Culture of Single and Clustered Oysters (*Magallana bilineata* Röding, 1798) in Hanging Net Pockets and Plastic Trays in Baleyadaan, Alaminos City, Pangasinan

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ABSTRACT

This study was conducted to determine growth and survivorship of single and clustered oysters in hanging net pockets and trays in Baleyadaan, Alaminos, Pangasinan for 98 days. Treatments tested were single oysters in net pockets (T1), single oysters in trays (T2), clustered oysters in pockets (T3), and clustered oysters in trays (T4). Single oysters were clustered using marine epoxy near the shell hinge of the left shell. T4 had the highest length gain (27.13 mm) followed by T1, T3, and T2 at 26.82 mm, 22.26, and 22.05, respectively. In terms of weight gain, T1 was highest at 32.50 g followed by T4, T3, and T2 at 28.93 g, 26.84 g, and 24.77 g, respectively. However, two-way ANOVA revealed no differences in length, width, and weight gains among different treatments and in terms of type of culture units and clustering. Treatment 4 had the highest mean survival after 98 days, but no significant differences ($P>0.05$) were observed among treatment means. Water quality parameters were within suitable range for culture, except for chlorophyll-*a*. The clustering was low enough to cause density-dependent effects on growth of single and clustered oysters either in pockets or trays. Allometric growth of shell dimensions was also described.

Keywords: Single oysters, clustered oysters, plastic trays, hanging net pockets, allometric growth

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INTRODUCTION

Magallana bilineata Röding 1798 (formerly *Crassostrea iredalei* Faustino 1932), is one of the several economically important species of oyster endemic to the Philippines (Rosell 1991; Salvi and Mariottini 2020). Oyster farming in the Philippines was first documented to have started in 1931 in Hinigaran, Negros Occidental where growers manually removed settled oysters from cultches and broadcasting them on the bottom along estuarine areas and left to grow until harvest size. Despite many developments in farming in other countries, oyster farmers in the Philippines still relyon using traditional and low-cost methods and remain dependent on wild spat as source of seed stocks. They still use the same types of substrates (e.g., bamboo, tires, oyster shells, ropes) for spat collection and grow-out of oysters.

Oysters grown using traditional methods usually form clumps or clusters, with some oysters settled upon later by younger spat, as in this method, tires and empty oyster shells are used as spat collectors. As oyster settlement of spat is unrestricted, density cannot be controlled. With uncontrolled settlement, the first settlers are usually the largest, and the last ones to settle on top are the smallest (Lebata-Ramos et al. 2021a). Moreover, these oysters utilized by other oysters as substrate exhibit delayed growth and morphological irregularities (Yapi et al. 2017; Lebata-Ramos et al. 2021a, 2021b). Hence, Lebata-Ramos et al. (2021b) compared the growth and survival of the slipper oyster *Magallana bilineata* (=*C.iredalei*) using traditional (tires and oyster shells) and new methods (pouches and trays) suspended from floating rafts. After six months of culture, they found that the mean shell length and body weight of oysters were significantly highest in those reared in pouches and trays than those grown on empty oyster shells and tires. Growing oysters using the new methods produced single, larger, meatier oysters of almost the same size at a shorter culture duration.

This study was a result of a field verification trial comparing the growth and survival rates of single and clustered oysters utilizing hanging net pockets and net trays in an existing oyster culture bed in Baleyadaan, Alaminos City, Pangasinan.

MATERIALS AND METHODS

Location of the Study

The study was conducted in an existing oyster ground (16°12’18” N 119°59’20” E) at Baleyadaan, Alaminos City, Pangasinan (Figure 1). Baleyadaan shares a common border with barangays Lucap, Mona, and Cayucay, Alaminos, Pangasinan.

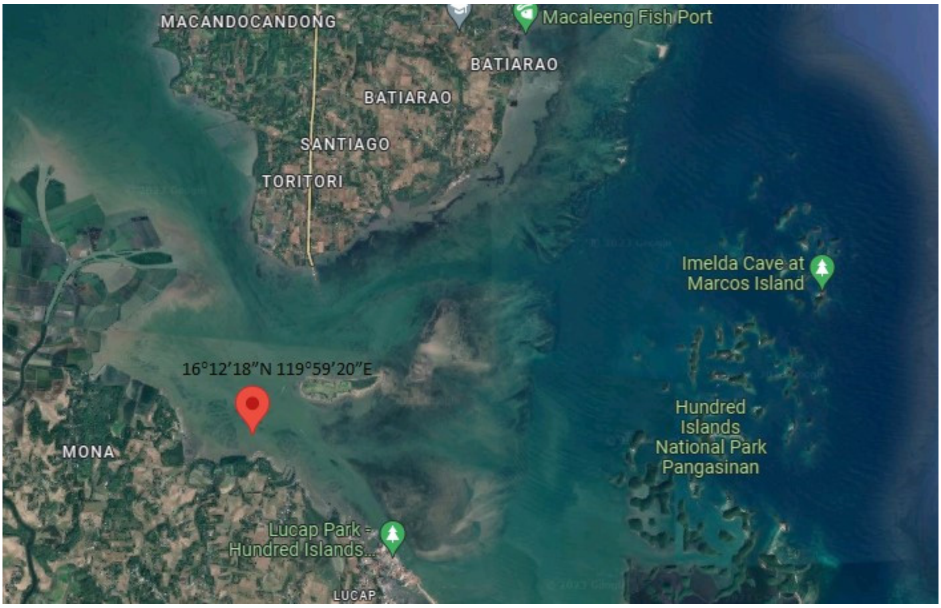

Figure 1. Google map of the oyster ground in Baleyadaan, Alaminos City, Pangasinan.

Experimental Animal

Three hundred sixty (360) single oysters (*Magallana bilineata*), with mean length, width, and weight (mean±SE) of40.53±0.78 mm, 29.39±0.61 mm, and 9.60±0.40 g, respectively, were used in the study. The oysters were farmed at the Regional Mariculture Technology and Development Center (BFAR-RMATDeC) in Alaminos City, Pangasinan.

Experimental Design and Treatments

The study was designed to determine the growth of single and clustered oysters grown in two types of experimental units, i.e., hanging net pockets and plastic trays. The study had four experimental treatments and three replicates. The growth experiment was conducted from January 14 to April 22, 2023, for a total of 98 days. Treatment 1 had hanging net pockets composed of 30 pockets, and each pocket had one single oyster. Treatment 2 had three plastic trays stacked on top of each other, and each tray having ten single oysters. Treatment 3 had hanging net pockets consisting of ten pockets with each pocket having three clustered oysters. Lastly, Treatment 4 had three plastic trays, and each tray had ten oysters clustered together. The net pockets and net trays were hung from bamboo poles set up in the area under a completely randomized design (Figure 2). Single oysters were clustered using marine epoxy near the shell hinge of the left shell.

Figure 2. Bamboo structures used to hang the experimental units in Baleyadaan, Alaminos.

Stocking and Sampling

Before stocking, the shell length and width were measured using an electronic Vernier caliper to the nearest 0.01 cm and initial body weight using a digital weighing scale. Shell length of the oyster was measured from the umbo (the high point near the hinge of the two shells) to the farthest point whereas the shell width was measured by the broadest distance roughly perpendicular to the shell length of the oyster.

About 50% of oysters in each experimental unit were sampled every 14 days until the end of the experiment by day 98. Unwanted particles and fouling organisms, as well as those that fouled the nets and trays, were cleaned from the oysters by brushing. After the oysters were measured and the experimental units were cleaned, the oysters were returned to their respective culture units and back to the oyster bed.

Determination of Growth Parameters

The oysters were sampled every two weeks by measuring the shell length and width using an electronic vernier caliper. At the same time, the body weight was recorded using a digital weighing scale with a 0.1-g precision. Figure 3 shows the oyster shell length and width as measured in this study. The growth parameters of oysters were calculated using the formulas below:

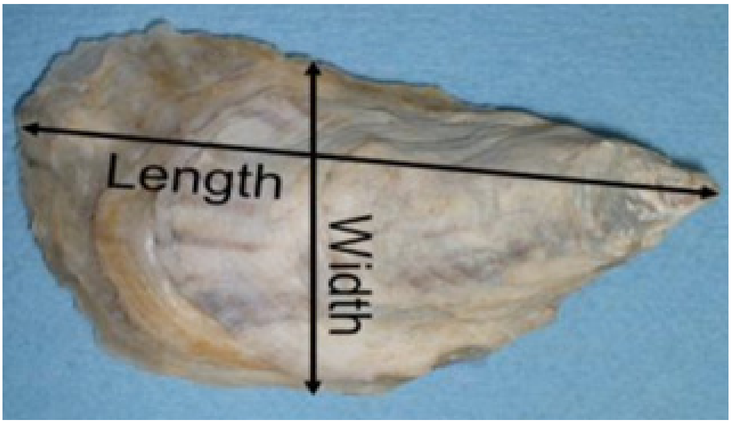

Figure 3. Oyster shell length and width.

Length Gain = Final length (mm) – Initial length (mm)
Width Gain = Final width (mm) – Initial width (mm)
Weight Gain = Final weight (g) – Initial weight (g)
Daily Growth Rate (mm/d or g/d) = ((Length gain (mm) or Weight gain (g)) / No. of days

Shell Allometric Growth Pattern

Allometric growth of *M. bilineata* shell dimensions was also described using linear morphometrics. Regression analyses were used to establish the shell width-shell length (SW-SL) relationships. Data were log-transformed and fitted in the linear function model provided by Gaspar et al. (2002). The *a* and *b* parameters of linear regression analysis were determined using the least squares method. The coefficient of determination (r^2) was used to estimate the degree of association between variables. Likewise, the confidence interval for *b* was set at 95% and the level of significance for r^2 was calculated. T-test (H_0:b = 1) at confidence level of 95% ($\alpha = 0.05$) was used to verify whether the *b* values derived from the established linear regression relationships were isometric (b = 1) or allometric ($b \neq 1$).

Water Quality Monitoring

Water quality parameters such as temperature, salinity, pH, and dissolved oxygen (DO) were monitored every two weeks using a ProQuatro YSI Multiparameter equipment whereas a Secchi disk was used to determine water transparency at mid-day (11:00 am). *In situ* water quality monitoring was always done during sampling. Water samples were also taken regularly for chlorophyll-*a* analysis by spectrophotometry at the BFAR-NFDC Bonuan-Binloc Dagupan City, Pangasinan.

Data Analysis of Growth Performance

Using Levene's test, the growth and survival of oysters were tested for homoscedasticity to satisfy parametric assumptions of subsequent statistical analyses. Growth performance and survival rate of oysters werethen analyzed using a two-way Analysis of Variance (ANOVA) in a Complete Randomized Design (CRD) using Microsoft Excel (2019) Analysis Tool Pack to determine whether significant differences exist among treatment means.

RESULTS AND DISCUSSION

Growth

Table 1 presents the mean length gain of the oysters grown in hanging net pockets and plastic trays during the 98 days of culture period. The data revealed that Treatment 4 had the highest value of 27.13 mm, followed by treatments 1, 3, and 2 at 26.82 mm, 22.26 mm, and 22.05 mm, respectively. However, two-way ANOVA on the mean length gain showed no significant differences (P>0.05) among treatment means which imply that the length gain of oysters grown in hanging pockets and plastic trays was comparable. It also showed no significant differences in length gain between single and clustered oysters as well as between culture systems. Likewise, there was no interaction between the clustering and the type of culture system. Figure 4 shows the growth curve of oysters in terms of shell length from the different experimental treatments for 98 days.

Table 1. Mean length gain of oysters grown in hanging net pockets and plastic trays during the 98-day culture period

Treatments	Replicates	Initial Length (mm)	Final Length (mm)	Length Gain (mm)
1	1	40.86	68.37	27.51
	2	37.92	61.23	23.31
	3	36.90	66.53	29.63
	Mean	38.56±1.19[a]	65.38±2.14[a]	26.82±1.86[a]
2	1	43.93	62.31	18.38
	2	43.35	66.84	23.49
	3	40.21	64.49	24.28
	Mean	42.50±1.16[a]	64.55±1.31[a]	22.05±1.85[a]

Treatments	Replicates	Initial Length (mm)	Final Length (mm)	Length Gain (mm)
3	1	36.17	61.89	25.72
	2	39.05	59.76	20.71
	3	35.24	55.59	20.35
	Mean	36.82±1.15[a]	59.08±1.85[a]	22.26±1.73[a]
4	1	39.14	67.55	28.41
	2	41.22	65.23	24.01
	3	40.53	69.50	28.97
	Mean	40.30±0.61[a]	67.43±1.23[a]	27.13±1.57[a]

Means having same superscripts are not significantly different at 5% level of significance.

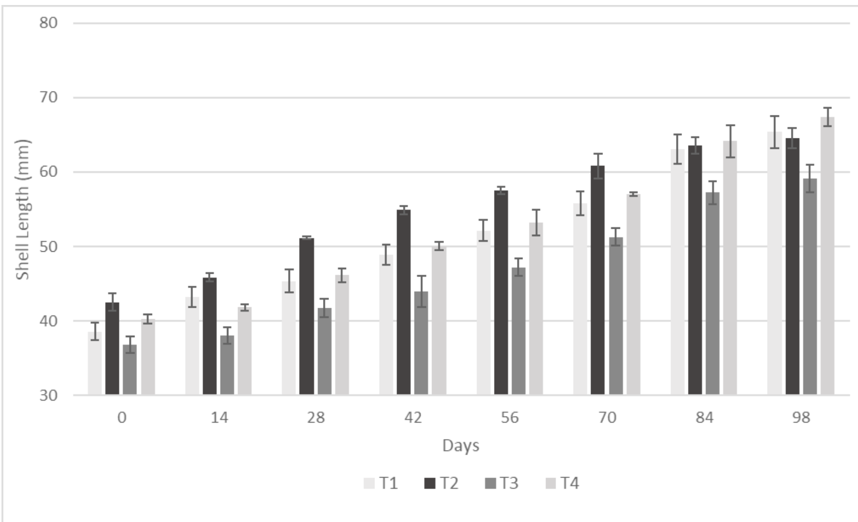

Figure 4. Growth curve of oysters in terms of shell lengths from the different experimental treatments for 98 days. Means ±SE.

The daily growth rate in length obtained in this study ranged from 0.23-0.28 mm/d, which was comparable to the findings of Chueachat et al. (2018) in their research on *Magallana bilineata* (=*Crassostrea iredalei*) reared in mangrove canals for two months. Oysters cultured in a mangrove canal achieved a length gain of 8.70 mm per month (0.29 mm/d) whereas oysters cultured in an earthen pond had a length gain of only 5.10 mm per month (0.17 mm/d), which was lower compared to the result obtained in this study. The present study also observed a comparable growth rate with the study of Lebata-Ramos et al. (2021b). They reported growth in shell length at 5.01 mm/month (0.167 mm/d) and 6.05 mm/month (0.21 mm/d) for trays and pouches, respectively.

Width Gain

Table 2 presents the mean width gain of the oysters grown in hanging net pockets and plastic trays during the 98-day culture period. The data revealed that Treatment 4 had the highest value of 22.51 mm followed by treatments 1, 3, and 2 with values of 21.24 mm, 21.08 mm, and 19.24 mm, respectively. However, two-way ANOVA on the mean width gain showed no significant differences ($P>0.05$) among treatment means. This result implies that the width of oysters grown in hanging pockets and trays is comparable. In terms of final width, it also showed no significant difference between single and clustered oysters and between culture systems. Likewise, there was no interaction between the clustering and the type of culture system. As shown in Figure 5, the spaces between the clustered oysters for Treatment 4 are large enough for growth of the shells in terms of length and width.

Table 2. Mean width gain of oysters grown in hanging net pockets and plastic trays during the 98-day culture period

Treatments	Replicates	Initial Width (mm)	Final Width (mm)	Width Gain (mm)
1	1	33.27	57.21	23.94
	2	29.59	50.88	21.29
	3	33.49	51.99	18.50
	Mean	32.12±1.26[a]	53.36±1.95[a]	21.24±1.57[a]
2	1	33.81	49.86	16.05
	2	31.27	53.58	22.31
	3	32.80	52.16	19.36
	Mean	32.63±0.74[a]	51.87±1.08[a]	19.24±1.81[a]
3	1	29.91	53.23	23.32
	2	28.01	49.53	21.52
	3	27.72	46.12	18.40
	Mean	28.55±0.69[a]	49.63±2.05[a]	21.08±1.44[a]
4	1	30.07	54.68	24.61
	2	31.82	52.73	20.91
	3	29.39	51.41	22.02
	Mean	30.43±0.72[a]	52.94±0.95[a]	22.51±1.10[a]

Means having same superscripts are not significantly different at 5% level of significance.

Figure 5. Clustered oysters in groups of ten from Treatment 4 after 98 days.

This study's width gain result is higher than the published reports of other related species of *Crassostrea*. The mean width gain obtained in this study for all treatments was 21.01 mm(0.21 mm/d) higher compared to the suspended technique used in Sonora, Mexico to culture the Pacific Oyster *C. gigas* that had isometric shell growth during the first 13 months, reaching a width of 25.1±0.8 mm (0.06 mm/d) (Chávez-Villalba et al. 2005).

Weight Gain

Table 3 presents the mean weight gain of single and clustered oysters *(Magallana bilineata)* reared in hanging net pockets and plastic trays for 98 days. The highest weight gain was observed in Treatment 1 with a mean value of 32.50 g, followed by Treatments 4, 3, and 2 with 28.93 g, 26.80 g, and 24.77 g, respectively. However, two-way ANOVA revealed no significant differences (P>0.05) among treatment means. It also showed no significant difference in final weight as well as weight gains between single and clustered oysters and between the culture units. There was no interaction between the clustering and the type of culture system. Therefore, the degree of clustering of oysters used did not create density-dependent effects on the growth performance of oysters in terms of weight as well as length.

The present study obtained mean weight gains of 24.77 g to 32.50 g equivalent to 0.25 g/d to 0.33 g/d which were slightly lower than the findings of Lebata-Ramos et al. (2021b). They obtained body weight gains of 10.77 g/month (0.36 g/d) and 10.98 g/month (0.37 g/d) for trays and pouches, respectively.

The slower growth in terms of weight gain observed in the present study was probably due to the low chlorophyll-*a* concentrations observed in the study site, indicating low primary productivity. In marine environments, chlorophyll-*a* is an indicator of primary production reflecting natural variations in biological productivity (Behrenfeld and Falkowski 1997; Zhang et al. 2007).

Table 3. Mean weight gain of single and clustered oysters grown in hanging net pockets and plastic trays during the 98-day culture period

Treatments	Replicates	Initial Weight (mm)	Final Weight (mm)	Weight Gain (mm)
1	1	9.93	50.23	40.30
	2	8.67	39.92	31.25
	3	10.33	36.28	25.95
	Mean	9.64±0.50[a]	42.14±4.18[a]	32.50±4.19[a]
2	1	12.33	35.15	22.82
	2	9.93	36.36	26.43
	3	10.27	35.34	25.07
	Mean	10.84±0.75[a]	35.62±0.38[a]	24.77±1.05[a]
3	1	7.53	35.58	28.05
	2	8.27	38.97	30.70
	3	7.33	29.11	21.78
	Mean	7.71±0.29[a]	34.55±2.89[a]	26.8±2.64[a]
4	1	9.60	34.62	32.54
	2	8.90	34.01	31.84
	3	9.60	46.27	34.08
	Mean	9.37±0.23[a]	38.30±3.99[a]	28.93±0.66[a]

Means having same superscripts are not significantly different at 5% level of significance.

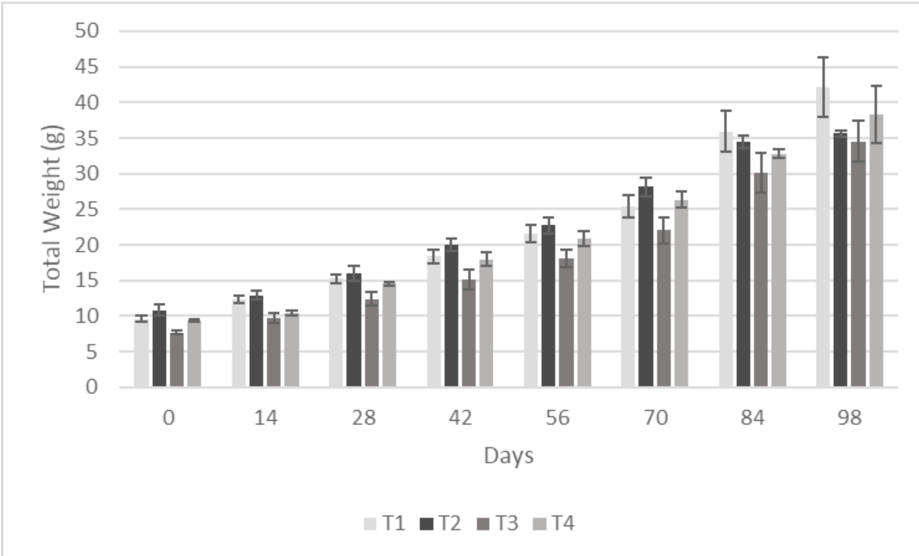

Figure 6. Growth curve of oysters in terms of total weight from the different experimental treatments for 98 days. Means±SE.

Table 4. Allometric growth parameters of *M. bilineata* individuals in various culture systems

Culture System	*N*	Linear Relation	Linear Equation	r^2	SE of *b*	Relationship
SingleOyster in Net Pockets	130	SW-SL	*Log* SW = 0.05 + 0.92*Log* SL	0.84*	0.0356	- Allometric
Single Oyster inPlastic Trays	132	SW-SL	*Log* SW = -0.03 + 0.95*Log* SL	0.76*	0.0467	Isometric
Clustered Oysters in Net Pockets	132	SW-SL	*Log* SW = -0.02 + 0.96*Log* SL	0.71*	0.0538	Isometric
Clustered Oysters in Plastic Trays	87	SW-SL	*Log* SW = -0.02 + 0.95*Log* SL	0.81*	0.0497	Isometric

N = number of individuals; SW = Shell Width; SL = Shell Length; r^2 = coefficient of determination; (*) = $P< 0.05$; SE = standard error; *b* = slope; (+) = positive; (-) = negative

Linear relationships between SW and SLwere established for *M. bilineata* cultured singly or clustered in net pockets and plastic trays (Table 4). Isometric growth was observed in most culture systems except in net pockets with single oyster, in which negative allometric shell growth was documented. All linear relationships were statistically significant.

Majority of the *M. bilineata* individuals investigated in this study exhibited isometric shell growth patterns. Bivalve shells with isometric growth patterns maintain a relatively stable form throughout time because the growth rate of the shell is constant during development (Okabe and Yoshimura 2017). According to Gaspar et al. (2002), this pattern is characterized by a linear relationship between shell width and length with a slope close to 1.The isometric growth of *M. bilineata* in various culture systems in this study can be advantageous because it suggests that the clams are growing successfully. This often leads to high survival rates and improved produce quality.

In this study, the negative allometric growth of single oysters grown in pockets may be attributed to the relatively reduced size of the pockets utilized in comparison to the clustered oysters. *M. bilineata* individuals may have encountered restricted food and nutrient availability as a result of the confined environment under experimental conditions. Consequently, these organisms might have deprioritized shell development in favor of other physiological processes, resulting in a reduction in shell growth relative to their body mass. In subsequent investigations, larger-sized compartments might be employed.

Survival Rate

Table 5 shows the survival rate of the oyster *(M.bilineata)* during the 98-dayculture period. With a mean value of 98%, Treatment 4 obtained the highest mean survival rate of slipper oysters, followed by Treatment 1 with 95%, Treatment 3 with 93% and Treatment 2 with 92%. However, two-way ANOVA on the survival rate failed to show a significant difference (*P*>0.05) among treatment means. It also showed no significant difference in the survivorship between single and clustered oysters. Likewise, there was no interaction between the clustering and the type of culture system.

Table 5. Survival rate of Slipper oyster *Magallana bilineata* during the 98-day culture period

Treatments	Survival			
	R1	R2	R3	Mean (%)
1	97	87	100	95±3.93[a]
2	100	97	80	92±6.23[a]
3	87	100	93	93±3.76[a]
4	93	100	100	98±2.33[a]

Means having same superscripts are not significantly different at 5% level of significance.

Compared with the 90% survival rate obtained in the study conducted by Lebata et al. (2021b), the survival rate in this study was higher. The enemies of oysters can be classified into four groups – predators, competitors (fouling animals), parasites, and red tide according to Fujiya (1970). As shown in Figure 7, competitors such as barnacles and windowpane oysters are observed attached to the oysters. However, as observed in this study, the few mortalities of the slipper oyster were attributed to predators such as crabs.

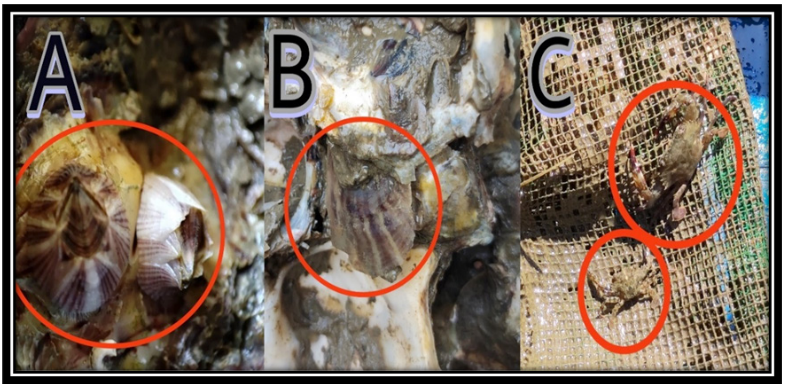

Figure 7. Barnacles (A), Windowpane Oyster (B), and Crabs (C).

Many factors influence the growth and survivorship of oysters such as genetic make-up, food availability, water quality, weather conditions, density, size or age of spat, predation, and air exposure as well as interactions of these factors.

Water Quality Parameters

Table 6 presents the results of water quality parameters such as salinity, temperature, water transparency, dissolved oxygen, and pH measuredin the oyster ground for the 84-day culture period.

Table 6. Water quality parameters monitored in the oyster ground during the culture period of 84 days

Day	Salinity (ppt)	Temperature (°C)	Water Transparency (m)	Dissolved Oxygen (ppm)	pH
1	36.46	29.00	0.60	4.98	7.39
14	37.02	27.03	0.56	5.73	8.32
28	37.21	29.00	0.66	4.67	8.38
42	38.88	28.40	0.58	4.88	8.58
56	37.73	29.70	0.66	4.18	8.46
70	37.18	30.40	0.60	5.90	8.47
84	38.40	31.5	0.66	4.21	8.05
Mean	37.55	29.33	0.62	4.93	8.30

The temperature recorded in the present study ranged from 27 to 31.5°C (mean= 29.33°C) whereas salinity ranged from 36.46 to 38.88 ppt (mean = 37.55 ppt). The slipper oyster *Magallana bilineata* (=*C.iredalei*) has a wide temperature tolerance of 25-33^{o}C (Garrido-Handog 1990), which shows that the water temperature measured in the oyster ground (Baleyadaan) is within the suitable range. The range of temperature in the present study was within the range (26-33°C) reported by Lebata-Ramos et al. (2021b) but the salinity is higher in the present study because the oyster ground is marine in nature. The high salinity observed in the present study may not have affected the oysters, which are very tolerant of fluctuating estuarine conditions (Angell 1986).

The water transparency in the oyster ground was measured using a Secchi disk and the recorded mean of 0.62 m indicates a high level of transparency. In Boyd's interpretation of Secchi disk visibility, 60 cm means phytoplankton is scarce, and more than 60 cm of water is clear and has inadequate productivity (Global Seafood Alliance 2004). The determination of Secchi disk visibility affords a practical estimate of plankton density provided plankton is the primary source of turbidity (Almazan and Boyd 1978).

The recorded mean concentration of dissolved oxygen in the oyster ground was 4.93 ppm. The recorded DOin this study isclose to the suitable range of DO levelsof at least 5 ppm recommended for oyster culture. Other studies have noted that oysters are capable of tolerating poor oxygen conditions (Sussarellu et al. 2010; Xie et al. 2023).

The mean concentration of pH level recorded in the oyster ground was 8.30, which is within the suitable range for oyster culture. The pH requirement in oyster culture ranges from 6.75 to 8.75 (Schumway 1996).

Table 7. Results of Chlorophyll-*a* concentration in the oyster ground of Baleyadaan, Alaminos City, Pangasinan.

Date	Water Samples	Chlorophyll-*a* (µg/L)
February 13, 2023	1	0.001
	2	0.001
February 27, 2023	1	0.000
	2	0.000
March 13, 2023	1	0.001
	2	0.001
March 27, 2023	1	0.001
	2	0.001
Mean		0.00075

The mean chlorophyll-*a* concentration in the oyster ground was 0.00075 μg/L which is considered low (Table 7). The chlorophyll-*a* concentration obtained in this study was lower compared to the study of Salonga et al.(2020) where they determined the abundance, composition, and diversity of phytoplankton in Leyte Gulf. The chlorophyll-*a* levels in their study area were higher and ranged from 0.001 to 0.727 μg/L (0.33 μg/L on average).Their results further revealed that the relationship between the chlorophyll-*a* and DO was strongly positively correlated to phytoplankton abundance.

Since all treatments have comparable results in growth performance in terms of length, width, and weight, it is recommended to conduct another experiment, but the clustering must be dense enough to imitate the natural clustering of oysters which restricts growth of oysters at the center of the cluster. An actual setting of oyster larvae was monitored using oyster shells in Himamaylan River, Negros Occidental with newly settled spats numbering from 101 to 186 per shell in August and September 1981 (Young et al. 1981). It was further mentioned in the same study that if 15 to 20 of these spats survived,then the yield was satisfactory. Meanwhile, a spat density of up to 129.07±9.3/m^2 was observed to have settled in a roughened tile substrate as compared to smooth tiles (66.95±7.0/m^2) placed in a natural oyster bed in New Washington, Aklan, Philippines from March 2015 to June 2017 (Lebata-Ramos et al. 2022). In a river mouth in San Fabian, Pangasinan, about 30-50 spats were observed to settle per oyster shell cultch from March to April 2022 but during grow-out only about half remained per cluster (2023 email message from N Santos of NFRDI to the author; unreferenced). For a follow-up experiment, a cluster of 20-25 oysters can be compared to single oysters in terms of growth and survivorship.

Some plastic trays were damaged, which made entry easy for predators, contributing to the oyster's mortality. Sturdier plastic trays or creating protection that can prevent damage are recommended. Regular maintenance and inspection of the hanging net pockets and plastic trays can also help identify and repair any damage promptly, reducing the risk of predator or competitor entry. We also observed fouling of the structures which would require weekly cleaning and brushing. Further study is recommended to evaluate the use of hanging net pockets and plastic trays in other culture sites that meet the water quality requirements for *Magallana bilineata* to assess the efficacy of these culture methods in promoting the growth and survival of the oysters.

Though no significant differences in growth were observed between single and clustered oysters in two types of culture units, the hanging net pockets offer great potential for adoption by oyster farmers in the country because these are easy to make, durable, and can be handled easier (Lebata-Ramos et al. 2021b). Moreover, the young oysters are protected from predators and fouling organisms which results in high survivorship.

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