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Original Article

Enhancing crab broodstock spawning and water quality using a recirculating aquaculture system with polychaete-assisted sand biofilter

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Abstract: The increasing global demand for mangrove crabs (genus *Scylla*) and threats to the wild populations highlight the urgency of fully rearing them in captivity. The supply of hatchery seedstock is still inadequate to meet the requirements for farming. One of the major causes is the inconsistent larval quality in the hatchery, which appears to be due to poor broodstock performance. The present study evaluated the efficiency of two broodstock holding systems: the Recirculating Aquaculture System (RAS) with a polychaete-assisted biofilter and the conventional system with regular water replacement, referred to as non-RAS (NRAS, control), based on the survival rate, spawning, and hatching of *S. serrata* captive broodstock. The percentage survival of mangrove crab broodstock was similar in the RAS (54.00±10.00) and NRAS (50.84±3.89) systems. However, a significantly higher mean percentage of spawning (28.61±6.65) and hatching (28.61±6.65) was observed in the RAS compared to the NRAS (15.18±7.41 and 15.18±7.41, respectively). Temperature levels were recorded in the RAS, ranging from 27.7-29.3°C, and that of NRAS was 26.7-28.5°C. Salinity in RAS ranged from 33.7-35.2 ppt, while in the NRAS, it ranged from 33.5-34.7 ppt. Dissolved oxygen (DO) concentrations ranged from 5.8 to 7.3 mg/L in RAS and 5.5 to 7.3 mg/L in NRAS. Additionally, pH values in the RAS ranged from 7.8 to 8.6, compared to 7.8-8.5 in the non-RAS. Moreover, ammonia levels were significantly lower in the RAS, 0.11-0.25 mg/L, compared to 0.31-1.18 mg/L in the NRAS. Similarly, nitrite concentrations were significantly lower in the RAS (0.001-0.007 mg/L) than in the NRAS (0.013-0.026 mg/L). This suggests that RAS with a polychaete-assisted biofilter enhances the reproductive performance of the mangrove crab *S. serrata*.

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Introduction

Mangrove crabs of the genus *Scylla* are highly valued seafood commodities in both local and export markets. Of the three common species of *Scylla* in the Philippines (*S. olivacea*, *S. serrata*, and *S. tranquebarica*), *S. serrata* is the preferred species by farmers due to its fast growth (Quinitio et al., 2011). SEAFDEC/AQD has developed the basic larval rearing and nursery protocols for *S. serrata* (Quinitio and Parado-Estepa, 2008). However, due to low survival rates, the supply of hatchery seedstock is still limited in meeting the major requirements for farming. One of the problems identified as a possible cause of low seed survival is inconsistent larval quality, which may be attributed to the quality of the broodstock. Larval culture is significantly improved by using broodstock that are maintained in an environment with optimum water quality (Pedapoli and Ramudu, 2014), nutrition (Millamena and Quinitio, 2000; Azra and Ikhwanuddin, 2016), and other environmental conditions (Díaz and Piferrer, 2015; Farhadi et al., 2021). It is, therefore, necessary to understand the factors affecting the reproductive performance of mangrove crab *Scylla* spp. in captivity.

Temperature is the dominant factor in controlling the metabolism and activity of aquatic organisms (Muranaka and Lannan, 1984). In the swimming crab, *Portunus trituberculatus*, temperature was a prominent environmental factor controlling ovarian

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development (Hamasaki et al., 2004). It has been linked to changes in the incidence and timing of spawning for some species of crustaceans (Waddy and Aiken, 1992). Other environmental factors like salinity (Gelin et al., 2009), photoperiod (Morales and Barba, 2015), and light intensity (Farhadi et al., 2021) also affect the performance of broodstock. In recent years, when the water temperature exceeded 31°C, there was a significant decrease in the spawning of *S. serrata* (SEAFDEC/AQD, 2017). With the increase in water temperature due to climate change, its effect on broodstock culture should be given attention.

Recirculating aquaculture systems (RAS) are utilized worldwide to cultivate various fish species in a controlled environment. RAS also provides optimum broodstock performance (enhances spawning ability) in most finfishes by maintaining optimum water quality and stable water temperature (Ranjan et al., 2019). However, the system requires high-cost production inputs associated with the reduction or removal of nutrient loads present in effluent water. Some marine polychaetes inhabit the sandy beaches of the Indo-West Pacific coastal regions, including the Philippines (Maceren-Pates et al., 2015). Aside from being a good source of nutrients for the mangrove crab broodstock, some species are known for their remarkable ability to recycle the waste nutrients from particulate organic matter in the aquaculture effluents (Jerónimo et al., 2020). The unique ability of these animals makes them a suitable species to use as bioremediators in the RAS. Therefore, this study describes the reproductive performance of mangrove crab, *S. serrata,* in captivity using a recirculating aquaculture system with a polychaete-assisted sand biofilter. The study results will provide information on an environmentally friendly approach for improving broodstock management to boost hatchery seed production.

Materials and Methods

Collection of mangrove crab broodstock: Adult maturing mangrove crab, *S. serrata*, broodstock were purchased from Kabasalan, Zamboanga Sibugay, and were maintained at the University of Science and Technology of Southern Philippines (USTP) hatchery in Panaon, Misamis Occidental. Upon arrival, the crabs were acclimated for 5-10 minutes and disinfected with 150 ppm formalin (Quinitio et al., 2018), then kept in concrete rectangular tanks with free-flowing seawater for at least 5 days before the experiment. The feeding and water management scheme was based on the protocol of Quinitio et al. (2018).

Broodstock management: To enhance the broodstock reproductive performance of *S. serrata* in captivity, two management systems were tested to determine which one is more suitable for *S. serrata* broodstock: RAS - *S. serrata* broodstock were maintained using the modified RAS with polychaetes incorporated into the bio-filter, and NRAS (control) - *S. serrata* broodstock were maintained using daily water replacement. The details of each are described below.

RAS set-up: A 1.2-ton rectangular fiberglass tank (FGT; 153x91x80 cm) filled with 1 ton of seawater was used as a broodstock holding tank. The exit water from the broodstock tank flowed directly into a 220-L, tight-head, blue plastic drum laid sideways with one side open. The drum was filled with layers of gravel (5.0-10.0 mm) approximately 6 inches thick on the bottom and a sand substrate (1.0-2.0 mm) approximately 12 inches thick on the topmost layer. The substrate layers were separated by a nylon screen and loaded with polychaetes at a density of 400 g/m² (Palmer, 2010). The filtered seawater flowed from the biofilter into a 155-L rectangular plastic container, which served as the settling tank. The water was pumped back into the broodstock holding tanks using the submersible pump (40 W). The RAS filtration system was set up to facilitate water flow from the broodstock to the sand biofilter and into the settling tank by gravity. The seawater used in the RAS was treated following Quinitio et al. (2018) at the beginning of the experiment. The schematic diagram of the broodstock recirculating system and its operation are shown in Figure 1.

Experimental procedure: The broodstock were held in 1.2-ton FGTs, each with one ton of seawater. For

Table 1. Mean percent survival, spawning, and hatching of *Scylla serrata* broodstock using the two management systems (RAS and NRAS). Values are means ± SE. Means with different letter superscripts in a column are significantly different (P<0.05).

Treatments	Mean % Survival ^{ns}	Mean % Spawned*	Mean % Broodstock with Successful Hatching*
RAS	54.00±10.00	28.61±6.65	28.61±6.65
NRAS	50.84±3.89	15.18±7.41	15.18±7.41

ns - no significant difference detected (P>0.05)
*- significantly different (P<0.05)

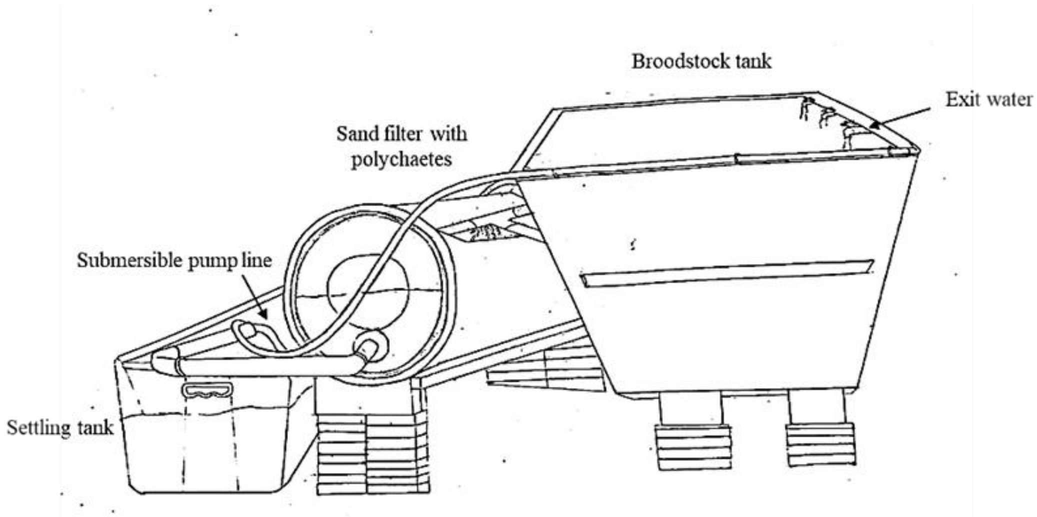

Figure 1. The Recirculating Aquaculture System with polychaete-assisted sand filter set-up.

RAS treatment, the culture tank was set up as a modified recirculating system with polychaetes incorporated into the biofilter. The crabs in both treatments were provided with cut PVC pipes as shelter. Each tank was stocked with five crabs. Each treatment had three replicates, and two runs were conducted. Only broodstock samples that tested negative for the bacterial and viral screening (luminescent vibriosis, WSSV, AHPND, etc.) were used in the study.

Adult maturing crab broodstock weighing 480-600 g with yellow to light orange gonads, determined by depressing the junction between the carapace and the first abdominal segment, were selected. The broodstock was fed alternately with natural food sources, including mussel meat, squid, fish, and polychaetes, at approximately 5% of their body weight (Quinitio et al., 2018). For the RAS treatment (T1), the rearing water was recycled at a rate of about 70% per day, and the same amount of water was changed daily for T2. Each tank was supplied with aeration throughout the study.

The broodstock was individually weighed and measured, and unilateral eyestalk ablation was done before the start of the experiment. The experiment was terminated after about 30 days. Other environmental parameters such as temperature, salinity, pH, and dissolved oxygen (DO) were measured twice daily. For the water quality analysis, measurements of the ammonia and nitrite levels in the broodstock rearing water were conducted at the Mindanao State University – Integrated Fishery Research and Development (MSU-IFRD) laboratory in Naawan, Misamis Oriental, using the spectrophotometric method. The water samples were taken from all treatments at the start of the experiment, weekly, and just before the experiment was terminated.

Statistical analysis: Survival, spawning, hatching, and water quality parameters, including seawater temperature, salinity, dissolved oxygen, pH, ammonia, and nitrite, were analyzed between the two culture methods using one-way analysis of variance

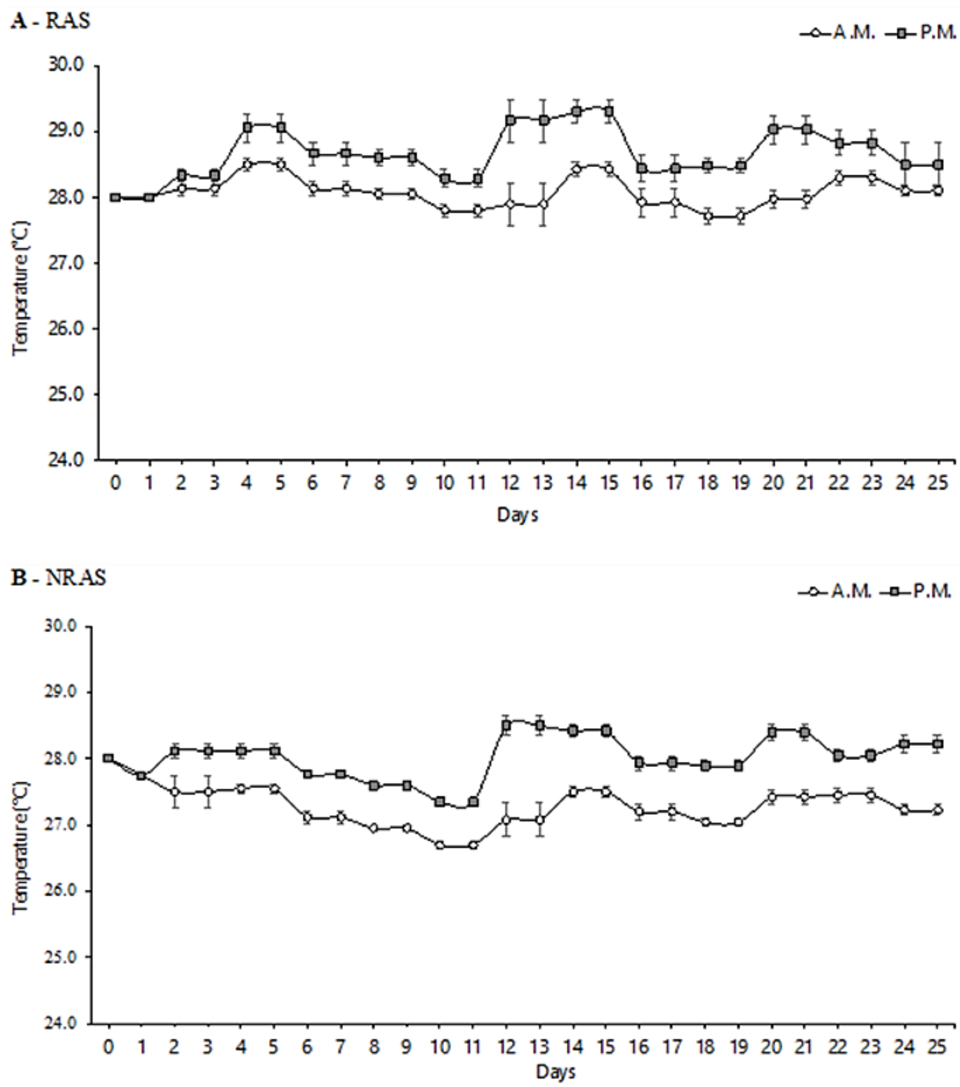

Figure 2. The Water temperature levels (AM and PM) in *Scylla serrata* broodstock tanks using a Recirculating Aquaculture System (RAS) with a polychaete-assisted biofilter (A) and NRAS-involving daily water change (B).

(ANOVA) and a t-test. Survival and spawning percentages were transformed using the arcsine function before ANOVA analysis.

Results

Survival, spawning, and hatching: The mean percent of survival and spawning of *S. serrata* cultured in RAS and NRAS are shown in Table 1. Mean percentage survival did not significantly differ (P>0.05) in RAS (54.00±10.00) and NRAS (50.84±3.89). However, RAS resulted in significantly (P<0.05) higher percentage of broodstock that spawned in RAS (28.61±6.65) and produced successful hatching (28.61±6.65) than NRAS (15.18±7.41 and 15.18±7.41, respectively).

Temperature, salinity, pH, and dissolved oxygen: The mean values of water parameters, including temperature, salinity, dissolved oxygen, and pH, are presented in Figures 2 to 5. Higher temperature levels were recorded in RAS, ranging from 27.7 to 29.3°C, while those in NRAS ranged from 26.7 to 28.5 °C (Fig. 2A, B). The salinity recorded in RAS ranged from 33.7-35.2 and 33.5-34.7 ppt in NRAS (Fig. 3A, B). The dissolved oxygen (DO) concentrations in RAS range from 5.8 to 7.3 and 5.5 to 7.3 mg/L in NRAS. Moreover, the pH values recorded in RAS were 7.8-8.6, while those in non-RAS were 7.8-8.5.

Ammonia and nitrite levels in RAS and NRAS: The mean concentrations of ammonia and nitrite are shown in Figure 3. The ammonia levels recorded for

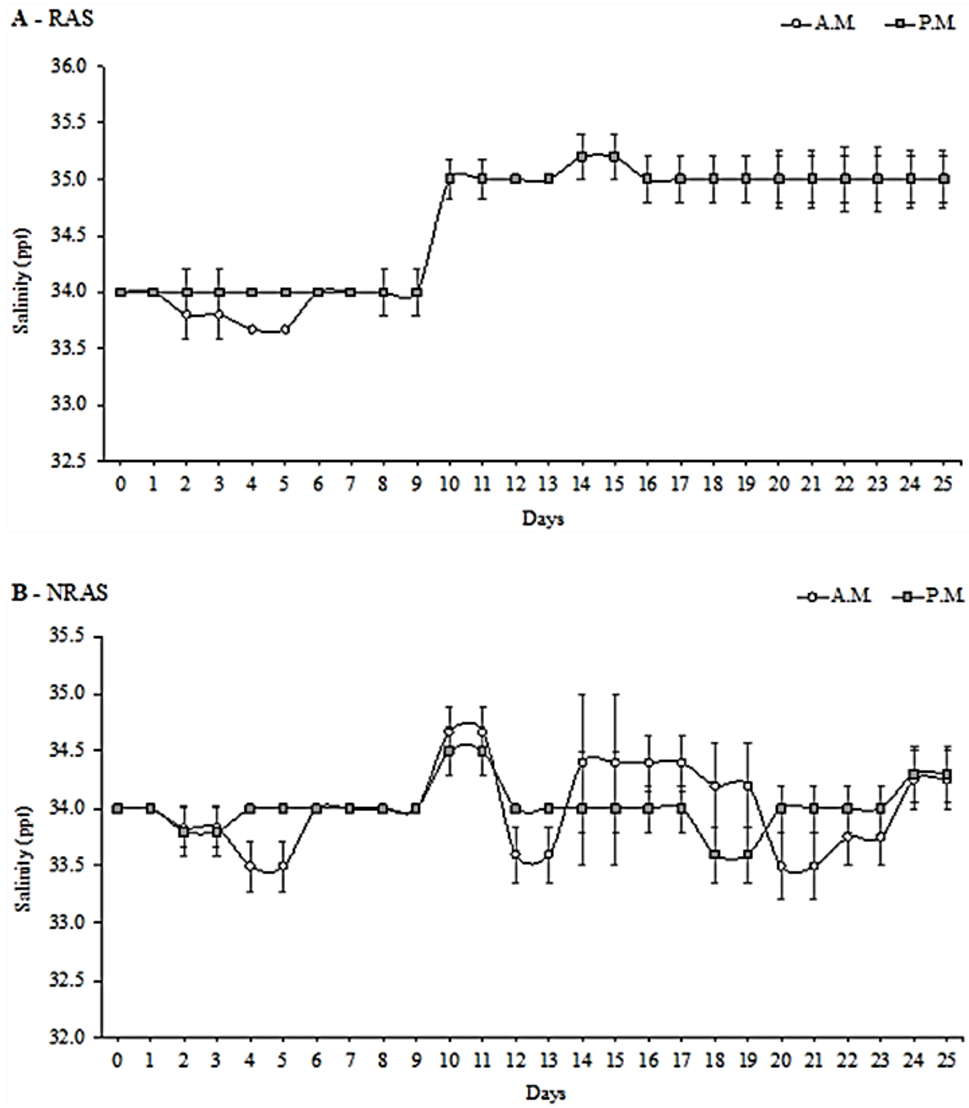

Figure 3. Salinity levels (AM and PM) in *Scylla serrata* broodstock tanks using Recirculating Aquaculture System (RAS) with (A) polychaete-assisted biofilter, and (B) NRAS- involving daily water change.

RAS ranged from 0.11 to 0.25 and 0.31 to 1.18 mg/L for NRAS. Significantly lower ammonia levels were observed in RAS than in NRAS. On the other hand, the nitrite concentrations in RAS were significantly lower (0.001-0.007 mg/L) than in NRAS (0.013-0.026 mg/L).

Discussions

The present study investigates the efficiency of the recirculating aquaculture system with polychaete-assisted biofilter (modified RAS) and the daily water change system (NRAS) on the survival and spawning performance of mangrove crab *S. serrata* broodstock in captive conditions. The low survival rate in the NRAS may be due to the cannibalistic behavior of the crabs, as all crabs were held together in one container. The high percentage of spawning and successful egg hatching of *S. serrata* in the modified RAS may have provided suitable conditions. The use of RAS on the broodstock culture of 2-year-old American shad (*Alosa sapidissima*) showed growth and survival rates of up to 93.50 and 94.67%, respectively, for a six-month culture period (Liu et al., 2018). Furthermore, the broodstock spawning performance was also enhanced, producing 65,930 eggs per kg body weight, more than those of a 5-year-old wild shad (Lie et al., 2018). Likewise, the growth, survival, and reproductive performance of the Kuruma shrimp

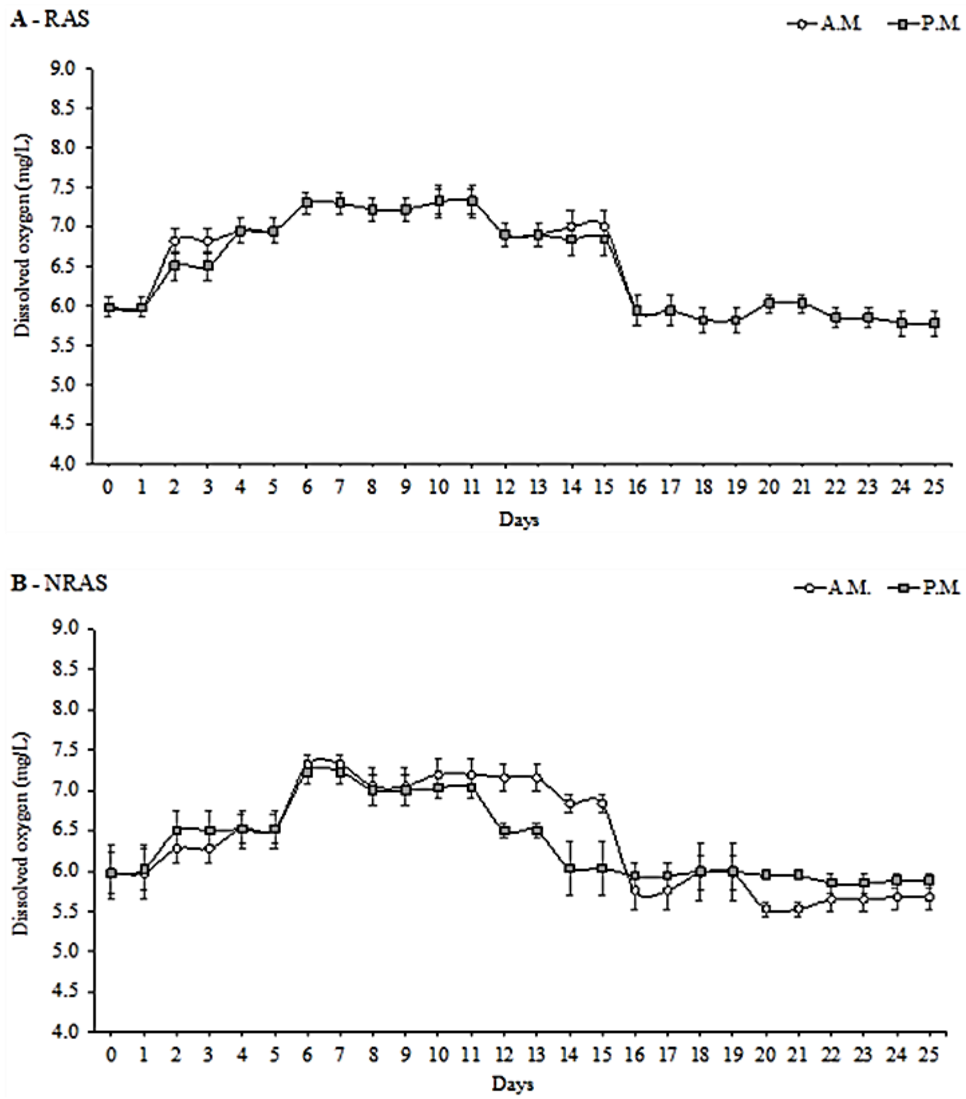

Figure 4. Dissolved oxygen levels (AM and PM) in *Scylla serrata* broodstock tanks using Recirculating Aquaculture System (RAS) with polychaete-assisted biofilter (A) and NRAS- involving daily water change (B).

Marsupenaeus japonicus improved with intensive culture in a closed recirculating aquaculture system (Suzuki, 2017). Millamena et al. (1991) also found that the better reproductive performance of *Penaeus monodon* broodstock in RAS for prawn broodstock maturation tanks than in the flow-through system. Otoshi et al. (2003) obtained better growth and reproductive performance of broodstock shrimp, *Litopenaeus vannamei*, reared in a bio-secure RAS than in a flow-through system.

Water rearing conditions, such as temperature, salinity, and nitrite levels, play a crucial role in the survival and physiological functions of crustaceans such as crabs. Fluctuations in these physical parameters can significantly impact how animals respond (Pati et al., 2023). Temperature is an important environmental factor in controlling the reproduction cycle of decapod crustaceans (Meusy and Payan, 1988; Waddy and Aiken, 1992). Excessively high seawater temperature and/or fluctuations can cause thermal stress in finfish and crustacean culture (Hyuk Ahn et al., 2019). In the present study, higher temperatures were observed in the modified RAS compared to NRAS, but they were within the ideal temperature range for *S. serrata* broodstock (Quinitio and Estepa, 2008). In this study, the improved spawning performance of *S. serrata* may be attributed to the high temperature in RAS. Hoang et al. (2002) found that increasing temperature from 23 to 27°C further improved the spawning

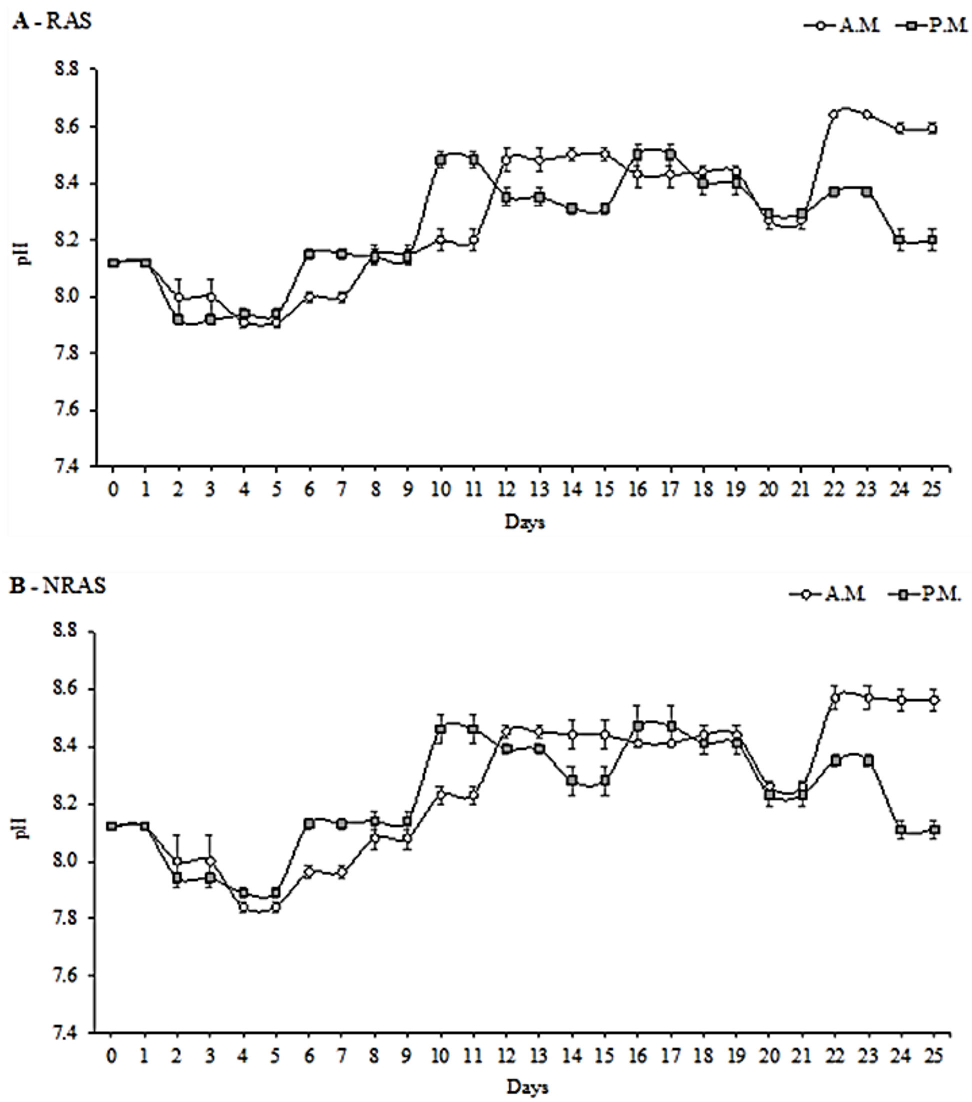

Figure 5. pH levels (AM and PM) in *Scylla serrata* broodstock tanks using Recirculating Aquaculture System (RAS) with polychaete-assisted biofilter (A) and NRAS- involving daily water change (B).

performance of ablated *Penaeus merguiensis*, while reducing the temperature from 27 to 23°C impaired spawning.

Salinity is another important abiotic factor in aquaculture, and many crustacean species have some degree of euryhalinity (Pequeux, 1995; Romano and Zeng, 2006). Moreover, salinity variations may affect phases in the crustacean reproductive cycle. For example, at low salinities, the ovarian development of several marine crustaceans was delayed (Gelin et al., 2001). In the present study, salinity variations were observed in the later days of culture in the NRAS treatment, which may be attributed to the rainy days during the experiment. This indicates that water changes in NRAS depend on the condition of the seawater source, which is prone to salinity fluctuations during rainy days unless a water reservoir is available to store seawater during favorable weather conditions. Higher variations in salinity may cause stress (Sun et al., 2023) and may result in poor broodstock reproductive performance in the NRAS culture method.

DO levels in RAS and NRAS fall within the optimum levels (≥ 5 ppm) in broodstock culture of *S. serrata* (Quinitio and Parado-Estepa, 2008). However, the DO level was expected to be higher in NRAS due to daily water changes. The acceptable pH range for mangrove crab is between 7.5 and 8.5 (Quinitio and Parado-Estepa, 2008). In the present study, both culture systems had pH levels within the

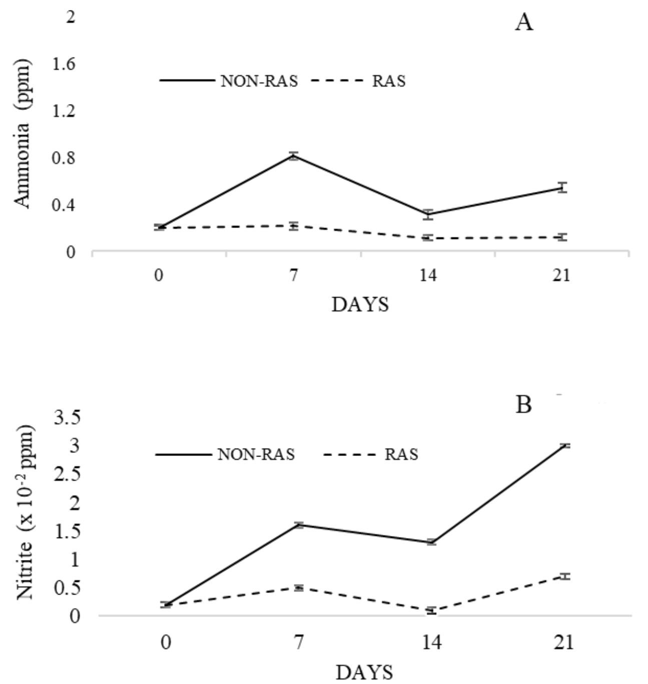

Figure 6. Mean ammonia (A) and nitrite (B) concentrations in *Scylla serrata* broodstock tanks using Recirculating Aquaculture System (RAS) with polychaete-assisted biofilter (A) and NRAS- involving daily water change (B). Values are means±SE. * indicates significant difference between RAS and NRAS within that day (P>0.05).

optimal range. Muthu et al. (1984) observed that ablated *P. indicus* females reached early maturation and then resorbed their ovaries when the pH of recirculated water declined from 8.2 to 7.2 in plastic-lined pools. Likewise, successful maturation, spawning, and hatching of viable nauplii were obtained only from ablated females kept in pools where pH was maintained at around 8.2 by daily addition of sodium carbonate (Muthu et al., 1984).

The lethality of ammonia and nitrite has been studied in various economically important aquaculture species (Cobo et al., 2014; Cui et al., 2017; Qiu et al., 2018). Ammonia is more toxic than nitrite, but regardless, their presence in the water has various negative effects on the growth and survival of aquatic animals (Romano and Zeng, 2013). Takeuchi (2017) described how RAS technology, utilizing a biological filter, efficiently removes ammonia and nitrite from the culture water. The present study also observed lower ammonia and nitrite concentrations in the RAS with polychaete-assisted biofilter. This may suggest that using polychaetes (*Perinereis* sp.) as a biofilter may efficiently remove metabolites in *S. serrata* broodstock culture. Honda and Kikuchi (2008) investigated the nitrogen budget of the polychaetes *Perinereis nuntia vallata* fed Japanese flounder feces and found that this species assimilated more than 60% of its nitrogen intake. Jerónimo et al. (2020) investigated the efficiency of polychaete-assisted sand biofilters in retaining particulate organic matter from aquaculture effluents. They found that wasted nutrients were converted into valuable worm biomass, suggesting that polychaetes would offer a sustainable solution to the existing problem of organic pollution. Moreover, Nereidid polychaetes can be reliably produced within a low-maintenance and straightforward sand filter with marine worms for wastewater treatment (Palmer, 2010).

Conclusion

The study showed that RAS with a polychaete-assisted biofilter enhanced the reproductive performance of the mangrove crab *S. serrata* broodstock in captivity compared to the usual daily water change (NRAS) crab management protocol. The system exhibits fewer fluctuations in environmental conditions, such as temperature, salinity, dissolved oxygen, and pH, which are within the ideal range for *S. serrata* broodstock culture. The lower concentrations of ammonia and nitrite in the RAS system suggest favorable conditions for mangrove crab broodstock culture. However, the study suggests that further modifications to the RAS are needed to enhance the system. This may include employing strategies such as individual compartments within the broodstock to address cannibalism in the culture tank. Additionally, other environmental parameters, such as temperature, light intensity, and spectrum, need to be optimized.

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