

## Original Research Articles

# Effects of densities and alkalinities on the rearing performance of all-male giant freshwater prawns (*Macrobrachium rosenbergii* De Man, 1879) postlarvae

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Farming all-male giant freshwater prawns (*Macrobrachium rosenbergii*) is becoming increasingly common in aquaculture due to the faster growth and larger maximum weights in males than females. However, the key factors influencing the development of all-male giant freshwater prawns (post-larval AMGFPs) under captive conditions remain insufficiently explored. This study evaluated the growth and survival of post-larval AMGFPs under varying stocking densities (200–600 ind m<sup>-3</sup>) and alkalinities (80–160 mg CaCO<sub>3</sub> L<sup>-1</sup>) across two completely randomized experiments in three replicates in 1 m<sup>3</sup> tank systems with a biofloc environment, where a carbon source (rice flour with a C:N ratio of 15:1) was added for biofloc formation. Post-larval AMGFPs (initial mean weight and length of 0.012–0.016 g and 1.13–1.53 cm, respectively) were maintained at a salinity 5‰ and fed commercial feed (40% crude protein) for 60 days. The stocking density in Experiment 2 was 500 m<sup>-3</sup> (the best stocking density obtained in Experiment 1). The results showed that after 45 rearing days under the experimental conditions, all growth parameters, such as mean weight (MW), daily weight gain (DWG), specific growth rate in weight (SGRw), mean length (ML), daily length gain (DLG), and specific growth rate in length (SGRL), increased as stocking density rose within 200–500 ind m<sup>-3</sup> but declined at 600 ind m<sup>-3</sup>. The highest survival rate (SR) was observed at a density of 500 ind m<sup>-3</sup>, which was significantly higher than at other densities. Additionally, the highest final biomass (FB) was in 500 and 600 ind m<sup>-3</sup> and differed from other densities. Besides, from rearing day 30 onward, within the alkalinity range of 80–160 mg CaCO<sub>3</sub> L<sup>-1</sup>, all growth parameters (weight and length), SR, and FB increased in proportion to both alkalinity level and rearing time, with the highest values at 160 mg CaCO<sub>3</sub> L<sup>-1</sup> and significantly differing from those at lower levels at the experimental end. A stocking density of 500 ind m<sup>-3</sup> and an alkalinity of 160 mg CaCO<sub>3</sub> L<sup>-1</sup> were recommended as optimal for rearing post-larval AMGFPs under the tested conditions.

## INTRODUCTION

The giant freshwater prawn (*Macrobrachium rosenbergii*) is the largest species in the *Macrobrachium* genus<sup>1</sup> and serves as a crucial nutritional and commercial food source worldwide.<sup>2,3</sup> Although this species primarily inhabits freshwater environments, its larvae require brackish water for complete development.<sup>4,5</sup> *M. rosenbergii* is native to Southeast Asia and the Pacific region, including countries such as the Philippines, Thailand, Malaysia, Indonesia, Vietnam,

Myanmar, India, Bangladesh, and Australia. It has also been introduced for aquaculture in regions beyond its natural range, such as China, the USA, and various Latin American countries. The species is favored in aquaculture due to its rapid growth rate, relatively short culture period, large harvest size, strong disease resistance, adaptability to polyculture with other freshwater fish species, and high market demand.<sup>3</sup> The global aquaculture production of giant freshwater prawns in 2019 reached 273,738 tons, with China

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leading (139,609 tons), followed by Bangladesh (52,197 tons), Thailand (31,345 tons), and Vietnam (20,129 tons).<sup>3</sup>

Notably, *M. rosenbergii* males possess faster growth rates and attain a larger maximum body size than females. In addition, as females reach sexual maturity, their growth rate significantly slows as energy is diverted to reproductive system development and egg production.<sup>6,7</sup> Numerous studies have shown that all-male giant freshwater prawn (AMGFP) cultures attained considerably higher yields than mixed-sex or all-female cultures.<sup>6-9</sup> These findings have driven the development of techniques to produce all-male prawn stocks.<sup>6,7,10-15</sup>

Stocking density, on the other hand, is another critical factor that directly influences the growth and survival of aquacultured species.<sup>16</sup> When stocking density is lower than optimal, it may lead to reduced productivity due to underutilization of space, whereas excessively high density can negatively affect survival rates and individual growth.<sup>17,18</sup> These effects are particularly pronounced in semi-intensive and intensive aquaculture systems, particularly in recirculating culture systems where space is limited.<sup>16</sup> For *M. rosenbergii*, issues such as cannibalism and aggressive behavior, especially among dominant males, can further raise these challenges.<sup>19,20</sup>

In addition, alkalinity is also a critical factor in the cultivation of *M. rosenbergii*, serving as an indicator of the water's buffering capacity and helping to stabilize pH levels against harmful fluctuations.<sup>21-24</sup> Moreover, together with total hardness, alkalinity is vital in crustacean farming because it indicates the levels of cations (e.g., calcium and magnesium) and anions (e.g., carbonates), which are essential for the proper mineralization of the prawn exoskeleton.<sup>25,26</sup>

The postlarval rearing phase of *M. rosenbergii* serves as an intermediary stage between larval rearing and grow-out, during which post-larvae are cultured at high densities from metamorphosis to juvenile stages.<sup>27,28</sup> The nursery aims to enhance the growth of postlarvae, making them larger and stronger, and reducing the risk of predation. This approach also contributes to greater survival and uniformity in market size at harvest.<sup>28,29</sup> Besides, intensive nurseries can serve as an effective biosecurity measure, helping to minimize crop losses due to disease outbreaks.<sup>30,31</sup> Practical rearing systems for freshwater prawns vary widely depending on the techniques applied, knowledge, and available resources to farmers. Each system offers distinct efficiency based on specific conditions. Common rearing models include traditional earthen ponds, polyethylene tanks, aquaponic setups, net cages,<sup>1</sup> and biofloc systems integrated with red seaweed.<sup>32-35</sup>

Based on the information mentioned above, this study aimed to examine the effects of various stocking densities and alkalinities on the development of post-larval AMGFPs within a biofloc environment for a 60-day rearing period. The goals are to improve rearing efficiency and support the expansion of commercial farming for AMGFPs.

## MATERIALS AND METHODS

### EXPERIMENTAL MATERIALS

Post-larval AMGFPs (initial mean weight and length of 0.012–0.016 g and 1.13–1.53 cm, respectively) were obtained through artificial reproduction by pseudo-female broodstock of *M. rosenbergii* using RNAi technology<sup>10,11,13</sup> from the Aquaculture Breeding Center of Tra Vinh Province. The prawns were transferred to the Experimental Aquaculture Hatchery at Tra Vinh University, where the animals were acclimated to experimental conditions using composite tanks of 1 m<sup>3</sup> for one week. During the acclimation period, the prawns were fed an experimental diet before being stocked into the experimental tanks.

The 5‰ salinity water used in the experiments was prepared by mixing freshwater and seawater. Freshwater was collected from the river, with sediments removed prior to disinfection using potassium permanganate (KMnO<sub>4</sub>) at a concentration of 5 mg L<sup>-1</sup>, followed by continuous aeration for three days. saltwater with a salinity of 120‰ was treated with 30 mg L<sup>-1</sup> chlorine and similarly aerated for three days. After treatment, the two water sources were combined to achieve a salinity of 5‰. The resulting mixture was then pumped into the experimental tanks through a filter bag with a 5 µm mesh size.

Five alkalinity levels were investigated in this study (80, 100, 120, 140, and 160 mg CaCO<sub>3</sub> L<sup>-1</sup>) and were adjusted following the method of Furtado et al.<sup>36</sup> and measured using the standard procedure suggested by APHA.<sup>37</sup> The initial water source had an alkalinity of 110 mg CaCO<sub>3</sub> L<sup>-1</sup>. To achieve the desired levels of 80, 100, and 120 mg CaCO<sub>3</sub> L<sup>-1</sup>, 1 M hydrochloric acid (HCl) (Synth<sup>®</sup>) was added. Meanwhile, for alkalinity levels of 140 and 160 mg CaCO<sub>3</sub> L<sup>-1</sup>, sodium bicarbonate (NaHCO<sub>3</sub> L<sup>-1</sup>) with 99% purity (Carbonor<sup>®</sup>) was used. Alkalinity in the rearing tanks was monitored weekly, and sodium bicarbonate was added in varying amounts (less than 0.15 g L<sup>-1</sup> per application) to maintain the desired alkalinity levels.

The experiments used Grobest pellet feed, which contains 40% crude protein and has a particle size range of 0.1–0.6 mm (made in Vietnam). The feeding regimes were based on the prawns' developmental stages (see Experiment 1).

The biofloc environment in the rearing tanks was created by using rice flour (AAAA brand, made in Vietnam), containing 73.43% carbohydrates and 0.26% nitrogen. The rice flour was mixed with water in a 1:3 ratio, heated to 40–60°C for 60 minutes, and then incubated for 48 hours.<sup>38,39</sup> The incubated rice flour solution was added to the feed every two days, in proportion to the amount of feed provided to the shrimp during that period.

### EXPERIMENTAL DESIGN

This study was conducted at the Aquaculture Experimental Hatchery of Tra Vinh University in southern Vietnam from May to July 2021. Two independent, completely randomized experiments, each with three replicates, were designed

to assess the effects of various densities and alkalinities on the growth and survival rates of postlarval AMGFPs during the 60-day rearing period. The details of the experiments are as follows:

In the first experiment, five stocking densities (200, 300, 400, 500, and 600 ind m<sup>-3</sup>) were tested. Post-larval AMGFPs (initial mean weight and length of 0.012 g and 1.13 cm, respectively) were randomly stocked in fifteen rearing tanks at a salinity of 5‰ with continuous aeration. The prawns were fed four times daily at 6:00H, 11:00H, 16:00H, and 20:00H based on their developmental stage: 10% of their body weight during the first 30 days and 7% during the final 30 days. Fecal matter and leftover feed were siphoned daily at 21:00H. All aeration faucets were turned off during siphoning to allow waste to settle. The water in the rearing tanks was changed 15 rearing days and then weekly thereafter, with 10-20% of the water volume changed.

The second experiment examined the effects of five different alkalinity levels (80, 100, 120, 140, and 160 mgCaCO<sub>3</sub> L<sup>-1</sup>) at a salinity of 5‰. Post-larval AMGFPs, with a mean weight of 0.016 g and 1.53 cm, were stocked in 15 rearing tanks at a stocking density of 500 ind m<sup>-3</sup> (the best stocking density obtained in Experiment 1). The care and management procedures in this experiment followed the same protocols as those implemented in the first experiment.

#### DATA COLLECTION AND CALCULATION

Water quality parameters were monitored regularly throughout the experiment. Temperature and pH were measured twice daily (at 7:00 and 14:00) using a pH meter. Alkalinity was assessed every seven days with a Sera test kit. Additionally, water samples for total ammonia nitrogen (TAN) and nitrite (NO<sub>2</sub><sup>-</sup>) analysis were collected weekly from each tank, stored at 4 °C, and later analyzed following the methods outlined by APHA.<sup>37</sup>

At the end of the experiment, 30 prawns were randomly sampled from each tank to assess growth performance. Individual wet weights were measured using a digital scale with 0.01 g precision. Total length was recorded to the nearest millimeter, measured from the tip of the rostrum to the end of the telson using a graduated ruler. Survival rate and total biomass were also documented and calculated at the end of the experimental period. Growth parameters, survival rate, and final biomass were determined based on the following equations.

- MW (g) = total weight of 30 individuals/30
- DWG (g day<sup>-1</sup>) = final weight – initial weight/culturing days
- DLG (cm day<sup>-1</sup>) = final length – initial length/culturing days
- SGR<sub>W</sub> (% day<sup>-1</sup>) = [(Ln final weight) – (Ln initial weight)]/culturing days × 100
- SGR<sub>L</sub> (% day<sup>-1</sup>) = [(Ln final length) – (Ln initial length)]/culturing days × 100
- SR (%) = (final number/initial number) × 100
- FB (ind m<sup>-3</sup>) = final number/culture volume

#### DATA ANALYSIS

Homogeneity of variance was tested using Levene's test, and percentage data were arcsine-transformed before statistical analysis. A one-way analysis of variance (ANOVA) was performed on all data, followed by Duncan's multiple range test to identify significant differences between treatments (with a significance level of  $p < 0.05$ ). All analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software, version 20.0 for Windows.

#### RESULTS

##### WATER QUALITY PARAMETERS

The monitored water quality parameters in both experiments had minimal variation and did not differ significantly between treatments ( $p > 0.05$ ) (Table 1).

##### REARING PERFORMANCE UNDER VARIOUS STOCKING DENSITIES

##### GROWTH PERFORMANCE

MW15 and MW30 did not significantly differ between stocking densities ( $p > 0.05$ ). However, from rearing 45 days, all growth parameters (MW, DWG, SGR<sub>W</sub>, ML, DLG, and SGR<sub>L</sub>) increased as stocking densities rose within the range of 200-500 ind m<sup>-3</sup>, and these parameters tended to decline at 600 ind m<sup>-3</sup> (Table 2). At the experimental end, the highest DWG and SGR<sub>W</sub> were observed at 500 ind m<sup>-3</sup>, which was significantly higher than values at 200 and 300 ind m<sup>-3</sup> ( $p < 0.05$ ), despite no significant differences between 400, 500, and 600 ind m<sup>-3</sup> ( $p > 0.05$ ) (Table 2).

On the other hand, the fortnightly data on length growth performance at five stocking densities showed that ML15 and ML30 had no significant differences between the stocking densities ( $p > 0.05$ ). However, the highest ML45 was observed at 500 ind m<sup>-3</sup>, which significantly differed from that at other stocking densities ( $p < 0.05$ ). At the end of the experiment, the highest ML60 was at 500 ind m<sup>-3</sup> and significantly higher than that at 200 and 300 ind m<sup>-3</sup> ( $p < 0.05$ ), but there was no significant difference from that at 400 and 600 ind m<sup>-3</sup>. Additionally, the highest DLG and SGR<sub>L</sub> were observed at 500 ind m<sup>-3</sup>, which were significantly higher than those at 200 and 300 ind m<sup>-3</sup> ( $p < 0.05$ ), despite no significant differences compared to 400 and 600 ind m<sup>-3</sup> ( $p > 0.05$ ) (Table 3).

##### SURVIVAL RATE (SR) AND FINAL BIOMASS (FB)

Data recorded at the experimental end showed that SR and FB ranged from 66.3 to 79.5% and 148 to 397 ind m<sup>-3</sup>, respectively. The highest survival rate (SR) was recorded at a stocking density of 500 ind m<sup>-3</sup>, which was significantly greater than the rates observed at other densities ( $p < 0.05$ ). Furthermore, the highest final biomass (FB) was found at 500 and 600 ind m<sup>-3</sup>, both of which were significantly higher compared to those at the other stocking densities ( $p < 0.05$ ). Furthermore, SR remained relatively stable across

**Table 1. Water quality parameters during the 60-day rearing periods at various stocking densities and alkalinities.**

| Parameters                                               | Test Time | Densities (ind m <sup>-3</sup> ) |          |          |          |          |
|----------------------------------------------------------|-----------|----------------------------------|----------|----------|----------|----------|
|                                                          |           | 200                              | 300      | 400      | 500      | 600      |
| Temperature (°C)                                         | 7:00      | 27.6±0.2                         | 27.3±0.3 | 27.6±0.5 | 27.4±0.7 | 27.2±0.4 |
|                                                          | 14:00     | 28.5±0.3                         | 28.1±0.4 | 28.4±0.5 | 28.3±0.8 | 28.1±0.5 |
| pH                                                       | 7:00      | 7.9±0.0                          | 7.8±0.1  | 7.9±0.0  | 7.8±0.0  | 7.8±0.0  |
|                                                          | 14:00     | 7.9±0.0                          | 7.8±0.0  | 7.8±0.0  | 7.8±0.0  | 7.9±0.0  |
| TAN (mg L <sup>-1</sup> )                                |           | 0.3±0.0                          | 0.3±0.0  | 0.3±0.0  | 0.4±0.0  | 0.4±0.1  |
| NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )       |           | 2.2±0.1                          | 2.4±0.1  | 2.4±0.1  | 2.2±0.1  | 2.3±0.2  |
| Alkalinities (mgCaCO <sub>3</sub> L <sup>-1</sup> )      |           | 93.7±0.5                         | 94.7±1.5 | 91.7±3.2 | 95.3±1.2 | 95.7±2.1 |
| Alkalinity levels (mgCaCO <sub>3</sub> L <sup>-1</sup> ) |           |                                  |          |          |          |          |
|                                                          |           | 80                               | 100      | 120      | 140      | 160      |
| Temperature (°C)                                         | 7:00      | 26.4±0.5                         | 26.6±0.1 | 26.6±0.1 | 26.6±0.1 | 26.6±0.3 |
|                                                          | 14:00     | 28.5±0.0                         | 28.4±0.1 | 28.5±0.0 | 28.5±0.0 | 28.6±0.1 |
| pH                                                       | 7:00      | 8.2±0.3                          | 8.3±0.2  | 8.8±4.9  | 8.5±0.1  | 8.5±0.1  |
|                                                          | 14:00     | 8.2±0.1                          | 8.3±0.2  | 8.4±0.1  | 8.5±0.0  | 8.5±0.2  |
| TAN (mg L <sup>-1</sup> )                                |           | 0.3±0.2                          | 0.3±0.3  | 0.3±0.1  | 0.3±0.1  | 0.4±0.1  |
| NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )       |           | 1.3±0.2                          | 1.5±0.3  | 1.3±0.6  | 1.1±0.1  | 1.2±0.2  |

Values are presented as mean±SD.

**Table 2. Mean weight (MW), daily weight gain (DWG), and specific growth rate in weight (SGR<sub>w</sub>) of post-larval AMGFPs during the 60-day rearing period at various stocking densities.**

| Parameters                              | Densities (ind m <sup>-3</sup> ) |                         |                         |                        |                        |
|-----------------------------------------|----------------------------------|-------------------------|-------------------------|------------------------|------------------------|
|                                         | 200                              | 300                     | 400                     | 500                    | 600                    |
| MW15 (g ind <sup>-1</sup> )             | 0.06±0.02 <sup>a</sup>           | 0.05±0.02 <sup>a</sup>  | 0.05±0.02 <sup>a</sup>  | 0.06±0.03 <sup>a</sup> | 0.07±0.03 <sup>a</sup> |
| MW30 (g ind <sup>-1</sup> )             | 0.15±0.07 <sup>a</sup>           | 0.19±0.08 <sup>a</sup>  | 0.19±0.08 <sup>a</sup>  | 0.17±0.10 <sup>a</sup> | 0.16±0.09 <sup>a</sup> |
| MW45 (g ind <sup>-1</sup> )             | 0.64±0.19 <sup>a</sup>           | 0.68±0.19 <sup>a</sup>  | 0.83±0.23 <sup>b</sup>  | 1.00±0.23 <sup>c</sup> | 0.87±0.18 <sup>b</sup> |
| MW60 (g ind <sup>-1</sup> )             | 1.12±0.37 <sup>a</sup>           | 1.17±0.34 <sup>ab</sup> | 1.34±0.30 <sup>bc</sup> | 1.51±0.40 <sup>c</sup> | 1.37±0.38 <sup>c</sup> |
| DWG (g day <sup>-1</sup> )              | 0.02±0.01 <sup>a</sup>           | 0.02±0.01 <sup>a</sup>  | 0.02±0.01 <sup>bc</sup> | 0.03±0.01 <sup>c</sup> | 0.02±0.01 <sup>c</sup> |
| SGR <sub>w</sub> (% day <sup>-1</sup> ) | 6.99±0.61 <sup>a</sup>           | 7.09±0.51 <sup>a</sup>  | 7.35±0.37 <sup>b</sup>  | 7.53±0.45 <sup>b</sup> | 7.36±0.46 <sup>b</sup> |

Values are presented as mean±SD. Values with different letters (a, b, c) in the same row show a significant difference ( $p < 0.05$ ).

**Table 3. Mean length (ML), daily length gain (DLG), and specific growth rate in length (SGR<sub>L</sub>) of Post-larval AMGFPs during the 60-day rearing period at various stocking densities.**

| Parameters                              | Densities (ind m <sup>-3</sup> ) |                         |                          |                         |                          |
|-----------------------------------------|----------------------------------|-------------------------|--------------------------|-------------------------|--------------------------|
|                                         | 200                              | 300                     | 400                      | 500                     | 600                      |
| ML15 (cm ind <sup>-1</sup> )            | 1.87±0.23 <sup>a</sup>           | 1.79±0.23 <sup>a</sup>  | 1.80±0.28 <sup>a</sup>   | 1.83±0.29 <sup>a</sup>  | 1.86±0.27 <sup>a</sup>   |
| ML30 (cm ind <sup>-1</sup> )            | 2.58±0.44 <sup>a</sup>           | 2.75±0.45 <sup>a</sup>  | 2.83±0.43 <sup>a</sup>   | 2.71±0.55 <sup>a</sup>  | 2.68±0.43 <sup>a</sup>   |
| ML45 (cm ind <sup>-1</sup> )            | 4.13±0.45 <sup>a</sup>           | 4.32±0.46 <sup>a</sup>  | 4.59±0.45 <sup>b</sup>   | 4.84±0.37 <sup>c</sup>  | 4.61±0.37 <sup>b</sup>   |
| ML60 (cm ind <sup>-1</sup> )            | 4.99±0.58 <sup>a</sup>           | 5.08±0.56 <sup>a</sup>  | 5.26±0.41 <sup>ab</sup>  | 5.51±0.48 <sup>b</sup>  | 5.26±0.52 <sup>ab</sup>  |
| DLG (cm day <sup>-1</sup> )             | 0.065±0.01 <sup>a</sup>          | 0.066±0.01 <sup>a</sup> | 0.069±0.01 <sup>ab</sup> | 0.073±0.01 <sup>b</sup> | 0.069±0.01 <sup>ab</sup> |
| SGR <sub>L</sub> (% day <sup>-1</sup> ) | 2.47±0.20 <sup>a</sup>           | 2.50±0.19 <sup>ab</sup> | 2.56±0.13 <sup>bc</sup>  | 2.63±0.15 <sup>c</sup>  | 2.56±0.17 <sup>bc</sup>  |

Values are presented as mean±SD. Values with different letters (a, b, c) in the same row show a significant difference ( $p < 0.05$ ).

200 to 400 ind m<sup>-3</sup> ( $p > 0.05$ ), while FB at 400 ind m<sup>-3</sup> was significantly higher compared to that at 200 ind m<sup>-3</sup> ( $p < 0.05$ ) (Table 4).

**Table 4. Survival rate (SR) and final biomass (FB) of post-larval AMGFs after 60 days of rearing at various stocking densities.**

| Parameters                | Densities (ind m <sup>-3</sup> ) |                       |                       |                       |                       |
|---------------------------|----------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|                           | 200                              | 300                   | 400                   | 500                   | 600                   |
| SR (%)                    | 74.3±1.5 <sup>b</sup>            | 74.3±2.1 <sup>b</sup> | 74.8±1.1 <sup>b</sup> | 79.5±1.3 <sup>c</sup> | 66.3±3.2 <sup>a</sup> |
| FB (ind m <sup>-3</sup> ) | 148±13 <sup>a</sup>              | 148±18 <sup>a</sup>   | 299±15 <sup>b</sup>   | 397±10 <sup>c</sup>   | 397±12 <sup>c</sup>   |

Values are presented as mean±SD. Values with different letters (a, b, c) in the same row show a significant difference ( $p < 0.05$ ).

**Table 5. Mean weight (MW), daily weight gain (DWG), and specific growth rate in weight (SGRw) of Post-larval AMGFs during the 60-day rearing period at various alkalinities**

| Parameters                  | Alkalinities (mgCaCO <sub>3</sub> L <sup>-1</sup> ) |                         |                        |                         |                        |
|-----------------------------|-----------------------------------------------------|-------------------------|------------------------|-------------------------|------------------------|
|                             | 80                                                  | 100                     | 120                    | 140                     | 160                    |
| MW15 (g ind <sup>-1</sup> ) | 0.08±0.02 <sup>a</sup>                              | 0.09±0.03 <sup>a</sup>  | 0.10±0.04 <sup>a</sup> | 0.09±0.03 <sup>a</sup>  | 0.10±0.03 <sup>a</sup> |
| MW30 (g ind <sup>-1</sup> ) | 0.18±0.08 <sup>a</sup>                              | 0.20±0.06 <sup>ab</sup> | 0.22±0.08 <sup>b</sup> | 0.24±0.07 <sup>b</sup>  | 0.24±0.07 <sup>b</sup> |
| MW45 (g ind <sup>-1</sup> ) | 0.77±0.38 <sup>a</sup>                              | 0.76±0.27 <sup>a</sup>  | 0.83±0.43 <sup>a</sup> | 0.96±0.28 <sup>ab</sup> | 1.08±0.42 <sup>b</sup> |
| MW60 (g ind <sup>-1</sup> ) | 1.25±0.49 <sup>a</sup>                              | 1.22±0.41 <sup>a</sup>  | 1.21±0.36 <sup>a</sup> | 1.42±0.68 <sup>a</sup>  | 1.67±0.39 <sup>b</sup> |
| DWG (g day <sup>-1</sup> )  | 0.02±0.01 <sup>a</sup>                              | 0.02±0.01 <sup>a</sup>  | 0.02±0.01 <sup>a</sup> | 0.02±0.01 <sup>a</sup>  | 0.03±0.01 <sup>b</sup> |
| SGRw (% day <sup>-1</sup> ) | 6.70±0.58 <sup>a</sup>                              | 6.69±0.55 <sup>a</sup>  | 6.70±0.47 <sup>a</sup> | 6.86±0.72 <sup>a</sup>  | 7.25±0.40 <sup>b</sup> |

Values are presented as mean±SD. Values with different letters (a, b) in the same row show a significant difference ( $p < 0.05$ ).

## REARING PERFORMANCE UNDER VARIOUS ALKALINITIES

### GROWTH PERFORMANCE

MW15 did not significantly differ among the investigated alkalinities ( $p > 0.05$ ). However, from 30 days of rearing to the end of the experiment, all growth parameters (weight and length), including MW, DWG, SGRw, ML, DLG, and SGR<sub>L</sub>, along with SR and FB, increased proportionally with both alkalinity levels and rearing time. These parameters reached their highest values at 160 mg CaCO<sub>3</sub> L<sup>-1</sup> and significantly differed from those in lower alkalinity levels (80–140 mg CaCO<sub>3</sub> L<sup>-1</sup>) at the experimental end (Table 5).

In a similar pattern in weight growth, the highest length growth parameters (ML, DLG, and SGR<sub>L</sub>) were observed at 160 mg CaCO<sub>3</sub> L<sup>-1</sup> throughout the rearing period. In detail, the highest ML30 was observed at 160 mg CaCO<sub>3</sub> L<sup>-1</sup>, which was significantly higher than at 80 mg CaCO<sub>3</sub> L<sup>-1</sup> ( $p < 0.05$ ). The highest ML45 and ML60 were observed at 160 mg CaCO<sub>3</sub> L<sup>-1</sup> and were significantly higher than those at 80, 100, and 120 mg CaCO<sub>3</sub> L<sup>-1</sup> ( $p < 0.05$ ). Moreover, both DLG and SGR<sub>L</sub> at 160 mg CaCO<sub>3</sub> L<sup>-1</sup> were significantly greater than those at 80, 100, 120, and 140 mg CaCO<sub>3</sub> L<sup>-1</sup> by the end of the experiment ( $p < 0.05$ ) (Table 6).

### SURVIVAL RATE (SR) AND FINAL BIOMASS (FB)

At the end of the experiment, SR and FB ranged from 67.1% to 81.0% and from 336 to 405 ind m<sup>-3</sup> respectively. These parameters reached their highest values at alkalinity 160 mg CaCO<sub>3</sub> L<sup>-1</sup>, which did not significantly differ from those at 100–140 mg CaCO<sub>3</sub> L<sup>-1</sup> ( $p > 0.05$ ); meanwhile, they were significantly higher than those at 80 mg CaCO<sub>3</sub> L<sup>-1</sup> ( $p < 0.05$ ) (Table 7).

## DISCUSSION

### WATER QUALITY PARAMETERS

Water parameters for both experiments showed that they remained stable throughout the rearing periods. The mean values included temperature ranging from 27.22 to 28.5°C, pH from 7.8 to 7.9, TAN from 0.3 to 0.4 mg L<sup>-1</sup>, nitrite from 2.2 to 2.4 mg L<sup>-1</sup>, and alkalinity from 91.7 to 95.7 mg L<sup>-1</sup> in the experiment 1, and 26.4–28.6°C, 8.2–8.8, 0.3–0.4 mg L<sup>-1</sup>, and 1.1–1.5 mg L<sup>-1</sup>, respectively, in the experiment 2 (Table 1), which were within the appropriate ranges for *M. rosenbergii*<sup>40–47</sup> and therefore did not considerably impact the main results.

### GROWTH AND SURVIVAL RATES OF THE AMGFs AT VARIOUS DENSITIES

Practical rearing systems for freshwater prawns vary according to techniques, producers' skills, knowledge, and available resources. These systems include biofloc integrated with red seaweed, tanks, aquaponic setups, net cages, etc., and each offers unique benefits depending on specific conditions.<sup>1,32–35</sup> On the other hand, optimizing stocking density is essential for enhancing aquaculture production.<sup>48–50</sup> While higher stocking densities can substantially increase production per unit area, they may also induce stress responses that compromise the health, growth, survival, and biomass of farmed animals.<sup>51–53</sup> Our study reared post-larval AMGF in 1 m<sup>3</sup> composite tanks with a biofloc environment in a density range of 200–600 ind m<sup>-3</sup> and showed that a stocking density of 500 ind m<sup>-3</sup> was the best choice for this rearing technique (Tables 2, 3 & 4).

**Table 6. Mean length (ML), daily length gain (DLG), and specific growth rate in length (SGR<sub>L</sub>) of Post-larval AMGFPs during the 60-day rearing period at various alkalinities**

| Parameters                              | Alkalinities (mgCaCO <sub>3</sub> L <sup>-1</sup> ) |                         |                         |                         |                        |
|-----------------------------------------|-----------------------------------------------------|-------------------------|-------------------------|-------------------------|------------------------|
|                                         | 80                                                  | 100                     | 120                     | 140                     | 160                    |
| ML15 (cm ind <sup>-1</sup> )            | 2.12±0.31 <sup>a</sup>                              | 2.21±0.29 <sup>a</sup>  | 2.24±0.33 <sup>a</sup>  | 2.16±0.25 <sup>a</sup>  | 2.26±0.30 <sup>a</sup> |
| ML30 (cm ind <sup>-1</sup> )            | 2.77±0.45 <sup>a</sup>                              | 2.90±0.33 <sup>ab</sup> | 2.92±0.28 <sup>ab</sup> | 3.04±0.46 <sup>b</sup>  | 3.05±0.36 <sup>b</sup> |
| ML45 (cm ind <sup>-1</sup> )            | 4.57±0.68 <sup>a</sup>                              | 4.49±0.51 <sup>a</sup>  | 4.58±0.67 <sup>a</sup>  | 4.81±0.56 <sup>ab</sup> | 5.04±0.69 <sup>b</sup> |
| ML60 (cm ind <sup>-1</sup> )            | 5.28±0.67 <sup>a</sup>                              | 5.22±0.58 <sup>a</sup>  | 5.26±0.54 <sup>a</sup>  | 5.45±0.82 <sup>ab</sup> | 5.76±0.49 <sup>b</sup> |
| DLG (cm day <sup>-1</sup> )             | 0.06±0.01 <sup>a</sup>                              | 0.06±0.00 <sup>a</sup>  | 0.06±0.00 <sup>a</sup>  | 0.07±0.01 <sup>ab</sup> | 0.07±0.00 <sup>b</sup> |
| SGR <sub>L</sub> (% day <sup>-1</sup> ) | 2.05±0.21 <sup>a</sup>                              | 2.04±0.17 <sup>a</sup>  | 2.05±0.17 <sup>a</sup>  | 2.10±0.24 <sup>a</sup>  | 2.20±0.20 <sup>b</sup> |

Values are presented as mean±SD. Values with different letters (a, b) in the same row show a significant difference ( $p < 0.05$ ).

**Table 7. Survival rate (SR) and final biomass (FB) of post-larval AMGFPs after 60 days of rearing at various alkalinities.**

| Parameters                | Alkalinities (mgCaCO <sub>3</sub> L <sup>-1</sup> ) |                        |                        |                        |                       |
|---------------------------|-----------------------------------------------------|------------------------|------------------------|------------------------|-----------------------|
|                           | 80                                                  | 100                    | 120                    | 140                    | 160                   |
| SR (%)                    | 67.1±6.5 <sup>a</sup>                               | 73.7±5.6 <sup>ab</sup> | 72.6±3.1 <sup>ab</sup> | 74.9±4.6 <sup>ab</sup> | 81.0±2.0 <sup>b</sup> |
| FB (ind m <sup>-3</sup> ) | 336±33 <sup>a</sup>                                 | 368±28 <sup>ab</sup>   | 363±16 <sup>ab</sup>   | 374±23 <sup>ab</sup>   | 405±10 <sup>b</sup>   |

Values are presented as mean±SD. Values with different letters (a, b) in the same row show a significant difference ( $p < 0.05$ ).

Inappropriate stocking densities are recognized as a major source of chronic stress in farmed teleost species.<sup>50</sup> Excessive stocking density can result in crowding stress, and therefore energy is diverted for maintaining homeostasis and restoring tissue and immune systems, which may negatively affect total productivity. If the stocking density is lower than optimal, it may reduce culture productivity due to the underutilization of available space.<sup>17,18</sup> Additionally, cannibalistic behavior in predatory species can further reduce growth and survival rates in farmed animals.<sup>54-56</sup> Cannibalism associated with molting has been observed in crustacean species,<sup>57-59</sup> and it is known that molting takes place throughout the life cycle of the giant freshwater prawn *M. rosenbergii*.<sup>60</sup> Moreover, territorial behavior tends to increase at high stocking densities in the prawns, potentially leading to cannibalism.<sup>61</sup> These results may account for the highest growth performance, survival rate (SR), and final body size (FB) of AMGFPs observed at a stocking density of 500 ind m<sup>-3</sup>, with declines noted at higher densities in the present study.

#### GROWTH AND SURVIVAL RATES OF AMGFP JUVENILES AT VARIOUS ALKALINITIES

The calcareous exoskeleton of crustaceans serves both protective and structural functions but also presents major constraints, as growth is restricted to periods of molting. In crustacean aquaculture, water total hardness and alkalinity are critical, as they indicate the concentrations of essential cations (such as calcium and magnesium) and anions (such as carbonates), which are necessary for proper exoskeleton mineralization and successful molting.<sup>62,63</sup> Additionally, alkalinity serves as a buffering system to help maintain a stable pH in the water.<sup>23</sup> Therefore, selecting an

appropriate site for farming *M. rosenbergii* is essential, as it must meet their specific requirements for alkalinity and total hardness.<sup>63</sup>

The present study indicated that 160 mgCaCO<sub>3</sub> L<sup>-1</sup> was the best alkalinity for rearing post-larval AMGFPs (**Tables 5, 6 & 7**). This finding is consistent with the results reported by Day et al.,<sup>64</sup> who indicated that juvenile AMGFPs showed the best culture performance at an alkalinity of 160 mgCaCO<sub>3</sub> L<sup>-1</sup>, compared to lower alkalinity levels (80–140 mgCaCO<sub>3</sub> L<sup>-1</sup>). Additionally, this alkalinity level falls within the range recommended for freshwater prawns. It was reported that culturing giant freshwater prawns in water with an adequate Ca<sup>2+</sup> concentration will help shorten their molting cycle and promote faster growth.<sup>62</sup> Low alkalinity levels in water bodies are considered less appropriate for farming prawns and fish. The upper limit of alkalinity is primarily determined by the specific requirements of each species and the extent to which it influences pH levels.<sup>24</sup> Although alkalinity levels of between 40 and 100 mgCaCO<sub>3</sub> L<sup>-1</sup> are suggested for the successful culturing of *M. rosenbergii*,<sup>62,65</sup> a range of 100–150 mgCaCO<sub>3</sub> L<sup>-1</sup> tends to take less energy on osmoregulation, resulting in better growth, while 205 mg CaCO<sub>3</sub> L<sup>-1</sup> or above was observed to reduce the growth rate of *M. rosenbergii*.<sup>62</sup>

Studies have shown that fish inhabiting low-calcium waters may lose calcium to their environment, requiring them to expend additional energy and utilize ions from their diet to reabsorb the lost calcium salts. A similar process may occur in prawns exposed to very low-calcium conditions, where calcium loss could take place through the exuviae.<sup>66</sup> Additionally, insufficient calcium concentration in water adversely affects calcareous exoskeleton mineralization, which in turn impacts the molting process of crustaceans.<sup>62,63</sup> This information may help explain why dur-

ing the first 30 culturing days, when the AMGFPs were still small, their mineral requirements for the outer shell were not significantly different, which is why no notable differences in growth parameters (weight and length) were observed across the various alkalinity levels. However, from the 45th rearing day, as the AMGFPs' body size increased, so did their mineral needs. The alkalinity of 160 mgCaCO<sub>3</sub> L<sup>-1</sup> may have met the increased mineral requirement, which may have been the reason for the best result observed.

SR ranges of 66.3–79.5% in Experiment 1 and 67.1–81.0% in Experiment 2 were higher than the range of 2.52–22.69% reported for mixed-sex postlarvae of *M. rosenbergii* reared in aquaponic systems over the same duration,<sup>67</sup> 53.5% for postlarval AMGFPs reared in ponds,<sup>34</sup> and they were within the range of 44.4–86.67% for postlarvae of *M. rosenbergii* reared in net cages installed in earthen ponds<sup>1</sup>; 80.3–83 for postlarvae of *M. rosenbergii* reared in a recirculatory system using types of artificial substrates.<sup>33</sup> Moreover, SGRw values of 6.99–7.53% day<sup>-1</sup> (Experiment 1) and 6.69–7.25% day<sup>-1</sup> (Experiment 2) were higher than the ranges reported 4.72–5.19% day<sup>-1</sup><sup>33</sup> and 2.52–2.69% day<sup>-1</sup>.<sup>67</sup> The differences in results between studies are likely closely related to variations in trial conditions, such as sex or rearing systems (techniques/methods), compared to the conditions in our study. This also shows the superiority of rearing post-larval AMGFPs with a biofloc tank system.

Based on the results of the two experiments, a stocking density of 500 ind m<sup>-3</sup> and an alkalinity of 160 mg CaCO<sub>3</sub> L<sup>-1</sup> were identified as the optimal conditions for rearing post-larval AMGFPs under the tested conditions. However, further research on alkalinity levels higher than 160 mg CaCO<sub>3</sub> L<sup>-1</sup> is needed to determine the most suitable alkalinity for rearing AMGFP post-larvae in a biofloc system.

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## AUTHORS' CONTRIBUTION

Conceptualization: Huynh K. Huong (Equal), Doan X. Diep (Equal). Methodology: Huynh K. Huong (Equal), Le H. Vu (Equal), Nguyen T.H. Nhi (Equal). Writing – original draft: Huynh K. Huong (Equal), Doan X. Diep (Equal). Writing – review & editing: Huynh K. Huong (Equal), Doan X. Diep (Equal). Supervision: Huynh K. Huong (Equal), Doan X. Diep (Lead). Formal Analysis: Le H. Vu (Equal), Nguyen T.H. Nhi (Equal). Investigation: Le H. Vu (Equal), Nguyen T.H. Nhi (Equal).

## COMPETING INTEREST – COPE

No competing interests were disclosed.

## ETHICAL CONDUCT APPROVAL – IACUC

The animal experiments complied with relevant national and international guidelines. Only the all-male giant freshwater prawns (*Macrobrachium rosenbergii*) underwent weighing and measuring during the experiment, ensuring no harm was caused. After the experiments, the prawns were returned to the storage tanks for grow-out culture.

## INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

## DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

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