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Performance of the winged pearl oyster *Pteria sterna* (GOULD, 1851), implanted for half-pearl (mabé) production in two size groups

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ABSTRACT

This study generated baseline information relative to overall performance of the winged pearl oyster *Pteria sterna* implanted at two groups (small and large) for half-pearl (mabé) production. This is a suitable technology to optimize the pearl production in the tropical coasts of Ecuador. The yield and quality of mabé pearl produced, as well as oyster condition index, increase in oyster survival, shell size and mass of tissues, and environmental variables were examined in this study. From 120 oysters used, 65 were of small size (25 nucleated, 25 non-nucleated and 15 for the initial sample; initial average shell length of 64.7 ± 5.3 mm; ~6 months old) and 55 were of large size (20 nucleated, 20 non-nucleated and 15 for the initial sample; 78.0 ± 5.8 shell length cm; ~10 months old). Hemispherical plastic nuclei (12.5 mm base diameter, 7 mm height) were used. All groups of oysters were anesthetized with 1% Eugenol and cultivated for five months (May through October 2017). At the end of the trials, only one dead oyster occurred in the small size group. A high percentage (29.2%) of adductor muscle over-growth on the nuclei occurred in small oysters, probably caused by the relatively fast growth rate (≥8 mm in 5 months). A lower percentage of muscle over-growth on the nuclei (5.6%), thicker nacre deposition, and higher occurrence of regular-shaped mabé pearls were observed in large oysters, compared to small oysters. This study suggests that large oysters (> 80 mm) should be preferably used for half-pearl production in *P. sterna* to ensure the highest quality standards for commercialization.

1. Introduction

Pteria sterna (Gould, 1851), commonly known as the rainbow pearl oyster or winged pearl oyster, is a marine bivalve (Pteriidae family) inhabiting the subtidal zone (Álamo and Valdivieso, 1997), from the lowest tide to 23 *m* depth (Mora, 1990). The species adheres to hard substrates (rocks, gorgonid corals and submerged metallic structures), coarse sand, and mangroves (Monteforte, 2005; Ordinola et al., 2010). Its distribution extends along a wide range of temperate, subtropical, and tropical coasts of the Pacific Ocean, from the upper Gulf of California (in Mexico) to Peru (Keen, 1971; Arízpe, 1992; Álamo and Valdivieso, 1997; Ordinola et al., 2010).

Since the 1900s, the ability of mantle tissue of different pearl oyster species to secrete prismatic nacre layers has been commercially exploited to produce cultured pearls. This process, which has changed little since then, provides the basis for an industry with increasing growth and value with different bivalve species (e.g. *Pinctada* spp., *Pteria* spp.) and gastropod species (*Haliotis* spp.). Today, pearl culture exists as a consolidated or growing industry in countries such as Fiji (Kishore et al., 2015), Tonga (Gordon et al., 2018, 2019), Mexico (Saucedo et al., 1998; Ruiz-Rubio et al., 2006), and Chile (Rojas-Figueroa et al., 2019). Despite this, the worldwide industry related to the production, sale, and marketing of cultured pearls has mostly relied on three pearl oyster species of the genus *Pinctada*: *Pinctada fucata*, *P. margaritifera*, and *P. maxima* (Taylor and Strack, 2008). Although Ecuador has high potential for the development of a similar industry due to the natural occurrence of *P. sterna* and mother-of-pearl *Pinctada mazatlanica*, there is little local information of their basic

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ecophysiological and reproductive biology in relation to pearl production in tropical areas of the Pacific (Serna-Gallo et al., 2014). In Mexico, for example, few studies have analyzed the factors influencing the production and quality of half pearls in the winged pearl oyster *P. sterna* (Saucedo et al., 1998; Ruiz-Rubio et al., 2006) or free and round pearls (Nava et al., 2000).

Half pearls produced from *Pteria penguin* are traditionally known as 'mabé' (Kripa et al., 2008). However, the term mabé is now used generically to refer to half pearls produced from a different number of bivalve species (Ruiz-Rubio et al., 2006; Kishore et al., 2015; Gordon et al., 2018, 2019). They are the result of a non-surgical procedure in which hemispherical plastic nuclei are cemented on the inner nacreous side of the shell and further covered by the mantle tissue that gradually secretes concentric layers of nacre material (Taylor and Strack, 2008). The cultivation of pearl oysters to produce mabé pearls requires a lower financial and technological investment than the cultivation of round pearls, and local communities can achieve this task by following adequate livelihood training programs (Haws et al., 2006; Southgate et al., 2006; Kishore et al., 2015).

For mabé production, the evidence indicates that in young, fast growing oysters the adductor muscle may begin to over-grow the nucleus, leading to its incomplete covering and reducing nacre thickness and lustre (Saucedo et al., 1998; Ruiz-Rubio et al., 2006). In turn, this effect can significantly reduce the value of resulting pearls. Similarly, nuclei placed too close to the shell edge may be covered with periostracum, favoring a high proportion of asymmetrical, dull, and low-quality mabé. Preliminary studies with *P. sterna* cultured in equatorial waters (Lodeiros et al., 2018; Treviño et al., 2019) show that the species can reach the minimum size required for pearl induction (70–80 mm shell height) within the first year (7–8 months), due to its high growth rates (> 8 mm/mo). Data describing shell growth rates also provide useful estimates of pearl growth, as shell growth and nacre deposition are positively correlated (Coeroli and Mizuno, 1985; Monteforte and Morales-Mulia, 2000; Kishore et al., 2018). This information illustrates the potential of *P. sterna* for mabé production, as occurs in Bahía de La Paz, Mexico (Saucedo et al., 1998; Ruiz-Rubio et al., 2006) and Bahía de Acapulco, Mexico (Serna-Gallo et al., 2014). These results contrast with the projection of 24 months that Gaytán-Mondragon et al. (1993) estimated for the species to reach the appropriate size for implantation and mabé pearl production in the upper Gulf of California, Mexico.

Previous studies with *Pteria* spp. also suggest that the number of implanted nuclei should remain between one to three, and on specific regions of shell (Saucedo et al., 1998; Kripa et al., 2008; Gordon et al., 2019). Similar evidence indicates that base diameter of nuclei used for mabé pearl production can vary from 7 to 9 mm in *P. sterna* (Monteforte et al., 1994; Ruiz-Rubio et al., 2006) and 10–14 mm in *P. penguin* (Kishore et al., 2015; Gordon et al., 2019). For example, nuclei placed from the central-pallial to the posterior area of the valve have a more homogeneous and thicker nacre covering (1.2–1.7 mm diameter in 14–16 months after implantation), and therefore, superior quality (Monteforte et al., 1994). In this shell region, the mabé show different colours and hues varying from silvery, golden, purple, blue and grey, while those formed near the margin or edge of the valves display darker hues (Saucedo et al., 1998).

Given the high growth rates of *P. sterna* in equatorial waters, the aim of this study was to generate baseline information relative to overall performance of two size groups of oysters implanted for mabé production. This as a strategy to advance the optimization of a suitable technology for the commercial use of the species, not only as a food source, but for mabé pearl production in Ecuador.

2. Material and methods

2.1. Implantation procedure and experimental design

A total of 120 *P. sterna* adults were used for the implantation trials. Of these, 65 were of small size (64.7 ± 5.3 mm shell length; ~6 months old) and were divided into 25 nucleated, 25 non-nucleated and 15 oysters for the initial sample. The remaining 55 oysters were of large size (78.0 ± 5.8 mm; ~10 months old) and were divided into 20 nucleated, 20 non-nucleated and 15 for the initial sample. Oysters from both groups (small and large) and treatments (nucleated and non-nucleated) were anesthetized for 15 min in a solution containing 1 mg L^{-1} Eugenol (clove oil) to facilitate nuclei implantation. Oysters were considered completely relaxed when they had no reaction to physical stimuli, or when the valves did not close when removed from the relaxant (Mamangkey and Southgate, 2009; Granados-Amores et al., 2018).

One hemispherical plastic nucleus (7 mm height, 12.5 mm base diameter) was cemented on the center of the left valve of each oyster using polycyanoacrylate glue (UHU™) (Haws et al., 2006; Ruiz-Rubio et al., 2006; Kripa et al., 2008; Kishore et al., 2015). Oysters were implanted following standard procedures described by Kishore et al. (2015). Each nucleus was located far from the adductor muscle in a place not affecting the normal closing of the valves. The possible effect of stress and mortality due to desiccation during implantation was minimized by limiting the process to approximately 3 min per oyster.

2.2. Recovery, cultivation, and monitoring of nucleated oysters

Following nucleus implantation, oysters from the two groups were separately placed in two 500-L plastic tanks containing freshly aerated seawater to allow their full recovery for 48 h. All surviving oysters were then transferred to a cultivation system consisting of a long-line anchored at 800 *m* from the coastline (1°59′12.19"S; 80°45′39.00"O) in Ayangue Bay, Santa Elena Province, Ecuador. The oysters from both groups (small and large) and treatments (nucleated and non-nucleated) were cultivated in three-floor baskets, previously described by Freites et al. (2019). Seven to eight oysters occupied the first floor or level of each basket, covering 27% and 34% of the bottom. Both groups of baskets (small and large) were suspended at 4-m depth. Oysters were deployed in May 2017 and harvested for mabé grading in October 2017 (see below). Oysters were counted on a fortnight basis to determine survival (%) after being implanted.

Before implantation, three replicates of five oysters each (n = 15), as representative of each group (small and large) and treatment (nucleated and not nucleated), were measured (± 0.01 mm) for shell length and thickness (Fig. 1). Similarly, oysters were weighed (± 0.01 g) to obtain the initial dry mass of the shell, dry mass of the adductor muscle, and total dry mass of the oyster (soft tissues and shell). The dimensionless condition index (CI) of the oysters was finally calculated as the ratio of dry meat weight to dry shell weight (Beninger and Lucas, 1984).

At the end of the study, the oysters from both groups and treatments were collected and randomly divided in three replicates of five oysters each to determine changes in the aforementioned biometric variables. They were carefully cleaned of shell fouling and the soft tissues were

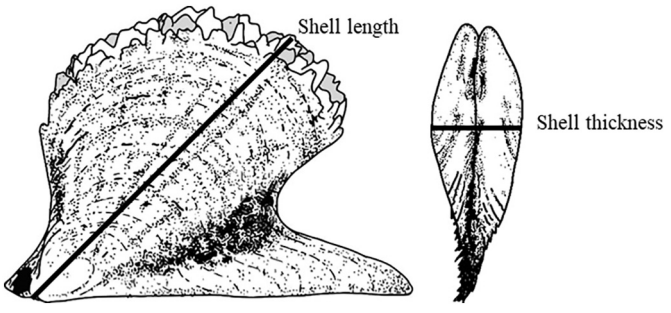

Fig. 1. Measurements used to determine the growth in shell length and shell thickness of the winged pearl oyster *Pteria sterna*.

removed to obtain the mass of the shell, adductor muscle and remaining somatic tissues. These body components were dehydrated in an oven (60 °C for 48 h) to obtain their dry mass (to the nearest 0.01 g). Data included the mean ± 95% Confidence Interval (CI).

Parallel to the monitoring of oysters, marine environmental variables were examined throughout the cultivation period. Water samples (in triplicate) were collected weekly between 0.5 and 1 m from the sea surface with 5-L plastic bottles. Water sub-samples were obtained for the determination of salinity (with a refractometer, ± 0.1 precision), as well as water samples filtered with 153-μm pore size mesh to eliminate macroplankton. The samples were transferred to 2-L dark plastic bottles and transported to the laboratory to estimate the phytoplankton biomass by the concentration of chlorophyll-*a* (Chl-*a*) and total seston. These analyses were performed by filtering the samples through Whatman GF/C filters (1.2 μm pore diameter) using Millipore vacuum filtration equipment. For the determination of Chl-*a*, the spectrophotometric method was used and the seston was determined using gravimetric techniques following Strickland and Parsons (1972). Water temperature was recorded every 30 min or 1 h using an electronic thermograph (Hobo, Onset, MA).

2.3. Evaluation of mabé pearl quality

After 5-months cultivation, the oysters were recovered and the quality of all mabé pearls produced was graded using the alphabetical nomenclature described by Matlins (1996) and Ruiz-Rubio et al. (2006). Prior to grading, the shells from each treatment were cleaned, the soft tissues removed, and all mabé were counted and photographed.

Mabé pearl quality was evaluated for key characteristics such as brightness (lustre), shape, and presence/absence of imperfections on the surface of nacre. The characteristics used to qualify the mabés are summarized in Table 1.

To determine the thickness of the nacre layer, the valve with the implanted nucleus was separated from the oyster and slowly carved with a Dremel drill, until the upper and central part of the nucleus was exposed. Scaled photographs of the mabés where then taken with a Sony TX30 camera, and the images were evaluated with Adobe Photoshop (v. 20.0.6), to digitally measure the thickness of the nacreous layer.

2.4. Statistical analysis of data

At the end of the trials, differences in shell growth, CI, and mass of soft tissues between nucleated and non-nucleated oysters, as well as differences in nacre thickness and quality of mabé pearls produced were analyzed using one-way ANOVA. The analyses were separately run for small and large oysters, after the verification of assumptions of normality and homogeneity of variances using the Bartlett test. When the effects measured were significant at $P < .05$, a Duncan post-hoc analysis was applied. Additionally, the interactions of oyster size group (small and large) and implanting treatment (nucleated and non-nucleated) on the shell growth, dry mass of soft tissues, and CI were analyzed using two-way ANOVA. Prior to analysis, all percentage data were arcsine transformed (Sokal and Rolhf, 1979).

3. Results

3.1. Effect of implantation on mabé yield and quality

Of all nucleated oysters, 92% and 100% of mabé pearls were produced by small and large oysters, respectively. Only one nucleus was lost after implantation in the small oyster group. The quality of mabé produced in small oysters (Fig. 2) was affected by their relative rapid growth rate in 5 months (> 8 mm in length increase), which resulted in the adductor muscle over-growing the nucleus (Figs. 2, 3E) in 29.2% of the cases. This value was significantly higher ($P < .05$) than that observed in oysters from the large size group (5.6%) (Fig. 2). Also in large oysters, a relatively higher, but not significantly different ($P = .133$) percentage of regular-shaped mabé occurred (72.2%, Figs. 2, 3A), compared to that from the small size group (45.8%).

No significant differences in the percentage of AA, A, B, and NC quality grade mabé occurred between oysters of both size groups ($P = .454$; $P = .180$; $P = .770$ and $P = .826$, respectively) (Fig. 2). Only one AAA mabé occurred in the large size group (Fig. 2). Some undesirable defects occurred in most of the mabés, such as folds and deformations (Fig. 3B) and incomplete coverage in response to mantle retraction on the corresponding side of the outer margin of the shell (3C). This was more evident in small oysters (41.7%) than in large oysters (38.9%). Thickness of the nacre layer on the top of the mabés was significantly higher ($P = .003$) in large oysters (1.15 ± 0.07 mm) than in small oysters (0.56 ± 0.05 mm) (Fig. 4).

3.2. Initial sampling before implantation of nuclei

The initial sampling before implantation showed that small oysters did not display significant differences in shell length ($P = .537$), CI ($P = .668$), soft muscle dry mass ($P = .154$) and total dry mass ($P = .460$) between nucleated and non-nucleated oysters. Similarly, differences in large oysters were not significant in shell length ($P = .625$), condition index ($P = .164$), muscle dry mass ($P = .244$) and total dry mass ($P = .907$) between nucleated and non-nucleated oysters.

3.3. Effect of implantation on oyster growth in shell size

Growth in shell length of small non-nucleated oysters (increase of 15.50 ± 3.53 mm) was significantly greater than that of small nucleated oysters (8.99 ± 2.09 mm) ($P < .05$) (Fig. 5A). In contrast, differences in shell length were insignificant ($P = .082$) between large nucleated (8.55 ± 1.64 mm) and non-nucleated oysters (7.29 ± 1.32 mm). In the small non-nucleated oysters (Fig. 5B), growth in the shell thickness (11.20 ± 1.33 mm) was significantly greater ($P < .05$) than that of small nucleated oysters (8.79 ± 1.76 mm). Similarly, growth in shell thickness in large non-nucleated oysters (6.21 ± 1.78 mm) was significantly greater than in large nucleated oysters (4.13 ± 0.76 mm) ($P < .05$).

In relation to the influence of oyster size (small and large), treatment (nucleated and non-nucleated), and their interactions (Table 2), only the length and thickness dimensions showed a significant interaction effect between both factors (ANOVA II, $P < .05$).

Table 1
Grading system used to evaluate pearl quality in this study (based from Matlins, 1996; Ruiz-Rubio et al., 2006; Kishore et al., 2015).

Grade	Mabé pearl characteristics
AAA	Highest quality of pearl. Perfect quality, with outstanding lustre, with at least 95% free from any forms of defect. Dark colours and good symmetry.
AA	Good quality. At least 75% of the surface does not have any form of defects. Good lustre with unvarying colours.
A	Medium quality. With at least 25% of defects. Varying colours with poorer symmetry and no over-tones.
B	Sufficient quality. Uneven surfaces, several flaws, but having good lustre.
NC	Pearls with no commercial value. Poorest lustre of all, with virtually all surface imperfections and very thin nacre.

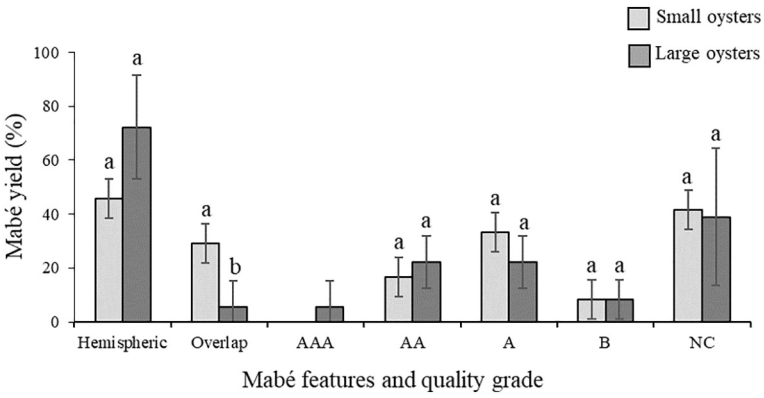

Fig. 2. Percentage in yield and quality grade of the mabé pearls produced by the two size groups (small and large) of the winged pearl oyster *Pteria sterna* in Ecuador. Data show the mean ± 95% Confidence Interval (CI). Different superscript letters denote significant differences between treatments using one-way (or two-way) ANOVA.

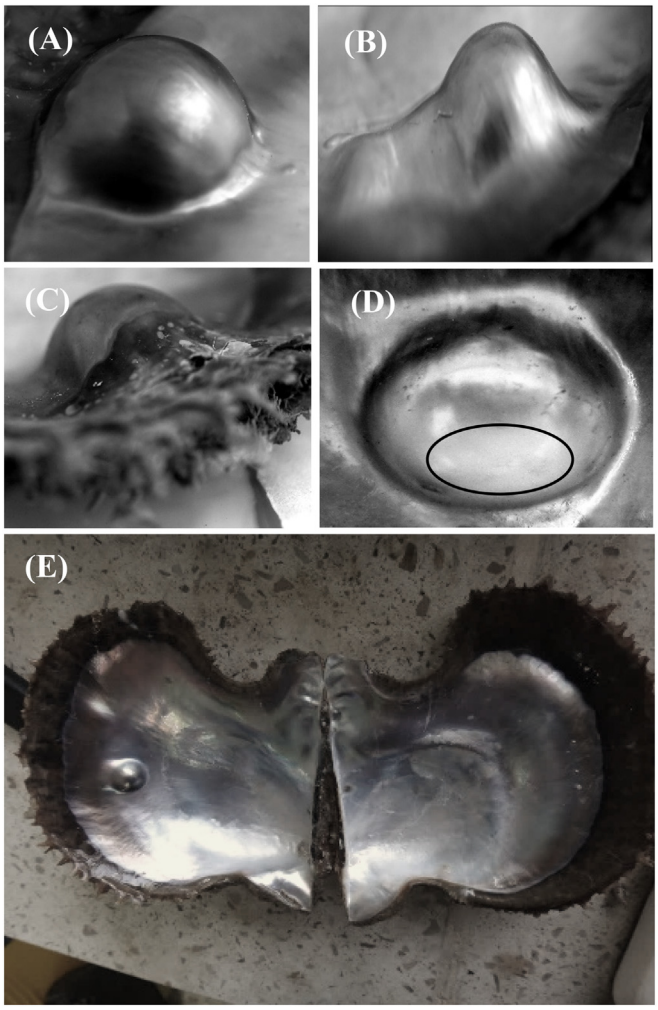

Fig. 3. Different qualities of mabé pearls produced in the winged pearl oyster *Pteria sterna* in Ecuador: Hemispherical mabé pearls with the most appropriate quality standards for commercialization (Fig. 3A). Mabé pearls with presence of folds and deformations (Fig. 3B); incomplete coverage in response to mantle retraction on the corresponding side of the outer shell margin (3C), and incomplete coverage of nacre by muscle over-growth, indicted by black circle (Fig. 3D). Mabé yield and quality increased using nuclei with lower height size (4 mm) and higher base diameter (9 mm) (Fig. 3E).

3.4. Effect of implantation on oyster growth in dry mass

Non-nucleated oysters from the small size group (Fig. 6A) gained significantly greater ($P < .05$) muscle dry mass (0.71 ± 0.27 g) than nucleated oysters (0.29 ± 0.22 g). In the large group, nucleated

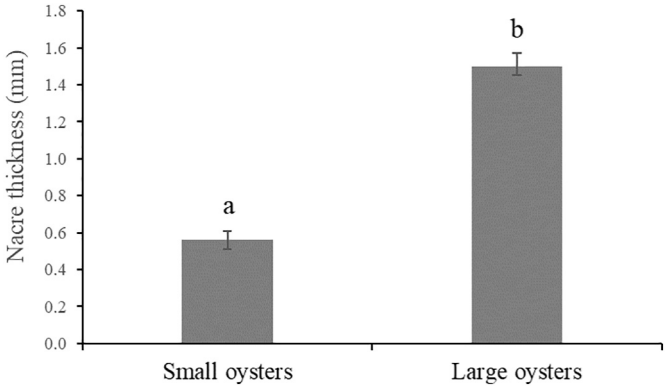

Fig. 4. Thickness of nacre layers deposited on the top of the nuclei in large and small oysters group.

oysters achieved significantly greater ($P < .05$) muscle mass (0.53 ± 0.24 g) than non-nucleated oysters (0.19 ± 0.35 g). In the large oysters, total dry mass was not significantly different ($P = .073$) between nucleated and non-nucleated oysters. Similarly, no significant differences ($P = .069$) occurred in small oysters in relation to total dry mass between nucleated (24.67 ± 6.57 g) and non-nucleated treatments (17.05 ± 4.30 g) (Fig. 6B).

3.5. Effect of implantation on oyster survival and condition index (CI)

Only one dead oyster occurred in the small size group. Here, the CI was significantly lower (ANOVA I, $P < .05$) in the implanted treatment (9.40 ± 1.15) than in the non-implanted treatment (12.59 ± 2.71) (Fig. 7). In the large size group, no significant differences in the CI (ANOVA I, $P = .122$) occurred between nucleated (9.98 ± 0.79) and non-nucleated oysters (11.17 ± 1.28).

3.6. Environmental variables

The variations in environmental factors that occurred during the study are summarized in Table 3. Chl-*a* showed the lowest values in August–September (1.6 μg L^{-1}) and the highest in October (4.6 μg L^{-1}). In general, POM showed similar variations than Chl-*a*, with lowest values in August and September (1.96 and 1.75 mg L^{-1}, respectively) and highest values also in August (4.29 mg L^{-1}). In contrast, TPM showed highest values in August and September samples (9.80 and 9.93 mg L^{-1}, respectively). Temperature peaked at the beginning of the study in May (27.52 ± 0.57 °C) and then steadily decreased until October (24.21 ± 0.49 °C). The average temperature for the entire study period was 25.48 °C.

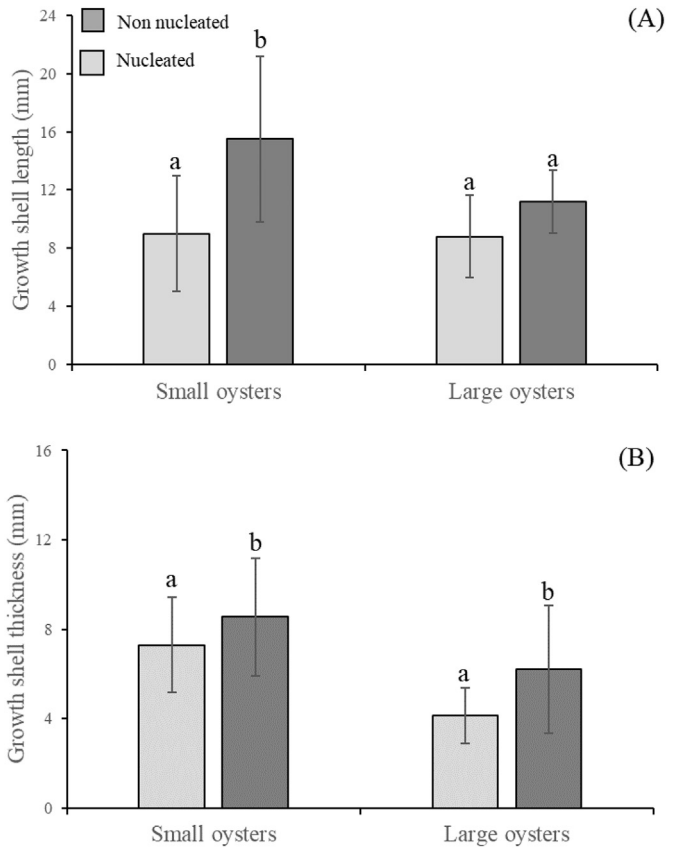

Fig. 5. Shell measurements of the two size groups (small and large) and implantation treatments (nucleated and non-nucleated) of the winged pearl oyster *Pteria sterna* in Ecuador. Data show the mean ± CI. Different superscript letters denote significant differences between treatments using one way ANOVA.

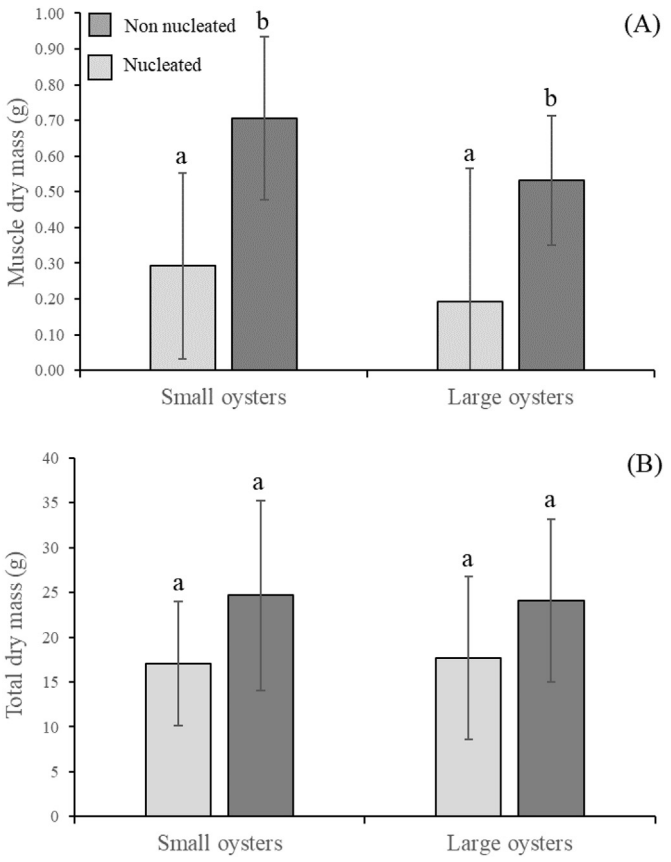

Fig. 6. Dry mass of soft body tissues of the two size groups (small and large) and implantation treatments (nucleated and non-nucleated) of the winged pearl oyster *Pteria sterna* in Ecuador. Data show the mean ± CI. Different superscript letters denote significant differences between treatments using one way ANOVA.

Table 2
Results of two-way ANOVA analysis evaluating the effects of oyster group (small and large) and treatment (nucleated and non-nucleated) on the shell growth and tissues dry mass of the winged pearl oyster *Pteria sterna* in Ecuador.

Variable	Source of variation	d.f.	Sum of squares	F-ratio
Muscle mass	A: Oyster group	1	3.2376	16.50***
	B: Treatment	1	1.0850	5.53***
	Interaction: A*B	1	0.2433	1.24 ns
	Error	36	7.0618	
Total soft tissues mass	A: Oyster group	1	25.2969	20.09***
	B: Treatment	1	0.0644	0.05 ns
	Interaction: A*B	1	0.1703	0.14 ns
	Error	36	45.3414	
Shell length	A: Oyster group	1	94.4026	4.03*
	B: Treatment	1	132.314	5.65*
	Interaction: A*B	1	117.958	5.04*
	Error	36	842.382	
Shell thickness	A: Oyster group	1	0.9278	0.16 ns
	B: Treatment	1	223.2180	38.05***
	Interaction: A*B	1	24.6364	4.20*
	Error	36	211.198	

ns: not significant.
* $P > .05$.
*** $P > .001$.

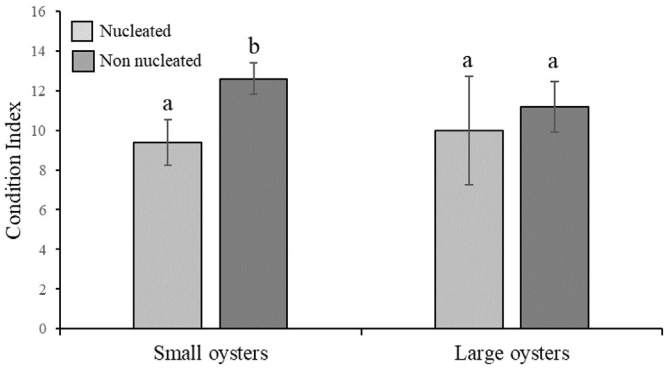

Fig. 7. Condition Index of the two size groups (small and large) and implantation treatments (nucleated and non-nucleated) of the winged pearl oyster *Pteria sterna* in Ecuador. Data show the mean ± CI. Different superscript letters denote significant differences between treatments using one way ANOVA.

4. Discussion

Our results showed that mabé production was 92% successful in small oysters and 100% in large oysters, with only one dead oyster and one nucleus lost after implantation in the small size group. Nuclei detachment and loss represented only 2.7% of total nuclei implanted in small and large oysters, which is relatively low compared to the 20% of nuclei loss reported by Kripa et al. (2008) in the pearl oysters *P. penguin* and *P. margaritifera*. These results demonstrate the potential for mabé production in Ecuador.

One of the factors that significantly reduced the quality of mabé pearls in this study was the higher occurrence of muscle over-growth upon the implanted nuclei in small oysters (5.6%), compared to that observed in large oysters (29.2%); this probably occurred because of the rapid growth of small oysters. Similar results were reported for *P. sterna* cultivated for mabé production in the Gulf of California (Saucedo

Table 3
Environmental variables registered during the experimental period in Ayangue Bay, Santa Elena Province, Ecuador.

Months	Chl-*a* (μg L^{-1})	POM (mg L^{-1})	TPM (mg L^{-1})	Temp. (°C)
May	2.43 ± 0.06	2.87 ± 0.12	6.23 ± 0.40	27.52 ± 0.57
June	2.20 ± 0.01	2.91 ± 0.12	7.13 ± 0.12	27.25 ± 0.39
June	3.17 ± 0.06	2.83 ± 0.26	8.23 ± 0.06	26.76 ± 0.32
July	2.99 ± 0.08	2.42 ± 0.13	6.73 ± 0.06	26.13 ± 0.27
July	2.20 ± 0.02	2.03 ± 0.10	7.75 ± 0.07	25.86 ± 0.38
August	3.25 ± 0.07	1.96 ± 0.03	6.85 ± 0.07	25.48 ± 0.34
August	2.70 ± 0.02	4.29 ± 0.19	8.80 ± 0.28	24.95 ± 0.66
August	1.60 ± 0.02	3.22 ± 0.03	9.80 ± 0.10	24.66 ± 0.54
August	3.40 ± 0.07	2.83 ± 0.11	8.57 ± 0.12	24.34 ± 0.63
September	1,87 ± 0.06	2.07 ± 0.08	9.93 ± 0.32	24.28 ± 0.46
September	1.60 ± 0.03	1.75 ± 0.07	6.33 ± 0.21	24.26 ± 0.33
October	4.60 ± 0.17	2.58 ± 0.03	7.33 ± 0.12	24.21 ± 0.49
Average	2.67 μg L^{-1}	2.65 mg L^{-1}	7.81 mg L^{-1}	25.48 (°C)

Chl-*a* = Chlorophyll *a*; POM = Particulate organic matter; TPM; Total particulate matter; Temp = Temperature of water.

et al., 1998; Ruiz-Rubio et al., 2006), where positioning of nucleus close to the adductor muscle in young oysters resulted in dull mabé with low commercial value. In contrast, the lower percentage of muscle over-growth and higher mabé yield in larger oysters is consistent with the lower increase in muscle mass (< 0.1 g) at larger sizes and the positioning of nuclei far from the muscle. In *P. penguin* implanted for mabé production at 96.5 ± 17.9 mm shell length, Kripa et al. (2008) did not observe muscle over-growth on the nuclei, regardless of their position on the shell. The results from this study suggest that the use of smaller nuclei (e.g. 4–5 mm height, 9–12 mm base diameter) could allow their placement closer to the shell margin (ensuring normal closing of the valves) and minimize muscle over-growth over implanted nuclei. Under these conditions, we estimate that good-to-high quality mabés, with good lustre, few defects on the surface, and an average nacre covering of 1.15 mm by large oysters, can be produced in less than six months. Previous studies have indicated that appropriate nacre thickness for commercialization is between 1.2 and 1.7 mm (Monteforte et al., 1994; Ruiz-Rubio et al., 2006).

Commercial nuclei for mabé implantation are available in high or low profiles, and various base diameters (5–16 mm) and forms (round, oval, drop-shape) (Haws et al., 2006; Kishore et al., 2015). These different characteristics determine the surface area of the nucleus and can influence how easily mantle tissue can deposit nacre layers over the nucleus. Gordon et al. (2019) showed that low profile nuclei (5.95 mm) produced a significantly higher number of good-quality mabé than high profile nuclei (9.17 mm), using 3.5 year-old (150 mm shell height) *P. penguin* oysters (two times greater than our large size group). Consequently, the occurrence of defects on the surface of the mabé, such as folds, bumps, cracks, stains, and incomplete coverage by the mantle tissue, may be minimized by reducing the height of implanted nuclei, which were relatively tall (7 mm height) for small oysters in our study. The use of low-profile nuclei may increase the likelihood that mantle tissue returns to its natural position and reduce the area of nuclei to cover following implantation (Gordon et al., 2019). Similarly, mantle tissue at the ventral-distal position folds and does not always cover a tall nucleus in the red abalone (*Haliotis rufescens*) used for mabé production in Chile, leaving it partially or totally exposed (Rojas-Figueroa et al., 2019). Gregori et al. (2020) carried out a study testing several nuclei sizes, with base diameters and heights as follows: “small” (9 mm × 4 mm), “medium” (11 mm × 6 mm), and “large” (14 mm × 13 mm), for the production of mabé pearls in *P. sterna* and reported a 23% occurrence of hemispherical mabés when using large nuclei, compared to the 70% of well-formed mabés observed with small and medium nuclei. Based on these results, we recommend implanting low profile nuclei (4–6 mm) and small base diameter (9–11 mm) to increase the occurrence of high-quality commercial mabés (Fig. 3E).

At the end of the trials, differences in the growth of oysters between nucleated and non-nucleated treatments were mainly reflected in the shell length, particularly towards the central margin where the nucleus was implanted. Saucedo et al. (1998) recommended this position as the best for nucleus placement in *P. sterna*, because the central pallial region, compared to the anterior and posterior regions, promotes a more homogeneous and thicker nacre covering (1.2–1.7 mm thickness in 14–16 months after implantation), and therefore, superior mabé quality. This positioning of the nucleus could explain the high percentage of muscle over-growth in the small size group, where a higher growth of the shell occurred. A similar frequency of muscle over-growth on the nucleus has also been observed in the red abalone (*H. rufescens*) in Chile, mostly in small specimens (55 mm shell length) and not in larger specimens (> 85 mm) (Rojas-Figueroa et al., 2019).

Growth performance of winged pearl oysters cultured under different environmental conditions is of interest to pearl farmers (Millione and Southgate, 2012). In this study, Chl-*a* and temperature fluctuated between 1.60 and 4.60 μg L^{-1} and 24.21 and 27.52 °C, respectively, resulting in a monthly increase of ~3.1 mm shell length (small group) and ~2.4 mm (large group) in non-implanted oysters. In others tropical environments (Acapulco, Mexico), *P. sterna* growth in shell height fluctuates between 2 and 7.7 mm, while Chl-*a* and temperature fluctuates between 2 and 3 μg L^{-1} and 25.0 and 29.8 °C, respectively (Serna-Gallo et al., 2014).

Our results show high survival of oysters from both size groups and implantation treatments, suggesting that transportation, anaesthesia with 1 ml L^{-1} Eugenol, and implantation in less than 3 min did not greatly affect overall performance of the oysters. Recently, it was reported that exposing adult *P. sterna* to similar Eugenol concentrations causes minimal interaction with antioxidant enzymes and little oxidative stress and oxidative damage to soft tissues (Granados-Amores et al., 2018). Another aspect of culture that could positively influenced survival was the maintenance and continuous monitoring of implanted oysters (fortnightly) that allowed regular removal of fouling and possible predators and competitors from the baskets.

This study provides new baseline information regarding mabé production from *P. sterna* in Ecuadorian coasts. Among the main results, the implantation process of nuclei should be highlighted, mostly because the production of mabés was 92% successful in small oysters and 100% in large oysters, with only one dead oyster and one nucleus lost after implantation. Additionally, using 1 mg L^{-1} Eugenol as anaesthetic was effective to fully relax the oysters and allow their recovery in a few minutes with a high survival percentage. We recommend a minimum implantation size of 80 mm shell length to prevent adductor muscle over-growth on the nuclei by the end of the pearl culture period. We finally suggest the use of smaller nuclei (lower profile, smaller diameter) to improve mabé quality yield.

Credit author statement

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

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

María Gregori: Management and coordination responsibility for the research activity planning and execution. Management activities to annotate (produce metadata), scrub data and maintain research data.

Adrián Márquez: Management and coordination responsibility for the research activity planning and execution. Management activities to annotate (produce metadata), scrub data and maintain research data.

Pedro E. Saucedo: Preparation, creation and/or presentation of the published work, including substantive translation.

Cesar Lodeiros: Development or design of methodology.

Preparation, creation and/or presentation of the published work, specifically writing the initial draft.

Declaration of Competing 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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