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GROWTH PERFORMANCE OF WHITELEG SHRIMP (*Litopenaeus vannamei*) AT DIFFERENT STOCKING DENSITIES IN A POLYCULTURE SYSTEM WITH SEA GRAPE (*Caulerpa* sp.)

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ABSTRACT

This study evaluated the growth performance, survival rate, and water quality improvement in a polyculture system integrating whiteleg shrimp (*Litopenaeus vannamei*) and sea grapes (*Caulerpa* sp.) with different shrimp stocking densities. This study employed a completely randomized design with three treatments, each at three levels of shrimp densities (15, 30, and 45 individuals per tank with a capacity of 0.06 m^3), integrated with sea grape (50 g) for 60 days. During maintenance, shrimp were fed commercial feed, and no water changes were performed. The results showed that the highest shrimp growth and survival were obtained at a density of 15 individuals per tank, with growth rates of 6.54% day^{-1} and survival rates of 98% for the shrimp, resulting in optimal growth in *Caulerpa* sp. In contrast, a higher stocking density (45 individuals per tank) was associated with lower observed growth and survival rate of whiteleg shrimp throughout the culture period. Water quality showed favourable conditions for both, with pH levels (6.5–9.4), dissolved oxygen concentrations (5.0–8.2 mg L^{-1}), and temperatures (26–38°C) within the optimal range. The polyculture system facilitated nutrient recycling, where shrimp waste was utilized by *Caulerpa* sp., effectively reducing nitrate and phosphate concentrations and preventing eutrophication in all treatments with increased stocking density. The conclusion of this study highlighted the potential of the polyculture system to improve ecological balance and productivity in aquaculture. Lower shrimp stocking densities resulted in high growth and survival, while integrating *Caulerpa* sp. contributed to environmental sustainability.

KEYWORDS: ***Caulerpa* sp.; growth; *Litopenaeus vannamei*; polyculture**

ABSTRAK: Performa Pertumbuhan Udang Vaname (*Litopenaeus vannamei*) pada Padat Tebar yang Berbeda dalam Sistem Polikultur dengan Anggur Laut (*Caulerpa *sp.)

*Penelitian ini mengevaluasi performa pertumbuhan, tingkat kelangsungan hidup, dan perbaikan kualitas air dalam sistem polikultur yang mengintegrasikan udang vaname (**Litopenaeus vannamei**) dan anggur laut (**Caulerpa** sp.) dengan kepadatan tebar udang yang berbeda. Penelitian ini menggunakan rancangan acak lengkap dengan tiga perlakuan, yaitu tiga tingkat kepadatan udang (15, 30, dan 45 ekor per wadah berkapasitas 0,06 m^3), masing-masing diintegrasikan dengan anggur laut (50 g) selama 60 hari. Selama pemeliharaan, udang*

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diberi pakan komersial dan tidak dilakukan pergantian air. Hasil penelitian menunjukkan bahwa pertumbuhan dan kelangsungan hidup udang tertinggi diperoleh pada kepadatan 15 ekor per wadah, masing-masing sebesar 6,54% per hari dan 98%, serta menghasilkan pertumbuhan ***Caulerpa*** *sp. yang optimal. Sebaliknya, kepadatan tebar yang lebih tinggi (45 ekor per wadah) menghasilkan pertumbuhan dan tingkat kelangsungan hidup udang vaname yang lebih rendah selama masa pemeliharaan. Kualitas air menunjukkan kondisi yang mendukung untuk keduanya, dengan pH (6,5–9,4), oksigen terlarut (5,0–8,2 mg L^{-1}), dan suhu (26–38°C) berada dalam kisaran optimal. Sistem polikultur ini memfasilitasi daur ulang nutrien, di mana limbah udang dimanfaatkan oleh* ***Caulerpa*** *sp., secara efektif mengurangi konsentrasi nitrat dan fosfat serta mencegah eutrofikasi pada semua perlakuan, bahkan dengan peningkatan kepadatan tebar. Kesimpulan dari penelitian ini menyoroti potensi sistem polikultur dalam meningkatkan keseimbangan ekologis dan produktivitas di bidang akuakultur. Kepadatan tebar udang yang rendah menghasilkan pertumbuhan dan kelangsungan hidup yang tinggi, sementara integrasi dengan* ***Caulerpa*** *sp. berkontribusi terhadap keberlanjutan lingkungan.*

KATA KUNCI: *Caulerpa* ***sp.;*** *Litopenaeus vannamei****; pertumbuhan; polikultur***

INTRODUCTION

One of the commodities that can be cultivated in polyculture with whiteleg shrimp is *Caulerpa* sp. Sea grapes, *Caulerpa* sp. or commonly called *latoh* (in East Nusa Tenggara), is a type of seaweed that is considered a local delicacy in many coastal communities in the region. It is often used as a food ingredient, eaten raw as a salad or as a vegetable by people living near the coast. This food ingredient has a fairly high nutritional content as a source of vegetable protein, minerals, and vitamins (Yustiati *et al*., 2018).

The whiteleg shrimp (*Litopenaeus vannamei*) is one of the potential aquaculture commodities for Indonesia, as it offers export opportunities and thus generates foreign exchange for the country. Indonesia was once the world's largest shrimp producer and exporter, a status it held since the National Shrimp Program was launched in 1982 (Dahuri, 2013). The Ministry of Marine Affairs and Fisheries also designated shrimp and seaweed commodities as key national commodities that must be developed to reach international markets.

In most polycultures of whiteleg shrimp and sea grape, the former serves as the primary commodity, while the latter functions as a side commodity to maintain water quality in the shrimp pond. The main principle of polyculture system is to recycle waste from the cultivation process produced by the main species into a source of energy and nutrients for the complementary farmed species (Omont *et al*., 2022). In an intensive system, whiteleg shrimp can be farmed at high stocking densities, ranging from 100 to 300 individuals m^{-2} (Nababan, 2015). However, the stocking density of whiteleg shrimp in an intensive system can be as high as 400 individuals m^{-3} when polycultured with sea grapes, while maintaining suitable water quality parameters and sustaining improved production efficiency in the culture unit (Anh *et al*., 2022). In addition, polyculture with seaweed has a significant impact on the absolute weight growth, survival, and feed conversion of farmed whiteleg shrimp (Samidjan *et al*., 2020). Ly *et al*. (2021) reported that sea grapes can be used at densities of 0.5–2 kg m^{-3} in polyculture with whiteleg shrimp, with 1 kg m^{-3} resulting in higher production and feed efficiency.

Sea grapes are currently being considered a potential new species for seaweed farming in Indonesia. Apart from being a food ingredient, Caulerpa racemosa has also been widely used in medical applications due to its antioxidant content (Gomez-Zavaglia *et al*., 2019). Each strain of *Caulerpa* sp. has different types of color pigments. A study by Putnarubun & Valentine (2022) reported that the color pigments successfully isolated from *Caulerpa* sp. algae using column chromatography were β-carotene, xanthophyll, carotene, chlorophyll-a, and chlorophyll-b. These difference means that the types of light colors absorbed also vary in the seaweed to achieve optimal photosynthesis. Sea grapes (*Caulerpa* sp.) are also known to contain amino acids and proteins, which can be utilized in the development of superior products (Nofiani *et al*., 2018). Raniello *et al.* (2004) stated that the *Caulerpa* sp. was first discovered in 1926 along the coast of Tunisia in the Mediterranean waters. *Caulerpa* sp. has been widely developed and cultivated in the community, although it is often found in protected places with clear water (Rahmawati *et al*., 2021).

The distribution of *Caulerpa* sp. is quite broad, especially in tropical regions such as in Indonesia, Thailand, Malaysia, Japan, China, the Philippines, and Korea (Nofiani *et al*., 2018). In Indonesia, this species can be found in eastern Indonesia, specifically in the waters of Maluku and its surroundings (Taputobon, 2018), as well as in the East Nusa Tenggara region (Oedjoe *et al*., 2019).

The utilization of sea grapes, *Caulerpa* sp., remains limited and is not yet widely recognized by the people of East Nusa Tenggara for its potential economic value. In this region, sea grapes are harvested by local communities from their natural habitats and predominantly used as fresh food, known as lawar, a traditional dish from East Nusa Tenggara (NTT). Due to their reliance on wild stock, which varies seasonally, it is necessary to undertake efforts to cultivate *Caulerpa* sp. to achieve sustainable production and meet market demands. Several previous studies have developed farming techniques for sea grapes in abandoned ponds to maximize the effects of high sunlight intensities. Based on these studies, we conducted further research to investigate the growth performance and biomass yield of *Caulerpa lentillifera* under polyculture systems with whiteleg shrimp with different stocking densities (Nasmia *et al*., 2022).

While several previous studies have explored the polyculture of *L. vannamei* with various aquatic organisms, such as milkfish (Putra *et al*., 2021), or even macroalgae such as *Gracilaria* (Azim & Little, 2006), there is limited research focusing specifically on the integration of *Caulerpa* sp. as a co-cultured species in whiteleg shrimp production systems. Most existing studies that include seaweed focus on improving water quality (Troell *et al*., 2003) or enhancing nutrient absorption efficiency (Chopin *et al*., 2001), rather than direct interactions with shrimp stocking density and productivity. In contrast, our study uniquely evaluates how different stocking densities of whiteleg shrimp affect the growth performance of both shrimp and *Caulerpa* sp., while simultaneously assessing the mutual benefits of this polyculture system. Additionally, the integration of EM4 as an immunostimulant and organic decomposer provides a novel approach that combines microbial bioremediation with integrated multitrophic aquaculture (IMTA) principles. This holistic evaluation of shrimp-seaweed-microbe interaction under varied stocking densities presents an innovative framework that has not been thoroughly examined in prior literature, contributing new insights into sustainable and efficient aquaculture practices.

This study aimed to examine the effect of polyculture systems on whiteleg shrimp and *Caulerpa* sp. with different whiteleg shrimp stocking densities on growth and survival, as well as improving the quality of cultured water. The results of this study are expected to enhance the sustainability and promote environmentally friendly shrimp farming practices. Additionally, the study could lead to innovations and the creation of new business fields for coastal communities in East Nusa Tenggara.

MATERIALS AND METHODS

Experimental Animals and Research Design

The experiment was conducted for three months at the Teaching Factory of Aquaculture, Marine and Fisheries Polytechnic of Kupang, located in East Nusa Tenggara, Indonesia. The organisms used in this research were the whiteleg shrimp postlarvae (PL12) and the macroalgae *Caulerpa* sp. Shrimps were purchased from a local supplier (CV. Raja Benur Situbondo). The experiment utilized a completely randomized design consisting of three treatments with three replications. The treatments consisted of three levels of shrimp densities (15, 30, and 45 individuals per tank) integrated with sea grape (50 g) each tank. All treatments were conducted in 0.06 m^3 fibre tanks. Sea grapes was acclimated for 3 days before being placed in experimental tanks. Shrimps were cultured for 60 days (Figure 1), and daily feeding was performed at a rate 5% of body weight using commercial feed. During the maintenance period, no water exchange was carried out.

Tools and Materials

The tools and materials used in this research included a water pump for water intake, and an aeration set to supply oxygen for the cultured organisms. Dissolved oxygen levels were measured using a DO meter (Lutron DO-5510, Taiwan), temperature was monitored with a thermometer, and water pH was recorded using a digital pH meter (Milwaukee MW102, USA). A refractometer (ATAGO, Japan) was used to measure water salinity. A digital scale was employed to weigh the shrimp, while a manual scale was used for measure the biomass of *Caulerpa* sp. A ruler was used to measure the length of *Caulerpa* branch-like structure (ramuli), and a hand counter was used to count the number of ramuli. A scoop net assisted in collecting test organisms, and a drop pipette was used to collect water samples for salinity testing. The organisms were cultured in 60 L fibre tanks, and polyethylene (PE) netting was used as the growing medium for *Caulerpa* sp. Light intensity was measured using a digital lux meter (AMTAST LX1330B, China). Feed was provided to *L. vannamei* as the test animal, while *Caulerpa* sp. was used as the cultured object. Distilled water was used for calibrating the refractometer prism. Commercial probiotic was applied at a concentration of 1–2 L per 10 m^3 of water on a weekly basis, functioning as an immunostimulant for shrimp, an organic fertilizer, and a bio-decomposer to enhance sea grapes growth.

Chlorophyll Analysis

The chlorophyll-a and chlorophyll-b content in *Caulerpa* sp. were analysed using a spectrophotometric method adapted from Valentine *et al.* (2021b), after 60 days cultivation. Fresh samples of sea grapes (5 g) were first homogenized and macerated using 99% acetone as the solvent until the pigments were completely extracted from the tissue. The extract was filtered through Whatman No. 42 filter paper. The remaining tissue was rinsed with acetone until it became colourless to ensure complete pigment recovery. The chlorophyll extract was then transferred into a quartz cuvette (4 mL), and the absorbance was measured at wavelengths of 663 nm and 645 nm using a UV-visible spectrophotometer.

Parameters Observed

The specific growth rate (SGR) was determined using the equation (1) suggested by Guo *et al*. (2014):

$$SGR = \frac{(LnWt)-(LnW0)}{t} \times 100 \quad ....(1)$$

Description:
SGR : Specific growth rate (% day^{-1})
Wt : Wet weight of macroalgae or whiteleg shrimp at the end of the study (g)
W0 : Wet weight of macroalgae or whiteleg

shrimp at the beginning of the study (g)
t : Rearing period (days)

The absolute weight growth was determined using the equation (2) suggested by Effendi (1979):

$$H = Wt - Wo \quad ......(2)$$

Description:
H : absolute weight growth (g)
Wt : Wet weight of macroalgae or whiteleg shrimp at the end of the study (g)
Wo : Wet weight of macroalgae or whiteleg shrimp at the beginning of the study (g)

The survival rate (SR) was determined using the equation (3) suggested by Effendi (1997):

$$SR\% = \frac{Nt}{No} \times 100 \quad ......(3)$$

Description:
SR : Survival rate (%)
No : Number of test animals at the beginning of the study (ind)
Nt : Number of test animals at the end of the study (ind)

Sea Grape Carrageenan

The carrageenan content in sea grapes was determined using the analytical method described by Glicksman (1979), with the percentage calculated based on the formula (4):

$$Kr = \frac{Wc}{Wm} \times 100 \quad ......(4)$$

Description:
KR : Carrageenan content (%)
Wc : Weight of carrageenan extract (g)
Wm : Dry sea grape weight (g)

Water Quality

Water quality parameters observed during the study included temperature, salinity, pH, dissolved oxygen (DO), phosphate, and nitrate. Temperature, salinity, pH and DO were measured daily using thermometer, refractometer, pH meter, and DO meter, respectively. Phosphate and nitrate concentrations were analyzed using commercial colorimetric test kits, following the manufacturer's protocols. The measurements were conducted daily (morning and evening) until the end of the study.

Ethical Clearance Statement

All experimental procedures and animal maintenance were conducted in accordance with the guidelines for whiteleg shrimp production in ponds using intensive technology based on SNI 01-7246-2006 (Badan Standardisasi Nasional, 2006).

Data Analysis

Growth, survival, water quality, chlorophyll and carrageenan data were presented descriptively in the form of tables and figures to illustrate observable trends and differences among treatments. The data were interpreted based on visual comparisons, and all data processing was carried out using Microsoft Excel.

RESULTS AND DISCUSSION

Effects of Integrating Different Densities of Whiteleg Shrimp with Sea Grape on Growth Performance of Whiteleg Shrimp

The results showed differences in the productivity of whiteleg shrimp and the growth of *Caulerpa* in a polyculture system. Based on growth parameters, Treatment A showed the highest absolute weight growth and specific growth rate, with values of 49.6 g and 6.54% per day, respectively. Treatment B followed with an absolute weight growth of 38.6 g and a specific growth rate of 5.98% per day. Meanwhile, Treatment C recorded the lowest values, with an absolute weight growth of 29.1 g and a specific growth rate of 5.75% per day (Figure 1a and b). These trends suggested that Treatment A appeared to support better shrimp growth, while treatment C showed comparatively lower growth performance.

Figure 1c presented the pattern of weight growth of whiteleg shrimp over 60 days of culture (DOC) under three different treatments in a polyculture system with *Caulerpa*. Throughout the culture period, shrimp in treatment A consistently gained more weight, reaching a final weight around 55 g by the end of the trial. Shrimp in treatment B reached about 45 g, while those in treatment C ended with approximately 35 g. Among the treatments, shrimp stocking density with 15 individuals per tank (Treatment A) showed the most favorable growth trend, indicating that this density supported better growth conditions in the polyculture system with *Caulerpa sp.*

Low stocking densities (Treatment A) yielded superior growth rates (6.54% per day), consistent with studies by Xu *et al.* (2020) and Rahman *et al.* (2019), which showed that higher stocking densities increase stress factor such as like hypoxia and ammonia toxicity, which in turn reduce growth and survival. The integration of *Caulerpa* sp. with shrimp demonstrated multiple benefits, including enhanced water quality through nutrient absorption, which supports shrimp health and contributes to better growth performance. Light intensity also influenced shrimp growth, with moderate levels supporting growth by enhancing biofilm formation and indirectly improving feed availability (Habaki *et al.*, 2016).

Growth Performance of *Caulerpa* sp.

The absolute weight growth and specific growth rate of *Caulerpa* sp. showed clear differences across the three treatments.

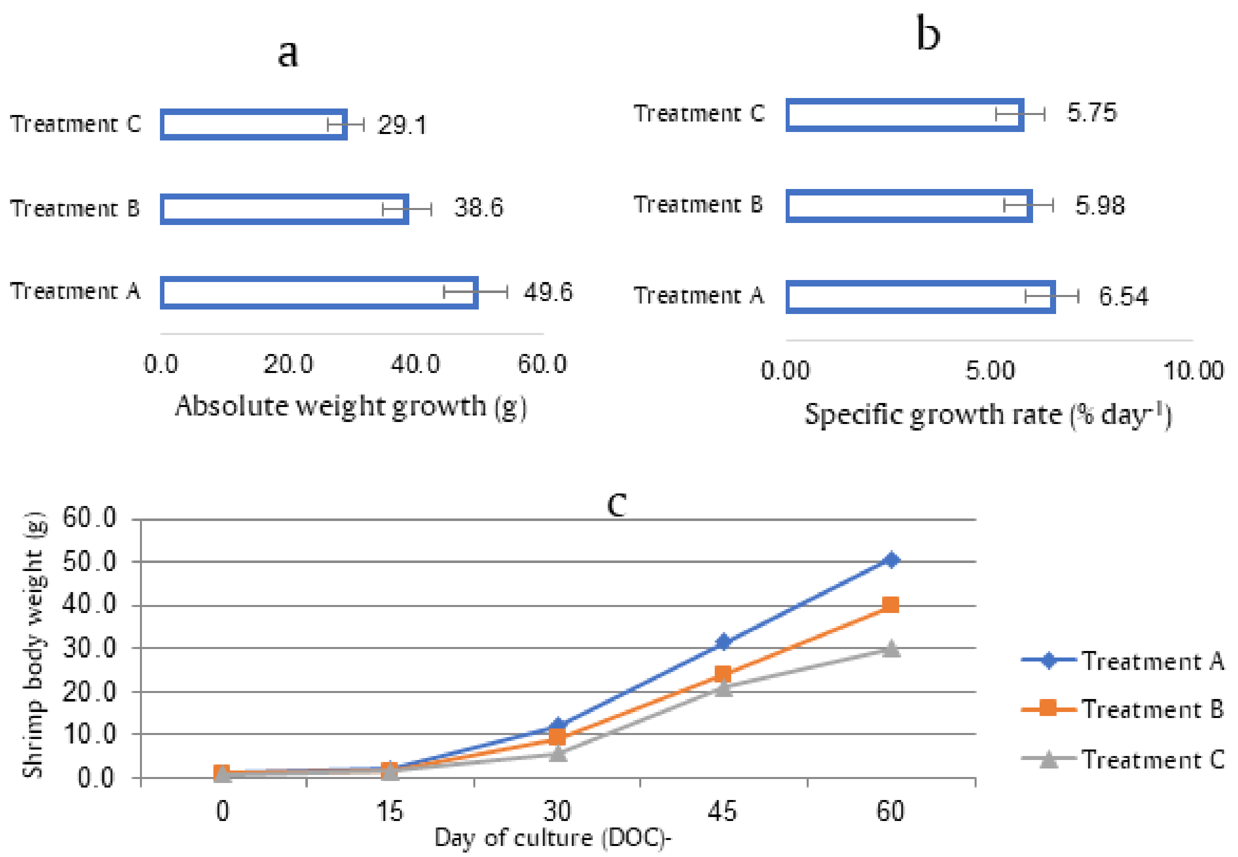

Figure 1. The growth of whiteleg shrimp reared in polyculture system with Caulerpa sp. based on absolute weight growth (a), specific growth rate (b), and shrimp body weight (c). Treatment A: 15 individuals per tank; B: 30 individuals per tank; and C: 45 individuals per tank

Treatment A exhibited the highest absolute weight growth, reaching 724 g in biomass and a specific growth rate of 4.54%, suggesting greater effectiveness in promoting biomass accumulation. Treatment B displayed moderate growth, with an absolute weight growth of 614 g, while treatment C resulted in the lowest growth, averaging 479 g. These trends suggested that treatment A supported more favorable conditions for the growth of *Caulerpa* sp., while treatment C showed more limited performance in biomass development (Figure 2a and 2b).

Figure 2c showed the growth progression of *Caulerpa* sp. over a 60-day culture period, with data recorded every 15 days for each treatment. Treatment A demonstrated the most substantial increase in biomass, reaching a final biomass of approximately 900 g by day 60. Treatment B reached around 750 g, and treatment C ended at approximately 600 g. The data indicate that *Caulerpa* in treatment A experienced more rapid and sustained growth, especially after day 30, when differences in biomass accumulation among the treatments became more noticeable.

In addition, Figure 2d illustrated the changes in thallus length of *Caulerpa* sp. throughout the culture period. At the beginning of the experiment, initial lengths varied slightly among treatments, with treatment A starting at approximately 8 cm, treatment B at 7 cm, and treatment C at about 6 cm. During the first 30 days, all treatments showed elongation of thalli, with treatment C displaying the fastest initial growth. However, from day 45 onwards, the growth rate of thallus length in all treatments appeared to stabilize, and by day 60, the lengths across treatments converged to approximately 12 cm. These observations suggested that while early-stage elongation varied, final thallus lengths tended to reach similar values under all treatments by the end of the culture period.

The growth of sea grapes (*Caulerpa* sp.) appeared to vary under different whiteleg shrimp stocking densities, as reflected in observable differences in parameters such as absolute weight growth, specific growth rate, biomass, and ramuli length. The highest values for these indicators were observed in treatment A, which corresponded to the lowest whiteleg shrimp density. This suggested that reduced competition for nutrients and lower disturbance from shrimp activity favored macroalgal development. Conversely, higher shrimp densities likely increased nutrient uptake competition and mechanical disturbance, thereby suppressing sea grape growth. Similar findings were reported by Troell *et al.* (2003), who demonstrated that seaweed growth in integrated aquaculture systems is highly sensitive to stocking density and nutrient availability. Moreover, the optimal ranges of temperature (26–38 °C), salinity (26–38 ppt), and nutrient levels observed in this study provided favorable environmental conditions for *Caulerpa* sp., supporting its physiological performance and photosynthetic efficiency, as also reported by Valentine *et al.* (2021a, 2021b).

Survival of Whiteleg Shrimp

The survival rate of whiteleg shrimp cultivated under different stocking densities in a polyculture system with *Caulerpa* sp. showed noticeable variation among the treatments. Treatment A recorded the highest survival rate at 98%, indicating that the conditions in this treatment supported optimal shrimp survival. Treatment B showed a slightly lower survival rate of 94%, which remained relatively close to that of Treatment A. Meanwhile, Treatment C exhibited the lowest survival rate at 71%, indicating a marked decline in shrimp survival compared to the other treatments. These observations suggest that lower stocking densities, as applied in Treatment A, were more favorable for maintaining shrimp survival

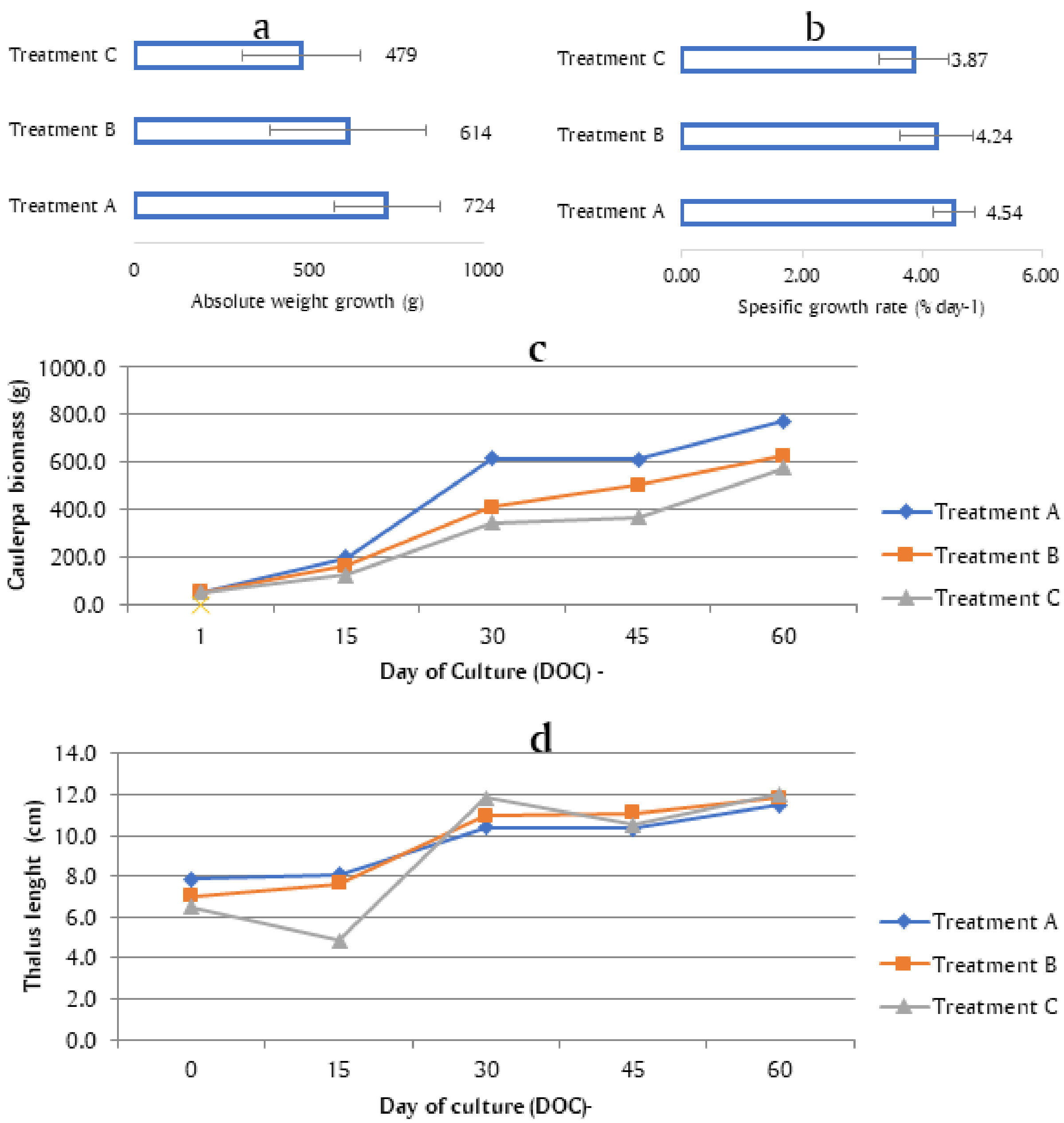

Figure 2. The growth of *Caulerpa* sp. cultured in polyculture system with whiteleg shrimp based on absolute weight growth (a), specific growth rate (b), biomass (c), and thallus length (d). Treatment A: 15 individuals per tank; B: 30 individuals per tank; and C: 45 individuals per tank

throughout the culture period (Figure 3).

The highest survival rate (98%) was recorded under low stocking density (Treatment A). This aligns with previous findings that higher densities elevate environmental stressors (e.g. reduced dissolved oxygen and increased ammonia), which negatively affect shrimp survival (Rahman *et al.*, 2019; Xu *et al.*, 2020). The presence of *Caulerpa* sp. also contributed to higher survival rates by improving water quality and providing a microhabitat that buffers against environmental stress.

Effects of Integrating Different Densities of Whiteleg Shrimp with Sea Grape on Carrageenan Level of *Caulerpa* sp.

Table 1 presented data on the carrageenan content of *Caulerpa* sp. cultivated in a polyculture system with whiteleg shrimp,

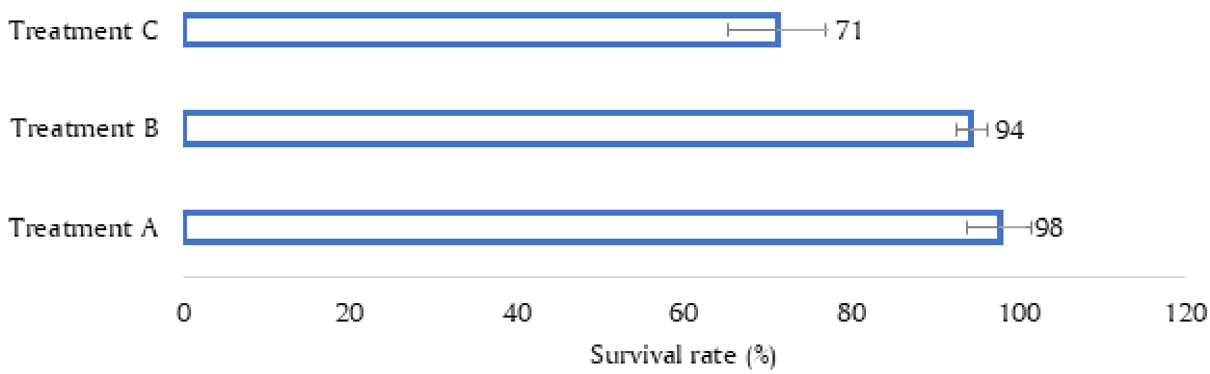

Figure 3. Survival rate of whiteleg shrimp reared in polyculture system with *Caulerpa* sp. Treatment A: 15 individuals per tank; B: 30 individuals per tank; and C: 45 individuals per tank

Table 1. Carrageenan levels in *Caulerpa* sp. in integrating different densities of whiteleg shrimp cultured with *Caulerpa* sp.

Treatment	Weight of empty cup (g)	Weight of cup + sample (g)	Final weight (g)	Water content (%)	Result of cage extraction (carrageenan yield) (%)
A	37.4566	39.4568	39.0109	22.29	6.75
B	42.8038	44.8055	44.2313	28.69	25.54
C	43.1053	45.1084	44.6421	23.28	24.33

Description: A = 15 individuals per tank; B = 30 individuals per tank; and C = 45 individuals per tank.

showing observable variation across the three treatments (A, B, and C). The initial weights of the empty cups ranged from 37.4566 g to 43.1053 g, while the combined weights of the cups and samples ranged from 39.4568 g to 45.1084 g. After processing, the final sample weights showed minimal differences, with treatment A recording the lowest final weight at 39.0109 g and treatment C as the highest at 44.6421 g. The water content of the samples varied across treatments. Treatment B had the highest water content at 28.69%, followed by treatment C at 23.28%, and treatment A at 22.29%. In terms of carrageenan yield—measured as the percentage of extractable material from the sample—treatment B showed the highest value at 25.54%, followed closely by treatment C at 24.33%. Treatment A had a notably lower carrageenan yield at 6.75%. These observations suggested that differences in water content and other cultivation-related conditions may have influenced the carrageenan yield, with treatments B and C appearing to support higher carrageenan production compared to treatment A.

The inclusion of *Caulerpa* in polyculture systems enhanced carrageenan production, with higher yield observed in nutrient-rich treatments such as treatment C and B. This finding aligns with previous studies indicating that nutrient availability is a critical factor in polysaccharide synthesis in seaweeds (Amaral *et al.*, 2021; Rohani *et al.*, 2022). Therefore, this polyculture system shows a potential source of valuable biochemical products alongside aquaculture operations.

Range of Chlorophyll Content of *Caulerpa* sp.

Table 2 illustrated the range of total chlorophyll content in *Caulerpa* sp. cultivated in a polyculture system with whiteleg shrimp

Table 2. Range of chlorophyll content of *Caulerpa* sp. in integrating different densities of whiteleg shrimp cultured with *Caulerpa* sp.

Treatment	Total chlorophyll (mg L^{-1})
A	1.485-44.390
B	1.806-44.752
C	3.297-45.937

Description: A = 15 individuals per tank; B = 30 individuals per tank; and C = 45 individuals per tank.

under three different treatments (A, B, and C). Treatment A exhibited the lowest chlorophyll range, with values ranging from 1.485 mg L^{-1} to 44.390 mg L^{-1}. Treatment B showed a marginally higher range, varying from 1.806 mg L^{-1} to 44.752 mg L^{-1}. In contrast, Treatment C demonstrated the highest chlorophyll content range, starting at 3.297 mg L^{-1} and reaching a maximum of 45.937 mg L^{-1}.

Treatment C had the highest chlorophyll levels, indicating optimal photosynthetic conditions in this group. Enhanced chlorophyll content reflects the macroalgae's physiological response to ideal environmental parameters such as light, nutrients, and water quality, as also supported by Valentine *et al.* (2021b).

Water Quality

Table 3 showed the water quality parameters observed during a study on the polyculture system of whiteleg shrimp and *Caulerpa* sp. across three treatments (A, B, and C). The light intensity ranged from 26 to 788 lux in treatment A, 71 to 911 lux in treatment B, and 70 to 790 lux in treatment C. The dissolved oxygen (DO) levels had varying value ranges for each treatment, which were 5 to 7.8 mg L^{-1} in treatment A, 4 to 7.4 mg L^{-1} in treatment B, and 5 to 8.2 mg L^{-1} in treatment C. Temperature varied from 28.0 to 32.67℃ in treatment A, 26.0 to 38.7℃ in treatment B, and 27.1 to 32.7℃ in treatment C. The pH levels across all treatments ranged from 6.5 to 9.4. Phosphate concentrations spanned from 0.0027 to 0.0073 ppm in treatment A, 0.0027 to 0.0104 ppm in treatment B, and 0.0012 to 0.0348 ppm in treatment C. Nitrate levels ranged from 0.1088 to 0.1434 ppm in treatment A, 0.0453 to 0.3512 ppm in treatment B, and 0.0482 to 0.3253 ppm in treatment C. Salinity was observed between 28.3 and 32.7 ppt in treatment A, 26 to 38.7 ppt in treatment B, and 27.7 to 38.3 ppt in treatment C.

Environmental parameters such as temperature (26–38 °C), dissolved oxygen (5.0–8.2 mg L^{-1}), pH (6.5–9.4), and salinity (26–38 ppt) remained within suitable ranges for both whiteleg shrimp and *Caulerpa* sp. These ranges are widely accepted as optimal for maintaining metabolic performance and physiological homeostasis in shrimp and macroalgae (Boyd & Tucker, 1998). Temperatures up to 38°C may be high, yet whiteleg shrimp is known to tolerate temperatures between 25°C and 38°C when other water quality parameters remain stable (Wang *et al.*, 2004). Similarly, pH between 6.5 and 9.4 falls within the physiological limits of both organisms, as shrimp perform well in the pH range of 7.5–8.5, and macroalgae like *Caulerpa* can tolerate broader pH variations without stress (Azim *et al.*, 2006; Rahman *et al.*, 2019).

Light intensity, an important abiotic factor, ranged from 26 to 911 lux across the treatments. Although there was a wide range in light intensity, the chlorophyll content and carrageenan levels showed relatively similar values among treatments, with no clear trends indicating substantial variation linked to light intensity differences. This can be attributed to the high adaptability of *Caulerpa* sp. to fluctuating light environments by modulating pigment composition and photosynthetic efficiency (Buschmann *et al.*, 2017). In other macroalgal studies, photosynthesis and growth in *Caulerpa lentillifera* remained stable

Table 3. Range of light intensity, dissolved oxygen (DO), temperature, pH, phosphate, nitrate, and salinity in the rearing polyculture tanks of whiteleg shrimp and *Caulerpa* sp. for 60 days of culture

Treatment	Light intensity (lux)	DO (mg L^{-1})	Temperature (°C)	pH	Phosphate (ppm)	Nitrate (ppm)	Salinity (ppt)
A	26-788	5-7.8	28.0-32.67	6.5-9.2	0.0027-0.0073	0.1088-0.1434	28-32.7
B	71-911	4-7.4	26.0-38.7	6.6-9.4	0.0027-0.0104	0.0453-0.3512	26-38.7
C	70-790	5-8.2	27.1-32.7	6.5-9.2	0.0012-0.0348	0.0482-0.3253	27.7-38.3

Description: A = 15 individuals per tank; B = 30 individuals per tank; and C = 45 individuals per tank.

under moderate variations in light up to 1000 lux, supporting our findings (Valentine *et al.*, 2021b). Additionally, moderate light supports the biosynthesis of bioactive compounds such as chlorophyll and polysaccharides (carbohydrates), but extremely high or low light intensity can suppress productivity (Rohani-Ghadikolaei *et al.*, 2012).

The data presented in Table 3 show that increasing shrimp stocking density from 15 to 45 individuals per tank did not result in deterioration of water quality throughout the 60-day culture period. The DO levels ranged from 4.0 to 8.2 mg L^{-1}, which are above the minimum required threshold of 3 mg L^{-1} to sustain aerobic metabolism in shrimp (Boyd, 2018). Salinity, ranging from 26–38.7 ppt, also remained within acceptable limits, as whiteleg shrimp is known to be euryhaline, tolerating salinities from 5 to 40 ppt (Bray *et al.*, 1994). Furthermore, the nitrate and phosphate levels in the system remained low ($\leq$ 0.35 and 0.01 ppm, respectively), indicating efficient nutrient assimilation by *Caulerpa* sp., which acted as a natural biofilter by absorbing excess nitrogen and phosphorus (Neori *et al.*, 2004; Troell *et al.*, 2003).

The nutrient-reducing effect of *Caulerpa* sp. was evident, particularly in treatment A (15 shrimp per tank), which showed the most stable and lowest nutrient concentrations. In contrast, treatments B and C showed slight increases in nitrate and phosphate, likely due to increased organic waste from shrimp. However, all nutrient values remained within environmentally acceptable levels and below thresholds associated with eutrophication (Boyd & Tucker, 1998). According to Chopin *et al.* (2001), macroalgae can significantly mitigate nutrient loading in integrated multitrophic aquaculture systems by converting dissolved inorganic nutrients into biomass, which was consistent with the increased *Caulerpa* growth observed in lower-density tanks.

The ability of *Caulerpa* sp. to maintain water quality is supported by its high nutrient uptake efficiency, particularly for ammonium, nitrate and phosphate, which enables it to function as an effective component of integrated systems (Lüning & Pang, 2003; Troell *et al.*, 2003). This explains why water quality remained within safe limits across all treatments, even as shrimp density increased. Furthermore, aeration during culture was continuously maintained to avoid oxygen depletion, especially at night, aligning with standard biosecurity protocols in semi-intensive and intensive shrimp systems (Boyd, 2018).

CONCLUSIONS

The polyculture of whiteleg shrimp (15 individuals per tank) with sea grape biomass (50 g) appeared to support higher growth and survival of the shrimp, as observed from the general performance trends during the culture period. Interestingly, increased in shrimp density improved water quality in the culture medium together with carrageenan content of sea grape (*Caulerpa* sp.). These findings highlighted the potential of polyculture systems as a sustainable approach to aquaculture development.

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AUTHOR CONTRIBUTION

RYV: conceptualization, data curation, formal analysis, project administration, methodology, supervision, writing—original draft, and writing—review and editing; DRH: visualization, resources, software, and project administration; ST: data curation, formal analysis, investigation, and validation.

DECLARATION OF COMPETING INTEREST AND USE GENERATIVE AI

The authors declare no competing interests. The authors did not use generative AI or AI-assisted technologies for writing or editing this manuscript beyond standard spelling and grammar checking.

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