Original Research Article

DENSITY OF GREEN MUSSELS (*Perna viridis*) AND ITS IMPACT ON GROWTH AND LEAD ACCUMULATION IN POLYCULTURE SYSTEMS IN TROPICAL WATERS

ABSTRACT

Aims: Green mussels (*Perna viridis*) are filter-feeding organisms known for accumulating heavy metals, including lead (Pb), in their tissues, making them valuable bioindicators of water quality.

Study design: This study aimed to assess the impact of different stocking densities on the growth and lead accumulation in *Perna viridis*.

Place and Duration of study: This study was conducted at a traditional pond in Ujungpangkah Subdistrict, Gresik, East Java, over a period of 21 days, from June 27 to July 18, 2024.

Methodology: A Completely Randomized Design (CRD) was employed with three treatment groups: Treatment A (10 mussels/m²), Treatment B (20 mussels/m²), and Treatment C (30 mussels/m²). The parameters measured included the absolute flesh width, daily growth rate, and lead content in the mussels.

Result: Statistical analysis using Analysis of Variance (ANOVA) revealed no significant differences in the daily growth rate among the treatments ($p > 0.05$), suggesting that stocking density did not significantly influence growth. However, significant differences in the absolute flesh width were observed, with Treatment C showing the largest growth at higher density. Additionally, lead accumulation increased with stocking density, with mussels in Treatment C accumulating the highest levels of lead (1.14 mg/L). These findings highlight the influence of stocking density on mussel growth and lead bioaccumulation, underscoring the need for effective management in aquaculture practices to minimize environmental contamination risks while maintaining sustainable mussel production.

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Keywords: Green mussels, stocking density, lead accumulation, growth, bioindicator

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1. INTRODUCTION

Green mussels (*Perna viridis*) are a highly valued seafood commodity, prized by consumers for their delicious Flesh and rich nutritional content. According to Eshmat et al. (2014), green mussels contain 40% water, 21.9% protein, 14.5% fat, 18.5% carbohydrates, and 4.3% ash, making them an excellent source of nutrition for human consumption. These qualities make green mussels a highly profitable commodity, both economically and in terms of health benefits.

Green mussels are filter-feeding organisms, meaning they filter microscopic particles from the water as their food source. As noted by Eshmat et al. (2014), the filter-feeding nature of green mussels makes them susceptible to accumulating heavy metals, including lead (Pb), which can build up in their tissues during the filtration process. Due to their limited mobility as filter feeders, green mussels can accumulate heavy metals over time, making them an important bioindicator for monitoring water quality (Bervoets et al., 2016). Lead accumulation in green mussels should be closely monitored, especially considering the potential health risks to consumers if the mussels become contaminated with heavy metals.

One of the factors that supports the success of green mussel farming is their relatively fast growth rate. According to Acosta et al. (2009), green mussels can reach marketable size in a short period, making them an attractive and low-cost commodity to farm. Mussel farming also has minimal environmental impact, as it does not degrade water quality, and is considered an environmentally friendly aquaculture activity (Ellis et al., 2002; Shumway et al., 2003). This farming method can be sustainably practiced, aligning with principles of sustainable aquaculture (Costa-Pierce, 2008).

Polyculture is an aquaculture method that integrates multiple fishery species within a single ecosystem, allowing for more efficient use of natural resources and reducing disease risks. Polyculture systems involving green mussels, milkfish (*Chanos chanos*), and vannamei shrimp (*Litopenaeus vannamei*) are widely practiced in tropical waters, including in Indonesia (Soon & Ransangan, 2014). In Pangkah Kulon, Ujungpangkah, Gresik, East Java, the area's favorable water conditions, such as adequate depth, stable salinity, and nutrient-rich content, make it an ideal location for developing green mussel farming through polyculture. Additionally, the local community has long been engaged in polyculture practices involving various aquaculture commodities, which suggests promising prospects for integrating green mussels into these systems.

Despite the region's significant potential, research on the morphometric characteristics of green mussels cultivated in polyculture systems in Pangkah Kulon remains limited. According to Wibowo and Handayani (2017), understanding the morphometric characteristics of green mussels, such as length, width, and weight, is crucial for selecting the right seedstock and determining the optimal harvest time. The morphology of green mussels can be influenced by environmental factors such as temperature, salinity, depth, and water quality, all of which can affect mussel growth and quality.

The primary aim of this study is to examine the effects of green mussel (*Perna viridis*) density on growth and lead accumulation within an extensive polyculture system in Pangkah Kulon, Ujungpangkah, Gresik. This research aims to provide deeper insights into the morphometric characteristics of green mussels cultivated in a polyculture system, as well as to understand the relationship between environmental factors and mussel growth. The results of this study are expected to contribute significantly to the management and development of sustainable green mussel farming in the Pangkah Kulon area and surrounding regions, while enhancing our understanding of how polyculture systems influence the quality and sustainability of green mussel production.

2. MATERIAL AND METHODS

2.1 Time and Location

This study was conducted at a traditional pond in Ujungpangkah Subdistrict, Gresik, East Java, over a period of 21 days, from June 27 to July 18, 2024. The research site was selected due to its favorable aquatic conditions for cultivating green mussels (*Perna viridis*). Additionally, this area has a long history of aquaculture practices involving polyculture, making it highly relevant to the objectives of this study. Polyculture, which integrates various species within a single system, is known to enhance the efficient use of natural resources and optimize fishery production (Wibowo & Handayani, 2017; Subandar, 2019). Therefore, the chosen location is well-suited to explore the potential for developing green mussel farming within such a system. For the measurement of lead (Pb) heavy metal content in the green mussels (*Perna viridis*), samples were analyzed at the Integrated Laboratory of Universitas Trunojoyo Madura, located in Bangkalan, East Java Province.

2.2 Experimental Design

This study employed a Completely Randomized Design (CRD) with three different treatments to assess the density of green mussels (*Perna viridis*), which were as follows:

Treatment A: Mussel density of 10 individuals/m²

Treatment B: Mussel density of 20 individuals/m²

Treatment C: Mussel density of 30 individuals/m²

Figure 1. Research Design

Each treatment was replicated three times, resulting in a total of nine experimental units. For each replicate, three green mussel samples were randomly selected to measure growth, length, and lead content. Testing the treatments with varying mussel densities is crucial for evaluating the effects of density on the growth and quality of green mussels, as excessively high densities may impact metabolism and water quality in the pond (Sutanto, 2020; Nurhidayati, 2021).

2.3 Observation Parameters

- Absolute Width of Green Mussel Flesh (cm)

According to Effendi (2003), the formula for the absolute width of green mussel Flesh is as follows:

PM = PLt – PL0

Where:

PM : Absolute Width (cm)

PLt : Final Total Mussel Flesh Width (cm)

PL0 : Initial Total Mussel Flesh Width (cm)

- Daily Growth Rate of Green Mussel Flesh (%/Day)

According to Setyobudiandi, I., & Hariati, T. (2018), the formula to calculate the daily growth rate of mussels is as follows:

DGR = [Ln (Final Flesh Weight) – Ln (Initial Flesh Weight)] / T

Where:

DGR : Daily Growth Rate (%/Day)

Ln (Final Flesh Weight) : Natural logarithm of the final mussel Flesh weight (g)

Ln (Initial Flesh Weight) : Natural logarithm of the initial mussel Flesh weight (g)

T : Observation Time (Days)

- Lead Content in Green Mussels (mg/L)

The Atomic Absorption Spectrophotometry (AAS) method was employed to evaluate the lead content in both the Flesh and shells of green mussels (*Perna viridis*). The mussel samples were separated into shell and Flesh components for analysis. Both components were dried in an oven at a temperature of 60–70°C until a constant weight was achieved. After drying, the samples were ground into a fine powder. Concentrated nitric acid (HNO_3) was then used to weigh and digest the sample powder until the solution became clear. The resulting solution was analyzed using AAS to measure the lead concentration at a specific wavelength (Effendi, 2003; Murtini et al., 2017).

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2.4 Statistical Analysis

Analysis of Variance (ANOVA) is utilized for statistical data analysis in this study, aiming to identify which treatment causes significant differences in the tested parameters, namely absolute width and daily growth rate. Meanwhile, lead content is assessed descriptively based on supporting literature.. The objective of this analysis was to assess whether different density treatments had a significant impact on the parameters studied ($P < 0.05$). If the ANOVA results indicated significant differences, a post-hoc Tukey test would be performed to identify which treatments had a significant effect. All statistical analyses were conducted using IBM SPSS Statistics version 20.0. ANOVA was selected because this method is effective in detecting significant differences among more than two treatment groups (Subandar, 2019).

3. RESULTS AND DISCUSSION

3.1 Daily Growth Rate of Green Mussel Flesh (%/Day)

(SGR) of green mussels (*Perna viridis*) at 7, 14, and 21 days. The purpose was to assess whether there were any significant differences in growth across different stocking densities (10, 20, and 30 mussels per square meter). The results from the ANOVA showed no significant differences in the growth rates between the different treatments at all observation points ($p > 0.05$). This indicates that mussel density, whether 10, 20, or 30 individuals per square meter, did not significantly impact the daily growth rate of the mussels during the study. In other words, despite varying densities, this factor did not appear to have a marked effect on mussel growth across the three observation periods.

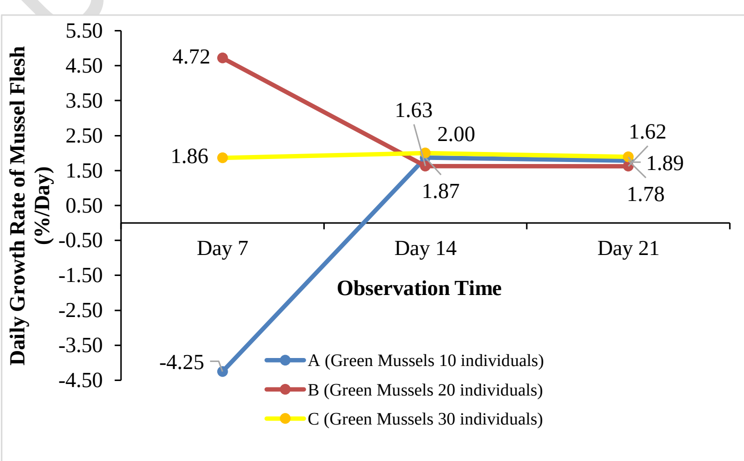

Figure 2. Daily Growth Rate of Mussel Flesh (%/Day) The analysis of variance (ANOVA) was conducted to examine the daily growth rate

On Day 7, Treatment A (10 mussels/m²) showed a negative growth rate of -4.25%, reflecting a decrease in mussel weight during the early phase of cultivation. This negative value, despite differences in growth rates between treatments, can be attributed to the stress caused by environmental acclimatization. At this point, the mussels were still adjusting to their new environment (Sugianto et al., 2020). Similar findings have been observed by other researchers, such as Agustin et al. (2018), who reported that early-stage mollusks often experience weight loss as they adapt to changes in environmental factors, including water quality and handling stress.

Treatments B (20 mussels/m²) and C (30 mussels/m²) recorded positive SGR values of 4.72% and 1.86%, respectively. These positive values indicate that, even at higher stocking densities, the mussels were able to adapt and begin showing stable growth. The lower SGR in Treatment A could be due to additional stress factors, such as limited space, which can negatively affect early growth. This observation is consistent with findings by Wen et al. (2019), who noted that higher stocking densities can lead to competition for resources, potentially limiting growth. In contrast, lower densities may reduce competition for food and space, enabling the mussels to grow more effectively in the early stages of cultivation.

By Day 14, all treatments showed improvements in their SGR, with Treatment A at 1.87%, Treatment B at 1.63%, and Treatment C at 2.00%. Despite the absence of significant differences based on the ANOVA, Treatment C exhibited a slightly higher growth rate. This could suggest that mussels in the higher-density group (30/m²) had begun to acclimate better to their environment and were growing slightly faster than those in lower-density treatments. The enhanced growth in Treatment C may also reflect improved efficiency in resource utilization, as the mussels adjusted to the polyculture system (Wibowo & Handayani, 2017). Similar trends were noted by Nguyen et al. (2019), who suggested that mussels in higher-density environments can more effectively utilize available space and nutrients after they have acclimated.

By Day 21, growth rates in all treatments had stabilized, with Treatment A at 1.78%, Treatment B at 1.62%, and Treatment C at 1.89%. These results indicate that, after the initial phase of adaptation, the growth rates of the mussels leveled out, regardless of density. This trend is consistent with findings from Hamzah et al. (2021), who observed that growth rates in polyculture systems tend to stabilize after an initial adjustment period, provided that the environmental conditions (e.g., water quality, food availability) remain optimal. The small variations between treatments in the final observation may reflect natural individual differences in growth response to environmental conditions, as highlighted by Kurniawan et al. (2018), who pointed out that mussels tend to exhibit more consistent growth in a stable environment, regardless of the stocking density. That mussel density did not significantly influence overall growth during the study period. However, early adaptation stress and competition for space may have influenced initial growth patterns. Once the mussels had acclimatized to their environment, their growth stabilized, demonstrating that green mussels are resilient to density variations as long as environmental factors such as water quality are adequately managed. These results are consistent with the work of Lestari et al. (2017), who found that mussels in well-managed polyculture systems can achieve similar growth rates, even at higher densities, after overcoming the initial adaptation phase. Future research could explore the long-term impacts of density on mussel growth, reproductive success, and disease resistance, helping to better understand the sustainability of green mussel farming under varying density conditions.

3.2 Absolute Flesh Width of Mussels (cm)

The analysis of the absolute Flesh width of green mussels (*Perna viridis*) through ANOVA revealed significant differences ($P < 0.05$) across different stocking densities at Day 7, 14, and 21. This suggests that stocking density plays a crucial role in influencing the body width growth of mussels over time. Treatment C, which had the highest stocking density of 30 individuals/m², showed significant differences compared to Treatment A (10 individuals/m²). However, Treatment B (20 individuals/m²) did not exhibit significant differences when compared to either Treatment A or C ($P > 0.05$). This suggests that while stocking density impacts growth, the increase in density may not always lead to proportional changes in growth, especially at moderate stocking densities.

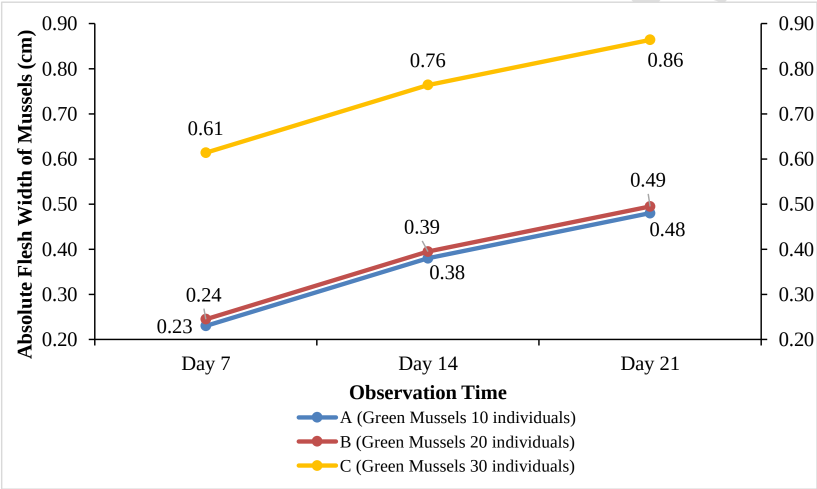

Figure 3. Absolute Flesh Width of Green Mussel (cm)

At Day 7, mussels in Treatment C exhibited a notably larger body width (0.61 cm) compared to those in Treatment A (0.23 cm) and Treatment B (0.24 cm). This finding underscores the influence of stocking density on growth during the initial stages. Mussels grown in higher-density conditions (Treatment C) appeared to develop faster, which could be attributed to competitive interactions. At higher densities, individuals are more likely to experience competition for space and food, which could lead to increased growth rates as they adapt to these competitive pressures. Such responses are commonly referred to as competitive stress, where mussels accelerate growth to outcompete others for resources. This phenomenon has been documented in other studies, where mussels in denser environments exhibited higher growth rates due to intensified competition (Feng et al., 2020; Sutanto, 2020).

Treatment B, with a moderate density of 20 individuals/m², did not show a significant difference in growth when compared to either Treatment A or C at Day 7. Although mussels in Treatment B grew slightly faster than those in Treatment A, this difference was not statistically significant. This suggests that the intermediate stocking density may not create the same level of competitive stress that promotes rapid growth in higher-density systems. Therefore, moderate densities may not always provide a significant growth advantage over lower or higher densities, possibly due to less intense competition for resources (Liu et al., 2019).

By Day 14, the body width of mussels in Treatment C remained significantly larger (0.76 cm) compared to both Treatment A (0.38 cm) and Treatment B (0.39 cm), although the difference in growth rate was less pronounced than at Day 7. This suggests that while stocking density influences early growth, the effect may diminish as mussels continue to grow. As mussels mature, the rate of growth may slow down, possibly due to factors such as limited available resources or reduced competitive pressures. This is consistent with other research, which suggests that the growth rate of marine bivalves typically slows after the initial growth phase as resources become more evenly distributed or as environmental conditions stabilize (Sun et al., 2021).

At Day 21, the trend observed earlier continued, with mussels in Treatment C still showing significantly larger body widths (0.86 cm) compared to those in Treatments A (0.48 cm) and B (0.49 cm). The persistent growth advantage for Treatment C suggests that higher stocking densities continue to facilitate faster development, especially during the early growth phases. However, this advantage is contingent on optimal environmental conditions, such as good water quality and adequate food availability. The results indicate that high stocking densities could be beneficial for maximizing mussel growth in aquaculture systems, particularly when the goal is to achieve rapid growth in a shorter period.

The findings of this study emphasize the complex relationship between stocking density, environmental conditions, and mussel growth. While higher stocking densities can drive faster growth due to competitive interactions, this approach is highly dependent on maintaining favorable environmental conditions. Specifically, water quality and food availability must be optimized to prevent growth inhibition due to overcrowding or resource depletion. As noted by Subandar (2020), ensuring high water quality and adequate food resources is essential in higher-density systems to support continued mussel growth without leading to adverse effects such as stunted growth or mortality. These findings are in agreement with other studies, which highlight the importance of maintaining optimal environmental conditions to allow mussels to thrive under higher stocking densities (Sutanto, 2020; Subandar, 2020). That higher stocking densities (30 individuals/m²) are more advantageous for promoting the growth of *Perna viridis*, particularly during the early stages. The competitive interactions fostered by higher densities may drive faster growth, but this effect is conditional upon the provision of suitable environmental conditions. Future research should explore the long-term effects of varying stocking densities on mussel growth and survival, as well as the broader ecological implications of such practices in sustainable aquaculture. The findings underscore the need for careful management of stocking density in commercial mussel farming to optimize growth while maintaining ecological balance and ensuring the sustainability of mussel populations.

3.3 Lead Content in Mussel Flesh (mg/L)

The results of this study revealed significant changes in the accumulation of lead (Pb) in the flesh of *Perna viridis* mussels. On the first day, mussels at a density of 10 individuals/m² (Treatment A) had a lead content of 0.87 mg/L, while those at densities of 20 individuals/m² (Treatment B) and 30 individuals/m² (Treatment C) showed higher lead concentrations of 0.95 mg/L and 1.14 mg/L, respectively.

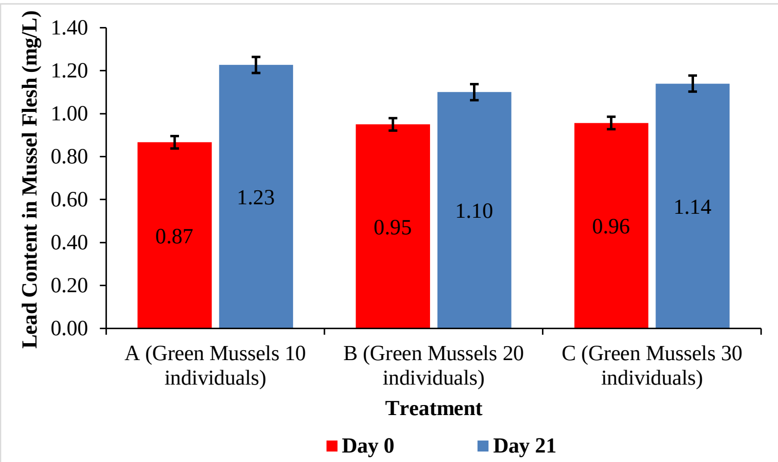

Figure 4. Lead Content in Mussel Flesh (mg/L)

These findings indicate that increased population density can enhance mussels' exposure to heavy metals, leading to higher lead accumulation in their tissues. Several factors can explain the increased lead concentration at higher population densities. The limited space in high-density environments or the decline in water quality due to increased metabolic activity in the mussels can exacerbate environmental stress, causing mussels in these conditions to remain in contact with contaminated water for longer periods. This prolonged exposure likely contributes to higher levels of lead accumulation. Previous studies have shown that higher population densities negatively affect water quality, including increases in pollution and decreases in dissolved oxygen levels, ultimately leading to the bioaccumulation of heavy metals in aquatic organisms (Sutanto, 2020). Furthermore, research by Tampubolon et al. (2013) emphasized that mussels at higher densities are more susceptible to contaminants from agricultural and industrial waste, which accumulate more rapidly in their tissues.

The lead content underwent significant changes after 21 days. After the treatment with a density of 20 individuals/m² (B), the lead concentration in the mussels dramatically decreased to 0.16 mg/L. This reduction suggests that mussels have mechanisms to regulate and reduce the accumulation of lead over time. Various factors may contribute to this decline, such as enhanced metabolic processes aimed at mitigating the impact of heavy metal accumulation through detoxification mechanisms. Alternatively, reduced exposure to contamination could also be a factor, potentially through decreased filtration activity or reduced interaction with polluted water. The decrease in lead content at a density of 20 individuals/m² could also be due to physiological compensation, where mussels adapt to the more crowded environment and consequently reduce the absorption of heavy metals.

Wibowo and Handayani (2017), green mussels tend to accumulate heavy metals more slowly and at lower densities, where they have more space and less environmental stress. Despite the larger space in low-density settings, environmental factors such as lower water quality and slower metabolic rates may inhibit the accumulation of lead. This observation suggests that environmental conditions, including water quality and metabolic rate, play critical roles in the bioaccumulation process.

The significant reduction in lead content observed after 21 days in mussels at a density of 20 individuals/m² (Treatment B) highlights the mussels' ability to adapt through various biological mechanisms. An increased metabolic rate could facilitate the excretion of lead, while reduced exposure to contamination could result from changes in feeding patterns or decreased interaction with polluted water. These mechanisms likely reflect an adaptive strategy to minimize the harmful effects of heavy metal accumulation. Sutanto (2020) reported similar findings, stating that mussels at moderate densities tend to adjust better to environmental changes, reducing heavy metal accumulation due to improved metabolic efficiency.

Mussels at densities of 10 and 30 individuals/m² demonstrated more stable lead concentrations. This stability may result from physiological compensation at both low and high density levels. Mussels at low densities might absorb lead more efficiently but reach a saturation point more quickly, while mussels at high densities may experience inhibited metabolic processes due to overcrowding or increased competition for resources. These findings are consistent with the research of Tampubolon et al. (2013), who found a correlation between the accumulation of heavy metals, particularly lead, and environmental factors such as population density. Their study concluded that mussels exposed to polluted environments for extended periods accumulate higher concentrations of heavy metals.

Wibowo and Handayani (2017) highlighted that excessive mussel density could lead to a decline in water quality due to the accumulation of biological waste, exacerbating environmental conditions and facilitating the bioaccumulation of heavy metals. Furthermore, Hidayat et al. (2022) demonstrated that detoxification mechanisms in mussels could help reduce heavy metal accumulation over time, even when there are variations in density and environmental conditions. This suggests that mussels can regulate heavy metal accumulation through internal physiological processes, such as metal sequestration and the synthesis of detoxifying proteins like metallothioneins, which reduce the toxic effects of metals. Effective management of mussel density is crucial for optimizing mussel growth and minimizing the accumulation of heavy metals in their bodies. The reduction in lead content at a density of 20 individuals/m² suggests that controlling population density may offer a viable strategy for mitigating heavy metal accumulation in aquaculture settings. These results are supported by previous research, which emphasizes the importance of population density and environmental management in minimizing the risk of heavy metal contamination in aquatic organisms.

4. CONCLUSION

This study demonstrated that while stocking density did not significantly impact the daily growth rate of *Perna viridis* across all observation periods, it did influence the absolute flesh width, with significant differences observed between the highest density (30 mussels/m²) and the lowest (10 mussels/m²). Higher densities, particularly at 30 individuals/m², resulted in increased growth during the initial stages, likely due to competitive interactions for resources. Additionally, mussels at higher densities accumulated more lead (Pb) in their tissues, highlighting the role of population density in enhancing exposure to heavy metals. The increased bioaccumulation can be attributed to prolonged exposure to contaminated water and the decline in water quality associated with denser stocking conditions. These findings underscore the importance of managing stocking densities to balance growth and minimize environmental stress, particularly in aquaculture settings.

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