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REMOTE SETTING OF *Nodipecten nodosus* (Linnaeus 1758) LARVAE*

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ABSTRACT

The present study aimed to evaluate the growth and survival of *Nodipecten nodosus* larvae grown in a remote settlement system. Two forms of transport were tested, one in a humid/wet, environment and one submerged in seawater, with a control treatment maintained at the Laboratory of Marine Mollusks (LMM). After transport treatments, individuals were populated simultaneously inside floating boxes directly at sea and in containers under controlled conditions in the Laboratory of the Experimental Center for Mariculture (CEMAR). No statistical differences were observed in larval survival relative to the method of transport in the different experiments. However, statistical differences were observed in Evaluation 1 (EVA1) for the survival and growth of larvae transported in submerged seawater and settled in the laboratory. No statistical difference was observed between the control and either wet/humid or submerged treatments in EVA2. The survival values in the control treatment in EVA3 were higher ($p<0.05$) in relation to the wet and submerged treatments cultivated in the laboratory. It was not possible to observe the presence of pre-seeds in treatments grown at sea. Is possible to transport larvae for 6 hours of travel to be settled in controlled conditions far from their place of origin.

Keywords: pectinid; transport; pediveliger; seeds; settlement; growth; survival.

ASSENTAMENTO REMOTO DE LARVAS *Nodipecten nodosus* (Linnaeus 1758)

RESUMO

Este estudo teve como objetivo avaliar o crescimento e a sobrevivência de larvas de vieiras *Nodipecten nodosus* cultivadas em sistema remoto de assentamento. Foram testadas duas formas de transporte, úmido e submerso em água do mar com um tratamento controle mantido no Laboratório de Moluscos Marinhos (LMM). Após serem expostos aos tratamentos de transporte os animais foram povoados dentro de caixas flutuantes, diretamente no mar e em recipientes em condições controladas no Laboratório do Centro Experimental de Maricultura (CEMAR). Não houve diferença estatística na sobrevivência das larvas em relação ao método de transporte nos diferentes experimentos. Observou-se diferenças estatísticas ($p<0,05$), na avaliação 1 (EVA1), para a sobrevivência e crescimento de larvas transportadas em meio submerso e assentadas no laboratório. Entretanto, não foi observada diferença estatística entre o tratamento controle, úmido e submerso na EVA2. Os valores de sobrevivência no tratamento controle na EVA3 foram superiores ($p<0,05$) em relação aos tratamentos úmido e submersos cultivados em laboratório. Não foi possível observar presença de pré-sementes nos tratamentos cultivados no mar. Conclui-se que é possível transportar larvas por seis horas de viagem para serem assentadas em condições controladas distante do seu local de origem.

Palavras-chave: vieiras; transporte; pediveliger; sementes; assentamento; crescimento; sobrevivência.

INTRODUCTION

Along the Brazilian coastline, pectinids are represented by six genera and 16 species (Rios, 1994). *Nodipecten nodosus* Linnaeus (1758) stands out for its zootechnical and economic potential in aquaculture (Rupp, 2009). This is the largest of the pectinids observed on the Brazilian coast (Rios, 1985), reaching 14 cm in height in wild individuals (Rupp and Parsons, 2006). Popularly known as scallops, they are found in small quantities surrounding Santa Catarina Island (Rupp et al., 2011). In contrast to the worldwide cultivation of some pectinids, low capture rates of natural scallop seeds make it necessary to produce them under controlled conditions (Uriarte et al., 2001).

Currently, the State of Santa Catarina produces 15,381.44 metric tons of mollusks, which corresponds to about 90% of national production and, of this quantity, scallops represent 26.9 tons (Santos et al., 2017). Although it is the least produced mollusk, its economic value is the highest, with a promising prospect of commercialization.

Recently, the world's production of pectinids has increased considerably, as have studies on this important biological group (Shumway and Parsons, 2016), providing improved seed production techniques in the laboratory to meet the commercial demand for this species (Román et al., 2001). Obtaining seeds is a process that includes the maturation of breeders, induction of gamete release, fertilization and embryonic development, larviculture and settlement (Uriarte et al., 2001). However, the most critical stage is larval settlement under controlled conditions (Bourne et al., 1989). At this stage, animals undergo metamorphosis characterized by a series of morphological and physiological changes (Gosling, 2004) and suffer considerable mortality (Helm et al., 2004). Thus, improved seed settlement management is required to obtain a higher yield in the laboratory production of pre-seeds (Carvalho et al., 2013) to make the practice more economically viable (Sühnel et al., 2008).

In view of this, one option would be to transfer larvae to the sea, followed by remote settlement. This technique consists of transporting larvae in the pediveliger stage to the cultivation units, or even land units, close to crops (Suplicy et al., 2017). This method started as an alternative to increase the supply of oyster larvae by producers in the USA and Canada (Jones and Jones, 1988; Roland and Broadley, 1990). It was later implemented for the sand mollusks *Venerupis japônica* Deshayes (1853) and *Mercenaria mercenaria* Linnaeus (1758) (Sturmer et al., 2003), as well as the Japanese species *Patinopecten yessoensis* Jay (1857) grown in Canada (Heath, 2001), and currently for mussels *Perna perna* Linnaeus (1758) in Santa Catarina (Novaes et al., 2016).

In the remote settlement of mollusks, larvae are transported in a humid environment, wrapped in a polyethylene mesh. However, the success of this process depends on several factors, such as water quality, temperature and metabolic compounds (Maeda-Martínez et al., 2000; Heath, 2001).

In order to improve the remote settlement of scallop larvae, reduce production costs in the laboratory and increase the cultivation of the species, this study evaluates the survival and growth of our model *N. nodosus* larvae settling at sea and in the laboratory, using different forms of transport (wet/humid and submerged in seawater). The hypothesis of this study is that survival and growth of larvae in remote settlement are affected by transportation.

MATERIAL AND METHODS

Study area

Research was carried out in the aquaculture area of the Experimental Center for Mariculture (CEMAR) at UNIVALI University in Penha, Santa Catarina (26º59'S; 48º38'W) and in the Laboratory of Marine Mollusks (LMM) in Florianópolis, Santa Catarina (27º35'S; 48º32'W), Brazil.

Experimental design

The experimental design was entirely randomized in a factorial scheme. Two factors were considered. Factor A involved transport (wet/humid and submerged in water), and factor B involved the method of cultivation (sea or laboratory). A control treatment was maintained at LMM. Treatments performed in the laboratories (LMM and CEMAR) consisted of four replicates, and the treatments maintained at sea consisted of three replicates. Three 40-day cultivations were made. The first evaluation (EVA1) was carried out in autumn (March 30, 2017 to May 09, 2017), the second (EVA2) between autumn and winter (June 12, 2017 to July 22, 2017) and the third (EVA3) between spring and summer (November 30, 2017 to January 09, 2018).

Obtaining larvae

Broodstock was conditioned in the maturation sector at LMM for 15 days at controlled temperature (19–21ºC) in a continuous flow of marine water (1 L min^{-1}) filtered at 1 µm. They were fed a mixture of microalgae species (*Chaetoceros muelleri* and *Isochysis galbana*) in the ratio of 9:1 at an average concentration of 3 x 10^4 cells mL^{-1}).

Spawning induction used 30 adult animals collected randomly from the maturation sector, grouped and submerged in a plastic container with 20 L with the same water as that in the conditioning tanks. The animals were transferred to a flat table where bio-encrustation was removed. After this procedure, the animals were transferred and distributed in a rectangular fiberglass tank with a capacity of 500 L. It was filled with only 100 L of marine water filtered at 1 µm and sterilized with ultraviolet radiation, sufficient to keep the animals submerged in a continuous flow of 20 L min^{-1} for about 10 min. The temperature was raised gradually from 21 to 25ºC. From the first spawning event, as verified by the expulsion of jets, all breeders were immediately transferred individually to containers with 2 L of water. Constant volume changes were carried out in these new containers, being emptied and refilled every minute, except in the case of new spawning events in the water. In these cases, the contents of the containers were separated by the color of the gametes present (orange characteristic of females and white of males) and subsequently transferred, separately, to another 20 L container, rinsed and filled in the same way. The procedure was performed repeatedly until the animals stopped spawning.

Subsequently, the 20 L containers with the oocytes were sieved at 60 µm to remove possible impurities. Three samples of 0.5 mL from the containers were duly homogenized and diluted in beakers with 5 mL of water. This 0.5 mL aliquot was transferred to a Sedgwick-Rafter® chamber and the oocytes counted with a LEICA® optical microscope. The value obtained was used to adjust the oocyte concentration and allow fertilization.

The fertilized solution was transferred to a fiberglass tank with a volume of 6,000 L of water with salinity of 35 and constant aeration, remaining there for 24 hours. After this period, a 45-µm

mesh sieve was placed at the outlet of the tank to retain larvae in the "D" stage. The retained content was subjected to new sieving, using 35, 50 and 70 µm mesh sieves to remove dirt and to separate the larvae, most of which were retained in the 50 µm mesh. The larvae were then transferred to a 20 L graduated bucket and homogenized. Following this, three 0.5 mL samples were collected, diluted in 5 mL, and counted under a microscope to estimate the total number of larvae and adjust the stocking density to 3 larvae mL^{-1}.

Larviculture was conducted in a static system. The experimental units were emptied daily by gravity using a sieve containing a 55 µm mesh for larvae retention. After the tank was exhausted, the retained larvae were concentrated in a 20-L container. The contents of the container were homogenized, and three 0.5 mL samples were collected, diluted in a 5 mL beaker, and homogenized again. A new 0.5 mL sample was then taken, fixed in 4% formaldehyde and transferred to a Sedgwick-Rafter® chamber to count the number of animals using a LEICA® optical microscope. At the end of the larviculture, a sample containing approximately 100 larvae suitable for settlement was withdrawn using a Pasteur® pipette. The sample was concentrated in an Eppendorf® tube, fixed in 4% formaldehyde and transferred to a Sedgwick-Rafter® chamber to be measured for height and length using a LEICA® optical microscope and LAS EZ 2.0.0® software.

Larvae were fed a mixture of *C. muelleri*, *I. galbana* and *Pavlova* spp. in the ratio of 1:1:1 at an initial concentration of $0.5x10^4$ cells mL^{-1} increasing to $3x10^4$ cells mL^{-1} at the end of the larviculture.

Collector maturation

The procedure for preparing the collectors and maturation was performed as described by Zanette et al. (2009) and adapted by LMM. As a substrate for the settlement, Netlon® collectors were used, rolled up into a ball shape, and filled with sheets of *Pinus elliotti* Engelm placed inside a Nitex® mesh bag with 500 µm mesh opening and a ballast weighing 1 kg to avoid fluctuation. They remained immersed in a glassfiber tank with 2000 L of fresh water containing sodium hypochlorite at 50 ppm for 24 hours. Subsequently, sodium thiosulfate was used to neutralize the chlorine and the tanks rinsed and then filled with filtered and sterilized seawater. The maturation of the collectors consisted of keeping them under the same conditions for the same duration as the larviculture with daily water exchange, constant aeration and supplies of *C. muelleri*, *I. galbana* and *Pavlova* spp. in the ratio of 1:1:1 at a concentration of $0.5x10^4$ cells mL^{-1}.

Larvae transport

The transport of larvae to CEMAR was carried out in two forms of storage: (a) submerged in seawater in 500 mL polyethylene terephthalate bottles and (b) wrapped in a 100-µm polyamide mesh covered with paper towels moistened with seawater. Five replicates per treatment were carried out, and 20,000 animals were used per experimental unit. During the trip, the experimental units were placed inside a polystyrene container, kept at room temperature and transported in a car. The route taken was from LMM, located in Barra da Lagoa, Florianópolis, to CEMAR, located in Armação do Itapocoroy, Penha; the journey took approximately 6 hours. Every 30 min, the temperature inside the container was measured using an Aquarium® digital thermometer.

Post-transport survival

After arrival at CEMAR, the larvae were transferred to 20 L containers with filtered and sterilized marine water and acclimatized for 20 minutes. The transport treatments (submerged and wet/humid) were performed simultaneously. After acclimatization, the larvae were concentrated in polyamide sieves with a mesh size of 100 µm and transferred to graduated beakers containing 1 L of seawater. After the homogenization process, three samples, consisting of 1 mL from each beaker, were collected, fixed in 4% formaldehyde and transferred to a Sedgewick Rafter® chamber to assess survival using a LEICA® optical microscope.

Settlement at sea

Part of the larvae transported in wet and submerged environments were transferred to nesting boxes. These units were built with wood forming a square (45 x 45 x 10 cm) with a total volume of 20.25 L and covered with a polyamide mesh containing two previously matured Netlon® collectors serving as fixing substrates. The units were populated with 1 larva mL^{-1}, totaling 20,000 individuals. Larvae from each transport treatment (wet/humid and submerged) were placed in separate nesting boxes with a mesh of initial porosity 120 µm throughout the entire EVA1. In the case of EVA2 and EVA3, the cultivation boxes had mesh sizes with initial porosities of 120 µm, 150 µm or 180 µm. The boxes remained distributed in a rectangular tank submerged in seawater and kept in the laboratory until transfer to the sea. At sea, they remained tied horizontally in floating cultivation structures approximately 1 m from the surface, located in the aquaculture area of CEMAR-UNIVALI.

Every 72 hours, the meshes were cleaned manually using circular movements with a brush. Every 10 days of cultivation, they were replaced by other meshes increasing by 30 µm in size. That is, for EVA2 and EVA3, the initial meshes were replaced by meshes with 150 µm, 180 µm and 210 µm; 180 µm, 210 and 240 µm; and 210 µm, 240 µm and 270 µm in the first, second and third handlings, respectively, until completing the 40-day experiment. In EVA1, the meshes were replaced in the first, second and third handlings by 150 µm, 180 µm and 210 µm meshes for all experimental units, respectively. The procedure for changing the meshes was carried out near the cultivation unit at sea in a boat. The boxes were removed from the anchorage and transferred gradually to marine water boxes inside the boat. After opening the box, the pair of collectors was manually transferred to another experimental unit.

Laboratory settlement

The cultivation system used for the settlement of animals in the laboratory was the static method with partial (70%) daily exchange of water (salinity 35, filtered at 1 µm and sterilized by UV light radiation). Containers (20 L) were used as cultivation

units with two submerged Netlon® collectors inside. Aeration was provided through a porous stone introduced with ceramic ballast fixed with a clamp next to the collectors to prevent fluctuation. Every 24 hours, the units were emptied manually through a 90 µm sieve. The retained individuals were re-allocated to their same unit after refilling with seawater.

The larvae from each transport treatment (wet/humid and submerged) were populated distinctly at a density of 1 larva mL^{-1} in the proportion of 10,000 for each collector and maintained in five replicates for each treatment. They were fed once a day with a mixture of microalgae containing *C. muelleri*, *Chaetoceros calcitrans*, *I. galbana* and *Pavlova lutheri* in the ratio of 1:1:1:1 and initial total concentration of $3x10^4$ cells mL^{-1} increasing to $20x10^4$ cells mL^{-1} at the end of the experiment, according to Carvalho et al. (2013).

Survival and growth assessments

The pre-seeds were detached after 40 days of settlement of all the experimental units kept at sea, in the laboratory and in the control, using brushes with Vonder® bristles, and transferred to containers with filtered and UV-sterilized seawater. To calculate the survival rates, the total volume of the animals was measured with a graduated cylinder, and the numbers of pre-seeds in three samples of 1 mL each were counted. The growth in length and height (mm) was evaluated according to Galtsoff (1964), using samples with 20 individuals per replicate, totaling 100 individuals per treatment (control, wet/humid and submerged), which were concentrated in an Eppendorf® tube, fixed in 4% formaldehyde, transferred to a Sedgwick-Rafter® chamber and counted under a LEICA® binocular stereomicroscope with LAS EZ 2.0.0® software.

Water quality parameters

During the settlement period in the laboratory, temperature and salinity were measured daily in the morning using an Incoterm® analog immersion thermometer and a Kasvi® portable refractometer, respectively.

At sea, the water was collected on the surface and inside the settlement boxes every 72 hours to analyze chlorophyll-a, total suspended solids (TSS) and salinity. In order to sample these parameters inside the settlement units, extra collection boxes were made, similar to those containing the larvae, which had a tap attached to their lateral structure to drain water from inside. These units were handled in the same way as the units that received animals.

The samples were collected in a 5-L polypropylene flask and subsequently filtered with a vacuum pump at CEMAR. The filters for analysis of TSS and chlorophyll-a were frozen and subsequently analyzed at the UNIVALI Chemical Oceanography and Marine Pollution Laboratory, following the recommendations described by APHA (2005).

Temperature was measured every 24 hours throughout all evaluations by the installation of a TID-BIT Hobo® digital device and salinity registered using the Kasvi® portable refractometer.

Statistical analysis

After testing for the basic assumptions (error normality and homogeneity of variances) the growth data were analyzed through analysis of variance (ANOVA), followed by Tukey's test of separation of means, when differences between treatments were verified by ANOVA. The survival data were analyzed using the Kruskal-Wallis test. All analyses were performed using the SAS® computational package (2003).

RESULTS

Temperature and survival during transport

The temperatures inside the isothermal boxes used to transport the larvae during the journey were 23.3 ± 2.3ºC, 22.1 ± 1.8ºC and 24.3 ± 2.6ºC for EVA1, EVA2 and EVA3, respectively, with no differences ($p>0.05$) between wet or submerged transport treatments.

The percentage of live larvae after transport in EVA1, EVA2, EVA3 were 91.7 ± 8.2%, 91.3 ± 9% and 95.1 ± 6.4% for larvae packed in a wet environment and 89.5 ± 7.1%, 86.4 ± 11% and 94.2 ± 6.7% for submerged larvae, respectively. No statistical differences ($p>0.05$) were observed in larvae survival between treatments immediately after transport.

Pre-seed survival and growth

Pre-seed survival rates were relatively low in settlements, ranging from 0.4 ± 0.25% to 3.3 ± 2.2% for those transported in a wet environment; from 1.81 ± 0.96% to 13.5 ± 10.1% for those transported in a submerged environment; and from 1.8 ± 2.4% to 27.4 ± 7.39% in the control treatment. Pre-seeds transported in a moist environment showed statistically lower survival rates in EVA1, in EVA2, together with the control treatment, and in EVA3, together with those pre-seeds transported in submerged medium (Table 1).

The initial height and length of the larvae were 191 ± 18.3 µm and 178.1±10.8 µm in EVA1; 176.1 ± 10.9 µm and 198.3 ± 14.7 µm in EVA2; and 164 ± 11.9 µm and 181 ± 16.6 µm in EVA3, respectively. After 40 days of cultivation, pre-seeds from the submerged showed statistically higher values of growth in height (1.89 ± 0.45 mm) and length (1.81 ± 0.39 mm) compared to the other treatments in EVA1. In EVA2 and EVA3,

Table 1. Survival (%) of *Nodipecten nodosus* pre-seeds after 40 days of cultivation by treatment of larvae and transport used.

Treatments	Evaluations		
	EVA1	EVA2	EVA3
Control	20.6±5.8^{a}	1.8±2.4^{ab}	27.4±7.39^{a}
Wet/humid	3.3±2.2^{b}	1.2±1.7^{b}	0.4±0.25^{b}
Submerged	13.5±10.1^{a}	4.6±2.3^{a}	1.81±0.96^{b}

Different letters in column indicate significant differences among means ($p\leq0.05$) by Kruskal-Wallis test.

no significant differences were noted between the different methods of transport (Table 2).

The average growth of scallops during the 40 days of settlement (Table 2) shows a variation between the lowest growth in length (7.5 µm day^{-1}) and height (8.1 µm day^{-1}) in the wet treatment of EVA2 and the highest growth in length (41.4 µm day^{-1}) and height (42.4 µm day^{-1}) in the submerged treatment of EVA1.

Table 2. Means (± standard deviation) of height and length of *Nodipecten nodosus* pre-seeds after 40 days of cultivation by treatment of larvae and transport used.

Treatments	Evaluations		
	EVA1	EVA2	EVA3
	Height (mm)		
Control	1.68±0.37^{b}	0.52±0.23	1.46±0.24
Wet/humid	1.65±0.47^{b}	0.50±0.15	1.48±0.43
Submerged	1.89±0.45^{a}	0.60±0.29	1.56±0.37
	Length (mm)		
Control	1.47±0.36^{b}	0.53±0.30	1.36±0.26
Wet/humid	1.53±0.39^{b}	0.49±0.90	1.56±0.37
Submerged	1.81±0.39^{a}	0.62±0.23	1.50±0.42

Different letters in column indicate significant differences among means ($p \leq 0.05$) by Tukey test.

Water quality parameters

Means (± standard deviation) of the temperature at cultivation sites ranged from 20.00 ± 1.58ºC to 24.24 ± 0.78ºC in EVA1, from 15.00 ± 1.58ºC to 20.14 ± 1.11ºC in EVA2, and from 15.00 ± 1.58ºC to 20.14 ± 1.11ºC in EVA3 (Table 3). Salinity at the cultivation sites ranged from 33.57 ± 2.51 to 35.71 ± 0.49 in EVA1, from 33.29 ± 0.49 to 35.57 ± 0.53 in EVA2, and from 32.80 ± 1.64 to 37.00 in EVA3 (Table 3).

Values of TSS found in the surface water and inside the settlement boxes in the three experiments ranged from 25.4 ± 11 mg L^{-1} in EVA2 to 47.4 ± 10.3 mg L^{-1} in EVA3. Chlorophyll-a in µg L^{-1} (mean ± standard deviation) recorded in the surface water and within the sea cultivation units ranged from 4.6 ± 3.1 in EVA1 to 7.4 ± 3.7 (collection box) in EVA2 (Table 4).

DISCUSSION

In the three experiments performed at LMM and CEMAR, the final survival rates after larval settlement varied between 0.4 and 27.4% (Table 1). The metamorphosing larvae and early settled spat are extremely fragile animals and high mortalities can occur at this stage of live (Bourne et al., 1989). The decrease in the spat average settlement was previously verified in laboratory and

Table 3. Weekly means (± standard deviation) of temperature (ºC) and salinity of water at the cultivation sites.

Week	Temperature (ºC)			Salinity		
	EVA1					
	Control (LMM)	Laboratory (CEMAR)	Sea	Control (LMM)	Laboratory (CEMAR)	Sea
1	23.83±0.68	24.24±0.78	23.86±1.07	33.57±2.51	35.00±0.82	35.14±0.69
2	23.77±0.84	23.34±0.94	25.14±1.07	35.43±0.98	35.14±0.69	34.86±0.90
3	23.09±0.52	21.91±0.25	22.91±0.11	36.14±0.69	34.71±0.49	35.43±0.79
4	21.64±0.93	22.70±0.63	22.71±0.76	35.57±0.79	34.43±0.53	34.86±0.38
5	21.97±0.58	20.00±1.58	22.21±0.86	35.71±0.49	34.86±0.38	34.57±0.53
6	22.00±0.71	22.72±0.22	22.88±0.11	35.20±0.45	34.60±0.55	35.00±0.00
	EVA2					
1	22.90±0.71	24.14±0.38	20.64±0.54	35.00±0.00	34.00±0.00	33.43±3.21
2	23.33±0.39	24.06±0.33	20.94±0.78	35.00±0.00	34.00±0.00	35.43±0.98
3	24.26±0.86	24.14±0.62	22.68±0.80	35.14±0.38	34.86±0.38	34.29±0.49
4	24.11±0.90	23.64±1.10	21.88±0.90	35.00±0.00	34.71±0.76	35.29±0.49
5	24.37±1.01	23.92±0.53	23.75±0.78	35.00±0.00	33.29±0.49	35.57±0.53
6	24.26±0.92	23.60±0.65	23.24±1.33	35.00±0.00	34.80±0.45	35.40±0.55
	EVA3					
1	18.57±1.13	19.43±0.61	19.64±1.35	35.43±0.53	35.14±0.38	37.00±0.00
2	17.61±1.20	19.00±1.71	20.07±0.93	35.00±0.00	35.43±0.53	36.71±0.49
3	17.71±1.89	19.43±1.69	20.14±1.11	35.86±0.38	35.71±0.49	36.00±0.00
4	19.33±0.75	19.07±1.54	19.43±1.59	36.14±0.38	35.29±0.49	35.43±0.53
5	17.11±3.32	20.07±1.17	19.93±1.30	37.00±0.00	34.57±0.53	34.29±0.49
6	16.86±0.77	15.00±1.58	17.20±0.91	36.20±0.45	35.00±0.00	32.80±1.64

Table 4. Means (± standard deviation) of chlorophyll-a (µg L^{-1}) and total suspended solids (TSS; mg L^{-1}) in the cultivation and at sea.

Evaluations	Local of sample	Chlorophyll-a (µg L^{-1})	TSS (mg L^{-1})
EVA1	Box	4.6±3.1	31.1±13.0
	Sea	5.5±3.6	27.4±10.6
EVA2	Box	7.4±3.7	32.2±10.9
	Box	5.1±3.7	28.8±1.5
	Box	6.4±2.7	34.3±23.7
	Sea	6.5±4.0	25.4±11.0
EVA3	Box	5.8±4.9	32.8±16.4
	Box	5.9±2.8	44.2±10.7
	Box	5.6±4.1	37.9±10.6
	Sea	5.4±3.2	47.4±10.3

in the sea study and this was attributed to a natural mortality of pectinids in this stage (Sühnel et al., 2008).

A significant part of early mortality can also be related to the aptitude of *N. nodosus* larvae in settling on collectors. Zanette et al. (2009), testing different vegetable and artificial collectors as substrates for fixing pre-seeds of *N. nodosus* larvae, reported settlement ranging from 0.04 to 64.92%. In fact, successful settlement of larvae depends on quantity and quality of food, water temperature, luminosity and shape of cultivation tanks. Therefore, Carvalho et al. (2013) analyzed factors (ambient light, cleaning of tanks and diet) affecting laboratory production and observed a variation from 2 to 88% of settled larvae for the *N. nodosus*.

An initial mortality in culture after settling is also common in other commercial species of scallops. A survival rates of post-settled spat varying from 10 to 35% for *Argopecten gibbus* Linnaeus (1758) was related on literature (Sarkis et al., 2006). The settlement of *Argopecten purpuratus* Lamarck (1819) from hatchery reared larvae, shows similar survival (6.31–24.94%) as observed in other scallops (Avendaño-Herrera et al., 2002). Though no causable effects were discussed for the related rates, for *A. purpuratus*, a positive correlation between temperature and the high concentration of larvae in water column are significant predictors for most successful survival rates for this species (Avendaño et al., 2019). The settlement rate in *Mimachlamys varia* Linnaeus (1758) ranging from 17.7 to 32.3% were described (De La Roche et al., 2005), being the best survival of spat found in larger sheltered areas at low luminosity in the inner center with spat attaching one on top of another. Juveniles and adults of *M. varia* exhibits a gregarious behavior, settling in a small area as a response to chemical attraction (Brand, 2006). In *Pecten maximus* Linnaeus (1758), metamorphosis and settlement are a slower process in which the number of spats increase after 2 to 3 weeks, with expected yields of 30-60% (Robert and Gerard, 1999).

The highest value found in the present study for the settlement rate of *N. nodosus* pre-seeds was 13.5%, as observed in EVA1 in the treatments maintained at CEMAR from larvae transported in the submerged treatment, those values being significantly higher than observed in the other treatments ($p<0.05$). Although the observed scallop pre-seed recovery results were lower than those described in the literature, our study was the first record in Brazil of the survival of pectinid larvae settled far from the breeding and larviculture sites.

In EVA2, low settlement rates were observed in the wet (1.2 ± 1.7%) and submerged (4.6 ± 2.3%) treatments, but especially in the control (1.8 ± 2.4%), which was not exposed to transport (Table 1). Magnesen et al. (2006) described that such performance of young scallop forms may be related to the low quality of gametes of the breeders used in spawning, an influence that is also verified in the post-larval cultivation of *N. nodosus* in spawning carried out in winter (Sühnel et al., 2008; Zanette et al., 2009). Pre-seed survival rates tend to improve, as shown in EVA3, in the spring-summer seasons owing to the better conditioning of *N. nodosus* breeders, which have mature gonadal stages for the release of gametes.

The ability of an organism to thrive in its environment is largely dependent on abiotic factors (Kinne, 1964). Among them, temperature and salinity affect directly the performance of young forms of pectinids (Hodgson and Burke, 1988; Chan, 1990; Rupp et al., 2005). The growth of cultivated bivalve post-larvae is positively correlated with higher temperatures (Widman and Rhodes, 1991), which can be associated with increased metabolic rate, as well as intensification of intake rates (Lu et al., 1999). In the present study, the average values of temperature and salinity recorded in laboratory cultivation (Table 3) varied from 17.9 to 23.9ºC and from 34.2 to 35.9ºC in the three experiments, similar to those found by Carvalho et al. (2013) (17 to 19ºC and 35). In addition, salinity levels close to 33 reflect lower mortality rates (Rupp and Parsons, 2004).

In studies with *P. maximus* seeds, the highest growth of cultivated individuals was reported at 20ºC and at salinities close to 30 (Laing, 2000, 2002), whereas lower mortality rates were verified at 18°C and a salinity of 30 (Christophersen and Strand, 2003). Similar results were reported for the species *Argopecten ventricosus* G.B. Sowerby II (1842), with the best performance at salinities greater than 27 (Navarro and Gonzalez, 1998).

The average duration of permanence of *N. nodosus* pre-seeds in the laboratory varied between 15 and 25 days of settlement. Better results are expected from both biological and economic points of view (Sühnel et al., 2008). However, the performance in our study was analyzed after completing only 40 days of settlement. We opted for this period based on the methodological precautions of evaluation, as the possible pre-seeds settled in the sea treatments would reach larger sizes with more likelihood of being observed in the middle of the fouling located next to the cultivation, allowing a comparison with results verified in the control (LMM) and laboratory (CEMAR) treatments. However, the total mortality of individuals of *N. nodosus* was observed in all experiments at sea.

After the settlement period in the laboratory, the pre-seeds in EVA1 showed the highest growth values. The submerged treatment was significantly higher ($p<0.05$) than other treatments, with a mean final height and length of 1.89 ± 0.45 mm and 1.81 ± 0.39 mm, respectively (Table 2). These values were higher than those observed by Velasco and Barros (2008), who reported a maximum length reached by *N. nodosus* pre-seeds of 1.14 mm after 50 days of cultivation. The total height increase verified

by Rupp et al. (2004) was similar to that reported in our study, reaching 403 ± 17 µm after 16 days of cultivation. Our results are corroborated by Zanette et al. (2009), who observed a mean final height increase of 711 ± 56.5 µm after 15 days of cultivation and by Carvalho et al. (2013), who tested density, handling and luminosity and found final growth values in height of 0.95 ± 0.23 mm, 0.90 ± 0.14 mm and 0.70 ± 0.06 mm, respectively.

The site of the present study, Armação do Itapocoroy, is an environment influenced by physical, biological, anthropic and climatic processes (Friedman et al., 1992), together with different aquaculture activities (Marenzi and Branco, 2005). This site is also influenced by the partial dilution of ocean waters and the continental contribution of the Itajaí-açu River (Resgalla Junior, 2011) with low water renewal rates, providing the maintenance of coastal primary production levels (Schettini et al., 1999).

In this context, a study by Resgalla Junior (2011) observed TSS values with temporal variations between 5.1 and 9.5 mg L^{-1}, which are lower than those observed in our study in EVA1, EVA2 and EVA3, varying from 25.4 to 47.41 mg L^{-1} (Table 4).

According to Rupp et al. (2004), chlorophyll-a values between 0.67 and 1.7 µg L^{-1} do not significantly influence pre-seed growth of *N. nodosus* scallops. Chauvaud et al. (1998) corroborate this, affirming that temperature and salinity affected the growth of *P. maximus* juveniles, but not the availability of food.

CONCLUSIONS

Based on our results, we conclude that it is possible to transport pediveliger *Nodipecten nodosus* larvae in moist and submerged treatments in seawater for approximately 6 hours and cultivate them far from their place of origin, albeit under controlled conditions. Higher rates of survival and growth of pre-seeds were verified when the larvae were transported submerged. No settlement was seen in the cultivation units directly at sea, indicating that it is not possible to settle scallops directly on these structures with the methodology herein employed.

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