

Research Article

Role of biofloc technology in hatchery efficiency for farming of mangrove crab (*Scylla serrata*)

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Abstract

Mangrove crab aquaculture is expanding in response to the growing global demand, however, production in the Philippines remains limited due to low survival rates, which are attributed to suboptimal water quality, insufficient nutrition, and disease outbreaks. This study assessed the overall performance of *Scylla serrata* from zoea to crab instar in various culture media: Biofloc water (BW), green water (GW), and clear water (CW). Survival from zoea 5 to crab instar was highest in GW; however, no significant differences were observed among treatments ($p>0.05$). Microbial analysis showed significantly higher total heterotrophic bacteria and presumptive *Vibrio* counts, but significantly lower luminous bacteria, in BW compared with GW and CW ($p<0.05$). Crabs reared in BW exhibited significantly higher survival after 12 hours of simulated transport and greater tolerance to abrupt low salinity exposure after 12 and 24 hours ($p<0.05$). Although survival after 24 hours of transport did not differ significantly among treatments, BW consistently maintained the highest mean survival. Overall, BW emerges as a promising alternative culture medium to traditional hatchery protocols, particularly during early larval stages (zoea 1–5). This study provides the first empirical evidence supporting the use of biofloc technology in mangrove crab larval hatchery systems, with the potential to enhance both resilience and sustainability.

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Introduction

Mangrove crabs of the genus *Scylla* are economically important crustaceans that inhabit mangrove ecosystems in the warm temperate and tropical regions of the Pacific and Indian Oceans (Alberts-Hubatsch *et al.*, 2016; Bir *et al.*, 2020; Lebata-Ramos *et al.*, 2024). Within this genus, *Scylla serrata*, commonly known as the giant mangrove crab, is the preferred candidate for aquaculture in the Philippines owing to its larger body size and more rapid growth rate (Quinitio *et al.*, 2011), as well as its greater economic value and widespread adoption among farmers (Nolial, 2024). In 2022, mangrove crab production in the Philippines reached 16,387 metric tons, ranking it as the sixth top aquaculture commodity in the country (Bureau of Fisheries and Aquatic Resources (BFAR, 2023). Traditionally, the supply of crab seed for aquaculture has relied heavily on wild populations; however, overexploitation and degradation have driven a shift toward the development of sustainable hatchery-based production systems (Waiho *et al.*, 2018; Paran *et al.*, 2022). Several countries have successfully completed the full life cycle of mangrove crabs under captive conditions to meet increasing global demand while reducing threats to wild populations (Quinitio and Parado-Estepa, 2017; Waiho *et al.*, 2018). In the Philippines, hatchery technology for *S. serrata* has been established and adopted at a commercial scale, primarily based on standardized protocols refined over the past two decades (Quinitio *et al.*, 2018; Lebata-Ramos *et al.*, 2024). Mangrove crab larvae are typically reared in hatcheries using either clear water or a microalgae-enriched

“green water” systems with periodic manual water exchange (Quinitio *et al.*, 2018; Waiho *et al.*, 2018). Despite technological advancements, larval rearing of *S. serrata* remains constrained by inconsistent survival, high levels of cannibalism, and difficulties in maintaining optimal water quality (Ganesh *et al.*, 2015; Kasan *et al.*, 2021). These limitations continue to impede large-scale production and highlight the need for more efficient, stable and environmentally sustainable rearing methods. Biofloc technology (BFT) has emerged as a promising approach for improving water quality and productivity in intensive aquaculture systems. In crustaceans such as the Pacific white shrimp (*Penaeus vannamei*) and mangrove crabs (*Scylla paramamosain*), BFT has been shown to stabilize pH, reduce toxic nitrogen compounds, and maintain adequate dissolved oxygen (Ong *et al.*, 2019; Kasan *et al.*, 2021). BFT is a microbial-based aquaculture system in which aggregates of bacteria, algae, protozoa, and organic matter are cultivated to improve water quality while providing additional nutritional benefits to the cultured species (Emerenciano *et al.*, 2025; Yadav *et al.*, 2025). This eco-friendly system, characterized by limited or zero water exchange, recycles nutrients such as ammonia into microbial biomass, thereby reducing the need for frequent water changes (Bossier and Ekasari, 2017; Zafar and Rana, 2022; Yadav *et al.*, 2025). Consequently, biofloc biomass serves as a continuous source of microbial protein and fatty acids that can support growth and survival while enhancing resistance to stress and pathogens (Khanjani and

Sharifinia, 2020; Yadav *et al.*, 2025). As such, BFT offers a sustainable approach to mangrove crab larval rearing, with the potential to mitigate persistent challenges such as high mortality and cannibalism, while improving production efficiency (Ong *et al.*, 2019; Kasan *et al.*, 2021; Zafar and Rana, 2022). Despite the recognized benefits of biofloc technology in crustacean aquaculture, its application to the larval rearing of *S. serrata* remains limited, and comprehensive evaluation of its effects on hatchery performance is lacking (Ong *et al.*, 2019; Kasan *et al.*, 2021).

Therefore, the present study aimed to evaluate the effects of biofloc water on the survival and growth performance of *S. serrata* larvae and to assess its impact on water quality and microbial composition in comparison with conventional green water and clear water systems. By examining BFT as a sustainable and environmentally friendly alternative for hatchery operations, this study addresses a critical gap in current rearing practices and provides regionally relevant solutions for improving larval survival and quality, thereby supporting food security and strengthening export markets for mangrove crab aquaculture in Southeast Asia.

Materials and methods

Animal ethics

This study was performed in compliance with all the principles of the Philippine National Standard: Code of Good Aquaculture Practices (PNS/BAFS 134:2014) and the SEAFDEC/AQD institutional guidelines for the care and use of experimental animal.

Experimental set up and inoculum preparation

This study was carried out at the mangrove crab hatchery of the Southeast Asian Fisheries Development Center, Aquaculture Department (SEAFDEC/AQD). Three different culture systems were tested in triplicates using twelve 1-ton fiberglass tanks: green water (GW), biofloc water (BW), and clear water (CW). Before the experiment, biofloc inoculum was prepared using a suspended-growth “green water” biofloc system (El-Sayed, 2021) using red tilapia (*Oreochromis* spp.). The red tilapia was utilized in the development of the microbial community consisting of algae and bacteria, with molasses added as a carbohydrate source to stimulate the growth of heterotrophic bacteria. Vigorous aeration was applied to maintain the suspension of the microbial community and enhance the phytoplankton productivity. Once the biofloc inoculum reached 2 mL/L sedimentable solids (Kasan *et al.*, 2021), it was transferred to the biofloc treatment tanks. Green water treatment followed the addition of *Nannochlorum* sp. (10^5 cells/mL) into the larval tanks (Quinitio *et al.*, 2018), which is the standard hatchery protocol at SEAFDEC/AQD. Clear water is a closed-water system that used dechlorinated seawater.

Experimental animals and growth performance

The standard hatchery protocol for *S. serrata* seed production (Quinitio *et al.*, 2018) was followed with slight modification. Hatchery-produced zoea 1 (Z1) was randomly collected and stocked at

100 zoea/liter per tank. The larval stage index (LSI) (n=10 per treatment) was monitored prior to thinning out, and was

$$\text{Larval stage index (LSI)} = \frac{(N1 \times n1) + (N2 \times n2) + (Ni \times ni)}{(n1 + n2 + ni)}$$

Where, N1, N2, Ni is the larval stage; while, n1, n2, ni is the number of larvae at each stage

The survival rate was measured once ~90% of the population reached zoea 5 (Z5) stage by visual examination and confirmed through LSI. After which, thinning was done once the larvae are entering the megalopa (M) stage (Syafaat *et al.*, 2019) by stocking 4,000 individuals/L per treatment and further reared until crab instar (C3) in the same 1-ton tank. Growth and survival of crab instar (C3) (n=30 per treatment) were recorded upon the termination of the experiment, when ~90%

calculated as (Nguyen *et al.*, 2021):

of the population was in crab instar stage checked visually. Because of the small size of the crablets, 30 samples were weighed by pooling in groups of 10 individuals using a digital weighing balance (Ohaus, USA), at a precision of 0.01 g. The carapace length was measured using a ruler scale with 0.01 cm precision. The survival rate was calculated following the formula of Kasan *et al* (2021):

$$\text{Survival (\%)} = \frac{\text{Number of individuals alive at the end of time period}}{\text{Number of individuals alive at the start of time period}} \times 100$$

Feeding and water management

Mangrove crab larvae in all treatments were fed with rotifers (15 ind/ml from Z1 to Z5) and newly hatched *Artemia* (0.5-1.0 ind/ml) from Z2 to megalopa, and 3–5-day old *Artemia* from Z4 to M. Additionally, a shrimp-formulated diet was incorporated into the feeding starting from Z2 stage. The GW treatment utilized the *Nannochlorum* sp. (10^5 cells/ml) replaced every four days during the water change (Quinitio *et al.*, 2018). The CW treatment used dechlorinated seawater, replaced every four days during water change. For the BW treatment, 10 L of filtered mature biofloc water was added once to each tank and mixed with dechlorinated seawater. Molasses was added as a carbon source

when the TAN levels, measured using API test kit® are higher than 1 ppm. The floc was measured using an Imhoff cone (Hargreaves, 2013) every 4 days to coincide with the water change of the other treatments, but water will only be added to BW tanks when sedimentable solids exceed 8 mL/L. Water parameters such as dissolved oxygen, pH, temperature, and salinity, was monitored daily every 0900h using a multi-parameter meter (YSI, USA), while total ammonia nitrogen (TAN), nitrite (NO₂-N), nitrate (NO₃-N) was monitored every four days before water change in GW and CW tanks, and every day for BW tanks using the API Master Test Kit®. To supplement the data, water samples collected in the mid-layer of each

treatment were analyzed weekly for TAN, NO₂-N and NO₃-N, Phosphate (PO₄-P) and alkalinity using a spectrophotometer following the standard procedures (American Public Health Association (APHA, 2017). Photoperiod followed the natural light with indoor conditions.

Microbial community monitoring

Microbial monitoring of total plate count (TPC), luminous bacterial count (LBC) and presumptive *Vibrio* count (PVC) in the tank water was done weekly until termination. Water samples were collected from the surface of each tank at 0900 h using sterile containers and immediately sent to Fish Health Section for analysis. Bacterial enumeration was done by plating tenfold serial dilutions using standard saline solution (NSS) in a separate nutrient agar (Pronadisa, Condalab) with 1.5% NaCl (RCI Labscan Ltd.) for TPC and LBC, and thiosulfate bile sucrose (TCBS) agar (Pronadisa, CondaLab) for PVC. Colonies on THC, LBC and PVC plates within the range of 30 – 300 were counted following incubation at 28°C for 24 h. Results were expressed as colony-forming units per milliliter (CFU/mL).

Stress response of crab larvae

At the termination of the experiment, larval quality was evaluated through transport stress and abrupt low salinity stress test (Palacios and Racotta, 2007). Ten crab instars per treatment group were stocked in triplicate 500-mL round plastic containers with 300 mL dechlorinated freshwater, with gentle aeration provided by a single air stone per container. After 24 and 48 h of exposure, crab instars that do not respond to

mechanical stimuli or when no movement was observed when touched were considered dead (Emerenciano *et al.*, 2011). For the transport stress test, C3 (ABW=0.03 g±0.02) from the different treatment groups (3 replicates each) were harvested and packed following the methods of Quinitio *et al.* (2018). Crablets were individually counted in basins prior to packing (100 instars/bag) in oxygenated double plastic bags (34 x 25 cm) with 1 L of water and straw shelters as substrate, and placed inside the styrofoam boxes provided with ice wrapped in paper. The styrofoam boxes were then placed in a mechanical shaker at 75 oscillations per minute (Mitamura Riken Kogyo, Inc.) to simulate movement during transport. Three replicates of each treatment were used to determine the survival rates after 12 and 24 h of simulated transport. The crab instars were considered dead once its color changed from transparent to opaque white or has no response when gentle pressure is applied.

Statistical analysis

Statistical analysis was conducted using SPSS version 23 (Statistical Tool package). The assumptions of normality and homogeneity of the variances were tested using Shapiro-Wilk and Levene's test, respectively. One-way analysis of variance (ANOVA) was used to determine significant differences among treatments, followed by Tukey's HSD test for post hoc comparison of mean between groups. All the data were expressed in the text, figures and tables as mean±standard error. Statistical significance was detected at $p < 0.05$ with a 95% confidence interval.

Results

Water quality parameters and microbial community

The mean values of the dissolved oxygen, temperature, pH, salinity, NO₃-N, NO₂-N and alkalinity recorded no significant changes ($p>0.05$) among the treatments (Table 1). However, TAN was observed to be significantly higher in GW ($p=0.015$)

than in CW and BW tanks, while PO₄-P was significantly lower in CW tanks ($p=0.044$). Significantly higher mean total heterotrophic bacterial counts ($p=0.010$) and mean presumptive *Vibrio* counts ($p=0.036$), and significantly lower presence of luminous bacteria ($p=0.009$) were observed in BW tanks (Table 2).

Table 1: Water parameters (mean \pm SE) in different culture medium during the larval rearing experiment of mangrove crab. CW: Clear water, GW: Green water, and BW: Biofloc water.

Water Parameters	CW	GW	BW
Dissolved oxygen (mg/L)	4.65 \pm 0.05	4.68 \pm 0.36	4.67 \pm 0.07
pH	8.11 \pm 0.04	8.12 \pm 0.05	8.11 \pm 0.05
Temperature ($^{\circ}$ C)	29.38 \pm 0.09	29.31 \pm 0.09	29.44 \pm 0.09
Salinity (ppt)	32 \pm 0.07	32 \pm 0.08	32 \pm 0.09
TAN (mg/L)	0.70 \pm 0.2 ^b	1.11 \pm 0.6 ^a	0.62 \pm 0.1 ^b
Nitrate-Nitrogen (mg/L)	0.21 \pm 0.1	0.14 \pm 0.1	0.10 \pm 0.1
Nitrite-Nitrogen (mg/L)	0.31 \pm 0.2	0.44 \pm 0.1	0.32 \pm 0.2
Phosphate (mg/L)	0.15 \pm 0.1 ^b	0.23 \pm 0.1 ^a	0.24 \pm 0.1 ^a
Total Alkalinity (mg/L)	169.55 \pm 31.8	177.30 \pm 24.4	175.84 \pm 31.4

Notes. Values are means (\pm SE) of three replicates. Mean values in the same row with different superscript letters are significantly different ($p < 0.05$) as determined by Tukey's HSD test.

Table 2: Microbiological results (mean \pm SE) of the larval rearing experiment in different culture medium. CW: Clear water, GW: Green water, BW: Biofloc water, THB: Total heterotrophic bacteria, LBC: Luminous bacterial count, and PVC: Presumptive *Vibrio* count.

Parameters	CW	GW	BW
THB (10 ⁴ CFU/mL)	2.15 \pm 1.62 ^b	3.16 \pm 4.89 ^b	10.2 \pm 5.36 ^a
LBC (10 ¹ CFU/mL)	196.3 \pm 1.30 ^b	15.0 \pm 19 ^b	0.17 ^a
PVC (10 ⁴ CFU/mL)	1.55 \pm 1.63 ^b	0.78 \pm 9.54 ^b	7.27 \pm 4.20 ^a

Notes. Values are means (\pm SE) of three replicates. Mean values in the same row with different superscript letters are significantly different ($p < 0.05$) as determined by Tukey's HSD test.

Growth performance and survival rate

The growth performance of mangrove crab reared in different culture medium from the Z1 to C3 stages demonstrated consistent development with LSI values that are relatively equal among treatments (Table 3). During the Z1 to Z5 stage, all treatments reached Z5 after 13 days of culture (DOC), while C3 was attained after DOC14 from Z5. The average carapace width (ACW) and average body weight (ABW) of C3 was

not significantly different among treatments ($p>0.05$). The larval survival from Z1 to Z5 were slightly higher in BW tanks compared to GW and CW (Fig.1a), but the differences were not statistically significant ($p>0.05$). In the Z5 to C3 stage, survival rates decreased across all treatments, with GW showing a higher survival than CW and BW (Fig. 1b), though no significant differences were observed ($p>0.05$).

Table 3: Growth parameters of mangrove crab from Z1 to C1 reared in different culture medium. CW: Clear water, GW: Green water, BW: Biofloc water, DOC: Days of culture, LSI: Larval stage index, SD: Stocking density, ACW: Average carapace width, and ABW: Average body weight.

Treatment	Stocking Density Z1 to Z5	DOC Z1 to Z5	LSI DOC13	SD Z5 to C3	DOC Z5 to C3	ACW (cm)	ABW (g)
CW	100,000	13	5	4,000	11	2.2±1.33 ^a	0.028±0.014 ^a
GW	100,000	13	5	4,000	11	2.4±1.28 ^a	0.033±0.016 ^a
BW	100,000	13	5	4,000	11	2.2±1.17 ^a	0.032±0.019 ^a

Notes. Values are means (\pm SE) of three replicates. Mean values in the same column with different superscript letters are significantly different ($p < 0.05$) as determined by Tukey's HSD test.

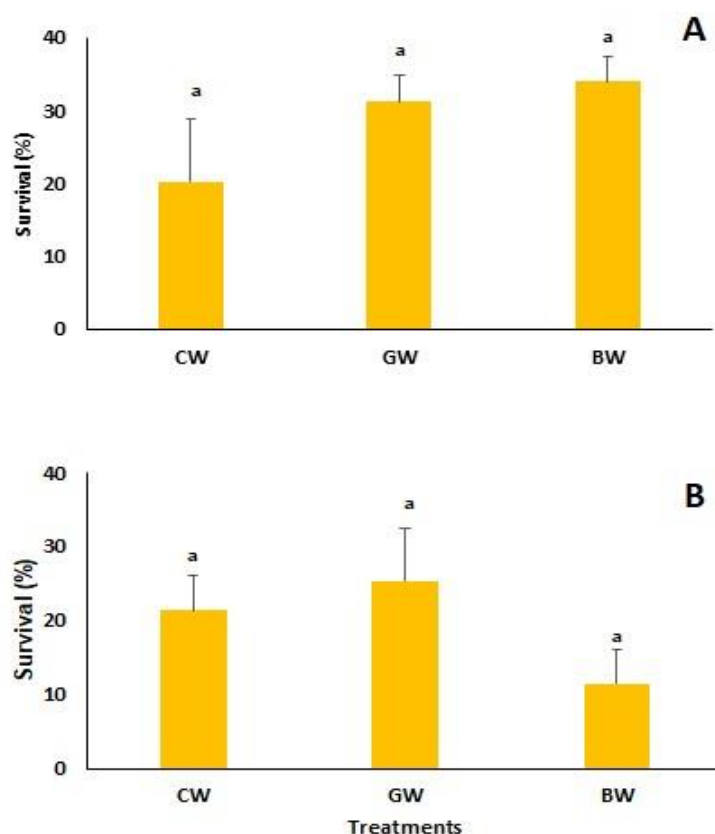


Figure 1: Mean survival (%) of mangrove crab larvae reared in different culture medium from: A) Z1 to Z5 and B) Z5 to C3. Means with the same superscripts indicate no statistically significant differences ($p > 0.05$). CW: Clear water, GW: Green water, and BW: Biofloc water.

Transport and salinity stress test

The mean survival (%) of crab instars reared in different culture medium after simulated transport at 12 and 24 hours is presented in Figure 2. After 12 hours of transport, the survival rate was significantly higher in BW ($p = 0.022$) compared to CW and GW treatments (Fig. 1a). After 24 hours, survival rates declined across all

treatments, with no significant differences among CW, GW, and BW ($p > 0.05$). For the salinity stress, survival was significantly higher in BW and GW tanks than in CW after 24 hours abrupt salinity change ($p = 0.048$) (Fig. 2b).

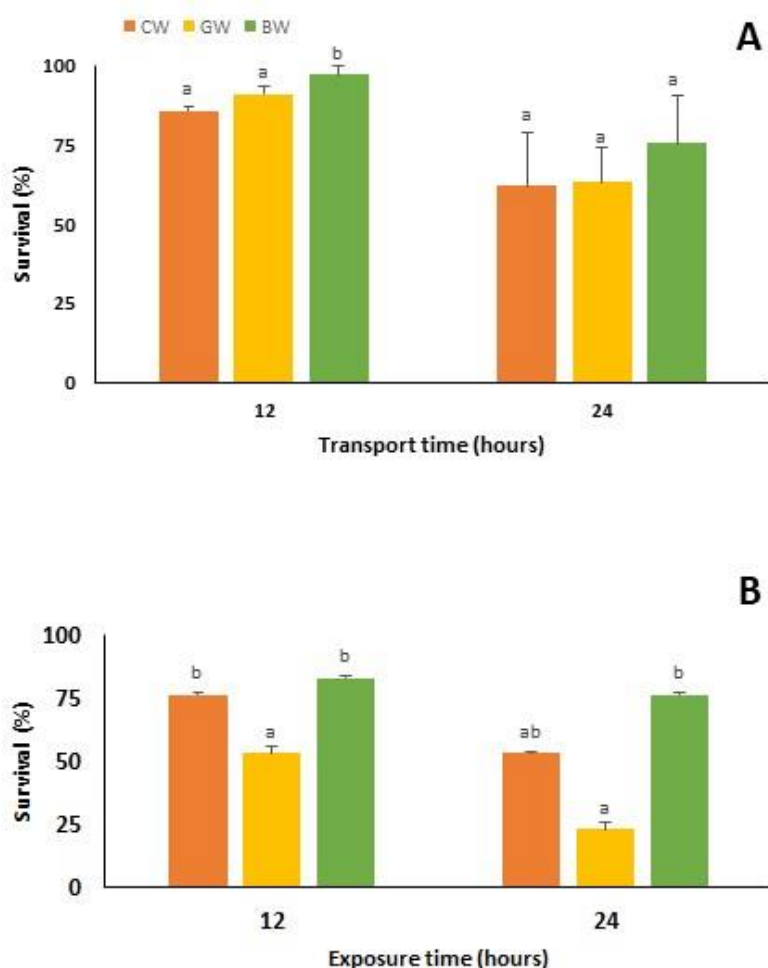


Figure 2: Mean survival (%) of crab instars reared in different culture medium after: A) simulated transport and B) low salinity stress test for 12 and 24 hours. Means with different superscripts indicate statistically significant differences ($p < 0.05$). CW: Clear water, GW: Green water, and BW: Biofloc water.

Discussion

A major challenge in aquaculture is ensuring proper care for farmed organisms during the early developmental stages, when they are highly sensitive to environmental fluctuations (El-Sayed *et al.*, 2024). This study demonstrates the influence of biofloc water on the hatchery performance of *S. serrata*, focusing on water quality, growth, survival, and stress tolerance. The water quality parameters (DO, pH, temperature, and salinity) in all treatments remained within the ranges reported as suitable for larval rearing of *S. serrata* under hatchery conditions (Ganesh

et al., 2015; Quintio *et al.*, 2018), as well as for related *Scylla* species (Herlinah *et al.*, 2021; Pati *et al.*, 2023). Notably, TAN was significantly higher in GW, likely reflecting phytoplankton-driven nitrogen dynamics in the green water systems and ammonia release during algal biomass decomposition (El-Sayed, 2021). In contrast, BW maintained lower TAN levels through heterotrophic microbial assimilation, efficiently converting nitrogenous wastes into bacterial biomass (Bossier and Ekasari, 2017; Zafar and Rana, 2022). The addition of a carbon source, such as molasses, has been shown to enhance this process and

significantly lower TAN and nitrite concentrations while improving the overall water quality and larval survival (Rajkumar *et al.*, 2016; Khanjani *et al.*, 2020; El-Sayed, 2021). Moreover, Quintio and Parado-Esteba (2000) reported that *S. serrata* larvae can occasionally tolerate TAN levels of up to about 4 ppm under hatchery conditions, which is consistent with the survival observed in GW tanks despite elevated TAN concentrations. Similar nitrogen dynamics under biofloc conditions have been reported for mud crab larvae by Kasan *et al.* (2021). Meanwhile, PO₄-P was lowest in CW due to minimal microbial activity, algal biomass, and reduced organic matter accumulation, whereas BW exhibited higher PO₄-P levels, characteristic of zero-water-exchange systems where nutrients are retained and recycled (Bossier and Ekasari, 2017; Kumar *et al.*, 2021). Microbial analysis showed that BW supported significantly higher THB (10.2×10^4 CFU/mL) and PVC (7.27×10^4 CFU/mL) than CW and GW, consistent with Cadiz *et al.* (2016) who observed higher *Vibrio* counts in BFT tanks (10^2 – 10^5 CFU/mL) compared to tilapia green water tanks (10^2 – 10^4 CFU/mL) for *P. vannamei*. The high THB community in BW tanks is likely due to the addition of carbon sources, which increases organic matter availability and promotes heterotrophic bacterial growth (Deng *et al.*, 2018; Panigrahi *et al.*, 2020; Kumar *et al.*, 2021). Importantly, the luminous bacterial count was significantly lower in BW (1.67×10^0 CFU/mL), emphasizing the ability of the biofloc system to suppress harmful microbial populations. Biofloc systems are known to improve water quality and foster

beneficial microbial communities that enhance immunity and suppress pathogens, thereby improving larval health and survival in cultured crustaceans (Ahmad *et al.*, 2017; El-Sayed, 2021; Kasan *et al.*, 2021). The dominance of the microbial community in BW tanks minimized pathogenic bacteria by acting as natural bioremediation candidates in the biofloc system. This microbial community also contributes to maintaining water quality and recycling nitrogenous wastes into microbial biomass that can serve as a supplementary food source in the rearing system (Panigrahi *et al.*, 2020; Kumar *et al.*, 2021). Similar microbial dynamics have been reported in biofloc-based larval rearing of mangrove crabs, where heterotrophic communities improved water stability and larval performance (Kasan *et al.*, 2021). Growth metrics (ACW and ABW) were similar among treatments, indicating that the different rearing media supported comparable larval growth. However, survival from Z1 to Z5 was highest in BW tanks, whereas GW outperformed CW and BW during the Z5–C3 stages. The early-stage advantage in BW may be related to stable water quality and beneficial microbial communities, while the improved performance in GW at later stages likely reflects the nutritional contributions of live microalgae and associated plankton in green water systems (Bossier and Ekasari, 2017; Panigrahi *et al.*, 2018; Basford *et al.*, 2021). The decline in BW survival beyond Z5 may be linked with floc volumes exceeding the 2 mL/L threshold recommended by Kasan *et al.* (2021), as excessive settleable solids can impair swimming, feeding, and oxygen

exchange. In zero-exchange systems, unconsumed feed and wastes accumulating on the tank bottom may further obstruct larval movement and contribute to increased mortality (Abakari *et al.*, 2021). Additionally, the rapid rise in ammonia from waste breakdown and larval excretion required further molasses addition, which stimulated bacterial growth and biofloc formation, thereby increasing settleable solids (Xu *et al.*, 2016; El-Sayed, 2021). Maintaining lower settleable solids provides more favorable conditions for larval swimming, development, and survival (Kasan *et al.*, 2021). Although biofloc improves water quality and provides nutritional benefits, excessive floc can interfere with molting and oxygen uptake, particularly in burrowing crab instars, potentially causing gill clogging, hypoxia, and subsequent mortality (Emerenciano *et al.*, 2013a; Schweitzer *et al.*, 2013; Ray *et al.*, 2017; El-Sayed, 2021).

After the hatchery phase, salinity and transport stress tests were conducted to evaluate larval quality following standard procedures used in crustacean hatcheries (Quinitio and Parado-Esteba, 2000; Estante-Superio *et al.*, 2023). These tests are particularly relevant for mangrove crab larvae, which experience abrupt salinity changes and handling stress during transport and stocking. Rapid salinity shifts impose osmotic stress that may exceed the limited regulatory capacity of early larval stages (Thabet *et al.*, 2017), while transport stress can disrupt physiological and immune balance in decapod crustaceans (Shi *et al.*, 2023).

Previous studies have shown that mud crab larvae are highly sensitive to abrupt

salinity reductions, especially during the zoeal stages, when survival and successful metamorphosis may be compromised (Bac and Tien, 2016; Misbah *et al.*, 2017). Although mangrove crabs are euryhaline, maintaining osmotic balance under low salinity requires substantial physiological adjustments, and tolerance varies among life stages (Xu *et al.*, 2023). To date, reports on the stress tolerance of crab instars reared in biofloc-based systems are lacking, indicating that the improved tolerance observed in BW-reared larvae represents new evidence under hatchery conditions. Crabs reared in BW exhibited significantly higher survival after 12 hours of simulated transport and greater tolerance to abrupt low salinity after both 12 and 24 hours. These results suggest that the beneficial microbial and algal communities present in biofloc systems contribute to the physiological resilience of the larvae (Emerenciano *et al.*, 2013b; Bossier and Ekasari, 2017), thereby improving their stress tolerance. Similar findings have been reported for other aquatic species, including *Cyprinus carpio* (Adineh *et al.*, 2019), *Macrobrachium rosenbergii* (Miao *et al.*, 2020), and *P. vannamei* (Esparza-Leal *et al.*, 2020), where biofloc systems enhanced tolerance to environmental stressors. Comparable responses have also been observed in shrimp, although species-specific differences may occur in brachyuran larvae. Overall, the improved survival and stress tolerance of BW-reared larvae indicate that biofloc systems may provide a more stable rearing environment than conventional clear water or green water systems for *S. serrata* hatcheries.

Conclusion

This study highlights the first trial of biofloc use in larval rearing of *S. serrata* and its potential to improve hatchery efficiency. Although biofloc technology has been widely applied in shrimp hatcheries, its use in mangrove crab larviculture remains limited (El-Sayed, 2021; Kasan *et al.*, 2021). The present findings indicate that biofloc water can help maintain water quality, improve survival and enhance the stress resistance of crab zoea. Given the increasing demand for mangrove crab seedstock in the Philippines and across Southeast Asia, integrating biofloc water into hatchery protocols could enhance production efficiency, improve seed quality for grow-out farms, and contribute to the sustainability of the regional aquaculture industry. However, further research is needed to optimize biofloc use beyond the megalopa stage, particularly focusing on sludge management, crab instar behavior, and the cost-effectiveness of the biofloc systems. Moreover, a deeper understanding of the physiological mechanisms underlying the improved resilience of BW-reared crabs, particularly the role of gut microbiota, immune function, and metabolic responses, remains unexplored.

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Conflicts of interest

The authors declare no conflict of interest.

References

- Abakari, G., Luo, G., Kombat, E.O. and Alhassan, E.H., 2021.** Supplemental carbon sources applied in biofloc technology aquaculture systems: Types, effects and future research. *Reviews in Aquaculture*, 13, 1193–1222. DOI:10.1111/raq.12525
- Adineh, H., Naderi, M., Hamidi, M.K. and Harsij, M., 2019.** Biofloc technology improves growth, innate immune responses, oxidative status, and resistance to acute stress in common carp (*Cyprinus carpio*) under high stocking density. *Fish and Shellfish Immunology*, 95, 440–448. DOI:10.1016/j.fsi.2019.10.045
- Ahmad, I., Babitha Rani, A.M., Verma, A.K. and Maqsood, M., 2017.** Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. *Aquaculture International*, 25, 1215–1226. DOI:10.1007/s10499-016-0108-8
- Alberts-Hubatsch, H., Lee, S.Y., Meynecke, J.O., Diele, K., Nordhaus, I. and Wolff, M., 2016.** Life-history, movement, and habitat use of *Scylla serrata* (Decapoda, Portunidae): Current knowledge and future challenges. *Hydrobiologia*, 763:5–21. DOI:10.1007/s10750-015-2393-z
- APHA., 2017.** Standard methods for the examination of water and wastewater. 23rd ed. Denver: American Public Health Association, American Water Works Association, Water Environment Federation.

- Bac, N.V. and Tien, N.T., 2016.** The effect of salinity on survival and metamorphosis of larvae mud crab (*Scylla paramamosain*). Proceedings of the 9th Vietnamese-Hungarian International Conference: Research for Developing Sustainable Agriculture, Tra Vinh University, Vietnam. pp 368-374.
- Basford, A.J., Makings, N., Mos, B., White, C.A. and Dworjanyn, S., 2021.** Greenwater, but not live feed enrichment, promotes development, survival, and growth of larval *Portunus armatus*. *Aquaculture*, 534, 736331. DOI:10.1016/j.aquaculture.2020.736331
- BFAR, 2023.** Philippine fisheries profile 2022. Quezon City: Bureau of Fisheries and Aquatic Resources. Philippines. 159 P.
- Bir, J., Islam, S.S., Sabbir, W., Islam, R. and Huq, K.A., 2020.** Ecology and reproductive biology of mud crab *Scylla* spp: A study of commercial mud crab in Bangladesh. *International Journal of Academic Research and Development*, 5, 01-07.
- Bossier, P. and Ekasari, J., 2017.** Biofloc technology application in aquaculture to support sustainable development goals. *Microbial Biotechnology*, 10, 1012–1016. DOI:10.1111/1751-7915.12836
- Cadiz, R.E., Traifalgar, R.F.M., Sanares, R.C., Andrino-Felarca, K.G.S. and Corre, V.L., 2016.** Comparative efficacies of tilapia green water and biofloc technology (BFT) in suppressing population growth of green *Vibrios* and *Vibrio parahaemolyticus* in the intensive tank culture of *Penaeus vannamei*. *Aquaculture, Aquarium, Conservation & Legislation*, 9, 195–203
- Deng, M., Chen, J., Gou, J., Hou, J., Li, D. and He, X., 2018,** The effect of different carbon sources on water quality, microbial community and structure of biofloc systems. *Aquaculture*, 482, 103–110. DOI:10.1016/j.aquaculture.2017.09.033
- El-Sayed, A.F.M., 2021.** Use of biofloc technology in shrimp aquaculture: A comprehensive review, with emphasis on the last decade. *Reviews in Aquaculture*, 13, 676–705. DOI:10.1111/raq.12494
- El-Sayed, H.S., Ghanem, S.F. and Barakat, K.M., 2024.** Role of recent feeding protocols, rearing water systems and microbial trends in improving marine larviculture: Insights into water quality and larval performance. *International Aquatic Research*, 16, 213–232. DOI:10.1007/s40071-024-00770-8
- Emerenciano, M., Ballester E.L., Cavalli, R.O. and Wasielesky, W., 2011.** Effect of biofloc technology (BFT) on the early postlarval stage of pink shrimp *Farfantepenaeus paulensis*: Growth performance, floc composition and salinity stress tolerance. *Aquaculture International*, 19, 891–901. DOI:10.1007/s10499-010-9408-6
- Emerenciano, M., Cuzon, G., Paredes, A. and Gaxiola, G., 2013a.** Evaluation of biofloc technology in pink shrimp *Farfantepenaeus duorarum* culture: growth performance, water quality, microorganisms profile and proximate analysis of biofloc. *Aquaculture*

- International*, 21(6), 1381-1394.
DOI:10.1007/s10499-013-9640-y
- Emerenciano, M., Gaxiola, G. and Cuzon, G., 2013b.** Biofloc technology (BFT): A review for aquaculture application and animal food industry. In: Matovic, M.D., (ed), Biomass Now – Cultivation and Utilization. Intech, United Kingdom. pp. 301-328.
- Emerenciano, M.G.C., Khanjani, M.H., Sharifinia, M. and Miranda-Baeza, A., 2025.** Could biofloc technology (BFT) pave the way toward a more sustainable aquaculture in line with the circular economy? *Aquaculture Research*, 2025, 1020045. DOI:10.1155/are/1020045
- Esparza-Leal, H.M., Ponce-Palafox, J.T., Álvarez-Ruiz, P., López-Álvarez, E.S., Vázquez-Montoya, N., López-Espinoza, M., Mejia, M.M., Gómez-Peraza, R.L. and Nava-Perez, E., 2020.** Effect of stocking density and water exchange on performance and stress tolerance to low and high salinity by *Litopenaeus vannamei* postlarvae reared with biofloc in intensive nursery phase. *Aquaculture International*, 28, 1473–1483. DOI:10.1007/s10499-020-00550-2
- Estante-Superio, E.G., Mandario, M.A.E., Santander-Avanceña, S.S., Geanga, T.M.M., Parado-Esteba, F.D. and Mamaug, R.E.P., 2023.** Inclusion of live mud polychaete (*Marphysa iloiloensis*) in the feeding regime improved the hatchery performance of domesticated Indian white shrimp (*Penaeus indicus*). *Regional Studies in Marine Science*, 62, 102923. DOI:10.1016/j.rsma.2023.102923
- Ganesh, K., Raj, Y.C.T.S., Perumal S., Srinivasan, P. and Sethuramalingam, A., 2015.** Breeding, larval rearing and farming of mangrove crab, *Scylla serrata* (Forsk., 1775). In: Perumal, S., Thirunavukkarasu, A.R. and Pachiappan, P. (eds) Advances in Marine and Brackishwater Aquaculture. Springer, New Delhi. pp 163–172.
- Hargreaves, J.A., 2013,** Biofloc production systems for aquaculture. SRAC Publication No. 4503. Southern Regional Aquaculture Center, United States. 11 P.
- Herlinah, Sulaeman, Gunarto, Parenrengi, A. and Rosmiati., 2021.** Effect of water salinity on survival and osmotic level of larval (Zoea Stage) of mud crabs *Scylla tranquebarica*. *IOP Conference Series: Earth and Environmental Science*, 890, 012032. DOI:10.1088/1755-1315/890/1/012032
- Kasan, N.A., Sze Yee, C., Manan, H., Ahmad Ideris, A.R., Kamaruzzan, A.S., Waiho, K., Lam, S.S., Wan Mahari, W.A., Ikhwanuddin, M., Suratman, S. and Tamrin, M.L.M., 2021.** Study on the implementation of different biofloc sedimentable solids in improving the water quality and survival rate of mud crab, *Scylla paramamosain* larvae culture. *Aquaculture Research*, 52, 4807–4815. DOI:10.1111/are.15313
- Khanjani, M.H. and Sharifinia, M., 2020.** Biofloc technology as a promising tool to improve aquaculture production. *Reviews in Aquaculture*, 12, 1836–1850. DOI:10.1111/raq.12412
- Khanjani, M.H., Sharifinia, M. and Hajirezaee, S., 2020.** Effects of different salinity levels on water quality,

- growth performance and body composition of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultured in a zero water exchange heterotrophic system. *Annals in Animal Science*, 20, 1471–1486. DOI:10.2478/aoas-2019-0106
- Kumar, V., Roy, S., Behera, B.K., Swain, H.S. and Das, B.K., 2021.** Biofloc microbiome with bioremediation and health benefits. *Frontiers in Microbiology*, 12, 741164. DOI:10.3389/fmicb.2021.741164
- Lebata-Ramos, M.J.H., Solis, E.F.D. and Sibonga, R.C., 2024.** The reestablishment of mangrove crabs (*Scylla* spp.) in an abandoned pond following natural mangrove recolonization. *Restoration Ecology*, 32, e14003. DOI:10.1111/rec.14003
- Miao, S., Hu, J., Wan, W., Han, B., Zhou, Y., Xin, Z. and Sun, L., 2020.** Biofloc technology with addition of different carbon sources altered the antibacterial and antioxidant response in *Macrobrachium rosenbergii* to acute stress. *Aquaculture*, 525, 735280. DOI:10.1016/j.aquaculture.2020.735280
- Misbah, I., Karim, M.Y. and Aslamyiah, S., 2017.** Effect of salinity on the survival of mangrove crab *Scylla tranquebarica* larvae at zoea-megalopa stages. *Aquaculture, Aquarium, Conservation & Legislation*, 10, 1590-1595.
- Nguyen, T.P., Nguyen, T.E., Nguyen, T.K.H., Le, Q.V. and Do, T.T.H., 2021.** Effects of different temperatures on the growth and survival of mud crab (*Scylla paramamosain*) larvae. *CTU Journal of Innovation and Sustainable Development*, 13, 9–16. DOI:10.22144/ctu.jen.2021.035
- Nolial, J.C.C., 2024.** Mangrove crab aquaculture practices and challenges: The case of Capalonga, Camarines Norte, Philippines. *International Journal of Biosciences*, 25, 201-208. DOI:10.12692/ijb/25.2.201-208
- Ong, Q.M., Fotedar, R., Ho, T.T.T., 2019.** Impact of different rearing systems on survival, growth and quality of mud crab (*Scylla paramamosain*) megalopae reared from early zoeae. *Aquaculture International*, 27, 1673–1687. DOI:10.1007/s10499-019-00432-4
- Palacios, E. and Racotta, I.S., 2007.** Salinity stress test and its relation to future performance and different physiological responses in shrimp postlarvae. *Aquaculture*, 268, 123–135. DOI:10.1016/j.aquaculture.2007.04.014
- Panigrahi, A., Saranya, C., Sundaram, M., Kannan, S. V., Das, R.R., Kumar, R.S., Rajesh, P. and Otta, S.K., 2018.** Carbon: Nitrogen (C: N) ratio level variation influences microbial community of the system and growth as well as immunity of shrimp (*Litopenaeus vannamei*) in biofloc based culture system. *Fish & Shellfish Immunology*, 81, 329-337. DOI:10.1016/j.fsi.2018.07.035
- Panigrahi, A., Saranya, C., Ambiganandam, K., Sundaram, M. and Sivakumar, M.R., 2020.** Evaluation of biofloc generation protocols to adopt high density nursery rearing of *Penaeus vannamei* for better growth performances, protective

- responses and immunomodulation in biofloc based technology. *Aquaculture*, 522, 735095. DOI:10.1016/j.aquaculture.2020.735095
- Paran, B.C., Jeyagobi, B., Kizhakedath, V.K., Antony, J., Francis, B., Anand, P.S.S., Radhakrishnapillai, A., Lalramchhani, C., Kannappan, S., Marimuthu, R.D. and Paulpandi, S., 2022.** Production of juvenile mud crabs, *Scylla serrata*: Captive breeding, larviculture and nursery production. *Aquaculture Reports*, 22, 101003. DOI:10.1016/j.aqrep.2021.101003
- Pati, S.G., Paital, B., Panda, F., Jena, S. and Sahoo, D.K., 2023.** Impacts of habitat quality on the physiology, ecology, and economical value of mud crab *Scylla* sp.: A comprehensive review. *Water*, 15, 2029. DOI:10.3390/w15112029
- Quinitio, E.T. and Parado-Esteba, F.D., 2000.** Transport of *Scylla serrata* megalopae at various densities and durations. *Aquaculture*, 185, 63–71. DOI:10.1016/S0044-8486(99)00349-2
- Quinitio, E.T. and Parado-Esteba, F.D., 2017.** Development of a sustainable mangrove crab industry through science-based research. *Fish for the People*, 15, 47–51.
- Quinitio, E.T., de la Cruz, J.J., Eguia, M.R.R., Parado-Esteba, F.D., Pates, G. and Lavilla-Pitogo, C.R., 2011.** Domestication of the mud crab *Scylla serrata*. *Aquaculture International*, 19, 237–250. DOI:10.1007/s10499-010-9360-5
- Quinitio, E.T., Parado-Esteba, F.D. and dela Cruz-Huervana, J.J., 2018.** Biology and hatchery of mangrove crabs *Scylla* spp. 3rd ed. SEAFDEC Aquaculture Dept., Philippines. 46 P.
- Rajkumar, M., Pandey, P.K., Aravind, R., Vennila, A., Bharti, V. and Purushothaman, C.S., 2016.** Effect of different biofloc system on water quality, biofloc composition and growth performance in *Litopenaeus vannamei* (Boone, 1931). *Aquaculture Research*, 47, 3432–3444. DOI:10.1111/are.12791
- Ray, A.J., Drury, T.H. and Cecil, A., 2017.** Comparing clear-water RAS and biofloc systems: Shrimp (*Litopenaeus vannamei*) production, water quality, and biofloc nutritional contributions estimated using stable isotopes. *Aquacultural Engineering*, 77, 9–14. DOI:10.1016/j.aquaeng.2017.02.002
- Schveitzer R., Arantes, R., Costódio, P.F.S, do Espírito Santo, C.M., Arana, L.V., Seiffert W.Q. and Andreatta, E.R., 2013.** Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. *Aquacultural Engineering*, 56, 59–70. DOI:10.1016/j.aquaeng.2013.04.006
- Shi, R., Yang, S., Wang, Q., Zhang, L. and Li, Y., 2023.** Effects of transport stress on immune response, physiological state, and WSSV concentration in the red swamp crayfish *Procambarus clarkii*. *Aquaculture and Fisheries*, 8, 498-508. DOI:10.1016/j.aaf.2022.01.001
- Syafaat, M.N., Muhammad, T., Abol-Munafi, A.B. and Ikhwanuddin, M.,**

2019. Data on population density, growth, survival, water quality, larval stage index and ingestion rate of selected microalgae of portunids crabs at different feeding regimes. *Data Brief*, 26,104438. DOI:10.1016/j.dib.2019.104438
- Thabet, R., Ayadi, H., Koken, M. and Leignel, V., 2017.** Homeostatic responses of crustaceans to salinity changes. *Hydrobiologia*, 799, 1-20. DOI:10.1007/s10750-017-3232-1
- Waiho, K., Fazhan, H., Quintio, E.T., Baylon, J.C., Fujaya, Y., Azmie, G., Wu, Q., Shi, X., Ikhwanuddin, M. and Ma, H., 2018.** Larval rearing of mud crab (*Scylla*): What lies ahead. *Aquaculture*, 493,37–50. DOI:10.1016/j.aquaculture.2018.04.047
- Xu, W.B., Zhang, Y.M., Li, B.Z., Lin, C.Y., Chen, D.Y., Cheng, Y.X. and Shu, M.A., 2023.** Effects of low salinity stress on osmoregulation and gill transcriptome in different populations of mud crab *Scylla paramamosain*. *Science of the Total Environment*, 867, 161522. DOI:10.1016/j.scitotenv.2023.161522
- Xu, W.J., Morris, T.C. and Samochoa, T.M., 2016.** Effects of C/N ratio on biofloc development, water quality, and performance of *Litopenaeus vannamei* juveniles in a biofloc-based, high-density, zero-exchange, outdoor tank system. *Aquaculture*, 453, 169–175. DOI:10.1016/j.aquaculture.2015.11.021
- Yadav, N.K., Paul, S., Patel, A.B., Mahanand, S.S., Biswas, P., Choudhury, T.G., Baidya, S., Meena, D.K., 2025.** The role of biofloc technology in sustainable aquaculture: Nutritional insights and system efficiency. *Blue Biotechnology*, 2, 7. DOI:10.1186/s44315-025-00025-x
- Zafar, M.A. and Rana, M.M., 2022.** Biofloc technology: An eco-friendly ‘green approach’ to boost up aquaculture production. *Aquaculture International*, 30, 51–72. DOI:10.1007/s10499-021-00794-0