INFLUENCE OF THE COLLECTOR TYPE AND AT-SEA CULTIVATION PERIOD ON SEEDS RECOVERY RATE AND GROWTH OUT OF *Pteria hirundo* IN SOUTHERN BRAZIL*

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ABSTRACT

The recovery rate of seeds and the growth of *Pteria hirundo* were evaluated in at-sea cultivation. It was evaluated a netlon collector and a red plastic netting collector, over cultivation periods of 60 and 90 days. Seeds were classified by size and were cultivated with occupation rates of 100% and 50% on the lantern plate. The recovery rates for netlon were 49.68% and 27.36% for 60 and 90 days, respectively. For the red collector, the recovery rates were 35.8% and 28.39% for the 60 and 90 day, respectively. No statistical differences were found between collectors for either the recovery rate or the growth rate during either experimental period. The recovery rate was statistically higher in the 60 days than for 90 days. After the final growth stage, the seeds removed from the sea 165 days after fecundation presented a predominate size of 30 to 40mm with a 90% survival rate and those removed after 195 days after fecundation were greater than 40mm. The time at-sea had a greater influence on the than the collector type. The netlon collector should be used with a 60 day period at-sea and a 100% occupation rate of the lantern plate surface.

Key words: Pteridae; culture system; spats; settlemen

INFLUÊNCIA DO TIPO DE COLETOR E DO TEMPO DE PERMANÊNCIA NO MAR EM CULTIVO NA TAXA DE RECUPERAÇÃO E NO CRESCIMENTO DE SEMENTES DE *Pteria hirundu* NO SUL DO BRASIL

RESUMO

Avaliou-se, neste trabalho, a taxa de recuperação e o crescimento de sementes de *Pteria hirundu* em sistema de cultivo no mar. Foram avaliados coletores de netlon e coletores de rede plástica vermelha após períodos de cultivo de 60 e 90 dias. As sementes foram classificadas e cultivadas com taxas de ocupação de 100% e 50% do prato da lanterna de cultivo. As taxas de recuperação nos coletores de netlon foram de 49,68% e 27,36% para 60 e 90 dias, respectivamente. Para os coletores vermelhos, as taxas foram de 35,8% e 28,39% para 60 e 90 dias, respectivamente. Não foi possível detectar diferenças estatísticas na comparação entre os coletores quanto ao crescimento e a taxa de recuperação. As taxas de recuperação foram estatisticamente maiores para 60 dias do que para 90 dias. Após o estágio final de crescimento avaliado, os animais retirados do mar depois de 165 dias após a fecundação apresentaram tamanho predominante de 30 a 40 mm, com 90% de taxa de sobrevivência, e as removidas após 195 dias após a fecundação, foram maiores que 40 mm. O tempo de permanência apresentou maior influência do que o tipo de coletor. Recomenda-se o uso do coletor de netlon com 60 dias de permanência no mar e taxa de ocupação de 100% do prato da lanterna de cultivo.

Palavras chave: Pteridae; sistema de cultivo; juvenis; assentamento

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INTRODUÇÃO

Aquatic organisms were first cultivated in order to contribute to local economic development, to redistribute wealth and to maintain traditional communities along the coastline. Cultivation is important in maintaining communities in their respective areas of origin, providing an increase in income and a significant increase in the quality of life of traditional and professional fishermen (VINATEA, 1999).

With the observed decline and the predicted collapse of world fisheries in the coming decades, the development of mariculture has been increased in all social and economic regions to meet human protein demands (FAO, 2006). In addition to providing food, the cultivation of mollusks is important to the production of jewels and was initiated with the cultivation of pearls in pearl oysters, principally in Asia (FASSLER, 1995).

The cultivation of bivalve mollusks is an established malacoculture in Brazil (SUPLICY, 2005). Despite the ample diversity of mollusks, commercial cultivation is limited to the oyster *Crassostrea gigas* (Thunberg, 1793) and the mussel *Perna perna* (Linnaeus, 1758), which are primarily produced in the Brazilian state of Santa Catarina (OSTRENSKY *et al*., 2008).

The total Brazilian aquaculture production was approximately 270 thousand tons in 2004. The state of Ceará had the largest national production with 37.6 thousand tons, followed by the states of Santa Catarina with 35.4 thousand tons; Rio Grande do Norte with 30.9 thousand tons; Rio Grande do Sul with 25.9 thousand tons; São Paulo with 21 thousand tons; Bahia with 18.3 thousand tons; Paraná with 17 thousand tons; and Mato Grosso with 16.6 thousand tons. Mollusks are responsible for 4.8% of the Brazilian aquaculture production, of which mussels (*P. perna*) account for 79.5%, followed by oysters, which account for 20.5% of production (OSTRENSKY *et al*., 2008).

Pearl oysters have not yet been commercially cultivated in Brazil. In recent years the Laboratory of Marine Mussels of the Universidade Federal de Santa Catarina (LMM – UFSC) has been studying the pearl oyster *Pteria hirundo* (Linnaeus 1758), a species that is native to the Brazilian coast. This oyster is also found in the United States from North Carolina to Florida and Texas, as well as in the Bermudas, Western India, Venezuela and along the coast of Brazil (RIOS, 2009).

Of the species belonging to the Pteriidae family that are found in the world's oceans, the genera *Pinctada* and *Pteria* are the species utilized in the commercial production of pearls. *Pinctada fucata* (Lamarck 1819) is cultivated in Iran, Sri Lanka, India, Thailand, China, Korea, Japan and Mexico; *Pinctada margaritifera* (Linnaeus 1758) in the Sudan, Australia, French Polynesia, Cook Islands, Philippines, China, Korea, Japan and Mexico; *Pinctada maxima* (Jameson 1901) in Australia, Burma, Malaysia, Indonesia and the Philippines; *Pteria penguin* (Röding 1798) in Japan and Thailand and the *Pteria sterna* (Gould 1851) in Mexico (TANAKA, 1990; FASSLER, 1994; 1995; CARINO and MONTEFORTE, 1995).

The greatest limitations to the development of the pearl cultivation industry in many parts of the world are the large variations in harvest of seeds and scarcity of natural stocks. This has promoted studies to obtain seeds in laboratories (GERVIS and SIMS, 1992; MARTÍNEZ-FERNÁNDEZ, *et al*., 2004).

Cultivation of pearl oysters and production of pearls represent the most economically favorable activity in the entire aquaculture industry (FASSLER, 1995). The production of pearl oyster seeds in the laboratory is common and practices originated in India and Japan (CHOI and CHANG, 1999). This technology helps to overcome the problem of the lack of suitable oysters for pearl production. Larviculture methods are economically viable and are easily implemented. In order to provide sustainability to the pearl industry, seeds should be produced in the laboratory (MARTÍNEZ – FERNANDEZ *et al*., 2004).

According to RIOS (2009), the pearl oyster *P. hirundo* is synonymous with the winged oyster or black oyster *Pteria colymbus* (Roding 1798). *Pteria colymbus* is a species with a high availability in the natural environment of Venezuela (LODEIROS, *et al*., 1997) and has a rapid growth with very low mortality under cultivation conditions. One disadvantage of this species is that it is unknown on the market (LODEIROS *et al*., 1999).

In addition to their capacity to develop pearls and semi-pearls, these mollusks provide nourishing foods for human consumption and the size of its muscle is equivalent to that of the pectinidae (LODEIROS *et al.*, 1997; 1999).

In addition to the experiments and the viability of induced spawning and larviculture performed at the LMM-UFSC, little information exists on the settlement, the viability of sea maintenance systems, the survival and growth rates of the pearl oyster. Given the scarce availability of seeds in the natural environment, increasing our knowledge of these factors is necessary to the development of a commercial system; those interested in production depend on larvae and seeds from the laboratory. Therefore, the present study aims to improve the current understanding of the settlement rate, at-sea cultivation period, detachment time, recovery rate, survival rate and the growth rate of *P. hirundo* in cultivated systems.

MATERIALS AND METHODS

The experiments in this study were performed from October 2007 to June 2008 at the base and experimental cultivation area of the Laboratory of Marine Mollusks (LMM) of the Universidade Federal de Santa Catarina (UFSC), located on Sambaqui Beach (North Bay 27º29'18.8'' S, 48º32'12.9'' W, of the Santa Catarina Island, Florianopolis, Southern Brazil) The *P. hirundo* species (Figure 1), a bivalve mollusk native to Brazil and known to be a pearl oyster species, was evaluated in the analyses.

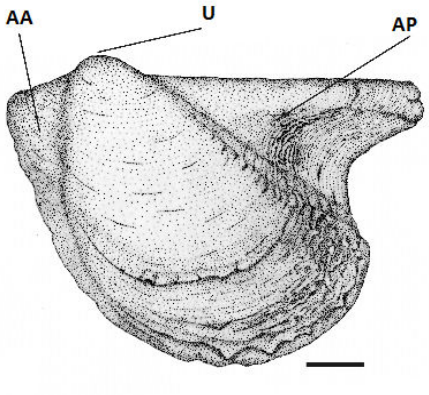

Figure 1. External view of *Pteria hirundo* shell. The bar represents 1 cm. The letters AA, the anterior auricule; U, the umbo; AP, the posterior auricule

The first step (larviculture and settlement) was carried out at a hatchery structure while in the second and third steps (cultivation), experimental cultivation was performed at Sambaqui Beach, situated in the North Bay of Santa Catarina Island.

Reproducers and Spawning

The broodstock used to produce seeds in this experiment were obtained from the Laboratory of Marine Mollusks-UFSC hatchery in 2006 and maintained in the experimental cultivation area of the LMM-UFSC. The reared larvae were obtained after induced spawning, by increased seawater temperature choke. The larviculture was conducted for 30 days in accordance with the methodology reported by GOMES *et al.* (2006). Settling was performed in the laboratory.

STAGE 1 - Settling: comparison between the two collectors types

The process of settling and larvae metamorphosis was performed using two collector types: a blue collector, a commercial netlon collector (used commercially with settling pectinidae); and a red collector, a plastic mesh (polyethylene) collector utilized nationally to package fruits.

The two collector types had the same dimensions, 140 cm in length and 68 cm in width, and were molded in the form of a sphere with 5 g of *Pinus sp*. leafs inside, according to the methodology described by ZANETTE *et al.* (2009).

In this stage of the experiment, 90 collectors of each type were randomly placed in the same 2,500 L fiber tank for a 15 day period for maturation (biofilm development) after which 900,000 reared larvae were added to the tank. The water, filtered, in the tank was drained, cleaned and renovated every 48 hours and food, consisting of a mixture of $3x10^4$ microalgae *Isochrysis galbana* (Parke, 1949) and *Chaetoceros muelleri* (Lemmermann, 1898) (1:1), was provided every 24 hours.

STAGE 2 – Transfer to the sea and seed recovery rate

After 15 days in the settling tanks, the 180 collectors were gathered in groups of three separated by collector type, within a 1mm nylon

mesh bag (used commercially for the cultivation of pectinidae), for a total of 60 bags and 30 of each collector type. On the same day, the collectors were transferred to the LMM-UFSC experimental cultivation site on Sambaqui Beach and maintained in a surface long line type cultivation system. The bags were tied to 1 m polyethylene cables (3 bags per cable) with 1 weight at the extremity, and these were tied to the long line for a total of 10 cables for each collector type. Bags were cleaned by brushing at the cultivation site every 15 days.

After 30 days in the sea, 6 collectors were removed for an initial evaluation of the separation process and to determine if any losses occurred at sea. This left 150 collectors for the analysis. The seeds in collectors were split into two periods; 60 and 90 days of at-sea cultivation. After 60 days, 28 bags containing 84 collectors were separated, of which 14 contained blue collectors and 14 contained red collectors. At 90 days, 22 bags containing 66 collectors were separated, in which 10 bags contained blue collectors and 12 contained red collectors.

Detachment was performed manually within basins. The seeds were sieved and separated by size (S, M, L, XL) in accordance with the following classification: S = 5 mm; M = 10 mm; L = 20 mm; XL = 30 mm. Seeds were quantified by volume. Previously counted samples in reduced volumes were used in order to extrapolate to greater volumes, as shown in Tables 1 and 2.

Table 1. Volume used in the samples and the mean quantity of seeds mL^{-1} after 60 days of cultivation at sea

	SAMPLE VOLUME (mL)	MEAN (mL^{-1})	DEVIATION	N
S	15	7.87	1.288	33
M	30	3.33	1.101	33
L	60	1.20	0.314	33

Table 2. Volume used in the samples and mean quantity of seeds mL^{-1} after 90 days of cultivation at sea

	SAMPLE VOLUME (mL)	MEAN(mL^{-1})	DEVIATION	N
S	15	7.87	1.288	33
M	50	2.56	0.267	24
L	100	0.85	0.087	24
XL	300	0.34	0.035	24

STAGE 3 – Evaluation of the effect of detachment separation period on survival and growth of individuals up to the juvenile phase

Seeds from each length of time at sea (60 and 90 days) were divided into three sizes and cultivated in two densities in lantern nurseries. For the first period of 60 days, seeds were categorized into sizes of S, M and L while seeds for the period of 90 days were categorized as M, L and XL. The experiment used 100% and 50% occupation of the total surface area of the cultivation structure units (a lantern plate with 35 cm in diameter), using 500 mL and 250 mL animals, respectively. Two nurseries with four trays (with seeds in three of the trays) were used for each size x density, with a total of 2 repetitions of 3 samples each. At the time of evaluation, an extra class was added due to growth, denominated as XXL (extra–extra-large), which included individuals greater than 40 mm.

After two months of cultivation, the seeds were sieved, again classified by size and later quantified manually.

Statistical Analysis

Comparisons were made between the recovery rates after 60 and 90 days of cultivation at sea and between rates observed for the blue and red collectors. The two detachment periods and the quantity of different seed sizes (S, M, L, XL) were compared to analyze growth. The means and deviations were analyzed for all comparisons. The significance level was fixed at

5%. Before statistical tests were conducted for each situation, normality of the data and homogeneity of the variances (Bartlett's test) were evaluated (VIEIRA and HOFFMANN, 1989). In all cases, despite the high variances, homogeneity allowed the means to be tested using a Student's t-test. In the case of growth comparisons, data were analyzed as percentages, which required an arc sin transformation (VIEIRA and HOFFMANN, 1989) before the Student's t-test was conducted.

RESULTS

During the experimental period, the sea water temperature in the region varied from 18.29 °C to 27.78 °C with a mean of 23.92 °C, as shown in Figure 2.

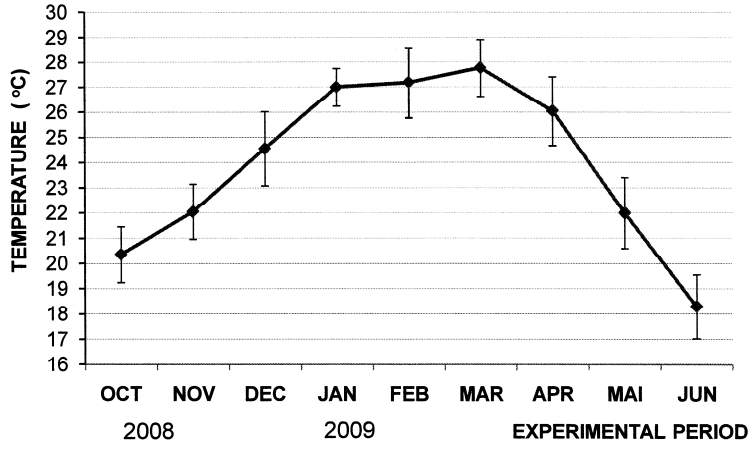

Figure 2. Variation of sea water temperature during the experimental period at the experimental site

A total of 900,000 reared larvae were placed in the settling tank with 180 collectors for the 60 and 90 day cultivation periods. Thus, there was a potential quantity of 5,000 reared larvae per collector; with 3 collectors per bag, this corresponded to 15,000 larvae per bag. Discounting the collectors for pilot analysis and those lost (30 collectors) and considering a total time of 6 months in the sea under cultivation conditions, 83.3% of the initial collectors were obtained at the end of the experiment. Therefore, 750,000 larvae and 150 collectors were considered in the analyses. The total quantity of seeds obtained in this experiment is shown in Table 3.

Table 3. Mean quantities of seeds recovered (absolute numbers) and recovery percentages for the two detachment periods (shown separately) which remained unattached or fixed to the bags and fixed to the collectors for the different analyses preformed

		60 DAYS		90 DAYS	
		NUMBER	%	NUMBER	%
BLUE	BAG	21,893	20.94	7,193	17.53
	COLLECTOR	82,653	79.06	33,844	82.47
	TOTAL	104,546		41,037	
RED	BAG	33,492	44.53	26,507	53.85
	COLLECTOR	41,724	55.47	22,712	46.15
	TOTAL	75,216		49,219	
OVERALL TOTAL		179,762		90,257	

At the end of the 60 day cultivation experimental period which used 2 bags containing 3 collectors of each type (blue and red) for the initial evaluation, 28 bags, 14 with blue collectors and 14 with red collectors remained, for a total of 84 collectors. Therefore,

of the 420,000 reared larvae, 179,762 total seeds were recovered, of which 104,546 were in the blue collector and 75,216 were in the red collector (28% fewer in the red collector).

For the cultivation period of 90 days at sea, 5 bags with blue collectors and 3 bags with red collectors were lost. Of the total of 30 bags, 22 bags with 66 collectors remained at the end of the experimental period. It should therefore be considered that of the 330.000 reared larvae, 90,257 seeds were recovered, of which 41,037 were from the blue collector and 49,219 were from the red collector.

For the two detachment periods, the seeds that were recovered were not all found to be fixed to the collectors. Approximately 19% of the seeds that were recovered were unattached in the bags containing blue collectors, while in the bags containing red collector, more than 49% of the seeds were not attached to the collectors.

Table 4 presents the mean recovery rates for seeds obtained by bags with blue and red collectors, considering potential quantities of 5,000 reared larvae per collector at the beginning of settling and the number of bags and collectors recovered at the time of the analysis. The blue collector had the greatest quantity of seeds per bag with 7,451 and showed a recovery rate of 49.68% for the 60 day experimental period. For the 90 day experimental period, these figures were 4,104 and 27.36%, respectively. For the 60 day period, the red collector recovered a mean of 5,370 seeds with a mean recovery rate of 35.8%, while for the 90 day period, a mean of 4,258 seeds were recovered with a recovery rate of 28.39%. According to the results of the statistical analysis comparing the recovery rate between the blue and red collectors for both for the 60 day (P = 0.082) and 90 day periods (P = 0.999), no statistical difference was detected at the 5% significance level.

Table 4. Mean quantity of seeds per bag (3 collectors per bag) and recovery rates for seeds in blue and red collectors after 60 and 90 days of cultivation at sea

		Blue	**Red**
60 Days	Mean ± sd	7,451 ± 3,284.95 [a]	5,370 ± 2,782.18 [a]
	percentage	49.68	35.8
90 Days	mean ± sd	4,104 ± 1,292.20 [b]	4,258 ± 2,668.22 [b]
	percentage	27.36	28.39

Letters with the same superscripts represent statistical equality for the different collectors compared separately in each period of maintenance at sea.

When comparing the two detachment periods, the recovery rate was statistically greater for the experimental period of 60 days (P<0.01). A large variance was found in all data analysis, though the variance was found to be homogeneous.

Growth was evaluated by quantifying the seeds in each of the size classes (S, M, L, XL). The mean quantity of seeds recovered is presented in absolute values in Table 5. To allow for statistical comparisons, the relative quantities (%) of the seed recovery rate were separated by size as presented in Figure 3.

Therefore, as indicated in Table 5, if we consider the removal of collectors for the initial analysis (pilot) and losses during the periods at sea, of the 750,000 larvae with 150 collectors, 270,000 seeds were recovered which resulted in an overall recovery rate of 36%. Considering these data, the settling rate (not quantified) was greater than 36%.

The analysis shown in Table 5 indicates that after 60 days of separation, only the S, M and L sizes were present; that is, seeds smaller than 30 mm largely between 10 to 20 mm in size. However after 90 days, fewer small seeds were present than after only 60 days; the majority of the seeds were concentrated in the M and L (10 to 30 mm) size classes and some seeds were XL (> 30 mm).

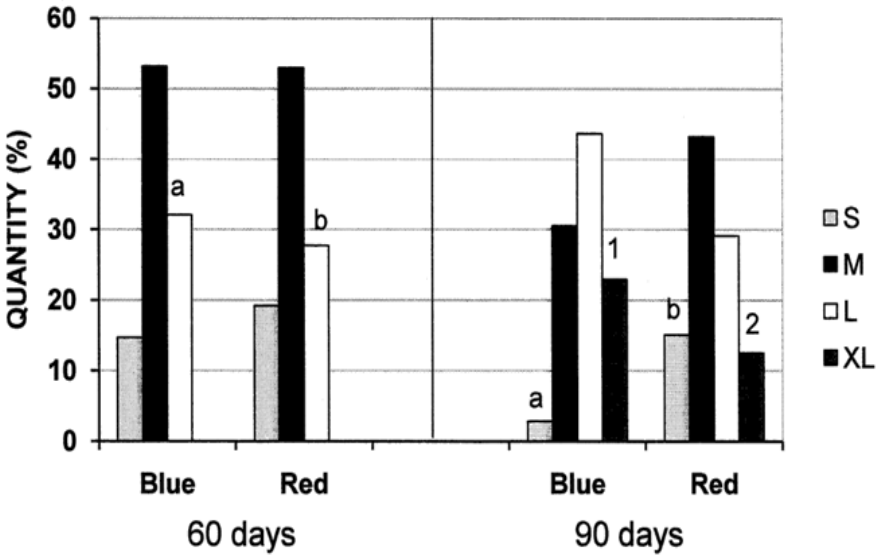

Figure 3. Growth in the function of the percentage of recovery (the quantities of seeds in each of the class sizes) of the seeds in the two experimental periods (60 and 90 days) for the blue and red collector. The lower case superscripts represent statistical differences in the quantities of seeds for the same size when comparing the treatment periods (60 vs. 90 days of treatment) for blue and red collectors separately. In the 60 days, L size grows more in the blue collector (a,b) and for 90 days, S size grows more in red collector (a,b) and XL size grows more in the blue (1,2).

Table 5. Mean quantities of seeds recovered at the end of the experimental periods of 60 and 90 days at sea, respectively, divided by size class

	60 days		90 days		Totals
	Blue	Red	Blue	Red	
S	15,362	14,469	1,163	7,429	38,423
M	55,597	39,866	12,545	21,276	129,285
L	33,587	20,881	17,904	14,341	86,713
XL	-	-	9,425	6,173	15,598
Total	104,546	75,216	41,037	49,219	270,019

As shown in Figure 3, when quantities of seeds in the blue and red collectors were compared for the experimental period of 60 days, seeds of the S and M sizes were not statistically different. However, the blue collector contained a significantly larger quantity of L seeds than the red collector (P<0.01). After 90 days, despite the apparent numerical differences in the graph, the blue collector presented a significantly lower quantity of S class seeds (P<0.01) and a significantly larger quantity of XL class seeds (P>0.05).

Results of tests evaluating whether the detachment period affected the survival are presented in Figures 4 and 5 and growth of individuals in the juvenile phase are presented in Figures 6 and 7. For the three seed size classes that were observed in the 60 day cultivation period (S, M and L), the survival rate was greater than 90%. After 90 days, the survival rate varied from 32.4 to 47.6% for M, L and XL .

No statistical differences were observed for the survival rate or growth of seeds between the 50 and 100% cultivation densities after 60 days. It was not possible to make meaningful comparisons after 90 days since, due to very heavy rain, there was an abrupt decline in salinity (from 32-33 to 10 g L^{-1}) which remained at 16 g L^{-1} for more than a week and contributed to the high mortality rate observed during this period.

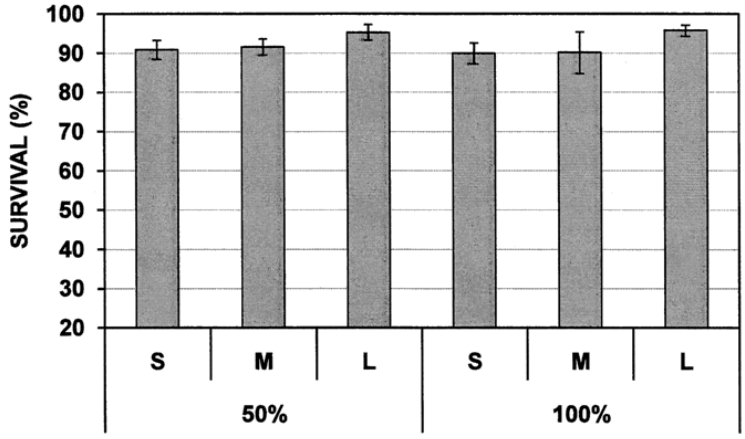

Figure 4. Survival rate (%) of seeds deployed in the 60 day period, after 2 months of cultivation in lantern nursery system

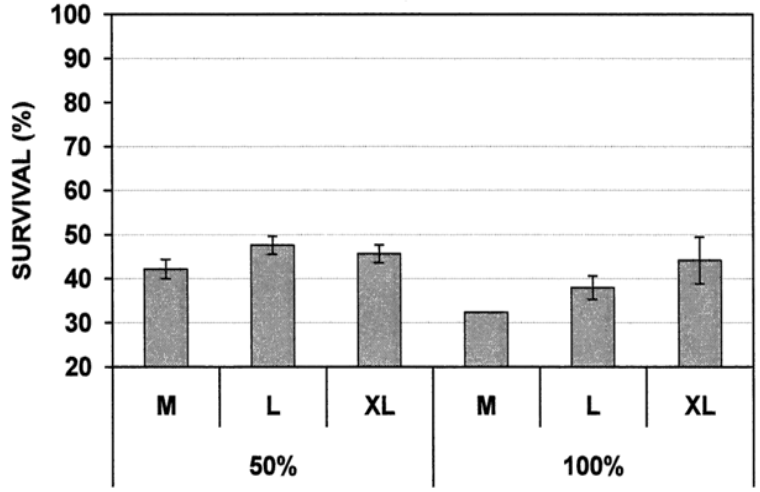

Figure 5. Survival rate (%) of seeds deployed in the 90 day period, after 2 months of cultivation in lantern nursery system.

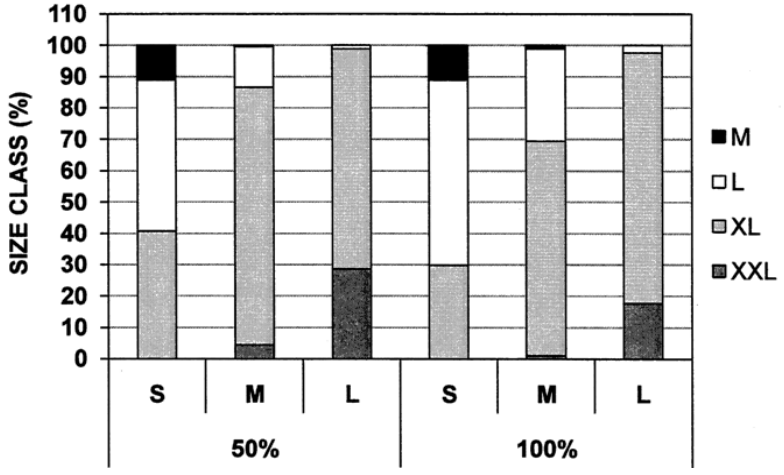

Figure 6. Size class (%) of seeds deployed in the 60 day period, after 2 months of culture in lantern nursery system

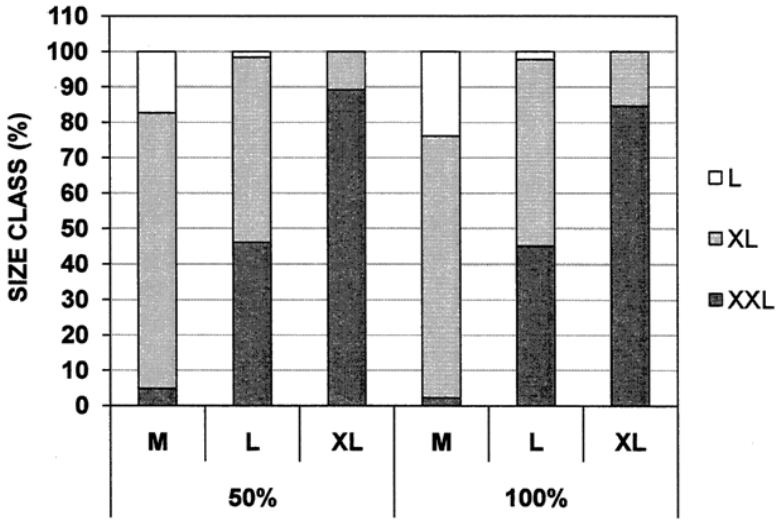

Figure 7. Size class (%) of seeds deployed in the 90 day period, after 2 months of culture in lantern nursery system

DISCUSSION

Larval behavior, substrate selection and settling are regulated by a complex interaction between different physical, chemical and biological factors; which serve different functions in different bivalves (CRISP, 1974; GRAY, 1974). Larvae of many bivalves found in nature (Pectinidae), do not present a rigorous preference for one substrate that they fix themselves to, whereas larvae of the sessile species (Ostreidae, Mytilidae, Pteriidae, Pinidae) developed a high degree of specificity for the substrates on which they are established. These sessile species are able to select between different material types and delay metamorphosis until they encounter an appropriate substrate (GRAY, 1974).

As in this study, various authors have performed laboratory studies evaluating different substrate materials for the fixation of bivalve larvae, such as asbestos sheets, plastic, glass and PVC (CRANFIELD, 1970; AJANA, 1979; ROSE and BACKER, 1994; TAYLOR *et al.*, 1998), bamboo (ALAGARSWAMI *et al.*, 1983), nylon monofilament mesh (ROSE and BACKER, 1994), nylon monofilament (ARAYA-NUNEZ *et al.*, 1995), onion sacks, mosquito nets and fishing nets (SAUCEDO *et al.*, 2005). The larvae can also be fixed to the surface of the settling tank, and can later be detached and allowed to re-settle on the collectors (ITO, 1999).

Results of the different studies on materials to be used for the collectors suggest that materials with a fibrous, rugged or porous surface are preferentially selected by larvae over those with smooth surfaces (PHELGER and CARY, 1983; ROSE and BACKER, 1994; TAYLOR *et al.*, 1998). In addition, substrates that offer smooth and/or raised edges are more attractive to the larvae (AJANA, 1979; ALAGARSWAMI *et al.*, 1983; TAYLOR *et al.*, 1998). Materials with an opaque color and with areas of low illumination are also favorable for larval settling in comparison to bright and well-illuminated materials (AJANA, 1979; ALAGARSWAMI *et al.*, 1983; TAYLOR *et al.*, 1998).

The two collectors used in the experiment were molded according to ZANETTE *et al.* (2009) for *Nodipecten nodosus* (Linnaeus 1758) in the form of a ball with 5 grams of *Pinus* sp. needles in the interior, providing a substrate with a fibrous mesh, smooth edges and a low illumination. The function of the pine needles was not made clear in the literature but the needles efficiently attracted enough settling scallop larvae to form a rigorous structure and introduced a large number of chemical compounds (ZANETTE *et al.*, 2009).

Providing an appropriate substrate is critical for larval settling success and metamorphosis in hatchery conditions (PAWLIK, 1992). SU *et al.* (2007) evaluated the effect of four different

substrate colors on the settling of *Pinctada martensii* (Dunker 1857). Their results showed that red and blue collectors attracted significantly greater quantities of larvae than those with green or yellow colors. These authors also verified that the use of the tissue extract from the pearl oyster attracts a greater number of larvae for settling. To reflect the results of this study, experiments performed in the current work used blue and red collectors.

DOROUDI and SOUTHGATE (2002) investigated the response of *P. margaritifera* larvae to different concentrations of chemical substances known to induce larval settling in some mollusks (GABA, epinephrine and norepinephrine) and which were thought to increase larval settling and the industrial yield of pearl production. Pharmacological inductive agents have been identified as powerful tools in the commercial cultivation of important species. However, in our experiments, the use of chemical substances was not necessary in order to successfully allow *P. hirundo* to settle under hatchery conditions.

After the metamorphosis, two critical factors controlling the survival of the seeds are the permanence time of pre-seeds in the laboratory and the time period before the detachment of the seeds from the collector. One of the important aspects of this process is the viability of larval or pre-seed transfer from the laboratory to marine production environments and the recovery rates and seed growth after this transport.

There is very little information with respect to the effects of the transfer of seeds from the laboratory to the sea for organisms in the Pteridae family. ALAGARSWAMI and DHARMRAJ (1984) transferred seeds of *P. margaritifera* nearly two months after the settling phase had begun. The seeds transferred to the cultivation farms presented a mean size of 3 mm and were placed in box type nursery cultivation structures with a 400 µm mesh. These same authors reported that a high mortality rate can occur if the transferred seeds are smaller than 3 mm.

PIT and SOUTHGATE (2000) transferred pre-seed collectors of *P. margaritifera* to the sea after 3, 5, 7 and 9 weeks of settling. The best recovery rate observed at 3.5 months was obtained from the pre-seeds that were transferred the earliest (3 weeks after settling). These results contradict the initial hypothesis, that pre-seeds that spent longer periods of time in the laboratory would be more resistant and would grow faster when transferred to the sea. The researchers suggested that this result was due to the higher quality and availability of microalgae in the sea than in the laboratory.

DYBDAHL *et al.* (1990) waited for seeds from the pearl oyster *P. maxima* to reach 6mm (2-3 months after settling) before transferring the seeds to the sea, while ROSE and BACKER (1994) transferred *P. maxima* seeds 3-4 weeks after settling. Seeds of *P. fucata* (Gould 1850) were allowed to reach 3mm in size before being transferred to the sea, with the aim of minimizing mortality caused by stress at this stage of cultivation (ALAGARSWAMI *et al.,* 1987; DHARMARAJ *et al.* 1991).

Similar to *P. hirundo*, *Nodipecten nodosus* also presents byssus and settles on "netlon" collectors. According to SÜHNEL *et al.* (2008), from an economic point of view, the pre-seeds of this scallop should spend as little time in the laboratory as possible. According to these authors, the laboratory environment offers several advantages, such as the control of pre-seed production and the maintenance of constant environmental conditions, but several other factors are also required. These include large quantities of microalgae and an aseptic environment, among other factors, which make the process of raising these organisms in the lab both onerous and laborious.

The seeds remain in the settling tank until they are transferred to the sea. However, duration of time that the seeds spent in the laboratory depends on the species and on factors such as food availability, seed condition and local cultivation conditions (DHARMARAJ *et al.*, 1991; ROSE and BACKER, 1994; SÜHNEL *et al.*, 2008).

In our experiment, we maintained seeds in the laboratory for two weeks before transfer, based on the few results in literature that suggest that shorter times spent in the laboratory are preferable for organisms which fix to collectors by byssus threads.

The two collectors evaluated in this study for the pearl oyster *P. hirundo* presented excellent results for the settling of larvae produced in the

hatchery and for seed recovery. The recovery rates for the two time periods evaluated were similar or superior to values for other mollusks that are commercially cultivated. Although the real settling and metamorphosis rate were not evaluated in order to limit the stress to the system, settling with metamorphosis in the collectors was deducted from the recovery rate data and was found to vary from 28 to 50%.

According to ALAGARSWAMI and DHARMARAJ (1984), the color of the tank influences larval settling, with the best settling rates observed in black tanks in comparison to the blue and white tanks. Aeration is necessary both in the larvae cultivation tanks as well as in the settling tanks, with negative effects verified in tanks of smaller volumes.

In the case of our experiments, white tanks were utilized and not all larvae were found to settle on the collectors. Those that settled on the tank were not considered in the analysis since they could not be recovered as seeds in the final process. Approximately 19% of the seeds were also recovered loose in the bags containing the blue collectors, while more than 49% of the seeds were found loose in the bags containing the red collectors. In the recovery rate calculations, these seeds were considered to have settled on the respective collectors.

Of the few data existing in the literature, those of SAUCEDO *et al.* (2005), which evaluated the settling of *Pinctada mazatlanica* (Hanley 1856) larvae, showed that the color and collector material used, as well as the depth of the collector, influenced larval settling. SÜHNEL *et al.* (2008) observed the best pre-seed recovery results of the scallop *N. nodosus* were found for those which remained in the laboratory for 15 to 25 days and at sea for 20 days.

MONTEFORTE and GARCÍA-GASCA (1994) considered that the initial cultivation of the young seeds was a critical strategy in processing *Pinctada mazatlantica*. Changes in the conditions within a semi-closed environment provided by the seed collectors or exposed boxes used to create the seeds represent considerable sources of stress to the pre-seeds (MONTEFORTE and GARCÍA-GASCA, 1994). The management, predation, stock density and colonization by associated species affect the growth and survival of the young pearl oysters (GERVIS and SIMS, 1992; MONTEFORTE, 1996; TAYLOR *et al.*, 1998).

In our case, the periods of 60 and 90 days of cultivation in the sea resulted in higher recovery rates than those described in the literature for similar organisms, showing the efficiency and applicability of the production system. We placed 900,000 reared larvae in the settling tank, providing a potential quantity of 5,000 reared larvae for each collector. Considering the collectors removed for the initial analysis (pilot) and the losses observed during the periods at sea, at the end of the experiment, 270,020 seeds were recovered of the 750,000 larvae observed within 150 collectors at the beginning of the experiment.

Of all seeds recovered, 179,762 were from a 60 day experimental period at sea, with 104,546 recovered from the blue collector and 75,216 recovered from the red collector. For the 90 day period, 90,257 seeds were recovered, with 41,037 recovered from the blue collector and 49,219 from the red collector. Cultivation for 60 days presented a quantity of seeds recovered in the blue collectors that was 28% higher than in the red collectors considering the total quantity of seeds recovered. This difference was not observed after cultivation for 90 days due to a different quantity of bag losses during cultivation. Five bags with blue collectors (15 collectors) and 3 bags with red collectors (9 collectors) were lost.

However, for the mean quantities of seeds per bag (3 collectors per bag), there were 7,451 and 4,104 seeds per bag for the blue collector after 60 and 90 days, respectively, and 5,370 and 4,258 seeds per bag for the red collector after 60 and 90 days, respectively. There was no statistical difference between the blue and red collectors, although the P value, $P = 0.082$, was very close to the critical p value. The difference was masked by the fact that three collectors were present in the bag and by the high deviation values since there was no preference in settling.

Statistically, seed survival in the 60 day detachment period was greater than that in the 90 day period. However, due to the shorter cultivation time, these seeds were predominantly in the M size class. For the 90 day experimental

period, seeds were predominantly M and L, and were also observed in a new class, size XL.

The effect of the detachment period on the survival and growth of the individuals until the juvenile phase for the seeds separated after 60 days of cultivation at sea and subsequent cultivation in lantern nurseries was excellent; survival rates for the three size classes were greater than 90%. These values cannot be compared with those for the 90 day period because there was an excess of rain in the experimental site during the 30 day interval, which caused a reduction in the salinity.

Seeds from the pearl oyster *P. margaritifera* reached a mean size of 40-45 mm in 12 months of cultivation (ALAGARSWAMI and DHARMARAJ, 1984). Survival of the *P. margaritifera* seeds, which were separated from the collectors in the natural environment for 4 to 6 months, was 58%. Survival rates of the seeds removed after 3 to 4 months that had a mean size of more than 15 mm and were cultivated in panel nets for two months were 93% and 82%, respectively (FRIEDMAN and BELL, 2000).

According to ALAGARSWAMI and DHARMARAJ (1984), there was 20% survival of seeds produced in a 500 L tank and 40-50% in a 50 L tank in the laboratory. The annual survival rate for the transfer of *P. margaritifera* seeds to the sea farm was 30%.

LODEIROS *et al.* (2002) showed in their studies with *Pinctada imbricata* (Röding 1798) that this species grows more rapidly when cultivated in suspension than when grown on the bottom. These authors found that the difference in growth is more evident in the weight of the tissues and shell than in the dimensions of the shell. In the first month of study, the survival rates were 91% in suspended cultivation and 88% for bottom cultivation. In the subsequent months of the experiment, survival increased to 100% in suspension and 93-97% on the bottom, and the differences between the cultivation methods were not significant. At the end of the experiment, the oysters in the suspended cultivation reached a mean size of 54.6mm (± 2.31), which was significantly greater than sizes observed from bottom cultivation (45.3 mm ± 2.48). The juveniles that initiated the experiment presented a mean length (dorsal-ventral axis) of 1.8 mm.

In it experiment, larvae produced in the laboratory and seeds separated after 60 days at sea showed a 90% survival rate and a growth of up to 30 mm after 165 days of fecundation, equivalent to the best rates obtained by the authors cited above. It was not possible to make comparisons with larvae and seeds separated after 90 days due to the intense rain which caused an abrupt drop in salinity (from 32-33 to 10 g L^{-1}, with a salinity value of 16 g L^{-1} maintained for more than one week) which resulted in a high mortality rate during this period. In spite of this event, the results for this stage produced seeds of up to 40mm after 195 days of fecundation.

Results of this study showed that the two evaluated collector types result in efficient settling of *P. hirundo*, with the blue collector (netlon) found to be more attractive. The quantity of seeds separated after 60 days of cultivation was found to be significantly greater than that observed after 90 days at sea, however seeds were found to be smaller. The time spent at sea had a greater influence on recovery than the collector type. The use of the netlon collector for a period of 60 days at sea with a 100% occupation area of the lantern nursery plate surface is recommended. The results of this experiment (high settling, recovery and growth rates) showed that it is possible to offer seeds of different sizes at a commercial scale to producers interested in cultivating this native bivalve species on the Santa Catarina coast.

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