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Original Article

Optimization of broodstock culture and larval quality of *Scylla serrata* in a modified recirculating aquaculture system incorporating a polychaete-assisted biofilter

Derly Nemeño[1,5], Gaudioso Pates, Jr.[1,2], Mercedes Maceren-Pates[*1], Charles Van Calunod[1], Emilia Quinitio[3], Fe Dolores-Estepa[3], Joseph Milfred Arcenal[4]

[1]Department of Marine Science, Mindanao State University-Iligan Institute of Technology, Iligan 9200, Philippines.
[2]Fishery Research Laboratory, Kyushu University, 4-46-24 Tsuyazaki, Fukutsu 811-3304, Japan.
[3]Integrated Services for the Development of Aquaculture and Fisheries (ISDA Inc.), Jaro, Iloilo City 5000, Philippines.
[4]Zamboanga del Sur Provincial Government College, Dimataling Campus, Dimataling, Zamboanga del Sur 7041, Philippines.
[5]University of Science and Technology of Southern Philippines (USTP) – Panaon Campus, Panaon, Misamis Occidental, Philippines.

Abstract: This study evaluated the performance of two culture systems: a recirculating aquaculture system (RAS) incorporating a polychaete-assisted biofilter, and a conventional non-recirculating aquaculture system (NRAS, control) with regular water replacement. Both systems were equipped with individual compartments to minimize cannibalism. The survival, spawning success, hatching rates, and larval quality of *Scylla serrata* broodstock were assessed. Broodstock maintained in the RAS exhibited significantly higher survival (70.37±6.41%), spawning (61.11±4.80%), and hatching (55.55±4.81%) rates compared to those in the NRAS (51.85±5.78%, 35.18±10.14%, and 20.37±8.01%, respectively), indicating that the RAS provided more favorable conditions for broodstock conditioning. Larvae from broodstock in both systems were subjected to salinity (0, 10, 15, 20, 25, and 30 ppt) and formalin (0, 10, 20, 30, 40, and 50 ppm) stress tolerance tests. Larvae produced from RAS-held broodstock displayed significantly higher LT_{50} values under both stressors, suggesting greater resilience. Overall, these results demonstrate that the RAS with a polychaete-assisted biofilter not only enhances the reproductive performance of *S. serrata* broodstock but also improves larval quality, providing a promising approach for sustainable and reliable hatchery production.

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Introduction

The mangrove crab, *Scylla* spp., is a valuable species in the aquaculture industry due to its high market demand and global commercial significance. In the Philippines, it has consistently ranked among the top aquaculture commodities in recent years. Production volume increased steadily from 1995 to 2021; however, between 2021 and 2022, it declined by 29.10% (PSA, 2023), a reduction that may be attributed to the depletion of the natural population (Quinitio et al., 2011).

To meet the growing demand in local and international markets, the establishment of mangrove crab hatcheries has become more prevalent across the Philippines over the years. The hatchery operators source crab broodstock either from the wild or trading centers and allow them to spawn, hatch the eggs, and rear the larvae until crab instar/crablet stage (Quinitio et al., 2018). However, despite the feasibility of culturing crab larvae within controlled hatchery conditions, the scale of commercial hatchery output remains constrained by inconsistent larval quality and low survival rates, partly stemming from the quality of broodstock (Quinitio et al., 2001; Pates et al., 2025). Among the common causes are variations in broodstock source and culture conditions, resulting in significant differences in reproductive performance that affect larval quality (Wu et al., 2010; Mirera and Moksnes, 2015). To address this, a culture system that provides complete control over water parameters offers advantages for producing high-quality broodstock.

The commonly used water management practice for holding broodstock in hatcheries is frequent water

*Correspondence: Mercedes Maceren-Pates
E-mail: mercedes.pates@g.msuiit.edu.ph
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exchange to maintain good water quality (Quinitio et al. 2008). However, regular water exchange may bring unstable water parameters, which can be stressful for the organisms. Recirculating aquaculture systems (RAS) have emerged as a more advanced alternative, designed to maintain stable water conditions through continuous water recycling and filtration (Wang, 2003). However, RAS setup incurs substantial costs, particularly for water treatment equipment to remove suspended solids, ammonia nitrogen, and nitrite (Xiao et al., 2019).

In this study, a RAS was modified by using polychaete marine worms as biofilters. Several reports have highlighted the potential of polychaetes for integrated aquaculture and nutrient recycling due to their ability to degrade organic waste (Jeronimo et al. 2020; Palmer 2010). Their feeding behavior contributes to the efficient removal of suspended solid waste and is accompanied by intriguing microbial processes. Polychaetes also possess bioactive compounds that stimulate gonad maturation and spawning in fish and crustaceans (Primavera and Gabasa, 1981; Millamena et al., 1986; Parado-Estepa et al., 2002). These unique capabilities of polychaetes make them highly effective and suitable biofilters for RAS applications.

In our previous study (Calunod et al., 2025), we compared the efficiency of a modified recirculating aquaculture system (RAS) with a polychaete-assisted biofilter against a conventional non-RAS system with daily water exchange for the broodstock culture of *Scylla serrata*. Both systems exhibited low survival, although the modified RAS demonstrated improved spawning and hatching performance. The low survival was primarily attributed to high levels of cannibalism, as crabs were kept together in a common compartment. Based on this, we hypothesized that incorporating individual compartments could further enhance the performance of the modified RAS. This led us to conduct subsequent experiments to optimize RAS performance. Furthermore, we extended our investigation by evaluating the larval viability of *S. serrata* produced from broodstock held in both systems.

Materials and Methods

Sourcing of mangrove crab broodstock: Maturing mangrove crab *S. serrata* broodstock with yellow or light orange ovary were sourced from Kabasalan, Zamboanga, Sibugay, and maintained in the hatchery of the University of Science and Technology of Southern Philippines (USTP), Panaon. Upon arrival, the crabs were acclimatized for 5-10 minutes and disinfected using 150 ppm formalin for 30 minutes (Quintio et al., 2008). After disinfection, the crabs were conditioned for at least 5 days in rectangular concrete tanks supplied with free-flowing seawater. The feeding and water management procedures were based on the protocol of Quinitio et al. (2018).

Experiment 1. Broodstock culture in modified RAS and NRAS with individual tank compartments: Broodstock were maintained in 1.2-ton fiberglass tanks (FGTs) under two systems: a recirculating aquaculture system (RAS) equipped with a polychaete-assisted biofilter, following the design and operation described by Calunod et al. (2025), and a non-recirculating aquaculture system (NRAS, control) with daily water replacement. Each tank contained 1 ton of seawater and was stocked with 6 crabs; each crab was housed individually in a separate compartment (Fig. 1). Each treatment had 3 replicates, and 3 experimental runs were conducted. The broodstock were alternately fed daily with low-value fish and blood cockle at 10% of their body weight. Once a broodstock spawned, it was transferred to a 155-L rectangular container filled with aerated seawater. Water in these containers was replaced daily at approximately 100% until the eggs hatched.

Experiment 2: Larval quality analysis from RAS and NRAS-held broodstock: A total of three batches of newly hatched larvae from broodstock maintained in RAS and NRAS were used in the stress test experiment. The batches originated from separate hatching events. Experiments were conducted in white plastic containers (300 mL capacity) using seawater prepared following Quinitio and Parado-Estepa (2008).

In the salinity stress test, 30 larvae were exposed to salinities of 0, 5, 10, 15, 20, 25, and 30 ppt. In the

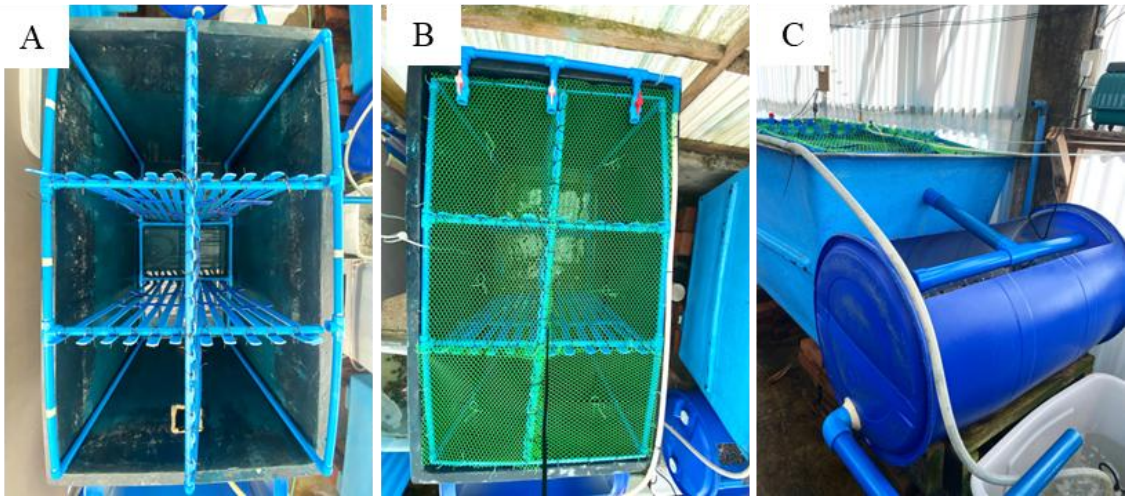

Figure 1. Overview of the Recirculating Aquaculture System (RAS). A. Broodstock tank showing individual compartments. B: Compartmentalized tank showing top cover. C: RAS operation layout.

formalin stress test, larvae were exposed to formalin concentrations of 0, 10, 20, 30, 40, and 50 ppm, using solutions prepared from chemical-grade formalin (37%). Each broodstock culture system yielded three spawnings, all used to assess larval performance. Each treatment was performed in triplicate, with three experimental runs conducted per test. Larvae were observed every four hours over a 24-hour period. Individuals that failed to respond to gentle stimulation with a fine glass rod were recorded as dead, with mortality confirmed by microscopic observation of abdominal movement. No feeding or water exchange was provided during the experimental period.

Spawning success was calculated as the percentage of females that spawned in each tank, based on the initial number stocked. Hatching success was calculated as the percentage of females that produced hatchlings in each tank, relative to the initial number stocked.

Statistical analysis: Survival, spawning, and hatching between the two culture methods were analyzed using one-way analysis of variance (ANOVA) and t-test. Survival and spawning percentages were arcsine-transformed prior to ANOVA. The median lethal time (LT50) of the larvae in each replicate was assessed and analyzed using probit regression in IBM SPSS version 20.0 (Chicago, IL, USA). To evaluate differences in LT50 among larvae from both systems exposed to multiple stress treatments, a two-way ANOVA was used. The two factors considered in the analysis were system type (RAS vs. NRAS) and concentration, both of which were tested for their individual and interactive effects on LT_{50}. The null hypothesis was rejected at the significance level of $P<0.05$. For post-hoc analysis, Bonferroni's test was applied to examine specific pairwise group differences.

Results

Survival, spawning, and hatching: The mean percent survival and spawning rates of *S. serrata* cultured in RAS and NRAS are presented in Figure 2. Survival rates differ significantly between the two systems ($P<0.05$), with RAS at 70.37±6.41% and NRAS at 51.85±5.78%. Similarly, spawning rates were markedly higher in RAS (61.11±4.80%) compared to NRAS (35.18±10.14%), and successful hatching rates followed the same trend (55.55±4.81% in RAS, while only 20.37±8.01% in NRAS). All differences were statistically significant ($P<0.05$).

Exposure of larvae to different salinity levels: LT_{50} values for each salinity level and system are given in Table 1. At lower salinity (<10 ppt), larval mortality was observed within a few minutes of exposure in both RAS and NRAS culture systems. The larvae obtained from broodstock held in NRAS had 100% mortality at 19 hours in all salinity levels, while larvae obtained from RAS-held broodstock continued to survive even until 24 hours in 15 to 30 ppt (Fig. 3). The results revealed that the interaction between system type and salinity was not significant ($P = 0.2028$). However, the

Table 1. Median lethal time (LT_{50}) of larvae obtained from broodstock held in RAS and NRAS after exposure to different salinity levels. Each value is the mean ± standard error.

Salinity Level (ppt)	LT_{50} (h)	
	RAS	NRAS
0	-	-
5	-	-
10	-	-
15	17.98±1.58	15.70±0.35
20	20.91±0.85	15.89±0.46
25	21.69±1.34	15.92±0.21
30	20.58±0.21	15.38±0.48

Table 2. Median lethal time (LT_{50}) of larvae obtained from broodstock held in RAS and NRAS systems after exposure to different formalin concentrations. Each value is the mean ± standard error.

Formalin concentration (ppm)	LT_{50} (h)	
	RAS	NRAS
0	19.26±0.26	12.97±0.57
10	19.98±0.49	20.34±0.26
20	23.06±0.12	21.79±28
30	0.38±0.10	0
40	-	-
50	-	-

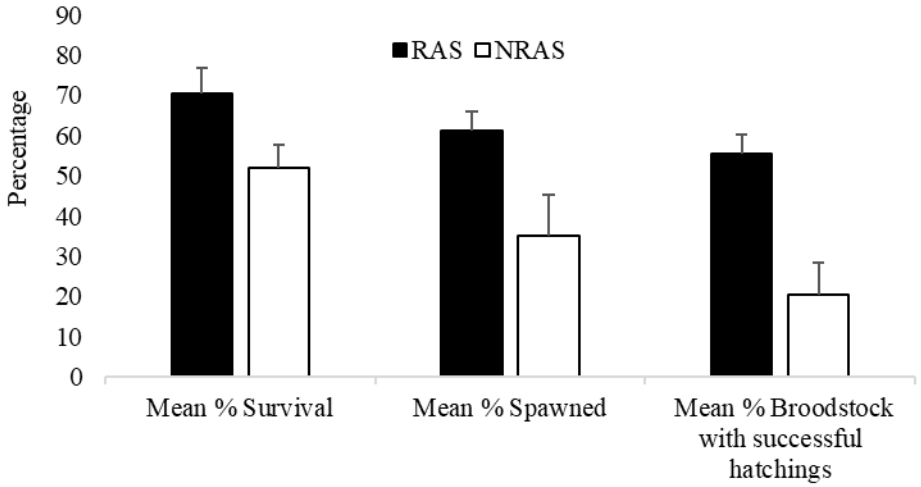

Figure 2. Mean percent survival, spawning, and hatching rates of *Scylla serrata* broodstock held in a single tank with multiple compartments under two management systems (RAS and NRAS).

main effect of culture system was highly significant (P<0.0001), with larvae from the NRAS system reaching 50% mortality faster (mean LT_{50} = 15.72 hours) compared to those from the RAS system (mean LT_{50} = 20.29 hours) (Fig. 3). In contrast, the main effect of salinity concentration was not significant (P = 0.1504), indicating that varying salinity concentrations (15, 20, 25, and 30 ppt) did not substantially influence the LT_{50} values.

Exposure of larvae to different formalin concentrations: LT_{50} values of larvae obtained from RAS-held and NRAS-held broodstocks after exposure to different formalin concentrations are shown in Table 2. No larvae survived at higher formalin concentrations (≥40 ppm) from broodstock held in both culture methods. The larval mortality rates after exposure to different formalin concentrations are shown in Figure 4. The results revealed that the interaction between the formalin concentrations and culture systems was highly significant (P<0.0001). Specifically, at 0 ppm and 20 ppm, there was a significant difference in survival between larvae

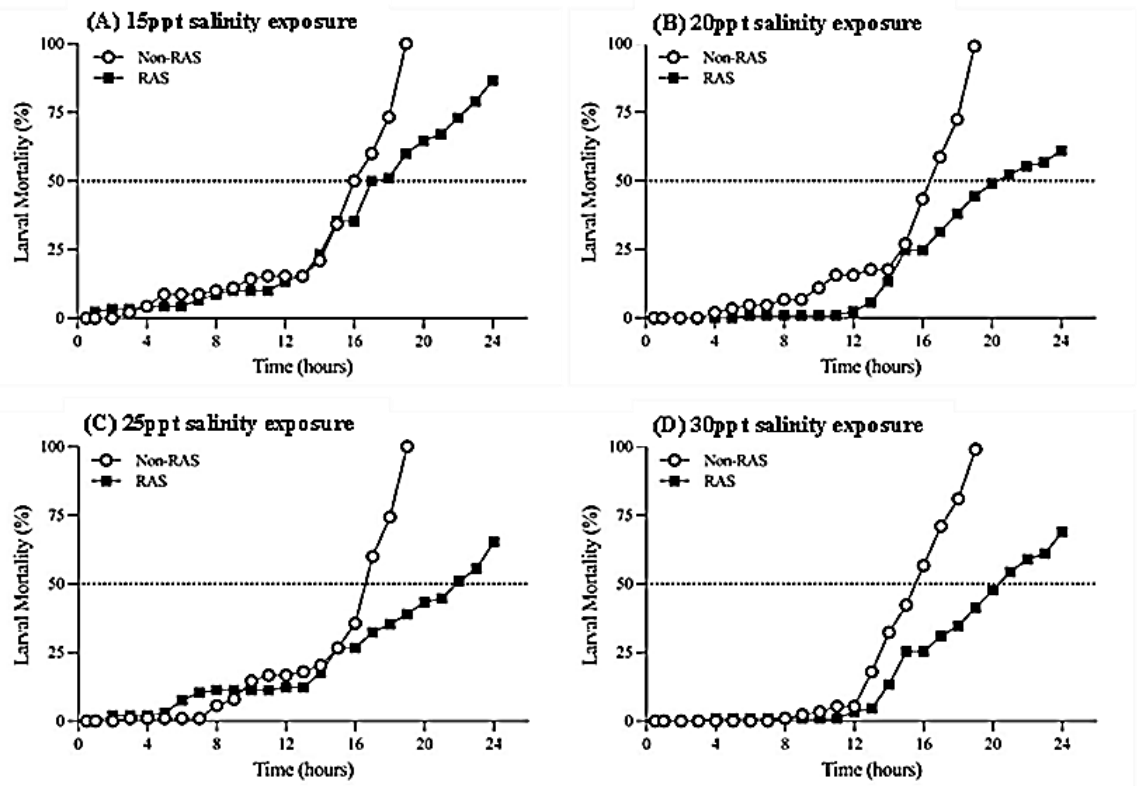

Figure 3. Cumulative mortality of *Scylla serrata* newly-hatched larvae (Z1) exposed to different salinity levels as a function of time in hours.

obtained from NRAS and RAS-held broodstock (see Table 2), with larvae from RAS-held broodstock surviving longer at these concentrations (mean difference = -6.291 for 0 ppm and -1.277 for 20 ppm). No significant differences were observed at 10 and 30 ppm. However, at 10 ppm, about 25% of larvae at the RAS survived even until 24h, while those from broodstock in NRAS all died within 21 h.

Discussions

In our previous study, we evaluated the effectiveness of a modified recirculating aquaculture system (RAS) incorporating a polychaete-assisted biofilter, compared with a conventional non-recirculating aquaculture system (NRAS), in improving the survival and reproductive performance of *S. serrata* broodstock under captive conditions. The results showed that both systems exhibited relatively low survival rates, although the RAS significantly enhanced broodstock spawning and hatching performance. In that study, the crab broodstock were maintained in a single common compartment, which likely led to intense cannibalism among individuals.

In the present study, we further examined this by comparing the two systems using tanks equipped with individual broodstock compartments. The results revealed that broodstock reared in the RAS exhibited significantly higher survival, spawning, and hatching rates than those maintained in the NRAS, in contrast to the findings of Calunod et al. (2025). The use of individual compartments likely minimized cannibalism, a major cause of mortality among mud crab broodstock in captivity (Quinitio et al., 2001; Laranja et al., 2010). Moreover, the stable and favorable environmental conditions maintained in the RAS may have further contributed to the enhanced survival and reproductive performance of *S. serrata* broodstock (Calunod et al., 2025).

We further extended our investigation by evaluating the larval quality of broodstock reared in the two culture systems under stress. Stress tests involve subjecting organisms to brief but intense external stress, with survival serving as an indicator of their physiological condition (Dhert et al. 1992; Fegan, 1992; Alvarez et al., 2004). In this study, larval survival was assessed using the LT_{50} value, defined as

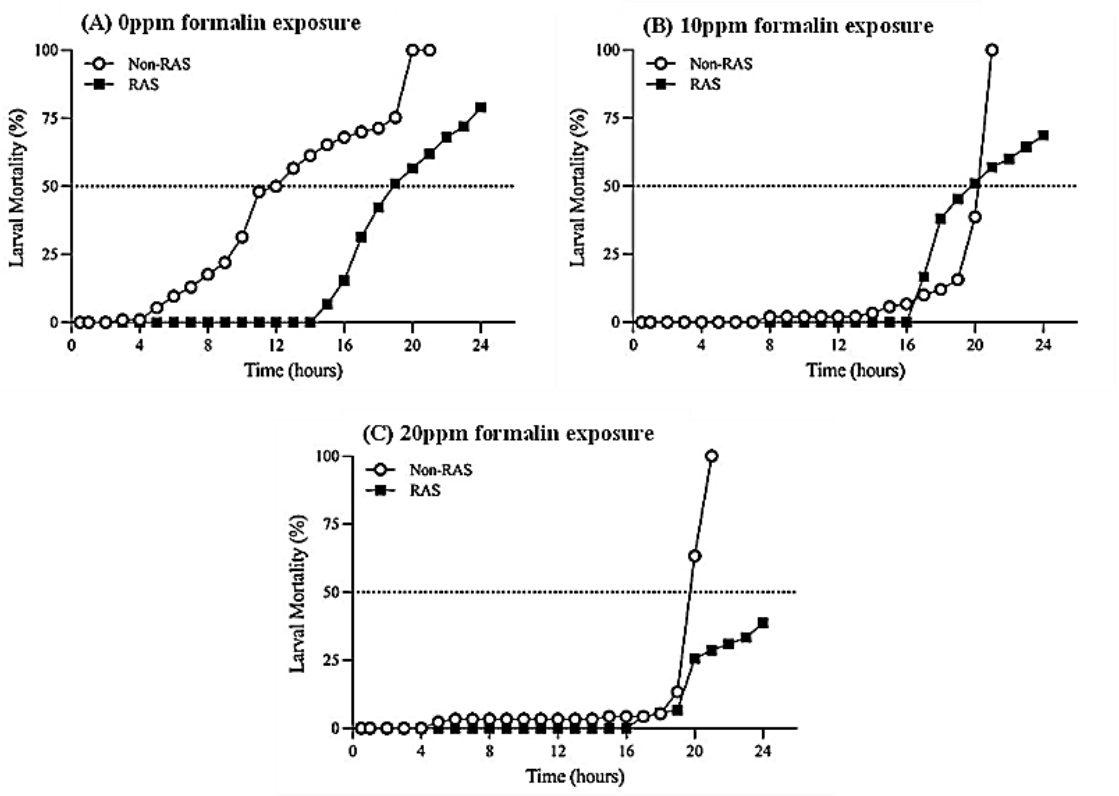

Figure 4. Cumulative mortality rate of *Scylla serrata* newly-hatched larvae (Z1) exposed to different formalin concentrations as a function of time in hours.

the time at which 50% of newly hatched larvae (zoea I) died under stress exposure. Higher LT_{50} values denote greater larval robustness (Parado-Estepa and Quinitio, 1999). Consistently, larvae derived from broodstock reared in the RAS exhibited higher survival rates and LT_{50} values than those from the NRAS when subjected to salinity and formalin challenges, indicating enhanced larval resilience.

Salinity is a critical abiotic factor that profoundly affects the survival and development of aquatic animal larvae (Fariasa et al., 2024). In *Penaeus vannamei* postlarvae, salinity has been employed as a stressor to assess the hardiness of hatchery-reared shrimp (Samocha et al., 1998). For mud crab, *S. serrata* larvae exhibit optimal development within a salinity range of 20-30 ppt (Nurdiani and Zeng, 2007) or 22-32 ppt (Quinitio and Parado-Estepa, 2008). Similarly, larvae of other mangrove crab species, such as *S. olivacea* and *S. tranquebarica*, thrive within comparable salinity ranges (Quinitio and Parado-Estepa, 2008). In the present study, *S. serrata* larvae survived at 15 ppt in both culture systems but not at 10 ppt, indicating that their lower salinity tolerance threshold lies near this level. This observation aligns with the findings of Nurdiani and Zeng (2007), who reported that the lower salinity tolerance of *S. serrata* larvae is between 15 and 20 ppt. Conversely, larvae in both systems failed to survive at 40 ppt, whereas Nurdiani and Zeng (2007) observed moderate survival at 35 ppt. Overall, larvae obtained from broodstock reared in the RAS exhibited higher LT_{50} values at 20, 25, and 30 ppt than those from broodstock maintained in the NRAS, suggesting enhanced tolerance to salinity stress.

Exposure to a single concentration of formalin (an aqueous solution of formaldehyde) as a stressor has been used to evaluate the hardiness of hatchery-produced *P. vannamei* postlarvae (Samocha et al., 1998). De Pedro et al. (2007) reported that newly hatched *S. serrata* larvae (zoea I) exhibited tolerance to formalin concentrations of 5, 10, and 15 ppm. Similarly, Quinitio et al. (2017) demonstrated that exposing newly hatched *S. serrata* zoeae to 40 mg/L formalin (37%) for three hours is an effective method for assessing larval quality. A mortality rate between

0 and 18% within three hours indicates a high-quality larval batch suitable for rearing, whereas a mortality rate exceeding 38% signifies poor-quality larvae that may be unsuitable for continued culture beyond the zoea I stage. In the present study, some larvae from RAS-held broodstock survived exposure to 30 ppm formalin, whereas those from NRAS-held broodstock did not, suggesting greater resistance among RAS-derived larvae. However, both groups showed high sensitivity to higher formalin concentrations. This finding is consistent with De Pedro et al. (2007), who observed a significant reduction in larval survival at 25-30 ppm formalin. Overall, larvae produced by broodstock reared in the RAS exhibited higher LT_{50} values than those from the NRAS, indicating greater larval resilience.

Seed quality can be substantially improved by maintaining optimally conditioned broodstock (Sudaryanto et al., 2004; Díaz and Piferrer, 2015). In this study, broodstock held in the RAS with a polychaete-assisted biofilter produced stronger larvae than those from the NRAS when exposed to both stressors. This suggests that the RAS provides a more favorable environment for *S. serrata* broodstock, thereby enhancing larval viability. Similar findings were reported in *Penaeus monodon*, where broodstock maintained in a recirculating system with a biological filter produced more viable nauplii than those in a flow-through system (Millamena et al., 1991).

Conclusion

This study demonstrates that maintaining mangrove crab (*S. serrata*) broodstock in a simple recirculating aquaculture system (RAS) incorporating a polychaete-assisted biofilter and individual compartments enhances broodstock performance and larval viability, thereby improving hatchery seed production. Further optimization of the modified RAS could yield even better outcomes in broodstock conditioning and larval performance. Potential enhancements include integrating additional biofiltration units and fine-tuning environmental parameters such as temperature, light intensity, spectrum, and wavelength. Moreover, identifying appropriate stressors and determining their optimal levels for assessing larval quality may provide valuable insights to improve hatchery management practices.

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