

Suspended culture of the winged oyster *Pteria sterna* (Gould, 1851) in tropical estuary

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Abstract. The growth and survival of the pearl oyster *Pteria sterna* were evaluated over a period of 7 months in suspended culture conditions, in the Chone river estuary, Manabí province, Ecuador. These variables were correlated with the fluctuations of the environmental conditions of the area, such as temperature, salinity, turbidity, oxygen concentration, seston and phytoplanktonic biomass. The initial antero-posterior length of the pearl oyster seeds was 15.2 ± 0.25 mm, which were confined in lantern nets at a density equivalent to 50% coverage of the area of the basket floors. Oysters were sampled every month to measure survival and perform biometric analyses to determine their growth parameters. The results show that cultivation of *P. sterna* under the conditions of the estuary is only feasible during dry weather periods, as these periods precede the low salinity environment provoked by rain, which causes high mortality in the oysters. During the dry period, environmental factors had no negative effects on the growth and survival of the oysters, allowing them to reach an antero-posterior length of ~ 60 mm, and a mass of ~ 4.6 g post-dehydration, equivalent to 20-25 g of wet soft tissue mass (meat), which is considered adequate for human consumption; however, they do not reach the sizes suggested for the development of pearls at through artificial implants.

Key Words: bivalve cultivation, estuary, nacre, salinity, tropical.

Introduction. The winged pearl oysters *Pteria sterna* (Gould, 1851) are distributed along the tropical and sub-tropical coastline of the western Pacific Ocean, from the state of Baja California (México) to Peru. Found in the sublittoral area, this oyster adheres to hard substrates via the byssus, particularly to gorgonian corals (Monteforte 2005; Coan & Valentich-Scott 2012). Due to their high-quality, multi-colored pearls, which are highly valued in international markets, the species has been considered of great productive importance in the gulf of California (Monteforte 2013; Kiefert et al 2004; Saucedo et al 2023).

In the tropical and subtropical eastern Pacific, *P. sterna* has also been considered important due to its dual production value; the oyster can be cultivated to harvest both its consumable soft tissues (meat) as well as their high-quality pearls (Lodeiros & Villegas 2018; Saucedo et al 2023). In previous studies, the species also proved to be highly feasible for cultivation at sea; individuals placed under suspended culture conditions in the coasts of the Santa Elena province reached a maximum antero-posterior length (maximum length from umbo to valve extreme) of 90-100 mm in just 11 months, the growth rate of these individuals showed little variation in relation to changes in the conditions of the environment, where salinity remained stable at 33-36‰ (Lodeiros et al 2018; Freites et al 2019; Jara et al 2022).

In the estuary of the Chone river, Manabí province, Ecuador, Treviño et al (2019) conducted a study on *P. sterna* individuals with an initial size of ~ 50 mm, and

determined that cultivation density had a significant and negative impact on the growth and fattening of the oysters studied. The present study aims to evaluate the early growth and survival rate of *P. sterna* individuals starting at < 20 mm in size under the conditions of the same estuary.

Material and Method

Cultivation. The study was conducted in the estuary of the Chone river, Bahía de Caráquez, Manabí Province, Ecuador (0°33'26.50"S; 80°25'22.71"W), using a floating platform placed near the docks of the Puerto Amistad Yacht Club. Beneath the platform, lantern nets containing the individuals were suspended for a period of 7 months at a depth of 1 m (Late July 2018 to mid-February 2019). All the procedures followed the guidelines for ethical and responsible research using *in vivo* animals for experiments (Kilkenny et al 2010). Furthermore, the animal bioethics procedures of this study were in accordance with the Institutional Committee of Bioethics of the Technical University of Manabí, registered in volume 021-5 page 21-5-1.

The seeds of *P. sterna* used had recruited on enclosures dedicated to the cultivation of the oyster *Magallana gigas* in the Centro Nacional de Acuicultura e Investigaciones Marinas of the Escuela Superior Politécnica del Litoral (CENAIM-ESPOL), Peninsula of Santa Elena, Ecuador.

The seeds (15.2±0.25 mm antero-posterior length) were too small to be placed directly onto the Japanese-style lantern nets used in this study (42 cm diameter, 20 cm height b/w floors, 20 mm mesh size), the individuals were first evenly distributed across 6 mesh sacks (totaling 120 seeds per sack), which had a mesh size of only 5 mm, the sacks were then placed inside 6 lantern nets (one sack per basket) until the seeds reached sizes > 20 mm (within the first month of cultivation). The sacks were then removed, leaving the oysters to rest directly on the lantern nets floors. The individuals were distributed across floors ensuring a cultivation density of 50%.

Cultivation density was determined by calculating the area of the lantern net floors, as well as the average area occupied by the oysters, which resulted in the number of individuals necessary to occupy 50% of the area available. As the individuals increased their size, these were "unfolded" (re-arranged and re-distributed) appropriately to maintain the same area coverage. Similarly, to compensate for the effects of mortality and oyster sampling, 2 of the 6 lantern nets deployed were used as replacement baskets: their individuals were utilized to replace oysters removed from the 4 remaining lantern nets. On every sampling, all living individuals were re-organized to preserve the cultivation density inside all 6 lantern nets; however, reserve baskets were excluded from measurements. All dead individuals were discarded.

Four samplings were performed throughout the experiment, each extracting 10-15 individuals from the 4 main lantern nets. Every organism extracted was measured to determine size in all axes: antero-posterior (maximum distance axis in the direction of the hinge at the end of the shell), dorso-ventral (maximum distance in axis perpendicular al antero-posterior) and maximum distance between valves with a digital caliper (0.01 mm precision), shells and soft tissues were separated, and their masses were measured using a balance (0.001 g precision) after dehydrating on a stove at 60°C for 72 h.

Environmental factors. To evaluate the influence which environmental factors had on the growth and survival rate of the oysters, several samplings were performed periodically. On a weekly basis, water temperature and oxygen concentration were measured using a YSI 550A multi-parametric probe, salinity was measured with a refractometer (0.1‰ precision) and water transparency was determined with a Secchi disk. Bi-weekly, three water samples were collected from a depth of 50 cm with 2 L Niskin bottles. These samples were sieved (153 µm) to eliminate macrozooplankton, and then filtered with Whatman GF/C filters (1.2 µm) in a vacuum system (Millipore) to determine total and inorganic seston. Phytoplanktonic biomass was estimated through chlorophyll-*a* concentration, following the recommendations from Strickland & Parsons (1972).

Statistical analysis. The averages of the growth parameters (oyster dimensions and soft tissue mass/shell mass) and the average survival rate were evaluated between samplings via a t-student distribution, following the recommendations in Zar (2010). To evaluate the relationship between these parameters and the environmental factors, averages of each factor were correlated with the size/mass increments registered on each sampling (increments were calculated in relation to their previous sampling). To this end, the growth and environmental variables were first gathered through a principal components analysis (PCA), then organized and studied through a biplot multi-variate dispersion analysis, as recommended in Johnson & Wichern (1992). Significant differences were calculated with $p = 0.05$.

Results. For the majority of the study period, mortality was practically non-existent, excepting early September, where the percentage of surviving individuals dropped to $89.0 \pm 4.53\%$ (Figure 1). However, at the end of the study period (February 2019) a total (100%) mortality of the oysters was registered.

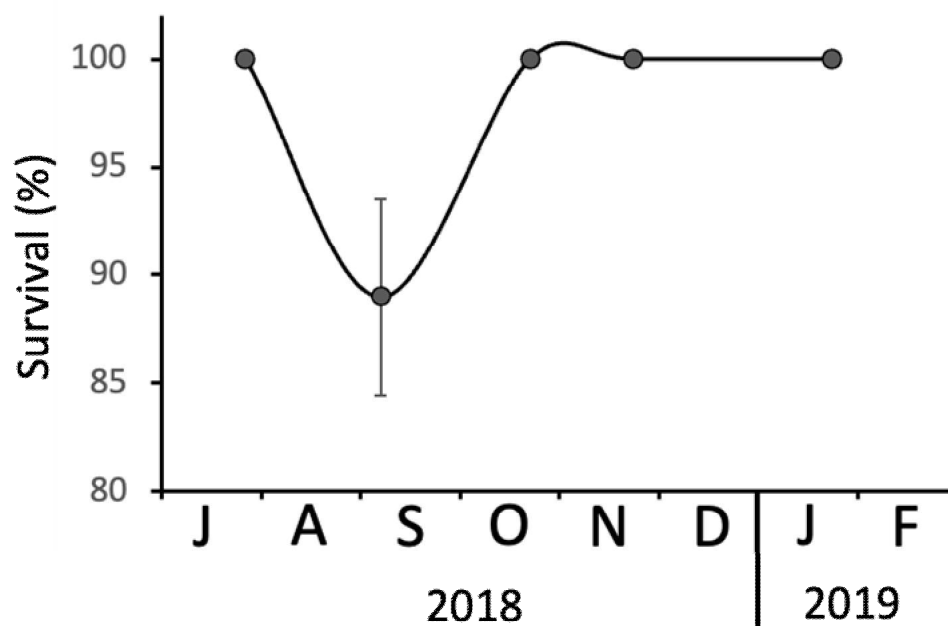


Figure 1. Survival of the pearl oyster *Pteria sterna* in suspended cultivation (estuary of Chone River, Manabí province, Ecuador, July 2018 to February 2019).

The oysters showed continuous shell growth in all dimensions until mid-November 2018, point after which no significant increments were observed (Figure 2A). The maximum average dimensions reached were 59.5 ± 4.91 mm antero-posterior, 57.9 ± 4.52 mm dorso-ventral and 28.4 ± 7.59 mm between valves. Dry mass growth of the shell and soft tissues was exponential, though highly variable. At the end of the experiment, the oysters reached a shell weight of 22.7 ± 4.80 g (Figure 2B), and a dry tissue weight of 4.7 ± 1.36 g (Figure 2C).

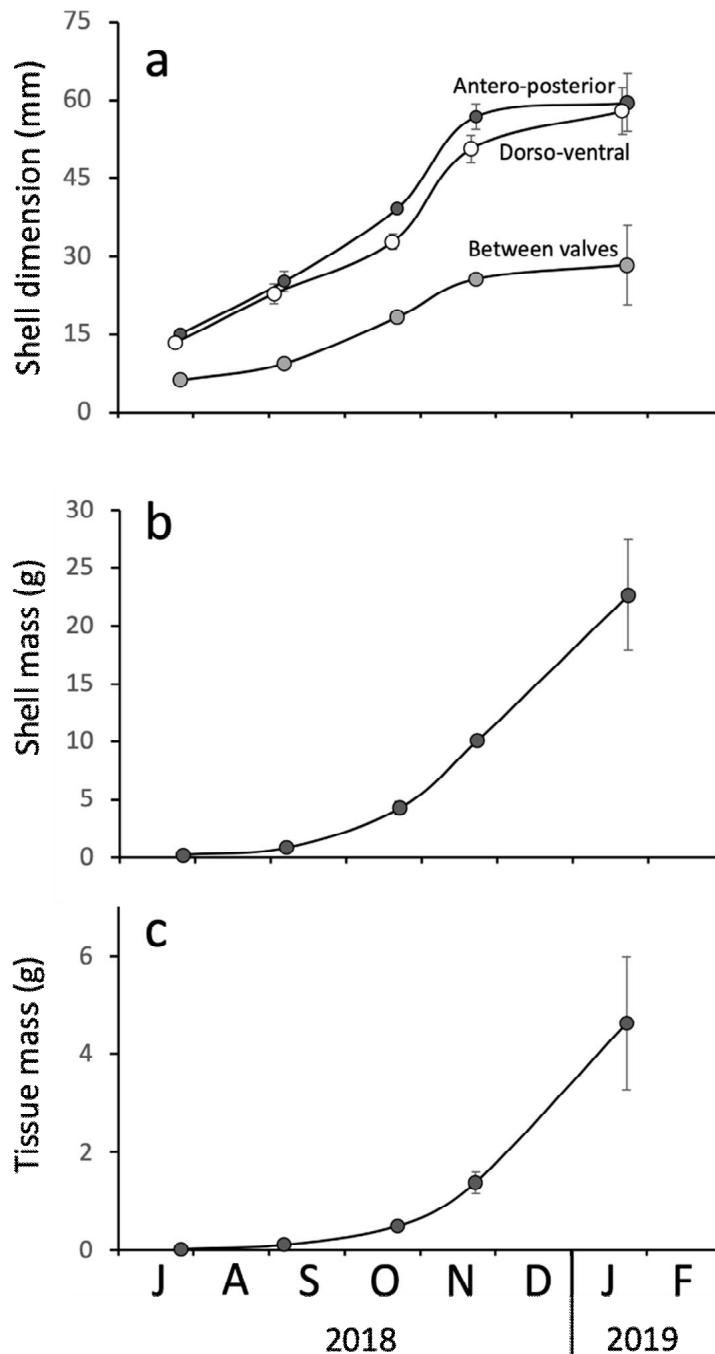


Figure 2. Average oyster shell growth in anterior-posterior, dorso-ventral, and intervalval dimensions (a), shell dry mass (b), and soft tissues dry mass (c) of the pearl oyster *Pteria sterna* in suspended cultivation (estuary of Chone River, Manabí province, Ecuador).

Environmental variables. Temperatures fluctuated within a range of 24-29°C, with the highest values observed in the last two months of the cultivation period (January-February 2019; Figure 3A). Salinity ranged between 23 and 33‰ until early February 2019, when they dropped abruptly to 3‰ in mid-February (Figure 3B). Water transparency ranged between 30 and 95 cm until mid-October 2018, where it began decreasing until the end of the study period, concluding in values of 30-40 cm (Figure 3C). Dissolved oxygen concentration presented values of 4.1-6.7 mg L⁻¹, the lowest values were recorded at both the beginning and specially the end of the study period (Figure 3D).

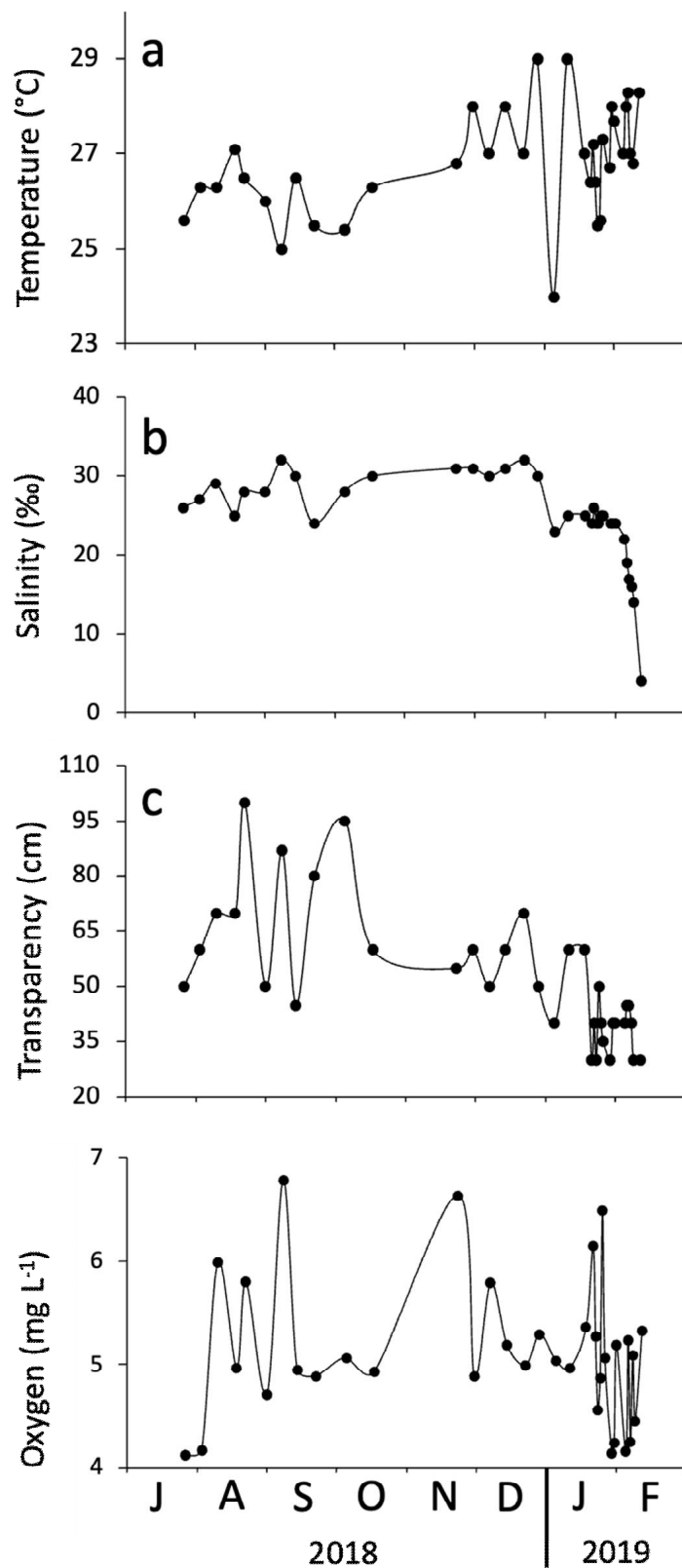


Figure 3. Temperature (a), salinity (b), transparency (c) and dissolved oxygen concentration (d) variability in the cultivation area of the pearl oyster *Pteria sterna* in suspended cultivation (estuary of Chone River, Manabí province, Ecuador).

Total and inorganic seston concentrations were 40-82 mg L⁻¹ and 24-80 mg L⁻¹, respectively. However, a relatively high increment was observed near the end of the study period, with values of 145 mg L⁻¹ and 117 mg L⁻¹, respectively. Similarly, organic seston stayed within a range of 14-25 mg L⁻¹ until the end of the study period, where it increased (Figure 4A). Chlorophyll-*a* ranged between 7 and 24 µg L⁻¹, reaching its highest values on both the beginning (July-August 2018) and the end (January-February 2019) of the study period (Figure 4B).

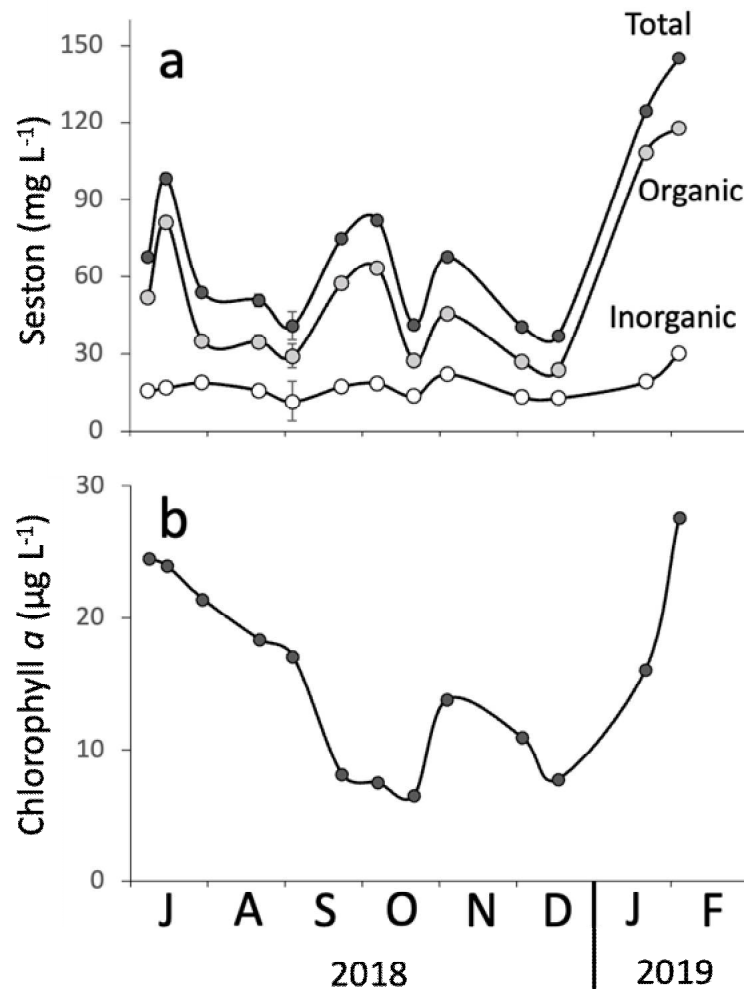


Figure 4. Organic, inorganic and total seston (a), and phytoplankton biomass estimated through chlorophyll-*a* (b) in the cultivation area of the pearl oyster *Pteria sterna* in suspended cultivation (estuary of Chone River, Manabí province, Ecuador).

Relationships between growth and environmental factors. The environmental variables and growth parameters (oyster dimensions and soft tissue mass/shell mass) found in the PCA were organized with a variance of > 80%, and their disposition on the biplot dispersion chart (Figure 5) showed a negative relationship between temperature and the growth parameters, particularly with shell dimensions. On the other hand, the chart suggests that growth parameters had a positive relationship with water transparency, chlorophyll-*a* and the total and organic seston.

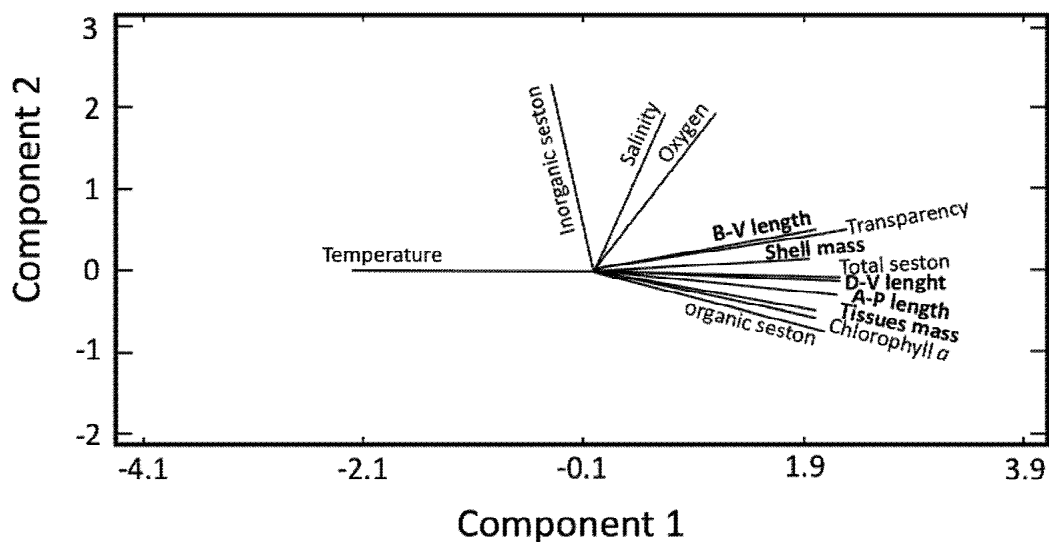


Figure 5. Biplot dispersion of the environmental variable components and growth parameters of the pearl oyster *Pteria sterna* in suspended cultivation in the estuary of Chone River, Manabí province, Ecuador, as given by a principal component's analysis-PCA (component variance was of > 80%).

Discussion. In the present study, it could be observed that in February 2019, water salinity dropped abruptly to < 5‰, which was associated to the 100% mortality rate suffered by the oysters, suggesting a low salinity environment is lethal for them. This observation agrees with Treviño et al (2019), who suggested that *P. sterna* is unable to adapt physiologically to levels of salinity below 12‰, producing mortality. Similarly, recent studies indicate that the lethal salinity threshold of *P. sterna* is 20‰ (Álvarez-Gracia et al 2021). This restricts cultivation of *P. sterna* to periods of dry weather, which suggests cultivation projects starting from April/May and ending in December/January (as was performed in this study) are a viable strategy for this oyster if cultivation is intended in the Chone river estuary. Alternatively, establishing *P. sterna* cultivation projects in the sea would also reduce the influence of elevated pluviosity periods on oyster survival.

Survival in the first sampling drops to 90%, this drop in survival could be caused by seed manipulation in the initial culture, so studies of the planting of *P. sterna* seeds should be developed to minimize the impact of cultivation in subsequent phases. On the contrary, during most of the dry period the survival remains practically 100%, it would appear that environmental conditions had no negative influence on *P. sterna*, and the species was capable of adapting to the environmental fluctuations of the estuary, such as the high temperatures registered, as well as the low transparency observed (Secchi disk depth of 30 cm). The scarcity of negative environmental influences corresponds with the exponential mass growth of both the shell and soft tissues of the oysters. This is a well described behavior in the biology of marine organisms when they are not disturbed by environmental stressors. However, despite shell growth being generally continuous, in the last sampling period (late November 2018 to late January 2019) the antero-posterior and dorso-ventral axes did not exhibit growth; during this same period, low levels of seston and chlorophyll-*a* were registered, which could suggest the low availability of food could have been associated to the equally low growth in these shell axes. This was reflected on the negative relationship between temperature and shell growth observed in the PCA, but this hypothesis did not appear to be supported, since *P. sterna* endured temperatures within a similar range in Lodeiros et al (2018) without suffering mortality or slowing its growth.

The lowest levels of chlorophyll-*a* registered in the present study surpassed 6 µg L⁻¹, and just as Saxby (2002) suggests, these concentrations do not limit the growth and development of bivalve mollusks in cultivation. This author considers that concentrations of chlorophyll-*a* > 1 µg L⁻¹ are still adequate to sustain intensive cultivation of these

organisms. Nevertheless, seston increased again in January (2019), which suggests that food availability was not a limiting factor for the growth of the oysters. This last hypothesis is supported by the absence of decrements in soft tissue mass during January.

The sudden stoppage in shell growth observed at the end of the cultivation period could be mostly associated with competition for space inside the baskets, as no “unfolding” was performed during this period. Monteforte et al (2005) showed that the final shell volume of *P. sterna* seed depended on the cultivation density arranged under semi-controlled conditions (inside a nursery), whereas Treviño et al (2019) showed that *P. sterna* juveniles reached higher shell lengths when the individuals were cultured at lower densities (50 and 75 oysters/enclosure) than at higher ones (100 oysters/enclosure). The overcrowding of the oysters could have prevented them from expanding in size. Observations of the lantern nets during the end of the study, which show the oysters having almost no space to grow any further, would support this hypothesis.

Epibionts, or organisms that live as part of the many materials that attach to baskets and oyster shells, are a factor that can negatively affect oyster growth. These organisms can either clog the baskets and limit the amount of food that reaches the oysters, directly compete against the oysters for food in the vicinity, and even impair their movement (Uribe et al 2001; Lodeiros & García 2004). Although the presence of these organisms was not measured in this experiment, high epibiont accumulations were not observed on the shells of the individuals, but they were observed on the baskets. Regardless of this, the growth of the pearl oysters remained constant. In the same way, Treviño et al (2020) with studies on the culture of oysters (*Magallana gigas*) report a high accumulation of fouling in the baskets, particularly due to the invasion of the mussel *Mytella strigata*, which negatively interferes in the culture process. Studies centered around the quantification of fouling on both shells and baskets, as well as other elements of cultivation systems, are required to elucidate their impact and develop possible countermeasures to their actions.

The cultivated *P. sterna* individuals grew at an accelerated rate, reaching antero-posterior lengths of ~ 60 mm and dry tissue masses of ~ 4.6 g. This could translate to 20-25 g of meat which is very attractive for human consumption. Tissue accumulation with an elevated proportion of meat and shell mass is characteristic of the Pteridae family, as it occurs with *Pinctada imbricata* (Semidey et al 2010), and allows *P. sterna* to be ready for consumption at a size of just 40-50 mm, which is achieved in only 3-4 months of cultivation (implying several cultivations could be completed within the dry weather period of the estuary). However, cultivating oysters for pearl production starting from seeds ~ 10-20 mm would appear unfeasible, as the periods of rain and their subsequent effects on salinity would prevent individuals from reaching the size (70-80 mm) suggested for this species to produce pearls (Saucedo et al 1998; Serna et al 2014; Freitas et al 2020). Considering this, the strategy for pearl formation in the estuary would require starting with larger seeds, as other studies show that half-pearls (mabé) can be obtained in around 3-4 months of cultivation (Espinoza-Vera et al 2023). These results can be extrapolated to the tropical estuarine areas of the Eastern Tropical Pacific, provided these are not influenced by periods of elevated pluviosity.

Conclusions. The culture of *Pteria sterna* in the conditions of the Chone river estuary, Manabí, Ecuador is only feasible during periods of dry weather, since in rainy periods the drop in salinity causes death in the cultured populations. During the dry period, environmental factors had no negative effects on oyster growth and survival, allowing them to reach an anteroposterior length of ~60 mm and a mass of ~4.6 g after dehydration, equivalent to 20-25 g of wet soft tissue (meat) mass, which is considered fit for human consumption; however, they do not reach the sizes suggested for the development of pearls at through artificial implants.

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Conflict of interest. The authors declare that there is no conflict of interest.

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