

**EVALUATION OF EASTERN OYSTERS, *Crassostrea virginica* (GMELIN, 1791), RESTORATION TECHNIQUES FOR USE IN INTERTIDAL SOUTHEASTERN UNITED STATES HABITATS CHARACTERIZED BY HEAVY SILTATION RATES**

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## Abstract

Restoration of eastern oyster, *Crassostrea virginica*, habitat within intertidal ecosystems bordering the southeastern United States provides a unique set of challenges. Heavy siltation can smother newly constructed reefs complicating restoration efforts. The purpose of this research was to assess intertidal restoration techniques in environments characterized by high levels of sediment deposition or migration. Experimental treatments were placed along the intertidal bank of Stacy Creek, Sapelo Island, Georgia prior to the oyster spawning season in April 2004. Treatment types employed were commercial spat sticks arranged in densities of 81 and 25/m<sup>2</sup>, crab traps containing whelk shell, fresh oyster shell, or washed (aged) oyster shell, and plastic mesh bags with fresh oyster shell. Two randomly selected replicates per treatment type were examined after one and two years. Commercial spat sticks in 81/m<sup>2</sup> densities out performed remaining treatment types and recorded the greatest biomass (119.5 ± 2.05 kg), live oyster density (4,794 ± 164/m<sup>2</sup>), oyster shell height (85.62 ± 0.02 mm), and the lowest oyster mortality rate (5.33 ± 0 %) per treatment replicate (mean ± S.E.). This research indicated that the vertical distance from the sediments and the refuge provided by commercial spat stick treatments enhanced oyster settlement and growth and reduced oyster mortality associated with predation and physiological stress from siltation.

## Introduction

The eastern oyster, *Crassostrea virginica* (Gmelin, 1791), is distributed from the Gulf of St. Lawrence in Canada to the Yucatan peninsula and the West Indies and has been reported along the coast of Brazil with introductions into the northwest Pacific of the U.S. (Bahr and Lanier 1981; Carriker and Gaffney, 1996). The geographical range and regional distribution of *C. virginica* result in regional differences in reproduction, shell morphology, and reef formation (Galtsoff 1964; Bahr and Lanier 1981; Carriker and Gaffney 1996; Thompson *et al.* 1996; White and Wilson 1996; Kennedy and Sanford 1999). *C. virginica* is distributed predominantly intertidally along the coast of states bordering the South Atlantic Bight (North Carolina, South Carolina, Georgia, and northeast Florida) and forms dense fringing or patch reefs (Stephenson and Stephenson 1952; Bahr and Lanier 1981; Kennedy and Sanford 1999; Coen and Luckenbach 2000).

As an indicator species, oysters not only reflect the health of the ecosystem in which they exist but serve a functional and ecological role within that ecosystem. Oyster reefs provide nesting habitat, settlement areas, and refuge for numerous species of fish and invertebrates (Wenner *et al.* 1996; Breitburg 1999; Coen *et al.* 1999; Coen and Luckenbach 2000; Posey *et al.* 1999; Peterson *et al.* 2003; Grabowski and Powers 2004). Oysters are filter feeders and significantly improve water quality by enhancing nutrient cycling and reducing turbidity through biodeposition (Brooks 1891; Newell 1988; Newell *et al.* 2002; Pietros and Rice 2003; Newell and Koch 2004; Newell *et al.* 2004). Research has also suggested that oyster reefs provide structural stability within estuaries by dispersing wave energy in a relatively soft mud environment and can shield salt marsh habitat from erosion associated with heavy boat traffic and winter storms (Coen and Luckenbach 2000).

It is generally perceived that Georgia's oyster populations were once immense, and that overfishing earlier in the last century, disease, storms, and alterations to water quality and natural flow regimes associated with coastal development, have reduced oyster populations by as much as 90% (Oemler 1894, Kirby 2004, Beck *et al.* 2009). This estimate is based on a few coast wide oyster surveys and reports (Drake 1981, Galstoff and Luce 1930, Harris 1980, Linton 1969, Oemler 1894, Walker and Cotton 2001). Recent GIS mapping of intertidal oyster habitat by the University of Georgia Marine Extension Service has however seriously undermined the confidence that should be

attributed to acreage losses based on the sampling methodologies and technologies used by these surveys (Power *et al.*, in press).

Several restoration techniques have been developed in states bordering the northeast Atlantic to address declining oyster populations such as oyster gardening and shell relaying (Kennedy and Sandford 1999; Wesson *et al.* 1999). In Georgia substrate availability is a restriction to re-establishing oyster reefs not larval supply, therefore oyster gardening may be viewed as an unnecessary practice. Oyster reefs reach their greatest density and biomass in the southeastern United States particularly in South Carolina and Georgia (Dame *et al.* 1984). The spawning season in Georgia is long and generally extends from April through October with two peaks in recruitment in late summer and in early to mid fall (Heffernan *et al.* 1989; O'Beirn *et al.* 1996a, b; Thoresen *et al.* 2005). There is intense oyster recruitment during this period and spat densities can reach an average of around 7,000 m<sup>2</sup> (O'Beirn *et al.* 1996a) with recorded recruitment rates as high as 204,700 m<sup>2</sup> per month (Thoresen *et al.* 2005).

Oyster habitat restoration programs in Georgia generally collect oyster shells from private roasts and restaurants (Bioremediation of Beach Creek, Jekyll Island, through Shellfish Restoration. Final Report to GA EPD 319 (h). November 2009. A. Power. University of Georgia Marine Extension Service.). Collected shell is then cured for 2-3 months and placed in plastic mesh bags for planting. Restored oyster reefs are constructed in the intertidal zone at a suitable oyster habitat site just prior to the natural oyster spawning season. Reefs constructed using the bag method have been very successful, however, there are some complications associated with this methodology. Bag-built reefs may undergo periods of heavy silt accumulation due to the increased sedimentation rate observed in the southeastern coastal salt marsh ecosystem and therefore can be covered with marsh mud over a relatively short period of time reducing or eliminating the total area available for oysters and other organisms to settle. The purpose of this research was to evaluate alternative oyster habitat restoration techniques for these challenging environments.

## Materials and Methods

Work was carried out from April 2004 to April 2006 at one site along the Duplin River within the Sapelo Island National Estuarine Research Reserve (SINERR), Sapelo Island, Georgia (Figure 1). The study was initiated in mid-April 2004 to take advantage of the spring oyster recruitment period (Heffernan *et al.* 1989, O'Beirn *et al.* 1997). The research site where artificial oyster habitat was constructed and tested was located at the mouth of Stacy Creek at N31°27.617 W081°16.873. Stacy Creek is a small tidal creek located on the western bank of the Duplin River. This site was selected because Stacy Creek is sheltered from storm and boat waves, had no naturally occurring live oyster reef, and drains directly into the Duplin River.

This study evaluated six different structural types of intertidal oyster cultch. Four replicates of each treatment were deployed. Three treatments included crab traps (61cm<sup>3</sup> with 3.8 cm diameter mesh plastic coated wire) with rebar bottom weights that were filled individually with either washed oyster shell (sun-bleached oyster shell washed onto the high marsh during winter storms) from natural deposits (Lunz 1958), fresh shell collected from oyster roasts or restaurants, or processed knobbed whelk, *Busycon carica*, shell. Treatment four included 4 mesh bags filled with fresh oyster shell. Treatments five and six included French style oyster spat collectors deployed in density arrays of 25 spat sticks/m<sup>2</sup> and 81 spat sticks/m<sup>2</sup>. The oyster spat collectors used in this study were longitudinally grooved P.V.C. infused with calcium carbonate (O'Beirn *et al.* 1997).

Four replicates of each of the six treatments were deployed according to random selection as established by a random numbers table. Treatments were placed on a mud creek bank at approximately two hours above the mean low water mark in a series parallel to Stacy Creek at a tidal height where oysters naturally occur in Georgia (Bahr and Lanier 1981). The replicates that incorporated crab traps were placed on two plastic Vexar oyster-growing bags (1 m x 0.5 m) that were filled with washed oyster shell to increase surface area under the treatments and offset the weight of the traps and shell material to prevent them from sinking into the mud substrate. Oyster bag treatments filled with fresh oyster shell were placed on Jute mesh to offset the weight of the treatments and prevent the treatment from sinking in the mud substrate. Commercial spat

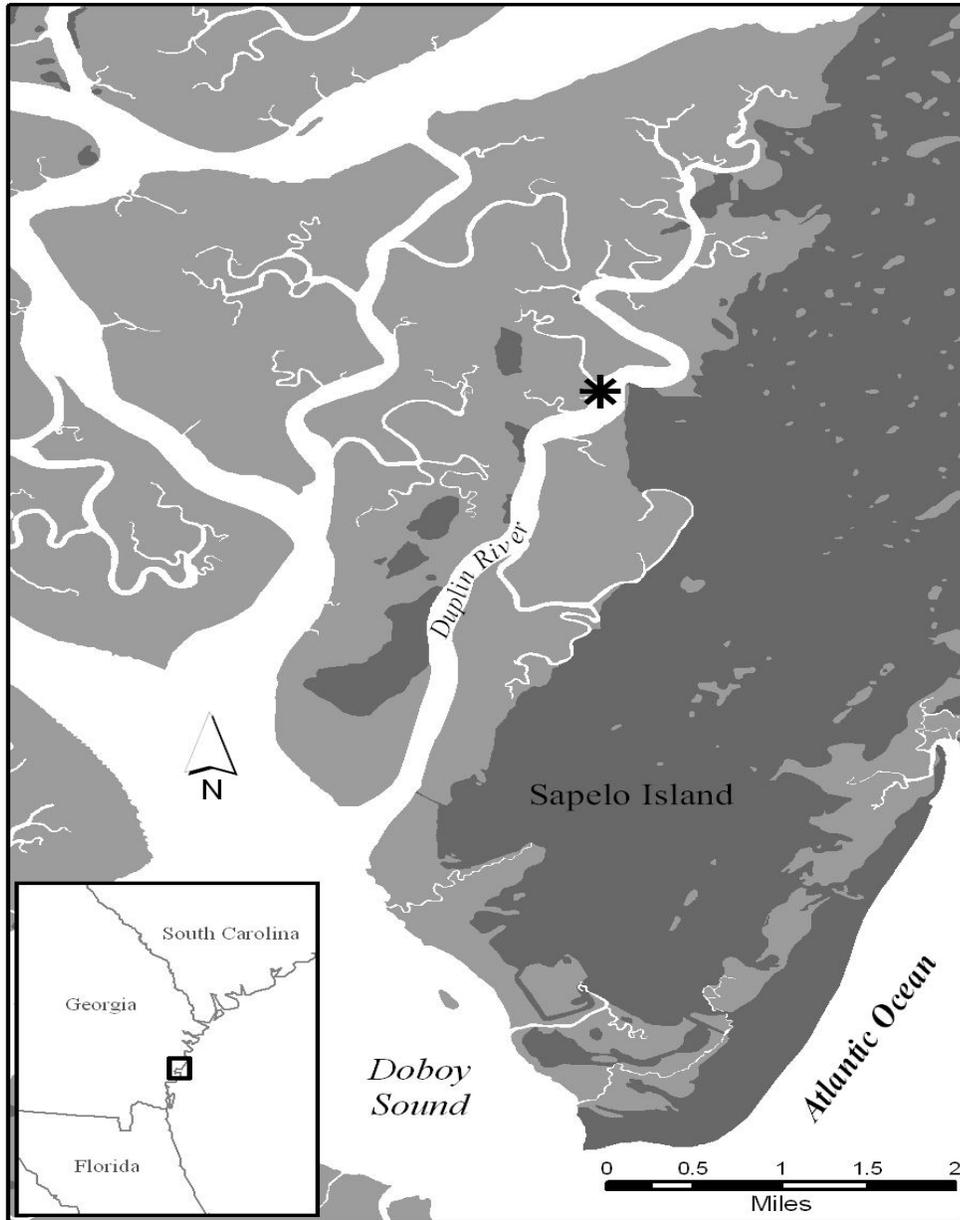


Figure 1. Map of the Duplin River; indicating the project site in Stacy Creek at N31°27.617 W081°16.873.

\* Denotes project site.

collectors 91.4 cm in length were placed in eight of the twenty-four plots with four replicates of spat sticks in densities of 25/m<sup>2</sup> (tubes every 15.2 cm apart per 1 m<sup>2</sup>) and four replicates of spat sticks in densities of 81/m<sup>2</sup> (tubes every 7.6 cm apart per 1 m<sup>2</sup>). The spat collectors were inserted into a wooden grid (to ensure accuracy of spacing during deployment) and collectors extended 61 cm out of the mud substrate to maintain a vertical height equal to that of the crab traps. Each replicate per treatment was weighed prior to and after deployment to determine biomass in kilograms. A standardized weight was determined for each replicate by calculating the mean weight in kilograms for each treatment type. Two replicates per treatment were randomly extracted after one year in April 2005. The remaining plots were terminated in April 2006.

Crab trap, bag, and spat stick treatments were processed by removing all contents within and on the treatments and placing the material in a sieve with 1 mm<sup>2</sup> mesh. Cultch material was cleaned in a sieve by spraying or washing with freshwater and collected fauna were weighed along with each of the constituent materials (i.e. crab trap and rebar weight, bag mesh, spat sticks). Sieved materials collected from each treatment were weighed along with each associated replicate. To determine total increase in biomass, the standardized weight for each treatment was subtracted from the total weight of each replicate collected during each sampling period.

Total number of live oysters was determined by counting all live oysters on cultch and structural materials for each treatment. One hundred oysters were randomly selected from each replicate and measured for oyster height and length in mm (Galtsoff 1964) to determine oyster size and shell morphology associated with each treatment. Oyster mortality was determined as a percentage by dividing the number of dead oysters from total oysters counted x 100 from a random sample within each replicate for each treatment. Oyster height was determined as the maximal dorsoventral dimension perpendicular to the hinge and length was the maximal anterior-posterior dimension of the shell parallel to the hinge (Carriker, 1996). Oyster shell morphology was compared between treatments to determine the level of density related crowding of oysters by calculating the mean height (mm) to length (mm) ratio as well as evaluating oyster shell morphology using linear regression.

All biological materials were closely inspected for associated oyster reef species and sieved materials were evaluated to determine general species and phylum richness between treatments; however total numbers of individuals for each species was not quantified.

Comparisons between treatments and for each treatment between years were conducted using an analysis of variance with a nested design (SAS Institute 1989). Data were rank transformed and outputs were express via Duncan's Multiple Range Test. Rank transformation was required due to the high number of individual treatments, limited number of replicates per year, and the effect of time. Spearman's rho correlation was used to examine the general relationships between all evaluated parameters. Microsoft Excel (Microsoft) was also used while analyzing data.

Physical water quality data was provided by the Sapelo Island National Estuarine Research Reserve, Sapelo Island, Georgia. Water temperature and salinity were collected in situ using an YSI Sonde remote sensing unit, which was stationed in the Duplin River, Georgia at Marsh Landing.

## **Results**

### **Physical Data**

Water temperature reflected seasonal patterns with mean highs of  $28.5 \pm 0.02^{\circ}\text{C}$  and  $30.3 \pm 0.01^{\circ}\text{C}$  occurring during July 2004 and August 2005, respectively and low temperatures reaching  $11.8 \pm 0.06^{\circ}\text{C}$  in January 2005 and  $12.3 \pm 0.03^{\circ}\text{C}$  in January 2006 (Figure 2). Mean salinity reflected typical estuarine salinity and ranged from a high of  $28.03 \pm 0.04$  PSU (May 2004) to a low of  $18.7 \pm 0.12$  PSU (April 2005) (Figure 2).

### **Number of Live Oysters, Growth, and Mean Biomass**

Spat sticks in densities of  $81/\text{m}^2$  had the greatest mean number of live oysters, mean oyster shell height, and mean biomass of all treatment types during 2005 ( $2,645 \pm 260$  oysters per replicate,  $77.09 \pm 1.74$  mm, and  $80.95 \pm 9.35$  kg) and 2006 ( $4,794 \pm 164$  oysters per replicate,  $85.62 \pm 0.02$  mm, and  $119.45 \pm 2.05$  kg) sampling periods (Figures 3 and 4). Oyster shell height increased significantly between years in  $81/\text{m}^2$  spat stick treatments ( $p = 0.0043$ ) and shell height was significantly greater ( $p = 0.0001$ ) in this treatment than any other treatment types, excluding spat

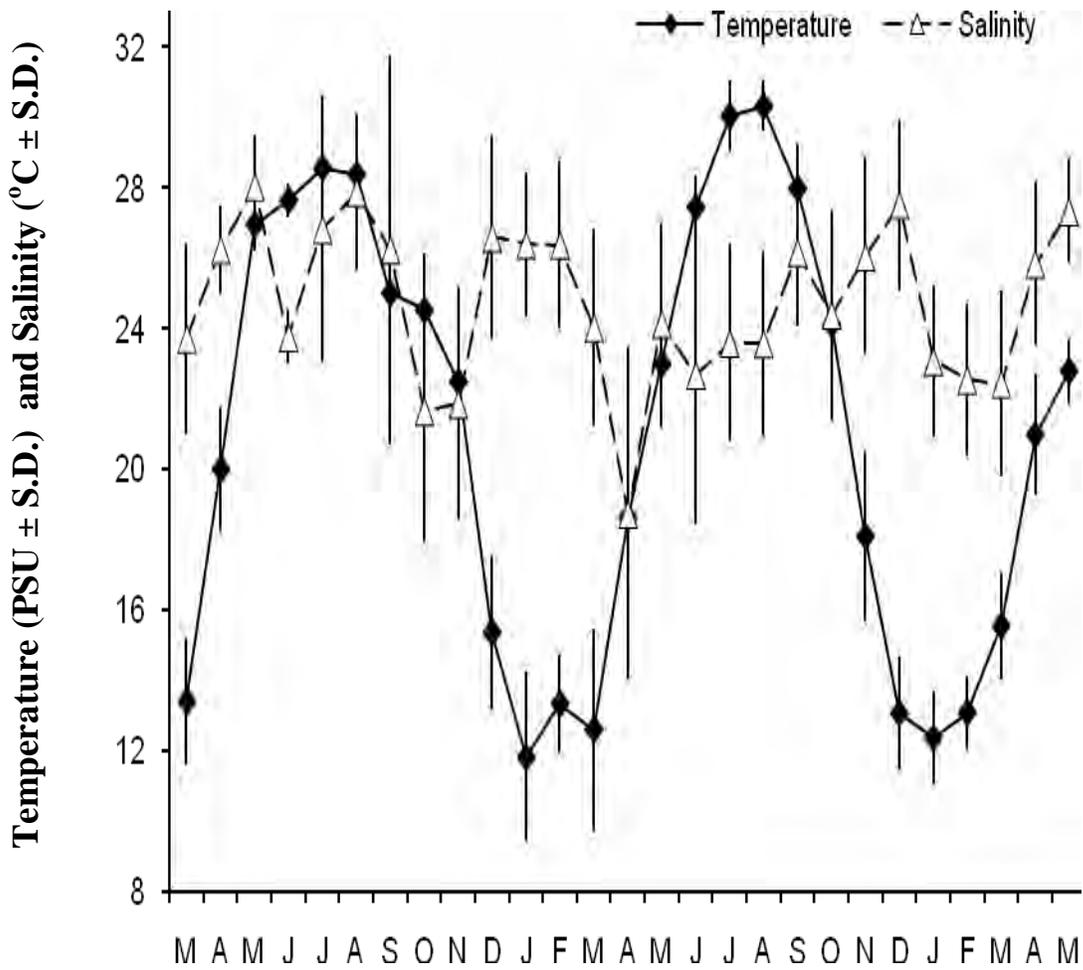


Figure 2. Mean salinity (PSU  $\pm$  S.D.) and temperature ( $^{\circ}$ C  $\pm$  S.D.) recorded in the Duplin River at Marsh Landing from March 2004-May 2006.

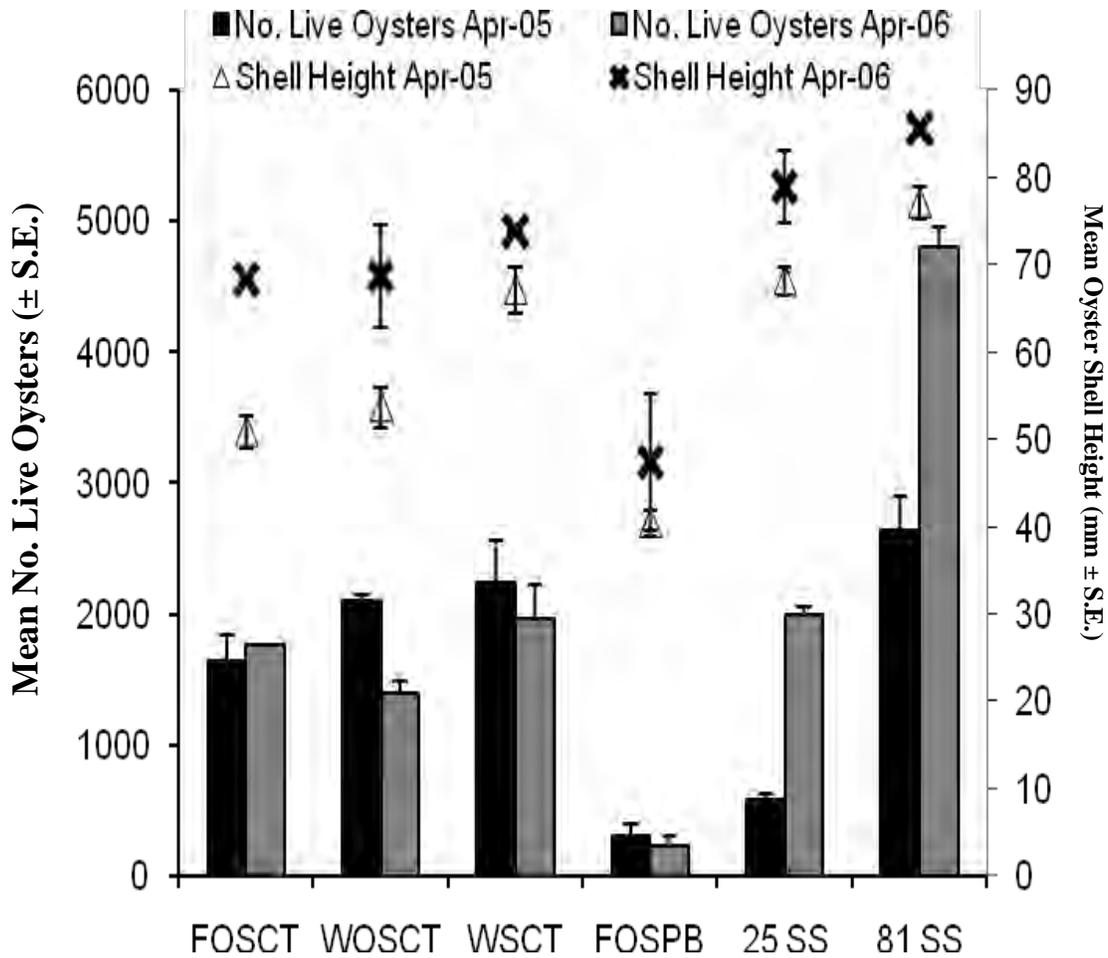


Figure 3. Mean number of live oysters ( $\pm$  S.E.) and mean oyster shell height (mm  $\pm$  S.E.) on crab trap with fresh oyster shell (FOSCT), crab trap with washed oyster shell (WOSCT), crab trap with whelk shell (WSCT), plastic mesh bags with fresh oyster shell (FOSP), 25 spat sticks/m<sup>2</sup> (25 SS), and 81 spat sticks/m<sup>2</sup> (81SS) treatment types during April 2005 and 2006 sampling periods.

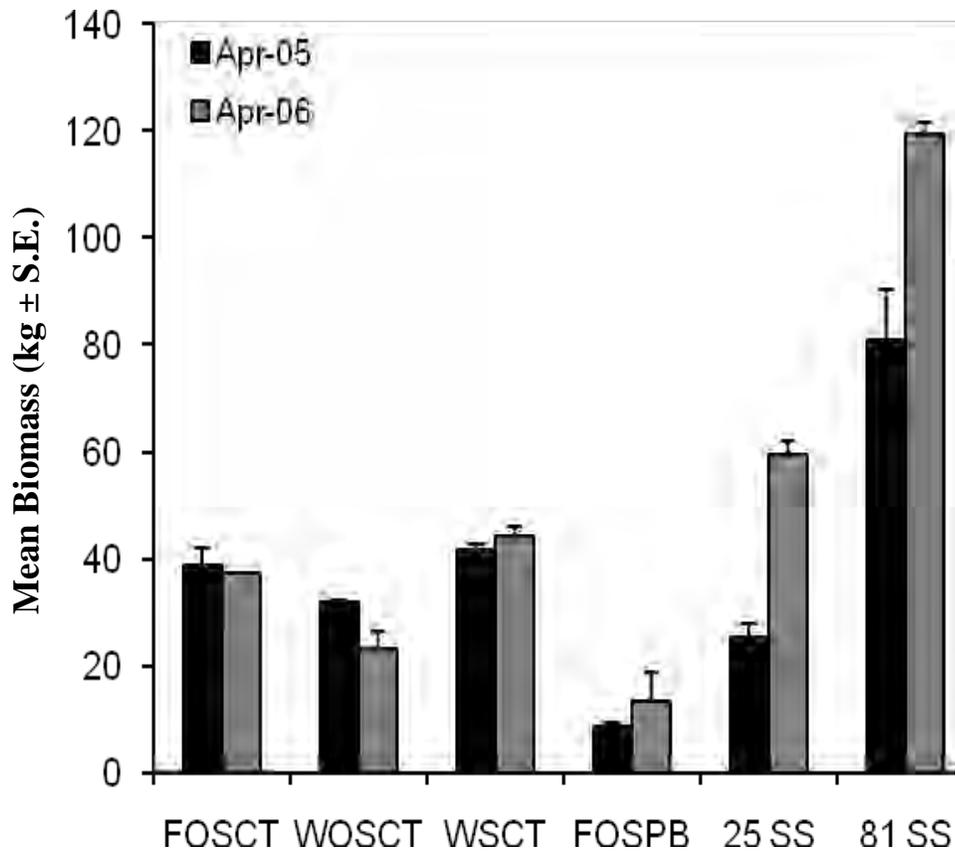


Figure 4. Mean biomass ( $\pm$  kg) for crab trap with fresh oyster shell (FOSCT), crab trap with washed oyster shell (WOSCT), plastic mesh bags with fresh oyster shell (FOSPB), crab trap with whelk shell (WSCT), 25 spat sticks/m<sup>2</sup> (25 SS), and 81 spat sticks/m<sup>2</sup> (81SS) treatment types at Stacy Creek during April 2005 and 2006 sampling periods.

sticks in densities of 25/m<sup>2</sup> (Tables 1 and 2). Oyster shell height increased significantly ( $p = 0.0001$ ) between April 2005 ( $68.18 \pm 1.60$  mm) and 2006 ( $78.98 \pm 4.14$  mm) in spat stick density treatments of 25/m<sup>2</sup> (Figure 3 and Table 1). Spat sticks in densities of 25/m<sup>2</sup> were the only treatment type to significantly increase in the mean number of live oysters ( $p = 0.0205$ ) and mean biomass ( $p = 0.0093$ ) between 2005 ( $578.5 \pm 54.5$  oysters per replicate;  $25.34 \pm 2.55$  kg) and 2006 ( $1,990 \pm 71$  oysters per replicate,  $59.55 \pm 2.55$  kg) (Figure 3 and 4 and Table 1).

Plastic mesh bags with fresh oyster shell had the lowest mean number of live oysters, oyster shell height, and biomass of all treatments during April 2005 ( $302 \pm 91.5$  oysters per replicate,  $40.34 \pm 1.44$  mm, and  $8.63 \pm 0.85$  kg) and 2006 ( $227 \pm 79$  oysters per replicate,  $47.35 \pm 7.87$  mm, and  $13.53 \pm 5.25$  kg) (Figures 3 and 4). Oyster shell height was significantly lower ( $p = 0.0001$ ) in plastic mesh bags with oyster shell than in any other treatment types during both years; however there was a significant increase ( $p = 0.0004$ ) in shell height within this treatment type between years (Figure 3 and Tables 1 and 2).

During the 2006 sampling period, one replicate representing the crab trap with fresh oyster shell treatment type was lost in transport from the field site to the processing facility henceforth values representing this particular treatment type will only represent a solitary replicate 2006. Oyster shell height was significantly greater ( $p = 0.0001$ ) in crab trap treatments with whelk shell than remaining crab trap treatments during 2005 (Table 2). Oyster shell height increased significantly between years for crab traps with whelk ( $p = 0.0001$ ) ( $67.12 \pm 2.67$  mm, 2005;  $73.87 \pm 0.31$  mm, 2006), fresh oyster ( $p = 0.0001$ ) ( $50.88 \pm 1.80$  mm and  $68.35$  mm, respectively), and washed oyster shell ( $p = 0.0001$ ) ( $53.66 \pm 2.31$  mm and  $68.69 \pm 5.83$  mm, respectively) (Figure 3 and Table 1). Crab traps with whelk shell experienced a decrease in live oysters between 2005 and 2006 ( $2,236.5 \pm 317.5$  and  $1,961.5 \pm 257.5$  oysters per replicate, respectively) and a slight increase in mean biomass ( $41.58 \pm 1.35$  kg and  $44.38 \pm 1.65$  kg, respectively) (Figures 3 and 4). Traps with fresh shell had a much lower mean number of live oysters ( $1,642.5 \pm 196.5$  oysters) and a slightly higher mean biomass ( $38.96 \pm 3$  kg) than traps with washed shell ( $2,093.5 \pm 59.5$  oysters per replicate;  $31.81 \pm 0.5$  kg) during 2005 (Figures 3 and 4). During 2006 the number of live oysters and biomass were greater in fresh shell than in washed shell trap treatments, which significantly decreased ( $p = 0.0299$ ) in oyster density between years (Figures 3 and 4).

Table 1. Mean number of live oysters (NLO), oyster shell height (H), shell height/length ratio (H/L), total biomass (BM), mortality (M), species richness (SR) and phyla richness (PR) between years for 81 spat sticks/m<sup>2</sup> (81SS), 25 spat sticks/m<sup>2</sup> (25 SS), crab trap with whelk shell (WSCT), crab trap with fresh oyster shell (FOSCT), crab trap with washed oyster shell (WOSCT), and plastic mesh bags with fresh oyster shell (FOSPB).

81SS	NLO	H (mm)	H/L	BM (kg)	M (%)	SR	PR
<i>P</i>	0.1548	0.0043*	0.8385	0.1056	0.1510	0.0001*	0.0001*
2005	<sup>A</sup> 2,645.0	<sup>B</sup> 77.09	<sup>A</sup> 2.12	<sup>A</sup> 80.95	<sup>A</sup> 3.33	<sup>B</sup> 17.0	<sup>B</sup> 5.0
2006	<sup>A</sup> 4,794.0	<sup>A</sup> 85.62	<sup>A</sup> 2.13	<sup>A</sup> 119.45	<sup>A</sup> 5.33	<sup>A</sup> 27.0	<sup>A</sup> 7.0
25SS	NLO	H (mm)	H/L	BM (kg)	M (%)	SR	PR
<i>P</i>	0.0205*	0.0001*	0.0001*	0.0093*	0.4482	0.0001*	0.0348*
2005	<sup>B</sup> 578.5	<sup>B</sup> 68.18	<sup>B</sup> 1.70	<sup>B</sup> 25.34	<sup>A</sup> 6.91	<sup>B</sup> 18.0	<sup>B</sup> 5.0
2006	<sup>A</sup> 1,990.0	<sup>A</sup> 78.98	<sup>A</sup> 2.05	<sup>A</sup> 59.55	<sup>A</sup> 9.66	<sup>A</sup> 26.0	<sup>A</sup> 7.0
WSCT	NLO	H (mm)	H/L	BM (kg)	M (%)	SR	PR
<i>P</i>	0.6470	0.0492*	0.9264	0.4929	0.0029*	0.0468*	0.0234*
2005	<sup>A</sup> 2,236.5	<sup>B</sup> 67.12	<sup>A</sup> 2.33	<sup>A</sup> 41.58	<sup>B</sup> 3.36	<sup>B</sup> 17.5	<sup>B</sup> 5.5
2006	<sup>A</sup> 1,961.5	<sup>A</sup> 73.87	<sup>A</sup> 2.32	<sup>A</sup> 44.38	<sup>A</sup> 18.66	<sup>A</sup> 27.5	<sup>A</sup> 7.0
FOSCT	NLO	H (mm)	H/L	BM (kg)	M (%)	SR	PR
<i>P</i>	0.8211	0.0001*	0.0001*	0.8790	0.0393*	0.0001*	0.0001*
2005	<sup>A</sup> 1,642.5	<sup>B</sup> 50.88	<sup>B</sup> 1.64	<sup>A</sup> 38.96	<sup>B</sup> 3.96	<sup>B</sup> 17.0	<sup>B</sup> 6.0
2006	<sup>A</sup> 1,760.0	<sup>A</sup> 68.35	<sup>A</sup> 2.15	<sup>A</sup> 37.26	<sup>A</sup> 15.33	<sup>A</sup> 27.0	<sup>A</sup> 7.0
WOSCT	NLO	H (mm)	H/L	BM (kg)	M (%)	SR	PR
<i>P</i>	0.0299*	0.0001*	0.0072*	0.0887	0.0560	0.0267*	0.0001*
2005	<sup>A</sup> 2,093.5	<sup>B</sup> 53.66	<sup>B</sup> 1.91	<sup>A</sup> 31.81	<sup>A</sup> 5.0	<sup>B</sup> 14.0	<sup>B</sup> 5.0
2006	<sup>B</sup> 1,397.0	<sup>A</sup> 68.69	<sup>A</sup> 2.08	<sup>A</sup> 23.36	<sup>A</sup> 17.66	<sup>A</sup> 26.5	<sup>A</sup> 7.0
FOSPB	NLO	H (mm)	H/L	BM (kg)	M (%)	SR	PR
<i>P</i>	0.5528	0.0004*	0.0024*	0.5528	0.0229*	0.0017*	0.0572
2005	<sup>A</sup> 302.0	<sup>B</sup> 40.34	<sup>B</sup> 1.58	<sup>A</sup> 8.63	<sup>B</sup> 9.0	<sup>B</sup> 3.0	<sup>A</sup> 2.0
2006	<sup>A</sup> 227.0	<sup>A</sup> 47.35	<sup>A</sup> 1.72	<sup>A</sup> 13.53	<sup>A</sup> 34.33	<sup>A</sup> 13.5	<sup>A</sup> 4.5

Results were evaluated using a non-parametric nested design ANOVA. Outputs from the Duncan's multiple range test are given. Treatments with the same letter designation were not significantly different. \* Indicates a significance at the  $P < 0.05$  range.

Table 2. Mean oyster height (H), height/length ratio (H/L), mortality (M), species richness (SR) and phyla richness (PR) between treatments types for 81spat sticks/m<sup>2</sup> (81SS), 25 spat sticks/m<sup>2</sup> (25 SS), crab trap with whelk shell (WSCT), crab trap with fresh oyster shell (FOSCT), crab trap with washed oyster shell (WOSCT), and plastic mesh bags with fresh oyster shell (FOSPB) during the 2005 and 2006 sampling period.

Year: 2005							
	<i>P</i> -Value	Highest Rank					Lowest Rank
H (mm)	0.0001*	81SS	25 SS	WSCT	FOSCT	WOSCT	FOSPB
H/L	0.0001*	WSCT	81SS	WOSCT	25 SS	FOSCT	FOSPB
M (%)	0.0302*	FOSPB	25 SS	WOSCT	FOSCT	WSCT	81SS
SR	0.00798*	FOSCT	WSCT	25 SS	81SS	WOSCT	FOSPB
PR	0.0002*	WSCT	25 SS	FOSCT	81SS	WOSCT	FOSPB
Year: 2006							
	<i>P</i> -Value	Highest Rank					Lowest Rank
H (mm)	0.0001*	81SS	25 SS	WSCT	FOSCT	WOSCT	FOSPB
H/L	0.0001*	WSCT	FOSCT	81SS	WOSCT	25SS	FOSPB
M (%)	0.007*	FOSPB	WSCT	WOSCT	FOSCT	25 SS	81SS
SR	0.03*	81 SS	FOSCT	WOSCT	WSCT	25 SS	FOSPB
PR	0.0001*	81 SS	25SS	FOSCT	WSCT	WOSCT	FOSPB

Data were rank transformed and evaluated using a non-parametric nested design ANOVA. A Duncan's multiple range test was used to determine statistical significance. Treatments types connected by the same line were not significantly different. \* Indicates a significance at the  $P < 0.05$  range.

Mean oyster shell height to length ratio was significantly higher ( $p = 0.0001$ ) in crab traps with whelk shell than on any other treatment type during 2005 ( $2.33 \pm 0.06$ ) and 2006 ( $2.32 \pm 0.08$ ) (Tables 1 and 2). Oysters on plastic mesh bags with fresh shell had a significantly lower ( $p = 0.0001$ ) mean oyster shell height to length ratio than all treatments excluding crab traps with fresh oyster shell during 2005 ( $1.58 \pm 0.03$ ) (Table 2). Oyster shell height to length ratio increased significantly ( $p = 0.0024$ ) between years in mesh bag treatments yet oysters on this treatment type had a significantly lower ( $p = 0.0001$ ) oyster shell height to length ratio than any other treatment type during 2006 ( $1.72 \pm 0.05$ ) (Tables 1 and 2). Shell height to length ratio was significantly greater ( $p = 0.0001$ ) in spat stick density treatments of  $81/\text{m}^2$  than in crab traps with washed oyster shell, and spat sticks in densities of  $25/\text{m}^2$  which were statistically equal to crab traps with fresh oyster shell treatments ( $2.12 \pm 0.04$ ,  $1.91 \pm 0.05$ ,  $1.70 \pm 0.03$ , and  $1.64 \pm 0.04$ , respectively) during 2005 (Table 2). There was a significant increase in shell height to length ratio between years for spat sticks in densities of  $25/\text{m}^2$  ( $p = 0.0001$ ), crab traps with fresh shell ( $p = 0.0001$ ) and washed shell ( $p = 0.0072$ ) (Table 1).

### **Oyster Mortality**

Oyster mortality rates increased significantly between years in plastic mesh bags with fresh oyster shell ( $p = 0.0229$ ), crab traps with whelk shell ( $p = 0.0029$ ), and crab traps with fresh oyster shell ( $p = 0.0393$ ) and was only marginally insignificant in crab traps with washed oyster shell ( $p = 0.0560$ ) (Table 1). Oysters on plastic mesh bags with fresh oyster shell experienced significantly greater mortality ( $p = 0.03$ ) ( $9.0 \pm 1.0$  %) than in crab traps with fresh oyster shell and whelk shell and spat sticks in  $81/\text{m}^2$  densities during 2005 (Figure 5 and Table 2). Oyster mortality during 2005 was significantly higher ( $p = 0.0302$ ) in spat stick densities of  $25/\text{m}^2$  ( $6.91 \pm 1.75$  %) than in spat sticks densities of  $81/\text{m}^2$  ( $3.33 \pm 1.33$  %) and crab traps with whelk shell ( $3.36 \pm 0.03$  %) (Figure 5 and Table 2). Only spat stick density treatments of  $25/\text{m}^2$  ( $9.66 \pm 1.66$ ) and  $81/\text{m}^2$  ( $5.33 \pm 0$  %) had a significantly lower ( $p = 0.007$ ) oyster mortality rate when compared to plastic mesh bags with fresh oyster shell ( $34.33 \pm 7.66$  %), which by far experienced the greatest oyster mortality during 2006 (Figure 5 and Table 2).

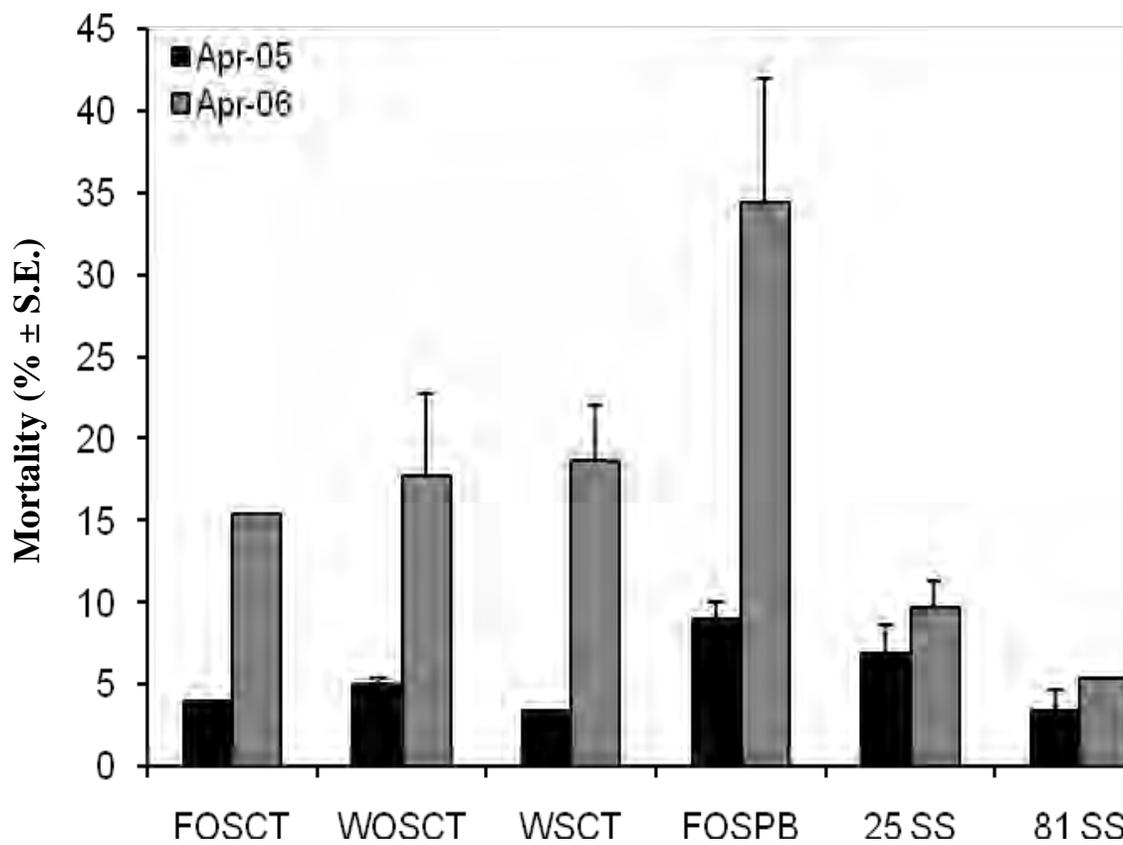


Figure 5. Mean oyster mortality ( $\% \pm$  S.E.) on crab trap with fresh oyster shell (FOSCT), crab trap with washed oyster shell (WOSCT), plastic mesh bags with fresh oyster shell (FOSPB), crab trap with whelk shell (WSCT), 25 spat sticks/m<sup>2</sup> (25 SS), and 81spat sticks/m<sup>2</sup> (81SS) treatment types at Stacy Creek during April 2005 and 2006 sampling periods.

## Species and Phyla Richness

Species richness was significantly higher in crab trap treatments with fresh oyster shell ( $17 \pm 0$ ) and lower in plastic mesh bags with fresh oyster shell ( $p = 0.00798$ ) ( $3 \pm 0$  species) than any other treatment type during the 2005 sampling period (Figure 6 and Table 2). During 2006 species richness was significantly lower in plastic mesh bags with fresh oyster shell treatments ( $p = 0.03$ ) ( $13.5 \pm 0.5$  species) than the spat stick densities of  $81/\text{m}^2$ , fresh oyster shell in crab traps, and washed oyster shell in crab traps (Figure 6 and Table 2). Species richness increased significantly between years for spat stick treatments in densities of  $25/\text{m}^2$  ( $p = 0.0001$ ) and  $81/\text{m}^2$  ( $p = 0.0001$ ), crab traps with whelk shell ( $p = 0.0468$ ), crab traps with fresh oyster shell ( $p = 0.0001$ ), crab traps with washed oyster shell ( $p = 0.0267$ ), and plastic mesh bags with fresh oyster shell ( $p = 0.0017$ ) (Figure 6 and Table 1). There were 22 species and 6 phyla total collectively observed on treatments during 2005, which increased to 30 species and 7 phyla during 2006 (Table 3). Mollusca was the dominant taxon during 2005 and was represented by 8 species followed by arthropoda (6 species), annelida (3 species), bryozoa (2 species), cnidaria (2 species), and chordata (1 species) (Tables 4). Arthropoda was the dominant taxon during 2006 and was represented by 10 species followed by mollusca (7 species), annelida (4 species), chordata (3 species), cnidaria (3 species), bryozoa (2 species), and porifera (1 species) (Table 4).

Phyla richness increased significantly between years in spat stick density treatments of  $25/\text{m}^2$  ( $p = 0.0348$ ) and  $81/\text{m}^2$  ( $p = 0.0001$ ), crab traps with whelk shell ( $p = 0.0234$ ), crab traps with fresh oyster shell ( $p = 0.0001$ ), and crab traps with washed shell ( $p = 0.0001$ ) (Table 1). Number of phyla associated with plastic mesh bag with fresh shell treatments during 2005 ( $p = 0.0002$ ) ( $2 \pm 0$  phyla) and 2006 ( $p = 0.0001$ ) ( $4.5 \pm 0.5$  phyla) was observed to be significantly lower than in any other treatment type (Figure 7 and Table 2). There were significantly fewer ( $p = 0.0002$ ) phyla associated with crab traps with washed oyster shell treatments during 2005 than crab traps with whelk shell and spat stick density treatments of  $25/\text{m}^2$  (Figure 7 and Table 2).

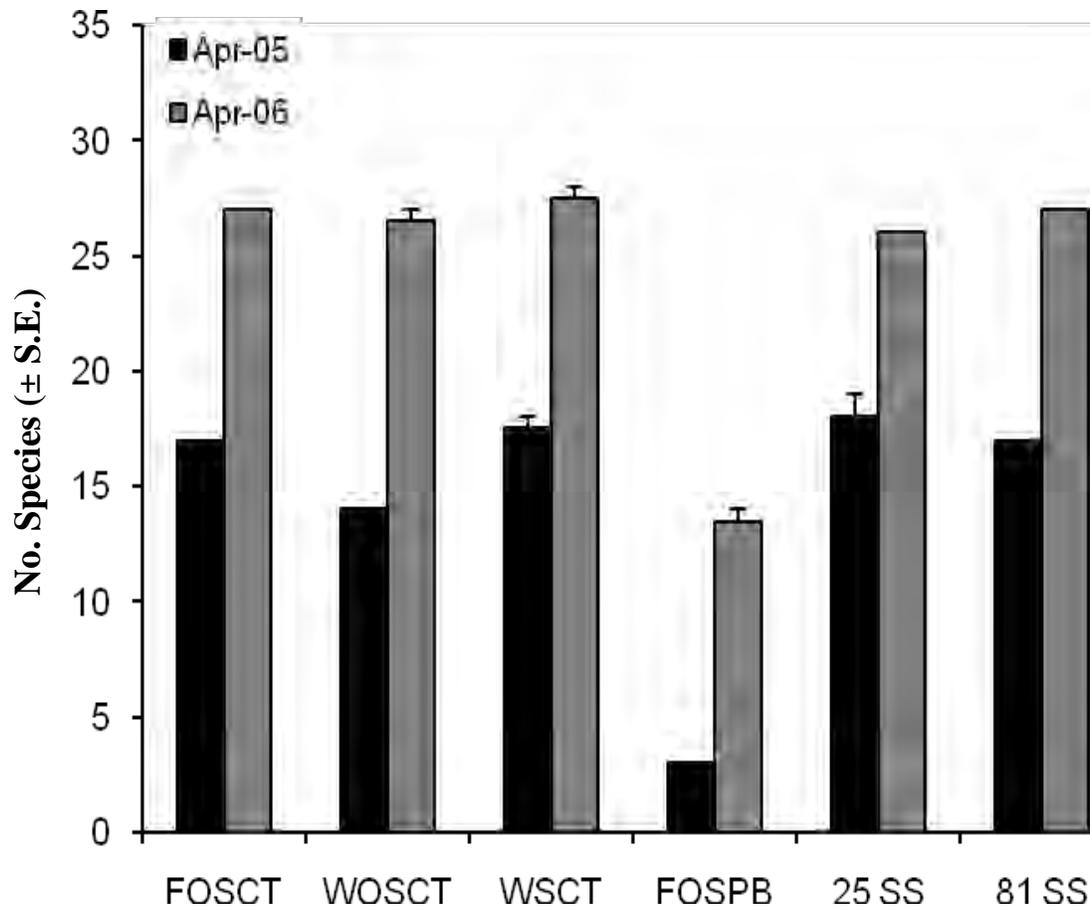


Figure 6. Mean number of species (No. individuals  $\pm$  S.E.) collected from crab trap with fresh oyster shell (FOSCT), crab trap with washed oyster shell (WOSCT), plastic mesh bags with fresh oyster shell (FOSPB), crab trap with whelk shell (WSCT), 25 spat sticks/m<sup>2</sup> (25 SS), and 81 spat sticks/m<sup>2</sup> (81SS) treatment types at Stacy Creek during April 2005 and 2006 sampling periods.

Table 3. Species and phyla list indicating the total number of species that were observed in the 81 spat sticks/m<sup>2</sup> (81SS), 25 spat sticks/m<sup>2</sup> (25 SS), crab trap with whelk shell (WSCT), crab trap with fresh oyster shell (FOSCT), crab trap with washed oyster shell (WOSCT), and plastic mesh bags with fresh oyster shell (FOSPB) treatment types during the 2005 sampling period. The number of species and phyla in each treatment type are indicated at the bottom of the table.

Year: 2005							
Month		81 SS	25 SS	WSCT	FOSCT	WOSCT	FOSPB
Species	Phyla						
<i>Diadumene lineata</i>	Cnidaria	+	+	+	-	-	-
Hydroid	Cnidaria	+	+	+	+	+	-
Filamentous bryozoa	Bryozoa	+	+	+	+	+	-
<i>Membranipora tenuis</i>	Bryozoa	+	+	+	+	+	-
<i>Boonea impressa</i>	Mollusca	+	+	+	+	+	-
<i>Brachidontes exustus</i>	Mollusca	+	+	+	+	+	-
<i>Crassostrea virginica</i>	Mollusca	+	+	+	+	+	+
<i>Crepidula maculosa</i>	Mollusca	+	+	+	+	-	-
<i>Geukensia demissa</i>	Mollusca	+	+	+	+	-	-
<i>Mulinia lateralis</i>	Mollusca	-	+	-	-	-	-
<i>Nassarius obsoletus</i>	Mollusca	+	+	+	+	+	-
<i>Sphenia antillensis</i>	Mollusca	+	+	+	+	-	-
<i>Hydroides</i> sp.	Annelida	-	-	-	-	+	-
<i>Nereis succinea</i>	Annelida	+	+	+	+	+	-
<i>Phyllodoce fragilis</i>	Annelida	-	-	+	-	-	-
<i>Balanus eburneus</i>	Arthropoda	+	+	+	+	+	+
Gammaridian	Arthropoda	+	+	+	-	+	-
<i>Menippe mercenaria</i>	Arthropoda	+	+	+	+	+	-
<i>Panopeus herbstii</i>	Arthropoda	+	+	+	+	+	+
<i>Petrolisthes armatus</i>	Arthropoda	+	+	+	+	+	-
<i>Sphaeroma quadridentata</i>	Arthropoda	-	+	-	-	-	-
<i>Gobiosoma bosci</i>	Chordata	-	-	+	+	-	-
Species		17	19	19	17	14	3
Phyla		5	5	5	6	5	2

+/- indicates species presence or absence during that particular sampling period.

Table 4. Species and phyla list indicating the total number species and phyla that were observed in the 81 spat sticks/m<sup>2</sup> (81SS), 25 spat sticks/m<sup>2</sup> (25 SS), crab trap with whelk shell (WSCT), crab trap with fresh oyster shell (FOSCT), crab trap with washed oyster shell (WOSCT), and plastic mesh bags with fresh oyster shell (FOSPB) treatment types during the 2006 sampling period. The number of species and phyla in each treatment type are indicated at the bottom of the table.

Year: 2006							
Month		81 SS	25 SS	WSCT	FOSCT	WOSCT	FOSPB
Species	Phyla						
<i>Cliona celata</i>	Porifera	+	+	+	+	+	+
Brown Anemone	Cnidaria	+	+	+	+	+	-
<i>Diadumene lineata</i>	Cnidaria	+	+	+	+	+	-
Hydroid	Cnidaria	+	+	+	+	+	-
Filamentous bryzoan	Bryozoa	+	+	+	+	+	-
<i>Membranipora tenuis</i>	Bryozoa	+	+	+	+	+	-
<i>Boonea impressa</i>	Mollusca	+	+	+	+	+	+
<i>Brachidontes exustus</i>	Mollusca	+	+	+	+	+	+
<i>Crassostrea virginica</i>	Mollusca	+	+	+	+	+	+
<i>Geukensia demissa</i>	Mollusca	+	+	+	+	+	+
<i>Littorina meleagris</i>	Mollusca	+	+	+	+	+	-
<i>Nassarius obsoletus</i>	Mollusca	+	+	+	+	+	+
<i>Sphenia antillensis</i>	Mollusca	+	+	+	+	+	-
<i>Hydroides</i> sp.	Annelida	+	+	+	+	+	-
<i>Nereis succinea</i>	Annelida	+	+	+	+	+	+
<i>Phyllodoce fragilis</i>	Annelida	+	+	+	+	+	+
<i>Polydora websteri</i> .	Annelida	+	+	+	+	+	+
<i>Alpheus</i> sp.	Arthropoda	+	+	+	+	+	+
<i>Balanus eburneus</i>	Arthropoda	+	+	+	+	+	+
<i>Callinectes sapidus</i>	Arthropoda	+	-	+	+	+	-
<i>Chthamalus fragilis</i>	Arthropoda	+	+	-	-	-	-
Gammaridian	Arthropoda	+	+	+	+	+	-
<i>Menippe mercenaria</i>	Arthropoda	-	-	-	+	+	-
<i>Palaemonetes pugio</i>	Arthropoda	-	-	+	-	-	-
<i>Panopeus herbstii</i>	Arthropoda	+	+	+	+	+	+
<i>Petrolisthes armatus</i>	Arthropoda	+	+	+	+	+	+
<i>Pinnotheres ostreum</i>	Arthropoda	+	+	+	+	+	+
<i>Gobiosoma bosci</i>	Chordata	+	+	+	+	+	+
<i>Molgula manhattensis</i>	Chordata	+	+	+	+	+	-
<i>Opsanus tau</i>	Chordata	-	-	+	-	-	-
Species		27	26	28	27	27	15
Phyla		7	7	7	7	7	5

+/- indicates species presence or absence during that particular sampling period.

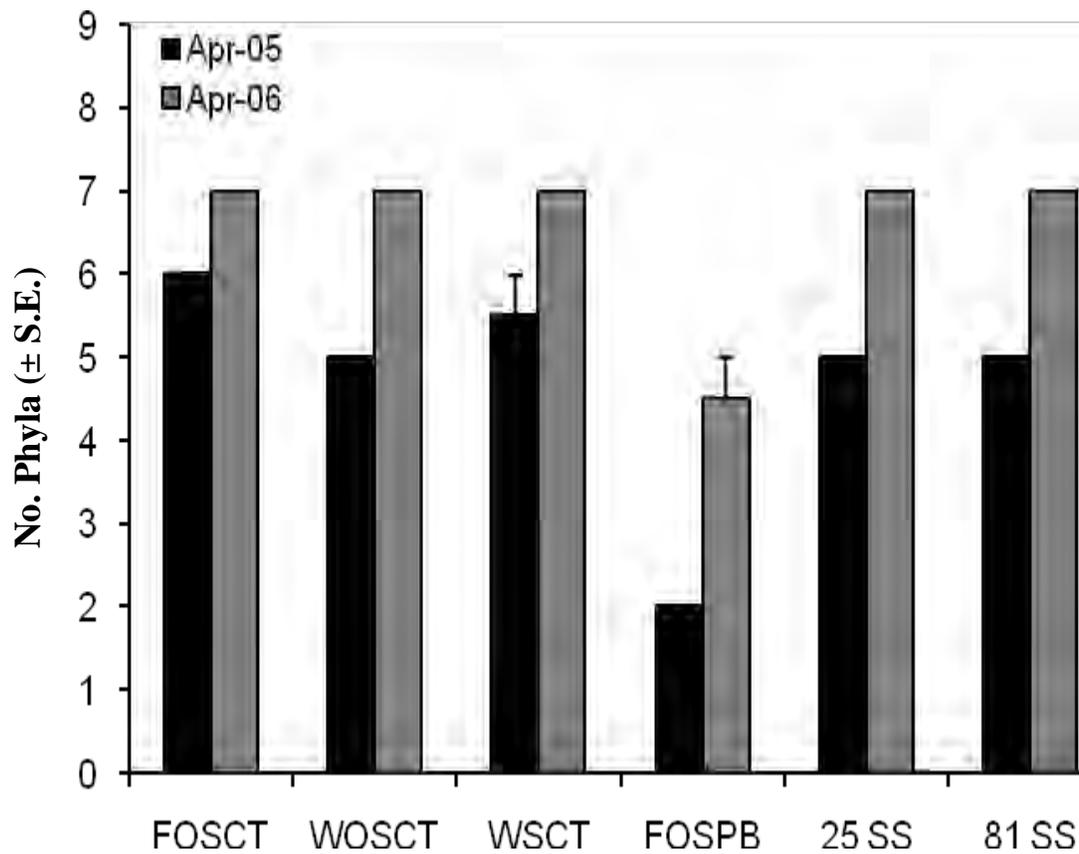


Figure 7. Mean number of phyla (# phyla  $\pm$  S.E.) observed on crab trap with fresh oyster shell (FOSCT), crab trap with washed oyster shell (WOSCT), plastic mesh bags with fresh oyster shell (FOSP), crab trap with whelk shell (WSCT), 25 spat sticks/m<sup>2</sup> (25 SS), and 81 spat sticks/m<sup>2</sup> (81SS) treatment types at Stacy Creek during April 2005 and 2006 sampling periods.

## Correlations

The number of live oysters was positively correlated to biomass ( $p = <0.0001$ ), oyster shell height ( $p = 0.001$ ), and oyster shell height to length ratio ( $p = 0.001$ ) (Table 5). The number of live oysters was negatively correlated to oyster mortality rate ( $p = 0.015$ ) (Table 5). There was a positive correlation between oyster shell height and oyster shell length ( $p = <0.0001$ ) as well as oyster shell height and oyster shell height to length ratio ( $p = <0.0001$ ) (Table 5). Biomass was negatively correlated with oyster mortality rate ( $p = 0.039$ ) and positively correlated with oyster shell height ( $p = <0.0001$ ), length ( $p = 0.005$ ), and height to length ratio ( $p = 0.001$ ) (Table 5). There was a positive correlation between species richness and oyster shell height ( $p = 0.007$ ), oyster shell height to length ratio ( $p = 0.014$ ), and oyster mortality rate ( $p = 0.016$ ) (Table 5). Phyla richness and oyster mortality rate were negatively correlated ( $p = 0.0001$ ) (Table 5).

Table 5. Results of Spearman's correlation evaluating the relationship between numbers of live oysters (NLO), oyster height (H), length (L), height/length ratio (H/L), total biomass (BM), mortality (M), species richness (SR), and phyla richness (PR) combined for all treatments.

Spearman's rho	+/- Relationship	P	Comparison	P-Value
	+	0.885	NLO : BM	<0.0001*
	+	0.352	NLO : PR	0.099
	+	0.270	NLO : SR	0.213
	-	0.449	NLO : M	0.015*
	+	0.655	NLO : H	0.001*
	+	0.377	NLO : L	0.077
	+	0.664	NLO : H/L	0.001*
	+	0.350	BM : PR	0.102
	+	0.379	BM : SR	0.075
	-	0.434	BM : M	0.039*
	+	0.719	BM : H	<0.0001*
	+	0.568	BM : L	0.005*
	+	0.634	BM : H/L	0.001*
	-	0.301	PR : SR	0.163
	-	0.757	PR : M	<0.0001*
	+	0.101	PR : H	0.645
	+	0.323	PR : L	0.133
	+	0.157	PR : H/L	0.475
	+	0.495	SR : M	0.016*
	+	0.549	SR : H	0.007*
	+	0.319	SR : L	0.183
	+	0.503	SR : H/L	0.014*
	-	0.013	M : H	0.952
	-	0.111	M : L	0.613
	-	0.060	M : H/L	0.784
	+	0.783	H : L	<0.0001*
	+	0.697	H : H/L	<0.0001*
	+	0.247	L : H/L	0.256

+/- indicate a positive or negative relationship between the parameters measured. P (rho) indicates the strength of the relationship between variables. \* Indicates statistical significance at the  $P < 0.05$  range

## Discussion

This research indicated that there were pronounced differences in the suitability of each experimental structure evaluated as restored oyster habitat when comparisons were made between treatments and within treatment types between years. Structures that accumulated less sediment and retained a greater degree of structural complexity yielded greater quantities of live oysters, overall biomass, and oyster growth and generally yielded lower oyster mortality. Structures comprised of commercial spat sticks (81 and 25/m<sup>2</sup>) provided a high level of habitat quality for newly settled and existing oysters. Spat stick densities of 81/m<sup>2</sup> consistently yielded the greatest oyster shell height, number of live oysters, mean biomass, and the lowest oyster mortality rate of all treatments types during both years.

Previous research (Lenihan and Peterson 1998; Lenihan 1999; Smith *et al.* 2005) has indicated a limited probability of success on restored oyster reef under situations of intense sediment loading and environmental characteristics that contribute to diminished habitat capacity. Oyster growth and the quantity of live oysters during this research were far greater on stick and crab trap versus mesh bags treatments. Similar to what was observed by Soniat *et al.* (2004), oyster mortality rate was much higher on structures that accreted more sediment within cultch materials and around the treatment perimeter such as bag treatments that had the least live oysters and the highest oyster mortality rate.

O'Beirn *et al.* (2000) has identified oyster shell as superior to alternative substrates during large scale restoration in Virginia. This study suggested oyster shell (washed and fresh) did not perform better than spat sticks and whelk shell. Lunz (1958) showed that washed oyster shell caught about half the number of spat than did fresh oyster shell from a steam cannery. During this research washed shell initially had greater oyster densities per treatment; however experienced greater oyster losses between years.

Oyster mortality rate did not significantly increase within either treatment type incorporating spat sticks suggesting that these treatments had a greater carrying capacity with respect to the

number of live oysters than other treatments. Reductions in young of the year oysters (on stick and other treatments) could have occurred as a result of predation by mud crabs *Panopeus herbstii*, which were ubiquitous (Bahr and Lanier 1981; Bisker and Castagna 1987; White and Wilson 1996). Grabowski and Powers (2004) indicated that habitat complexity associated with oyster reef increased mud crab foraging behavior and predation on clams. It is possible that dense oyster growth on stick treatments totally restricted the access of macropredators, such as oyster toadfish (Grabowski 2004), to interior portions of the reef and provided a stable environment for mud crab foraging free of disruption by predators.

Biomass decreased in crab traps with washed and fresh oyster shell and increased in crab traps with whelk shell and plastic mesh bags with fresh oyster shell, which can be explained by observed trends in oyster mortality rate and oyster growth. Crab traps with fresh shell may have had a slightly higher number of live oysters (~100 oysters) between years but it was not enough to offset the relatively high mortality rate (~15.33 %). New settlement on fresh oyster shell was restricted to live oysters growing on treatments containing this cultch type. There were high numbers of relict oyster shells from new and older recruits from the previous reproductive seasons below the sediment line within crab trap and mesh bag treatments. Buried oysters were not considered in assessment of mortality since death was directly related to sediment smothering and these recruits were no longer considered part of the living reef. Relict oysters on our experimental treatments containing oyster shell were also difficult to discern from original cultch so it would not be prudent to consider them during evaluation of oyster mortality. Oyster mortality rates and loss of oysters due to sediment covering were high enough between years in plastic mesh bags with fresh oyster shell and crab trap treatments to account for any losses in biomass; however not all treatments experiencing significant oyster mortality between years exhibited decreased biomass. Reductions in the number of live oysters within crab traps and plastic mesh bag with fresh oyster shell were not significantly different between years. Therefore any increases in biomass on these treatments could be accounted for by increases in live oyster growth. Crab traps with whelk shell and plastic mesh bags with fresh shell had a slight biomass increase between years regardless of reductions in the number of live oysters, which could be accounted for by oyster growth.

Comparison of oyster shell length versus height via linear regression was employed to determine the level of consistency in oyster shell morphology between replicates within treatments and was used as an indication of crowding. Oyster settlement on 25/m<sup>2</sup> spat stick treatments during year two of this research appeared to be greatest and most intense on oysters that settled during year one, which may explain an increase in H/L ratio on this treatment between years. Accretion of oysters onto crab traps and mesh bag treatments was limited to only pre-existing oysters by the 2005 oyster reproductive season due to heavy silt accumulation on and between cultch materials. Live oysters that persisted between years on crab trap and bag treatments were substantially larger and provided the only substrate for new oyster recruits. Similarities in the oyster settlement and growth environment on mesh bag and crab trap treatments were supported by a level of consistency in oyster morphology between these treatments during year two of this research.

In general species and phyla richness was lowest in plastic mesh bag with fresh shell treatments. Statistically, species richness was positively correlated to oyster mortality rate, oyster shell height, and oyster shell height to length ratio. When collectively observing all treatments, there was a shift in the dominant phylum from mollusca during year one to arthropoda during year two of research as greater refuge areas were created. Hackney *et al.* (1976) suggested that constant environmental fluctuation, such as that observed in tidal creeks, can create a situation favorable to the persistence of species that are poor competitors under stable conditions and create increased species richness.

Physical habitat structure is an important factor in controlling species distribution and abundance on oyster reef creating a range of habitat types in a localized area (Lenihan 1999). Vertical distance was cited as important for certain species of mussels (Soniati *et al.* 2004) as well as oyster abundances on reefs (Luckenbach *et al.* 2005). Though there was some variation in the performance of stick and crab trap treatments as habitat for reef associated species, there were no statistical differences between these treatment types by year two of research. Quantitative differences in the availability of diverse microhabitat between stick and crab traps was polarizing; however the persistence of an approximately equal number of species and phyla between the treatments suggested that there is still enough refuge on both treatment types to maintain a functionally diverse reef community. Species richness on bag treatments was limited when compared to remaining

treatments; however oyster growth between years on bag treatments could have accounted for increased species and phyla richness on these treatments through greater microhabitat provision.

Interstitial space is repeatedly touted as critical to providing microhabitat for the recruitment and survival of several invertebrate and vertebrate species (Chestnut 1974; Bahr and Lanier 1981; Bartol and Mann 1999; Coen and Luckenbach 2000; Luckenbach *et al.* 2005). This was supported by this research in that the growth and perpetuation of oysters on experimental constructed oyster reef resulted in significant increases in species richness between years for all treatments evaluated. Though mesh bags were observed to have a lower number of species than stick and crab trap treatments, these treatments still yielded substantial increases in species richness and mean oyster height between years suggesting that, to some degree, there was greater availability of microhabitat for settlement of interstitial fauna. Tolley and Volety (2005) found that habitat value of oyster clusters was high for decapod crustaceans and fishes regardless of the oyster being live or dead as long as the shells remained articulated. Observations during this study were consistent with those of Tolley and Volety (2005) since even treatments with declining oyster abundance experienced increased species richness due to remaining patches of clustered oysters and associated refuge.

## **Conclusion**

In summary, prolonged provision of clean substrate by spat stick treatments enabled greater settlement and growth of species essential to the structural integrity of healthy oyster reef. Crab trap treatment, though sustaining an equivalent degree of species richness as spat sticks, incurred greater habitat losses due to oyster mortality as a result of sediment blanketing and cultch subsidence. Plastic mesh bag treatments experienced very little oyster settlement and performed poorly as restored oyster habitat when compared to the other treatment types. It should be noted that in more recent years, the University of Georgia Marine Extension Service's community-based GEORGIA (Generating Enhanced Oyster Reefs in Georgia's Inshore Areas) program has moved towards planting shell bags on wooden pallets in these soft sediment environments and has recorded much greater success than when the bags are directly planted on the substrate.

Oysters are commonly cited as ecological engineers (Coen and Luckenbach 2000) and beneficially manipulate local environmental conditions by generating 3-dimensional reefs that alter

water flow, sedimentation patterns, and create habitat for a number of invertebrate and vertebrate species. Therefore, constructed oyster habitat that supports high levels of structural complexity and vertical distance from the sediments improves habitat quality for oysters and associated reef species by reducing silt smothering and providing greater microhabitat via oyster growth and reef proliferation. When employing alternative techniques for oyster habitat restoration, it is important to take into consideration tidal amplitude, local currents, sediment type and rate of deposition. This research highlighted the relationship between vertical relief on constructed reef and rate of success and indicated that techniques using vertical stick cultch materials are viable for restoration in areas characterized by heavy sediment deposition rates.

### **Implications for Practice**

For *Crassostrea virginica* habitat restoration:

- Restoration techniques should reflect the local habitat type due to variability in environmental characteristics between geographical regions.
- Overall success of restored oyster habitat is highly dependent on tidal placement of cultch materials as is the timing of the deployment of cultch materials to ensure adequate recruitment and survival of young of the year oyster spat.
- In estuaries characterized by high levels of sediment deposition or migration it is advantageous to use cultch materials that allow sufficient water exchange as well as provide adequate elevation from sediments.

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