

Larval settlement and spat recovery rates of the oyster *Crassostrea brasiliana* (Lamarck, 1819) using different systems to induce metamorphosis

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Abstract

This study aimed at the assessment, in the laboratory, of the larval settlement and spat recovery rates of oysters of the species *Crassostrea brasiliana* using plastic collectors, epinephrine (C₉H₁₃NO₃ C₄H₆O₆) and shell powder in settlement tanks. Polypropylene was used attached to bamboo frames. The material was chosen due to its pliability – that favours the spat detachment. Two experiments were carried out; the first between February and April 2008, and the second between November and December 2008 at the Marine Mussel Laboratory of Santa Catarina Federal University (*Laboratório de Moluscos Marinhos da Universidade Federal de Santa Catarina*). In the first experiment, the scratched plastic collectors were tested consorting them with shell powder; on the second, the plastic collectors were tested consorted with shell powder, only shell powder and epinephrine as the metamorphosis stimulator. The quantification was carried out of the larvae settled in the plastic collectors, and of the recovery and integrity of the spats after their detachment. The first experiment has shown a recovery rate of 48.83% of the spats in comparison with the *D* larvae used. From this percentage, 4.9% settled in the plastic collectors and 43.93% in shell powder. The second experiment revealed 55.78% regarding the settled spats in comparison with the total of larvae used (using epinephrine), 78.62% in the treatment with the collector plus shell powder and 58.33% in the treatment only with shell powder. Thus, the use of the collector plus shell powder resulted in a greater spat recovery when compared to the other treatments.

Keywords: settlement, larvae, *Crassostrea brasiliana*, collectors, spats.

Taxas de assentamento larval e recuperação de sementes da ostra *Crassostrea brasiliana* (Lamarck, 1819) com uso de diferentes sistemas de indução à metamorfose

Resumo

O presente estudo teve como objetivo avaliar a taxa de assentamento larval e recuperação de sementes de ostras da espécie *Crassostrea brasiliana*, em laboratório, através do uso de coletores plásticos, epinefrina (C₉H₁₃NO₃ C₄H₆O₆) e pó de concha em tanques de assentamento. Foram utilizados coletores plásticos de polipropileno, presos a armações de bambu. O material foi escolhido devido à boa maleabilidade, o que facilita o destacamento das sementes. Foram realizados dois experimentos, o primeiro entre fevereiro e abril de 2008, e o segundo entre novembro e dezembro de 2008 no Laboratório de Moluscos Marinhos da Universidade Federal de Santa Catarina. No primeiro experimento, testaram-se coletores de plástico arranhado consorciado com pó de concha em um tanque de assentamento, enquanto que no segundo foram utilizados dois tanques de assentamento, um contendo os coletores de plástico consorciado com pó de concha e apenas pó de concha, e, no outro tanque, utilizou-se epinefrina como estimulador da metamorfose. Foi realizada a quantificação das larvas assentadas nos coletores plásticos e a taxa de recuperação e integridade das sementes após o destacamento. No primeiro experimento, recuperaram-se 48,83% de sementes em relação às larvas *D* utilizadas. Deste percentual, 4,9% assentaram em coletores plásticos e 43,93% em pó de concha. No segundo experimento, a porcentagem de sementes assentadas em relação ao total de larvas utilizadas foi de 55,78% com o uso de epinefrina, 78,62% no tratamento com coletor mais pó de concha e de 58,33% no tratamento só com pó de concha. Assim, verifica-se que o uso de coletor mais pó de concha resulta em maior recuperação de sementes se comparado com os demais tratamentos.

Palavras-chave: assentamento, larva, *Crassostrea brasiliana*, coletores, sementes.

1. Introduction

The use of clay tiles, shell powder or whole oyster shells, several plastic materials (bottles of poly ethyl terephthalate (PET), poly vinyl chloride plates (PVC), collectors made of bamboo, coconut shell, asbestos, and tyres may be used as substrate for bivalve mollusc larval settlement (Devakie, 1993; Helm et al., 2006). In some studies, such materials, before their use, endured different types of treatment to attract the larvae for fixation and posteriorly, promote their easy detachment from the collectors.

Devakie and Ali (2002) tested the use of plastic plates of different textures in the presence and absence of biofilm as substrate for the oyster larval settlement (*Crassostrea iredalei*). The best settlement larval rate was observed in rough plastic without the biofilm (success of $37.6 \pm 2.2\%$), followed by the use of rough plastic with biofilm ($27.1 \pm 1.8\%$) and by the use of smooth plastic with biofilm ($22.0 \pm 2.1\%$). The worst rate was presented by the smooth plastic without the biofilm. The plastic substrate with an extract of tissue of several bivalve species was also tested by Devakie and Ali (2002) for larval settlement. Plastic slides with extract of tissue of *C. iredalei* and *Crassostrea belcheri* have promoted superior settlement rates ($28.3 \pm 3.9\%$ and $26.1 \pm 5.5\%$, respectively) when comparing those with the extract of the tissue of the mussel *Perna viridis* ($15.7 \pm 1.4\%$) and the oyster *Saccostrea cucullata* ($12.9 \pm 3.6\%$).

In another study, Holliday (1996), using PVC plates in several positions with or without coating of cement paste upon the settlement of the larvae *Saccostrea commercialis*, showed that the settlement rate in such plates horizontally positioned was 76.6% (23.8 larvae/cm^2). However, in plates positioned vertically, the settlement was only 0.71% (0.2 larvae/cm^2). The larval settlement in plates horizontally positioned covered with cement past was 60.2% (18.7 larvae/cm^2).

Collet et al. (1999) tested PVC plates covered with wax and a second layer of polyester resin to assess the time length taken by the larvae of *Crassostrea gigas* in the fixation on the collectors. The results obtained showed that the larvae with ages of 17, 20, 23, and 26 days take 1, 4, 2, and 4 days, respectively, for settlement on the collectors.

In a study to test different plastic collectors, it was concluded that polypropylene rope is superior to the PVC plates for settlement (Taylor et al., 1998). However, the authors reinforced that the PVC collectors offer advantages regarding the spat growth due to a greater area. It was also observed that the horizontal position favours the larval settlement.

The use of PET bottles as collectors in the natural environment is widely spread due to its flexibility and low cost. In this sense, a study carried out with *Crassostrea rhizophorae* in Venezuela with 20 plastic bottles made of such material, longitudinally cut forming a superficial area of 1.8 m², obtained 326 spats settled per m² in a period of one month in the natural environment (Buitrago and Alvarado, 2005).

Despite the use of substrates to promote the oyster larval settlement, neurotransmitters such L-DOPA (L-3,4-dihydroxi-phenylalanine), epinephrine, and ammonia are used. Among them, epinephrine is the most used.

Beiras and Widdows (1995) using the epinephrine as the chemical component for the metamorphosis of the larvae of *Crassostrea gigas*, concluded that the exposition to epinephrine under a concentration of 10^{-4} M for 15 minutes was sufficient to promote more than 80% of larval settlement. In another study (Garcia-Lavandeira et al., 2005), epinephrine dissolved in 0.005 of NHCl and diluted (1:9) in sterile water was used for inducing the metamorphosis of *Ostrea edulis*. The authors obtained a settlement rate of 55.86% exposing the larvae to 10^{-5} M of epinephrine for 48 hours. On the other hand, Doroudi and Southgate (2002) tested the epinephrine at concentrations of 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} M for 24 hours upon the effect in the larval settlement rates of *Pinctada margaritifera* and has not presented any satisfactory results.

However, for an efficient action of epinephrine, more than one application is needed on the larvae able to settle and this process causes strong stress on the larvae contributing, therefore, to an increase of mortality that may cause contamination in the metamorphosis tanks (Silva, 2007). The use of shell powder as a substrate for settlement also put the settlement tanks under risk of contamination. The shell powder hinders the cleaning of the debris in the water change and may favour the development of a noxious environment for the larvae and pre-spats. Thus, technical alternatives and settlement material of low density are searched to reach the maximum recovery rate of the larvae from small hatchery. In this context, an alternative is the use of artificial collectors in the settlement tanks.

The use of plastic collectors in the larval settlement is an interesting option since they can be arranged in the water column, without contact with the bottom, offering a huge superficial settlement area, and due to its great pliability, they allow the easy detachment of the spats, avoiding losses due to competition or contact during growth. Another fundamental characteristic of such collectors is that they allow the settlement of larvae at different ages, letting them free, not causing any stress and contamination – observed when using shell powder and epinephrine.

Thus, the objective of this study was to test the use of polypropylene collectors, shell powder and epinephrine upon the native oyster larval settlement rate in the laboratory.

2. Material and Methods

2.1. Spawning

In the spawning, animals of the species *Crassostrea brasiliiana* (Lamarck, 1819) from the Laboratory of Marine Molluscs (LMM) from the Santa Catarina Federal University (Universidade Federal de Santa Catarina – UFSC), Florianópolis, Brazil ($27^{\circ} 35' \text{ S}$ and $48^{\circ} 32' \text{ W}$) were used.

Four hundred and 310 animals were used in the first and second experiment, respectively. Firstly, they were scraped

and brushed. Afterwards, they were kept for 1 hour in fresh water with 25‰ sodium hypochlorite in a 500 L tank for sanitation. Later, they were rinsed with fresh water and treated brine (filtered at 1 µm and sterilised with ultraviolet radiation – UV) and kept in the tank for another 40 minutes under a minimal output of 10 L/minute of seawater for removing the impurity and residues (faeces and pseudo-faeces). At this moment, 10 animals were separated from the group, have their shell manually removed using a knife and their sex determined using a microscope to assess the maturation condition of the lot. With respect to the sexing, a sample of little portions of gonadal material was sampled using the blade of a scalpel and place on a microscope slide for observations using a microscope. After sexing the animals, the females and males were placed in different buckets and scraped using a scalpel to extract the gamete. Afterwards, the solutions obtained were filtered to separate the gametes from the somatic tissues using a 60 µm sieve. The rest of the animals were submitted to spawning at four intervals – from 3 to 5 minutes – to exposition to the air intercalated with 20 minutes in flowing filtered seawater (minimum of 10 L/minute). The temperature was raised by 2 °C at every immersion in brine until reaching 28 °C, for then transferring the oysters to the 6,000 L tank filled with treated seawater at 26 °C. In the tank, four doses of approximately 1.5 L of concentrated gamete were placed upon the reproducer (extracted from the ten sexed animals previously) every 20 minutes, finishing the stimuli for the spawning.

2.2. Spawning assessment

Twenty-four hours after induction, the 6,000 L tanks were emptied and the solid content was gathered in sieves with mesh of 35 µm. This content was selected using a set of sieves of mesh of 120, 55, and 35 µm. From this screening, only the larvae held on screens with mesh of 55 µm were kept, and then collected in a bucket filled with 12 L of treated seawater. Regarding the quantification of the *D* larvae, three aliquots of 0.5 mL were collected using a pipette. Following that, each aliquot was diluted in a beaker containing 5 mL of seawater. After homogenisation, three new samplings of 0.5 mL from the solution were carried and placed on Sedwick-Rafter slides, where the quantification was performed. The number of *D* larvae was calculated by the mean of the three countings.

2.3. Larviculture

The larviculture were carried in 6,000 L tanks using a static system of larviculture (batch). The seawater was filtered at 1 µm and radiated with ultraviolet (UV). The water exchange and tank cleaning occurred every 48 hours. Concerning the cleaning, the juice of two Tahiti lemons (*Citrus aurantifolia*) were crushed in a blender and diluted in 3 L of freshwater. For their nourishment, different types of unicellular algae were supplied. In the first experiment, until the third day of life, the larvae were fed on *Isochrysis* sp. TISO (CCMP 1324) at a density of 3×10^4 cells/mL. Between the third and the fifth day of larviculture they

were supplied with a mixture of *Isochrysis* sp. TISO (CCMP1324) and *Pavlova* sp. (CCMP 459) at a density of 2×10^4 cells/mL. From the sixth day on, they were supplied with a mixture of *Isochrysis* sp. TISO (CCMP 1324), *Skeletonema* sp. (CCMP 795), *Pavlova* sp. (CCMP 459), and *Chaetoceros calcitrans* (CCMP 1315). In this period, the density supplied was increased gradually from 4.0×10^4 until 10×10^4 cells/mL. In the second experiment, three species of microalgae were used (excepting the *Isochrysis* sp. TISO) (CCMP 1324). On the first day, the larvae were fed on a mixture of *Chaetoceros calcitrans* (CCMP 1315), *Pavlova* sp. (CCMP 459), and *Chaetoceros muelleri* at a concentration of 2.0×10^4 cells/mL. Between the second and the last day of larviculture, the larvae were supplied with a mixture of *Pavlova* sp. (CCMP 459) and *Chaetoceros muelleri*. In this period, the density offered was increased gradually from 2×10^4 until 9×10^4 cells/mL.

The selections of larvae able to settle (pedivéliger) was carried out using sieves with meshes of 145, 210, 230, and 390 µm. The number of larvae able to settle, selected in sieves with meshes of 210 and 230 µm, was estimated through the weight (where 1 g corresponded to approximately 57,000 larvae). The larvae were gathered in pieces of nylon screen, had the excess water removed using a towel and then were weighed, not taking into account the weight of the fabric to identify the amount of larvae.

After the quantification, the larvae gathered in the nylon screen were placed in beakers with seawater and kept in a refrigerator under a temperature ranging from 4 to 11 °C for 72 hours in the first experiment and 48 hours in the second.

The larvae were kept in the refrigerator (at low temperature) to decrease their metabolism, causing stress and accelerating their settlement. After this period, they were taken out from the refrigerator and approximately 2,244,000 and 630,000 pedivéliger larvae were used in the settlement of the first and second experiment, respectively.

2.4. Settlement

After being removed from the fridge, the larvae were transferred and distributed equally into 20 L settlement buckets, whose bottom was replaced by nylon mesh of 170 µm. For the first experiment, 12 buckets with approximately 187,000 larvae per bucket were used. For the second, 21 buckets with nearly 30,000 larvae per bucket were used. The buckets were placed into 1,300 L tanks with *Air Lift* system – a method consisting of the elevation of water in the capitation through the injection of compressed air through tubes of reduced diameter. The compressed air injected creates an emulsion with water, making the water circulate within the tank.

The tank water changes and the cleaning of the bucket screens are performed every 24 hours. The appropriate salinity for the species was guaranteed through filtration of the seawater through screen of 1 µm, irradiation with ultraviolet (UV) during the settlement and through daily measurement with further corrections to approximately 27‰ adding freshwater. The rapid chloride removal in

the freshwater was guaranteed through the use of nearly 1 g of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) in each settlement tank ($\text{Na}_2\text{S}_2\text{O}_3$).

Plastic collectors made of polypropylene comprised of 8×8 cm squares were used for the larval settlement. Each collector was comprised of thirty plastic plates with 0.5 cm spacing in between them, joined by a bamboo stick passed through the centre of the plates. The 20 L buckets were added to the collectors. Besides the collectors, there was added a thin layer of shell powder (approximately 15 g) upon the screens of the buckets. The shell powder was comprised of ground oyster shells, which were sifted; only the powder with particulate size between 230 and 390 μm was used; it was chlorinated and matured with treated seawater to form the biofilm.

For the first experiment, 48 plastic collectors were used among shell powder in twelve 20 L buckets. In this experiment, the collectors received three treatments: 1) Collectors were bathed in chalk solution (proportion of 1:2 of chalk and water, respectively), without maturation (CSM); 2) Collectors bathed in chalk solution with maturation (CCM); 3) Collectors not bathed in chalk solution and without maturation (C). The collectors bathed in chalk solution were previously prepared and were kept drying for approximately 30 hours before being used. The maturation of the collectors to be used in the biofilm creation was carried out for four days in a tank filled with treated seawater, food and aeration. The shell powder remained 72 hours under maturation. With regard to the feeding during the settlement stage, two types of microalgae – *Isochrysis* sp. TISO (CCMP1324) and *Chaetoceros muelleri* – were offered in the proportion 3:7 at a final concentration of 6×10^4 cells/mL.

In the second experiment, two 1,300 L tanks were used. In one, there were tested the collectors of the treatments CSM, CCM, and control (from the first experiment) put into 15 buckets, totalising three collectors of each treatment per bucket. In the other three buckets, only shell powder was used upon the mesh (without collectors). In this experiment, the maturation of the collectors for the biofilm creation was performed in the same way as the first, however during five days. The shell powder was chlorinated for 96 hours and matured in treated seawater for approximately 72 hours. Three buckets were put into the other tank, where the settlement was carried out using epinephrine ($\text{C}_9\text{H}_{13}\text{NO}_3$, $\text{C}_4\text{H}_6\text{O}_6$) as metamorphosis stimulator. The epinephrine was dissolved in distilled water in the proportion of 0.33 g of epinephrine for one litre of distilled water and diluted (1:9) in treated seawater. Three epinephrine baths of three hours (homogenised every hour) were performed every 48 hours.

Regarding the feeding, during the settlement stage, two types of microalgae were offered – *Pavlova* sp. (CCMP 459) and *Chaetoceros muelleri* – in the proportion of 3:7 at the final concentration of 6×10^4 cells/mL. On the last settlement day (18th day in the first experiment and 17th day in the second), all the settled spats were detached manually and placed in flasks containing absolute alcohol.

Posteriorly, using an optical microscope, they were quantified and assessed regarding their integrity (broken shell). The settled spats in shell powder were also placed in flasks containing absolute alcohol for further assessment per sampling. Ten samples of each bucket were used to obtain the mean number of settled spats on the shell powder per bucket.

2.5. Statistical analyses

The experiments were carried out on an entirely randomised delineation. The statistical analyses were made using the minimum square method and the computational package SAS (2003). The comparison of the means was carried out using the *t*-test through permutation at 5% of probability.

3. Results

There were obtained approximately 23,000,000 and 74,600,000 *D* larvae 24 hours after fecundation in the first and second experiment, respectively. At the end of the larvicultures, 7,847,960 and 10,102,680 larvae able for settlement were obtained, presenting an efficiency of 34.1% and 13.5% in each experiment, respectively. Among such larvae, 2,244,000 and 630,000 were used in experiments 1 and 2, respectively.

The larvicultures of the first and second experiment took, respectively, 18 and 24 days from the fecundation of the oocytes until the larvae reached the pediveliger stage.

The mean temperature of the water tanks in the larviculture was $25.3 \text{ }^\circ\text{C} \pm 0.80$ in the first experiment and $23.1 \text{ }^\circ\text{C} \pm 1.3$ in the second; the salinity of both larvicultures was approximately 27‰. The larvae remained in the settlement tank for 18 and 17 days during the first and second experiment, respectively.

The mean temperature of the water in the settlement tank in the first experiment was $26.1 \text{ }^\circ\text{C} \pm 1.19$ and the salinity was approximately 27‰. In the second experiment, the mean temperature and salinity of the settlement tank with the collectors were $24 \text{ }^\circ\text{C} \pm 0.93$ and $27.4\text{‰} \pm 1.79$, respectively. In the tank with epinephrine, the mean temperature and the salinity of the water tank were $24 \text{ }^\circ\text{C} \pm 0.91$ and $26.9\text{‰} \pm 1.89$, respectively.

No significant difference ($p > 0.05$) was presented by the first experiment between the mean ($3,043 \pm 1,125$) detached spats of the control treatment (collectors not bathed with chalk solution and without maturation) and the mean ($2,357 \pm 989$) detached spats from the CSM treatment (collectors bathed in chalk solution with maturation). However, a significant difference ($p < 0.05$) was found between the mean ($2,357 \pm 989$) detached spats of the CSM treatment and the mean ($3,737 \pm 989$) detached spats of the CCM treatment. The collectors bathed in chalk and without maturation have presented an inferior number of settled spats in comparison to the others. The best larval settlement rate was found for the treatment CCM. The non-damaged spat percentage in the collectors in relation to the total of larvae used was $1.3\% \pm 0.53$ in the CSM treatment, $2.0\% \pm 0.70$ in the CCM treatment and $1.6\% \pm 0.60$ in the control. A mean settlement rate

of $43.93\% \pm 12.72$ to the total was observed in the shell powder (added to the bottom of the bucket). Thus, a total settlement rate of 48.83% was noticed. No significant difference was observed in the comparison of the mean of damaged shells and the used collectors.

In the second experiment, no significant difference ($p > 0.05$) was found between the mean ($17,500 \pm 9,758$) of spats detached from the control (only shell powder) and the mean ($16,733 \pm 5,777$) of spats detached from the treatment with epinephrine. However, a significant difference ($p < 0.05$) was found between the mean ($23,586 \pm 3,607$) of the spats from the treatment with collector and shell powder and the means ($17,500 \pm 9,758$) from the control treatment and from the treatment with epinephrine ($16,733 \pm 5,777$). The percentage of spats (undamaged) settled in the shell powder to the total was $55.78\% \pm 3.33$ in the treatment with epinephrine, $78.62\% \pm 12.02$ in the treatment with collector and shell powder, and $58.33\% \pm 32.53$ in the control (only with shell powder). In the comparison of the means of the damaged shells among the treatments, a significant difference ($p < 0.05$) was found between the treatment with epinephrine and the others. The treatment with epinephrine presented $10.00\% \pm 3.33$ of damaged spats; in the other treatments, this percentage was lower – $5.90\% \pm 2.48$ in the treatment with collector and shell powder and $3.67\% \pm 1.41$ in the control.

Higher homogeneity was found in the treatments without the use of epinephrine ($C_9H_{13}NO_3$, $C_4H_6O_6$) with regard to the size of the detached spats.

4. Discussion

The settlement rates for the collectors with chalk and without maturation ($1.3 \pm 0.53\%$), for those with chalk with maturation ($2.0 \pm 0.70\%$), and for the collectors without chalk and without maturation ($1.6 \pm 0.60\%$) obtained using plastic collectors, on the first experiment, were inferior to those obtained by Devakie and Ali (2002). These authors obtained, in their study with the oyster *Crassostrea iredalei*, larval settlement rates of $37.6 \pm 2.2\%$ using rough plastic without biofilm, $27.1 \pm 1.8\%$ using rough plastic and biofilm, $22.0 \pm 2.1\%$ using smooth plastic with biofilm, and the worst rate of $11.0 \pm 1.2\%$ when using smooth plastic without biofilm. However, considering the percentage of settled spats in shell powder to the total of larvae used in the experiment, we found a settlement rate of $43.93 \pm 12.72\%$ for the first experiment, $78.62 \pm 12.02\%$ for the treatment with collector and shell powder, and $58.33 \pm 32.53\%$ for only shell powder in the second experiment. Results like these, superior to those reported by Baker and Mann (1998) when using ground adult shells ($3 \times 3 \times 0.2$ cm) and matured as substrate for the larval settlement of *Crassostrea virginica*, presented approximately 11% of settlement. Su et al. (2007) have shown superior results using rough plastic with biofilm, followed by smooth plastic with biofilm, rough plastic without biofilm, rough plastic without biofilm, and smooth plastic without biofilm when assessing the settlement rate of larvae of *Pinctada martensii*.

The preferential settlement rate of the larvae on the shell powder to the other artificial collectors made of polypropylene can be due to the habit of the species *Crassostrea brasiliiana* – fixing on the bottom – since it is generally found on the infralittoral areas. Contextually, it was noticed in the first experiment that the plastic plates located in the bottom showed a higher rate regarding the larval settlement.

Epinephrine presented an efficient settlement rate in larvae settlement – $55.78 \pm 3.33\%$. However, when the mean damaged shells among the treatments was compared, a significant difference ($p < 0.05$) was found among the collectors for the treatment with epinephrine.

A traditional method to stimulate and increase oyster larval metamorphosis success is the employment of a biofilm on the surface or the use of chemical products. The use of biofilm on the surface of oyster shell (Tamburri et al., 1992; Satuito et al., 1995) and the use of neurotransmitters such L-DOPA (L-3,4-dihydroxy-phenylalanine), epinephrine, and ammonia (NH_3) has shown efficiency in increasing the settlement of larvae of the oyster from the genus *Crassostrea* (Coon et al., 1985, 1990a, b; Fitt and Coon, 1992). Contextually, Beiras & Widdows (1995), when testing chemical compounds for the metamorphosis of the larvae of *Crassostrea gigas*, concluded that the exposition to epinephrine at a concentration of 10^{-4} M for 15 minutes was sufficient for promoting more than 80% of larval settlement. On the other hand, Kingzett et al. (1990), when testing the effect of different concentrations of epinephrine – 10^{-4} , 10^{-4} , and 10^{-4} M – upon the metamorphosis of the larvae of *Patinopecten yessoensis*, presented settlement rates between 10 and 16%. In this context, Doroudi and Southgate (2002), studying the effect of epinephrine at concentrations of 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-2} M for 24 hours upon the larval settlement of *Pinctada margaritifera*, have not shown any satisfactory result.

Gamma-aminobutyric acid (GABA), dissolved in sterile water (10^{-3} M), and epinephrine dissolved in 0.005 NHCl and diluted (1:9) in sterile water (10^{-3} M) were used to stimulate the metamorphosis of *Ostrea edulis* (García-Lavandeira et al., 2005). The authors presented 59% of metamorphosis using GABA at a concentration of 10^{-4} M and 55.86% using epinephrine at a concentration of 10^{-5} M.

In this study, 18-day old larvae took two days to start settlement. Collet et al. (1999), in their study with 17, 20, 23, and 26-day old larvae, observed that they took 1, 4, 2, and 4 days, respectively, to be settled on the collectors.

Holliday (1996), studying the position of the collectors with or without chalk for the settlement of the larvae of *Saccostrea commercialis*, has shown that the PVC plates, positioned horizontally, presented very high rates (60.2 and 76.6%), when compared with the 0.71% from those positioned vertically. In another study, Taylor et al. (1998) observed that the PVC collectors offer advantages regarding the growth of the spats of *Pinctada maxima* due to a higher surface and the ease to settle horizontally. In our studies, the collector plates were all placed horizontally, which could explain, in part, the settlement success.

Holliday et al. (1991) reported settlement rates varying from 77 to 85% for *Saccostrea commercialis* kept refrigerated

at 11 °C for 98 hours and 68% for *Crassostrea gigas* kept at 6 °C for 98 hours. Comparing these values, in the first experiment (with 72 hours under refrigeration), an inferior value to the other two species was presented. In the second, which kept the spats under refrigeration for 48 hours, the *C. brasiliensis* present settlement rates as high as those detected by Holliday et al. (1991).

The possible contamination problems in the settlements with epinephrine and shell powder were not observed in this experiment. The high percentages of spats obtained with these two kinds of material prove it.

5. Conclusions

The results obtained show that the use of artificial collectors is not needed, since most of the larvae settled on shell powder. The use of epinephrine was efficient on larval settlement; however, higher heterogeneity was found for spats settled using this product in relation to the other obtained for the other treatments.

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