Arquivos de Ciências do Mar

THE COMBINED EFFECTS OF SALINITY, STOCKING DENSITY AND FREQUENCY OF WATER EXCHANGE ON GROWTH AND SURVIVAL OF MANGROVE OYSTER, *Crassostrea rhizophorae* (GUILDING, 1828) LARVAE

Efeito conjunto da salinidade, densidade de estocagem e tempo de troca de água, sobre o crescimento e sobrevivência larval da ostra-do-mangue, *Crassostrea rhizophorae* (Guilding, 1828)

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ABSTRACT

This paper reports the combined effects of salinity, stocking density and frequency of water exchange on the growth and survival of Crassostrea rhizophorae *from first to 6th day (trial 1) and 7th to 14th day of development (trial 2). Two salinities (25 and 35) and three densities (3, 6 and 12 larvae ml^{-1} in trial 1; and 2, 4 and 8 larvae ml^{-1} in trial 2) were tested at three different frequencies of water exchange (24, 48 and 72 h). Larvae reared in the salinity of 35 showed the highest survival in trial 1, but it was not significantly different from the salinity of 25 with water exchange of 72 h. In the second trial, survival was higher in the salinity of 25. Growth was higher in both trials in the salinity of 25. Overall, water exchanges of 48 and 72 h significantly improved growth and survival. Stocking densities had no significant effect on individual length, but its height was improved at 3 and 6 larvae ml^{-1}. Survival was higher at 6 (33.13%) and 12 (36.22%) larvae ml^{-1} in trial 1 and at 2 (16.69%) larvae ml^{-1} in trial 2.*

***Key words**: larval rearing, management practices, growth, mangrove oyster, physical factors.*

RESUMO

O efeito conjunto da salinidade, densidade de estocagem e tempo de troca de água no crescimento e sobrevivência larval da Crassostrea rhizophorae *foram estudados em dois experimentos correspondentes ao período do 1º ao 6º dia (experimento 1) e 7º ao 14º dia de desenvolvimento (experimento 2). Duas salinidades (25 e 35) e três densidades (3, 6 e 12 larvas ml^{-1} no experimento 1 e 2, 4 e 8 larvas ml^{-1} no experimento 2) foram testadas sob três tempos de troca de água (24, 48 e 72 h). Larvas cultivadas sob a salinidade de 35 apresentaram maior sobrevivência no experimento 1, porém esta não foi significativamente diferente da salinidade de 25 com trocas de água a cada 72 h. No segundo experimento a sobrevivência foi maior na salinidade de 25. O crescimento foi superior na salinidade de 25 em ambos experimentos. Trocas de água de 48 e 72 h aumentaram significativamente o crescimento e a sobrevivência. As densidades de estocagem não apresentaram efeito significativo no crescimento em comprimento, porém a altura foi incrementada nas densidades de 3 e 6 larvas ml^{-1}. A sobrevivência apresentou os maiores resultados nas densidades de 6 (33,13%) e 12 (36,22%) larvas ml^{-1} no experimento 1 e 2 (16,69%) larvas ml^{-1} no experimento 2.*

***Palavras-chaves**: larvicultura, práticas de manejo, crescimento, ostra-do-mangue, fatores físicos.*

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INTRODUCTION

The mangrove oyster, *Crassostrea rhizophorae*, is an important species for commercial exploitation along the Brazilian coast, mainly in the Northeast region. Extensive beds of this species, especially around urban areas, are overexploited, and natural production has been decreasing significantly (Lemos *et al*., 1994). Oyster culture could help to overcome this problem, but the supply of seeds for commercial purposes, must be assured by hatchery production as the collection of wild seeds will unlikely meet the demands for this kind of venture. However, few studies on seed production of *C. rhizophorae* are available in the literature and a well-established rearing protocol for this species has not yet been defined.

Ecological studies have clearly demonstrated that development, growth and survival of bivalves are affected by physical parameters, particularly salinity (Kinne, 1964). Salinity represents one of the most important environmental factors that influence the biology of planktonic stages of estuarine organisms, such as oyster larvae. Furthermore, salinity can be easily measured, manipulated and controlled in hatcheries (Devakie & Ali, 2000). The influence of salinity on the larval growth and survival has been studied for *C. rhizophorae* (Dos Santos & Nascimento, 1985; Lemos *et al*., 1994; Miranda & Guzenski, 1999).

Loosanoff & Davis (1963) established the general principles for culturing larvae of bivalve mollusks, but recommended rearing densities substantially higher than those normally tolerated by different species. While an inverse relationship between growth and stocking density occurs for the hard clam, *Mercenaria mercenaria* (Linnaeus), and for the American oyster, *Crassostrea virginica* (Gmelin), these authors found that survival was not significantly affected. Subsequently, a few studies have been undertaken to determine optimal stocking densities for different mollusk species (Ibarra *et al*., 1997).

Commercial hatcheries usually use a static-water culture system, where the water is periodically exchanged after a certain period of time. During this procedure, larvae are usually drained from the culture tanks into a mesh screen and returned after the tank is cleaned and refilled (Southgate & Ito, 1998). Helm & Millican (1977) verified that when water is exchanged more frequently than every 48 h, larval growth is affected. Since then, several authors (Ibarra *et al*., 1997; Labarta *et al*., 1999; Doroudi & Southgate, 2000; Ponis *et al*., 2003a; Ponis *et al*., 2003b) have exchanged water every 48 h during larval rearing of bivalve molluscs rather than daily (Doroudi *et al*., 2002) or 72 h exchanges (Thompson *et al*., 1996).

The aim of the present study was to examine the combined effects of salinity, stocking density and frequency of water exchange on growth and survival of *Crassostrea rhizophorae* larvae during the first two weeks of development.

MATERIAL AND METHODS

This study was carried out in September, 2006 at the Laboratório de Maricultura Sustentável, Universidade Federal Rural de Pernambuco (LAMARSU–UFRPE), Recife, Brazil. Mature mangrove oyster broodstock were collected in a farm located in the municipality of Goiana (7º40'33"S, 34º50'34"W), Pernambuco, Brazil and transferred to LAMARSU.

Spawning

One hundred mature broodstock were induced to spawn by thermal stimulation (Breese & Malouf, 1975). After the oocytes and spermatozoids were released, the total number of gametes was estimated and then oocytes were fertilized *in vitro*. Approximately 12 million eggs were incubated at a density of 30 eggs ml^{-1} for 24 h in a fiberglass tank containing water at 25 ºC and salinity of 30.

After 24 h of fertilization, D-veliger larvae were collected using a 35-µm mesh sieve and the survival was estimated by counting three 1-ml aliquots. A 40 ml sample was preserved in a 4% formaldehyde solution to determine initial size (shell length and height) of 30 larvae.

Larval rearing

Veliger larvae were reared at three frequency of water exchange (FWE): 24, 48 and 72 h, divided in two trials regarding the larval stages of *C. rhizophorae*. In the first trial, first to 6th day - D-veliger larvae were reared in the densities of 3, 6 and 12 larvae ml^{-1}. In the second trial, 7th to 14th day – umbo larvae were reared in the densities of 2, 4 and 8 larvae ml^{-1}. Regardless of stocking density and FWE, both larval stages were exposed to the salinities of 25 and 35. Each trial was composed by 18 treatments (combinations of 2 salinities, 3 stocking densities and 3 FWE) with 3 replicates each.

In both trials, larvae were reared in 3-L polypropylene containers with 3-µm filtered seawater. Gentle aeration was provided via individual air diffusers, keeping mean values of dissolved oxygen at 6.78 ± 0.16 mg l^{-1}. At each water exchange, larvae were drained onto 35 and 90-µm mesh sieves in the first trial and 50 and 120-µm sieves in the second.

Mean water temperature during the 14 days of culture was 24.9 ± 0.4 ºC.

Feed was offered twice a day (40% in the morning and 60% after water exchange) and consisted of *Thalassiosira pseudonana* (30 cells μl^{-1}) from days 1 to 6 and a mixture of 35 cells μl^{-1} of *Chaetoceros muelleri* and 15 cells μl^{-1} of *T. pseudonana* from days 7 to 14. Microalgae species were cultured in a batch system using Conway medium (Walne, 1964) and were offered to the larvae during the exponential growth phase. In both trials, the same concentrations of microalgae were fed to larvae regardless of density.

Larvae used in trial 2 were reared during 7 days in two 500-L fiberglass tanks at salinities of 25 and 35 each, stocking density of 6 larvae ml^{-1}, water exchange of 48 h, gentle aeration and the same concentration of food used in trial 1.

At the end of each trial, all larvae were drained and preserved in a 4% formaldehyde solution for further analysis. Survival was estimated as a percentage of the initial population in each unit.

Growth measurements in terms of shell length (maximum antero-posterior dimension) and height (maximum dorso-ventral dimension) were determined in 30 larvae per experimental unit. Larvae were observed under a microscope (100 x) connected to a digital camera. Images were captured, digitized and measurements were performed using the software ImageTool version 2.0 for Windows (The University of Texas Health Science Center in San Antonio, TX, USA).

Statistical analyses

Data were analyzed using Statistica 7.0 (StatSoft Inc., USA). The sample size (30 larvae) for growth analysis was defined according to the method described by Nomura (1960). Percentage data were arcsine transformed to satisfy requirements for homogeneity of variance before being analyzed. Normality and homogeneity were verified by the Kolgomorov-Smirnov and Cochran tests (Zar, 1984), respectively.

A multi-factorial ANOVA (2 x 3 x 3) was used to verify the effects of salinity, stocking density, frequency of water exchange and their interactions on growth and survival. In the cases where significant differences ($P<0.05$) were observed, the Student-Newman-Keuls test ($P<0.05$) was performed.

RESULTS

Trial 1

Mean (±SD) initial length and height of the larvae was 64.42 ± 2.78 and 56.71 ± 2.98 µm, respectively. Salinity and FWE presented significantly different effect on length, but only salinity affected height. The effects of salinity-FWE and salinity-stocking density interactions on larval growth are shown in Figures 1 and 2, respectively.

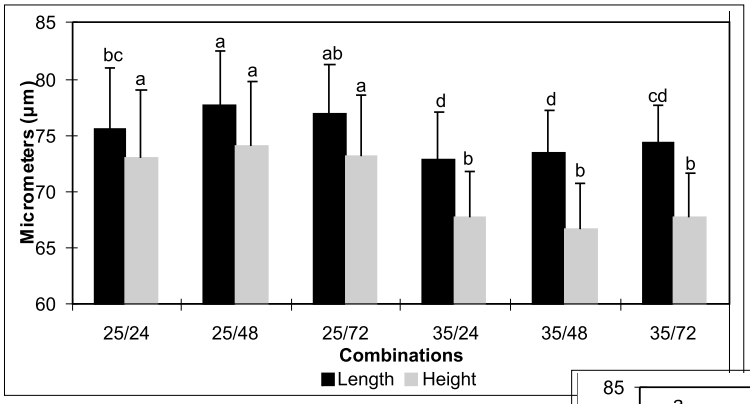

Figure 1 - Mean values (± SD) of *C. rhizophorae* larvae length and height obtained in the 6th day of culture in the combinations of salinity (25 and 35) and frequency of water exchange (24, 48 and 72 h). Values with the same letter do not differ significantly according to the Student-Newman-Keuls test (P=0.05).

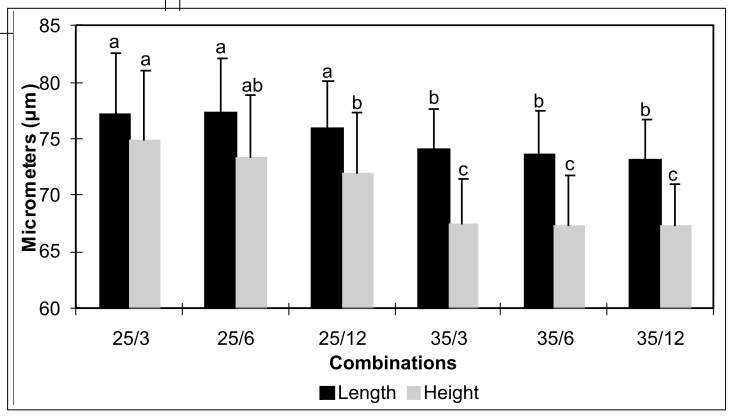

Figure 2 - Mean values (± SD) of *C. rhizophorae* larvae length and height obtained in the 6th day of culture in the combinations of salinity (25 and 35) and stocking density (3, 6 and 12 larvae ml^{-1}). Values with the same letter do not differ significantly according to the Student-Newman-Keuls test (P=0.05).

With the exception of the salinity-stocking density and stocking density-FWE interaction, all other factor significantly affected survival. The effects of the salinity-FWE and salinity-stocking density interactions on survival are shown in Table I. Overall, the higher results of survival were verified in the salinity of 35, stocking densities of 6 and 12 larvae ml^{-1} and FWE of 48 e 72 h.

The growth performance (length and height) was improved when larvae were reared in the salinity of 25 and FWE of either 48 or 72 h. Stocking densities had no significant effect in terms of length, but height was improved at 3 and 6 larvae ml^{-1}. Daily water exchange presented negative effect on growth and survival.

Trial 2

Initial larval size for trial 2 of 82.44 ± 4.67 and 82.13 ± 6.77 µm (length) and 78.89 ± 3.31 and 74.91 ± 4.49 µm (height), respectively. As these values differed significantly, the percentage growth rate (length and height) was used as a response variable. The percentage growth rate in length and height were significantly influenced by the salinity and the salinity-FWE interaction (Figure 3).

Survival was influenced by all other factors and their interactions, except the salinity-stocking density interaction. Table I shows the final survival in the salinity-FWE and salinity-stocking density interactions.

The highest relative growth rates were observed when larvae were reared in the salinity of 25 at all densities (2, 4 and 8 larvae ml^{-1}) and water exchanges of 48 and 72 h. For survival, highest results were verified in the salinity of 25 at the density of 2 larvae ml^{-1} and water exchange of 48 h. The use of daily water change and salinity of 35 presented a negative effect on larval growth and survival.

Table I -. Mean (± standard deviation) survival (%) of *C. rhizophorae* larvae obtained in the 6th (trial 1) and 14th day (trial 2) of culture in combinations of salinity (25 and 35), stocking density (trial 1 = 3, 6 and 12 larvae ml-1; trial 2 = 2, 4 and 8 larvae ml-1) and FWE (24, 48 and 72 h).

Combinations Salinity-FWE	Survival (trial 1)	Survival (trial 2)
25/24	10.14 ± 1.93^{c}	13.66 ± 2.04^{bc}
25/48	27.01 ± 3.35^{b}	22.36 ± 2.86^{a}
25/72	45.16 ± 8.50^{a}	15.15 ± 0.99^{b}
35/24	23.80 ± 2.90^{b}	11.52 ± 1.38^{c}
35/48	45.75 ± 4.97^{a}	15.65 ± 1.27^{b}
35/72	42.36 ± 3.62^{a}	12.16 ± 1.23^{c}
Salinity-Stocking density		
25/3 (2)	24.58 ± 3.66^{c}	19.01 ± 1.25^{a}
25/6 (4)	27.34 ± 5.56^{bc}	15.68 ± 1.89^{b}
25/12 (8)	30.40 ± 4.55^{b}	16.49 ± 2.76^{b}
35/3 (2)	30.92 ± 2.89^{b}	14.37 ± 0.74^{bc}
35/6 (4)	38.94 ± 5.04^{a}	12.79 ± 1.36^{cd}
35/12 (8)	42.05 ± 3.52^{a}	12.17 ± 1.78^{d}

Numbers inside brackets represent the stocking densities used in trial 2. Survival means with the same letter do not differ significantly according to the Student-Newman-Keuls test (P<0.05).

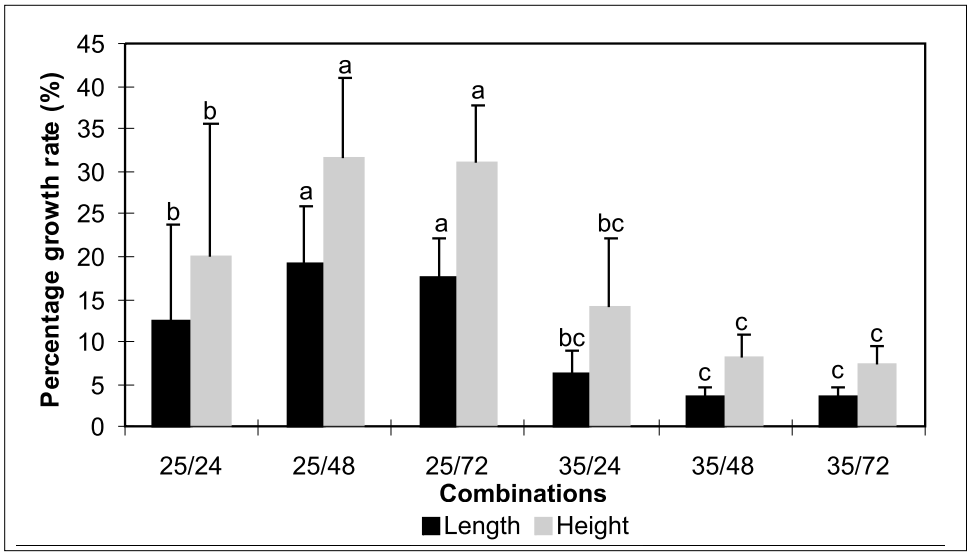

Figure 3 - Mean values (± SD) of percentage growth rate for *C. rhizophorae* larvae length and height obtained in the 14th day of culture in the combinations of salinity (25 and 35) and frequency of water exchange (24, 48 and 72 h). Values with the same letter do not differ significantly according to the Student-Newman-Keuls test (P=0.05).

DISCUSSION

In the present study, the embryogenesis of *C. rhizophorae* under the temperature of 25 ºC and the salinity of 30 agreed with previous study that verified for this same species the highest metamorphosis rate of D-veliger larvae in the temperatures of 20 and 25 ºC and salinity of 25 to 37 (Dos Santos & Nascimento, 1985). Tan & Wong (1996) observed for *Crassostrea belcheri* the optimum salinity range for the embryonic development of 24 to 30 and concluded that tropical oysters in general need higher salinities for eggs to D-larvae development. Likewise, Sudrajat (1990) found the optimum salinity of 25 for the embryonic development of *Crassostrea iredalei* and *Saccostrea cucullata*.

In the first trial, the highest survivals (mean of 37.30%) were obtained in salinity of 35. Our results corroborate with Lemos *et al*. (1994) who recommended salinities over 30 until the seventh day of culture of *C. rhizophorae*. Nevertheless, in the second trial (7th-14th day) the salinity of 25 resulted in a higher survival (mean of 17.06%). This result is supported by several studies that suggested salinities under the marine value as ideal for the larval development and survival for oysters such as *Ostrea edulis* (22.5 to 27) (Davis & Ansell, 1962), *C. virginica* (17.5 to 27) (Davis & Calabrese, 1964), *Crassostrea rivularis* (20) (Breese & Malouf, 1977), *Crassostrea gigas* (25) (Helm & Milican, 1977), *Saccostrea echinata* (20 to 30) (Coeroli *et al*., 1984), *C. iredalei* (20 to 30) and *S. cucullata* (25 to 30) (Sudrajat, 1990) and *Crassostrea belcheri* (12 to 24) (Tan & Wong, 1996). The largest size (length and height) in both trials were observed in the salinity of 25, which is within the range (25-30) suggested to improve larval growth of *C. rhizophorae* (Lemos *et al*., 1994).

Helm & Millican (1977) observed that water exchanges more frequently than 48 h affected negatively larval growth of *C. gigas*. Yan *et al*. (2006) verified that water exchanges of 50% every 72 h is ideal for larval growth of *Ruditapes philippinarum*. Water exchange every 48 h has been used for *C. rhizophorae* larval rearing (Lemos *et al*., 1994; Miranda & Guzenski, 1999). In the present study the highest larval growth and survival performance in both trials were verified when the culture water was exchanged at either 48 or 72 h. In accordance, Oliveira (1998) found the highest survival of *C. gigas* larvae during the first week of culture by exchanging water every 48 and 72 h. Water exchange every 48 h has been commonly used in the larval culture of bivalve molluscs (Ibarra *et al*., 1997; Ponis *et al*., 2003a; Labarta *et al*., 1999; Doroudi & Southgate, 2000). The utilization of water exchanges every 48 or even 72 h could reduce production costs, management practices and larval stress. On the contrary, daily water exchange usually reduces growth and survival probably due to the severe stress that larvae are exposed during sieving and washing.

The effect of stocking density on larval growth of bivalves has been reported as an inverse relationship, as higher growth performance is usually achieved at lower densities (Davis, 1953; Gruffydd & Beaumont, 1972; Rojas *et al*., 1988; Ibarra *et al*., 1997; Miranda & Guzenski, 1999). The present study confirmed this tendency in the first trial for densities of 3 and 6 larvae ml^{-1}, whereas in the second trial no effect of density in terms of relative growth rate was observed. This fact probably occurred because the densities used in this study were within a short interval, commonly utilized in bivalve hatcheries.

Results from this study suggest that during the first week of development *C. rhizophorae* larvae should be cultivated in the salinity of 25, density of 12 larvae ml^{-1} and total water exchange every 72 h. For the second week of culture, the same salinity level is recommended, but the stocking density of 2 larvae ml^{-1} and water exchange every 48 h. These information may provide a quantitative basis for optimizing hatchery production of *C. rhizophorae* seeds.

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