

# Hanging culture of juveniles of the winged oyster *Pteria sterna* in two baskets of different design

Cultivo suspendido de juveniles de la ostra alada Pteria sterna en dos cestas de diferente diseño

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# Keywords Growth,

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#### **Palabras clave**

Crecimiento, Cultivo suspendido, Ostras perleras, Depredación, Pacifico Tropical Oriental.

ABSTRACT | The aim of this study was to determine the growth and survival of juveniles of the winged oyster Pteria sterna, culture in two types of baskets manufactured with local materials. These were 'one floor baskets' (OFB) and 'three floor baskets' (TFB), hung from two 'long lines' in two study sites, Ayangue and Palmar, Province of Santa Elena, Ecuador, over a five months experimental culture period. Environmental variables monitored included total particulate matter and its organic and phytoplanktonic fraction (chlorophyll a), temperature, and dissolved oxygen. Individuals showed an initial length, height (dorso-ventral) and thickness (inter-valvar) shell axis of  $18.7 \pm 0.32$ ;  $18.2 \pm$ 0.29 and 7.4  $\pm$  0.18 mm, respectively; and the soft tissue mass, shell mass and total mass were:  $1.03 \pm 0.04$ ;  $0.11 \pm 0.005$ ;  $1.14 \pm 0.04$  g, respectively. At the end of the period, biometric and survival parameters were recorded. The individuals cultured in Ayangue using TFB showed increments in shell, soft tissues and total mass that were significantly higher than those from the OFB. Similar results, but with lower increments, were obtained by the individuals cultivated at Palmar site. The ANOVA II showed that the site, type of basket and the interaction between the two had a significant effect on the growth of the oysters. Survival exceeded 80% at both sites and for both basket types, but no significant differences were observed between site and basket type. Considering the growth and survival obtained for both types of baskets, we recommend the TFB baskets for winged oyster cultivation in these regions.

**RESUMEN** | El objetivo de este estudio fue determinar el crecimiento y la sobrevivencia de juveniles de la ostra alada Pteria sterna, cultivadas en dos tipos de cestas manufacturadas con materiales locales. Estas fueron 'cestas de un piso' (OFB, en sus siglas en inglés) y 'cestas de tres pisos' (TFB, en sus siglas en inglés), suspendidas en dos líneas largas o 'long lines' ubicados en dos sitios de estudio, Ayangue y Palmar, Provincia de Santa Elena, Ecuador, por un periodo experimental de cinco meses de cultivo. Las variables ambientales monitoreadas incluyeron la materia total particulada y su fracción orgánica, Clorofila a, temperatura y oxígeno disuelto. Los individuos mostraron tallas iniciales de los axis de la concha: largo, alto (dorso-ventral) e intervalvar de 18.7  $\pm$  0.32; 18.2  $\pm$  0.29 and 7.4  $\pm$  0.18 mm, respectivamente; y masa seca de los tejidos suaves, concha y total de  $1.03 \pm 0.04$ ;  $0.11 \pm 0.005$ ;  $1.14 \pm 0.04$  g, respectivamente. Al finalizar el periodo de cultivo, los parámetros biométricos y la sobrevivencia fueron documentadas. Los individuos cultivados en Ayangue en las cestas TFB mostraron incrementos en masas de la concha, tejidos suaves y total significativamente más altos que la de los cultivados en las OFB. Resultados similares, pero con menores incrementos, fueron obtenidos por los individuos cultivados en Palmar. El ANOVA II mostró que el sitio, tipo de cesta y su interacción tuvieron un efecto significativo en el crecimiento de las ostras. La sobrevivencia fue mayor al 80% en ambos sitios de cultivo y tipos de cestas, aunque sin diferencias significativas. Considerando el crecimiento y la supervivencia obtenidas en ambos tipos de cestas, se recomienda el uso de las TFB tipo de cestas, para el cultivo de las ostras en esta región.

#### INTRODUCTION

*Pteria sterna* is commonly known as the rainbow-lipped pearl oyster or the Pacific wing-oyster. It is a marine bivalve mollusc of the family Pteriidae, currently found in shallow water (2.6 to 20 m) along the tropical and subtropical Pacific coast of America, extending from Bahía La Choya, Gulf of Baja California, Mexico (31.4°N), to Ancón, Lima, Perú (11.8°S) (Paredes et al., 1998; Coan and Valentich-Scott, 2012). In the Eastern Tropical Pacific coast, a high but periodic availability of *P. sterna* spat has been observed through natural catch using artificial collectors (up to 4–5 juveniles of 10 mm length over 5 cm<sup>2</sup> of sardine fishing net (Lodeiros et al., 2017). This may support a growing industry in the production of pearls and/or food, as well as for the by-products from the shells. Preliminary studies (Jara et al., 2016; Lodeiros et al., 2017, Treviño et al., 2019) reported high growth rate of *P. sterna* in equatorial waters (0° and 2°S). However, more studies are necessary to examine *P. sterna* growth and survival in tropical waters to determine the feasibility of cultivation.

Growth and survival of cultured bivalves is influenced by many factors including stocking density, predation, fouling, cleaning regime and type of culture unit (Pit and Southgate, 2003). With regards to this last aspect, numerous studies shown that the design of culture enclosures significantly effects the growth and survival of winged and pearl oysters in hanging culture conditions (Gaytan-Mondragon et al., 1993; Southgate and Beer, 1993; Friedman and Southgate, 1999; Ruiz-Rubio et al., 2006; Millione and Southgate, 2011). Thus, the development of optimal culture methods can maximize growth and survival rates in juvenile pearl oysters and reduce the time required to reach operable size for pearl production (Millione and Southgate, *op cit.*)

On the other hand, the mortality caused by the action of some predators is an important factor that affects production rates and economic profitability in the aquaculture industry (Hickman, 2001). Predation could be minimized by suspending oysters in mid-water meshes of appropriate size, hence inaccessible to benthic predators (Quayle and Newkirk, 1990). However, suspended culture may increase the vulnerability of bivalves to other predators, particularly some species of fish of the Families Balistidae, and Monocanthidae (Alagarswami, 1987). Accordingly, Southgate and Beer (1996) showed that predation is a factor that contributes to high mortality of blacklip pearl oyster juveniles, *Pinctada margaritifera* (L.), during nursery culture in Kiribati, Micronesia.

Similar predation activity occurs on the Ecuadorian coast in the cultivation of *Crassostrea gigas* using hanging lantern nets on a long line (Lodeiros et al., 2018). This has been inferred by damaged mesh on the lantern nets only on the side in which the oysters were accumulated (Fig. 1). For this reason, the cultivation nets (Japanese pearl nets and lantern nets) have been covered with sacks made with "anchovetera"-type nets (1/2" mesh) to protect oysters from predatory fish (Sonnenholzner et al., 2017). With the protective sack (used traditionally in some hanging cultures of *Crassostrea gigas*) fouling was reduced by up to 40%. However, oyster growth in the protected nets was decreased, presumably because reduced water exchange inside the baskets limited food availability (Sonnenholzner et al., *op cit.*). In addition, the protective meshes require greater financial investment, labor and maintenance costs and therefore reduce the profitability of the crop. Given the decrease in the availability of food in the double mesh baskets, and the consequent decrease in growth of the bivalves cultivated, optimization of the culture method and studies on materials that are resistant to predator are essential.

In the present study, the growth and survival of juveniles of the winged oyster *Pteria sterna* were determined for two types of baskets manufactured with local materials.



Figure 1. Evidence of predatory action on the mesh of a lantern net. Freites et al.

#### MATERIALS AND METHODS

#### Study area

Juveniles were obtained by means of natural recruitment using catchers made with "anchovetera" fishing net. These were installed on the Ayangue long line in May 2017 until the end of July 2017. The grow-out study was carried out between 10 August 2017 and 17 December 2017. Culture baskets were deployed on two long lines anchored 800 m from the shore at Palmar (2°01'46.44"S, 80° 44'45.71"O) and Ayangue (1°59'16.85", 80°45'38.61" O), Santa Elena Peninsula, Ecuador. The water depth at the longlines was 8 m and 18 m, at Ayangue and Palmar, respectively. Palmar was located at the mouth of and estuary with notable amounts of total material particulate matter (TPM) and low salinity (especially in the rainy season).

#### **Experimental culture**

At the beginning of the experimental period, a pre-selection of the initial size of juveniles based on shell axis length was made to exclude very large or small specimens. Size measurement of the shell axis was made with a digital calliper. Individuals showed an initial size of length, height (dorso-ventral) and intervalvar (thickness) shell axis of  $18.7 \pm 0.32$ ;  $18.2 \pm 0.29$  and  $7.4 \pm 0.18$  mm, respectively (Fig. 2). Initial soft tissue mass, shell mass and total mass were:  $1.03 \pm 0.04$ ;  $0.11 \pm 0.005$ ;  $1.14 \pm 0.04$  g, respectively. At the end of the experimental period, three baskets were subsampled randomly. The number of live oysters in each basket was recorded to determine the survival rate. Selected individuals were carefully cleaned of fouling on the shells. Soft tissues were then carefully dissected from each individual to obtain the mass of shell and soft tissues. All components were dehydrated in an oven (60 °C for 48 h) to obtain dry mass with an uncertainty of 0.001 g. The dimensionless condition index (CI) of the oysters was then calculated as the ratio of dry meat weight to dry shell weight (Beninger and Lucas, 1984).

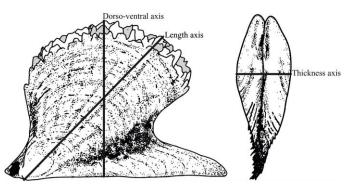


Figure 2. Shell axis measurements used to determine the growth: Shell length (from the umbo to the ventral edge of the shell), height (dorsal-ventral), and thickness axis (distance between the maximum convexity of the valves), as applied to *Pteria sterna*. Freites *et al.* 

#### **Basket design**

The cylindrical baskets used were manufactured with two layers. One layer was a 20-mm mesh plastic screening of dimensions 45 x 130 cm, tied to 3 or 4 circular frames (electrical PVC pipeline) with nylon fishing lines (80 pounds resistance). Each circular frame was hung from three plastic rope segments placed in an equidistant manner (Fig. 2). The baskets were built with two different designs. The first consisted of one floor or level (**OFB**), in which juvenile oysters were attached to three sections of 2-mm mesh plastic screening 40 cm in length x 15 cm width suspended in the central part of the upper basket level (Fig. 3A). In each mesh section, 15 juveniles of the oyster were placed and fastened with biodegradable classic rayon used for the stringing of mussel seeds in the Spanish method, for a total of 45 juveniles per basket. The second basket design had three floors or levels (**TFB**) separated from each other by 15 cm (Fig. 3B). Each floor was made with a circular piece of 20 cm radius (1256 cm<sup>2</sup> area) of the same mesh used for the body cylinder of the baskets, with three layers for the bottom of the basket and one in the other the levels. In each internal level, 15 juveniles were wrapped with the biodegradable net to avoid the loss of juveniles since the initial size of these was less than that of the mesh size. The groups of juveniles were placed in each of the three floor levels, giving a total of 45 juveniles per basket. Four replicate were used per basket type and deployed at 4 m depth on the long lines in Palmar and Ayangue.

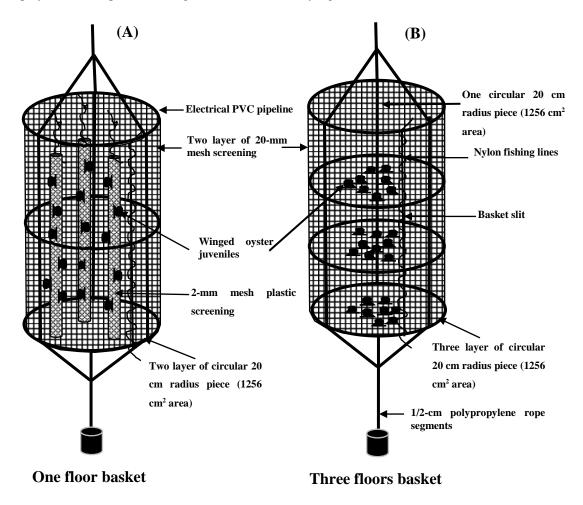


Figure 3. Two different design of the experimental baskets studied. A) one floor basket (OFB) and B) three floor basket (TFB).

#### **Environmental variables**

All variables were samples for three weeks. Water temperature was monitored with an electronic YSI 550A-12 CC. Samples for phytoplankton biomass and total seston were taken using a Niskin bottle and

transferred on board to an opaque plastic bottle. All samples were transported to the laboratory in isothermal containers. Then, two 1 L replicates were pre-filtered (153  $\mu$ m) to remove large particulate matter and zooplankton and used to determine chlorophyll *a* and total particulate matter for each water depth. These samples were filtered on pre-combusted (450 °C for 4 h) and weighed GF/F 0.7- $\mu$ m filters and rinsed with isotonic ammonium formate (0.5 M). Phytoplankton abundance was estimated as chlorophyll *a* using the spectrophotometric method following Strickland and Parsons (1972). Total dry particulate matter (TPM) was established as the weight measured after drying the filters to constant weight at 80 °C for 48 h. Particulate organic seston (POM) corresponded to the weight loss after ignition at 450 °C for 4 h in a muffle furnace.

#### **Predatory action of fishes**

At the end of the experimental period, the baskets were inspected for evidence of the predatory triggerfish on the mesh plastic screening and on the winged oyster individuals. Predation for each basket type and their respective replicates was gauged by the number of damaged sites on the mesh that allow the fish to insert their jaws and access the oysters, and in the number of broken or lost juveniles.

#### Statistical analysis

At the end of the experiment, the growth, condition index, and survival of winged oysters were analyzed using a one-way ANOVA after the verification of the assumptions of normality and homogeneity of variances. If any of these factors showed a significant effect (p<0.05), a Duncan post-hoc analysis was applied. Further, biometric data (shell, soft tissue and total dry mass, length, height, intervalvar shell axis), CI and survival were also analyzed using two-way ANOVA, where biometric parameters were considered as dependent variables and two culture sites and basket design were used as fixed factors. The biometric oyster data were log-transformed and survival percentages transformed into arcsine (Sokal and Rohlf, 1979).

#### RESULTS

#### **Environmental variables**

Similar trends in environmental variables were observed at the Ayangue and Palmar sites (Fig. 3). The average values of Chl-*a*, POM, TPM and temperature were also similar. The largest differences between sites were noted for TPM, although not were significantly different (Fig. 3D).

#### Growth

#### Shell, soft tissues and total mass increment

Individuals of the winged oyster cultured in the long line located at Ayangue in the TFB (Fig. 4A) obtained increases in shell mass (16.60  $\pm$  1.73 g), soft tissue mass (2.93  $\pm$  0.21 g) and total mass (18.98  $\pm$  1.77 g) that were significantly higher (*p*<0.05) than for the oysters cultured in the OFB (11.60  $\pm$  1.12 g, 1.77  $\pm$  0.29 g and 12.36  $\pm$  1.38 g, respectively). Similar results but to a lesser magnitude were obtained for the individuals cultivated at Palmar site (Fig. 4B). With the exception of the soft tissue mass for the oysters grown in the TFB (1.43  $\pm$  0.36 g), no significant differences were observed between the oyster of the OFB (0.83  $\pm$  0.28 g).

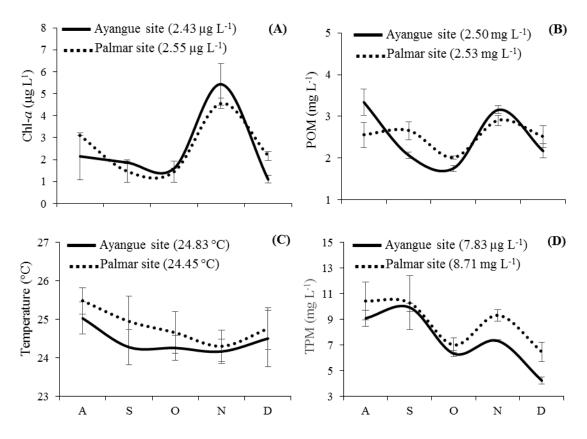


Figure 4. Environmental variables during the experimental period. A) Chlorophyll *a*; B) POM; C) Temperature; D) TPM. Freites et al.

#### Shell axis increment

No significant differences were observed between the shell axes lengths in individuals cultivated in both basket designs at Ayangue (Fig. 5A). At Palmar, however, a significantly higher increment in the length  $(35.58 \pm 4.21 \text{ mm})$  and height  $(30.90 \pm 3.97 \text{ mm})$  of the oysters grown in the TFB was observed (Fig. 5B) compared to the oysters cultured in the OFB  $(32.90 \pm 1.93 \text{ mm} \text{ and } 28.67 \pm 2.01 \text{ mm}$ , respectively). Two-way ANOVA analysis showed that culture sites and basket design had a significant effect on the increment of the different biometric variables studied including shell mass, soft tissues mas, total mass, shell length and height (p < 0.05; Table 1), whereas their interactions had a non-significant effect, except for the total mass.

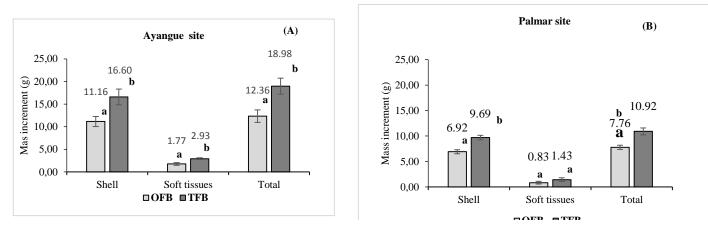


Figure 5. Increment in shell axis, soft tissues, and total mass of juveniles of the winged oyster *P. sterna* cultivated in OFB and TFB at the two different sites (Ayangue and Palmar). Different letters above the bars indicate significant differences among treatments (ANOVA, P < 0.05). Values over the bars represent the biometric mean.

Table 1. Results of two-way ANOVA analysis evaluating the effects of 'sites' (Ayangue and Palmar) and 'basket design' (one floor and three floors) factors on the growth of shell tissue and total dry mass; besides, length, height and thickness axis of shell, condition index and survival).

Variable	Source of variation	d.f.	Sum of squares	F-ratio
Shell mass	A: Site	1	93.287	81.41***
	B: Treatment	1	50.520	44.09***
	Interaction: A*B	1	5.3681	4.68NS
	Error	8	9.1670	
Soft tissue mass	A: Site	1	4.450	53.74***
	B: Treatment	1	2.313	27.93***
	Interaction: A*B	1	0.236	NS
	Error	8	0.662	
Total mass	A: Site	1	120.295	84.78***
	B: Treatment	1	71.668	50.51***
	Interaction: A*B	1	9.024	6.36*
	Error	8	11.352	
Shell length	A: Site	1	544.033	104.63***
	B: Treatment	1	52.920	10.18**
	Interaction: A*B	1	0.963	0.19NS
	Error	8	41.60	
Shell height	A: Site	1	444.083	45.63***
	B: Treatment	1	33.333	3.42NS
	Interaction: A*B	1	1.920	0,20NS
	Error	8	35.217	
Thickness shell	A: Site	1	29.079	6.61*
	B: Treatment	1	19.969	4.54NS
	Interaction: A*B	1	8.200	1.86NS
	Error	8	35.217	
Condition index	A: Site	1	1.613	0.65NS
	B: Treatment	1	12.102	4.87NS
	Interaction: A*B	1	8.736	3.52NS
	Error	8	19.881	
Survival	A: Site	1	0	0.00NS
	B: Treatment	1	80.658	0.34NS
	Interaction: A*B	1	0	0.00NS
	Error	8	1902.880	

NS, not significant; \* *p* > 0.05; \*\* *p* > 0.01; \*\*\* *p* > 0.001.

# **Condition Index**

Oysters cultivated in the TFB suspended at Ayangue site showed significantly higher (p<0.05) condition index (14.34 ± 1.92) than those cultivated in the OFB (10.63 ± 1.36) (Fig. 6A). In contrast, oysters grown in at Palmar site in the TFB (11.92 ± 1.66) did not show significant differences with oysters grown in the OFB (11.60 ± 1.29) (Fig. 6B). Two-way ANOVA analysis showed that at the end of the study period, basket design, culture site and their interaction had a non-significant effect on the CI (p<0.05; Table 1).

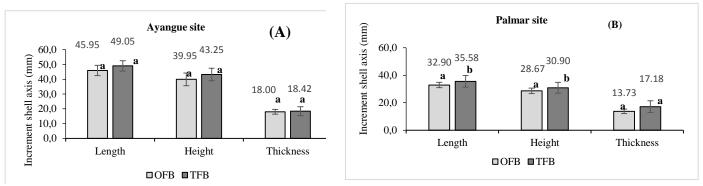


Figure 6. Increments of length, height and intervalvar axis of the shell of juveniles of the winged oyster *P. sterna* cultivate in OFB and TFB at the two different sites (Ayangue and Palmar). Different letters above the bars indicate significant differences among treatments (ANOVA, P < 0.05). Values over the bars represent the biometric mean.

## Survival

Survival of the cultured oysters (Fig. 7) exceeded 80% at both sites and in both basket designs with no significant differences between sites. Two-way ANOVA analysis showed that at the end of the study period basket design, culture site and their interaction had a non-significant effect on survival (p<0.05; Table 1).

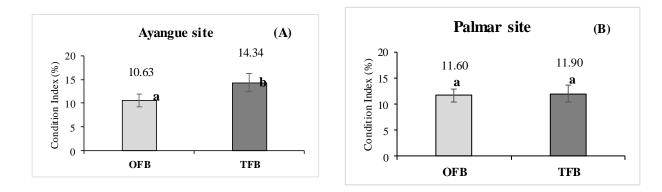


Figure 7. Condition Index (CI) of the juveniles of the winged oyster *P. sterna* cultivated in OFB and TFB at two different sites (Ayangue and Palmar). Different letters above the bars indicate significant differences among treatments (ANOVA, P < 0.05). Values over the bars represent the biometric mean.

#### **Predatory activity**

In neither case (site or basket design), the observed mortality could not be attributed to the predatory action of the triggerfish *Pseudobalistes naufragium* (Jordan & Starks, 1895) because the damage on the external net was of low (Fig. 8A) to medium magnitude (Fig. 8B). Neither were any juveniles of this fish species observed inside the baskets. However, juveniles of predatory gastropods of the Ranellidae and Muricidae Families, specifically individuals of *Linatella caudata* (Gmeling, 1791) and *Stramonita* (*Thais*) *biserialis* (Blainville, 1832), were observed.

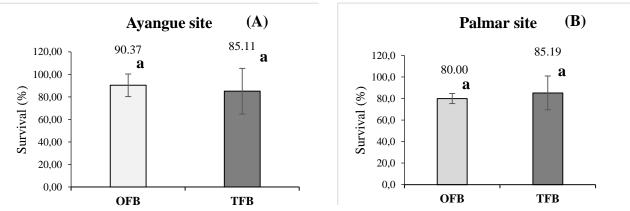


Figure 8. Survival of the juveniles of the winged oyster *P. sterna* cultivated in OFB and TFB at two different sites (Ayangue and Palmar). Different letters above the bars indicate significant differences among treatments (ANOVA, P < 0.05). Values over the bars represent the survival mean.

#### Discussion

Our results showed that the growth rate in the shell length of juveniles cultivated at Ayangue was 11.40 mm month<sup>-1</sup> (TFB) and 10.69 mm moth<sup>-1</sup> (OFB). These values could be considered to be relatively high compared with the 7.15 mm month<sup>-1</sup> shown by Lodeiros et al. (2018) for *P. sterna* (cohort II) at the same site and over the same cultivation period (August-December). These workers used pearl net baskets protected with a cover bag made of sardine fishing net (10-mm mesh screening) to avoid predation by fishes of the family Balistidae. The lower growth rate observed by Lodeiros et al. (*op cit.*) could be attributed to the type of basket used, to the smaller mesh screening of the pearl nets and mesh used to

protect the baskets, or to inter-annual differences in the environmental variables such as food availability and temperature. The growth rates shown in the present study were also higher than those for *P. sterna*  $(0.70-7.65 \text{ mm month}^{-1})$  cultivated in Acapulco, Mexico, by Serna-Gallo et al. (2014). Growth rates of *P. sterna* (8.1 mm month}^{-1}) cultivated in lantern nets at Palmar by Jara et al. (2016) were similar to those in the present study in TFB (8.27 mm month}^{-1}). With respect to the growth rate observed in other winged oyster species in Australia, Millione and Southgate (2000) reported a shell growth rate of *Pteria penguin* of 4.4-5.4 mm month}^{-1}, while Beer (1999) reported a growth rate of 5.9 mm month}^{-1}. Both growth rates are relatively lower than shown here for Ecuador.

Furthermore, the rate of growth of 11.40 mm month<sup>-1</sup> for the winged oyster cultured in the TFB at Ayangue suggests that shell length could reach 80 mm in only six months, as follows: Initial shell length (18.7 mm) + size increment obtained in TFB (49.05 mm) + one month of culture (11.40 mm) = 79.15 mm

This size is within the minimum size range required for pearl production (70–80 mm; Saucedo et al., 2014), and contrasts with the 24 months projection that Gaytán-Mondragon et al. (1993) estimate to reach the appropriate size for nuclei implantation in this species in the Mexican Pacific.

The conditions at the culture site can influence the growth of pearl oysters (Yoo et al., 1986; Pouvreau and Prasil, 2001) and winged oyster juveniles (Millione and Southgate, 2011, 2012; Freites et al., 2017). In accordance with the results of these authors, the culture site had a significant effect on the increment of the different biometric variables studied. These results suggest that, in spite of the short distance between the two sites ( $\approx$ 5 km), small changes in the culture conditions affect the growth rate of the cultured oysters. In this way, information on the growth performance of the winged oyster cultured under different environmental conditions is important to pearl farmers (Millione and Southgate, 2012). Shell growth rates also provide information on pearl growth, as shell increment and nacre deposition are positively correlated (Coeroli and Mizuno, 1985).

In contrast, the CI did not show significant differences between sites, suggesting that both groups of individuals had a similar physiological condition. This could be explained if the environmental variables showed similar values and trends in addition to similar average values of the experimental period. Only a minor difference in the TPM concentration was observed (<0.9 mg L<sup>-1</sup>), while the differences in POM concentration were 0.03 mg L<sup>-1</sup>.

The results show that the cultivated individuals did not suffer the predatory activity by the fishes of the Family Balistidae, judging by the magnitude of the damage caused to the outer layer of the basket only. Hence, the fish would not have had access to the oysters. Neither were observed any juvenile triggerfish inside the baskets. In addition, the dead oysters within the two types of baskets showed no evidence of fracture or rupture of the shells, or missing shells, as would be case if triggerfish were present. The empty shell of the dead individuals was present and without any obvious damage, which is instead characteristic of the predatory action of gastropods observed in the two basket types. These belong to the Family Rannelidae and Muricidae, specifically individuals of *Linatella caudata*. This species has been studied in the Western Pacific (Morton, 1990; Zhou and Pan, 2000), Indo-Pacific (Dharmaraj et al., 1987; Muthiah et al., 1987) and the Caribbean Sea (Freites et al., 2000; Malavé et al., 2012). *Stramonita (Thais) biserialis*, observed in almost all of the culture baskets, have also been blamed as a predator of marine bivalves (Herbert, 2004).

Although the oysters were exposed for five months to the potential action of predators, the survival observed in both sites and types of baskets was 80% (OFB in Palmar site) whereas survival was >90% in the OFB at Ayangue site. Over the five month study period, no maintenance was carried out to eliminate predators, or cleaning of biofouling. Freites et al. (2000) observed a mortality rate of 25 scallop/months (>90% scallop) that was caused by a single individual from the gastropod predator *Cymatium poulseni* (*Synonym Linatella caudata*).

Based on the relatively high growth rates (>10 mm month<sup>-1</sup>) and survival (>80%), in addition to easily available cheap materials from national markets for manufacturing the baskets (<5 USD by basket), we recommend routine use of three floor baskets for the cultivation of *P. sterna*. However, new studies with the objective to determine culture condition such as management of density, depth and culture site, and influence of the environmental variables on the rate of growth, need to be carried out.

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