LARVICULTURE OF THE SAND CLAM CULTIVATED IN DIFFERENT DENSITIES

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ABSTRACT

The development of technologies for the cultivation of larvae and post-larvae in the laboratory is an alternative to supply the reduction of natural stocks of *Anomalocardia brasiliana*. In order to improve the technology of mollusc production in the laboratory, the present work evaluated the effect of culture density on the survival and growth of larvae and post-larvae of *A. brasiliana*. Two experiments were conducted, the first evaluated the density in the veliger larvae culture, testing densities of 2, 6 and 10 larvae mL^{-1} for seven days. In the second, densities of 60 and 120 post-larvae cm^{-2} were tested, for 38 days. In the culture of veliger larvae, better results were found for survival and growth in the densities of 2 and 6 larvae mL^{-1}. When evaluated for density in post-larvae culture, the best results for both survival and length were obtained at the density of 60 post-larvae cm^{-2}, with 93.81 ± 0.68% and 2.18 ± 0.18 mm, respectively. Thus, to optimize productivity, it is recommended a density of 6 larvae mL^{-1} (veliger larvae) and 60 post-larvae cm^{-2} for the cultivation of *A. brasiliana* in the laboratory.

Key words: berbigão; growth; survival; larvae; post-larvae; *Anomalocardia brasiliana*.

LARVICULTURA DE MOLUSCO DE AREIA CULTIVADO EM DIFERENTES DENSIDADES

RESUMO

O desenvolvimento de tecnologias para o cultivo de larvas e pós-larvas em laboratório é uma alternativa para suprir a redução dos estoques naturais de *Anomalocardia brasiliana*. Com o objetivo de aprimorar a tecnologia de produção de moluscos em laboratório, o presente trabalho avaliou o efeito da densidade de cultivo sobre a sobrevivência e o crescimento de larvas e pós-larvas de *A. brasiliana.* Foram realizados dois experimentos, no primeiro avaliou-se a densidade no cultivo de larvas véliger, testando as densidades de 2, 6 e 10 larvas mL^{-1} durante sete dias. No segundo, foram testadas as densidades de 60 e 120 pós-larvas cm^{-2}, durante 38 dias. No cultivo de larvas véliger, os melhores resultados para sobrevivência e crescimento foram encontrados nas densidades de 2 e 6 larvas mL^{-1}. Quando avaliada a densidade no cultivo de pós-larvas, os melhores resultados, tanto para a sobrevivência quanto para o comprimento final, foram obtidos na densidade de 60 larvas cm^{-2}, com 93,81 ± 0,68% e 2,18± 0,18 mm, respectivamente. Dessa forma, para otimizar a produtividade, recomenda-se as densidades de 6 larvas mL^{-1} (larvas véliger) e de 60 pós-larvas cm^{-2} para o cultivo de *A. brasiliana* em laboratório.

Palavras-chave: berbigão; crescimento; sobrevivência; larva; pós-larva; *Anomalocardia brasiliana*.

INTRODUCTION

World aquaculture production was approximately 101 million tons in 2014, of which shellfish production represented 15.9% (16.11 million tons) (FAO, 2016a). In that same year, the world production of Veneridae by catch was 774 thousand tons, while aquaculture production was 5,360 thousand tons (FAO, 2016b).

The species *Anomalocardia brasiliana* (Gmelin, 1791) (Bivalvia: Veneridae), is considered a fishing resource of great importance for Brazil, especially for the coastal communities of artisanal fishermen in the northeast region (EL-DEIR, 2009; PEZZUTO *et al*., 2010). This species is distributed from the Gulf of Mexico to Brazil, observed along the entire Brazilian coast (TURGEON *et al*., 2009). It inhabits sandy

beaches, mangroves, coves, bays and estuaries (BOEHS *et al.*, 2008) of sandy-muddy and sandy sediments (CANTERA, 1991).

In Brazil, the commercialization of *A. brasiliana* is based on extractivism in natural banks, being carried out mainly by coastal communities (LAGREZE-SQUELLA *et al.*, 2015). However, uncontrolled exploitation and environmental degradation can compromise stocks of this mollusk. In the State of Pernambuco, in the Northeast of Brazil, the traditional communities that capture the species have identified the reduction of the size of the individuals, even though there are no recent data on production or fishing in the state (SILVA-CAVALCANTI and COSTA, 2011). This decline in the natural banks of bivalve mollusks throughout the world has become an incentive for studies on larviculture (PRADO *et al.*, 2010), since the production of juveniles in a laboratory is an alternative to mitigate and supply the extraction demand for mariculture, as well as helping to recover natural stocks (HELM *et al.*, 2004; LAGREZE-SQUELLA *et al.*, 2015).

The development of *A. brasiliana* passes through the larval phases of trochophore and veliger (D-larva, umbonate and pediveliger) until it becomes post-larva, also known as plantigrade or juvenile (MOÚEZA *et al.*, 1999; OLIVEIRA *et al.*, 2016b). MOÚEZA *et al.* (1999) reported that nine hours after the onset of fertilization, trochophorous larvae emerged from D larvae after 24 hours, and from the seventh day these larvae begin to metamorphose, which concludes with the differentiation of siphons. Between 8 and 10 days of life, the larvae are in the pediveliger stage, where the larvae swim and crawl in small intervals, and present rudimentary foot and premature gill. At 15 days, the larval phase is completed, nutritional and respiratory functions are assumed by the gills, the locomotor function by the foot, and at that moment the organisms settle and are considered post-larvae (MOÜEZA *et al.*, 1999).

The success of larval and juvenile cultivation is influenced by variables such as temperature, salinity, feeding and stocking density (FRÉCHETTE, 2005; LAGREZE-SQUELLA *et al.*, 2015). Several studies have already been carried out to evaluate the effect of stocking density on bivalve mollusk larvae in relation to survival and growth in the veliger phase until the beginning of metamorphosis, as reported for the species: *Meretrix meretrix* (LIU *et al.*, 2006), *Ruditapes philippinarum* (YAN *et al.*, 2006), *Mulinia edulis* (OLIVA *et al.*, 2014) and even for *A. brasiliana* (OLIVEIRA *et al.*, 2016a). However, few have focused on this effect on post-larvae cultivation. *Anomalocardia brasiliana* presents a critical phase with high mortality from the seventh day of larval development due to the metamorphosis process (MOUÈZA *et al.*, 1999), because in this period the bivalve larvae are fragile and susceptible to diseases (OLIVEIRA, 2014).

For the reasons given above, the present study aimed to evaluate the effect of stocking density on the growth and survival of *A. brasiliana* larvae and post-larvae in the laboratory.

MATERIALS AND METHODS

Experimental design

Two experiments were carried out at the Sustainable Mariculture Laboratory (LAMARSU) of the Department of Fisheries and Aquaculture (DEPAq) of the Federal Rural University of Pernambuco (UFRPE), Recife, Brazil. The first was completely randomized with three treatments: D2 (stocking density of 2 veliger larvae mL^{-1}); D6 (stocking 6 veliger larvae mL^{-1}) and D10 (stocking 10 veliger larvae mL^{-1}), with three replicates each and a duration of 7 days, since the morphological differentiations for metamorphosis begin at seven days of life (MOÜEZA *et al.*, 1999). The second experiment was also completely randomized with two treatments: D60 (stocking density of 60 post-larvae cm^{-2}) and D120 (stocking 120 post-larvae cm^{-2}), with three replicates each and duration of 38 days.

Collection and acclimatization

A total of 800 breeding *A. brasiliana* with 20 ± 2.7 mm length were collected at the Mangue Seco beach, Igarassu, Pernambuco, Brazil (07º50'1.19''S, 034º50'39.1''W). In the laboratory, the specimen were washed and sanitized with a 5 g L^{-1} sodium hypochlorite bath for five minutes, then acclimatized in tanks with 400 liters of marine water, filtered through a mechanical filter with five and three micrometer openings, and sterilized with ultraviolet light, in a salinity of 35, a temperature of 22 °C and 5 g L^{-1} dissolved oxygen, during 24 h of acclimatization and a density of 1 ind L^{-1}.

Release of gametes

After acclimatization, breeders were induced to release gametes by adding *Chaetoceros calcitrans* (Heterokontophyta) microalgae (200,000 cells mL^{-1}) and raising the temperature to 25 °C and then 28 °C in a 400-liter tank at a density of 2 ind L^{-1}. The release of gametes was observed after approximately 20 minutes from the beginning of the application of the stimuli. After fertilization, the eggs were collected in 35 μm aperture meshes, counted and placed in 30-liter incubators (50 eggs mL^{-1}) with seawater, previously filtered in a 1.5 μm mesh and sterilized with sodium hypochlorite, at a salinity of 35 and temperature of 24 °C, with a moderate aeration point. The eggs were kept in the incubators for 24 h until they reached stage D larva.

Management of larvae

The D-larvae used in the first experiment had an initial length of 88.76 ± 3.75 μm and an initial height of 68.85 ± 4.18 μm (n=50), where the length was considered as the maximum antero-posterior dimension, and height the maximum dorso-ventral dimension (OLIVEIRA and OLIVEIRA, 1974). These larvae were cultivated in a static system with total water exchange and 50 μm mesh filtration every 48 hours, to maintain nitrogen compounds at low concentrations. The experimental units were plastic cylinders 18 cm in diameter and 18 cm in height with 2 liters of useful volume, with filtered sea water (1.5 μm) and sterilized in 5 g L^{-1} sodium

hypochlorite solution in the salinity of 35 and temperature of 24 °C, under a constant aeration point.

Plantigrades (post-larvae), of 12 days of culture, had a length of 197.09 ± 1.03 µm and a height of 188.83 ± 0.97 µm (n = 50) at the beginning of the second experiment. These were grown in six PVC cylindrical structures 10 cm in diameter and 90 cm high (78.5 cm² area) in two 30L tanks (three culture structures per tank), in a static system with total water exchange and filtration every 48 hours, to keep nitrogen compounds in low concentrations. Inside the tanks there was a constant aeration point and an airlift system for water and food circulation. The meshes used were 80 µm (first to the sixteenth day of culture) and 185 µm (seventeenth to the thirty-eighth day of culture).

Feeding of larvae

Feeding of the D-larvae was given daily through a diet composed of the microalgae *C. calcitrans* (Heterokontophyta) and *Isochrysis galbana* (Haptophyta), in a 1:1 ratio, with concentrations of 15,000 mL⁻¹ cells of each species (OLIVEIRA *et al*., 2016a) by the fifth day, and 25,000 mL⁻¹ cells of each species, from the sixth day to the end of the experiment. For the feeding of post-larvae, *C. calcitrans* and *I. galbana* microalgae were also used daily in a 1:1 ratio, with concentrations of 25,000 mL⁻¹ cells of each species, until the twentieth day, of 35,000 mL⁻¹ cells of each species until the thirtieth day, and 50,000 mL⁻¹ cells of each species until the end of the experimental period. The feeding, in both experiments, was adjusted by observing the algal consumption, by means of daily counts of algal residual in a Neubauer chamber.

Water quality

Dissolved oxygen, temperature, salinity, and pH were monitored with the help of a multiparameter (YSI model 556, Yellow Springs, Ohio, USA), twice a day. Total ammonia and nitrite were measured every two days using the Visocolor alpha kit.

Larval development

In order to evaluate the survival of the veliger larvae, random sampling of the larvae of each experimental unit was carried out to evaluate the survival, every 48 hours. The larvae were analyzed using the Sedgwick-Rafter chamber and an optical microscope (Coleman-N107 LED). For post-larvae, survival was measured by a volumetric method, where the organisms were concentrated in a beaker for withdrawal of three one-milliliter (mL) samples with a Pasteur pipette, and then the post-larvae were counted on a plate using a stereoscopic binocular zoom microscope (Coleman - XTB - 2B), every 48 hours.

In relation to the growth of larvae and post-larvae, length and height measurements were evaluated with the help of the Fiji software, using images taken by an optical microscope (Coleman - N107 LED) with a photographic camera, every 48 hours.

The larval growth rate was estimated using the following formula:

$$SGR = 100 \ (\ln L2 - \ln L1) \ / \ T \tag{1}$$

Where SGR is the specific growth rate (% day⁻¹); L1 and L2 represent the lengths (in µm) at the beginning and end of the experiment, respectively, and T is the duration time of the experiment (URBAN *et al*., 1983).

In the second experiment, the surface coverage rate – SCR was estimated from the covered area, with the following formula:

$$AO = [(L/2) \times (H/2) \times \pi] \times N \tag{2}$$

Where AO is the area occupied by shells, L is the average length of the shells, H is the mean height and N is the total number of post-larvae per experimental unit. The result was correlated to the background area to find the surface coverage rate (SCR) given in percentage (%); methodology used by LIU *et al*. (2011).

Statistical analysis

The data were previously checked for homogeneity (Cochran; $p<0.05$) of the variances and normality (Shapiro-Wilk; $p<0.05$). Subsequently, they were submitted to one-way ANOVA for Experiment I. For Experiment II, two-way analysis of variance (ANOVA) was applied, and the factors were density (60 and 120 post-larvae cm⁻²) and time (2-day intervals). The Tukey's test ($p<0.05$) was performed to compare and classify the means, when differences between treatments were observed. All statistical analyses were performed using Statistica Version 10 software.

RESULTS

Water quality

The water quality variables were adequate for the cultivation of the species throughout the two experiments, and no significant differences were observed for water temperature (23.91 ± 0.19 °C), dissolved oxygen (5.37 ± 0.39 mg L⁻¹), salinity (34.19 ± 1.88) and pH (8.22 ± 0.33). Nitrogen compounds, total ammonia and nitrite, were maintained in concentrations below 0.016 and 0.25 mg L⁻¹, respectively, due to the total exchange of water every 48 h in both experiments

Experiment I

Survival from the third day of cultivation in the D2 treatment (86.75 ± 6.36%) was significantly different ($p<0.05$) from the other treatments. At the end of the experiment, D2 (55.91 ± 3.57%) and D6 (65.55 ± 6.74%) presented similar survival values, however, significantly different ($p<0.05$) from the results observed in D10 (45.58 ± 3.74%) (Figure 1).

The length and height of larvae in D2 (166.8 ± 4.73 µm and 155.69 ± 5.19 µm) and in D6 (155.95 ± 16.49 µm and 134.54 ± 8.77 µm) were significantly different ($p<0.05$) than those observed in D10 (129.57 ± 27.92 µm and 127.39 ± 16.22 µm). In relation to SGR, significant differences ($p<0.05$) were observed between the treatments (Table 1).
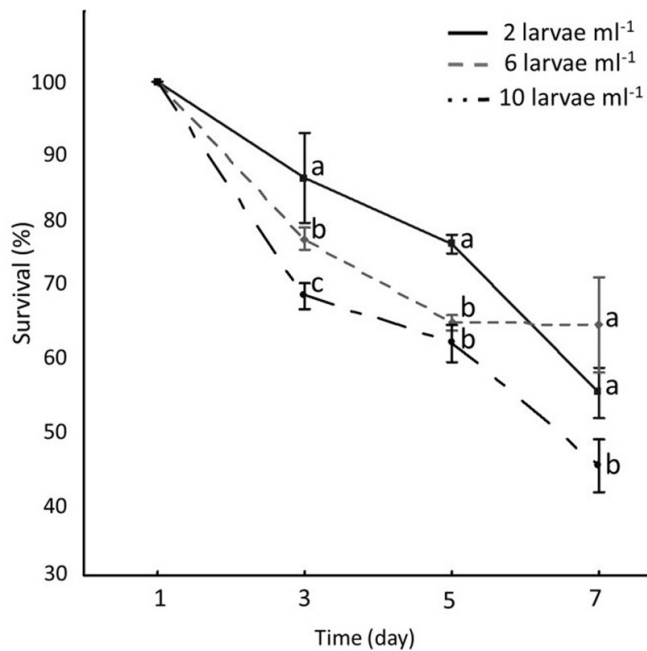
Figure 1. Mean and standard deviation of larval survival (%) in experiment I. Different letters indicate significant difference by Tukey's test (p <0.05).

Experiment II

The results of survival, length and height of post-larvae were significantly influenced (p<0.05) by stocking density and culture time (Table 2).

Survival in D60 treatment (93.81 ± 0.68%) was significantly different from D120 (82.81 ± 0.31%). A similar result was also observed for length, where in the D60 treatment (60 post-larvae cm^{-2}) provided an increase of 48%. The post-larvae cultured in the D60 treatment reached 1 mm in length on the thirtieth day of the experiment. However, in the D120 treatment (120 post-larvae cm^{-2}), only 66.7% of the 1 mm long organisms were observed on the thirty-eighth day of the experiment. Regarding the height of the shells, at the end of the experiment the height of the cultivated plantigrades in the D120 treatment was 41% smaller when compared to the D60 treatment.

For the specific growth rate (SGR), the effects of different densities, growing time, and their interaction were all significant (p<0.05), both for length and height. During the whole experimental period (1-38 days), the post-larvae of the D60 treatment remained with the highest daily specific growth rate for both length and height, being significantly higher (p<0.05) than the post-larvae growth rate of the D120 treatment. From the twentieth day, all post-larvae, regardless of density, had a growth rate of less than 6% day^{-1} (Table 2).

Table 1. Production parameters of the véliger larvae of *Anomalocardia brasiliana* cultivated at different densities, during the experimental period of seven days.

Parameters	Treatments		
	D2 2 larvae mL^{-1}	D6 6 larvae mL^{-1}	D10 10 larvae mL^{-1}
Survival (%)	55.91 ± 3.57[a]	65.55 ± 6.74[a]	45.58 ± 3.74[b]
Length (μm)	166.80 ± 4.73[a]	155.95 ± 16.49[a]	129.57 ± 27.91[b]
Height (μm)	155.69 ± 5.19[a]	134.54 ± 8.77[b]	127.39 ± 16.22[b]
SGR[1] of Length (% day^{-1})	7.88 ± 0.36[a]	6.98 ± 1.37[ab]	4.47 ± 2.75[b]
SGR[1] of height (% day^{-1})	9.98 ± 1.08[a]	8.35 ± 0.81[ab]	7.60 ± 1.65[b]

The results are mean of three replicates per treatment ± standard deviation. The data were analyzed by measures of variance analysis and Tukey's test. The mean values of the same vertical line with different exponents differ significantly (p <0.05). [1]SGR: specific growth rate.

Table 2. Survival and growth of post-larvae of *Anomalocardia brasiliana* cultivated at different densities, during the experimental period of 38 days.

Parameters	Treatments		ANOVA		
	D60 60 larvae cm^{-2}	D120 120 larvae cm^{-2}	d	t	d x t
Survival (%)	93.81 ± 0.68[a]	82.81 ± 0.30[b]	*	ns	ns
Length (μm)	2177.34 ± 184.36[a]	1127 ± 163.86[b]	*	*	*
Height (μm)	1918.62 ± 244.26[a]	1113.95 ± 230.40[b]	*	*	*
SGR[1] of Length (% day^{-1})	6.37 ± 0.24[a]	5.14 ± 0.39[b]	*	*	*
SGR[1] of height (% day^{-1})	6.09 ± 0.34[a]	5.24 ± 0.18[b]	*	*	ns

Results are mean of three replicates per treatment ± standard deviation. Data were analyzed by two-way analysis of variance (ANOVA) and by Tukey's test. d - Effect of density; t - time. * - p <0.05; ns - not significant. [1]SGR: specific growth rate.

Table 3. Rate of surface coverage, in percentage, for post-larvae of *Anomalocardia brasiliana* cultivated at different densities, during the experimental period of 38 days.

SCR[1] (%)	Treatment		ANOVA		
	D60 60 post-larvae cm^{-2}	D120 120 post-larvae cm^{-2}	d	t	d x t
1° day	1.75 ± 0.36^a	3.50 ± 0.25^b	*	*	*
18° day	17.07 ± 1.02^a	21.66 ± 2.01^b	*	*	*
38° day	108.49 ± 0.78^a	166.72 ± 0.63^b	*	*	*

Results are mean of three replicates per treatment ± standard deviation. Data were analyzed by two-way analysis of variance (ANOVA) and Tukey's test. d - Effect of density; t- time. * - p<0,05; ns - not significant. [1]SCR: Surface coverage rate.

Due to the lower density, the surface coverage rate (SCR) of the D60 treatment, throughout the experimental period (38 days of cultivation), was significantly lower (p<0.05) than the D120 treatment; however, at the end of the experiment, both exceeded the maximum surface coverage rate (100%) (Table 3).

DISCUSSION

The survival and growth of mollusk larvae are strongly affected by temperature and salinity (KINNE, 1964; LIU *et al*., 2006), however, in the experiments, these variables remained stable throughout the entire cultivation period. In cultures, high densities are generally detrimental due to the amount of metabolic waste generated, which, in turn, affect larval growth (YAN *et al*., 2006). In the present study, periodic water renewals (every 48 h) used in the experiments were considered efficient in the removal of these nitrogenous residues.

The static system with total water exchange every 48 hours is commonly used in larval bivalve cultivation (ROBERT and GERARD, 1999), however, RICO-VILLA *et al*. (2009) and RAGG *et al*. (2010) recommend the continuous flow of water and food to larvicultures using small volumes and high densities. The continuous flow cultivation system is more efficient than the static system, with or without changing the water, presenting in general higher survival results; however, even in this system, the high density damages the yield of the larvae (TURINI *et al*., 2014). In the present study, satisfactory results were obtained using the static system, since all of the survival rates were higher to 45%.

High stocking densities in the culture of bivalve larvae may lead to a decrease in survival and growth (LIU *et al*.,2006), as the high density causes discontinuous consumption of food resulting from frequent collisions between the animals, since the larvae close their valves and stop feeding due to the mechanical shock (LOOSANOFF and DAVIS, 1963). LAGREZE-SQUELLA *et al*. (2015), when testing different densities in *A. brasiliana* larvae in the veliger phase, observed that increasing the stocking density of 10 larvae mL^{-1} to 50 larvae mL^{-1} negatively influences both survival and growth of the organisms. LIU *et al*. (2006), YAN *et al*. (2006) and OLIVA *et al*. (2014), for Veneridae *M. meretrix, R. philippinarum and M. edulis* mollusks, also found the best results of larval culture in the veliger phase at the lowest densities tested.

OLIVEIRA *et al*. (2016a) found a survival rate of approximately 13.5% when studying *A. brasiliana* veliger larvae fed *C. calcitrans* and *I. galbana*, in a closed system with 5 larvae mL^{-1} for 15 days. In the same study, the best results were found for larvae fed with *Phaeodactylum tricornutum* and an algal mix of *C. calcitrans* and *Pavlova lutheri*, of 24.8 and 31.5%, respectively. These results were lower than those found in the present study, where the highest density tested (10 larvae mL^{-1}) had a survival of 45.6%. This difference between the results may be related to the feeding routine of the larvae, since in the present study feeding was adjusted as a function of algal consumption, while OLIVEIRA *et al*. (2016a) offered a 30,000 cells mL^{-1} diet throughout the experiment (15 days). Another factor that may have interfered in the results obtained by these authors is the fact that the larvae began to metamorphose during the experimental period, a process that starts on the seventh day of cultivation (MOÜEZA *et al*., 1999) and demands energy, possibly resulting in the mortality of the larvae.

Regarding growth, for the cultivation of veliger larvae of *A. brasiliana*, LAGREZE-SQUELLA *et al*. (2015), in a seven-day experiment under static system and feeding composed of the microalgae *I. galbana* and *C. muelleri*, observed that the larvae of the treatments with densities of 30 and 50 larvae mL^{-1} (higher densities tested), at the end of the experiment, were smaller than 120 μm. Meanwhile, in the present study where the densities were 2, 6 and 10 larvae mL^{-1} under similar culture conditions, the larvae of all tested densities reached measurements greater than 120 μm, for both length and height; showing that lower densities may favor the growth of cultured organisms. In the larvae of *Mulinia edulis*, OLIVA *et al*. (2014), who showed that the density influences the length, when testing the densities of 10 and 20 larvae mL^{-1} also found that the lower density tested resulted in a longer length (223 ± 22.9 μm), while the larvae of the treatment with density of 20 larvae mL^{-1} larvae reached an average length of 216 μm.

In the cultivation of *A. brasiliana* plantigrades in a static system with water exchange every 48 hours and fed with *P. lutheri* and *C. calcitrans* microalgae, OLIVEIRA (2014) found better survival results (less than 60%) in the densities of 40 and 80 post-larvae cm^{-2}, for larvae cultured for 28 days, while survival at the highest density (160 post-larvae cm^{-2}) was approximately 32%. In the second experiment, the survival rates were higher than 80% for the two studied densities (60 and 120

post-larvae cm^{-2}). The development of post-larvae of *A. brasiliana* does not suffer interference as a result of the search for a substrate to settle in, a factor that causes mortality in other bivalve species, such as *M. edulis* (BAYNE *et al*., 1976), since they do not need this in their metamorphic process (MOUËZA *et al*., 1999). For this reason, the survival results of these post-larvae cannot be tied to the presence of a substrate for fixation. On the other hand, in the present experiment, after the beginning of the metamorphosis process it was observed that the larvae become more fragile and their food consumption was reduced, even without the need for substrate search, contributing to lower survival in the early stages (larvae veliger and pediveliger) in comparison with the later stage (plantigrade), when the metamorphosis was already complete.

OLIVEIRA *et al*. (2016b), when cultivating the same species as the present study, at low density (1 larvae mL^{-1}) for 84 days, from the D larvae stage to juvenile, found that from the 10th day of cultivation all larvae were found in the pediveliger stage with a length of 183.2 ± 20.8 μm, and that in 15 days all animals were already plantigrades, although the final survival in this study was only 7%. However, these authors found good results when evaluating the survival by stage, these being 58.8% for D larvae, 60% for pediveliger larvae and 100% for plantigrades. In the phase of umbonate larvae, OLIVEIRA *et al*. (2016b) found the lowest survival (20%), probably due to the metamorphosis, since the authors report that this phase occurred between the sixth and the tenth day of cultivation. In the present study, even at higher densities, similar results were found for both D larvae cultivation and plantigrade cultivation.

In the cultivation of larvae and post-larvae of *A. brasiliana*, which lasted 28 days, OLIVEIRA (2014) found a similar result to the present study, where the best results obtained were with the lowest density of 40 post-larvae cm^{-2}, with a length greater than 1.2 mm, while the highest density of 160 post-larvae cm^{-2} reached approximately 800 μm. In the cultivation of pediveliger larvae of *Mulinia edulis* at densities of 5, 10, 20 and 40 cm^{-2} larvae, over a 20-day period, the best survival was found in the treatment with lower density (5 cm^{-2} larvae), greater than 60%, and a length greater than 2 mm. However, the highest density (40 larvae cm^{-2}) showed survival of 32% and growth of less than 1.5 mm (OLIVA *et al*., 2014).

When observing the SGR of the treatments in the two experiments, it was verified that the treatments with the lowest densities presented the highest specific growth rates throughout the experimental periods. Studies by LIU *et al*. (2006), also demonstrated that the SGR of *M. meretrix* veliger larvae was reduced from the third day regardless of density, being close to < 2%. OLIVEIRA (2014), in post-larvae culture of *A. brasiliana*, also observed a decrease in SGR from the first to the second week, however, the largest reductions were observed in the highest densities tested (80 and 160 cm^{-2}). In addition, this author found results similar to those of the present study, where the lowest SGRs were found at the highest densities.

The background coverage area may have been a limiting factor in *A. brasiliana* juvenile growth in the present study, because at the end of the cultivation the surface cover rate (SCR) was greater than 100% for both densities. According to LIU *et al*. (2011),

densities between 20 and 320 larvae cm^{-2} in the cultivation of *Clinocardium nutallii* larvae result in shorter specimens due to the high coverage rate, therefore, they suggest that the surface cover rate should not be greater than 100% of the useful area for settlement in a downwelling flow cultivation system. Similar results were found by HEASMAN *et al*. (2002), after observing that occupying 70% of the useful area for settlement is a factor that limits the growth of juveniles of the scallop *Pecten fumatus*.

In the larviculture, starting from the veliger phase, the density must be adjusted, as it influences the survival and growth of bivalve mollusk larvae (LIU *et al*., 2006; YAN *et al*., 2006; LIU *et al*., 2011). OLIVEIRA (2014), for post-larvae cultivation of *A. brasiliana*, recommends using a density of 160 post-larvae cm^{-2} from the pediveliger phase until the individuals reach 600 μm in length, with a subsequent reduction to 40 post-larvae cm^{-2} until they reach 1 mm. Meanwhile, for *Clinocardium nuttallii* larvae in a downstream well-flow system, a stocking density of 160 pediveliger cm^{-2} is recommended until the individuals reach 1 mm in length, moving to 40 larvae cm^{-2} until they reach 2 mm (LIU *et al*., 2011).

CONCLUSION

Density interferes in the development of larvae and post-larvae of *A. brasiliana* grown in a static system with full water exchange every 48 hours, requiring density management during cultivation. Thus, it is concluded that the density of veliger larvae should be 6 larvae mL^{-1} until the beginning of metamorphosis and, after settlement, 60 post-larvae cm^{-2}, until juveniles occupy a maximum of, 70% of available background area.

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