

Farming Pacific oysters using the spat-on-shell system in a shallow area in the subtropical coast of Brazil

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ABSTRACT

The Santa Catarina Island Bay (SCIB) contributes with the largest production of cultivated oysters in Brazil, which is almost entirely based on hatchery produced cultchless oyster spat, whose metamorphosis is induced by an epinephrine solution. A production scale experiment was carried out to analyze the technical feasibility of the spat-on-shell system in SCIB. The experiment was carried out for 47 weeks, involving an internal phase, the larval settlement, and an external phase, encompassing nursery and growth. Nursery periods varied according to the experimental treatments: T3W - 3 weeks; T5W - 5 weeks; and T12W - 12 weeks. T3W and T5W showed lower survival during the two-week period shortly after transfer of the cultch from the nursery to the grow-out phase, likely linked to premature exposure of the spat in the cultch strings to predators such as fish. The 12-week nursery period showed the best results with clusters with a median of 6 animals and 11.4 g of meat per oyster at the end of the experimental period. Future studies could verify whether longer nursery periods or alternative methods to avoid exposure to predators can outperform these results.

Keywords: *Crassostrea*, Oyster farming, Oyster meat, Oyster cluster, Mariculture in Brazil.

China, France, U.S., South Korea, Japan, and Canada are the main global oyster producers (FAO, 2016). North American and European countries primarily focus on farming oysters to be sold in the raw, half-shell market (McKean and Whitbeck, 2000), whereas Asian countries, including China, focus on shucked meat products used in canning and frozen meals and oyster sauce (Botta et al., 2020). A large proportion of oysters in these countries are produced using the spat-on-shell system, a farming technique ideal for shucking (Kusuki, 1991). Under this cultivation system, larvae are provided with cultch, which is the substrate (e.g. adult mollusk shells) upon which oysters settle

(Webster et al., 2019), metamorphose, and grow, forming clusters. Longlines (long ropes tethered to buoys) or rafts can be deployed in deep areas of sheltered bays to suspend cultch strings, commonly up to 10 meters in length (Ventilla, 1984).

Santa Catarina (SC), a state in the south of Brazil, contributes the largest production of cultivated mollusks in the country, with yearly production over the last decade at times exceeding 3,000 tons of Pacific oysters (*Crassostrea gigas*) (www.infoagro.sc.gov.br). Santa Catarina Island Bay (SCIB) concentrates 96% of the state's oyster production, nearly all of which is based on hatchery-produced cultchless oyster spat, whose metamorphose is induced using a solution of epinephrine (Coon et al., 1986). Local farmers use lantern nets to grow the oysters (in some cases other types of structures are used in the initial/nursery phase) to commercial size and sell them mostly raw for the

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half-shell market (Suplicy, 2020). This end-product commands a higher price per unit weight than oyster products that are sold in other markets (e.g., shucked meat, clumps for char grilling) (Botta et al., 2020), but the system used to produce oysters with attractive and uniform shells requires more labor and maintenance than cultch production. The farming practices adopted in SC can involve weekly to monthly (depending on the farming phase) removal of the lantern nets from the sea for net cleaning, oyster grading, and farming density adjusting, a routine that accounts for approximately 34% of the cost of production (Suplicy, 2020; 2021).

In SC, *C. gigas* develop gonads during the winter months and reach the highest meat yield during the spring. Oysters tend to spawn during the summer months, negatively impacting meat yield, flavor, and appearance during the tourist season, when demand is highest (Sühnel et al., 2017). This pattern has increased the interest from farmers for alternative systems that allow harvesting and shucking at the time of best meat yield (spring), and conservation (e.g., freezing) for trading during the tourist season. A production-scale experiment was performed to analyze the technical viability of the spat-on-shell system in the SCIB.

This study involved an indoor phase, the larval settlement, and an outdoor phase, encompassing the nursery and grow out. The settlement was conducted at the facilities of the Laboratory of Marine Mollusks (Laboratório de Moluscos Marinhos - LMM) (27°35'4.42"S, 48°26'29.92"W) at the Federal University of Santa Catarina. The nursery and grow out phases were carried out in a marine farm (27°42'10.02"S, 48°33'31.45"W) located in the SCIB, which is a shallow (average depth = 3.4 m) and microtidal body of water located between the mainland and Santa Catarina Island. The marine farm is 4 meters deep, with a sandy-muddy bottom. A one-year study carried out between 2009-2010 monitored the location's salinity fortnightly and revealed a mean salinity of 28.4 g kg⁻¹ (sd= 5.7, range = 10.56 - 33.95) (Epagri, unpublished data, available per request to the corresponding author).

Clean, sun-dried, and perforated adult oyster shells were used as cultch. Groups of 100 shells were placed in 1.8 m tubular bags, 100 mm in

diameter, made of 35 mm nylon mesh netting. The bags containing the shells were closed using string and submerged in a tank containing 2,000 L filtered (5 µm filter) seawater, where they remained for five days, in order to form a biofilm that favors the settlement of oyster larvae (Webster et al., 2019). After this period, *C. gigas* larvae in the pediveliger stage were transferred to the tank containing cultch (100 larvae shell⁻¹, or 52 larvae L⁻¹). The water temperature in the tank when the larvae were released was 20.6 °C, while the salinity was 35. Strong aeration was maintained during the following 48 hours. After this period, the bags containing shells with the settled larvae were transferred to the sea and attached to a longline, beginning the nursery phase. The bags remained hanging vertically during different periods according to the experimental treatments: T3W - 3 weeks; T5W - 5 weeks; and T12W - 12 weeks. By the end of the nursery phase, the bags were removed from the longlines, and the shells were removed from the bags and used to assemble the cultch strings. A 5 mm cable was passed through the shell perforations and interspersed with 5 cm spacers made of plastic hose. Twelve strings, containing fifteen shells each, were mounted (four per treatment). The strings were attached to longlines and remained hanging for eleven months of farming, after which the oysters were harvested, and the experiment was concluded. The experiment duration was 47 weeks, starting with settlement on 06/30/2020 and ending with harvest on 05/23/2021.

The first inspection of the strings to count settled oyster seeds occurred at the end of the nursery phase. A sample of 90 shells per treatment were inspected (Figure 1-A). Two weeks after the installation of the cultch strings, a new inspection was conducted (90 shells per treatment), and the oyster seeds were counted. At the end of the experiment, the strings were removed from the sea and the oyster clusters were steamed in an industrial cooker for 30 minutes. After steaming, the oysters from each cluster were counted and shucked, and the oyster flesh was weighed. Average flesh weight per oyster was inferred by dividing the total weight of oyster flesh by the number of oysters in each cluster.

Water temperature during the experiment was monitored hourly using a temperature logger (Star Oddi CTD-CT) installed on a longline. The water temperature during the study averaged 21.1 °C (range: 14.6 - 29.5°C). The temperature throughout the experimental period is described in Figure 1-B.

Shapiro-Wilk tests applied on the ANOVA residuals indicated that normality is violated for the data series of number of surviving oysters per cluster and amount of oyster flesh per cluster ($p < 0.001$). As such, the analyses of these parameters were conducted using non-parametric statistics. The treatment comparisons were performed using the Kruskal-Wallis H test followed by multiple Wilcoxon-Mann-Whitney tests. The Shapiro-Wilk normality test did not indicate normality violations for the data series of amount of flesh per oyster, but the Levene's test indicated a significant variance (p -value = 0.003) across groups. Accordingly, the treatment comparisons were performed using the Welch's one-way ANOVA.

The results show that prolonging the nursery period significantly reduced survivorship for settled oyster seeds. T3W and T5W, with nursery periods of three and five weeks respectively, showed significantly higher survivorships (Kruskal-Wallis chi-squared = 13.489, $df = 2$, p -value = 0.001) than

T12W, which was subjected to a 12-week nursery period (Figure 2-A). However, this pattern inverted two weeks after transferal oyster seeds to the cultch strings, with a higher survivorship in T12W compared to T3W and T5W (Kruskal-Wallis chi-squared = 95.235, $df = 2$, p -value $< 2.2e-16$). The median number of seeds per shell, 19 and 16.5 in T3W and T5W during the first inspection, declined to 3 and 2, during the second inspection (Figure 2-B). The median number of oyster seeds per shell in T12W was 14 in the first inspection, declining by half, still a much smaller reduction compared to other treatments. As of the second inspection, the survivorship data distribution was similar (Figure 2-C), and T12W continued to show higher survivorship than T3W and T5W (Kruskal-Wallis chi-squared = 72.806, $df = 2$, p -value $< 2.2e-16$). The median survivorships at harvest were 2, 2, and 6 for T3W, T5W, and T12W, respectively. Shells without surviving oysters were only observed in T3W and T5W (18.3% and 13.3%, respectively). The highest survivorship in T12W resulted in the highest amount of oyster flesh produced per cluster at harvest (Kruskal-Wallis chi-square = 69,144, $df = 2$, p -value = 9,674e-16) (Figure 2-E). It is important to note that this difference was exclusively due to the number of surviving oysters, since there was no significant difference (Welch's ANOVA,

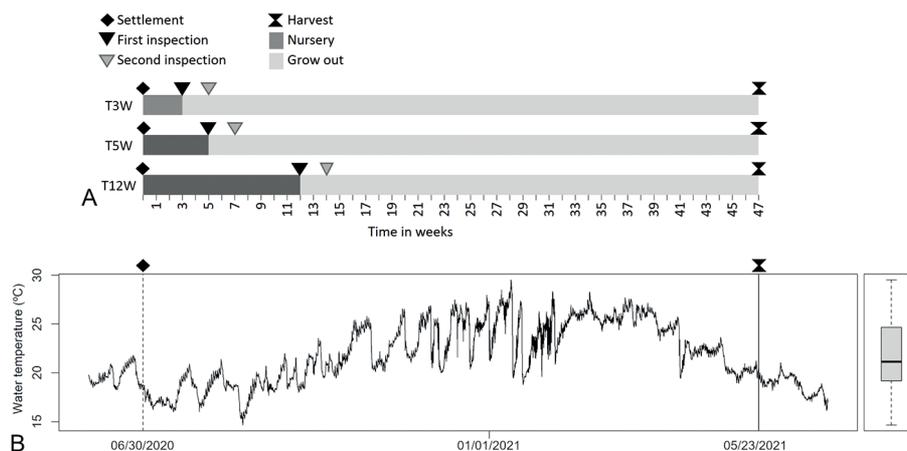


Figura 1. Schematic of the experimental design (A) and the seawater temperature during the experiment (B). The duration of the nursery period varied between treatments T3W, T5W and T12W. The first inspection for surviving oysters was performed when the shells were transferred to the grow out structures, the second inspection two weeks after this transference and the final count of survivors was performed in the day the oyster clusters were harvested. The line plot (B) indicates the seawater temperature variations during the experiment. The boxplot on the right summarizes the data in terms of median, interquartile and total range.

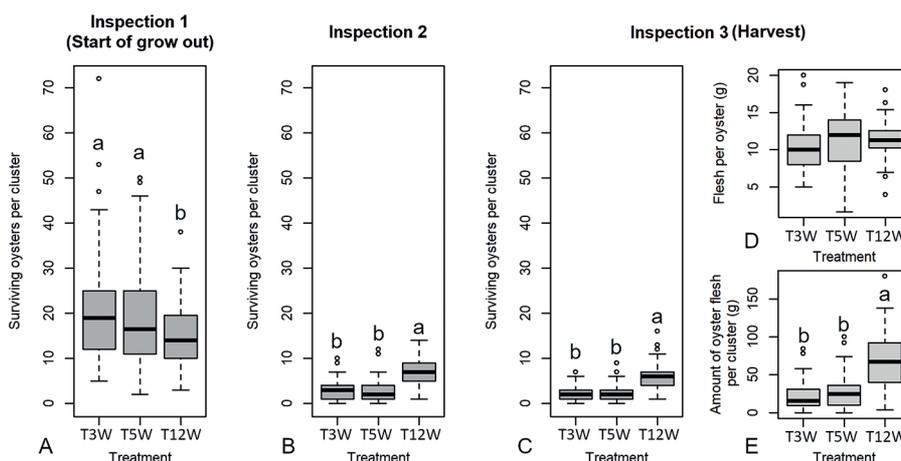


Figure 2. Number of survivor oysters observed in the different treatments (T3W, T5W and T12W) at the beginning of the grow out phase (inspection 1) (A), two weeks later (inspection 2) (B) and at the harvest (inspection 3) (C). A comparison of the weight of the flesh per oyster (D) and per cluster (E) at the harvest is also presented. Superscript lowercase letters indicate homogeneous groups according to pairwise comparisons using Wilcoxon rank sum test (A, B, C and E).

$F = 1.57$, p -value = 0.21) between the amount of flesh per oyster observed for the different treatments (Figure 2-D), whose averages were 10.5 g, 11.5 g, and 11.4 g for treatments T3W, T5W, and T12W, respectively.

The experiment succeeded in showing that is possible to farm Pacific cupped oysters using the spat-on-shell system in the SCIB. The oysters reached ~11g of flesh after 11 months, considered satisfactory, but oyster development seems slower than in farms using the lantern-net system. For reference, an experiment carried out on the coast of SC, ~100 km north of the study location, showed oysters reaching an average length of 73.5 cm (commercial size) and average meat weight of 9.58 g after six months of farming (168 days) (Manzoni and Schmitt, 2006). It is important to note that in that case the raw meat was weighed, therefore the results are not strictly comparable.

The experiment was planned to reach a density of 10 oysters per cluster by the end of the farming period, as recommended by Webster et al. (2019). In the treatment that performed best (T12W), the median number of oysters per cluster was six. The results suggest that two periods were most critical for survivorship, settlement and the two weeks following transferal of the cultch with settled oyster seed from the nursery to the grow out phase.

Regarding the remote setting, the adopted methodology is, generally, in line with recommendations from remote setting manuals from Webster et al. (2019) and Bohn et al (1995) for the Atlantic coast of the USA. Oyster shells are indicated as one of the best cultch materials, and the minimum salinity registered in the experiment site is above the 9 g kg⁻¹ limit, under which oysters do not set reliably (Bohn et al., 1995; Webster et al., 2019). On the other hand, water temperature when the larvae were released in the settling tank was lower than recommended ($\geq 24^{\circ}\text{C}$). According to Bohn (1995), this could result in slower settling. The survivorship observed during this phase in the present study seems satisfactory compared to literature. After nursery periods varying between two to twelve weeks in the sea, results ranged between 14 to 19 spat shell⁻¹ (medians) for the different treatments, representing 14% to 19% setting efficiency (setting with 100 larvae shell⁻¹). For comparison, Ozbay et al. (2020) reported average setting efficiency of oyster, *Crassostrea virginica*, ranging from 17% to 29% in different years (from 2009 to 2015). The spat produced by those authors supply small-scale oyster enhancement efforts and a citizen oyster gardening program in the east coast of the USA. They adopted a three-week nursery period in settling tanks and feed larvae/spat with

seawater containing naturally produced microalgae. Experimental assays at smaller scales report higher setting efficiency. Holiday et al. (Holiday et al., 1991) achieved 68% setting efficiency eight days after settling for Pacific oyster (*C. gigas*). In their study, larvae were maintained in PVC screens (450 mm diameter, 150 mm deep, 200 µm mesh size), subjected to a 100% water exchange regime, and tanks were fed a mix of microalgal species.

The experimental protocol adopted in the present study focused on reducing human labor and maintenance needs in the settling tank to the extent possible. As such, we opted not to feed the larvae during the settling but instead to transfer the spat to the sea as soon as possible. Feeding larvae with natural microalgae by renewing the tank seawater and prolonging the maintenance of spat in the settling tanks can be tested for improved survivorship in the future. According to Webster et al. (2019), spat can be removed from the setting tanks as early as a couple of days after they have set or several months later. In most cases, spat will grow faster and survive more once they are moved out of the setting tank. It is also possible to consider simply using higher larval densities in the settlement stage than the 100 larvae shell⁻¹ or 52 larvae L⁻¹ used in the present study, to achieve higher final oyster densities in the clusters.

Another aspect that could be improved in future attempts of farming oysters using the spat-on-shell system in SC is related to the two-week period right after the transference of the cultch from the nursery to the grow out phase. The lower survival during this period in the treatments with the shortest nursery times (T3W and T5W) is likely linked to premature exposure of the spat in the cultch strings to predators such as fish. This phenomenon is well-described for brown mussels (*Perna perna*), as the species is commonly farmed using spat collectors in SC. The capture and stomachal analysis revealed that at least seven fish species, two swimming crabs, two gastropods, one cephalopod, and one Asteroidea species are mussel seed predators (Leite, 2007). Future attempts could consider testing longer nursery periods or other methodologies that prevent exposure of oyster seeds to predators. For example,

in the USA, nurseries are commonly staked on pallets in a shallow area of the intertidal zone until seeds reach 1.3 cm to 2 cm, at which point they are transferred to the grow out structures (Webster et al. 2019).

Santa Catarina has 485 marine farms (available in: www.infoagro.sc.gov.br). These are generally located in shallow areas, with 46 % of the farms at <3 m and an average depths of 3.5 m (Souza et al., 2016). Their water salinity is high (median = 31.7 g kg⁻¹; 75% of the results > 28.3 g kg⁻¹), and the average water temperature is 21.78 °C in SC (Epagri, unpublished results). Despite the much higher market price and favorable environmental conditions, only 20% of marine farms in SC currently produce oysters. The less laborious farming practices for Brown mussels are probably a major factor. Our experiment showed that it is possible to produce oysters dedicating only four major efforts (cultch preparation, remote setting, transference for nursery, and cultch strings preparation/installation) compared to the fortnightly/monthly management required by traditional systems adopted in SC. Therefore, oyster farming with the spat-on-shell system could be considered an alternative to increase production. There is a need for economic studies comparing the spat-on-shell and traditional systems and analyzing the different aspects of the techniques (e.g. the lesser reliance on human labor, the most inexpensive farming structures, the longer farming time, and less valuable end product - shucked meat) to assess if there are significant revenue benefits.

In conclusion, the study showed it is possible to farm Pacific oysters using the spat-on-shell system in shallow coastal zones in SC, Brazil. The experiment also showed it is possible to obtain animals with good condition indices by settling them in the winter and harvesting eleven months later. Nursery periods of 84 days yielded the highest survivorship, and future studies could verify if longer nursery periods, or alternative methods to avoid exposure to predators, can exceed these results.

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AUTHOR CONTRIBUTIONS

F.M.S.: Conceptualization; Funding acquisition; Project administration; Investigation; Methodology; Writing - original draft; Writing - review & editing.

R.V.S: Formal Analysis; Visualization; Writing - original draft; Writing - review & editing.

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