Nursery rearing freshwater prawn, *Macrobrachium rosenbergii*, in biofloc system integrated with red seaweed, *Gracilaria tenuistipitata*, at different stocking densities under zero water exchange

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Abstract:

This study was conducted to assess the effects of stocking densities on the water quality, survival and growth of the freshwater prawn *Macrobrachium rosenbergii* postlarvae (PL) cultured in a biofloc system integrated with red seaweed (*Gracilaria tenuistipitata*). Postlarvae prawns weighing 0.012±0.001 g were stocked at densities of 1,000, 1,500, 2,000, and 2,500 PL/m^3, noted as D1, D1.5, D2, and D2.5, respectively. Each density treatment was replicated three times and randomly assigned to 12 plastic tanks of 150 l at a salinity of 10 ppt. Red seaweed was added at a rate of 1.5 kg/m^3, and molasses was used as a carbon source to maintain a C:N ratio of 15:1. No water exchange occurred during the 30-day rearing period. Results indicated that water quality parameters, including TAN and NO_2^-, biofloc volume, total heterotrophic bacteria, and *Vibrio* spp. counts, increased at higher prawn densities but remained within the appropriate range for prawn performance. Growth rate in weight and survival of prawns decreased as stocking density increased, with D1 and D1.5 showing comparable and significantly higher results compared to D2 and D2.5. Prawn production increased with rising stocking density, with a significant difference among treatments ($p<0.05$). Furthermore, prawns in D1 and D1.5 exhibited significantly higher feed efficiency than those in D2 and D2.5. These findings suggest that nursery rearing of prawn PL at a density of 1,500 ind/m^3 in a biofloc system integrated with red seaweed offers optimal growth and feed efficiency, while maintaining good water quality and conserving water resources.

Keywords: **biofloc, feed efficiency, freshwater prawn, *Gracilaria tenuistipitata*, growth rate, water quality.**

Classification numbers: **3.1, 5.3**

1. Introduction

The freshwater prawn, *Macrobrachium rosenbergii*, is a tropical species commonly cultured in Asia, including Vietnam, due to its ability to thrive in diverse environments, ranging from freshwater to brackish water [1]. This species demonstrates optimal development within a salinity range of 0-15 ppt [2]. As noted by T.N. Hai, et al. (2020) [3], freshwater prawn emerges as a significant candidate for culture in the brackish waters of the Mekong Delta in Vietnam, particularly in response to salinity intrusion induced by climate change.

Biofloc technology has emerged as a sustainable aquaculture approach, contributing to reduced feed input, water consumption, and enhanced water quality [4, 5]. It has found widespread application in nursery phases of shrimp and prawns in recent years [6-9]. According to M.E. Hosain, et al. (2021) [8], rearing *M. rosenbergii* PL at a salinity of 10-15 ppt using biofloc technology resulted in improved survival and prawn production. Additionally, integrated aquaculture systems that co-culture organic extractive aquaculture species (such as seaweed) with fed aquaculture species (like fish/shrimp/prawn) offer both environmental sustainability and economic stability [10, 11]. N.T.N. Anh (2019) [12] revealed that co-culturing freshwater prawns with red seaweed *Gracilaria tenuistipitata* enhanced growth rates and survival, reduced feed costs, and maintained good water quality. Similar research on red seaweed integration with postlarvae of whiteleg shrimp [13] and black tiger shrimp [14] during the nursery phase significantly improved water quality and shrimp survival at high

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stocking densities compared to monoculture. Another study found that using red seaweed *G. tenuistipitata* in rearing crablets, *Scylla paramamosain*, greatly enhanced survival and provided an ideal culture medium for crab performance [15].

Several investigations highlight the benefits of the nursery phase for postlarvae of shrimps and prawns, including increased size uniformity, reduced predation, shorter pond grow-out cycles, more harvests per year, improved feed efficiency, and enhanced biosecurity [6, 9, 16]. Stocking density is a critical factor influencing the growth, survival, productivity, and production efficiency of cultured species during the nursery phase [7, 13, 14, 16, 17]. This study aims to determine the optimal stocking density of prawns concerning water quality and prawn performance in a biofloc system integrated with red seaweed under zero water exchange conditions.

2. Materials and methods

2.1. Biological materials

High-quality all-male prawn *M. rosenbergii* postlarvae (PL12) were procured from a commercial hatchery in Bac Lieu province, Vietnam, and acclimatised in a 2 m^3 tank for three days to adjust to experimental conditions. Red seaweed, *G. tenuistipitata*, was sourced from an improved extensive pond in Bac Lieu province. The specimens underwent thorough cleaning to remove sediments and fouling before acclimatisation to an experimental salinity of 10 ppt over three days.

2.2. Experimental design, management and monitoring

Prawn PL15 were reared in a biofloc system integrated with *G. tenuistipitata* under a transparent roof at the College of Aquaculture and Fisheries, Can Tho University, Vietnam. Four treatments were randomly assigned in triplicate with different stocking densities: 1,000 (D1), 1,500 (D1.5), 2,000 (D2), and 2,500 PL/m^3 (D2.5). Initial prawn sizes were 0.012±0.001 g body weight and 1.13±0.05 cm total length. They were reared in 150 l tanks at a salinity of 10 ppt with continuous aeration to maintain dissolved oxygen levels of 5-6 mg/l. Red seaweed was added to the rearing tanks at a biomass of 1.5 kg/m^3. During nursery rearing, water temperature, pH, and alkalinity in the rearing tanks fluctuated within the ranges of 25.1-28.3°C, 7.9-8.13, and 90-126 mg $CaCO_3$/l, respectively.

To promote biofloc formation, carbohydrates (molasses containing 46.7% carbohydrate and 0.95% protein) were added to the rearing tanks after prawn stocking to maintain a C:N ratio of the biofloc system at 15:1, following Avnimelech's protocol [4]. Prawn postlarvae were fed four times daily (at 7:00, 11:00, 16:00, and 21:00 h) with commercial feed containing 40% protein (Grobest, Vietnam). Feeding ration was set at 8-10% of the estimated prawn biomass, monitored, and adjusted based on feed presence or absence after one hour of feeding. Throughout the rearing period, no water exchange occurred, and freshwater was only added to the rearing tank to compensate for evaporation and maintain initial salinity and culture volume. The experiment lasted for 30 days.

2.3. Data collection

Water temperature and pH were monitored every three days at 07:00 and 14:00 hours using a multi-channel meter (Mettler Toledo, USA). Alkalinity level was measured using a test kit (Sera, Germany), while the concentrations of total ammonia nitrogen (TAN) and NO_2- were determined at 10-day intervals using the standard method of APHA [18]. Biofloc volume was measured every ten days by collecting a 1l water sample in an Imhoff cone and allowing it to settle undisturbed for 30 minutes; settled floc was recorded as ml/l [4].

At the experiment's conclusion, total heterotrophic bacterial and *Vibrio* spp. densities in water were analysed using Zobell marine agar medium and TCBS (thiosulphate-citrate-bile salts-sucrose agar), respectively, following standard procedures [19, 20]. The red seaweed biomass in each tank was harvested, excess water was removed, and it was weighed to calculate the percentage biomass increment (BI).

BI (%) = (Final biomass - Initial biomass)/Initial biomass x 100

The initial weight and total length of prawns were determined by randomly selecting 40 animals from the conditioning tank and measuring them with a 0.01 g precision balance and callipers, respectively. At the experiment's conclusion, the total prawn biomass in each rearing tank was weighed to record the final weight and growth rate, and survival rate was determined by counting all harvested prawns. Forty

shrimp were randomly selected from each rearing tank to measure the final length of the prawn. Growth performance of prawns, such as specific growth rate in length (SGR_L) and weight (SGR_W), survival, feed conversion ratio (FCR), and feed efficiency (FE), were calculated as follows:

SGR_L (%/day) = [ln(Final length) - ln(Initial length)]/ Day of culture x 100

SGR_W (%/day) = [ln(Final weight) - ln(Initial weight)]/ Day of culture x 100

Survival (%) = Final number of prawn/Initial number of prawn x 100

FCR = Consumed feed (g)/(Final weight - Initial weight) (g)

FE (%) = [Consumed feed/(Final weight - Initial weight)] x 100

2.4. Statistical analysis

All percentage values were arcsine transformed, and variance homogeneity was tested using Levene's test. A one-way ANOVA was employed to determine the overall effect of the treatment. The Duncan test was used to identify significant differences between the mean values at a significance level of $p<0.05$ (SPSS, version 20.0).

3. Results and discussion

3.1. Water quality parameters

Water quality parameters during the experiment are shown in Table 1. Results indicated that water temperature, pH, and alkalinity in the rearing tanks fluctuated within the ranges of 25.1-28.3°C, 7.9-8.13, and 90-126 mg$CaCO_3$/l, respectively. These values remained consistent across density treatments, falling within the appropriate range for prawn culture, as suggested by M.B. New (2012) [1].

Figure 1 shows that the concentrations of TAN and NO_2^- increased with rearing duration, with higher levels observed at higher prawn density. The D1 and D2.5 treatments had the lowest and highest values, respectively, and were significantly different ($p<0.05$) from the other density treatments (Table 1). Although the highest prawn density (2,500 PL/m^3) resulted in the highest levels of TAN (0.39 mg/l) and NO_2^- (1.68 mg/l) at the experiment's conclusion, these values are considered safe for prawn rearing [21, 22].

Notably, the water quality in the culture medium remains within an acceptable range for prawn performance under a no-water-exchange system in the present study. This could be attributed to (1) The presence of red seaweed *G. tenuistipitata* in the rearing tanks, which absorbed nutrients for its growth [10, 13, 14]; and (2) The addition of molasses as a carbon source to stimulate the growth of heterotrophic bacteria in the biofloc. Ammonia levels could be reduced more rapidly via assimilation than by the nitrification process, which primarily controlled the increase in nitrogenous compound levels in the water [4, 5, 23].

Table 1. Average parameters of water quality in the rearing tanks.

Density treatment		1,000 PL/m^3 (D1)	1,500 PL/m^3 (D1.5)	2,000 PL/m^3 (D2)	2,500 PL/m^3 (D2.5)
Temperature (°C)	7:00 h	25.1±0.8	25.4±0.8	25.3±0.9	25.3±0.9
	14:00 h	26.5±0.6	28.3±1.8	27.8±1.8	27.1±1.0
pH	7:00 h	8.08±0.07	8.05±0.09	8.07±0.07	8.04±0.08
	14:00 h	8.22±0.06	8.23±0.11	8.25±0.10	8.20±0.06
Alkalinity ($CaCO_3$/l)		110.3±13.9	117.0±13.3	115.5±9.3	116.3±7.1
TAN (mg/l)		0.09±0.01^a	0.16±0.05^b	0.19±0.02^{bc}	0.24±0.03^c
NO_2^- (mg/l)		0.57±0.08^a	0.86±0.16^b	0.99±0.05^b	1.33±0.06^c
Biofloc volume (ml/l)		1.68±0.37^a	1.91±0.42^b	2.30±0.52^c	2.59±0.64^d
Total heterotrophic bacteria (x10^3 CFU/ml)		1.98±0.73^a	2.63±0.29^a	3.23±0.62^{ab}	4.42±1.03^b
Vibrio spp. (x10^2 CFU/ml)		3.60±0.5^a	5.13±0.35^{ab}	6,23±0.22^b	7.20±0.21^b

*: values are expressed as mean±SD (n=3). Mean values in with different superscripts within the same row are significantly different at $p<0.05$.

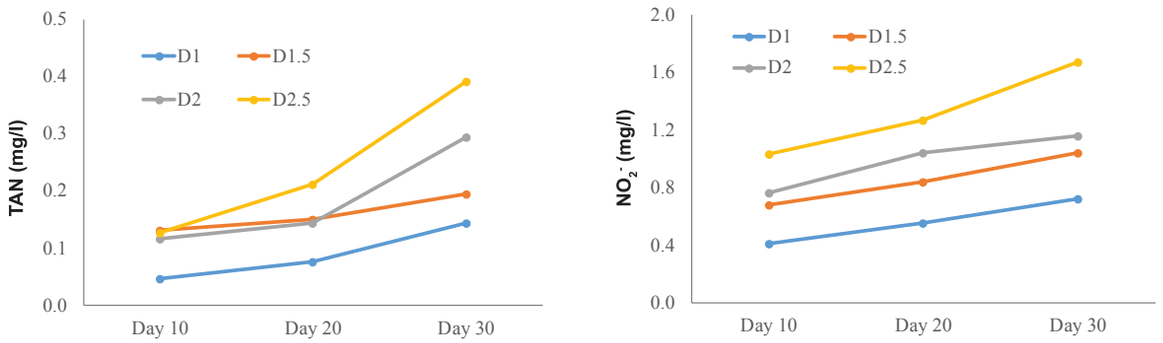

Fig. 1. Variations in the contents of TAN and NO_2^- during 30 days of nursery rearing.

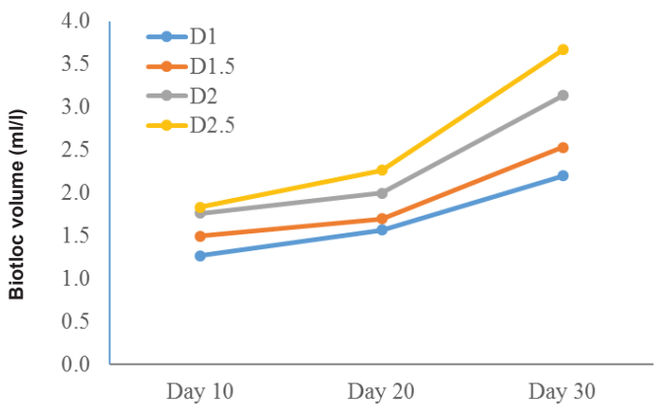

Fig. 2. Variations in biofloc volume during 30 days of nursey rearing.

At the termination of the experiment on day 30, biofloc volume ranged from 2.20 to 3.63 ml/l, with the lowest and highest values observed for the D1 and D2.5 treatments, respectively (Fig. 2). There was a statistically significant difference ($p<0.05$) in average biofloc volume among the four density treatments (see Table 1). Furthermore, biofloc volume increased with shrimp density and tended to rise with rearing time. This could be related to the addition of carbohydrates based on the amount of pellet feed fed to shrimp [4]. In this scenario, more commercial feed and molasses (carbonhydrate) were delivered at greater prawn densities, and with no water exchange during the rearing phase, resulting in the accumulation of shrimp excrement and inorganic suspended particles in the rearing tanks. This, in turn, stimulated the proliferation of heterotrophic bacteria, resulting in an increase in the overall bacterial population, which aggregated to form massive bioflocs, thereby increasing biofloc volume in the culture medium [4]. Previous researchers [5-9] have shown that bioflocs, comprising a variety of microbial organisms such as bacteria, algae, zooplankton, and protozoa, play a crucial role in maintaining good water quality and providing an additional food source for shrimp.

Similarly, total heterotrophic bacteria and *Vibrio* spp. counts increased with prawn density, reaching 1.98-4.41x10^3 CFU/ml and 0.36-0.72x10^2 CFU/ml, respectively, with the D2.5 group having significantly higher values than other treatments ($p<0.05$). In this study, the presence of low densities of total bacteria and *Vibrio* in the rearing tank was not detrimental to prawn performance [6, 7]. Previous research revealed that shrimp cultured in biofloc systems significantly reduced Vibrio density and boosted shrimp disease resistance [6, 9].

3.2. Biomass of red seaweed G. tenuistipitata

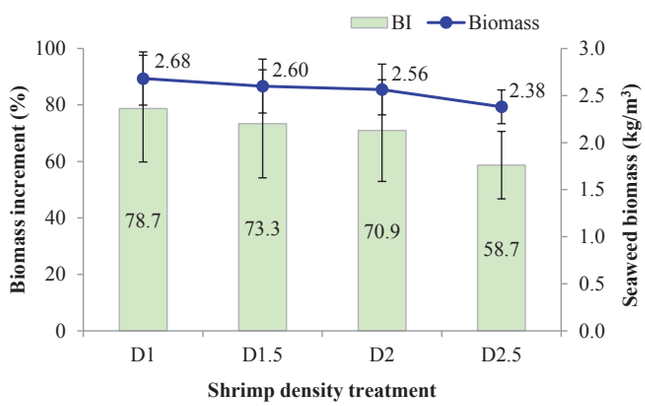

Fig. 3. Growth rate of red seaweed *G. tenuistipitata* after 30 days of experiment. *: values are expressed as mean ±SD (n=3).

Figure 3 shows that the average red seaweed biomass of *G. tenuistipitata* at the end of the experiment in all treatments ranged from 2.38 to 2.68 kg/m^3, which was

higher than the initial quantity (1.5 kg/m^3) and equivalent to a biomass increment (BI) of 58.7-78.7%. Although the D2.5 treatment had lower values than other treatments, no significant difference was observed among treatments ($p>0.05$). This indicates that red seaweed grows well in co-culture with prawns in the same tanks, which helps maintain good water quality in the culture medium. Analogous findings were found in recent investigations [13, 14, 24].

3.3. Prawn performance

Prawn performance after 30 days of rearing is presented in Table 2. Results showed that the final length of the prawn ranged from 3.39 to 3.64 cm, and the final weight ranged from 0.25 to 0.32 g, corresponding to a specific growth rate in length (SGR_L) of 3.66-3.89%/day and in weight (SGR_W) of 9.93-10.89%/day. There was no statistical difference ($p>0.05$) between the D1 and D1.5 groups, both of which had significantly higher values than the D2 and D2.5 groups ($p<0.05$). This illustrates that high stocking densities (2,000 and 2,500 PL/m^3) resulted in significantly poorer growth rates than low stocking densities (1,000 and 1,500 PL/m^3).

The mean survival of prawns in D1 and D1.5 treatments was highest (91.67%), and it tended to decline at higher stocking density, with the D2.5 treatment having the lowest value (73.00%) and statistical difference ($p<0.05$) from other density treatments.

The average biomass and production of prawns ranged from 299.3 to 446.8 g/m^3 and 917 to 1,825 ind/m^3, respectively, and showed a tendency to increase with stocking density. Statistical analysis indicated that prawn biomass in the D1.5, D2, and D2.5 treatments was comparable ($p>0.05$), and all were significantly higher than the D1 treatment, while production (number of harvested prawn) was significantly different among rearing density treatments ($p<0.05$).

Feed conversion ratio (FCR) was 0.68-0.93, equivalent to feed efficiency (FE) of 108.6-146.0%, with higher stocking densities resulting in a higher FCR and lower FE. There was no statistical difference ($p>0.05$) between D1 and D1.5 groups or between D2 and D2.5 groups.

Stocking density is known to be one of the key technical parameters influencing growth, survival, and production of prawn and shrimp in the nursery phase, and the proper stocking density depends on the species, rearing method, duration, and stage of culture. When applying too high stocking density, living space is limited, cannibalism increases, and more feed is needed, which leads to poor water quality, crowding stress, delayed growth, high mortality, and pathogen outbreaks. low stocking density

Table 2. Prawn postlarvae performance after 30 nursery rearing.

Treatment	D1	D1.5	D2	D2.5
Initial length (cm)	1.13±0.12	1.13±0.12	1.13±0.12	1.13±0.12
Final length (cm)	3.64±0.07^b	3.63±0.08^b	3.46±0.09^a	3.39±0.11^a
SGR_L (%/day)	3.89±0.06^b	3.88±0.07^b	3.72±0.09^a	3.66±0.10^a
Initial weight (g)	0.012±0.001	0.012±0.001	0.012±0.001	0.012±0.001
Final weight (g)	0.32±0.02^b	0.31±0.01^b	0.27±0.02^a	0.25±0.02^a
SGR_W (%/day)	10.89±0.20^b	10.73±0.12^b	10.24±0.22^a	9.93±0.28^a
Survival (%)	91.67±0.84^c	91.67±4.45^c	81.67±1.91^b	73.00±2.34^a
Biomass (g/m^3)	299.3±16.1^a	427.2±17.5^b	437.9±25.8^b	446.8±25.5^b
Production (ind/m^3)	917±9^a	1.375±67^b	1.633±38^c	1.825±59^d
FCR	0.68±0.04^a	0.69±0.02^a	0.85±0.05^b	0.93±0.07^b

*: values are expressed as mean ±SD (n=3). Mean values in with different superscripts within the same row are significantly different at $p<0.05$.

gives high growth and survival but higher expenses and a lower production output per culture unit [16, 17, 25].

According to previous investigations, the co-culture of red seaweed with shrimp or prawn not only improved water quality but also functioned as natural food and a shelter, thereby increasing shrimp and prawn survival and production [16, 17, 25]. Additionally, biofloc formation in the rearing tanks had high nutrition content, which is a source of supplementation for the prawn diet [4, 7, 9].

Overall, the results showed that prawn performance in terms of growth rate, survival, and feed efficiency at a stocking density of 1,500 PL/m^3 was comparable to that of prawns reared at a density of 1,000 PL/m^3 and significantly higher than that of prawns reared at stocking densities of 2,000 and 2,500 PL/m^3.

4. Conclusions

The freshwater prawn *M. rosenbergii* cocultured with red seaweed *G. tenuistipitata* in a biofloc system maintained good water quality under no water exchange conditions, as shown by TAN and NO_2^- levels as well as total bacteria and *Vibrio* spp. counts that were within the suitable range for prawn performance.

Freshwater prawn postlarvae co-cultured with red seaweed in a biofloc system at a stocking density of 1,500 ind/m^3 achieved optimal growth rates and feed efficiency at the nursery phase.

CRediT author statement

Tien Hai Ly: Methodology, Formal analysis, Original draft preparation, Visualisation; Le Hoang Vu: Conceptualisation, Data curation, Investigation; Ly Van Khanh: Investigation, Visualisation, Formal analysis; Nguyen Thi Ngoc Anh: Supervision, Validation, Writing - Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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