

Grow-out Culture of Oyster *Magallana bilineata* (Röding, 1798) Using Pouches: A Comparison of Growth and Survival in the River and Earthen Pond

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Oysters are among the most in-demand aquaculture commodities in the Philippines and worldwide. With the decreasing culture area and the deteriorating water quality of oyster beds, there is a need to explore new culture sites and techniques to address the problems of dwindling stocks and the increasing demand for better quality oysters. This study compared the growth and survival of the oysters *Magallana bilineata*, the new accepted name of *Crassostrea iredalei*, cultured in an earthen pond and the river using pouches suspended from rafts. Mean growth rates of oysters in length and weight were significantly higher in those reared in the river (0.56 ± 0.02 cm mo⁻¹; 10.27 ± 0.42 g mo⁻¹) than those in the pond (0.41 ± 0.03 cm mo⁻¹; 5.99 ± 0.22 g mo⁻¹), but the meat yield of oysters reared in the river ($25.96 \pm 0.92\%$) was almost the same as in the pond ($24.05 \pm 1.41\%$). Likewise, the proximate composition was the same for oysters coming from the river and the pond, respectively — $58.04 \pm 0.14\%$ and $53.86 \pm 0.38\%$ crude protein, $7.00 \pm 0.08\%$ and $7.93 \pm 0.38\%$ crude fat, $24.68 \pm 0.07\%$ and $28.54 \pm 0.74\%$ nitrogen-free extract, and $10.27 \pm 0.01\%$ and $9.68 \pm 0.01\%$ ash. Oyster survival at harvest was significantly higher in the river ($70.21 \pm 2.84\%$) than in the pond ($13.10 \pm 1.57\%$). However, with some interventions, ponds may still be utilized for oyster culture despite the low survival results in this study. Extending oyster culture in these aquaculture facilities may serve as a basis for interventions to make the pond more habitable for oysters and may help boost production in the country.

Keywords: *Crassostrea iredalei*, *Magallana bilineata*, oysters, pond culture, pouch, SEAFDEC/AQD

INTRODUCTION

Oysters play an essential role in the marine and estuarine ecosystems by providing habitat to other organisms, maintaining healthy marine environments, and contributing to the local economy. The slipper-shaped oyster or *talabang tsinelas*, long been referred to as *Crassostrea iredalei* (Faustino 1932) by the Philippine scientific community and recently as *Magallana bilineata* (Röding 1798) (del Norte-Campos et al. 2020; Lebata-Ramos et al. 2022; WoRMS 2022), is the most popular and commercially desirable among the four species of oysters belonging to the family Ostreidae (Lovatelli 1988; Samsin 1988). The recent separation of the phylogenetically distinct cupped oysters from two biogeographic regions (Indo-Pacific and Atlantic) into *Magallana* and *Crassostrea*, respectively, was based on comprehensive taxon sampling, independent datasets, and varied analytical methods. In addition, the new maximum likelihood

phylogenetic analysis of oysters, based on mitochondrial gene order data representing molecular phenotypes above the sequence level, has provided strong support for this reclassification (Salvi and Mariottini 2016; Salvi and Mariottini 2020).

M. bilineata is very ideal for culture as it is the largest among the *Magallana*/*Crassostrea* species in the country, capable of growing at a faster rate to a maximum of 15 cm shell length in a favorable environment (Poutiers 1998; PCAARRD 2021) and with a straight shell margin making it easier to open (Samsin 1988). It was believed to be endemic to the Philippines (Rosell 1991; Poutiers 1998); hence, it has not been reported in other countries until the mid-1990s (Garrido-Handog 1990; Rosell 1991). The culture of *M. bilineata* dates back to as early as the 1930s in Hinigaran, Negros Occidental (Delmendo 1989) and Bacoar, Cavite (Blanco et al. 1950), with transplantation of adults or spat in the 1970s to areas where they do not naturally thrive in to promote farming (Juinio-Meñez

2004). Known for its high tolerance to fluctuating estuarine conditions (Angell 1986) and its abundance in tidal rivers, channels, gulfs, and land-locked bays, *M. bilineata* grows on different substrates (e.g., bamboo, recycled bike tires, hanging ropes with oyster shells, or coconut husks) where its spat can settle and left to grow for nine to 12 mo until harvestable size (Rosell 1991; Lebata-Ramos et al. 2021a).

M. bilineata is an important aquaculture species as it is a major source of food and livelihood in many coastal communities (Siar et al. 1995; Lebata-Ramos et al. 2021b). Its flesh is considered a sought-after food commodity due to its sweetness and creaminess, while its shells are hung on ropes and suspended in water columns to be used as settlement substrates for competent larvae to produce the next generations of oysters (Lebata-Ramos et al. 2021a). Studies have also found that *M. bilineata* shells are good sources of calcium carbonate (Atienza and Cruz 2019), chitin and chitosan (Cadano et al. 2021), and lime (Chilakala et al. 2019). The increasing demand for oysters, both for their flesh and shells, and the decreasing culture areas due to the seaward expansion of commercial, industrial, and residential properties have caused dwindling production. Unstable oyster production of oysters does not only affect the Philippines but also most of the oyster-producing countries worldwide, which have their own shares of booms and busts, both in capture and aquaculture production (Botta et al. 2020; FAO 2021a, 2021b).

Aside from unstable production, another issue that local oyster growers are facing is the high-end market preference for large and meaty individual oysters, typically produced using newer methods such as the use of Stanway oyster cylinders (Robert et al. 1993); sticks, trays, and baskets (Nell 2001; Leavitt 2017); and pouches (Tanyaros et al. 2008; Lebata-Ramos et al. 2021b), as opposed to those produced using cheaper traditional methods which yield oysters that are clumped together in a wide range of sizes and that usually end up in small stalls on the roadsides or in local markets.

Hence, to boost production and improve oyster quality, other culture systems and new technologies must be explored to support the growing market and the increasing demand for higher-quality oysters. Although it has been observed in other localities in the country that oysters are reared on elevated bamboo racks inside ponds, this is the first report on an attempt to compare the growth and survival of *M. bilineata* cultured in the pond and the adjacent river. Moreover, pouches suspended from rafts were used (Lebata-Ramos et al. 2021b) instead of the traditional culture methods to

produce higher-quality oysters that are larger, individually separated, and almost identical in size.

MATERIALS AND METHODS

Study Site

The experiment was conducted in Panay, Capiz, Philippines in a portion of Panay River (11°33'30"N, 122°50'22"E) and in one of the adjacent milkfish (*Chanos chanos*) grow-out culture ponds (11°33'34"N, 122°50'25"E) (Fig. 1). Panay River, the longest river in Panay Island, measures approximately 152 km (Mapcarta 2022) with a catchment area of 1,985 km² (Villalba et al. 2020). The spat used in the experiment were sourced from this river on March 10, 2019, through the pond caretaker's deployment of recycled bike tires used as cultches for the competent oyster larvae to settle. After three months, when enough spat were available, the experiment commenced on June 13, 2019.

Experimental Set-up

Six bamboo rafts, each measuring 2.0 x 1.5 m, were set-up in the pond and the adjacent Panay River. For each raft, 10 pouches made from black B-nets (0.5 cm mesh size), each with 25 pockets, were designated. Before stocking them in pouches, the spat growing on cultches were removed individually and then randomly distributed into 120 groups at 25 each (6 rafts per treatment x 10 pouches per raft x 25 pockets per pouch = 1,500 oyster spat per treatment). From each group, five were randomly taken, blotted dry, and measured for shell length to the nearest tenth of a centimeter (cm SL)

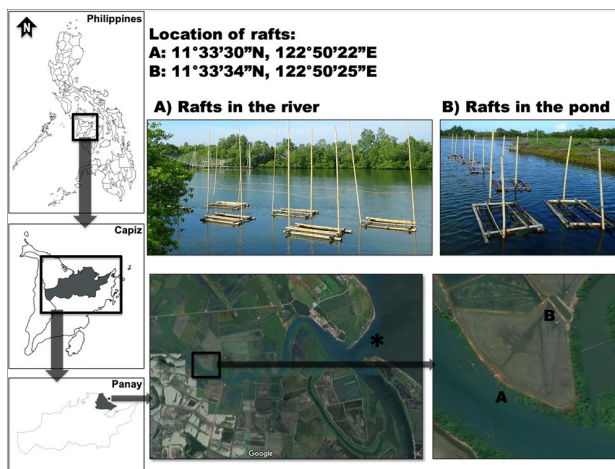


Fig. 1. Map showing the location of the study site in Panay, Capiz, Philippines, where the oysters *Magallana bilineata* were reared in grow-out culture in Panay River (A) and the adjacent milkfish pond (B) using pouches suspended from rafts. Photos of the study sites were taken by MJHL Lebata-Ramos, while some images were generated from Google Maps (<https://maps.google.com>) and QGIS (www.qgis.org).

and total body weight to the nearest tenth of a gram (g BW) using a plastic Vernier caliper and the Ohaus CS200 digital balance, respectively. Then, together with the remaining 20, the five were placed in the individual pockets of each pouch and hung at 10 per raft. Oyster spat in the river setup ranged 3.79 – 7.41 cm SL, means $\pm$ S.E. = 5.59 $\pm$ 0.04 cm; and 7.20 – 34.20 g BW, means $\pm$ S.E. = 19.46 $\pm$ 0.32 g, while those in the pond ranged 3.63 – 8.06 cm SL, means $\pm$ S.E. = 5.66 $\pm$ 0.04 cm; and 7.90 – 38.10 g BW, means $\pm$ S.E. = 19.72 $\pm$ 0.35 g.

Monthly Monitoring

Monthly monitoring of stocks commenced one month after stocking and every 30 d thereafter until the oysters were harvested. Five oysters, which amounted to 600 per mo, were randomly taken from each of the 10 pouches per replicate raft and measured for shell length and body weight, as in the previous section. After taking the necessary measurements, the oysters were returned to their respective pouches.

Temperature and salinity were measured daily using the laboratory alcohol thermometer and Atago hand refractometer, respectively. *In situ* measurements of dissolved oxygen (DO), sulfide, phosphate, nitrate, nitrite, and ammonia levels were done during monthly samplings using the LaMotte Smart2 colorimeter following procedures 3688-SC, 3654-01-SC, 3653-SC, 3649-SC, 3650-SC, and 3659-01-SC, respectively, as described in the operator's manual. The total suspended solids (TSS) concentration was determined according to the standard method 2540 D by filtering 100 ml from a well-mixed 1.0 L water sample collected from the two treatment sites through a glass fiber filter and weighing the dried residues (Baird and Bridgewater 2017). Plankton were sampled and counted following the methods of Baird and Bridgewater (2017) and Suthers et al. (2009). All measurements and water samples were taken approximately 1.0 m from the water surface at three points in each culture site to represent three replicates and reported as means $\pm$ S.E.

Harvest

After 6 mo of culture, all surviving oysters were harvested and counted. Following monthly monitoring protocols, five oysters from each pouch were measured for shell length and body weight, as previously described. Another 30 samples were randomly selected from each treatment for meat yield determination. Each was weighed and shucked, then the whole oyster flesh was removed, blotted dry with a paper towel, and weighed to the nearest tenth of a gram. Meat yield (MT)

was obtained following Betanzos-Vega et al. (2018) and Sorio and Peralta (2017) as:

$$MT = (\text{fresh flesh weight} / \text{total body weight}) \times 100 \quad (1)$$

The flesh samples of the 30 oysters from each treatment dissected for meat yield determination were then pooled and submitted to the Laboratory Facilities for Advanced Aquaculture Technology of the Southeast Asian Fisheries Development Center Aquaculture Department (SEAFDEC/AQD) for proximate composition analysis following the Official Methods of Analysis of AOAC International (Latimer 2016).

Statistical Analysis

Statistical analysis and graphs were done using the Minitab 17.0 software package (Minitab, State College, Pennsylvania, USA, <http://www.minitab.com>). Growth, meat yield, proximate composition, and survival between oysters reared in the river and the pond were tested for normality using the Anderson-Darling Test and compared using a two-sample *t*-test at $p < 0.05$ statistical significance. Meat yield, proximate composition, and survival in % were arcs in transformed prior to the test.

Monthly growth rates, both for length (GR_L) and weight (GR_W), were obtained using the following equations:

$$GR_L = (L_f - L_i) / (T_f - T_i) \times 30 \quad (2)$$

$$GR_W = (W_f - W_i) / (T_f - T_i) \times 30 \quad (3)$$

where GR = growth rate; *L* = length (cm); *W* = weight (g); *T* = sampling date; *f* = present sampling; *i* = preceding sampling; 30 = constant, days in a month.

Monthly growth rates were also correlated with mean monthly readings of temperature, salinity, DO, TSS, plankton count, sulfide, phosphate, nitrate, nitrite, and ammonia using the Pearson correlation at $p < 0.05$ statistical significance. Correlation coefficients were interpreted using Schober et al. (2018) as follows:

< 0.10 = negligible correlation;
0.10 – 0.39 = weak correlation;
0.40 – 0.69 = moderate correlation;
0.70 – 0.89 = strong correlation;
0.90 – 1.00 = very strong correlation.

RESULTS

Growth

Monthly means $\pm$ S.E. of length and weight of *M. bilineata*, reared for 6 mo in Panay River and the adjacent pond using pouches, are shown in Fig. 2. Both monthly

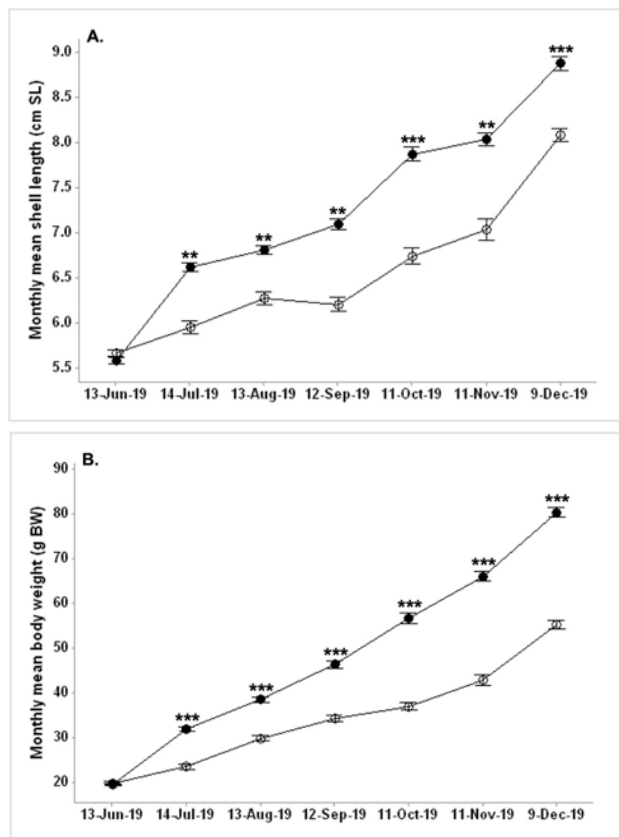


Fig. 2. Monthly means $\pm$ S.E. of (A) shell length and (B) body weight of oysters *Magallana bilineata* reared in grow-out culture in Panay River, Panay, Capiz, Philippines (black-filled circles), and the adjacent milkfish pond (white-filled circles) using pouches suspended from rafts. Two-sample *t*-test: *** $p < 0.001$; ** $p < 0.01$.

mean shell lengths and weights were significantly higher or much higher in oysters reared in the river than in the pond starting from the first month of culture until harvest. Monthly mean growth rates of oysters, both for length and weight, during the six-month culture duration were higher in those reared in the river (SL range = 0.18 – 1.00, means $\pm$ S.E. = 0.56 ± 0.02 cm mo^{-1} ; BW range = 6.81 – 15.59, means $\pm$ S.E. = 10.27 ± 0.42 g mo^{-1}) than in the pond (SL range = -0.08 – 1.17, means $\pm$ S.E. = 0.41 ± 0.03 cm mo^{-1} ; BW range = 2.75 – 13.77, means $\pm$ S.E. = 5.99 ± 0.22 g mo^{-1}). However, growth rates showed a very high significant difference in oysters reared in the river than in the pond only during the first [two-sample *t*-test: $p < 0.001$, $T = -9.97$ (1.00 vs. 0.25 cm mo^{-1}); $p < 0.001$, $T = -12.30$ (12.07 vs. 3.36 g mo^{-1})] and the third month of culture [two-sample *t*-test: $p < 0.01$, $T = -3.84$ (0.31 vs. -0.08 cm mo^{-1}); $p < 0.05$, $T = -3.09$ (8.33 vs. 4.41 g mo^{-1})] (Fig. 3).

The meat yield of oysters reared in the river (range = 13.86 – 32.86%; means $\pm$ S.E. = $25.96 \pm 0.92\%$) did not

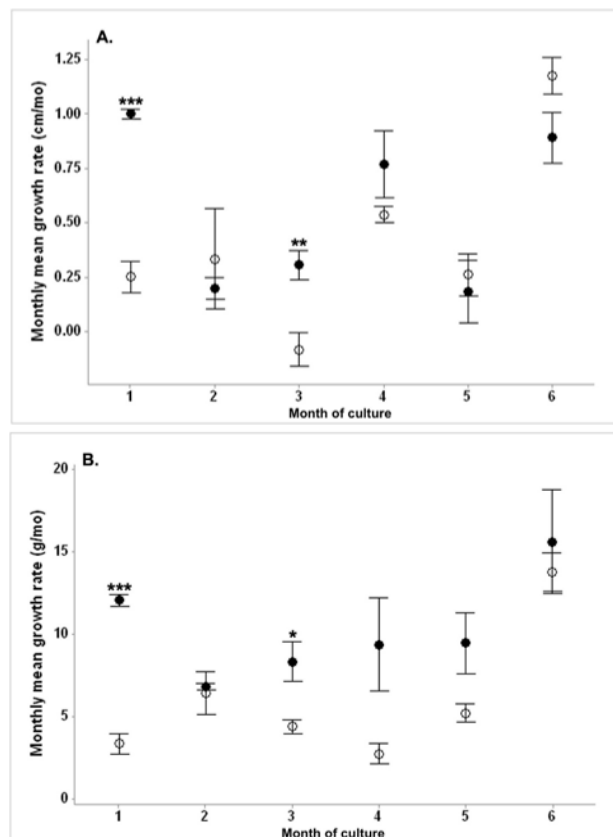


Fig. 3. Monthly means $\pm$ S.E. of growth rates in terms of (A) cm/mo shell length and (B) g/mo body weight of oysters *Magallana bilineata* reared in grow-out culture in Panay River, Panay, Capiz, Philippines (black-filled circles), and the adjacent milkfish pond (white-filled circles) using pouches suspended from rafts. Two-sample *t*-test: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

significantly differ from those in the pond (range = 9.30–40.00%; means $\pm$ S.E. = $24.05 \pm 1.41\%$) (two-sample *t*-test: $p > 0.05$). Moreover, the proximate composition of dried whole flesh samples showed almost the same amounts of crude protein ($58.04 \pm 0.14\%$, $53.86 \pm 0.38\%$), crude fat ($7.00 \pm 0.08\%$, $7.93 \pm 0.38\%$), nitrogen-free extract ($24.68 \pm 0.07\%$, $28.54 \pm 0.74\%$), and ash ($10.27 \pm 0.01\%$, $9.68 \pm 0.01\%$) in both river and pond-reared oysters, respectively. In addition, crude fiber was not detected in both samples.

Survival

Mean survival at harvest was significantly very high in oysters reared in the river at $70.21 \pm 2.84\%$ than those in the pond at $13.10 \pm 1.57\%$ (two-sample *t*-test: $p < 0.001$, $T = 17.60$). During the first and second months of culture, during monthly monitoring of stocks, some pouches in the pond had peregrine crabs (*Varuna litterata*) preying on the young oysters, while others were already empty and had large holes with some remnants of oyster shell fragments. There was no evidence of crab predation in

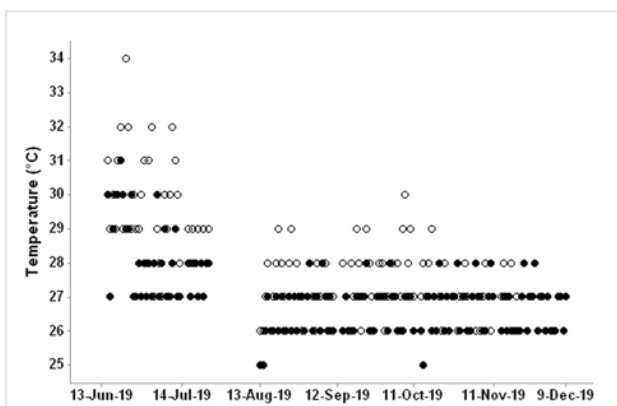


Fig. 4. Daily water temperature (°C) recorded from Panay River, Panay, Capiz, Philippines (black-filled circles) and the adjacent milkfish pond (white-filled circles) from 13 June to 9 December 2019, during the grow-out culture of oysters *Magallana bilineata* reared using pouches suspended from rafts.

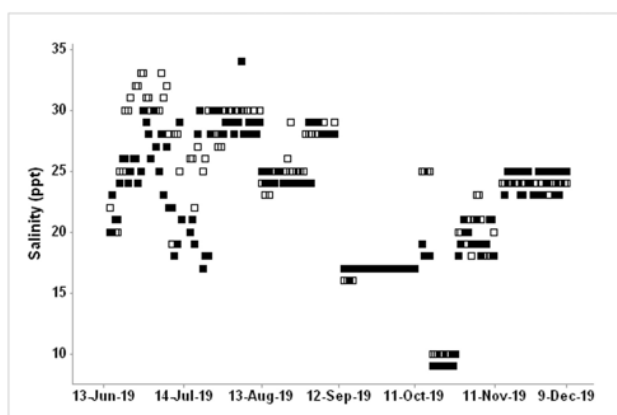


Fig. 5. Daily salinity (ppt) recorded from Panay River, Panay, Capiz, Philippines (black-filled squares) and the adjacent milkfish pond (white-filled squares) from 13 June to 9 December 2019, during the grow-out culture of oysters *Magallana bilineata* reared using pouches suspended from rafts.

oysters reared in the river. However, within the duration of culture, some pouches got lost, which contributed to the relatively lower survival of oysters in the river.

Environmental Variables

From June 13 to December 9, 2019, daily water temperature ranged from 25 – 31°C (means $\pm$ S.E. = 27.00 $\pm$ 0.09°C) in Panay River and 26 – 34°C (means $\pm$ S.E. = 27.76 $\pm$ 0.12°C) in the pond (Fig. 4), while salinity ranged from 9 – 34 ppt (means $\pm$ S.E. = 22.32 $\pm$ 0.42 ppt) and 10 – 33 ppt (means $\pm$ S.E. = 23.43 $\pm$ 0.44 ppt) (Fig. 5), respectively. Means of temperature, salinity, dissolved oxygen, TSS, plankton, sulfide, phosphate, nitrate, nitrite, and ammonia, recorded monthly for 6 mo from the first month of culture until harvest, are shown in Table 1. The top two most dominant plankton were the same in both

Panay River and the pond and varied only in concentrations; mean concentrations of *Coscinodiscus* sp. and *Navicula* sp. were 2.22 $\pm$ 0.26 and 1.86 $\pm$ 0.17 cells ml⁻¹ in Panay River, respectively, and 1.78 $\pm$ 0.37 and 0.51 $\pm$ 0.08 cells ml⁻¹ in the pond, respectively.

Pearson correlation analysis showed significant correlations in growth rates of oysters reared in the river and the pond with some water parameters tested in both culture areas. Growth rates in both length and weight of those reared in the river had significantly strong negative correlations with phosphate concentration (Pearson correlation: $p < 0.05$, $r = -0.80$; $p < 0.05$, $r = -0.82$, respectively). On the other hand, those reared in the pond had significantly moderate and strong positive correlations with TSS (Pearson correlation: $p < 0.05$, $r = 0.52$; $p < 0.01$, $r = 0.72$, respectively) and ammonia concentrations (Pearson correlation: $p < 0.01$, $r = 0.53$; $p < 0.001$, $r = 0.74$, respectively), and significantly strong and moderate positive correlations with plankton concentration (Pearson correlation: $p < 0.001$, $r = 0.75$; $p < 0.001$, $r = 0.66$, respectively).

Table 1. Means $\pm$ S.E. of monthly temperature, salinity, dissolved oxygen, total suspended solids, plankton, sulfide, phosphate, nitrate, nitrite, and ammonia recorded in Panay River and the adjacent milkfish pond in Panay, Capiz, Philippines, during the six-month grow-out culture of oyster *Magallana bilineata* in pouches. Two-sample *t*-test: $p < 0.05$ denotes significant differences readings from Panay River and the pond.

Parameters	Panay River	Pond	Two-Sample <i>T</i> -test
Temperature (°C)	26.77 $\pm$ 0.11	27.48 $\pm$ 0.17	$p < 0.01$, $T = -3.50$
Salinity (ppt)	21.82 $\pm$ 0.89	22.83 $\pm$ 0.92	$p > 0.05$, $T = -0.78$
Dissolved oxygen, DO (ppm)	4.58 $\pm$ 0.11	5.48 $\pm$ 0.43	$p > 0.05$, $T = -2.00$
Total suspended solids, TSS (ppm)	45.21 $\pm$ 13.02	10.38 $\pm$ 2.20	$p < 0.05$, $T = 2.64$
Plankton (cells ml ⁻¹)	18.08 $\pm$ 1.48	8.00 $\pm$ 1.04	$p < 0.001$, $T = -5.55$
Sulfide (ppm)	0.031 $\pm$ 0.002	0.079 $\pm$ 0.021	$p < 0.05$, $T = -2.22$
Phosphate (ppm)	0.210 $\pm$ 0.014	0.135 $\pm$ 0.024	$p < 0.05$, $T = 2.70$
Nitrate (ppm)	0.054 $\pm$ 0.009	0.029 $\pm$ 0.005	$p < 0.05$, $T = 2.47$
Nitrite (ppm)	0.066 $\pm$ 0.011	0.067 $\pm$ 0.014	$p > 0.05$, $T = -0.02$
Ammonia (ppm)	0.047 $\pm$ 0.008	0.018 $\pm$ 0.004	$p < 0.01$, $T = 3.19$

DISCUSSION

This study compared the growth of *M. bilineata* wild spat when reared in grow-out culture in the river and the adjacent earthen pond using pouches (Leбата-Ramos et al. 2021a, 2021b). Mean growth rates of oysters, both for length and weight, were higher in those reared in the river than in the pond. These are comparable with the results of Leбата-Ramos et al. (2021b) on wild spat *C. iredalei* cultured in pouches in Batan Bay (0.60 cm SL mo^{-1} ; 11.0 g BW mo^{-1}) and three other different sites (0.54 cm SL mo^{-1} , 9.87 g BW mo^{-1}) (Leбата-Ramos 2019). Similarly, Songkeao et al. (2006) reported a higher growth rate in *Crassostrea belcheri* reared in the natural waters of Khlong Natap (0.69 cm SL mo^{-1}) than in the shrimp pond (0.39 cm SL mo^{-1}). Likewise, in a study conducted by Chueachat et al. (2018), *C. iredalei* cultured inside earthen ponds had lower growth rates (0.51 cm mo^{-1}) compared with those reared in mangrove canals (0.87 cm mo^{-1}). Oyster growth is greatly affected by the culture environment, and it is evident in this study that better growth is achieved in an open natural environment than in a closed system. Both growth rates in the length and the weight of oysters reared in the pond had significantly moderate and strong positive correlations with TSS and ammonia which, during the whole duration of culture, ranged from 2.0 – 31.3 ppm (means $\pm$ S.E. = 10.38 ± 2.20) and 0.00 – 0.08 ppm (means $\pm$ S.E. = 0.018 ± 0.004), respectively. Also noted were the significantly strong and moderate positive correlations with plankton concentration which, during the whole duration of culture, ranged from 2.08 – 22.60 cells ml^{-1} (means $\pm$ S.E. = 8.00 ± 1.04).

In the river, where TSS (45.2 ± 13.02 ppm), ammonia (0.047 ± 0.01 ppm), and plankton (18.08 ± 1.48 cells ml^{-1}) were significantly higher, no correlations with growth were observed. Plankton are an important food for filter-feeding organisms like oysters. Oyster growth rates are primarily regulated by food supply and are highly site-specific depending on the availability of food (Brown 1988; Grangeré et al. 2009). In the pond, where plankton supply is limited, the oysters' need for food is clearly shown in the positive correlations of plankton concentrations with growth rates (Fig. 6a), while in the river, where they are abundant, no correlation with growth was evident (Fig. 6b).

The increasing plankton concentration may be one of the contributing factors to the increasing TSS in the pond where water is more stagnant as compared with the running water in the river. The increasing concentration of plankton with the increasing TSS concentration may also explain the significant positive

correlation of oyster growth rates with both plankton and TSS levels in the pond. In a study in the Pearl River Estuary comparing phytoplankton in the turbidity maximum zone (TMZ) (mean TSS = 171 mg L^{-1}) and the adjacent non-TMZ (mean TSS = 45 mg L^{-1}), results showed a positive correlation of phytoplankton abundance with TSS in the non-TMZ, where phytoplankton concentration continuously increased until it peaked at around 40 mg L^{-1} (Shi et al. 2017). This study shows that the positive correlation of plankton concentration with TSS has limitations because high concentrations of suspended substances may affect plankton growth due to light limitation. On the other hand, the positive correlation of growth rates with ammonia may also be indicative of the relationship between plankton density and ammonia, with the latter being utilized by the rapidly regenerating plankton (Pietros and Rice 2003). In the river, where most environmental factors affecting growth were not

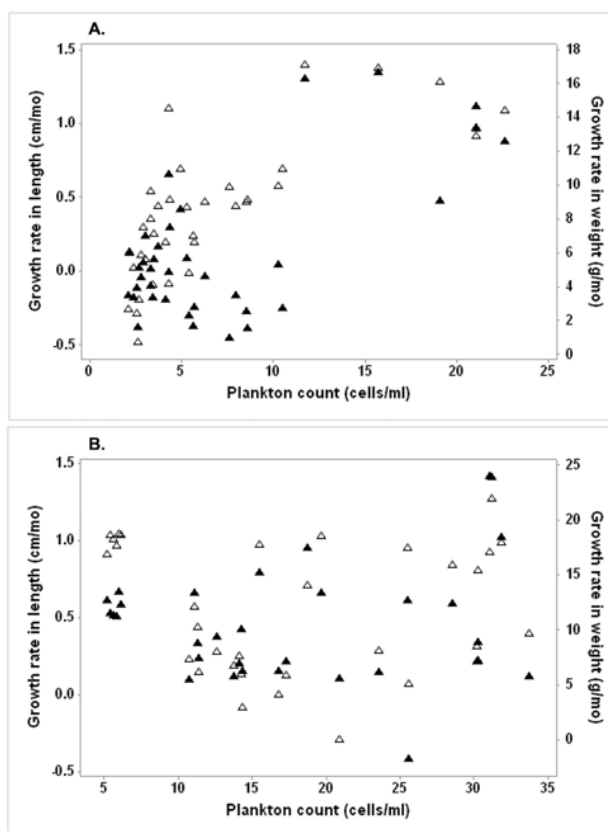


Fig. 6. A) Positive correlations of plankton concentrations with growth rates in terms of length (Pearson correlation: $p < 0.001$, $r = 0.75$; white-filled triangles) and weight (Pearson correlation: $p < 0.001$, $r = 0.66$; black-filled triangles) of oysters *Magallana bilineata* reared in grow-out culture in a milkfish pond using pouches suspended from rafts. B) No significant correlation was observed between plankton concentrations and growth rates of oysters reared in Panay River, Panay, Capiz, Philippines.

limiting, no significant correlations with growth were observed. However, phosphate, which exceeded the maximum acceptable level for marine waters of 0.2 ppm (PHILMINAQ 2013), showed significantly strong negative correlations with growth rates for both length and weight. This negative effect of phosphate on the growth of oysters was not observed in the pond, where phosphate concentrations ranged from 0.04 – 0.22 ppm and were mostly below the maximum acceptable level for marine waters. Kunigelis and Wilbur (1987) also reported the negative effects of high levels of phosphate on *Crassostrea virginica*, where trochophore larvae exposed to 15 and 150 ppm of different phosphate compounds resulted in morphological developmental changes, increased mortality, and inhibition of shell growth.

The meat yield of oysters varies greatly between species but is almost the same within species. *Crassostrea gigas* in the United Kingdom and Brazil had almost the same meat yield of 5 - 14% and 11.0%, respectively (Stroud 1981; Muniz et al. 1986). The same was reported for *Ostrea edulis* in the United Kingdom (6 – 18%) and Turkey (6 – 16%) (Stroud 1981; Yildiz et al. 2011). In this study, the meat yield of oysters cultured in the river ($25.96 \pm 0.92\%$) was higher but not significantly different from that in the pond ($24.05 \pm 1.41\%$). These were a bit higher than the meat yield obtained by Lebata-Ramos et al. (2021b) for the same species of oysters reared in Batan Bay using the same methods ($22.60 \pm 0.86\%$). However, Sorio and Peralta (2017) reported a lower meat yield of 14.5 – 18.9% for *C. iridalei* obtained from a local producer and reared using the traditional method. Aside from the variations by species, differences in meat yield, especially for *M. bilineata*, may be attributed to the differences in the culture environment and methods. With a very high meat yield of almost 25%, when using the pouch method both in the river and the pond, the latter may be considered for culturing oysters.

Lebata-Ramos et al. (2021a) reported the first proximate composition of dried whole flesh samples of wild and hatchery-bred *M. bilineata*. Earlier, Peralta et al. (2018) reported the first proximate composition of *C. iridalei* residue, a by-product of oyster extract processing. Although the results of the present proximate composition analysis of oysters reared in the river and the adjacent pond slightly vary from the values obtained earlier (Lebata-Ramos et al. 2021a), it is understandable and acceptable since the proximate composition of oysters may vary within species between sizes (Woke et al. 2016), depths of culture area (Ngo et al. 2006), and seasons (Martino and da Cruz 2004; Kim et al. 2014).

Mean survival at harvest was relatively low at $70.21 \pm 2.84\%$ in the river and only $13.10 \pm 1.57\%$ in the pond. Although Treviño et al. (2020) considered 70% as adequate survival for cultivation, survival obtained in the present study was relatively low compared with previous reports on *C. iridalei* regardless of culture methods (Lebata-Ramos et al. 2021b) or source of seed stocks (Lebata-Ramos et al. 2021a). Similarly, high survival rates have been reported in other species of *Crassostrea*— 86.5 – 98.7% in *C. virginica* cultured subtidally for 9 mo and subjected to varying levels of aerial exposure (La Peyre et al. 2018), and 98.6% for triploid and 76.7% for diploid *C. gigas* reared in trays for 10 mo (Villanueva-Fonseca et al. 2017). However, many factors can affect survival. In some species, survival can be affected by size and density. *Crassostrea gigas* had lower survival (31.5 – 57.3%) when initially stocked at 20 – 30 mm shell height and higher survival (63.2 – 89.7%) when stocked at 40 – 50 mm (Brown and Hartwick 1988). In *C. belcheri*, depending on the stocking density, survival rates varied from 55.8 – 98.3% (Tanyaros et al. 2008). In this study, the very low survival in the pond may be attributed to predation by peregrine crabs *V. litterata* during the early months of culture, leaving some pouches with large holes and remnants of oyster shell fragments. On the other hand, lower survival in the river was due to some missing pouches, which may have been stolen or accidentally dropped from the raft because of strong river currents. The results showed that using pouches suspended from rafts to culture oysters in shallow ponds is not advisable. Pouches may only be used in deeper ponds, suspended from the raft, with at least a distance of 0.5 m from the pond substrate to minimize mortalities caused by bottom-dweller predators. Similarly, the length of the pouches may be adjusted with the depth of the pond, maintaining the recommended distance of 0.5 m from the pond bottom.

CONCLUSION

As culture areas become less productive due to intensive culture exceeding their carrying capacity, thus deteriorating water quality, and as they get smaller due to industrialization and urbanization expanding seaward, new culture methods and areas need to be explored to meet the increasing demand for oysters. This study investigated the use of ponds, both abandoned and productive, as potential culture areas for oysters. Despite the significantly lower growth rate of oysters in the pond than those cultured in the river, the meat yield of almost 25% in both culture areas is very promising. Culture in the pond may be extended from 1 – 1.5 mo to

compensate for the slower growth rates and attain the size of oysters reared in the river for only 6 mo. The low survival of oysters reared in the pond may be addressed by suspending pouches in deeper ponds or adjusting their length to maintain at least a distance of 0.5 m from the pond substrate and minimize predation. Moreover, the culture of oysters in ponds may be done using other methods and must not be limited to the use of suspended pouches from rafts.

In a closed system, such as the pond where nutrients are lower and limited, oyster growth rates showed a positive correlation with some parameters. No such correlation between oyster growth rates and the environmental parameters in the river were observed. Hence, it is recommended to culture oysters in ponds with fed aquaculture commodities that may help provide ample nutrients for the oysters. In this study, oysters were cultured with milkfish in an extensive system where formulated feeds were given only towards the end of the culture—when *lab-lab* or algal mat, which is technically known as periphyton (Fortes and Piñosa 2010), the natural food in the pond, had been consumed. Based on the results of the present work and as supported by earlier studies (Songkeao et al. 2006; Chueachat et al. 2018), oysters may be cultured in an impoundment, and growth rates and survival may be improved through some refinements in the culture techniques. For the pond to be utilized in oyster culture, food for the filter-feeding oysters and the presence of predators are among the most important factors to be considered when refining the culture method.

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