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Optimization of seaweed farming: The impact of *Perna viridis* density on *Gracilaria verrucosa* in extensive ponds

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Abstract
The cultivation of *Gracilaria verrucosa* seaweed is a significant aquaculture endeavor, particularly within polyculture systems with green mussels (*Perna viridis*). The number of green mussels in ponds can influence water quality and the growth of planted seaweed. This study seeks to evaluate the influence of *Perna viridis* density on the development and water quality in the cultivation of *Gracilaria verrucosa* in vast ponds located in Pangkahkulon Village. A fully randomized design (CRD) was implemented, including three treatments with green mussel densities of 10, 20, and 30 mussels/m², each duplicated three times, yielding nine experimental units. The observed characteristics comprised the specific growth rate (SGR), thallus length (both horizontal and vertical), and water quality. The findings indicated that the density of green mussels had a significant impact on the specific growth rate of *Gracilaria verrucosa*, with treatment A (10 mussels/m²) demonstrating the greatest growth rates of 6.65±0.11 on day 7 and 5.08±0.01 on day 21. Treatment C (30 mussels/m²) had the lowest growth rate, measuring 0.96±1.08 on day 7 and 4.07±0.19 on day 21. Mussel density affected thallus length, although the impact was not substantial. On day 21, treatment A exhibited the greatest vertical thallus length (4.2±4.05 cm) and the largest horizontal thallus length (5.2±2.25 cm). This work offers significant insights into the regulation of green mussel density in polyculture systems to enhance seaweed development and preserve water quality.

Keywords: Density of green mussels, *Gracilaria verrucose*, specific growth rate, polyculture, thallus length

Introduction
The predominant portion of the populace in Ujung Pangkah Subdistrict, Gresik Regency, Indonesia, relies on aquaculture for their sustenance. The predominant agricultural technique in this region is vast or traditional pond cultivation. Polyculture systems have recently become popular as a sustainable agricultural practice. Polyculture is cultivating various species within a single ecosystem, such as Vannamei shrimp (*Litopenaeus vannamei*), green mussels (*Perna viridis*), seaweed (*Gracilaria verrucosa*), and milkfish (*Chanos chanos*). This technique improves biodiversity, maximizes pond space utilization, and offers economic advantages to farmers. Nonetheless, difficulties such as feed waste, metabolic waste, and pesticide application pose risks to water quality and the health of cultivated organisms [1].

Perna viridis, often known as green mussels, are exceptionally nutritious and constitute a significant agricultural asset. The composition includes of 11.84% protein, 78.86% water, 3.60% ash, 0.70% fat, and 4.70% carbs [2]. Seaweed, especially *Gracilaria verrucosa*, has emerged as a principal aquaculture commodity in Indonesia. Gracilaria considerably contributes to food security and socio-economic development as both a food source and a raw material for non-food businesses [3, 4]. In 2022, Indonesia accounted for 44.46% of global seaweed exports, making it one of the major exporters worldwide [5].

Gracilaria verrucosa has several advantages, particularly as a vital raw material in the food sector (notably for agar manufacturing), cosmetics, and medicines. Enhanced seaweed yield is essential to satisfy the elevated worldwide demand. There are two principal methods of cultivation: extensive and intense systems. The extensive system depends on natural circumstances, whereas the intensive system employs organic fertilizers, chemical fertilizers, and artificial habitats [6].

Green mussels have dual functions in polyculture systems: they generate revenue and function as natural biofilters, enhancing water quality and facilitating the establishment of other planted species [7]. The influence of green mussel density on the development and quality of *Gracilaria verrucosa* in vast pond systems has not been well investigated.

This study aims to investigate the impact of *Perna viridis* density on the growth and water quality in the cultivation of *Gracilaria verrucosa* in wide ponds. The outcomes are anticipated to promote sustainable agricultural practices that improve production, tackle environmental concerns, and advance more eco-friendly aquaculture techniques.

Materials and Methods

Time and Location

The investigation was carried out for 21 days, from June 27 to July 18, 2024, at the Polyculture Extensive Pond in Pangkahkulon Village, Ujungpangkah Subdistrict, Gresik Regency.

Research Methodology

This study employed a Completely Randomized Design (CRD) consisting of three treatments and three replications, yielding nine experimental units. The research was conducted in the Extensive Pond region of Pangkahkulon Village, Ujungpangkah Subdistrict, Gresik Regency, for a period of 21 days. Each pond measured 1 x 1 x 1 meter. The initial weight of the green mussels was roughly 2.2 g, with an average length of around 19.2 mm, whereas the initial weight of the seaweed was 250 g per square meter. Sampling occurred weekly, with an initial seaweed weight of 10 g per square meter for each treatment. The fluctuations in green mussel density are as follows:

Treatment A: Green mussel density of 10 individuals

Treatment B: Green mussel density of 20 specimens

Treatment C: Green mussel density of 30 individuals

B3	A2	C1
B2	A1	C2
B1	C3	A3

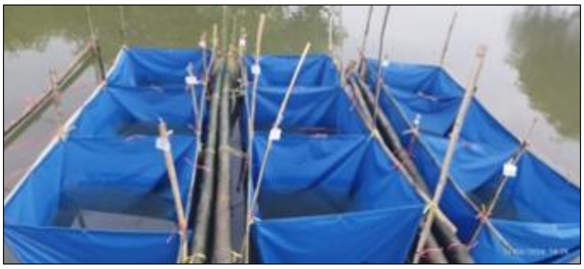

Fig 1: Research Design

Specific Growth Rate (% Day^{-1})

The specific growth rate (SGR) of the seaweed is calculated using the following formula [8]:

SGR (% Day^{-1}) = Ln Wt – Ln W0/t x 100

Explanation:

SGR : Specific Growth Rate (% day^{-1})

LnWo : Natural Logarithm of Initial Weight (g)

LnWt : Natural Logarithm of Final Weight (g)

T : Duration of Maintenance (Days)

Absolute Thallus Length (cm)

The total length of the seaweed is determined using the formula established by [8]:

$\Delta L = Lt - L_0$

Explanation

ΔL = Absolute Horizontal or Vertical Length (cm)

Lt = Final Horizontal or Vertical Length (cm)

L_0 = Initial Horizontal or Vertical Measurement (cm)

Water Quality

Throughout the maintenance period, water quality was assessed weekly to confirm that the pond environment satisfied the growth requirements for *Gracilaria verrucosa*. Calibrated instruments were employed to measure temperature, pH, and salinity. Temperature was assessed using a digital thermometer, pH was determined with a portable pH meter, and salinity was evaluated using a refractometer. Morning water samples were collected to provide representative data on the wide pond's conditions. Water quality is essential in seaweed production, since environmental alterations can greatly impact growth and productivity [9];[4].

Statistical Examination

The influence of green mussel density on the specific growth rate (SGR) and absolute thallus length (both vertical and horizontal) of *Gracilaria verrucosa* was examined by one-way analysis of variance (ANOVA). Before the analysis, the data were assessed for normality and homogeneity of variances. This was executed to guarantee the accuracy of the results. Tukey's post-hoc test was employed to discern significant differences across treatments when ANOVA revealed significant differences ($p < 0.05$). Furthermore, water quality parameters including temperature, pH, and salinity were subjected to descriptive analysis to elucidate the environmental circumstances during the research. This approach was employed to analyze the environmental attributes of the culture system and investigate the correlation between green mussel density and seaweed growth efficacy.

Results and Discussion

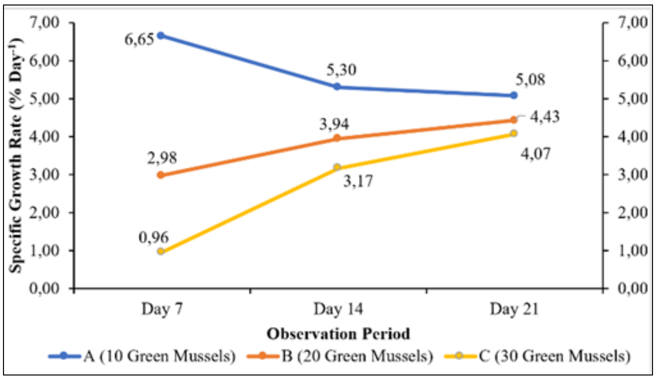

Fig 2: Specific Growth Rate (% Day^{-1})

Gambar 2. Seaweed Specific Growth Rate (SGR)

The results indicated that the specific growth rate (SGR) of *Gracilaria verrucosa* in polyculture over a 21-day period varied between 4.07% and 5.08% day^{-1}. Treatment A, characterized by a green mussel density of 10 individuals/m², demonstrated the highest specific growth rate (SGR), recording values of 6.65±0.11% on day 7 and 5.08±0.01% on day 21. Treatment B (20 mussels/m²) exhibited lower specific growth rate (SGR) values of 2.98±0.70% on day 7 and 4.43±0.10% on day 21. In contrast, Treatment C (30 mussels m^{-2}) demonstrated the lowest growth rates, with measurements of 0.96±1.08% on day 7 and 4.07±0.19% on day 21. Although there was a minor decrease in SGR for Treatment A over time, the values continued to be significantly higher than those observed in Treatments B and C. ANOVA indicated that mussel density had a significant impact on SGR ($p < 0.05$), with Tukey's test confirming that Treatment A differed significantly from Treatments B and C ($p < 0.05$). No significant difference exists between Treatments B and C ($p > 0.05$).

This study demonstrates a significant correlation between green mussel density and the specific growth rate (SGR) of *Gracilaria verrucosa*. With an increase in mussel density, the specific growth rate (SGR) of the seaweed typically exhibited a decline. The specific growth rate (SGR) was highest in Treatment A, which had 10 individuals/m² of mussels. This suggests that lower mussel densities make the environment better for the growth of *Gracilaria verrucosa*. This indicates that decreased mussel density lowers competition for critical resources, including light, space, and nutrients, which are essential for optimal photosynthetic activity and growth. Studies show that putting a lot of filter-feeding species in an area, like green mussels, can make resources scarce, which can slow the growth of co-cultured seaweeds [10, 11].

In Treatment C, where mussel densities reached 30 mussels/m², a reduced specific growth rate (SGR) was observed in *Gracilaria verrucosa*. The decline is attributable to heightened competition between mussels and seaweed, potentially diminishing the availability of nutrients and light necessary for seaweed growth. As filter feeders, mussels can make it harder for *Gracilaria verrucosa* to get the planktonic nutrients it needs for photosynthesis [2]. High mussel densities may result in the accumulation of organic waste, such as feces, which can degrade water quality and impede the growth of seaweed [12]. To improve the specific growth rate of *Gracilaria verrucosa* and support sustainable aquaculture practices, this study shows how important it is to control the density of mussels in polyculture systems.

The findings indicate that reduced mussel density facilitates the accelerated development of *Gracilaria verrucosa*, suggesting that habitats with diminished mussel populations are more conducive to the growth of both *Gracilaria verrucosa* and other aquaculture species, including fish and shrimp. In contrast, elevated mussel concentrations may impede the development of seaweed. This aligns with the findings of [10], which indicated that initial seed weight influences competition among thalli in seaweed development, affecting space usage, light absorption for photosynthesis, and nutrient acquisition. Moreover, [11] indicate that reduced initial seed weights often lead to accelerated development due to less competition for resources among thalli, facilitating improved nutrient allocation. This study highlights the significance of regulating mussel density in polyculture systems to enhance the development of *Gracilaria verrucosa* and promote sustainable aquaculture methods. Reduced mussel concentrations not only promote the development rate of seaweed but also foster a more balanced and healthier ecology in aquaculture environments.

Absolute Thallus Length (cm)

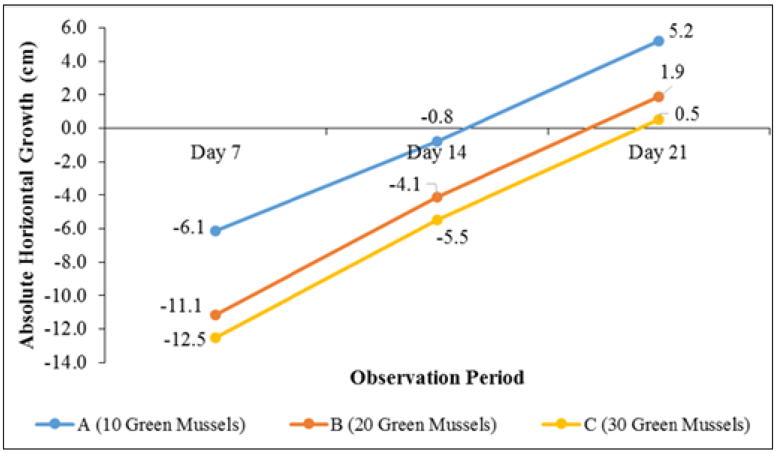

Fig 3: Absolute Horizontal Growth of Seaweed

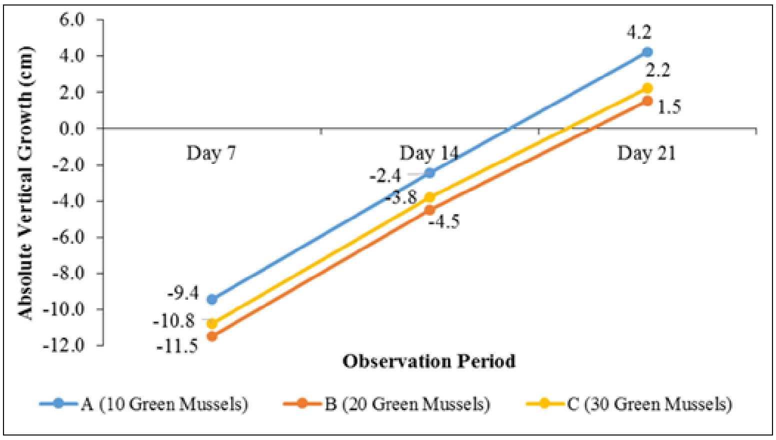

Fig 4: Absolute Vertical Growth of Seaweed

Following 21 days of development with the polyculture method, the horizontal thallus length of *Gracilaria verrucosa* ranged from -0.8 to 11.1 cm, whereas the vertical thallus length fluctuated between -9.4 and 4.2 cm. ANOVA analysis indicated that green mussel density substantially influenced horizontal thallus length on day 7 ($p < 0.05$) but did not have a significant effect on days 14 and 21 ($P > 0.05$). We observed no significant changes in vertical thallus length on any of the days ($P > 0.05$). Tukey's post-hoc test indicated that the horizontal thallus length on day 7 was substantially superior in the 10 mussels m^{-2} treatment relative to the 30 mussels m^{-2} treatment ($p < 0.05$). No significant changes were seen between the 10 and 20 mussels m^{-2} treatments or between the 20 and 30 mussels m^{-2} treatments on day 7.

The results demonstrate that heightened green mussel density significantly affects the development of *Gracilaria verrucosa* thallus. The drop seen on days 7 and 14 could be because of competitive interactions between species in the polyculture system. For example, the green mussels could change the availability of nutrients or the conditions in the microenvironment that affect the seaweed. This aligns with the findings of [13], which indicate that high densities of green mussels in aquatic environments might result in elevated fecal output and a deterioration of water quality. Moreover, stocking density affects competition for oxygen and space, thereby inducing stress in organisms and leading to mussel death. The good growth seen on day 21 could be because the seaweed is able to adapt to its surroundings or because the different parts of the polyculture system are now in balance after the first stress phase.

The intricate dynamics of nutrient cycling within polyculture systems may influence the observed decline in growth on days 7 and 14 in higher mussel density treatments. Green mussels, via their filter-feeding behavior, can modify the levels of dissolved nutrients, especially nitrogen and phosphorus, essential for seaweed growth. High mussel densities can cause nutrient imbalances or too much nutrient uptake, which makes these important resources less available for *Gracilaria verrucosa*. The accumulation of organic matter, such as mussel feces and pseudofeces, may lead to hypoxic conditions in the water column, thereby inhibiting seaweed development [7]. Environmental stressors may cause a temporary decline in growth until system stabilization occurs, as noted in the later stages of the experiment.

The spatial competition for light and space in polyculture systems significantly influences the growth of mussels and seaweeds. Having too many green mussels in one place can create shading effects that make it harder for *Gracilaria verrucosa* to get the light it needs to photosynthesise. [10] assert that in environments where multiple species coexist, the equilibrium of light exposure and spatial distribution is crucial for providing optimal growth conditions for each species. In this study, the initial competition for space and light likely influenced the early growth stages; however, by day 21, the system seemed to achieve an equilibrium, enabling the seaweed to recover and sustain growth. This finding backs up the idea that polyculture systems are changing places where species can interact with each other in new ways over time to make them more sustainable [7, 11]. The assessed water quality during the study yielded significant insights regarding its influence on organism growth.

The pH levels for all three treatments were between 7.7 and 8.6, falling within the optimal range for the growth of aquatic organisms.

Water Quality

Table 1: Water Quality Measurements During the Study

Treatment	Range of Water Quality		
	Water pH	Salinity (ppt)	Temperature (°C)
A (10 Green Mussels)	7.8-8.4	17.3-27.0	28.4-31.2
B (20 Green Mussels)	7.8-8.5	11.7-26.0	28.1-31.1
C (30 Green Mussels)	7.7-8.6	14.0-25.0	28.1-30.3

The indicated pH range suggests that the water conditions were stable and favorable for the growth of both Vannamei shrimp and milkfish. The pH level indicates the acidity of water; extreme acidity or alkalinity can interfere with the metabolic and respiratory functions of the organisms present. The findings of [14] indicate that the optimal pH for the growth of *Gracilaria verrucosa* ranges from 6 to 9.

Salinity levels varied from 11.7 to 27.0 ppt in Treatment A, 14.0 to 25.0 ppt in Treatment C, and were marginally higher in Treatment B, ranging from 11.7 to 26.0 ppt. The values indicate that the salinity fell within the appropriate range for growth. Salinity variations influence organism adaptation and the ecological equilibrium of pond systems. The elevated salinity levels observed suggest the study site's proximity to the ocean [15] indicate that *Gracilaria verrucosa* is a marine species characterized by its euryhaline nature, allowing it to survive in salinities ranging from 5.2 to 38.1 ppt.

Water temperature varied between 28.1 and 31.2 °C, a range conducive to the health and growth of the organisms. The stable temperature, maintained within the optimal range for both Vannamei shrimp and milkfish, facilitates positive growth outcomes. The assessed water quality parameters at the research site fell within the acceptable range for seaweed cultivation [16] reported that *Gracilaria verrucosa* exhibits growth in water temperatures between 26 and 33°C. The temperature conditions in the ponds of Pangkah Kulon Village, Ujung Pangkah Subdistrict, were conducive to the growth of *Gracilaria verrucosa*. Temperature influences the physiological processes of seaweed, including respiration and metabolism, thereby affecting organism growth [17].

The findings of this study demonstrate that green mussel density is essential for the success of polyculture farming. It is better for *Gracilaria verrucosa* to grow when there are fewer mussels (10 individuals/m²), but when there are more mussels, growth and environmental quality may get worse, which can hurt farming results. Modifying mussel density is essential for the optimal functioning of integrated aquaculture systems rooted in the Blue Economy, improving production, and preserving water quality. Green mussels function as effective biofilters, as indicated by [18]; however, their density requires careful management to prevent adverse environmental effects.

Conclusion

The findings of this study indicate that the density of green mussels, also known as *Perna viridis*, has a substantial impact on the development of *Gracilaria verrucosa* in polyculture systems. Higher concentrations of mussels led to decreased growth, most likely as a result of greater competition for space, nutrients, and light. We found that lower densities of mussels facilitated better seaweed development, while higher densities resulted in reduced growth. The findings emphasize the significance of managing mussel density in order to improve seaweed output and guarantee a balanced ecosystem for aquaculture. In addition, the findings highlight the fact that the management of mussel density not only benefits the general sustainability and health of polyculture systems, but it also helps to improve the growth of *Gracilaria verrucosa*. The maintenance of this equilibrium is necessary for the preservation of water quality, the advancement of biodiversity, and the enhancement of the effectiveness of integrated aquaculture systems.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Research highlights

- Study the effect of green mussel density on *Gracilaria verrucosa* growth and water quality in polyculture systems.
- Mussel densities of 10, 20, and 30 mussels m^{-2}, with three replications.
- Highest specific growth rate (SGR) at 10 mussels m^{-2}; lowest at 30 mussels m^{-2}.
- Greater horizontal and vertical thallus length at 10 mussels m^{-2} by day 21.
- Mussel density influences water quality, affecting seaweed growth.
- Lower mussel densities (10 m^{-2}) promote better growth of *Gracilaria verrucosa*.

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