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RESEARCH PAPER

Performance of the Winged Pearl Oyster *Pteria sterna* (Gould, 1851), Maintained in Hanging Culture at Three Depths, in the Eastern Equatorial Pacific

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Abstract

This study evaluated the performance of *P. sterna* under suspended culture at three depths (2, 6, and 10 m). Juveniles (dorso-ventral length 42.6±0.94 mm) were placed in enclosures suspended on a long line in Palmar (Ecuador) from October 2018 to October 2019. The antero-posterior axis of the shells, the shell, softs tissues, and biofouling masses, and the survival rate, were determined during one year of culture. Chlorophyll-*a*, total particulate matter (TPM), particulate organic matter (POM), salinity and temperature were studied at each depth. At the end of the cultivation period, growth and survival rates did not show significant differences between water depths, with all oysters attaining dorso-ventral lengths of 102-106 mm. Principal components analysis (PCA) revealed a direct and significant relationship between the variance of soft tissue dry mass growth and chlorophyll *a* concentration, salinity, particulate organic matter (POM) concentration, and temperature, during the culture period evaluated. Thus, the results obtained indicate that the suspended culture of the winged oyster *P. sterna* in Ecuador can be carried out effectively within the range of 2 and 10 m of depth, obtaining relatively high yields (growth-survival).

Introduction

Mollusks are a well-known fishery resource, cultivated to supply the great demand for animal protein worldwide (FAO, 2016). Principally, the cultivation of bivalves has been a model for sustainable economic development and marine conservation, as it provides coastal communities with an alternative economic activity to fishing (Cartier et al., 2012; Castiñeira, 2013). Among the activities related to bivalve aquaculture, pearl cultivation is an art that encompasses the biological production of pearls and is described as one of the most profitable. Pearls have been considered to be a representation of the beauty and perfection of nature in many cultures throughout history (Alagarswami and Dharmaraj, 1984). Currently, the cultivation of pearl oysters is a large industry that contributes to international exchange activities in countries where such cultivation is carried out at high capacity, such as Japan. Japan is considered the leading producer of pearls worldwide, reporting annual exports that exceed $500 million in revenue (Southgate and Lucas, 2008).

In the Americas, the pioneer country in the cultivation of pearl oysters is Mexico, with the winged pearl oyster *P. sterna* and the pearl oyster *Pinctada*

mazatlanica supporting the pearl industry (Serna-Gallo et al., 2014). Of these two species, *P. sterna* is of particularly excellent quality because it produces nacre with strong iridescence and a great variety of exotic colors (blue, green, pink, purple, and golden hues). *P. sterna* can also be used to produce high quality half pearls (mabé pearls) through different techniques such as the implantation of spherical or semi-spherical nuclei (Monteforte, 1990; 1991; Kiefert et al., 2004, Freites et al., 2020a).

The biology and vital processes of bivalves are controlled by environmental factors such as seston, temperature, and food availability, which together condition their growth, physiological status, and reproductive cycles (Gómez-Robles et al., 2013; Meza-Buendía, 2017). Thus, a deeper understanding of the how the environment affects bivalves is essential to optimize their cultivation and the production of high-quality proteins and pearls (Pit and Southgate, 2003; Saucedo and Southgate, 2007). Culture site conditions must be investigated thoroughly to examine their influence on the growth of juvenile pearl oysters (Yoo, 1986; Pouvreau and Prasil, 2001). Similarly, the depth at which the bivalves are placed must also be considered carefully, as it may impact growth and mortality due to the variability of environmental factors through the water column (García et al., 2016; Gasca-Carreon, 2019, Freites et al., 2020b).

Additionally, some authors suggest that bivalve growth could be hindered by intense biofouling on the cultivation meshes and shells of bivalves grown in enclosures (Claereboudt et al., 1994; Taylor et al., 1997; Lodeiros and García, 2004). These studies show that the development of tissue mass in cultivated scallops was 68% higher in clean enclosures than in those affected by biofouling, along with less biofouling at greater cultivation depths (Claereboudt et al., 1994). This highlights the importance of culture depth as a factor to consider in oyster farming and, together with temperature and food availability, forms the group of main physiological regulators in mollusk bivalves (Côté et al., 1993; Smitasiri et al., 1994; Lodeiros et al., 1998; García et al., 2016).

Other studies conducted on pearl oysters, such as *Pinctada fucata martensii* and *Pinctada margaritifera*, show that the growth rate of the oysters decreases as cultivation depth increases (Tomaru, et al., 2002; Gasca-Carreon, 2019). In these cases, however, the inverse relationship between growth and depth was attributed to a decrease in phytoplanktonic biomass, and the variation in temperature, which decreases with depth. It is known that temperature plays an important role in the physiology and metabolism of oysters and the regulation of vital processes, whereas food availability, composition, and quantity regulate reproductive events (Vite and Saucedo, 2008; Cáceres-Puig et al., 2009; Helmuth et al., 2010). As such, comparative growth studies based on depth and biological-environmental variables are needed to allow the determination of the optimal yields for pearl oyster cultivation.

Currently in the Ecuadorian coast, several studies seek to establish a baseline, to begin optimizing the cultivation of *P. sterna* in the sea (including the dual-purpose production of both pearls and soft tissues as a source of protein for humans). The cultivation of this oyster is pursued to diversify aquaculture, as this activity is currently centered almost exclusively on shrimp farming, which takes place on land-based facilities.

As further contribution to the knowledge on the cultivation of the winged pearl oyster *P. sterna*, the present study aims to determine the effect of the culture depth on the performance of this oyster on Ecuadorian waters of the Eastern Tropical Pacific Ocean.

Materials and Methods

Culture Conditions

A total of 690 winged oysters (recruited in artificial collectors deployed in Palmar, Santa Elena Province, Ecuador) (2°01'46.44"S, 80° 44'45.71"O; Figure 1) were placed a total of 15 baskets. At each experimental depth (2, 6 and 10 m) was assigned 5 of these enclosures. The baskets used were handcrafted following the recommendations described by Freites et al. (2019), that is, each basket was subdivided into three floors with

Figure 1. Location of the hanging cultivation area for the winged oyster *P. sterna* subjects at Palmar, Santa Elena Province, Ecuador

15 oysters on each floor, giving a total of 45 oysters per basket, and 225 oysters at each depth. Three additional replicates of five oysters each were used to determine the initial dorso-ventral height, obtaining a size of 42.6±0.94 mm.

Also, on each sampling, carried out between October 2018 to October 2019, three replicates of five oysters (randomly selected) were taken from each of the three depths (totaling 45 oysters per sampling), and the remaining oysters were re-distributed to a consistent cultivation density as these samples were taken. After each sampling, the oysters were transferred to the laboratory and the biofouling mass was detached by scraping the shell. The dorso-ventral length was measured with a Vernier (0.01 mm accuracy), and the soft tissues were separated from the shells by dissection. Body tissue and biofouling were dehydrated in a Nemmer brand stove at 80°C for 48 h. Once dehydrated, the dry weight of the soft tissues and shell mass was weighed with an analytical balance with 0.01 g precision. The condition index was calculated based on the proportion of soft tissue dry mass divided by the individual's dry mass, following the formula described by Narváez et al. (2008).

$$\mathrm{CI} = \frac{\text{Soft tissue dry mass x}100}{\text{Total dry mass}}$$

Additionally, the number of predatory gastropods *Stramonita* (=*Thais*) *biserialis* (Blainville, 1832), as well as other environmental factors were recorded.

Two more samplings were carried out in July and October (2019) to maintain a record of their performance after a complete year of culture. At these times, only the antero-posterior length, shell and soft tissues dry mass, CI and survival rates of the oysters were measured.

Environmental Variables

Parallel to the monitoring of the oysters' performance, the environmental variables at the three depths of the water column were examined to determine their influence on the oysters throughout only the first six months of the study period (October 2018 to March 2019), as financing problems did not allow the project to continue the sampling for the rest of the study period (six additional months). Salinity was measured a hand refractometer ATAGO S/Mill (range 0–

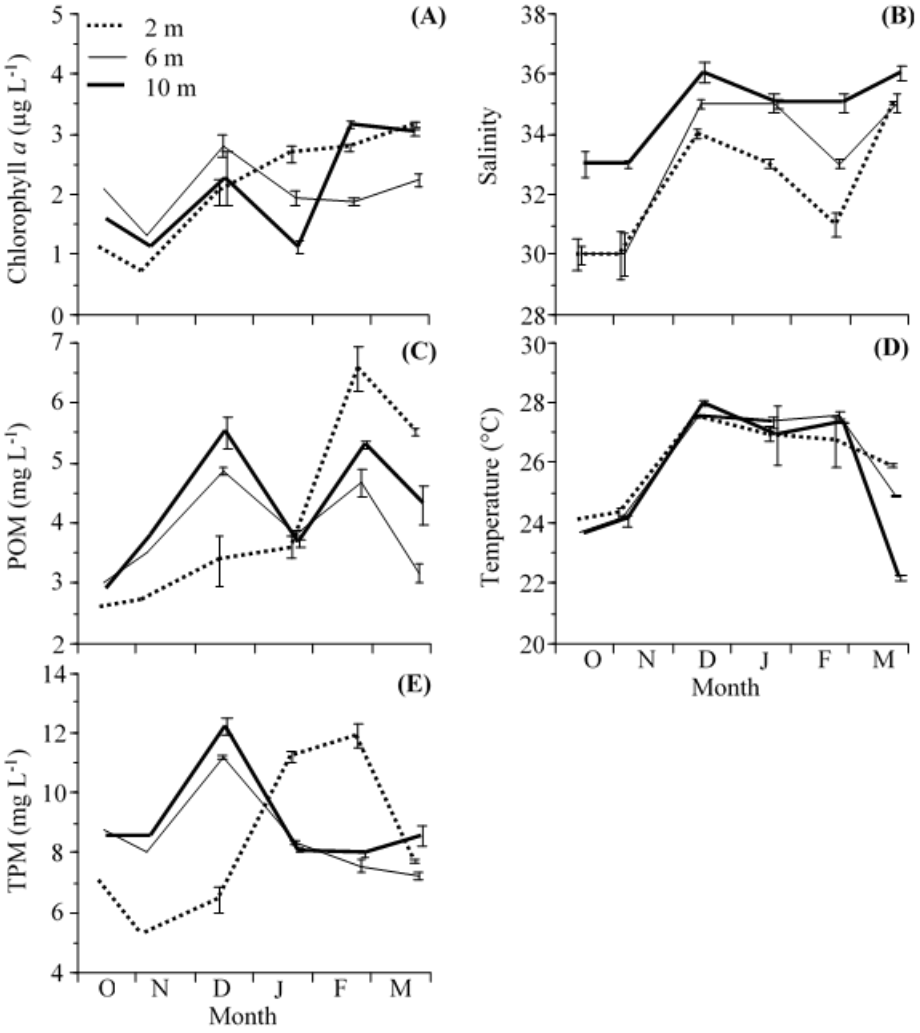

Figure 2. Phytoplanktonic biomass estimated by chlorophyll *a* (A), salinity (B), POM (C), TPM (D), temperature (E), observed between October 2018 and March 2019 on the Ecuadorian coast. The vertical lines indicate the standard deviation.

100 UPS) and Temperature were recorded with a HANNA HI 9829 multi-parameter instrument. For the quantification of seston and phytoplankton biomass, three liters of water were sampled from each depth using a Niskin bottle, transferred to opaque plastic bottles onboard. These water samples were pre-filtered (153 µm) to remove large particles and zooplankton and transferred to opaque plastic containers for transport to the laboratory. These water samples were passed through Whatman GF/F 0.7 µm filters that had been pre-washed and dried at 450°C for 4 h. After the samples were filtered, they were rinsed with isotonic ammonium formate (0.5 M) to remove marine salts. The total particulate matter (TPM) was obtained with the gravimetric method using an analytical balance after dehydration (65°C/24 h). The inorganic fraction (PIM) was determined after the combustion of the samples in a muffle furnace (450°C/ 4 h), and the organic fraction (POM) was calculated by subtracting the PIM mass from the TPM. Phytoplankton abundance was estimated from chlorophyll *a* using the photometric technique described by Strickland and Parsons (1972). These analyses were carried out at the National Center for Aquaculture and Marine Research (CENAIM), Ecuador.

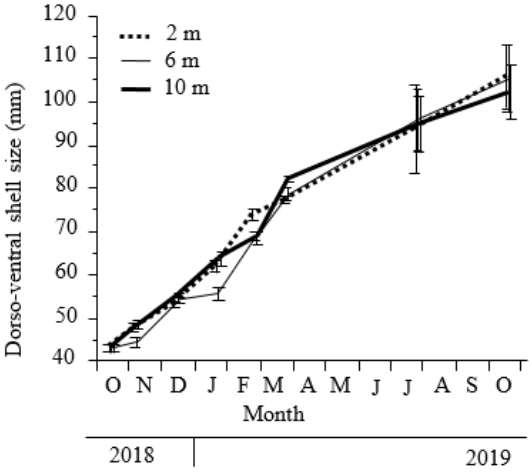

Figure 3. Growth of the dorso-ventral size of *P. sterna*, cultivated at different depths on the Ecuadorian coast. The vertical lines indicate the standard deviation.

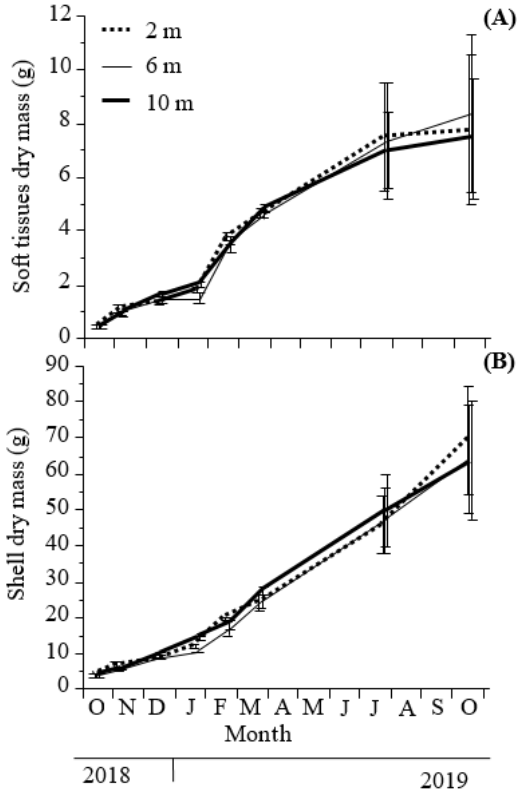

Figure 4. Soft tissue (A) and shell dry mass (B) of *P. sterna* cultivated at different depths on the Ecuadorian coast. The vertical lines indicate the standard deviation.

Statistical Analysis

To determine differences in parameters such as shell growth, condition index and survival rates, as well as the dry masses of the shell, soft tissues, and biofouling, the data were evaluated using ANOVA one-way analysis after verifying the assumptions of normality and homoscedasticity of the variances (Zar, 1984). If any of these factors showed a significant effect ($p<0.05$), a Duncan post-hoc analysis was employed.

Additionally, the influence that the environmental variables exerted on these biometric parameters during the first six months of the experiment (October 2018-March 2019) was studied using Principal Component Analysis (PCA) as an exploratory method of graphic ordination (Chatfield and Collins, 1980; Clarke and Warwick, 2001). The values of each environmental variable were contrasted with their own average values corresponding to the same period. These analyses were performed using the statistical software R (R Core Team 2015).

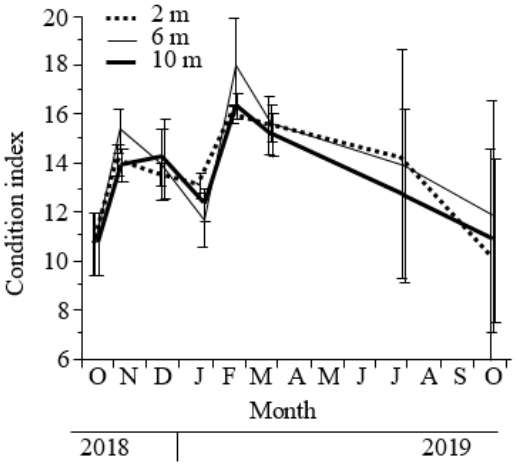

Figure 5. Condition index of the *P. sterna* (A), cultivated at different depths on the Ecuadorian coast. The vertical lines indicate the standard deviation.

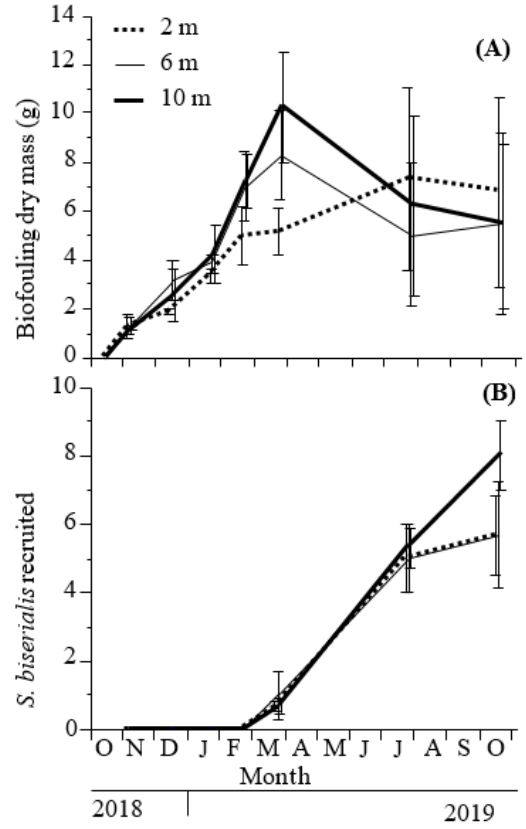

Figure 6. Biofouling dry mass fixed on *P. sterna* shell (A). Gastropod *S. biserialis* recruitment on the culture baskets (B), cultivated at different depths on the Ecuadorian coast. The vertical lines indicate the standard deviation.

Results

Environmental Variables

Phytoplankton: Chlorophyll *a* concentration showed a series of alternating increases and decreases (Figure 2A). In the period between October and December 2018, the highest concentrations were obtained at 6 m depth (2.80±0.17 µg L^{-1}), and minors values at 10 (2.27±0.46 µg L^{-1}) and 2 m depths (2.03±0.21 µg L^{-1}. The lowest concentrations of chlorophyll *a* were observed at the beginning of the study (October 2018), with values ranging between 0.7 and 1.3 µg L^{-1}. Salinity: this variable maintained similar fluctuations at the 2 and 6 m depths, varying between 30 and 36 PSU. A minimum of 30 PSU was recorded in February 2019. The following month, both depths showed an increase to 35 PSU (Figure 2B). Salinity at 10 m showed the lowest variability, and remained between 33-36 PSU. Seston: monthly variations in the concentration of POM (Figure 2C) showed a similar behavior to that of TPM (Figure 2E), but with different magnitudes throughout the experiment. Highest value of POM was recorded in February and March 2019 (6.6 mg L^{-1}) at 10 m depth. The total seston contents recorded in October 2018 were relatively low at all three depths, and then increased to values exceeding 10 mg L^{-1} in December 2018 (6 and 10 m) and February 2019 (2 m). Temperature: between November and December 2018, all three depths displayed temperatures between 23.5 and 24.3°C (Figure 2D) followed by an increase to a maximum of 28°C. Between December 2018 and February 2019, the temperature remained high, varying between 26.9 and 28°C, followed by a drop in March 2019 to 25.8°C (2 m), 24.8°C (6 m) and 22.1°C (10 m).

Shell Growth

In general, after 12 months of culture (finalized in October 2019) the antero-posterior axis of the shell (Figure 3) presented similar tendencies and showed sustained growth patterns in all of the depths studied, showing a dorso-ventral size of 105.57±7.08 mm (2 m), 105.27±7.66 mm (6 m) and 102.20±6.13 mm (10 m). Thus, at the end of the study, the differences in growth between depths were not considered significant ($p>0.05$).

Oyster Mass

Soft tissues dry mass, shell dry mass and the total dry mass of the oysters showed similar exponential growth patterns (Figure 4A, B). An increase was noticeable in the soft tissue dry mass growth curves at all depths from January 2019 until July 2019 (Figure 4A). At the end of the study, all the oysters presented a similar amount of soft tissue dry mass: 7.72±3.30 g (2 m), 8.31±3.00 g (6 m) and 7.40±2,27 g (10 m), without significant differences between depths ($p>0.05$). Similarly, at the end of the study no significant differences were observed in shell dry mass: 69.21±14.93 (2 m), 63.38±12.56 (6 m) and 63.38±16.58 g (10 m).

Condition Index

In general, the condition indices (CI) show high variability (Figure 5). This resulted in non-significant differences between depths for almost the whole experimental period. Further, two peaks were observed in November 2018 and February 2019, with a downward trend in the last months of the study.

Survival

The average percentage of survival of the oysters remained above 90% in the three cultivation depths until October 2019. At the end of the culture period, no significant differences were observed ($p>0.05$) between the depths.

Biofouling and *Stramonita biserialis* Recruitment

Biofouling mass fixed in the *P. sterna* shells sustained a similar increase at all 3 depths (Figure 6A). At the end of the study, biofouling did not show significant differences ($p>0.05$) between 2 m (5.12±0.93 g), 6 m (8.25±1.82 g) and 10 m depths (10.25±2.27 g). Regarding the recruitment of the gastropod *Stramonita biserialis*, no individuals were observed during the first 5 months of the study (Figure 6B). By March 2019, the average number of individuals was close to 1 gastropod/basket. However, in July and October, the average number of individuals at the 2 and 6 m depths was greater than 5 gastropods/basket, while the baskets placed at the 10 m depth showed average values exceeding 7 gastropods/basket. Nevertheless, these differences were not statistically significant ($p>0.05$).

Influence of Environmental Variables on Tissue Mass

In the first six months of culture at the three depths, the PCA analysis can be summarized as relationships that demonstrate the influence of the environmental variables on the observed variance in the soft tissues dry mass and CI:

2m = 0.104*soft tissue mass + 0.454*CI + 0.537*Chlorophyll *a* + 0.359*POM + 0.427766*Salinity + 0.427828*Temperature.

6m = 0.337*soft tissue mass + 0.439*Chlorophyll *a* + 0.403*POM + 0.465*CI+ 0.499855*Salinity – 0.251661*Temperature.

10m = 0.558*Soft tissues mas + 0.402*CI + 0.622*Chlorophyll *a* + 0.328*POM + 0.126*Salinity + 0.127*Temperature.

In these equations, the variables that contribute most to the variance in the soft tissue dry mass were chlorophyll *a*, POM, temperature, and salinity, generally with a positive correlation. Further, this analysis showed that the first two or three components explained 83.11%, 80.85% and 64.18% of the soft tissue dry mass variation at the 2, 6, and 10 m depths, respectively, indicating that these are acceptable graphical representations (Table 1).

Discussion

The trends in growth parameters (shell size and tissues mass) and survival were qualitatively and quantitatively very similar with no significant differences between depths. This suggests that the suspended culture of the winged oyster *P. sterna* can be carried out over the entire water column at the study site (at least up to 10 m) with >90% survival rate. The development of intensive cultivation of this species may be possible, guaranteeing high production levels (high growth/high survival). On the other hand, it was observed that in just 6 months (October 2018 to March 2019) the oysters at all depths reached the minimum size (75-80 mm) recommended for the implantation of both half pearls (mabés) and regular pearls (Ruiz -Rubio et al., 2006, Freites et al., 2020a). Similar growth rates to those observed here have been registered on the coasts of Santa Elena Province, Ecuador (Lodeiros et al., 2018, Freites et al., 2019, 2020a). This accelerated growth differs from studies carried out in Baja California, Mexico, where the minimum size (75-80 mm) was reached between 15 and 17 m water depth (Gaytan-Mondragón, 1992; Gallo et al., 2014). The relatively high growth rate of the individuals in this study is indicative of the high potential of the species to obtain the minimum sizes for pearl production.

The results showed in this study regarding the growth of *P. sterna* do not agree with those shown for the pearl oysters *Pinctada fucata martensii*, *Pinctada margaritifera*, and *Pteria colymbu*s, where lower growth rates were associated with an increment of the cultivation depth, due to a low availability of food (Tomaru et al., 2002; Gasca-Carreón, 2019, Freites et al., 2020b). In our case, the concentrations of chlorophyll *a* recorded between October 2018 and December 2019 were not very different between the three depths. Rather, the concentrations of chlorophyll *a* observed at 10 m depth were highest in February and March 2019, which might have favored the oysters grown at this depth.

The lack of differences in the growth of cultured winged oysters at the three water depths could be attributed to the water column mixing in the shallow areas close to the Ecuadorian coasts. This is caused by changes in semi-diurnal tides (>2.5 m), and wave action (height 1.4 and 1.6 m) perpendicular to the coast. In addition, the coast of Ecuador, or the equatorial front, is a transition zone where tropical waters flowing southward from the Bay of Panama mix together with the Humboldt current flowing northward from Peru. The coast is thus strongly influenced by coastal upwelling. Mixing of these two water masses takes place between Manta and Punta Santa Elena (Pennington et al., 2006; Fiedler & Lavin, 2017).

Regarding the variations observed in the condition index of the individuals cultivated at the three depths, two peaks were recorded: November 2018 and February 2019. Considering that the sexual maturity of this species is reached once their dorso-ventral height exceeds 50 mm (Saucedo and Monteforte, 1997), and that our cultivated individuals presented sizes of 50 mm in November, it is likely that several spawning events occurred during that month. This is probably be related

Table 1. Eigenvalues and variance percentages for each component of the PCA data corresponding to soft oyster tissue dry mass for all cultivation depths and their corresponding environmental variables: Particle organic material (POM), chlorophyll *a*, temperature and salinity (Evaluated period October 2018-March 2019).

2 m depth culture			
Component Number	*Eigenvalue*	*Percent of Variance*	*Cumulative Percentage*
1	2.92624	48.771	48.771
2	2.06044	34.341	83.111
3	0.59168	9.861	92.973
4	0.27488	4.581	97.554
6 m depth culture			
Component Number	*Eigenvalue*	*Percent of Variance*	*Cumulative Percentage*
1	3.19055	53.176	53.176
1	3.19055	53.176	53.176
2	1.6606	27.677	80.853
3	0.52054	8.676	89.528
4	0.46644	7.774	97.302
10 m depth culture			
Component Number	Eigenvalue	Percent of Variance	Cumulative Percentage
1	2.27114	37.852	37.852
2	1.57966	26.328	64.180
3	1.20932	20.155	84.335
4	0.80324	13.387	97.723

to the decrease in the CI in December 2018 and March 2019. Gallo *et al*. (2014) observed that spawning events in *P. sterna* were related to environmental variables. High primary productivity, such as those observed from December onwards, can trigger spawning in *P. sterna*, according to Gregori et al. (2019). Similar cases have been recorded in other marine invertebrate species, and a direct relationship was found between phytoplankton outcrops and a high recruitment rate of juveniles of commercial mollusks under high primary productivity conditions (Soria et al., 2014). On the other hand, in February 2019, the CI increased notably at all depths, agreeing with a period of temperatures close to 28°C and high availability of food. These conditions have been associated with periods of maturation and subsequent spawning in *P. sterna* (Cáceres-Puig et al., 2009; Treviño et al., 2019).

The relatively high survival rate (>90%) observed at the three depths was probably due to the use of cultivation baskets specifically designed and manufactured to prevent predation (Freites et al., 2019). However, although low but sustained mortality was observed between December 2018 and March 2019, it was not until March that recruitment of the predatory gastropod *Stramonita* (=*Thais*) *biserialis* was observed. Although the number of snails increased appreciably for July and October 2019, mortality did not show a notable increase during this last period. According to observations taken during the sampling, the gastropods may have led their predatory activity to other recently recruited bivalves, judging by the empty shells of juveniles observed inside the baskets.

Gastropod species of the genus *Stramonita*, belonging to the family Muricidae, have been reported as bivalve predators and the cause of significant mortalities in cultivations of oysters and other marine invertebrates (Brown et al., 2004; Herbert, 2004; Ramírez et al., 2009). On the other hand, although it has been reported that fish from the Balistidae family (*Balistes polylepis* and *Pseudobalistes naufragium*) attack and perforate culture baskets (Sonnenholzner et al., 2017; Lodeiros et al., 2018), attacks from these individuals were not recorded in this study, which probably contributed to a relatively high survival rate.

The principal component analysis performed between soft tissue dry mass, the CI, and the environmental factors, showed that the observed variance was directly and significantly related to chlorophyll *a*, POM, and temperature. In this sense, although the fluctuation of the phytoplankton biomass concentration was notable, it exceeded 1 µg L^{-1} for almost the entire study period. The average annual phytoplankton biomass values were 2-3 µg L^{-1}, which is the range of phytoplanktonic biomass established by Saxby (2002) for commercially exploitable bivalve growth.

Our results show that shell size growth was sustained throughout the study period, and exceeded 100 mm in October 2019. The relatively high growth rates of *P. sterna* during our study highlight the potential of this species to quickly obtain the minimum recommended size for pearl production; between 70 and 80 mm (Saucedo et al., 1998; Serna-Gallo et al., 2014, Freites et al., 2020a). The oysters achieved this in approximately 7 to 8 months according to our growth curves. These results contrast with those shown by Gaytan-Mondragon et al. (1993) who considered that *P. sterna* in the Mexican Pacific requires 24 months to reach the adequate size for nuclei implantation.

On the other hand, Cranford and Grant (1990) reported that organic debris may contribute to the oyster's diet in periods when phytoplankton concentrations are too low to meet their energetic demands. Other studies have performed comparative trials between different cultivation sites and cultivation depths for some bivalve species, and highlighted the importance of organic seston concentration (Toro et al., 1995; Kleinman et al., 1996, Freites et al., 2003).

Regarding salinity, a direct relationship was observed between this environmental factor and the soft tissue dry mass. Studies carried out on the effect of salinity on the metabolism of the pearl oysters *Pinctada fucata* (Liu et al., 2011) and *Pteria penguin* (Chen et al., 2012), showed that, depending on the rate of dissolved oxygen consumption and the O:N ratio, the optimum salinity lies between 26 and 36 PSU. These results suggest that the observed salinity range (30-36 PSU) at the cultivation site was probably insufficient to cause metabolic stress and negatively affect the performance of the oysters, as demonstrated by the growth and survival rates achieved.

The maximum water column depth in the study area was approximately 14 m, which restricted the study depths to 10 m. The close proximity of the deepest baskets to the seabed may affect the experimental design. For this reason, we recommend that future studies for *P. sterna* cultivation in deeper waters should also examine the effect of water depth on the performance of this species under suspended culture conditions.

Ethical Statement

All the procedures followed the guidelines for ethical and responsible research using in vivo animals for experiments (Kilkenny et al., 2010).

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Author Contribution

Franklin Jara: Provision of study materials, reagents, materials, laboratory samples, animals, instrumentation, or other analysis tools. Management

activities including annotation (produce metadata), and maintenance of research data.

Luis Freites: Conducting research and investigation, performing the experiments, data curation. Preparation, creation and/or presentation of the published work, specifically writing the initial draft.

María Gregori: Management and coordination responsibility for the research activity, planning and execution. Acquisition of the financial support for the project leading to this publication.

Adrián Márquez: Management and coordination responsibility for the research activity planning and execution. Management activities and maintain research data.

Jimmy Villón: Management and coordination responsibility for the research activity, planning and execution. Management activities and maintain research data.

Luis Trocolli: Management and coordination responsibility for the research activity, planning and execution. Management activities and Statistical analysis of the data.

Daniel Rodríguez-Pesantes: Provision of study materials, laboratory samples, animals, instrumentation, or other analysis tools. Management activities and maintain research data.

Cesar Lodeiros: Development or design of methodology preparation, creation and/or presentation of the published work.

Short title: *Pteria sterna* culture at three depths

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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