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Research Article

Growth and survival of *Anomalocardia brasiliana* larvae (Bivalvia: Veneridae) fed with microalgal diets

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ABSTRACT. Effects of different microalgal diets on the growth and on the survival of *Anomalocardia brasiliana* larvae between trochophore and pediveliger stages were evaluated. Diets were evaluated in two separate experiments. The first tested the microalgae *Isochrisys galbana* (Ig), *Phaeodactylum tricornutum* (Phaeo), *Chaetoceros calcitrans* (Cca) and the combinations (Ig+Cca), (Ig+Phaeo) and (Cca+Phaeo). The second tested the microalgae *C. calcitrans* (Cca), *Pavlova lutheri* (Pl) and the combination (Cca+Pl). When provided alone, *I. galbana* resulted in survival and growth rates lower than the rest of the diets, and the best results achieved were obtained with *P. tricornutum* and *C. calcitrans* supplied alone or combined with other microalgae. However, in the second experiment the diet Cca+Pl resulted in better growth and survival rates (261.67 ± 9.64 μm and 31.50 ± 0.87%) than all diets tested in both experiments, therefore it is recommended for *A. brasiliana* larvae.

Keywords: *Anomalocardia brasiliana*, larval culture, microalgae diets, aquaculture.

Crecimiento y supervivencia de larvas de *Anomalocardia brasiliana* (Bivalvia: Veneridae) alimentadas con dietas de microalgas

RESUMEN. Se evaluó el efecto de diferentes dietas de microalgas en el crecimiento y supervivencia de larvas de *Anomalocardia brasiliana* entre etapas trocófera y pedivelíger. Las dietas se evaluaron en dos experimentos separados. En el primero se probaron la microalgas *Isochrisys galbana* (Ig), *Phaeodactylum tricornutum* (Phaeo), *Chaetoceros calcitrans* (Cca) y las combinaciones (Ig+Cca), (Ig+Phaeo) y (Cca+Phaeo). En el segundo, se probaron las microalgas *C. calcitrans* (Cca), *Pavlova lutheri* (Pl) y la combinación (Cca+Pl). Cuando *I. galbana* se administró sola, dió menor supervivencia y crecimiento que el resto de las dietas, y los mejores resultados se obtuvieron con *P. tricornutum* y *C. calcitrans* suministradas solas o combinadas con otras microalgas. Sin embargo, en el segundo experimento la dieta Cca+Pl dio un mayor crecimiento y supervivencia (261,67 ± 9,64 μm y 31,50 ± 0,87%) que todas las dietas probadas en ambos experimentos y, por lo tanto, se recomienda para las larvas de *A. brasiliana*.

Palabras clave: *Anomalocardia brasiliana,* cultivo larvario, dietas de microalgas, acuicultura.

INTRODUCTION

The entire production of cultured marine mollusks in 2010 was of 13.9 million ton, and represented 75.5% of all cultured marine organisms worldwide (FAO, 2012). However, mollusk culture in Brazil is limited to a few species including the Mytilidae *Perna perna*, the Ostreidae *Crassostrea gigas*, *C. rhizophorae*, *C. brasiliana* and the Pectinidae *Nodipecten nodosus*. Taking this into account, there is a great potential for cultivating other species, diversifying the national aquaculture.

Northeastern Brazil has favorable climatic conditions for the development of mollusk culture, but there is still a need for new technologies to grow native species, such as the bivalve *Anomalocardia brasiliana*

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(Gmelin,1791). *A. brasiliana* is an important fishery resource for Brazilian coastal communities (Silva-Cavalcanti & Costa, 2009; Oliveira *et al.*, 2011), and laboratory production of its seed could serve for restocking heavily exploited populations along Brazilian coastline.

One of the obstacles to establish successful larval cultures is the availability of an appropriate diet (Ponis *et al.*, 2006; Liu *et al.*, 2009; Pettersen *et al.*, 2010). Microalgae are the main food source for larvae and seeds in bivalve hatchery (Helm & Bourne, 2004). The promotion of greater shellfish larvae survival and growth rates depends directly on the offered food. There is no information on the best or at least adequate algae species for *A. brasiliana,* being the purpose of this study to evaluate survival and growth of larvae of *A. brasiliana* when fed with different microalgal diets.

MATERIALS AND METHODS

A total of 500 clams, longer than 20 mm, were collected on the beach of Mangue Seco (7º49.70'S, 35º50.05'W), Igarassu municipality, 30 km away from Recife, State of Pernambuco, Brazil. They were transported to the Laboratory of Sustainable Mariculture (LAMARSU), and kept during 24 h in 500 L tanks at 25ºC, 30 salinity and 6 mg L^{-1}mean dissolved oxygen.

After this period, they were fed twice daily with a mixture of *Isochrysis galbana* and *Chaetoceros calcitrans* at a cell ratio of 1:1, with a total ration of $20x10^4$ cells mL^{-1} day^{-1}. In the first experiment, after 10 days spawning occurred spontaneously, and the fertilized eggs were filtered through a 50 µm mesh. Concerning the second experiment, animals arrived with fully mature gonads, so that the acclimation process in the laboratory was not necessary, spawns occurred on the same day, through induction; by releasing gametes into the warter and gradually raising water temperature (1ºC h^{-1}).

After 24 h D-veliger larvae (n = 30) had an average length of 69.94 ± 1.54 mm and were stocked with an initial density of 5 larvae mL^{-1}, in triplicate plastic containers with two liters of seawater (3 µm filtered and UV-treated).

Temperature and salinity were measured daily, oxygen twice a day and the water of larval cultures was renewed completely in each third day. Feeding was carried out once a day; the microalgae supplied were from three days-old cultures, in exponential growth.

The microalgae used were obtained from LAMARSU stock strains. The seawater with salinity of 32 ± 2 went through 1 µm porous filter paper, autoclaved, and enriched with Conway sterilized medium (Walne, 1966), supplemented with sodium metasilicate (40 mg L^{-1}) to provide a silica source for the two diatoms.

The effect of microalgal diets on larval growth of *A. brasiliana* was evaluated in two completely randomized experiments. The first experiment tested the microalgae *I. galbana* (Ig), *Phaeodactylum tricornutum* (Phaeo) and *Chaetoceros calcitrans* (Cca) and the combinations (Ig+Cca), (Ig+Phaeo) and (Cca+Phaeo). The larval rearing period was of 15 days, starting with the D-veliger larval stage and ending with pediveliger larvae. The total algal density provided was $30x10^3$ cells mL^{-1} and for bialgal diets a 1:1 ratio was used. The second experiment evaluated the algae *C. calcitrans* (Cca), *P. lutheri* (Pl) and the combination (Cca+Pl). The methodology and algal density provided in the second experimet were the same adopted for the first experiment.

To assess larval survival at the end of experiment the entire volume of each experimental unit was filtered. The larvae were concentrated in a 50 mL container, from which 1 mL samples were drawn. The larval counting was done with a Sedgewick-Rafter counting chamber and optical microscope, three samples of each experimental unit were analyzed.

For the evaluation of larval growth, 1 mL samples were taken from each experimental unit on the first and last days of the experiment; images of 30 larvae randomly chosen were obtained using an optical microscope coupled to a camera, and their length (maximum anterior-posterior dimension) and width (maximum dorsal-ventral dimension) were measured using the software ImageTool, version 2.0 (Texas University, Health Science Center, San Antonio, USA).

Relative growth (K) was calculated using the equation $K = (\ln L2 - \ln L1) / t$, where *L1*, *L2* respectively stand for the lengths in µm at the beginning and end of the experiment, while *t* stands for the duration of the experiment in days.

Data on survival, length, width and relative growth in each type of diet was generated in both experiments; the data was previously checked for normality using the Kolmogorov-Smirnov test and for homogeneity of variance by Cochran's C test. Analysis of Variance (ANOVA) was used to determine the effect of diets on the growth and survival of larvae through out the time of cultivation. Duncan's test was performed to detect the mean levels which differed significantly between treatments. The level of significance was $P < 0.05$.

RESULTS

The temperature (ºC), salinity and dissolved oxygen (mg L^{-1}) of the water were maintained within acceptable limits for shellfish growing. The tempe-rature ranged from 25.05 to 25.60ºC; salinity from 29.88 to 30.18, and dissolved oxygen had a minimum of 5.89 mg L^{-1} and a maximum of 6.66 mg L^{-1}.

First experiment

The highest survival rate, of approximately 25%, was achieved with the *Phaeo* diet, and the lowest with diets Ig and Cca+Phaeo, 6.8% and 5.3% respectively. Intermediate values were achieved with the other diets (Table 1). No significant variation in shell width was found in larvae fed with the different algal diets tested (Table 1), but diets Cca and Ig+Phaeo accomplished higher growth rate ant shell legth than the Ig diet.

Second experiment

The highest survival rate, 31.5%, was achieved with the Cca+Pl diet, and the lowest with Cca diet. Shell width, shell legth and growth rate were always higher with the Cca+Pl diet, significantly different from Cca diet (Table 2); Pl diet achieved intermediate values in all growth data.

DISCUSSION

The diet nutritional value is of great importance for growing shellfish larvae. The relative growth of larvae fed with Cca and Ig+Phaeo diets were higher only than that of larvae fed with Ig diet; no significant differences to the other diets tested were found. *C. calcitrans* is the most suitable for feeding bivalve larvae (Brown & Robert, 2002), not only because of its biochemical composition, but also because of its cell size, digestibility and absence of toxins (Pettersen *et al.*, 2010).

Studies have found that the use of *P. tricornutum* for feeding other bivalves causes slow growth (Epifanio *et al.*, 1981; Albentosa *et al.*, 1996; Rivero-Rodriguez *et al.*, 2007); it is difficult to digest (Rivero-Rodriguez *et al.*, 2007), probably due to its lack of tryptophan (Epifanio *et al.*, 1981). Tang *et al.* (2006) achieved a relatively low growth in *Meretrix meretrix* larvae fed with *Phaeodactylum tricornutum* and *Pavlova viridis*.

At the end of the cultivation, longer shells were achieved with a diet composed of the microalgae *C. calcitrans*. *Crassostrea corteziensis* seeds showed significant growth when feed with *C. calcitrans* alone or in combination with other algae; when this microalga is present in the diet, the seeds' growth was up to twice bigger. This is probably related to the fact that *C. calcitrans* contains high levels of arachidonic acid (Rivero-Rodriguez *et al.*, 2007). Similar results were found by Liu *et al.* (2009)

In the second experiment, findings indicate that there was an increase in the survival of *A. brasiliana* larvae when *C. calcitrans* and *P. lutheri* microalgae were used in combination, reaching average survival rates above 31%. Ponis *et al.* (2008), evaluating the effect of *P. lutheri* in *Crassostrea gigas* larvae, obtained survival rates above 78% when *C. calcitrans* was added to the diet. This sustains our findings that after 15 days of culture, larvae fed with Cca+Pl diet had better survival. This is significantly different from the other diets. Other studies have also found positive results in adding other species of diatom microalgae to the diet (Epifanio, 1979; Romberger & Epifanio, 1981; Laing & Millican, 1986; O'Connor & Heasman, 1997); this has been attributed to better essential nutrient balance (Webb & Chu, 1983).

Bialgal diets are often used in feeding bivalve larvae, and it is common to combine species, using a flagellate with a diatom, to maximize growth and larval development (Martínez-Fernández & Southgate, 2007; Liu *et al.*, 2009; Galley *et al.*, 2010). The flagellated species commonly used are *Isochrysis galbana* and *Pavlova lutheri* and the diatoms include *Chaetoceros calcitrans*. Spolaore *et al.* (2006) affirm that the combination of different algae species offers a better nutritional balance and improves animal growth when compared to monoalgal diets; this is in accordance whith the result of this study, which found that the bialgal diet (Cca+Pl) led to better growth. Martínez-Fernández & Southgate (2007) suggest that feeding *P. margaritifera* larvae with a single specie of microalgae may be more practical during the first 10 days in a hatchery. However, the addition of diatom microalgae to the diet increased growth rate and survival in umbonate larvae of *P. margaritifera* in comparison with treatments without diatoms.

Protein and vitamin content are important factors for determining the nutritional value of microalgae. Furthermore, high amounts of polyunsaturated fatty acids (*e.g.*, eicosapentaenoic [EPA], arachidonic acid [ARA] and docosahexaenoic acid [DHA]) (Hemaiswarya *et al.*, 2011) can lead to better larvae growth and survival when fed with the microalgae *P. lutheri*, which is rich in DHA/EPA, and *C. calcitrans*, which is used to increase vitamin levels (Hemaiswarya *et al.*, 2011).

This study found that monoalgal and bialgal diets present satisfactory results in terms of survival. Prymnesiophyceae *P. lutheri* is commonly used in aquaculture as live food for marine invertebrates and particularly for bivalves (larvae, juveniles and breeding stock) (Webb & Chu, 1983; Borowitzka, 1997; Wikfors & Onho, 2001; Brow, 2002; Rico-Villa *et al.*, 2006), but its use alone may achieve a low growth rate when compared to the use in combination with other diatoms (Rico-Villa *et al.*, 2006; Ponis *et al.*, 2008).

Table 1. Mean (± SE) survival, width, length and relative growth (K) of larvae of *A. brasiliana* fed different diets over 15 days of culture. Cca: *Chaetoceros calcitrans*, Ig: *Isochrysis galbana*; Phaeo: *Phaeodactylum tricornutum*, mixed diets Cca+Ig: *C. calcitrans* and *I. galbana*; Cca+Phaeo: *C. calcitrans* and *P. tricornutum*, and Ig+Phaeo: *I. galbana* and *P. tricornutum.* Different letters in the same column indicate significant difference (one-way ANOVA, $P < 0.05$).

Diets	Survival (%)	Width (µm)	Length (µm)	K (µm day^{-1})
Ig	6.83 ± 1.92^{b}	212.33 ± 6.79	$230{,}00 \pm 7{,}42^{b}$	0.0779 ± 0.0022^{b}
Cca	12.33 ± 5.84^{ab}	229.00 ± 5.19	$251{,}33 \pm 5{,}66^{a}$	0.0848 ± 0.0015^{a}
Phaeo	24.83 ± 3.03^{a}	222.33 ± 5.27	$246{,}33 \pm 5{,}98^{ab}$	0.0830 ± 0.0020^{ab}
Ig+Cca	13.50 ± 4.91^{ab}	226.33 ± 5.04	$241{,}33 \pm 6{,}39^{ab}$	0.0821 ± 0.0020^{ab}
Ig+Phaeo	18.27 ± 2.42^{ab}	226.00 ± 7.81	$249{,}00 \pm 7{,}16^{ab}$	0.0839 ± 0.0019^{a}
Cca+Phaeo	5.27 ± 1.36^{b}	220.00 ± 6.49	$239{,}67 \pm 6{,}24^{ab}$	0.0807 ± 0.0020^{ab}

Table 2. Mean (±SE) survival, width, length and relative growth (K) of larvae of *A. brasiliana* fed different diets over 15 days of culture. Cca: *Chaetoceros calcitrans*, Pl: *Pavlova lutheri*, mixed diet Cca+Pl: *C. calcitrans* and *P. lutheri*. Different letters in the same column column indicate significant difference (one-way ANOVA, $P < 0.05$).

Diets	Survival (%)	Width (µm)	Length (µm)	K (µm day^{-1})
Cca	4.42 ± 1.58^{c}	211.67 ± 8.60^{b}	$211.67 \pm 8{,}60^{b}$	0.0781 ± 0.0028^{b}
Pl	16.80 ± 5.63^{b}	219.33 ± 6.69^{ab}	$219.33 \pm 6{,}69^{ab}$	0.0805 ± 0.0021^{ab}
Cca+Pl	31.50 ± 0.87^{a}	242.00 ± 10.02^{a}	$242.00 \pm 10{,}02^{a}$	0.0867 ± 0.0024^{a}

Our findings confirm the potential of the microalgae *C. calcitrans* in the growth of mollusc larvae. The combination of the microalgae *C. calcitrans* and *P. lutheri* proved to be an excellent diet for the larval culture of *A. brasiliana*; there seem to be a synergistic effect when they combined.

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