### **EFFECTS OF MESH SIZE AND**



### STOCKING DENSITY ON GROWTH AND SURVIVAL OF MERCENARIA MERCENARIA SEED CULTURED UNDER BOTTOMLESS CAGE ENCLOSURES IN COASTAL GEORGIA

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## Effects of Mesh Size and Quahog Stocking Density on Growth and Survival of *Mercenaria mercenari*a Seed Cultured under Bottomless Cage Enclosures in Coastal Georgia

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### **ABSTRACT**

The optimal quahog stocking density and mesh size of bottomless cage enclosures were determined for field-cultured *Mercenaria mercenaria* seed in coastal Georgia. Replicate mesh enclosures (8.9 m<sup>2</sup>) placed on a sandy-mud intertidal flat on Four Mile Island, Georgia were stocked with seed clams of a mean shell length (SL) size of  $26.6 \pm 1.7$  mm (SE) harvested from a mesh-bag-line culture system. From December 1996 until harvesting in September 1997, enclosures were sampled on a bi-monthly basis to determine clam size. Clams stocked at a density of 750 clams/m<sup>2</sup> were cultured under enclosures with mesh sizes of 6.7, 12.6 and 19.0 mm. Additional clams were stocked in densities of 750 and 1,000 clams/m<sup>2</sup> and placed under enclosures of two mesh sizes (6.7 mm and 12.6 mm). By September 1997, clams cultured under enclosures with mesh sizes of 12.6 and 19.0 mm (SL:  $\bar{x} = 48.3 \pm$ 0.27 mm and  $\bar{x} = 47.5 \pm 0.25 \text{ mm}$ , respectively) were not significantly different in size, however both

were significantly larger (p < 0.0001) than clams in the 6.7 mm mesh treatment (SL:  $\bar{x} = 46.0 \pm 0.23$ mm). By September 1997, clams stocked in 6.7 mm mesh enclosures in densities of 750/m<sup>2</sup> and 1,000/  $m^2$  were not significantly different in size (SL:  $\bar{x} =$  $46.0 \pm 0.23$  mm and  $\bar{x} = 45.7 \pm 0.27$  mm, respectively), however both were significantly smaller (p < 0.0001) than clams stocked at either density in 12.6 mm mesh (SL:  $\bar{x} = 48.3 \pm 0.22$  mm and  $\bar{x} = 47.4 \pm 0.02$ 0.22 mm, respectively). Survival among all treatments ranged from 74% to 100%. The optimal mesh size of 12.6 mm and seed stocking density of 750 clams/m<sup>2</sup> were determined for *M. mercenaria* cultured in bottomless cage enclosures. Bottomless cage enclosures demonstrate a promising potential for the Georgia quahog aquaculture industry.

### **Key Words:**

Mercenaria, aquaculture, mesh enclosure, stocking density, growth, survival

### INTRODUCTION

Recent advances in northern quahog, Mercenaria mercenaria, aquaculture have precipitated many new culturing techniques which are specific to geographical areas. These field culturing techniques rely primarily upon the purchase of hatchery-reared seed which are then cultured to marketable size in natural environments. Current field culture methods include mesh bags placed in cages for seed culture (Rheault, 1995). Fablock or soft bag culture (Vaughan and Creswell, 1988; Hadley et al., 1997), bottom bag culture (Fernandez et al., 1997), cage culture (Walker, 1984; Vaughan and Creswell, 1988; Walker and Heffernan, 1990a), trav culture (Eldridge et al., 1979; Vaughan et al., 1987; Vaughan and Creswell, 1988; Crenshaw et al., 1996), baffle-aggregate pens (Castagna and Kraeuter, 1981), oyster-belt bag culture (Vaughan and Creswell, 1988), plastic mesh covers (Walker and Heffernan, 1990b), and mesh-bag-line culture (Walker and Hurley, 1995). The commercial value

of each technique is dependent upon factors associated with location (tides, currents and substrate types), operational costs (material expenses and longevity coupled with labor costs), and biological yields (growth and survival rates of the clams).

The University of Georgia Marine Extension Service developed a mesh-bag-line system (Walker and Hurley, 1995) for culturing quahogs in the soft-bottom substrates which predominate in coastal Georgia. This culturing method greatly improved the survival rates of small quahog seed (Walker and Hurley, 1995), however, in terms of growth to harvestable size, the rates were lower than expected. Results of a study comparing growth and survival of quahogs cultured in the mesh-bag-line system versus trays and bottom cages showed that clam growth was slower in the mesh-bag-line system but survival rates were higher (Walker, 1997).



The present study was carried out to determine an alternative strategy for culturing quahogs that utilizes the advantages of the mesh-bag-line system (good growth and increased survival of small seed) without limiting the growth rate of larger seed. Previous studies revealed that seed greater than 25 mm experienced reduced growth rates in the meshbag-line system, which in turn increases culture time to harvestable size (Walker, 1997). The new culture system used the mesh-bag-line system to culture small seed to 25 mm size. At that size, quahogs are afforded physical protection from most natural predators (e.g., Gibbons and Blogoslawski, 1989). The larger quahog seed were then removed from the bags, placed directly on the bottom, and covered with a protective mesh enclosure. In previous experimental trials, mesh covers had proven effective at protecting large seed clams from predation (Walker and Heffernan, 1990b). The objective of this study was to determine the optimal mesh size and stocking density for Mercenaria mercenaria seed cultured in bottomless cage

enclosures in coastal Georgia. This work was performed in conjunction with Sapelo Sea Farms, Inc. of McIntosh County, Georgia.

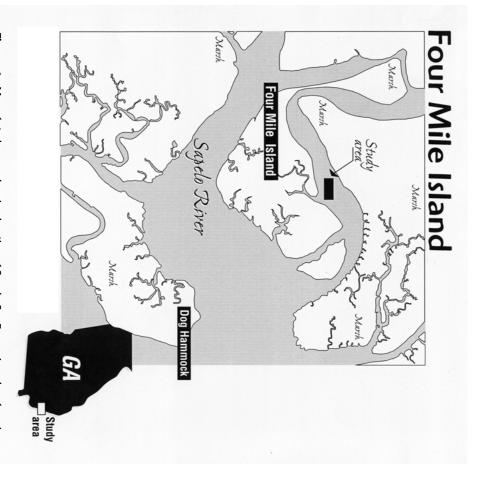
### METHODS AND MATERIALS

Mercenaria mercenaria seed clams acquired from a hatchery were field-planted on an intertidal sandymud flat adjacent to Four Mile Island in Sapelo Sound, Georgia, on June 3, 1996 (Fig. 1). Initial mean clam size (n=200) was  $9.0 \pm 0.9$  (S.E.) mm in shell length (SL=maximum anterior-posterior length). Seed clams were stocked based upon volumetric displacement counts at 9,300 clams per 0.5 m<sup>2</sup> mesh bag. Clams were placed into 65, 3-mm mesh bags with five bags attached per mesh-bagline system (Walker and Hurley, 1995). On July 26, 1996, the seed density in each bag was reduced by half, and 3-mm mesh bags were replaced with 6.7mm mesh bags. Additional bags were deployed to accommodate the seed thinning (density reduction) process. Seed numbers again were reduced by half on September 24, 1996 when the 6.7-mm mesh bags were exchanged for 12.7-mm mesh bags. A final thinning on October 30, 1996 reduced bag

density to approximately 1,163 clams/0.5m<sup>2</sup>. This density was maintained until clams were transplanted to bottomless cage enclosures.

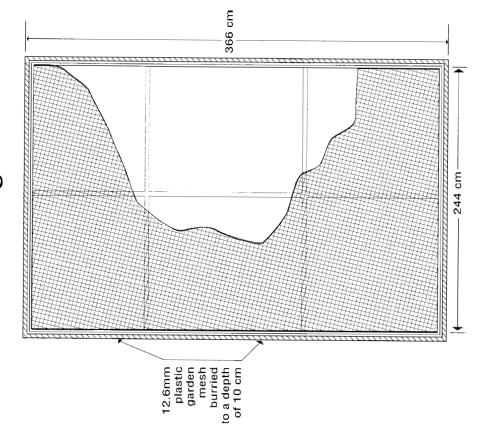
On December 10 and 11, 1996, two field culture experiments were initiated. Both were designed to test the effects of bottomless cage enclosure mesh size and stocking density on quahog growth and survival. The experiments were terminated in September 1997. Rectangular bottomless cage enclosures (Fig. 2) constructed of 12.7-mm diameter, welded, re-bar frames with length/width dimensions of 3.7 x 2.4 m were stocked with seed clams [SL:  $26.6 \pm 1.7$  mm (SE)] harvested from the mesh-bag-line system. Replicate enclosures were assigned placement randomly in one of three series containing four enclosures each, and arranged parallel to the river at mean-low water. Enclosures were oriented in a straight line with the 3.7-m sides





Mdntosh County, Georgia Figure 1. Map of study area showing location of Sapelo Sea Farms, Inc. clam farm in

# Schematic diagram of bottomless cage enclosure



Schematic of a plastic mesh enclosure used for culturing quahogs in the coastal waters of Georgia Figure 2.

perpendicular to the river channel. Plastic garden screening (12.6 mm mesh) was cut into 10-cm wide strips, 6.1-meters long. The screening was bent at a right angle at a length of 3.7 meters. Two L-shaped screens were formed into a perimeter around the enclosure and pushed down into the sandy-mud sediment. This precluded predators from burrowing into the enclosures from underneath. A total of six enclosures were built for the mesh size experiment - 2 replicates in each of the following mesh sizes: 6.7, 12.6 and 19 mm. The polyethylene mesh was stretched over re-bar frames and the resulting enclosures were stocked with clams at a density of 750 per m<sup>2</sup>. The stocking density/mesh size experiment consisted of two mesh size treatments (6.7 and 12.6 mm); in two stocking densities (750 clams/m<sup>2</sup>; 2 replicates each mesh size and 1,000 clams/m<sup>2</sup>; 3 replicates each mesh size). At 60-day intervals, 30 clams per enclosure (15 clams were selected from the upper and lower halves of each cage as related to tidal placement) were measured

for shell length ( $\pm$  0.1 mm) to determine the effect of tidal placement on clam growth. At the end of the study, clam size was estimated for each enclosure by measuring ( $\pm$  0.1 mm) 100 randomly selected clams from the upper and lower areas of each replicate enclosure. Survival estimates at termination of the study (September 1997) were based upon volumetric displacement estimates per replicate. Final volumetric displacement estimates were based upon number of clams per replicate per 18.9 liters displaced.

In the mesh size experiment, differences in clam size among the various treatments were determined by Analysis of Variance ( $\alpha$ = 0.05) and Tukey's Studentized Range test (SRT) ( $\alpha$ = 0.05). In the mesh size/ stocking density experiment, effects of the individual factors of mesh size and stocking density, and the mesh size by stocking density interaction were determined by Two-Way Analysis of Variance ( $\alpha$ = 0.05). Percentage survival data are

based upon volumetric displacement estimates and counts per replicate conducted at initiation and termination dates of the study, respectively. Kruskal-Wallis Tests were used to determine significant differences in clam survival between treatments. Statistical analysis was performed on SAS for PC (SAS Institute Inc., 1989).

### **RESULTS**

### **Mesh Experiment**

For the mesh enclosure experiment, we detected no significant differences in quahog mean SL among treatments sampled during February 1997 and April 1997 (Table 1). In July, clams from the 6.7-mm mesh enclosures were significantly smaller than clams from the 12.6-mm mesh enclosures. Clams from the 19.0-mm mesh enclosures were statistically the same size as clams from the 6.7-mm and 12.6-mm mesh enclosures. By September 1997, quahogs in the two larger mesh treatments (12.6 and 19.0 mm) were significantly larger (p < 0.0001) than clams in the 6.7-mm mesh treatment (Table 1).

Mean survival among mesh treatments at termination of the study in September was comparable, ranging from  $90.6\pm9.8\%$  to  $100.0\pm16.6$  % (19.0 and 12.6 mm mesh, respectively). Mean clam survival in the 6.7- mm mesh enclosure was  $91.4\%\pm$ 

18.4%. No significant differences (Chi-square = 0.857; p = 0.65) in survival occurred among the mesh treatments.

### **Density/Mesh Experiment**

Neither mesh size, stocking density nor the interaction of the two had any significant effects on quahog size from the study's initiation in December 1996 through the April 1997 sampling periods (Table 2). In the July sample period, both mesh size (p < 0.0001) and stocking density (p < 0.0001) significantly affected quahog shell length (Table 2). By September 1997, both mesh size (p < 0.0001) and stocking density (p = 0.0123) had significantly affected quahog shell length (Table 2). No significant interaction effects were observed in either the July 1997 (p = 0.2809) or September 1997 (p = 0.1285) sampling periods (Table 2).

**Table 1.** Clam shell length in mm ( $\pm$  SE) of quahogs cultured under various mesh sizes in bottomless cage enclosures at a stocking density of 750/m<sup>2</sup> by sample period. Letters under means represent Tukey's SRT rankings ( $\alpha$ =0.05), with common letters indicating no significant difference between mean size.

Mesh size	6.7	12.6	19.0
December*	26.6 ± 1.7	26.6 ± 1.7	26.6 ± 1.7
February (p = 0.5105)	29.1 ± 0.46 (a)	$28.9 \pm 0.45$ (a)	28.4 ± 0.43 (a)
April (p = 0.8446)	$35.4 \pm 0.45$ (a)	35.3 ± 0.45 (a)	35.0 ± 0.49 (a)
July (p = 0.0381)	41.0 ± 0.61 (b)	$42.8 \pm 0.48$ (a)	41.3 ± 0.5 (ab)
September (p < 0.0001)	$46.0 \pm 0.23$ (b)	$47.2 \pm 0.27$ (a)	47.5 ± 0.25 (a)

<sup>\*</sup> mean based upon collective sample experimental cohort (n = 200)

**Table 2.** Combined effects of stocking densities (750 and 1,000/m<sup>2</sup>) and mesh sizes (6.7 and 12.6 mm) upon quahog shell length in mm  $\pm$  (SE) by sample date as analyzed by Two-Way ANOVA.

Treatment mesh size(mm):	6.	7	12	.6
stocking density :	750	1000	750	1000
Dec 96 (n = 200)	26.6 ± 1.70	26.6 ± 1.70	26.6 ± 1.70	26.6 ± 1.70
Feb 97 ANOVA (n = 300)		=0.4454)	$28.9 \pm 0.45$ density: (nteraction: (p=0.357)	p=0.6649)
April 97 ANOVA (n = 300)	35.4 ± 0.45 mesh: (p	=0.4613)	22.0 = 0	p=0.5312)
July 97 ANOVA (n = 300)	41.0 ± 0.61 mesh: (p	<0.0001)	$42.3 \pm 0.48$ density: (nteraction: (p=0.286)	p<0.0001)
Sept 97 ANOVA (n = 2000)	46.0 ± 0.23 mesh: (p	45.27± 0.20 <0.0001) mesh/density ir		p<0.0123)

Lower stocking density (750 clams/m²) treatments in both the 6.7- mm mesh (94.1%  $\pm$  18.4%) and 12.7-mm mesh (100.0%  $\pm$  16.6%) enclosures yielded higher quahog survival over both higher stocking density (1,000 clams/m²) in 6.7-mm mesh (74.1%  $\pm$  1.1%) and 12.7-mm mesh enclosures (90.0%  $\pm$  4.9%: Fig. 3). No significant differences (Chi-square = 6.527; p = 0.1099) in clam survival occurred among treatments.

mean growth of quahogs between intertidal placements was significant, the minute difference is meaningless to the clam farmer.

### Tidal Placement vs. Clam Size

No significant differences in clam size as related to tidal placement occurred from December 1996 (p = 0.62) to April 1997 (p = 0.88). During July 1997, clam size ( $\bar{x}$ = 43.2 mm) in the high intertidal zone was significantly greater (p < 0.02) than clam size in the lower intertidal zone ( $\bar{x}$ = 42.1 mm), but by September 1997, clam size ( $\bar{x}$  = 46.9 mm) in the lower intertidal zone was significantly greater (p < 0.01) than clam size ( $\bar{x}$ = 46.4 mm) in the higher intertidal zone. Although the difference (0.5 mm) in



### DISCUSSION

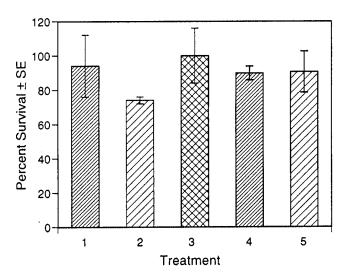
Predation on *Mercenaria mercenaria* seed clams is recognized as the primary factor contributing to mortality in both naturally recruited stocks (Carriker, 1961; MacKenzie, 1977; Peterson, 1982; Walker and Tenore, 1984) and cultured stocks (Castagna and Kraeuter, 1981; Kraeuter and Castagna, 1985; Flagg and Malouf, 1983; Walker, 1984). However, growth in field-cultured Mercenaria mercenaria is dependent upon a large spectrum of biotic and abiotic factors which, acting in concert, affect clam size (Kraeuter and Castagna, 1989). In this study, the integration of the meshbag-line technique used in conjunction with the bottomless cage enclosure technique demonstrated high predator protection and increased growth of seed, evidenced by high survival and rapid growth associated with each culturing phase (Walker and Hurley, 1995; Fig. 3 and Tables 1 and 3, respectively).

For the mesh enclosure study (750 clams/m<sup>2</sup>), clam

size was not significantly different among the three mesh sizes (6.7, 12.6 and 19.0 mm; Table 1), until harvesting in September 1997, when greater clam size was associated with the larger mesh sizes. This result is not unexpected since a reduction in mesh aperture proportional to clam size could negatively affect clam siphon extrusion through the mesh. This restriction could affect feeding efficiency and negatively impact growth. Furthermore, it is a standard industrial culturing practice to match mesh size proportionally with seed size. This allows increased water movement and flow of food particles through the mesh (Castagna and Kraeuter, 1981; Vaughan et al., 1987; Hadley et al., 1997).

In the mesh experiment, quahog survival rates were high and also similar among the various treatments (from 90.6% to 100%; Fig. 3). Two primary factors affecting cultured clam survival are predation (Hadley et al., 1997; Castagna and Kraeuter, 1981;

**Figure 3.** Percent survival of *Mercenaria mercenaria* seed of different stocking density and mesh size treatments in bottomless cage enclosures on Four Mile Island, Georgia. Error bars represent one SE above and below the mean.



Treatment	stock density (clams / m <sup>2</sup> )	mesh diameter (mm)
1	750	6.7
2	1000	6.7
3	750	12.6
4	1000	12.6
5	750	19.0

Kraeuter and Castagna, 1985; Flagg and Malouf, 1983; Walker, 1984) and sedimentation (Vaughan et al., 1987; Hadley et al., 1997; Castagna and Kraeuter, 1981). Mortality due to predation was minimal in both experiments reported here (Fig. 3). Clam predation by *Panopeus herbstii*, *Busycon* carica and Eupleura caudata was evident. Deleterious sedimentation and subsequent clam suffocation were controlled by lifiting the mesh covers bimonthly when routine growth data was collected. We hypothesize that lifting the mesh covers allowed for a positive vertical migration of clams within the enclosures. This upward movement could increase clam feeding efficiency, and thus lead to increased growth and survival. Lifting and shaking the mesh to remove accumulated sediment was found to be an efficient process that took one man less than two minutes per enclosure to complete.

In the mesh/density experiment, the effect of mesh sizes (6.7 versus 12.6 mm) and stocking density (750 versus 1,000/ m²) on quahog shell length

(Table 2) and survival (Fig. 3) were compared to better understand the individual and combined effects of these culturing practices. By the July sampling period, both mesh size and stocking density had a highly significant effect upon quahog shell length (Table 2). However, the interaction of these variables in July was insignificant(p = 0.2809; Table 2). The same trend continued through termination of the study in September 1997, with both mesh size (p < 0.0001) and stocking density (p = 0.0123) significantly affecting quahog shell length. No significant interaction effects (p = 0.1285; Table 2) were noted at termination. In this experiment, stocking density did not play a significant role in clam size until the July (p < 0.0001) and September (p < 0.0001) sampling dates (Table 2). Over time and exposure to culturing conditions, stocking density effects increased along with quahog size and biomass/area. Quahog survival was comparable between equal stocking density treatments (Fig. 3). Thus, these results demonstrate that stocking density directly affected clam growth rates within the treatments. Presumably, an increase in clam biomass/ area resulted in an increased demand that available algal resources be utilized for clam growth and maintenance. In intensive clam culturing operations, this results in greater competition for proportionally decreasing resources since bivalve food reguirements increase exponentially with body size/ age (Malouf and Bricelj, 1989). The growth data (Tables 1 and 2) indicate that this biomass/resource relationship demonstrated significant effects on clam size by the July sampling period (p = 0.0381; p < 0.0001; Tables 1 and 2, respectively), when animals approached 40 mm in length. This trend continued in both experiments until the clams were harvested in September (p < 0.0001, p = 0.0123; Tables 1 and 2, respectively). At that time, mean clam size in all treatments for both experiments exceeded 45.3 mm (Tables 1 and 2).

The effects of mesh size upon quahog shell length followed the same trends as stocking density with significant effects occuring by the last two sample periods of July (p < 0.0001) and September 1998 (p < 0.0001) (Table 2). By September, quahogs cultured under the 12.6-mm mesh in both density treatments (750 and 1,000/m<sup>2</sup>) had shell lengths comparable to those of quahogs cultured under the 6.7-mm mesh in equal densities (Table 2). These results further reinforce the findings of the mesh enclosure experiment (Table 1), in that the 12.6-mm mesh demonstrated superior aquacultural application in terms of clam biomass or yield. Additionally, lower stocking density in the 12.6-mm mesh size resulted in higher survival rates and greater clam size. These results concur with similar field studies in which increased stocking density negatively affect clam growth rates (Fernandez et al., 1997; Crenshaw et al., 1996; Walker, 1984; Eldridge et. al., 1979).

Although no significant differences in clam survival occurred in either experiment, overall clam survival per mesh size and stocking density yielded higher overall survival associated with the lower stocking density of 750 clams/m<sup>2</sup> in both 6.7 and 12.6 mm



mesh (Fig. 3). Likewise, the larger mesh size (12.6) mm) yielded increased survival over the smaller mesh size (6.7 mm) in both the higher and lower stocking densities (1,000 clams/m<sup>2</sup>, 12.6-mm mesh > 1,000 clams/m<sup>2</sup>, 6.7-mm mesh and 750 clams/m<sup>2</sup>, 12.6-mm mesh > 750 clams/m<sup>2</sup>, 6.7-mm mesh; Fig 3). These results suggest that different processes are responsible for survival differences among the mesh sizes utilized in the final grow-out stages of culturing. We hypothesize that two processes primarily account for survival differences observed among the different mesh sizes. First, a smaller mesh size is much more likely to be affected by fouling and sedimentation than a larger mesh size (Hadley et al., 1997; Castagna and Kraeuter, 1981; Vaughan et al., 1998). This was observed but not quantified during the routine sample periods of the study. Secondly, while small mesh is more prone to the effects of sedimentation, it also excludes a greater range of predators. The converse holds for larger mesh - it is less susceptible to sedimentation, but is more accessible to a wide range of predators. Greater

predator numbers and sizes may account for the reduced survival associated with the largest mesh treatment (19.0 mm; Fig. 3).

In the study evaluating the effects of tidal placement on clam size, it was found that in all teatments significantly larger clams (p < 0.01) occurred lower in the intertidal zone compared to the size of those higher in the intertidal zone ( $\bar{x}$ = 46.9 mm versus = 46.4 mm; respectively). Although these results represent significant statistical differences, the relatively small differences in clam size (0.5 mm) in each tidal zone do not demonstrate meaningful values from an applied aquacultural perspective.

This study determined that in terms of both clam growth and survival, the optimal treatment was a 12.6-mm mesh and a stocking density of 750 clams/ m² (Tables 1 and 2). Survival rates in both the larger and smaller mesh sizes (89% in 19.0-mm and 92% in 6.7-mm mesh treatments, respectively) were lower than that for clams from the 12.6-mm mesh

enclosures. We believe that the initial clam stocking size  $(26.6 \pm 1.7 \text{ mm}; \text{Table 1})$  was a critical factor in selecting the optimal mesh size (12.6 mm) for commercially culturing *Mercenaria mercenaria* in this study. Additionally, stocking density within an optimal mesh size played a significant role in expected clam growth in commercial applications (Walker, 1984; Anderson et al., 1982; Eldridge et al., 1976) and should approximate 750 clams/  $m^2$ , if bottomless cage enclosures are employed as a final grow-out technique.

The practice of culturing small seed in the mesh-bag-line system to a size of > 25 mm, and then transferring them to bottomless cage enclosures has excellent commercial potential along Georgia inshore waters. The mesh-bag-line system yields excellent seed survival rates (Walker and Hurley, 1995; Walker, 1997); however, animals exhibit a reduction in growth as they approach a 20-25 mm size. A comparative growth and survival study of quahogs cultured in three different grow-out

systems (mesh-bag-line, bottom cage and off bottom trays) showed significantly greater clam size (p < 0.0001) in trays and bottom cages compared to bags. Clam survival, however, was significantly higher in bags (p = 0.0054) versus trays and cages (Walker, 1997). Studies by Walker (1984) and Crenshaw et al. (1996) have shown that it takes approximately two years for clams to reach marketable size ( > 45 mm) in tray and cage cultures in Georgia. In this study, clams were field-planted at a size of 9.0 mm in the mesh-bag-line system on a commercial lease of Sapelo Sea Farms in June of 1996. At initiation of these experiments in December of 1996, clams were removed from the mesh-bag-line system and restocked under bottomless cage enclosure treatments. By September 1997, 68% of the animals cultured under the optimal mesh size and stocking density (12.6 mm and 750 clam/m<sup>2</sup>, respectively) had attained market size (45 mm). Thus, seed clams planted at a size of 9.0 mm had reached marketable size in 15 months using the conjunctive techniques of the mesh-bagline system and bottomless cage enclosures.



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