COMPARATIVE EFFICIENCY AND YIELD IN DIFFERENT SYSTEMS AND DENSITIES AT THE NURSERY CULTURE PHASE OF THE OYSTER *Crassostrea gigas* IN SOUTHERN BRAZIL[1]

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ABSTRACT: This study was conducted to compare different aquaculture systems and seed densities for the nursery phase of *Crassostrea gigas* in Brazil. Small oyster seeds (2 mm in height) reared in three nursery systems (same area/floor), a lantern net, a bouncing bucket or a floating box were compared with three initial stocking densities (50, 100 and 200 mL of seeds/system floor). As density increased, there was a significant decline in seed growth and yield, mainly in the lantern nets (P=0.0002). Better yields were observed in the box (P=0.005) and bucket systems (P=0.02) under densities of 50 and 100 mL. However, at the highest density, the nursery box system had the highest seed yield when compared to the lantern net and bucket systems (P=0.003). The results showed that the floating box was the most efficient system tested with a survival of 54% at a density of 100 mL. This was more than twice the survival for the same volumes used in the nursery lantern nets (24%), which was the most common culture method in Brazil prior to this experiment (2002). An additional advantage is the reduced cost of starting with 2.0 mm seeds that cost 50% less than the usual 4.0 to 8.0 mm seeds. These results showed the efficiency of the floating box system, which is currently used by over 90% of oyster producers in Brazil.

Keywords: *Crassostrea gigas*, growth, nursery, production, seeds.

COMPARAÇÃO DE EFICIÊNCIA E RENDIMENTO DE DIFERENTES SISTEMAS E DENSIDADES NA FASE DE CULTIVO BERÇÁRIO DA OSTRA Crassostrea gigas NA REGIÃO SUL DO BRASIL

RESUMO: Neste trabalho, foram comparados diferentes sistemas de cultivo e densidades na fase de berçário da *Crassostrea gigas* no Sul do Brasil. Sementes de ostra (2 mm de altura), mantidas em três sistemas de berçário (de mesma área de piso), lanternas, baldes flutuantes e caixas flutuantes foram comparadas começando com três densidades de estocagem (50, 100 e 200 mL de sementes por piso de cada sistema). Com o aumento da densidade houve significativa diminuição do crescimento e rendimento, principalmente nas lanternas berçário (p=0,0002). As melhores sobrevivências foram observadas na caixa (p=0,005) e nos baldes (p=0,02) em densidades de 50 e 100 mL. Na maior densidade, o sistema de caixa flutuante apresentou o melhor rendimento quando comparado ao sistema de balde e de lanterna (p=0,003). Os resultados mostraram a caixa flutuante como o mais eficiente dos sistemas testados, chegando a rendimentos de 54% na densidade de 100 mL, mais do que duas vezes a sobrevivência alcançada no sistema de lanternas (24%), que era o mais utilizado no Brasil até o momento deste experimento (2002). Uma vantagem adicional é a redução no custo ao se iniciar o cultivo com sementes pequenas (2mm) que custam 50% menos do que sementes de tamanho normal entre 4 e 8 mm. Esses resultados mostram a eficiência do sistema de caixa que é utilizada, atualmente, por mais de 90% dos produtores no Brasil.

Palavras-chave : *Crassostrea gigas*, crescimento, berçário, produção, sementes.

INTRODUCTION

World aquaculture production attained an all-time high in 2010 of approximately 60 million tons (excluding aquatic plants) worth US$ 119 billion. Mollusks were the second highest group produced at 23.6% of total production of aquaculture (FAO, 2012).

In Brazil, oyster culture is an industry characterized by production based on the family unit, which offers alternative income options and economic benefits to the local fishery communities. Thanks to the increased development of employment opportunities, those taking part in oyster culture are settling down in their areas of origin (Brandini *et al.* 2000).

Crassostrea gigas is a non-native species in Brazil, and because of its inefficient natural reproduction, it is necessary to use hatchery production of larvae and seeds for commercial purposes (Ferreira *et al*., 2011). The nursery phase in a natural sea water environment is very important in order to supply the seeds with a suitable biological habitat, as well as to reduce the costs of laboratory procedures (Gomes, 1986; Ferreira *et al*., 2007).

The systems used during the nursery phase can be organized into three categories: raceways, upwellers and natural environment systems. The natural environment system is the least expensive. However, there is less control of the biophysical parameters to which the seeds are submitted, such as temperature and food concentration in the culture site. According to Anon (1993), the control of these parameters can lead to better growth and survival.

The nursery and intermediary phases have the highest mortality rates in the culture process for seeds and juveniles (Walne, 1979). Rheault Jr (1995) and Kraeuter *et al*. (1998) attained good survival and growth rates in natural nursery systems with *Mercenaria mercenaria* seeds. Specifically, in Santa Catarina ecosystems, where water column and flow rate are relatively small, the production loss can reach 80% (Oliveira Neto *et al.* 2003). These conditions directly influence the increase in the seed and juvenile mortality in oyster cultures (Walne, 1979).

Previous studies that describe the effect of density on mollusk growth in different nursery systems include that of Holliday *et al.* (1991) with *Saccostrea commercialis* in trays, Widman and Rhodes (1991) with *Argopecten irradians irradians* in pearl nets, Bricelj *et al.* (1992) with *C. virginica* in floating trays, and Mondragon *et al*. (1993) with *Pinctada mazatlanica* and *Pteria sterna* seeds in lantern nets, pearl nets, pocket nets and boxes. Other relevant studies were conducted by Taylor *et al.* (1997) with *Pinctada maxima* in a suspended nursery, Southgate and Beer (1997) with *P. margaritifera* in mesh bags and trays, Honkoop and Bayne (2002) with *C. gigas* and *Saccostrea glomerata* cultured in trays, and by Walker (2001) with *Spisula solidissima* cultured in mesh bags and boxes.

Although there are some published studies related to the performance of *C. gigas* culture in Brazil, there is a lack of information regarding the optimal period to initiate cultivation, as well as seed survival and growth during cultivation (Robbs, 2000).

Silva (1998) studied the performance of *C. gigas* culture in two Santa Catarina areas (Sambaqui and Palhoça). Maccacchero (2001) tested the growth and survival of *C. rhizophorae* at two initial densities with two cleaning and handling variations. A previous experiment carried out at EPAGRI (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina) compared bucket and lantern net systems using *C. gigas* seeds at densities of 10,000 seeds per tray (Oliveira Neto *et al*., 2003).

The Brazilian oyster production rates are directly related to seed availability (Ostrenk *et al*., 2000). While the demand for oyster seeds has been increasing, seed production still depends on a single public hatchery laboratory. The lack of an adequate and constant supply of seeds is worsened by the low yields achieved by the producers, due to poor handling techniques during the small seed phase (Oliveira Neto *et al*., 2003). As a result of these difficulties, producers used to acquire seeds that were 5.0 to 8.0 mm or more in height, which ensured fewer losses during the grow-out phase. However, the seeds cost double compared to smaller seeds due to the higher risks and lower production capabilities in the laboratory.

The purpose of nursery rearing bivalves is to culture small seeds in a minimum amount of time, at high densities, with minimal costs and risks, until the seeds reach a size suitable for transfer to grow out lanterns (Claus *et al*., 1981). Moreover, the supply could be regulated if the producer had a way to increase yield and was able to acquire smaller seeds, which would reduce seed manual labor and residence time in the laboratory, thereby, increasing the production flow.

In Southern Brazil, increasing attention has been focused on evaluating better handling systems for small *Crassostrea gigas* oyster seeds that are 2.0 mm in height instead of the normal 5.0 to 8.0 mm seeds used in the traditional lantern nets culture method. This could lead to better profits for the producers and improved laboratory production and distribution capabilities.

This study aimed to test the feasibility of three different culture methods and to evaluate the most efficient seed density and yield for each system.

MATERIAL AND METHODS

The experiment was conducted in two trials, each lasting two months between February and April 2002. The experimental trials were carried out at an Experimental Cultivation Unit located at Sambaqui Beach (48°32'W-27°35'S), Florianópolis, North Bay of Santa Catarina Island, Southern Brazil. The culture area belongs to the Laboratory of Marine Molluscs (LMM) of Santa Catarina Federal University and is characterized as a sheltered bay inside the strait between the Santa Catarina Island and the mainland, with a sandy mud bottom and a predominant south or northeast wind. The bay is 3 to 4 meters deep, with no waves and a tidal variation of approximately 1 meter. The hatchery seeds of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) used in this study were obtained from five different LMM production batches, all with an initial height of 2.0 mm (selected with 1.5 mm mesh nets).

Seeds were manually counted and the average seed number per mL was calculated to determine the initial amount of seeds used in the different systems and the different densities.

In order to compare the efficiency and yield of the nursery systems and the stock densities for *C. gigas* seeds, five experimental procedures (Table 1) were conducted. In addition to the well-known bouncing buckets and nursery lantern nets used in many culture areas, a floating box system was also tested. The floating box system consists of two exterior screened wooden frames (base and lid) attached to each other with screws containing nine compartments. Rubber enclosures were used to prevent seed losses either to the exterior environment or to other box compartments. Floats and anchors were used to maintain system buoyancy and stability, respectively (Figure 1). The buckets had the same design as those that are commonly used by producers. They were 50 cm in height and had a floor area of 0.13 m^2, with mesh on the floor and a small round mesh cover (10 cm diameter) in the center. The lantern nets used were also similar to those used by the producers with 5 floors that were 1 m in height with an area of 0.13 m^2. We used all floors that had the same size and density of seeds in addition to the seeds on the middle floor for the analyses.

For each nursery system, the three different seed densities were carried out in triplicate. Therefore, nine lantern nets, nine buckets, and one floating box with nine compartments were used in each experimental procedure. All of the tested systems had 1.0 mm mesh nets, and all of the nursery floors had the same area of 0.13 m^2. A long-line was used to hang the lantern nets and buckets.

With practical application and posterior utilization as the goal, the oyster farmers measure densities in milliliters (mL). However, to obtain the yield percentages for statistical analysis, the volume was converted to seed number/mL.

Although all of the seeds used to start the experiments were classified as 2 mm, they were obtained from different production batches and selected in 1.5 mm mesh nets, hence, there were small differences in height and shape. Therefore, to standardize, the seed quantities that were 1.0 mL per lot are shown in Table 2.

Once the nursery systems were installed, handling was carried out every three days by cleaning the structures with a high pressure washer. Afterwards any damage was verified and repaired or substituted if necessary.

Every ten days the structures were removed for handling, as described above, and the screening was removed to examine survival and yield. The seeds that did not reach a bigger size class returned to the sea (in the same structures) to be sieved again after another ten-day period. To standardize the experiments only two mesh screens were utilized in each experimental batch.

Table 1. Stock densities and nursery culture systems in each trial and experimental procedure

Trial	Experimental procedure	Stock densities (mL)	Nursery culture systems
01	01	50 and 100	Box and lantern net
01	02	50 and 100	Box, bucket and lantern net
02	03	50, 100 and 200	Box, bucket and lantern net
02	04	50, 100 and 200	Box, bucket and lantern net
02	05	50, 100 and 200	Box, bucket and lantern net

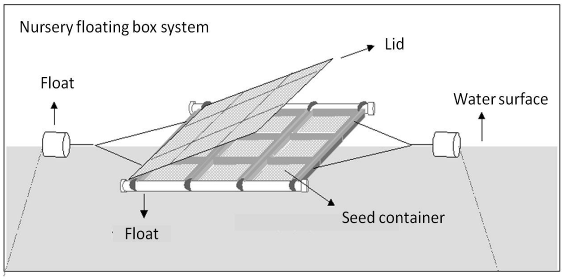

Figure 1. Model of the nursery floating box system.

Table 2. Average seed number of size 1.5 mm (mesh nets) in 1.0 mL used to start the experimental trials

Production batch	Quantity by mL
01	178.66 ± 13.36
02	279.0 ± 21.01
03	254.0 ± 18.56
04	272.2 ± 25.09
05	310.0 ± 34.61

The seeds submitted to screening were placed into freshwater tanks and passed through a group of sieves with different mesh sizes (1.5 mm - 2.0 mm- 3.0 mm- 4.0 mm and 5.0 mm), superimposed in crescent order. To facilitate the analysis, the sieves' mesh sizes were used as seed size and then, after being graded, volume was determined for each class size.

From each class size, three samples were taken (in triplicate) for posterior manual counting and calculation of the approximate average seed number. The seed classes determined the volume taken (Table 3). To verify the seed quantity in each class size obtained in the experimental trials, the average seed number was divided by the sampled volume and multiplied by the total volume found.

Table 3. Size classes and sampled seed volume at each sieve mesh size

Mesh nets size (mm)	Height (mm)	Sampled volume (mL)
1.5	2.0	2
2.0	3.0 to 4.0	2
3.0	4.0 to 5.0	10
4.0	5.0 to 7.0	15
5.0	7.0 to 8.0	30

The seeds with a size equal to or greater than 3.0 mm (selected with mesh nets) represented the more highly commercialized seeds commonly sold to producers. The seed yield was calculated as a percentage of the sum of the seed numbers for the 3.0 mm, 4.0 mm and 5.0 mm (mesh nets) (from 4.0 to 8.0 mm in height) seeds compared to the initial seed number size of 1.5 mm (mesh nets) (2.0 mm in height) at each density.

All of the percentage data were arccosine transformed for statistical analysis (Steel and Torrie, 1980). The results were initially analyzed with Friedman and Bartlett tests. An analysis of variance (ANOVA) was employed, followed by Tukey's test.

RESULTS

The results of each independent trial were analyzed together due to the standardization of the methodologies used and of the statistical analyses.

Figure 2 shows the average percentage yields of 1.5, 2.0 and ≥3.0 mm (mesh nets) seeds obtained over the nine treatments. Table 4 shows the average percentage yield results and statistical comparisons for the different densities tested in each nursery system.

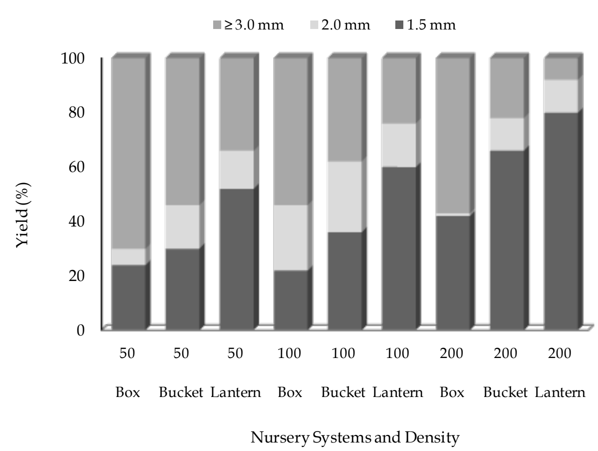

Figure 2. Final average yield percentages of 1.5mm, 2.0mm and ≥3.0mm (mesh nets) seeds in the nine treatments.

Table 4. Final average yield percentages of ≥3.0 mm (mesh nets) seeds in the densities tested (mL) and nursery system

System[2]	Average yield percentages		
	Density[1] (mL)		
	50	100	200
Box	69.73aA	53.61bA	58.94abA
Bucket	54.84aB	41.56abAB	21.02bA
Lantern net	35.59aAB	24.45aB	8.41bB

[1]exponential lowercase letters in line indicates significant differences (P<0.05) between densities in each nursery system. [2]exponential capital letters in column indicate significant differences (P<0.05) between nursery systems in each density.

Significant differences were observed in the yield of seeds that were ≥3.0 mm (mesh nets) at the end of the experiment for the different densities in the floating box system (P=0.005), bucket system (P=0.02) and lantern net system (P=0.0002).

In the floating box system, no significant differences were observed between the densities of 50 and 200 mL, nor between the 100 and 200 mL densities. A significant difference was detected in the final seed ≥3.0 mm (mesh nets) yield (P=0.04) when comparing the 50 mL to 100 mL densities.

We did not observe significant differences between the 50 and 100 mL and the 100 and 200 mL densities, whereas a higher yield was found for the 50 mL density compared to the 200 mL (P=0.003) in the bucket system. In the lantern net system, the 200 mL density had a lower yield compared to the 50 mL (P=0.001) and the 100 mL (P=0.02).

For the yield of seeds ≥3.0 mm (mesh nets), significant differences were detected for the densities of 50 mL (P=0.00001), 100 mL (P=0.0005), and 200 mL (P=0.00002) among different the nursery systems.

Significant differences were not found between the floating box and bucket systems at densities of 50 and 100 mL. However, lower yields were observed in lantern nets, at both the 50 and the 100 mL densities, when compared to the floating box (P=0.00014 and P=0.0009) and the buckets (P=0.017 and P=0.04).

At the 200 mL density, the floating box had a higher yield compared to lantern nets (P=0.0001) and buckets (P=0.0001). However, significant difference between buckets and lantern nets, was not observed.

DISCUSSION

When the seed density increased, a reduction in growth, and a decrease in yield of *Crassostrea gigas* seeds was observed, although no significant difference was found between the 50 and 100 mL densities. Overall, the 50 and 100 mL densities showed higher yields when compared to the 200 mL density, specifically in the lantern net system.

Unexpectedly, the 50 mL density did not differ statistically from the 200 mL in the floating box. This could be attributed to an overestimation of the average percentage yield, due to some repetition, despite the Friedman test showing that the different experimental batches could be considered as replicates. However, as expected, there was a higher yield of seeds ≥3.0 mm (mesh nets) (representing seeds from 4 to 8 mm in height) at the 50 mL density compared to the 100 mL density. The bucket system met its expectations: higher yields were achieved at the 50 mL density when compared to the 200 mL density.

Contrary to the current results, Widman and Rhodes (1991) did not find significant differences among densities using 4.0 mm seeds of *Argopecten irradians irradians* cultured in natural pearl net system. Similar results were reported by Honkoop and Bayne (2002) for *C. gigas* and *Saccostrea glomerata* with larger seeds (5.0 mm) in trays, and the densities did not influence oyster survival and growth.

Holliday *et al*. (1991) achieved lower *S. commercialis* seed growth with increasing density in trays in a natural nursery. Additionally, the survival (approximately 97.5%) did not significantly decrease with a higher seed volume. Reductions in growth and survival were recorded at higher culture densities of *C. virginica* that had initial sizes of 6.4 and 31.7 mm (Bricelj, 1992).

Taylor *et al*. (1997) also found lower growth and survival of 5.0 - 6.0 mm seeds of the pearl oyster *Pinctada maxima* when they were cultured in high densities. The authors attributed the decrease in growth to increased food competition as density increased.

Honkoop and Bayne (2002), reported that the lack of a density effect on growth and survival was merely due to the variations in the tested densities being too small to promote such effect.

In the present study, at densities of 50 and 100 mL in the floating box and bucket systems resulted in similar yields, which were superior to the lantern nets. Lantern nets with a seed density of 100 mL have been the most widely used system by oyster farmers in Santa Catarina. Thus, incorporating other tested nursery systems may increase efficiency and productivity of oyster farming.

Furthermore, at the higher density, the floating box system was superior in yield of seeds ≥3.0 (mesh nets) (representing seeds from 4 to 8 mm in height) compared to the buckets and lantern nets, which did not significantly differ.

Mondragon *et al.* (1993) compared nursery systems (lantern nets, pearl nets, pocket nets and bottom cages) for *P. mazatlantica* and *Pteria sterna*, and the net cages nursery system is recommended higher survival of pearl oysters.

The growth of 1.38 mm *P. margaritifera* seeds was lower in higher density treatments, and in the tray nursery system the growth was superior to growth in the pearl net system that had been traditionally used for oyster culture (Southgate and Beer, 1997).

The negative effect of density in relation to growth and survival was more enhanced in 5.0 mm *Spisula solidissima* seeds (surfclam) raised in the standard mesh bag nursery compared to the bottom cage nursery (Walker, 2001).

Our study showed results similar to Oliveira Neto *et al*. (2003), when EPAGRI attained better results in buckets when compared to lantern nets on the yield of 2.0 mm (1.5 mm mesh nets) *C. gigas* seeds. The authors concluded that the compactness and sedimentation on the lantern net base floors could be one of the factors responsible for the seeds' mortality and growth delay, while the buckets, which allow vertical water circulation, produce the opposite effect of higher growth.

Unlike the buckets and lantern nets that stay closer to the bottom, the floating box occupies a superior position in the water column, and could be submitted to larger water flows, which according to Walne (1979), are an important influence on bivalve growth. Food availability is equally important and is directly associated to water flow (Brown and Hartwick, 1988). Moreover, according to Southgate and Beer (2000), for pearl oysters, pocket bags in the surface position, undergoing constant agitation increases the water oxygenation received by the seeds and leads to less fouling. According to Mallet *et al.* (2013), floating systems are efficient for growing oyster seeds in a less dynamic environment, and this is the case in this study at the Santa Catarina bay site with no waves and low variation in tide.

The washing and handling carried out in this study prevented the incidence of fouling, which, according to Bardach *et al.* (1972), may hinder growth by food competition or clogging the nursery mesh.

Some structural damages occurred in the floating box and bucket systems, mainly due to rough weather conditions. As reported by Silva (1998), the lantern net nursery used in the early experimental stage did not withstand the strength of the sea, which highlights that issues early in the experiment can greatly affect the outcome.

It is important to mention that the present study evaluated yields using only two screenings, and the remaining 1.5 and 2.0 mm (mesh nets) seeds were returned to the culture system to be revaluated later (at the end of the two months experiment time), which led to higher yields of seeds ≥3.0 mm (mesh nets) (representing seeds from 4 to 8 mm in height) at the end of the experiment.

CONCLUSIONS

In this study, the floating box and buckets had superior efficiencies and yields when compared to the lantern nets, which are currently the most widely used nursery system in Brazil. Furthermore, the floating box system was more beneficial for farmers compared to the other systems because it reduced handling and labor.

Although the results showed the floating box at a density of 100 mL to be the most efficient nursery system tested, the choice of seed density is directly related to the purchasing power and labor availability to handling and screening.

Beyond the cost-benefit advantages to the farmers, this methodology allows the laboratory to give smaller size seeds to the producers, which may increase productivity and production, as well as reduce the delivery time and cost of seeds, due to their reduced maintenance time in the laboratory.

Since 2004, and including the results of this experiment, the Laboratory of Marine Molluscs has been testing the floating box system in many experiments and using it as a control for seed production. Additionally, because of these results, the producers started to use 2.0 mm (1.5 mm mesh nets) seeds that cost half the price of the larger 4.0 to 8.0 mm seeds, with more than 80% yield from 2 to 10 mm in 3 weeks. Due to these positive results more than 90% of the producers of Santa Catarina State (southern Brazil), the largest oyster production area in the country, are currently using the floating box system.

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