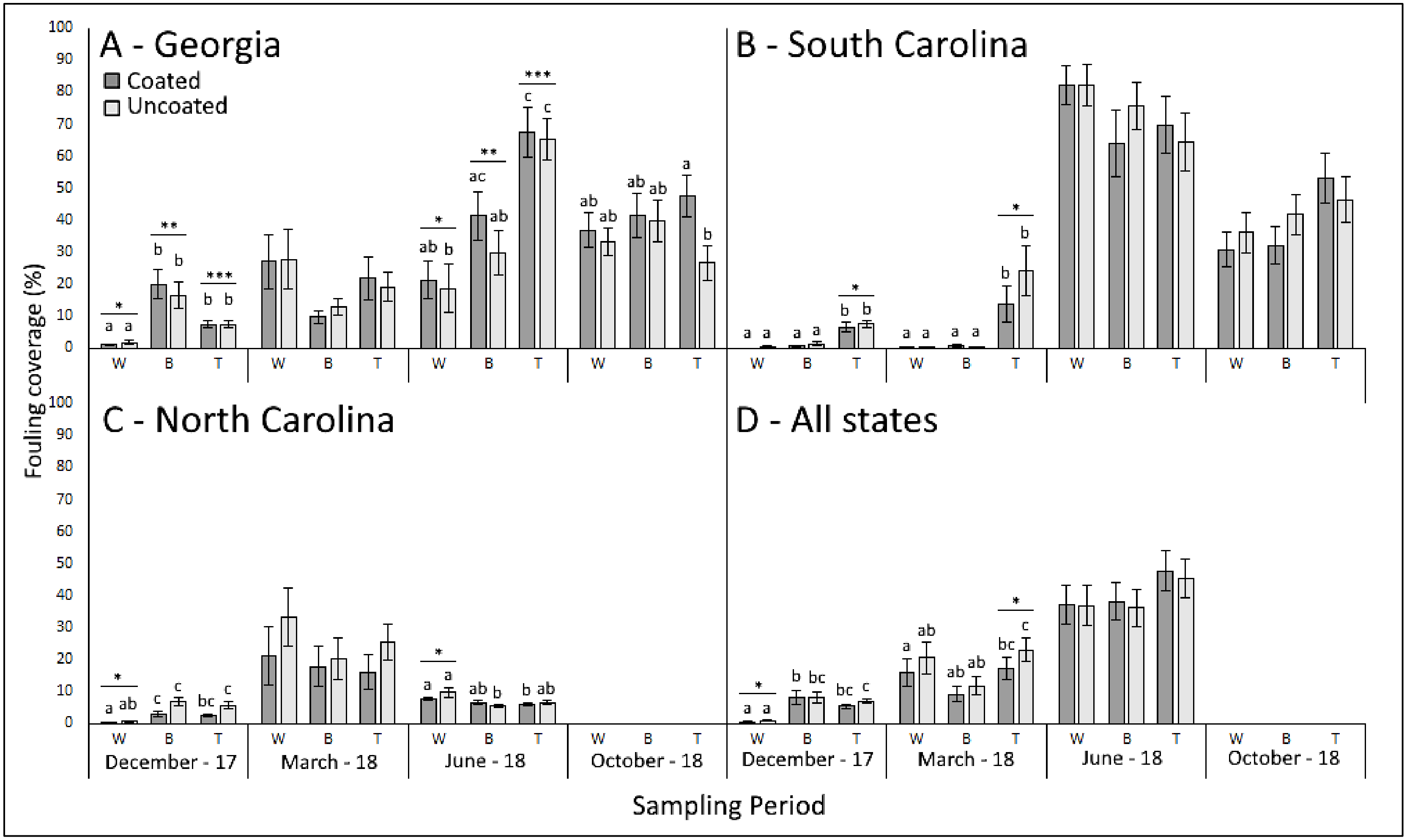

Figure 2.2: Biofouling percent coverage means (± standard error) for oysters grown in floating cages in Georgia (A), South Carolina (B), North Carolina (C), and all states' data combined (D) under different drying (W = weekly, B = biweekly, T = triweekly) and bag coating treatments (coated, uncoated) over four sampling periods. Oysters were deployed in October 2017. Error bars are standard error. North Carolina bags were lost in the October sampling period because of Hurricane Florence and no data can be presented. Figure D shows December-June sampling periods because of the loss of North Carolina oysters. Lowercase letters denote differences among drying and bag treatments as whole effects in accordance to Dunn's tests. Bars with asterisks represent differences among drying treatments only (Kruskal-Wallis tests, α = 0.05).

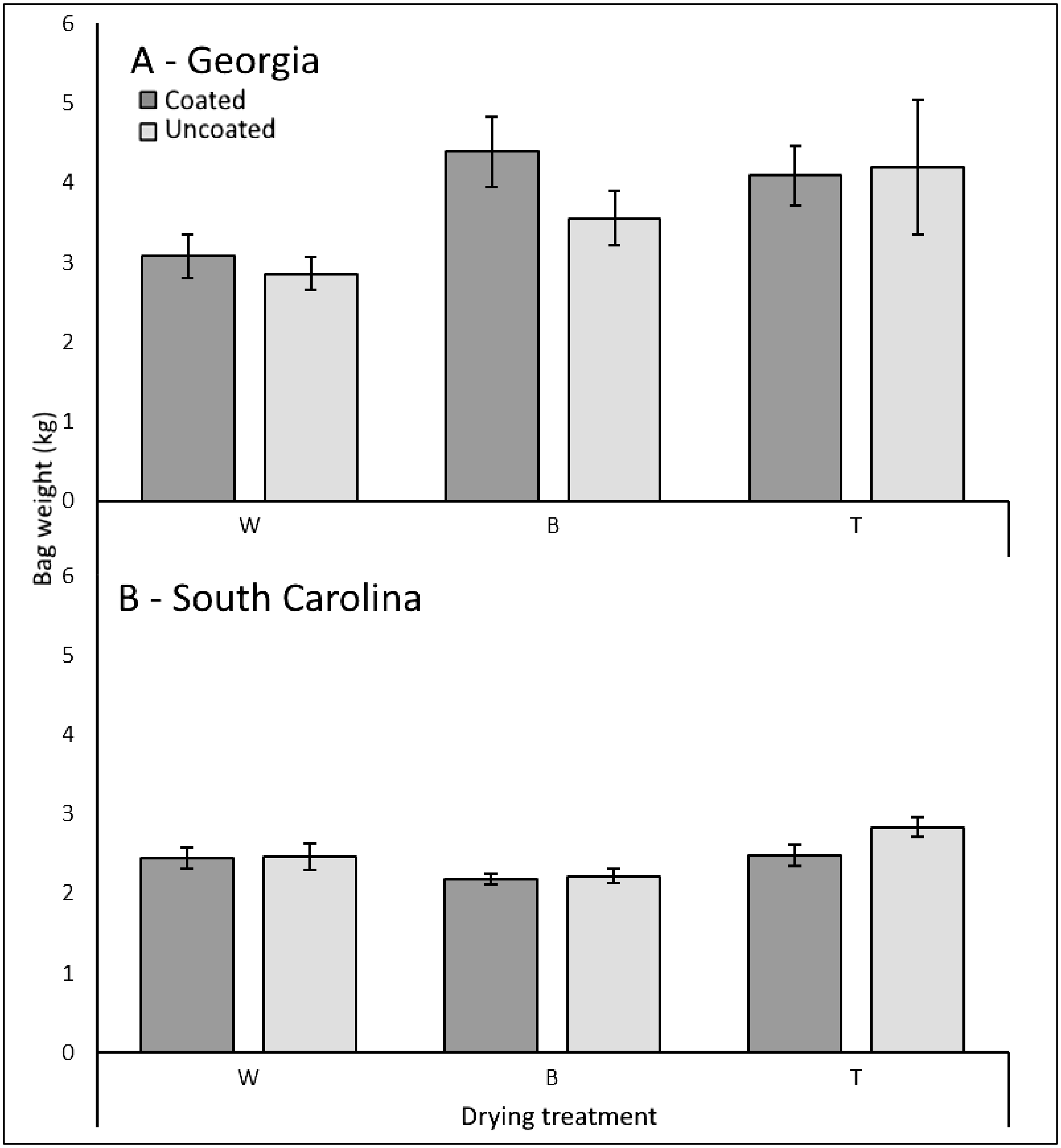

Figure 2.3: Mean (± standard error) final weights of Vexar bags in kg deployed in floating cages in Georgia (A) and South Carolina (B) under different drying regimes (W = weekly, B = biweekly, T = triweekly) and bag coating treatments (coated, uncoated). Bags were deployed from October 2017 until October 2018. Error bars are standard error. North Carolina bags were lost prior to weighing because of Hurricane Florence and no data could be presented. No significant differences are represented because there was no difference among any treatments (α = 0.05).

Table 2.1: Mean (± standard error) fouling ratios of oysters grown in floating cages in Georgia with differing drying (W = weekly, B = biweekly, T = triweekly), bag coating (CB = coated, NB = uncoated), and combined drying and bag coating treatments over two sampling periods (June and October 2018). Initial oyster stocking occurred in October 2017. Total fouling ratio (total fouling weight / clean wet weight), hard fouling ratio (hard fouling weight / clean wet weight), and soft fouling ratio (soft fouling weight / clean wet weight) are reported. Weights were measured in grams. Letters represent significant differences between groups in accordance to Tukey's HSD analysis results (α = 0.05).

Period	Treatment			Total fouling ratio	Hard fouling ratio	Soft fouling ratio
June	Drying	W		0.02 ± 0.00 a	0.01 ± 0.00 a	0.01 ±0.00 a
		B		0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ±0.00 a
		T		0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ±0.00 a
	Coating	CB		0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ±0.00 a
		NB		0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ±0.00 a
	Drying x coating	W	CB	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ±0.00 a
			NB	0.02 ± 0.00 a	0.01 ± 0.00 a	0.01 ±0.00 a
		B	CB	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ±0.00 a
			NB	0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ±0.00 a
		T	CB	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ±0.00 a
			NB	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ±0.00 a
October	Drying	W		0.68 ± 0.08 a	0.67 ± 0.08 a	0.02 ±0.00 a
		B		0.79 ± 0.12 a	0.77 ± 0.11 a	0.02 ±0.00 a
		T		0.62 ± 0.10 a	0.61 ± 0.10 a	0.02 ±0.00 a
	Coating	CB		0.79 ± 0.08 a	0.77 ± 0.08 a	0.02 ±0.00 a
		NB		0.62 ± 0.08 a	0.61 ± 0.08 a	0.02 ±0.00 a
	Drying x coating	W	CB	0.85 ± 0.10 a	0.83 ± 0.10 a	0.02 ±0.00 a
			NB	0.53 ± 0.10 a	0.52 ± 0.11 a	0.02 ±0.00 a
		B	CB	0.89 ± 0.17 a	0.86 ± 0.17 a	0.02 ±0.00 a
			NB	0.70 ± 0.16 a	0.69 ± 0.15 a	0.02 ±0.00 a
		T	CB	0.61 ± 0.14 a	0.59 ± 0.14 a	0.02 ±0.00 a
			NB	0.64 ± 0.15 a	0.62 ± 0.15 a	0.02 ±0.00 a

Table 2.2: Mean (± standard error) percent occurrence of fouling on oysters grown in floating oyster gear in Georgia with differing drying (W = weekly, B = biweekly, T = triweekly), bag coating (CB = coated, NB = uncoated), and combined drying and bag coating treatments over two sampling periods (June and October 2018). Initial oyster stocking occurred in October 2017. Fouling types included wild oyster spat, barnacles, ascidians, mussels, and bryozoans. Letters represent significant differences among groups (Dunn's test, α = 0.05).

Period	Treatment			Spat (%)	Barnacle (%)	Ascidian (%)	Mussel (%)	Bryozoan (%)
June	Drying	W		86.7 ± 4.8 a	47.5 ± 4.3 a	0.0 ± 0.0 a	0.0 ± 0.0 a	5.0 ± 2.3 a
		B		85.7 ± 3.8 a	38.3 ± 4.9 a	0.0 ± 0.0 a	0.0 ± 0.0 a	16.7 ± 7.6 ab
		T		84.1 ± 5.3 a	42.3 ± 7.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	27.5 ± 7.5 b
	Coating	CB		88.9 ± 2.9 a	40.0 ± 4.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	8.0 ± 3.0 a
		NB		82.1 ± 4.4 a	45.6 ± 4.8 a	0.0 ± 0.0 a	0.0 ± 0.0 a	23.6 ± 6.5 b
	Drying x coating	W	CB	87.5 ± 5.9 a	48.3 ± 6.5 a	0.0 ± 0.0 a	0.0 ± 0.0 a	2.5 ± 1.6 a
			NB	85.8 ± 7.7 a	46.7 ± 5.8 a	0.0 ± 0.0 a	0.0 ± 0.0 a	10.0 ± 5.8 ab
		B	CB	91.7 ± 2.1 a	35.8 ± 6.7 a	0.0 ± 0.0 a	0.0 ± 0.0 a	10.0 ± 5.8 ab
			NB	79.1 ± 7.3 a	40.9 ± 7.3 a	0.0 ± 0.0 a	0.0 ± 0.0 a	23.3 ± 14.5 ab
		T	CB	87.3 ± 6.6 a	35.5 ± 8.2 a	0.0 ± 0.0 a	0.0 ± 0.0 a	17.5 ± 8.5 ab
			NB	80.9 ± 8.6 a	49.1 ± 11.6 a	0.0 ± 0.0 a	0.0 ± 0.0 a	37.5 ± 11.1 b
October	Drying	W		96.7 ± 1.6 a	96.7 ± 1.6 a	13.3 ± 4.1 a	53.3 ± 6.0 a	1.7 ± 1.7 a
		B		100.0 ± 0.0 a	100.0 ± 0.0 a	2.5 ± 1.8 b	54.2 ± 5.0 a	6.7 ± 3.1 ab
		T		98.0 ± 1.4 a	98.0 ± 1.4 a	0.0 ± 0.0 b	51.0 ± 6.1 a	15.0 ± 5.6 b
	Coating	CB		98.2 ± 1.0 a	98.2 ± 1.0 a	8.2 ± 2.9 a	56.5 ± 4.4 a	7.1 ± 3.4 a
		NB		98.2 ± 1.0 a	98.2 ± 1.0 a	2.9 ± 1.7 a	49.4 ± 4.7 a	7.6 ± 2.7 a
	Drying x coating	W	CB	95.0 ± 2.6 a	95.0 ± 2.6 a	21.7 ± 6.7 a	58.3 ± 9.0 a	3.3 ± 3.3 ab
			NB	98.3 ± 1.7 ab	98.3 ± 1.7 ab	5.0 ± 3.6 b	48.3 ± 8.0 a	0.0 ± 0.0 a
		B	CB	100.0 ± 0.0 b	100.0 ± 0.0 b	1.7 ± 1.7 b	56.7 ± 6.4 a	1.7 ± 1.7 ab
			NB	100.0 ± 0.0 b	100.0 ± 0.0 b	3.3 ± 3.3 b	51.7 ± 8.0 a	11.7 ± 5.8 b
		T	CB	100.0 ± 0.0 b	100.0 ± 0.0 b	0.0 ± 0.0 b	54.0 ± 7.9 a	18.0 ± 10.1 b
			NB	96.0 ± 2.7 ab	96.0 ± 2.7 ab	0.0 ± 0.0 b	48.0 ± 9.5 a	12.0 ± 5.3 b

Table 2.3: Mean (± standard error) fouling ratios of oysters grown in floating oyster gear with differing drying (W = weekly, B = biweekly, T = triweekly), bag coating (CB = coated, NB = uncoated), and combined drying and bag coating treatments over two sampling periods (June and October 2018). Initial oyster stocking occurred in October 2017. Total fouling ratio (total fouling weight / clean wet weight), hard fouling ratio (hard fouling weight / clean wet weight), and soft fouling ratio (soft fouling weight / clean wet weight) are reported. Weights were measured in grams. Letters represent significant differences between groups in accordance to Dunn's test results (June total fouling ratios, June hard fouling ratios) and Tukey's HSD results (October total fouling ratios, October hard fouling ratios, June and October soft fouling ratios) (α = 0.05).

Period	Treatment			Total fouling ratio	Hard fouling ratio	Soft fouling ratio
June	Drying	W		0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		B		0.02 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		T		0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
	Coating	CB		0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		NB		0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
	Drying X coating	W	CB	0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
			NB	0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		B	CB	0.02 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
			NB	0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		T	CB	0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
			NB	0.02 ± 0.00 a	0.00 ± 0.00 a	0.02 ± 0.00 a
October	Drying	W		0.10 ± 0.03 a	0.08 ± 0.02 a	0.02 ± 0.00 a
		B		0.10 ± 0.02 a	0.09 ± 0.02 a	0.02 ± 0.00 a
		T		0.12 ± 0.02 a	0.10 ± 0.02 a	0.02 ± 0.00 a

Table 2.4: Mean (± standard error) percent occurrence of fouling on oysters grown in floating oyster gear in Georgia for differing drying (W = weekly, B = biweekly, T = triweekly), bag coating (CB = coated, NB = uncoated), and combined drying and bag coating treatments over two sampling periods (June and October 2018). Initial oyster stocking occurred in October 2017. Fouling types include wild oyster spat, barnacles, ascidians, mussels, and bryozoans. Letters represent significant differences among groups (Dunn's test, α = 0.05). Only drying treatments were assessed in October because stocks were reduced after June sampling and bag coating treatments could not be assessed.

Period	Treatment			Spat (%)	Barnacles (%)	Ascidians (%)	Mussels (%)	Bryozoans (%)
June	Drying	W		2.9 ± 1.8 a	2.1 ± 1.0 a	64.2 ± 4.3 a	0.0 ± 0.0 a	1.7 ± 1.3 a
		B		1.7 ± 1.0 a	2.1 ± 0.8 a	64.6 ± 5.5 a	0.0 ± 0.0 a	0.4 ± 0.4 a
		T		1.7 ± 0.8 a	7.5 ± 1.8 b	66.7 ± 5.5 a	0.0 ± 0.0 a	0.8 ± 0.8 a
	Coating	CB		2.5 ± 0.8 a	3.3 ± 1.1 a	64.7 ± 4.4 a	0.0 ± 0.0 a	0.8 ± 0.6 a
		NB		1.7 ± 1.2 a	4.4 ± 1.2 a	65.6 ± 4.0 a	0.0 ± 0.0 a	1.1 ± 0.9 a
	Drying x coating	W	CB	1.7 ± 1.1 a	1.7 ± 1.7 abc	62.5 ± 6.8 a	0.0 ± 0.0 a	0.8 ± 0.8 a
			NB	4.2 ± 3.4 a	2.5 ± 1.3 a	65.8 ± 5.7 a	0.0 ± 0.0 a	2.5 ± 2.5 a
		B	CB	3.3 ± 1.9 a	1.7 ± 1.1 ab	68.3 ± 7.7 a	0.0 ± 0.0 a	0.0 ± 0.0 a
			NB	0.0 ± 0.0 a	2.5 ± 1.3 abc	60.8 ± 7.9 a	0.0 ± 0.0 a	0.9 ± 0.9 a
		T	CB	2.5 ± 1.3 a	6.7 ± 2.2 bc	63.3 ± 8.7 a	0.0 ± 0.0 a	1.7 ± 1.7 a
			NB	0.8 ± 0.8 a	8.3 ± 3.0 c	70.0 ± 7.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a
October	Drying	W		97.5 ± 2.5 a	97.5 ± 2.5 a	65.0 ± 19.4 a	5.0 ± 5.0 a	2.5 ± 2.5 a
		B		100.0 ± 0.0 a	100.0 ± 0.0 a	25.0 ± 5.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
		T		100.0 ± 0.0 a	100.0 ± 0.0 a	22.5 ± 10.3 a	0.0 ± 0.0 a	2.5 ± 2.5 a

Table 2.5: Mean (± standard error) fouling ratios for oysters grown in floating oyster gear in North Carolina with differing drying (W = weekly, B = biweekly, T = triweekly), bag coating (CB = coated, NB = uncoated), and combined drying and bag coating treatments in June 2018. Initial oyster stocking occurred in October 2017. Total fouling ratio (total fouling weight / clean wet weight), hard fouling ratio (hard fouling weight / clean wet weight), and soft fouling ratio (soft fouling weight / clean wet weight) are reported. Weights were measured in grams. Letters represent significant differences between groups in accordance to Tukey's HSD results (total fouling ratio, soft fouling ratio) and Dunn's test results (hard fouling ratios) (α = 0.05). North Carolina bags were lost prior to October 2018 sampling because of Hurricane Florence and no data could be presented.

Period	Treatment			Total fouling ratio	Hard fouling ratio	Soft fouling ratio
June	Drying	W		0.02 ± 0.00 a	0.00 ± 0.00 ab	0.01 ± 0.00 a
		B		0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		T		0.02 ± 0.00 a	0.00 ± 0.00 b	0.01 ± 0.00 a
	Coating	CB		0.02 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		NB		0.02 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
	Drying x coating	W	CB	0.02 ± 0.00 a	0.00 ± 0.00 abc	0.01 ± 0.00 a
			NB	0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		B	CB	0.01 ± 0.00 a	0.00 ± 0.00 ab	0.01 ± 0.00 a
			NB	0.01 ± 0.00 a	0.00 ± 0.00 abc	0.01 ± 0.00 a
		T	CB	0.02 ± 0.00 a	0.00 ± 0.00 bc	0.01 ± 0.00 a
			NB	0.02 ± 0.00 a	0.01 ± 0.00 c	0.01 ± 0.00 a

Table 2.6: Mean (± standard error) percent occurrence of fouling on oysters grown in floating oyster gear in North Carolina for differing drying (W = weekly, B = biweekly, T = triweekly), bag coating (CB = coated, NB = uncoated), and combined drying and bag coating treatments in June 2018. Initial oyster stocking occurred in October 2017. Fouling types included wild oyster spat, barnacles, ascidians, mussels, and bryozoans. Letters represent significant differences among groups (Dunn's test, α = 0.05). North Carolina bags were lost prior to October 2018 sampling because of Hurricane Florence and no data could be presented.

Period	Treatment			Spat (%)	Barnacles (%)	Ascidians (%)	Mussels (%)	Bryozoans (%)
June	Drying	W		0.4 ± 0.4 a	52.9 ± 4.8 a	0.0 ± 0.0 a	0.0 ± 0.0 a	6.2 ± 3.2 a
		B		0.8 ± 0.6 a	50.4 ± 5.6 a	0.0 ± 0.0 a	0.0 ± 0.0 a	16.3 ± 4.0 b
		T		0.4 ± 0.4 a	55.4 ± 4.5 a	0.0 ± 0.0 a	0.0 ± 0.0 a	14.0 ± 4.4 ab
	Coating	CB		0.6 ± 0.4 a	53.9 ± 4.6 a	0.0 ± 0.0 a	0.0 ± 0.0 a	9.3 ± 2.6 a
		NB		0.6 ± 0.4 a	51.9 ± 3.4 a	0.0 ± 0.0 a	0.0 ± 0.0 a	14.0 ± 3.6 a
	Drying x coating	W	CB	0.0 ± 0.0 a	53.3 ± 6.7 a	0.0 ± 0.0 a	0.0 ± 0.0 a	3.3 ± 3.3 a
			NB	0.8 ± 0.8 a	52.5 ± 7.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	8.3 ± 5.1 ab
		B	CB	0.8 ± 0.8 a	50.8 ± 9.6 a	0.0 ± 0.0 a	0.0 ± 0.0 a	15.7 ± 5.7 b
			NB	0.0 ± 0.0 a	50.0 ± 6.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	16.7 ± 5.8 b
		T	CB	0.8 ± 0.8 a	57.5 ± 7.7 a	0.0 ± 0.0 a	0.0 ± 0.0 a	10.0 ± 4.3 ab
			NB	0.8 ± 0.8 a	53.3 ± 5.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	18.9 ± 8.4 b

Table 2.7: Mean (± standard error) combined states (Georgia, South Carolina, North Carolina) fouling ratios for differing drying (W = weekly, B = biweekly, T = triweekly), bag coating (CB = coated, NB = uncoated), and combined drying and bag coating treatments in June 2018. Initial oyster stocking occurred in October 2017. Total fouling ratio (total fouling weight / clean wet weight), hard fouling ratio (hard fouling weight / clean wet weight), and soft fouling ratio (soft fouling weight / clean wet weight) are reported. Weights were measured in grams. Letters represent significant differences between groups in accordance to Tukey's HSD results (total fouling ratio, soft fouling ratio) and Dunn's test results (hard fouling ratios) (α = 0.05). Only June results are presented because of changes in management practices among states and loss of North Carolina bags in October 2018.

Period	Treatment			Total fouling ratio	Hard fouling ratio	Soft fouling ratio
June	Drying	W		0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		B		0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		T		0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
	Coating	CB		0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		NB		0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
	Drying x coating	W	CB	0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
			NB	0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		B	CB	0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
			NB	0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
		T	CB	0.01 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
			NB	0.02 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a

Table 2.8: Mean (± standard error) percent occurrence of fouling on oysters grown in floating oyster gear in three states states (Georgia, South Carolina, North Carolina) for differing drying (W = weekly, B = biweekly, T = triweekly), bag coating (CB = coated, NB = uncoated), and combined drying and bag coating treatments in June 2018. Initial oyster stocking occurred in October 2017. Fouling types included wild oyster spat, barnacles, ascidians, mussels, and bryozoans. Letters represent significant differences among groups (Dunn's test, α = 0.05). Only June results are presented because of changes in management practices among states and loss of North Carolina bags in October 2018.

Period	Treatment			Spat (%)	Barnacles (%)	Ascidians (%)	Mussels (%)	Bryozoans (%)
June	Drying	W		30.0 ± 5.0 a	34.2 ± 3.4 a	21.4 ± 3.9 a	0.0 ± 0.0 a	4.0 ± 1.4 a
		B		28.5 ± 4.9 a	30.1 ± 3.5 a	21.5 ± 4.0 a	0.0 ± 0.0 a	8.2 ± 2.1 a
		T		27.3 ± 4.9 a	34.9 ± 3.7 a	22.2 ± 4.2 a	0.0 ± 0.0 a	10.0 ± 2.4 a
	Coating	CB		30.1 ± 4.1 a	32.3 ± 2.9 a	21.6 ± 3.3 a	0.0 ± 0.0 a	5.1 ± 1.2 a
		NB		27.1 ± 4.0 a	33.8 ± 2.8 a	21.9 ± 3.3 a	0.0 ± 0.0 a	9.5 ± 2.0 a
	Drying x coating	W	CB	29.7 ± 7.2 a	34.4 ± 5.0 a	20.8 ± 5.4 a	0.0 ± 0.0 a	2.1 ± 1.2 a
			NB	30.3 ± 7.2 a	33.9 ± 4.8 a	21.9 ± 5.6 a	0.0 ± 0.0 a	6.1 ± 2.5 ab
		B	CB	31.9 ± 7.2 a	29.4 ± 5.2 a	22.8 ± 6.0 a	0.0 ± 0.0 a	6.4 ± 2.4 ab
			NB	24.9 ± 6.7 a	30.9 ± 4.7 a	20.3 ± 5.5 a	0.0 ± 0.0 a	10.0 ± 3.3 b
		T	CB	28.6 ± 7.1 a	33.1 ± 5.1 a	21.1 ± 5.8 a	0.0 ± 0.0 a	7.4 ± 2.4 ab
			NB	26.0 ± 6.9 a	36.6 ± 5.4 a	23.3 ± 6.0 a	0.0 ± 0.0 a	12.8 ± 4.3 b

CHAPTER 4

DISCUSSION

Recovery and development of eastern oyster (*Crassostrea virginica*) aquaculture industries in the southeastern US states has been substantial in recent years. Off-bottom oyster aquaculture has the potential to be a major economic contributor in the Southeast. However, biofouling of cages and cultured oysters themselves can diminish culture success and decrease economic yields. Appropriate biofouling mitigation methods are necessary for farmers to produce high-quality oysters while minimizing cost and effort. The goal of this study was to investigate the efficacy of two biofouling mitigation methods on growth and quality of oysters as well as on fouling of gear and oysters. We investigated the effects of three aerial drying frequencies and a fouling-release coating on oysters in floating cages in Georgia (GA), South Carolina (SC), and North Carolina (NC). Across all the metrics we measured, trends differed among states but within each state, we were able to identify some general trends in oyster growth.

In chapter 2, we found that drying frequency and fouling-release coating treatments resulted in differences in shell metrics throughout sampling periods, but they were not consistent among the three states. However, some trends were identifiable – higher drying frequencies tended to have a negative relationship with shell height (SH) as oysters grew to harvest size. Early in the study (December), when fouling coverage was low (<20%), lower SH among oysters dried weekly may be attributed to the oysters spending less time in the water and therefore having reduced feeding times. Oysters with longer feeding times may show faster growth in colder months when fouling isn't as prevalent (Bishop and Peterson 2006). In December, we also observed higher cup and fan ratios in oysters dried weekly; this growth pattern may result from the additional handling caused by frequent cage flipping, which can break off new shell growth and encourage shell thickening (Stone et al. 2013). Some growers find that higher cup ratios imply high oyster quality among consumers, and may therefore be a desirable outcome (Brake et al. 2003). In congruence with other work evaluating suspended oysters (Manley et al. 2009; Mallet et al. 2013; Thomas et al. 2019), it is likely that the collisions among oysters within the bags and the additional tumbling from weekly cage flipping chipped oyster shells more frequently, ultimately resulting in a shell shape that is more desirable to consumers.

As oysters grew beyond harvest size, shell metric trends changed; prior to the October (~1 yr) measurement, SH trended higher among oysters dried biweekly and triweekly in GA, but cup ratio and fan ration were higher among oysters dried weekly. However, in October there were no significant differences among drying treatments among any shell metrics. In SC, oysters showed a similar shift in trends, except that in October cup and fan ratios were significantly higher within biweekly and triweekly drying treatments than in weekly treatments.

In chapter 3 we found that fouling ratios observed in this study were unexpectedly unaffected by drying regimes as well as bag coatings. Tidal aerial exposure has been shown to decrease fouling coverage on wild oysters (Bishop and Peterson 2006), while coating bags with fouling-release agents has been shown to increase fouling on cultured shellfish as organisms settle on the next hard uncoated substrate they encounter – in this case, the cultured oysters

(Tettelbach et al. 2014; Sievers et al. 2017). Although we initially observed a negative relationship between drying frequency and fouling accumulation, this trend was not consistent among all states or sampling seasons. We observed a significant difference in hard fouling among drying treatments only in NC in June, but no differences for soft fouling or total fouling were observed. Overall, there were no noticeable differences in cultured oyster fouling between bag coating treatments in this study. These results suggest that fouling on cultured oysters may not be affected by the aerial drying frequencies or bag coating treatments we evaluated.

Although the amount of fouling generally did not vary among treatments, the type and occurrence of fouling organisms on cultured oysters varied by treatment and season. Our results suggest that aerial drying frequency may have more of an effect on fouling assemblages than on fouling density. Over the duration of the study, fouling on oysters was higher GA than SC or NC. Wild oyster spat was the most common type of fouling, which was not unexpected, as this has been previously reported for oysters grown off-bottom in GA (Adams et al. 1991; O'Beirn et al. 1996; Moroney and Walker 1999). In GA, there was a negative correlation between drying frequency and fouling occurrence for the first three sampling periods. However, after ~1 year (October) the pattern changed: bryozoans, wild oyster spat, and barnacles occurred more frequently in biweekly treatments compared to weekly and triweekly treatments. Also, ascidians were observed more frequently in weekly treatments in October, when other fouling organisms weren't as abundant, which is likely because of ascidians nature towards settling on available substrate (Carman et al. 2010). Mussel occurrence did not seem to be influenced by any treatment. Only bryozoans were abundant in the June and October sampling periods in triweekly drying treatments in GA. Fouling in SC was predominately composed of ascidians in June across all treatments. Barnacles were the only organism that seemed to be affected by any treatment in SC in June, as occurrence in triweekly drying treatments was higher than both biweekly and weekly drying. In October, the fouling community shifted towards higher occurrences of barnacles and wild oyster spat across all treatments. In NC in June, barnacle occurrence was higher than any other fouling type. There were no differences in occurrence of any fouling type among any treatments.

Overall, our findings suggest that the aerial drying frequencies and bag coating treatments we tested resulted in few differences among shell metrics, mortality, fouling coverage, fouling ratios, and fouling occurrences. Some of our results contrast with previously published studies, but there are several factors that could have influenced our results. We expected that the weekly aerial drying treatment would result in lower fouling coverage, but we generally did not see this trend. For example, in October in GA, there were no differences in bag coverage among treatments. This might have been due to the overall lack of success of the cage flipping regimes; for instance, GA oysters grew heavier than the cages could handle, causing them to flip back over before the full 24-hour drying cycle was complete, thus potentially decreasing the efficacy of the drying regimes. In SC in that same time period, bags were removed and restocked with smaller seed, thereby reducing the weight within the cages, ensuing that drying regimes could continue successfully.

The length of time between quarterly sampling periods may have also masked differences in growth and fouling. Although GA oysters were not overly fouled in June, October fouling was so dense that the bags were completely full of fouling organisms and cultured oysters needed to be broken apart within the bags prior to harvest. We also froze oysters prior to fouling analysis, which may have affected our results. For example, ascidians tended to fall off the oysters during harvest and lost water while traveling back to the lab for placement in the freezers, decreasing both their occurrence and mass. If we had been able to assess fouling biweekly or monthly and with live organisms, it may have been easier to identify seasonal trends in fouling.

Generally, oysters dried biweekly and triweekly with no bag coating performed best in terms of growth, and growth was poorest in oysters dried weekly with coated bags. However, condition indices and meat weights were not affected by any treatment or combination of treatments. Our results indicate that the Netminder® coating as applied in this study did not affect fouling accumulation or assemblage on gear or cultured oysters. If these results hold true with additional testing, farmers could avoid this treatment and reduce costs without compromising oyster quality. The optimal aerial drying regime for floating cage oyster aquaculture is less clear. A weekly drying regime may result in more aesthetically pleasing oyster shell shape but may also take more time to reach harvest size. Frequent drying and longer growth time may also increase risk of damage to gear and oysters as well as costs associated with labor, fuel, and gear management. Less frequent drying may result in faster growth and lower costs, while potentially compromising ideal oyster shape. However, any treatment may need to be coupled with supplemental fouling mitigation to prevent cages from becoming too heavy and sinking or flipping unexpectedly.

Fouling was minimal for the first three quarters of our sampling, although there were still instances of early set hard fouling that occurred despite aerial drying treatments. A practical additional mitigation method for fouling control would be to pressure wash gear and cultured oysters periodically during barnacle and oyster spawning seasons (Carman et al. 2010). Sinking oyster cages to the bottom during fouling recruitment periods may also be a successful mitigation method (Adams et al. 1991; Moroney and Walker 1999).

We found that oysters at each of our three sites varied in growth metrics and fouling accumulation. Growers should account for site dynamics, such as environmental parameters, ecology of the region, and market demands when deciding on oyster culture management strategies (Mallet et al. 2009). By limiting handling of oysters, decreasing the number of trips for management (i.e. flipping cages), and avoiding additional costs of a fouling-release coating, growers may be able to see higher economic returns upon harvest. In contrast, increased handling may result in shell thickening and higher cup ratios, increasing oyster aesthetics and marketability (Brake et al. 2003; Manley et al. 2009; Mallet et al. 2013; Stone et al. 2013; Thomas et al. 2019). Our results demonstrated little difference in survival, condition, and fouling quantities among our selected drying and coating treatments, but suggest potential aesthetic benefits and costs associated with aerial drying methods that should be taken into consideration by growers determining fouling mitigation practices.

Additional research regarding fouling mitigation techniques should be conducted to determine optimal management strategies. In this study, we exposed oysters to 24 hour drying regimes once every week, two weeks, or three weeks. Future studies should explore different drying durations and frequencies. Additionally, more frequent monitoring may help identify fouling trends during peak fouling seasons that our quarterly observations overlooked. Regarding fouling release coatings, future research should investigate alternate active ingredients, application methods, and reapplication. Finally, future studies should examine the efficacy of fouling mitigation methods in proximal locations with differing site dynamics.

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