Recruitment And Restoration Of The Oyster Crassostrea Virginica In Areas With Intense Boating Activity In Mosquito Lagoon, Florida

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RECRUITMENT AND RESTORATION OF THE OYSTER
CRASSOSTREA VIRGINICA IN AREAS WITH INTENSE BOATING ACTIVITY IN
MOSQUITO LAGOON, FLORIDA

by

LISA MICHELE WALL
B.S. University of Central Florida, 1996
M.Ed University of Central Florida, 2001

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

Summer Term
2004
ABSTRACT

Productivity, diversity and survival of estuaries are threatened by explosive coastal population growth and associated recreational activities. One major area of recreational growth has been the number of small pleasure craft motoring in shallow waters at high speeds. On the east coast of Central Florida in the Indian River Lagoon system, intense boating activity occurs year-round and intertidal reefs of the eastern oyster *Crassostrea virginica* with dead margins (piles of disarticulated shells) on their seaward edges are commonly found adjacent to major boating channels. The cause(s) of the dead margins is unclear. However, the disarticulated shells may be reducing reef sustainability if these surfaces are unavailable for larvae. Recruitment trials were run on eight reefs (4 with dead margins, 4 without) in three 8-week trials in 2001/2002. Significant differences were found for location on reef and season. For survival of recruits, significant differences were found for reef type, location on reef, and season. Sediment loads, percent silt/clay, and relative water motion were all found to be significantly higher on impacted reefs. Spring months were found to be the optimal time for larval recruitment to increase larval set and survival and to also decrease the effects of sedimentation and water motion. Based on these results, experimental restoration began May 2003 to develop an ecologically and economically feasible restoration protocol for this intertidal region. Four different densities of shells (0, 16, 25, 36) were attached to vexar mesh mats (45 X 45 cm) displaying shells perpendicular to the substrate. 360 mats were randomly deployed at one of six identified optimum recruitment locations. Recruitment increased through June and was significantly higher on mats with 36 shells. This was followed by a large, expected decline in recruitment and
survival in July/August, due to competition, predation and/or extreme high temperatures. Total live oysters on the restoration mats significantly increased during October 2003 through February 2003. These newly-created oyster reefs are moveable and provide optimal substrate and larval set to be transported post-recruitment to areas resource managers have slated for restoration to aid in reef sustainability. To determine the potential negative effects of flow and sediment levels on oyster larval settlement, which may be associated with an increase in boating activity, laboratory experiments were conducted. Eighteen trials, with competent oyster larvae, nine in flowing-water and nine in still-water were run at three sediment levels: no sediment, low sediment, and high sediment loads. Larval settlement was significantly higher in the still-water trials and both high and low sediment loads significantly reduced larval settlement.
# TABLE OF CONTENTS

LIST OF TABLES........................................................................................................................................ vi

LIST OF FIGURES........................................................................................................................................ vii

INTRODUCTION ............................................................................................................................................. 1

Study Location............................................................................................................................................... 3

Biology of the Eastern Oyster *Crassostrea virginica* .................................................................................. 7

General Anatomy.......................................................................................................................................... 7

Reproduction................................................................................................................................................ 8

Larval Dispersal and Recruitment ............................................................................................................. 9

Sediment Impacts......................................................................................................................................... 10

Restoration.................................................................................................................................................. 10

METHODS: RECRUITMENT AND SURVIVAL......................................................................................... 13

Recruitment and survival on impacted versus unimpacted oyster reefs ............................................... 13

Sediment loads ........................................................................................................................................ 14

Relative water motion ............................................................................................................................... 14

RESULTS: RECRUITMENT AND SURVIVAL......................................................................................... 20

Statistical Analyses ..................................................................................................................................... 20

Recruitment................................................................................................................................................. 20

Percent Survival........................................................................................................................................ 24

Sediment loads ........................................................................................................................................ 28

Percent silt and clay ................................................................................................................................. 33
LIST OF FIGURES

Figure 1: The Indian River Lagoon system ............................................................... 5
Figure 2: Fellers House Field Station ..................................................................... 6
Figure 3: Oyster reefs in Mosquito Lagoon ............................................................. 16
Figure 4: Aerial photo of reefs in Mosquito Lagoon .............................................. 17
Figure 5: Locations within each oyster reef ......................................................... 18
Figure 6: Experimental frame ............................................................................... 19
Figure 7: Mean oyster recruitment on impacted and unimpacted reefs ............... 22
Figure 8: Mean oyster recruitment within each reefs ............................................ 23
Figure 9: Percent oyster survival on impacted and unimpacted reefs ................. 26
Figure 10: Percent oyster survival within reef locations ....................................... 27
Figure 11: Mean sediment load: Summer 2001 ..................................................... 30
Figure 12: Mean sediment load: Winter 2001-2002 .............................................. 31
Figure 13: Mean sediment load: Spring 2002 ...................................................... 32
Figure 14: Percent silt/clay: Summer 2001 ............................................................ 35
Figure 15: Percent silt/clay: Winter 2001-2002 ...................................................... 36
Figure 16: Percent silt/clay: Spring 2002 .............................................................. 37
Figure 17: Mean dissolution of plaster spheres: Summer 2001 ......................... 40
Figure 18: Mean dissolution of plaster spheres: Winter 2001-2002 .................... 41
Figure 19: Mean dissolution of plaster spheres: Spring 2002 ............................. 42
Figure 20: Experimental restoration mats ............................................................. 49
Figure 21: Randomized design of restoration mats ......................................................... 50

Figure 22: Oyster recruitment on restoration mats. ...................................................... 53

Figure 23: Mean larval settlement in varying flows and sediment loads ....................... 64
LIST OF TABLES

Table 1: Timeline of research efforts.......................................................... 12
Table 2: Results of a three-way ANOVA: Recruitment ........................................ 21
Table 3: Results of a three-way ANOVA: Percent Survival .............................. 25
Table 4: Results of a three-way ANOVA: Sediment loads .............................. 29
Table 5: Results of a three-way ANOVA: Percent silt/clay .............................. 34
Table 6: Results of a three-way ANOVA: Dissolution rates ......................... 39
Table 7: Results of two-way ANOVA: Live oysters on restoration mats .......... 52
Table 8: Experimental conditions for flume trials. ....................................... 60
Table 9: Experimental conditions for still-water trials. ................................. 61
Table 10: Results of a two-way ANOVA: Larval settlement in varying flow rates and sediment loads ................................................................. 63
INTRODUCTION

Estuaries of the world are increasingly exposed to multiple stressors from anthropogenic sources. Stressors, such as increasing numbers of recreational and commercial vessels may affect nearly all organisms within an estuarine system (Breitburg et al. 1999). Susceptibility of organisms within estuaries to stressors vary among species (e.g., Magnuson et al. 1989; Sanders and Riedel 1998; Diaz and Rosenberg 1995; Williamson et al. 1999), and may be influenced by the presence of other stressors (i.e. sedimentation or water motion) or the intensity of other variables (i.e. extreme temperatures) (e.g., Folt et al. 1999; Lenihan 1999). As human population growth and activities increase, the intensity, geographic extent, and number of anthropogenic stressors generated also increases.

Environmental threats, primarily the result of human population growth along the east coast of central Florida over the last 100 years, have encroached on local estuaries (Grizzle et al. 2002). In trying to make areas more suitable for human habitation, the threats have become increasingly difficult to control. These problems include nutrient loading, creation of impoundments for mosquito eradication, large-scale channeling for flood control, and commercial and recreational usage (IRLNEP 1994). Federal and state legislation now protects many areas in Florida where estuaries are of particular concern (Walters et al. 2001b).

One area of particular interest is the Indian River Lagoon system (IRL). In 1990, the U.S. Environmental Protection Agency designated the IRL as “an estuary of national significance (IRLNEP 1994). Mosquito Lagoon, the northernmost section of the IRL, is additionally designated as an Outstanding Florida Water (Rule 62-302.700(9) F.A.C.) This state designation,
under the Clean Water Act, is intended to afford the highest level of protection to existing high quality waters (Walters et al. 2001b). Additionally, Mosquito Lagoon and its surrounding area, are part of Canaveral National Seashore (CANA). First established in 1975 by Public Law 93-626, the National Park Service must preserve and protect the outstanding natural, scenic, scientific, ecologic, and historic values of lands, shorelines and waters of CANA and to provide public outdoor recreational use of these same waters.

In an attempt to protect the Indian River Lagoon system from further destruction, the Aquatics Preserves Act (1975) set aside state-owned submerged lands and associated waters to be maintained in their natural condition. Canaveral National Seashore (CANA), in which several hundred reefs of *C. virginica* are found, is an ideal location for the observation of intertidal oyster reef communities.

The southern-most geographic limit on the Atlantic coast for intertidal oyster reefs of the eastern oyster *Crassostrea virginica* is found within Mosquito Lagoon (Grizzle and Castagna 1995). Using aerial photography and field surveys, uncharacteristic dead margins were first noted on oyster reefs within CANA as early as 1943, and total reef area has decreased by over 50% (Grizzle et al. 2002). These dead margins, found along the seaward edges of reefs, consist of disarticulated shells mounded above the adjacent living reef. This type of dead zone differed from the long-term growth pattern of a dead middle reef area surrounded by living oysters (Bahr and Lanier 1981). These uncharacteristic dead margins occupy a surface area covering approximately 10% of the reefs studied (Grizzle et al. 2002). The aerial photographs used in the study showed that in 1943 none of the reefs away from the Intercoastal Waterway had dead
margins. All of these reefs with dead margins in 2000 occurred adjacent to major boating channels (Grizzle et al. 2002).

Whether resulting from anthropogenic influences, (i.e. increasing boating activity) or natural fluctuations in ecosystem components, reefs of the eastern oyster in the Indian River Lagoon have begun to decline. However, little is known about the effects of increased water motion by motor boats on intertidal reef systems of *C. virginica*. To better understand the hypothesized impact of increased boating activity on the decline of oyster reefs in Mosquito Lagoon, I monitored recruitment, early juvenile survival, sediment loads, relative water motion, and the interactions between settling larvae, sediment levels, and relative water motion on oyster reef with and without dead mounds. Using the data collected, I then help develop, deploy, and monitor experimental restoration mats for one year.

**Study Location**

Research was conducted in Mosquito Lagoon, the within Canaveral National Seashore (Fig. 1). The Lagoon system is a series of three distinct, but connected, estuaries which extend 251 kilometers (156 miles) from Ponce de Leon Inlet to Jupiter Inlet on the east coast of central Florida. The University of Central Florida research facility, Fellers House Field Station (28° 54’ N, 80° 49’ W) is located within the bounds of the National Park (Fig. 2).

The average depth of the Lagoon is less than 1 meter in most areas and the current is primarily wind-driven (Grizzle et al. 2002). Annual salinity ranges between 25 and 45psu, depending on rainfall (Grizzle 1990; Walters et al. 2001a). Oyster reefs are intertidal, often
adjacent to seagrass beds of *Halodule wrightii* that are extensive in some areas (Morris et al. 2000). Most of the lagoon within CANA is a complex system of shallow and open water areas with nearly 100 mangrove (*Rhizophora mangle* and *Avicennia germinans*) dominated islands (Walters et al. 2001b). Intertidal oyster reefs are found throughout this region and often adjacent to these mangrove-dominated islands.

The IRL generates over $800 million in revenue annually to the local economy (IRL Comprehensive Conservation and Management Plan 1996). Oyster reefs are an important component of this diverse estuary and have significant economic importance as they are harvested both recreationally and commercially (Walters et al. 2001b). Harvesting in the IRL is confined almost entirely to Mosquito Lagoon, where harvesting is conditionally approved, depending on weather conditions (IRLNEP 1994).

In 2001, there were over 90,000 registered boats within the counties that border the northern IRL system, and this number has increased nearly 10% annually since 1986 (Hart 1994; ANEP 2001). Although many concerns with the increasing number of boaters have been well documented, little is known about the impact of rapidly increasing boat activity on important benthic organisms, including the oyster *Crassostrea virginica* (Walters et al. 2001b).
Figure 1: The Indian River Lagoon system runs 251 kilometers along the east coast of Florida. Research was conducted in Mosquito Lagoon, the northern-most section of the IRL system.
Figure 2: Fellers House Field Station. This University of Central Florida research facility is located within the bounds of Canaveral National Seashore.
Biology of the Eastern Oyster Crassostrea virginica

The eastern oyster *Crassostrea virginica* (phylum Mollusca, class Bivalvia, order Ostreoid, family Ostreidae) was first described by Gmelin in 1791. *Crassostrea virginica* can be found from the Gulf of the St. Lawrence in Canada to the Gulf of Mexico, Caribbean and coasts of Brazil and Argentina (Newball and Carriker 1983; Garcia-Cubas et al. 1987; Andrews 1991). *Crassostrea virginica* is common in estuaries and coastal areas of reduced salinity or in the intertidal zone. It occurs in some areas as extensive reefs on hard to firm bottoms, both intertidally and subtidally (MacKenzie 1983, 1996a, 1996b; Kennedy et al. 1996).

The eastern oyster is extensively cultivated in many areas of its range (Canada, Virginia, Florida, Texas, and Louisiana) (Galtsoff 1964; Garcia-Cubas et al. 1987; Quayle and Newkirk 1989; Andrews 1991; Menzel 1991). *Crassostrea virginica* has been introduced to the west coast of North America, Hawaii, Australia, England and Japan, but has not become established in any of these locations (Quayle 1988; Arakawa 1990).

**General Anatomy**

The eastern oyster is a monomyarian lamellibranch with a pronounced bilateral asymmetry and a restricted coelom (Seed 1983). The shell of the oyster consists of two calcareous valves joined by a resilient hinge ligament (Carriker 1996). The oyster settles on its left side, the right valve is always uppermost; valves are asymmetrical and the left is larger and more deeply cupped than the right (Kennedy et al. 1996). The mantle is joined at the posterior
margin and forms a cap that covers the mouth and labial palps (Eble and Scro 1996). The adductor muscle, situated at the posterior region of the body, functions to close the shell (Kennedy et al. 1996). Gills of the eastern oyster consist of four demibranchs (folds) of tissue that occupy much of the ventral portions of the mantle cavity. Together with the mantle, they are the chief organs of respiration. They create water currents and move food particles to the labial palps for sorting. The labial palps function as the major conduit for getting food to the mouth (Newell and Langdon 1996).

Reproduction

*Crassostrea virginica* is a protandric species; when individuals first mature they function as males (Mackie 1984). As individuals grow, the proportion of functional females in each size class increases with an excess of females occurring among larger oysters (Galtsoff 1964).

*Crassostrea virginica* is a broadcast spawner with external fertilization (Thompson et al. 1996). Gametogenesis is synchronized so that eggs and sperm are released concurrently to ensure fertilization and maximize the number of zygotes (Thompson et al. 1996). Spawning is induced by environmental cues in the surrounding water and the presence of gametes that stimulate or signal the onset of spawning in adjacent oyster (Kennedy et al. 1996). The resulting planktotrophic larvae develop in the water column (Mann et al. 1994).

*Crassostrea virginica* larvae respond to dissolved chemical cues by moving downward in the water column (Tamburri et al. 1992). The stimuli are peptides released into the water column by adult conspecifics (Zimmer-Faust and Tamburri 1994). These waterborne chemical cues
evoke settlement behavior in oyster larvae and influence oyster settlement in flowing water, as well as, in still water (Tamburri et al. 1996).

**Larval Dispersal and Recruitment**

Oyster larvae live in a complex and dynamic environment. They are subject to macroscale and microscale physical forces (Turner et al. 1994). On a macroscale, they are relatively weak swimmers (ranges: 0.7 – 2 mm s$^{-1}$) in the water column where water is moving in many directions (Mileikovsky 1973). Thus, maximum distribution of larvae and their survival to settlement will be governed by the length of their pelagic existence and the rate and direction of transporting currents (Scheltema 1986). On the microscale, because of their small size, larvae exist in a viscous environment that influences their ability to swim and capture food (Scheltema 1986). Oyster larvae inhabit a world in which they have a very low Reynolds number and are thus affected greatly by flow, sediment loads and other physical factors (Nowell and Jumars 1984; Butman 1987). Flow environments may influence: 1) the delivery of larvae to the substrate, 2) the maintenance of position during and after settlement, and 3) subsequent survival and growth (Nowell and Jumars 1984). Nelson (1953) reported that oyster larvae tend to settle on the undersurfaces of experimental shells in the field to avoid sediment, silt and turbidity. Therefore, oyster larval recruitment is easily influenced by forces such as wind-driven or locally-forced circulations (Fischer et al. 1979).

Planktotrophic larvae of *Crassostrea virginica* feed on phytoplankton, detritus and bacteria (Kennedy et al. 1996). Temperature and food supply affect the length of the larval
period; oyster larvae may settle for up to two weeks after reaching the eyed-larval stage. However, this leads to decreased survival due to increased exposure to predators and disease (Underwood and Fairweather 1989). When competent to settle, pediveliger larvae crawl over hard surfaces sensing cues associated with oyster shells. These shells may have live adult conspecifics or disarticulated shells (Zimmer-Faust and Tamburri 1994). If appropriate stimuli occur, the larva cements its left valve to the shell and metamorphoses. At this stage, it is called a spat (Zimmer-Faust and Tamburri 1994).

**Sediment Impacts**

Oyster settlement and recruitment on oyster beds is greater where shells are abundant and silt deposits and fouling organisms on shells are scarce (MacKenzie 1983). In addition, MacKenzie (1977) and Gunter (1979) found that oyster beds covered with a layer of sediment several centimeters thick and covering less than 2.5 cm on the surface of the shells reduced oyster settlement. This field study compared reefs found in Mississippi where predation in concert with high sediment and silt levels may have contributed to the decline of recruits. It was hypothesized that the settling larvae were killed by abrasion (Gunter 1979).

**Restoration**

Before oyster reef restoration protocols can be established, important questions regarding oyster reef degradation must be addressed. Have sources of larval mortality been identified? If
so, how can the source be deterred? Has there been a change in habitat variables, such as an increase in wave action or sediment loads? If so, what influences do these changes have on larval recruitment? Are these influences negatively impacting the overall productivity of the reef? Answering these questions and establishing best management practices will help determine optimal restoration techniques.

To overcome obstacles believed to contribute to reef decline (such as over-harvesting and habitat decline), several management techniques have been identified. First, settling sites for oyster larvae must be increased (Hargis and Havan 1988). Second, competition, and predation need to be controlled (MacKenzie 1977). In 1991, MacKenzie established guidelines for managing the eastern oyster, which include the following:

1. Accumulation of data on negative physical and biological features of the beds.
2. Evaluation of available and relevant technologies and methods needed to increase oyster abundance.
3. A plan to accomplish the objectives (as determined by the community or park management specialists) within a certain time period, through use of the best strategies with available resources.

Coen and Luckenbach (2000) stated that ecologically motivated restoration of oyster reef habitat will continue be a growing practice in the United States for at least four reasons: 1) resource-based economics alone do not always justify the practice; 2) increasing aquaculture production of native oysters will continue to ease some of the fishing pressure on wild oyster stock; 3) there is a growing recognition of the ecological importance of oyster reefs in estuarine
and near-shore environments; and 4) some public agencies have begun to require mitigation for disturbance to shallow water habitats, and oyster reefs are a viable option for enhancement and or restoration.

In the long term, restoration protocols can be established once important questions are answered based on quantitative data. Therefore, the primary objectives of this research were to quantify recruitment, survival, relative water motion, and sediment loads within and between oyster reefs of Mosquito. Using data collected, a new restoration protocol was then developed for the conditions specific to Mosquito Lagoon. Additionally, laboratory experiments were conducted to determine the effects of sediment levels and water motion on larval settlement.

Table 1: Timeline of research efforts

<table>
<thead>
<tr>
<th>Timeline of study</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season and Year</td>
<td></td>
</tr>
<tr>
<td>Summer 2001</td>
<td>Settlement and survival field study</td>
</tr>
<tr>
<td>Winter 2002 - 2002</td>
<td>Settlement and survival field study</td>
</tr>
<tr>
<td>Spring 2002</td>
<td>Settlement and survival field study</td>
</tr>
<tr>
<td>Summer 2002</td>
<td>Prepare restoration mats</td>
</tr>
<tr>
<td>Winter 2002 – Spring 2003</td>
<td>Prepare restoration mats</td>
</tr>
<tr>
<td>Spring 2003</td>
<td>Deploy restoration mats</td>
</tr>
<tr>
<td>Summer 2003 – Spring 2004</td>
<td>Monitor restoration mats</td>
</tr>
<tr>
<td>Fall 2003</td>
<td>Larval settlement study in lab</td>
</tr>
</tbody>
</table>
Recruitment and survival on impacted versus unimpacted oyster reefs

Recruitment and survival were monitored within Mosquito Lagoon to determine if these variables differ between impacted reefs (reefs with uncharacteristic dead margins) and unimpacted (pristine) reefs (Fig. 3). Eight oyster reefs were chosen for this study; four replicate reefs had large dead margins and four reefs were in pristine condition (Fig. 4). Additionally, recruitment and survival were monitored at three locations on each reef: 1) seaward (exposed) edge of reef (adjacent to channel), 2) middle reef area (within the dead margin of impacted reefs or the center of the pristine reefs), and 3) protected, back-reef region (Fig. 5).

Recruitment and survival were monitored over 12 months in three eight-week trials. The first trial began on May 6, 2001. The second began on December 15, 2001 and the third trial began on March 6, 2002. Twenty-four 0.5 m$^2$ frames were created using 1.9 cm (0.75 inch) PVC pipe and black plastic mesh (Vexar: 1.0 cm opening) that was attached to the PVC with cable ties (Ortega and Sutherland 1992) (Fig. 6). Twenty-five, clean oyster shells were drilled and attached with cable ties to the Vexar mesh of each frame in a 5 X 5 array. Half of the shells were attached with the inside of the valve facing the Vexar. The remaining shells had the inside of the valve exposed for recruitment. All frames were suspended, with shells facing downward, 15 cm off the benthos by cable ties and concrete blocks (20 height X 20 width X 40 cm length). One frame was placed at each of the three locations on each reef (Ortega and Sutherland 1992). Oyster recruitment and survival were monitored weekly using maps created from clear plastic
transparencies and waterproof markers for all eight weeks of each trial. In our analysis however, an individual “survived” only if it remained on the substrate for a minimum of four weeks. Thus, only individuals that settled during weeks one through four were monitored for survival.

**Sediment loads**

Sediment loads were monitored weekly using 24 replicate, cylindrical PVC pipe sediment traps (10 cm diameter X 25 cm deep) submerged flush with substrate using a design described by Lenihan (1999). Traps were capped underwater at the time of retrieval. They were retrieved every 7 days and new traps deployed to replace them. One trap was deployed on the seaward (exposed) region, middle and protected regions on each reef (Fig. 5) on all eight study reefs (Fig. 4). Total sediment mass was determined by drying samples at 60° C for 24 hours in a drying oven (Econotherm Model Number 51221126) and weighing the contents on a top loading balance (O’Haus Scout Model Number SC6010). Grain size was determined by grinding dried sediment and sorting samples with a sieve (0.062 mm) to separate the silt/clay fraction from the sand/grain fraction.

**Relative water motion**

Relative water motion was recorded by measuring the dissolution of replicate calcium sulfate (plaster-of-Paris) spheres each week (Doty 1971; Muus 1968; Thompson and Glenn 1994) that were attached to concrete blocks suspending the oyster frames. To create the spheres,
plaster-of-Paris (Botanicals Art and Science) was poured into 5 cm diameter round candle molds (Candle Crafting Molds, Model Number 110791). A 15 cm long metal rod was placed in the mold while the plaster was setting to create a narrow diameter hole through the center into which a cable tie was later inserted. Spheres were dried at 60° C for 24 hr prior to start of trial in a drying oven and weighed on top-loading balance.

At each 1-week sampling interval, the plaster spheres were removed, dried, and reweighed to determine weight loss (as described above). New spheres were deployed each week on sites 1 (impacted), 4 (unimpacted), 5 (impacted), and 8 (unimpacted) (Fig. 5). Dissolution rates were compared to dissolution at known flow rates in a recirculating flow tank. This provided a relative measure of water motion at each location that incorporated tidal currents, wind-generated waves, and boat wakes.
Figure 3: Oyster reefs. Left: An impacted oyster reef in Mosquito Lagoon with uncharacteristic dead margin. Right: A pristine (unimpacted) reef in Mosquito Lagoon.
Figure 4: This aerial photo shows the eight oyster reefs used in this study. Reefs 1, 2, 5, and 7 were impacted reefs; reefs 3, 4, 6, and 8 were unimpacted reefs. Sediment traps were placed on all eight reefs. Plaster spheres and temperature probes were placed on reefs 1, 4, 5, and 8.
Figure 5: Locations within each oyster reef where experimental frames, plaster-of Paris spheres, temperature probes, and sediment traps were deployed.
Figure 6: Each experimental frame (0.5 m²) was created from plastic mesh (Vexar 1.0 cm opening) and 1.9 cm (0.75 inch) PVC pipe.
RESULTS: RECRUITMENT AND SURVIVAL

Statistical Analyses

Data was analyzed using a 3-way complete analysis of variance (ANOVA) to separately test whether recruitment, percent survival, sediment load, or relative water motion differed among our test reefs. Factors in each ANOVA were: reef type (impacted or unimpacted), location on reef (exposed, middle, or protected), season (summer, winter, spring), and 1) recruitment, 2) percent survival, 3) total sediment load, 4) percent silt/clay, or 6) relative water motion. To test for normality and heterogeneity, Levene’s F and Kolmogorov-Smirnov tests were run. *A posteriori* Bonferroni’s pairwise comparisons were then used to determine differences among the treatments with the level of significance at $\alpha = 0.05$ with 95% confidence intervals.

Recruitment

Mean recruitment did not differ between impacted and unimpacted reefs during this study (Table 2). Recruitment was, however, significantly higher during the summer 2001 trial than the spring 2002 trial ($p = 0.03$; Fig. 7). No recruitment occurred during the winter 2001-2002 trial, so it was not included in the analysis. Recruitment also differed significantly within reef locations for both seasons ($p < 0.0001$); the exposed and protected regions had significantly higher recruitment than the middle regions (Fig. 8).
Table 2: Results of a three-way ANOVA testing whether recruitment varied as a function of reef type (impacted or unimpacted), location on reef (exposed, middle, exposed), and season (summer 2001, spring 2002).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>$F$</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reef type (T)</td>
<td>1</td>
<td>588</td>
<td>0.18</td>
<td>0.6712</td>
</tr>
<tr>
<td>Location on reef (L)</td>
<td>2</td>
<td>6547.5</td>
<td>20.55</td>
<td><strong>&lt; 0.0001</strong></td>
</tr>
<tr>
<td>Season (S)</td>
<td>1</td>
<td>16354</td>
<td>5.1</td>
<td>0.0302</td>
</tr>
<tr>
<td>T X L</td>
<td>2</td>
<td>1469.8</td>
<td>0.46</td>
<td>0.6362</td>
</tr>
<tr>
<td>T X S</td>
<td>1</td>
<td>1656.8</td>
<td>0.52</td>
<td>0.4771</td>
</tr>
<tr>
<td>L X S</td>
<td>2</td>
<td>10343.5</td>
<td>3.22</td>
<td>0.0516</td>
</tr>
<tr>
<td>T X L X S</td>
<td>2</td>
<td>1110.4</td>
<td>0.35</td>
<td>0.7099</td>
</tr>
</tbody>
</table>

*Note:* Significant values are in bold. Data are shown in Fig. 7.
Figure 7: Mean oyster recruitment (± SE) on impacted and unimpacted reefs during the summer 2001 and spring 2002 trials. Letters represent means that are significantly different at the $p \leq 0.05$ level when compared with Bonferroni’s pairwise comparisons.
Figure 8: Mean oyster recruitment (± SE) on three locations within reefs during the summer 2001 and spring 2002 trials. Letters represent means that are significantly different at the $p \leq 0.05$ level when compared with Bonferroni’s pairwise comparisons.
Percent Survival

Percent survival differed significantly for reef type, location within reef, and season, and the interaction between reef type and season (Table 3). Impacted reefs (reefs adjacent to major boating channels) had significantly lower survival than unimpacted reefs (Figure 9; p < 0.0001). The exposed and protected regions of each reef had higher percent survival (Fig. 10; p = 0.0032). In the spring trial, percent survival of oyster recruits was significantly higher than those of the summer trial (p = 0.0001).
Table 3: Results of a three-way ANOVA testing whether percent survival of oyster recruits varied as a function of reef type (impacted or unimpacted), location on reef (exposed, middle, exposed), season (summer 2001, spring 2002)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reef type (T)</td>
<td>1</td>
<td>12875.66</td>
<td>52.48</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Location on reef (L)</td>
<td>2</td>
<td>1662.44</td>
<td>6.78</td>
<td>0.0032</td>
</tr>
<tr>
<td>Season (S)</td>
<td>1</td>
<td>4570.97</td>
<td>18.63</td>
<td>0.0001</td>
</tr>
<tr>
<td>T X L</td>
<td>2</td>
<td>354.78</td>
<td>1.45</td>
<td>0.2488</td>
</tr>
<tr>
<td>T X S</td>
<td>1</td>
<td>4420.07</td>
<td>18.02</td>
<td>0.0001</td>
</tr>
<tr>
<td>L X S</td>
<td>2</td>
<td>24.77</td>
<td>0.10</td>
<td>0.9042</td>
</tr>
<tr>
<td>T X L X S</td>
<td>2</td>
<td>97.04</td>
<td>0.40</td>
<td>0.6762</td>
</tr>
</tbody>
</table>

*Note: Significant values are in bold. Data are shown in Figs. 9 and 10.*
Figure 9: Percent oyster survival (± SE) for the summer and spring trials on impacted and unimpacted reefs. Letters represent means that are significantly different. Capitol letters refer to seasonal differences, lower case letters refer to differences between reef type (impacted vs. unimpacted) at the p ≤ 0.05 level when compared with Bonferroni’s pairwise comparisons.
Figure 10: Percent oyster survival (± SE) during the summer and spring trials on the exposed, middle, and protected reef regions. Letters represent means that are significantly different at the $p \leq 0.05$ level when compared with Bonferroni’s pairwise comparisons.
Sediment loads

Total dried sediment load was significantly higher on impacted reefs adjacent to major boating channels (impacted reefs; p = 0.050; Table 4). In addition to sediment loads being significantly higher on impacted reefs, sediment loads differed significantly within reef locations (exposed, middle, and protected). The exposed regions of impacted reefs had greater sediment loads than the protected regions and the middle regions (exposed > protected > middle; p < 0.0001; Table 4; Fig. 11). This significant pattern was also observed for the unimpacted reefs, however the exposed regions experienced similar sediment loads as the protected regions (exposed = protected > middle; p < 0.0001). Comparing seasonally, sediment loads were significantly higher during summer 2001 trial than winter 2001-2002 and spring 2002 (Figs 11, 12, 13). Sediment loads were similar during the winter 2001-2002 and spring 2002 trials.
Table 4: Results of a three-way ANOVA testing whether sediment loads on oyster reefs varied as a function of reef type (impacted or unimpacted), location on reef (exposed, middle, exposed), season (summer 2001, winter 2001-2002; spring 2002)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>$F$</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reef type (T)</td>
<td>1</td>
<td>740.38</td>
<td>4.02</td>
<td>0.0500</td>
</tr>
<tr>
<td>Location on reef (L)</td>
<td>2</td>
<td>6195.40</td>
<td>33.64</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Season (S)</td>
<td>2</td>
<td>1726.69</td>
<td>9.38</td>
<td>0.0003</td>
</tr>
<tr>
<td>T X L</td>
<td>2</td>
<td>1062.91</td>
<td>5.77</td>
<td>0.0540</td>
</tr>
<tr>
<td>T X S</td>
<td>1</td>
<td>120.35</td>
<td>0.65</td>
<td>0.5243</td>
</tr>
<tr>
<td>L X S</td>
<td>4</td>
<td>182.07</td>
<td>0.99</td>
<td>0.4216</td>
</tr>
<tr>
<td>T X L X S</td>
<td>4</td>
<td>75.57</td>
<td>0.41</td>
<td>0.8004</td>
</tr>
</tbody>
</table>

*Note:* Significant values are in bold. Data are shown in Figs. 11, 12, 13.
Figure 11: Mean sediment load (± SE) during the summer (2001) on the exposed, middle, and protected reef regions of impacted and unimpacted reefs. Impacted reefs are those adjacent to major boating channels; unimpacted reefs are not adjacent to boating channels. Capitol letters represent means that are significantly different between reef type. Lower case letters represent means that are significantly different of the within reef locations at the $p \leq 0.05$ level when compared with Bonferroni’s pairwise comparisons.
Figure 12: Mean sediment load (± SE) during the winter (2001-2002) on the exposed, middle, and protected reef regions of impacted and unimpacted reefs. Impacted reefs are those adjacent to major boating channels; unimpacted reefs are not adjacent to boating channels. Capitol letters represent means that are significantly different between reef type. Lower case letters represent means that are significantly different of the within reef locations at the $p < 0.05$ level when compared with Bonferroni’s pairwise comparisons.
Figure 13: Mean sediment load (± SE) during the spring (2002) on the exposed, middle, and protected reef regions of impacted and unimpacted reefs. Impacted reefs are those adjacent to major boating channels; unimpacted reefs are not adjacent to boating channels. Capitol letters represent means that are significantly different between reef type. Lower case letters represent means that are significantly different of the within reef locations at the $p \leq 0.05$ level when compared with Bonferroni’s pairwise comparisons.
**Percent silt and clay**

Silt and clay are components of sediment that are smaller than sand and grain particles. Sand and grain particles have an effective diameter between 2 mm and 63 µm; silt/clay diameters are < 62 µm (Levinton 2001). Percent silt/clay of the total sediment load differed significantly between reefs adjacent to major boating channels with uncharacteristic dead margins (impacted), and unimpacted reefs, with greater percent silt/clay on impacted reefs (p = 0.0139; Table 5; Figs. 14, 15, 16). Percent silt/clay amount was similar between summer, winter and spring trials even though total sediment loads were much greater during the summer trial than the winter and spring (p = 0.4321). Percent silt/clay differed significantly within reef locations (p = 0.0025). There were also significant interactions between reef type, location on reef and reef type and season (Table 5).
Table 5: Results of a three-way ANOVA testing whether percent silt/clay of sediment loads on oyster reefs varied as a function of reef type (impacted or unimpacted), location on reef (exposed, middle, exposed), season (summer 2001, winter 2001-2002; spring 2002)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reef type (T)</td>
<td>1</td>
<td>258.42</td>
<td>6.46</td>
<td><strong>0.0139</strong></td>
</tr>
<tr>
<td>Location on reef (L)</td>
<td>2</td>
<td>268.19</td>
<td>6.71</td>
<td><strong>0.0025</strong></td>
</tr>
<tr>
<td>Season (S)</td>
<td>2</td>
<td>34.09</td>
<td>0.85</td>
<td>0.4321</td>
</tr>
<tr>
<td>T X L</td>
<td>2</td>
<td>264.85</td>
<td>6.62</td>
<td><strong>0.0027</strong></td>
</tr>
<tr>
<td>T X S</td>
<td>1</td>
<td>287.50</td>
<td>7.19</td>
<td><strong>0.0017</strong></td>
</tr>
<tr>
<td>L X S</td>
<td>4</td>
<td>61.66</td>
<td>1.54</td>
<td>0.2032</td>
</tr>
<tr>
<td>T X L X S</td>
<td>4</td>
<td>70.18</td>
<td>1.75</td>
<td>0.1515</td>
</tr>
</tbody>
</table>

*Note: Significant values are in bold. Data are shown in Figs. 14, 15, 16.*
Figure 14: Percent silt/clay (± SE) during the summer (2001) on the exposed, middle, and protected reef regions of impacted and unimpacted reefs. Impacted reefs are those adjacent to major boating channels; unimpacted reefs are not adjacent to boating channels. Capitol letters represent means that are significantly different between reef type: A > B (p = 0.139); Lower case letters represent means significantly differ on the within reef locations at the p ≤ 0.05 level when compared with Bonferroni’s pairwise comparisons.
Figure 15: Percent silt/clay (± SE) during the winter (2001-2002) on the exposed, middle, and protected reef regions of impacted and unimpacted reefs. Impacted reefs are those adjacent to major boating channels; unimpacted reefs are not adjacent to boating channels. Capitol letters represent means that are significantly different between reef type. Lower case letter represent means that are significantly different on within reef locations at the p < 0.05 level when compared with Bonferroni’s pairwise comparisons.
Figure 16: Percent silt/clay (± SE) during the spring (2002) on the exposed, middle, and protected reef regions of impacted and unimpacted reefs. Impacted reefs are those adjacent to major boating channels; unimpacted reefs are not adjacent to boating channels. Capitol letters represent means that are significantly different between reef type. Lower case letters represent means significantly different on within reef location at the p ≤ 0.05 level when compared with Bonferroni’s pairwise comparisons.
Relative water motion

Dissolution rates of plaster-of-Paris spheres, used to determine relative water motion, differed significantly between reef type (impacted > unimpacted; \( p < 0.0001 \)) during all seasons (Table 6). Dissolution rates also differed significantly within reef locations (\( p < 0.0001 \)), where the exposed regions experienced greater relative water motion than the protected or middle regions (\( p < 0.0001 \)). Dissolution rates were significantly higher during the summer 2001 trial, while these rates were similar for winter and spring trials (\( p = 0.0291 \); Figs. 17, 18, 19). A significant interaction was identified for reef type and location on reef.
Table 6: Results of a three-way ANOVA testing whether dissolution rates, used to determine relative water motion on oyster reefs varied as a function of reef type (impacted or unimpacted), location on reef (exposed, middle, exposed), season (summer 2001, winter 2001-2002; spring 2002).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reef type (T)</td>
<td>1</td>
<td>6679.59</td>
<td>33.79</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Location on reef (L)</td>
<td>2</td>
<td>48695.22</td>
<td>246.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Season (S)</td>
<td>2</td>
<td>856.74</td>
<td>4.33</td>
<td>0.0291</td>
</tr>
<tr>
<td>T X L</td>
<td>2</td>
<td>5241.32</td>
<td>26.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T X S</td>
<td>2</td>
<td>292.59</td>
<td>1.48</td>
<td>0.2540</td>
</tr>
<tr>
<td>L X S</td>
<td>4</td>
<td>330.63</td>
<td>1.67</td>
<td>0.2000</td>
</tr>
<tr>
<td>T X L X S</td>
<td>4</td>
<td>125.97</td>
<td>0.64</td>
<td>0.6426</td>
</tr>
</tbody>
</table>

*Note: Significant values are in bold. Data are shown in Figs. 17, 18, 19.*
Figure 17: Mean dissolution of plaster spheres (± SE) used to determine relative water motion during the summer (2001) on the exposed, middle, and protected reef regions of impacted and unimpacted reefs. Impacted reefs are those adjacent to major boating channels; unimpacted reefs are not adjacent to boating channels. Capitol letters represent means that are significantly different between reef type. Lower case letters represent means significantly different on within reef locations at the $p \leq 0.05$ level when compared with Bonferroni’s pairwise comparisons.
Figure 18: Mean dissolution of plaster spheres (± SE) used to determine relative water motion during the winter (2001-2002) on the exposed, middle, and protected reef regions of impacted and unimpacted reefs. Impacted reefs are those adjacent to major boating channels; unimpacted reefs are not adjacent to boating channels. Capitol letters represent means that are significantly different between reef type. Lower case letters represent means significantly different on within reef locations at the p < 0.05 level when compared with Bonferroni’s pairwise comparisons.
Figure 19: Mean dissolution of plaster spheres (± SE) used to determine relative water motion during the spring (2002) on the exposed, middle, and protected reef regions of impacted and unimpacted reefs. Impacted reefs are those adjacent to major boating channels; unimpacted reefs are not adjacent to boating channels. Capitol letters represent means that are significantly different between reef type. Lower case letters represent means significantly different on within reef locations at the p ≤ 0.05 level when compared with Bonferroni’s pairwise comparisons.
DISCUSSION: RECRUITMENT AND SURVIVAL

**Impacted versus unimpacted reefs**

Using aerial photography, Grizzle et al. (2002), found 60 oyster reefs within CANA with significant declines in total reef area. All of these reefs had dead margins consisting of disarticulated shells. The shells created mounds of shells above the living portion of the reef (Grizzle, pers. obs.). These dead margins of shells packed tightly together are uncharacteristic of oyster reefs. All of these reefs were adjacent to major boating channels; including reefs found in narrow channels which they hypothesized were not affected by wind waves. Their historical assessment hypothesized a direct correlation between increased boating activity and total reef area decrease. In their study, Grizzle et al. (2002) provided strong correlative evidence that boating activity has had a detrimental effect on oyster reefs within CANA, and suggested that increased water motion and sediment loads may be major contributors to reef decline. My results support these hypotheses.

One of the primary goals of this study was to determine oyster recruitment and survival differences between intertidal reefs adjacent to major boating channels and reefs not exposed to boating effects in an estuarine system of coastal Florida. In addition to determining if these two types of reefs have significant oyster larval recruitment and survival differences, other variables, including sediment loads, relative water motion, and temperature, within and between each reef were monitored. Knowledge of recruitment, survival, and additional abiotic factors that affect
oyster reefs is critical to understanding oyster reef habitats and ultimately to restoration efforts (Whitlatch and Osman 1999; Coen and Luckenbach 2000).

Recruitment was predicted to be lower on impacted reefs compared to unimpacted reefs, concurring with the results of Lenihan (1999) where recruitment decreased with flow on intertidal reefs in the Neuse River Estuary, North Carolina. Also, O’Beirn et al. (1995) found that oyster recruitment decreased with wave exposure on subtidal oyster reefs in Georgia. In our study, however, recruitment was found to be higher on reefs with an increase in relative water motion. This agrees with studies by Mullineaux and Garland (1993) and Sandford et al. (1994) who found larval recruitment to be greatest where flow rate is faster due to enhanced larval supply in the marine benthic community. Another additional explanation for the higher recruitment on impacted reefs may be the design of recruitment frames placed on each reef. The experimental frames, suspending the oyster shells, provided ample space for larval recruitment on both types of reefs (Fig 6). This design did not accurately represent shell orientation on impacted reefs. Shells on impacted reefs may have reduced surface area caused by shells packed tightly together horizontal to the benthos (Fig 3). Also, impacted reefs have increased shell movement potentially due to increased relative water motion (Walters et al., unpublished) and increased sediment loads (Figs. 11, 12, 13). Additionally, experimental frames restricted shell movement by securing shells to frames. The combination of these factors may have increased larval recruitment on impacted reefs when in fact, recruitment may actually be lower.

Flow and sedimentation had a major influence on oyster survival in this study. On impacted reefs adjacent to major boating channels, percent survival was significantly lower (Figure 10). During all trials, low oyster survival correlated with high relative water motion, high
sediment loads, and high percent silt and clay levels. The incidence of lower percent survival allied to an increase in sediment loads concur with the findings of MacKenzie (1977, 1983, 1996), Gunter (1979), Kennedy et al. (1996), and Perret et al. (1999). MacKenzie (1996b) hypothesized that the increase in sediment load may kill settled larvae by abrasion. Additionally, these results are similar to those found by Konar and Roberts (1996), and Airoldi and Cineli (1997) on sessile organisms on rocky habitats, and by Loya (1976) in coral reef communities where burial and abrasion by sedimentation increased mortality of sessile invertebrates.

Dead margins of impacted reefs in Mosquito Lagoon differed from the well-documented, long-term growth pattern of a dead region surrounded by live oysters on unimpacted reefs (Grizzle et al. 2002). Differences observed within reef locations, particularly the middle of the dead region on impacted reefs, highlights the almost complete lack of recruitment and survival on these mounds of disarticulated shells. The hypothesis that the middle region of impacted reefs was unsuitable for larval recruitment was confirmed as significantly lower relative water motion was observed on the middle regions of impacted reefs compared to the middle regions of unimpacted reefs (Figs. 17, 18, 19). Few, if any, larvae would not be able to reach, nor settle on this region.
Implications for restoration

Seasonal differences were identified for recruitment, percent survival, sediment loads, and relative water motion (Tables 2, 3, 4, 6). These differences strongly suggest three major components of restoration in this unique area. First, to avoid significantly higher sediment loads and relative water motion, and to increase the survival of oyster larvae, restoration efforts should begin during the spring months. Sediment loads and relative water motion were significantly lower during the spring, whereas percent survival was significantly higher. Second, to increase larval set, restoration efforts should be focused on the exposed regions of unimpacted reefs, where oyster recruitment is greatest, avoiding the negative effects of sedimentation and water flow. Finally, as shell retention is critical in providing available substrate for larval recruitment, shells must be attached to the substrate in a manner that mimics shell attachment and orientation of the unimpacted reefs. To maintain and restore oyster reefs in Virginia, North Carolina, and South Carolina, piles of shells or other material have been deployed with little attention given to the physical structure of the habitat being created (Lenihan 1999). This physical structure is critical to restoring intertidal oyster reefs in this unique location as the impact of boat wakes negatively effects shell retention (Walters et al. 2002).

The motivations for this study were: 1) determine differences between impacted and unimpacted reefs, and 2) determine if any seasonal differences were identified for recruitment, percent survival, sediment loads, and relative water motion that may influence restoration protocols. The major findings of this study highlight significant differences between impacted reefs, those adjacent to major boating channels and unimpacted reefs, while providing a
dependable foundation on which restoration efforts and protocol may be built. More importantly, this study provides strong evidence that boating activity has detrimental effects on the intertidal oyster reefs of this location.
METHODS: RESTORATION

Restoration

6,930 disarticulated oyster shells were used to create restoration mats. Half of the shells used were collected from the surrounding lagoon area and half were donated by Mr. Harry Price, a local oysterman. Shells ranged in size from 6 – 13.5 cm with an mean size of 8.9 cm (± 1.9 S.E). After removing all attached organisms with a toothbrush and running water, a 1.2 cm diameter hole was drilled in the middle each shell with a drill press.

A total of 360 restoration mats (45 X 45 cm) were created using Vexar mesh (1.0 cm openings), cable ties, and cleaned, drilled oyster shells (Fig. 20). Shells were randomly placed and tightly attached perpendicular to the mesh using cable ties in one of three densities: 16, 25, and 36 shells. Mats with no shells were used as controls.

Equal numbers of mats were deployed on April 6, 2003 on six oyster reefs previously determined to provide optimal larval settlement. Mats were placed at each of the six sites in the same randomized experimental design (Fig. 21). Equal numbers of mats of each shell density were placed on the exposed regions of reefs. Two circular, irrigation weights (diameter: 20 cm) and cable ties (length: 28 cm; strength: 80kg) were used to secure each mat in the field. At each site, 24 mats were monitored for recruitment each month. The same mats were monitored at 1, 2, 3, 4, 6 and 9 months. The total number of oysters on each mat were visually counted and recorded.
Figure 20: Experimental restoration mats (45 cm X 45 cm) with 3 different shell densities (16, 25, and 36 shells). Mats with no shells (controls) were also included in this study (not pictured).
Figure 21: Randomized design of restoration mats on the seaward edge of 6 reefs in Mosquito Lagoon. Numbers indicate shell densities on mats. Stars (*) show mats monitored for oyster recruitment.
RESULTS: RESTORATION

*Restoration Mats*

Data was analyzed using a 2-way ANOVA with month and number of shells per mat as fixed factors. To test for normality and heterogeneity, Levene’s F and Kolmogorov-Smirnov tests were run. Bonferroni comparisons of means on the number of recruited live oysters at each date were conducted to determine which groups were significantly different. Knowing this, first a one-way ANOVA was run to show no difference between sites (p = 0.2048).

Significant differences were identified for shell number (p = 0.0001) and date (p < 0.0001) (Table 7). June, October, and February had significantly higher numbers of live oysters than May, July, and August (Fig. 22). Additionally, the 36 density shell mats showed significantly higher number of live oysters during all six monitoring periods than the 25, 16, and 0 shell densities (36 > 25 > 16 > 0). An interaction was identified between shell density and month.
Table 7: Results of two-way ANOVA testing whether total number of live oysters on restoration mats varied over time.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell Density (A)</td>
<td>9837132</td>
<td>5</td>
<td>1967426</td>
<td>7.029466</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Month (B)</td>
<td>17430746</td>
<td>3</td>
<td>5810249</td>
<td>20.75958</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>A * B</td>
<td>11190769</td>
<td>9</td>
<td>563267</td>
<td>12.62968</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>4198241</td>
<td>15</td>
<td>279882.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>42656888</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 22: Settlement of live oysters (± SE) on experimental restoration mats during 2001-2002. Total number of oysters monitored on mats with 0, 16, 25, and 36 shell densities. No oysters attached to mats with 0 shells. Capitol letters represent means that are significantly different between months at the p ≤ 0.05 level when compared with Bonferroni’s pairwise comparisons.
DISCUSSION: RESTORATION MATS

The motivation for this study was to increase oyster reef habitat and potentially reduce degradation of oyster reefs that have declined over the last 50 years in Mosquito Lagoon. Oyster reef restoration has been recognized as an important need by resource management agencies in many states along the Atlantic and Gulf of Mexico coasts of the United States (Breitburg et al. 2000). Most efforts have been directed at increasing or maintaining oyster habitat (MacKenzie 1989; Luckenbach et al. 1999; Coen and Luckenbach 2000). Our study focused on creating small reefs that mimicked the natural three-dimensional structure of intertidal oyster reefs in Mosquito Lagoon. Coen and Luckenbach (2000) state that providing sufficient quantities of substrate with three-dimensional relief is necessary to sustain oyster populations in areas with adequate recruitment rates and is critical to oyster reef restoration.

The experimental design of this study tested which shell density was optimum for restoration mats placed on reefs where oyster larval set is highest. As hypothesized, the 36-shell density restoration mats provided the maximum shell area for larval recruitment while retaining the ability to be moved, post-recruitment, to areas slatted for restoration. My specific recommendation to use the 36-shell density design will allow for significantly greater larval recruitment than the 25 or 16 shell density design over the ten month period studied.

The sharp decline in live oysters on all restoration mats (36, 25, and 16 shell densities) during July and August 2002 is mostly likely due to predation by crabs and competition with barnacles and other sessile invertebrates (Fig. 22). All densities of shells experienced similar effects of predation and competition. However, the restoration mats were of sufficient size and
shell density for the oyster population to recover was apparent by the total increase in live oysters on the mats from October 2002 through February 2003.

Previous restoration efforts have shown that intertidal oyster reefs can be restored as long as the substrate resists subsidence and extends above bottom waters (Lenihan and Peterson 1998; Lenihan 1999; Leard et al. 1999; Perret et al. 1999). More specifically, these oyster reefs restoration efforts in North Carolina, South Carolina, Florida, and Louisiana, have increased the productivity of depleted reefs and the longevity of reef habitat. In our study, the unique design of shells attached perpendicular to the vexar mesh, met this requirement while allowing for the mats to be placed in different locations as restoration needs change. Coen and Luckenbach (2000) stressed the importance of long-term monitoring in evaluating the success of any reef habitat restoration project. Because oyster recruitment and survival patterns vary on both a spatial and temporal scale, continued monitoring is required to ensure effective restoration practices (Christensen et al. 1996; Peterson and Lubchenco 1997; Lenihan and Peterson 1998; Lenihan 1999; Coen et al. 1999; Coen and Luckenbach 2000). The restoration efforts mentioned above and our current study, help to improve existing reef productivity and maintain biodiversity in estuarine systems through continued monitoring and evaluation of restoration protocol.

Results from this restoration experiment indicated how a restoration protocol for this unique intertidal region encompasses: 1) appropriate substrate and substrate orientation, 2) shell density which maximizes surface area and oyster larval recruitment without increasing mortality, 3) timing and placement of restoration mats to achieve optimal larval set, and 4) the ability for mats to be moved post-recruitment to areas where restoration is needed. The relationship between oyster reef restoration and the benefits to both ecological and economic functions
provide unparallel opportunities to improve our understanding of future restoration efforts and the ecological role of oyster reefs in coastal systems. Critical to this relationship is continued monitoring in quantifiable ways that express both the benefits of oyster reef restoration and directions of future studies.
METHODS: LARVAL SETTLEMENT IN THE LABORATORY STUDY

Experiments were performed at the University of Central Florida’s Research Station in Canaveral National Seashore (28° 54’ N, 80° 49’ W) on September 20 - 21, 2003. Larvae from the Middle Peninsula AquaCulture (MPA, North, VA) were used in both still-water and flowing-water experiments. Competent larvae, 16 days old and with a distinct eyespot, were shipped on ice via overnight courier on September 19, 2003 from MPA to the University of Central Florida. Larvae were kept refrigerated at 10° C until experiments were run (18 – 36 hrs after delivery). Cold storage of up to 98 hours has been shown to be effective in maintaining oyster larvae with no decrease in setting success (Holiday et al. 1991).

One hour before each run, larvae were brought up to 24° C by placing them in filtered lagoon water (salinity 30-33‰; 24-28° C). Over the course of approximately 60 minutes, larvae were repeatedly observed under a dissecting microscope (2.5 X) to determine larval activity. At least 50% of the observed larvae had to be swimming or crawling on the bottom of the observation chamber before experimentation began (Tamburri et al. 1996). Estimates of larval abundance in each trial were made by averaging counts of six 0.5 ml aliquots pipetted from a suspension of larvae in 1000 ml of filtered lagoon water (Tables 8, 9). For all trials, larvae were suspended in a beaker and slowly poured from the beaker (over 5-10 sec) into the container 4 cm above the bottom (Tamburri et al. 1996).

Sediment was collected from the exposed regions of oyster reefs of Mosquito Lagoon. Sediment loads were normalized by total volume of water in the tank and tub. Three replicate trials were conducted with three levels of sediment for both the still-water and flowing water
experiments. Three trials had no sediment, 3 trials had low sediment loads (8 g/ml wet weight for flowing-water and still-water), and 3 trials with high sediment loads, (16 g/ml wet weight for flowing-water and still-water). The order of trial runs was randomly selected.

Prior to running the still and flow trials, 1,890 disarticulated oyster shells were cleaned to remove any and all macroflora or macrofauna and placed into the Lagoon for two weeks to establish a natural biological film. Immediately prior to use, shells were visually inspected, and any with attached macroflora or macrofauna were not used. Lagoon water, obtained from the waters adjacent to the research station (salinity 30-33‰; 24-28 °C), was filtered with a 25 micron mesh bag filter (Aquatic Eco-systems, Model number N1025) to remove sediments. New filtered lagoon water was added for each run. The duration of each trial was 60 min.

Still-water experiments were conducted simultaneously with the flowing-water experiments. Nine trials were conducted in a plastic tub (Sterilite Clearview 63 L, Model number 1753; 55 X 37 X 30 cm). For each trial, 20.0 liters of filtered lagoon water were added. The depth of water added was 10 cm. Seventy oyster shells, half of which had the inside of the valve facing up and half had the outside of the valve facing up, covered the bottom of the container.

Flowing-water experiments were conducted in a recirculating raceway flume at a flow rate of 5 cm/s. The flow rate is the average flow rate found within Mosquito Lagoon on a calm, spring day (L.Walters, pers. com.). The flume was 20-cm wide, consisting of two semicircular ends (20-cm radius at inner walls) and two straight sections, 120-cm long (Tamburri et al. 1996). Water flow was generated through the use of a motor-driven paddle wheel. To reduce across-stream fluid motion, polycarbonate sheeting was place parallel to the curved flume walls upstream of the working area to act as flow straighteners. 140 oyster shells, half of which had the
inside of the valve facing up and half had the outside of the valve facing up, covered the bottom of the container. For the flowing-water trials, 80 L of filtered lagoon water was added for a depth of 10 cm.

After each trial, shells were gently removed from water and observed under a dissecting microscope to identify newly settled individuals. Settlers were counted as individuals in which there was plantigrade attachment to the shell with the foot (Turner et al. 1994). After each trial, the plastic container and the flume were rinsed with freshwater and dried completely to remove any remaining larvae and sediment.
Table 8: Experimental conditions for flume trials.

1 = no sediment (0 g/ml)

2 = low sediment (8 g/ml)

3 = high sediment amount (16 g/ml)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Date</th>
<th>No. of Larvae added (± SD)</th>
<th>No. of Larvae added / L water</th>
<th>Speed (cm s⁻¹)</th>
<th>Sediment load</th>
<th>Temp. ºC</th>
<th>Salinity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9/20/2003</td>
<td>13,005 ± 5505</td>
<td>162 ± 92</td>
<td>5</td>
<td>1</td>
<td>28</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>9/20/2003</td>
<td>15,000 ± 8591</td>
<td>188 ± 144</td>
<td>5</td>
<td>3</td>
<td>28</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>9/20/2003</td>
<td>14,500 ± 6197</td>
<td>182 ± 104</td>
<td>5</td>
<td>1</td>
<td>28</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>9/20/2003</td>
<td>11,750 ± 5232</td>
<td>147 ± 88</td>
<td>5</td>
<td>2</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>9/20/2003</td>
<td>12,750 ± 1103</td>
<td>160 ± 139</td>
<td>5</td>
<td>2</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>9/21/2003</td>
<td>10,500 ± 5282</td>
<td>132 ± 89</td>
<td>5</td>
<td>3</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>9/21/2003</td>
<td>12,500 ± 3507</td>
<td>157 ± 59</td>
<td>5</td>
<td>2</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>9/21/2003</td>
<td>10750 ± 2208</td>
<td>135 ± 37</td>
<td>5</td>
<td>3</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>9/21/2003</td>
<td>6750 ± 3643</td>
<td>85 ± 61</td>
<td>5</td>
<td>1</td>
<td>24</td>
<td>30</td>
</tr>
</tbody>
</table>

Average 11,975 ± 4585  150 ± 90
Table 9: Experimental conditions for still-water trials.

1 = no sediment (0 g/ml)

2 = low sediment (8 g/ml)

3 = high sediment amount (16 g/ml)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Date</th>
<th>No. of Larvae added (± SD)</th>
<th>No. of Larvae added / L water</th>
<th>Speed (cm s^{-1})</th>
<th>Sediment load</th>
<th>Temp. ºC</th>
<th>Salinity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9/20/2003</td>
<td>4,333 ± 611</td>
<td>216 ± 69</td>
<td>0</td>
<td>1</td>
<td>28</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>9/20/2003</td>
<td>5000 ± 955</td>
<td>250 ± 94</td>
<td>0</td>
<td>3</td>
<td>28</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>9/20/2003</td>
<td>4833 ± 689</td>
<td>241 ± 119</td>
<td>0</td>
<td>1</td>
<td>28</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>9/20/2003</td>
<td>3917 ± 581</td>
<td>195 ± 89</td>
<td>0</td>
<td>2</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>9/20/2003</td>
<td>4250 ± 1228</td>
<td>212 ± 104</td>
<td>0</td>
<td>2</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>9/21/2003</td>
<td>3500 ± 587</td>
<td>175 ± 128</td>
<td>0</td>
<td>3</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>9/21/2003</td>
<td>4167 ± 390</td>
<td>207 ± 96</td>
<td>0</td>
<td>2</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>9/21/2003</td>
<td>3583 ± 245</td>
<td>179 ± 88</td>
<td>0</td>
<td>3</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>9/21/2003</td>
<td>2250 ± 405</td>
<td>113 ± 76</td>
<td>0</td>
<td>1</td>
<td>24</td>
<td>30</td>
</tr>
</tbody>
</table>

Average 3981 ± 632  198 ± 95
RESULTS: LARVAL SETTLEMENT IN THE LABORATORY STUDY

Data was analyzed using a 2-way full model analysis of variance (ANOVA) to test whether settlement of larvae of *Crassostrea virginica* differed due to flow rate and sediment load. Factors were: flow rate (flowing-water: 5 cm/s and still-water: 0 cm/s) and sediment load (high, low, and no sediment). To test for normality and heterogeneity, Levene’s F and Kolmogorov-Smirnov tests were run. A Bonferroni’s pairwise comparison was then used to determine levels of significant differences between factors at a significance level at $\alpha = 0.05$ with 95% confidence intervals.

Larval settlement differed significantly due to both flow rate and sediment load (Table 10). Larval settlement was significantly lower in flowing-water compared to still-water ($p = 0.002$, Fig. 23). The highest sediment load tested significantly impacted larval settlement. Larval settlement was significantly higher in no sediment compared to low and high sediment loads ($p < 0.0001$). Larval settlement in low sediment loads was similar to settlement in no sediment trials.
Table 10: Results of a two-way ANOVA testing whether larval settlement varied as a function of flow rate (5 m/s or 0 m/s), sediment loads (no, low, high)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>$F$</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment Load (S)</td>
<td>2</td>
<td>164786.17</td>
<td>180.28</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Flow Rate (F)</td>
<td>1</td>
<td>23544.5</td>
<td>15.47</td>
<td>0.0020</td>
</tr>
<tr>
<td>S X F</td>
<td>2</td>
<td>17470.5</td>
<td>11.48</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*Note: Significant values are in bold. Data are shown in Fig. 10.*
Figure 23: Mean larval settlement (± SE) in flowing-water and still-water trials. Letters represent means that are significantly different. Capitol letters refer to flow rate differences, lower case letters refer to differences due to sediment loads at the $p \leq 0.05$ level when compared with Bonferroni’s pairwise comparisons.
DISCUSSION: LARVAL SETTLEMENT IN THE LABORATORY STUDY

Our results show larval settlement of *Crassostrea virginica* is negatively affected by increased water flow and increased sedimentation. These findings concur with the findings of Shelbourne (1957), Seliger et al. (1982), Nowell and Jumars (1984), and Lenihan (1999), where larval settlement decreased with either greater flow rates or higher sediment loads. In our study, flow had a major influence on oyster larvae settlement in a laboratory setting. The still-water trials had almost twice as many larvae settle per trial compared to the flowing-water results (Fig. 23). Often, changes in the flow rates explain differences in sedimentation rates on oyster reefs (Lenihan 1999). However, few studies have quantified their negative effects (Bahr and Lanier 1981; Kennedy and Sandford 1999; Grizzle et al. 2002). Understanding and quantifying the negative effects of increased water motion and high levels of sedimentation on larval settlement and potentially oyster survival is critical to determining which mechanism(s) cause oyster reef declines (Grizzle et al. 2002).

Wave action (i.e. increased flow rate) may lead to an accumulation of sediment eventually smothering oysters and high turbidity may decrease larval set (Kennedy and Sandford 1999; Bartol et al. 1999). Additionally, Bahr and Lanier (1981) state flow rates higher than a threshold level comprised of both water current or wave energy will prevent the development of a reef. In our study, that threshold, where settlement was significantly lower than still-water, was 5 cm/s. This determination is the first step in quantifying the potentially negative effects of increased water motion, by motor boats for example, on larval settlement in field conditions. The flow rate used in our study was similar to flow rates found within Mosquito Lagoon. In a recent
study by Grizzle et al. (2002), they urge future studies to quantify the negative effects of increased flow rate and high levels of sedimentation which may be the mechanism(s) causing oyster reef decline in Mosquito Lagoon. They hypothesized that boating activity may have detrimental effects on some of the oyster reefs in their historical study.

Another hypothesized mechanism for inhibiting reef development is smothering caused by sediment transport (Churchill 1920; Marshall 1954; Bahr and Lanier 1981) and sediment movement inhibiting larval settlement (Gunter 1979). In our study, larval settlement decreased with increasing sediment loads. These high levels of sedimentation reduced larval set by altering surface topology. Altering surface topography influences larval settlement (Walters 1992) and sediment in constant motion may cause mortality of settling larvae by abrasion (MacKenzie 1996b). Either one of these explanations is plausible and may explain the significant difference between treatments in this study.

One interesting and noteworthy point of this study is the similarity of larval settlement in response to high and low levels of sedimentation. Both treatments significantly reduced settlement compared to the ‘no sediment’ treatment. This response was similar in flowing-water and in the still-water trials. These results strongly suggest the extreme detrimental effects sedimentation may have even in low amounts. The negative effects of sedimentation on oyster reef have been noted in field studies (MacKenzie 1977; Gunter 1979; MacKenzie 1983; MacKenzie 1996a, 1996b; Bartol et al. 1999; Lenihan 1999). In all of these studies, researchers suggest that productivity of oyster reefs is much higher when sediment levels are low.

This study was the first step in identifying and quantifying the potential negative effects of increased sedimentation levels and flow rates similar to those found in Mosquito Lagoon.
These conditions may be associated with the increase in boating activity of recent years.

Knowledge of larval settlement in site-specific conditions is critical to understanding regional population dynamics (Coen and Luckenbach 2000) and will aid in the development of management and restoration plans for this unique intertidal system.
REFERENCES


Kennedy VS, Newell RIE and Eble AF 1996. The eastern oyster. Maryland Sea Grant, College Park, MD.


70


