Effects of Plastic Debris on Macrofaunal Diversity in Green Mussel (*Perna viridis* Linnaeus, 1758) Bed Assemblages in the Northern Straits of Malacca

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Abstract

Mussel beds are vital ecosystem engineers that enhance coastal productivity and provide habitat for diverse macrofauna. Green mussels (*Perna viridis*) form dense aggregations that support rich macrofaunal communities, but these habitats are increasingly threatened by plastic debris, which can alter habitat structure and disrupt benthic ecosystems. Plastic pollution is a growing concern because it can modify habitat complexity, alter species interactions, and potentially disrupt benthic community structure and functioning.

This study aimed to assess the richness and abundance of macrofauna associated with green mussel beds. Seven replicates of polyvinyl chloride (PVC) platforms (50 × 50 cm) were set up, each containing 30 mussel specimens with layers of pre-weathered plastic bags, fishing lines, or without any plastics as a control. The platforms were enclosed by a plastic mesh to prevent specimen loss and deployed along the jetty of the Centre for Marine and Coastal Studies (CEMACS) in the northern Straits of Malacca. After six weeks, the platforms were collected, and the associated macrofauna were preserved in 70% ethanol for identification. A total of 3,622 individual organisms representing 68 species were recorded. The macrofauna community was dominated by Mollusca (33.88%), followed by Vertebrata (12.81%), Crustacea (5.99%), Porifera (1.93%), Echinodermata (0.52%), and Cnidaria (0.47%). While plastic debris did not significantly affect species richness and evenness, moderate amounts of film-type plastics were associated with higher richness, suggesting subtle effects on habitat complexity. These findings highlight the resilience of *Perna viridis* assemblages to short-term plastic exposure, while underscoring the need for longer-term studies.

Keywords: Straits of Malacca, Biodiversity, Coastal Waters, Green Mussel, Plastic Pollution

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Introduction

Mussel beds are globally recognized as ecosystem engineers that profoundly shape coastal habitats and enhance biodiversity. These biogenic structures play a crucial role in modifying benthic and littoral environments by providing essential substrates for attachment, refuge from predation, and complex three-dimensional habitat for a wide array of marine organisms (Asaduzzaman *et al*., 2025). Among the prominent mussel species, the green mussel *(Perna viridis)*, native to the tropical and subtropical Indo-Pacific, stands out for its significant contribution to coastal ecosystem functioning (Gardner *et al*., 2023). *P. viridis* beds enhance local biodiversity, promote nutrient cycling, and support diverse communities of associated macrofauna, including crustaceans, molluscs, polychaetes, and both sessile and mobile organisms (de Messano *et al*., 2024).

Recent studies highlight the ecological and economic importance of *Perna viridis*, noting its rapid growth, tolerance to a range of environmental conditions, and its ability to thrive in both native and introduced habitats (de Messano *et al*., 2024). The species is also recognized for its capacity to improve water quality through filtration and nutrient cycling, making it valuable in polyculture and integrated aquaculture systems (Setyarini and Adharini, 2022). Despite these benefits, *P. viridis* can also outcompete native species, alter community structure, and impact trophic relationships, especially in areas where it becomes invasive (de Messano *et al*., 2024).

In recent years, plastic debris has emerged as a significant modifier of coastal habitats, often interacting with biogenic structures like mussel beds. Due to their durability and varied shapes, plastic materials can increase the available surface area for colonization by marine organisms, effectively providing additional substrate for attachment and refuge (Wright *et al*., 2020). Organisms that settle on floating marine plastic can be transported across oceans and, in some cases, become invasive species in vulnerable ecosystems (Wright *et al*., 2020).

Additionally, the accumulation of plastics can create shading effects that reduce light availability in benthic zones, thereby limiting photosynthesis and potentially disrupting the composition and functioning of benthic communities (Mueller and Schupp, 2020).

Beyond shading, plastic debris can also cause direct physical impacts by smothering benthic habitats, including mussel beds, which may reduce oxygen exchange, inhibit feeding activity, and interfere with sediment-related processes (Oceana, n.d.). This dual role of plastics, both as a novel substrate for colonization and as a physical stressor, highlights the complex interactions between plastic pollution and mussel bed ecosystems, with significant implications for biodiversity, ecological functioning and habitat resilience.

Furthermore, coastal ecosystems worldwide face increasing threats from plastic pollution, which can alter habitat quality and negatively impact marine communities. Plastic pollution has emerged as a significant global concern, with an estimated 350 million metric tons of plastic waste generated annually. A considerable proportion of this waste originates from land-based sources and eventually enters the marine environment, contributing to the accumulation of approximately 51 trillion microplastic particles. These persistent materials can suffocate benthic habitats, reduce light penetration and oxygen availability, and disrupt key ecological processes such as nutrient cycling. Driven by the prevalence of single-use items, and inadequate waste management, plastic debris threatens marine life through ingestion, entanglement, and chemical exposure (Pilapitiya and Ratnayake, 2024).

Nonetheless, investigations into the biodiversity associated with *Perna viridis* bed assemblages in the northern Straits of Malacca remain limited. Although plastic debris is abundant in coastal waters, its specific influence on the richness, abundance, and community structure of macrofauna associated with *Perna viridis* beds remains poorly understood. Addressing this gap is essential for conservation and habitat restoration efforts, particularly in regions like the northern Straits of Malacca where plastic contamination coexists with important mussel bed habitats. Therefore, this study aims to assess the community composition of species inhabiting *P. viridis* bed.

Method

This study was conducted along the jetty at the Centre of Marine and Coastal Studies (CEMACS), Universiti Sains Malaysia, located in the northern Straits of Malacca. The site features coastal waters that support diverse marine ecosystems, with salinity ranging from 30 to 32 ppt and a temperature of approximately 30°C making it an ideal location for studying green mussel (*Perna viridis)* bed assemblages.

Experimental design

To investigate biodiversity within *Perna viridis* bed assemblages, a total of 35 submerged suspension platforms were deployed. Each platform was constructed using a 50x50 cm polyvinyl chloride (PVC) sheet as a base for mussel bed formation. The platforms were divided into three treatments, which were control (no plastic), film (20% and 40% pre-weathered plastic bags), and filament (20% and 40% pre-weathered fishing line) with seven replicates for each treatment. Plastic materials for the film (plastic bags) and filament (fishing lines) treatments were commercially sourced to standardize material properties, and subsequently pre-weathered in aerated seawater tanks for two weeks to allow biofilm development and simulate environmentally weathered plastic debris.

Each platform was assembled with 30 green mussel specimens, positioned between layers of the designated plastic materials in the film and filament treatments, while specimens in the control group remained unaltered. The platforms were enclosed within plastic mesh nets to prevent specimen loss during deployment. After seven days of aggregation in tanks, the mussels were transferred to the study site.

Deployment and retrieval

Due to spatial constraints at the jetty, not all platforms could be deployed simultaneously. The experimental platforms were deployed along the jetty at CEMACS, suspended at a depth of 3-4 meters during two seasonal periods in between April to June and September to November 2021, each for six weeks. During this timeframe, seawater temperatures generally ranged between 28°C to 31°C, with salinity consistently between 30 to 33 ppt. After the deployment period, all platforms were retrieved and transported to the laboratory for analysis. Upon arrival, the plastic mesh was carefully removed, and the green mussel clusters were gently rinsed with seawater to remove debris, while preserving the integrity of mobile fauna further analysis. The mobile fauna was then sieved through a 50 µm mesh sieve. Retained organisms were collected and kept alive in seawater until further processing.

Collected mobile fauna were identified based on external morphological characteristics observed under a stereomicroscope. Where possible, specimens were identified to the lowest feasible taxonomic level (species or genus) by referring to the World Register of Marine Species (WoRMS) and the Malaysia Biodiversity Information (MyBIS) which provided updated identification keys, taxonomic names, and regional distribution data relevant to Malaysian marine fauna. Taxa were classified into major groups, including Crustacea, Mollusca, Polychaeta, Vertebrata, and Ascidiacea. Crustaceans and polychaetes were mostly identified to the family level, while molluscs were generally identified to species or genus. Vertebrates and ascidians were identified to the lowest possible taxonomic level, typically genus or species, where taxonomic resolution permitted. Species richness was quantified as the total number of species observed across all replicates, while abundance was determined by counting individuals within each taxonomic group.

Photographs of each specimen were taken individually with a ruler placed beneath for scale to document morphological features.

All collected specimens were preserved in 70% ethanol to maintain morphological integrity for subsequent analysis. Organisms were carefully transferred into labeled glass jars filled with ethanol, sealed tightly with Parafilm to prevent evaporation and contamination, and stored in a dry, cool, and dark environment to ensure long-term preservation. Data on identified mobile fauna were organized in an Excel spreadsheet. Species richness was calculated using the spec number function from the *vegan* package in R.

Results

Effects of rigidity and amount on species richness

Macrofaunal diversity associated with *Perna viridis* mussel aggregates was assessed using species richness, Shannon diversity index (H′), Simpson's diversity index (D), and evenness (Pielou's J'). A total of 22 macrofauna species were identified across all treatments, representing the overall diversity observed in this study.

Species richness, defined as the number of different species present per sample, ranged from 7 to 19 (Table 1), with a median of 11 and a mean of 12.3 (±3.2 SD) , suggesting a modest skew toward samples with more species. The interquartile range (IQR), spanning from 10 (Q1) to 14 (Q3), reflects moderate consistency among samples, with the middle 50% falling within this range. The highest species richness was observed under both the 20% Film and 40% Filament treatments (maximum=19), with 20% Film also exhibiting the highest mean richness. This distribution indicates that, although some variability was present, most mussel aggregates supported a broadly similar level of biodiversity. These patterns suggest that plastic shape and amount might subtly influence habitat complexity.

In addition to species richness, Shannon diversity index (H′) and Simpson's index (D) were calculated to assess diversity patterns across treatments. These indices exhibited similar trends, with the highest H′ value (2.61) observed in the 20% Film treatment, and moderately elevated values also recorded in the 20% Filament and 40% Film treatments. Similarly, Simpson's index peaked at 0.91 in the 40% Film treatment, with relatively high values also associated with other Film treatments, suggesting slightly greater macrofaunal diversity under film-type plastic substrates.

Table 1: Summary statistics of species richness in aggregates of *P. viridis* under treatments under 20% and 40% treatments with Film (plastic bags) and Filament (fishing line).

Statistic	Value
Minimum	7
1st Quartile (Q1)	10
Median	11
Mean	12.3
3rd Quartile (Q3)	14
Maximum	19

However, two-way ANOVA revealed that neither plastic rigidity (F=1.571, p=0.223) nor plastic amount (F=0.007, p=0.934) significantly affected species richness, and no significant interaction was found (F=0.637, p=0.433). Likewise, ANOVA results for both Shannon and Simpson indices indicated no statistically significant effects of plastic rigidity or amount (all *p-values*> 0.32).

These findings suggest that macrofaunal diversity within mussel aggregates is relatively stable and not strongly influenced by the physical characteristics of plastic debris under the tested conditions. This may reflect the ability of mussel beds to buffer environmental variability and maintain complex habitats, even in the presence of different plastic forms and concentrations.

Based on Figure 1, the Filament shape shows slightly higher species richness in the 40% treatment compared to the 20% treatment. Specifically, the mean species richness for Filament was 13.4±1.5 at 40%, compared to 11.9±1.2 at 20%, indicating a 12.6% increase in richness with hogher plastic amount. Additionally, the 40% Filament group displays greater variability, as indicated by a wider interquartile range and longer whiskers, suggesting more variation in species composition among replicates.

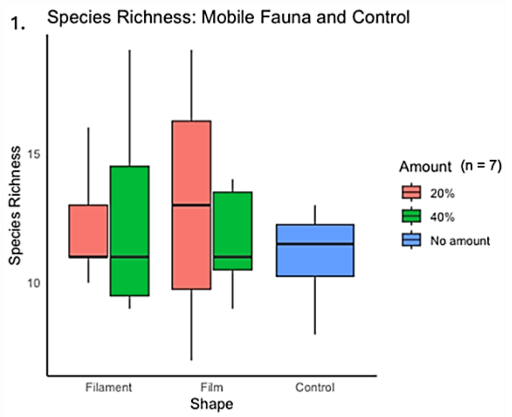

Figure 1*:* Species richness of mobile fauna across treatment groups defined by plastic shape (Film and Filament), and amount (20% and 40%) with a Control (no plastic) included for comparison (n=7 replicates per treatment).

For Film treatment, species richness averaged 14.1±2.0 at 20%, but declined to 11.3±1.7 at 40%, indicating a potential reduction in diversity and variability with higher plastic levels. Meanwhile, the Control treatment with no plastic added, shows relatively consistent species richness across replicates. It is characterized by a narrower interquartile range and median values similar to or slightly lower than most treatment groups

These patterns suggest that introducing plastic, especially in the Film at a moderate amount (20%), may enhance habitat complexity and support a more diverse mobile fauna community. However, increasing the plastic amount to 40% does not consistently lead to higher species richness and may even reduce diversity, potentially due to negative impacts such as habitat obstruction or degradation.

ANOVA test to examine the effect on species richness

To statistically assess the influence of plastic substrate characteristics on species richness, a two-way ANOVA was performed. This analysis tested the effects of plastic rigidity (Film vs. Filament), amount (20% vs. 40%), and their interaction on species richness in *Perna viridis* aggregates (Table 2).

Table 2: ANOVA results for species richness.

Source of variation	df	SS	MS	F	p
Rigidity	1	0.0	0.03	0.000	0.987
Amount	1	1.48	1.478	0.129	0.723
Rigidity × Amount	1	4.15	4.149	0.361	0.554

The results indicated no statistically significant effects of plastic rigidity ($F_{1,x}$=0.000, p=0.987), plastic amount ($F_{1,x}$=0.129, p=0.723), or their interaction ($F_{1,x}$=0.361, p =0.554) on species richness. These findings suggest that neither the shape nor the amounts of plastic added significantly affected the number of species present within mussel aggregates.

Although visual inspection of Figure 1 revealed slight differences, such as higher richness in the 20% Film group, these were not statistically supported, indicating that the observed variability is likely due to natural variation rather than treatment effects.

Evenness (Pielou's J') of mobile fauna across treatments

In Figure 2, the evenness of mobile fauna, measured by Pielou's J', across treatment groups defined by plastic shape (Film and Filament) and amount (20% and 40%), as well as the control group without plastic. Pielou's J' indicates how evenly individuals are distributed among the species present, with values closer to 1 reflecting more even communities. Filament showed relatively high median evenness, especially at 40%, where values were both high and consistent, suggesting more balanced species distributions. In contrast, the 20% Filament treatment exhibited greater variability, with a wider interquartile range and a few low outliers, indicating that evenness was more inconsistent across replicates. Film had slightly lower median evenness compared to Filament. Notably, the 40% Film had relatively high evenness with low variability, while the 20% Film group showed broader spread and a low outlier, suggesting that some samples had communities dominated by one or few species. The Control displayed moderate evenness with a median slightly lower than most treatment groups. However, the range and variability were comparable to the 20% Film group, suggesting that plastic presence alone did not drastically disrupt or improve evenness.

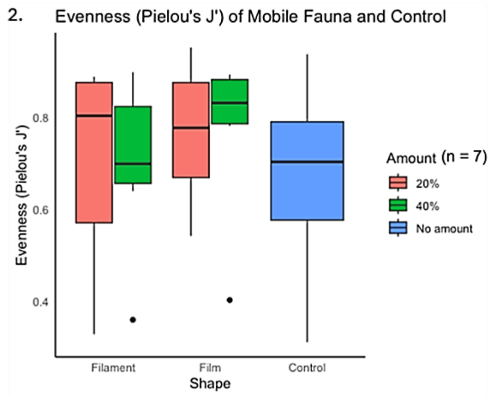

Figure 2: Pielou's evenness (J') of mobile fauna under different treatments defined by plastic shape and amount, including a control (no plastic). Error bars represent standard error. Each treatment group includes n=7 per treatment.

These results indicate that plastic treatments, particularly Filament at higher amount (40%) may promote more evenly structured communities, the overall differences in evenness among treatments are subtle. These findings align with earlier results on species richness, where no statistically significant effects of shape or amount were detected. Together, they suggest that macrofauna communities exhibit a degree of resilience to the presence and variation of plastic debris in mussel beds.

Discussion

This study examined how plastic debris, differing in shape (Film and Filament) and treatment (20% and 40%), affects the biodiversity of mobile macrofauna associated with green mussel (*Perna viridis*) beds. The primary biodiversity metrics evaluated were species richness, Shannon diversity index (H′), Simpson's diversity index (D), and Pielou's evenness (distribution of individuals among species).

The macrofauna community was taxonomically dominated by Mollusca (33.88%), followed by Vertebrata (12.81%), Crustacea (5.99%), Porifera (1.93%), Echinodermata (0.52%), and Cnidaria (0.47%). Molluscs, particularly gastropods and bivalves, commonly dominate mussel bed communities because they can exploit the structural complexity of mussel aggregates and utilize diverse feeding strategies, including grazing and filter feeding (Chowdhury *et al*., 2024). Vertebrate presence likely reflects small fish or juvenile stages utilizing the mussel matrix for shelter and foraging. Crustaceans, although less abundant in this study, are frequently associated with mussel beds due to their high mobility and ability to exploit interstitial spaces within mussel aggregates (Callaway, 2018).

Across all treatments, species richness ranged from 7 to 19 species per sample, with a median of 11. The 20% Film treatment exhibited the highest median richness, whereas the 40% Filament group showed the greatest variability. This may be because film-type plastic bags create shaded microhabitats or more complex surfaces that facilitate settlement and refuge for small invertebrates (Clemente *et al*., 2018). In contrast, filament- type plastics may allow easier movement between mussels, reducing competitive exclusion and supporting evenness (García-Gómez *et al*., 2021a). Evenness (J') was generally high in Filament treatments, particularly at 40%, indicating more balanced communities. This may reflect reduced dominance by opportunistic species, suggesting that filamentous plastic supports a more even community structure despite its presence. The 20 % Film treatment displayed the highest richness but also greater variability in evenness. Statistical analysis (two-way ANOVA) revealed no significant effects of plastic shape, amount, or their interaction on species richness or evenness (p>0.05).

The absence of statistically significant differences suggests that the presence and variation of plastic debris, regardless of the forms or amount tested, do not drastically alter taxonomic richness or evenness of mobile macrofauna in green mussel beds. However, this study was limited by a relatively small sample size (n=7 per group) and short deployment duration (6 weeks), which may have reduced statistical power to detect subtle community shifts. This points to a degree of resilience in mobile invertebrate communities, likely stemming from their adaptability to microhabitat heterogeneity and tolerance to minor substrate modifications. This adaptability aligns with observations that plastic debris with limited structural complexity often exerts only minor or variable effects on benthic fauna (Torn *et al*., 2022a).

Nonetheless, the higher richness observed in the 20% Film treatment and greater evenness in the 40% Filament treatment suggest subtle influences on habitat complexity. Moderate amounts of Film-type plastic (plastic bags) may create additional crevices or shaded microhabitats that facilitate species coexistence by modifying sediment dynamics (Clemente *et al*., 2022; Torn *et al*., 2022a). Conversely, the linear nature of Filament plastics (fishing line) could promote space partitioning or movement within aggregates, potentially reducing competition and supporting greater biological diversity (García-Gómez *et al*., 2021b).

While this short-term study did not find significant impacts of plastic presence on biodiversity metrics, ecological consequences remain complex. Over longer periods or at higher amount, plastics may contribute to habitat degradation, altered species interactions, or increased contaminant exposure. Additionally, variability among replicates and relatively small sample sizes may have obscured subtle but biologically meaningful effects.
Similar to (Torn *et al*., 2022a), who found no significant effects of discarded plastic bags on benthic macrofauna, our results suggests that limited amounts of plastic do not immediately disrupt community structure. Conversely, larger-scale studies have (Clemente *et al*., 2022). observed biodiversity loss in more heavily polluted systems, highlighting the importance of plastic amount and exposure duration.

Conclusion

The study demonstrates that the mobile macrofauna associated with *Perna viridis* beds in the northern Straits of Malacca exhibit resilience to the presence and moderate variation of plastic debris. Although minor trends suggest that plastic type and amount may subtly influence habitat complexity and community structure, these effects were not statistically significant within the scope of this experiment. Despite the absence of statistically significant effects, our results provide valuable baseline information on macrofaunal assemblages in *Perna viridis* beds and can inform monitoring and habitat restoration strategies in plastic-impacted coastal environments. Long-term and larger-scale studies are necessary to assess chronic effects of plastic accumulation on coastal benthic biodiversity, which may manifest beyond the short-term scale of this experiment.

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