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Nature Environment & Pollution Technology

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Original Research

Optimizing green mussel density for enhanced growth and agar production in *Gracilaria verrucosa* within extensive pond aquaculture

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Key Words	Aquaculture systems, Bioremediation strategies, Algae-Mussel interactions, Environmental sustainability, Brackish water ecosystems
DOI	https://doi.org/10.46488/NEPT.2025.v24i04.D1759 (DOI will be active only after the final publication of the paper)
Citation of the Paper	Rahim, A. R., Rosmarlinasiah, Jumadi, R., Asari, S. and Utami, D. R., 2025. Optimizing green mussel density for enhanced growth and agar production in *Gracilaria verrucosa* within extensive pond aquaculture. *Nature Environment and Pollution Technology*, 24(4), p. D1759. https://doi.org/10.46488/NEPT.2025.v24i04.D1759

Abstract: *Gracilaria verrucosa*, a red alga that is used in the manufacturing of agar, has a significant impact on coastal ecosystems and is important in several industrial uses. This study examines the effects of different densities (10, 20, and 30 individuals) of green mussels (*Perna viridis*) on the growth, agar yield, viscosity, gel strength, and ability to remove lead from water in brackish water pond systems. The research used a randomized complete block design with three replications. The findings indicate that the density of green mussels does not have a substantial impact on agar production, gel strength, or the effectiveness of lead removal. The density of green mussels affects the daily growth rate (DSGR) and viscosity. Lower densities (10 mussels) show better early performance, but these differences diminish over time. The presence of consistent salt levels indicates that salinity has less impact on production outcomes. This study elucidates the dynamics between algae and mussels in aquaculture systems, providing recommendations for enhancing production practices and implementing bioremediation strategies.

1. INTRODUCTION

Gracilaria verrucosa, a species of red algae that inhabits maritime habitats, is being increasingly acknowledged for its wide range of applications in several industries. The significance of this alga lies in its capacity to synthesize agar, a viscous substance that is highly sought after in the food, pharmaceutical, and biotechnology industries because of its exceptional ability to form gels and thicken solutions (Lee et al., 2022; Roseline and Sachindra, 2018). In addition to its economic uses, *G. verrucosa* plays a crucial role in coastal environments by contributing to ecological stability and promoting marine biodiversity (Widyartini and Hidayah, 2023; Widowati et al., 2021).

To improve the cultivation of *G. verrucosa*, a novel method involves incorporating green mussels (*Perna viridis*) into the aquaculture systems used for growing the algae. Green mussels has inherent filtration properties, enabling them to enhance water quality through the elimination of surplus nutrients and particulate matter (Ruangchuay et al., 2022; Widowati et al., 2021). Optimizing growing conditions and raising the total productivity of *G. verrucosa* can potentially be advantageous for algal production. Recent research indicates that manipulating the density of mussels can have a substantial impact on the rate of algal development and the quality of agar generated. Therefore, this is an important field of study.

Moreover, *G. verrucosa* has exhibited considerable potential for bioremediation, particularly in the elimination of heavy metals, such as lead, from contaminated water sources. Given the urgency of lead pollution as an environmental concern, it is imperative to discover efficient approaches for diminishing lead concentrations in order to safeguard aquatic ecosystems and human well-being (Raj et al., 2022; Kang et al., 2023). An investigation into the impact of varying densities of green mussels on the efficacy of lead removal by *G. verrucosa* could yield significant information regarding its contribution to environmental management.

This study seeks to examine the impact of different densities of green mussels (10, 20, and 30 individuals per pond) on various important parameters of *G. verrucosa* cultivated in pond habitats. The metrics encompass algal proliferation, agar synthesis, fluid thickness, gel potency, and the ability to eliminate lead from the water. The research aims to better our comprehension of how to optimize algal cultivation and boost methods in both aquaculture and environmental cleanup by analyzing these characteristics.

The findings of this study are anticipated to enhance the efficiency and long-term viability of aquaculture methods. The project aims to provide practical advice for enhancing algal cultivation and bioremediation procedures by studying the impact of mussel density on *G. verrucosa*. This has the potential to drive progress in both the commercial cultivation of algae and the endeavors to protect the environment. The primary objective of this research is to address the current gaps in information surrounding the interactions between green mussels and seaweeds in large-scale pond systems. The results will provide vital knowledge for improving growing methods and incorporating algae-based solutions for sustainable aquaculture and environmental management.

2. MATERIALS AND METHODS

2.1. Experimental

The research was carried out in large pond habitats located in Pangkahkulon Village, Ujungpangkah District, Gresik Regency, East Java, Indonesia, over the period of October to November 2024. A Completely Randomized Design was employed to assure the random assignment of treatments and to avoid bias. The media containers were regulated using tarpaulin, with dimensions of 1x1 m, a water depth of 50 cm, and a soil thickness of 10 cm at the base. The installation was situated in a 2.5 hectare area specifically designated for brackish water pond systems (Figs.1). Three different treatments were administered, utilizing different concentrations of green mussels. Three replicates of each treatment were conducted, resulting in a total of nine experimental ponds. Every pond was carefully managed with consistent environmental conditions to guarantee precise outcomes.

Explanation:

Treatment A: 10 green mussels

Treatment B: 20 green mussels

Treatment C: 30 green mussels

1,2,3 : Replication

2.2. Cultivation

The green mussels (*Perna viridis*) were sourced from a floating cage aquaculture facility situated in Banyuurip Village, Ujungpangkah District, Gresik Regency (Rahim et al. 2024). The mussels were classified based on their size into three categories: small (10-20 g), medium (21-30 g), and giant (31-40 g) (Fauzi and Safitri, 2022; Madin and Ransangan, 2016). Only mussels that were tiny in size, weig`hing between 10-20 grams, were used in this investigation. The mussels were categorized into three density treatment groups: low density (10 mussels per square meter), medium density (20 mussels per square meter), and high density (30 mussels per square meter) (Widowati et al., 2021). To maintain consistent environmental conditions among all groups, each treatment group was allocated to separate experimental ponds that were clearly labeled. Prior to being placed in the ponds, the mussels were acclimated in a controlled environment for one week. The algae were uniformly dispersed around the ponds to ensure similar development conditions and were meticulously cleansed to eliminate any attached moss and other types of algae **(**Rendaje et al., 2019) (Figs.2). The seaweed seedlings used in this study were *Gracilaria verrucosa***,** with an initial weight of 250 grams per square meter and an age of 1–2 months (Rahim et al. 2024). The mussels' growth, health, and survival were closely observed, and their weight and size were measured at regular intervals (Toralde et al., 2021).

Figure 2. (A) *Gracilaria verr* ... used for monitoring in tarpaulin ponds.

2.3. Water Quality Monit

The study included continuous monitoring of crucial water quality factors to provide optimal circumstances for the growth of both algae and mussels. Salinity levels were assessed on a weekly basis using a refractometer (Melendres and Largo, 2021). Lead contents were evaluated every two weeks using atomic absorption spectroscopy (Ananda Kumar and Vijaya Kumar, 2024).

2.4. Data Analysis

2.4.1 Daily Specific Growth Rate (DSGR) (% day^{-1})

The Specific Growth Rate is a measure of the organism's relative growth over a period of time, represented as a percentage. The formula for determining the Daily Specific Growth Rate (Yang et al., 2015), is:

DSGR (% day^{-1}) = ln (Wt)-Ln (Wo)/t x 100

Explanation:

ln (Wt) : Natural logarithm of the final weight.

ln (Wo) : Natural logarithm of the initial weight.

t : Duration of the observation period in days.

2.4.2 Agar Production

- Agar Yield (%)

Employ a systematic approach to measure the agar yield in their investigation. To prepare the seaweed samples, cleanse and desiccate them at a consistent weight by subjecting them to a temperature of 60°C. To obtain agar, submerge desiccated seaweed in distilled water, elevate the temperature to 90°C, and then segregate the liquid using filtration. In order to cause agar precipitation, simply decrease the temperature of the filtered solution to 4°C, which will result in the production of agar gel (Mendes et al., 2024). Afterwards, divide and dry out the gel. Calculate the yield using the given formula:

Agar Yield (%) = Weight of Dry Agar/Weight of Dry Seaweed Sample x 100

- Viscosity (cps)

Prepare the Gracilaria seaweed by washing and drying it. To extract the gel, immerse a predetermined amount of dehydrated seaweed in distilled water for a duration of 24 hours. Perform gel filtration to separate the solid remains from the viscous gel solution. To measure the viscosity of the gel solution, employ a viscometer for viscosity measurement. Make sure to calibrate the viscometer in accordance with the instructions provided by the manufacturer. Perform the necessary calculations and provide the viscosity measurement in centipoise (cps). Modify measurements as needed, taking into account temperature and gel concentration (Jayasinghe and Pahalawattaarachchi, 2016).

Gel Strength (g.cm^{-2})

Produce a gel using the extract obtained from the Gracilaria seaweed. Combine a certain quantity of dehydrated seaweed with distilled water and raise the temperature to 80°C in order to create a gel. Allow the gel to cool and solidify at a temperature of 4°C for a duration of 24 hours. After the gel has hardened, extract it from the mold and divide it into pieces of uniform size. Utilize a texture analyzer to quantify the strength of a gel. Exert a uniform force using a probe to penetrate the gel until it reaches a predetermined depth, such as 1 cm. Measure the highest amount of force needed to pierce the gel and quantify it as the gel's strength in grams per square centimeter (g cm^{-2}) (Vuai, 2022).

2.4.3 Calculation of Lead Waste Reduction Rate (mg L^{-1} day^{-1})

Determine the difference in lead concentration for each reactor between successive days. This is achieved by calculating the difference between the concentration of lead on day n and the concentration on the previous day, n-1 (Tan et al., 2016).

Determine Concentration Change (ΔC) = $C_{n-1} - Cn$

Explanation:

C_{n-1} : Concentration on day n-1 and

C_n : Concentration on day n

Convert the concentration change to the rate of reduction per day:

Reduction Rate = $\Delta C/\Delta t$

Explanation:

ΔC : Change in concentration in mg L^{-1}

Δt : Time interval in day^{-1}

2.5 Statistical Analysis

We employed a rigorous statistical methodology to examine the impact of various green mussel densities on the Specific Growth Rate (SGR), Agar Yield, Viscosity, Gel Strength, and Lead Removal Rate of *Gracilaria verrucosa*. Initially, a one-way analysis of variance (ANOVA) was employed to see if there were any significant variations in these attributes among the different mussel densities. Subsequently, Tukey's Honest Significant Difference (HSD) test was employed to assess the significant differences between the various mussel density treatments. This method guarantees a comprehensive evaluation of the impact of varying mussel density on crucial parameters in seaweed farming. Furthermore, a descriptive research was conducted to examine the impact of different salinity levels on the growth viability of *G. verrucosa*, as well as its capacity to produce agar and remove lead efficiently. This approach offers a precise and thorough understanding of the ideal circumstances required to maximize the efficiency and yield of seaweed. The regression analysis between seaweed agar quality and green mussel density was conducted using a quadratic regression model to determine the optimal mussel density for maximizing agar quality.

3. RESULTS AND DISCUSSIONS

3.1 Daily Specific Growth Rate (DSGR) (% day^{-1})

An analysis of variance (ANOVA) revealed statistically significant variations in the Daily Specific Growth Rate (% day^{-1}) across different green mussel densities on days 7, 14, and 21 ($p < 0.05$). The Tukey's HSD test revealed that Treatment A, consisting of 10 mussels, had a substantially greater growth rate of 6.65% day^{-1} compared to Treatments B (20 mussels) and C (30 mussels), which displayed lower rates of 2.98% per day and 0.96% day^{-1}, respectively (Figs.3). The increased growth rate seen in Treatment A can be attributed to the favorable conditions resulting from decreased mussel populations. These conditions likely improved nutrient availability and reduced competition, leading to the enhanced growth of seaweed. On the 14th day, Treatment A had the largest growth rate, while the differences between treatments decreased. Treatment B had a moderate growth rate of 3.94% day^{-1}, and Treatment C had a growth rate of 3.17% day^{-1}. These findings suggest that whereas lower mussel densities initially enhance development rates, these benefits may decrease with time due to changing environmental conditions or nutrient dynamics. On the 21st day, the growth rates of Treatments B and C had become equal, but Treatment A maintained its advantage with a growth rate of 5.08% day^{-1}.

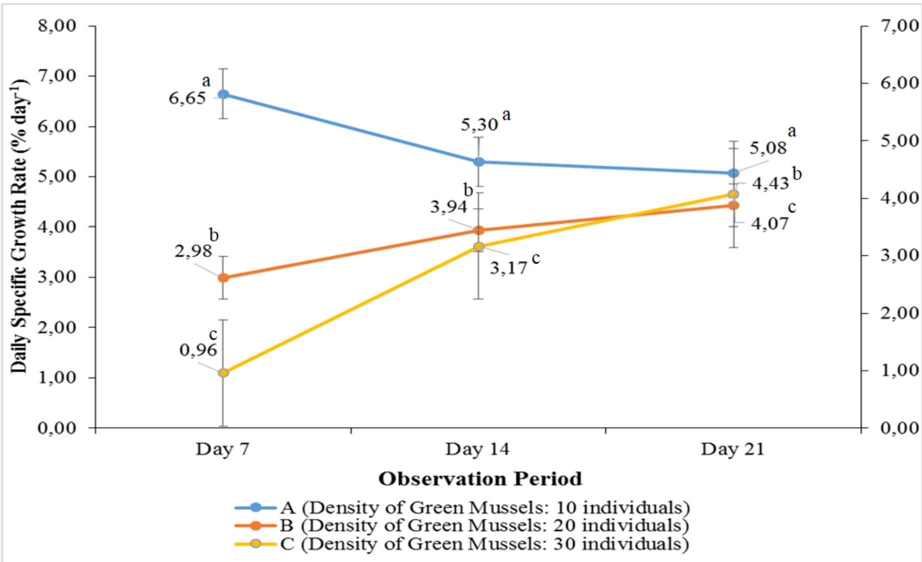


Figure 3. Daily ... gnificant differences betwe... rror (SE).

ANOVA analysis and Tukey's HSD test showed that there was a significant effect caused by different treatments, supporting green mussels as good biofilters; these findings also pointed out the optimization of mussel densities to maximize growth of *G. verrucosa* at low to moderate levels of mussel density, especially in the early stages of cultivation. These results indicate that the specific growth rate of *Gracilaria verrucosa* is closely related to the density of organisms in the aquaculture system. In Treatment A, with only 10 green mussels, the nutrient availability for a unit biomass of *Gracilaria* is higher, hence allowing superior nutrient uptake and faster growth. This is supported by the fact that Widowati et al. (2021) proved that low-density *Perna viridis* combined with *Gracilaria verrucosa* has improved water quality due to reduced organic matter, leading to optimum growth of seaweed. In support, Rahim et al. (2023) also reported that low-density conditions are good for enhancing the gel quality of *Gracilaria verrucosa* biomass and increasing its growth. Chebil et al. (2018) reported that low density reduces competition for space and nutrient availability, enabling the growth rate of *Gracilaria* to become higher.

]On the other hand, at low density, its advantages do not last very long after Day 14; this is highly indicated by Treatment A's negative SGR. This may result from the general decrease in the nutrient levels of the water column, which could reduce growth rates despite lesser competition. On the other hand, medium density, Treatment B (20 green mussels), has higher growth rates, possibly with supplementary nutrients released by the waste of green mussels. Lim et al. (2019) suggested that changes in nutrient availability have dynamic impacts on *Gracilaria* SGR through time. Indeed, Susilowati et al. (2019) found in their work that medium density strikes an ideal balance in competition for nutrition and resource availability to maintain better growth performance among *Gracilaria*. Where this density is kept at even higher levels, dramatic competition for space and nutrients significantly lowers the growth of seaweed. Amalia et al. (2022) found that very highly dense conditions at the polyculture level reduce the SGR of *Gracilaria* to less than 1.0% day^{-1}.

3.2 Agar Production

3.2.1Agar Yield (%)

The ANOVA analysis indicated that there were no statistically significant variations in agar yield (%) among the various densities of green mussels on days 0, 7, 14, and 21 ($p > 0.05$). The Tukey's HSD test indicated that there were no statistically significant differences between the treatments (A: 10 mussels, B: 20 mussels, C: 30 mussels) at any of the time points. At the start of the experiment (day 0), Treatment A produced an agar yield of 9.68%, whereas Treatments B and C yielded 7.05% and 7.83%, respectively. On the seventh day, the yields for Treatment A, Treatment B, and Treatment C were 15.68%, 13.05%, and 13.83% respectively. Treatment A reached a yield of 21.68% on day 14, whereas Treatment B and Treatment C had yields of 19.05% and 19.83% respectively. Treatment A had the highest yield of 28.62% on day 21, while Treatments B and C had yields of 26.56% and 24.07%, respectively (Figs.4). However, the variations in yields did not show any statistically significant changes.

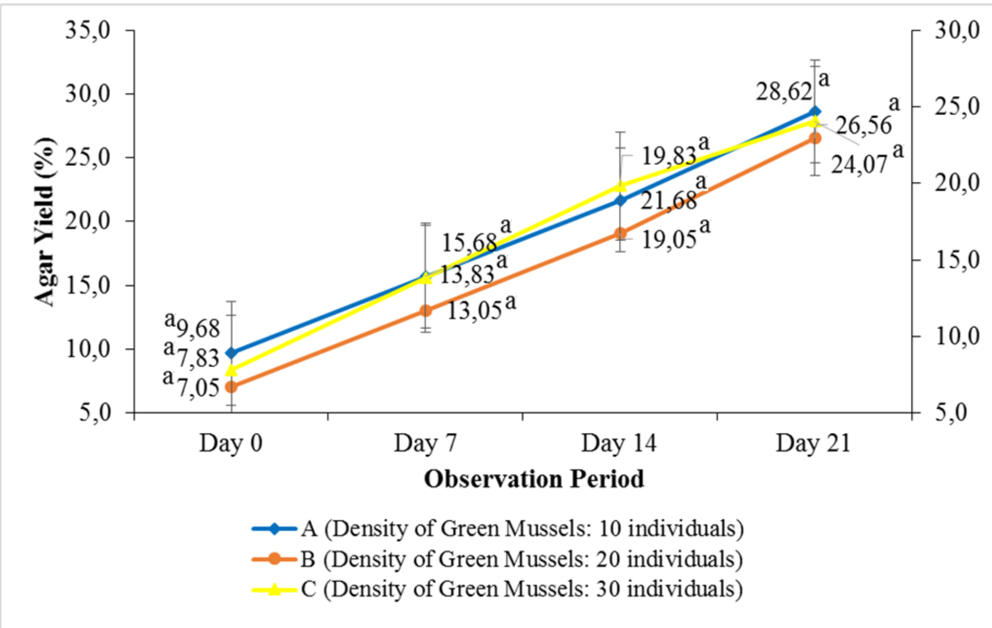


Figure 4. Agar ... treatments based on

This s... *...aria* highlights the potential for optimizing integrated aquaculture systems. Although the results did not show significant differences in agar yield, this may indicate *Gracilaria's* ability to adapt to a wide range of environmental conditions. The trends observed align with previous studies emphasizing the importance of nutrient availability and competition in influencing productivity. Large ponds offer a controlled environment to study these interactions, enhancing the understanding of species dynamics in aquaculture systems (Ajjabi et al., 2018). The findings show that lower mussel densities result in slightly higher agar yields, likely due to reduced competition for nutrients and improved light penetration, which is essential for the photosynthetic efficiency of *Gracilaria* (Anh et al., 2023). Additionally, the gradual increase in agar yield over time reflects natural growth and the accumulation of secondary metabolites, key factors influencing agar quality and quantity.

Recent advancements in integrated multitrophic aquaculture (IMTA) have outlined ecological benefits, particularly when combining seaweed and bivalve farming. Green mussels act as efficient biofilters, removing suspended organic particles from water, reducing pollution, and indirectly creating favorable conditions for *Gracilaria* growth. The density-dependent effects observed in this study highlight the importance of balancing species interactions to optimize productivity while maintaining ecological integrity (Stenton-Dozey et al., 2021).

The adaptability of *Gracilaria* in diverse aquaculture conditions emphasizes its potential as a renewable source of agar. Increased productivity in systems with lower mussel densities supports findings on the synergistic effects of integrating seaweed and bivalve farming, where nutrient recycling plays a crucial role. These insights are essential for scaling up IMTA, particularly in regions where *Gracilaria* serves as a primary agar source (Peng et al., 2018). Although this study did not reveal statistically significant differences in agar yield among treatments, the observed trends provide valuable insights for optimizing integrated aquaculture practices. Future research should focus on exploring the mechanisms underlying species interactions and evaluating the scalability of these findings in commercial aquaculture settings.

The lack of significant differences in this study, particularly in the agar yield of *Gracilaria verrucosa*, may be attributed to several factors. *Gracilaria* exhibits a high degree of adaptability to environmental conditions, which may have allowed it to maintain stable productivity despite variations in green mussel density. Additionally, the brackish water pond system used in this study may have had sufficient nutrient availability, minimizing the competitive effects of mussel density on seaweed growth. Another contributing factor is the relatively short duration of the study, which lasted less than one month due to transitional seasonal changes and tidal flooding (banjir rob) during the research period. Environmental instability, including fluctuations in temperature, salinity, and extreme tidal variations, likely influenced *Gracilaria*'s growth and introduced natural variability that masked the effects of the treatments. Therefore, future studies with longer experimental durations and more stable environmental conditions are recommended to further evaluate the impact of mussel density on agar production.

3.2.2 Viscosity (cps)

Distinct patterns are seen in the viscosity data analysis of *Gracilaria verrucosa* at different green mussel densities and time intervals. On day 0, there were no notable variations in viscosity amongst the treatments. Treatment A, consisting of 10 mussels, had a viscosity of 60.00 cps. Treatment B, containing 20 mussels, had a viscosity of 43.33 cps. Treatment C, with 30 mussels, had a viscosity of 40.00 cps (Figs.5). The absence of statistical significance ($p > 0.05$) at this preliminary stage indicates that mussel density does not exert a significant influence on the early viscosity of the seaweed. This phenomenon can be ascribed to the first stage of development, during which the physiological impacts of mussel density on *G. verrucosa* are not yet evident. On the seventh day, there were noticeable variations in viscosity, which were validated by ANOVA ($p < 0.05$) and further examined using Tukey's HSD test. Treatment A demonstrated the maximum viscosity at 120.00 cps, which was much higher than the viscosity of Treatment C, which was 55.00 cps. Treatment B, with a viscosity of 83.33 cps, had values that were between those of Treatment C and Treatment A, although there was no significant difference between Treatment B and Treatment A. The observed pattern indicates that when mussel densities are lower (Treatment A), there is a greater viscosity, possibly because there is less competition for nutrients and light.

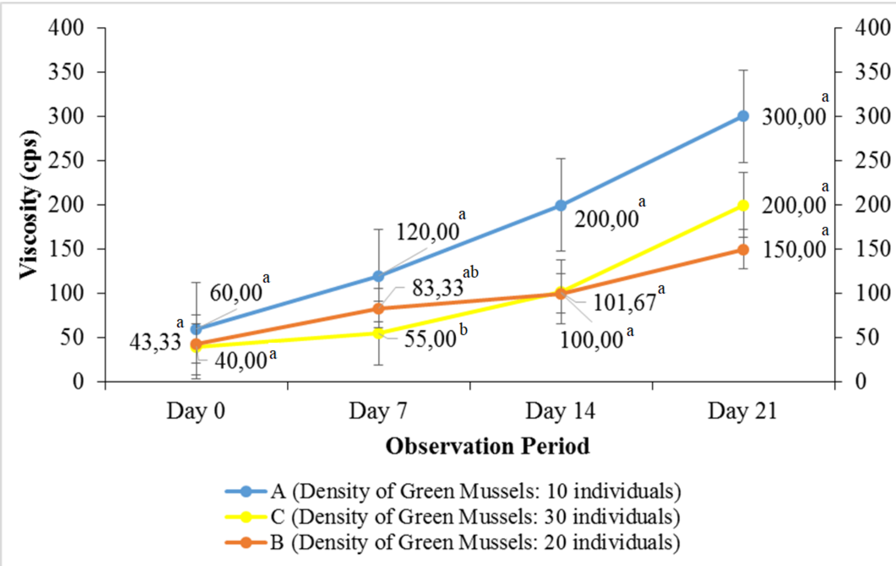


Figure 5. Viscosity (c[illegible]en treatments based on

The viscosity trends of *Gracilaria verrucosa* in relation to green mussel (*Perna viridis*) density reveal a complex interaction between nutrient availability and macroalgal physiology. On day 0, no significant differences in viscosity were observed across treatments, indicating that during the early stages of co-culture, *Gracilaria* retains stable biochemical properties. This finding aligns with studies suggesting that macroalgae often require acclimation periods to exhibit physiological responses to environmental changes (Neori et al., 2004). The similar viscosity levels observed at this stage likely reflect the minimal impact of mussel density during the initial cultivation phase.

By day 7, significant changes in viscosity emerged, with lower mussel density (Treatment A) correlating with higher viscosity. This supports the hypothesis that reduced competition for nutrients and light promotes optimal physiological performance in *Gracilaria*. Lower densities enable increased photosynthetic activity and polysaccharide synthesis, which are critical factors influencing viscosity (Reddy et al., 2017). Furthermore, the higher viscosity observed in Treatment A may be attributed to less nutrient depletion in the water column, ensuring greater resource availability for *Gracilaria's* metabolic processes (Stenton-Dozey et al., 2021). Interestingly, the moderate performance of Treatment B suggests that intermediate mussel densities provide a balance between nutrient competition and environmental stability. This underscores the critical role of density thresholds in co-culture systems for determining optimal outcomes.

In contrast, Treatment C, with the highest mussel density, exhibited significantly lower viscosity levels. This may result from nutrient competition between *Gracilaria* and mussels, which can limit resources and disrupt metabolic pathways responsible for producing high-viscosity polysaccharides (Peng et al., 2018). Additionally, shading caused by dense mussel populations could reduce photosynthetic efficiency in *Gracilaria*, impairing its ability to synthesize hydrocolloids that contribute to viscosity (Widowati et al., 2024). These findings highlight the importance of optimizing species density in co-culture systems to balance productivity and environmental stability. This study provides valuable insights into the temporal dynamics of *Gracilaria* viscosity under varying mussel densities, offering a foundation for advancing sustainable aquaculture practices. Further research is needed to investigate the biochemical pathways and environmental interactions influencing *Gracilaria's* viscosity, particularly in commercial-scale systems.

3.2.3 Gel Strength (g cm^{-2})

The experiment including different densities of green mussels and Grac*ilaria verrucosa* shows that the gel strength is not substantially influenced by mussel density, as evidenced by the ANOVA findings ($p > 0.05$). During the investigation, although there were numerical fluctuations, the gel strength values were generally stable across the varied mussel densities. On day 0, Treatment A measured a gel strength of 433.33 g cm^{-2} using 10 mussels. On the other hand, Treatments B (consisting of 20 mussels) and C (consisting of 30 mussels) had somewhat decreased values of 400.00 g cm^{-2} each (Figs.6). Based on the available data, it appears that the density of mussels does not have a major impact on the gel strength during the early stages of development. On the seventh day, Treatment A maintained the maximum gel strength at 500.00 g cm^{-2}, but Treatments B and C had gel strengths of 416.67 g cm^{-2} and 456.67 g cm^{-2}, respectively. While Treatment A exhibited the highest values, these disparities did not reach statistical significance. Treatment A exhibited the maximum gel strength of 550.00 g cm^{-2} on day 14, surpassing Treatment B's gel strength of 500.00 g cm^{-2} and Treatment C's gel strength of 430.00 g cm^{-2}. The ANOVA analysis consistently indicated that there were no statistically significant variations seen among the different treatments. On the 21st day, Treatment A exhibited the maximum gel strength of 600.00 g cm^{-2}, whereas Treatments B and C had values of 500.00 g cm^{-2} and 450.00 g cm^{-2}, respectively. No discernible disparities were identified.

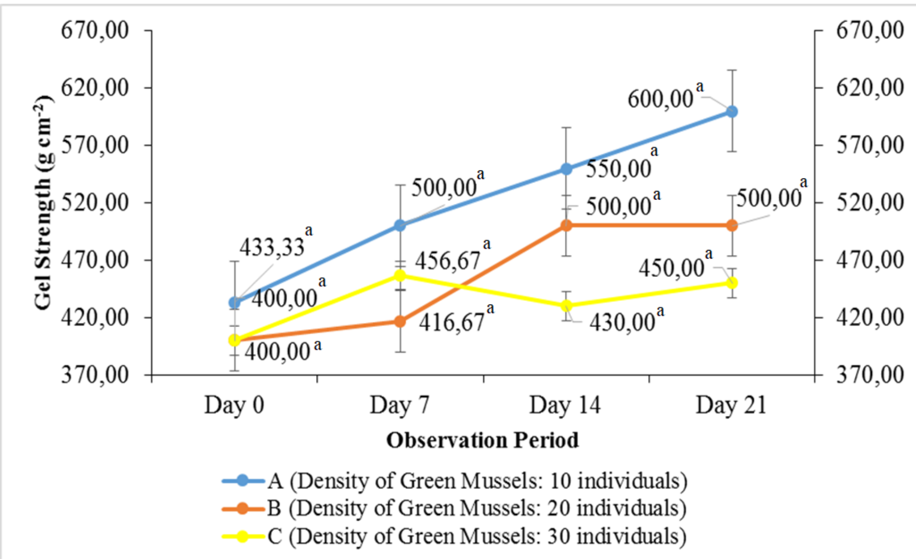


Figure 6. Gel Stren… between treatments

The relatio… ensity in co-culture systems highlights important physiological responses under varying environmental pressures. At day 0, no significant differences in gel strength were observed among treatments, suggesting that mussel density has minimal impact on the initial physical properties of *Gracilaria*. This stability aligns with findings by Nguyen et al. (2015), which indicated that macroalgal thalli require a lag period to adapt to stressors such as nutrient competition and shading. The biochemical composition responsible for gel strength remains unaltered during the initial cultivation phase, ensuring early-stage stability.

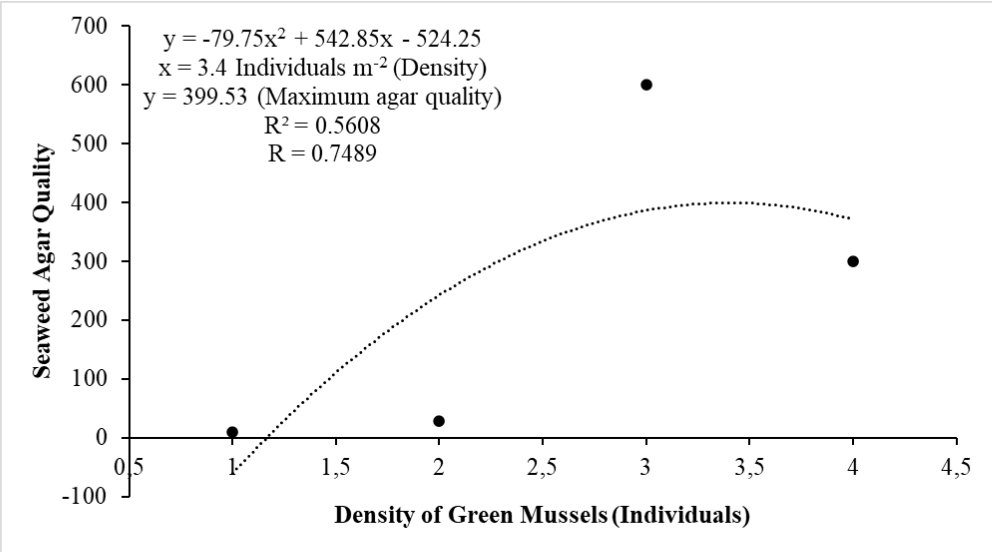
By day 7, the highest gel strength was recorded in Treatment A (lowest mussel density), although the differences were not statistically significant. This trend suggests that reduced mussel density minimizes competition for nutrients and light, enabling *Gracilaria* to focus on synthesizing hydrocolloids responsible for gel formation. These results are consistent with Peng et al. (2018), who found that macroalgal polysaccharide production is influenced by nutrient availability and environmental stress. In contrast, the reduced gel strength observed in higher-density treatments (B and C) may be due to nutrient depletion and shading effects caused by mussel activity, which disrupt critical metabolic pathways.

By day 14, the differences in gel strength became more pronounced, with Treatment A consistently outperforming higher-density treatments. This stage highlights the cumulative effects of resource availability on hydrocolloid accumulation, as suggested by Dellatorre et al. (2022). The superior gel strength in low-density systems supports the hypothesis that reduced interspecies competition optimizes conditions for producing agarose and related compounds critical to gel strength.

At day 21, Treatment A maintained its lead, achieving a peak gel strength of 600.00 $g \cdot cm^{-2}$. This sustained superiority underscores the importance of nutrient dynamics in co-culture systems. Syahrir et al. (2024) emphasized how mussel density influences water quality and nutrient flux, directly impacting *Gracilaria's* physiological performance. The diminishing gel strength in Treatments B and C further underscores the need for optimizing mussel density to balance ecosystem services with productivity in integrated aquaculture systems. This study provides valuable insights into the temporal and density-dependent variations in *Gracilaria verrucosa* gel strength under co-culture conditions with green mussels. Unlike previous research focusing mainly on growth metrics, this study highlights the physical properties of the harvested biomass, offering critical information for industries reliant on high-quality agar. Future research should explore the biochemical mechanisms linking environmental stressors to hydrocolloid synthesis and evaluate the scalability of these findings for commercial aquaculture applications.

3.3 Regression Seaweed Agar Quality and Green Mussel Density

The regression analysis illustrated in the graph depicts the relationship between green mussel density (individuals m^{-2}) and seaweed agar quality. The quadratic equation $y = -79.75x^2 + 542.85x - 524.25$ exhibits a parabolic trend, indicating that agar quality initially improves with increasing mussel density, reaches an optimal threshold, and subsequently declines. The estimated optimal mussel density for maximizing seaweed agar quality is $x = 3.4$ individuals m^{-2}, representing the peak of the regression curve. At this optimal density, the maximum agar quality achieved is 399.53**,** highlighting the importance of balancing mussel density to optimize hydrocolloid production. The coefficient of determination ($R^2 = 0.5608$) suggests that 56.08% of the variability in agar quality can be attributed to mussel density, while the correlation coefficient ($R = 0.7489$) reflects a moderate to strong positive association between these variables. These findings indicate the existence of an optimal mussel density that maximizes agar quality.



Fi ith

These findings are consistent with previous studies indicating that interactions among aquatic organisms play a crucial role in influencing product quality. For example, Yousefi et al. (2013) reported that processing parameters, particularly extraction time, significantly impacted the quality of agar obtained from *Gracilaria*. Although the focus of that study differed, its findings highlight the importance of environmental conditions and biotic interactions in determining the final quality of the product. In this context, the enhancement of agar quality at moderate green mussel densities may be attributed to the increased nutrient flow facilitated by the mussels' filtration activity. This enhanced nutrient availability supports the growth and secondary metabolite production of *Gracilaria verrucosa*. However, at excessively high mussel densities, competition for nutrients and space may arise, potentially inhibiting seaweed growth and diminishing agar quality. These results indicate the existence of an optimal green mussel density that maximizes the quality of agar derived from *Gracilaria verrucosa*.

3.4 Lead Heavy Metal Removal Rate (mg L^{-1} day^{-1})

The investigation of lead removal rates by *Gracilaria verrucosa* under different densities of green mussels demonstrates that the effectiveness of lead removal is not substantially influenced by mussel density on Day 7, Day 14, and Day 21 ($p > 0.05$). Treatment A, consisting of 10 mussels, had a lead elimination rate of 0.0124 mg L^{-1} on Day 7, which subsequently fell to 0.0016 mg L^{-1} by Day 21. On Day 7, Treatment B (20 mussels) exhibited clearance rates of 0.0048 mg L^{-1}, which slightly decreased to 0.0044 mg L^{-1} by Day 21. The concentration of Treatment C decreased from 0.0062 mg L^{-1} on Day 7 to 0.0013 mg L^{-1} on Day 21, with 30 mussels being used in the treatment (Figs.7). Although there are numerical differences, the absence of statistical significance suggests that changes in green mussel density do not significantly affect the effectiveness of lead removal. *G. verrucosa* is renowned for its capacity to bioaccumulate and excrete heavy metals, such as lead, from aquatic habitats. The efficacy of lead removal is influenced by factors such as the growth rate of seaweed, the availability of nutrients, and the interactions with related species such as green mussels. While the density of green mussels may have an impact on nutrient dynamics and competition, the rates of lead removal that were found were similar regardless of the mussel densities. These findings indicate that the main factors influencing the removal of lead are most likely the physiological traits of the seaweed and the overall ambient circumstances, rather than just the density of mussels.

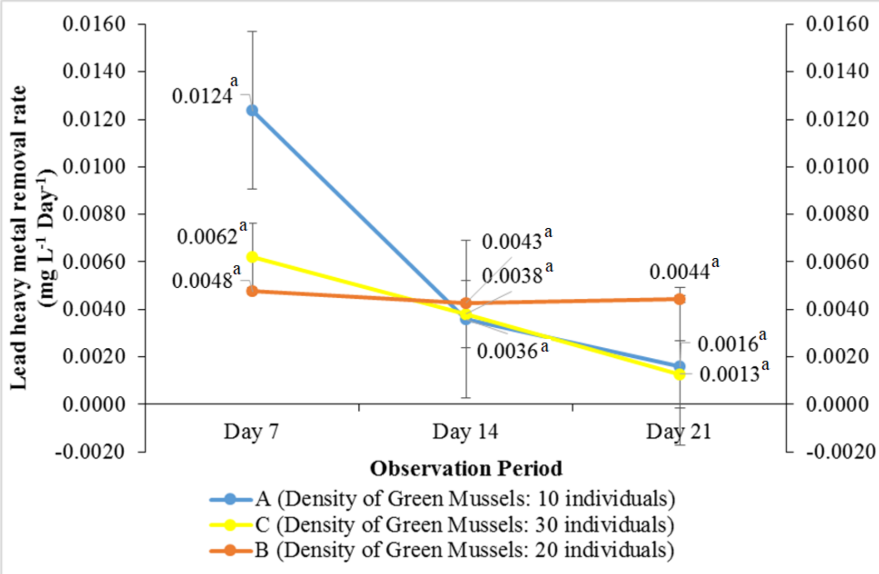


Figure 8. Lead Hea… indicate significant differences betw… dard error (SE).

The study findi… *sa* is not significantly affected by variations in green mussel density, as indicated by consistent ANOVA results across multiple time points. On Day 7, numerical differences were observed, with Treatment A (the lowest mussel density) exhibiting the highest initial Pb removal rate at 0.0124 mg L^{-1}. However, these differences were not statistically significant. This outcome reinforces the idea that *Gracilaria verrucosa* primarily relies on its intrinsic bioaccumulative properties for metal uptake, with minimal influence from co-cultivation factors such as mussel density (Widowati et al., 2024).

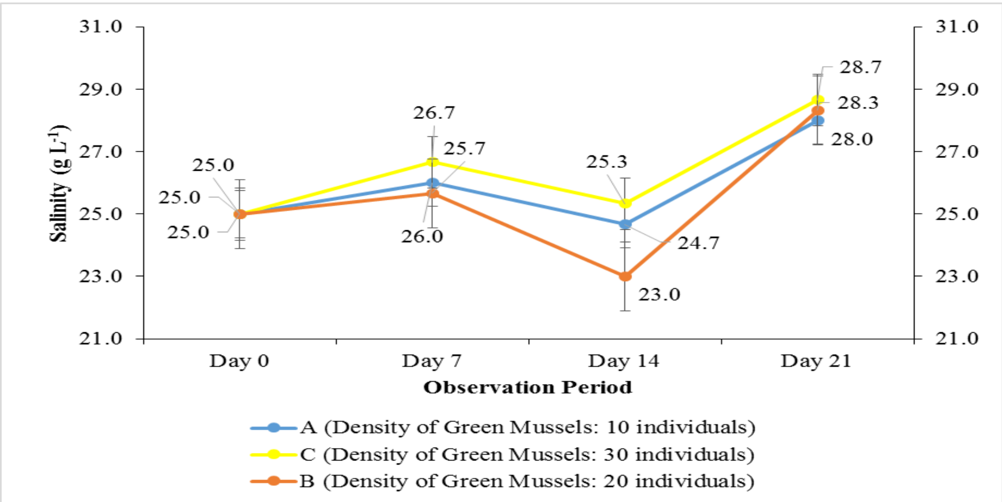
By Day 21, all treatments showed a gradual decline in Pb removal efficiency, indicating the natural physiological saturation of *Gracilaria*'s metal uptake capacity. This decline suggests that *Gracilaria* may have reached its maximum metal absorption threshold, where the available binding sites on cell wall polysaccharides became fully occupied. Once saturation occurs, its ability to continue Pb absorption diminishes, leading to a reduction in removal rates across all treatments. This pattern aligns with previous studies on macroalgal bioaccumulation, which highlight that metal uptake follows a saturation curve rather than a continuous linear increase. Interestingly, despite its higher mussel density, Treatment C (30 mussels) exhibited a decline similar to that of Treatment A (10 mussels), further suggesting that mussel density has little impact on heavy metal bioaccumulation in *Gracilaria*. These findings are in accordance with Kim et al. (2014), who demonstrated that the biofiltration efficiency of *Gracilaria* is predominantly influenced by environmental conditions and its metabolic activity rather than density-induced interactions.

The consistent Pb removal efficiency across treatments underscores the fundamental role of *Gracilaria verrucosa*'s physiological mechanisms in metal uptake. As an efficient bioaccumulator, *Gracilaria* sequesters heavy metals through cell wall polysaccharides, which act as natural binding sites for contaminants. However, an additional factor that may contribute to Pb cycling in the system is mussel excretion. While green mussels primarily function as biofilters, they also release metabolic byproducts that can influence the chemical behavior of heavy metals in the water column. Mussel excretion may alter the speciation of Pb, facilitating its redistribution between dissolved and particulate phases. Similar mechanisms have been documented in other filter-feeding bivalves, where excreted waste products can modulate metal bioavailability. Although this study did not directly quantify the impact of mussel excretion on Pb cycling, future research should explore its role in influencing heavy metal dynamics in aquaculture systems. Additionally, the co-cultivation of green mussels may indirectly enhance water quality, thereby creating optimal conditions for *Gracilaria*'s bioaccumulation processes (Anh et al., 2023).

This study provides a novel perspective by investigating the combined effects of macroalgal physiology and mussel density on Pb remediation in aquaculture systems. Unlike prior research focusing on individual species or fixed-density setups, this study highlights the minimal influence of mussel density while reinforcing *Gracilaria*'s dominant role in Pb uptake. However, to comprehensively evaluate the effectiveness of *Gracilaria* in Pb bioremediation, it is essential to compare its performance against other widely used remediation methods, such as activated carbon and biochar. While macroalgae offer a sustainable and environmentally friendly alternative, materials like activated carbon and biochar possess higher adsorption capacities and are extensively used in wastewater treatment. A comparative analysis between these approaches could provide valuable insights into the efficiency, cost-effectiveness, and scalability of *Gracilaria*-based remediation strategies in commercial aquaculture settings. These findings align with emerging research emphasizing species-specific bioaccumulation mechanisms over interspecies competition in integrated aquaculture systems (Syahrir et al., 2024).

3.5 Salinity of the Cultivation Water for *G. verrucosa*

The salinity levels observed during the growth of *Gracilaria verrucosa* under different densities of green mussels were consistently steady with little variations throughout the investigation. On Day 0, the salt level was consistently recorded at 25.0 g L^{-1} for all treatments, suggesting that the beginning circumstances were consistent and unlikely to affect the growth and production results that followed. On the seventh day, there was a modest rise in salinity across the different treatments. Treatment A had a salinity of 26.0 g L^{-1}, Treatment B had a salinity of 25.7 g L^{-1}, and Treatment C had a salinity of 26.7 g L^{-1} (Figs.8). The study recorded a consistent salinity level of 25.0 g L^{-1} at the start of the experiment across all treatments, highlighting the controlled conditions under which it was conducted. This uniformity is crucial in ensuring that any variations in the growth or production of *Gracilaria verrucosa* can be directly linked to treatment effects rather than external environmental factors. According to Kim et al. (2014), maintaining stable salinity levels in aquaculture systems is essential for accurately studying species interactions and their impacts on water quality.



By Day 7, a slight increase in salinity was observed across treatments. Treatment A (10 mussels) recorded a salinity of 26.0 g L^{-1}, Treatment B (20 mussels) measured 25.7 g L^{-1}, and Treatment C (30 mussels) reached 26.7 g L^{-1}. This small variation can be attributed to the metabolic activities of green mussels, such as excretion and filtration, which can subtly alter water composition. Banik et al. (2023) observed that bivalves in integrated aquaculture systems contribute to salinity changes by influencing the ionic balance in the surrounding water, a process that becomes more pronounced with higher stocking densities.

The slight rise in salinity in higher-density treatments may have implications for *Gracilaria verrucosa's* physiological responses. This species is known for its adaptability to a range of salinity conditions, and the observed levels remained within the optimal range for its metabolic processes and nutrient uptake. Widowati et al. (2024) emphasized that such stability, even under different densities of co-cultivated species, demonstrates the resilience of *Gracilaria*, making it an ideal candidate for integrated multi-trophic aquaculture (IMTA) systems. The significance of this study lies in its exploration of how bivalve density influences salinity modulation in IMTA systems. Unlike earlier research, which primarily focused on nutrient cycling, this study sheds light on the role of green mussel density in shaping salinity dynamics and ensuring the stability of aquaculture environments. These findings are particularly relevant for regions prone to salinity fluctuations caused by environmental factors like evaporation, which can significantly affect aquaculture productivity.

4. CONCLUSIONS

This study reveals a significant impact of mussel density on the daily specific growth rate (DSGR) of *Gracilaria verrucosa* on days 7, 14, and 21. Initially, lower mussel density (10 mussels) resulted in higher growth rates, though these benefits diminished over time. However, no significant differences were found in agar yield, viscosity on days 0, 14, and 21, gel strength, or lead removal rates across different mussel densities. Water quality, specifically salinity, remained within acceptable conditions for seaweed growth throughout the study. The practical implications of these findings suggest that optimizing mussel density in aquaculture systems can enhance early-stage growth of *Gracilaria verrucosa*. To improve agar quality and extend the positive effects on biomass production, further research is needed with longer trial periods, larger pond sizes, and deeper exploration of multi-metal remediation potential. Longer trials will allow for the observation of long-term effects of mussel density on environmental dynamics and *Gracilaria* productivity. Future studies should also investigate the cumulative impact of mussel density on water quality and remediation capacity, especially in terms of heavy metal reduction in aquaculture systems.

AUTHOR CONTRIBUTIONS: Author Andi Rahmad Rahim designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author Dwi Retnaningtyas utami managed literature review, planning additional experiments, and manuscript revisions.. Author Rosmarlinasiah managed data collection, statistical analysis, and writing of the methodology section, Author Rahmad djumadi managed field data collection, preliminary data analysis, and contribution to the writing of the results section. Author Slamet Asari managed to development and refinement of the theoretical model, as well as contribution to the discussion section. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Acknowledgements : This research was supported by the Ministry of Higher Education, Science, and Technology of the Republic Indonesia. We would like to express our sincere gratitude for their financial assistance, which made this study possible. Their continued commitment to advancing research and development is deeply appreciated.

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