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Larviculture of *Anomalocardia flexuosa* under different conditions of salinity and temperature

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ABSTRACT. On the Brazilian coast, the commercialization of *Anomalocardia flexuosa* is based on fishing in natural banks, but its stocks have been declining. As an alternative to this limitation, laboratory production is promising for plans of repopulation and cultivation of *A. flexuosa*. Aiming to develop techniques for this purpose, the current study evaluated the influence of temperature and salinity on the rearing of *A. flexuosa* larvae. Two experiments were conducted: (1) the effect of different salinities (25, 30, and 35 g L^{-1}) on survival and growth in the larval stage of *A. flexuosa*, lasting seven days; and (2) the effect of temperature (22 and 26 ºC) on larval growth and survival, lasting ten days. In both experiments, the D larvae were stored in experimental units (2 L) with a density of 10 larvae mL^{-1}. At salinities of 25 and 30 g L^{-1} there were greater survival rates. When analyzing the final length and the daily growth rate, the higher values were observed at salinity of 25 g L^{-1}, 126.08 ± 1.45 µm and 8.33±0.20 µm day^{-1}, respectively. Regarding temperature, 26 ºC showed better values both for survival (62.25 ± 3.48%) and larval development (210.19 ± 0.56 µm). Thus, to optimize productivity, it is recommended to maintain salinity between 25–30 g L^{-1} and a temperature of 26 °C.

Keywords: clams; berbigão; veliger larvae; abiotic factors.

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Introduction

On the Brazilian coast, the commercialization of the West Indian pointed Venus *Anomalocardia flexuosa* is conducted mainly by coastal communities, being, in some cases, the most important source of protein in the diet of local families, in addition to being based on artisanal fishing from natural stocks (Lagreze, Albuquerque, Araujo, Sühnel, & Melo, 2015). On the northern coast of the state of Pernambuco, due to the disorderly exploitation of *A. flexuosa*, traditional communities are reporting a decrease in terms of size and volume of the capture in recent years (Silva-Cavalcanti & Costa, 2009; Silva-Mourão, Baracho, de Faria Lopes, Medeiros, & Diele, 2021). In addition, the availability of this species in the natural environment is affected by anthropogenic factors, such as water pollution, as well as by biotic (Lagreze et al., 2015) and abiotic factors (Deng, Fu, Liang, Du, & Xie, 2013; Carneiro, Soares, Manso, & Pagliosa, 2020; Nowland, O'Connor, Elizur, & Southgate, 2021).

Considering the importance of this species for fishing activities and the current situation of stocks, aquaculture can contribute by mitigating the demand caused by fishing through the production of seeds in the laboratory (Food and Agriculture Organization [FAO], 2020). Although there is no commercial cultivation of *A. flexuosa* there is great potential for the development of mariculture, especially in the Brazilian Northeast (Lagreze-Squella, Sühnel, Vieira, Langdon, & Melo, 2018). The cultivation of *A. flexuosa* in that region could represent an important advance in the mollusk productive sector in Brazil, since most of the national production becomes food. Among the abiotic factors, salinity is the one that most affects the growth in the larval phase of marine bivalves (Albuquerque et al., 2012; Oliveira et al., 2016b), impairing physiological processes, such as feeding, as well as life span in the planktonic phase (Legat, Puchnick-Legat, Gomes, Sühnel, & Melo, 2017). Salinity variations affect the osmotic balance of mollusks, resulting in energy expenditure to readjust their osmotic balance (Cheng, Yeh, Wang, & Chen, 2002; Deaton, 2008).

Another important factor in mollusk cultivation is temperature, especially in the pelagic phase (Helm, Bourne, & Lovatelli, 2006). This variable also accelerates all metabolic functions, including breathing and ingestion (Nie et al., 2016; Carneiro et al., 2020), so it influences the energy needs of individuals. The rise in temperature was also able to interact with factors related to food, affecting the mechanisms involved in lipid deposition.

In view of the above facts, the current study aimed to evaluate the growth and survival of *A. flexuosa* larvae cultivated under different conditions of salinity and temperature in the laboratory.

Material and methods

Capture and management

The breeding of broodstock was conducted out on the beach of Mangue Seco, Northern coast of Pernambuco state, in Igarassu city (07°49' 44,19''S, 035°50' 03,06''W). A total of 400 adult individuals with a minimum size of 20 mm (maximum anteroposterior dimension of the shell) were captured to obtain gametes in each experiment, as they had already reached the size of their first sexual maturation (Barreira & Araújo, 2005).

In the Laboratory of Sustainable Mariculture (LAMARSU), the breeders were placed in tanks containing 400 L at a density of 1 ind L^{-1} in sea water (35 g L^{-1}) and constant aeration, where they were observed for 48 hours and fed daily with the microalgae *Chaetoceros calcitrans* at a concentration of 300,000 cells mL^{-1}.

Obtention of Larvae D

The breeders were induced to release gametes through stimuli in cycles with temperature variation, through addition of algae and gametes in the water, following the methodology described by Lavander et al. (2014). Then, the water containing the gametes was filtered through 90 and 35 µm screens, for retention and quantification of eggs and verification of fertilization, respectively. This verification was carried out with the aid of a Sedgwick-Rafter chamber and optical microscope, then the eggs were placed in incubators with 30 L of useful volume at the density of 20 eggs mL^{-1}, where they remained for 24 hours, until the stage of D veliger larvae (Figure 1).

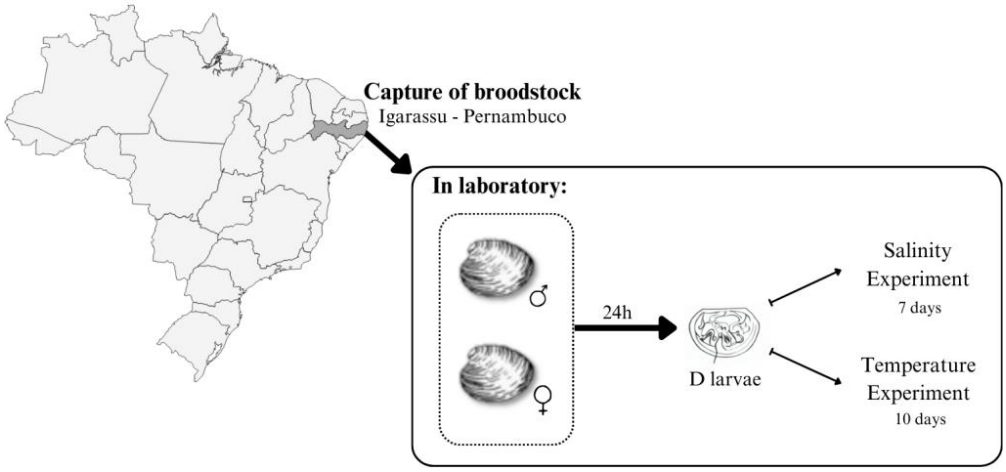

Figure 1. Schematic diagram of the steps for carrying out the experiments.

Experimental design

Two experiments were conducted at the Sustainable Mariculture Laboratory (LAMARSU), at the Department of Fisheries and Aquaculture (DEPAq), at the *Universidade Federal Rural de Pernambuco* (UFRPE), Recife, Brazil. Experiment I lasted 7 days, with a completely randomized design, where three different salinity gradients were tested, namely: 25, 30 and 35 g L^{-1}, with four replicates of each.

In experiment II, two temperatures were tested: 22 and 26 ºC, with four replications each, in a randomized block design, an it continued until the D larvae reached the veliger foot stage, which occurred on the tenth day.

In both experiments, the D larvae were stored in beakers with a useful volume of 2L, at a density of 10 larvae mL^{-1} (Lagreze et al., 2015). In the second experiment, at a temperature of 22 °C, the units were stored in an air-conditioned room, while at a temperature of 26 °C, the units were kept at room temperature.

Larvae feeding

Food was offered daily, using a microalgae mix composed of *Chaetoceros calcitrans* and *Isochrysis galbana* in a 1:1 cell ratio, at a concentration of 30,000 cells mL^{-1} in the experimental units (Oliveira, Silva Neto, Lavander, Lima, & Gálvez, 2016a). Before the offer, algal residual counts were carried out in each unit, to observe algal consumption and determine the volume of food.

Physicochemical analysis of water

The variables that indicate water quality, such as temperature (°C), salinity (g L^{-1}), pH and dissolved oxygen (mg L^{-1}), were measured daily with the aid of a multiparameter (YSI model 556, Yellow Springs, Ohio, USA). Total ammonia and nitrite levels were measured using the Visocolor Alpha Colorimetric kit, following the manufacturer's instructions. The maintenance of water quality during the experimental period was carried out through total water changes, performed on the 2nd and 5th days (48 and 120 hours), in experiments 1 and 2, respectively.

Survival and growth

For the evaluation of survival, every two days, a population survey was carried out by counting the larvae with the aid of a Sedgwick-Rafter chamber under an optical microscope (Coleman - N107 LED, Brazil) and calculated using the formula expressed in Equation (1).

$$S(\%)=\left(\frac{P_f}{P_i}\right)\times 100 \quad (1)$$

Where P_f is the final population and P_i is the initial population.

Growth was evaluated by measuring the length (maximum anteroposterior dimension) (Oliveira & Oliveira, 1974) of 30 veliger larvae from each unit at the end of experiment. Measurements were performed using the Fiji image software, through photographs taken under an optical microscope.

The daily length increment and the specific growth rate of the larvae were estimated using the following formulas expressed in Equations (2 and 3).

$$\text{DLI } (\mu\text{m day}^{-1}) = \left(\frac{C_1 - C_2}{t}\right) \quad (2)$$

Where DLI is daily length increment, C1 is initial length, C2 is final length and t is cultivation time.

$$\text{SGR} = 100 \times \left[\frac{(lnC_2 - lnC_1)}{t}\right] \quad (3)$$

Where SGR is the specific growth rate (% day^{-1}); C1 and C2 represent the lengths at the beginning and end of the experiment in µm, respectively, and t is the duration of the experiment (Oliveira et al., 2019).

Statistical analysis

To confirm the homogeneity and normality of the variables of experiments I and II, the Cochran and Shapiro-Wilk tests were used. Then, analysis of variance (ANOVA) was applied, followed by Tukey's test, for experiment I, and Student's t-test, for experiment II, to compare and classify the averages. Water quality data were analyzed by repeated measures ANOVA followed by Tukey's test to classify the averages, when significant differences were observed between treatments. Statistical tests were conducted using the STATISTICA 10.0. For all statistical analyses, p <0.05 was considered for significance.

Results

Experiment I

The water quality variables remained adequate for the species and for larviculture of bivalve mollusks during the experiment, and no significant differences were observed between treatments. Ammonia and nitrite levels also remained within the ideal conditions for the cultivation of bivalve larvae (Table 1).

At the end of the experiment, the survival of *A. flexuosa* larvae was significantly higher (p <0.05) in salinities of 25 and 30 g L^{-1} , with values higher than 39%, differing from the treatment with salinity 35 g L^{-1} , as described in Figure 2.

Table 1. Water quality variables in the cultivation of *Anomalocardia flexuosa* larvae subjected to different salinity gradients.

Variables	Treatments (g L^{-1})		
	25	30	35
Temperature (°C)	26.00 ± 0.70[a]	26.03 ± 0.73[a]	26.13 ± 0.63[a]
Dissolved oxygen (mg L^{-1})	5.15 ± 0.31[a]	5.10 ± 0.33[a]	4.90 ± 0.25[a]
pH	8.37 ± 0.11[a]	8.36 ± 0.15[a]	8.45 ± 0.06[a]
Total ammonia (mg L^{-1})	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]
Nitrite (mg L^{-1})	0.25 ± 0.27[a]	0.25 ± 0.27[a]	0.25 ± 0.27[a]

Mean of values sampled over the entire experimental period ± standard deviation. Means followed by the same letters do not differ from each other by Student's t test (p >0.05).

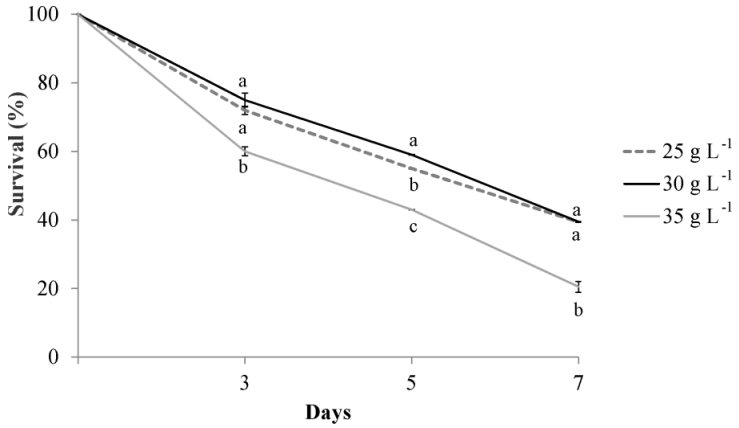

Figure 2. Larval survival (%) during the experiment with different salinities. Different letters indicate significant difference by Tukey's test (p <0.05).

The data related to the specific growth rate behaved in the same way, where higher values were observed for salinities of 25 and 30 g L^{-1} (Table 2). The highest values of total length and daily growth were found in the salinity of 25 g L^{-1}, differing significantly from the other treatments (p < 0.05).

Table 2. Veliger larvae growth of *A. flexuosa* cultivated in different salinities, during seven days.

Variables	Treatments (g L^{-1})		
	25	30	35
Survival (%)	39.35 ± 0.06[a]	39.47±0.01[a]	20.48 ± 0.58[b]
Total length (µm)	126.08 ± 1.45[a]	121.68 ± 1.57[b]	118.65 ± 0.93[b]
DLI (µm day^{-1})	8.33 ± 0.20[a]	7.66 ± 0.21[b]	7.26 ± 0.13[b]
SGR (% day^{-1})	8.79 ± 0.16[a]	8.24 ± 0.17[a]	7.94 ± 0.11[b]

The data correspond to the mean ± standard deviation. [a,b] - Different letters on the same line indicate statistical differences by Tukey's test (p<0.05). Abbreviations: DLI= daily length increment; SGR= specific growth rate.

Experiment II

The water quality variables also remained adequate for the cultivation of the species during the experiment, with significant differences being observed for dissolved oxygen and pH, where the highest results were found in the treatment with 22°C. For nitrogen compounds, ammonia differed statistically between treatments, while for nitrite no differences were found, although both were maintained at appropriate concentrations for the species throughout the 10 days of the experiment. (Table 3).

Table 3. Water quality variables in the cultivation of veliger larvae of *Anomalocardia flexuosa* cultivated under different temperature conditions.

Variables	Treatments (ºC)	
	22 ºC	26 ºC
Salinity (g L^{-1})	30.77 ± 0.09[a]	30.81 ± 0.19[a]
Dissolved oxygen (mg L^{-1})	5.78 ± 0.05[a]	5.09 ± 0.05[b]
pH	8.66 ± 0.01[a]	8.61 ± 0.11[a]
Total ammonia (mg L^{-1})	0.09 ± 0.04[b]	0.25 ± 0.04[a]
Nitrite (mg L^{-1})	0.84 ± 0.23[a]	0.92 ± 0.22[a]

Mean of values sampled over the entire experimental period ± standard deviation. [a,b] – Different letters on the same line indicate statistical differences by Tukey's test (p<0.05).

The survival, total length, daily growth and specific growth rate of *A. flexuosa* larvae at the end of the experiment were significantly higher (p <0.05) at ambient temperatures. (26 °C) (Table 4; Figure 3).

Table 4. Productive variables of *Anomalocardia flexuosa* veliger larvae cultivated under different temperature conditions for ten days.

Variables	Treatments (ºC)	
	22	26
Survival (%)	50.00 ± 2.33[b]	62.25 ± 3.48[a]
Total length (µm)	162.71 ± 0.33[b]	210.19 ± 0.56[a]
DLI (µm day^{-1})	7.28 ± 0.03[b]	12.02 ± 0.06[a]
SGR (% day^{-1})	5.93 ± 0.02[b]	8.49 ± 0.03[a]

The data correspond to the mean ± standard deviation. [a,b] - Different letters on the same line indicate statistical differences by Tukey's test (p<0.05). Abbreviations: DLI= daily length increment; SGR= specific growth rate.

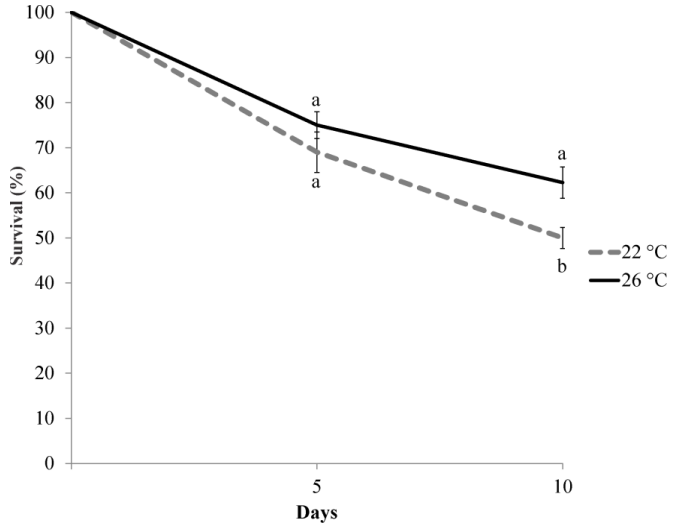

Figure 3. Larval survival (%) during the experiment with different temperatures. Different letters indicate significant difference by Tukey's test (p <0.05).

Discussion

Anomalocardia flexuosa is characterized as a species tolerant to variation in environmental variables, therefore, it can adapt to different environmental conditions (Lagreze-Squella et al., 2018). However, it was observed that temperature and salinity can affect the development and growth of larvae of bivalve mollusk (Ng, Teh, Poi, Yasin, & Tan, 2016) as early as from spawning (oocyte fertilization) and embryogenesis (Lopes et al., 2023). In the present study, these variables significantly altered the survival and growth of *A. flexuosa* larvae.

The results found for the development of the larvae in treatments with 25 and 30 g L^{-1} of salinity and at both temperatures were similar to other studies with the species, reaching length > 120 µm and survival between 45.58% and 77.78% in 7 days of cultivation with a density of 10 larvae mL^{-1} (Lagreze et al., 2015; Lima, Lavander, Silva, & Gálvez, 2018), in the others, lower results were observed. For specific growth rate, we found similar values to that reported by Oliveira et al. (2016a), when cultivating veliger larvae of *A. flexuosa*, while Lima et al. (2018) found lower daily growth rates (about 4.47 % day^{-1}), under a stocking density of 10 larvae mL^{-1} at 35 g L^{-1} in comparison to the current study.

Water quality during larval rearing was considered ideal for bivalve mollusk species, where all variables remained at ideal levels for cultivation. Metabolic residues generated in the cultivation may also negatively affect the development of the larvae (Yan, Zhang, & Yang, 2006). Studying the growth of *Crassostrea rhizophorae* larvae, Antônio, Guimarães, Peixoto, and Gálvez (2009) reported greater survival with water change frequencies of 48 or 72 hours, when compared to changes every 24 hours. In experiment II, where the

water exchange was performed on the fifth day (120 hours) of the experiment, survival of *A. flexuosa* was higher to that observed in experiment I, where water exchanges happened every two days (48 hours). This finding shows that water changes at short intervals (i.e., 24 or 48 hours) negatively affect the survival of *A. flexuosa* larvae, probably due to the greater fragility of the larvae.

Knowing that the survival and growth of bivalve mollusk larvae may be affected by water quality parameters, such as salinity and temperature, Huo et al. (2014) evaluating the effects of salinity on the development, survival and growth of oyster larvae of *Crassostrea hongkongensis,* reported that although the larvae of this bivalve tolerate a wide range of salinities (ranging from 15 to 30 g L^{-1}), higher growth and survival rates were observed at low salinities. This fact can also be observed in the current study for *A. flexuosa*, where in lower salinities we have found better growth and survival rates for the larvae. Probably higher salinities can influence the development of organisms due to a higher metabolic energy expenditure (Deaton, 2008), even if in the Brazilian Northeast — natural environment of this species — the average salinity was reported to be between 23 and 45 g L^{-1} (Lavander et al., 2011; Nascimento, Silva, Silva, & Maia, 2022). On the other hand, higher survival rates of *Pteria hirudo* were reported for salinity of 35 g L^{-1} (Albuquerque et al., 2012). These differences may be associated with the life cycle of these mollusks, in which *A. flexuosa* inhabits, mainly, estuarine regions and near coast areas, where oscillations in the salinity gradient are more frequent.

Like salinity, temperature can also affect the larval development of mollusks (Liu, Gurney-Smith, Beerens, & Pearce, 2010). As the temperature rises, the metabolic rate increases, and the bivalve larvae acquire more energy by increasing the consumption of microalgae to maintain a positive energy consumption (Nair & Appukuttan, 2003; Rico-Villa, Poureau, & Robert, 2009). However, when these temperatures exceed the thermal tolerance limit of the cultivated species, they negatively influence the survival and growth of organisms.

In tests previous to this work, it was observed that the lower and upper thermal limits reported for *A. flexuosa* are close to 17 and 35 ºC, respectively, resulting in total mortality of the larvae. When evaluating the effects of temperature on larvae of *Crassostrea gigas*, Kheder, Moal and Robert (2010) reported a reduction in the larval stage at high temperatures (32 °C) as well as increased growth and overall metabolism stimulation. Marcelino, Macia, Mafambissa, Castejón, and Andrade (2023), when evaluating the combined effects of temperature and salinity on the embryonic and larval development of the oyster *Saccostrea cucullata*, observed that low temperatures (24 ºC) negatively affect growth regardless of the salinity level, and that survival decreased linearly salinity increased. According to Gadomski, Moller, Beentjes, and Lamare (2015), while studying the larval development of *Paphies ventricosa*, the smaller size of the larvae at colder temperatures reflects the delay in larval development, however no physiological damage was observed. Thus, the results obtained in the present study and others indicate that there is an optimal range of temperature and salinity to achieve the best larval development of bivalve mollusks.

Conclusion

Salinity and temperature influence the growth and survival of *Anomalocardia flexuosa* veliger larvae. In this sense, it is recommended that larvae of this species be cultivated in salinities of 25 to 30 g L^{-1} and initially at 26°C of average water temperature. Other experiments should be carried out to test the effect of the periodicity of water exchange on larval culture and its consequences on the survival of larvae of the species.

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