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# Effects of Trophic Relationships on Oyster Reef Restoration Success in the Mississippi Sound

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# EFFECTS OF TROPHIC RELATIONSHIPS ON OYSTER REEF RESTORATION SUCCESS IN THE MISSISSIPPI SOUND

by

Virginia Fleer

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# EFFECTS OF TROPHIC RELATIONSHIPS ON OYSTER REEF RESTORATION SUCCESS IN THE MISSISSIPPI SOUND

by Virginia Fleer

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# ABSTRACT

# EFFECTS OF TROPHIC RELATIONSHIPS ON OYSTER REEF RESTORATION SUCCESS IN THE MISSISSIPPI SOUND

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Natural and anthropogenic changes resulting from altered hydrology, hurricanes, variable precipitation, and the BP oil spill have all taken their toll on oyster reefs in Mississippi. In response, oyster reef restoration efforts are currently underway within the Northern Gulf of Mexico. In order to understand why these efforts succeed or fail, it is crucial to consider predator-prey relationships within the context of the trophic dynamics of oyster reefs. Thus, for this dissertation study I integrated a multidisciplinary approach to understanding key trophic interactions affecting oyster recruitment, growth and survival, comprising field sampling, manipulative lab experiments, and individual-based modeling. Spat settlement density and abundances of key members of the trophic web were quantified at twelve oyster reef sites over the course of seven months. Peak spat settlement at each reef occurred in September, with Linear Mixed Model analyses indicating a significant difference between mean spat settlement per day between reef types ($F = 29.229$, $p < 0.005$), among regions ($F = 81.203$, $p < 0.005$), and among sampling time periods ($F = 35.190$, $p < 0.001$), as well as in all two and three-way interactions among these factors. Additionally, PERMANOVA revealed spatial-temporal differences in the oyster trophic assemblage attributable to region and season. A suite of manipulative laboratory experiments examined: (1) the predatory effects of both oyster drills and mud crabs on various sizes of oyster spat; (2) oyster reef trophic interactions relative to different substrate types; and 3) the effects of key predator removals on trophic interactions. Mesocosm experiments showed that mud crabs and oyster drills greatly reduced oyster spat survival. Moreover, size selectivity by both of these predators was evident. Complex experiments involving different substrates and multiple predators revealed that limestone substrate used for restoration potentially leads to greater spat mortality in contrast to oyster shell. Information from field study and mesocosm experiments was then used to construct an individual-based model simulating oyster production and recruitment at reefs in the Mississippi Sound. Results from each component of this dissertation study were instrumental to the overall project goal of understanding trophic relationships affecting oyster reef restoration in the Mississippi Sound.

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# ACKNOWLEDGMENTS

Partial funding and vital information for this doctoral project were provided by the Mississippi Department of Marine Resources. Specifically, I want to thank Dr. Paul Mickle for believing in my research prospectus and Erik Broussard for historical oyster reef information and ensuring I received the necessary supplies for the field and laboratory portions of this project. I also owe a tremendous amount of thanks to my advisor Dr. Chet Rakocinski who also provided financial support for this project and spent countless hours consulting with me and working on data analysis. He always managed to turn our conversations into a discussion rather than a lecture, which pushed me to think critically and ultimately made me a better scientist. I will always be thankful for our stimulating talks about experiments, ecology, and life in general. Additionally, I want to thank my knowledgeable committee members Dr. Robert Leaf, Dr. Megan LaPeyre, Dr. Read Hendon, and Dr. Kevin Dillon for their time, support, and invaluable wisdom. Finally, this project would not have been possible without my boat captains and field helpers, Chris Lapniewski, Scott McIntosh, Krystin Estes Forbes, and Erik Schweiss.

# DEDICATION

This dissertation is dedicated to the memory of Christopher Langel, a beloved friend and fellow marine scientist who inspired me to chase my wildest dreams and pushed me to become a better scientist. Chris was a NOAA fisheries observer who lost his life aboard the Lady Cecilia on March 10, 2012, though his zest for life and adventurous spirit lives on through the lives of all who knew him. Additionally, I owe a tremendous amount of thanks to my loving parents, Robin and Jane Fleer, supportive siblings, friends (especially Emily Satterfield and Heather Wylie), adoptive parents Dr. Darryll and Marcia Barksdale, undergraduate mentor Dr. Peter Adam, and specifically my husband Erik Schweiss who stuck with me through a long-distance relationship the past two years and contributed immensely to the fifth chapter of this dissertation. Although they will never read this, I also want to thank Callie and Scamper for their unconditional love, emotional support and making me smile at the end of every day.

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# CHAPTER I - INTRODUCTION

Due to the commercial and ecological importance of the eastern oyster, *Crassostrea virginica*, and its ongoing decline in coastal waters of the northern Gulf of Mexico (nGOM), the use of artificial and restored oyster reefs has become as a potential tool to enhance oyster population growth (La Peyre et al. 2014). Oyster reefs serve as essential habitat for sessile benthic invertebrates that select or require hard substrate during their settlement. Habitat is considered essential when it is "physically discrete and indispensable for the survival of at least one life stage of the target species" (Langton et al. 1996). In the Gulf of Mexico, the engineering species making up the oyster benthic invertebrate community mainly includes the eastern oyster, several barnacle species of the genus *Amphibalanus* (*A. improvises*, *A. eburneus*, and *A. amphitrite*), and three mussel species (*Brachidontes exustus*, *Ischadium recurvum*, and *B. domingensis*). Additional ecologically relevant organisms in oyster reef food webs in terms of the survival and growth of oysters include the mud crab (*Panopeus simpsoni)*, stone crab (*Menippe adina)*, blue crab (*Callinectes sapidus*), southern oyster drill (*Stramonita haemastoma*), and Gulf toadfish (*Opsanus beta*); and the trophic dynamics among these species potentially determine the long-term production success of restored and artificial oyster reefs (Grabowski et al. 2005).

Throughout the last century the use of artificial reefs has emerged as an appealing management tool to enhance recreational and commercial fisheries (Sato 1985; Seaman and Sprague 1991), potentially altering the trophic dynamics of the original ecosystem. The appeal of using artificial reefs is based on the assumption that they serve to increase catch rates of commercially and recreationally important fish stocks (Sato 1985). Additionally, such efforts play a public relations role by fostering better relations between management agencies and the various fishing sectors (Bohnsack and Sutherland 1985; Nakamura 1985; Santos and Montiero 1998). Unfortunately, there are major knowledge gaps in assessing the condition of the original undisturbed ecosystem and how the addition of substrate material affects the trophic dynamics of the existing ecosystem. The question remains whether artificial reefs enhance fish production or simply attract fish (Baine 2001; Bortone 1998; Brickhill et al. 2005; Grossman et al. 1997; Pickering and Whitmarsh 1997; Powers et al. 2003); and the implication that such reefs may be disrupting existing ecosystems without enhancing production. However, artificial reefs continue to be used as a management tool for promoting commercially and recreationally important fish species and engaging stakeholders.

## Oyster Reef Restoration

In recent years, existing oyster reefs within the nGOM have been negatively impacted by natural and anthropogenic changes to coastal environments, caused by river damming, hurricanes, variable precipitation, the *Deepwater Horizon* (DWH) oil spill, and commercial harvesting (Livingston et al. 1999; La Peyre et al. 2014). Oyster reef area has decreased by two orders of magnitude within southeastern US estuaries (Rothschild et al. 1994; Lenihan and Peterson 1998). Consequently, a great deal of oyster reef restoration has occurred in the northern Gulf of Mexico in the past few years (La Peyre et al.

2014). Oyster reef restoration proponents purport that the overall goal of the enhancement of oyster habitats is to enhance commercially and recreationally important fisheries; however, there is little evidence to support the success of these efforts (Baine 2001; Kennedy et al. 2011; Geraldi et al. 2013; La Peyre et al. 2014). There is a knowledge gap in the quantitative assessment of the amount of hard substrate available and the presence or absence of live oysters occurring at artificial reef sites. In order to understand artificial oyster reef productivity, further study is needed to document parameters affecting oyster ecosystem function (i.e. oyster abundance, reef substrate quality, and long-term success), especially considering the economic and ecological importance of the eastern oyster in this region.

Ecosystem services provided by oyster habitat are directly and indirectly beneficial to humans. The term, ‘ecosystem service’ most commonly represents, “the benefits humans receive from ecosystems” (MEA 2005; Dempsey and Robertson 2012). Oysters serve as a model species because they provide numerous benefits to humans. The ecosystem services of oyster reefs contribute substantially to the inshore marine ecosystem at both local and global scales. At regional scales, oyster filtration serves to ameliorate eutrophication effects (Jackson et al. 2001), enhance water clarity by filtering suspended inorganic particles, phytoplankton, and detritus (Dame and Allen 1996), and enhance benthic production through the biogeochemical breakdown of pseudofeces (Newell 2004). At the global scale, due the $CaCO_3$ composition of oyster shell, oyster reefs contribute to the global carbon budget (Hargis and Haven 1999).

Additionally, the barrier protection provided by oyster reefs serves as a living shoreline along coastal areas (Meyer et al. 1996).

In addition to biogeochemical services provided by oyster reefs, a trophically diverse community of associated organisms utilize oyster reefs, including various prey and intermediate level predators (polychaetes, mollusks, decapods, and various other invertebrates), as well as apex predators (fishes, large mobile crustaceans, and whelks) (Wells 1961; Breitburg 1999; Coen et al. 1999; Peterson et al. 2003; Lenihan et al. 2001; Grabowski et al. 2005). Fishes inhabiting oyster reefs are categorized as residents (dependent on reef), facultative residents (use reef resources), or transients (feed at reef but may not be permanently associated with it). The benthic community supported by the provisioning of hard oyster substrate likely incorporates key trophic cascades, as mediated by variation in prey, predators and habitat complexity (Shurin et al. 2002).

The primary objective of this project is to examine oyster productivity and the effect of trophic dynamics on artificial and historic reefs in the Mississippi Sound. Samples were taken from various natural and artificial reefs to monitor oyster spat settlement and to obtain organisms for model validation and mesocosm experiments. In addition, an individual-based model was parameterized and calibrated using the literature and findings from field samples and mesocosm experiments. It is anticipated that such models can serve as tools for determining the most promising locations for future oyster reef restoration projects. Specific primary objectives for this dissertation project were:

1. To conduct field sampling to document oyster spat settlement abundance and to document the presence of key (i.e., representing major trophic linkages to oysters) oyster reef organisms at twelve reefs in the Mississippi Sound (chapter two).
2. To conduct manipulative experiments to inform the IBM model by (a) quantifying direct predator/prey interactions among mud crabs, oyster drills, and various stages of oysters; and (b) determining what stages of oysters are most vulnerable to each type and size class of predator (chapter three).
3. To conduct manipulative experiments to (a) examine how various combinations of predators affect the survival of oyster spat within the context of alternative substrate types and (b) consider the potential role of trait-mediated intermediate interactions (TMIIs) involving two top resident predators within the trophic web of oyster reefs (chapter four).
4. To construct an individual-based model designed to forecast oyster production in the context of oyster life stages, abiotic factors, and predation pressure for the Mississippi Coast (chapter five).

# CHAPTER II – GEOGRAPHICALLY EXTENSIVE ASSESSMENT OF SPAT SETTLEMENT AND RESIDENT ABUNDANCE AND BIOMASS AT HISTORIC AND ARTIFICIAL REEFS ALONG THE MISSISSIPPI COAST

## Introduction

Three methods are commonly used by management agencies for restoration of oyster reefs: 1) addition of reef material, 2) harvest closures and season limits, and 3) transplantation of oysters from other areas (Coen et al. 1998). Reef materials such as limestone and shell cultch are commonly added to existing oyster reefs or used to create artificial reefs in the Mississippi Sound to promote oyster settlement and recruitment. It is imperative for the hard substrate to be deployed at a time relative to the oyster settlement and recruitment season because otherwise the substrate can get covered by silt or competing encrusting organisms such as barnacles and mussels (MacKenzie 1981; Osman et al. 1989). Hayes and Menzel (1981) indicated that fall oyster spawning in the Gulf of Mexico is preceded by a rapid decrease in water temperature; therefore, monitoring of water temperature near oyster settlement sites can help determine when to deploy additional reef material. However, dates of spawning vary from year to year (Kennedy 1980; Hargis and Haven 1988), and the Mississippi Sound may be too shallow to show much temperature variation among sites so monitoring reefs is still necessary to ensure proper timing of material deployment each spawning season.

Despite extensive oyster reef restoration efforts, existing assessments are insufficient for determining the long-term success of implemented reef habitats (Bohnsack and Sutherland 1985; Baine 2001; Mann and Powell 2007; Seaman 2007). A variety of factors, such as reef complexity and substrate type have been examined in a few studies in order to determine the most advantageous reef design for successful restoration efforts. Gregalis et al. (2008) described a comparison between high versus low relief reefs in Mobile Bay, Alabama, and their results indicated an increase in oyster recruitment success on high profile reefs. The authors reported that oyster production and recruitment are affected by a variety of factors in Mobile Bay, such as dissolved oxygen concentration, oyster drill predation, disease, hurricanes, and overharvesting. Reefs with a higher vertical profile may be less prone to hypoxic conditions that often occur on lower elevation reefs; however, high profile reefs cost almost ten times the amount to construct, which may outweigh the benefit these reefs provide (Gregalis et al. 2008). The effect of reef substrate type has also been studied with conflicting results; however, the majority of evidence supports the conclusion that the three-dimensional structure of oyster shell cultch provides the best habitat for oyster settlement (Soniat and Burton 2005). Additionally, it has been suggested that reef substrate should be composed of a calcium-based material (Hidu et al. 1974), and calcium-carbonate materials (limestone, shell, etc.) attract a greater abundance of spat than gravel (Soniat et al. 1991). While these studies have provided useful information for determining advantageous reef designs, there is not enough existing information to estimate how much these reefs are enhancing oyster production over longer time scales.

There has been a detailed study conducted along the Gulf Coast to describe the condition of reefs as well as their potential for oyster production. La Peyre et al. (2014) documented the number of restored oyster reefs in the northern Gulf of Mexico and determined the restoration success of selected reefs. The authors identified 259 reefs, 72 of which were located along the Mississippi coast. Three of these reefs near Bay St. Louis (one historic and two artificial) were quantitatively examined, and both the historic (i.e. naturally occurring) and artificial reefs (rock or shell substrate) supported live oysters. However, there are numerous other reefs along the Mississippi coast where oyster production potential has not been documented. Such information would be invaluable for ensuring the success of future reef restoration efforts (i.e. increase oyster production). The work by La Peyre et al. (2014) illustrated the need for further assessments of the habitat function of restored oyster reefs, especially considering the economic importance and valuable ecosystem services of oyster reefs.

While the addition of hard substrate is the first step for oyster reef restoration, it is critical to quantify spat settlement and survival and persistence on these restored and constructed reefs to potentially help determine long-term sustainability. Saoud et al. (2000) conducted a spat settlement study on four reefs in Mobile Bay, Alabama, using squares of cement fiber board as spat collectors that were replaced every two weeks from May through mid-November. They found a difference in spat abundance between the two reefs in the western region versus the two in the eastern region of Mobile Bay but also a difference within the reefs in the eastern region. Peak spat settlement occurred between the end of August and the end of September, and the authors indicated this settlement occurred approximately three weeks following a fast decline in water temperature at the reef sites (Saoud et al. 2000). O’Beirn et al. (1995) conducted a similar spat settlement study in Wassaw Sound, Georgia, at three reefs varying in hydrodynamic and abiotic factors to examine how location and sampler deployment length affected spat abundance. They used PVC tubing for settlement and sampled at bi-weekly, monthly, and seasonal intervals. Overall, peak spat settlement occurred from July to mid-September and varied at each site; however, all three sites had a recruitment window from May through September. Recruitment was significantly increased at the site sheltered from currents and wave action, and two of the reef sites showed a negative relationship between oyster settlement and sampler deployment length (O’Beirn et al. 1995).

In addition to reef location and length of sampler deployment, Bartol and Mann (1997) indicated spat sampling methods can affect the results of oyster spat settlement studies. They examined spat settlement on artificial reefs composed of oyster cultch in the intertidal region of Chesapeake Bay over a period of two years. Three sampling methods were executed: 1) shell strings that were sampled and replaced at weekly time intervals, 2) tray samplers containing shells that were also sampled weekly, and 3) a destructive method where shells were physically removed from the reef structure every week. Among these three methods, shell strings had the most amount of spat settlement, and the destructive method was documented to have the least amount of oyster spat throughout the entire sampling period (Bartol and Mann 1997). Based on these results, it may be inaccurate to compare spat settlement studies that use different sampling methods, making the task of documenting oyster spat abundance at reef sites more difficult.

### *Competition/Predation on Oyster Reefs*

The processes of competition and predation can also affect oyster spat settlement and recruitment. Oyster larvae compete with other encrusting resident species, such as barnacles, bryozoan, and mussels for settlement space on oyster reefs, which is often limited by the availability of hard substrate or siltation of existing reef material (Osman et al. 1989; Saoud et al. 2000). Additionally, oyster recruitment and survival is limited by predation via key predators, such as mud crabs, stone crabs, blue crabs, and oyster drills (Brown and Stickle 2002; Grabowski 2004; O’Connor et al. 2008). Oyster spat and juvenile oysters are particularly vulnerable to these predators, and consumption of newly settled oysters can potentially derail oyster reef restoration progress (O’Beirn et al. 2000). Therefore, it is critical to monitor the presence of these key competitors and predators on oyster reefs in addition to quantifying oyster life stages.

### *Objectives*

While the above studies have provided useful information regarding the effects of reef location, sampler deployment length, and sampling method on oyster spat settlement, a detailed oyster spat study on reefs in the Mississippi Sound that encompasses the entire oyster spawning season and geographic region is currently lacking. Accordingly, the focus of this chapter is on the assessment of oyster spat abundance and biomass on nearshore artificial and historic oyster reefs in the Mississippi Sound. Additionally, the insights gained in this chapter were essential for initializing oyster and predator abundance in IBM simulations and providing information for managers regarding the suitability of various geographic regions for successful restoration (i.e. increased oyster productivity).The objectives of this chapter were: 1) quantify spat settlement throughout the oyster reproductive season (April to October) for one year at twelve reefs along the Mississippi coast, 2) examine the spatial and temporal variation of key species potentially affecting oyster production among reef sites, and 3) document abiotic variables at reef sites to detect seasonal and regional differences during the sample period.

## Methods

### *Field Sampling locations*

To provide a thorough geographic representation of reef conditions along the Mississippi Coast, the coast was divided into three regions (west, central, and east), with two artificial reefs and two historic oyster reefs per region for a total of twelve reef locations.  Sites were selected with various depths, water flow conditions, and distances from shore (Table 1 and Figure 1). With the exception of the natural historic reefs, all of these sites are low-profile near-shore artificial reefs established by the Mississippi Department of Marine Resources (MSDMR). Each historic reef is a naturally occurring oyster reef that was previously harvested and which has had no artificial reef substrate augmentation. Reefs were divided into three regions that represent different hydrology: west, east, and central, defined by the Mississippi Department of Environmental Quality (MDEQ) and the National Fish and Wildlife Foundation (NFWF) in the 2016 Mississippi Gulf Coast Restoration Plan.

### *Field Sampling Units*

Sampling to determine the abundance, biomass, and trophic connections of fauna on the reefs was performed using a multi-stage sampling routine. Trays made of plastic measuring 52 cm x 52 cm x 11 cm and lined with self-closing mosquito netting bags (0.5 mm mesh) were filled with oyster shell and deployed at each site. Forty-eight trays (four per site) were each filled with approximately 6,000 g of oyster shell. Oyster shell was obtained from Crystal Seas processing plant in Pass Christian, MS. Attached to each of the four corners of the trays was a four-point harness, which was attached to a float line. Settlement plates (ceramic tiles with dimensions of 15 cm x 15 cm x 0.5 cm) were attached using zip ties to the topsides of cinder blocks with separate buoy lines from the trays.

There was a total of four settlement plates per site, with each being attached to a different cinder block.

### *Sampler Deployment and Retrieval*

At each of the twelve reef sites, four trays were deployed and sampled twice at 10-week intervals starting in the middle of May and ending at the end of October (i.e., total time period of 20 weeks). Sampling over two 10-week periods allowed for adequate replication and seasonal representation. Before placing trays, a PVC pole was used to probe the reef bottom to ensure deployment on hard substrate. The trays were lowered onto the reef from a boat using a two-point harness to ensure that no shell was lost from the tray during placement. During retrieval, the buoy line was pulled up and the mesh bag cinched closed over the top of the tray to minimize loss of contents. Tray material was immediately rinsed into a plastic tub containing filtered seawater (0.5 mm) at the sampling location. Each shell was rinsed and carefully inspected to ensure the collection of any organisms hiding in the shell itself. The contents in the tub were poured into a 0.5 mm sieve. Organisms needed for the mesocosm study (toadfish, oyster drills, and mud, blue, and stone crabs) were quantified, collected and placed in aerated containers. The remaining fauna were poured into sample jars and stored on ice until returning to the lab. After each shell was rinsed, the shells were transferred back into the clean tray and redeployed at the same location.

### *Settlement Plate Deployment and Retrieval*

Settlement plates were used to document spatial-temporal variation in spat settlement throughout the entire spat season. At each site, four settlement plates, each attached to a separate cinder block with a buoy line were deployed, starting at the end of April. Every two or three weeks (depending on weather and boat availability), the settlement plates were retrieved and placed into zip lock bags for processing. Four new settlement plates were attached to the tops of cinder blocks using zip ties and lowered for the next two weeks. This process was repeated until the end of October for a total of eleven two-week time periods.

### *Abiotic Characteristics*

The habitat and abiotic characteristics of the site were recorded using a YSI, including measurements of dissolved oxygen (mg/l), oxygen saturation (%), salinity (ppt), and water temperature (C). These characteristics were recorded during every sampling event at both the surface of the water and near the reef bottom. A time-series of the salinity, temperature, and dissolved oxygen at each region was constructed to examine how these physical variables correspond with oyster spat abundance and biomass.

### *Laboratory Processing*

Upon arrival at the lab, the tray fauna samples were placed in the freezer at 0 °C to be used for abundance and biomass determination. Settlement plates were carefully stored at 0 °C for spat and mussel quantification (number settled and size). Live organisms collected for laboratory manipulations were transferred into holding tanks containing seawater of the same temperature and salinity and allowed to acclimate before using them for experiments.

### *Abundance and Biomass*

In order to calculate the abundance and biomass of influential fauna, samples were sorted, with fauna of interest (listed below) being identified and weighed. Samples were rinsed through nested 2.0 mm and 0.5 mm sieves to separate organisms by size, making it simpler to quantify smaller organisms that may otherwise be overlooked. The focus of this sorting process was solely to quantify and obtain the biomass for the most abundant organisms on oyster reefs in the Mississippi Sound (mussels, toadfish, oyster drills, blue crabs, mud crabs, and stone crabs). For each of these organisms, shell length, carapace width, or total length and wet weight were recorded in order to calculate both the abundance and biomass of each agent (i.e., key types of organisms potentially affecting oyster production through their trophic connections), as well as the total agent biomass of each reef. Linear regressions (log-log scale) were performed using length and weight data from these tray organisms in order to calculate the biomass of any specimens used in manipulative experiments that were not weighed (weight at length relationship).

### *Settlement Plate Processing*

Quantification of settlement plate coverage documented settlement via the number and size of oyster spat and ribbed mussels at each sampling date at the various reef sites. Every spat and mussel found by eye and microscopy was carefully extracted using forceps to scrape the spat (shell and tissue) from both the front and back of the tiles and preserved in 5% formalin. The length of each oyster spat was measured using calipers, and the total mass of oyster spat tissue was derived from shell length (L) by an empirical relationship (log-log) determined by measuring and weighing fifty spat of various sizes (Figure 2). This calculated average plate biomass was used to 1) compare oyster biomass in each of the three regions (west, central, east) and at historic versus artificial reef sites, and 2) determine whether the locations supporting oyster growth in the IBM actually produced oyster spat in 2015.

### *Statistical Analysis*

Using SPSS (version 18), a linear mixed model (LMM) separately examined two response variables, spat abundance per day and spat biomass per day, with reef type, region, and period serving as fixed main factors. Individual plates served as subjects. The period factor was represented by the six sampling times for which spat settlement occurred consistently between 28 July and 16 October. In addition, the time period served as a repeated effect for which the diagonal covariance structure was fitted. All two-way interactions and the three-way interaction among the main factors also served as fixed factors in the model, and an intercept was included. Following significant overall effects, custom Emmeans statements compared marginal means among levels of main factors and their interactions.

A PERMANOVA was conducted within PRIMER v.6 to compare dissimilarity in assemblages of the key fauna between types of reef, and among regions, and periods, and among all two-way as well as the three-way interaction as fixed factors. Data for PERMANOVA consisted of Bray Curtis similarity on fourth-root transformed abundances of the key taxa for each recovered tray. Significance for effects of main factors and interactions was determined by comparison to 999 permutations of residuals under a reduced model. As a follow-up, Two- and One Way SIMPER (Similarity Percentages) procedure within PRIMER v.6 helped ascribe rankings of key taxa contributing to differences between reef types and among regions and periods. In addition, non-metric multidimensional scaling (nMDS) ordinations of Bray Curtis dissimilarity values based on fourth-root transformed abundances aggregated by site-event illustrated differences in assemblages of key taxa among site-events, as well as among species, based on the transposed matrix.

The null hypotheses for the settlement plate analysis:

Ho: There is no difference in spat abundance and/or biomass between artificial and historic reefs.

Ho: There are no differences in spat abundance and/or biomass among regions (west, central, east).

Ho: There are no differences in spat abundance and/or biomass among sampling time periods.

The null hypotheses for the tray assemblage analysis:

Ho: There is no difference in the assemblage of key fauna between artificial and historic reefs.

Ho: There are no differences in the assemblages of key fauna among regions (west, central, east).

Ho: There is no temporal difference in assemblage structure between the two-time periods.

## Results

For every time point there was a minimum of two settlement plates retrieved (usually at least three) retrieved from every sampling site. From April through June, not many spat occurred, and settlement plates were either mostly clean or had barnacles and/or bryozoan attached. Spat settlement generally increased throughout the summer months and then decreased in October.

### *Spat Abundance*

Spat abundance differed significantly for all three main factors, as well as for all two-way and three-way interactions among them within the LMM (all $P < 0.001$; Table 2). The Diagonal covariance structure fit the model well, as shown by significant Wald Z values for all six repeated levels (i.e., periods) of the model (all $P < 0.001$), and accounting for heterogeneous variance among the six time points. In addition, the model intercept was significant ($F = 156.380$; $P < 0.001$).

Spat number per day differed overall between reef types ($F = 29.229$, $P < 0.005$). Historic reefs exhibited a mean abundance of 4.582 ± 0.362 spat per day, while artificial reefs only had a mean abundance of 1.816 ± 0.362 spat per day

(±1 standard error). Likewise, spat abundance differed overall among regions (F = 81.203, P < 0.001). Mean abundance of spat settling per day (Figure 3) was highest at the west region (7.721 ± 0.433) and lower in the central and eastern regions (1.437 and 0.438 ± respectively). Similarly, the number of spat settling per day was significantly higher in the west than at both the central and the east regions (P< 0.001). However, the number of spat was not significant between the central and east regions (P = 0.120).

Spat abundance also differed overall among sampling time periods (F = 35.190, P < 0.001). Between late July and mid-October, mean spat settlement per day was highest for the September 2nd time period (12.139 ± 1.353), second highest on September 16th (4.410 ± 0.651), and lowest for the October 16thtime period (0.118 ± 0.016) (Figure 4). On September 2nd and September 16th, spat abundance was significantly higher than at all the other time periods (P < 0.001), except abundance was also significantly higher on September 2nd than on September 16th (P < 0.001). Conversely, spat abundance was significantly lowest on October 16th, compared to all other time periods (all P < 0.001).

In addition to significant main effects, all three main factors had significant interactions with every other factor: type × region (F = 39.205, P < 0.005), type × period (F = 10.054, P < 0.005), region × period (F = 19.499, P < 0.005), and there was a significant three-way interaction among the main factors (F = 9.431, P < 0.005) (Table 2). Type of reef differed between the west and central regions (Figure 5)—in the west historic reefs had a higher spat abundance (12.235 ±

0.613) than artificial reefs (3.208 ± 0.612); while in the central region artificial reefs had more spat settling per day (2.046 ± 0.613) compared to historic reefs (0.828 ± 0.654). For artificial reefs, the west region sampling sites had significantly higher spat abundance (3.208 ± 0.612) than the sites in the east region (0.194 ± 0.654) (P = 0.001), and central sites had significantly more spat settling per day than east sites (P = 0.043). Historic reefs showed a slightly different interaction among regions—west sites had significantly higher spat abundance settling per day (12.235 ± 0.613) than those sites in the central (0.828± 0.654) and east regions (0.683 ± 0.612) (P < 0.005). However, spat number per day was not significantly different between historic reefs in the central versus east regions (P= 0.871). In general, the effects of region were significant at artificial reefs (F= 5.716, P= 0.005) and at historic reefs (F= 114.710, P < 0.001).

At artificial reef sites, spat number per day was significantly higher on September 2nd (7.810 ± 1.931) than during every other time period (P < 0.005). September 16th (2.176 ± 0.920) had significantly more spat than the August 12th (p= 0.042), September 2nd (P = 0.010), and October 16th (P = 0.029) sampling time points. Likewise at historic reefs, the September 2nd time period (16.468 ± 1.913) had significantly more spat settlement per day than every other time period (P< 0.001), and the September 16th time period (6.644 ± 0.920) had significantly more spat than all but the September 2nd sampling time point (P < 0.001). Additionally, on October 16th there was significantly less spat than the rest of the sampling periods (P < 0.001). Overall, the effect of period was significant at artificial reef sites (F= 4.742, P= 0.001) and at historic reef sites (F= 40.479, P < 0.001).

In the west and central regions, the September 2nd time period had significantly more spat settling per day (28.034 ± 2.290 in the west and 7.702 ± 2.370 in the central region) than any other time period (P < 0.001). However, in the east region the spat settlement numbers were all less than one spat per day, with the October 5th time point having the most spat (0.715 ± 0.305). In the west region, the September 16th time point had significantly more spat than all time periods besides September 2nd (P < 0.001), and on October 16th there was significantly less spat than the other sampling time points (P < 0.001). The effects of time period were significant in the west and east regions (F= 71.424, P < 0.001 and F= 59.079, P = 0.018 respectively), but not in the central region (F= 2.126, P = 0.074).

There were significant differences in spat abundance during multiple sampling time periods on both artificial and historic reefs and in all three regions. Historic reefs in the western region had the most spat settlement of all reefs during peak season with 44.344 ± 3.238 and 19.096 ± 1.558 spat settling per day on the September 2nd and 16th time points respectively. The next highest spat settlement was seen on September 2nd at artificial reefs in the west (11.725 ± 3.238) and central (11.375 ± 3.238) regions. Regardless of reef type and period, the eastern region never had greater than 1.242 spat settling per day, with peak settlement occurring on October 5th at historic reefs. Comparisons among artificial reefs in the three regions revealed the September 2nd time point had significantly more spat settling per day than every other period besides September 16th in the western region, and in the central region the September 2nd time point also had significantly higher spat settlement per day than all other time periods ($P < 0.01$). However, spat abundance did not differ significantly among any periods at artificial reefs in the eastern region. A different trend in pairwise comparisons occurred among regions at historic reefs. Whereas the September 2nd and September 16th periods showed significantly more spat than all other time periods at historic reefs in the western region ($P < 0.001$); spat settlement was not significantly different among any periods at historic reefs in the central region. Abundances at historic reefs in the east region were significantly lower for the October 16th time point than for either the August 12th ($P < 0.001$) or October 5th ($P =0.006$) time periods. Overall, the effects of period at artificial reefs were significant in the west ($F= 6.070$, $P < 0.001$) and central ($F=2.500$, $P = 0.040$) regions but not in the east region ($F=0.137$, $P = 0.983$). Conversely, the effects of period at historic reefs were significant in the west ($F= 92.511$, $P < 0.001$) and east ($F= 4.572$, $P = 0.001$) but not in the central ($F= 0.278$, $P = 0.924$) region.

### *Spat Biomass*

Spat biomass per day differed significantly for the two main factors, region and period, as well as for all two-way and three-way interactions among the three main factors within the LMM (all significant P < 0.001). The type main factor was not different (F = 0.768; P = 0.386), although spat biomass per day averaged higher at historic reefs (12.164 ± 2.282g) than artificial reefs (9.335 ± 2.282g). Again, the Diagonal covariance structure fit the model well, as shown by significant Wald Z values for all six repeated levels (i.e. periods) of the model (all P < 0.005), and accounting for heterogeneous variance among the six-time points. In addition, the model intercept was significant (F = 44.374; P < 0.001).

Overall, spat biomass per day (Figure 6) differed significantly among the three regions (F = 11.771, P < 0.001). The west region had the highest spat biomass per day (20.580 ± 2.731g), and the central region had approximately half as much spat biomass (10.099 ± 2.827g). Spat biomass settling per day averaged 13 times lower in the east region (1.569 ± 2.826g) compared to the west. Differences in spat biomass were significantly higher in the west than either the central (P = 0.011) or the east (P < 0.001), and significantly higher in the central compared to the east (P = 0.039).

The time period factor (Figure 7) was significant for spat biomass settlement per day (F = 13.121, P < 0.005). The September 2nd settlement period had the most biomass (51.000 ± 9.570g), while the October 5th period had the lowest spat biomass per day (0.898 ± 0.157g). Like for abundance, mean spat biomass was significantly higher on September 2nd and September 16th than on all the other periods (P < 0.001), except biomass was also significantly higher on September 2nd than on September 16th (P < 0.001).

Significant interactions relative to spat biomass occurred between type and region (F = 9.578, P < 0.005), type and period (F = 3.725, P = 0.005), and region and period (F = 8.640, P < 0.005). In addition, the three-way interaction among type, region, and period was significant (F = 5.226, P < 0.005) (Table 3). When comparing the interaction between type and region, artificial reefs in the central region exhibited significantly higher biomass than those in the east region (P = 0.006). On historic reefs, there was significantly higher spat biomass in the western region than in the central or eastern regions (P < 0.001). Overall, the effect of region was significant at both artificial (F= 4.122, P = 0.023) and historic (F= 17.257, P < 0.001) reefs. Likewise, there were significant differences among numerous time periods on both artificial and historic reefs (Figure 8), with more significant differences among periods occurring on historic reefs. On artificial reefs, the September 2nd time period (50.425 ± 13.533g) had significantly more spat biomass than all other periods (P = 0.001), and on historic reefs, the July 28th (3.862 ± 0.832g) in addition to the September 2nd (51.755 ± 13.533g), and September 16th (13.237 ± 1.796g) periods all had significantly more spat biomass than other time periods. The effect of period overall was significant at both artificial and historic reefs (F = 3.835, P = 0.004 and F = 13.024, P < 0.001 respectively).

Additionally, spat biomass was significantly different among multiple time periods within the west and central regions, but there were no significant differences in spat biomass among periods in the east region. The highest spat biomass seen among all regions was in the western region during the September 2nd period (90.112 ± 16.194g), followed by the September 2nd period in the central region (58.616 ± 16.762g). Overall, the effects of period were significant in the west (F= 28.325, p< 0.001) and central (F= 2.512, P = 0.038) regions, but not in the eastern region (F= 0.424, P = 0.831).

When comparing the three-way interaction involving type, region, and period, the highest spat biomass was seen at historic reefs in the western region on September 2nd (132.103 ± 22.902g), and the second highest was seen at artificial reefs in the central region (100.225 ± 22.902g). The smallest amount of spat biomass per day was seen at artificial reefs in the central region on August 12th (0.010 ± 0.731g) (Figure 12). A significant three-way interaction was seen for artificial reefs in the west (F=3.832, p= 0.004) and central (F=3.865, p=0.004) regions, but there were no significant differences in spat biomass among periods on artificial reefs in the east region (F=0.024, p= 1.000). On historic reefs, there were significant differences in spat biomass among periods, in the west region (F= 37.359, p< 0.001), but not in the central (F= 0.220, p= 0.953) or east (F= 0.936, p= 0.464) regions.

### *Oyster Associated Fauna*

There were 24 and 20 trays retrieved for the July and October sampling time points respectively (50% and 42% of trays deployed). The remaining trays were potentially tripped on accident by crabbers or carried away in the current following strong storms. In July there was a minimum of two trays retrieved from at least one historic and one artificial site in each region; however, in October only one tray was recovered from both historic sites in the West region. For both time periods, no trays were recovered at SSH or Graveline sampling sites so these reefs were omitted from the tray fauna analysis, leaving ten sites for data analysis.

### *Tray Fauna Analysis*

PERMANOVA revealed significant differences in faunal assemblages among regions (F= 2.4567, p= 0.037) and between the two sampling periods (F= 11.444, p= 0.001); however, reef types did not differ (F= 1.769, p= 0.165). In addition, there were no significant two or three-way interactions (Table 4). A non-metric multidimensional scaling (nMDS) plot of the two sampling periods shows a clear seasonal distinction between the species assemblages at period one and period two (Figure 9). When comparing tray species, all six species are within a 20% similarity envelope, as circumscribed by an agglomerative hierarchical cluster analysis in PRIMER v.6 (Figure 10), which indicates all the species are 20% similar. Mud crabs and mussels are within a 35% similarity envelope, as are stone crabs, drills, and toadfish; however, blue crabs are singularly most dissimilar. Additionally, stone crabs and drills are within a 50% similarity envelope, and mud crabs and mussels are inside a different 50% similarity ellipsis as well.

Figures of species abundance proportions (Figure 11) illustrated trends in the relative abundances of oyster associated species at the various reefs within the three regions. In the western region, there were relatively fewer mussels at period one and more mud crabs at period two on each of the reefs. A similar trend in mud crab and mussel relative abundance was seen at all three reefs in the central region, except MDBH. Additionally, oyster drills occurred only at Kuhn and MLH reefs in the central region. In the east region, blue crabs were only present during period one and oyster drills occurred only at HIST reef. Overall the analysis of oyster associated fauna revealed significant assemblage differences between time periods and regions, as substantiated by differences in relative abundances of key oyster associated species relative to specific reefs, regions, and sampling times.

### *Abiotic Characteristics*

Temperature increased from May until it peaked in July/August; and water temperature steadily declined from September until November at the four reefs in the western region. The highest temperature recorded throughout the sampling season was at USM reef (32.4°C) on July 28th and the lowest temperature was at SSH reef (17.8°C) on November 20th. All four reefs in the central region followed a similar overall temporal trend in temperature as the west region; however, peak temperatures occurred at different times for various reefs: Kuhn reef in June (31.1°C), FB reef and MDBH reef in July (31.6°C), and MLH reef in August (31.4°C). Also, there was an anomalous increase in temperature in October followed by a drop in November at the central reefs. In the eastern region, there was a general trend of increasing temperature until August followed by decreasing temperatures thereafter. But like the central region, there was an anomalous increase in temperature in October followed by a drop in temperature (Figure 12).

Salinity was more variable than temperature among the reefs within the three regions (Figure 12). On the western reefs salinity was low in May and June, but then rose, except for SSH reef which dropped below 5ppt at the end of July. All four reefs in the western region peaked in salinity at the end of September, and USM reef exhibited higher salinity than the other three sites at all time points. In the central region salinity was low in May and June, but then rose and remained high; however, there was not a general decrease from August to November, and MDBH and FB reefs actually peaked in November (33.9ppt and 29.7ppt respectively). Salinity was most variable in the eastern region, but all sites increased in July. Grav reef typically exhibited the highest salinity, except the highest salinity was observed at Hist reef (34ppt). The PWRM site typically exhibited the lowest salinity (e.g., 1.5ppt).

Dissolved oxygen (DO) at depth was arguably the most variable among regions and sampling sites (Figure 13). In the western region, the lowest dissolved oxygen level was recorded at USM reef (4.08mg/L) and highest at SSH reef (8.91mg/L). A clear difference between sites was seen in November, when SSH and USM reefs showed increasing trends in dissolved oxygen; but DO at AL and BSLH reefs remained constant or decreased in November. In the central region DO was lowest at FB reef (2.37mg/L) and generally highest at MLH reef, with a peak of 9.62mg/L in April. PWRM, Hist, and Grav reefs in the eastern region followed parallel patterns of increasing and decreasing DO; but PH reef showed a different trend. Dissolved oxygen was lowest at Grav reef (2.79mg/L) and highest at PWRM reef (10.08mg/L) within the eastern region.

## Discussion

The results from this field study provided useful insights into the abundance of spat settlement throughout the oyster spawning season. Spat abundance per day was greatest at historic reefs and in the western region, which makes logical sense considering historic reefs are generally older and the western region of the Mississippi Sound generally supplies more harvestable oysters than the central or east regions due to hydrology. La Peyre et al. (2014) found similar results when quantitatively determining total oyster density at historic reefs versus created rock and shell reefs. The authors found when looking at total oyster density (spat and adult), historic reefs had a significantly higher density than rock or shell reefs, and the overall size distribution on historic reefs ranged from spat (< 25mm) to over 100 mm. However, when dividing overall oyster density by size class, adult oyster density (> 25mm shell height) was significantly greater at artificial rock reefs versus historic and artificial shell reefs, while spat oyster density (< 25mm shell height) was significantly greater on historic reefs versus artificial rock and shell reefs. The findings by La Peyre et al. (2014) support the findings from this study, as both studies found significantly higher oyster spat abundance at historic reefs versus artificial reefs. This study did not quantify adult oyster abundance, so future studies focusing on the recruitment of spat to the adult harvestable stage in the Mississippi Sound would be beneficial to determine if the increased abundance of spat on historic reefs translates into increased abundance of harvestable oysters compared to artificial reefs.

Additionally, spat abundance varied significantly seasonally, with peak spat settlement occurring in September regardless of reef type or region. This finding was not surprising considering past research in the study area has indicated higher spat abundance during the fall spawning season compared to the spring spawning season (pers. obs.). In other geographic regions, similar results have been reported. A study by Saoud et al. (2000) examined spat abundance in Mobile Bay from May through mid-November 1999 with sampling of spat collectors occurring every two weeks (a similar timeline and sampling regime to this study). The authors indicated peak spat settlement occurred between the end of August and the end of September, which is similar to the results of this study, and previous work in Mobile Bay that indicated the fall oyster spawning peak is higher than the spring spawning peak (Hoese et al. 1972; Hayes and Menzel 1981). In addition, O'Beirn et al. (1995) indicated a peak in spat settlement from July to mid-September at their sampling sites in Wassaw Sound, Georgia. Therefore, regardless of exact geographic location, it appears that *Crassostrea virginica* spawning in the southeast Atlantic and eastern Gulf regions tends to peak in the early fall. Hayes and Menzel (1981) indicated that peak spawning season in the fall is generally preceded by a rapid decrease in water temperature. In this study, a rapid temperature drop (relative to peak summer temperature) occurred at the end of August/beginning of September, which supports Hayes and Menzel's (1981) finding that a rapid temperature decrease precedes fall spawning. However, dates of spawning as well as shifts in water temperature vary from year to year (Kennedy 1980; Hargis and Haven 1988), so yearly monitoring of spat settlement is still necessary to determine the peak oyster spawning period in any given year.

Spat abundance per day and spat biomass per day were not always parallel. For example, while spat abundance was significantly different between artificial and historic reefs, spat biomass was not significantly different between reef types. This discrepancy can be explained as an inverse relationship between the number of spat and spat shell diameter (i.e., higher biomass per individual) on some settlement plates from artificial reef sites. As spat grow, they take up more surface area, which leaves less settlement plate area for additional spat to settle, or an alternative explanation for this relationship is an increased growth rate when fewer individuals are present (i.e. density dependent growth).

In terms of species assemblage, the proportion of each species varied to an extent at each reef, which may translate to varying oyster spat survival on the reefs. The species assemblage did not differ significantly between historic and artificial reefs in this study; however, other studies have shown significant differences in cryptic fish assemblages (Fleer and Rakocinski, in prep) and transient fish assemblages (Rooker et al. 1997) on natural versus artificial reefs in the Gulf of Mexico. Likewise, Walters and Coen (2006) determined differences in the similarity of benthic macrofaunal community assemblages at natural versus artificial reefs near Charleston, SC. Using multiple approaches, the authors failed to show convergence in the assemblages between reef types, even seven years following artificial reef construction. Another study, Dillon et al. (2015) reported equal or greater oyster number and faunal richness on constructed intertidal *Crassostrea virginica* reefs versus natural reefs in a northern Gulf of Mexico estuary within two years of reef construction. The above studies do not support the findings from this study, but this difference may be attributed to location, season, and sampling regime.

In addition, while this study was restricted to shallow subtidal oyster habitat, Glancy et al. (2003) found assemblages of oyster reefs was distinct from those of seagrass and marsh-edge habitats. In this study species assemblages did vary to some extent among reefs even though all reefs were classified as the same type of habitat. For example, in the western region of the Sound, mud crab proportion increased in the fall sampling period, while mussels decreased in proportion in the fall. This finding could mean that the oyster spat experienced less competition for settlement space in the fall due to decreased mussel proportion but increased predation pressure by mud crabs after settlement. A similar trend was seen in the central region with the exception of MDBH reef. In addition, specific species occurred only at some reefs in each region. In the western region, both drills and blue crabs were only found at USM reef; in the central region drills, toadfish, and stone crabs were only found at Kuhn and MLH reefs; and in the eastern region drills and stone crabs only occurred at Hist reef. It is difficult to decipher why these species were only found at specific reefs, but every reef varies in location, physical characteristics, and surface area so variability in species assemblage is not unexpected. Likewise, the differences in species assemblage likely affected the survival of oysters on each reef, such as the increased spat settlement during the fall could be related to a decreased proportion of mussels competing for the oysters' settlement area, but this finding could also be related to many other factors as well. Overall, the tray faunal assemblage helped to illustrate the proportion of each species on oyster reefs, and this information was used to initialize starting values of each species in the individual-based model (Chapter 5).

One tool for deciphering the changes in spat abundance and biomass is to analyze the abiotic data and look for any apparent trends. Unfortunately, there were not continuous data recorders at the reef sites as part of this study, and abiotic measurements taken every two weeks leave large gaps in the abiotic data. Therefore, for interpretation purposes, for each region, the mean temperature and salinity was taken from the nearest USGS sensor and plotted from the beginning of May until the end of October. When comparing temperature between the three regions (Figure 14), the East generally had the highest temperature and the West usually had the lowest temperature (five-degree Celsius difference in July and late August). For salinity values (Figure 15), the East region always had the highest salinity, while the West and Central region followed similar lower trends in salinity. The rate of predation and disease infection of *Perkinus marinus* on oysters has been shown to increase with increasing temperature and salinities (Mackenzie 1970, Manzi 1970, La Peyre et al. 2003). Likewise, necessary physiological functions, such as growth, filtration, and reproduction are also effected by temperature and salinity being too high or too low (Galstoff 1964, Shumway 1996), with 15-18 ppt being the salinity for optimal oyster physiological performance (Newell 1985). The higher salinity and temperature values seen in the Eastern region likely contributed to the lower spat abundance and biomass seen in this region, whereas more moderate temperature and salinity values occurred in the Central and especially the West region. Overall, the abiotic factors of temperature and salinity, along with the hydrology of the western MS Sound likely resulted in the significantly higher spat abundance and biomass of oyster spat in this region.

Several caveats pertaining to this study in terms of spat settlement seem warranted. First of all, the factor of “region” was used to compare the East, Central, and western areas of the Mississippi Sound as a simplification to discussing the hydrology and physical properties of the different areas. The significant differences in spat abundance and biomass among regions is not attributed to the region per se, but rather the difference in spat number reflects the numerous streams and rivers entering into the western region of the MS

Sound which aids oyster growth and recruitment. Additionally, the tile samplers used to quantify spat settlement were ceramic, which is not the same chemical composition as oyster cultch or artificial calcium carbonate materials such as limestone. However, pilot studies determined ceramic tiles attracted oyster spat as well as did cement board, and there is conflicting information on the most suitable material for oyster settlement. For example, Cole and Knight-Jones (1949) showed less settlement of *Ostrea edulis* spat on cleaned shells versus uncleaned shells, which would lead to the conclusion that the clean tiles replaced every two weeks for in this study may have favored low rates of spat settlement, depending on how long it takes for a proper biofilm to develop. However, Pineda and Caswell (1997) found spat collectors such as tiles and plates elevated off the bottom might actually overestimate spat settlement, because the increase in height of the spat collector decreases predation, sedimentation, and exposure to low oxygen levels. Spat collectors in this study comprised tiles attached to the tops of cinder blocks which increased the height of the settlement plate off the bottom by approximately 19 cm. However, hypoxic bottom conditions were not measured during this study and none of the bricks appeared to be affected by sedimentation, even though hypoxic conditions could have occurred in between abiotic measurements and decreased predation may have still been a factor.

Additionally, due to inclement weather and gear-related problems, consistent retrieval of settlement plates did not occur every two weeks. Consequently, we normalized the spat data by the number of days soak time.

However, tiles that were deployed for more than two weeks may have incurred relatively more fouling by barnacles and other encrusting organisms, potentially deterring spat settlement (Osman et al. 1989). While we made every attempt to conduct and analyze this study in an unbiased manner, it is likely that some sources of bias were not fully rectified.

Table 1

*Reef sampling names, location, and reef type.*

| **Region** | **Reef Name** | **Reef type** | **Latitude** | **Longitude** |
|---|---|---|---|---|
| West | American Legion | Artificial | 30.30030 N | -89.32930 W |
| | Saint Stanislaus | Historic | 30.30000 N | -89.32584 W |
| | Bay Saint Louis | Historic | 30.31077 N | -89.31542 W |
| | University of Southern MS | Artificial | 30.34720 N | -89.13365 W |
| Central | Kuhn Street | Artificial | 30.39127 N | -88.87250 W |
| | Mud Lumps | Historic | 30.38437 N | -88.86115 W |
| | Fort Bayou | Artificial | 30.42090 N | -88.85948 W |
| | Mouth of Davis Bayou | Historic | 30.39237 N | -88.80748 W |
| East | Graveline | Artificial | 30.34723 N | -88.67091 W |
| | Historic | Historic | 30.34725 N | -88.61649 W |
| | Pascagoula West River Mouth | Artificial | 30.36453 N | -88.59988 W |
| | Pascagoula Historic | Historic | 30.34505 N | -88.59158 W |

Note. Twelve sampling locations in the Mississippi Sound used for this study. Reefs are divided by region (West, Central, or East) in the first column and distinguished by type (historic versus artificial) in the fourth column.

Table 2

*Mixed model analysis values of oyster spat abundance per day.*

| **Factor** | **df** | **F-value** | **P-value** |
|---|---|---|---|
| Reef Type | 1 | 29.229 | < 0.001 |
| Region | 2 | 81.203 | < 0.001 |
| Period | 5 | 35.190 | < 0.001 |
| Type x Region | 2 | 39.205 | < 0.001 |
| Type x Period | 5 | 10.054 | < 0.001 |
| Region x Period | 10 | 19.499 | < 0.001 |
| Type x Region x Period | 10 | 9.431 | < 0.001 |

Note. Results of the LMM analysis of spat number settling per day for reef type, region, and period, as well as the interactions between these factors.

Table 3

*Mixed model analysis values of oyster spat biomass per day.*

| **Factor** | **df** | **F-value** | **P-value** |
|---|---|---|---|
| Reef Type | 1 | 0.768 | 0.386 |
| Region | 2 | 11.771 | < 0.001 |
| Period | 5 | 13.121 | < 0.001 |
| Type x Region | 2 | 9.578 | < 0.001 |
| Type x Period | 5 | 3.725 | 0.005 |
| Region x Period | 10 | 8.640 | < 0.001 |
| Type x Region x Period | 10 | 5.226 | < 0.001 |

Note. Results of the LMM analysis of spat biomass settling per day for reef type, region, and period, as well as the interactions between these factors.

Table 4

*PERMANOVA values of organismal abundance within sampling trays.*

| **Factor** | **df** | **F-value** | **P-value** |
|---|---|---|---|
| Reef Type | 1 | 1.769 | 0.165 |
| Region | 2 | 2.457 | 0.037 |
| Period | 1 | 11.444 | 0.001 |
| Type x Region | 2 | 2.258 | 0.066 |
| Type x Period | 1 | 0.062 | 0.89 |
| Region x Period | 2 | 1.047 | 0.406 |
| Type x Region x Period | 2 | 0.900 | 0.504 |

Note. PERMANOVA output for dissimilarity in key oyster species assemblage relative to reef type, region, and sampling period, as well as the interactions between these factors.

*Figure 1.* Map of the twelve sampling sites in the Mississippi Sound.

Note. Map of the twelve sampling locations along the Mississippi Coast. The reefs are color-coded based on region (yellow = western, pink = central, blue = eastern). Within each region, historic reefs are denoted by balloon-star symbols and artificial reefs are shown as pincushion symbols. The reefs are American Legion Artificial Reef (AL), St. Stanislaus Historic Reef (SSH), Bay St. Louis Historic Reef (BSLH), USM Artificial Reef (USM), Kuhn Street Artificial Reef (KS), Mud Lumps Historic Reef (MLH), Fort Bayou Artificial Reef (FB), Mouth of Davis Bayou Historic (MDBH), Graveline Artificial Reef (GRAV), Historic Reef (HIST), Pascagoula West River Mouth Reef (PWRM), and Pascagoula Historic Reef (PH). Geographic coordinates obtained from MSDMR.

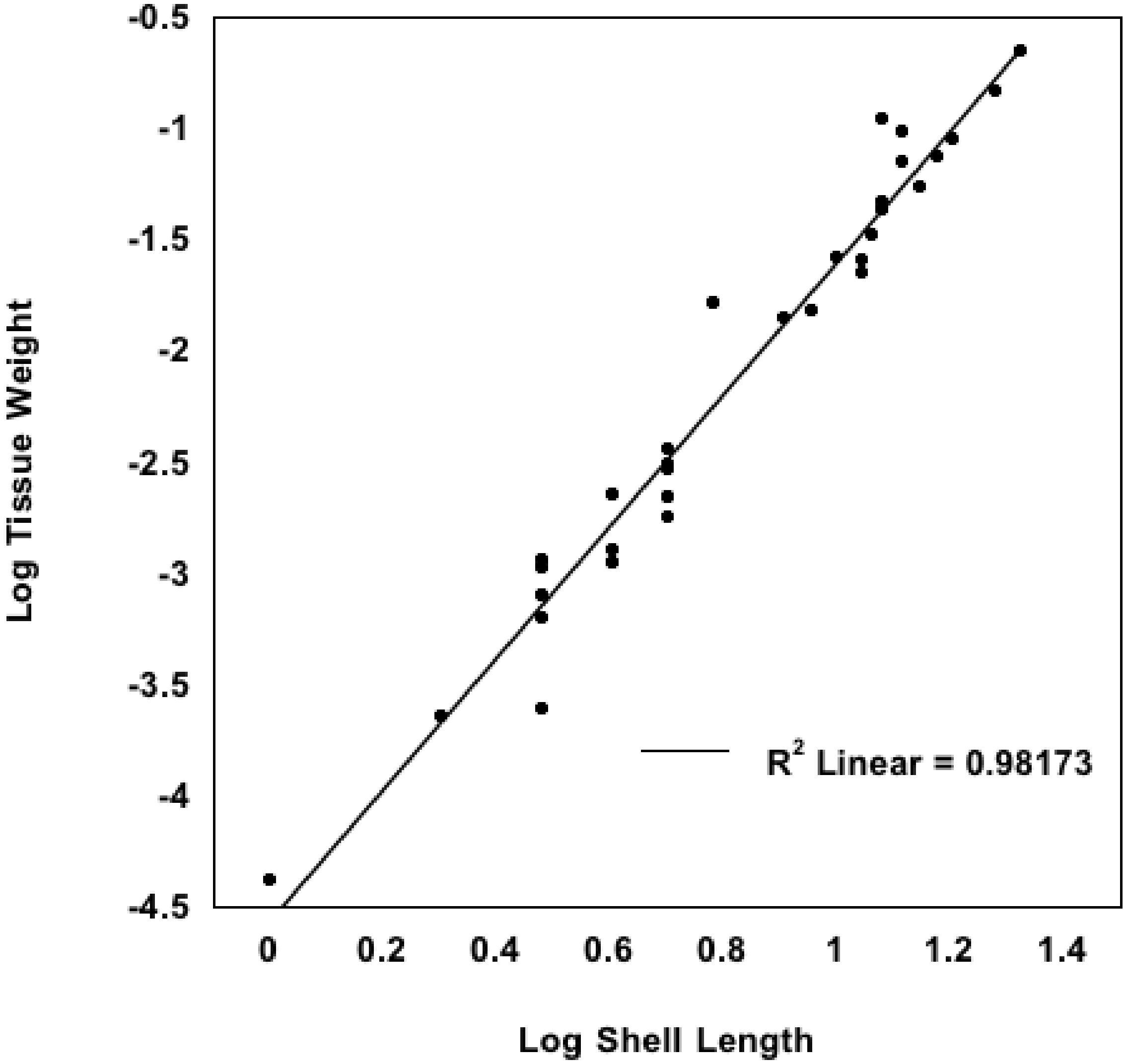


*Figure 2.* Eastern oyster shell length wet tissue weight regression.

Note. Log transformed Eastern oyster shell length wet tissue weight relationship used to calculate spat settlement biomass (Weight (grams) = 2.9582 (length in mm) – 4.5542).

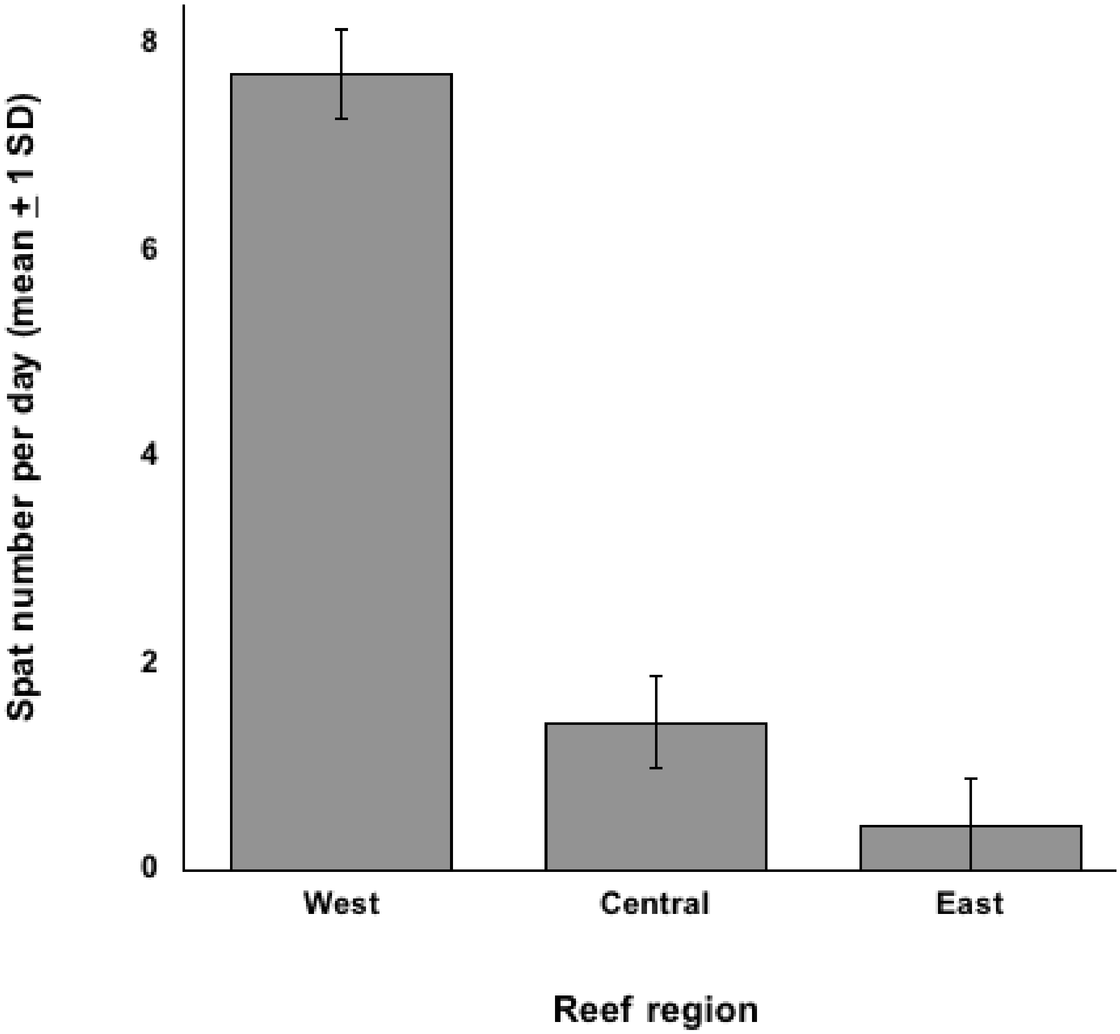


*Figure 3.* Comparison of oyster spat abundance in the three sampling regions.

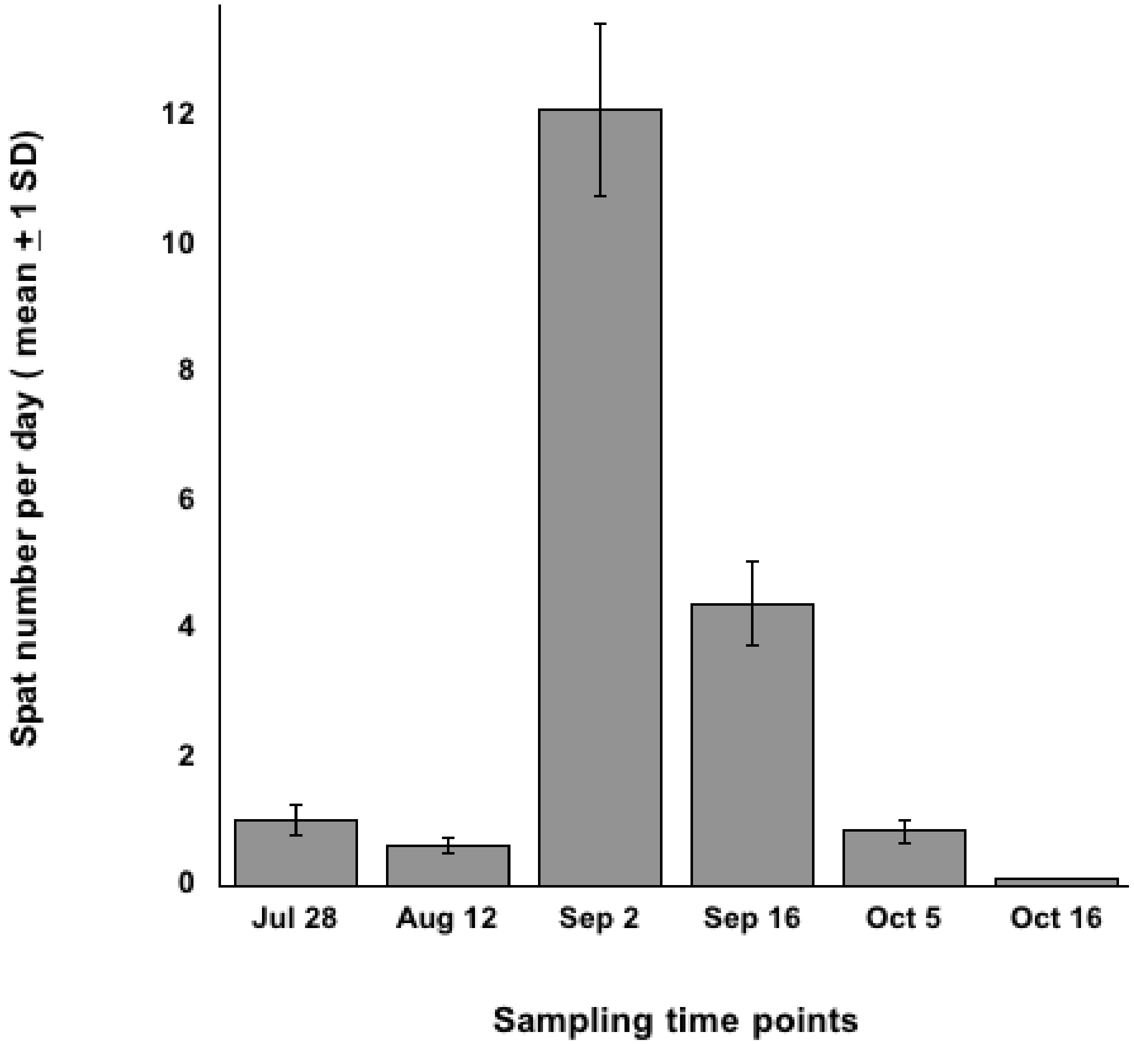


*Figure 4.* Relationship of oyster spat abundance and sampling periods.

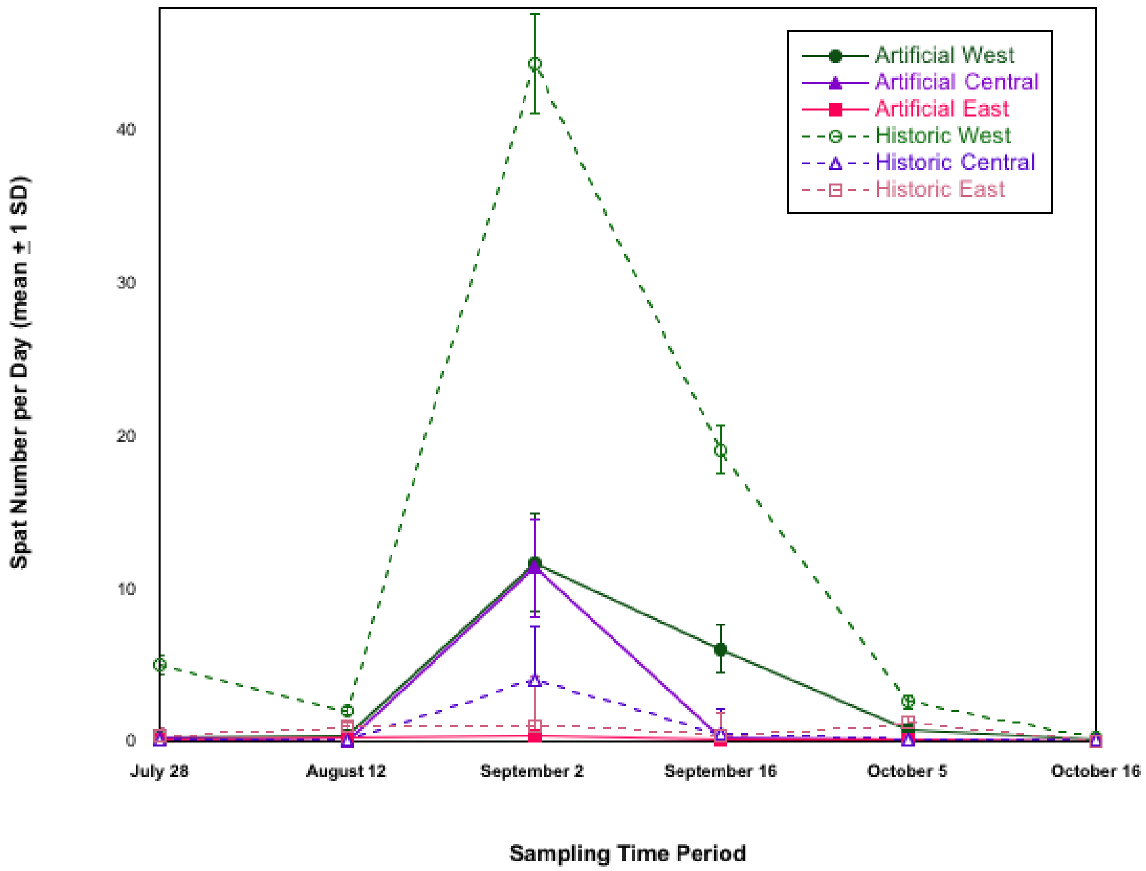


*Figure 5.* Temporal change in the mean number of spat per day.

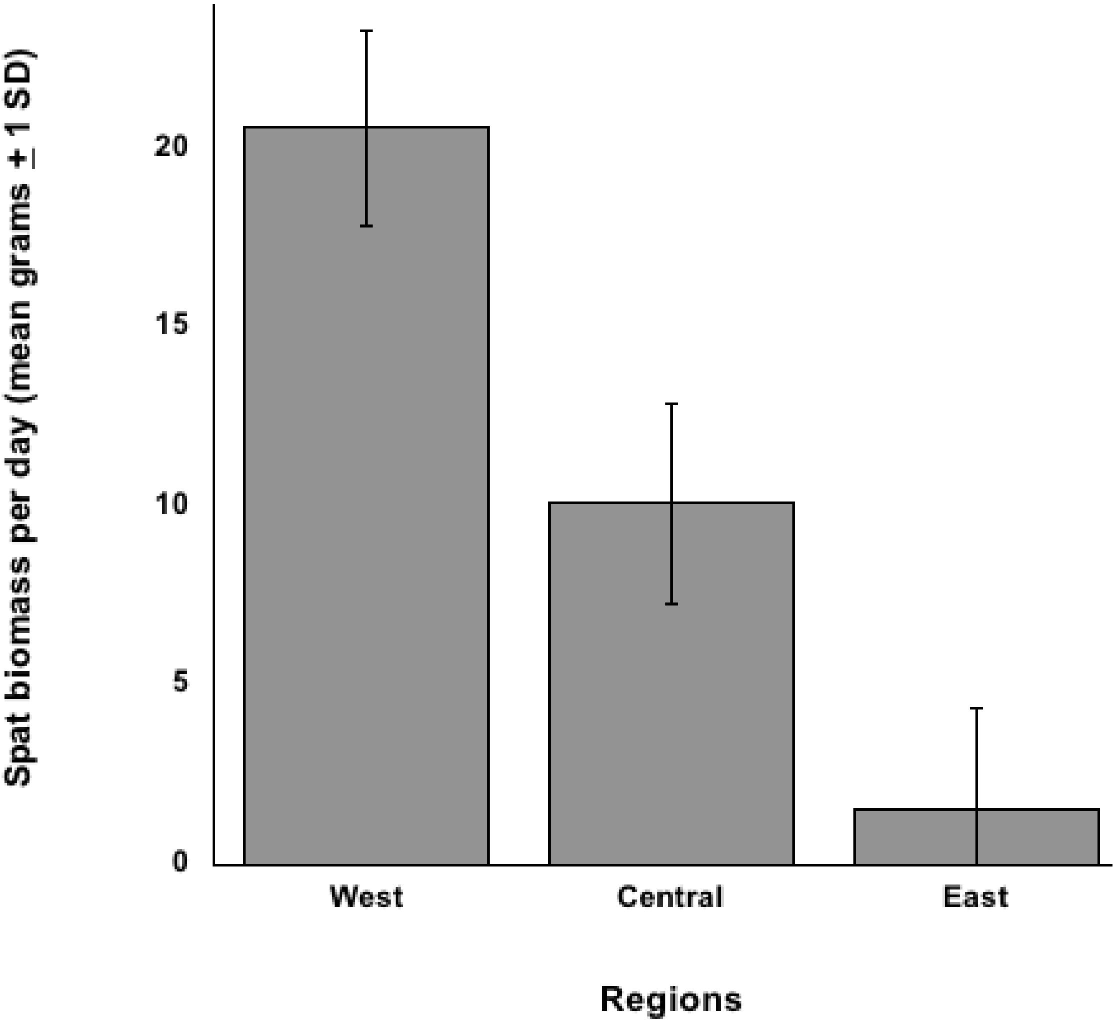


*Figure 6.* Relationship of oyster spat biomass (grams) and regions.

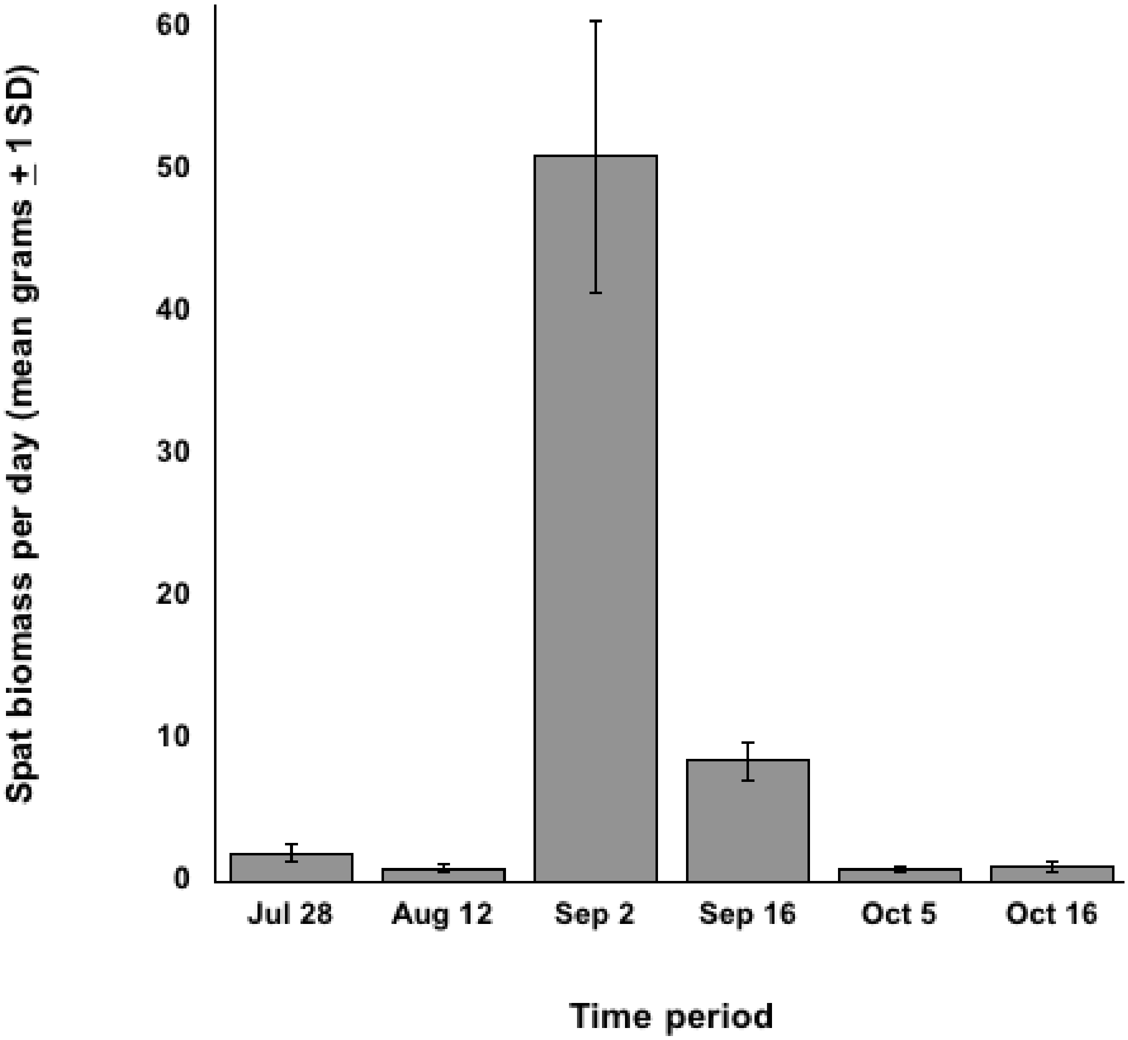


*Figure 7.* Comparison of oyster spat biomass (grams) and sampling periods.

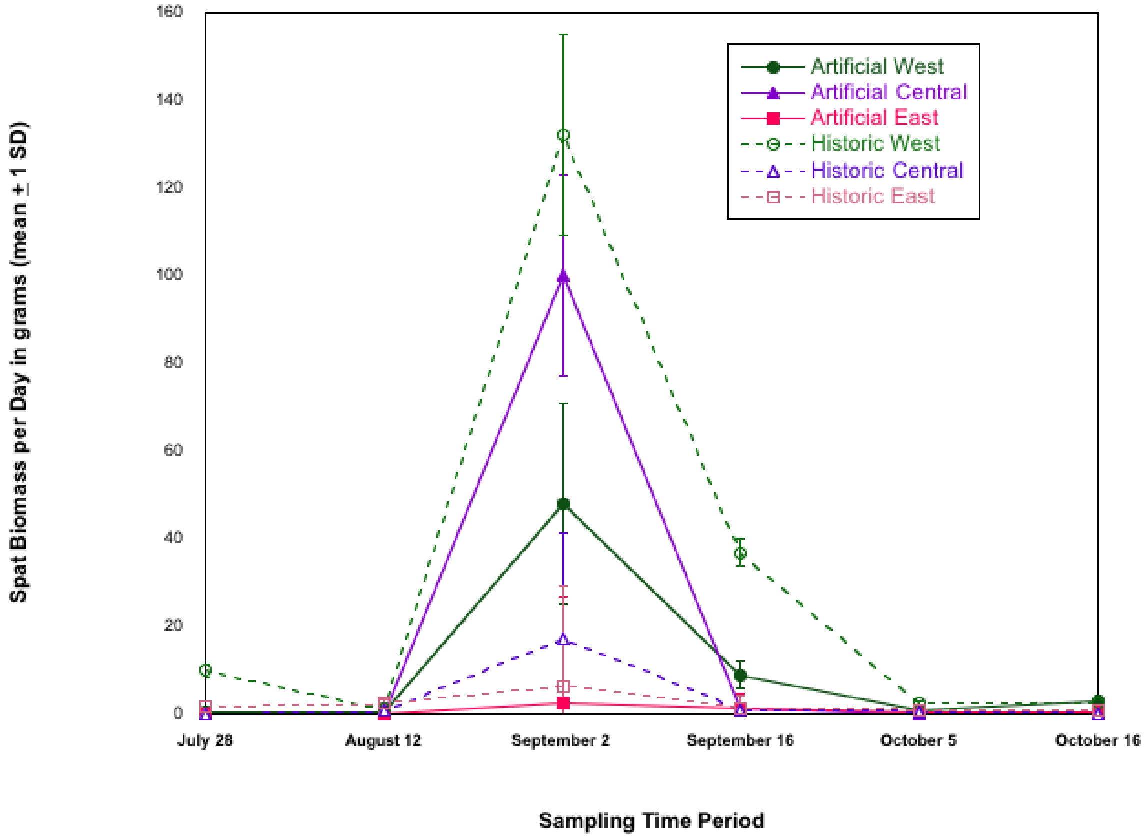


*Figure 8.* Temporal change in mean spat biomass (grams) per day.

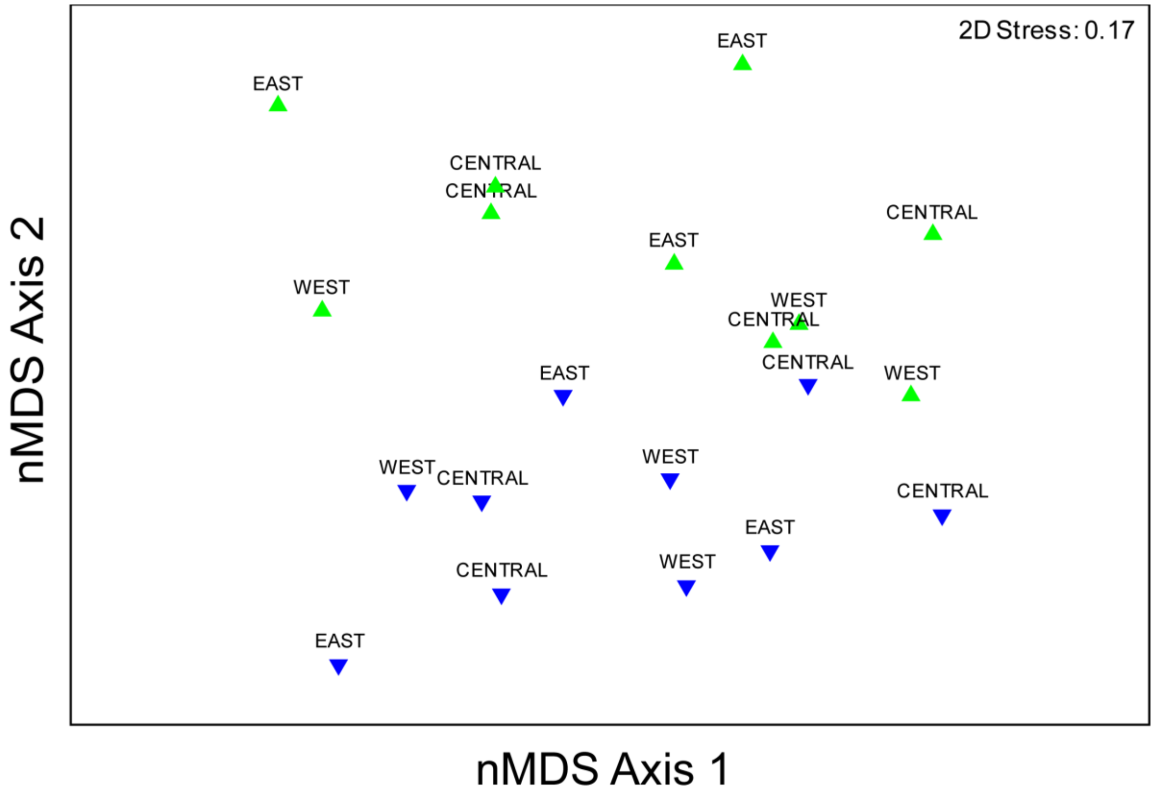


*Figure 9.* Non-metric multidimensional scaling plot of site events based on species dissimilarity during the two sampling time periods.

Note. Bright green trianges represent the first sampling time point in July, and blue upside-down trianges show the second sampling time point in October.

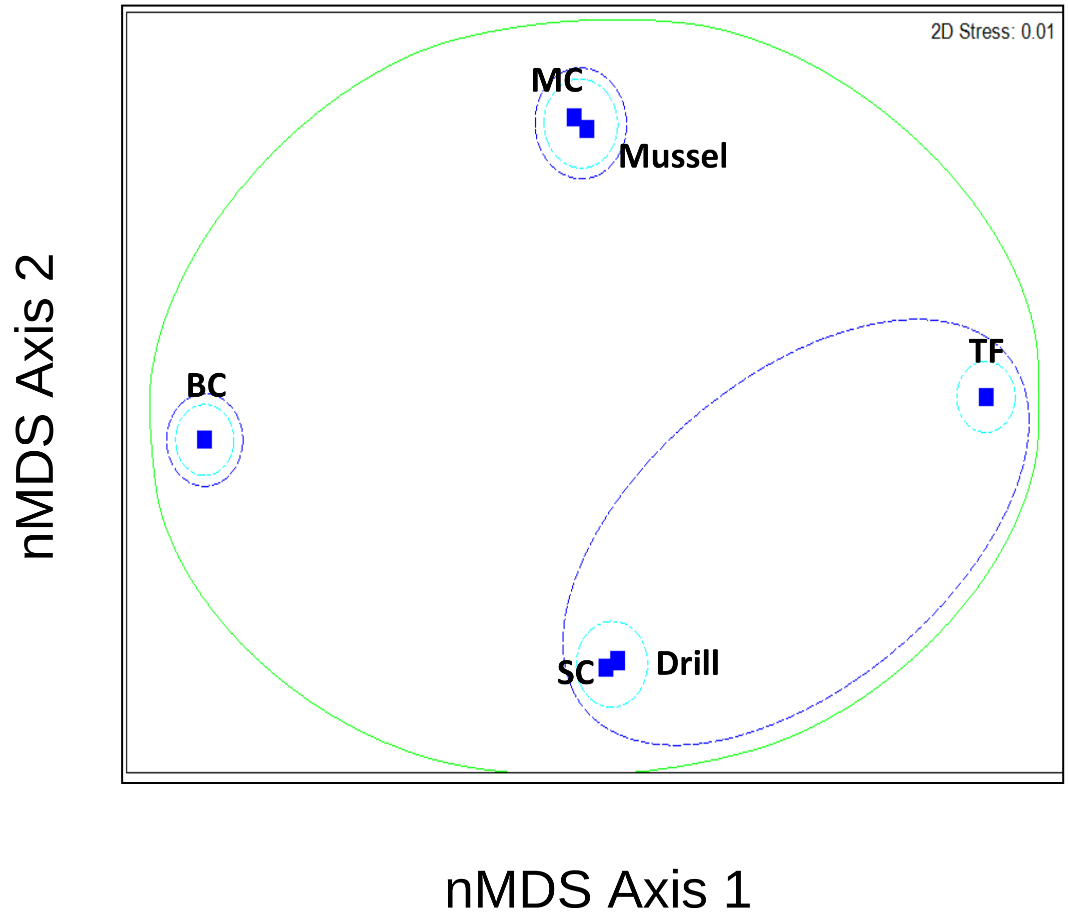


*Figure 10.* Non-metric multidimensional scaling plot of species assemblage.

Note. MC = mud crab, BC = blue crab, SC = stone crab, TF = toadfish, and Drill = oyster drill. Ellipses are used to show the degree of similarity: bright green = 20%, dark blue = 35%, and bright blue = 50% similarity.

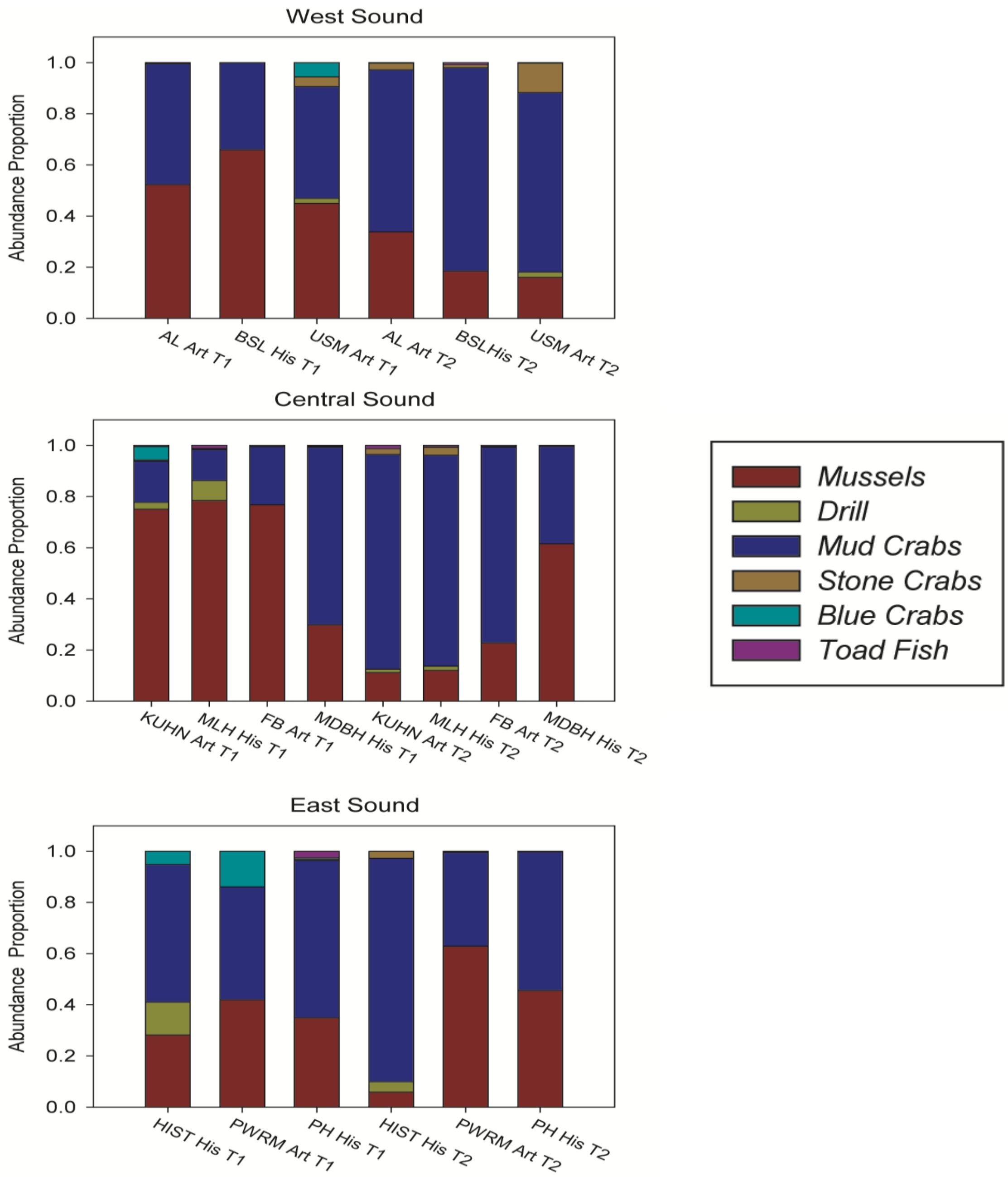


*Figure 11.* Abundance proportion figure of species composition at each reef.

Note. The top figure shows the West region: AL = American Legion, BSLHis = Bay Saint Louis, USM = University of Southern Mississippi. The middle figure shows the Central region: KUHN = Kuhn Street, MLH = Mud Lumps, FB = Fort Bayou, and MDBH = Mouth of Davis Bayou. The bottom figure depicts the East region: HIST = Historic Reef, PWRM = Pascagoula West River Mouth, and PH = Pascagoula Historic. Reef type is designated by Art = artificial reefs and His = historic reefs. Time points are represented by T1 = July sampling time point and T2 = October sampling time point.

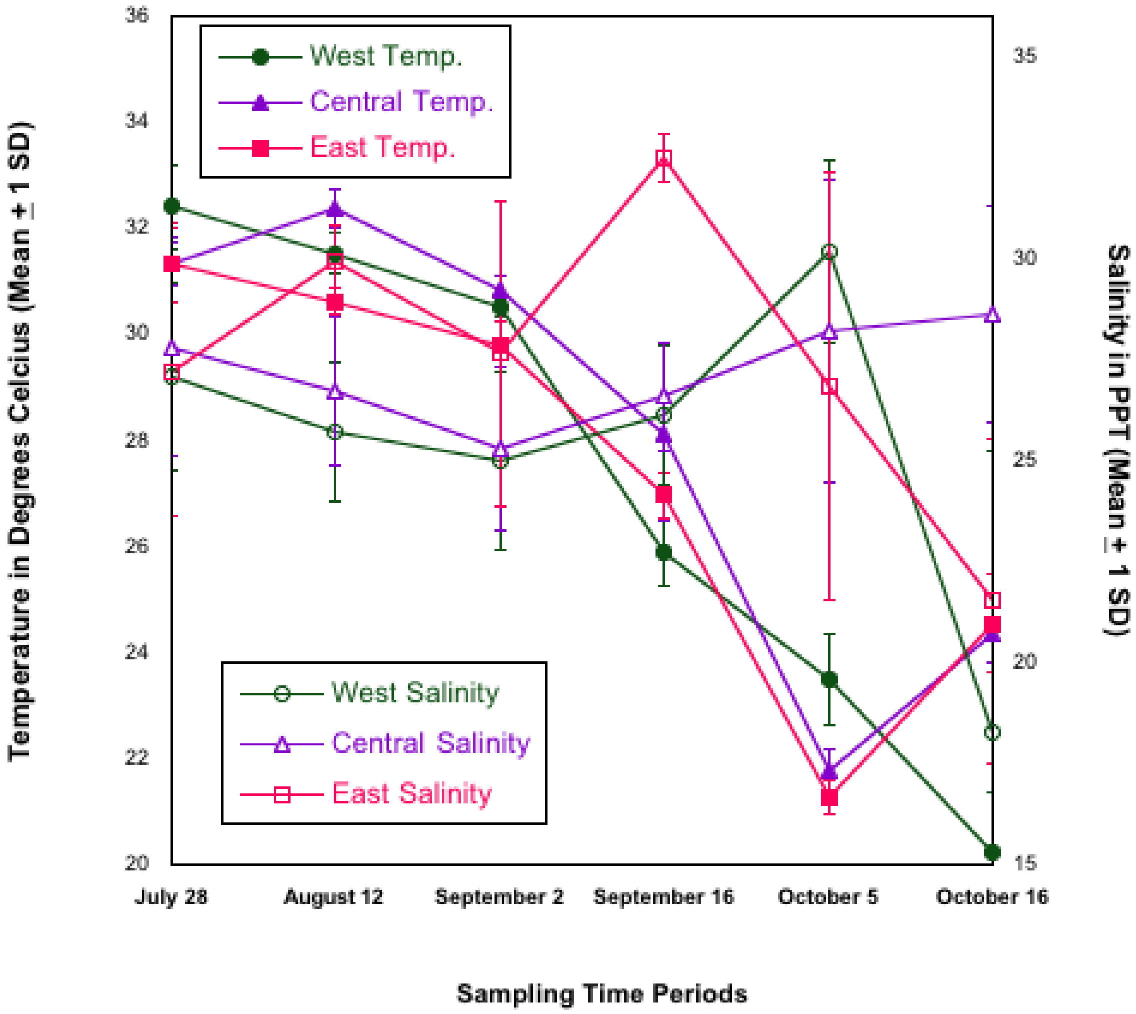


*Figure 12.* Temporal change in mean temperature and salinity in each region.

Note. The temperature and salinity values for the four sites within each region were averaged and plotted as mean + 1 Standard Deviation. The dates shown on the x-axis correspond to the peak settlement period (other dates were omitted from this graph and data analysis).

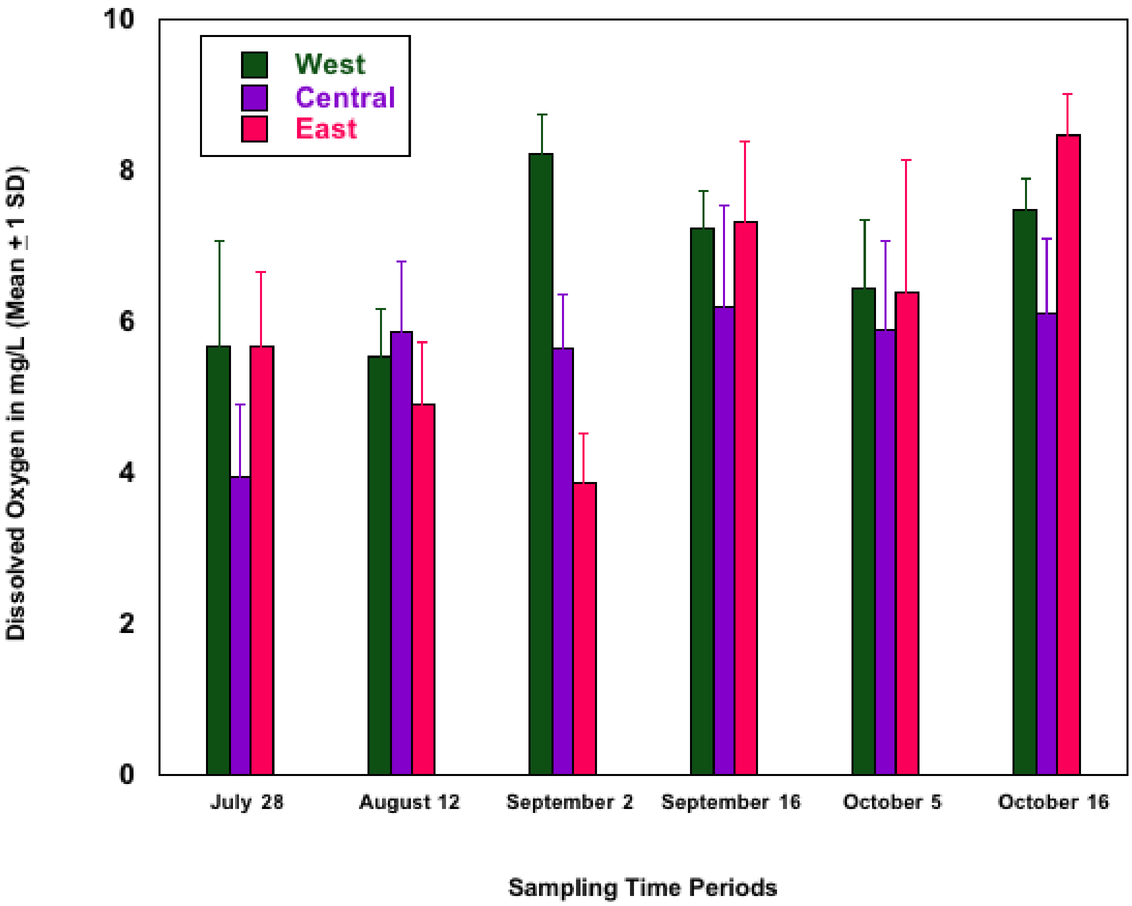


*Figure 13.* Temporal change in dissolved oxygen in each region

Note. Data from each reef was averaged by region and plotted as mean ± 1 Standard Deviation.

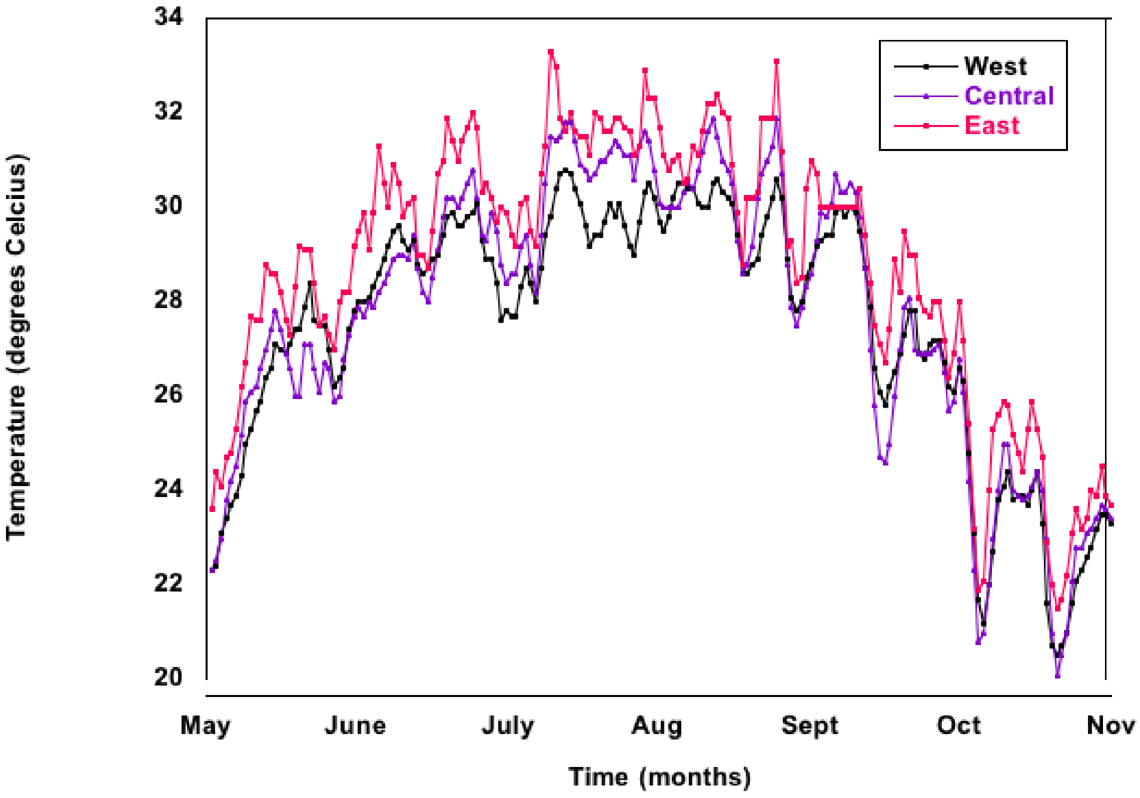


*Figure 14.* Temporal change in mean temperature from USGS sensor data.

Note. Temperature values were obtained from the USGS website: https://maps.waterdata.usgs.gov/mapper/index.html.

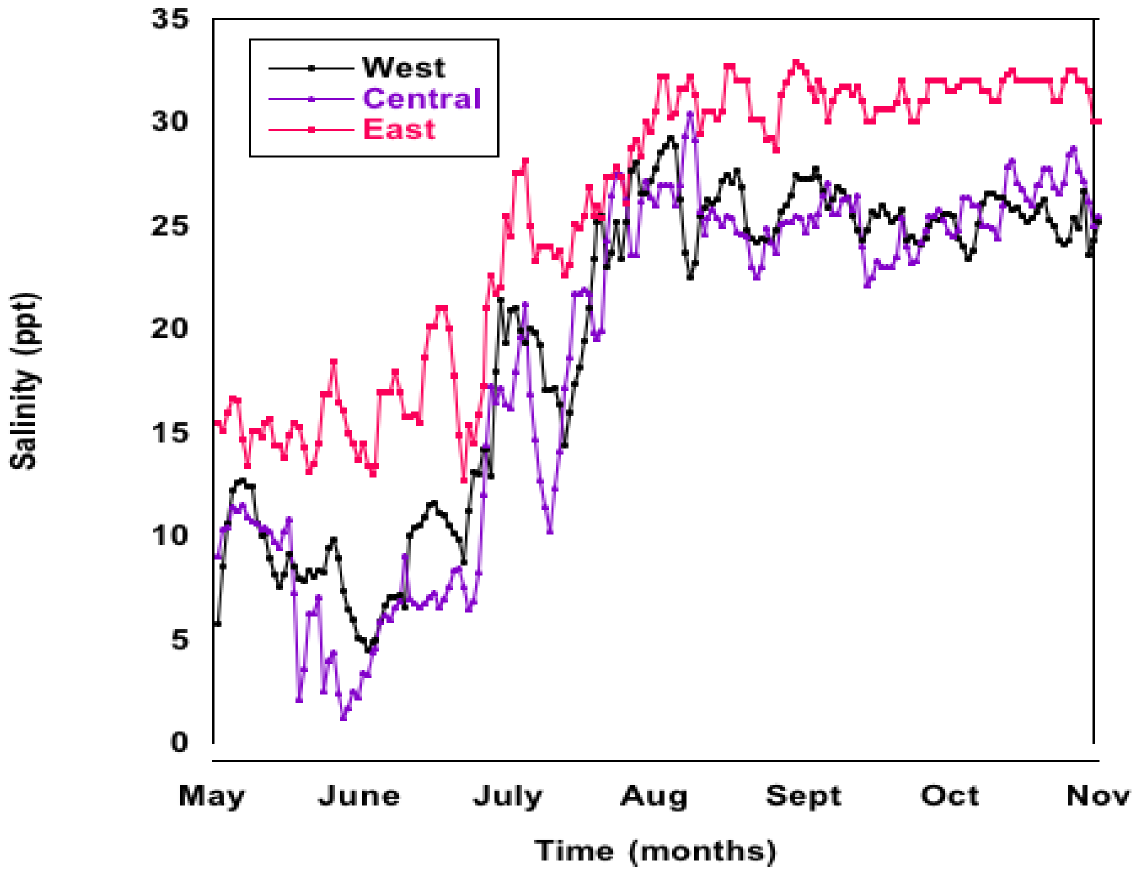


*Figure 15.* Temporal change in mean salinity from USGS sensor data.

Note. Data values were obtained from the USGS website: https://maps.waterdata.usgs.gov/mapper/index.html.

# CHAPTER III – EXAMINATION OF DIRECT PREDATION ON OYSTER SPAT BY SOUTHERN OYSTER DRILLS (*Stramonita haemastoma*) AND MUD CRABS (*Panopeus sp*.)

## Introduction

The ability of predators to structure food webs and the means by which they do so is well noted in the literature (Abrams 1982; Sih et al. 1985, 1998; Lima and Dill 1990; Bruno and O'Conner 2005; Grabowski et al. 2008). Apex predators directly affect lower trophic levels via consumption of prey or indirectly affect prey feeding behavior and the way they interact with other organisms (Carpenter et al. 1985; Strong 1992; Schmitz et al. 2004; Werner and Peacor 2003; Grabowski et al. 2008). Trophic cascades can ensue, involving alternating positive and negative effects of apex predators upon successively lower trophic levels (McQueen et al. 1989). Additionally, interactions between predator species at both upper and intermediate trophic levels can affect lower level prey (Soluk and Collins 1988; Wissinger and McGrady 1993; Morin 1995, Crowder et al. 1997; Eklov and Vankooten 2001). Keystone predators also enhance species diversity via consumption of dominant organisms (Paine 1966).

On oyster reefs in the nGOM, various types of organisms directly consume juvenile and adult oysters (Figure 16). The oyster drill (*Stramonita haemastoma)* is one such major predator of juvenile and adult oysters that has been studied. Brown (1997) documented the ability of Southern oyster drills to consume oysters less than 150 g (shell and tissue weight), and revealed a preference for smaller oysters by all sizes of oyster drills (24 to 60 mm shell length (SL)), regardless of which prey size was most energetically beneficial. However, prey value concerns tradeoffs between costs and benefits (McQueen et al. 1989). In contrast, Walker (2014) found that all sizes (15 to 42 mm SL) of the Atlantic oyster drill, *Urosalpinx cinera*, consumed all sizes of oysters (26 to 65 mm SL), but larger drills preferred larger oyster prey. Regardless of the specific predator-prey size ratios in Brown (1997) and Walker (2014), both studies demonstrated the ability of oyster drills to consume a large number of oysters in a short time period.

In addition to oyster drills, resident mud crabs (*Panopeus sp.*) and transient blue crabs (*Callinectes sapidus*) are capable of consuming large numbers of juvenile oysters on oyster reefs. Eggleston (1990) examined the predatory response of blue crabs to six different densities and three different sizes (15, 25, and 35 mm SL) of oysters, and he found blue crab predation depends upon the size and density of oysters available. His results also indicated blue crabs tend to be more selective when densities of oysters increased, and that regardless of oyster density, large blue crabs can completely consume all available oysters. Newell et al. (2007) compared the predation rates of four species of mud crabs and blue crabs on the Eastern oyster and the Suminoe oyster, *Crassostrea ariakensis*. Their results illustrated the ability of the crabs to predate on both species of oysters; however, there was significantly more predation by all five crab species on the Suminoe oyster versus the Eastern oyster, which is likely due to the shell strength of *C. ariakensis* being 64% as strong as *C. virginica* (Newell et al. 2007). In an effort to compare how crab type affects oyster survival, Hill and Weissburg (2013) tested the oyster consumption rate of mud crabs versus blue crabs, and in their mesocosm experiments mud crabs ate more oysters (10 to 20 mm shell length) than blue crabs. All of the above studies suggest that predatory responses of mud crabs and blue crabs can significantly impact oyster recruitment and survival; however, none of these studies focused on small spat (5 mm). The objectives of this chapter were: 1) examine the predatory responses of various sizes of oyster drills relative to different sizes of spat, and 2) examine the predatory relationship between various sizes of mud crabs and oyster spat.

## Methods

### *Collection of Organisms*

Organisms for manipulative experiments were obtained from sampling trays deployed during the field portion of this study (Chapter Two). Collected organisms included oyster drills (sizes 5 mm to 70 mm) and mud crabs (sizes 10 mm to 35 mm). All organisms were removed from the trays and placed into a labeled bucket containing seawater from the collection site and an aerator. Back at the laboratory each organism was identified, measured, counted, and placed into isolation within tanks containing only one species and then acclimated for a period of three days. All mud crabs were kept in individual containers to avoid cannibalism. Oyster spat were obtained from the University of Auburn Shellfish

Laboratory in Dauphin Island, AL to ensure the proper sizes and numbers of oyster spat were available for the experimental manipulations.

### *Mesocosm Experiments*

Aquariums (76.2 x 33 x 33 cm) were filled with 75 l filtered seawater from the northern Gulf of Mexico and amended to keep abiotic factors constant (salinity = 29 ppt, temperature = 25 ° C, twelve-hour light cycle). Heaters were placed in each tank to regulate the water temperature and LED aquarium hood lights were placed on a 12-hour light cycle using automatic timers. For each treatment combination, unless otherwise mentioned, there were a minimum of three replicates, a randomized layout, and blocking in time due to limited tank and resource availability. Factors and treatment levels for the various experiments are illustrated in Table 5. Organisms were not reused (i.e., subjected to more than one experimental block) in an effort to prevent the occurrence of learning behaviors and ensure independence.

### *Oyster Drill Predation Experiment in Fall 2015*

To examine the predatory responses of oyster drills on oyster spat, two factors were examined, oyster spat size and oyster drill size. A preliminary pilot study initially assessed the logistics of this experiment in terms of predator-prey size ratios and spat density. For this preliminary experiment, only one fully crossed block was completed due to the limited availability of oyster spat. Thus, results of this trial were analyzed using a Chi-Square test.

The predator-prey size ratio between oyster drill and oyster spat was the focus of the preliminary drill predation experiment. Three treatment levels of the spat size factor (5, 25, and 50 mm) were represented by ten individuals placed into tanks within a randomized treatment layout. The oyster drill-size factor also included three treatment levels (10, 30, and 65 mm). Selected sizes represented the size ranges of spat and drill in nature. One drill predator was placed randomly within each tank containing ten spat, for a total of nine treatment combinations of spat and drill sizes; each experimental unit initially contained ten spat and one oyster drill predator. The experiment was conducted for twelve days, with replacement of any predated spat occurring every 24 hours.  At the end of the experiment, the number of spat eaten each day was quantified for every experimental unit. A Chi-square analysis examined differences in whether different sizes of oyster drills could consume various sizes of oyster spat.

### *Oyster Drill Predation Experiment in Fall 2016*

Additional drill predation experiments were conducted to further examine feeding selectivity vis-à-vis the drill-spat predator-prey size ratio. The sizes of oyster drills and oyster spat differed slightly from those sizes used in 2015: the levels of the spat size factor were represented by 5, 15, and 25 mm and the levels of the drill size factor included 10, 30, and 50 mm. Each experimental unit contained ten spat and one oyster drill. Factors were fully crossed, experimental units randomized, and the experiment blocked in time four times. Each experimental block continued for ten days, with replacement of consumed spat occurring every 24 hours.

The Generalized Linear Model (GLM) procedure within SPSS (version 18), examined spat consumption by oyster drill within a Two-Way ANOVA comprising the two main factors, drill size and spat size, as well as their interaction, as fixed factors. Following overall significance, differences in marginal means were compared among levels of main factors and their interaction based on Least Significant Difference (LSD) tests, as specified by custom EMMEANS statements. Because the variance as tested by Levene's test was slightly heterogeneous, a two-way PERMANOVA using Manly's approach—Unrestricted Permutation of Observations in R was used to confirm effects of each factor and their interaction.

### *Mud Crab Predation Experiment*

Another trophic interaction for which the effect of predator-prey size ratio on feeding selectivity was examined involved the consumption of various sizes of oyster spat by different sizes of mud crabs. There were two treatment levels the spat size factor (5 mm and 25 mm) and three treatment levels the mud-crab size factor (small - 13-15 mm, medium - 21-23 mm, and large - 28-31 mm). Treatment combinations were fully crossed. Ten spat and one mud crab were placed within each randomly assigned experimental unit (spat were placed in the tank just prior to the mud crab). Tanks were checked every 12 hours, at which time predated spat shells were removed, recorded, and replaced with new oyster spat. Four experimental blocks were completed, with each block lasting five days.

The Generalized Linear Model (GLM) procedure within SPSS (version 18), examined spat consumption by mud crabs within a Two-Way ANOVA comprising the two main factors, drill size and mud crab size, as well as their interaction, as fixed factors. Following overall significance, differences in marginal means were compared among levels of main factors and their interaction based on Least Significant Difference (LSD) tests, as specified by custom EMMEANS statements. Because the variance as tested by Levene’s test was significantly heterogeneous, omnibus tests of main factors and their interaction were made with a two-way PERMANOVA using Manly’s approach—Unrestricted Permutation of Observations in R.

## Results

### *Oyster Drill Predation Experiment in Fall 2015*

The results of a Chi-square analysis revealed a significant difference in frequencies of size of oyster spat eaten across the drill sizes (Chi-Square = 85.73, df = 10, $p < 0.001$). The proportions of observations vary among drill sizes in association with spat size (Fig. 17). Small drills consumed small spat and large drills consumed large spat, while medium drills consumed both small and medium spat. Visual observations and video revealed multiple cases where large drills moved past small spat, as well as instances of small and medium drills unsuccessfully attempting to drill through large spat, illustrating potential costs underlying prey size preferences.

### *Oyster Drill Predation Experiment in Fall 2016*

Both main factors, drill size (F = 4.362, p = 0.025), and spat size (F = 6.173, p = 0.008), significantly related to the number of spat eaten per day. There was also a significant interaction effect between the two factors (F = 62.814, p < 0.001) (Table 6). In this experiment, all sizes of drills consumed all sizes of spat; however, prey size selectivity was evident with small drills primarily consuming small spat, medium drills eating medium spat, and large drills preferentially consuming large oyster spat. Medium drills ate significantly more spat than small drills (p = 0.007), but generally, all three drill sizes consumed comparable mean numbers of spat in ten days (5.167 ± 0.60 vs. 7.667 ± 0.60 vs. 6.250 ± 0.60, respectively). Small drills ate mostly 5mm spat (13.25 ± 1.493) compared to 15mm (0.75 ± 0.479) or 25mm spat (1.5 ± 0.289) (mean ± 1 standard error). Medium drills consumed primarily 15mm spat (13.75 ± 1.182), some 25mm spat (8.00 ± 1.291), and very few 5mm spat (1.25 ± 0.479). On average large drills consumed relatively few and the same number of both 5mm and 15mm spat (2.00 ± 1.155), but mostly 25mm spat (14.75 ± 1.109) (Figure 18). Overall, more 25mm spat were consumed than either 5mm (p = 0.005) or 15mm spat (p = 0.005). Pairwise interaction comparisons of marginal means showed small drills ate significantly more 5mm than 15mm or 25mm spat (p < 0.001), the consumption of 15mm versus 25mm spat did not differ (p = 0.614). Medium drills consumed significantly more 15mm spat than 5mm or 25mm spat (both $p < 0.001$), and they ate significantly more 25mm spat than 5mm spat ($p = 0.001$). Large drills ate significantly more 25mm spat than either 5mm or 15mm spat (both $p < 0.001$).

### *Mud Crab Predation Experiment*

Mud crabs also selected various sizes of oyster spat, depending on the predator-prey size ratio (Fig. 19). Once again, a PERMANOVA was run to confirm the significance of F-values, as Levene's Test of Equality of Variances was significant ($F = 8.601$, $p < 0.005$) for the conventional Two-Way ANOVA. Accordingly, both mud crab size ($F = 32.99$, $p < 0.001$) and spat size ($F = 20.57$, $p < 0.001$) factors were significant, as was the interaction between the two main factors ($F = 36.10$, $p < 0.0051$) (Table 7).

Overall there were significantly more 5mm spat eaten than 25mm spat ($p < 0.005$), and medium mud crabs ate significantly more spat than either small ($p < 0.005$) or large mud crabs ($p < 0.005$). Small mud crabs did not consume any 25mm spat in any of the experimental blocks; whereas they consumed 15.2 ± 4.188 (mean ± 1 standard error) 5mm spat in five days. Medium mud crabs primarily consumed 5mm spat (65.8 ± 9.074), but they were also able to predate on 25mm spat (16.0 ± 3.141). Large crabs preferentially consumed 25mm spat (21.6 ± 2.542); they only consumed 1.4 ± 0.872 (mean ± 1 s.e.) 5mm spat in the five-day experiment. Pairwise interaction comparisons of marginal means revealed medium mud crabs ate significantly more 5mm spat than both small or large mud crabs (both $p < 0.001$), and small mud crabs ate significantly more 5mm spat than large mud crabs ($p = 0.033$). Additionally, small mud crabs ate significantly fewer 25mm spat than medium ($p = 0.008$) or large mud crabs ($p = 0.001$), but there was no significant difference between medium and large mud crabs in the number of 25mm spat eaten ($p = 0.348$). Small and medium mud crabs consumed more small spat than large spat; whereas, large mud crabs consumed more large spat than small spat.

## Discussion

The experimental results helped elucidate trophic relationships occurring on oyster reefs by identifying key predator-prey size ratios and potential prey preferences displayed by predators. Although it has been well-noted that oyster drills are important predators of oysters on oyster reefs (Brown and Richardson 1988, Brown and Alexander 1994, Brown 1997), meaningful details regarding the prey-size preferences and feeding rates of different sizes of drills on different sizes of oysters are lacking. Brown (1997) indicated that large oysters (>150g whole oyster wet weight) were not consumed by even the largest drills (60mm shell length), and a preference was shown for small spat (21g) by all sizes of drill, regardless of whether larger spat would be more energetically favorable (i.e. in terms of cost/benefit analysis). Preferences by oyster drills for small spat were also supported by Butler (1985), Burrows and Hughes (1991), and Hughes et al. (1992). However, Walker (2014) reported all sizes of Atlantic oyster drill, *Urosalpinx cinera,* capably consumed all sizes of oysters, but larger drills preferred larger oysters. Although studies by Brown (1997) and Walker (2014) focused on two different species of oyster drills (Southern vs. Atlantic), conflicting data between these studies concerning preferences of oyster prey size by sizes of drills inspired this study. Although the present study focused more on oyster spat than large adult oysters, there was some overlap in both predator and prey sizes used in study and those studies conducted by Brown (1997) and Walker (2014). In the 2015 drill predation study, large drills (65mm) consumed few to no small (5mm) or medium (25mm) spat, but they actively preyed upon large (50 mm) spat. A similar trend emerged in the 2016 drill predation experiment, with large (55-60mm) drills eating few or no small (5mm) spat; however, in the 2016 experiment, large drills consumed considerable 25mm spat (i.e., roughly 15) while in 2015 large drills consumed very few 25mm spat. An additional observation included the potential display of optimal foraging by large oyster drills—on film large drills were shown preferentially passing by numerous small spat and only consuming medium and large spat. This could be an example of optimal foraging behavior because the drills have to expend a great deal of energy to drill through the spat shell and digest its inside contents; therefore, the energy gained from a small spat may not be worth the cost in terms of the energy required for handling time and consumption.

Although it is difficult to make direct comparisons between this study and Brown (1997) due to differences in sizes of drill and oysters used, the same general trend of all drill sizes preferring small spat was not supported by either of the two drill predation experiments conducted in the present study. This contradiction is likely due to the use of much larger oyster prey in Brown (1997) than the spat used the present study, and potentially the use of attached spat in Brown (1997) versus the unattached spat used in the present study. In contrast, the present study used a similar range of both predator and prey sizes as used by Walker (2014), and both of these studies observed larger drills preferentially prey upon larger spat, while capably consuming other sizes of spat when large spat are not available. This agreement indicates that prey-size preferences by the Atlantic oyster drill and Southern oyster drill may be similar; however, further studies investigating feeding generalities involving the two drill species are needed.

In addition to oyster drills, mud crabs can decimate oyster populations in nature by cutting off recruitment (Hill and Weissburg 2013). In this study, the abundant mud crab, *Panopeus sp.,* was the representative species used to examine predator-prey dynamics between mud crabs and oyster spat. All three sizes of mud crabs (ranging from 15 to 32mm CW) capably consumed small 5 mm spat; however, medium mud crabs (21-23mm) ate significantly more small spat than the other sizes of mud crabs. Although small mud crabs were apparently unable to consume 25mm spat, both medium and large mud crabs actively fed on 25mm spat. For both the mud crab predation experiment and the treatment in the predator combination experiment containing only mud crabs and spat, mud crabs actively predated on oyster spat and affected oyster spat survival. Other studies have documented the ability of mud crabs, as well as blue crabs, to predate on juvenile oysters. In mesocosm experiments, Hill and Weissburg (2013) demonstrated mud crabs ate significantly more oysters than blue crabs, suggesting intermediate predators like mud crabs may exhibit more control over oyster recruitment and juvenile survival than large apex predators (Hill and Weissburg 2013). In accordance with Hill and Weissburg (2013), the present study demonstrated the ability of mud crabs ≥ 25mm to consume spat ranging in size from 10 to 20mm, the size range of oyster prey used by Hill and Weissburg (2013).

Various other studies that have examined mud crab and blue crab predation on oysters. Upon comparing the predatory response of blue crabs relative to six densities and three sizes of oysters, Eggleston (1990) showed how blue crab predation heavily and jointly depends upon both prey size and density. Blue crabs were more size-selective of prey at higher oyster densities. Eggleston also discovered that large blue crabs completely decimated available oysters, regardless of oyster density. This finding supports the idea that blue crabs can be major predators of young oysters in nature. Similarly, Newell et al. (2007) documented substantial predation by blue crabs and four species of mud crabs on both the Eastern and the Suminoe oyster, *Crassostrea ariakensis.* Additionally, O’Connor et al. (2008) documented negative effects of mud crabs and blue crabs on oyster recruitment. These three former studies agree with the present study in that they all show mud crabs and blue crabs readily consume juvenile oysters. However, one unique aspect of this dissertation study is the use of 5 mm spat as a prey size. Former studies used 10mm oysters (e.g. Hill and Weissburg 2013) and 15mm oysters (e.g. Eggleston 1990); however, all these studies neglected to focus on smaller 5mm spat. The survival of small 5mm spat are is vital to overall oyster recruitment. While the sizes of crab predators and juvenile oysters used in the present and former studies varied somewhat, they all support the notion that mud crabs and blue crabs are significant predators of young oysters.

Overall the experiments conducted in this chapter provided insight that prey size selectivity by drill and mud crabs disproportionately affects survival of spat sizes. Study results revealed predator-prey size ratios and potential prey preferences shown by predators, as well as the potential trophic cascade that could possibly result from the removal of top predators from oyster reef ecosystems. Additionally, this study was the first to use predation of oyster spat as small as 5 mm, which is an important size for spat as they are recruiting to the seed stage.

Table 5

*Experimental factors and treatments for direct predation experiments.*

| **Experiment** | **Factor 1** | **Levels** | **Factor 2** | **Levels** | **Duration** |
|---|---|---|---|---|---|
| Drill predation 2015 | Spat size | 5 mm | Drill size | 10 mm | 12 days |
| | | 25 mm | | 30 mm | |
| | | 50 mm | | 65 mm | |
| Drill predation 2016 | Spat size | 5 mm | Drill size | 10 mm | 10 days |
| | | 15 mm | | 30 mm | |
| | | 25 mm | | 50 mm | |
| Mud crab predation | Spat size | 5 mm | Crab size | 13-16 mm | 5 days |
| | | 25 mm | | 21-23 mm | |
| | | | | 28-31 mm | |

Table 6

*PERMANOVA values from oyster drill predation experiment in Fall 2016.*

| **Factor** | **df** | **F-value** | **P-value** |
|---|---|---|---|
| Drill Size | 2 | 4.36 | 0.024 |
| Spat Size | 2 | 6.17 | 0.008 |
| Drill Size x Spat Size | 4 | 62.81 | < 0.001 |

Table 7

*PERMANOVA values from mud crab predation experiment.*

| **Factor** | **df** | **F-value** | **P-value** |
|---|---|---|---|
| MC Size | 2 | 32.99 | < 0.001 |
| Spat Size | 1 | 20.57 | < 0.001 |
| MC Size x Spat Size | 2 | 36.10 | < 0.001 |

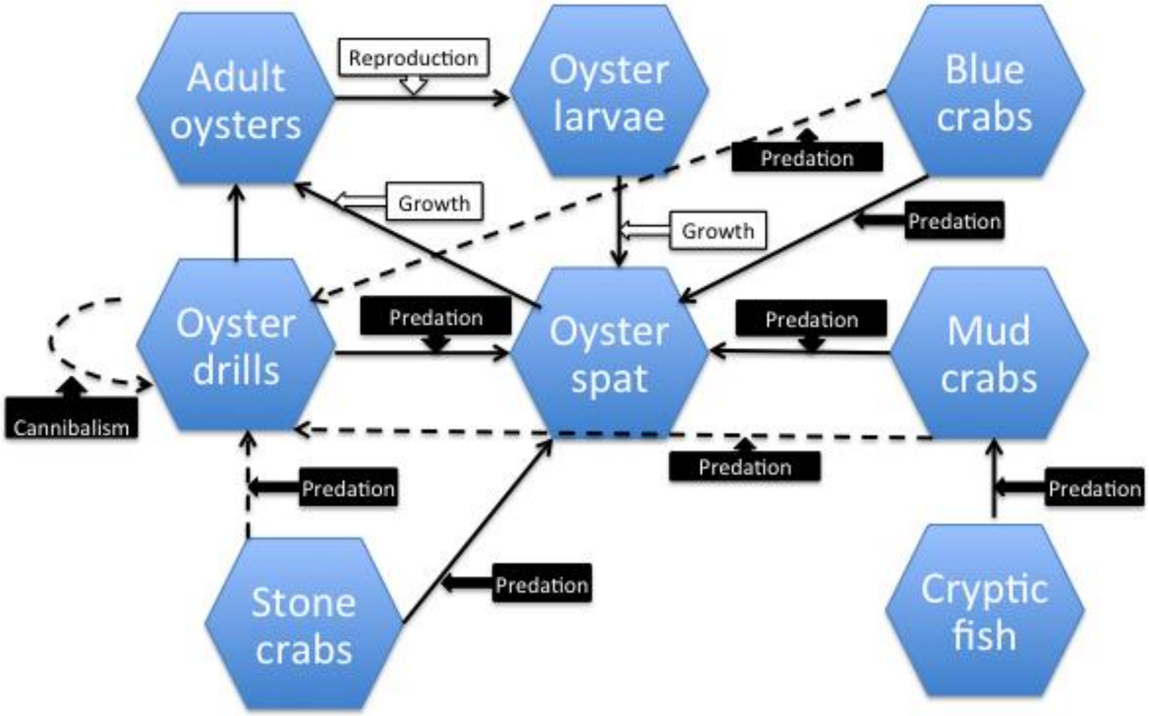


*Figure 16.* Diagram of trophic relationships on oyster reefs.

Note. Blue hexagons represent each organism and solid arrows show relationships between them, while dashed arrows show hypothetical relationships between organisms.

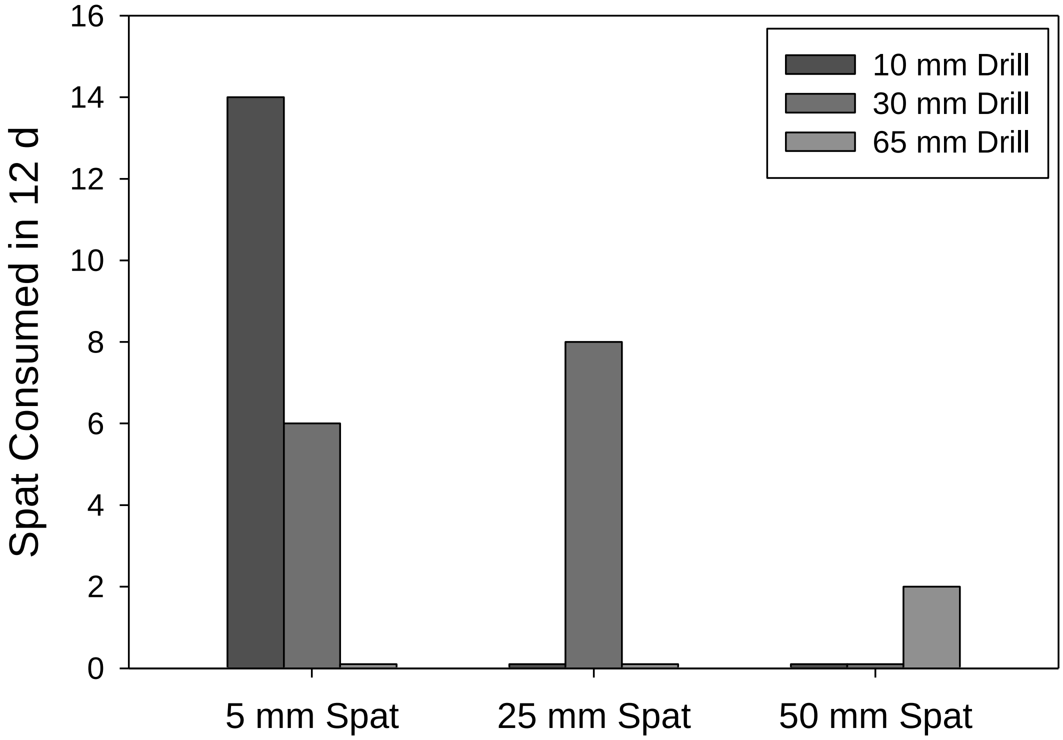


*Figure 17.* Oyster drill predation experiment results from 2015.

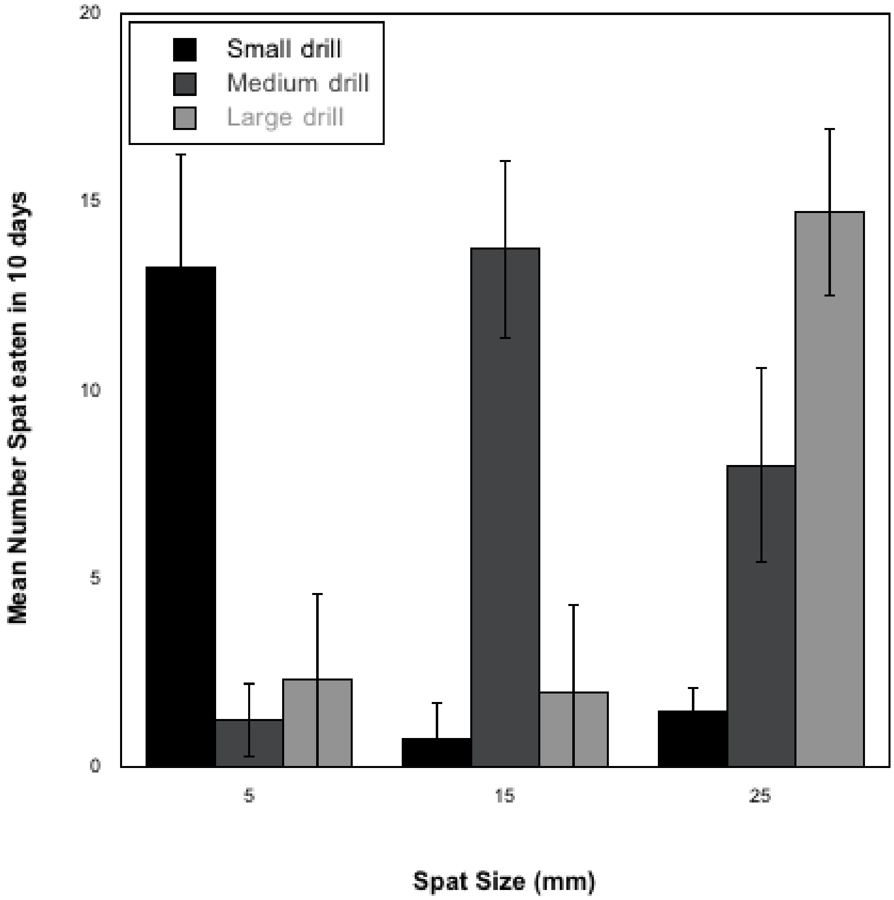


*Figure 18.* Oyster drill predation experiment results from 2016.

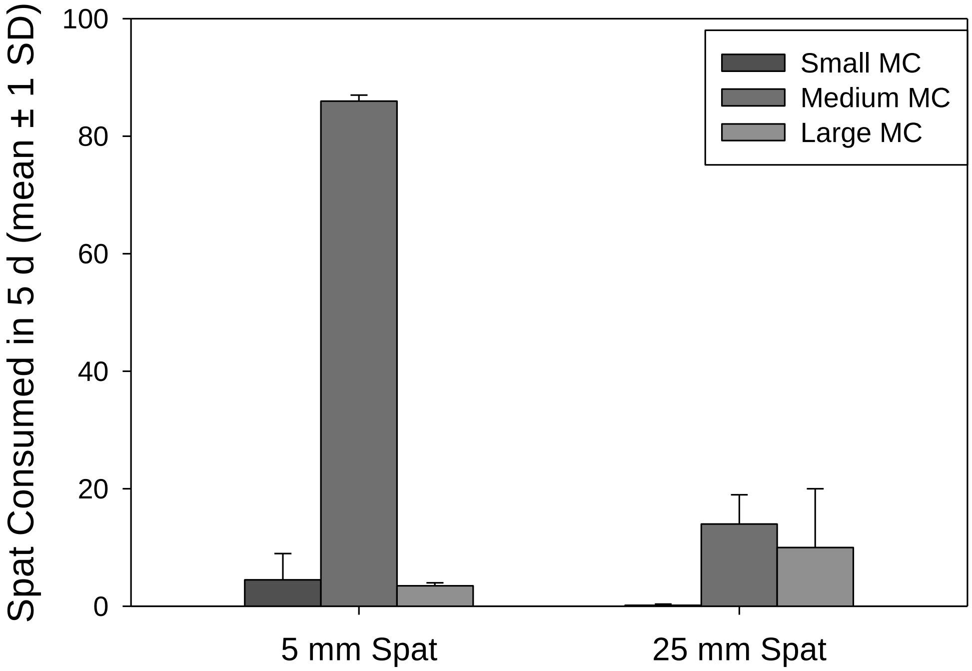


*Figure 19.* Mud crab predation experiment results.

# CHAPTER IV – EFFECTS OF INDIRECT PREDATION AND HABITAT COMPLEXITY ON TROPHIC RELATIONSHIPS ON OYSTER REEFS

## Introduction

Often a top predator (e.g. toadfish) can affect the behavior of an intermediate predator (e.g. mud crabs), in turn affecting various prey via trait-mediated indirect interactions (TMIIs). There are many types of indirect interactions, and studies often fail to separate the difference between density-mediated indirect interactions (DMIIs) and TMIIs (Werner and Peacor 2003). A trait-mediated indirect interaction is a result of a prey changing its behavior in response to the addition or removal of its predator (Schmitz et al. 2004). Such TMIIs can have a major bearing on trophic dynamics, potentially leading to a trophic cascade (Schmitt 1987; Wootton 1993). Conversely, a predator can change prey behavior in order to alter the likelihood of it being eaten (Morin 1995; Crowder et al. 1997). An example of this kind of TMII effect is seen on oyster reefs, where the oyster toadfish (*Opsanus tau*) primarily consumes mud crabs, which forage on juvenile oysters (Menzel and Nichy 1958; Wilson et al. 1982). A mesocosm manipulation by Grabowski et al. (2008) demonstrated that the presence of oyster toadfish benefited oysters by depressing mud crab and blue crab foraging on spat; therefore, the toadfish indirectly aided oyster recruitment whether it was in the presence of just one, or both blue and mud crabs. Ecological communities may comprise multiple TMIIs, which can considerably affect species interactions and thereby the trophic dynamics of the entire system (Werner and Peacor 2003).

Habitat complexity can mediate interaction effects involving predators, thereby altering trophic cascades (Murdoch and Oaten 1975; Crowder and Cooper 1982). Increasing habitat complexity generally creates additional refuge, which can alter trophic dynamics by decreasing predator efficiency (Pace et al. 1999; Littler et al. 1989; Diehl 1992; Schriver et al. 1995; Beukers and Jones 1997). Some past studies have shown lower encounter frequencies with predators in complex habitats, leading to less interference interactions between predators and weaker predator effectiveness (Swisher et al. 1998; Grabowski and Powers 2004; Griffen and Byers 2006; Hughes and Grabowski 2006). Other studies have shown that higher predator density as well as the occurrence of multiple types of predators can translate into increased predation in more structurally complex habitats (Grabowski and Powers 2004; Hughes and Grabowski 2006).

Oyster reefs are a useful model system conducive for mesocosm studies of habitat complexity because they have vertical relief, a faunal assemblage that is relatively easy to manipulate, and variable habitat complexity in the natural setting (Lenihan 1999; Lenihan et al. 2001). An experiment using common oyster reef organisms by Grabowski (2004) found that increasing habitat complexity decreased the strength of trophic interactions, had no effect on avoidance behavior, and led to increased oyster survival. Humphries et al.

(2011a) tested the effect of habitat complexity on the survival of grass shrimp (*Palaemonetes pugio*) prey in the presence of red drum (*Sciaenops occellatus*). They found decreasing predation with increasing habitat complexity, but a threshold occurred at which increasing complexity did not depress predation any further (Humphries et al. 2011a). However, another study documented an increase in oyster predation by blue crabs and mud crabs when the two species of crabs were together, as a result of interference competition in conjunction with habitat complexity (Grabowski et al. 2008). Hughes and Grabowski (2006) found a similar effect in their study of predation by stone crabs (*Menippe mercenaria*) and knobbed whelks (*Busycon carica*) on hard clams (*Mercenaria mercenaria*) and the ribbed mussel (*Geukensia demissa*) in two types of habitat (oyster reef and sand). When the two types of predators were isolated, less predation occurred in the sand habitat; however, when the two types of predators were together, the overall extent of predation did not differ between sand and oyster reef habitats. These results suggest habitat complexity was less important when the two predators occurred together as a result of interference competition occurring between the stone crabs and knobbed whelks. Furthermore, Humphries et al. (2011b) found that reef habitat complexity had no bearing on nekton abundance or diversity. These conflicting results necessitate further examination of critical trophic interactions relative to oyster reef complexity to understand oyster vulnerability to predation better in order to inform habitat restoration efforts. The objectives of this chapter were: 1) examine how various combinations of predators affect the survival of oyster spat within the context of alternative substrate types and 2) consider the potential role of trait-mediated intermediate interactions (TMIIs) involving two top resident predators within the trophic web of oyster reefs.

## Methods

### *Effect of Various Predator Combinations on Oyster Spat Survival Relative to Substrate Type*

This experiment involved multiple members of the oyster reef community to examine trophic interactions and oyster spat survival within the context of substrate type. Substrate type was manipulated by using either shell cultch, obtained from the same processing plant as the substrate used in tray samplers, or #57 limestone, a source of limestone commonly used for oyster reef restoration projects. Both substrate types were soaked and cleaned to remove excess dust and any potential organisms or debris before use. Experimental units consisted of small mesocosms (35 x 19 x 13 cm tubs) filled with 200 l 0.5 mm filtered MS Sound water (29 ppt). Each mesocosm contained 30 spat (fifteen 15 mm and fifteen 30 mm) randomly placed on top of the bottom substrate, one hour prior to the introduction of other organisms. Heaters kept the water at 25°C, aerators provided water circulation and adequate oxygen, and a handheld YSI model #85/25 monitored abiotic factors. Sizes of other types of organisms were roughly: mud crabs (MC) = 23mm CW, stone crabs (SC) = 60mm CW, and toadfish (TF) = 120mm total length. The sizes of these organisms were the most commonly recorded sizes in the tray sampling portion of this study (Chapter II), and the number of spat and each organism was determined by conducting pilot studies to find the ratio of predators to prey that resulted in at least one prey organism of each species remaining at the end of 24 hours. The goal was to ensure the predators were never prey-limited and had access to at least one prey of each species at all times during the experiment. Four replicates were obtained for each of the following different presence/absence treatment combinations:

1. Shell substrate: 30 spat (control)
2. Shell substrate: 30 spat, 7 MC
3. Shell substrate: 30 spat, 7 MC, 1 TF
4. Shell substrate: 30 spat, 7 MC, 1 SC
5. Shell substrate: 30 spat, 7 MC, 1 TF, 1 SC
6. Shell substrate: 30 spat, 1 SC
7. Shell substrate: 30 spat, 1 SC, 1 TF
8. Limestone substrate: 30 spat (control)
9. Limestone substrate: 30 spat, 7 MC
10. Limestone substrate: 30 spat, 7 MC, 1 TF
11. Limestone substrate: 30 spat, 7 MC, 1 SC
12. Limestone substrate: 30 spat, 7 MC, 1 TF, 1 SC
13. Limestone substrate: 30 spat, 1 SC
14. Limestone substrate: 30 spat, 1 SC, 1 TF

Again, the use of 30 spat included 15 of each of the two size classes. The layout of each experimental block was randomized, and blocks continued for 24 hours, after which numbers of predated small spat (15mm spat), large spat (30mm), and mud crabs were recorded.

Differences in the number of spat consumed per hour were compared among treatments using the linear mixed models (LMM) procedure in SPSS (version 18). Main fixed factors included substrate type (2 levels), Stone crab presence (2 levels), Toadfish presence (2 levels), and Mud crab presence (2 levels), as well as six two-way interactions and five three-way interactions among the four main factors. An intercept was also included. Spat size (2 levels) served as a repeated effect for which the Diagonal covariance structure was used. Experimental units (mesocosms) served as subjects.

### *Trait-mediated Indirect Interactions (TMIIs)*

TMII experiments helped determine the effects of indirect interactions on major predator behavior on oyster reefs. In order to prevent direct predation from occurring while still exposing prey sensory perceptions of the predator, cylindrical wire mesh cages (6 cm diameter x 12 cm tall) contained the predator and precluded any physical interactions. Aquariums (76.2 x 33 x 33 cm) were filled with 75 l filtered seawater from the northern Gulf of Mexico and amended to keep abiotic factors constant (salinity = 29 ppt, temperature = 25 ° C, twelve-hour light cycle). Heaters were placed in each tank to regulate the water temperature and LED aquarium hood lights were placed on a 12-hour light cycle using automatic timers. Additional treatments tested for a cage effect, for which results were non-significant relative to all other treatments. Factors and treatment levels for the various experiments are illustrated in Table 8. Organisms were not reused (i.e., subjected to more than one experimental block) in an effort to prevent the occurrence of learning behaviors and ensure independence. TMII experiments focused on the effects of two resident top predators, Stone crab and toadfish, on mud crab behavior in terms of oyster spat predation.

### *Trait-mediated Effect of Stone Crabs on Mud Crab Predation of Oyster Spat*

The goal of this experiment was to determine if *Menippe sp.* indirectly alleviated oyster spat survival by mediating the predation behavior of *Panopeus sp.* In each aquarium, ten 15mm oyster spat were randomly distributed just prior to mud crab introduction. The three sizes of mud crabs were: small (13-15mm), medium (21-23mm), and large (28-31mm). One mud crab belonging to one of the three size classes was randomly assigned to each experimental tank. Each of the four blocks also included three units with empty cages, and a mud crab for each size class which did not face any threat of predation pressure (i.e., empty cage). For each of the four blocks, three other treatment combinations contained ten spat and one mud crab of each size class; however, one *Menippe sp.* (approximately 65mm carapace width) was housed within the cages of these units. The duration of this experiment was 24 hours, after which the number of predated spat was recorded.

The Generalized Linear Model (GLM) procedure within SPSS (version 18), examined spat consumed per hour by mud crabs within a Two-Way ANOVA comprising the two main factors, Stone crab presence and mud crab size, as well as their interaction. Following overall significance, differences in marginal means were compared among levels of main factors and their interaction based on Least Significant Difference (LSD) tests, as specified by custom EMMEANS statements. Because the variance as tested by Levene's test was somewhat heterogeneous (F = 3.348; p = 0.026)), results of omnibus tests of main factors and their interaction were confirmed with a two-way PERMANOVA using Manly's approach—Unrestricted Permutation of Observations in R.

### *Trait-mediated Effect of Toadfish on Mud Crab Predation of Oyster Spat*

Like for the stone crab experiment, this purpose of the Toadfish TMII experiment was to determine the effects of indirect interactions on predation behavior at oyster reefs. However, *Opsanus beta* was used as the caged predator. Once again, ten 15mm oyster spat were randomly distributed just prior to mud crab introduction and one mud crab belonging to one of the three designated size classes as defined before was randomly assigned to each experimental tank. Cages for the Toadfish present treatment level contained single toadfish (approximately 120mm TL), whereas cages were empty for the Toadfish absent treatment level. Treatment combinations were fully crossed and randomized for each block, which was replicated four times.

The Generalized Linear Model (GLM) procedure within SPSS (version 18), examined spat consumed per hour by mud crabs within a Two-Way ANOVA comprising the two main factors, toadfish presence and mud crab size, as well as their interaction. Following overall significance, differences in marginal means were compared among levels of main factors and their interaction based on Least Significant Difference (LSD) tests, as specified by custom EMMEANS statements. Because the variance as tested by Levene's test was significantly heterogeneous ($F = 9.914$; $p < 0.0001$), results of omnibus tests of main factors and their interaction were based on a two-way PERMANOVA using Manly's approach—Unrestricted Permutation of Observations in R.

### *Trait-mediated effect of Toadfish on Mud Crab versus Blue Crab Spat Predation*

Given that larger crabs are known to cannibalize smaller crab individuals, another TMII experiment was performed for which three small mud crabs (approximately 14-15mm) were housed along with one large mud crab (28-30 mm), and three small blue crabs (15mm) were housed along with and one larger blue crab (30 mm). This combination of crab treatment levels was repeated twice for each block, including complete sets either with or without caged toadfish (120 mm TL). Ten spat (8-10mm) were randomly placed in each tank, and the experiment continued for 24 hours, with spat replacement occurring at 12 hours. Four experimental blocks were completed.

The Generalized Linear Model (GLM) procedure within SPSS (version 18), examined spat consumed per hour by mud crabs and blue crabs within a Two-Way ANOVA comprising the two main factors, toadfish presence and mud crab vs. blue crab, as well as their interaction. Because the variance as tested by Levene's test was homogeneous ($F = 0.458$; $p = 0.717$), a two-way PERMANOVA was not needed to confirm omnibus tests from the ANOVA.

### *Synthesis*

The synthesis of information from the forgoing experiments helped to obtain a better understanding of direct and indirect interactions occurring at oyster reefs in the nGOM.  Using this information, a more detailed picture of trophic relationships helped to depict key interactions and their strengths among key oyster reef organisms potentially affecting oyster survival, as well as positive and/or negative effects of each species.

## Results

### *Effect of Substrate with Mixed Predators*

In the LMM analysis of predator combination mesocosm experiments, substrate significantly affected the survival of spat (F = 6.993, p = 0.009). Numbers of both large and small spat consumed were higher in the limestone substrate compared to the shell substrate (Fig. 20). In addition, there was a significant interaction between substrate type and stone crab presence (F = 6.274, p = 0.013). Perhaps as a reflection of this interaction, intermediate-level predators (mud crabs) also survived better in shell versus limestone substrate (1.667 + 1.667 mud crabs consumed on average in shell versus 12.333 + 4.055 mud crabs consumed in limestone).

### *Effects of Various Predator Combinations*

Mixed model results of predator combination mesocosm experiments revealed significant effects of mud crab presence (F = 67.190, p < 0.001), stone crab presence (F = 76.719, p < 0.001), and toadfish presence (F = 28.738, p < 0.001) on the survival of oyster spat (Table 9). On average, mud crabs while in the absence of their predators consumed a large number of spat in both shell (17.8 ± 2.48) and limestone (19.8 ± 4.99) substrates. However, in the presence of stone crabs, mud crabs consumed fewer spat in shell (13 ± 2.39) and limestone (15.2 ± 4.81). When toadfish were present, mud crabs consumed particularly low numbers of spat in shell (6.2 ± 1.93) and limestone (1.2 ± 0.97).

Ironically, spat predation averaged higher in the presence of all three types of predators (Fig. 20) in shell (14.2 ± 4.16) and limestone (18.2 ± 4.5). Additionally, significant interaction effects occurred between stone crab and toadfish presence (F = 45.582, p < 0.001), as well as between stone crab and mud crab presence (F = 43.732, p < 0.001).

### *Trait-mediated Effect of Stone Crabs on Mud Crab Predation of Oyster Spat*

The presence of caged stone crab appeared to reduce the rate of consumption of spat by mud crabs (Figure 21). A PERMANOVA was run to confirm the significance of F-values based on a conventional Two-Way ANOVA, as Levene's Test of Equality of Variances was mildly significant (F = 3.348, p = 0.026). Both stone crab presence (F = 78.913, p < 0.001) and mud crab size (F = 25.591, p < 0.001) factors significantly affected the survival of spat (Table 10). In addition, a significant interaction occurred between these two main factors (F = 12.548, p < 0.001).

Overall, small mud crabs ate significantly fewer spat per hour than either medium (p = 0.001) or large mud crabs (p < 0.001), and large mud crabs consumed significantly more spat per hour than medium mud crabs (p = 0.008). In the presence of a caged stone crab, all sizes of mud crabs consumed fewer spat per hour: small (0.00 + 0 vs. 0.042 + 0.030), medium (0.010 + 0.010 vs. 0.250+ 0.038), and large (0.063 + 0.024 vs. 0.354 + 0.021). Pairwise interaction comparisons revealed that when the stone crabs were present, there were no significant differences among the three mud crab sizes in the number of spat eaten per hour (P = 0.111 – 0.783). However, when the stone crabs were absent, small mud crabs ate significantly fewer spat than medium and large mud crabs (p < 0.001), and medium mud crabs ate significantly fewer spat per hour than large mud crabs (p = 0.012).

### *Trait-mediated Effect of Toadfish on Mud Crab Predation of Oyster Spat*

The presence of caged toadfish (F = 51.07, p < 0.005) significantly affected spat survival (Fig. 22), as confirmed by a PERMANOVA. However, PERMANOVA also confirmed that the mud crab size factor was marginally non-significant (F = 3.49, p = 0.052), and the interaction between the toadfish presence and mud crab size factors was non-significant (F = 2.04, p = 0.159) (Table 11).

Consumption of spat by all sizes of mud crabs was strongly suppressed by the presence of toadfish. In the presence of toadfish, both small and medium mud crabs did not consume any spat, and large mud crabs barely consumed any spat (0.021 ± 0.012). By comparison, when the toadfish were absent, small mud crabs consumed0.115 ± 0.039, medium crabs consumed0.198 ± 0.031, and large mud crabs consumed 0.260 ± 0.057 spat per hour (mean ± se). Overall, large mud crabs ate significantly more spat than small mud crabs (p = 0.017); however, spat consumption rates did not differ between small and medium mud crabs (p = 0.203) or between medium and large mud crabs (p = 0.203).

### *Trait-mediated Effect of Toadfish on Mud Crab versus Blue Crab Spat Predation*

The presence of the caged toadfish significantly reduced oyster spat predation by both mud crabs and blue crabs (F = 14.663, p = 0.002) (Fig. 23). However, neither the crab type factor (mud vs. blue) (F = 0.200, p = 0.662) or the interaction of stonefish present and crab type factors were significant (F = 1.091, p = 0.317) (Table 12). Thus, toadfish presence suppressed the consumption of spat equally between blue crabs and mud crabs. Blue crabs ate more spat per hour in the absence of toadfish (0.635 ± 0.101) than when the caged toadfish were present (0.344 ± 0.087). Similarly, mud crabs consumed more spat per hour in the absence of caged toadfish (0.700 ± 0.135 vs. 0.188 ± 0.088).

## Discussion

The predator combination experiments in this dissertation study revealed how different assemblages of predators can change oyster spat survival patterns. O’Connor et al. (2008) studied the effect of predator combinations involving blue crabs, stone crabs, and mud crabs on juvenile oyster survival and recruitment in experimental plots at Hoop Pole Creek, North Carolina. Their results were similar to the present study, as both studies demonstrated negative separate effects of stone crabs and mud crabs, as well as different effects on oyster survival when the two crabs are together. Additionally, O’Connor et al. (2008) specified how the type of crab mattered, and how negative effects of mud crabs on oyster recruitment can be as dramatic as effects of blue or stone crabs. The present study also supports this, because the treatment containing just mud crabs and spat showed as low or lower survival of spat than the treatment combination containing just stone crabs and spat. However, the present study is unique in that it focused on the use of both spat (< 25mm) and seed (26-50mm) oysters, while the study conducted by O'Connor et al. (2008) only included juvenile oysters < 20 mm, and this study included the Gulf toadfish, a well-known keystone predator on oyster reefs in the Gulf of Mexico.

A tacit expectation of the predator combination experiment was that the concurrence of all predator types (mud crabs and stone crabs) would culminate in the lowest oyster spat survival, because both mud crabs and stone crabs can consume large quantities of spat. However, this was not the case because treatments containing only mud crabs exhibited higher spat predation than the treatment combining both mud crabs and stone crabs. In addition, the treatment combining all three predators (mud crabs, stone crabs, and toadfish) also resulted in less spat consumed per hour compared to the treatment containing only mud crabs. One explanation for this finding is that when all three predators are together, the toadfish predates on the mud crabs; whereas the stone crab focuses its predation efforts on oyster spat. The toadfish/mud crab treatment combination showed such low spat predation because toadfish suppress mud crab predation, while not consuming oyster spat. Accordingly, the stone crab/mud crab treatment exhibited less spat consumption than the three-predator treatment combination, because stone crabs can consume both spat and mud crabs. Another possible interpretation is the suppression of movement by mud crabs caused by toadfish within the mesocosms reduced the encounters of mud crabs as prey for stone crabs, which consequently had to rely on oyster spat for prey. Grabowski et al. (2008) reported a similar interaction between toadfish, mud crabs, and blue crabs. When toadfish were absent, blue crabs significantly reduced the number of mud crabs; however, the mortality of mud crabs paradoxically decreased in the presence of toadfish. The authors postulated this result reflected refuge seeking by mud crabs when toadfish were present (Grabowski et al. 2008). Because stone crabs are more prevalent residents on reefs than blue crabs in the nGOM (pers. obsv.), the present study focused on stone crabs as predators. The resulting different predator combination makes this study distinct from that of Grabowski et al. (2008). The findings from the present study allude to a potential trophic cascade that could occur on oyster reefs. If toadfish decrease in abundance, then mud crab density will likely increase and potentially lead to decreased recruitment of juvenile oysters to the adult population. Often when monitoring oyster production, only abiotic factors and substrate availability are taken into consideration; however, the findings from this study suggest trophic dynamics can also play a key role in mediating oyster survival and recruitment.

Whereas the aforementioned predator-combination experiments were conducted in a laboratory setting, Abbe and Breitburg (1992) conducted a field study in the Chesapeake Bay to examine how common oyster reef predators (mud crabs, blue crabs, and oyster toadfish) affected the survival of oyster spat.

They used mesh cages to prevent blue crabs and toadfish from eating mud crabs and oyster spat in their treatments. As with the present study, they found a negative relationship between mud crab abundance and spat survival. Additionally, the lowest spat survival rate occurred in the presence of solely blue crabs, and there was no effect on oyster spat survival in the presence of solely toadfish. Because toadfish apparently do not consume oyster spat, the present study did not include a predator treatment with solely toadfish and spat. Further studies need to examine the predator-prey relationships to gain a better understanding of oyster reef trophic dynamics, especially in a natural setting.

Another aspect of the predator combination experiment was to compare the survival of oyster spat in oyster shell cultch versus limestone substrate. Previous studies have examined the suitability of limestone for attracting spat, and found that the calcium component in the substrate matters (Hidu et al. 1974) and calcium carbonate materials attract more spat than river gravel (Soniat et al. 1991). This goal of the present study focused on how two calcium carbonate based substrates mediate predator-prey interactions relative to oyster spat survival. Oyster-shell cultch substrate presumably exhibits greater habitat complexity than limestone gravel, due to the three-dimensional matrix it forms, including many crevices between shells that provide refuge for prey items. The results of the present study demonstrated that spat survived better in oyster shell than in limestone substrate, likely reflecting the positive effects of a more complex habitat in terms of lower predation. Other laboratory mesocosm studies have also looked at ramifications of oyster habitat complexity; Hill and Weissburg (2013) showed that higher habitat complexity negatively influenced blue crab predation on mud crabs, and Humphries et al. (2011a) demonstrated decreasing predation by red drum on grass shrimp with increasing habitat complexity. However, other studies have shown a more complex effect of habitat complexity, in that it depends on prey density (Grabowski and Powers 2004) and the degree of interference competition between predators (Grabowski and Powers 2004; Hughes and Grabowski 2006). Although mesocosm experiments in the present study did not focus on varying densities of predators; treatment combinations involved predators in isolation with spat, as well as every possible combination of other predators to help determine the role of potential interference interactions on oyster survival between the two substrates.

In both of the mud crab TMII experiments, the presence of a caged predator (stone crab or toadfish) significantly enhanced the survival of oyster spat. Many experiments focus solely on how intermediate predators directly affect the survival of a basal resource, for example the predator combination experiment in the present study. However, TMII experiments like those in the present study help elucidate effects of indirect interactions on trophic dynamics. Presumably, chemical or other sensory perceptions of the caged predator by mud crabs influenced their behavior such that their predatory response was reduced, which in turn increased oyster spat survival. Similarly, Turner et al. (2000) showed that the freshwater snail (*Physella gyrina*) shifted its habitat use in response to two caged predators: molluscivorous pumpkinseed sunfish (*Lepomis gibbosus*) and crayfish (*Orconectes rusticus*), an effect which cascaded by enhancing periphyton abundance. Like in the present study, snail density was the same for each treatment used by Turner et al (2000), in order to ensure observed indirect effects were trait-mediated and not density-mediated (DMII). Additionally, using laboratory mesocosm manipulations, Trussell et al. (2006) found that the strength of TMIIs was significant and equal to or greater than DMII strength for the interaction involving a predatory crab (*Carcinus maenas*) and an intermediate consumer snail (*Nucella lapillus*) in the rocky shore habitat. Because the snail spent more time in refuge habitat, it spent less time predating on the more basal barnacle (*Semibalanus balanoides*). Such recognition of the two types of indirect effects is rarely distinguished in experiments (Werner and Peacor 2003); but the design of the present study enables such a separation and distinguishes it because densities were not altered.

Previous laboratory studies also demonstrated the importance of TMIIs on the trophic dynamics of oyster reefs (Grabowski 2004, Grabowski and Kimbro, 2005, and Grabowski et al 2008). While Grabowski (2004) and Grabowski and Kimbro (2005) examined the effect of toadfish presence on mud crab predation, the effects of both blue crab and toadfish presence were investigated by Grabowski et al. (2008). However, unlike the present study neither of these studies focused on the effect of stone crab presence on mud crab predation, which adds to the existing knowledge of trophic dynamics on oyster reefs because stone crabs are key resident predators on oyster reefs in the Gulf of Mexico that are capable of consuming two dominant predators of juvenile oysters, mud crabs and oyster drills.

Although laboratory experiments cannot accurately portray interactions occurring in the wild, they do facilitate testing of specific hypotheses and mechanisms. It is impossible to control for every extraneous variable that may affect experimental results, especially in the field where few variables can be manipulated. Various efforts were undertaken to make laboratory experiments in the present study more realistic by using densities similar to those recorded in the wild during field studies. All experiments used water from the Mississippi Sound, kept at a constant temperature, and maintained salinity consistent with field conditions in the summer season. However, the in situ field salinity varies and aerators do not provide the same water circulation and flow patterns found in nature. Additionally, in the wild oyster spat settle upon hard substrate and are incapable of movement, while in the present study aquaculture un-attached spat were used that could be moved by the predator. This un-attached spat could have increased or decreased handling time depending on how the predator utilized this unique form of its usual prey. These predators also experienced restricted movement due to the small size of mesocosms compared to oyster reefs in the wild. The use of larger mesocosms with circulating water flow would likely have reduced potential tank effects, but using larger mesocosms to perform the plethora of varied treatments examined in the present study was prohibitive.

Overall, the results of this study were very informative, and findings helped to elucidate trophic interactions occurring on inshore reefs in the Mississippi Sound. A key finding from the experiments in this chapter is the reduced survival of oyster spat in un-natural limestone substrate versus natural shell cultch. A unique aspect of this study is the use of experiments examining just one predator at a time, as well as all predators in combination, which allows a more comprehensive view of how each predator individually affects the trophic dynamics on oyster reefs and how each predator affects one another.

Table 8

*Experimental design for indirect predation experiments*

| **Experiment** | **Factor 1** | **Levels** | **Factor 2** | **Levels** | **Duration** |
|---|---|---|---|---|---|
| SC/MC TMII | Mud crab size | 13-16 mm<br>21-23 mm<br>28-31 mm | Stone crab | Present<br>Absent | 24 hours |
| TF/MC TMII | Mud crab size | 13-16 mm<br>21-23 mm<br>28-31 mm | Toadfish | Present<br>Absent | 24 hours |
| TF/MC/BC TMII | Toadfish | Present<br>Absent | | | 24 hours |

Table 9

*Mixed model analysis values from substrate/predator removal experiment.*

| **Factor** | **df** | **F-value** | **P-value** |
|---|---|---|---|
| Substrate | 1 | 6.993 | 0.009 |
| SC Presence | 1 | 76.719 | < 0.001 |
| TF Presence | 1 | 28.738 | < 0.001 |
| MC Presence | 1 | 67.190 | < 0.001 |
| Substrate x SC Presence | 1 | 6.274 | 0.013 |
| SC Presence x TF Presence | 1 | 45.582 | < 0.001 |
| SC Presence x MC Presence | 1 | 43.732 | < 0.001 |

Table 10

*PERMANOA values from Stone Crab/Mud Crab TMII experiment.*

| **Factor** | **df** | **F-value** | **P-value** |
|---|---|---|---|
| MC Size | 2 | 25.59 | < 0.001 |
| SC Presence | 1 | 78.91 | < 0.001 |
| MC Size x SC Presence | 2 | 12.55 | < 0.001 |

Table 11

*PERMANOVA values from Toadfish/Mud Crab TMII experiment.*

| **Factor** | **df** | **F-value** | **P-value** |
|---|---|---|---|
| MC Size | 2 | 3.49 | 0.052 |
| TF Presence | 1 | 51.07 | < 0.001 |
| MC Size x TF Presence | 2 | 2.04 | 0.156 |

Table 12

*PERMANOVA values from Toadfish/Mud Crab/ Blue Crab TMII experiment*

| **Factor** | **df** | **F-value** | **P-value** |
|---|---|---|---|
| Crab Type | 1 | 0.200 | 0.662 |
| TF Presence | 1 | 14.663 | 0.002 |
| Crab Type x TF Presence | 1 | 1.091 | 0.317 |

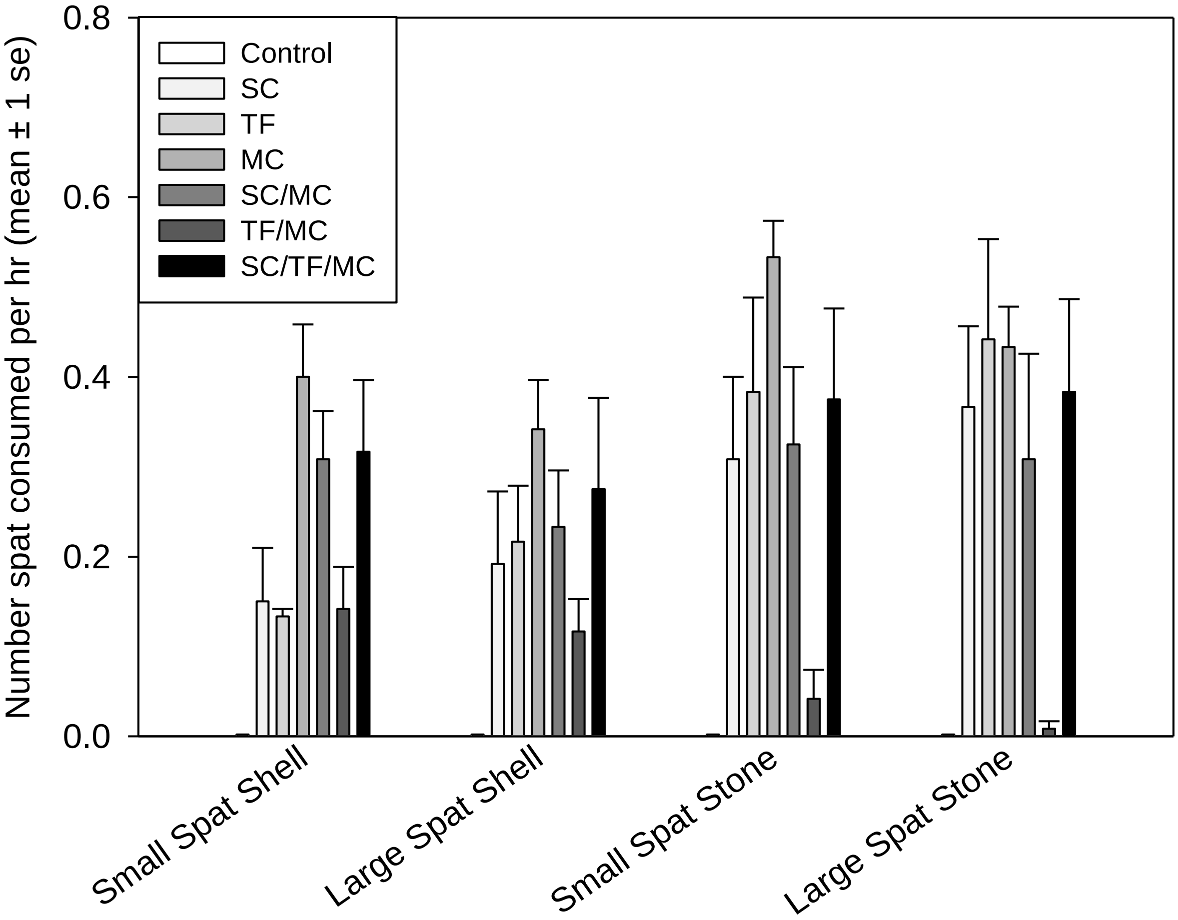


*Figure 20.* Habitat complexity/predator removal experiment results.

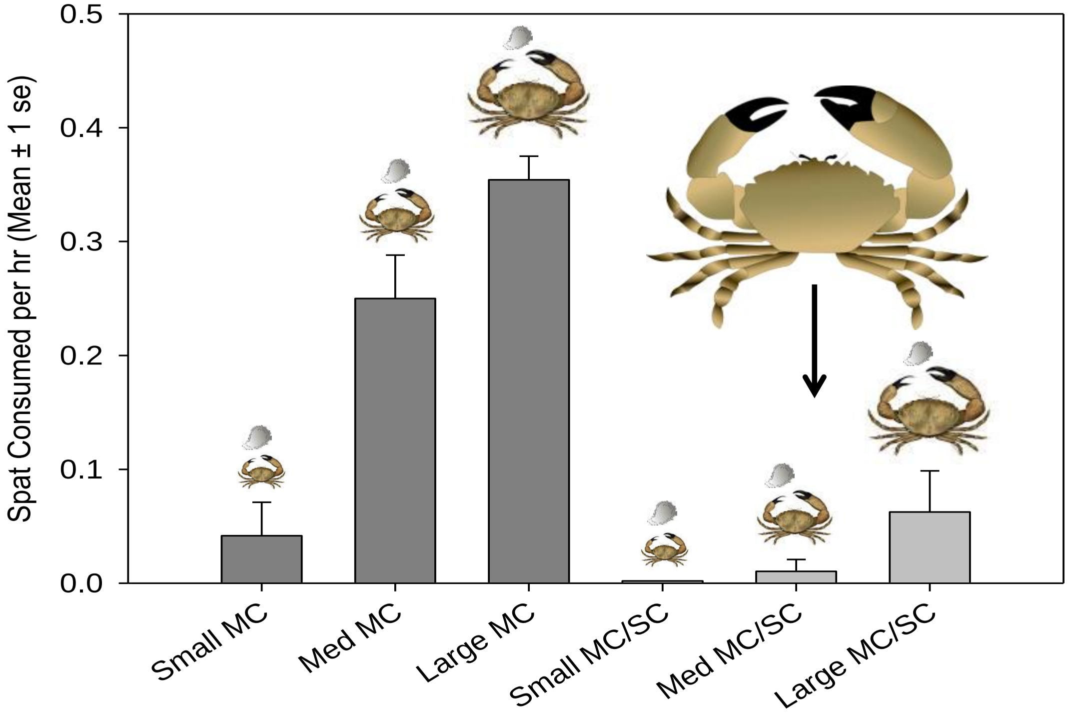


*Figure 21.* Stone Crab/Mud Crab TMII experiment results.

Note. In the absence of stone crab results are shown on the left and in the presence of a caged stone crab results are shown on the right.

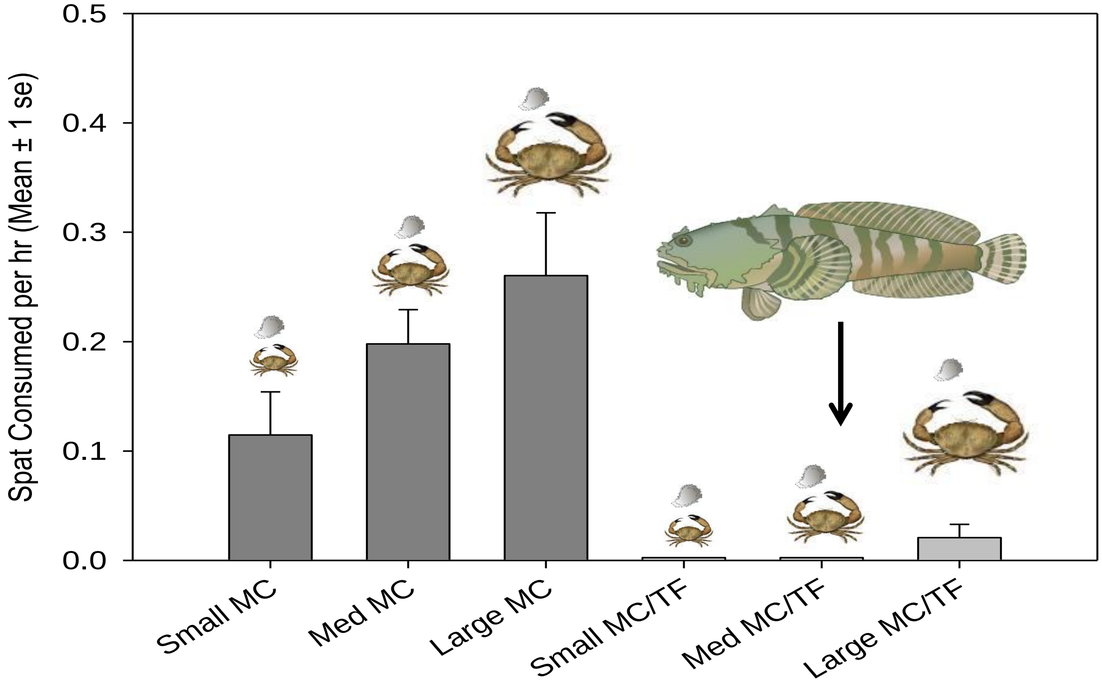


*Figure 22.* Toadfish/Mud Crab TMII experiment results.

Note. Results show numbers of spat consumed per hour by the three sizes of mud crabs in the absence (left) and presence (right) of a caged toadfish predator.

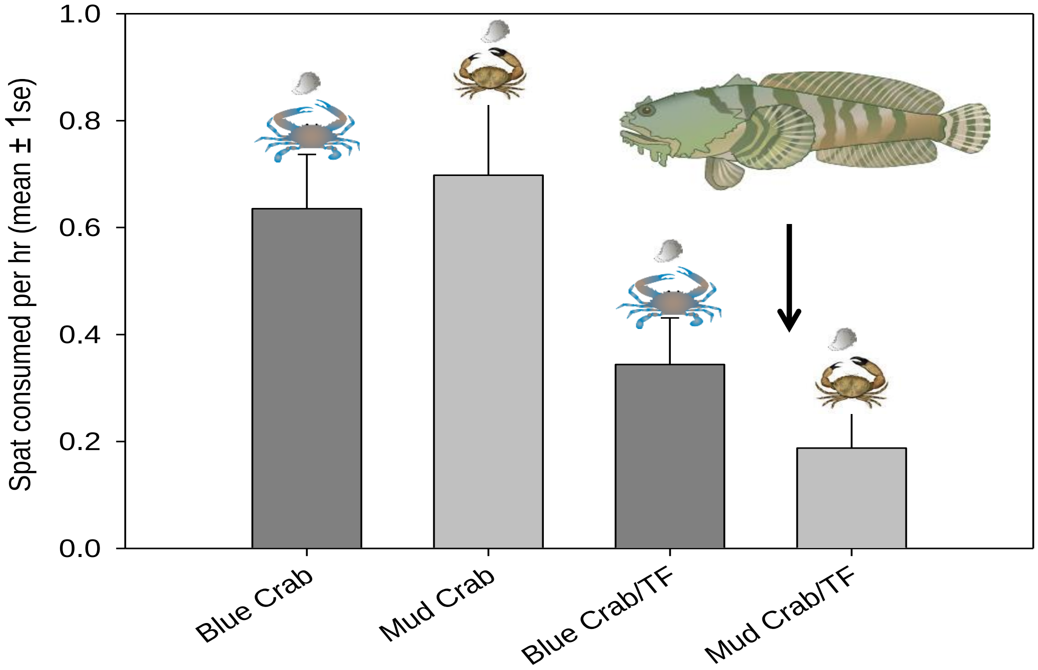


*Figure 23.* Toadfish/Mud Crab/Blue Crab TMII experiment results.

# CHAPTER V – INDIVIDUAL-BASED MODEL FOR EXPLORING OYSTER PRODUCTIVITY AT REEFS IN THE MISSISSIPPI SOUND AND IMPLICATIONS FOR MANAGEMENT

## Introduction

### *Individual-based Models*

A model is a “purposeful representation of some real system” (Starfield et al. 1990), and while population-level models are more common, individual-based models (IBMs) have become more frequently used over the last twenty-five years in a variety of disciplines focusing on complex ecological systems (DeAngelis and Gross, 1992; Grimm 1999; Grimm and Railsback 2005; Grimm et al.  2006). In the context of ecology, IBMs can be characterized as a bottom-up approach to understanding population dynamics (Grimm 1999), because the IBM approach begins with simulated individuals (known as agents in IBMs) within the ecosystem, and interactions among these agents ensue based on probabilistic-driven outcomes (DeAngelis and Mooij 2005; Grimm and Railsback 2005). This modeling approach contrasts with other community and population-level modeling approaches because IBM agents are discrete and have at minimum one trait that varies during the life cycle (Huston et al. 1988; Grimm 1999), whereas the community-level scale of population models do not generally incorporate individual variability (Grimm 1999). IBMs are used to examine how the specific adaptive traits of each individual affect the overall population and system, as well as how each individual is probabilistically affected by the system

(Railsback 2001; Grimm et al. 2006). Thus by using IBMs, connections emerge between population dynamics, ecological consequences, and bionomic traits (DeAngelis and Mooji, 2005).

Earlier versions of IBMs neglected habitat spatial variation (Van Winkle et al. 1993) because the models focused strictly on stationary homogeneous systems such as forests (Gross et al. 1995). More recently, many IBMs are spatially explicit (Railsback et al. 1999), especially in open patchy environments such as the ocean where planktonic life stages are dependent on unbounded physical conditions involving water flow, temperature, salinity, turbidity, and other spatially dynamic factors (Werner et al. 1997). With this added spatial structure, movement rules become a critical feature of IBMs (Hughes 1998; Railsback et al. 1999) because responses to physical and biotic forces often occur via movement within the spatial landscape. Thus, movement becomes integral for the simulation of growth, reproduction, mortality, and larval dispersal (Werner et al. 2001). Generally, when the growth or condition of an agent is hindered due to abiotic factors or prey concentration, movement rules dictate when the agent will leave (departure rules) as well as their new location (destination rules) (Clark and Rose 1997).

Numerous IBMs have been created to simulate trophic interactions involving community processes in a variety of ecosystems. Giacomini et al. (2009) developed a predation IBM for natural systems specifying energetics, life-history parameters, movement, and encounters between model agents. The authors created assembly rules for the functional traits associated with each agent in order to examine interspecific interactions occurring at both local and regional scales.  More recently, Campbell et al. (2011) created a spatially explicit IBM to simulate interactions among three fish species in the Gulf of Mexico (GOM), including effects of density-dependent competition on artificial reefs. Their simulations demonstrated an increase in the number of artificial reefs generally leads to increases in overall biomass at the expense of slower growth rates and smaller individual sizes. Both Giacomini et al. (2009) and Campbell et al. (2011) provided trophic information for natural, as well as artificial systems.

### *Oyster Models*

Numerous modeling approaches have been used to simulate oyster dynamics; however, most of these models are population-based. For example, Hofmann et al. (1992) developed a time-dependent population dynamics model to examine how both temperature and food concentration affect oyster spawning. From environmental conditions for Laguna Madre, Galveston Bay, and Chesapeake Bay, they showed reproductive effort decreased with increasing latitudes. Additionally, their simulations showed discrete spawning times in higher latitudes versus more continuous spawning in lower latitudes. Overall their population-based model indicated that small changes in temperature or the timing of the spring/fall blooms can alter spawning dynamics, and that salinity and turbidity are not as important to oyster population growth and reproduction as is temperature and food concentration (Hofmann et al. 1992). Another population-based model developed by Hofmann et al. (1994) simulated the energy flow in post-settlement oysters in order to determine what factors primarily influence adult size and reproductive effort. Within the same geographic range as Hofmann et al. (1992), simulations showed relatively more net production allocated towards somatic tissue growth than reproductive tissue development with increasing temperature. Furthermore, the authors report this result may explain why northern oysters are larger than southern oysters in the Gulf of Mexico (Wilson et al. 1992). However, simulations also revealed more net production allocated towards reproductive tissue at lower latitudes where there is higher food concentration, despite increasing temperatures (Hofmann et al. 1994).

While the models by Hofmann et al. (1992 and 1994) focused solely on oyster energetics such as respiration, assimilation, and filtration, Wang et al. (2008) developed a model by combining oyster energetics with hydrodynamics to examine how salinity affects oyster growth rates. This model integrated population-level processes of ingestion, assimilation, respiration, reproduction, spawning, recruitment, and mortality with a hydrodynamic model specific to Apalachicola Bay. Simulations focused specifically on oyster growth rates at two reefs inside the Bay. Model simulations indicated salinity significantly affects oyster growth rates, and the lowest rate of growth occurred in mid-spring which corresponds to the timing of increased river flows (i.e. reduced salinity) (Wang et al. 2008). Another model developed for the Apalachicola Bay area by Pine et al.

(2015) used field data to determine what caused the collapse of the Apalachicola Bay oyster fishery in 2012. This model also simulated various management restoration actions to evaluate how useful they were for restoring oyster populations. Their results revealed oyster harvest did not induce poor recruitment. Instead, decreasing abundance and survival of spat in the years leading up to the fishery collapse lowered the amount of dead shell available for settlement of early stages.

While the aforementioned oyster models provide useful information regarding oyster reproduction, growth, and recruitment, none of these models are individual-based. There is one individual-based model that considers a single life stage of oysters in conjunction with circulation patterns to simulate the distribution and growth of oyster larvae in Delaware Bay (Narvaez et al. 2012). These authors found reduced growth corresponds with lower salinity, and that larvae are primarily exported in the upper estuary, whereas larvae mostly self-recruited in the middle and lower estuary. However, simulations by Narvaez et al. (2012) did not correlate larval settlement with harvestable oyster production. There still is no IBM that forecasts the various life stages of oysters and the accrual of total reef biomass in light of abiotic and biotic factors for restored and artificial oyster reefs. Such a model for Mississippi Sound would be useful for determining the likelihood of reef restoration success and as a means of identifying the best locations for reef restoration efforts. Thus, the primary objective of the IBM proposed herein is to develop an IBM capable of specifying the potential for restoration of individual reefs by factoring in key stages of the oyster life cycle, predation pressure, and abiotic factors. To accomplish this objective, model simulations were first run using a literature value of natural mortality, and then simulations were run in presence of a specified number of predators (based on tray data from Chapter II) in order to show how predation pressure influences the survival and recruitment of juvenile oysters.

## Methods

### *Model Framework and Overview*

IBM development follows the guidelines for the overview, design concepts, and details (ODD) protocol for describing individual-based models recommended by Grimm et al. (2006). Numerous past IBM descriptions have been difficult to interpret and implement; therefore 28 authors from a variety of disciplines met to test the original PSPC + 3 protocol (Grimm and Railsback 2005), and the changes implemented in this meeting led to the creation of the ODD protocol (Grimm et al. 2006). The overview includes the specification of model purpose, states variables and scales, and process overview and scheduling, which should generally describe the overarching focus and complexity of the IBM. The design concepts detail the foundational framework of the IBM as well as agent interaction and the role of stochasticity. The details portion of the guidelines includes initialization and inputs (Grimm et al. 2006).

### *Overview: Purpose*

The primary objective of this IBM is to forecast the potential of a given location for oyster reef restoration, with regards to abiotic variables, as well as natural mortality rates for each oyster life stage. The interactions of each agent within the context of the simulated reef habitat reflect potential trade-offs inherent in utilizing these structures. For example, oyster larvae require hard surfaces on which to settle, while also facing a relatively short settlement window and predation pressure by oyster drills and small crabs inhabiting oyster habitats (Sellers and Stanley 1984). This IBM focuses specifically on how the survival, growth, and reproduction of oysters at reefs under conditions varying in space and time influence the potential for successful restoration (i.e. increased oyster production) at different locations along the Mississippi Coast, both with literature values for natural mortality rates and in the presence of specific densities of predators (i.e. implicit versus explicit predation).

### *Overview: State Variables and Scales*

In this IBM there are four hierarchical levels: individual, reef population, total harvestable population, and environment. There are four agents representing the four major stages of the Eastern oyster life cycle: larvae, spat, seed, and oyster. Agents are characterized by the state variables: age, sex, shell length (also determines if they can be harvested), biomass, reproductive maturity, and on which reef they reside. In turn, each of these variables can be mediated by the environmental variables of salinity, temperature, and bottom substrate. Individuals that are the result of fertilization following spawning are characterized as larvae. Once these larvae settle, they are referred to as spat. The variable of age begins when the larvae settle and become spat—larvae do not have an age but rather a settlement window, and the length of the settlement window is dependent on water temperature upon spawning (Kennedy 1996).

All oysters start out as males because eastern oysters are sequential hermaphrodites that exhibit protandry (Lorio and Malone 1994). The exact timing at which oysters switch from male to female varies in nature, but for the purpose of this model 365 days after settling, each male oyster has a 1% chance each day of becoming a female. Harvestable oysters are characterized as an oyster agent that is greater than 75 mm shell length. The main focus of the IBM is on the survival and growth of oyster larvae, spat, and seed as well as their recruitment to the harvestable oyster population (Figure 24).

Oyster reefs harbor many species, and the interactions between these organisms in the wild have both direct and indirect effects on the survival of oysters. Key species and the resulting processes of competition and predation are factored into this IBM; model agents in addition to oysters include: oyster drills, mud crabs, blue crabs, ribbed mussels, stone crabs, and toadfish. Each of these species is divided into three size classes (small, medium, and large), and their interactions with each oyster life stage (Table 13) is coded based on experimental results from the mesocosm experiments in chapters three and four. Predators randomly interact with oyster agents of the grid, and if they can consume the oyster agent they do so; however, predators do not consume each other, because they cannot die or reproduce.

A spatial grid was constructed by importing a digital map of the Mississippi Sound into the IBM program domain. The spatial grid for the IBM is rectangular in shape with a length of 16,896 m and width of 76,032 m, and it is comprised of 4,718,592 square cells (each cell = 16.5 m x 16.5 m), for a total spatial domain of roughly 1.28 x $10^9$ $m^2$. The coordinates and dimensions of approximately 75 reefs in the nearshore Mississippi Sound region were obtained from the MS-DMR and were also stored within the model domain grid within which patch types of reef, mud, and land are defined. For the simulations presented herein, the twelve reefs sampled in chapter two of this study are simulated.

*Overview: Process Overview and Scheduling*

The functions executed for agents within this IBM include: simulate, move (for larvae and predators only), settle, grow, reproduce, and die. These functions, as well as the entire model framework are outlined in a conceptual diagram for clarity (Figure 25). Larvae are the only oyster agents in the model for which movement rules are necessary, because spat, seed, and adult oysters are immobile. The larval stage is particularly important for the IBM, because larvae require hard substrate in order to recruit to the spat stage; therefore, the locations of reefs are essential to larval recruitment and oyster production. Planktonic oyster larvae movement is broadly based upon particle motion obtained from water velocity, with the general assumption that larvae do not drift very far from the vicinity where they are spawned (Levin 2006). This complex and variable movement requires knowledge of tides, currents, and river/stream outflow velocities in order to accurately simulate larval movement in the water column. Thus, because the exact movement of larvae is considered beyond the scope of this IBM, larvae move randomly in direction according to movement rules adapted from equations in Campbell et al. (2011). Distance moved is also based on a random exponential equation; larvae can move a maximum of 103 grid cells each day or they may not move at all in a day (average movement is approximately 10 grid cells each day). In addition, daily movements of predators are determined in the same random manner, though predators only move a maximum of six grid squares each day and always remain within the reef boundaries (considered to be reef residents).

The settlement time window is based on the water temperature on the day fertilization occurs. At the close of the settlement window, larvae will settle successfully if hard substrate is encountered within one week. Once settled, a larva becomes a spat with an age of 0 days and size of 0.3 mm. Larvae which do not encounter proper conditions within the settlement window are removed from the model simulation.

The major processes of growth, reproduction, and mortality for individual larvae, spat, and oysters were specified using parameter values obtained from the literature (Table 14). Growth equations based on salinity and temperature values (Lowe et al. In prep) were used to determine the daily growth of each oyster agent (equations are specific to the spat, seed, and adult stages). For reproduction to occur, water temperature must be above 25°C and salinity must be above 10 ppt. All female oysters have a 100% chance of reproducing every day between April (> 90 days into the year) and October (< 270 days) if the abiotic conditions are satisfied. Each spawn per individual releases 20,000 eggs, and spawning can occur once every 45 to 75 days. To decrease model complexity and run time, the release of one egg within the model is equal to a potential five spat (i.e. if one larva settles, it is equivalent to five spat settling). Larvae have a 10% chance of dying each day; each spat and seed have a 2.7% chance of dying each day; and oysters have a 0.2% chance of dying each day due to natural mortality.

This model runs in discrete time steps (days), and the model is projected over two years to account for seasonal variation in salinity and temperature regimes. Special attention is focused on the Gulf of Mexico oyster-spawning period, from April to October (Sellers and Stanley 1984). Additional years can be easily simulated by specifying the number of years in the prompt code when starting the model. At the end of each day, the total number of larvae, spat, seed, and harvestable oysters is calculated for each selected reef in the map, as well as for the entire map, and this data is exported as a csv file.

*Design Concepts*

*Emergence:* The life cycle of the oyster and the degree to which each functional process is affected by environmental variation is imposed by empirical rules in the model. Inherent behaviors of the agents, such as larvae settling onto hard substrate, are included in these empirical rules, and are not defined as adaptive or fitness-seeking behaviors. However, the population dynamics of reefs and the entire system emerge from all of these empirical rules.

*Sensing:* Oyster larvae settle onto the reef patches during their settlement window if there is sufficient substrate and the ambient conditions favor their recruitment. It is assumed competent larvae can sense whether patches are mud or reef.

*Interaction:* Interactions occur between environmental variables and the processes of settlement, growth, and reproduction. These interactions were discussed above in the process overview and scheduling portion and are shown in Figure 24.

*Stochasticity:* Every day, each agent has a defined probability of survival, movement (larvae and predators only), settlement (larvae only), growth, and reproduction (for oyster stage only). These probability values were obtained from published literature and were used in this IBM to simulate these processes directly, without the addition of complex current patterns, stream outflow, and predation.

*Collectives:* Each agent has its own properties; however, agents can also be grouped into individual reef populations, historic vs. artificial populations, and region-specific populations when aggregating the output data.

*Observation:* Each day the total abundance and biomass of all oysters and harvestable oysters is calculated for each reef, region, and the entire system. This information is outputted in a csv file containing individual spreadsheets for each reef, and one large spreadsheet for the entire system. Using these data, trends in abundance and biomass throughout the year or across multiple years can be visualized.

*Details: Initialization*

At the beginning of each simulation, the model loads the map from Google Earth, and it uses it to create a spatially explicit grid. The spatial grid layout mimics realistic spatial and physical properties of oyster reef habitat, including distances between reefs, reef patchiness and abiotic fluctuations. The model can be manually initialized to include all of the reefs on the grid or just selected specific reefs, as well as the number of days or years to simulate. Because the primary focus is dynamics of oyster reefs, areas outside of mapped reef substrate zones are all designated as soft substrate (i.e., with no settlement and recruitment). For this study, a time period of two years and reef configurations of the sampling sites from the field portion of this study (Chapter II) were simulated.

*Details: Inputs*

Various environmental drivers are input into the model, including temperature and salinity directly from the USGS website. These drivers are used in growth rate equations to calculate the growth per day of each oyster spat, seed, and adult agent. The surface area of each specified reef is also an input;

their coordinates and surface areas are stored in the IBM code, which was written in Python programming language. The surface area was calculated using maps provided by MSDMR.

The initialization values for the density of each life stage of oyster as well as all predator agents were determined from data on spat settlement abundance, tray samples, estimates of reef production from the sites, and data from MSDMR sampling. Table 15 shows initialization values used for each reef in the simulations used for this study. In addition, the input of initialization values for the number of oysters and predators on each reef at the start of each simulation can be customized, as well as the probabilities for mortality, settlement, growth, and reproduction.

### *Details: Submodels*

Each model simulation was replicated a minimum of ten times, from which means and standard deviations were calculated. For detailed descriptions of each process (i.e. submodel) the reader may consult the process overview and scheduling portion (See Figure 24) for detailed descriptions of each process (i.e. submodel), and the list of equations used and their sources (see Table 14).

## Results

### *Simulations Using a Literature Value of Natural Mortality*

Simulations run with a natural mortality parameter and no predator agents had a similar output after two years in the number of oyster agents as the results of the spat settlement study in Chapter II. In general, eastern reefs (GRAV = 27.8

± 13.345, HIST = 932.8 ± 197.446, PWRM = 64.6 ± 22.798, and PH = 738.5 ± 108.199) yielded lower total oyster abundances than most reefs in the central and west regions; and historic reefs in the western region of the MS Sound (SSH = 6403.7 ± 622.991 and BSLH = 9234.4 ± 773.087) showed the highest oyster abundances at the end of the two-year simulations. Peak oyster abundances occurred at reefs following the spawning season in the late summer/early fall, and the lowest abundances occurred in the winter, when oyster agents were experiencing natural mortality and not reproducing to replenish their population. The mean number of spat per meter squared at each reef (Figure 26) followed a similar trend as overall oyster abundance, lowest in the east region and highest at historic reefs in the west region during peak spawning season (mid-July to early August).

### *Simulations Including Common Predators*

Multiple simulations were run varying the number of predators on each reef to produce comparable oyster abundances as was seen in simulations using literature-based natural mortality rates for each oyster agent. For oyster spat per meter squared (Figure 27), explicit predation output values for the entire map (all twelve reefs combined) were somewhat lower than those using an overall natural mortality rate, but overall the outputs were similar, suggesting results using specific predator interactions were realistic. A similar pattern occurred when comparing implicit and explicit predation seed oyster values for the whole map (Figure 28). For total oyster abundance (all four stages), historic reefs in the western region exhibited the highest abundances of oysters per reef (SSH = 66666.5 ± 342.668 and BSLH = 9375 ± 755.357), and artificial reefs in the eastern region showed the lowest abundances of oysters (GRAV = 25.1 ± 7.141 and PWRM = 55.2 ± 19.054). The start of spawning season was evident from the peak in mean oyster abundance in the spring/summer of each year, and the lowest oyster values occurred in the winter months.

Further simulations were run to test the effect of multiplying the initial number of predators on each reef by a factor of 10 and 100. Increasing the number of predators did not seem to affect the seasonal trends—clear spawning peaks and winter lows were still evident for each of the reefs in the months of May and February, respectively. However, mean harvestable oyster abundance most visibly decreased under amplified predation (Figure 29). For example, at the end of a two-year simulation run from the beginning of 2015 to the end of 2016, for the explicit predation scenario there were 3134.333 ± 97.572 harvestable oysters, but only 2892.667 ± 17.01 and 2348 ± 66.506 harvestable oysters for the 10 and 10 times amplified predation scenarios respectively.

## Discussion

Although it may seem intuitive that oyster reefs initialized with higher numbers of spat and seed will have a higher abundance of harvestable oysters after two years, this is not necessarily the case. Spat and seed recruitment to the adult phase is problematic due to the increased mortality at these stages. Every size of predator included in this model is able to eat spat and most can consume seed;

however, only a couple of large-sized predators can consume harvestable oysters. The field study portion of this dissertation did not quantify recruitment to the adult stage. Likewise, Narváez et al. (2012) constructed an individual-based model of oyster larvae in Delaware Bay that left out the later stages of the oyster life cycle. In contrast, the IBM in the current study links information on earlier stages from the field portion of the present study to recruitment of the adult stage based on reasonable and flexible probabilistic outcomes.

One pitfall with the current IBM is the lack of incorporation of realistic hydrodynamics within Mississippi Sound as a transport mechanism within the model. Oyster population models of Apalachicola Bay by Wang et al. (2008) and Pine et al. (2015) incorporated hydrodynamic models to simulate oyster recruitment patterns; however, neither of these models were individual-based. Narváez et al. (2012) did include circulation patterns within their IBM. Thus, their results on larval survivorship and settlement were likely more realistic than shown by the current model based on random transport along with literature-derived values of dispersal to determine larval movements. However, that the end results of the current IBM were comparable to observed data suggests the general recruitment pattern was captured by the simplified approach. The main goal of the current IBM was to illustrate the potential effects of trophic dynamics on post-settlement oyster recruitment. Indeed, amplified predation can make a large economic impact in terms of harvest. A future goal is to combine the current IBM with a circulation pattern model in order to provide a clearer picture of where larvae may be expected to settle, and how many recruit to the spat and adult stages.

Another deficit within the current IBM is its omission of oyster bioenergetics, including respiration, assimilation, and ingestion (filtration rates). Two oyster population models by Hofmann et al. (1992 and 1994) factored in bioenergetics to simulate energy flow and growth; however, these models were not individual based, and did not follow individual oysters from birth to death. The inclusion of bioenergetics considerations within the current IBM would have greatly complicated the model to the extent that simulation run time would have been in days instead of hours. Instead, because a subsidiary goal of the current IBM was to capture effects of abiotic factors, empirical regressions developed by Lowe et al. (2017) linking salinity and temperature to oyster growth were adopted.

Despite various caveats associated with the current IBM as identified above, the model also has much strength. The coding foundation for the current IBM has the capacity and flexibility to simulate hundreds of reefs, and the model can be expanded to simulate additional reefs through inputs of their geographic coordinates and surface areas. This function will allow managers to simulate potential regions under planning for the placement of artificial reefs or restored reefs in terms of oyster production. Such a tool could save a lot of time and money in the end. Managers can also easily incorporate fishing mortality into the IBM as an additional component to the natural or predation mortality components, which could consider the effects of various harvest limits within each region of the Sound. Additionally, many other parameters can be varied in addition to predation, such as reproduction, growth, abiotic factors, number of days, and initial oyster numbers. While the model outputs are presented as means for entire reefs, the current model actually tracks single larvae from birth until death, as well as their daily movement until they settle and their daily growth once they become spat. Considering that each oyster releases 20,000 eggs within the model it would not be feasible to monitor the billions of larvae, along with all the other stages of oysters produced within the Mississippi Sound. Thus, using the current IBM tool in conjunction with field monitoring would provide a more efficient approach to understanding oyster recruitment and production. Therefore, the complexity, feasibility, and functionality of the current IBM is a crucial step towards a, “purposeful representation of some real system” (Starfield et al. 1990).

Table 13

*Predator sizes and the oyster stages they consume.*

| Organism | Size Class | Process | Oyster agent affected |
|---|---|---|---|
| Mussels | All | Competition | Larvae and spat |
| Mud crab | Small | Predation | Spat |
| | Medium | Predation | Spat and seed < 35 mm |
| | Large | Predation | Spat and seed < 50 mm |
| Stone crab | Small | Predation | Spat and seed < 35 mm |
| | Medium | Predation | Spat and seed < 35 mm |
| | Large | Predation | Spat, seed, and adult |
| Blue crab | Small | Predation | Spat |
| | Medium | Predation | Spat and seed < 50 mm |
| | Large | Predation | Spat and seed |
| Oyster drill | Small | Predation | Spat |
| | Medium | Predation | Spat and seed < 50 mm |
| | Large | Predation | Spat, seed, and adult |

Table 14

*Literature values of model parameters*

| Stage | Variable | Value/Equation | Reference |
|---|---|---|---|
| Larvae | Larval period | Larval Period (in days) = 110.76*e(-0.0825*T) | Kennedy (1996) |
| | Mortality | 10% daily mortality | Drinnan & Stallworthy (1979) |
| | Size at settlement | 300 uM | Kennedy (1996) |
| | Dispersal | 1 to several hundred km | Siegel et al. (2003) |
| | | Increases with longer larval period | Kinlan & Gaines (2003) |
| Spat | Growth | = (-26.18+2.91*(T)+0.055*(T)^2+0.012*(S)^2)/30 | Lowe et al. (2017) |
| | Mortality | 2.7% per day | Krantz & Chamberlin (1978) |
| | Recruitment to seed | At 26 mm | Rybovich (2014) |
| Seed | Growth | Growth= (-19.49+1.97*(T)+ -0.036*(T)^2)/30 | Lowe et al. (2017) |
| | Mortality | 2.7% per day | Krantz & Chamberlin (1978) |
| | Recruitment | above 75 mm | Rybovich (2014) |
| Adult | Growth | Growth (mm/day) = (-2.81+0.29*(T)+0.22*(S)+ -0.0074*(T)^2+0.0068*(S)^2)/30 | Lowe et al. (2017) |
| | Mortality | 0.2% per day | Manzi et al. (1977) |
| | Reproduction | Spawning can occur between day 90 and 270 | Kennedy (1996) |
| | | Temperature ≥ 25 degrees Celcius to spawn | Lorio & Malone (1994) |
| | | Salinity > 10ppt for spawning | Lorio & Malone (1994) |

Table 15

*Model simulation initialization values.*

| Reef | Lat (N) | Long (W) | Area (m2) | Oysters | Mussels | Drills | MC | SC | BC | TF |
|---|---|---|---|---|---|---|---|---|---|---|
| AL | 30.2981667 | -89.33 | 54403 | 674 | 282 | 1 | 409 | 12 | 2 | 2 |
| SSH | 30.30034 | -89.3222 | 410905 | 2570 | 366 | 1 | 363 | 3 | 1 | 2 |
| BSLH | 30.3106 | -89.1576 | 486653 | 2570 | 366 | 1 | 363 | 3 | 1 | 2 |
| USM | 30.3472 | -89.1336 | 54832 | 674 | 65 | 6 | 200 | 31 | 4 | 1 |
| KUHN | 30.3912667 | -88.8725 | 54856 | 430 | 199 | 9 | 234 | 6 | 12 | 4 |
| MLH | 30.334235 | -89.1576 | 486653 | 174 | 275 | 29 | 225 | 8 | 3 | 6 |
| FB | 30.4209 | -88.8594 | 55243 | 430 | 160 | 1 | 504 | 1 | 1 | 3 |
| MDBH | 30.395758 | -88.8277 | 109934 | 174 | 469 | 1 | 386 | 1 | 1 | 4 |
| GRAV | 30.3472333 | -88.6709 | 55235 | 41 | 41 | 26 | 466 | 14 | 2 | 1 |
| HIST | 30.348881 | -88.6208 | 421930 | 144 | 41 | 26 | 466 | 14 | 2 | 1 |
| PWRM | 30.3645333 | -88.5998 | 56622 | 41 | 344 | 1 | 224 | 2 | 18 | 1 |
| PH | 30.336328 | -88.5877 | 297484 | 144 | 989 | 1 | 155 | 2 | 1 | 2 |

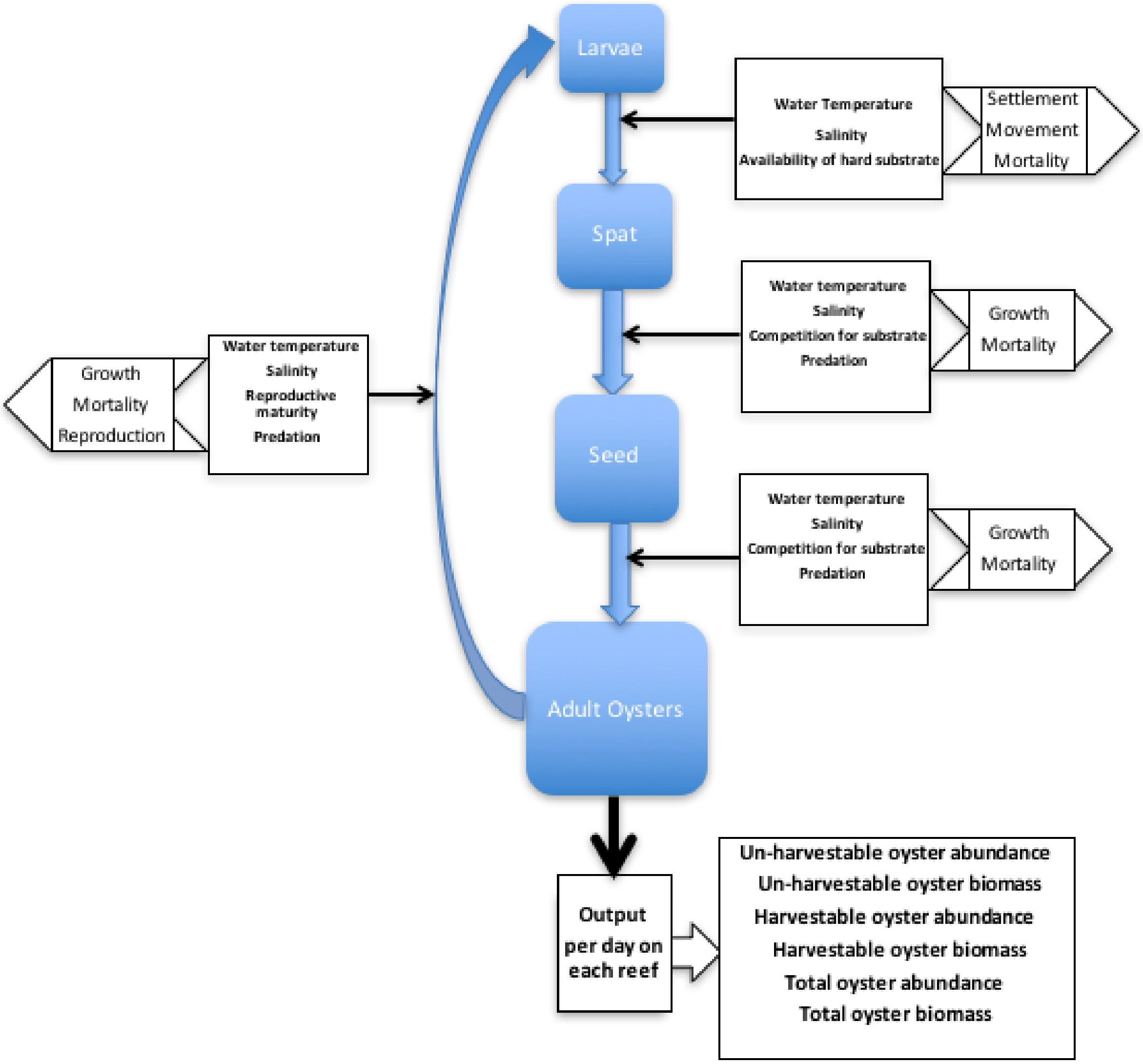


*Figure 24.* Flow diagram of oyster life stages.

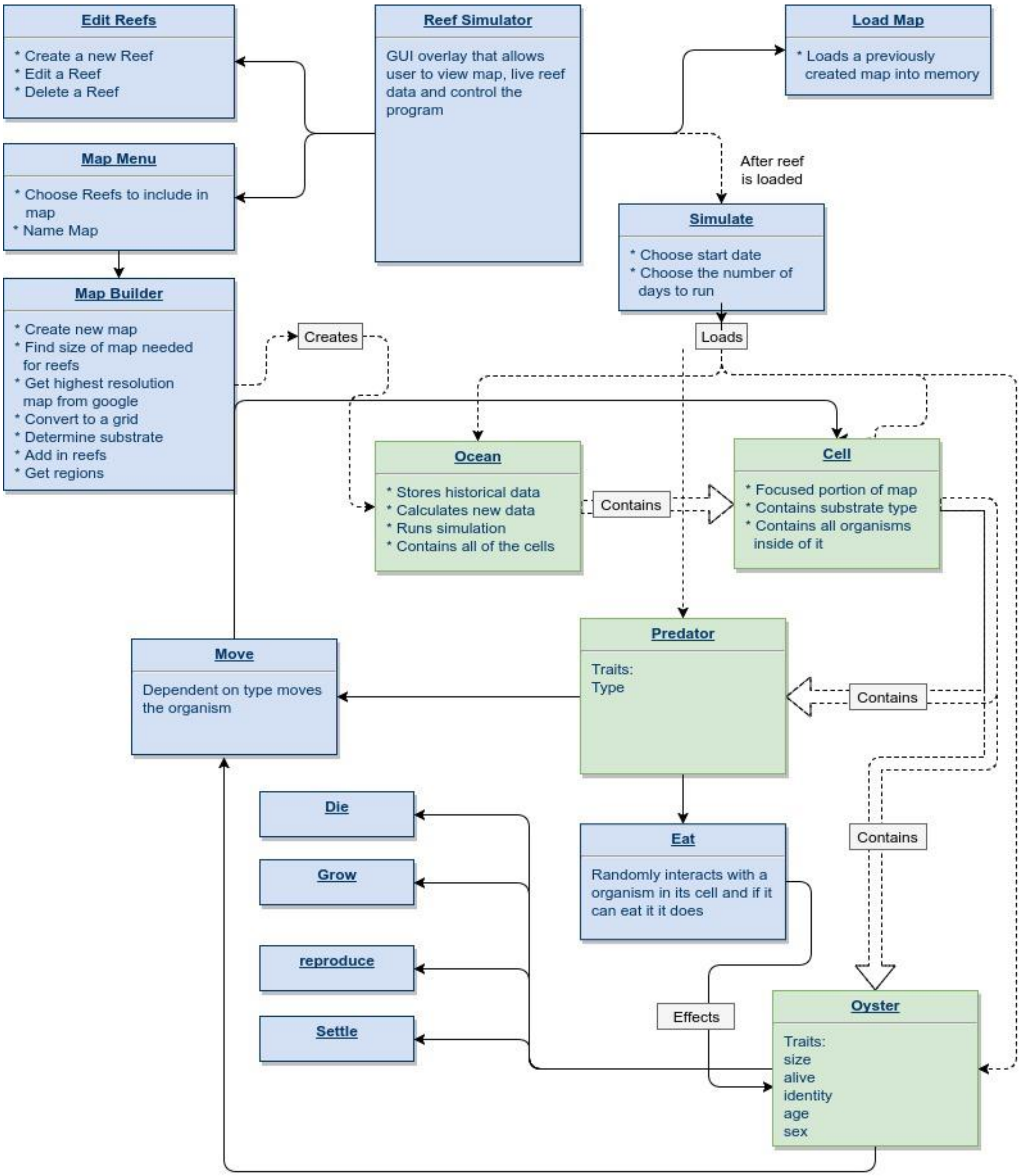


*Figure 25.* Conceptual map of model framework.

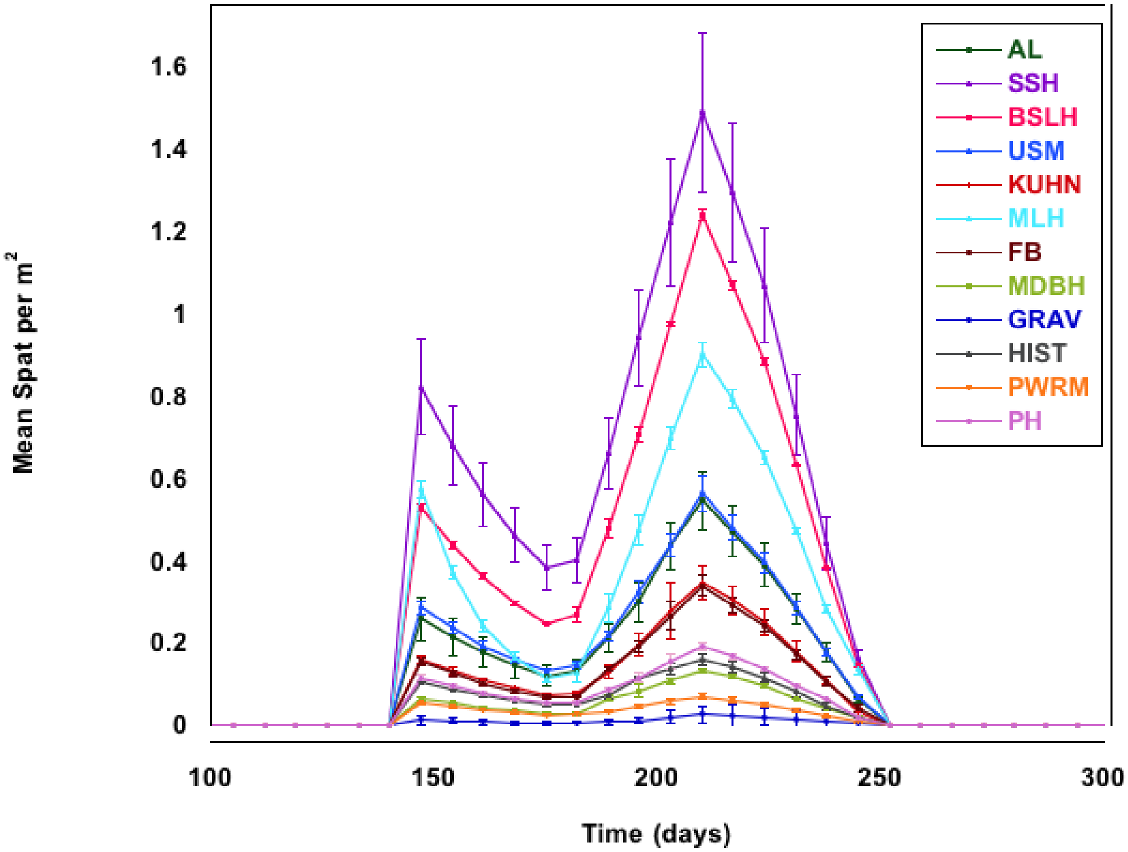


*Figure 26.* Temporal change in spat per square meter at each reef.

Note. The x-axis depicts day 100 through 300 of the 2015 spawning season to focus in on the time when spat are settling. Mean data points and standard deviation bars are plotted every 14 days.

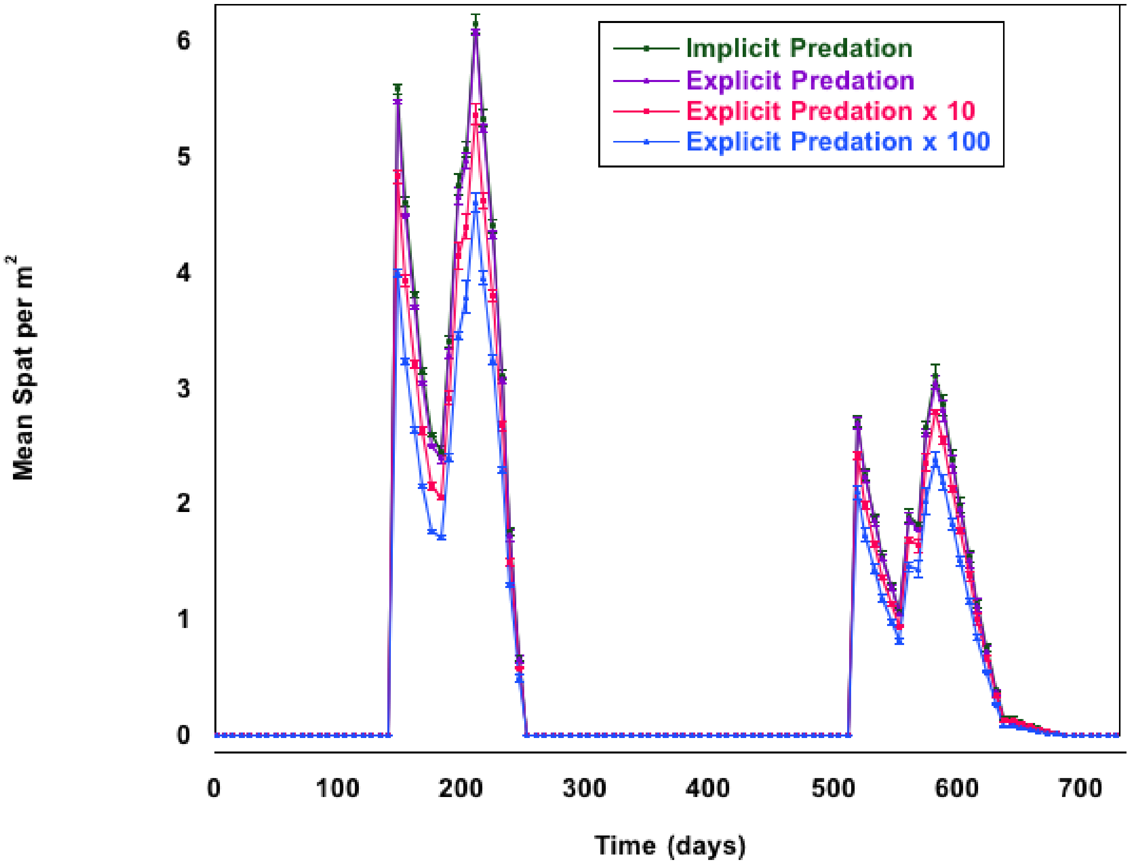


*Figure 27.* Relationship of spat per meter squared and predation scenarios.

Note. Values are plotted from the beginning of the simulation in January 2015 to the end of the simulation (December 30, 2016). Mean data points and standard deviation bars are plotted every 14 days.

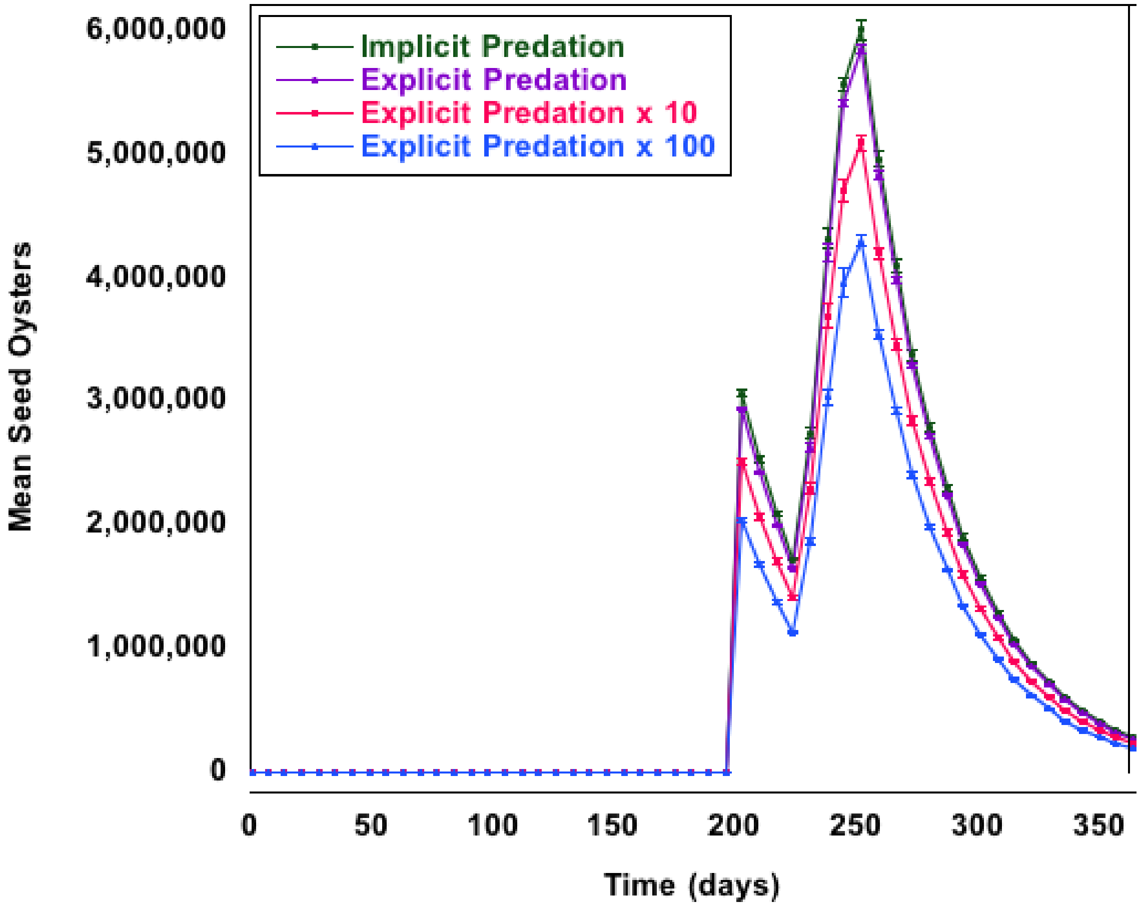


*Figure 28.* Relationship of oyster seed and predation scenarios.

Note. Points are plotted every 14 days for 2015.

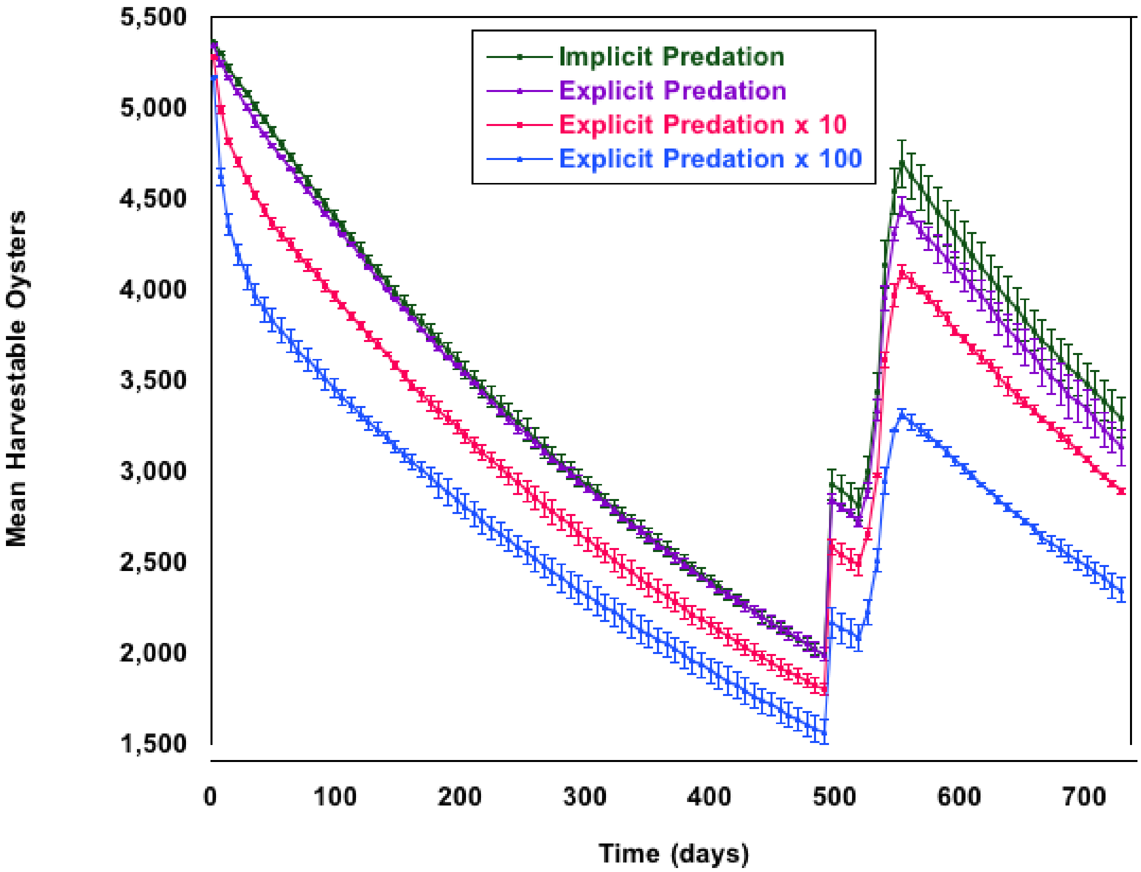


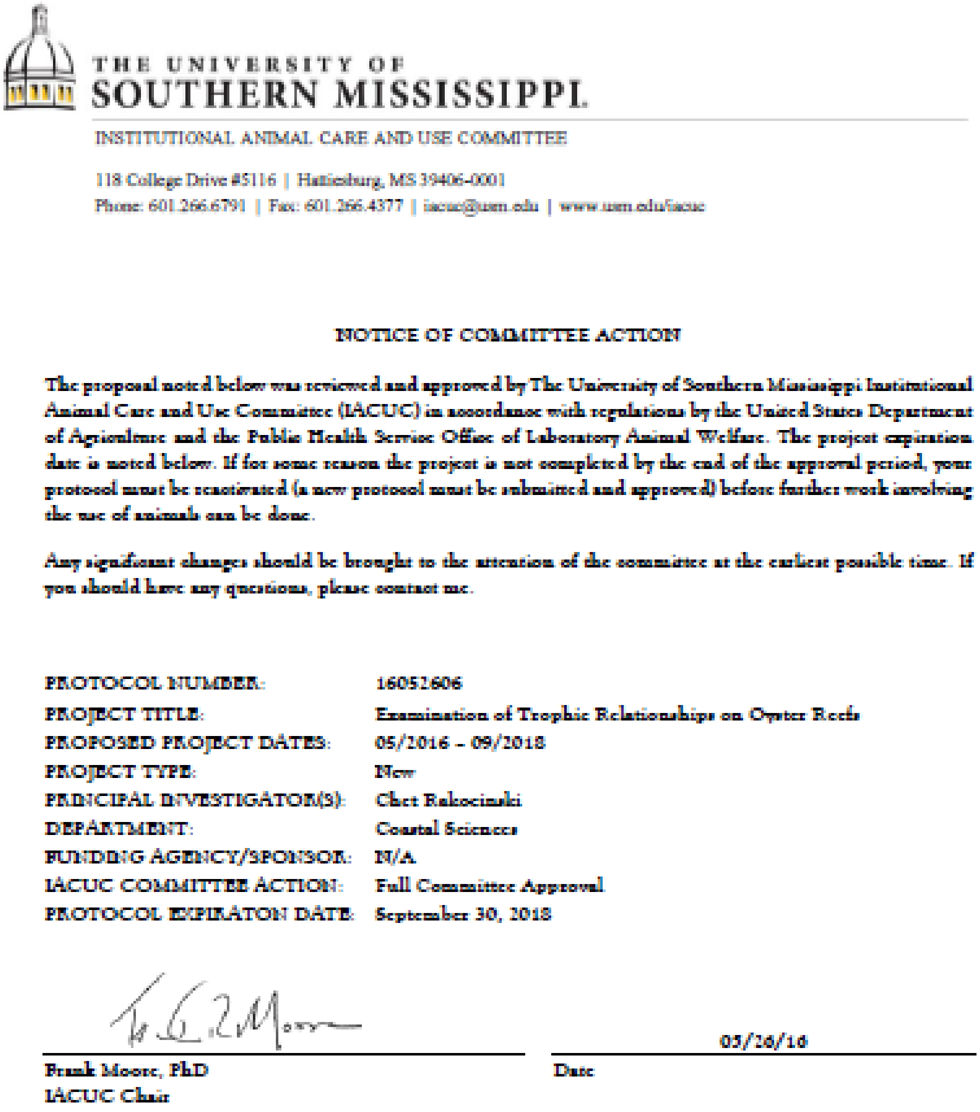
*Figure 29.* Comparison of harvestable oysters from four predation scenarios.

Note. Harvestable oysters are 75 mm shell length or above. Points are plotted every 14 days from 1/1/2015 to the end of 2016.

# CHAPTER VI CONCLUDING REMARKS

An overall goal of the current study was to examine how trophic dynamics at artificial and historic oyster reefs in the Mississippi Sound affect oyster survival and recruitment to the adult harvestable stage. Chapter II provided insight into variability in the abundance and biomass of spat settling at twelve reefs in the Mississippi Sound. This data showed that historic reefs in the western region exhibited the highest abundance of oyster spat settling per day. Spat settlement abundance was also generally greater at historic versus artificial reefs ($p < 0.001$) and in western versus other regions ($p < 0.001$). Peak seasonal spat abundance and biomass occurred in the month of September. When comparing variability in key faunal organisms potentially affecting oysters among reefs, reef region ($p = 0.037$) and sampling period ($p = 0.001$) most significantly affected the species assemblage. Chapter III experimentally addressed relative prey size selection relative to oyster spat, interactions related to different predator combinations, and TMII effects within the oyster system. Prey size selection by mud crabs and oyster drills on oyster spat was significant. Both drill size and spat size factors varied significantly in relation to the number of spat consumed per day ($p = 0.0248$ and $p = 0.0084$ respectively), and both mud crab size and spat size factors also varied significantly relative to oyster spat consumption ($p < 0.001$). Moreover, oyster cultch substrate significantly reduced spat mortality compared to limestone substrate ($p = 0.009$). The presence of both stone crabs and Gulf toadfish significantly enhanced spat survival by mediating mud crab predation on oyster spat ($p < 0.001$), even in the absence of direct predation. Finally, chapter IV presented an IBM capable of simulating recruitment, survival, and growth of the four main life stages of oysters. This IBM was further used as a tool to compare scenarios incorporating literature-based natural mortality versus explicit probabilistic predation effects. The capacity to track larval movement, oyster growth, reproduction, and mortality for twelve reefs in Mississippi Sound makes this the first known IBM that can simulate and output oyster abundance and biomass across multiple reefs and years. In conclusion, this dissertation clearly shows how trophic dynamics can play a key role in affecting oyster survival and recruitment at both historic and artificial oyster reefs in the Mississippi Sound.

# APPENDIX A – IACUC Approval Letter



THE UNIVERSITY OF
SOUTHERN MISSISSIPPI
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

118 College Drive #5116 | Hattiesburg, MS 39406-0001
Phone: 601.266.6791 | Fax: 601.266.4377 | iacuc@usm.edu | www.usm.edu/iacuc

**NOTICE OF COMMITTEE ACTION**

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

| | |
|---|---|
| PROTOCOL NUMBER: | 16052606 |
| PROJECT TITLE: | Examination of Trophic Relationships on Oyster Reefs |
| PROPOSED PROJECT DATES: | 05/2016 – 09/2018 |
| PROJECT TYPE: | New |
| PRINCIPAL INVESTIGATOR(S): | Chet Rakocinski |
| DEPARTMENT: | Coastal Sciences |
| FUNDING AGENCY/SPONSOR: | N/A |
| IACUC COMMITTEE ACTION: | Full Committee Approval |
| PROTOCOL EXPIRATON DATE: | September 30, 2018 |

05/26/16

Frank Moore, PhD
IACUC Chair

Date

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