Comparison of Techniques for Off-Bottom Culture of the Eastern Oyster, *Crassostrea virginica* (Gmelin, 1791), in Georgia

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Abstract

Three intertidal off-bottom culture methods were tested to determine if a quality single oyster, *Crassostrea virginica* (Gmelin, 1791), could be produced for sale in the Georgia winter oyster roast season. Five hundred 17-mm seed oysters in three replicates per treatment were stocked in 9-mm mesh bags laid flat on a trestle, in 9-mm mesh boxes attached flat to a trestle, or in 9-mm mesh bags hung straddling the trestle in October 2006. Growth, mortality, condition index, intensity of *Perkinsus marinus* infection, mud worm (*Polydora websteri*), and pea crab (*Pinnotheres ostreum*) infestation in oysters were monitored through March 2007. Oysters grew significantly ($p<0.0001$) larger in the mesh bags straddling the trestles ($\bar{x} = 48.5 \pm 0.83$ mm), than those in boxes ($\bar{x} = 45.1 \pm 0.72$ mm) which in turn were significantly larger than those in the mesh bags laid flat on the trestle ($\bar{x} = 42.5 \pm 0.58$ mm). No significant differences in oyster mortality ($p=0.2586$) or intensity of disease ($p=0.6541$) which were very low occurred between treatments. No parasitic mud worms or symbiotic pea crabs were found in oysters. Oysters from boxes had a significantly ($p=0.0068$) lower condition index than oysters in the other treatments, while those in hanging bags had a significantly ($p<0.0001$) higher amount of chalky deposits in their shells. While the study showed that quality single oysters could be grown by gathering clusters of early young-of-the-year spat and breaking them into singles, oysters did not grow sufficiently large enough to achieve the legal minimal size (76 mm) for harvesting by the end of the winter oyster roast season. Rationale for reducing the minimal-legal-size limit for harvesting oysters in Georgia is given.

Introduction

Shellfish growers in Georgia have expressed interest in diversifying the northern quahog, *Mercenaria mercenaria* (Linnaeus, 1758), aquaculture industry by culturing oysters, *Crassostrea virginica* (Gmelin, 1791). Georgia has expansive intertidal oyster reefs that once supported a viable commercial fishery (Harris 1980). Georgia led the nation in oyster harvesting in 1908 (Table 1), but the industry began declining after the 1930's due to overharvesting and labor shortages (Harris 1980). Presently, wild harvest of oysters is but a small business in Georgia mostly for the fall/winter sack trade with landings of only 7,247 kg of meat valued at $65,250 in 2007.

Oysters from natural reefs generally require 18 months to reach the legal minimal size of 76 mm in shell height. While oyster aquaculture in Georgia has promise (Manley 2007), two major hurdles must be overcome. The major problem is an overabundance of recruitment each year. Oysters in Georgia begin spawning in April/May and spawn throughout summer and into fall (Heffernan *et al.* 1989). Recruitment rates of 10,000 to 30,000 spat per meter squared per month are common (O'Beirn *et al.* 1995; O'Beirn *et al.* 1996a; Thoresen *et al.* 2005, Manley *et al.* 2008b) with a rate of 204,700 spat per meter square per month reported in the Duplin River, Sapelo Island, GA (Thoresen *et al.* 2005). Early of the year recruits will sexually mature in two to three months, then spawn and contribute to the late summer/fall recruitment (O'Beirn *et al.* 1996b).

This abundance of spat results in heavy setting of oysters which in turn results in overcrowded conditions on oyster reefs (O'Beirn *et al.* 1996a; Thoresen *et al.* 2005). Recruiting spat require a clean substrate for settlement and attachment and existing oysters provide an ideal surface. As a result of intense competition for space and food, oysters grow long, thin and narrow, have poor meat quality and are not aesthetically suitable for the lucrative halfshell market. Markets want well-formed single or doublet oysters. In Georgia the main sale of oysters is for the fall and winter oyster roast season. Thus, due to extremely high recruitment rates, a single oyster from year one will not be marketable for the halfshell market by year two because of complications associated with spat fouling. By fall the single oyster will be covered with fast-growing spat.

Perkinsus marinus is a protozoan parasite that plagues commercial oyster operations along the Gulf of Mexico and the east coast of the United States including coastal Georgia. *P. marinus* is prevalent in most oysters in Georgia (Lewis *et al*. 1992; O'Beirn *et al*. 1996a; Thoresen *et al*. 2005; Power *et al*. 2006) and under optimal environmental conditions can result in heavy mortalities (Lewis *et al*. 1992). Along the eastern seaboard and Gulf Coast of the United States, *P. marinus* typically infects oysters during summer of their first year, with mortalities generally not occurring until fall of year two (Ford and Tripp 1996). Effects of the disease are combined with the physiological stress of both spawning during summer and the effects of high summer water temperatures. Oysters may die just prior to the fall/winter harvesting period.

The solution to overcoming problems associated with disease and oyster spat fouling is to produce a marketable size oyster within a shorter time period. In an oyster reef restoration project (Manley 2007) that featured commercial spat sticks, spat settlement occurred in April/May, and oysters grew to a marketable size by the following March. Oysters grown off-bottom on spat sticks began to attain market size at nine months, and the majority of oysters reached market size at eleven months (Manley 2007). Unfortunately, it is difficult to remove oysters from these commercial spat sticks without breaking their shells. Techniques for gathering spat from PVC pipe coated with cement does allow for successful collection and subsequent separation of spat into singles (Manley *et al*. 2008a). At the request of industry, this study compared three off-bottom methods for culturing single oyster spat grown intertidally off-bottom to a marketable size in hopes of producing single oysters for the fall/winter oyster roast season.

Table 1. Eastern Oyster, *Crassostrea virginica*, production in kgs of meat landed and monetary value in dollars from 1880 to 2008 in Georgia. Data are from Georgia Department of Natural Resources (1979-2008) and National Marine Fisheries Service (1880-1978).

Year	kg of meat	Value	Year	kg of meat	Value	Year	kg of meat	Value
1880	178,262	NA	1954	98,430	65,160	1982	8,297	24,016
1887	280,774	NA	1955	78,471	51,990	1983	2,008	6,013
1888	307,536	NA	1956	54,522	35,716	1984	2,560	8,590
1889	415,944	NA	1957	50,938	27,401	1985	16,737	67,832
1890	571,980	NA	1958	38,374	20,634	1986	1,788	7,878
1897	1,240,576	NA	1959	54,068	28,737	1987	4,119	17,889
1902	3,120,262	NA	1960	104,961	58,831	1988	16,108	71,145
1908	3,660,490	NA	1961	71,894	47,650	1989	30,920	137,278
1910	1,287,749	NA	1962	66,633	51,415	1990	32,518	50,469
1918	404,151	NA	1963	106,821	82,425	1991	38,393	167,459
1923	626,411	NA	1964	88,813	68,536	1992	38,846	182,020
1927	275,784	NA	1965	112,355	86,696	1993	16,195	76,697
1928	381,925	NA	1966	82,508	63,563	1994	6,180	29,764
1929	200,488	NA	1967	92,125	114,007	1995	2,876	15,571
1930	72,121	NA	1968	86,455	106,619	1996	1,936	9,227
1931	139,706	NA	1969	24,267	30,349	1997	3,393	18,428
1932	266,712	NA	1970	34,473	40,364	1998	3,155	17,212
1934	258,094	NA	1971	62,823	72,870	1999	2,997	17,325
1936	149,685	NA	1972	68,999	86,812	2000	1,724	9,733
1937	108,409	NA	1973	48,080	65,122	2001	3,868	22,254
1938	69,853	NA	1974	29,331	36,040	2002	3,627	19,997
1939	106,141	12,353	1975	20,003	25,613	2003	4,979	31,019
1940	120,202	15,178	1976	32,586	49,240	2004	2,317	14,859
1945	115,666	50,026	1977	39,562	75,009	2005	2,390	20,473
1950	139,706	76,735	1978	9,497	18,792	2006	6,568	55,231
1951	132,903	72,155	1979	5,160	11,459	2007	7,247	65,250
1952	100,244	55,300	1980	15,022	42,112	2008*	3,153	30,021
1953	100,698	58,400	1981	11,294	35,715			

NA = data not available
*Preliminary data and subject to change

Materials and Methods

This research was performed on the commercial shellfish lease of MacClam Inc. of Crescent, GA. The lease is located adjacent to Creighton Island in Sapelo Sound, GA (Figure 1). Salinity in Sapelo Sound is high, because it receives no major freshwater inflow. Salinity and water temperature were collected at this site monthly using a refractometer and hand held thermometer, respectively.

Oysters were gathered from an oyster spat collection study performed in 2006 (Manley *et al.* 2008a). Oyster spat were separated into single oysters. Two-hundred oysters were measured for shell height (from hinge to lip), length (maximal anterior-posterior dimension of the shell parallel to the hinge) and width (across two valves) to the nearest 0.1 mm with Vernier calipers. Oysters ranged in size from 3 to 35 mm in shell height and had a mean size of 17.07 ± 0.38 (SE) mm. Oysters were divided in 9 groups of 500 oysters each.

Oysters were planted in the field in October 2006 using three culture methods: 9-mm mesh Vexar plastic oyster bags (1.0 x 0.5 m) laid flat; 9-mm mesh Vexar oyster bags formed into a box (1.0 X 0.5 x 0.3 m), and in 9-mm mesh Vexar plastic bags straddled across a trestle. Three replicates were deployed per treatment. Bags and boxes of oysters were placed on three different trestles made of 12.7-mm diameter reinforcement rods. The dimensions for the top of each trestle that supported each mesh enclosure were 3 m length x 1 m width. The four legs that supported each trestle were 1.3 m in length and were stabilized with four crossbeams located 0.61 m below the top of each trestle. Trestles were placed in the intertidal zone on a mud flat parallel to the northeast corner of Creighton Island, Georgia. Trestles were stabilized in the mud by sinking the legs of the trestle until the crossbeams were flush with the mud substrate which left the enclosures approximately 0.61 m off-bottom and aligned with the adjacent natural oyster reef. Bags and boxes were spaced approximately 0.5-m apart on each trestle and were affixed to trestle frames with cable ties. Oysters in the bags straddling the trestle were hanging lower in the intertidal zone than were those in the other two treatments. Oysters were evaluated for growth and survival in November, January and February. The experiment was terminated in March 2007.

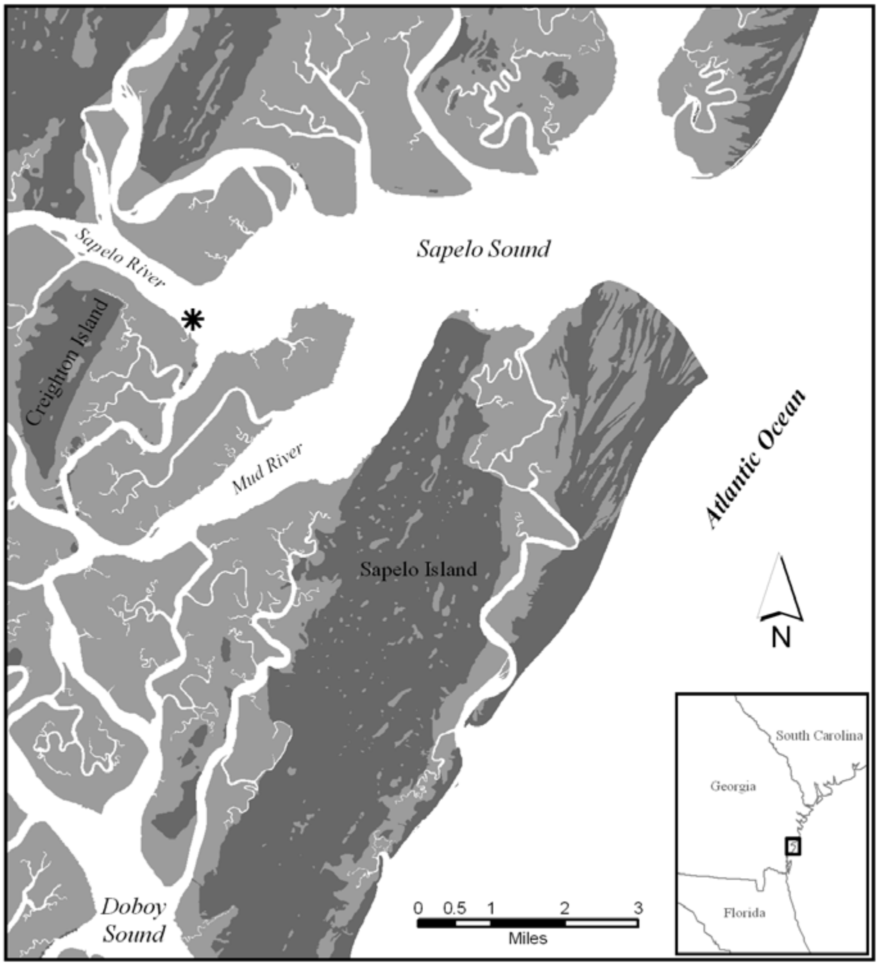

Figure 1: Experimental site for oysters cultured on the MacClam, Inc. Shellfish Lease, Creighton Island, Sapelo Sound, GA.

Thirty-two oysters per replicate, per treatment type were measured monthly for shell height, length and width to the nearest mm with Vernier calipers. Monthly, fifty oysters per replicate were assessed to determine oyster mortality rate. At the conclusion of the experiment in March, 32 oysters per treatment type were evaluated to determine index of condition. An additional 32 oysters per treatment type were evaluated for prevalence and intensity of the oyster pathogen *Perkinsus marinus*. Shell morphology and characteristics were also taken into account, and 32 oysters from each treatment type were assessed for shell height to length ratio, shell height to width ratio, percent cover of pigmentation on the exterior of the shell, chalky deposits on the interior of the shell, number of pea crabs, *Pinnotheres ostreum* Say, 1817, found internally, and the number of mud blisters caused by the polychaete, *Polydora websteri* Hardman, 1943, on the interior of the shell.

Condition index was determined based on the methods of Lawrence and Scott (1982). Each oyster was measured for shell length (umbo to tip, *mm*) and weighed (total weight, *g*). Subsequently, the shell was opened, and the meat was placed on a pre-weighed aluminum foil dish and dried in an oven at 67ºC for forty-eight hours to obtain a dry weight. Empty oyster shells were also dried at room temperature for twenty-four hours and subsequently weighed again. The internal cavity volume was determined by subtracting shell weight (*g*) from the total weight (meat and shell). The condition index was calculated by the following formula: (dry meat weight in grams) (100) / (internal cavity volume in centimeters cubed).

Ray's fluid thioglycollate medium assay (Ray 1952) was used to assay rectal tissue from oysters according to procedures outlined by Powell & Ellis (1998). Each sample was examined microscopically with a dissecting scope and an intensity index of *Perkinsus marinus* infection assigned according to Craig *et al*.'s (1989) semi-quantative scale. Mean infection intensity indices were calculated for each site by averaging the intensity index score of each oyster including un-infected individuals (score = 0) at each site. Values range from a low of 0 to a high intensity level of 5.

One-way Analysis of Variance (ANOVA) and Tukey's Studentized Range Test (α =0.05) using SAS for PC (SAS Institute Inc., 1989) were carried out. All proportional data were arcsine transformed prior to statistical analysis (Sokal and Rohlf 1981)

Results

Water temperature and salinity levels during the experiment were below normal for Georgia's coastal waters. Water temperature ranged from a low of 10°C in January to a high of 20.1°C in March 2007. Salinity ranged from a high of 36 PSU in January and February to a low of 27 PSU in March (Table 2).

Table 2. Water temperature and salinity data for Creighton Island in Sapelo Sound, GA.

	Nov 2006	Jan 2007	Feb 2007	Mar 2006
Salinity (PSU)	28.9	36	36	27
Temperature (ºC)	19.6	10	12	20.1

Oyster shell morphology changed significantly over time. By the end of the experiment oyster height was significantly different ($P<0.0001$) between each treatment (Figure 2) with greatest size obtained in the hanging bags ($\bar{x}$ = 48.5 ± 0.83 mm), intermediate size in boxes ($\bar{x}$ = 45.1 ± 0.72 mm) and smallest size in the flat bags ($\bar{x}$ = 42.5 ± 0.58 mm). By March oysters cultured in the flat mesh bags had a significantly (P = 0.008) lower shell height:shell length ratio (1.46 ± 0.02) than the other two treatments (1.58 ± 0.03 hanging vs 1.55 ± 0.03 boxes) (Figure 3). Shell height to shell width ratios by March (Figure 4) showed that oysters in hanging bags ($\bar{x}$ = 4.2 ± 0.15) were equal to those in the boxes ($\bar{x}$ = 3.94 ± 0.12). Oysters grown in flat bags ($\bar{x}$ = 3.68 ± 0.09) were significantly (P = 0.0096) different from those grown in hanging bags, but were not significantly different from those in boxes.

The effect of *P. marinus* was negligible, as was overall oyster mortality (Figures 5, 6 and 7). *P. marinus* prevalence was 9.4% ± 0.02 in oysters from the hanging bags and 6.25% ± 0.01 in the other treatments (Figure 6). No significant differences (P = 0.6541) in *P. marinus* intensity occurred with intensity (N-values) being very low ranging from 0.021 to 0.042 (Figure 5). Oyster mortality was low ranging from 0 in boxes to a maximum of 2% in oysters in hanging bags in March with no significant difference observed between treatments (P = 0.2586) (Figure 7). Neither pea crabs nor mud blisters were found in any of the oysters.

Oyster conditions differed in terms of the amount of pigmentation (Figure 8), and the chalky condition of the shell (Figure 8). Condition index in oysters in the flat bags ($\bar{x}$ = 15.1 ± 0.50) and hanging bags ($\bar{x}$ = 15.0 ± 0.58) was equal while those in the boxes were significantly (P = 0.0068) lower ($\bar{x}$ = 13.1 ± 0.34). Oysters grown in hanging bags had significantly (P < 0.0001) higher ($\bar{x}$ = 38.3% ± 3.55) chalky shells compared to those in flat bags ($\bar{x}$ = 15.9% ± 1.83) and boxes ($\bar{x}$ = 11.9% ± 1.03) which were not significantly different. The percent pigmentation was significantly different (P= 0.0329), with oysters from the flat bags ($\bar{x}$ = 21.2% ± 2.89) being higher than those from the hanging bags ($\bar{x}$ = 18.4% ± 1.94). Oysters from the boxes ($\bar{x}$ =19.7% ± 3.11) were not statistically different from the other two treatments.

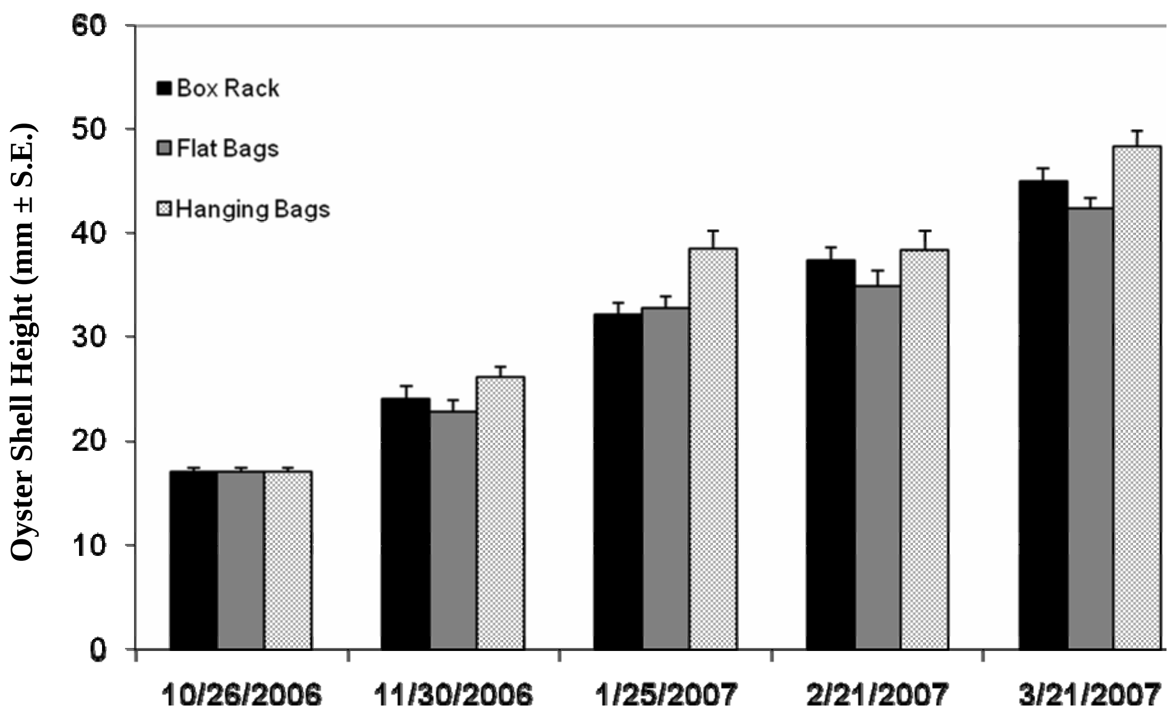

Figure 2: Mean oyster shell height ± S.E. in mesh boxes, flat clam bags and hanging mesh bags on rebar frames in Sapelo Sound, GA.

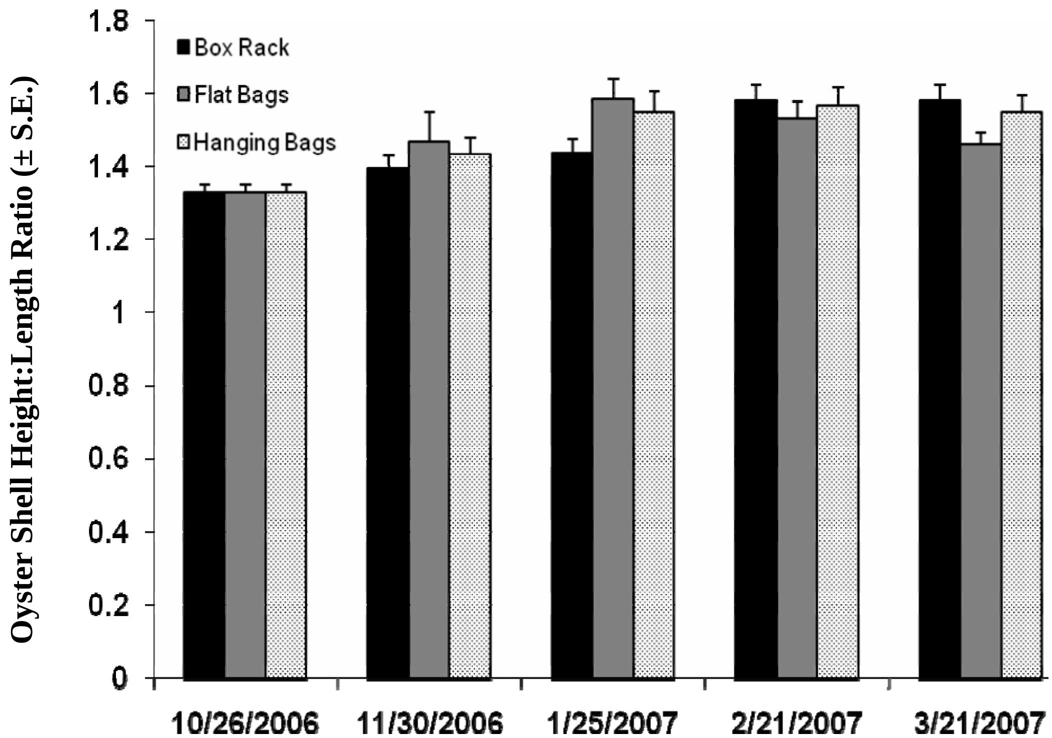

Figure 3: Mean oyster shell height:shell length ratio ± S.E. in mesh boxes, flat clam bags and hanging mesh bags on rebar frames in Sapelo Sound, GA

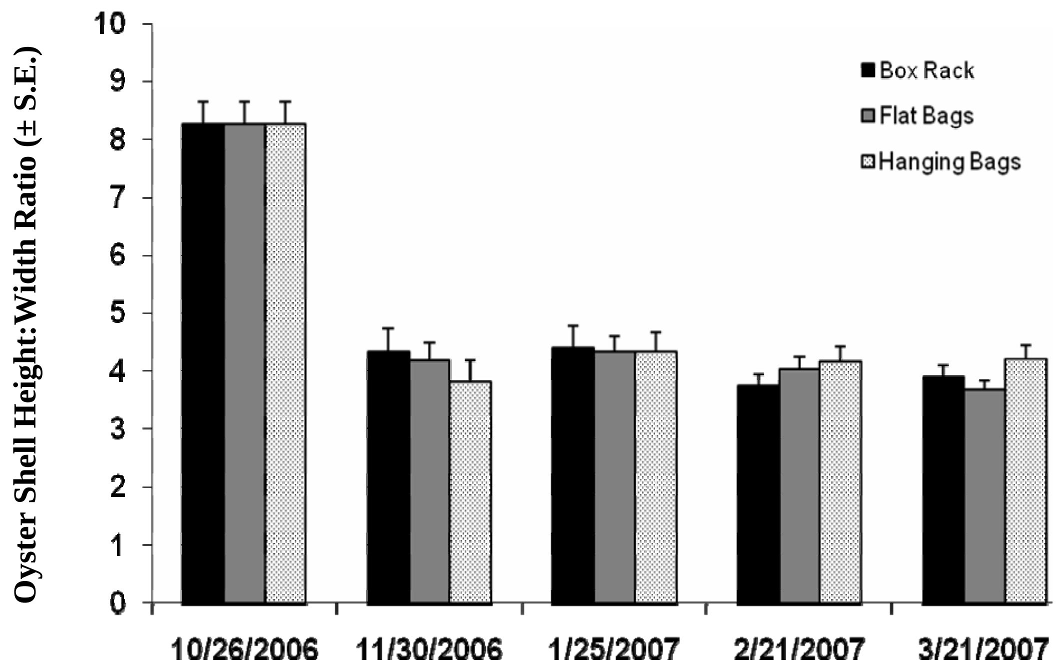

Figure 4: Mean oyster shell height:shell width ratio ± S.E. in mesh boxes, flat clam bags and hanging mesh bags on rebar frames in Sapelo Sound, GA

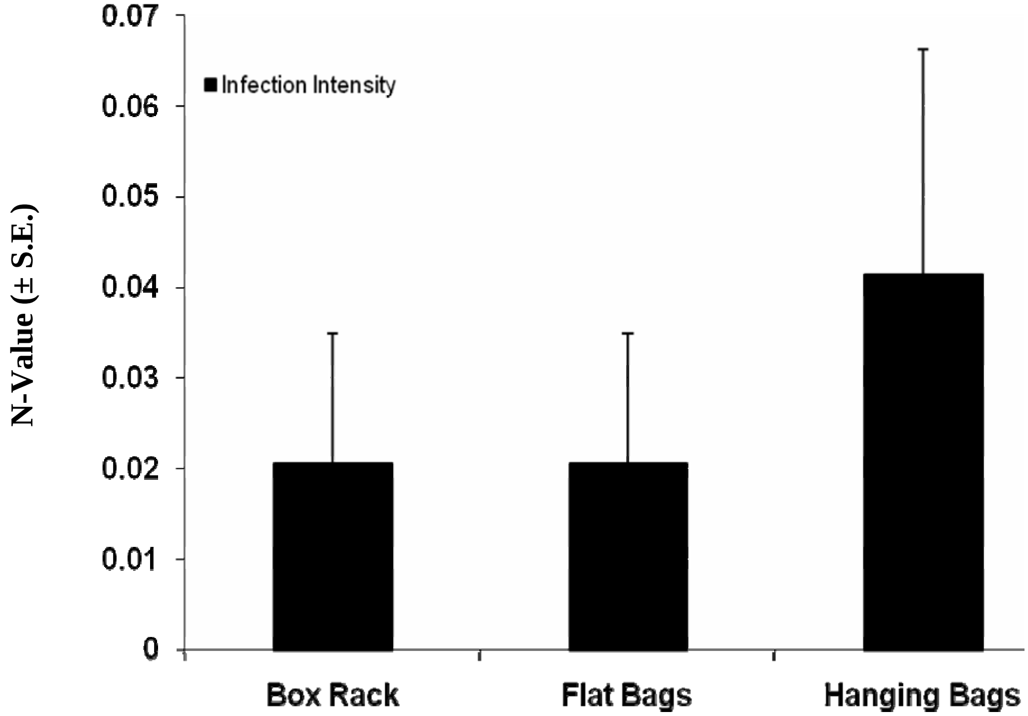

Figure 5: Mean oyster shell height:shell width ratio ± S.E. in mesh boxes, flat clam bags and hanging mesh bags on rebar frames in Sapelo Sound, GA

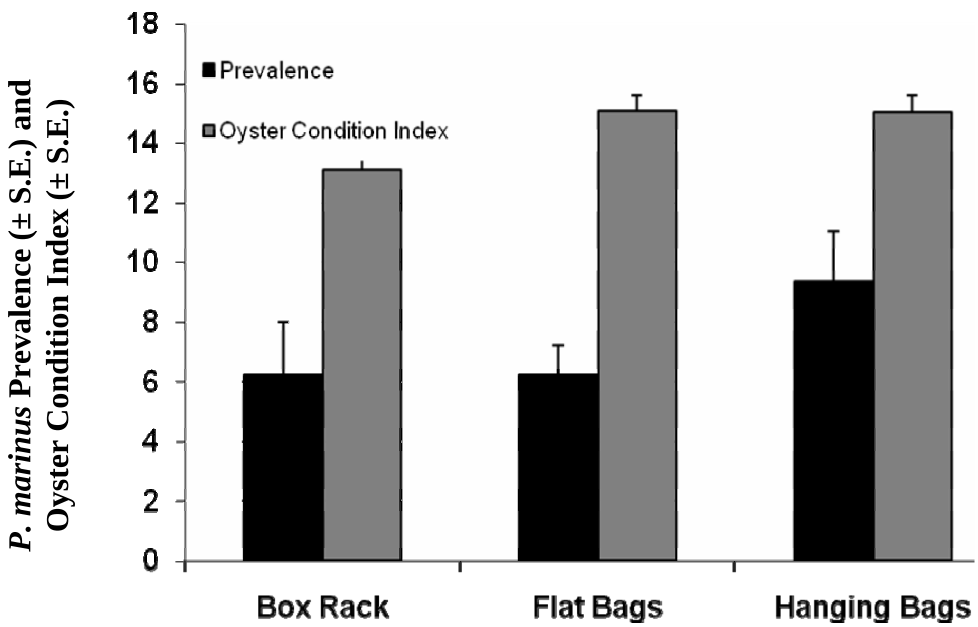

Figure 6: Mean *Perkinsus marinus* prevalence ± S.E. and oyster condition index ± S.E. for oysters in mesh boxes, flat clam bags and hanging mesh bags on rebar frames in Sapelo Sound, GA.

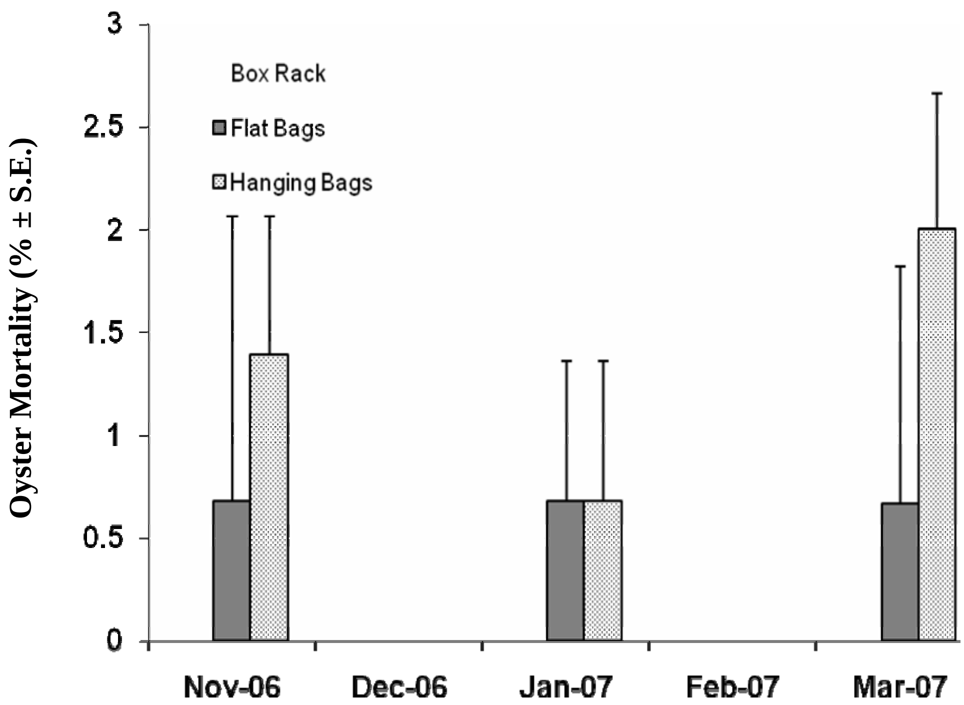

Figure 7: Mean oyster mortality ± S.E. for oysters in mesh boxes, flat clam bags and hanging mesh bags on rebar frames in Sapelo Sound, GA.

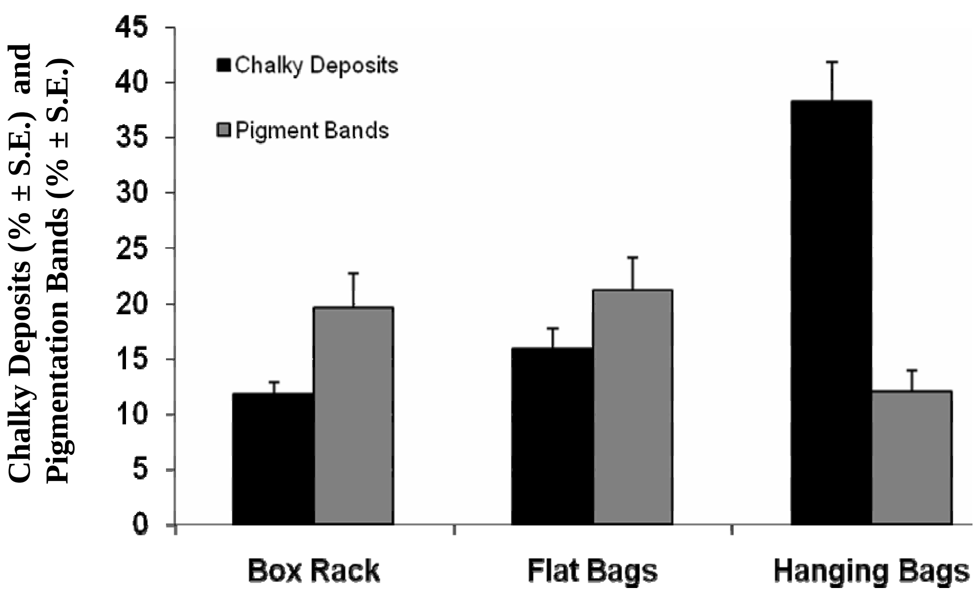

Figure 8: Mean percent cover of chalky deposits ± S.E. and pigment ± S.E. of oysters grown in mesh boxes, flat clam bags and hanging mesh bags on rebar frames in Sapelo Sound, GA

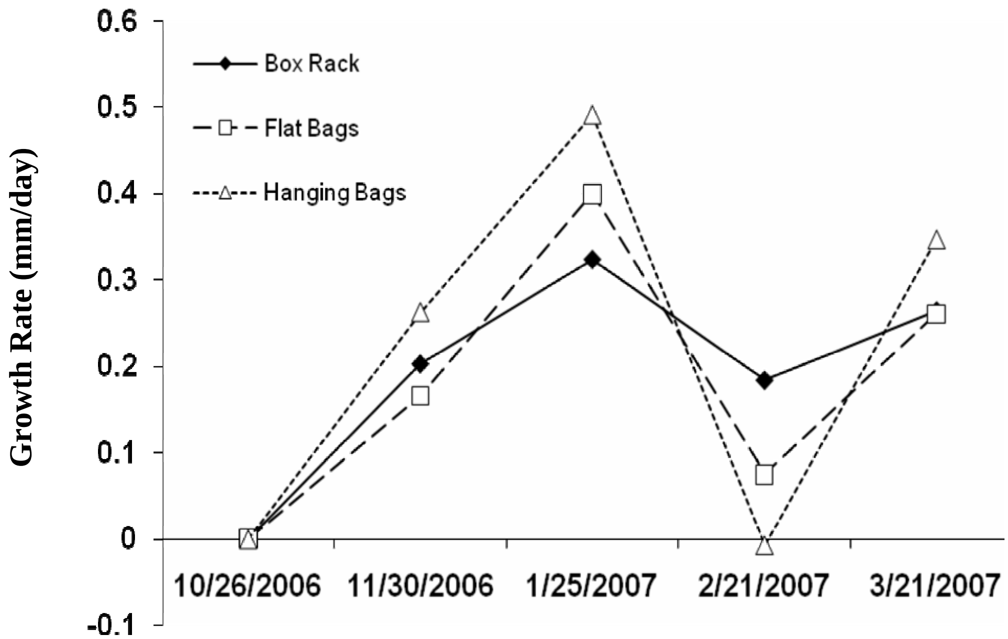

Figure 9: Oyster growth rate (mm/day) in box rack, flat clam bags and handing bags on rebar frames in Sapelo Sound, GA.

Discussion

This study sought to determine the feasibility of producing high-quality legal-size (76 mm) oysters in a nine month period. Although oysters failed to reach marketable size, we nevertheless believe there is potential for an aquaculture fishery for 50-mm oysters here in Georgia.

Differences in growth between treatments may be explained. Greater growth in the hanging bags may well be a result of the oysters being suspended lower in the intertidal zone, or less emphasis may have been placed on shell thickness, since bag-grown oysters exhibited a thinner more fragile shell. Oysters in hanging bags had greater opportunities for feeding due to longer submersion time at low tide, as well as less physical disturbance by wave action (Spencer 2002). The bottom of the hanging bags where all the oysters would have collected were at least 0.25 m (suspended from the center of the bag) lower in the intertidal zone than those on the trestles. Oysters in the boxes would have had a less stable environment due to the wave action of storms and boats. Oysters in hanging bags were piled on top of each other. Due to the crowding of individual oysters in the pockets of hanging bag treatments, oyster growth characteristics may have resembled those observed in oysters grown in high densities. Oysters in the bags laid flat on the trestle had the top of the bag lying on the oysters to hold them in place; however, oysters in the boxes would have been subject to movement within the box by wave action. Wave action has been shown to negatively affect oyster growth (Ortega 1981, Quayle and Newkirk 1989) as well as mussels (Harger 1970); however it has been essential in improving shell shape and shelf life (Matthiessen 2001, Daly 2003, Handley and Jeffs 2003).

Although the condition index of oysters from the box treatments was significantly lower than the other two treatments, aesthetic appearance was enhanced. The shell shape appeared more finished and undistorted by the enclosure with greater amounts of shell ridging as opposed to both bag treatments in which oysters grew partially into the mesh. Oysters grown in box enclosures had thicker shells and a cleaner appearance with greater contrast in external shell pigmentation and a greater degree of pearly nacre internally as opposed to chalky deposits. Again being tossed about within box enclosures by wave action could account for poor condition; however in the global halfshell oyster market there is usually a process implemented to yield a final marketable product. Oyster industries in France, Australia, and the west coast of the United States for years have used a multi-step system to raise aesthetically pleasing oysters for the halfshell industry. Operations in New South Wales, Australia, for instance, are known to collect wild Sydney rock and Pacific oyster spat on lime coated PVC slats and move them to bags (Matthiessen 2001) and then finish adult oysters in Stanway tubes that rotate and tumble oysters into a final product (Dave DeAndre Taylor Shellfish Farms Inc. personal communication). Therefore it is possible that a similar multi-step process that focuses on off-bottom oyster culture deserves further investigation.

Oyster growth was consistent with seasonal trends. The lowest values occurred between January and February (Figure 9). This is explained by the drop in water temperature to 10ºC in January. Oysters also were exposed to extremely low air temperatures during low tide, as well as increased wave action associated with winter storms. In addition, January marks the beginning of gametogenesis in Georgia oysters (Heffernan *et al.* 1989), a time when energy is transferred from growth to reproduction.

Although oysters of harvestable size were not obtained in this study, the results are promising. Moroney and Walker (1999) showed that oysters cultured in mesh bags off-bottom in the intertidal zone grew faster and had higher survival rates than oysters grown on-bottom. However, during spawning season oysters from both on and off-bottom were covered with newly settling spat, with significantly higher spat settlement occurring on off-bottom oysters. Adams *et al.* (1994) found no growth differences in oysters stocked in densities of 100, 250 and 500 oysters per bag in Georgia; however, the high density treatment had significantly lower spat attachment during the next spawning season. It may be possible to increase the growth rate of oysters by placing them lower in the intertidal zone. For clam filter feeders *Mercenaria mercenaria* and *Spisula solidissima* cultured at various intertidal heights, growth was significantly reduced with increases in aerial exposure at low tide (Walker and Heffernan 1990). This study showed that oysters grown in bags hanging 0.25 m lower in the intertidal zone grew significantly larger than oysters in bags or boxes on a trestle. By moving the trestles lower in the intertidal zone, oysters would have a longer period for feeding. Oysters in Georgia naturally occur at the two hours above mean low water mark. Predation by a host of organisms is believed to control oyster distribution in the lower intertidal and subtidal regions. The protective mesh bags will eliminate most natural predation, especially if the bags are suspended above the intertidal bottom. Culturing oysters in the subtidal zone does not work in Georgia due to the massive fouling of bags and oysters by a host of invertebrate species: bryozoans, hydroids, sea squirt, etc. Some intertidal exposure is needed to control this fouling or growth and survival of oysters will greatly decline. Extensive labor costs would also be involved in constantly cleaning bags and oysters to reduce fouling and associated mortalities if placed subtidally.

An oyster aquaculture industry could still be developed. Shell shape is becoming of greater importance in reference to product quality, as opposed to size (Jacobsen 2007). There is a rapidly growing market for smaller-sized ovate and deeply cupped oysters as is observed with the Kumamoto oyster, *Crassostrea sikamea* (Amemiya 1928), that is commonly sold at 38 to 50.8 mm (1.5-

2 inches) in shell height (Brake *et al.* 2003; Jacobsen 2007). Small-sized gemlike oysters are usually preferred by first-time oyster eaters and are increasingly common in raw oyster bars (Jacobsen 2007). A market trend towards a smaller oyster is very beneficial for Georgia's oyster industry, since a nicely shaped smaller oyster can be grown easily in under a year. With the productivity of Georgia's estuaries, Georgia has the potential to dominate the oyster industry as it once did in the early 1900's (Harris 1980). To accomplish this, a change in the legal minimum size for the harvesting of oysters is required.

Usually, a minimum-legal size limit is imposed to ensure that stocks have at least one opportunity to reproduce prior to harvest. This is however unnecessary for oysters in Georgia. Georgia still has vast areas of oyster reefs, most of which occur in areas that are off limits to commercial or recreational fishermen. Secondly, there is a vast overabundance of naturally occurring spat due to the prolonged spawning season of oysters in Georgia. Furthermore, young-of-the-year spat can sexually mature within two months and spawn by summer/fall. The problem in Georgia is not the natural production of spat, but the lack of suitable clean sites for successful settlement. As a result, there is massive overcrowding of oysters on natural reefs. The State should investigate either eliminating the minimal-legal size limit or reduce it to 38 mm or 50.8 mm to encourage the development of an oyster aquaculture industry.

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