RESEARCH ARTICLE

Growth and Survival of Black-lip Pearl Oyster *Pinctada margaritifera* (Linnaeus, 1758) in Bamboo and Metal-framed Pocket Net Baskets Subjected to Cleaning and Without Cleaning Conditions

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ABSTRACT

The farming of black-lip pearl oyster *Pinctada margaritifera* (Linnaeus, 1758) has been a viable industry for small and large-scale farmers in the South Pacific, but not in the Philippines, where it is monopolized by large-scale farms primarily based on the gold-lip pearl oyster *Pinctada maxima*. To promote the industry among small-scale players, we simplified the culture method by using bamboo slats as frame materials and compared them to the common material used in pearl oyster culture, metal rods. A total of 400 individuals seven-month-old hatchery-produced *P. margaritifera* were used as experimental animals, distributed in the following treatments with five replications: T1 (metal-framed pocket net basket with monthly cleaning), T2 (metal-framed pocket net basket without monthly cleaning), T3 (bamboo-framed pocket net basket with monthly cleaning), and T4 (bamboo-framed pocket net basket without monthly cleaning). Growth was fast in the first four months and slowed down after that. Two-way analysis of variance found no significant differences between the average anteroposterior shell (APS) length increments of pearl oysters between two types of frame, and between two cleaning conditions. Survival rates did not significantly differ between types of basket frame, and between cleaning conditions. Parameters of the "Typical" von Bertalanffy growth model (L_∞ = 118.41 mm, K = 1.03 year^{-1}, and t_0 = 0.12) suggested that oyster would take about 23.16 months to reach 100 mm APS length, a size suitable for nucleus implantation. The life span of bamboo slats as basket frame was half of the metal frame, and the absence of cleaning has reduced the operational cost by up to 82.08%.

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1. INTRODUCTION

The black-lip pearl oyster *Pinctada margaritifera* (Linnaeus, 1758), found in almost all the seas of the tropical and sub-tropical belt (Victor et al. 1995), is well-known in producing black pearls, which are considered the queen of pearls (Libini et al. 2014). They are harvested for pearls and their thick nacreous shell layer known as mother-of-pearl to produce buttons and jewelry pieces (Ellis and Haws 1999; Kimani and Mavuti 2002).

Since 1976, the farming of *P. margaritifera* has been a viable industry in the tropical Pacific region (Ellis and Haws 1999; Johnston et al. 2019). In 1998, the French Polynesia produced around 5 tons of pearls worth over USD 150 million. The success in the culture of black pearls in the South Pacific, particularly French Polynesia and the Cook Islands, has led to intensified research on the ecology and biology of tropical pearl oysters (Fitridge et al. 2012; Gervis and Sims 1992; Kimani and Mavuti 2002; Le Moullac et al. 2012).

Black-lip pearl oyster farming in the South Pacific ranged from family or community arrangements to commercial-scale enterprises. Certain aspects of pearl farming do not require large capital outlay and use low technology and sustainable methods suitable for rural and under-developed areas (Ellis and Haws 1999). However, pearl oyster farming is beset with problems like biofouling. Various organisms growing on the nets and the oysters may reduce the growth and survival of the cultured species (Lacoste et al. 2014; Su et al. 2007); hence, regular removal of biofouling species makes pearl farming a labor-intensive industry (Haws 2002; Sims 1994). Furthermore, cleaning cultured pearl oysters in large-scale farms requires machines which may cause stress and mortality (Gervis and Sims 1992; Lacoste et al. 2014).

There are 30 registered pearl farms in the Philippines (Bondad-Reantaso et al. 2007), and 11 of these are found in Palawan (Baltazar and Dalusong-Rodriguez 2016). However, in a lucrative business monopolized by large companies, published papers about the growth of pearl oysters in the Philippines is almost nonexistent (see Bondad-Reantaso et al. 2007), even as of today. In these farms, gold-lip pearl oysters are grown in pocket net baskets with metal frames and are subjected to monthly cleaning, one of the major labor costs within the industry (Watson et al. 2009). These practices make pearl farming expensive and not well-suited for small-scale growers. To simplify and possibly reduce the operational cost in the culture method for this species, we partnered with a newly established small-scale pearl farm to evaluate the growth and survival of *P. margaritifera* using bamboo and metal-framed pocket net basket subjected to cleaning and without cleaning conditions.

2. MATERIALS AND METHODS

2.1. Source of stocks

The 400 individuals seven-month-old pearl oysters used in the experiment were obtained from the grow-out facility of Krisjewels Pearl Oyster Culture and Hatchery Incorporated (KPOCH Inc.) in Honda Bay, Puerto Princesa City, Palawan, Philippines. The average (± sd) anteroposterior shell (APS) length of all pearl oysters was 41.01 ± 5.54 mm (Table 1).

Table 1. The experimental setup and the average (± sd) anteroposterior shell (APS) lengths (mm) of the black-lip pearl oyster *Pinctada margaritifera* used in the study. T1: Metal-framed pocket net basket with cleaning; T2: Metal-framed pocket net basket without cleaning; T3: Bamboo-framed pocket net basket with cleaning; T4: Bamboo-framed pocket net basket without cleaning

Treatments	Basket Frame	Cleaning Condition	Shell per basket or replicate	Total shell per treatment	Initial average (± sd) APS length of oyster (mm)	Age at the start of culture (month)	Length of Culture (month)
T1	Metal rod	Monthly	20	100	43.96 (± 4.64)	7	9
T2		Without	20	100	37.36 (± 6.68)		
T3	Bamboo slat	Monthly	20	100	41.52 (± 5.41)		
T4		Without	20	100	41.20 (± 5.45)		

2.2. Experimental set-up

The pearl oysters were stocked in 20-pocket net baskets grouped into four treatments with five replications: T1 – metal-framed pocket net basket with monthly cleaning; T2 – metal-framed pocket net basket without monthly cleaning; T3 – bamboo slat-framed pocket net basket with monthly cleaning, and T4 – bamboo slat-frame pocket net basket without monthly cleaning (Table 1). On the assumption that the water conditions were homogenous within the 10 m x 10 m area, the pocket net baskets or replicates for each treatment were grouped to facilitate in situ cleaning and sampling. The first 10 pocket net baskets belonging to T1 and T2 were tied with 3 m rope (12 mm diameter) and hanged at 1 m intervals at the last 10-m portion of the long line rope (24 mm diameter) of KPOCH Inc. The other 10 pocket net baskets belonging to T3 and T4 were hanged in separate long line rope, installed 10 m away from the first long line. Floaters were attached at the end of each long line to maintain the desired position and depth of the pearl oyster baskets. During the first three months of the experiment, all pocket net baskets were covered with b-net to protect the pearl oyster from predators.

The experiment was conducted at the grow-out facility of KPOCH Inc. in Honda Bay, Palawan. The area is situated far from any river system, has moderate waves, and is about 8 m deep. The average water temperature and salinity during the conduct of the study were 31.73 ± 1.54 °C and 34.66 ± 0.36 ppt, respectively. A place like this is known to support good oyster growth compared to atoll lagoons (Pouvreau and Prasil 2001).

2.3. Samplings

The pearl oysters' APS lengths (Gervis and Sims 1992) were measured monthly using calipers to the nearest 0.1 mm. Because of their fragile shells, only 50% of the stock were measured for initial shell length, 75% were sampled on the next four months, and total sampling after that until the study ended. During the first two months, T1 and T3 were manually cleaned, after which, once a month cleaning using the pressurized water jet sprayer was applied to every pocket net basket (without removing the pearl oysters from the net) until the end of the experiment (Table 1). For treatments without monthly cleaning (T2 and T4), only the basket net covers were changed during the first three months to allow the normal flow of water at each framed net basket and left to the longline without changing the metal frame during the subsequent months. The bamboo frame was replaced on the 6th month. The number of live pearl oysters was counted every sampling. The nine-month study was conducted between January and October 2017.

2.4. Simple cost analysis

The following costs were estimated to determine the favorable method for the local farmers: a) materials in fabricating the metal and bamboo-framed net baskets, and b) labor cost during cleaning. A 6 mm steel bar (6 m long) would cost PHP 100 each and can be used as the frame for two net baskets, which would last for a year, thus PHP 500 as the total cost for 10 net baskets. A single-pole of bamboo costing about PHP 100 could be enough to fabricate the needed 10 frames for the first six months (life span of bamboo slats) and replacement from six months onwards. Each b-net basket would require 1 m^2 b-net valued at PHP 70 m^{-2}, hence PHP 1,400 cost for 20 baskets. The green net that held the oyster costs PHP 30 per meter, thus a PHP 600 for 20 framed nets. Other expenses are sinkers (PHP 50) and nylon twines (PHP 100), paint for the metal frames (PHP 100), and labor in fabricating the 20 framed net baskets (PHP 1,000). The bamboo frame needs replacement on the 6th month, adding a PHP 500 labor cost. The cost for the cleaning of 10 frames would require labor for two individuals (PHP 500 for two persons per sampling or PHP 6,000 per year). The rentals for banca and pressurized jet sprayer would each cost PHP 250 per sampling or PHP 3,000 per year. The cost for long lines, ropes, and floats was not included in the simple cost estimate as we only used a small portion of the KPOHC Inc. set-up.

2.5. Data analyses

Shapiro-Wilk normality test was performed prior to two-way analysis of variance, which tested the effects on growth (APS length increment) of cleaning, framing material, and interaction. In the analysis, the average monthly APS length increments per treatment were treated as a dependent variable, and the rest (cleaning condition and frame materials) were fixed factors. The survival data violated the assumption of normality even after data transformations, so we used the non-parametric equivalent to independent samples, the Mann Whitney U test, to compare whether there is a difference in the survival rate between types of frame and between cleaning methods. The analyses were performed using the trial version of SPSS 25 (IBM Corp. 2017). RStudio 4.0.4 (R Core Team 2021) was used to fit the "Typical" von Bertalanffy growth model (Ogle 2013) into the data to generate the L_∞ and K values. In addition, four sets of growth data taken before the start of the experiment (at ages 0.08, 0.22, 0.30, and 0.39 years) were added to the analysis. To predict the time required in rearing the pearl oyster until reaching 100 mm, the estimated $L\infty$ and K values were substituted to the typical von Bertalanffy growth formula (VBGF) $L_t = L_\infty\ (1\text{-}e^{-k(t-t0)})$, where: Lt - length at time t, L_∞ - mean asymptotic APS length, K - growth coefficient, and t_0 - mean length at time zero (Cailliet et al. 2006; Ogle 2016).

3. RESULTS

3.1. Growth and survival

The growth for all treatments was generally greater at the start of the study until May, before slowing down towards October (Figure 1). From an overall initial average (± sd) APS length of 41.01 ± 5.54 mm, the pearl oyster reached 81.51 ± 0.95 mm (range: 80.20 to 82.29 mm) in nine months, representing an average 4.5 mm monthly increment. However, during the first four months, the overall average (7.45 ± 1.38 mm) APS length increment was three times higher than the average increment for the last five months (2.14 ± 1.68 mm). The average (± sd) APS length increment in nine months (T1: 4.24 ± 3.31; T2: 4.76 ± 3.04; T3: 4.43 ± 3.41; and T4: 4.56 ± 3.11 mm, respectively) did not significantly differ between cleaning conditions ($P > 0.05$), and between the type of basket frame ($P > 0.05$). In addition, the interaction between cleaning conditions and type of basket frame was not significant ($P = 0.859$). Furthermore, survival rates (Figure 2) after nine months were very high (T1: 96 ± 2.24%; T2: 96 ± 6.52; T3: 97 ± 4.47; and T4: 94 ± 6.52) and did not differ significantly between types of frame ($P > 0.05$) and between cleaning conditions ($P > 0.05$).

Parameters of the "Typical" von Bertalanffy growth (VBG) model were $L_\infty = 118.41$ (± 3.2 at 95% CI), K = 1.03 (± 0.05 at 95% CI), and $t_0 = 0.12$ (± 0.01 at 95% CI). Substituting these values to the typical VBG formula would take about 23.16 months for the pearl oyster to reach a 100 mm grafting size (Figure 3).

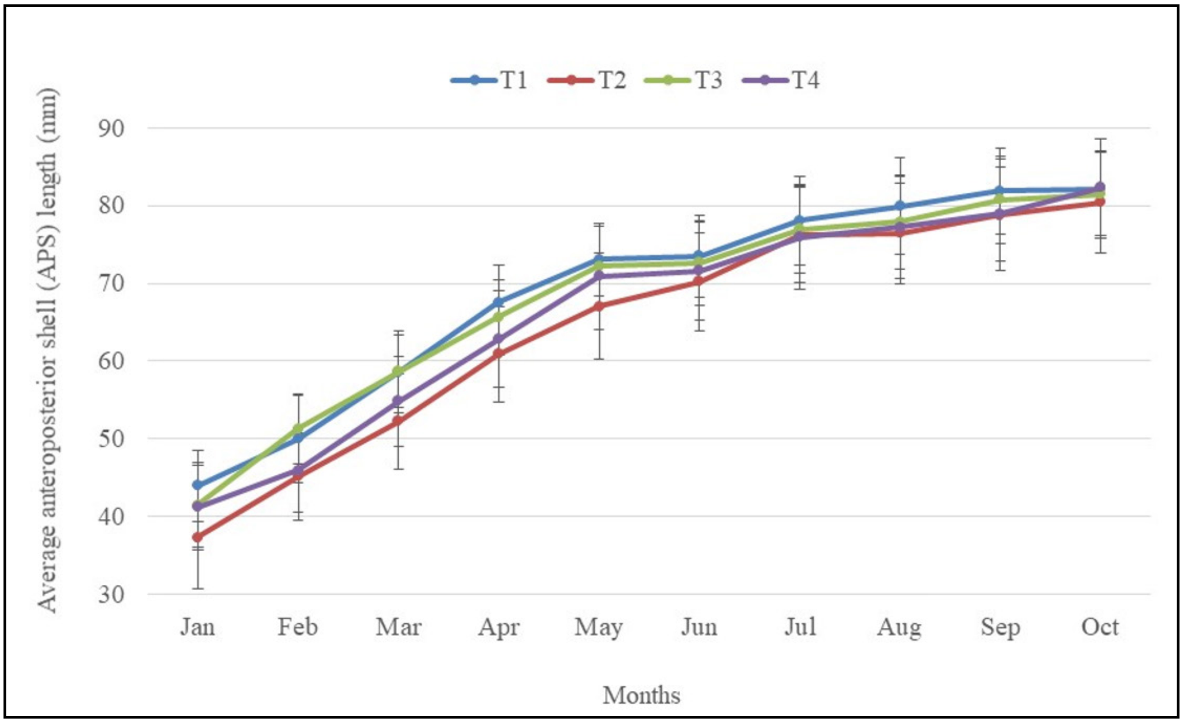

Figure 1. Monthly average (± sd) anteroposterior shell (APS) length (mm) of *Pinctada margaritifera* in the different treatments. T1: Metal-framed pocket net basket with cleaning; T2: Metal-framed pocket net basket without cleaning; T3: Bamboo-framed pocket net basket with cleaning; T4: Bamboo-framed pocket net basket without cleaning.

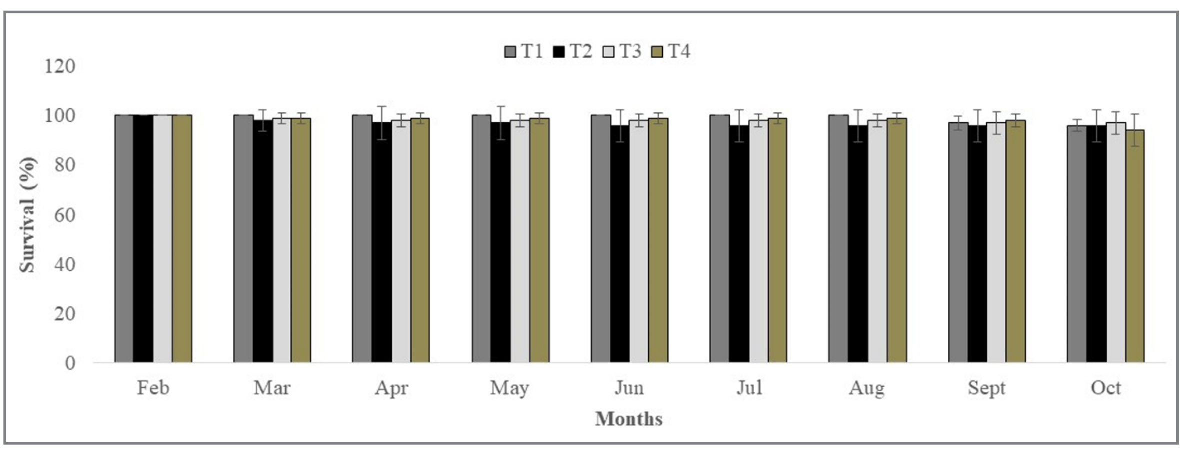

Figure 2. Monthly percent survival of *Pinctada margaritifera* in the different treatments. T1: Metal-framed pocket net basket with cleaning; T2: Metal-framed pocket net basket without cleaning; T3: Bamboo-framed pocket net basket with cleaning; T4: Bamboo-framed pocket net basket without cleaning

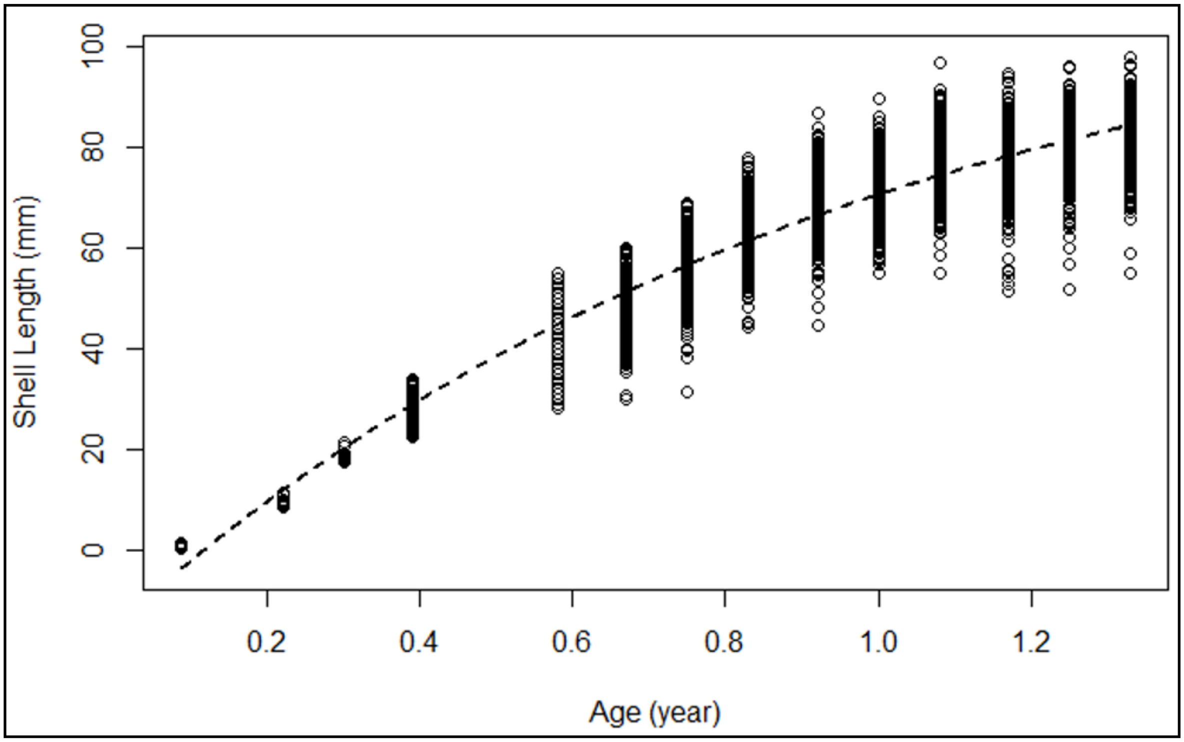

Figure 3. Pooled "Typical" von Bertalanffy growth curve of the black-lip pearl oyster *Pinctada margaritifera* in all four treatments. The first four sets of data were measurements prior to the start of the experiment.

3.2. Simple cost analysis

The total cost estimate for treatments without cleaning ranged between PHP 1,075 and PHP 1,100, while treatments having monthly cleaning ranged between PHP 7,075 and PHP 7,100. The cost estimate for treatments with a metal frame (T1 and T2) was PHP 25 lesser than the bamboo framed net baskets (T3 and T4). The life span of bamboo slats is only six months, thus doubling the labor cost. The absence of monthly cleaning has reduced the operational cost by 82.08% and 81.67% for metal (T2) and bamboo-framed (T4) pocket net baskets, respectively (Table 2).

4. DICUSSION

4.1. Growth and survival

As observed in other studies, the shell increment decreased with age (Pouvreau et al. 2000; Pouvreau and Prasil 2001; Lodeiros et al. 2002). The average 4.50 mm monthly shell length increment in this study is comparable to the report of Southgate and Beer (2000). They held an eight-month-old hatchery-produced *P. margaritifera* in five types of culture techniques. The oysters raised for five months in 24-pocket nets reached 65.8 mm from 41.5 mm initial dorsoventral height, representing a 4.86 mm average monthly growth increment. In French Polynesia, Le Moullac et al. (2012) obtained a 4.94 mm monthly growth increment within 11 months of culture. In spite of these similarities, there was inconsistent growth on our oysters as they had smaller initial (41.01 ± 6.03 mm) and final (81.51 ± 0.95 mm) APS lengths compared to the oysters in the study of Le Moullac et al. (2012), which measured 89.1 ± 9.1 mm at the start and reached 119.7 ± 10.8 mm after 11 months. Also, the average APS length increment (7.45 ± 1.38 mm) of oysters in this study during the first four months was slightly lower than the 10 mm monthly increment observed for fast-growing oysters (shooters) in the KPOHC Inc. pearl farm.

The average water temperature (31.73 ± 1.54°C) during the conduct of the study was within the normal range for the pearl oysters. The study site was far from any river mouth and had a stable water salinity (34.66 ± 0.36 ppt) even during the rainy season. As part of a large bay, the site is known to promote good oyster growth (Pouvreau and Prasil 2001). We also used the suspended culture method, known to promote rapid growth because of the greater availability of food (at the water column) than the bottom culture method (Lodeiros et al. 2002; Southgate and Lucas 2008). We, therefore, suspect that

Table 2. Actual cost comparison between bamboo and metal framed pocket net basket subjected to cleaning and without cleaning condition. T1: Metal-framed pocket net basket with cleaning; T2: Metal-framed pocket net basket without cleaning; T3: Bamboo-framed pocket net basket with cleaning; T4: Bamboo-framed pocket net basket without cleaning

Treatments	Materials (PhP/year)								Cleaning (PhP/year)			TOTAL	Percent cost reduction
	Steel bar	Bamboo	Green net	B-net	Nylon twine	Sinkers	Paint	Labor in Fabrication/repair	Labor	Boat rent	Jet sprayer rent		
T1	250	-	150	350	25	-	50	250	3000	1500	1500	7075	-
T2	250	-	150	350	25	-	50	250	-	-	-	1075	82.08
T3	-	50	150	350	25	25	-	500	3000	1500	1500	7100	-
T4	-	50	150	350	25	25	-	500	-	-	-	1100	81.67
Total	500	100	600	1400	100	50	100	1500	6000	3000	3000	-	-

the reduction in growth during the last five months of culture was size and density related. The pearl oysters were kept at 20-pocket net baskets throughout the study, while in a pearl farm in Palawan, 70-80 mm, and 100 mm oysters are transferred in 15 and 6-pocket net baskets, respectively. Taylor et al. (1997) obtained an inverse proportion between growth and density and a proportional relationship between deformities and density of early juvenile (initial length 6.2 ± 1.8 mm) *Pinctada maxima*.

The predicted L_∞ = 118.41 mm in this study was lesser than the common (130 mm) and the maximum (250 mm) size of the species (Carpenter and Niem 1998). Our estimate was also lower than those in the Cook Islands, where L_∞ ranged between 130.7 mm and 309.7 mm (Sims 1994). Growth could be highly variable between individuals, culture methods, and geographic locations (Pouvreau and Prasil 2001; Sims 1994; Southgate and Beer 2000). Our low L_∞ estimate could have been affected by the slow growth in the last five months of rearing, and this estimate may change depending on culture methods, density, and locality. The estimated 23.16 months required to reach a 100 mm grafting size is comparable to the slow-growing oysters in KPOCH Inc. Fast-growing oysters at the farm would reach 100 mm size in 16 to 18 months of rearing, while 21 months for the slow-growing group as observed in KPOHC Inc. Information on L_∞ and K values are essential when selecting favorable farm sites and culture methods for pearl oysters.

It is interesting to note that treatments without cleaning (T2: 4.76 ± 3.04; T4: 4.56 ± 3.11 mm) had slightly higher (although not significantly different) average APS length increment than treatments with regular cleaning (T1: 4.24 ± 3.31; T3: 4.43 ± 3.41 mm). The absence of significant variation in the growth of pearl oysters in treatments with and without cleaning for nine months was an indication that biofouling did not hinder the growth of cultured oysters. These findings agree with that of Lacoste et al. (2014), who reported the absence of effect of biofouling in both with or without regular cleaning on the growth of adult *P. margaritifera* (81-90 mm shell length) been raised for 20 months. Hulot et al. (2019) also reported the absence of negative effects of biofouling on *P. margaritifera* been raised for 14-15 months. By contrast, smaller oysters seem vulnerable to biofouling. Pit and Southgate (2003) reported the lowest final dorsoventral height of *P. margaritifera* juveniles in uncleaned trays (16.2 ± 1.0 mm) compared to treatments that have been cleaned every eight weeks (21.2 ± 0.8 mm) and every four or eight weeks (19.4 ± 1.2 mm).

Common biofouling organisms found during the culture trials were sponges, algae, and hydroids that grow directly on the pocket net baskets and pearl oyster shells. The predatory gastropods *Cymatium* spp. were also found inside the net pockets. The monthly sampling which dislodged these predatory gastropods from the nets could have helped reduce their negative effects on the cultured oysters. Walker (1984) improved the survival of clams *Mercenaria mercenaria* in cages upon removing the predatory crabs. The barnacles, one of the notorious biofouling organisms which can reduce growth and cause mortalities on pearl oysters (Fitridge et al. 2012), were less noticed on our pocket net baskets and cultured pearl oysters. This could be one of the reasons for having similar growth and survival between cleaned and uncleaned treatments. Lodeiros et al. (2002) did not observe any adverse effect of heavy fouling on *Pinctada imbricata*. They suspect that the hanging culture method position the shell vertically and facilitates the valves to easily open and filter foods even in the presence of numerous biofouling organisms.

While biofouling had been reported to have high and devastating impacts in marine aquaculture (Fitridge et al. 2012; Watson et al. 2009), these effects may vary according to the sizes of cultured species, types of fouling organisms, localities, and methods of culture (Fletcher et al. 2013; Watson et al. 2009). Pit and Southgate (2003) had reported the highest survival in the uncleaned trays for 16 weeks (75 ± 8%) compared to the other two treatments, which received cleaning (30 ± 5 and 63 ± 4%). The high survival rates (94%-97%) of *P. margaritifera* in this study further indicated its tolerance against biofouling organisms. Our result is comparable to the 90.6%-100% survival rates of Southgate and Beer (2000) for eight-month-old hatchery-produced juvenile black-lip pearl oysters (41.4 ± 0.6 mm initial shell length) been raised for five months with a monthly cleaning.

These findings suggest that monthly cleaning could be minimized without affecting the growth of certain size groups. While reducing the cleaning of oysters could help reduce labor costs and disturbance to the environment, it is important to regularly monitor the cultured stock as other types of dominant biofouling organisms may have adverse effects even for large pearl oysters.

4.2. Simple cost analysis

The absence of monthly cleaning, which reduced the operational cost by 82.08% (metal-framed) and 81.67% (bamboo-framed pocket net baskets), were higher than the estimates of Haws (2002), where around 40% of the total cost goes to the cleaning of biofouling itself. Some estimates, however, were much lower such as those of Johnston et al. (2019), who reported 41.63% and 24.72% annual cleaning labor costs for chaplet-based and panel-net-based culture methods for *P. margaritifera*. In Tonga, oyster cleaning required per production cycle to operate the modeled mabé pearl farm constitute 39.18% of the total labor requirements (Johnston et al. 2020).

The life span of bamboo slats was 50% shorter than the metal used as a frame for net baskets. Nevertheless, bamboo frames may have less impact on the environment, while the use of steel could affect or damage aquatic life. Jakimska et al. (2011) reported the harmful effect of metal on aquatic life. Steel contains cadmium which can cause skeletal deformities in fish that result in the impaired ability of fish to find food and avoid predators. Also, cadmium and chromium can harm aquatic plants, affecting the entire ecosystem because green plants are at the base of all food chains (Solomon 2008). A detailed cost analysis in operating a hectare of pearl farm is needed to include all other materials such as long lines and floats, which may slightly affect the current estimate.

5. CONCLUSION

As expected, the pearl oysters grew relatively fast in the first four months before slowing down towards the 9th month. The slow growth could be size and density-related, and could be corrected using low-density pocket net baskets (e.g., 15-pocket net baskets). The growth increment did not significantly vary between the two cleaning conditions, and between bamboo and metal as basket frames. No interaction was also observed between the cleaning conditions and the type of basket frame. In addition, the survival rates of pearl oysters were high, ranging between 94 ± 6.52 and 97 ± 4.47%, and did not significantly vary between types of frame and between cleaning conditions. The absence of monthly cleaning on the pearl oyster has vastly reduced the cost of the farm operation. Bamboo slats have the potential use as a frame for net baskets. These results are essential in reconsidering the usual management practices in a pearl farm.

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AUTHOR CONTRIBUTIONS

Cueba FRA: Investigation, Data Curation, Writing—Original Draft. **Diaz RD:** Resources, Supervision, Project Administration. **Villanueva EG:** Project Administration, Formal Analysis. **Mecha NJMF:** Formal Analysis, Visualization, Writing—Review and Editing. **Dolorosa RG:** Conceptualization, Formal Analysis, Writing—Review and Editing, Funding Acquisition.

CONFLICTS 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.

ETHICS STATEMENT

The researchers followed all precautionary measures to ensure the survival of the cultured pearl oysters.

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