

Polyculture of red seaweed (*Gracilaria tenuistipitata*) with different stocking densities of whiteleg shrimp (*Litopenaeus vannamei*): Effects on water quality and shrimp performance

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Abstract:

This study aimed to determine the impact of co-culturing red seaweed (*Gracilaria tenuistipitata*) with varying densities of whiteleg shrimp (*Litopenaeus vannamei*) on water quality and shrimp performance. The experiment was conducted in indoor tank systems in triplicate. Five shrimp densities (100, 200, 300, 400, and 500 shrimp/m³) were integrated with red seaweed (2 kg/m³) over 60 days. The results demonstrated that the polyculture of red seaweed with shrimp maintained appropriate levels of total ammonia nitrogen (TAN) and NO₂⁻ in rearing tanks at high shrimp densities ranging from 100 to 300 shrimp/m³. Shrimp growth rates tended to decline as density increased, but there were no statistical differences (p>0.05) between treatments at stocking densities ranging from 100 to 300 shrimp/m³. Shrimp survival declined dramatically as stocking densities increased from 400 shrimp/m³ upwards. The lowest and highest shrimp yields were achieved at densities of 100 and 300 shrimp/m³, respectively. Furthermore, the sensory quality of cooked shrimp meat was highly rated by the panellists. These findings demonstrate that the polyculture of red seaweed with whiteleg shrimp can be applied at stocking densities of up to 300 shrimp/m³ while maintaining appropriate water quality parameters and improving production efficiency in the culture unit.

Keywords: *Gracilaria tenuistipitata*, *Litopenaeus vannamei*, polyculture, survival, yield.

Classification numbers: 3.1, 5.1

1. Introduction

Whiteleg shrimp (*Litopenaeus vannamei*) have recently been widely farmed in Asia, including Vietnam, due to their low protein requirements and rapid growth at high stocking densities in both indoor and outdoor conditions [1]. However, the intensification of shrimp farming has resulted in environmental degradation and widespread disease, causing significant losses, particularly due to antibiotic use, which lowers the quality of commercial shrimp products for export. Consequently, research into improving productivity and product quality, as well as developing sustainable, environmentally friendly shrimp farming practices, is a top priority [2].

Seaweeds provide marine organisms with habitat, shelter, and natural food, as well as the ability to absorb excess nutrients (N and P) in aquaculture wastewater and absorb CO₂. These abilities contribute to reducing water contamination and mitigating the negative effects of climate change [3, 4]. Polyculture of seaweed and aquatic species has been believed to be an environmentally friendly approach to green aquaculture technology [3].

The application of polyculture in shrimp farming provides numerous advantages, including reduced pollution of surrounding ecosystems, increased shrimp growth and health, and efficient use of the various natural resources in the culture model [3, 5]. The red seaweed, *Gracilaria corticata*, is commonly found in marine environments and is a main source of agar, food for humans, and animal feed [6]. It also possesses high biofiltration capabilities that can improve the production, water quality, and economic efficiency of shrimp farming through polyculture systems [7, 8]. Using *Gracilaria* in an integrated system with white-leg shrimp *L. vannamei* reduces dissolved inorganic nitrogen and phosphorus levels, as well as shrimp dietary protein content [7, 9].

Stocking density is a key factor affecting the survival, growth, and production efficiency of farmed shrimp throughout the grow-out phase, especially in indoor tanks with limited space. Additionally, the appropriate breeding density can provide the highest shrimp performance and increase production efficiency per culture unit [10, 11]. In a polyculture of *L. vannamei* and red seaweed *G. corticata* in a zero water exchange system, shrimp densities of 25-

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50 shrimp/m² or *Penaeus monodon* shrimp were integrated with *G. tenuistipitata* at a density of 100 shrimp/m³ [12]. Similarly, it was discovered that whiteleg shrimp co-cultured with sea grape (*Caulerpa lentillifera*) at up to 400 shrimp/m³ increased shrimp yield while maintaining an appropriate culture medium for 8 weeks [13, 14]. Until now, no studies have been conducted on the polyculture of red seaweed *G. tenuistipitata* and whiteleg shrimp at high stocking density for the grow-out phase in indoor tanks. The current study aims to evaluate the effects of combining red seaweed with various stocking densities of whiteleg shrimp on water quality and shrimp performance in order to determine the optimal stocking density for polyculture applications. The study's results could serve as a solid foundation for developing a more environmentally friendly shrimp culture system on a large scale.

2. Materials and methods

2.1. Experimental animal and conditions

Red seaweed, *G. tenuistipitata*, was collected from the improved extensive pond in Bac Lieu province, and separated from other seaweeds before being acclimated to the desired salinity of 10 ppt. Whiteleg postlarvae from one single batch were purchased from a commercial hatchery in Bac Lieu province and reared for four weeks to obtain an average weight of about 0.43 g for the experiment. Saline water (80 ppt) originated from Bac Lieu saltworks, was treated with chlorine at 30 g/m³ with strong aeration for 3 days, then diluted with freshwater to achieve the experimental salinity of 10 ppt, before being pumped into the culture tanks.

2.2. Experimental design and management

The investigation was performed at the College of Aquaculture and Fisheries, Can Tho University. The experiment had five treatments in randomly designed triplicate tanks, which comprised integrating five different shrimp densities (100, 200, 300, 400, and 500 shrimp/m³) with red seaweed density of 2 kg/m³ [15] and were denoted as T100, T200, T300, T400, and T500, respectively. The culture system was placed under a transparent roof with a natural photoperiod. Juvenile shrimp with a mean weight of 0.43±0.04 g were stocked in 150 l tanks filled with 130 l seawater at a salinity level of 10 ppt and provided continuous aeration.

Shrimps were initially fed at 7% biomass per day with Vannamei shrimp commercial feed (Grobtest, protein ≥40%, lipid ≥6%). The quantity of feed provided was determined by the stocking density of each treatment, and feeding was adjusted based on the presence or absence of unseated feed in the culture tank to ensure satiation. Feeding was done four times a day at 7:00, 11:00, 16:00, and 20:00 h. The feeding was performed in accordance with the shrimp feed

manufacturer's recommendation for adjusting the crumble size (pellet size) and feeding rate throughout the culture period. For tank management, the rearing tanks were siphoned every two days and refilled with fresh seawater to the original volume. Water was exchanged every 15 days (about 50% of tank volume), and the experiment lasted 60 days.

2.3. Data collection

Water temperature and pH were recorded daily at 7:00 and 14:00 h using a multi-channel meter (Mettler Toledo, USA). The alkalinity level was measured using a test kit (Sera, Germany), and the concentrations of total ammonia nitrogen (TAN) and NO₂⁻ were measured at 15-day intervals following the standard method of APHA (2000). The variation in light intensity during daytime was measured close to the culture tank's water surface every three days with a light meter (Extech EA31, Taiwan, China) at 7:00, 11:00, 14:00, and 17:00 h.

Red seaweed biomass was determined every 15 days during the culture period. Seaweed in each tank was collected, the excess water in the biomass was removed, and the entire biomass was weighed using an electronic balance and returned to the original tank.

Shrimp's initial weight and total length were evaluated by randomly choosing 40 animals from the conditioning tank and measuring them with a 0.01-g precision balance and callipers, respectively. Shrimp weight was determined every 15 days by randomly taking 10 shrimp from each culture tank and weighing them in groups, then returning them to their original tanks. At the end of the experiment, the total shrimp biomass in each culture tank was weighed and counted to determine the final weight for calculating the growth rate and the survival rate, respectively. The growth performance of shrimps, such as weight gain (WG), daily weight gain (DWG) specific growth rate weight (SGR_w), and in length (SGR_L), survival, and feed conversion ratio (FCR) were calculated as follows:

$$\text{WG (g)} = \text{Final weight} - \text{Initial weight}$$

$$\text{DWG (g/day)} = (\text{Final weight} - \text{Initial weight})/\text{day of culture}$$

$$\text{SGR}_w (\%/day) = [(\ln \text{ final weight}) - (\ln \text{ initial weight})]/\text{day of culture} \times 100$$

$$\text{SGR}_L (\%/day) = [(\ln \text{ final length}) - (\ln \text{ initial length})]/\text{day of culture} \times 100$$

$$\text{Survival (\%)} = \text{Harvested number of shrimp}/\text{Initial number of shrimp} \times 100$$

$$\text{FCR} = \text{Consumed feed (g)}/(\text{Final weight} - \text{Initial weight}) (\text{g})$$

$$\text{Yield (kg/m}^3\text{)} = \text{Total biomass (kg)}/\text{Culture volume (m}^3\text{)}$$

Sensory assessments of experimental shrimp were carried out at the end of the culture. Nine shrimp were selected at random from each culture tank and boiled for five minutes. Five cooked shrimp from different treatments were photographed together to compare colour among the density treatments. The cooked shrimp samples were then deshelled for sensory evaluation. The sensory properties of cooked shrimp were evaluated using the old method [16]. In brief, a panel of nine trained panellists was shown fresh and cooked shrimp samples. Panellists were asked to rate samples on a 9-point hedonic scale based on colour, taste, flavour, and toughness. Scores of six or higher were considered acceptable, and vice versa.

2.4. Statistical analysis

Data in percentage were arcsine transformed, and variance homogeneity was tested using Levene’s test. A one-way ANOVA was applied to determine the overall impact of the treatment. The Tukey test was employed to detect 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 and seaweed biomass

The water quality in the rearing tanks during shrimp culture is presented in Table 1. Water temperature, pH, and alkalinity in the culture tanks varied in the ranges of 26.3-31.7°C, 7.22-8.00, and 116-125 mg CaCO₃/l, respectively. Light intensity during the day fluctuated from 2,647 to 15,832 lux, with the highest value found at 14:00 h. These values were similar among treatments and were in suitable ranges for whiteleg shrimp culture [17]. Red seaweed *G. corticata* species have a high tolerance to a wide range of environments, indicating an acceptable limit for the development of this species [6].

Table 1. Temperature, pH, alkalinity, and light intensity.

Treatment	Temperature (°C)		pH		Alkalinity (mgCaCO ₃ /l)
	7:00 h	14:00 h	7:00 h	14:00 h	
T100	26.3±0.9	31.4±1.5	7.56±0.27	8.00±0.30	122±11
T200	26.3±0.8	31.1±1.3	7.38±0.23	7.81±0.26	116±14
T300	26.3±0.9	31.5±1.4	7.40±0.29	7.79±0.29	119±17
T400	26.3±0.9	31.7±1.4	7.32±0.26	7.72±0.29	117±14
T500	26.3±0.8	31.2±1.2	7.22±0.23	7.64±0.25	125±11
Light intensity (lux)	7 h	11 h	14 h	17 h	
	2,647±1,445	14,136±2,941	15,832±3,437	3,103±1,830	

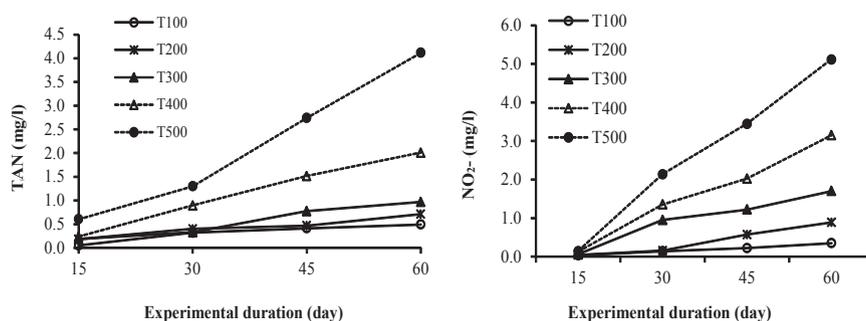


Fig. 1. Variations in the concentrations of total ammonia nitrogen and NO₂⁻ during shrimp culture.

Figure 1 indicated that the concentrations of TAN and NO₂⁻ increased with shrimp density and tended to increase over time. Particularly, two of these parameters in the T400 and T500 treatments showed sharp increases at day 60 (2.01-4.12 and 3.15-5.12 mg/l, respectively) compared to day 45.

The toxicity of TAN and NO₂⁻ in shrimp culture depends on water salinity, with NO₂⁻ being less harmful at high salinity than low salinity. Moreover, the toxicity effect of TAN is temperature- and pH-dependent, with greater pH and temperature resulting in higher quantities of non-ionised ammonia (NH₃), which is more hazardous to penaeid shrimp [18]. The ideal TAN content for shrimp culture is less than 2 mg/l. The safe NO₂⁻ content for shrimp is less than 4.5 mg/l [19]. As a result, TAN and NO₂⁻ levels at densities ranging from 100 to 300 shrimp/m³ were adequate for shrimp performance; however, these two values at densities of 400 and 500 shrimp/m³ exceeded the threshold, potentially harming the experimental shrimp [20].

Previous research has discovered that seaweeds have widely been used in integrated mariculture systems to absorb inorganic nutrient (N and P) wastes, which have acted as a nutrient source for seaweed growth and led to lower nutrient load and improved water quality [7, 9, 12]. Similarly, the co-culture of *Gracilaria* with *L. vannamei* reduced nitrogen and phosphorus levels in water while improving water quality in shrimp culture [9, 12]. In this study, TAN and NO₂⁻ concentrations increased dramatically at higher shrimp densities (400 and 500 shrimp/m³) from day 45 onward. This phenomenon could be influenced by red seaweed growth, as explained below.

From days 15 to 30, the biomass of red seaweed (*G. tenuistipitata*) in all treatments increased relative to its original weight. At day 45, the T100, T200, and T300 treatments showed a slight reduction in weight, which was further reduced at day 60. Notably, T400 and T500 treatments exhibited a significant decline in biomass at day 60 (Fig. 2). Red seaweed *Gracilaria* species are well-adapted to extreme environmental conditions (temperature, salinity, and light) [6]. However, high concentrations of suspended solids in their habitat have negative impacts on *Gracilaria* development.

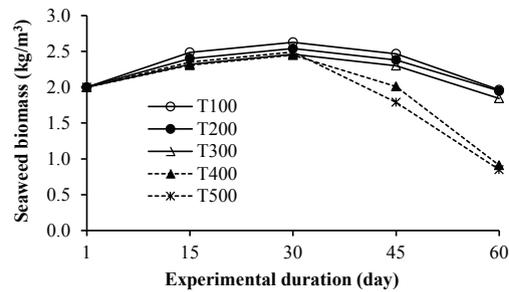


Fig. 2. Change of red seaweed *G. tenuistipitata* biomass cocultured with different shrimp densities over 60 days.

Our results indicated that shrimp densities had a significant impact on red seaweed growth. As shown in Fig. 1, the amounts of TAN and NO_2^- increased during the shrimp culture period, indicating increasing nutrient levels for seaweed development. However, it produces more suspended solids (excreted products and feces from shrimp) in the water column over time that may attach to the seaweed thallus, particularly at high shrimp densities (400 and 500 shrimp/m³). This might suppress red seaweed photosynthesis and nutrient absorption efficiency, which would make them lose their biofilter function. As a result, a part of the red seaweed biomass collapsed and decomposed, releasing nutrients into the water. These factors could explain the substantial increase in TAN and NO_2^- levels, as well as the marked reduction in seaweed biomass at higher shrimp density on day 60. Additionally, when shrimp and red seaweed (*G. tenuistipitata*) are co-cultured in the same culture unit, the red seaweed serves as extra food for the shrimp, potentially contributing to a reduction in seaweed biomass during the latter culture period [13].

3.2. Shrimp performance

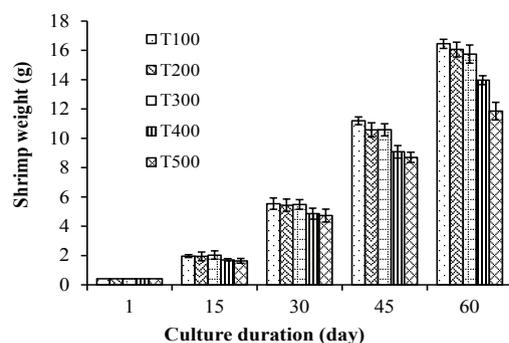


Fig. 3. Variations in shrimp weight co-cultured with red seaweed over 60 days.

Figure 3 showed that shrimp weight was affected by stocking density from day 15 onwards, with higher shrimp density resulting in lower shrimp growth. The performance of whiteleg shrimp co-cultured with red seaweed *G. tenuistipitata* over 60 days is presented in Table 2.

Table 2. Shrimp performance after 60 days of co-culture with red seaweed.

Treatment	T100	T200	T300	T400	T500
Initial length (cm)	4.07±0.08	4.07±0.08	4.07±0.08	4.07±0.08	4.07±0.08
Final length (cm)	14.30±0.35 ^d	13.09±0.21 ^c	12.96±0.19 ^c	11.64±0.45 ^b	9.68±0.72 ^a
SGR _L (%/day)	2.09±0.04 ^a	1.94±0.03 ^c	1.93±0.03 ^{bc}	1.74±0.07 ^b	1.43±0.13 ^a
Initial weight (g)	0.43±0.04	0.43±0.04	0.43±0.04	0.43±0.04	0.43±0.04
Final weight (g)	16.45±0.31 ^c	16.06±0.50 ^c	15.74±0.62 ^c	13.96±0.31 ^b	11.86±0.59 ^a
SGR _w (%/day)	6.07±0.03 ^c	6.03±0.06 ^c	6.00±0.08 ^c	5.79±0.04 ^b	5.52±0.09 ^a
WG (g)	16.03±0.28 ^c	15.63±0.51 ^c	15.31±0.62 ^c	13.54±0.31 ^b	11.43±0.60 ^a
DWG (g/day)	0.267±0.005 ^c	0.261±0.008 ^c	0.255±0.010 ^c	0.225±0.005 ^b	0.190±0.010 ^a
Survival (%)	81.25±6.25 ^b	74.41±5.08 ^b	73.17±5.74 ^b	36.36±4.97 ^a	29.90±4.24 ^a
FCR	0.96±0.04 ^a	1.01±0.08 ^a	1.06±0.04 ^{ab}	1.19±0.06 ^b	1.54±0.05 ^c
Yield (kg/m ³)	1.65±0.16 ^a	2.59±0.27 ^b	3.54±0.22 ^c	2.15±0.20 ^{ab}	1.84±0.23 ^{ab}

*: Mean values in the same row with different letters are significantly different ($p < 0.05$). SGR_L and SGR_w: Specific growth rate in length and in weight, respectively; WG: Weight gain, DWG: Daily weight gain; FCR: Feed conversion ratio.

It was 1.43-2.09%/day, of which the lowest and highest values were observed at the T500 and T100 treatments, respectively, and were significantly different from other density treatments ($p > 0.05$). The final weight was 11.86-16.45 g, with a specific growth rate (SGR_w) of 5.52-6.07%/day. The T100, T200, and T300 groups did not differ statistically ($p > 0.05$), but had significantly higher values than the T400 and T500 groups ($p < 0.05$). This finding revealed that high stocking densities (400 and 500 shrimp/m³) resulted in significantly slower growth rates than low stocking densities (100-300 shrimp/m³). A similar trend was observed for weight gain and daily weight gain.

Shrimp survival ranged from 29.90 to 81.25% and decreased with increasing stocking density. The T500 treatment had the lowest value and a statistical difference ($p < 0.05$) from other density groups. There was no significant difference among the T100, T200, and T300 groups. Feed conversion ratio (FCR) was 0.96-1.54, with higher stocking densities resulting in a higher FCR. The T500 group had the highest value and was significantly different from the remaining density treatments. There was no statistical difference in FCR ($p > 0.05$) between T100, T200, and T300 treatments. The average shrimp yield was 1.84-3.54 kg/m³, which increased with stocking density from 100 to 300 shrimp/m³ while declining at higher shrimp densities (400-500 shrimp/m³). The T300 had the highest shrimp production and significantly differed from other stocking density treatments ($p < 0.05$). Furthermore, polynomial regression analysis displayed a significant increase in shrimp yields from 100 to 300 shrimp/m³, followed by a decrease from 400 shrimp/m³ onwards (Fig. 4).

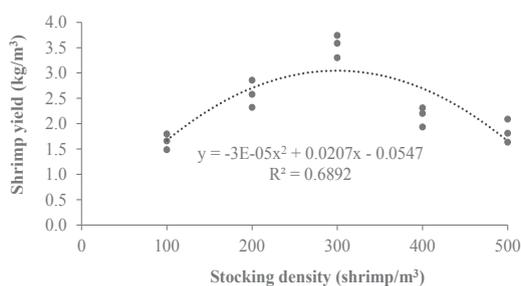


Fig. 4. Polynomial regression between shrimp yield and stocking density in polyculture with red seaweed after 60 days of culture.

Stocking density is a critical technical factor influencing shrimp development, survival, and production; the optimal stocking density varies depending on the species being grown, the rearing method employed, and the culture duration. An excessively high stocking density leads to limited living spaces, increased cannibalism, and more feed input, all of which cause the rearing tanks to exceed their carrying capacity, as indicated by poor water quality, a slow growth rate, high mortality, and disease outbreaks [10, 11, 21]. Whiteleg shrimp co-cultured with sea grape *C. lentiliifera* can be applied at different shrimp densities, with 400 shrimp/m³ obtaining the highest yield, while 500 shrimp/m³ showed a dramatically reduced shrimp yield [13]. The findings of this study revealed that shrimp density had a significant effect on shrimp growth rates, survival, FCR, and production. These results are consistent with the majority of the previous findings. Seaweeds function as bioremediators since they absorb nutrients for their development in the form of biomass. Introducing seaweed in intensive shrimp farms may be of interest as a bioremediator in order to improve water quality, reduce nutrient loads in effluent, and provide a natural food source that improves overall shrimp productivity at high stocking density [13].

3.3. Sensory assessment of cooked whiteleg shrimp

Sensory evaluation is a scientific method of assessing the consumption quality of food under controlled conditions [16]. For cooked shrimp meat, colour, taste, flavour, and toughness are the main criteria in sensory evaluating product quality for human consumption [22].

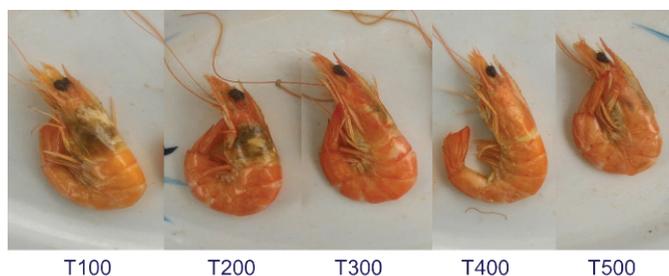


Fig. 5. Colour of the cooked whiteleg shrimp after boiling for 5 mins.

Table 3. Sensory scores of cooked shrimp meat after 60 days of culture.

Parameters	Colour	Taste	Flavour	Toughness
T100	7.59±0.13 ^a	8.44±0.34 ^a	8.43±0.25 ^{bc}	8.43±0.29 ^b
T200	8.30±0.23 ^b	8.55±0.25 ^a	8.48±0.16 ^c	8.48±0.21 ^b
T300	8.29±0.17 ^b	8.39±0.26 ^a	8.52±0.21 ^c	8.43±0.29 ^b
T400	7.71±0.17 ^a	8.28±0.25 ^a	7.76±0.30 ^{ab}	7.28±0.25 ^a
T500	7.67±0.11 ^a	8.22±0.25 ^a	7.62±0.33 ^a	7.14±0.15 ^a

*: Mean values in the same column with different letters are significantly different (p<0.05).

Figure 5 indicated that the colour of whiteleg shrimp after boiling did not distinctly differ across treatments. Furthermore, Table 3 showed that the average sensory score (colour, taste, flavour, and toughness) of cooked shrimp meat in various density treatments ranged from 7.14 to 8.55, indicating that these products were highly acceptable to the panellists [16]. Particularly in terms of colour, flavour, and texture, the T400 and T500 treatments had significantly lower scores than other density treatments (p<0.05) except for the colour criteria.

4. Conclusions

The polyculture of red seaweed (2 kg/m³) and whiteleg shrimp at densities ranging from 100 to 300 shrimp/m³ maintained acceptable levels of TAN and NO₂⁻ during the culture period. However, at higher shrimp densities (400 and 500 shrimp/m³), these parameters exceeded the threshold level by the end of the culture.

Polyculture of red seaweed with whiteleg shrimp can be applied at different shrimp densities, with 300 shrimp/m³ producing the highest output. However, increasing shrimp density beyond 400 shrimp/m³ resulted in reduced growth rate, survival, and shrimp yield. To improve the culture system’s production efficiency, shrimp density (300 shrimp/m³) is considered the optimal stocking density for polyculture with red seaweed. Furthermore, the sensory quality of cooked shrimp meat (colour, taste, flavour, and toughness) in all density treatments was highly acceptable to the panellists, with lower densities receiving higher scores than higher densities that meet the quality for human consumption.

CRedit author statement

Hai Ly Tien: Methodology, Formal analysis, Original draft preparation, Visualisation, Validation, Writing - Reviewing and Editing; Bich Tuyen Cao: Investigation, Visualisation, Formal analysis; Hoang Vu Le: Supervision, Writing - Reviewing and Editing; Huu Yen Nhi Nguyen: Investigation, Visualisation, Formal analysis; Thi Ngoc Anh Nguyen: Conceptualisation, Data curation, Investigation, 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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