UNDERSTANDING SEDIMENT BIOGEOCHEMISTRY AND THE ROLE OF JUVENILE OYSTERS ON RECENTLY RESTORED EASTERN OYSTER REEFS

by

BRYAN JOHN LOCHER

B.S. University of Miami, 2014

A thesis submitted in partial fulfillment of the requirements
for the Degree of Master of Science
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

Summer Term

2019

Major Professor: Lisa G. Chambers

ABSTRACT

In recent decades, goals for the restoration of eastern oyster (*Crassostrea virginica*) populations along the eastern coast of the United States have shifted from increasing harvestable oyster fisheries to enhancing the range of ecosystem services provided by oyster reefs. By filtering large volumes of water and releasing nutrient-rich feces and pseudofeces, oysters can locally enhance sediment biogeochemical cycling compared to that of unstructured benthic environments. An ongoing restoration program in Mosquito Lagoon, FL was leveraged to assess the immediate impacts (< 1 year) of restoration on sediment biogeochemical properties of oyster reefs. The first study measured both short-term and long-term pools of carbon, nitrogen and phosphorus on dead, natural and restored reefs periodically over one year. The second study investigated one of the contributions to sediment nutrient pools by comparing feeding and feces/pseudofeces nutrient content of juvenile and older oysters. Results show that inorganic nitrogen and phosphorus pools can change within weeks after restoration and total nutrient pools by 6 months post-restoration. Restored reefs experienced a 136 % increase in ammonium, 78 % increase in total nitrogen, 46 % increase in total phosphorus, and 75 % increase in organic matter concentrations after 12 months of restoration. These nutrient increases were all positively correlated with oyster density, shell length and reef height measured on each reef. When standardized to grams of dry tissue weight, juvenile oysters showed significantly higher rates of chlorophyll-a removal, release of ammonium, and biodeposits with higher concentrations of dissolved organic carbon, nitrite + nitrate, and ammonium. The short-term changes to biogeochemical cycling on eastern oyster reefs within the first year of restoration are important to managers seeking to monitor ecosystem service recovery and overall coastal ecosystem health.

ACKNOWLEDGMENTS

I would like to express the deepest appreciation for my advisor, Dr. Lisa G. Chambers, for her unwavering support, positivity, and scientific foresight throughout the process of creating this work. Her seemingly endless patience and understanding granted me the space I needed to develop as a scientist. I was able to push through the busiest times and navigate my entrance to the world of biogeochemistry with her personal and professional advice at every step of the way. I would also like to thank the committee members Dr. Linda J. Walters and Dr. Kelly Kibler for reminding me that not everyone is a biogeochemist and providing support in experimental design and ideas for improving this work.

I would like to recognize University of Central Florida's Coastal and Estuarine Ecology Lab for their hard labor monitoring and restoring oyster reefs in Mosquito Lagoon and helping with fieldwork logistics. I would like to thank the University of Central Florida's Surf Team for reminding me to fit in fun between research and maintaining my stoke for surfing which is my main inspiration for going into this field of study of coastal ecosystems.

I would like to recognize all members of the University of Central Florida's Aquatic Biogeochemistry Lab for their field and lab support and fun conversations in the lab. I would like to thank undergraduate lab members Jennifer Bennet, Angela Ferebee, Jessica Chevallier, Tasnim Mellouli, Hayden Denton, Kevin McCarthy, Chelsea Nitsch, and Evan Duga for their help with fieldwork and sample processing. Working with our group of undergrads has been one of the most rewarding parts of my time at UCF.

I would like to thank the lab's first master's student, Janet Ho, for being my defacto class advisor and helping me to figure out our lab and master's program. I would like to thank our newly minted PhD student, Dr. Havalend Steinmuller, for explaining what was a horrible idea or

not in fieldwork, labwork or statistics and for providing comic relief about our grad student lives with our late-night lab conversations. I would like to thank fellow grad student Sarah Harttung for her constant positivity and for providing the cheesiest of jokes and the richest of baked goods. I would like to thank our lab's postdoc Josh Breithaupt for talking through my random soil or chemistry questions and showing us that any science question can be fun to analyze to the point of not knowing. I would like to give a special thanks to our lab manager, Nia Hurst, who patiently answered all of my questions on analytical instruments, gave me plenty of laughs with her pointed sarcasm, and worked with me every step of the way on the BACI study to ensure success in the project.

Finally, I would like to deeply thank my parents and my brother for listening to me and sharing in my struggles and successes throughout my master's degree. I appreciate all of the knowledge and inspiration I've sourced from the genuine, intellectual people surrounding me during my time exploring science at the University of Central Florida.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

Eastern Oysters in Coastal Ecosystems

Across the United States, coastal counties comprise less than 10 % of the total land area, yet counties directly on the shoreline were home to 39 % of the U.S. population (NOAA 2018). This number is expected to increase by 8 % in 2020. The great portion of the population that lives on the coast depends on coastal ecosystems as a source of food, areas for recreation and a source of income. Factors such as rapidly growing coastal development, overfishing, and altered freshwater inflow have degraded many coastal areas over the past several decades (Jackson et al. 2001, Kirby 2004). Among the most affected areas are estuaries, which are regions where freshwater carried by rivers meets saltwater carried by tides. Estuarine ecosystems serve as important nurseries for marine life and provide essential habitat for waterfowl, sportfish, shellfish and thousands of other unique plants and animals (Beck et al. 2001, Dybas 2002). An ecologically and economically significant estuarine species is *Crassostrea virginica*, commonly known as the eastern oyster. The geographic range of this species extends along the Atlantic Coast from the bays of Newfoundland to the coastal lagoons of Texas in the Gulf of Mexico (Kennedy 1996).

Oysters are a keystone species in coastal habitats worldwide and are components for both healthy coastal ecosystems and economies. In the United States marine aquaculture industry, oyster production was valued at $215 million in 2015 (NOAA Fisheries 2018). Oysters are farmed commercially as a food source and provide habitat for other commercially valuable fish species (Tolley and Volety 2005, Humphries and La Peyre 2015). Reefs of oysters have been

shown to enhance the estuarine ecosystem by supporting higher densities of invertebrates and fish than other habitats, such as seagrass or salt marsh (Coen & Grizzle 2007, Gain et al. 2017).

Once abundant in estuaries, oyster populations have precipitously declined in the past two centuries due to overharvesting, disease and habitat degradation, such as changes in freshwater inflow, increased sedimentation, and nutrient loading (Beck et al. 2001, Jackson et al. 2001, Kirby 2004). The collapse of oyster reefs on the Atlantic coast of the United States was accelerated in the nineteenth century with the rise of dredge harvesting methods coupled with increases in coastal development and pollution (Mackenzie 1996). New York City was once known for the abundance of oysters on its shorelines but demand for oysters as food caused the depletion of most native oysters in New York by the early 19th century (Mackenzie 1996). Continual harvesting for human demand led to a depletion of oyster fisheries along the east coast for the remainder of the 19th century (Kirby 2004). In places such as the Chesapeake Bay and New York Harbor, less than 1% of oyster reefs remain today (Beck et al. 2011). Modern declines in the Chesapeake Bay have severely affected profits from oyster harvesting. The total value of the oyster harvest in the Chesapeake Bay declined from $60.1 million in 1980 to $4.3 million in 2001 (expressed in 2001 dollars), a total drop of 93% in 21 years (NRC 2004). One of the primary factors that drives reef decline is the rapid loss of reef complexity due to commercial harvesting (Kirby 2004). This makes oyster reefs more susceptible to other stressors, such as anoxia and disease from nonnative species (Lenihan et al. 1999). In addition, coastal development causes alterations to freshwater inflows that lead to changes in salinity and increases in both sedimentation and surface water runoff that carries nutrients and toxins (Jackson et al. 2001, Lenihan and Peterson 1998). The process of overharvesting, followed by disease and other anthropogenic stressors, have often lead to dramatic population declines (Kirby

2004). It is estimated that shellfish reefs have declined by about 85% worldwide in the past 130 years (Beck et al. 2011).

Restoration of Eastern Oyster Reefs

In order to mitigate this trend and improve the health of human-impacted coastal habitats, local, state and federal organizations have been implementing oyster reef restoration projects in the United States in recent decades (Baggett et al. 2015). The goals of restoration typically include water quality improvement, creation of benthic habitat, and shoreline stabilization (Grabowski et al. 2012). Various substrate, such as disarticulated shells, concrete reef balls, and shell mats, are strategically placed in suitable benthic habitat in order to encourage recruitment of new oysters. After appropriate substrate is deployed it is recommended that, regardless of the goals, monitoring the performance of restoration projects includes four universal oyster metrics: reef area, reef height, oyster density and shell height distribution (Baggett et al. 2015).

The principal motivation behind most restoration efforts is the enhancement of oyster populations degraded by commercial exploitation and poor water quality (Coen and Luckenbach 2000). Research on oyster reefs in the past two decades has seen a rise in the interest on ecosystem services that achieve a broader goal of ecological restoration associated with restoring oyster reef habitat (Coen and Luckenbach 2000, Coen et al. 2004). Because of both ecologic and economic considerations, multiple studies have investigated the effects of restoring oysters on fish and invertebrate communities. Comparing restored reefs to non-restored reefs or unstructured sand/mud bottoms, multiple studies have demonstrated that restoration can enhance these reef-associated fish and invertebrate communities (Grabowski et al. 2005, Peterson et al. 2003, Tolley and Volety 2005). Restoration has been shown to increase species diversity and even increase macrofauna densities by one to two orders of magnitude compared to non-restored

areas (Dillon et al. 2015, Rodney and Paynter 2006). Over two years in coastal Louisiana, restoration efforts led to a dramatic 226% total increase in local fisheries value compared to mud-bottom habitats (Humphries and La Peyre 2015).

Recent research has also focused on the impacts of restoration on other ecological functions, such as improving water clarity, enhancing nutrient cycling, and altering food web dynamics. As oyster reefs grow following restoration, more nutrients are sequestered from the water column via filter-feeding (La Peyre et al. 2014). Over time, a restored reef can transfer significant stores of carbon and nitrogen into reef sediments (Chambers et al. 2017, Kellogg et al. 2013, Newell et al. 2005, Pollack et al. 2013) and increase benthic primary productivity (Falcão et al. 2007, Blomberg et al. 2017). Other studies have also looked at the sequestration of nitrogen or carbon into oyster tissue, shells, and other reef organisms (Blomberg et al. 2017, Dalrymple & Carmichael 2015, Kellogg et al. 2013, Westbrook et al. 2019). It has been demonstrated in different estuaries that changes in the physical structure of reefs after restoration can alter community structure and trophic dynamics within months (Grabowski and Powers 2004, Lenihan et al. 2001). Considering that measurable improvements in oyster populations and associated ecological functions can occur within one year, and that restored reefs can recover their median restoration costs in an estimated 2-14 years (Grabowski et al. 2012), restoration of oyster reefs can rapidly produce benefits to both humans and estuarine ecosystems. Studies indicating the time frame of recovery of certain ecological benefits are shown in the table below.

Table 1: Prior studies on the timeframes of the various impacts of eastern oyster restoration.

Time post-restoration	Post-restoration effects	Study
weeks	Oyster recruitment and linear increase in filtration capacity in LA restored reefs	La Peyre et al. 2014
2 months +	Changes in physical structure alters community structure and trophic dynamics	Grabowski and Powers 2004, Lenihan et al. 2001
5 months	Similar food resources and food chain length as natural reefs	Rezek et al. 2017
6-8 months	Fish abundances similar to that of 4-6 year old natural reefs in NC	Pierson and Eggleston 2014
12-15 months	More diverse and evenly distributed fish and invertebrate community similar to a natural reef	Rezek et al. 2017

Ecosystem Services of the Eastern Oyster

Oysters are classified as ecosystem engineers because of the variety of ecosystem services they provide that maintain estuarine habitats, such as top-down control of phytoplankton, water clarity improvement, and feeding and nesting habitat for both mobile and sessile species (Coen et al. 2007, Grabowski et al. 2005, Newell et al. 2007). An ecosystem engineer is defined as an organism that significantly modifies or controls resources in its environment (Jones et al. 1994). Oysters control the availability of resources through building three-dimensional reef structures that can enhance food web complexity and predator foraging efficiency (Grabowski & Powers 2004, Rezek et al. 2017).

The oyster reproduces through a life cycle that includes fertilization then planktonic larval development in the water column for up to three weeks. In the final larval pediveliger stage, a foot that detects substrate typically chooses oyster shell and cements itself in place, after which point the larvae develops into spat (Kennedy 1996). Mature oysters provide the shell substrate where new generations will set and grow. This accretion of reef structure contributes to

architectural complexity and heterogeneity in the benthic environment and positively affects biodiversity and abundance across both the population and community levels (Lenihan and Peterson 1998, La Peyre et al. 2014). Oyster reef habitat enhances species density and richness because these shells provide safe areas to nest and hide from predation (Lenihan et al. 1999). Species such as fish and decapod crustaceans can be found in higher densities on oyster reefs than on structureless sand- or mud-bottom habitats (Grabowski et al. 2005, Tolley and Volety 2005). The most recent estimate for the valuation of ecosystem services of oyster reefs is between $5,500 and $99,000 per hectare per year, excluding oyster harvesting (Grabowski et al. 2012).

Arguably, the most important ecosystem service *C. virginica* provides is water quality improvement by measurably reducing suspended sediment, detritus and phytoplankton in the water column through filter feeding (Dame et al. 1984). Suspension feeding highly influences trophic structure by exerting top-down control on phytoplankton abundance, thus regulating nutrient availability for primary consumers (Newell et al. 2007). Oysters have the capacity to reduce total suspended solid and chlorophyll-a concentrations in the water column above reefs by up to 75% (Nelson et al. 2004). Modeling efforts show that historic population numbers (circa 1880-1910) in several estuaries in the Gulf of Mexico had the ability to filter the entire estuary's volume of water within the residence time of the water (Ermgassen et al. 2013). During feeding, they assimilate what is needed for metabolic requirements and then release digested and unused material in the form of feces and pseudofeces (collectively called biodeposits). These mucus-bound biodeposits can be buried in the sediment, transformed into new nutrients, or assimilated by grazers and microbes in the benthos (Dame 1999, Newell et al. 2005). This flux of materials between the benthic and pelagic interface is called benthic-pelagic coupling and is an important

ecosystem service of oysters that has implications for nutrient burial and availability at multiple trophic levels (Humphries et al. 2011).

The Role of Eastern Oysters in Biogeochemical Cycling

Biogeochemical cycling comprises the transport, transformation, and storage of biologically important elements or substances that cycle between biotic and abiotic compartments of the Earth. Changes in the components of these cycles can be observed in organic (produced by organisms) and inorganic (mineral) forms and with samples of the three main states of matter - solid, liquid and gas. Samples collected in the experiments presented here analyze the carbon (C), nitrogen (N), and phosphorus (P) content of sediments and surface waters collected in the field (Chapter 2) and in a laboratory experiment (Chapter 3). Measurements of C, N and P concentrations in the sediment and surface waters of oyster reefs reveal how organic and inorganic elemental forms are transported and transformed between the dissolved fraction (< 45 µm) of the water column above oysters and solid states (sediment and biodeposits) and are stored in oyster reef sediments over time. Short-term pools of nutrients are those that are bioavailable and readily exchanged between organisms. These are the inorganic forms of nutrients that we refer to here as "extractable nutrients" that comprise inorganic forms of C, N and P in the porewater and adsorbed to the surface of sediment particles (Alva 1993). These nutrient pools were extracted from fresh, wet soil samples by dissolving organic C and inorganic ions into solution with the addition of salts. Long-term pools of nutrients are composed of both organic and inorganic elemental forms that are more recalcitrant and are not easily taken up by organisms (Reddy & Delaune 2008). Total nutrient pools include nutrients both adsorbed and occluded with sediment particles and in these studies are measured on dried, ground sediment or biodeposit samples.

The coastal environment is the recipient of significant external inputs of N, P and C from terrestrial sources (Oelsner and Stets 2019, Regnier et al. 2013, Seitzinger et al. 2010). High nutrient loads to coastal ecosystems can exacerbate issues such as hypoxic waters, harmful algal blooms and habitat loss (Bricker et al. 2007, Gilbert and Burford 2017, Kang et al. 2015). It is well-documented that estuarine habitats such as mangrove forests, salt marshes, and seagrass beds play an important role in transforming and storing these nutrients carried by freshwater sources (McGlathery et al. 2007, Mcleod et al. 2011, Regnier et al. 2013). Within the past decade, oyster reefs have also become recognized as important habitats for the transformation and storage of nutrients (Chambers et al. 2017, Smyth et al. 2013, Kellogg et al. 2013). Structured estuarine habitats, such as seagrass beds, salt marsh or oyster reefs, have been shown to have higher rates of denitrification and processing of estuarine materials than intertidal or subtidal flats (Piehler and Smyth 2011, Smyth et al. 2016). Relative to other structured estuarine habitats, oyster reefs have been measured to produce the highest net N (N_2) flux and ammonium (NH_4^+) production (Smyth et al. 2013), evidence that they play a key role in regulating nutrient cycles in estuaries.

It is well documented that *C. virginica* influences phytoplankton abundance through filter feeding and provides habitat for coastal species by creating three-dimensional reef structures (Nelson et al. 2004, Grabowski et al. 2005). Less is known about the influence of eastern oysters on sediment biogeochemistry. The process of benthic-pelagic coupling makes oyster reefs biogeochemical hotspots, which are areas of enhanced transformation and storage of biologically important elements (McClain et al. 2003). Previous research on biogeochemical cycling mainly focuses on the deposition of organic matter and on the benthic N cycle (e.g., Westbrook et al. 2019, Smyth et al. 2015, Southwell et al. 2017). Multiple studies have documented that the

physical structure of reefs influences water velocities which can cause sedimentation and increase sediment organic matter content (Lenihan 1999, Reidenbach et al. 2013, Southwell et al. 2017). Whether oyster reefs are net C sources or sinks has not yet been established and seems to depend on whether a reef is subtidal or intertidal and the type of adjacent habitat, among other factors (Fodrie et al. 2017). Shallow subtidal reefs and saltmarsh-fringing reefs with more organic-rich sediments can be net C sinks whereas ten-year-old reefs on sandflats can be net C sources with carbonate dissolution from shells (Fodrie et al. 2017, Waldbusser et al. 2011). Oyster reefs can develop aerobic zones in sediment surface layers facilitated by the introduction of oxygen via bioturbation by invertebrates, tidal fluctuations (intertidal reefs only), and benthic microalgae (Mermillod-Blondin and Rosenberg 2006, Smith et al. 2016, Volaric et al. 2018). This can promote the oxidation of organic material by increasing the availability of oxygen, the most thermodynamically favorable electron acceptor for microbial redox reactions that transform nutrients (Reddy and DeLaune 2008).

Through filter feeding, oysters can transfer significant portions of N loads from the water column to the sediment surface (Pollack et al. 2013, Newell et al. 2007). Their metabolism converts organically-bound N into the inorganic form of ammonium that has been measured in much higher concentrations above oyster reefs than other benthic systems (Dame et al. 1984, Kellogg et al. 2013). N deposited by oysters into sediments can then be removed by enhanced nitrification-denitrification processes in the sediment (Humphries et al. 2016, Kellogg et al. 2013, Pollack et al. 2013). Nitrification is the process by which ammonium (NH_4^+) is biologically oxidized to nitrate (NO_3^-). Infaunal communities and phytobenthic communities can enhance sediment nitrification by increasing the delivery of oxygen, a necessary ingredient for this process (Mermillod-Blondin and Rosenberg 2006, Volaric et al. 2018). Denitrification is the

microbially-mediated respiration of nitrate to atmospheric dinitrogen gas (N_2) that occurs under anaerobic conditions. Recent evidence suggests that the deposition of labile C and N from oyster biodeposits as well as alternating oxic and anoxic conditions promotes coupled nitrification-denitrification in sediments both on and adjacent to reefs (Hoellein et al. 2015, Smyth et al. 2016). However, several studies have reported high N_2 fluxes from oyster reefs that differ by orders of magnitude based on site and season (Kellogg et al. 2013, Mortazavi et al. 2015, Smyth et al. 2015, Smyth et al. 2018, Westbrook et al. 2019), highlighting the need for clarification of the reef and habitat characteristics at play in oyster-mediated nutrient cycling. There is also little to no data available on the dynamics of organic matter deposition, long-term nutrient burial in reef sediments, and the role of reef physical characteristics in the fate of labile nutrients from biodeposits.

Site Description

The subsequent studies were conducted in the Indian River Lagoon (IRL), which is a shallow coastal lagoon that averages about 1 m deep and stretches for 251 km on the Atlantic coast of central Florida (Dybas 2002). The IRL sits at the border of warm-temperate and subtropical climates (Figure 3). Because the biota can include a mix of more southern-dwelling and northern-dwelling Atlantic coast species, the IRL is one of the most biodiverse estuaries in North America (Dybas 2002). This body of water also provides an estimated $7.6 billion in revenue and 19,000 jobs to the Florida economy (East Central Florida Regional Planning Council 2016).

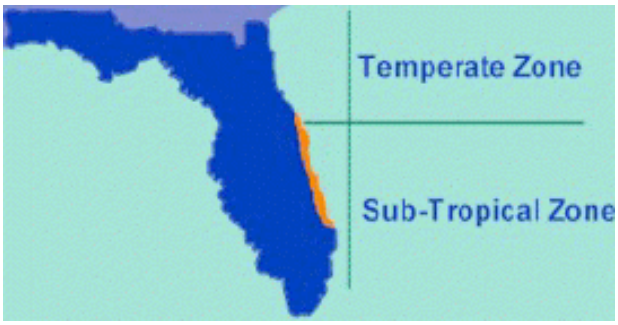

Figure 1: IRL in relation to the temperate and subtropical zones border (Dybas 2002)

The reefs of *C. virginica* for this study are located in the northernmost portion of the IRL, known as Mosquito Lagoon. Water currents in this lagoon are primarily wind-driven and secondarily driven by tidal exchange (Smith 1993). The Ponce de Leon Inlet to the north creates shorter residence times in the northern parts of the lagoon and longer residence times in the south (Smith 1993). With low tidal exchange in the southern end, salinities in Mosquito Lagoon can be as high as 45.2 parts per thousand (Phlips et al. 2015).

The oyster reefs in Mosquito Lagoon have become degraded in recent decades due to year-round boating activity (Grizzle et al. 2002, Wall et al. 2005). Reefs located along popular boating channels frequently have dead margins composed of disarticulated shell piles on their seaward side (Garvis et al. 2015). The wakes produced by passing boats do not significantly affect oyster spat recruitment, but significantly reduce the survival of juveniles through the dislodgement of oyster clusters (Wall et al. 2005). The earliest known appearance of these dead margins was found in 1943 imagery of a reef adjacent to the Intracoastal Waterway (Garvis et al. 2015). Using aerial imagery, Garvis et al. (2015) found that oyster reef coverage in the entire lagoon had decreased by 24 % since 1943. By 2000, 60 reefs had dead margins, representing 9.1 % of the total areal coverage by oyster reefs within the grounds of Canaveral National Seashore (Grizzle et al. 2002). Since 2007, there has been a community-based restoration program to

restore the affected margins and dead reefs. As of Summer 2018, about 3.25 acres of reef area have been restored on 89 individual reefs (L.J. Walters, pers. comm.).

CHAPTER 2: BEFORE-AFTER-CONTROL-IMPACT STUDY OF SEDIMENT BIOGEOCHEMISTRY ON RESTORED OYSTER REEFS

Introduction

Oyster populations have severely declined worldwide due to overharvesting, disease and habitat degradation, such as alterations in freshwater inflow and increased sedimentation (Beck et al. 2011, Jackson et al. 2001, Kirby 2004). From 1900 to 1995, the annual harvest of the eastern oyster, *Crassostrea virginica,* in millions of pounds of meat has decreased by more than 90 % in most Atlantic Coastal States (Mackenzie 1996). In order to mitigate this trend, oyster restoration projects have attempted to restore degraded populations. Recently, restoration goals have begun to shift away from the creation of harvestable populations toward improving habitat health and mitigating the effects of coastal degradation through increased seston filtration, sediment stabilization, and provisioning of benthic habitat (Coen and Luckenbach 2000, Coen et al. 2007, Grabowski et al. 2012, Volety et al. 2014).

Previous research on restored oyster reefs has found that the recovery of certain ecosystem services begins directly following recruitment, with reef-dependent faunal communities changing within one year of restoration (Dillon et al. 2015, Humphries et al. 2011, Humphries and La Peyre 2015, La Peyre et al. 2014, Pierson and Eggleston 2014, Rezek et al. 2017). Specifically, within the timeframe of weeks following restoration, oyster recruitment can begin and a linear increase in filtration capacity was observed on restored reefs in Louisiana (La Peyre et al. 2014). Within just five months, restored reefs in Texas displayed similar nutrient availability and food chain length as natural reefs (Rezek et al. 2017). Within six months to a year, fish abundances on restored reefs in North Carolina were found to be similar to that of 4- to

6-year-old restored reefs (Pierson and Eggleston 2014). By one to two years post-restoration, restored reefs can attract more diverse and evenly distributed resident macrofauna communities that are comparable to natural reefs (Dillon et al. 2015, La Peyre et al. 2014, Rezek et al. 2017). Despite the numerous studies on the impacts of restoration on habitat ecosystem services, very little is understood about the recovery of other services such as benthic-pelagic coupling and enhanced biogeochemical cycling.

Oyster reefs exert significant control over nutrient cycling in coastal waters by grazing on phytoplankton and often facilitating higher rates of denitrification than many other estuarine habitats (Dame et al. 1984, Kellogg et al. 2013, Smyth et al. 2013, Smyth et al. 2015). As ecosystem engineers, oysters can sequester large amounts of suspended organic matter and deposit it in the underlying sediment, thereby developing a coastal biogeochemical hot spot, which is an area with high rates of biogeochemical cycling and increased storage of biologically important elements (Chambers et al. 2017, Dame et al. 1989, McClain et al. 2003). Oysters reduce suspended sediment, detritus, and phytoplankton in the water column through filter-feeding (Dame et al. 1984, Nelson et al. 2004, Newell et al. 2007), which is then excreted in the form of feces and pseudofeces, collectively called biodeposits. These mucus-bound biodeposits can be buried within the sediment, transformed or recycled by sediment microbial communities, flux back into the water column, or be assimilated by grazers and microbes in the benthic environment (Dame 1999, Newell et al. 2005). Biodeposition supplies labile nutrients for microbially-mediated transformations of carbon (C), nitrogen (N) and phosphorus (P) (Chambers et al. 2017, Newell et al. 2005, Smyth et al. 2016). The availability of labile nutrients can promote coupled nitrification/denitrification in sediments both on and adjacent to reefs,

depending on the habitat context (Hoellein et al. 2015, Humphries et al. 2016, Pollack et al. 2013, Smyth et al. 2013).

Despite the growing acknowledgement of the role of oyster reefs in estuarine nutrient cycling, there have been few investigations into how oyster reef restoration can impact sediment physical characteristics, as well as nutrient concentrations (Chambers et al. 2017, Kellogg et al. 2013, Southwell et al. 2017). Specifically, there is a need to improve the understanding of the rapid (first year post-restoration) response of oyster reef sediment biogeochemistry to restoration, and how the response relates to metrics of oyster reef development (e.g., oyster density, shell length, and reef height). This study sought to fill this knowledge gap by monitoring the progression of biogeochemical properties on intertidal oyster reefs in central Florida during the transition from a dead reef to a one-year-old restored reef. Changes in three treatment groups – dead reefs, natural reefs, and restored reefs – were measured in a Before-After-Control-Impact (BACI) study. The enhancement of sediment biogeochemical properties in restored reefs is hypothesized to occur during the first year of oyster growth based on prior work in this system by Chambers et al. (2017), which indicated dramatic changes in extractable (bioavailable) and total nutrient pools occur during the first year post-restoration.

The goal of this study was to quantify the change in sediment nutrients in the twelve-months immediately following restoration and to correlate the effect of oyster growth on C, N and P nutrient pools on control reefs with dead reefs as a negative control and natural reefs as a positive control. This research was based on the following predictions: (1) all sediment nutrient pools at the restored sites will be similar to dead reefs prior to restoration but will increase to concentrations comparable to natural reefs by the end of the first year. However, extractable nutrient pools will increase most rapidly (within 6 months post-restoration) in response to

increased deposition of labile nutrients, from live oysters and the water column, while total nutrient pools will increase more slowly, reaching the levels of natural reefs by 12 months post-restoration; (2) the density of live oysters will be a better predictor of sediment nutrient concentrations than average shell length or reef height. Monitoring the development of reef biogeochemical properties soon after restoration is crucial to understanding the ecological impacts and timescale in which restoration can replace the ecosystem services provided by oyster reefs.

Methods

Site Description and Restoration History

This study was conducted in Mosquito Lagoon, a shallow (average 1.7 m deep), microtidal body of saline water separated from the Atlantic Ocean by mangrove-dominated barrier islands that are surrounded by oyster reefs (Smith 1993). Circulation is driven by both wind and tidal exchange, water temperatures can range from 4 to 33° C, and salinities can range from 22.6 to as high as 45.2 ppt due to long water residence times (Phlips et al. 2015, Sheng and Davis 2003). Historically, Mosquito Lagoon contained an abundance of intertidal reefs colonized by *Crassostrea virginica*, but many of these reefs have experienced degradation in the past several decades due to year-round boating activity (Grizzle et al. 2002, Wall et al. 2005). Reefs located along popular boating channels develop dead margins composed of disarticulated shell piles on their seaward side that accumulate as oyster clusters become dislodged by boat wakes (Garvis et al. 2015). Within the boundaries of Canaveral National Seashore, where the southern reefs for this study are located, 40% of oyster reef coverage has been lost since 1943 (Garvis et al. 2015).

Since 2007, there has been a large community-based restoration effort to restore the affected margins and dead reefs. The restoration process involves leveling piles of shell in the selected area to a low intertidal height equal to the elevation of adjacent live oyster clusters. Mats with attached oyster shells are deployed over the leveled area and held in place with cement weights. This layer of stabilized shells provides substrate for natural oyster larvae to recruit and form a restored section of reef. As of summer 2018, 3.25 acres of reef area have been restored on 89 individual reefs (L.J. Walters, pers. comm.).

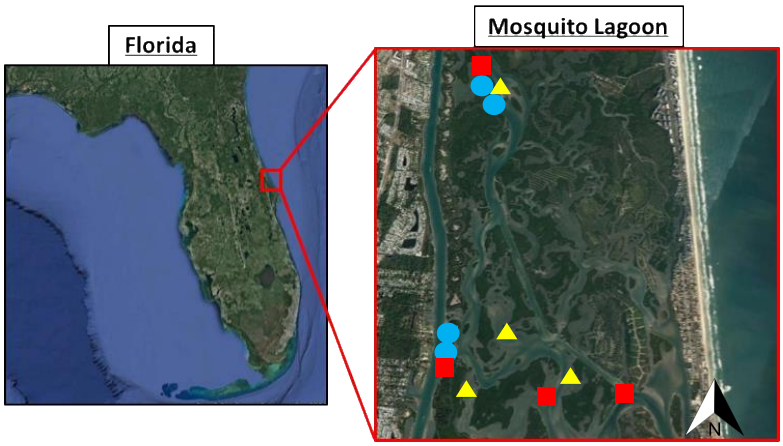

Figure 2: Reef study sites in Mosquito Lagoon, FL. Locations of dead reefs are marked in red, natural reefs in green, and restored reefs in blue.

Experimental Design and Field Sampling

This study utilized a before-after-control-impact (BACI) design where samples were collected from twelve oyster reefs at timepoints before the restoration, post-leveling of the restored reefs, and one-week, one-month, six-months, nine-months, and twelve-months after restoration. Twelve reefs, four in each of three treatment groups were utilized for this study: dead, restored, and natural (Figure 2). Restored sites were selected based on obtaining the largest latitudinal spread of sites within Mosquito Lagoon's restoration area. Natural and dead reefs adjacent to restored reefs were selected to serve as positive and negative controls, while also

allowing for assessment of confounding environmental variables (e.g., seasonal shifts in water chemistry and temperature) at each restored reef.

Sample collection from all reefs started in May 2017 before restoration activities occurred. For the post-leveling timeframe, samples were collected only from the four restored reefs to observe effects of the perturbation of surface layer sediments by the removal of dead shell and decrease in reef elevation. Restoration occurred in June 2017 and samples were collected from all twelve reefs at one-week, one-month, six-months, nine-months, and twelve-months post-restoration. For each sampling, four replicate sediment samples from each reef were collected at haphazardly selected points within the intertidal zone of the reef over the course of two consecutive days during low tide (± 3 hours). Large pieces of shell (> 3 cm diameter) that interfered with penetration of the coring tube were removed from the surface before coring. On restored reefs, oyster mats were pulled back by hand in order to access the sediment below. Each core was collected using the push core method with a beveled 7 cm diameter polycarbonate tube. Each sample consisted of two 0-5 cm cores collected within 0.5 m from each other and composited into one sample. The cores were field-extruded, placed into sterile Whirl-Pak bags, placed on ice, and transported to the laboratory. At the time of sampling, one surface water grab sample was collected from the top 10 cm of the water column 2-4 m adjacent to the reef in a 500 mL acid-washed Nalgene bottle and immediately placed on ice. At every reef, a handheld sonde was used to record surface water temperature, pH, dissolved oxygen (DO), salinity and conductivity at a 10 cm depth (ProDSS, YSI Inc., Yellow Springs, OH, USA).

Biophysical oyster monitoring data were collected at the same locations where sediment samples were collected once countable oysters appeared on the restored reefs (starting six-months post-restoration). To accomplish this, marking flags were placed at the exact location of

each sediment sample during the six-, nine- and twelve-month timeframes to identify the area for biophysical measurements. Within four days of sediment collection, these sites were revisited and 0.25 m^2 quadrats were placed directly on top of flagged locations. The number of live oysters, shell lengths of the first fifty oysters, and reef height (of the tallest point from the reef sediment and five additional random points) were recorded in each quadrat.

Surface Water Properties

Upon return to the laboratory, surface water samples were vacuum filtered through a 0.45 μm membrane filter and acidified with distilled, deionized H_2SO_4 to a pH < 2. Samples were stored at 4 °C until analysis for nitrate + nitrite (hereafter referred to as NO_3^-), ammonium (NH_4^+), soluble reactive phosphorus (SRP) and dissolved organic carbon (DOC). Concentrations of NO_3^-, NH_4^+ and SRP were determined colorimetrically on a Seal AQ2 Automated Discrete Analyzer (Seal Analytical, Mequon, WI) using EPA methods 353.2 Rev. 2.0, 350.1 Rev. 2.0, and 365.1 Rev. 2.0, respectively (USEPA 1993). A Shimadzu TOC-L Analyzer (Shimadzu Scientific Instruments, Kyoto, Japan) was used to measure the concentration of nonpurgeable DOC.

Sediment Physicochemical Properties

Upon return from the field, sediment samples were weighed and homogenized by hand in the laboratory. All samples contained some amount of shell; any shell fragments > 2 cm diameter were excluded in sample processing. Sediment bulk density was determined using the combined volume of the two replicate 0-5 cm cores. Moisture content was determined by weighing a ~50 g subsample in an aluminum tin and drying at 70 °C in a gravimetric drying oven for at least 3 days until a constant weight was achieved. Soil pH was determined by mixing field moist sediment with DI water in a 1:5 ratio by mass, allowing the slurry to equilibrate at room

temperature for 30 min, and then measuring the pH of the solution with an Accumet benchtop pH probe (Accumet XL200, Thermo Fisher Scientific, Waltham, MA, USA).

Sediment Nutrient Pools

Extractable pools of nutrients comprise the inorganic and bioavailable forms of nutrients in the porewater and adsorbed to the surface of sediment particles removed by the addition of salts. Extractable nutrient pools were determined within 72 h of collection for NO_3^-, NH_4^+, SRP and DOC by placing 3-4 g of sediment into a 40 mL centrifuge tube. Two M KCl was added for NO_3^-, NH_4^+ and SRP extraction, and 0.5 M K_2SO_4 for DOC extraction, and the samples were agitated on an orbital shaker at 100 rpm for 1 h. Samples were then centrifuged at 4000 rpm at 10 °C for 10 min. The supernatant was filtered through a Supor 0.45 μm filter (Pall Corporation, Port Washington, NY), acidified with distilled, deionized H_2SO_4 to a pH < 2 and stored at 4 °C. Subsequent analysis for NO_3^-, NH_4^+ and SRP was performed on the Seal AQ2 using EPA methods 353.2 Rev. 2.0, 350.1 Rev. 2.0, and 365.1 Rev. 2.0, respectively (USEPA 1993). Nonpurgeable DOC concentration was determined using the Shimadzu TOC-L Analyzer.

Total nutrient pools include both inorganic and organic nutrients adsorbed and occluded with sediment particles. After sediment samples were dried, they were ground in a stainless-steel ball mill container with a SPEX Sample Prep 8000M Mixer/Mill (SPEX Certiprep, Metuchen, NJ). Ground subsamples were used to determine total C and N on a Vario Micro Cube CN Analyzer (Elementar Americas Inc., Mount Laurel, NJ). A subsample of dried, ground sediment was also combusted at 550 °C for 5 h to determine organic matter content via loss-on-ignition. Following loss-on-ignition analysis, solid-phase total P was determined by boiling the resulting ash in 1 M HCl on a hot plate for 1 h and filtering through Whatman #41 filter papers (Anderson

1976). Samples were then analyzed for total P on a Seal AQ2 via method 365.1 Rev. 2.0 (USEPA 1993).

To determine the relative amounts of organic versus inorganic carbon, 15 subsamples were chosen based on previously measured total C content: 5 with relatively low total C values (11.8 - 36.4 g C kg^{-1}), 5 with mid-range values (51.9 - 75.6 g C kg^{-1}), and 5 with relatively high values (86.2 - 110.4 g C kg^{-1}). These samples were re-run for total C using the Vario Micro Cube CN Analyzer following combustion at 550 °C for 5 h to determine total inorganic C. The inorganic C measurement was subtracted from the non-burn total C measurement to determine the organic C fraction.

Statistical Analyses

Data analysis was performed in R version 3.5.1 (R Core Team 2018) using R Studio (R Studio Team 2016). Linear mixed-effects models were used for nutrient pools and physicochemical data with the command 'lmer' in the lme4 package (Pinheiro et al. 2016). The interaction of treatment (dead, natural or restored reef) and sampling time were applied as fixed effects, and individual reef was a random effect. Homogeneity of variance was assessed using Levene's tests and normality of the model residuals was assessed by visually inspecting Q-Q plots. P-values were obtained from the models by using the lmerTest package and running ANOVA on the models (Kuznetsova et al. 2017). A least squares means post hoc test was used to identify significant differences among the pairwise comparisons between the treatments and between the time frames using the package lsmeans (Lenth 2016). All results were considered statistically significant at $\alpha = 0.05$. Additionally, principal component analysis was utilized to assess the variability between individual reefs with the package FactoMineR (Le et al. 2008). In order to assess the effect of geography on sediment properties, Google Earth (Google, 2019) was

utilized to calculate distance to the nearest inlet and channel width for each reef. Distance to the nearest inlet was calculated using lines drawn through major boating channels and channel width was calculated as the nearest shoreline perpendicular to the reef.

Correlation tables were used to assess the strength of correlations between sediment nutrients and physicochemical parameters of all treatments, as well as between sediment properties and geographical parameters. A separate correlation table was also produced for the correlations of sediment properties with biophysical data at the six-, nine- and twelve-months samplings when oyster reef biophysical data was collected. All correlation tables were computed with Microsoft Excel and the critical value was calculated using the r-statistic to assess the significance of the coefficients at $\alpha = 0.05$ and $\alpha = 0.01$.

Results

Surface Water Properties

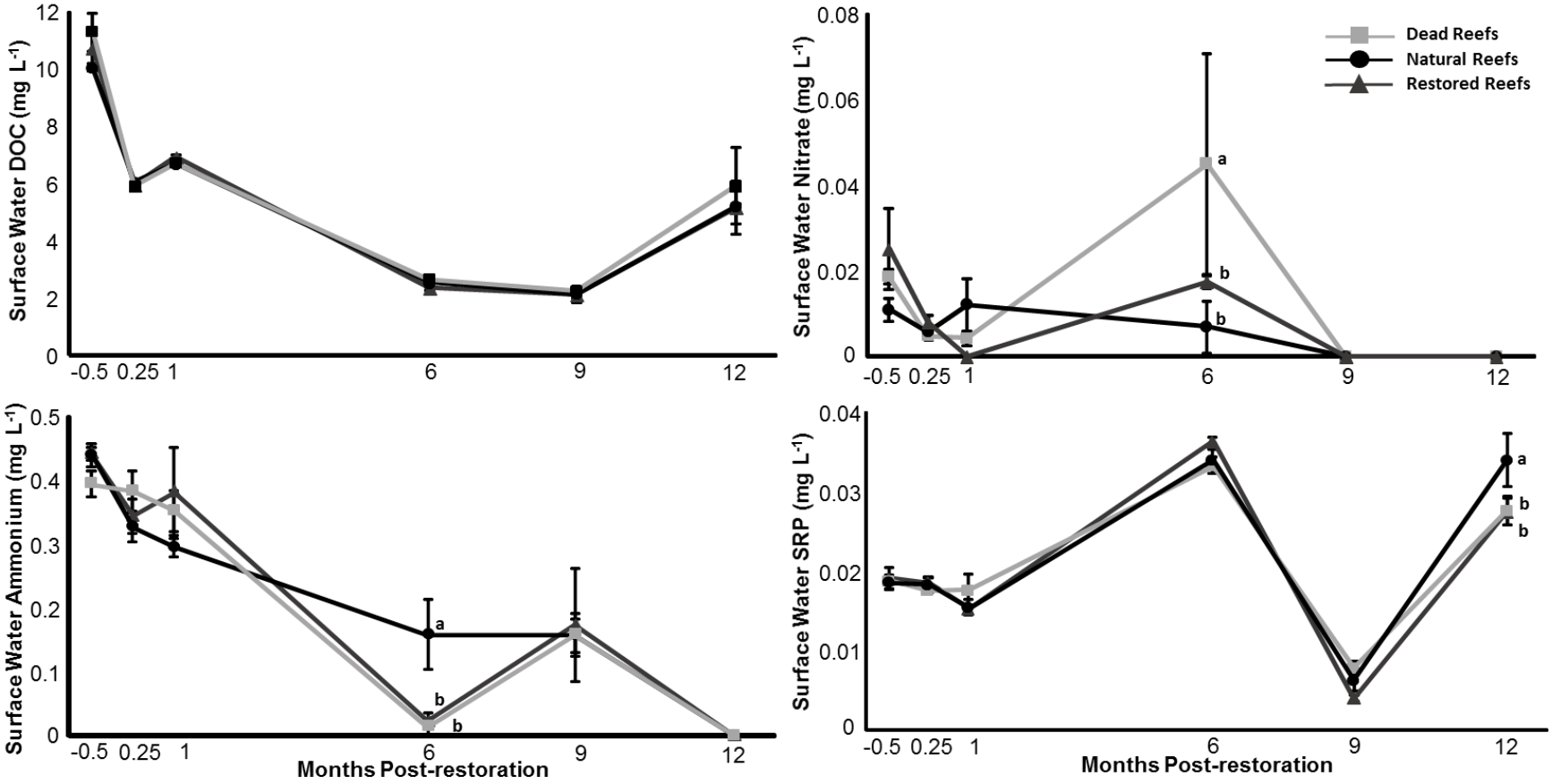

Figure 3: Surface water nutrients over time measured from the top 10 cm of the water column 2–4 m adjacent to dead, natural, and restored reefs. Error bars indicate standard error and letters denote significantly different means ($p \leq 0.05$) between treatments according to a post hoc least squares means pairwise comparison.

The linear mixed effects model for surface water nutrients showed that the fixed effect of time was a significant predictor for all water quality and nutrient parameters measured ($p < 0.001$) and treatment was not significant for any of these parameters (Table 1). Post hoc tests showed significant differences between treatments at one sampling each for surface water NO_3^-, NH_4^+, and SRP (Appendix Table 1).

The random effect of reef was only significant for the variables of temperature, % DO, salinity, and SRP (Table 2). DOC concentrations changed on every reef from the before to one-week, one-month to six-months, and nine-months to twelve-months sampling times (Appendix Table 2). Surface water NO_3^- concentrations were consistently near the detection limit or below detection (BD) for the one-month, nine-months and twelve-months time frames (detection limit 0.003 mg NO_3^- L^{-1}). During the six-months timeframe, NO_3^- was higher in the dead reef samples (0.05 ± 0.03 mg NO_3^- L^{-1}) (mean ± standard error) than in the natural (0.01 ± 0.01 mg NO_3^- L^{-1}) and restored reef samples (0.02 ± 0.002 mg NO_3^- L^{-1}) (Figure 3). Surface water NH_4^+ averaged 0.22 ± 0.02 mg L^{-1} throughout the study, showed a general decrease to an average of 0.11 ± 0.02 mg L^{-1} between the winter samplings (six-months and nine-months), and was below detection at both six-months and twelve-months post-restoration (detection limit 0.07 mg NH_4^+ L^{-1}). Surface water SRP concentrations changed for every reef from one-month to six-months, six-months to nine-months, and nine-months to twelve-months post-restoration (Appendix Table 2). SRP concentrations averaged $0.020 \pm .001$ mg L^{-1} on all reefs throughout the study, were highest on all reefs at six-months ($0.035 \pm .001$ mg L^{-1}) and twelve-months ($0.030 \pm .002$ mg L^{-1}) post-restoration, and lowest at nine-months post-restoration ($0.006 \pm .001$ mg L^{-1}). The average temperature during the summer samplings was 29.0 ± 0.3 ºC and during the winter samplings was 16.5 ± 0.3 ºC. The average % DO in surface waters was 94.1 ± 1.9 % over twelve months

and was not different between treatments (Appendix Table 1). The only change over time occurred on restored reefs from one-month (95.7 ± 0.3 %) to six-months (64.2 ± 5.3 %) and six-months to nine-months (105.3 ± 2.1 %) post-restoration (Appendix Table 2). Conductivity measurements for all reefs was 61.4 ± 424 ms cm^{-1} from before to one-month post-restoration and then decreased for all three treatments to 50.3 ± 191 ms cm^{-1} from six-months to twelve-months post-restoration (Appendix Table 2). Average salinity was 37.0 ± 0.6 ppt for all reefs over twelve months (Figure 3). There were no differences between treatments, but salinity changed between every timeframe for all three treatments (Appendix Table 2), with a high of 44.6 ± 0.1 ppt on all reefs before restoration (June 2017) and a low of 29.7 ± 0.2 ppt at twelve-months post-restoration (June 2018). Surface water pH did not change over time and averaged 7.96 ± 0.03 for all reefs over twelve months (Appendix Table 2). pH was lowest on all reefs at one-week post-restoration (7.84 ± 0.03) and highest at twelve-months post-restoration (8.28 ± 0.05) (Figure 3). There were no correlations of surface water quality or nutrients data with sediment properties except for a negative correlation between surface water SRP and both sediment pH and NO_3^- (Appendix Table 1).

Table 2: P-values from the results of linear mixed effects models of all water quality parameters. The effects of treatment, time, interaction of treatment with time, and the random effect of individual reef were calculated using the package lmerTest and an ANOVA on each model. Values in bold denote significance at $p \leq 0.05$ and values in bold and italics denote significance at $p \leq 0.01$.

	Temp	DO	Cond	Salinity	pH	DOC	NO_3^-	NH_4^+	SRP
Treatment	0.683	0.503	0.228	0.141	0.746	0.527	0.157	0.808	0.722
Time	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***
Treatment:Time	0.085	***0.005***	0.717	0.541	0.288	0.867	**0.012**	0.109	***0.002***
Reef	***0.003***	***0.005***	0.154	**0.029**	0.143	0.273	1	0.803	***0.006***

Temp - temperature, DO - % dissolved oxygen, Cond - conductivity, DOC - dissolved organic carbon, NO_3^- - nitrate, NH_4^+ - ammonium, SRP - soluble reactive phosphorus

Sediment Physicochemical Properties

Table 3: P-values from the results of linear mixed effects models of all sediment properties measured. The effects of treatment, time, interaction of treatment with time, and the random effect of individual reef were calculated using the package lmerTest and an ANOVA on each model. Values in bold denote significance at $p \leq 0.05$ and values in bold and italics denote significance at $p \leq 0.01$.

	BD	pH	DOC	NO_3^-	NH_4^+	SRP	OM	TC	TN	TP
Treatment	0.421	0.224	0.194	0.806	0.082	**0.0497**	0.079	0.124	**0.049**	***0.004***
Time	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	0.070	***<0.001***	***<0.001***
Treatment:Time	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***0.004***	**0.045**	***<0.001***	**0.032**	***<0.001***
Random Reef	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***

BD - bulk density, DOC - dissolved organic carbon, NO_3^- - nitrate, NH_4^+ - ammonium, SRP - soluble reactive phosphorus, OM - organic matter, TC - total carbon, TN - total nitrogen, TP – total phosphorus

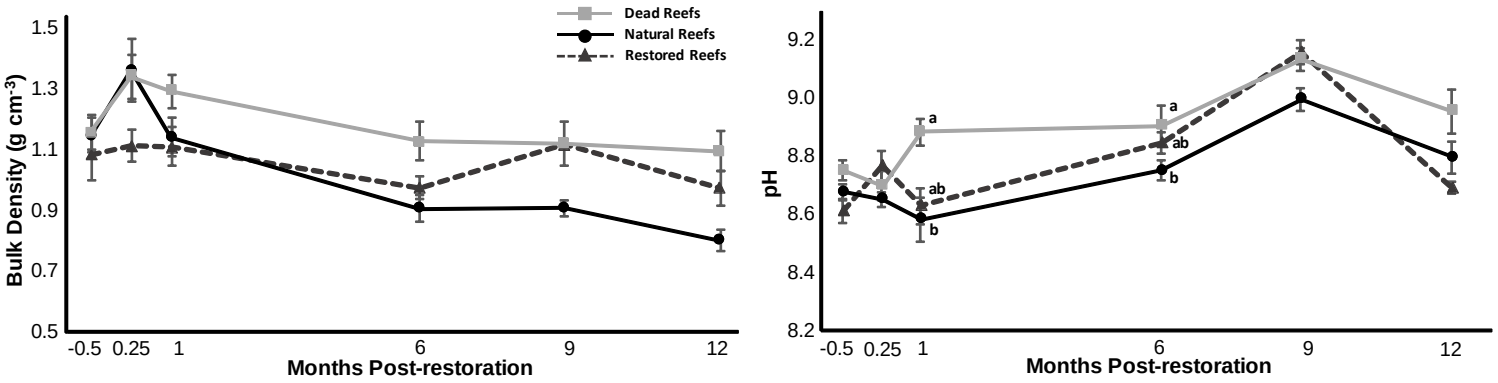

Figure 4: Bulk density and sediment pH over time in dead, natural, and restored reefs. Error bars indicate standard error and letters denote significantly different means ($p \leq 0.05$) between treatments according to a post hoc least squares means pairwise comparison.

There were no significant differences between treatments for sediment bulk density (Table 4) but the restored and natural reefs both showed changes over time around the restoration. Bulk density was lower ($p < 0.001$) in the restored reefs after leveling, changing from a mean of 1.08 ± 0.08 g cm^{-3} before leveling to 1.04 ± 0.06 g cm^{-3} after leveling. Bulk density changed again in the restored reefs after the deployment of oyster mats, increasing by a total of 8 % from 1.04 ± 0.06 g cm^{-3} to 1.11 ± 0.05 g cm^{-3} one-week after this disturbance. Bulk density on natural reefs also changed from before restoration to one-week post-restoration by a total increase of 19 % (Table 5). Bulk density was negatively correlated with organic matter, total C and total N (Appendix Table 3). Time, the interaction of treatment with time, and the random effect of reef were significant predictors of sediment bulk density and pH (Table 3). Treatment was not a significant predictor for either physicochemical property (Table 3).

Sediment pH changed in all reef types from six- to nine-months and from nine- to twelve-months post-restoration (Table 5). Natural reef sediments generally showed lower pH levels (8.79 ± 0.21) than dead reefs (8.92 ± 0.22) throughout the study and were significantly lower than dead reefs at both the one-month and six-months sampling times (Table 4). pH was negatively correlated with organic matter and total N (Appendix Table 3).

Extractable Nutrient Pools

The random effect of reef was very significant for all extractable nutrients (Table 3). The main effect of treatment was only a significant predictor for extractable SRP (Table 3), but the interaction of treatment with time was a significant predictor for all the extractable nutrients (Table 3).

Dead and natural reef DOC concentrations were not statistically different throughout the study (Table 4) and averaged 0.083 ± 0.004 g DOC kg^{-1} in dead reef sediments and 0.095 ± 0.004 g DOC kg^{-1} in natural reef sediments (Figure 7). Restored reef DOC concentrations decreased after the leveling of loose shells and then started to increase between the one-month and six-months samplings (Table 5). This trend continued between six months and nine months (Table 5) and DOC concentrations increased by a total of 60 % from before the restoration to the peak at nine months (Figure 7). Restored reefs became significantly higher than both dead and natural reefs at nine-months and twelve-months post-restoration (Table 4). Extractable DOC was positively correlated with total N (Appendix Table 3).

Throughout the study, most sediment samples had generally low extractable NO_3^- concentrations (< 0.1 mg NO_3^- kg^{-1}) that were just above the minimum detection limit of 0.015 mg NO_3^- kg^{-1}. Most measurements were BD during both the one-month and six-months samplings. Restored reef NO_3^- concentrations changed ($p < 0.001$) from 1.74 ± 0.29 mg NO_3^- kg^{-1} before the restoration to 0.84 ± 0.04 mg NO_3^- kg^{-1} after the leveling of reefs (Table 5). Both dead and restored reefs showed a similar pattern of change from before restoration to one-week, increasing between six and nine months, and decreasing from nine- to twelve-months post-restoration (Table 5, Figure 5). Natural reef NO_3^- concentrations were more stable through time and only changed significantly from six- to nine-months post-restoration (Table 5). Extractable NO_3^- was positively correlated with extractable SRP (Appendix Table 3).

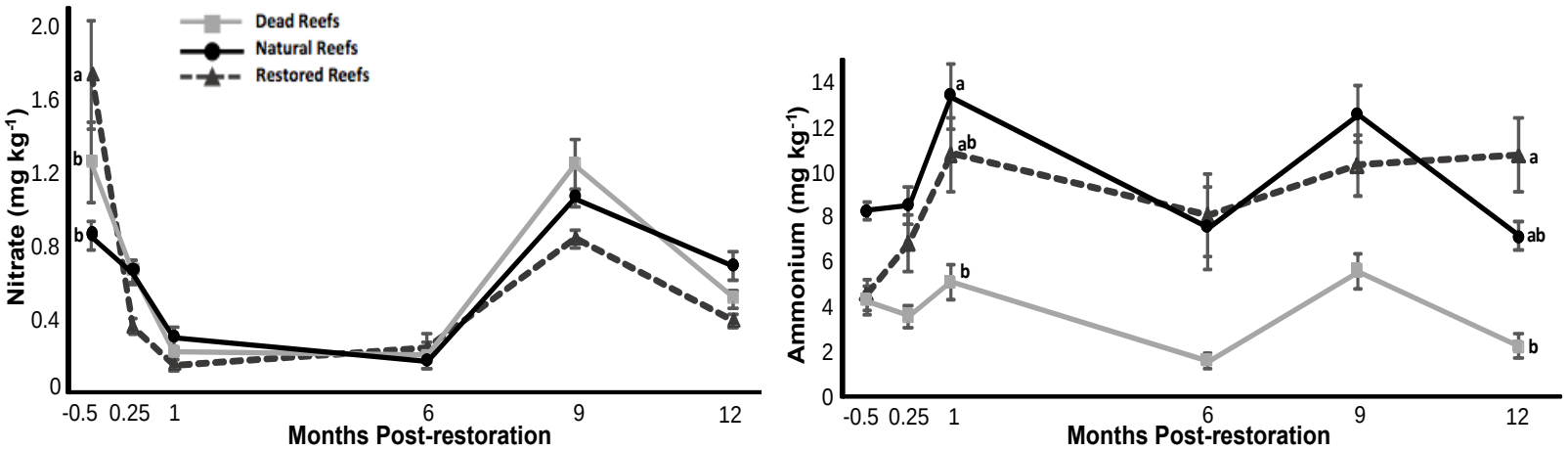

Figure 5: Sediment NO_3^- and NH_4^+ pools over time in dead, natural, and restored reefs. Error bars indicate standard error and letters denote significantly different means ($p \leq 0.05$) between treatments according to a post hoc least squares means pairwise comparison.

After the oyster mats were deployed, extractable NH_4^+ concentrations generally increased in restored reefs and changed significantly between one-week to one-month post-restoration from an average of 6.83 ± 1.20 to 10.78 ± 1.61 g NH_4^+ kg^{-1} (Figure 5). However, both restored and natural reefs also changed significantly during this time with a 58 % increase in restored reef sediments and a 57 % increase in natural reef sediments. From one-month onwards, restored reef NH_4^+ concentrations did not change over time and averaged 9.98 ± 0.81 g NH_4^+ kg^{-1} from one-month to twelve-months post-restoration. They remained similar to natural reefs which averaged 10.16 ± 0.77 g NH_4^+ kg^{-1} and consistently higher than dead reefs which averaged 3.63 ± 0.38 g NH_4^+ kg^{-1}. Natural reef extractable NH_4^+ concentrations were the most variable over time, changing between each sampling time from one-week onwards (Table 5). Sediment NH_4^+ on all reefs was positively correlated with extractable DOC, OM, total N and total P (App. Table 3).

Extractable SRP was generally higher in dead reef sediments, which averaged 1.29 ± 0.08 g PO_4^{3-} kg^{-1} throughout the study, compared to natural reef sediments with an average of 0.51 ± 0.05 g PO_4^{3-} kg^{-1} (Figure 11). Dead and natural reefs were significantly different at one-month, six-months and nine-months post-restoration (Table 4). Restored reef sediments generally decreased from an average of 1.55 ± 0.24 g PO_4^{3-} kg^{-1} before the restoration, to 1.19 ± 0.16 g PO_4^{3-} kg^{-1} at twelve-months post-restoration (Figure 11). Restored reef SRP concentrations were higher than natural reefs before the restoration and then were similar to natural reef levels for all post-restoration samplings (Table 4). Extractable SRP was positively correlated with total C (Appendix Table 3).

Table 4: Results of post hoc least squares means pairwise comparisons between treatments. Values in bold denote significance at $p \leq 0.05$ and values in bold and italics denote significance at $p \leq 0.01$.

		BD	pH	DOC	NO_3^-	NH_4^+	SRP	OM	TC	TN	TP
Before	Dead - Natural	0.996	0.815	0.999	**0.064**	0.223	0.245	0.171	0.814	0.109	**0.024**
Before	Dead - Restored	0.833	0.416	0.995	**0.029**	0.997	0.590	0.872	0.055	0.828	0.912
Before	Natural - Restored	0.873	0.777	0.988	***<.001***	0.252	**0.040**	0.361	0.161	0.269	**0.011**
1 Wk	Dead - Natural	0.988	0.904	0.742	1.000	0.144	0.257	0.324	0.975	0.113	**0.023**
1 Wk	Dead - Restored	0.224	0.816	0.875	0.231	0.441	0.962	1.000	**0.043**	0.992	0.416
1 Wk	Natural - Restored	0.178	0.562	0.452	0.225	0.729	0.381	0.314	0.066	0.094	**0.002**
1 Mo	Dead - Natural	0.497	**0.041**	0.714	0.913	**0.019**	**0.012**	0.059	0.944	0.073	***0.005***
1 Mo	Dead - Restored	0.369	0.089	0.981	0.894	0.082	0.341	0.929	**0.018**	0.966	0.468
1 Mo	Natural - Restored	0.969	0.913	0.823	0.649	0.715	0.208	**0.029**	**0.033**	0.110	***0.001***
6 Mo	Dead - Natural	0.253	**0.041**	0.932	0.968	0.107	***0.008***	**0.049**	0.807	**0.031**	***0.008***
6 Mo	Dead - Restored	0.496	0.089	0.138	0.964	0.081	0.330	0.782	0.485	0.341	0.919
6 Mo	Natural - Restored	0.877	0.913	0.074	0.869	0.987	0.153	0.159	0.206	0.324	**0.017**
9 Mo	Dead - Natural	0.237	0.405	0.544	0.587	0.053	**0.015**	0.118	0.800	**0.041**	***0.005***
9 Mo	Dead - Restored	1.000	0.980	***<.001***	0.063	0.199	0.577	0.482	0.982	0.677	0.460
9 Mo	Natural - Restored	0.241	0.311	**0.003**	0.397	0.718	0.111	0.611	0.697	0.169	**0.044**
12 Mo	Dead - Natural	0.101	0.358	0.901	0.676	0.147	0.226	**0.034**	0.990	**0.015**	***0.002***
12 Mo	Dead - Restored	0.637	0.076	***0.008***	0.679	**0.018**	0.655	0.204	0.711	0.326	0.215
12 Mo	Natural - Restored	0.424	0.621	**0.020**	0.206	0.512	0.692	0.571	0.629	0.185	**0.044**

Table 5: Results of post hoc least squares means pairwise comparisons between sampling times. Values in bold denote significance at $p \leq 0.05$ and values in bold and italics denote significance at $p \leq 0.01$. PL denotes the post-leveling sampling time where only restored reefs were sampled after the displacement of shell to level the area.

Reef	Time pair	BD	pH	DOC	NO_3^-	NH_4^+	SRP	OM	TC	TN	TP
Restored	Before – PL	***<.001***	0.983	***<.001***	***<.001***	1.000	0.083	1.000	1.000	1.000	1.000
Restored	1 Week – PL	***<.001***	0.612	1.000	**0.022**	0.661	1.000	0.920	1.000	0.610	1.000
Restored	1 Wk – 1 Mo	1.000	0.291	1.000	0.712	**0.010**	1.000	0.074	0.499	1.000	0.973
Restored	1 Mo – 6 Mo	0.477	1.000	***<.001***	0.987	0.073	0.625	0.996	**0.022**	***<.001***	***<.001***
Restored	6 Mo – 9 Mo	0.398	***<.001***	**0.015**	***0.001***	0.446	0.282	0.994	0.452	**0.039**	1.000
Restored	9 Mo – 12 Mo	0.381	***<.001***	0.060	**0.025**	1.000	1.000	0.844	0.773	0.681	0.977
Dead	1 Wk – Before	0.124	0.975	**0.042**	***<.001***	0.997	1.000	1.000	1.000	1.000	0.224
Dead	1 Wk – 1 Mo	0.994	0.055	0.785	0.059	0.892	**0.030**	**0.013**	0.968	0.994	0.926
Dead	1 Mo – 6 Mo	0.215	1.000	0.154	1.000	0.092	**0.025**	0.758	0.984	0.868	0.784
Dead	6 Mo – 9 Mo	1.000	***0.001***	0.071	***<.001***	**0.035**	0.768	0.632	1.000	0.866	0.503
Dead	9 Mo – 12 Mo	1.000	**0.050**	1.000	***<.001***	0.121	1.000	1.000	1.000	1.000	1.000
Natural	1 Wk – Before	**0.040**	0.998	0.615	0.802	1.000	1.000	0.983	0.958	1.000	0.195
Natural	1 Wk – 1 Mo	**0.033**	0.935	0.844	0.147	***0.004***	**0.013**	***<.001***	0.926	1.000	0.979
Natural	1 Mo – 6 Mo	**0.018**	1.000	1.000	0.967	***<.001***	0.998	0.839	0.915	0.095	0.992
Natural	6 Mo – 9 Mo	1.000	***<.001***	1.000	***<.001***	***<.001***	0.455	0.132	1.000	0.563	0.926
Natural	9 Mo – 12 Mo	0.866	**0.027**	0.958	0.111	***<.001***	0.254	0.810	0.891	0.813	0.976

Total Nutrient Pools

The random effect of reef was significant for every total nutrient measured (Table 3). Treatment was a significant predictor for total N and total P. Total C was the only sediment property for which time was not a significant predictor. The interaction of treatment with time had a significant effect on all total nutrients (Table 2).

Organic matter (OM) content was generally higher in natural reefs compared to dead reefs with an average of 0.95 ± 0.03 g OM kg^{-1} for natural reefs and 0.55 ± 0.03 g OM kg^{-1} for dead reefs (Figure 8). Both dead and natural reefs increased significantly from one-week to one-month post-restoration and then fell back to starting levels (Table 5). Restored reef sediments averaged 0.63 ± 0.03 g OM kg^{-1} over twelve months and were only significantly lower than natural reefs at one-month post-restoration (Table 4). After this time, restored reef sediments generally increased in OM content and approached natural reef levels, reaching 0.80 ± 0.08 g OM kg^{-1} by twelve-months post-restoration compared to 0.98 ± 0.05 g OM kg^{-1} in natural reef sediments (Figure 8). OM content was positively correlated with total N and total P (Appendix Table 3).

For total C pools, dead reef sediments maintained a consistent average of 54.6 ± 2.1 g C kg^{-1}, which was nearly the same as the natural reef average of 53.9 ± 1.6 g C kg^{-1} throughout the study (Figure 8). Total C concentrations on restored reefs were generally higher than that of both dead and natural reefs from before the restoration to one-month post-restoration (Table 4). Total C decreased on restored reefs from one-month to six-months post-restoration (Table 5) and a total of 12 % from before the restoration to twelve-months post-restoration. Total C was negatively correlated with bulk density and positively correlated with SRP (Appendix Table 3).

Table 6: Results of organic and inorganic carbon measurements on 15 selected samples. Total carbon is represented in grams per kilogram of sediment and the individual fractions are expressed as a percentage of total carbon.

High Carbon	**Time**	**TC (g kg^{-1})**	**% OC**	**% IC**
Live 1C	Before	86.2	17	83
Restored 1A	Before	90.3	2	98
Dead 1C	9 Months	93.6	17	83
Restored 4D	1 Month	93.9	21	79
Restored 3B	1 Week	110.4	0	100
Medium Carbon				
Restored 2C	1 Month	51.9	2	98
Restored 3C	9 Months	52.0	4	96
Dead 3C	1 Month	55.8	30	70
Dead 1C	6 Months	63.2	14	86
Live 1B	6 Months	75.6	20	80
Low Carbon				
Dead 4B	1 Week	11.8	26	74
Live 3A	6 Months	17.7	72	28
Dead 4D	Before	19.2	27	73
Live 3C	1 Week	31.1	39	61
Live 2D	9 Months	36.4	0	100

An analysis of 15 sediment samples that represented low, medium and high ranges of total C concentrations and every sampling time showed that oyster reef sediments were composed of a large portion of inorganic C. The selected samples contained an overall average of 81 % inorganic C and 19 % organic C. The high total C samples contained the highest average inorganic C at 91 %, the mid-range contained 86 % average inorganic C, and the low total C samples had the lowest at 67 %. Conversely, % organic C was lowest in the high total C samples (11 %), higher in the mid-range total C (14 %), and highest in the low total C samples (33 %).

Total N concentrations were generally higher in natural reefs (1.40 ± 0.04 g N kg^{-1}) compared to dead reefs (0.58 ± 0.05 g N kg^{-1}) throughout the entire study and were significantly higher at six-months, nine-months, and twelve-months post-restoration (Figure 10, Table 4).

Restored reef sediments increased a total of 79 % from the one-month to six-month samplings (Table 5). From six-months onwards, restored reef sediments measured an average 0.98 ± 0.07 g N kg^{-1}, compared to 0.57 ± 0.07 in dead reef sediments and 1.52 ± 0.07 g N kg^{-1} in natural reef sediments (Figure 10). Total N was positively correlated with total P (Appendix Table 3).

Natural reef total P concentrations were significantly higher than both dead and restored reefs at every sampling (Table 4). Natural reefs averaged 0.76 ± 0.01 g P kg^{-1} and dead reefs averaged 0.46 ± 0.01 g P kg^{-1} throughout the study (Figure 10). Restored reef sediments maintained concentrations similar to dead reefs with an average of 0.46 ± 0.02 g P kg^{-1}. However, restored reef sediments increased by 48 % from one-month to six-months post-restoration (Table 5). Although restored reefs still differed from natural reefs at 9 months ($p = 0.044$) and twelve-months ($p = 0.044$), they began to approach natural reef levels during this time.

Correlation coefficients were also calculated across all of the sediment physicochemical and nutrient measurements for all time points (Appendix Table 3). Total pools of C and P were highly positively correlated. Total pools of C and N were not significantly correlated (Appendix Table 3). Total N was also significantly correlated with more parameters (eight total, seven significant at $p \leq 0.01$) than any other measured variable. Total C and total P were each significantly correlated with four other parameters. OM content was significantly correlated with seven other parameters, five of which were significant at $p \leq 0.01$.

Reef Geographical and Biophysical Variables

Table 7: Results of Pearson correlation coefficients between reef geographical parameters and sediment properties. For n = 48, a two-tailed test and $p < 0.05$ significance, the absolute value of the correlation coefficient must be > 0.285; for $p < 0.01$ the correlation coefficient must be > 0.368. This applies to all subsequent correlation tables. Values in bold denote significance at $p \leq 0.05$ and values in bold and italics denote significance at $p \leq 0.01$.

	Distance to Inlet (m)	**Channel width (m)**
BD	**0.334**	0.042
pH	0.276	0.189
NO_3^-	-0.158	-0.068
NH_4^+	**-0.323**	-0.194
SRP	**-0.353**	-0.001
DOC	***-0.419***	-0.207
OM	-0.222	-0.273
TC	***-0.618***	-0.004
TN	-0.215	-0.218
TP	0.084	-0.068
Water NH_4^+	0.016	-0.024
Water SRP	-0.081	-0.073
Water NO_3^-	-0.138	0.001

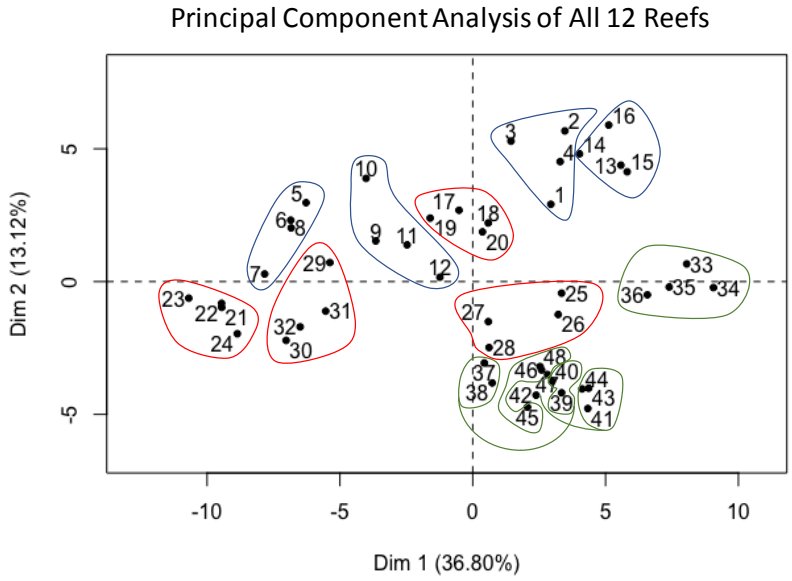

Figure 6: Results of principal component analysis of all 48 samples taken from oyster reefs (4 samples per reef, 4 reefs per treatment) for all sediment properties. Restored reefs are circled in blue, dead reefs in red, and natural reefs in green.

In order to evaluate the random effect of reef, correlation coefficients between the geographical properties of distance to the nearest inlet and channel width for individual reefs with sediment nutrients were calculated (Table 7). Sediment bulk density was positively correlated with distance to the inlet. Extractable NH_4^+, SRP, DOC, and total C were all negatively correlated with distance to the inlet.

Principal component analysis (PCA) provided further insight into the significant random effect of reef (Figure 6). PCA was performed on all sediment properties from all twelve reefs. In this PCA, dimensions one and two accounted for 49.92 % of variation in the data and samples showed a separation amongst the individual reefs and amongst the three treatments (Figure 5). The natural reef treatment showed the most similarity between its four separate reefs with three of those reefs grouped closely together (Figure 5).

Table 8: Pearson's correlation coefficients between sediment properties and reef biophysical characteristics. Values in bold denote significance at $p \leq 0.05$ and values in bold and italics denote significance at $p \leq 0.01$.

	Months	BD	pH	DOC	NO_3^-	NH_4^+	SRP	OM	TC	TN	TP
Oyster	6	***-0.468***	***-0.419***	0.043	-0.067	0.203	***-0.480***	***0.390***	0.014	***0.643***	***0.622***
Density	9	***-0.375***	-0.154	***0.519***	-0.195	0.229	-0.273	***0.548***	0.179	***0.488***	***0.465***
	12	***-0.425***	***-0.400***	***0.596***	0.132	***0.657***	-0.158	***0.531***	0.181	***0.540***	***0.512***
Reef	6	***-0.534***	***-0.414***	0.072	-0.057	**0.352**	***-0.599***	***0.368***	0.033	***0.715***	***0.650***
Height	9	**-0.351**	-0.197	***0.384***	-0.227	***0.462***	***-0.452***	***0.485***	-0.035	***0.538***	***0.548***
	12	***-0.460***	**-0.332**	***0.416***	0.186	***0.584***	-0.138	***0.593***	0.150	***0.577***	***0.575***
Shell	6	***-0.455***	***-0.485***	-0.091	-0.105	0.100	***-0.596***	***0.374***	-0.124	***0.634***	***0.559***
Length	9	***-0.463***	**-0.290**	0.119	0.033	**0.357**	***-0.614***	***0.645***	-0.066	***0.634***	***0.595***
	12	***-0.502***	**-0.303**	**0.346**	0.182	***0.469***	-0.245	***0.607***	0.216	***0.586***	***0.495***

Correlations of sediment nutrients with oyster biophysical parameters measured at six, nine and twelve months showed that sediment bulk density was negatively correlated with oyster density at all timeframes, with reef height at six- and twelve-months post-restoration, and with shell length at all time frames (Table 8). Sediment pH was negatively correlated with oyster density at both six- and twelve-months, with reef height only at six-months and twelve-months, and with shell length at all time frames.

Extractable DOC showed a highly significant positive correlation with both oyster density and reef height at nine-months and twelve-months and with shell length at twelve-months only. Extractable NO_3^- concentrations yielded no significant correlations with the oyster parameters. Sediment NH_4^+ concentrations generally showed a positive correlation with all of the oyster parameters and the correlation was significant at twelve-months post-restoration for all three parameters, at nine-months post-restoration for reef height and shell length only, and at six-months post-restoration for reef height only. Extractable SRP showed a negative correlation with all three oyster parameters. This correlation was significant at $p \leq 0.01$ with all three parameters at six-months, with reef height and shell length at nine-months, and with none of the oyster parameters at twelve-months (Table 8).

OM content was highly correlated with all three oyster parameters at each time frame (Table 8). The total pool of C showed varying positive or negative correlations and was not correlated with any of the oyster parameters. The total pools of both N and P were highly correlated with all three of the oyster parameters at each time frame (Table 8). There were highly positive correlations of total P and OM with all three biophysical parameters at every single time frame (Table 8). Between the three parameters of oyster density, shell length and reef height, there is no one metric that correlates with sediment physicochemical and biogeochemical

properties clearly better than the other (prediction 2). Oyster density showed 21 significant correlations with the sediment parameters, shell length showed 23 significant correlations and reef height showed 24 significant correlations (Table 8).

Discussion

Sediment Physicochemical Properties

Sediment bulk density reflected physical changes on restored reefs that was likely due to restoration activities. Bulk density decreased ($p < 0.001$) after leveling of the restored reefs and then increased ($p < 0.001$) after the placement of oyster mats (Table 5). The lack of observed differences in sediment bulk density between natural, dead and restored reefs was most likely due to the high amounts of oyster shells observed in surface sediments. Bulk density was generally lower in natural reefs which is supported by higher OM measurements in natural reefs compared to the dead reefs as well as a negative correlation of bulk density with percent organic matter that has been recognized as a general property of terrestrial and wetland soils (Chambers et al. 2013, Saini 1966).

Despite no observed differences in sediment pH due to restoration activities (Table 3), there was some evidence of biogenic influence as natural reefs generally had lower pH levels than dead reefs (Figure 4). This is most likely due to decreases in alkalinity on natural reefs from the process of calcium carbonate formation releasing CO_2 into surface water and net increases in alkalinity on dead reefs due to shell dissolution releasing bicarbonate ions (HCO_3^-) (Waldbusser et al. 2013, Gutierrez et al. 2002). Biophysical data also showed a negative correlation of pH with oyster density and shell length, reaffirming the idea that high rates of shell calcification by

oysters releases dissolved CO_2, forming carbonic acid and decreasing sediment pH (Waldbusser et al. 2011).

Carbon Dynamics

Previous research on the impacts of restoration on sediment biogeochemistry mainly focused on the enhancement of benthic N cycling; very limited studies exist on oyster reef sediment C and P dynamics (Chambers et al. 2017, Dame et al. 1989, Kellogg et al. 2013, Southwell et al. 2017). This study found that DOC concentrations in restored reef sediments can become significantly higher than both natural and dead reefs by nine-months post-restoration (Table 4). This repeats the findings in this estuary by Chambers et al. (2017) where one-year-old restored reefs had significantly higher DOC concentrations than both natural and dead reefs. However, results differ here because natural and dead reefs had similar DOC concentrations over the entire study, indicating that DOC levels were influenced by factors other than just oyster biodeposition. DOC concentrations in restored reef sediments increased by a total of 60 % from pre-restoration to peak levels at nine-months post-restoration and were an order of magnitude greater than both natural and dead reef concentrations from six months onwards (Figure 7). The only other study to our knowledge to compare organic C content of restored versus control sites showed that seven-year-old restored reefs in the Chesapeake Bay also contained organic C levels an order of magnitude higher than sites lacking shell and oysters (Kellogg et al. 2013).

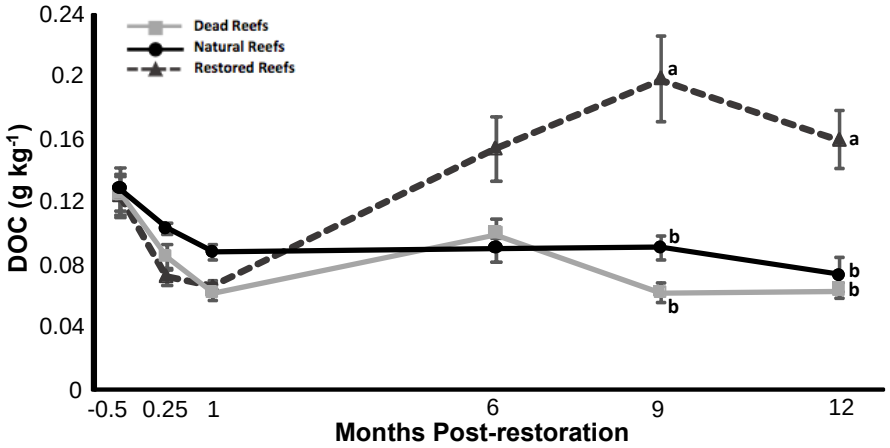

Figure 7: Dissolved organic carbon over time in dead, natural, and restored reefs. Error bars indicate standard error and letters denote significantly different means ($p \leq 0.05$) between treatments according to a post hoc least squares means pairwise comparison.

These increases in surface sediment DOC concentrations can be due to both biological and physical changes on restored reefs (Dame et al. 1989, Smyth et al. 2016). Within the first year of restoration, increases in oyster feeding and deposition of organic-rich sources can result in three-fold increases in sediment chlorophyll-*a* concentrations (Southwell et al. 2017) and the introduction of reef structure can lead to four-fold increases in organic C content of settled particles (Falcão et al. 2007). Prior studies have also demonstrated that increased light penetration due to improved water clarity and the deposition of labile organic matter can stimulate primary production in micro- and macro-benthic algal layers in the sediment, which can be a contributing factor to this significant increase in DOC pools within months after restoration (Blomberg et al. 2017, Boucher and Boucher-Rodoni 1988, Dame et al. 1992, Falcão et al. 2007, Leguerrier et al. 2004, Newell et al. 2002). Furthermore, if organic matter from biodeposition is non-limiting for microbial processes and sediments are anoxic due to stimulated microbial action and tidal submersion, organic C and N from biodeposits can accumulate (Dame et al. 1989, Hoellein and Zarnoch 2014, Reddy and Delaune 2008). The increases in organic

matter content on restored reefs can also lead to more DOC held within sediments by sorption processes between organic molecules (Wang et al. 2007).

Restored reef sediments increased 75 % in organic matter content from pre-restoration to twelve months, whereas dead reefs and natural reefs only showed 12 % and 32 % increases, respectively (Figure 8). This disproportionate increase in organic matter on restored reefs could be a result of the growth of oysters, as well as the increasing complexity of reef structure. The process of biodeposition on restored and natural reefs can increase the amount of organic particles that settle to the sediment (Haven and Morales-Alamo 1966, Smyth et al. 2016, Hoellein et al. 2015) due to the release of mucus-bound feces and pseudofeces that can settle to the reef surface several times faster than natural particle deposition (Dame 1999, Widdows et al. 1998). The influence of oysters on sediment organic matter content is also supported by the positive correlations of organic matter with extractable NH_4^+ and total N (Appendix Table 3), which have been shown to increase due to oyster biodeposition (Chambers et al. 2017, Mortazavi et al. 2015, Newell et al. 2005, Pollack et al. 2013). The development of architectural complexity on restored reefs can also reduce water velocity and increase sedimentation as reefs develop (Lenihan 1999, Lenihan et al. 2001).

Organic matter sources such as chlorophyll-a and suspended particulate matter can vary seasonally in estuarine systems and mask the trends observed within a one-year timeframe (Ubertini et al. 2012). All reefs showed seasonal fluctuations in organic matter in surface sediments, yet still maintained the relative levels of higher organic matter on natural reefs, lowest organic matter on dead reefs, and increasing concentrations on restored reefs (Figure 8), indicating that sampling surface sediment (0-5 cm) was effective in catching the signal of differences in organic matter content due to oyster reef condition. Chambers et al. (2017) found

no significant differences in OM content between one-, four- and seven-year-old restored reefs and dead reefs, suggesting that restoration in Mosquito Lagoon can steadily increase sediment organic matter content, but not significantly above background levels. This study only found a difference of 3.0 % in organic matter between restored and dead treatments after twelve months of restoration, but collected sediments from 0-10 cm, possibly diluting the signature of increased concentrations at the surface. Kellogg et al. (2013) found a similar difference of 6.1 % in organic matter content between seven-year-old restored reefs and sites without oysters.

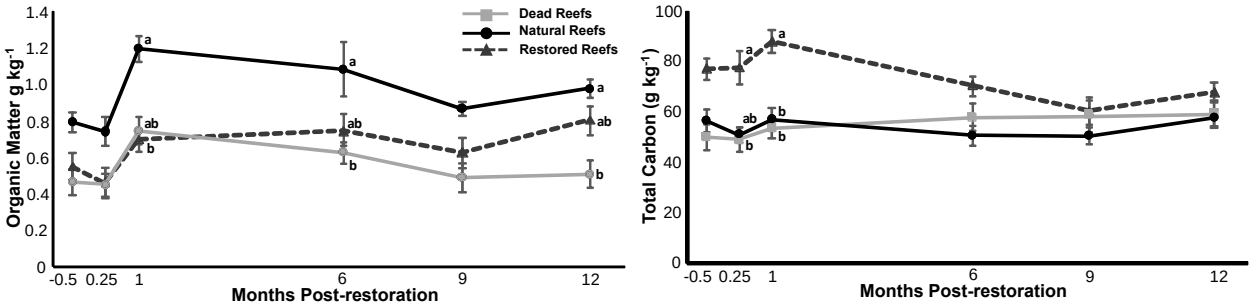

Figure 8: Organic matter and total C concentrations over time in dead, natural and restored reef sediments. Error bars indicate standard error and letters denote significantly different means ($p \leq 0.05$) between treatments according to a post hoc least squares means pairwise comparison.

The influence of allochthonous nutrient sources is apparent in total C measurements where total C did not increase significantly on restored reefs after twelve months. The only change measured in restored reef total C pools was from one-month to six-months post-restoration where there was a significant decrease (Figure 8). The effect of time was significant for all nutrient pools except for total C (Table 3). This study and Chambers et al. (2017) both demonstrated that total C does not increase linearly with age in restored reefs, indicating that increased organic matter content on restored reefs does not necessarily lead to higher stores of C. Unlike the other total pools of nutrients, Total C pools in surface sediments do not seem to be highly influenced by oyster density, reef height and shell length, as total C did not have any

significant correlations and showed both positive and negative correlations. These measurements differ from the results of Chambers et al. (2017) in that dead reefs did not have significantly lower concentrations of total C than natural reefs. There is not enough evidence provided in this study to demonstrate that intertidal oyster reefs in Mosquito Lagoon enhance C pools above background levels in surface sediments. Similar results were found in Louisiana oyster reefs by Westbrook et al. (2019) where adjacent productive wetlands released high amounts of organic N and C, masking the effects of C and N burial via biodeposition. The variables that influence C pools in oyster reef sediments are poorly understood and has been evaluated by few studies (Dame et al. 1989, Fodrie et al. 2017, Westbrook et al. 2019). Whether oyster reefs are net C sources or sinks has not yet been established and seems to depend on whether a reef is subtidal or intertidal and the type of adjacent habitat, among other environmental factors (Fodrie et al. 2017).

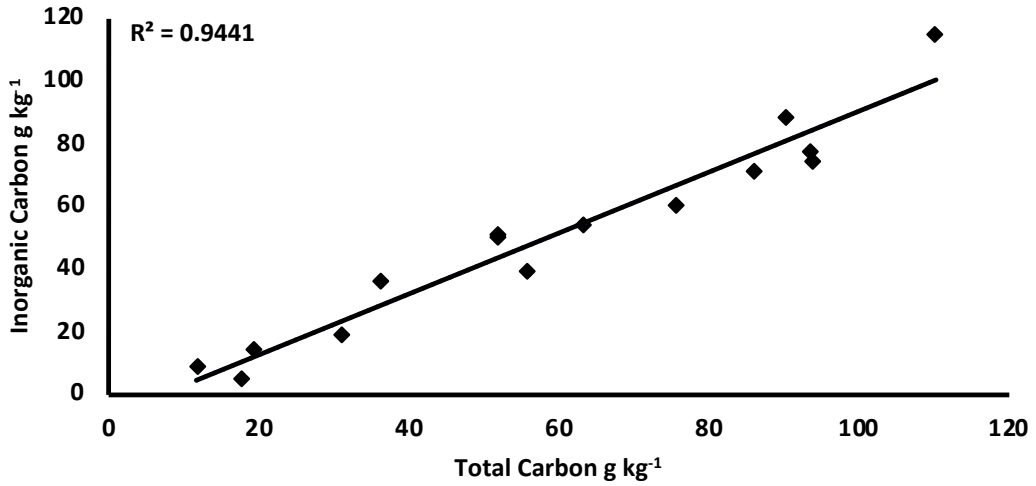

Figure 9: Relationship of total C versus inorganic C for the 15 sediment samples selected for C analysis.

Total C measurements include both organic and inorganic C pools. Based on visual observations, as well as an analysis of inorganic C content in select samples, total C pools on these intertidal reefs appear to be primarily composed of inorganic C from calcium carbonate

originating from oyster shells. Analysis of 15 representative samples (5 high total C, 5 mid-range total C, and 5 low total C) showed that a large majority of the C in surface layer sediments is inorganic. The high inorganic C content (average 81 %) and low organic C (average 19 %) is assumed to be mostly from C in calcium carbonate ($CaCO_3$) shells being deposited at high rates on the reef surface (Fodrie et al. 2017, Kellogg et al. 2013, Waldbusser et al. 2013, Gutierrez et al. 2002). This amount nearly matches the 86 % of carbonate C and 14 % of organic C by weight of total C on the same type of intertidal sandflat reefs in North Carolina calculated by Fodrie et al. (2017). Although shells larger than 2 cm were removed for all sediment analyses, 13 out of 15 samples contained more than 70 % inorganic C. Prior studies have demonstrated that the deposition of calcium carbonate by mollusks can make large contributions to overall sedimentation rates in estuaries and oysters can make even larger contributions because they produce calcium carbonate at rates much higher than other marine mollusks (Gutierrez et al. 2002 and citations within, Powell et al. 1989). However, these calculations are likely not translatable to samples from other types of oyster reefs. Relative amounts of organic and inorganic C can vary according to the depth of the sample, as a higher proportion of organic C can be found below 5 cm where shells are more degraded (Fodrie et al. 2017). Organic C varies between reef types and can be higher on reefs that are subtidal or bordering saltmarshes or seagrasses (Fodrie et al. 2017, Smyth et al. 2015).

Nitrogen Dynamics

NO_3^- concentrations were BD or nearly BD for most sediment samples, which corresponds with very low NO_3^- availability in surface waters over the entire twelve-month period (Figure 5). Sediment N flux rates were not measured in this study, but any NO_3^- present on these reefs could be removed by either an inhibition of nitrification due to sediment anoxia or enhanced rates of N transformations due to available organic matter and NH_4^+ (Smyth et al. 2013, Smyth et al. 2016, Kellogg et al. 2013). Sediment redox conditions were not measured in this experiment, but organic matter from oyster biodeposition can stimulate microbial communities enough to create anoxic sediments, which leads to reduced nitrification due to the lack of oxygen necessary for the microbially-mediated oxidation of NH_4^+ to NO_3^- (Carlsson et al. 2012, Hoellein and Zarnoch 2014).

The other form of inorganic N measured in this study responded differently to restoration of the reefs. As early as one-month post-restoration, restored reef sediments showed significant increases in NH_4^+ pools and remained at levels similar to natural reef sediments thereafter (Tables 4, 5). However, during the one-month sampling in July 2017, an increase was also measured in natural reefs (Table 5). An average 58 % increase in NH_4^+ pools in restored reefs was matched by a 57 % increase in natural reefs, indicating possible influence from estuary-wide allochthonous sources. In comparing trends between sediment and surface water, NH_4^+ in surface layer sediments seem to follow similar seasonal patterns as surface water concentrations (Figures 3 & 5). Seasonal differences in NH_4^+ dynamics were also measured in sediment on other subtidal oyster reefs in the Chesapeake Bay (Kellogg et al. 2013), intertidal oyster reefs in South Carolina (Dame et al. 1989), and caged oysters in Jamaica Bay, New York (Hoellein and Zarnoch 2014).

Despite the influence of seasonal trends, the increase in NH_4^+ pools on restored reefs was sustained past the one-month timeframe and remained similar to natural reefs. This sustained increase corresponds with observations of the colonization of reefs during summer months when water temperatures averaged 31.4 °C for the one-month sampling (Appendix Table 4). Although relatively smaller, juvenile oysters can still contribute measurable amounts of N to sediments (Mortazavi et al. 2015).

The rapid increase in sediment NH_4^+ pools can also be attributed to oyster biodeposition because prior studies demonstrate that a majority of the particulate nitrogen deposited by oyster feeding can be rapidly mineralized to NH_4^+ on intertidal reefs (Dame et al. 1985, Dame et al. 1989, Smyth et al. 2013b). Additionally, the availability of organic C and N from biodeposits can stimulate microbial communities enough to cause sediment anoxia, which shifts N mineralization processes towards enhanced rates of NH_4^+ production (Carlsson et al. 2012, Christenson et al. 2000, Lunstrum et al. 2017). Compared to sediments from mudflats, submerged aquatic vegetation and salt marsh, oyster reef sediments had the highest rates of dissimilatory NO_3^- reduction to NH_4^+ in intertidal oyster reefs in Bogue Sound, North Carolina (Smyth et al. 2013b). Enhanced NH_4^+ concentrations on Mosquito Lagoon reefs is supported by positive correlations of extractable NH_4^+ with organic matter, DOC, and total N (Appendix Table 3).

After twelve-months of reef development, restored reef NH_4^+ pools measured 136% higher than pre-restoration levels, compared to a negative % change in both dead and natural reefs (Figure 5). Other studies on restored reefs have measured significant increases in sediment NH_4^+ flux and NH_4^+ concentrations in the water column above restored reefs compared to control sites (Kellogg et al. 2013, Southwell et al. 2019, Smyth et al. 2013b, Plutchak et al. 2010). Analogous to Smyth et al.'s (2013a) experimental oyster, sediment, and oyster plus sediment

microcosms, increases in both NH_4^+ and organic matter pools on Mosquito Lagoon's restored reefs could indicate the conversion of reef sediments from a N source with net N fixation, to a N sink, can occur within six-months post-restoration and be largely due to the initial colonization and growth of oysters.

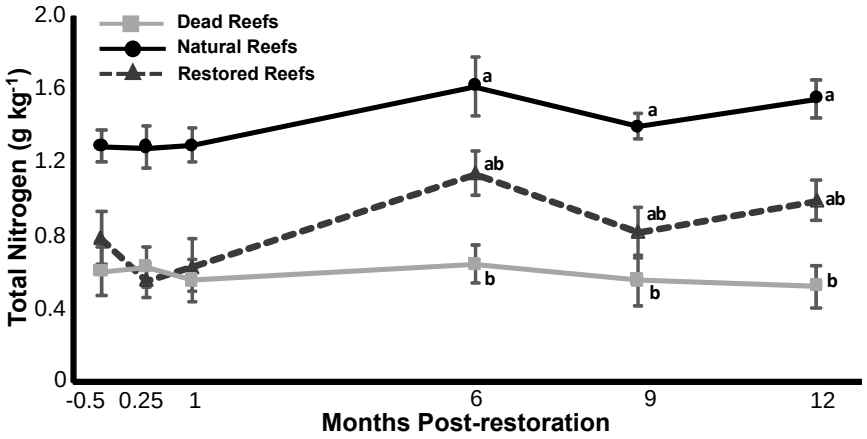

Figure 10: Total N concentrations over time in dead, natural and restored reef sediments. Error bars indicate standard error and letters denote significantly different means ($p \leq 0.05$) between treatments according to a post hoc least squares means test.

Utilizing the oyster mat restoration method, Mosquito Lagoon reefs experienced an overall 78 % increase in total N after twelve months with the greatest increase occurring between one-month and six-months post-restoration ($p < 0.001$, Table 5). In addition to N inputs from oyster biodeposition, the trapping of particles due to the physical structure of the reef can enhance total N pools (Falcão et al. 2007). Experimental evidence has demonstrated that the aerobic sediments and sufficient light present on intertidal reefs can stimulate microphytobenthos growth and sequester available NH_4^+ into organic pools of N, thus increasing the total pool of N (Newell et al. 2002). This is supported by a positive correlation of NH_4^+ with total N (Table 2). Results here and in Chambers et al. (2017) both showed that total N is highly influenced by reef

type (Table 3) and significantly different between the treatments of dead, restored and natural reefs (Table 4). Both studies also suggest that sediment total N does not increase linearly over time (Figure 10), indicating that a variety of non-oyster factors such as hydrodynamics and shifts in microbial processes may be at play in determining the availability of organic and inorganic N. Although restored reef sediments were not statistically different than natural reefs by six-months post-restoration (Table 5), results presented here and in Chambers et al. (2017) suggest that total N pools in intertidal reefs in Mosquito Lagoon likely take > 4 years to become equivalent to or exceed that of natural reefs.

Phosphorus Dynamics

Extractable SRP concentrations also demonstrated the impact of oyster growth and reef structure, maintaining levels statistically equivalent to natural reefs after one-month post-restoration (Table 4). There is a notable pattern of declining SRP immediately following restoration activities, which is supported by past studies demonstrating that bivalves excrete low concentrations of SRP and bivalve reef sediment are a net source of SRP (Dame et al. 1989, Hoellein et al. 2015, Magni et al. 2000, Asmus et al. 1995, Newell et al. 2005). One of the few studies of SRP fluxes on eastern oyster reefs found that only 8 % of the annual total P flux into sediments is deposited as SRP from oyster metabolism (Dame et al. 1989), accounting for the lack of increase in SRP measured here. In addition, concentrations of sediment SRP measured in this study were 1 order of magnitude lower than NH_4^+. Magni et al. (2000) also measured SRP in sediment pore water underneath venus clam and green mussel populations at 1-2 orders of magnitude lower than that of NH_4^+.

Natural reef sediments generally contained the lowest concentrations of sediment SRP (Figure 11) and the effect of treatment was significant for SRP (Table 3). These results are

similar to subtidal reefs in Kellogg et al. (2013) where seven-year-old restored sites were not sinks of SRP and had significantly higher SRP fluxes compared to control sites. A study of P dynamics in natural mussel beds also found a net release of inorganic P from the sediments (Asmus et al. 1995). Furthermore, in a study of lake sediments, Wang et al. (2007) found an inverse relationship between organic matter content and sorption efficiency of SRP, which helps explain the negative correlation between organic matter and extractable SRP on Mosquito Lagoon reefs (Appendix Table 3). This trend of lower SRP concentrations in natural reefs is supported by studies of other bivalve reefs where higher densities of bivalves caused sediments to become anaerobic due to increased microbial metabolism (Lunstrum et al. 2017, Newell 2004 and references therein). Although sediment oxygen conditions were not measured in this study, a lack of oxygen due to microbial activity in natural reef sediments could shift redox conditions and decrease the availability of iron oxides and sulfate, thus releasing more SRP from iron and sulfur complexes (Correll 1998, Lunstrum 2017).

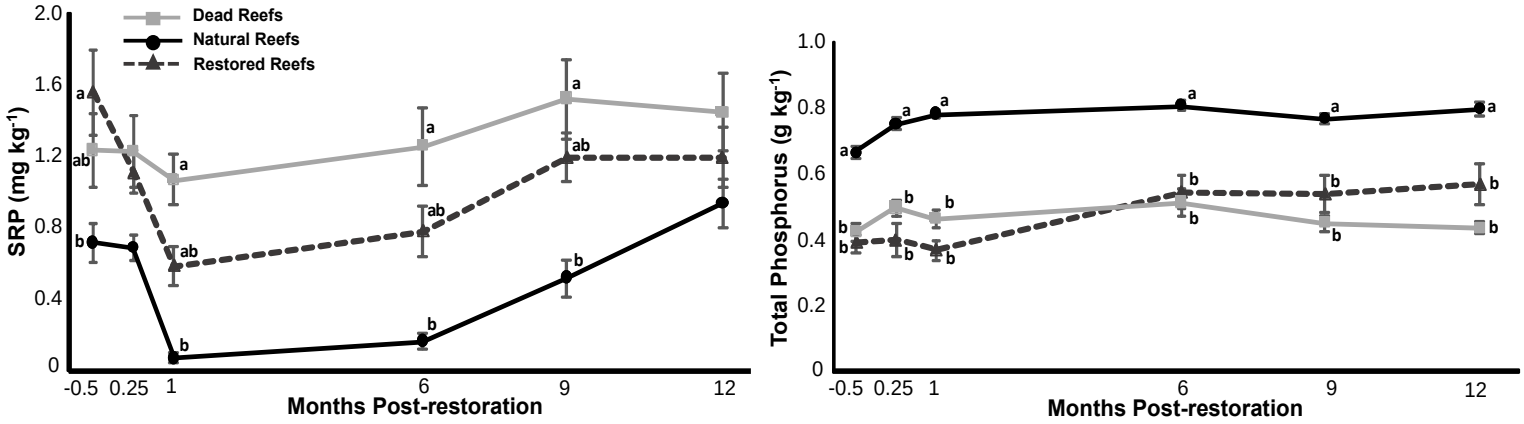

Figure 11: Extractable SRP and total P over time in dead, natural, and restored reefs. Error bars indicate standard error and letters denote significantly different means ($p \leq 0.05$) between treatments according to a post hoc least squares means pairwise comparison.

Despite the lack of deposition of inorganic P, the total pool of P (inorganic plus organic pools) showed the opposite trend and increased by an average of 48 % on restored reefs from before to twelve-months post-restoration (Figure 11) and was significantly affected by reef type (Table 3). Total P concentrations increased steadily in restored reef sediments with the only significant increase occurring during the longest stretch of time, from 1 month to 6 months post-restoration (Table 5). Between the total pools of nutrients, total P displayed the closest approximation to a linear increase which repeats the results measured in this lagoon by Chambers et al. (2017). Although twelve-month-old restored reefs were statistically equivalent to dead reefs in both studies, this investigation into changes within one year showed that restored reefs increased in total P pools by 6 months post-restoration and they start to approach natural reef levels at nine-months ($p = 0.044$) and twelve-months ($p = 0.044$) post-restoration (Table 4).

Total P pools in the sediments of restored intertidal reefs most likely need greater than one year to accumulate to levels similar to natural reefs. Natural reef sediments maintained total P concentrations that were significantly higher than dead reef sediments over the entire study (Figure 9). This trend can be attributed to the increased sorption of organic P with higher organic matter content (Debicka et al. 2016). The sorption of organic P increases more than the sorption of SRP with increasing organic matter content (Wang et al. 2007), which is demonstrated in the negative correlation between organic matter and SRP (Appendix Table 3). This relationship has also been demonstrated in natural mussel beds where inorganic P was released into the water column and organic P was taken up by mussel bed sediments, bound in particles and organic matter (Asmus et al. 1995).

Greater deposits of P are most likely due to the sedimentation of organic P found in organic matter (Berner et al. 1993) and is supported by the positive correlation of total P with

organic matter content (Table 2). Studies on reefs of *Crassostrea gigas* in France showed 97 % of the total P filtered by pacific oysters is released in the form of particulate organic matter in biodeposits (Sornin et al. 1986). One of the few studies of total P fluxes affirms these results; intertidal reefs in South Carolina retained P at a rate of 98 g P m^{-2} yr^{-1}, which was much lower than the deposition of C or N (Dame et al. 1989). A prior study by Newell et al. (2005) also found that P pools in oyster reef sediments accumulate more slowly than N pools. Additionally, intertidal reefs in Mosquito Lagoon and subtidal reefs in the Chesapeake Bay both measured sediment total C content as two orders of magnitude higher than total P, and total N as one order of magnitude higher than total P (Chambers et al. 2017, Kellogg et al. 2013).

Chambers et al. (2017) measured much larger increases in nutrient pools between dead reefs and one-year-old restored reefs than what was measured here between pre-restoration to twelve-months post-restoration reefs. After one year of restoration, there was an 89 % difference between the two studies in the measured increases of organic matter, a 182 % difference in the increases of total N and a 249 % difference in the increases of total C. Measurements likely differ between the two studies because this study utilized 0-5 cm depth cores and Chambers et al. utilized 0-10 cm depths. My twelve-month study is reflective of the nutrient content of only surface layer sediments that are directly involved in benthic-pelagic coupling and are more dynamic due to differences in water velocities and sedimentation rates across the reef (Boynton et al. 2017, Reidenbach et al. 2013). These differences also highlight the large stores of C, N, and P that may be buried below 5 cm of depth on intertidal oyster reefs. However, total C and N content was not significantly different by depth in 30 cm cores taken by Westbrook et al. (2019) on subtidal reefs, indicating that C and N burial in oyster reef sediments is influenced substantially by subtidal or intertidal hydrodynamics and nutrient inputs from adjacent habitats.

Reef Geographical and Biophysical Variables

The random effect of the four individual reefs in each treatment had a significant impact on sediment properties based on the model results ($p < 0.001$) for every single sediment parameter (Table 3). The PCA on sediment properties further confirms this effect of reef where samples generally separated between the three treatments and clustered around samples from the same reef (Figure 6). Correlations with geographical parameters showed that as distance to the inlet increases for a reef, extractable SRP, NH_4^+, DOC, and total C concentrations decrease (Table 7). This indicates a possible influence of the coastal ocean bringing in nutrient-rich seawater (Boynton et al. 2017) thus feeding the production of organic matter through higher primary production rates (Cloern et al. 2014).

The random effect of reef was more likely influenced by the size and architectural complexity of oysters on each reef (Table 8). Sites closer to the inlet experience greater tidal amplitude which results in greater reef heights and oyster densities on the sites that are clustered in the north (K. Kibler and L.J. Walters, personal communication). This can change the amount of biodeposits released or sediment deposited on the reef and thus sediment nutrient concentrations (Smyth et al. 2013, Lenihan 1999). Negative correlations of bulk density with all three biophysical parameters measured support the notion that there is a direct relationship between oyster reef development and increases in sediment organic matter, and therefore decreases in bulk density (Chambers et al. 2013, Saini 1966). Biophysical data also showed a negative correlation of sediment pH with oyster density and shell length (Table 8), reaffirming the negative relationship between sediment pH and biophysical characteristics previously measured in this system (Chambers et al. 2017).

The contribution of oyster biodeposition to increases in extractable NH_4^+ pools are supported by positive correlations with oyster density, reef height and shell length (Table 8). Reef height was the only biophysical parameter that was significantly correlated with NH_4^+ at every timeframe likely because this biophysical variable varied the least between reefs of the same treatment. The other parameters, shell length and oyster density, can vary greatly between sites during the first year of growth (Dillon et al. 2015, Munroe et al. 2017) and can vary within one reef (Hanke et al. 2017, Lenihan 1999, Luckenbach et al. 2005), leading to weaker correlations between NH_4^+ and these variables. When biophysical data was collected, a 33% increase in NH_4^+ on restored reefs from six to twelve-months post-restoration corresponded with a 146% increase in oyster density, a 79% increase in shell lengths, and a 36% increase in reef height (Figure 12).

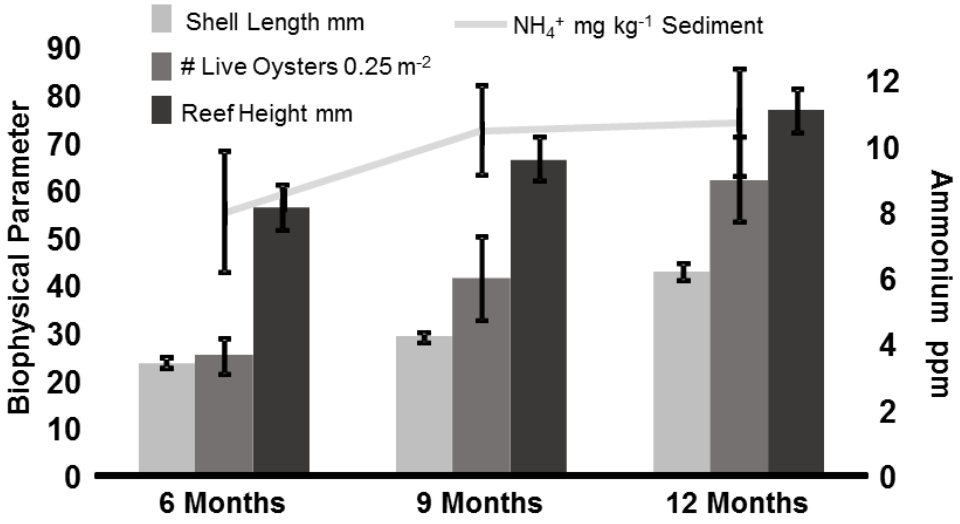

Figure 12: Sediment NH_4^+ and oyster biophysical parameters on restored reefs at six-, nine-, and twelve-months post-restoration.

Positive correlations of organic matter with all three biophysical parameters at each time frame indicate that oyster size and reef structure have a large influence on organic matter in

surface sediments (Table 8). Measurements of reef height, shell length and oyster density show increases in architectural complexity on restored reefs that can trap more organic-rich sediments and phytoplankton, thus contributing to food sources for oysters and also leading to the increases measured in extractable NH_4^+, DOC, organic matter and total N (Blomberg et al. 2017, Fãlcao et al. 2017, Southwell et al 2017).

The condition of oyster reefs also appears to impact both organic and inorganic pools of P on Mosquito Lagoon reefs as increases in oyster density, reef height and shell length correlate to decreases in SRP concentrations and increases in total P concentrations (Table 8). This trend is reflected in the relative levels of sediment SRP and total P concentrations between dead, natural and restored reefs (Figure 11). The significant impact of oysters on total pools of nutrients is demonstrated by positive correlations of biophysical variables at every time frame with both total N and total P (Table 8). The effects of oyster reefs on sediment total N and total P is relatively understudied, but restoration of subtidal reefs was shown to enhance total N and P content by an order of magnitude after seven years, whereas Chambers et al. (2017) found an increase of an order of magnitude in total N, but not total P. Beyond the storage of total N and P in sediments, Kellogg et al. (2013) found that oyster tissue and shell composed greater than 50 % of the total N and P stocks among invertebrates, indicating that oyster reefs can be significant sinks for N and P in the estuarine environment.

Conclusion

This study evaluated how the structural condition and development of intertidal oyster reefs can affect sediment physicochemical and biogeochemical properties and is the first to describe the impacts of restoration on oyster reef sediment properties on the time scale of months. Comparisons between dead, natural and restored reefs revealed clear differences in

sediment nutrient pools, as well as the influence of estuary-wide seasonal trends that occurred within a period of twelve months. The lack of significant differences of certain variables between treatments and sampling times can be attributed to high within treatment variability and to non-biogenic influences, such as sediment deposition and water velocity, which affect the surface of reefs. Although not all of the variables measured on restored reefs became equivalent to natural reef levels within one year, significant changes over time were observed in every sediment property on restored reefs:

- Bulk density of restored reefs changed significantly after the leveling of disarticulated shells and the deployment of oyster mats.
- Sediment pH was largely determined by seasonal trends and showed no effect of treatment.
- Extractable NH_4^+ and SRP responded immediately to restoration activities and measured concentrations similar to natural reefs by one-month post-restoration.
- Extractable SRP was found in significantly lower concentrations in natural reefs relative to dead reefs.
- Extractable NO_3^- only showed a response to seasonal variation and no effect of treatment.
- Extractable DOC was the only parameter to actually exceed the concentrations of both dead and natural reef sediments within the first year post-restoration.
- Interestingly, total C showed no effect of treatment and the large majority (average 81 %) of the C comprising the surface layer of intertidal reefs is inorganic C, indicating that carbonate-C from oyster shells likely is the biggest contributor to total C pools
- Organic matter in restored reef sediments reached levels similar to natural reefs at one-month post-restoration and was significantly correlated with oyster density, reef height and shell length.

- Total N and total P pools were also all significantly correlated with every oyster biophysical parameter at every time frame.
- Total N and P both increased significantly within restored reefs by six-months post-restoration, but only total N reached levels equivalent to that of natural reefs.

Increases in sediment nutrient pools within months after restoration matches the timeframe of recovery of other ecosystem services such as water filtration capacity and habitat provision for fish and invertebrates found in other coastal areas (Dillon et al. 2015, La Peyre et al. 2014, Pierson and Eggleston 2014, Rezek et al. 2017). Universal metrics for monitoring the performance of restoration projects include reef area, reef height, and shell length and the environmental variables of water temperature, salinity and dissolved oxygen (Baggett et al. 2015). The monitoring of additional ecosystem-service based metrics depend on restoration goals which can include the enhancement of sediment nutrient cycling (Kellogg et al. 2014). In this study of intertidal reefs in a shallow lagoon, sediment organic matter content and total N held the most consistent measurements over time, as well as clearest differences between treatments (Figures 8 & 10, Table 4). Based on the results of this study on intertidal reefs, we propose that the sediment nutrient pools of organic matter and total N may be the best candidates for monitoring the ecosystem service of biogeochemical cycling on restored reefs.

CHAPTER 3: COMPARATIVE LABORATORY STUDY OF JUVENILE AND OLDER OYSTERS

Introduction

Estuarine waters contain a wide range of organic and inorganic matter composed of living microorganisms, detritus and inorganic nutrients from both external and internal sources (Baldwin and Newell 1991, Blomberg et al. 2017, Oelsner and Stets 2019). Bivalve filter feeders, such as oysters, can remove both dissolved and particulate constituents in the water column at rapid rates, leading to top-down control on phytoplankton abundance and measurable improvements in water clarity that is beneficial for submerged aquatic vegetation (Coen et al. 2007, Dame et al. 1992, Newell et al. 2007, Peterson and Heck 1999, Newell and Koch 2004). They sort and preferentially ingest food particles through a digestive system lined with cilia that filters out particles according to size and nutrient content (Loosanoff and Engle 1947, Newell and Jordan 1983). Oysters are omnivorous and their diet includes detritus, both autotrophic and heterotrophic microflagellates, organic matter sourced from benthic microalgae, and organic matter from the sediment (Blomberg et al. 2017, Dame et al. 2002). Oysters and other bivalve filter feeders have such a measurable influence on chlorophyll-*a* concentrations in the water column that chlorophyll-*a* can be utilized as a predictor of the distribution of filter-feeders in an estuary (Ubertini et al. 2012). Oysters also have the capacity to reduce total suspended solids and chlorophyll-a concentrations in the water column above reefs by up to 75% (Dame et al. 1984).

Through filtration and subsequent excretion, oysters play an important role in converting large quantities of particulate matter in the water column into both organic and inorganic nutrients (Newell 1988, Dame et al. 2002, Dame et al. 1989). During feeding, they assimilate

what is needed for metabolic requirements and then release both digested and unused material in the form of feces and pseudofeces (herein referred to collectively as biodeposits). These mucus-bound biodeposits can then be buried in the sediment, assimilated by grazers and microbes in the benthos, or transformed into new forms of nutrients (Dame et al. 1984, Newell et al. 2005). This exchange of materials from the water column to the sediment mediated by oysters plays a role in estuarine-wide benthic-pelagic coupling, and represents an important ecosystem service of oyster reefs (Coen et al. 2007, Smyth et al. 2013).The process of biodeposition has an influence on nutrient availability at various trophic levels and subsequently effects microbially-mediated transformations in oyster reef sediments (Humphries et al. 2011, Prins et al. 1997, Smyth et al. 2016).

Relative to other invertebrates, oysters can assimilate large amounts of N and P in shells and tissue that can then be removed from the estuary through harvesting (Carmichael et al. 2012, Dalrymple and Carmichael 2015, Kellogg et al. 2013, Pollack et al. 2013). Of the N not manually removed during harvest, Pollack et al. (2013) estimated that oyster N transformation into biodeposits alone can remove 754 kg N km^2 annually in a Texas estuary, through either burial or denitrification. Experimental studies have demonstrated that oyster biodeposits can have a significant effect on N dynamics, benthic microalgal production, and zooplankton communities (Newell et al. 2002, Porter et al. 2018, Smyth et al. 2013). Mucus-bound biodeposits can aggregate individual particles of diameters < 100 µm into diameters of a few mm (Sornin et al. 1983) and increase particle deposition by up to seven times more than normal gravity-driven deposition in the water column (Dame 1999), but exactly how much is resuspended or transferred to surface sediments is unknown because tidal currents and waves may distribute biodeposits off the reef (Haven and Morales-Alamo 1968). The extent to which

biodeposition contributes to sediment nutrient pools and nutrient removal from estuaries remains poorly characterized (Newell et al. 2005). Studies usually describe oyster biodeposits as "high quality organic matter" (Smyth et al. 2013, Smyth et al. 2016) or "C- and N-rich" (Hoellein et al. 2015), but provide no quantification of C, N and P concentrations in biodeposits. At present, no studies have been performed to characterize of inorganic forms N and P, or DOC, in oyster biodeposits. This is of particular interest because previous studies of oyster reefs in Mosquito Lagoon found that even though the density of live oysters on a one-year-old restored reef was significantly less than the density on older reefs, restored sediments still showed higher concentrations of DOC, total C, and NH_4^+ than sediments on *all* the older reefs; natural reefs, four-year-old and seven-year-old restored reefs (Chambers et al. 2017). This suggests that oysters one-year-old or less may have a greater ability to sequester nutrients from the water column or release biodeposits with higher nutrient concentrations than older oysters.

The aim of this study was to investigate the transfer of nutrients and chlorophyll-*a* (chl-a) from the water column into oyster biodeposits from young (juvenile) oysters (≤ 12-14 months old) compared to older oysters. This age-nutrient paradox presented in Chambers et al. (2017) will be tested in the following hypotheses: (1) Juvenile oysters have a greater capacity to remove particulate matter and dissolved nutrients from the water column than older oysters; and (2) Juvenile oysters produce more nutrient-rich biodeposits per gram of body tissue than older oysters.

Methods

Specimen Collection

This study was conducted in Mosquito Lagoon (ML), a microtidal coastal lagoon where water circulation is primarily wind-driven and exchange with the Atlantic Ocean only occurs through one northern inlet (Ponce de Leon Inlet), leading to long water residence times (Smith 1993). Water temperatures in the lagoon range from 4 to 33° C and salinities range from 22.6 to 45.2 ppt (Phlips et al. 2015). There is an abundance of intertidal reefs of *C. virginica* bordered by mangrove-dominated islands. Aerial photography from 2009 revealed a total of 2,542 natural reefs covering a total of 46.34 hectares of benthic habitat (Garvis et al. 2015). Many of these reefs have experienced degradation within the past century due to the action of boat wakes from year-round boating activity (Grizzle et al. 2002, Wall et al. 2005). The single reef of *C. virginica* utilized for this study is a fringing reef found parallel to a mangrove-dominated shoreline, composed of a dead margin on the channel side and an intact inner portion that is protected from boat wakes and lies adjacent to mangroves. A section of dead margin on this reef was selected for restoration in June 2017 and a 65.5 m^2 area was restored utilizing the oyster mat method described in Chapter 2. This created a situation where two reef types: natural (composed of a mix of oysters of all sizes/ages, but dominated by larger adult oysters) and restored (composed of only oysters less than or equal to 12-14 months old, based on the time since restoration), were juxtaposed and otherwise experienced the same environmental conditions.

Oysters for this lab experiment were collected from both the restored section and the intact/natural section of the reef to create two treatment groups: maximum 12-14 months old, herein referred to as juvenile, and a mix of ages with mainly larger oysters, herein referred to as older oysters. Oyster length measurements were conducted at the end of each of the experiments

(herein referred to as rounds) to confirm the juvenile treatment group consisted of significantly smaller mean shell sizes than the adult treatment. During four separate rounds of experiments (June 1, July 5, July 14, and August 7, 2018), seven groups of 20-40 oysters were collected from each section of reef (14 total groups) during low tide and placed on ice for transport back to the laboratory at the University of Central Florida (UCF). A total of 20-30 older oysters and 30-40 juvenile oysters were collected to attempt to obtain similar dry tissue weights in each feeding tank. At the time of oyster collection, a handheld sonde (ProDSS, YSI Inc., Yellow Springs, OH, USA) was placed at 10 cm depth 2-3 m off the reef edge to measure water temperature, percent dissolved oxygen (DO), pH, salinity, and turbidity. A chl-a probe (Manta plus, Eureka Water Probes, Austin, TX, USA) was also deployed at 10 cm depth to measure chlorophyll concentrations as a proxy for phytoplankton concentration. Directly after oyster collection, 1,325 L of lagoon surface water was collected 4-5 m off shore at the Canaveral National Seashore kayak ramp (0.48 km from the site of oyster collection) and transferred into a PVC-coated Kolaps-A-Tank (Burch Inc., Fort Dodge, IA, USA) for transport to UCF.

Feeding Experiments

Upon return to UCF, oysters were scrubbed clean of barnacles, algae and sediment particles and groups of oysters were placed in individual 75.7 L (20-gallon) aquaria filled with the collected site water. A 120 gallon per hour recirculation pump was used to ensure even suspension of particulates during the filtration period. Groups of 20-40 oysters were placed on top of a 25 cm wide plastic funnel and biodeposits were collected in a glass mason jar attached to the bottom of the funnel with 3M™ electrical tape (Figures 13 & 14). No adjustment period was given for the oysters as water inside the tanks had similar characteristics as reef water. Pilot studies demonstrated that groups of about 30 oysters in these funnels begin feeding and

producing biodeposits within minutes after placement in tanks. Three tanks without oysters were used as controls to observe the effects of particulate organic matter deposition that occurs regardless of oyster filtration (Reidenbach et al. 2014).

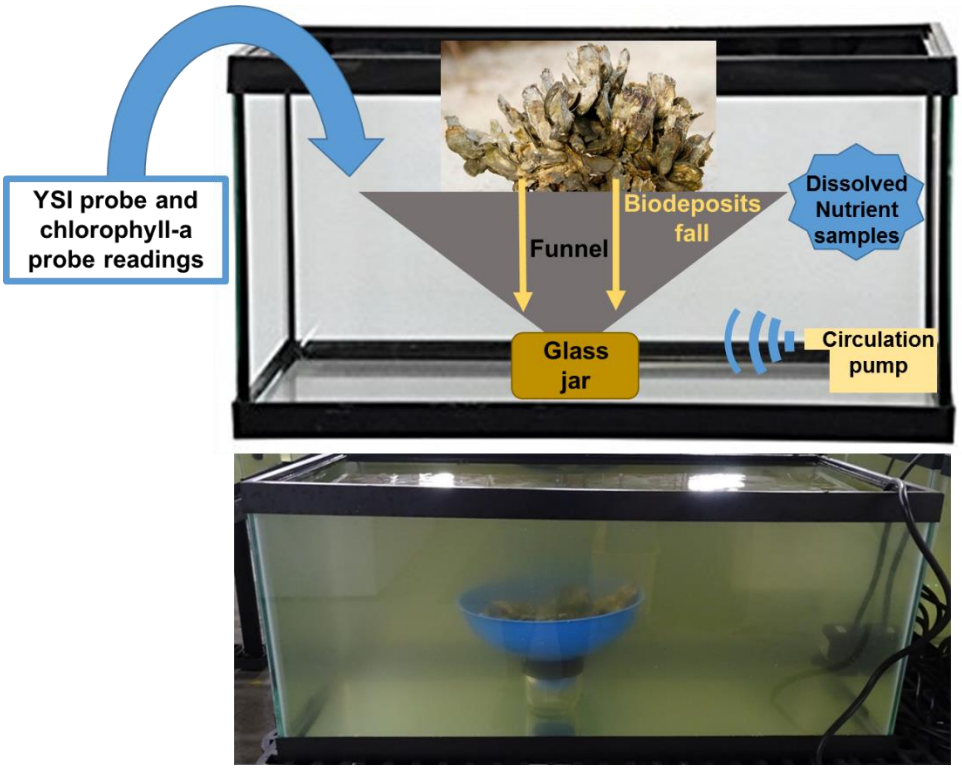

Figure 13: Laboratory set up of an individual tank for the Funnel Drop method. The placement of oysters and locations for taking water quality measurements and grab samples in each tank are indicated.

Oysters were allowed to feed for a total of 24-hours and during that time water quality parameters, chlorophyll-a concentration, and surface water were sampled five times in each tank at time points immediately before oyster placement, and at 2 h, 6 h, 12 h, and 24 h after placement into tanks. Water quality parameters (water temperature, DO, pH, salinity, turbidity) were collected with the YSI handhelde sonde, chlorophyll concentration with the chlorophyll probe and grab water samples were collected in 20 mL scintillation vials at the tank's mid-depth beside the water recirculation pump for later analysis of dissolved nutrient content. Water tank samples were immediately filtered through a 0.45 μm syringe filter and preserved at a pH < 2

with distilled, deionized sulfuric acid and refrigerated at 4 °C for later quantification of nitrate (NO_3^-), ammonium (NH_4^+), soluble reactive phosphorus (SRP) and dissolved organic carbon (DOC) concentrations within 28 days of collection. A Shimadzu TOC-L Analyzer (Shimadzu Scientific Instruments, Kyoto, Japan) was used to measure the concentration of nonpurgeable DOC. Concentrations of NO_3^-, NH_4^+ and SRP were determined colorimetrically on a Seal AQ2 Automated Discrete Analyzer (Seal Analytical, Mequon, WI) using EPA methods 353.2 Rev. 2.0, 350.1 Rev. 2.0, and 365.1 Rev. 2.0, respectively (USEPA 1993).

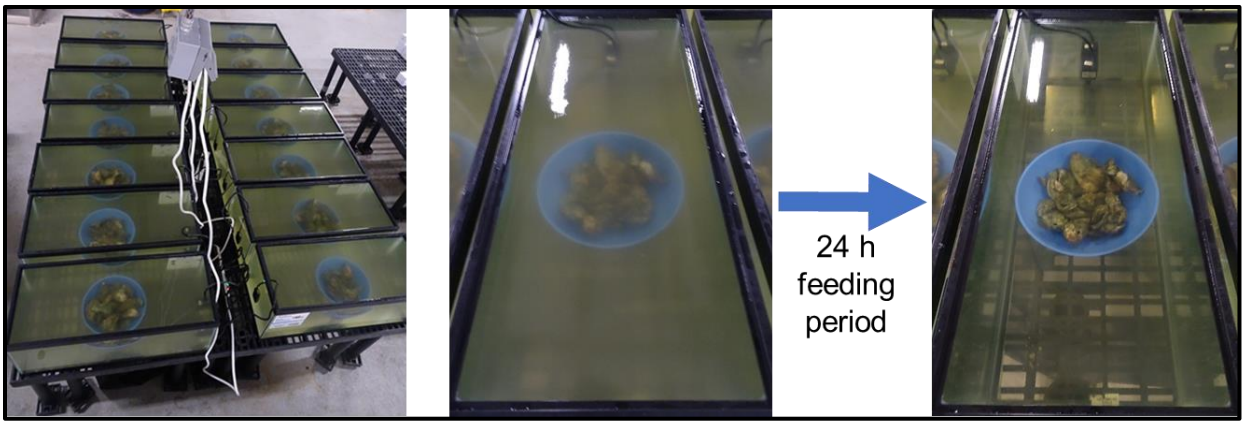

Figure 14: Laboratory set up of multiple tanks for the Funnel Drop method and an example of the typical reduction in turbidity before and after the 24 h feeding periods.

Biodeposit Extraction

After the 24-hour feeding period, water was siphoned out of the tanks ensuring no disturbance to biodeposits and glass containers were extracted. Both feces and pseudofeces were separated from water in 40 mL centrifuge tubes by centrifuging at 4000 RPM and 10 °C for 5 min. The saltwater supernatant was removed with a 5 mL pipette. Separation of feces and pseudofeces was not possible as both components mixed well to form an aggregate of biodeposits at the bottom of the containers (Figure 15). For the extraction of DOC, NO_3^-, NH_4^+ and SRP, 0.5 g of biodeposits were placed in clean 40 mL centrifuge tubes and mixed with 2 M

KCl. The samples were agitated on an orbital shaker at 100 rpm for 1 h. Samples were then centrifuged at 4000 rpm and 10 °C for 10 min. The supernatant was filtered through Supor 0.45 μm filters (Pall Corporation, Port Washington, NY), acidified to a pH < 2 with distilled, deionized H_2SO_4 and stored at 4 °C. Nonpurgeable DOC concentration was determined using the Shimadzu TOC-L Analyzer. Subsequent analysis for NO_3^-, NH_4^+ and SRP was performed on the Seal AQ2 using EPA methods 353.2 Rev. 2.0, 350.1 Rev. 2.0, and 365.1 Rev. 2.0, respectively (USEPA 1993). After weighing for extractable nutrients, the remainder of the biodeposits were placed in aluminum tins, weighed for total wet weight, and dried at 70 °C in a gravimetric drying oven for at least 3 days until a constant weight was achieved (Figure 15).

Figure 15: Left photo – example of a biodeposit sample after removal from the tank and before centrifuging to separate biodeposits from site water. Right photo – example of biodeposit color and texture after removal from centrifuge tubes and before nutrient extraction.

After biodeposit processing, all oysters were measured for shell length and wet tissue was removed and dried to obtain total dry body weight in order to standardize to g dry body weight in each tank. The total process of oyster collection, 24 h feeding, and biodeposit analysis was repeated four times to reach an n = 28 for each treatment group.

Data Analysis

In order to standardize measurements made in tanks with different sized oysters and different total tissue weights, measurements that showed an effect of oyster feeding were standardized to g dry tissue weight (herein referred to as g dry wt). For the measurements of tank waters over the 24 h filtration period, end (24 h) measurements were subtracted from start (0 h) measurements for every water quality and nutrient parameter. Where the change over time appeared to be linear, the rate of removal or addition (slope) was calculated from 0-6 h, 0-12 h, or 0-24 h (based on the linear phase) for each tank and used as the measurement of comparison between juvenile and older oysters. Where appropriate, R^2 values were compared between 0-6 h or 0-12 h slopes in order to decide which timeframe was more linear and should be used for the comparison.

Statistical analysis was performed in R version 3.5.1 (R Core Team 2018) using R Studio (R Studio Team 2016). Data was tested for the assumptions of a normal distribution with Shapiro-Wilk Test and for homogeneity of variance with Levene's Test. Water quality parameters and surface water nutrients met both of these assumptions and a logarithmic transformation was applied to the biodeposit nutrient data in order to meet assumptions. A two-way ANOVA was used to assess differences between the juvenile and older treatment groups and the interaction between treatment with rounds 1-4 of the experiment. Where the interaction of treatment with rounds 1-4 was significant, Tukey's HSD post hoc test was used to assess the differences between individual rounds of the experiment for each age treatment. All results were considered statistically significant at $\alpha = 0.05$.

Results

Oyster Age Class

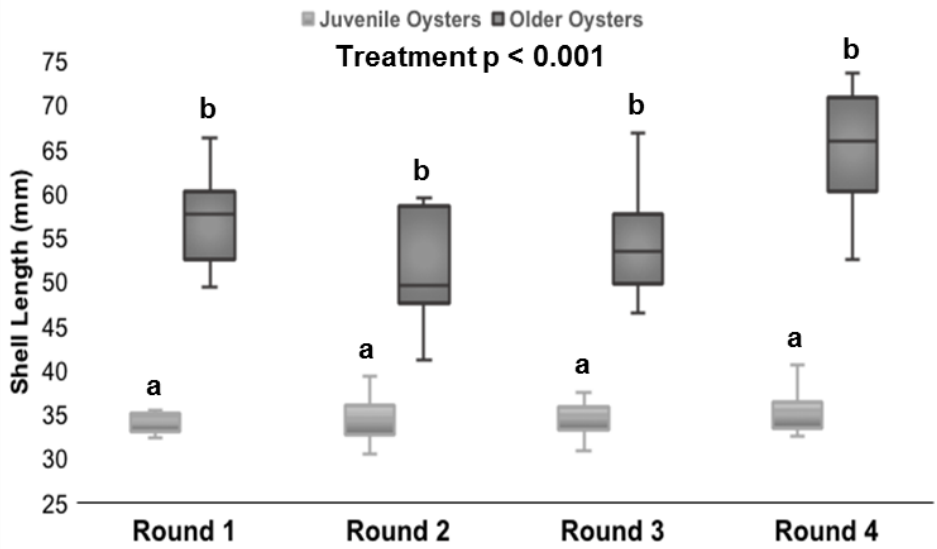

Figure 16: Shell length of juvenile and older oysters in each round of the experiment. Middle lines of the boxes indicate medians, edges of the boxes indicate quartile 1 and 3 boundaries, and error bars indicate the minimum (Q1 – 1.5*interquartile range) and maximum (Q3 + 1.5*interquartile range).

Table 9: ANOVA and Tukey HSD results for oyster shell lengths.

		P value
	Treatment	< ***0.001***
	Round	***0.002***
Round 1	Young - Older	< ***0.001***
Round 2	Young - Older	< ***0.001***
Round 3	Young - Older	< ***0.001***
Round 4	Young - Older	< ***0.001***

Over four repetitions of the experiment, the oysters collected from the 12-14 month old section of reef averaged 34.7 ± 0.4 mm (mean ± SE) in shell length and the oysters collected from the intact portion of the reef averaged 57.1 ± 1.6 mm (Figure 16). ANOVA for shell length

showed that the difference between juvenile and older treatments was highly significant with $p < 0.001$ (Table 9). The difference in shell lengths between juvenile and older treatments in each round of the experiment was also very significant with p values < 0.001 (Table 9). Round 4 older oysters were 65.0 ± 7.8 mm compared to 54.5 ± 6.9 mm in round 3, 51.8 ± 7.2 mm in round 2, and 57.0 ± 5.9 mm in round 1 (Figure 13).

Tank Water Quality Parameters

Table 10: Tukey HSD p values for treatment tank to blank tank pairwise comparisons in individual rounds. Values bolded and italicized indicate $p < 0.01$ and bolded values indicate $p < 0.05$.

		DO ES	DO 6h	pH 24h	Cond ES	Temp ES	Sal ES	Turb ES	Turb 6h
Round 1	Older - Blank	***<0.001***	0.050	**0.023**	**0**	**0.031**	1	1	1
	Juvenile - Blank	***<0.001***	0.222	**0.003**	**0**	**0.038**	1	1	1
Round 2	Older - Blank	**0**	**0**	**0**	0.969	0.989	1	0.670	**0.008**
	Juvenile - Blank	***<0.001***	***<0.001***	**0**	0.566	0.880	1	0.202	**0.007**
Round 3	Older - Blank	**0**	**0**	**0**	0.986	0.996	1	***<0.001***	***<0.001***
	Juvenile - Blank	**0**	**0**	**0**	0.887	0.824	0.879	***<0.001***	***<0.001***
Round 4	Older - Blank	**0**	**0**	**0**	**0**	**0**	1	***<0.001***	***<0.001***
	Juvenile - Blank	**0**	**0**	**0**	**0**	**0**	1	***<0.001***	***<0.001***

DO ES (% dissolved oxygen end-start) DO 6h (% dissolved oxygen over 6h) Cond ES (conductivity end-start) Temp ES(temperature end-start), Sal ES(salinity end-start), Turb ES(turbidity end-start), Turb 6h(turbidity over 6h)

The blank tanks without oysters differed from the treatment tanks for dissolved oxygen, pH, and turbidity in rounds 3 and 4. Conductivity and temperature were different in the blank

tanks only during rounds 1 and 4. Salinity stayed consistent between the blank and treatment tanks over all 4 rounds of the experiment (Table 10).

Table 11: ANOVA and Tukey HSD p values for water quality parameters over all four rounds. Values with time units indicate slopes that were used, otherwise the measurements were End-Start (24 h – 0 h) concentrations. Values bolded and italicized indicate $p < 0.01$ and bolded values indicate $p < 0.05$.

Parameter	Treatment	Round	Round 1	Round 2	Round 3	Round 4
Salinity	0.219	0.050	0.890	1	0.995	1
Conductivity	0.310	***< 0.001***	1	0.955	1	1
Temperature	0.453	***< 0.001***	1	1	0.984	1
% DO	***< 0.001***	***< 0.001***	***< 0.001***	0.697	0.088	1
% DO 6 h^{-1}	***< 0.001***	***< 0.001***	***< 0.001***	0.844	0.813	0.973
pH 24 h^{-1}	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***0.002***	**0.025**
Turbidity	***< 0.001***	***< 0.001***	0.372	***0.001***	***0.007***	**0.016**
Turbidity 6 h^{-1}	***< 0.001***	***< 0.001***	0.848	0.123	0.125	0.216

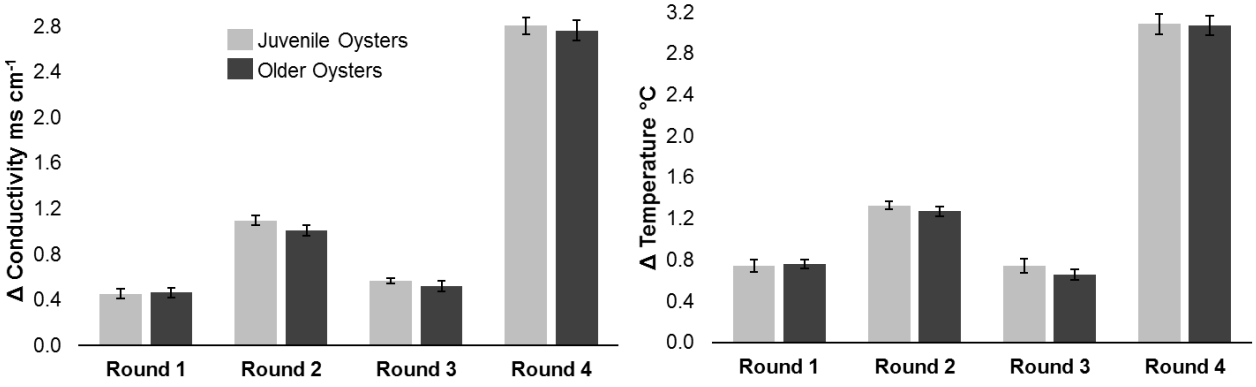

Figure 17: Change in conductivity and change in temperature (24 h – 0 h) in tank waters over all rounds with no significant differences between treatment means.

The water quality parameters of salinity, conductivity, and temperature showed no effects of treatment throughout the experiment (Table 11). Salinity did not change significantly between rounds of the experiment and averaged 33.8 ± 0.1 ppt for all tanks.

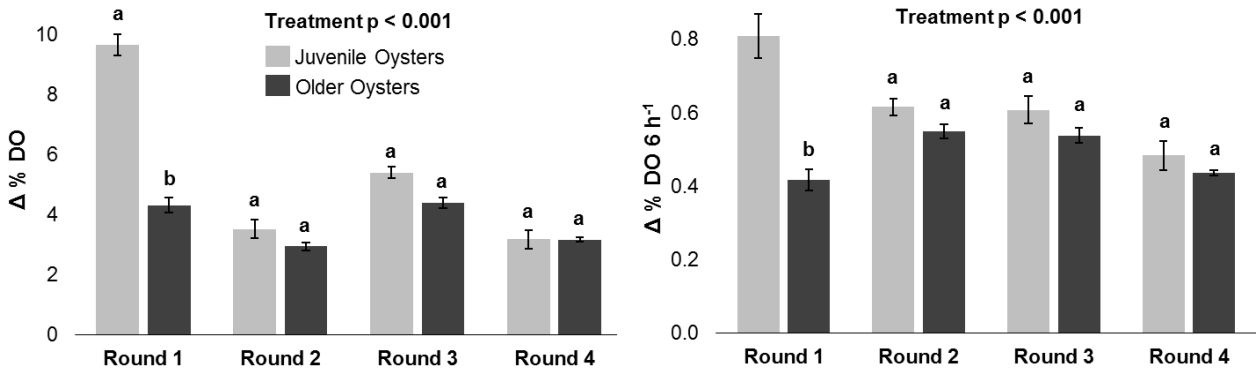

Figure 18: Change in % DO (24 h – 0 h) and % DO slopes in tank waters over 6 h for all rounds. Letters indicate significant differences between treatment means in each round.

The parameters of % DO, pH and turbidity differed between treatment and between rounds within each treatment (Table 11). End-start differences for % DO averaged -5.4 ± 0.5 % DO g dry wt^{-1} for juvenile oysters and -3.7 ± 0.1 % DO g dry wt^{-1} for older oysters. Measurements of % DO approximated a linear relationship from 0-6 h with r-squared values averaging 0.935 ± 0.006. The change in % DO averaged -0.63 ± 0.03 % DO 6 hrs^{-1} g dry wt^{-1} for juvenile oysters and -0.49 ± 0.01 % DO 6 hrs^{-1} g dry wt^{-1} for older oysters (Figure 18).

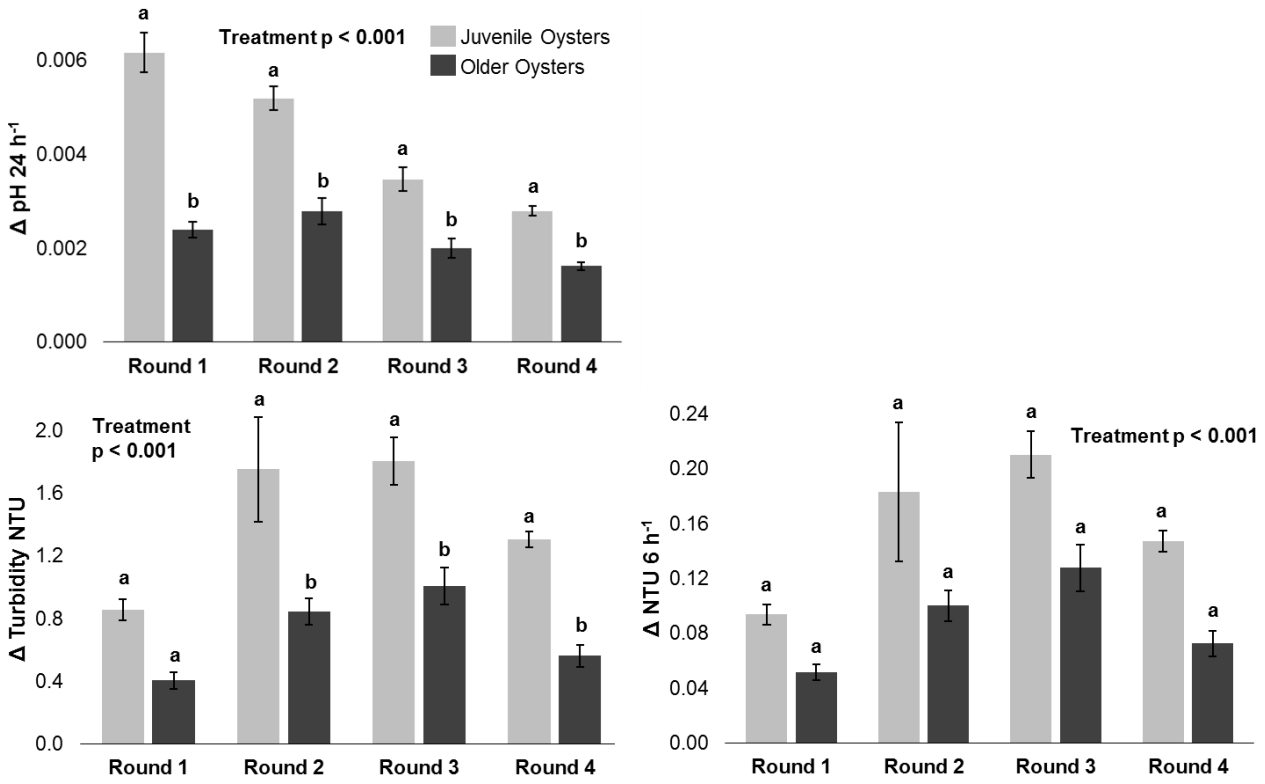

Figure 19: pH slopes over 24 h, change in turbidity (24 h – 0 h) and turbidity slopes in tank waters over 6 h for all rounds. Letters indicate significant differences between treatment means in each round.

Surface water pH showed an approximate linear decrease over 24 h in the treatment tanks with r-squared values averaging 0.873 ± 0.011. pH was different between treatments and averaged -0.0044 ± 0.0003 pH g dry wt^{-1} for juvenile oysters and -0.0022 ± 0.0001 pH g dry wt^{-1} for older oysters over all rounds of the experiment (Figure 19). End-start differences for turbidity over the 24 h feeding period were different between juvenile and older oysters (Table 11). Overall differences in turbidity after 24 h of feeding was also significantly different between treatments with overall decreases of -1.43 ± 0.12 NTU g dry wt^{-1} for juvenile oysters and -0.71 ± 0.06 NTU g dry wt^{-1} for older oysters (Figure 19). The rate of decrease in turbidity over 6 h also approximated a linear relationship with r-squared values of 0.935 ± 0.007. Turbidity over 6 h was different between treatments with decreases of -0.16 ± 0.02 NTU 6 hrs^{-1} g dry wt^{-1} for juvenile oysters and for -0.09 ± 0.01 NTU 6 hrs^{-1} g dry wt^{-1} older oysters (Figure 19).

Tank Water Nutrients

Table 12: Tukey HSD p values for treatment tank to blank tank pairwise comparisons in individual rounds. Values bolded and italicized indicate $p < 0.01$ and bolded values indicate $p < 0.05$.

		Chl-a ES	Chl-a 6h	DOC ES	NO_3^- ES	NH_4^+ ES	NH_4^+ 24h	SRP ES	SRP 24h
Round 1	Older - Blank	0.990	0.996	1	1	***<0.001***	***<0.001***	1	0.996
	Juvenile - Blank	0.930	0.992	1	1	**0.029**	**0.024**	1	1
Round 2	Older - Blank	**0**	**0**	0.999	1	0.942	0.938	1	1
	Juvenile - Blank	**0**	**0**	0.999	1	1	1	1	1
Round 3	Older - Blank	**0**	**0**	0.539	0.328	***<0.001***	***<0.001***	***<0.001***	**0.002**
	Juvenile - Blank	**0**	**0**	0.314	0.155	**0.005**	**0.012**	0.598	0.685
Round 4	Older - Blank	**0**	**0**	1	1	**0**	**0**	***<0.001***	***<0.001***
	Juvenile - Blank	**0**	**0**	1	1	**0.002**	**0.001**	0.135	0.108

Chl-a ES (chlorophyll-a end-start) Chl-a 6h (chlorophyll-a over 6h) DOC ES (dissolved organic carbon end-start) NO_3^- ES (nitrate end-start) NH_4^+ ES (ammonium end-start) NH_4^+ 24 h (ammonium over 24h) SRP ES (soluble reactive phosphorus end-start) SRP 24h (soluble reactive phosphorus over 24h)

Both juvenile and older oysters reduced chlorophyll-a concentrations in the treatment tanks to levels significantly lower than that of the blank tanks in rounds 2, 3 and 4 (Table 12). The treatment tanks also contained levels of NH_4^+ that were higher than the blank tanks in rounds 1, 3 and 4 of the experiment. SRP concentrations were higher than blank tanks in only the tanks containing older oysters in rounds 3 and 4. DOC and NO_3^- concentrations did not differ from the blank tank concentrations (Table 12).

Table 13: ANOVA and Tukey HSD results for chl-a and dissolved nutrients in tank waters. Values with time units indicate slopes that were used, otherwise the measurements were End-Start (24 h – 0 h) concentrations. Values bolded and italicized indicate $p < 0.01$ and bolded values indicate $p < 0.05$.

Parameter	Treatment	Round	Round 1	Round 2	Round 3	Round 4
Chl-a	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	0.018
Chl-a 6 h^{-1}	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	0.049
DOC	0.551	0.280	1	1	1	0.993
NO_3^-	0.086	***< 0.001***	0.997	1	0.309	0.999
NH_4^+	***< 0.001***	***< 0.001***	***< 0.001***	1	0.795	0.995
NH_4^+ 24 h^{-1}	***0.002***	***< 0.001***	***< 0.001***	1	0.882	0.996
SRP	0.205	***< 0.001***	1	0.993	0.979	1
SRP 24 h^{-1}	0.521	***< 0.001***	1	0.988	0.995	1

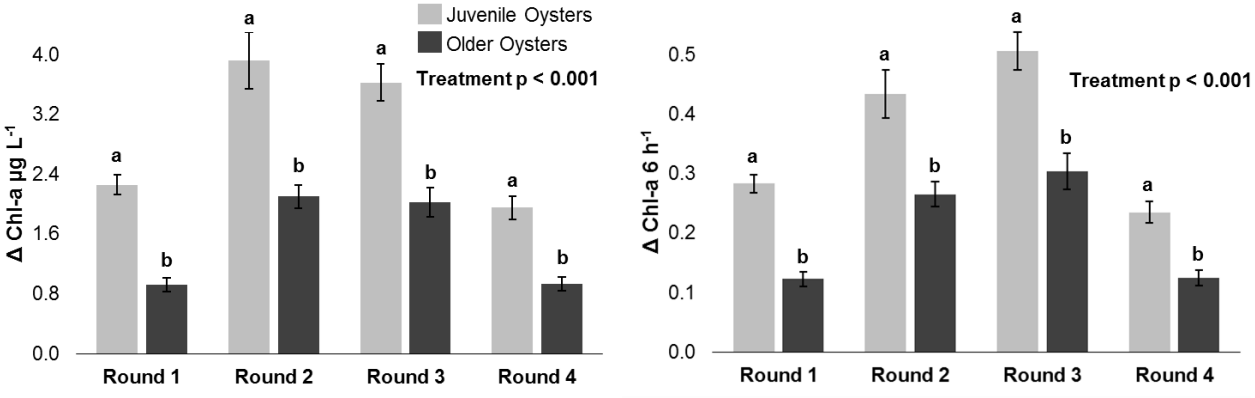

Figure 20: Change in chl-a (24 h – 0 h) and chl-a slopes in tank waters over 6 h for all rounds. Letters indicate significant differences between treatment means in each round.

Chl-a concentrations decreased by -36 ± 3 % in blank tanks over the entire experiment, by -81 ± 2 % in the juvenile oyster tanks and -76 ± 2 % in the older oyster tanks. When concentrations of chl-a are standardized to grams of dry body weight in each tank, juvenile oysters decreased chl-a concentrations over the 24 h feeding period by 2.9 ± 0.2 µg L^{-1} g dry wt^{-1} and older oyster tanks decreased by 1.5 ± 0.1 µg L^{-1} g dry wt^{-1} (Figure 20). Change in chl-a over the first 6 h of feeding in the oyster tanks approximated a linear decrease with r-squared values of 0.959 ± 0.006 for all treatment tanks. The change in chl-a over 6 h was different between

treatments (Table 13) with a mean decrease of -0.36 ± 0.02 µg L^{-1} 6 h^{-1} g dry wt^{-1} in juvenile oyster tanks and -0.20 ± 0.02 µg L^{-1} 6 h^{-1} g dry wt^{-1} in older oyster tanks over all four rounds (Figure 20). The starting concentration of chl-a at time 0 h changed from a mean of 2.3 ± 1.0 µg L^{-1} g dry wt^{-1} for all oyster tanks in round 1, to 3.6 ± 1.4 µg L^{-1} g dry wt^{-1} for all oyster tanks in round 2, 3.1 ± 1.1 µg L^{-1} g dry wt^{-1} in round 3 and 1.9 ± 0.7 µg L^{-1} g dry wt^{-1} in round 4 (Figure 20). Water column chl-a concentrations that are not standardized to dry tissue wt were analyzed to see if the treatment tanks had significantly different chl-a concentrations at the start of the 24 h feeding. The chl-a values were not significantly different between juvenile and older oyster tanks at time 0 h for all four rounds of the experiment (Table 14).

Table 14: Tukey HSD p values for starting chl-a concentrations in tank waters. Values bolded and italicized indicate $p < 0.01$ and bolded values indicate $p < 0.05$.

Chl-a comparison	Round 1	Round 2	Round 3	Round 4
Young - Older	1	1	0.940	1
Young - Blank	0.999	**0.014**	0.057	0.881
Older - Blank	1	***0.008***	0.543	0.922

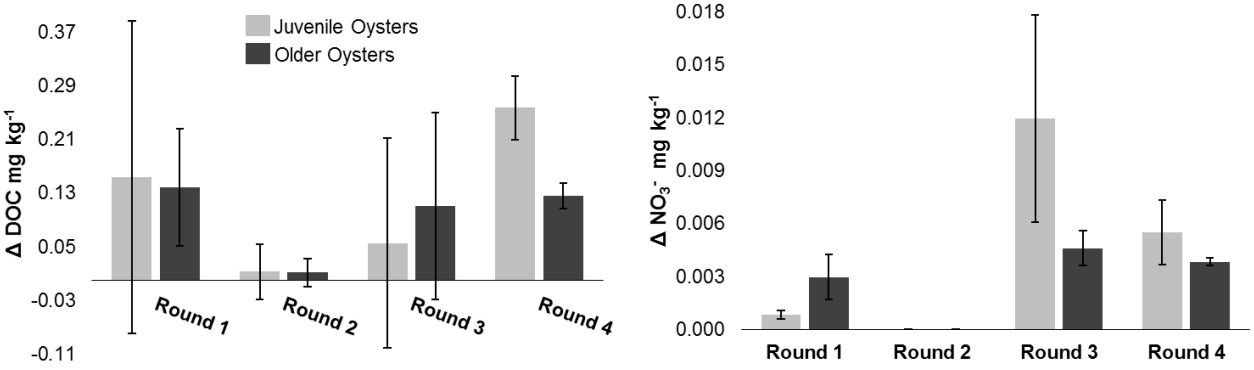

Figure 21: Change in tank water DOC and NO_3^- (24 h – 0 h) concentrations in tank waters for all rounds with no significant differences between treatment means in each round.

Differences in tank water DOC concentrations between 0-24 h were not impacted by treatment (Table 13). Differences in DOC over all four rounds between 0-24 h averaged 0.09 ±

0.07 mg L^{-1} g dry wt^{-1} for juvenile oysters and 0.04 ± 0.04 mg L^{-1} g dry wt^{-1} for older oysters (Figure 21). Concentrations of NO_3^- between 0-24 h were also not impacted by treatment but there was a significant interaction of treatment with rounds (Table 13). Tank water samples did not have any measurable concentrations of NO_3^- in round 2, from 0 - 6 h in round 1, and from 2 - 24 h in round 3. Most water samples that contained measurable concentrations of NO_3^- were at or near the minimum detection limit of 0.003 mg L^{-1}. Concentrations of NO_3^- decreased over all rounds by an average of -0.004 ± 0.002 mg L^{-1} g dry wt^{-1} for juvenile oysters and by -0.001 ± 0.001 mg L^{-1} g dry wt^{-1} for older oysters (Figure 21).

Both 0-24 h differences in tank waters and 0-24 h slopes of NH_4^+ concentrations differed by treatment and treatment also interacted significantly with rounds 1-4 (Table 13). Over all four rounds, juvenile oysters increased NH_4^+ concentrations after the 24 h feeding period by 0.15 ± 0.02 mg NH_4^+ L^{-1} g dry wt^{-1} and older oysters by 0.11 ± 0.01 mg NH_4^+ L^{-1} g dry wt^{-1} (Figure 22). The increase in NH_4^+ concentrations in tank waters over 24 h was approximately linear with r-squared values of 0.853 ± 0.024 for all oyster tanks. Over all four rounds of the study, juvenile oysters increased by 0.006 ± 0.001 mg NH_4^+ L^{-1} 24 h^{-1} g dry wt^{-1} and older oysters increased by 0.004 ± 0.001 mg NH_4^+ L^{-1} 24 h^{-1} g dry wt^{-1} (Figure 22).

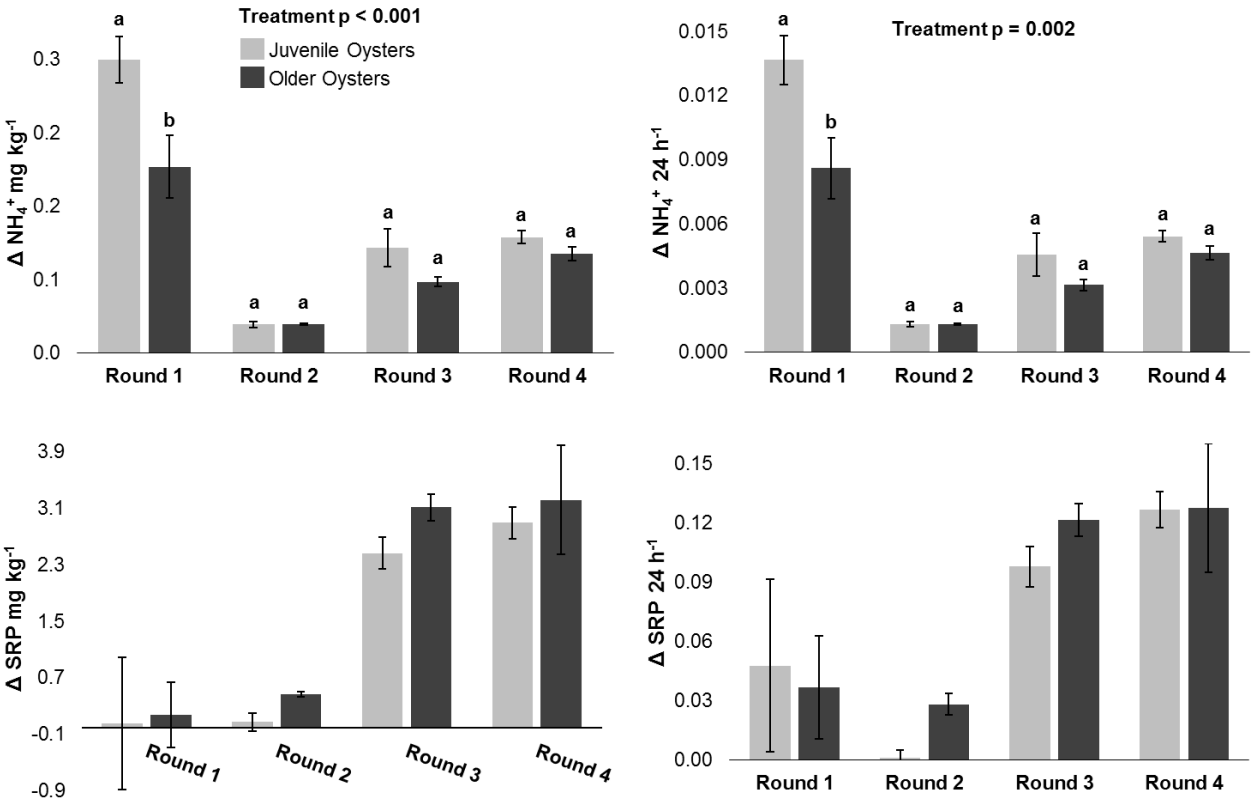

Figure 22: From left to right, change in NH_4^+ (24 h – 0 h) NH_4^+ slopes in tank waters over 24 h, change in SRP (24 h – 0 h), and SRP slopes over 24 h for all rounds with letters indicating significant differences between treatment means in each round.

Both 0-24 h differences and 0-24 h slopes of SRP in tank waters did not have an effect of treatment but treatment did interact significantly with round (Table 13). After the 24 h feeding periods, SRP increased by 1.3 ± 0.4 µg L^{-1} g dry wt^{-1} for juvenile oysters and by 1.7 ± 0.3 µg L^{-1} g dry wt^{-1} for older oysters over all four rounds (Figure 22). Changes in SRP concentration over 24 h were approximately linear with an r-squared of 0.647 ± 0.048. The rate of increase in SRP concentrations were 0.068 ± 0.014 µg L^{-1} 24 h^{-1} g dry wt^{-1} for juvenile oysters and 0.079 ± 0.013 µg L^{-1} 24 h^{-1} g dry wt^{-1} for older oysters over the entire study.

Biodeposit Nutrient Content

Table 15: Number of oysters and biodeposits collected in each tank. Top number- number of individual oysters per tank, middle number in italics - dry tissue weight per tank (g), bottom number - wet biodeposit weight per tank (g). The 7 tanks containing juvenile oysters and 7 tanks containing older oysters are shown.

Tank	Juv 1	Juv 2	Juv 3	Juv 4	Juv 5	Juv 6	Juv 7
Round 1	33	25	26	21	27	25	20
	3.91	*3.15*	*2.96*	*2.49*	*3.56*	*2.81*	*3.09*
	2.61	1.90	2.27	1.94	2.44	2.20	1.99
Round 2	32	36	35	30	31	34	37
	4.80	*5.07*	*3.67*	*3.94*	*3.32*	*5.26*	*5.15*
	5.21	2.78	2.07	5.99	2.49	2.74	2.70
Round 3	42	33	40	38	41	40	46
	7.74	*5.40*	*6.00*	*6.13*	*8.40*	*6.16*	*8.38*
	8.41	7.64	8.27	7.37	8.50	9.00	7.78
Round 4	44	52	41	37	44	42	43
	6.67	*7.22*	*7.60*	*7.15*	*7.22*	*6.82*	*6.48*
	2.49	3.56	3.23	2.29	2.65	3.45	2.42
Tank	Older 1	Older 2	Older 3	Older 4	Older 5	Older 6	Older 7
Round 1	21	16	18	18	17	14	14
	7.76	*8.21*	*7.53*	*8.16*	*6.84*	*7.03*	*5.09*
	2.36	2.50	2.77	2.46	2.38	2.28	2.39
Round 2	26	33	24	27	25	24	26
	7.83	*7.71*	*7.32*	*10.17*	*9.02*	*5.42*	*9.59*
	3.31	3.93	2.43	4.33	3.84	13.60	3.01
Round 3	25	28	31	32	28	30	32
	18.37	*13.47*	*12.46*	*11.40*	*10.19*	*8.46*	*15.96*
	10.30	10.80	8.31	9.26	10.53	8.61	9.72
Round 4	28	28	24	24	27	29	22
	14.90	*14.61*	*12.54*	*16.60*	*10.74*	*16.87*	*15.03*
	3.42	4.21	5.17	5.51	3.74	4.20	3.56

Table 16: Mean total wet biodeposit weights (g) collected per tank with standard deviations.

	Round 1	Round 2	Round 3	Round 4
Juvenile Tanks	2.19 ± 0.27	3.42 ± 1.52	8.14 ± 0.57	2.87 ± 0.53
Older Tanks	2.45 ± 0.16	4.92 ± 3.88	9.65 ± 0.96	4.26 ± 0.80

Over all four rounds of the experiment, tanks with juvenile oysters contained a total of 21-52 individual oysters per tank compared to 14-33 individuals per tank in the tanks with older oysters (Table 15). The total dry tissue wt per tank averaged 5.38 ± 1.84 g for the juvenile treatment compared to 10.69 ± 3.77 g for the older treatment (Table 15). Despite the difference in number of individual oysters and dry tissue wt between the treatments, the total wet wt of

biodeposits collected over all four rounds was a mean of 4.16 ± 2.52 g overall for juvenile tanks and 5.32 ± 3.32 g for the older oyster tanks.

Table 17: Tukey HSD p values for treatment tank to blank tank pairwise comparisons in individual rounds. No measurable amount of sediment was recovered from the blank tanks in Round 1. Bolded values indicate $p < 0.05$.

		NO_3^-	NH_4^+	SRP	DOC
Round 2	Older - Blank	1	1	0.958	1
	Juvenile - Blank	1	1	1	1
Round 3	Older - Blank	0.924	0.993	0.726	0.430
	Juvenile - Blank	0.949	0.726	1	0.147
Round 4	Older - Blank	0.749	0.914	**0.022**	0.982
	Juvenile - Blank	0.860	0.241	0.676	0.994

Enough sediment was collected in rounds 2, 3 and 4 to analyze extractable nutrients on water column deposits in the three blank tanks. Sediment deposited into jars in the blank tanks was not significantly different in nutrient content from biodeposits collected in the treatment tanks (Table 17). The only detectable difference was in SRP concentrations between biodeposits from older oysters and the blank tanks in round 4.

Table 18: ANOVA and Tukey HSD results for standardized biodeposit nutrients in concentrations of mg kg^{-1} g dry wt^{-1}. Values bolded and italicized indicate $p < 0.01$ and bolded values indicate $p < 0.05$.

	Treatment	Round	Round 1	Round 2	Round 3	Round 4
DOC	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	0.900	0.986
SRP	0.077	***< 0.001***	0.148	0.746	0.903	0.516
NO_3^-	***< 0.001***	***0.008***	***0.004***	0.069	0.102	0.152
NH_4^+	**0.046**	***< 0.001***	0.224	0.241	1	0.955

Biodeposit nutrient concentrations were significantly different by treatment for DOC, NO_3^-, and NH_4^+. The interaction between treatment and round was significant for all four extractable nutrients (Table 18). The average DOC concentration in biodeposits excreted by juvenile oysters over all four rounds was 183 ± 27 mg DOC kg^{-1} biodeposit g dry wt^{-1} and by older oysters was 108 ± 12 mg DOC kg^{-1} biodeposit g dry wt^{-1} (Figure 23). SRP concentrations did not differ by treatment but did have a significant interaction between treatment and round

(Table 18). Over all four rounds, juvenile oysters excreted biodeposits with 9.8 ± 1.3 mg SRP kg^{-1} biodeposit g dry wt^{-1} and older oysters excreted biodeposits with 7.7 ± 0.8 mg SRP kg^{-1} biodeposit g dry wt^{-1} (Figure 23).

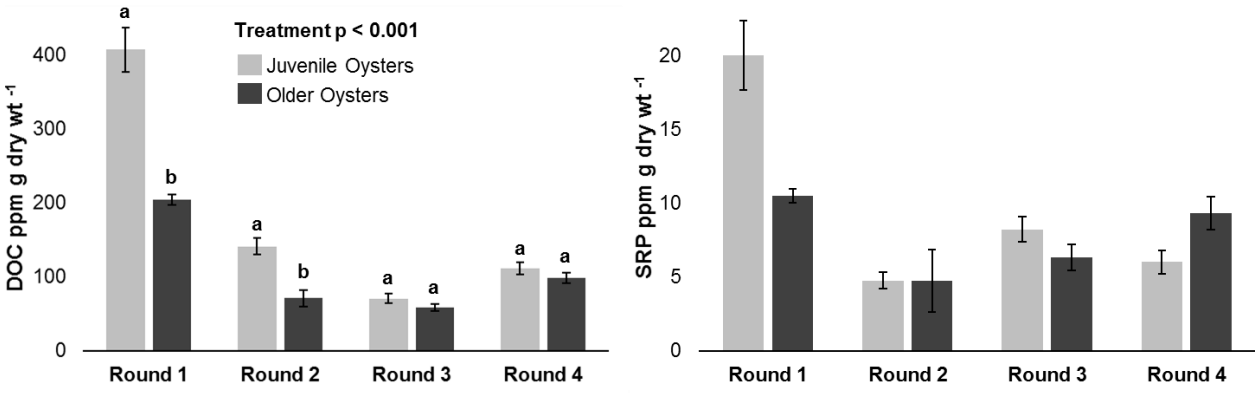

Figure 23: Standardized biodeposit DOC and SRP (mg kg^{-1} g dry wt^{-1}) concentrations for all rounds with letters indicating significant differences between treatment means in each round.

NO_3^- concentrations in oyster biodeposits were also significantly different between treatments (Table 18). Over the entire study, NO_3^- concentrations were 2.0 ± 0.2 mg NO_3^- kg^{-1} biodeposit g dry wt^{-1} for juvenile oysters and 1.1 ± 0.1 mg NO_3^- kg^{-1} biodeposit g dry wt^{-1} for older oysters (Figure 24). Over the entire study, juvenile oyster biodeposits contained 37 ± 5.3 mg NH_4^+ kg^{-1} biodeposit g dry wt^{-1} and older oysters biodeposits contained 31 ± 5.0 mg NH_4^+ kg^{-1} biodeposit g dry wt^{-1} (Figure 24).

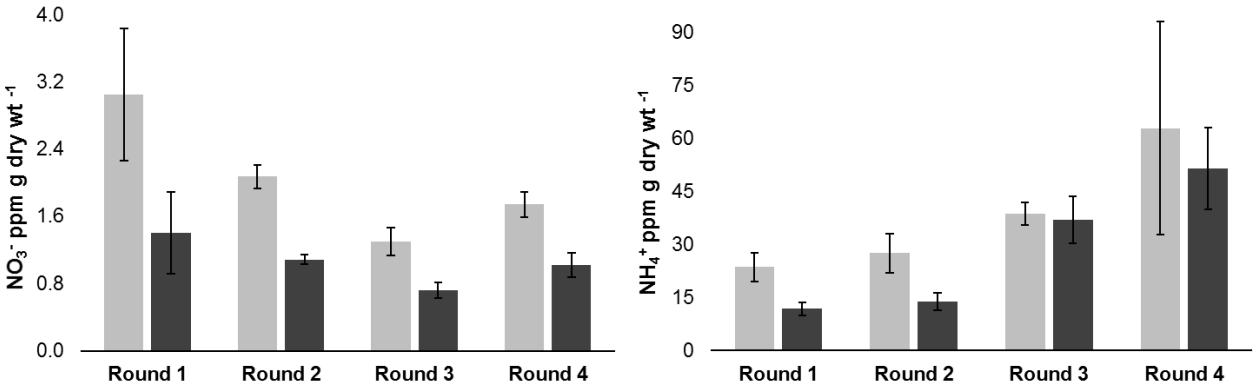

Figure 24: Standardized biodeposit NO_3^- and NH_4^+ (mg kg^{-1} g dry wt^{-1}) concentrations for all rounds with letters indicating significant differences between treatment means in each round.

Discussion

Oyster Age Class

Generally, spat oyster is ≤ 25 mm length and adult oyster is > 25 or 30 mm length (Blomberg et al. 2017, La Peyre et al. 2014). For all juvenile oyster tanks throughout the study, spat composed anywhere from 9 – 37 % of the individuals, with an average of 19 % of the oysters in each tank. Average shell length in juvenile oyster tanks ranged from 31 – 41 mm, which is near the mean shell length of 42.4 ± 0.3 mm for oysters in Mobile Bay, AL used for the juvenile treatment in the age class comparison conducted by Dalrymple and Carmichael (2015). The average length in older tanks ranged from 41 – 74 mm with an average of 63 % of the oysters in each tank measuring > 50 mm (Figure 16). Shell length is used to provide evidence for the difference in age for the two treatment groups because oysters generally have linear growth rates in the first year of life (Munroe et al. 2017) and then decrease as oysters approach maximum size (Dalyrmple & Carmichael 2015, Kennedy 1996). Dalyrmple and Carmichael (2015) demonstrated that juvenile oysters grew at a quantifiable rate per day and continually assimilated N into soft tissues, while adult oysters did not have measurable shell growth and even lost soft tissue weight and N content over ~150 d. Observing the 31 – 41 mm size range in the juvenile treatment after a possible 12-14 months of growth (time since restoration) shows that with high temperatures in ML, *C. virginica* can grow at greater rates; as oyster growth has been shown to be positively correlated with temperature and salinity (Kennedy 1996). Oysters in the Delaware Bay with slightly colder temperatures (21.7 – 24.9 °C) and salinities of about half ML (13.5 – 18.2 ppt) reached a size of 27 – 33 mm in their first year of growth (Munroe et al. 2017). Other estimates of the first-year growth of *C. virginica* exceed shell lengths here but are from oysters that were spawned and raised in optimal laboratory conditions. These oysters reached

shell lengths of 50 mm (Harding 2007) and 50 – 70 mm (Paynter et al. 2010) in the first year of life.

Additional factors that can influence oyster growth and recruitment patterns include reef area and type such as fringing versus patch reefs (Hanke et al. 2017). For this reason, oysters used in this study were collected from the same reef and assumed to increase in shell length at similar rates. Given that variability in reef size and complexity does not cause significant differences in the food resources available to eastern oysters (Blomberg et al. 2017), this study assumes of exposure to similar conditions between the restored section and intact section of the reef is credible. Larval development is significantly influenced by both food concentrations and temperature (Rico-Villa et al. 2009) and both temperature and chl-a (phytoplankton) concentration have been found to not vary significantly above an oyster reef on the meter scale (Lenihan 1999, Plutchak et al. 2010, Dalyrmple & Carmichael 2015). Furthermore, environmental conditions that affect growth rates such as temperature, salinity, and dissolved oxygen, as well as duration and depth of water inundation, vary less in an individual reef than between reefs at different sites (Byers et al. 2015, Bartol et al. 1999, Dillon et al. 2015, Hanke et al. 2017, Lenihan 1999). The variations in shell length and g dry body wt for each treatment/age class could be attributed to the fact that oyster growth and reproduction is greatest at temperatures between 20 – 30 °C (Kennedy 1996) and reproduction can occur year-round in central Florida (Grizzle 1990), thus spat can settle and begin growth on both sections of this reef at any time of the year.

Water Quality Parameters

Salinity ranged from 32.3 – 36.8 ppt at time 0 h in the oyster tanks, which is within the range of ML's 22.6 – 45.2 ppt salinity, according to measurements taken monthly from May

2006 – July 2013 (Phlips et al. 2015). Salinity did not change due to the action of oyster feeding over all four rounds of the experiment (Table 11). The slight decreases in conductivity observed in both treatment change was most likely due to oyster feeding reducing the concentrations of the dissolved ions that contribute to resistivity of an electrical current in the water. Conductivity measurements are not typical of oyster feeding experiments, either in the field or the laboratory, as environmental parameters such as temperature, salinity, particulate matter concentrations, and particle size are the primary drivers of oyster feeding behavior (Baldwin and Newell 1985, Loosanoff and Engle 1947, Newell and Jordan 1983). This study is one of the few, if any, to measure the effects of oyster feeding on reducing conductivity in the water column. Conductivity and temperature differed between the 3 blank tanks and 14 treatment tanks during rounds 1 and round 4 (Table 10). This is most likely due to uneven filling of the 20-gallon tanks from the large tank reservoir of site water. The large Kolaps-a-Tank used for this experiment was mixed during the filling of the 20-gallon tanks however it was still possible that inconsistencies in the particulate matter or temperature of the water existed.

Over all rounds of the experiment, starting temperature in the tanks ranged from 24.9 – 27.9 °C. This is in the upper half of the 4 – 33 °C temperature range for ML measured by monthly samplings in Phlips et al. (2015). Over all four rounds of the experiment, the average drop in temperature was -1.5 ± 0.1 °C with a minimum drop of -0.4 °C and a maximum drop of -3.4 °C (Figure 20). This was most likely due to the gradual decrease of water temperature as it was transferred from the Kolaps-A-Tank into the oyster tanks that were indoors. According to one of the only studies on oyster pumping rates in L h^{-1} in response to temperature, Loosanoff (1958) determined that eastern oysters of 100 to 110 mm in length rapidly change feeding rate between the temperatures measured in this experiment, therefore it is entirely likely that a

temperature drop of ~1.5 °C over 24 h can measurably change feeding rate. However, chl-a concentrations started to plateau after only 6 h of feeding and the effects of temperature changes < 1.0 °C within 6 h likely did not contribute to differences between the treatments.

DO use by oysters in all treatment tanks created DO levels that were significantly lower than that of the blank tanks over the 24 h feeding periods (Table 10). Juvenile oysters used up oxygen at a rate that was 0.14 % DO g dry wt^{-1} faster than older oysters. This indicates that when presented with the same food resources, the juvenile oysters utilized more O_2 per g dry body wt than older oysters and their metabolism was respiring at faster rates. Since the juvenile oysters were a maximum of 12-14 months old, most of the oysters were in a phase of rapid growth that occurs during the first year of life (Munroe et al. 2017) and metabolic requirements for growth during the juvenile stage are greater than the adult stage (Kennedy 1996). The differences in oxygen consumption between rounds can be attributed to differences in water temperature as oxygen uptake is positively correlated with temperature (Shumway & Koehn 1982). In addition, microbial activity alone cannot account for these large drops in % DO because on oyster reefs, oxygen uptake is largely attributed to respiration by oysters and not microbial respiration (Reidenbach et al. 2013). When oyster biomass is not accounted for, DO decreased by a mean of 26 ± 2 % for juvenile oyster tanks and 39 ± 3 % for older oyster tanks over 24 h. Despite declining DO concentrations, the oysters continued to feed during all 24 h (Figures 18 & 20). According to work by Shumway & Koehn (1982), as oxygen concentration declines, *C. virginica* is able to regulate oxygen consumption in a variety of temperatures and salinities. The effects of oysters on oxygen concentrations in this experiment are not translatable to oysters exposed to other temperatures and salinities as acclimation to temperature and salinity has a significant effect on oxygen consumption (Shumway and Koehn 1982).

The greater decrease in % DO in the juvenile oyster tanks matches the response that occurred in water pH levels. This created pH levels in the treatment tanks that were significantly lower than that of the blank tanks over all four rounds of the experiment (Table 10). Higher rates of respiration by the juvenile oysters releases more CO_2 into the water column as a byproduct of respiration. At the pH range of 7.24 – 8.25 in the oyster tanks throughout the experiment, the dominant form of dissolved CO_2 in water of a salinity near that of seawater (35 ppt) is bicarbonate (HCO_3^-) and in the process of CO_2 bonding with a molecule of H_2O to form HCO_3^-, one H^+ is produced (Pavlova 1990). Although respiration rates or CO_2 concentrations were not measured in this study, the greater drop in % DO in the juvenile treatment indicates greater respiration rates per g body wt and thus greater rates of CO_2 release and decreases in pH levels (Figure 19). The drops in both % DO and pH observed over the 24 h feeding period are similar in magnitude and duration to the fluctuations that occur at night when rates of photosynthesis decrease and DO concentrations decrease, CO_2 concentrations increase and pH decreases due to respiration (Keppel et al. 2016). Bayne (2002) demonstrated that other oyster species modify their feeding behavior in order to compensate for fluctuations in environmental conditions such as temperature, DO and pH. Under conditions of low pH (generally < pH 7.5), Keppel (2014) observed increases in adult oyster feeding that compensated for instantaneous lower growth rates. However, chl-a and turbidity concentrations in my study show that available food resources were depleted by ~12 h which didn't allow for measuring this signal of increased feeding by older oysters. The juvenile treatment was likely impacted less by drops in % DO and pH because Keppel et al. (2016) demonstrated that juvenile oysters can withstand the short-term negative effects of diel cycling of hypoxia and pH levels as low as 7.0.

This consistency in reductions of turbidity between rounds 1-4 indicate that feeding by *C. virginica* can be consistent despite significant changes in other water parameters such as temperature, DO concentration, and chl-a concentrations (Table 11). Between 0-6 h, turbidity changed more in the treatment tanks compared to the blank tanks due to the action of oyster feeding reducing particulate matter in the water column (Table 10). Measurements of turbidity combined with chl-a concentration can provide a strong indication of food resources available to filter feeders (Iglesias et al. 1998) because turbidity can measure the presence of other particulate organic matter such as detritus that the fluorescence method used by chl-a probes cannot measure (Falkowski and Kiefer 1985).

Water Nutrients

Water column chl-a concentration is a useful indicator of oyster feeding because a main food source for oysters is photosynthetic plankton of various sizes (Ward et al. 1998) and chl-a concentration is used as a proxy for phytoplankton concentration in water (Andersson and Rudehäll 1993). As chl-a is a direct measure of the food available to oysters, this variable proved to be the most useful in indicating differences in clearance rates, and thus oyster feeding, between treatments. Since previous studies indicate food resource use by oysters does not change with oyster reef condition or among reefs from different habitats or over time (Blomberg et al. 2017), we do not anticipate the field conditions of the two treatment groups used in this study contributed to the observed differences in the tanks.

Both treatments reduced water column chl-a concentrations in rounds 2, 3 and 4 of the experiment (Table 13). A significant in reduction was not observed in round 1 likely because this round occurred earliest in the summer season when chl-a concentrations were lowest, creating fewer food resources during the 24 h feeding period. Reductions in chl-a concentrations between

0-24 h and rates of chl-a reduction over 6 h were both higher in juvenile oysters (Figure 20). These differences in filtration rates can be attributed to the differences in energy allocation between juvenile and older oysters. Since the juvenile age class is in a period of more rapid growth than the older oysters (Munroe et al. 2017) and their smaller size limits their energy reserves, they most likely have to compensate by feeding at more rapid rates (Keppel et al. 2016). This is supported by calculations based on in situ measurements of filtration rates on restored reefs in Louisiana, where La Peyre et al. (2014) calculated that oysters < 75 mm accounted for 70 % of filtration capacity on a reef while oysters > 75 mm accounted for the remaining 30 %.

This study found clear reductions in chl-a concentrations over time because tank waters contained a limited amount of phytoplankton. There are few field measurements of chl-a reductions above oyster reefs and these studies have shown varying results with no standard for oyster densities or temporal scales (Cressman et al. 2003, Dame et al. 1992, Dame and Libes 1993, Grizzle et al. 2018, Nelson et al. 2004, Wilson-Ormond et al. 1997) and wide variability of chl-a measurements even on an individual reef (Grizzle et al. 2008). Indications of significant reductions in chl-a have been observed between upstream and downstream of a reef at 5-10 cm from the surface (Dame et al. 1992, Grizzle et al. 2008, Nelson et al. 2004), 10-20 m off the reef (Grizzle et al. 2018) and were not observed at ~50 cm from the reef surface (Plutchak et al. 2010). Based on the slopes of chl-a clearance from 0-2 h for the most realistic field concentrations of phytoplankton and the assumption that oysters feed for 12 h out of 24 h per day due to tidal cycling, calculations showed that *C. virginica* under these experimental conditions can remove chl-a at a rate of 2.3 mg L^{-1} m^{-2} of reef in one day for 25 g dry wt^{-1}, which supports numerical model results by Newell and Koch (2004) that even modest abundances (25 g dry wt^{-1}

m^{-2}) of oysters over an entire estuary can significantly reduce suspended particle concentrations in shallow estuaries. Using the same assumptions and an average of 196 g m^{-2} dry weight of oyster, a total annual removal of 6.6 g m^{-2} of reef was calculated which is 72% lower than the 23.7 g m^{-2} estimate of annual chl-a reduction by Dame et al. (1992) in which a regression equation that accounted for 85 % of variation in chl-a provided their estimate. This difference highlights the lack of realistic estimates from laboratory-held oysters and the importance of taking into account environmental variables such as variations in tidal cycles, water velocities, phytoplankton concentrations and water temperatures to produce accurate estimates of oyster filtration (Dame et al. 1992).

Concentrations of particulate matter fractions are typically used to characterize the food available to oysters (Iglesias et al. 1998) as oysters feed exclusively on particulates and microorganisms suspended in the water column (Dame et al. 1984). Bivalve filter feeders indiscriminately intake particulate matter and can capture particles greater than 5 µm in diameter with ~50-90 % efficiency (Dunphy et al. 2006, Riisgård 1988) and particles up to 200-300 µm in diameter (Paulmier 1972). In this study, dissolved organic C and dissolved inorganic N and P were measured in tank water and are in the < 45 µm range. This size range still encompasses microphytoplankton (20-75 µm) and nanophytoplankton (2-20 µm), which are more nutritious for adult eastern oysters (Langdon and Newell 1996), and picophytoplankton (<2 µm) (Newell 2004). I expected to see a consistent decrease in DOC concentrations over time since oysters feed upon phytoplankton assemblages < 45 µm (Dunphy et al. 2006, Newell and Jordan 1983). Despite significant reductions in chl-a concentrations, DOC concentrations did not show a proportional rapid decrease. There were no significant differences between treatments in DOC concentrations in any round of the experiment (Table 13). In theory, *C. virginica* will remove

almost all of the particles that contain organic C < 45 µm from the water column (Newell and Jordan 1983), but results here indicate that resuspension of DOC from biodeposits could be the only source of the slight additions of DOC on the order of 10^{-2} mg L^{-1} g dry wt^{-1} that were observed after 24 h of feeding (Figure 21).

Both DOC and NO_3^- in the water column were unaffected by the presence of oysters (Table 12). Almost all NO_3^- measurements were below detection (BD). Prior studies on the composition of nitrogenous wastes in bivalves show that inorganic NO_3^- is not a waste product found in urine (Bayne & Hawkins 1992). The other possible pathway for NO_3^- production in the water column is through the oxidation of NH_4^+ into NO_2^-, and then NO_3^-, by aerobic nitrifying bacteria (Heiss & Fulweiler 2017). Despite the availability of the two reactants, O_2 and NH_4^+, necessary for the production of NO_3^-, the BD values indicate that nitrification may be negligible in this closed system. As NH_4^+ became increasingly available by oyster excretion, the depletion of % DO by respiration also reduced the likelihood that nitrifying bacteria can oxidize NH_4^+ to form NO_3^- (Dame et al. 1992, Reddy and Delaune 2008, which seems plausible based on decreases in NO_3^- for rounds 3 and 4 (Figure 21). Furthermore, NO_3^- is often a limiting nutrient for algae growth and was assimilated so rapidly in aquaculture oyster ponds in France that it was also measured as BD (Robert et al. 1982). The lack of treatment differences for tank water NO_3^- and SRP is in agreement with data from Jackson et al. (2018) that showed no significant relationship between oyster biomass with fluxes of SRP and NO_3^- directly from oyster clusters.

However, multiple studies have demonstrated that fluxes of NH_4^+ in both sediments and the water column on oyster reefs have a significant positive relationship with oyster biomass, which can be attributed to oyster excretion and biodeposition (e.g., Chambers et al. 2017, Dame et al. 1984, Jackson et al. 2018, Kellogg et al. 2013, Smyth et al. 2016). The presence of oysters

increased water column NH_4^+ concentrations above that of tanks without oysters in three out of four rounds of the experiment (Table 12). The source of this form of inorganic N comes from their diet of N-rich phytoplankton, bacteria and flagellates (Bayne & Hawkins 1992, Kreeger & Newell 2001). The majority of N assimilated by oysters is used for tissue growth and the rest is excreted as urine, 70 % of which is NH_4^+ (Bayne & Hawkins 1992). In the field, concentrations of NH_4^+ downstream of a reef have been recorded at much higher levels than in the water column above other benthic systems (Hammen et al. 1966, Nelson et al. 2004, Dame et al. 1984, Dame et al. 1992).When enclosed in 20-gallon tanks, oysters increased NH_4^+ concentrations over time and the juvenile oysters produced NH_4^+ at rates that were significantly higher than adult oysters (Figure 22). This can be attributed to higher rates of growth in juvenile oysters (Munroe et al. 2017) leading to higher rates of feeding (Figure 20), and thus more production of NH_4^+ by nitrogenous wastes. In some tidal creeks, oysters alone do not produce enough NH_4^+ to satisfy phytoplankton productivity (Dame et al. 2002), so at the scale of 20-gallon aquaria and with phytoplankton removal, there should be no sinks for NH_4^+, allowing it to continually accumulate over the 24 h period (Figure 22). Additionally, there were no sediments in these tanks, so the amount of NH_4^+ in the water column released by the mineralization of particulate organic N to NH_4^+ by microbes can be considered negligible (Dame et al. 1989).

On a g dry wt basis, dissolved SRP increased by an order of magnitude less than DOC after 24 h (Figures 21 & 22). The small concentration of SRP (1.5 ± 0.2 µg L-1 g dry wt-1) released into tank waters after 24 h is likely because significantly more organic P than inorganic P is released by oysters (Dame et al. 1989, Newell et al. 2005, Porter et al. 2018). A study of P flux on an intertidal oyster reef demonstrated that very little of total P is mineralized into SRP by oyster metabolism (Dame et al. 1989). Despite differences in growth rates between juvenile and

older oysters (Dalrymple & Carmichael 2015), no differences were found between treatments for 0-24 h changes in DOC and SRP (Table 13). This can be partially attributed to the variability of negative and positive increases in both DOC and SRP (Figures 21 & 22). However, the older and large oysters did increase SRP concentrations above that of the blank tanks in rounds 3 and 4 (Table 12), showing that in high enough densities oysters can increase local inorganic P concentrations.

Biodeposit Nutrient Content

Biodeposit nutrient measurements using the funnel drop method comprise both feces and pseudofeces as all solid excreta from oysters settled into the collection jars. Since defecation occurred within 24 h, all of the material ingested by oysters was considered a part of these measurements, including some waste from food consumed in the field prior to the experiment (Dalyrmple & Carmichael 2015, Newell 2004). The extractable nutrient content of excreted material from oysters and material that fell out of suspension in the blank tanks was not significantly different (Table 17). This indicates that oysters may not significantly reduce NO_3^-, NH_4^+, SRP or DOC concentrations of ingested organic matter via digestion and assimilation and supports the idea that *C. virginica* plays a critical role in connecting water column nutrients to benthic nutrients (Newell et al. 2005, Smyth et al. 2016). In comparing the relative concentrations of the extractable nutrients in biodeposits, the concentrations differed by orders of magnitude. When biodeposit nutrient concentrations are standardized to g dry wt, this study found concentrations of DOC one order of magnitude higher than NH_4^+ and two orders of magnitude higher than both SRP and NO_3^- (Table 19).

Table 19: Comparison of mean biodeposit nutrient concentrations (mg kg^{-1} g dry wt^{-1}) between treatments and overall average value presented for all oysters. Values bolded indicate significant differences between treatment means according to ANOVA results.

	DOC	SRP	NO_3^-	NH_4^+
Juvenile	**183 ± 26.7**	9.77 ± 1.33	**2.04 ± 0.23**	**36.7 ± 5.30**
Older	**108 ± 11.7**	7.74 ± 0.75	**1.06 ± 0.13**	**31.4 ± 4.97**
All oysters	146 ± 15.3	8.76 ± 0.77	1.55 ± 0.15	34.1 ± 3.62

The uptake of nutrients from food in bivalves are largely driven by food quality and current metabolic requirements (Bayne 2009, Deslous-Paoli et al. 1992, Wilson-Ormond 1997). Following nutrient uptake, biodeposit production is related to soft tissue mass, physiological activities, and particulate matter concentration (Dalrymple & Carmichael 2015, Songsangjinda et al. 2000) and both organic C content and biodeposition rates can vary seasonally (Haven & Morales-Alamo 1966, Mitchell 2006). The greater reductions of water column chl-a by the juvenile oysters indicate higher clearance rates per g dry tissue, likely fueled by a need to sustain higher tissue growth rates compared to the older oysters (Munroe et al. 2017). This could lead to the higher concentrations of DOC, NO_3^-, and NH_4^+ observed in the juvenile oyster biodeposits (Table 19) as a result of more particles rejected as pseudofeces, thus enriching total biodeposits in inorganic particles that are deemed not nutritious enough by the labial palps (Newell & Jordan 1983). Additionally, higher clearance rates could indicate that juvenile oysters have higher success in ingesting organic-rich particles to then convert them into a higher concentration of inorganic particles present in feces (Jordan 1987). The first scenario of greater pseudofeces production seems to be more likely based on a prior study by Dalrymple & Carmichael (2015) where juvenile pseudofeces had significantly higher N content than adult pseudofeces but the percentage of N in feces did not differ between age classes. Dalrymple & Carmichael (2015) also found that pseudofeces weights were greater than feces weights in both age classes, leading to

overall higher N contributed by pseudofeces than feces. Furthermore, a separate study of sediment enrichment underneath juvenile and adult oysters found significantly higher chl-a concentrations in the sediment underneath the juvenile oysters (Mortazavi et al. 2015).

Haven & Morales-Alamo (1966) found that the largest group of oysters in their study (mean weight 73.3 g oyster^{-1}) deposited less material per unit weight than the three smaller groups, supporting the notion that juvenile oysters can capture more particles and have higher concentrations of inorganic nutrients in pseudofeces. These results were repeated by Dalrymple & Carmichael (2015), where both feces and pseudofeces production increased up to 1.2-1.5 g dry wt oyster^{-1}, and then decreased for adult oysters larger than this. Concentrations of particulate organic C and N in biodeposits could help determine if juvenile oysters did indeed ingest more large particulate particles but the particulate size fraction (> 0.7 μm) of C and N was not measured in this study.

Biodeposit to Sediment Nutrient Comparison

Table 20: Comparison of mean nutrient concentrations (mg kg^{-1}) between biodeposits and natural reef sediments in Mosquito Lagoon (Ch. 2 values). Orders of magnitude between the two values are shown.

	DOC	SRP	NO_3^-	NH_4^+
Natural Reef Sediments	95.2 ± 3.94	0.512 ± 0.047	0.620 ± 0.041	9.58 ± 0.55
All oysters	928 ± 58.7	64.9 ± 5.81	10.1 ± 0.605	311 ± 47.0
Magnitude difference	1	2	2	2

Bivalve filter feeders can assimilate 40-94 % of captured organic matter for physiological needs with a portion of that excreted in urine, leaving 6-60 % of the ingested matter released as

feces and pseudofeces (Deslous-Paoli et al. 1992, Kreeger & Newell 2001). At a range of environmentally relevant particulate matter concentrations, Deslous-Paoli et al. (1992) demonstrated that the nutrient content of feces and pseudofeces is less than that of the ingested suspended particles, yet still biodeposits are known to enrich sediments with labile C and N (Dame et al. 1989, Newell et al. 2005, Pollack et al. 2013, Smyth et al. 2016). With the average of both juvenile and adult oysters taken to attain a more realistic reef-wide comparison, the extractable nutrient content of biodeposits (non-standardized) are 1 to 2 orders of magnitude higher than sediments collected from natural reefs in Mosquito Lagoon (Table 20). The large differences in SRP, NO_3^-, and NH_4^+ concentrations between biodeposits and sediments indicate that these nutrients are likely transported away from the reef by water currents and are deposited elsewhere or stay suspended in the water column (Haven & Morales-Alamo 1966, Newell et al. 2005, Porter et al. 2018). It is likely that the nutrients are utilized in primary production as SRP and NO_3^- in particular are limiting nutrients for algae growth (Robert et al. 1982, Plutchak et al. 2010). However, DOC and SRP concentrations in the water column varied widely (Figures 21 & 22).

Table 21: Comparison of mean nutrient concentrations (ppm) between time 0 h tank water and oyster biodeposits. The orders of magnitude between the two values are shown.

	DOC	SRP	NO_3^-	NH_4^+
0 h Tank Water	5.11 ± 0.225	0.008 ± 0.001	0.032 ± 0.006	0.002 ± 0.002
All oysters	928 ± 58.7	64.9 ± 5.81	10.1 ± 0.605	311 ± 47.0
Magnitude difference	2	4	3	5

When extracted from the water column and repackaged into oyster biodeposits, DOC and NO_3^- increased in concentration by a magnitude of 2 and 3, respectively, concentrations of SRP increased by a magnitude of 4 and NH_4^+ by a magnitude of 5 (Table 21). This indicates that biodeposition can significantly transform water column nutrients and not only have an impact on N cycling, but also significantly impact estuarine C and P cycles which are elemental cycles that are much less studied on oyster reefs (Fodrie et al. 2017, Newell et al. 2005).

The results from this laboratory experiment are not fully translatable to field conditions as food resources on oyster reefs can vary over time due to seasonal shifts in phytoplankton communities (Kreeger & Newell 2001, Phlips et al. 2015). The magnitude and relative concentrations of biodeposit extractable nutrients in this study will likely vary for eastern oysters experiencing different physical and biological conditions, such as particulate matter concentrations (Newell and Jordan 1983, Wilson-Ormond et al. 1997), water temperature (Dame et al. 1992, Haven & Morales Alamo 1966), and life stage (Dalrymple & Carmichael 2015). These are some of the main drivers identified in previous studies that affect both feeding and biodeposition. In the field, differences in water flow velocity and duration of inundation between reefs can affect the growth of oyster biomass (Byers et al. 2015), thus feeding and subsequent biodeposit production likely varies greatly between reefs with different hydrodynamic properties. Furthermore, nutrients from biodeposits are not all deposited into one sink; biodeposits can only reach the sediment surface when bottom water velocities are low enough (Newell et al. 2005, Porter et al. 2018). Areas with high flow speeds distribute biodeposits off the reef and can encourage these nutrients to enter the water column (Haven & Morales-Alamo 1968, Mitchell 2006, Widdows et al. 1998) or be remineralized on the sediment surface to sustain the growth of more phytoplankton (Newell 2004 and references therein, Newell et al. 2005).

Conclusion

Measurements here are a collection of 24 h snapshots of dissolved C, N and P and chl-a in the water column and subsequent release in oyster biodeposits during the summer season. The treatment (age class) of eastern oysters in this study had a profound effect upon % DO, pH, and turbidity, the reductions of chl-a in the water column and both DOC and inorganic N released in biodeposits. Results support both hypotheses that juvenile oysters have a greater capacity than older oysters to remove chl-a and decrease turbidity (hypothesis 1), as well as produce biodeposits that have higher concentrations of DOC, NO_3^-, and NH_4^+ (hypothesis 2), thus beginning to answer the age-nutrient paradox of the observed higher sediment nutrients in reefs with lower oyster densities in ML (Chambers et al. 2017). Evidence from this study indicate that eastern oysters play a significant role in transforming C, N and P, but further characterization of the fate of oyster biodeposits will allow for understanding the full magnitude and extent of this role.

CHAPTER 4: SUMMARY

Evidence presented in these two studies supports the theory that eastern oysters play a significant role in the cycling of C, N and P in estuarine ecosystems at both the estuary scale (Ch. 2) and reef scale (Ch. 3). These studies represent an important contribution to the body of science on the restoration of eastern oyster reefs, as the past two decades have seen restoration goals shift from the augmentation of commercially-viable oyster populations to enhancing oyster reef ecosystem services (e.g., Coen & Luckenbach 2000, Coen et al. 2007, Bagget et al. 2015, Grabowski et al. 2012). The expected recovery time of these ecosystem services is important to restoration managers aiming to improve water quality and reduce nutrient loading with eastern oyster populations (Kellogg et al. 2014 and references therein). With the recognition of oyster reefs as areas of enhanced C, N and P cycling and storage, measuring changes in biogeochemical properties on oyster reefs should be incorporated into restoration monitoring plans where the ecosystem-level impacts of oyster recovery are a primary objective.

A prior study by Chambers et al. (2017) identified the rapid increase in sediment biogeochemical properties with increasing age of restored reefs in Mosquito Lagoon, FL. The before-after-control-impact study presented in Chapter 2 focused on the response time of organic matter content and both extractable SRP and NH_4^+ to within weeks to six months, and provided a comparison of dead, natural and restored reef sediment biogeochemistry over a year of measurements. Dalrymple & Carmichael (2015) compared N content in bidoeposits from juvenile and adult oysters but to our knowledge, no studies to date have measured extractable nutrient concentrations in biodeposits or investigated C and P content of biodeposits between age classes. Both studies add understanding to the broad knowledge gap of C and P cycling on eastern oyster reefs, as numerous investigations of N cycling have been conducted on oyster

reefs across the Gulf of Mexico and the Atlantic Coast of the United States (e.g., Carmichael et al. 2012, Dame et al. 1989, Kellogg et al. 2013, Mortazavi et al. 2015, Pollack et al. 2013, Smyth et al. 2013b, Southwell et al. 2017).

Within twelve-months post-restoration, eastern oysters can impact sediment biogeochemical properties in the following ways:

- Extractable SRP, NH_4^+, and organic matter in surface sediments can respond within weeks and reach concentrations similar to natural reefs by one-month post-restoration.
- DOC was the only sediment property to exceed the levels of natural reefs within one-year post-restoration and can be attributed to the ability of juvenile oysters to produce biodeposits with higher DOC content than older oysters, as well as concentrations of DOC that are 2 orders of magnitude higher than surface water DOC and 1 order of magnitude higher than that of natural reef sediments.
- Total N and P pools both increased significantly in restored reef sediments by six-months post-restoration and can be attributed to the greater ability of juvenile oysters to deposit inorganic N as well as proportional increases in sediment organic matter content containing organic N and P.
- Despite SRP concentrations in oyster biodeposits that are 4 orders of magnitude higher than that of lagoon water and 2 orders of magnitude higher than surface sediments on natural reefs, SRP on restored reefs decreased over time, indicating that there may be a large sink for inorganic P or change in SRP sorption on oyster reefs.
- Several sediment nutrient pools such as total N, total P, C:N ratios and organic matter are positively correlated to oyster density, shell length, and reef height, indicating that the

biological activity and/or physical structure of intertidal oysters significantly influences nutrient pools.

- Sediment organic matter and total N concentrations appear to be the best candidates for monitoring sediment biogeochemistry on restored intertidal reefs; these measurements held the most consistent increases on restored reefs and clearest differences between reef types.
- The study presented in chapter 3 developed and confirmed the utility of a new method, "the funnel drop method", for studying the transformation of water column nutrients to oyster biodeposits and for collecting an appropriate amount (2-8 g wet wt) of oyster biodeposits for extractable nutrient and total nutrient analyses
- The method of placing 30-40 oysters inside of wide funnels in tanks, the funnel drop method, and funneling biodeposits into glass collection jars proved effective for both allowing oysters to feed and collecting biodeposits for subsequent nutrient analyses.
- When standardized to g of dry tissue, juvenile oysters can reduce water column chl-a concentrations and increase NH_4^+ concentrations at rates greater than that of older oysters, as well as release significantly higher concentrations of DOC, NO_3^-, and NH_4^+ in biodeposits.
- Overall, eastern oysters can reduce chl-a concentrations at rapid rates and release low concentrations of SRP and high concentrations of NH_4^+ into the water column.
- Eastern oysters can produce feces and pseudofeces containing concentrations of DOC, SRP, NO_3^-, and NH_4^+ that are 2 to 5 orders of magnitude higher than concentrations in the water column and 1 to 2 orders of magnitude higher than natural reef sediment concentrations.

APPENDIX: SUPPLEMENTARY TABLES

Supplementary Table 1 Results of post hoc least squares means pairwise comparisons between treatments for surface water data. Values in bold denote significance at $p \leq 0.05$ and values in bold and italics denote significance at $p \leq 0.01$.

		DOC	NO_3^-	NH_4^+	SRP	Temp	DO	Cond	Salinity	pH
Before	Dead - Live	0.107	0.587	0.623	0.990	0.538	0.904	0.993	0.973	0.995
Before	Dead - Restored	0.638	0.669	0.601	0.978	0.904	0.888	0.518	0.142	0.997
Before	Live - Restored	0.485	0.164	0.999	0.939	0.303	0.648	0.451	0.091	0.985
1 Week	Dead - Live	0.975	0.991	0.497	0.913	0.827	0.999	0.943	0.866	0.814
1 Week	Dead - Restored	0.998	0.918	0.719	0.851	0.998	0.998	0.910	0.763	0.617
1 Week	Live - Restored	0.987	0.960	0.932	0.990	0.798	0.995	0.996	0.980	0.942
1 Month	Dead - Live	0.999	0.556	0.478	0.449	0.443	0.996	0.879	0.763	**0.028**
1 Month	Dead - Restored	0.930	0.842	0.810	0.410	0.767	0.999	0.904	0.686	0.072
1 Month	Live - Restored	0.915	0.255	0.183	0.998	0.855	1	0.634	0.285	0.916
6 Months	Dead - Live	0.980	***<0.001***	**0.012**	0.913	0.131	0.053	0.798	0.495	1
6 Months	Dead - Restored	0.861	***0.004***	0.981	0.220	0.443	0.066	0.836	0.613	0.942
6 Months	Live - Restored	0.942	0.367	**0.019**	0.410	0.736	***<0.001***	0.997	0.980	0.942
9 Months	Dead - Live	0.982	1	1	0.697	0.880	0.996	0.969	0.909	0.999
9 Months	Dead - Restored	0.974	1	0.948	0.118	0.443	0.927	0.751	0.463	NA
9 Months	Live - Restored	0.999	1	0.943	0.449	0.214	0.893	0.605	0.252	NA

12 Months	Dead - Live	0.494	1	1	***0.005***	0.413	0.906	0.192	0.566	1
12 Months	Dead - Restored	0.438	1	1	1	0.926	0.708	0.600	0.508	0.573
12 Months	Live - Restored	0.995	1	1	***0.003***	0.234	0.448	**0.023**	0.093	0.587

Supplementary Table 2 Results of post hoc least squares means pairwise comparisons between sampling times for water quality and nutrient data. Values in bold denote significance at $p \leq 0.05$ and values in bold and italics denote significance at $p \leq 0.01$. PL denotes the post-leveling sampling time where only restored reefs were sampled after the displacement of shell to level the area.

Reef	Time pair	DOC	NO_3^-	NH_4^+	SRP	Temp	DO	Cond	Salinity	pH
Restored	Before - PL	***<0.001***	0.319	**0.020**	0.973	1	1	***<0.001***	***<0.001***	0.999
Restored	1 Wk - PL	***<0.001***	1	0.787	0.855	***<0.001***	0.694	***<0.001***	***<0.001***	0.857
Restored	1 Wk - 1 Mo	0.641	0.958	0.986	0.376	***<0.001***	1	**0.022**	***<0.001***	0.868
Restored	1 Mo - 6 Mo	***<0.001***	0.273	***<0.001***	***<0.001***	***<0.001***	***0.004***	***<0.001***	***<0.001***	0.971
Restored	6 Mo - 9 Mo	1	0.273	**0.045**	***<0.001***	***<0.001***	***<0.001***	0.332	***0.006***	NA
Restored	9 Mo - 12 Mo	***<0.001***	1	**0.013**	***<0.001***	***<0.001***	0.996	0.909	***<0.001***	NA
Dead	Before - 1 Wk	***<0.001***	0.546	1	0.984	***<0.001***	0.212	***<0.001***	***<0.001***	0.987
Dead	1 Wk - 1 Mo	0.827	1	0.995	1	***0.002***	1	**0.023**	***<0.001***	0.302
Dead	1 Mo - 6 Mo	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	0.832	***<0.001***	***<0.001***	0.477
Dead	6 Mo - 9 Mo	0.991	***<0.001***	0.061	***<0.001***	***<0.001***	0.396	**0.014**	***<0.001***	0.840
Dead	9 Mo - 12 Mo	***<0.001***	1	**0.031**	***<0.001***	***<0.001***	0.987	0.973	***<.0001***	0.069
Live	Before - 1 Wk	***<0.001***	0.992	0.252	1	***<0.001***	0.064	***<0.001***	***<0.001***	0.721
Live	1 Wk - 1 Mo	0.931	0.979	0.994	0.524	***<0.001***	1	0.182	***<0.001***	0.833
Live	1 Mo - 6 Mo	***<0.001***	0.993	0.091	***<0.001***	***<0.001***	0.675	***<0.001***	***<0.001***	0.957
Live	6 Mo - 9 Mo	0.992	0.967	1	***<0.001***	***<0.001***	0.971	**0.044**	***<0.001***	0.866
Live	9 Mo - 12 Mo	***<0.001***	1	**0.032**	***<0.001***	***<0.001***	0.813	0.988	***<0.001***	0.063

Supplementary Table 3 Pearson correlation coefficients between sediment properties and surface water nutrients. The critical correlation value was calculated to assess the significance of the correlation coefficients at α =0.05 and α =0.01. For n=48, a two-tailed test and $p \leq 0.05$ significance, the absolute value of the correlation coefficient must be ≥ 0.285 and for $p \leq 0.01$ the correlation coefficient must be ≥ 0.368. Values in bold denote significance at $p \leq 0.05$ and values in bold and italics denote significance at $p \leq 0.01$.

	BD	pH	NO_3^-	NH_4^+	SRP	DOC	OM	TC	TN	TP
pH	0.174									
NO_3^-	-0.210	0.053								
NH_4^+	-0.194	-0.154	0.034							
SRP	-0.077	0.025	***0.400***	-0.182						
DOC	-0.159	-0.131	0.180	***0.441***	0.111					
OM	**-0.333**	**-0.286**	-0.041	***0.412***	-0.209	0.244				
TC	***-0.476***	-0.249	0.181	0.217	**0.346**	0.201	0.192			
TN	***-0.419***	***-0.380***	0.126	***0.514***	-0.270	**0.330**	***0.633***	0.188		
TP	-0.186	-0.149	0.012	**0.296**	-0.227	0.103	***0.527***	-0.101	***0.626***	
Water NH_4^+	0.251	-0.211	0.215	-0.048	-0.021	-0.076	-0.139	0.031	-0.148	-0.200
Water SRP	-0.206	**-0.319**	**-0.310**	-0.203	0.000	0.040	0.187	0.076	0.198	0.121
Water NO_3^-	0.033	-0.133	0.043	-0.183	0.161	0.121	-0.077	0.058	-0.058	-0.073

Supplementary Table 4 Water quality data recorded at 10 cm depth and 2-4 m distance from each reef at each sampling time.

Time	**Reef**	**Temperature ºC**	**% DO**	**Conductivity ms/cm**	**Salinity ‰**	**pH**
Before	Restored 1	25.4	73.8	65.7	44.56	7.95
Before	Restored 2	26.7	93.9	65.3	44.20	7.99
Before	Restored 3	26.7	87.8	64.9	43.93	7.97
Before	Restored 4	26.0	76.8	65.5	44.43	7.92
Before	Dead 1	25.4	72.6	65.7	44.53	7.85
Before	Dead 2	26.7	87.4	65.8	44.61	8.00
Before	Dead 3	25.4	70.6	66.0	44.78	7.98
Before	Dead 4	26.5	84.4	66.3	45.01	8.03
Before	Live 1	25.4	69.0	65.5	44.39	7.92
Before	Live 2	25.9	79.9	66.0	44.81	8.01
Before	Live 3	24.9	70.7	66.4	45.12	7.97
Before	Live 4	25.8	79.5	66.0	44.82	8.00

Post-rake	Restored 1	25.8	68.7	57.3	38.12	7.76
Post-rake	Restored 2	26.2	96.2	57.7	38.42	8.01
Post-rake	Restored 3	26.6	99.5	58.4	38.95	8.07
Post-rake	Restored 4	26.6	75	57.9	38.6	7.82
1 Week	Restored 1	28.8	97.6	61.3	41.11	7.64
1 Week	Restored 2	29.9	95.7	61.8	41.45	7.89
1 Week	Restored 3	30.1	98.2	61.6	41.24	7.95
1 Week	Restored 4	28.9	97.5	61.9	41.5	7.68
1 Week	Dead 1	29.4	96.9	61.6	41.32	7.91
1 Week	Dead 2	29	96.2	61.6	41.28	7.81
1 Week	Dead 3	29.7	98.9	62.0	41.61	7.91
1 Week	Dead 4	29.5	99	62.2	41.75	7.93
1 Week	Live 1	29	97.5	61.4	41.12	7.79
1 Week	Live 2	29	99.7	62.0	41.64	7.88
1 Week	Live 3	29.2	98	61.6	41.27	7.75
1 Week	Live 4	29.3	97.3	61.8	41.45	7.88
1 Month	Restored 1	31.0	96.2	60.2	40.15	7.86
1 Month	Restored 2	31.8	95.2	59.8	39.86	7.95
1 Month	Restored 3	31.7	95.3	59.7	39.73	7.95
1 Month	Restored 4	31.1	96.1	60.3	40.26	7.89
1 Month	Dead 1	31.0	96.3	60.2	40.15	7.71
1 Month	Dead 2	31.5	95.6	59.8	39.86	7.99
1 Month	Dead 3	30.9	96.4	60.5	40.40	7.91
1 Month	Dead 4	30.9	96.4	60.5	40.37	7.08
1 Month	Live 1	31.0	96.3	60.2	40.18	7.87
1 Month	Live 2	31.8	95.3	60.8	40.57	7.96
1 Month	Live 3	32.3	94.5	60.2	40.11	8.06
1 Month	Live 4	31.5	95.6	60.8	40.58	7.93
6 Months	Restored 1	14.7	58.2	49.8	32.59	7.79
6 Months	Restored 2	16.0	70.8	49.3	32.29	7.83
6 Months	Restored 3	15.2	75.2	49.2	32.17	7.88
6 Months	Restored 4	14.5	52.6	49.7	32.50	7.80
6 Months	Dead 1	14.4	47.7	49.7	32.49	7.69
6 Months	Dead 2	15.3	82.0	49.0	32.07	7.89
6 Months	Dead 3	14.0	106.2	48.7	31.77	7.92
6 Months	Dead 4	14.4	106.9	49.4	32.33	7.94
6 Months	Live 1	15.4	65.8	49.7	32.55	7.77

6 Months	Live 2	15.4	119.7	49.5	32.37	7.89
6 Months	Live 3	15.7	128.0	49.7	32.57	7.88
6 Months	Live 4	15.3	118.8	49.3	32.24	7.90
9 Months	Restored 1	16.7	100.6	50.5	33.15	-
9 Months	Restored 2	18.1	108.7	50.5	33.12	-
9 Months	Restored 3	17.8	109.0	50.6	33.25	-
9 Months	Restored 4	17.6	102.7	50.6	33.21	-
9 Months	Dead 1	16.4	93.1	50.4	33.07	-
9 Months	Dead 2	17.8	99.8	50.6	33.24	7.85
9 Months	Dead 3	18.7	102.9	51.2	33.72	8.10
9 Months	Dead 4	19.6	111.4	51.4	33.82	8.10
9 Months	Live 1	17.7	104.9	51.0	33.52	-
9 Months	Live 2	18.9	103.9	51.1	33.60	8.12
9 Months	Live 3	18.0	96.8	50.9	33.42	7.84
9 Months	Live 4	18.8	98.5	51.2	33.7	8.07
12 Months	Restored 1	29.1	87.3	49.0	29.17	8.10
12 Months	Restored 2	30.1	111.2	50.5	29.70	8.33
12 Months	Restored 3	30.2	114.2	50.3	29.47	8.37
12 Months	Restored 4	29.9	89.1	50.1	29.56	8.03
12 Months	Dead 1	28.6	84.8	47.3	28.46	8.03
12 Months	Dead 2	29.5	93.4	49.9	29.65	8.27
12 Months	Dead 3	31.5	130.0	52.6	30.26	8.43
12 Months	Dead 4	30.4	123.2	52.1	30.58	8.53
12 Months	Live 1	29.6	88.9	49.5	29.33	8.08
12 Months	Live 2	30.3	116.9	51.5	30.22	8.40
12 Months	Live 3	30.7	106.9	51.7	30.11	8.32
12 Months	Live 4	31.8	134.5	52.9	30.25	8.45

REFERENCES

Alva, A.K. 1993. Comparison of Mehlich 3, Mehlich 1, Ammonium Bicarbonate-DTPA, l.OM Ammonium Acetate, and O.2M Ammonium Chloride for Extraction of Calcium, Magnesium, Phosphorus, and Potassium for a Wide Range of Soils. Comm. Soil Sci. Plant Anal. 24(7): 603–612.

Asmus H., Asmus R., Zubillaga G. 1995. Do mussel beds intensify the phosphorus exchange between sediment and tidal waters? Ophelia. 41(1): 37-55.

Baggett, L. P., Powers, S. P., Brumbaugh, R. D., Coen, L. D., Deangelis, B. M., Greene, J. K., Ermgassen, P. S. E. 2015. Guidelines for evaluating performance of oyster habitat restoration. Restoration Ecology, 23(6): 737–745.

Baldwin, B. S., Newell, R. I. E. 1991. Omnivorous feeding by planktotrophic larvae of the eastern oyster Crassostrea virginica. Mar. Ecol. Prog. Ser. 78: 285-301.

Bayne, B.L. & A. J. S. Hawkins. 1992. Ecological and physiological aspects of herbivory in benthic suspension-feeding molluscs. In: D. M. John, S. J. Hawkins & J. H. Prince, editors. Plant-animal interactions in the marine benthos. Systematics Association Special Volume No. 46. Oxford: Clarendon Press, pp. 265-388.

Bayne B. 2002. A physiological comparison between Pacific oysters Crassostrea gigas and Sydney Rock oysters Saccostrea glomerata: food, feeding and growth in a shared estuarine habitat. Marine Ecology Progress Series 232: 163–178.

Beck, M. W., Brumbaugh, R. D., Airoldi, L., Carranza, A., Coen, L. D., Crawford, C., Guo, X. 2011. Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. BioScience.

Beck, M. W., Jr, K. L. H., Able, K. W., Daniel, L., Eggleston, D. B., Gillanders, B. M., Weinstein, M. P. 2001. The Identification, Conservation, and Management of Estuarine and Marine Nurseries for Fish and Invertebrates. BioScience, 51(8): 633–641.

Berner R.A., Ruttenberg K.C., Ingall E.D., Rao JL. 1993. The Nature of Phosphorus Burial in Modern Marine Sediments. In: Wollast R., Mackenzie F.T., Chou L. (eds) Interactions of C, N, P and S Biogeochemical Cycles and Global Change. NATO ASI Series, vol 4. Springer, Berlin, Heidelberg.

Blomberg, B. N., Lebreton, B., Palmer, T. A., Guillou, G., Pollack, J. B., & Montagna, P. A. 2017. Does reef structure affect oyster food resources ? A stable isotope assessment. Marine Environmental Research, 127: 32–40.

Bricker, S., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2007. Effects of Nutrient Enrichment In the Nation's Estuaries: A Decade of Change. NOAA Technical Memorandum. Silver Spring, MD. 328 pp.

Chambers, L.G., T.Z. Osborne, and K.R. Reddy. 2013. Effect of salinity pulsing events on soil organic carbon loss across an intertidal wetland gradient: A laboratory experiment. Biogeochemistry 115: 363– 383.

Chambers, L., Gaspar, S., Pilato, C., Steinmuller, H., McCarthy, K., Sacks, P., Walters, L. 2017. How well do restored intertidal oyster reefs support key biogeochemical functions in a coastal lagoon? Estuar. Coasts. 1-16.

Coen, L. D., & Grizzle, R. E. 2007. Atlantic States Marine Fisheries Commission The Importance of Habitat Created by Molluscan along the Atlantic Coast. Atlantic States Marine Fisheries Commission Habitat Management Series, 8(7): 1–108.

Cressman, K.A., Posey, M.H., Mallin, M.A., Leonard, L.A., Alphin, T.D. 2003. Effects of oyster reefs on water quality in a tidal creek estuary. Journal of Shellfish Research 22: 753–762.

Dame, R. F., Zingmark, R.G., & Haskin, E. 1984. Oyster reefs as processors of estuarine materials. Journal of Experimental Marine Biology and Ecology, 83(3): 239–247.

Dame, R.F. 1985. The summer uptake and release of nitrogen by an intertidal oyster reef. Netherlands Journal of Sea Research. 19: 3-4.

Dame R.F., Spurrier J, Wolaver T. 1989. Carbon, nitrogen and phosphorus processing by an oyster reef. Mar Ecol Prog Ser 54: 249−256

Dame, R.F., Spurrier, J.D., & Zingmark, R.G. 1992. In situ metabolism of an oyster reef. Journal of Experimental Marine Biology and Ecology. 164(2): 147–159.

Dame, R., Libes, S. 1993. Oyster reefs and nutrient retention in tidal creeks. J. Exp. Mar. Biol. Ecol. 171: 251–258.

Dybas, C.L. 2002. Florida's Indian River Lagoon: An Estuary in Transition. BioScience, 52(7): 555–559.

Deslous-Paoli J-M. Lannou A-M. Geairon P. Bougrier S. Raillard O. Heral M. 1992. Effects of the feeding behaviour of Crassostrea gigas (Bivalve Mollusc) on biosedimentation of natural particulate matter. Hydrobiologia 231: 85-91.

Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1): 12-19.

East Central Florida Regional Planning Council. 2016. Indian River Lagoon Economic Valuation Update 2016. https://loveourlagoon.com/IRL-Economic-Valuation-Update-07252016.pdf.

Ermgassen, P. S. E., Spalding, M. D., Grizzle, R. E., & Brumbaugh, R. D. 2013. Quantifying the Loss of a Marine Ecosystem Service : Filtration by the Eastern Oyster in US Estuaries. Estuaries and Coasts, 36: 36–43.

Falcão, M., Santos, M. N., Vicente, M., & Monteiro, C. C. 2007. Biogeochemical processes and nutrient cycling within an artificial reef off Southern Portugal. Marine Environmental Research, 63: 429–444.

Falkowski, P., Kiefer, D.A., 1985. Chlorophyll a fluorescence in phytoplankton: relationship to photosynthesis and biomass, J. Plankton Research, 7(5): 715–731.

Fodrie F.J., Rodriguez A.B., Gittman R.K., Grabowski JH, Lindquist NL, Peterson CH, Piehler MF, Ridge JT. 2017 Oyster reefs as carbon sources and sinks. Proc. R. Soc. 12(3): 62-79.

Gain, I. E., Brewton, R. A., Reese, M. M., Keith, R., Smee, D. L., & Stunz, G. W. 2017. Macrofauna using intertidal oyster reef varies in relation to position within the estuarine habitat mosaic. Marine Biology, 164(1): 1–16.

Garvis, S. K., Sacks, P. E., & Walters, L. J. 2015. Formation, Movement, and Restoration of Dead Intertidal Oyster Reefs in Canaveral National Seashore and Mosquito Lagoon, Florida. Journal of Shellfish Research, 34(2): 251–258.

Google Earth Pro V 7.3.2.5776. (March 10, 2019). Mosquito Lagoon, Florida. 28.940459, -80.866784, Eye alt 11000 feet. Google 2019.

Grabowski, J. H., & Powers, S. P. 2004. Habitat complexity mitigates trophic transfer on oyster reefs. Marine Ecology Progress Series, 277: 291–295.

Grabowski, J. H., Hughes, A. R., Kimbro, D. L., & Dolan, M. A. 2005. How habitat setting influences restored oyster reef communities. Ecology, 86(7): 1926–1935.

Grabowski, J. H., Brumbaugh, R. D., Conrad, R. F., Keeler, A. G., James, J., Peterson, C. H., Smyth, A. R. 2012. Economic Valuation of Ecosystem Services Provided by Oyster Reefs. BioScience, 62(10): 900–909.

Grizzle, R. E. 1990. Distribution and abundance of Crassostrea virginica (Gmelin, 1791) (eastern oyster) and Mercenaria spp. (quahogs) in a coastal lagoon. J. Shellfish Res. 9: 347–358.

Grizzle, R. E., Adams, J. R., & Walters, L. J. 2002. Historical changes in intertidal oyster (Crassostrea virginica) reefs in a Florida lagoon potentially related to boating activities. Journal of Shellfish Research, 21(2): 749–756.

Grizzle, R.E., Greene, J.K., Coen, L.D. 2008. Seston removal by natural and constructed intertidal eastern oyster (Crassostrea virginica) reefs: a comparison with previous laboratory studies, and the value of in situ methods. Estuar. Coast 31: 1208–1220.

Grizzle, R.E., Rasmussen, A., Martignette, A.J., Ward, K., Coen, L.D. 2018. Mapping seston depletion over an intertidal eastern oyster (Crassostrea virginica) reef: implications for restoration of multiple habitats. Estuar. Coast Shelf Sci. 2: 265-272.

Gutierrez, J. L., Jones, C. G., Strayer, D. L., & Iribarne, O. 2003. Mollusks as Ecosystem Engineers: The Role of Shell Production in Aquatic Habitats. Oikos, 101(1): 79–90.

Hammen, C. S., Miller, H. F., Geer, W. H. 1966. Nitrogen excretion of Crassostrea virginica. Comp. Biochem. Phy siol. 17: 1199-1200.

Harding, J.M. 2007. Comparison of growth rates between diploid DEBY eastern oysters (Crassostrea virginica, Gmelin 1791), triploid eastern oysters, and triploid Suminoe oysters (C. ariakensis, Fugita 1913). Journal of Shellfish Research 26(4): 961–972.

Haven DS, Morales-Alamo R. 1966. Aspects of biodeposition by oysters and other invertebrate filter feeders. Limnology and Oceanography 11: 487−498.

Haven DS, Morales-Alamo R. 1968. Occurrence and transport of faecal pellets in suspension in a tidal estuary. Sediment Geology 2: 141−151.

Hoellein, T. J., Zarnoch, C. B., & Grizzle, R. E. 2015. Eastern oyster filtration, biodeposition, and sediment nitrogen cycling at two oyster reefs with contrasting water quality in Great Bay Estuary. Biogeochemistry, (122): 113–129.

Humphries, A. T., La, M. K., Kimball, M. E., & Rozas, L. P. 2011. Journal of Experimental Marine Biology and Ecology Testing the effect of habitat structure and complexity on nekton assemblages using experimental oyster reefs. Journal of Experimental Marine Biology and Ecology, 409(1): 172–179.

Humphries, A. T., & Peyre, M. K. La. 2015. Oyster reef restoration supports increased nekton biomass and potential commercial fishery value. Peer J: 3.

Humphries, A. T., Ayvazian, S. G., Carey, J. C., Hancock, B. T., Grabbert, S., Cobb, D., Fulweiler, R. W. 2016. Directly Measured Denitrification Reveals Oyster

Aquaculture and Restored Oyster Reefs Remove Nitrogen at Comparable High Rates. Frontiers in Marine Science, 3(74).

Ivanina, A.V., Gary H. Dickinson, Omera B. Matoo, Rita Bagwe, Ashley Dickinson, Elia Beniash, Inna M. Sokolova, Interactive effects of elevated temperature and CO2 levels on energy metabolism and biomineralization of marine bivalves Crassostrea virginica and Mercenaria mercenaria, Comparative Bioch. and Phys. Part A: Volume 166, Issue 1, 2013, pp. 101-111.

Jackson, J. B. C., Kirby, M. X., Berger, W. H., Bjorndal, K. A., Botsford, L. W., Bourque, B. J., Warner, R. R. 2001. Historical Overfishing and the Recent Collapse of Coastal Ecosystems. Science, 293(5530): 629–639.

Jones, C. G., Lawton, J. H., & Shachak, M. 1994. Organisms as Ecosystem Engineers. Oikos, 69(3): 373–386.

Jordan, S.J. 1987. Sedimentation and remineralization associated with biodeposition by the American oyster Crassostrea virginica (Gmelin). Doctoral dissertation. University of Maryland. College Park.

Kellogg, M. L., Cornwell, J. C., Owens, M. S., & Paynter, K. T. 2013. Denitrification and nutrient assimilation on a restored oyster reef. Marine Ecology Progress Series, 480: 1–19.

Kellogg ML, Smyth AR, Luckenbach MW, Carmichael RH, Brown BL, Cornwell JC, Piehler MF, Owens MS, Dalrymple DJ, Higgins CB. 2014. Use of oysters to mitigate eutrophication in coastal waters. Estuar Coast Shelf Sci 151: 156–168

Kennedy, V.S. & Sanford, Lawrence. 1999. Characteristics of relatively unexploited beds of the eastern oyster, Crassostrea virginica, and early restoration program. Oyster Reef Habitat Restoration: A Synopsis and Synthesis of Approaches. 25-46. Keppel A (2014) The effects of co-varying diel-cycling hypoxia and pH on disease susceptibility, growth, and feeding in Crassostrea virginica. College Park, MD: University of Maryland Center for Environmental Science.

Keppel AG, Breitburg DL, Burrell RB 2016. Effects of Co-Varying Diel-Cycling Hypoxia and pH on Growth in the Juvenile Eastern Oyster, Crassostrea virginica. PLoS ONE, 11(8): 372-390.

Kirby, M. X. 2004. Fishing down the coast : Historical expansion and collapse of oyster fisheries along continental margins, 101(35): 13096–13099.

Kreeger DA, Newell RI. Seasonal utilization of different seston carbon sources by the ribbed mussel, Geukensia demissa (Dillwyn) in a mid-Atlantic salt marsh. 2001. J Exp Mar Bio Ecol. 260(1):71-91.

Kuznetsova A, Brockhoff PB, Christensen RHB 2017. "lmerTest Package: Tests in Linear Mixed Effects Models." Journal of Statistical Software, 82(13): 1-26.

La Peyre, M. K., Humphries, A. T., Casas, S. M., & La Peyre, J. F. 2014. Temporal variation in development of ecosystem services from oyster reef restoration. Ecolog. Engineering, 63: 34–44.

Lapointe, B. E., Herren, L. W., Debortoli, D. D., & Vogel, M. A. 2015. Evidence of sewage-driven eutrophication and harmful algal blooms in Florida's Indian River Lagoon. Harmful Algae, 43: 82–102.

Langdon CJ, Newell RIE. 1996. Digestion and nutrition in larvae and adults. In: Kennedy VS, Newell RIE, Eble AF (eds) The Eastern oyster Crassostrea virginica. Maryland Sea Grant, College Park, pp 231–269.

Lenihan, H. S. 1999. Physical-biological coupling on oyster reefs: How habitat structure influences individual performance. Ecological Monographs, 69(3): 251–275.

Lenihan, H. S., Peterson, C. H., Byers, J. E., Grabowski, J. H., Thayer, G. W., & Colby, D. R. 2001. Cascading of habitat degradation: Oyster reefs invaded by refugee fishes escaping stress. Ecological Applications, 11(3): 764–782.

Lenth, R.V. 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software, 69(1): 1-33.

Loosanoff, V.L., Engle, J.B. 1947. Feeding of oysters in relation to density of microorganisms. Science 105: 260–261.

Lunstrum, A., K. McGlathery, and A. Smyth. 2017. Oyster (Crassostrea virginica) aquaculture shifts sediment nitrogen processes toward mineralization over denitrification. Estuaries and Coasts. 1–17.

Mackenzie, C. L. 1996. History of Oystering in the United States and Canada , Featuring the Eight Greatest Oyster Estuaries. Marine Fisheries Review, 58(4).

Mann & J. A. Wesson, editors. Oyster reef habitat restoration: a synopsis and synthesis of approaches. Gloucester Point, VA: Virginia Institute of Marine Science Press. pp. 25–46.

Mcclain, M. E., Boyer, E. W., Dent, C. L., Gergel, S. E., Grimm, N. B., Groffman, P. M., Pinay, G. 2003. Biogeochemical Hot Spots and Hot Moments at the Interface of Terrestrial and Aquatic Ecosystems. Ecosystems, (6): 301–312.

Mermillod-Blondin, F., & Rosenberg, R. 2006. Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. Aquatic Sciences, 68: 434–442.

Mitchell IM. 2006 In situ biodeposition rates of Pacific oysters (Crassostrea gigas) on a marine farm in Southern Tasmania (Australia). Aquaculture, 257: 194−203.

Mortazavi, B., A. C. Ortmann, L. Wang, R. J. Bernard, C. L. Staudhammer, J. D. Dalrymple, R. H. Carmichael, and A. A. Kleinhuizen. 2015. Evaluating the impact of oyster (Crassostrea virginica) gardening on sediment nitrogen cycling in a subtropical estuary. Bulletin of Marine Science, 91: 323–341.

Munroe, D., Borsetti, S., & Bushek, D. 2017. Early Post-Settlement Growth in Wild Eastern Oyster (Crassostrea virginica Gemlin 1791) Populations. Estuaries and Coasts, 40: 880–888.

National Coastal Condition Assessment 2010. 2016. (EPA 841-R-15-006) (Office of Water and Office of Research and Development) Washington, D.C. http://www.epa.gov/national-aquatic-resource-surveys/ncca.

National primary drinking water regulations. EPA. 2019. https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations. Accessed 07 May 2019.

Nelson, K. A., Leonard, L. A., Posey, M. H., Alphin, T. D., & Mallin, M. A. 2004. Using transplanted oyster (Crassostrea virginica) beds to improve water quality in small tidal creeks : a pilot study. Journal of Experimental Marine Biology and Ecology, 298: 347–368.

Newell, R. I. E., Kemp, W. M., Iii, J. D. H., Cerco, C. F., Testa, M., Boynton, W. R., Boynton, W. R. 2007. Top-down control of phytoplankton by oysters in Chesapeake Bay , USA : Comment on Pomeroy et al . (2006). Marine Ecology Progress Series, 341: 293–298.

Newell, R.I.E., Jordan, S.J., 1983. Preferential ingestion of organic material by the American oyster Crassostrea virginica. Mar. Ecol. Prog. Ser. 13: 47–53.

Newell RIE., CJ Langdon. 1996. Mechanisms and Physiology of Larval and Adult Feeding. In The Eastern Oyster, Crassostrea virginica, VS Kennedy RIE Newell A Eble (Eds). Maryland Sea Grant Publication, College Park, MD, USA, pp 185-230.

Newell RIE, Cornwell JC, Owens MS. 2002. Influence of simulated bivalve biodeposition and microphytobenthos on sediment nitrogen dynamics: a laboratory study. Limnol Oceanogr. 47: 1367–1379

Newell RIE. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. J Shellfish Res 23: 51−62

Newell, R.I.E. & Koch, E.W. 2004. Modeling seagrass density and distribution in response to changes in turbidity stemming from bivalve filtration and seagrass sediment stabilization. Estuarie 27: 793.

Newell RIE, Fisher T, Holyoke R, Cornwell J. 2005. Influence of eastern oysters on nitrogen and phosphorus regeneration in Chesapeake Bay, USA. In: Dame RF, Olenin S (eds) The comparative roles of suspension-feeders in ecosystems. NATO Advanced Research Workshop on the Comparative Roles of Suspension-Feeders in Ecosystems, Nida, Lithuania, pp. 93−120

NOAA Fisheries, Office of Habitat Conservation, 2018. https://www.fisheries.noaa.gov/national/habitat-conservation oyster-reef-habitat. Accessed 15 Jan 2019.

NOAA. 2013. National Coastal Population Report. What percentage of the American population lives near the coast?, https://oceanservice.noaa.gov/facts/population.html, 06/25/18.

Paulmier, G. 1972. Seston, phytoplancton et microphytobenthos en rivière d'Auray. Leur rôle dans le cycle biologique des huîtres (Ostrea edulis L.). Rev. Trav. Inst. Pêches Marit. 36: 373–506.

Pavlova, G., & Tishchenko, P. 1990. Calculation of carbon-dioxide equilibrium by pitzer method. Okeanologiya, 30(6): 1013-1021.

Paynter, K.T., V. Politano, H.A. Lane, S.M. Allen, and D. Meritt. 2010. Growth rates and prevalence of Perkinsus marinus in restored oyster populations in Maryland. Journal of Shellfish Research 29(2): 309– 317.

Peterson, C. H., Grabowski, J. H., & Powers, S. P. 2003. Estimated enhancement of fish production resulting from restoring oyster reef habitat: quantitative valuation. Marine Ecology Progress Series, 264: 249–264.

Phlips EJ, Badylak S, Lasi MA, Chamberlain R, Green WC, Hall LM, Hart JA, Lockwood JC, Miller JD, Morris LJ, Steward JS. 2015. From red tides to green and brown tides: bloom dynamics in a restricted subtropical lagoon under shifting climatic conditions. Estuar Coast. 38: 886–904.

Piehler, M. F., & Smyth, A. R. 2011. Habitat-specific distinctions in estuarine denitrification affect both ecosystem function and services. Ecosphere, 2(1): 12.

Pierson, K. J., & Eggleston, D. B. 2014. Response of Estuarine Fish to Large-Scale Oyster Reef Restoration. Transactions of the American Fisheries Society, 143: 273–288.

Pollack, J. B., Yoskowitz, D., Kim, H., & Montagna, P. A. 2013. Role and Value of Nitrogen Regulation Provided by Oysters (Crassostrea virginica) in the Mission-Aransas Estuary. PLoS ONE, 8(6).

Porter ET, Franz H, Lacouture R. 2018. Impact of Eastern oyster Crassostrea virginicabiodeposit resuspension on the seston, nutrient, phytoplankton, and zooplankton dynamics: a mesocosm experiment. Mar Ecol Prog Ser 586: 21-40.

Powell, E. N., Staff, G. M., Davies, D. J. and Callender, W. R. 1989. Macrobenthic death assemblages in modern marine environments: formation, interpretation, and application. Crit. Rev. Aquat. Sci. 1: 555-589.

Prins, T.C., A.C. Smaal, and R.F. Dame. 1997. A review of the feedbacks between bivalve grazing and ecosystem processes. Aquatic Ecology 31: 349-359.

R Core Team 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reddy, K.R., and R. DeLaune. 2008. Biogeochemistry of wetlands: Science and applications. New York: CRC.

Redfield, A. C., Ketchum, P. H., Richards, F. A. 1963. The influence of organisms on the composition of seawater. In: Hill, M. (ed.) The sea, Vol. II. Wiley, London, p. 26.

Rezek, R. J., Lebreton, B., Roark, E. B., Palmer, T. A., & Pollack, J. B. 2017. How does a restored oyster reef develop? An assessment based on stable isotopes and community metrics. Marine Biology, 164(3): 1–17.

Rico-Villa, B., S. Pouvreau, R. Robert. 2009. Influence of food density and temperature on ingestion, growth and settlement of Pacific oyster larvae, Crassostrea gigas, Aquaculture, 287(3): 395-401.

Riisgård, H.U. 1988. Efficiency of particle retention and filtration-rate in 6 species of northeast American bivalves. Mar. Ecol. Prog. Ser. 45: 217–223.

Rodney, W. S., & Paynter, K. T. 2006. Comparisons of macrofaunal assemblages on restored and non-restored oyster reefs in mesohaline regions of Chesapeake Bay in Maryland. Journal of Experimental Marine Biology and Ecology, 335: 39–51.

RStudio Team 2016. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Saini, G. 1966. Organic Matter as a Measure of Bulk Density of Soil Nature. 210 (5042): 67-78.

Sebastien Le, Julie Josse, Francois Husson. 2008. FactoMineR: An R Package for Multivariate Analysis. Journal of Statistical Software, 25(1): 1-18.

Sheng, Y.P., and J.D. Davis. 2003. Indian River Lagoon pollution load reduction (IRLPLR) model development. Final Report to the St. Johns River Water Management District, Volume 1: A 3-D IRL hydrodynamic/salinity model (UF-CH3D). St. Johns River Water Management District, Palatka, Florida.

Schumway, S.E., Koehn, R.K. 1982. Oxygen consumption in the American oyster Crassostrea virginica. Mar. Ecol. Prog. Ser. 9: 59–68.

Smith, N. P. 1993. Tidal and nontidal flushing of the Florida's Indian River Lagoon. Estuaries 16: 739–746.

Smyth, A. R., Geraldi, N. R., & Piehler, M. F. 2013. Oyster-mediated benthic − pelagic coupling modifies nitrogen pools and processes. Marine Ecology Progress Series, 493: 23–30.

Smyth AR, Piehler MF, Grabowski JH. 2015. Habitat context influences nitrogen removal by restored oyster reefs. J Appl Ecol 52: 716−725.

Smyth, A.R., N.R. Geraldi, S.P. Thompson, and M.F. Piehler. 2016. Biological activity exceeds biogenic structure in influencing sediment nitrogen cycling in experimental oyster reefs. Marine Ecology Progress Series 560: 173–183

Songsangjinda P, Matsuda O, Yamamoto T, Rajendran N, Maeda H. 2000. The role of suspended oyster culture on nitrogen cycle in Hiroshima Bay. J Oceanogr 56: 223−231.

Sornin, J. M., M. Feuillet, M. Heral & J. M. Deslous-Paoli. 1983. Effets des biodep6ts de 1'huitre Crassostrea gigas (Thunberg) sur l'accumulation de matieres organiques dans les pares du bassin de Marennes-Oleron. J. moll. Stud. 12: 185-197.

Southwell MW, Veenstra JJ, Adams CD, Scarlett EV, Payne KB. 2017. Changes in Sediment Characteristics upon Oyster Reef Restoration, NE Florida, USA. J Coast Zone Manag 20: 442.

St. Johns River Water Management District. 2019. The Indian River Lagoon. https://www.sjrwmd.com/waterways/indian-river-lagoon/. Accessed 15 Apr 2019.

Tolley, S. G., & Volety, A. K. 2005. The role of oysters in habitat use of oyster reefs by resident fishes and decapod crustaceans. Journal of Shellfish Research, 24(4): 1007–1012.

Volety, A.K., Tolley, G., Loh, A.N., Abeels, A., 2010. Oyster Monitoring Network for the Caloosahatchee Estuary. Final Report, South Florida Water Management District. 145 pp.

Wall, L. M., Walters, L. J., Grizzle, R. E., & Sacks, P. E. 2005. Recreational boating activity and its impact on the recruitment and survival of the oyster Crassostrea virginica on intertidal reefs in Mosquito Lagoon, FL. J. of Shellfish Research, 24(4): 965–973.

Ward, J.E., Levinton, J.S., Shumway, S.E., Cucci, T. 1998. Particle sorting in bivalves: in vivo determination of the pallial organs of selection. Mar. Biol. 131: 283–292.

Westbrook, P., Heffner, L. & La Peyre, M.K. 2019. Measuring carbon and nitrogen bioassimilation, burial, and denitrification contributions of oyster reefs in Gulf coast estuaries. Mar Biol. 166: 4.

Widdows J, BrinsleyMD, Salkeld PN, Elliott M. 1998. Use of annular flumes to determine the influence of current velocity and bivalves on material flux at the sedimentwater interface. Estuaries 21: 552−559

Wilberg MJ, Livings ME, Barkman JS, Morris BT, Robinson JM. 2011. Overfishing, disease, habitat loss, and potential extirpation of oysters in upper Chesapeake Bay. Mar Ecol Prog Ser 436: 131-144

Wilson-Ormond, E.A., E.N. Powell, and S.M. Ray. 1997. Short-term and small-scale variation in food availability to natural oyster populations: food, flow and flux. Marine Ecology 18: 1–34.