

Grow out of the Pacific oyster *Magallana gigas* (Thunberg, 1793) within culture ponds of the shrimp *Penaeus vannamei* Boone, 1931

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Abstract. The biological feasibility of cultivating Magallana gigas, within semi-intensive culture ponds of the shrimp, Penaeus vannamei in the Cojimíes estuary, Esmeraldas province, Ecuador, was evaluated from July 2021 to February 2022. A batch of *M. gigas* (45.4±2.52 mm) was seeded in lantern nets with a diameter of 50 cm (4-tier; 68 organisms/tier - 40% coverage of the base area) until reaching commercial size. Water temperature, salinity, and transparency were recorded, and inorganic and organic seston levels as well as phytoplankton biomass, were evaluated. At the end of the study, a 40% survival of oysters was obtained, with a length of 92.9±1.86 mm, a dry shell mass of 40.0±3.21 g, and a dry soft tissue mass of 1.2±0.06 g. Abrupt temperature increases with high intraday intervals, coinciding with low chlorophyll-a concentration and high seston content, were associated with periods of little or no shell growth, suggesting a negative influence of these factors on the productive performance of *M. gigas*. Average growth rates of 0.22 mm day⁻¹ were recorded, which are lower than those reported for this oyster in tropical and subtropical regions of the American Pacific. However, adult oysters can be harvested at commercial size (80 mm) within 7 months of growing out. M. gigas is a species with the potential for incorporation into multi-trophic aquaculture in shrimp ponds, which brings benefits such as nitrogen capture and reduction of microalgae and seston concentrations. Further research is recommended to optimize farming techniques and enhance the productive performance of shrimp farms. Key Words: Crassostrea gigas, lantern net, mollusc culture, multitrophic culture, shrimp culture, tropic.

Introduction. The Pacific oyster *Magallana gigas* (Thunberg, 1793), formerly known as *Crassostrea gigas* (Thunberg, 1793), identified as a species of temperate climate, has been successfully cultivated in various regions worldwide due to its high adaptability to different environmental factors and its wide market acceptance (Martínez-García et al 2022). The annual global production of *M. gigas* is estimated to be > 600,000 t, with the highest production in temperate regions (FAO 2023). However, recent studies have shown the high feasibility of cultivation in tropical areas, particularly in equatorial waters (Chávez-Villalba et al 2021).

In coastal areas of the equatorial region, there have been reports of cultivations in which the Pacific oyster reaches commercial size (80 mm) in 10 months (Lodeiros et al 2018), or in 5 months in estuarine waters (Treviño et al 2020). This suggests that the growth of *M. gigas* in tropical waters is favored by estuarine environmental conditions associated with high levels of seston concentration, particularly high phytoplankton concentration. Given that shrimp farming systems contain high concentrations of particulate organic matter, suspended solids, and bacteria that can be filtered by bivalve mollusks (Tendencia 2007; Martínez-Córdova et al 2011; Barraza-Guardado et al 2013),

it has been suggested that coupling bivalve mollusks in shrimp farms can contribute to increasing the profitability of the cultivation and reducing eutrophication that typically occurs in shrimp farming systems (Ramos et al 2008; Strand et al 2019; Mazón-Suástegui et al 2022).

In this context, Lombeida-Terranova (1999) reported the results of several studies on the growth and survival of Pacific oysters in reservoirs, drainage channels, and shrimp culture ponds, determining certain feasibility of *M. gigas* cultivation in a shrimp farm located in the province of Santa Elena, Ecuador. In contrast, Vergara-López et al (2006) showed that *M. gigas* developed with low profitability in experimental cultivations in shrimp farm reservoirs in the province of Coclé, Panama.

This discrepancy between studies and the limited information regarding the production of *M. gigas* in shrimp ponds in tropical climates underscores the need to increase research efforts in this area. The objective of this research was to evaluate the biological feasibility of cultivating the Pacific oyster *M. gigas* in shrimp culture ponds in the Cojimíes estuary, Esmeraldas, Ecuador.

Material and Method

Cultivation. The study was conducted within a semi-intensive shrimp culture pond for *Penaeus vannamei* located in the Cojimíes estuary, San José de Chamanga Parish, Esmeraldas Province, Ecuador ($0^{\circ}17'25.4"$ N; 79°58'36.9" W). The pond was set up with a system of suspended Japanese lantern nets on a long line for growing out oysters with an average size of 45.4±2.52 mm in length, for a period of 7 months (July 7, 2021 - February 12, 2022). The oysters were obtained from local cultivation from the Chone River estuary area, following the recommendations of Treviño et al (2020), and transported dry at a temperature of 15°C.

The bioassay was carried out with 5 experimental units, each consisting of a 4-tier lantern net. Following the recommendations of Vélez-Falcones et al (2020), the cultivation started with 68 organisms, equivalent to 40% coverage of the base area of the tier. For each unit, the second tier was considered as the experimental replicate, and the remaining tiers were used as reservoirs for the replacement of sampled organisms.

Monthly, the live organisms were counted to estimate the survival rate, and 15 randomly selected individuals from each experimental replicate were measured for antero-posterior length, dorso-ventral height, and shell width or thickness, corresponding to the maximum length between valves. These measurements were taken using a digital caliper (with a precision of 0.01 mm). Additionally, every two months, 5 randomly selected individuals per experimental replicate were collected to determine the dry mass of the shell and soft tissues. The dry mass of the shell and soft tissues was dehydrated at 65°C for 48 h and weighed using a balance (with a precision of 0.001 g).

Environmental variables. To assess the effect of environmental factors on the cultivated oysters, the temperature was recorded daily at 30-minute intervals using a submersible thermograph (Onset HOBO, USA), and salinity was measured using a refractometer (with a precision of 1‰), with water samples collected on an intra-weekly basis. Simultaneously, water transparency was determined using a Secchi disk. Every fifteen days, water samples were taken and filtered (through an 80 µm filter) to determine the seston in its organic, inorganic, and total fractions, as well as the estimated phytoplankton biomass through chlorophyll-a analysis, following the recommendations in Strickland & Parsons (1972).

Statistical analysis. According to the Shapiro-Wilk and Levene tests, the data distribution for some variables did not meet the assumptions of homogeneity of variances. Therefore, the Welch-corrected ANOVA was used, when necessary, to evaluate the response variables associated with growth parameters, considering the sampling time as a factor. Differences between samplings were determined using *post-hoc* Games-Howell analysis. The significance level for all tests was set at p = 0.05.

Results. Survival gradually decreased until October 2021, reaching values of $54\pm17.81\%$ (Figure 1). It then decreased to $42.4\pm18.34\%$ at the middle of November and remained without significant differences until the end of the study ($39.2\pm22.8\%$).



Figure 1. Survival of Pacific oyster *Magallana gigas* cultivated in suspended lantern nets within a shrimp farm pond in a semi-intensive culture system, in the Cojimíes estuary, Esmeraldas Province, Ecuador. The vertical lines indicate the 95% confidence intervals.

Growth. The growth in shell dimensions of the oyster showed a continuous pattern, except for the period from mid-November to mid-December when there were no significant differences in the average values of the three shell length dimensions (Figure 2). In the final period of the study, from mid-December 2021 to mid-February 2022, the growth rate in anteroposterior length was 0.50 mm day⁻¹, dorsoventral growth was 0.30 mm month⁻¹, and between-valve growth was 0.21 mm day⁻¹, which was notably higher than the growth rate observed in previous periods (< 0.25 mm day⁻¹ anteroposterior length). At the end of the study, the average anteroposterior length of the shell was 92.9±1.86 mm (Figure 2a), the dorsoventral length was 51.6±2.21 mm (Figure 2b), and the between-valve length was 29.2±3.71 mm (Figure 2c).

Both, the shell mass (Figure 3a) and tissue mass (Figure 3b) exhibited a continuous growth pattern. There was a slightly lower increase in mass during the first period (early July to early September 2021) compared to the subsequent periods. At the end of the experiment, the average dry mass of the shell reached 25.2 ± 3.21 g, while the tissue mass was 1.3 ± 0.07 g.

The relationship among the between-valves and the anteroposterior lengths of the shell did not show a distinct pattern during the experimental period (Figure 4). Generally, the values were above 0.3 or close to that value. At the end of the study, when the organisms reached commercial sizes, the relationship was 0.33 ± 0.027 .



Figure 2. Growth of the shell in anteroposterior length (a), dorsoventral length (b), and between valves length (c) of the Pacific oyster *Magallana gigas* cultivated in suspended lantern nets within a shrimp farm pond in a semi-intensive culture system, in the Cojimíes estuary, Esmeraldas Province, Ecuador. The vertical lines indicate the 95% confidence intervals.









Environmental factors. The daily average temperature ranged from approximately 25 to 29.5°C throughout the study (Figure 5a). The highest values were recorded at the beginning of the experiment, during July 2021. These increases were associated with a high thermal variation of up to an interval of 6°C per day. There was a high-temperature variation during the early months of the experiment, with sudden decreases of more than 10°C in mid-December 2021. Towards the end of December, the temperature started to gradually increase with lower daily variations (< 3°C).

The transparency of the water varied throughout the study, with generally variable values ranging from 20 to 120 cm. The transparency remained variable throughout the entire study period, with values generally above 70 cm. However, there was a trend of decreasing transparency towards the end of the experiment (Figure 5b).

Salinity generally remained within the range of 24 to 26‰ throughout the study period (Figure 5c). However, there were notable decreases at the beginning of June 2021, towards the end of October 2021, and at the end of the experiment, when it dropped significantly to 11-14‰.



Figure 5. Average daily temperature (thick line) and observed minimum and maximum values (thin lines) (a); intraweekly record of water transparency measured with Secchi disk (b); and salinity (c) in the experimental culture zone.

The phytoplankton biomass (Figure 6a) remained above 5 μ g L⁻¹ of chlorophyll-*a*, reaching up to 87 μ g L⁻¹ in mid-September 2021. The values were lower, below 45 μ g L⁻¹, during July-August, and then increased during August-September 2021. Subsequently, there was a decrease until October 2021, with values around 5 μ g L⁻¹ in late December, and then increased again from January 2022.

The total sector registered values between 150 and 300 mg L⁻¹, with the organic component being more representative, ranging from 110 to nearly 200 mg L⁻¹ (Figure 6b). The inorganic component of the sector was always lower than the organic component and generally ranged from 32 to 65 mg L⁻¹, except in mid-September when it reached values close to 100 mg L⁻¹.



Figure 6. Seston in its components total, organic and inorganic (a) and estimated phytoplankton biomass by chlorophyll-*a* (b) in the experimental culture zone.

Discussion. The biological attributes of *M. gigas* described by various trials in different regions, establish a positioning of this species as a suitable economic alternative for aquaculture in different parts of the world, including Latin American regions (Martínez-

García et al 2022). However, its potential to be integrated into multitrophic systems in tropical areas has been scarcely explored.

The results of our study suggest that *M. gigas* (with an initial length of 45 mm) can reach the commercial size (80 mm) in 6-7 months with a survival of 40% and a growth rate of 0.22 mm day⁻¹ after being integrated into semi-intensive shrimp farming ponds. This allows us to suggest *M. gigas* as a species with certain feasibility to be cultured in shrimp ponds in a tropical region. However, the integration of the culture of both species requires further investigation since the productive parameters obtained in the present study can be considered of low yield, compared to other studies carried out in seas, coastal lagoons, or estuaries in the tropics and subtropics of the American Pacific (Chávez-Villalba et al 2021).

There are few published studies on the growth of *M. gigas* in shrimp farms in tropical areas. One of the first studies conducted in Ecuador was carried out by Lombeida-Terranova (1999), who, after several experiments, showed, in the most successful trial, that using Pacific oyster seeds measuring 4 to 11 mm, could obtain individuals with an antero-posterior length of 70 mm in about 4.2 months, with a survival rate of 60%, which represents a growth rate of 0.47-0.53 mm day⁻¹. These parameters were higher than those obtained in the present study. In contrast, Vergara-López et al (2006) conducted experimental cultivation of juvenile oysters in a reservoir located in the province of Coclé, Panama, and obtained low yields. Oysters reached a harvest size of 72 mm in 9.3 months after seeding juvenile oysters of 4.2 mm, with a growth rate of 0.24 mm and a survival rate of 28%. Unlike Lombeida-Terranova (1999), who used a system of trays on tables, Vergara-López et al (2006) used a suspended system, similar to the one used in this study, suggesting that the suspended cultivation system might not to be suitable for the production of *M. gigas* in shrimp ponds. Comparative studies of various types of baskets and farming systems must be tested to verify the aforementioned hypothesis.

Another important factor to consider is the environmental parameters of the cultivation system, as they govern the physiological performance of oysters and, consequently, the profitability of production (Smaal et al 2019). The average temperature recorded in this study ranged from 25 to 29.5°C, with maximum values of up to 35.5°C during certain hours of the day. Mann et al (1991) establish an optimal temperature range of 11-34°C for *M. gigas*; however, other authors suggest a greater growth at temperatures below 24°C, a decrease in growth at 30°C, and a negative effect on growth at temperatures between 33 and 37°C (Friedman et al 1998). Additionally, for iuvenile M, gigas, a temperature of 32° C is associated with a survival rate of less than 50% (Flores-Vergara et al 2004). This information suggests that the temperatures reported in the present study during the first 3 months of cultivation, may have caused unfavorable physiological changes in the current and future performance of the cultivated oysters at that time. This was reflected in the high mortality observed during this cultivation period and can be explained by the fact that the biological performance of marine bivalves is closely related to thermal stress, which affects physiological responses and the productive performance of organisms. Additionally, high intraday temperature variability was observed, which was greater during the warmer periods, reaching amplitudes of up to ~10°C in some cases, which could have induced drastic physiological changes. This greater temperature amplitude occurred during the period from November to December 2021 when the lowest concentrations of phytoplankton biomass (~5 μ g L⁻¹ chlorophyll-a) were recorded, and it was when the organisms ceased in shell growth. Probably, during this period, the organisms did not grow because they could not meet the metabolic demands due to the high temperatures and the drastic changes associated with a low availability of food.

On the contrary, when the temperature does not exceed 28°C and there is low intraday variability (< 3°C), the oysters showed high growth rates and higher survival. The differences between the years 2021 and 2022 in terms of high temperatures with equally high intraday temperature intervals, and during the rainy season in early 2021 compared to the rainy season in 2022, could be associated with the global climate phenomenon of La Niña prevailing since the second half of 2021 (NOAA 2023). This effect

of better performance of *M. gigas* cultivated within a shrimp farm at lower temperatures and lower performance at higher temperatures was also pointed out by Lombeida-Terranova (1999).

It is important to note that the period during which a reduction in shell growth was observed (November-December) did not reflect in the soft tissue of the organisms. Taking into account that *M. gigas* is considered a species of temperate zones and its first maturity in tropical areas has been reported in organisms with lengths of ~20 mm (Kasmini et al 2019), it is not ruled out that during this period, the reproductive-capable organisms allocated energy reserves to gonad formation. Similar results have been reported by Mazón-Suástegui et al (2022) in the Cortez oyster, *Crassostrea corteziensis* (Hertlein, 1951) cultured with shrimp effluents. These authors associated the reproductive season of the species with periods where the organisms showed constant weight growth accompanied by a reduction in shell growth. In bivalve mollusks, when the gametogenesis process is initiated, most of the organism's available energy is allocated to gamete development rather than shell growth (Barber & Mann 1991; Wadsworth et al 2019).

Considering the recorded values of environmental factors, it can be established that the oysters were exposed to eutrophicated cultivation environment, reaching high loads of suspended matter (average transparency < 60 cm with seston typically > 150 mg L⁻¹), which could have affected the physiological processes of *M. gigas*. A seston concentration of ~90 mg L⁻¹ in this species leads to regulation of ingestion rates through the production of pseudofeces, and concentrations above 90 mg L⁻¹ result in a reduction of ingestion rates (Barille et al 1997). This implies particle selection and mucus secretion to compact them, which undoubtedly incurs an energy cost that oysters must bear to thrive in such an environment. Additionally, in high seston concentrations, oysters may close their valves to avoid excessive or clogged gills.

It appears that the high levels of eutrophication would likely not allow optimal performance of the oysters. Studies related to these aspects regarding oyster performance in different shrimp ponds with varying levels of eutrophication are necessary to elucidate the aforementioned hypothesis. An alternative strategy is to cultivate oysters in areas with a lower suspended matter, such as reservoirs of shrimp farms or other shrimp cultivation systems with lower seston load, as described for shrimp farm effluents in Mexico, where the native species *C. corteziensis* achieves high growth rates (Mazón-Suástegui et al 2022).

Another strategy could be to increase the integration of trophic levels into cocultivation. This means employing multitrophic systems associated with macroalgae, which can reduce nutrients and seston load by decreasing nitrogen and phosphorus levels, as well as microalgal load. This approach could provide suitable conditions for oyster growth and bioremediation (Brito et al 2014; Song et al 2020).

It is also possible that long-term responses or higher stocking densities in oyster cultivation may yield more favorable results than our experimental cultivation. With a larger number of oysters, the filtration capacity increases, which in turn enhances the possibility of reducing seston levels in the cultivation ponds. Whether through one way or another, future research should focus on elucidating the aforementioned hypotheses.

The results demonstrate that starting culture with 45 mm oyster in shrimp farm ponds operated under semi-intensive systems, it is feasible to achieve a length of 100 mm with a survival rate of 40% in 7-8 months of cultivation. Additionally, individual specimens with appropriate morphology for commercialization can be obtained, as indicated by the favorable morphometric relationship between valve length and anteroposterior length (> 0.25) (Brake et al 2003; Mizuta & Wolfors 2018).

Considering the potential of oyster-shrimp co-cultivation and its possible advantages for implementation in Ecuadorian shrimp farms, such as its multitrophic action in capturing nitrogen, reducing microalgal concentrations, and lowering seston levels, our analysis based on experimentation in a shrimp farm in the Cojimies estuary suggests that future studies should focus on the development and optimization of zootechnical practices for *M. gigas* production in co-cultivation with *P. vannamei* to improve and optimize the observed performance.

Conclusions. According to our results, the biological feasibility of cultivating *Magallana gigas* in shrimp farms is not particularly high compared to other cultivation areas in tropical and subtropical regions. However, we suggest considering its cultivation due to the potential benefits it may bring to shrimp production, such as the possible reduction in nitrogen content and concentrations of microalgae and seston.

Acknowledgements. This research was funded through the project "Feasibility of Pacific oyster (*Crassostrea gigas*) and winged oyster (*Pteria sterna*) cultivation in the estuary of the Chone River, Manabí, Ecuador" by IMBICUM - Universidad Técnica de Manabí and *AquaCEAL* Corporation. The collaboration of J. A. López-Carvallo is acknowledged for reviewing the manuscript and providing valuable comments.

Conflict of interest. The authors declare that there is no conflict of interest.

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Received: 02 June 2023. Accepted: 21 June 2023. Published online: 29 June 2023. Authors:

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How to cite this article:

Vélez-Giler E., Vélez-Falcones J., Cáceres-Farias L., Espinoza-Vera M. M., Cedeño-Zambrano J., Mazón-Suásteguil J. M., Lodeiros C., 2023 Grow out of the Pacific oyster *Magallana gigas* (Thunberg, 1793) within culture ponds of the shrimp *Penaeus vannamei* Boone, 1931. AACL Bioflux 16(3):1769-1780.