



BASELINE ASSESSMENT OF STREAM MACROINVERTEBRATE COMMUNITIES AND SEDIMENT NUTRIENT CONTENT IN PANTI FOREST RESERVE, MALAYSIA: IMPLICATIONS FOR BIODIVERSITY AND ECOSYSTEM FUNCTION

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ABSTRACT

Protected regions, such as Malaysia's Panti Forest Reserve (PFR), play a vital role in preserving freshwater biodiversity. However, the absence of baseline data on stream macroinvertebrates and sediment nutrient contents presents a significant challenge. This study, conducted from August 21-24, 2022, as part of the Panti Expedition organized by the Johor State Forestry Department, aimed to investigate the current nutrient flows and stream macroinvertebrates in PFR, providing preliminary assessments. Utilizing kick and grab sampling techniques, we collected stream macroinvertebrates and benthic sediments along the entire upstream-to-downstream gradient of Pelepah Kiri and Gunung Muntahak. Our findings revealed a diverse assemblage of 385 macroinvertebrates from 28 distinct families, with notable abundance observed in Baetidae (14.0%), Simuliidae (10.6%), and Hydropsychidae (10.6%). The analysis of overall taxonomic composition indicated a Jaccard index similarity of 0.64, suggesting overlapping communities between the two sites. Predators were significantly more abundant in Pelepah Kiri, while collectors were more abundant in Gunung Muntahak. The expedition trails exhibited varying levels of moisture content (ranging from 17.2% to 31.3%), total organic carbon content ($\leq 0.4\%$), and total nitrogen levels (ranging from 1,330 mg/kg to 3,180 mg/kg), while phosphorus loading remained undetectable due to low concentration levels. Nutrient analyses indicated no significant impact of total nitrogen on macroinvertebrate family richness, abundance, and diversity. The study's biological metrics demonstrated good stream quality, with taxa richness ranging from 21 to 20 and EPT taxa richness ranging from 6 to 10. These results highlight the diverse freshwater macroinvertebrate communities in PFR, suggesting the maintenance of favorable water quality for biodiversity preservation. Overall, this study provides critical baseline data for future conservation management strategies in PFR, contributing to our understanding of the intricate connections between nutrient dynamics and stream macroinvertebrates in protected areas.

Keywords: Benthic sediments; Biodiversity Preservation; Stream macroinvertebrate; Freshwater biodiversity; Nutrient flows; Pantı Forest Reserve; Protected regions; Stream macroinvertebrates

1. INTRODUCTION

Biodiversity loss poses a grave global concern, driven by human activities, leading to rapid declines in ecosystems worldwide (IPBES, 2019). The alarming rates of species loss, habitat degradation, and ecosystem fragmentation necessitate immediate and decisive conservation actions to mitigate the detrimental impacts on Earth's biodiversity and the crucial services it provides (Cardinale et al., 2012; Ceballos et al., 2020). Thus, protected areas have emerged as indispensable tools in the global conservation strategy, playing a pivotal role in safeguarding biodiversity, particularly for endangered species (Watson et al., 2014; Hoffman, 2022).

Forest reserves, specifically designed to protect and preserve natural forests and their ecosystems, play a crucial role in this overarching effort (Geldmann et al., 2013). By preventing deforestation and promoting the sustainable use of forest resources, these reserves positively influence the recovery of endangered populations, enhancing their chances of survival and resilience against ongoing threats. Moreover, forest reserves actively maintain biodiversity and ecological balance by providing habitat for a wide range of plant and animal species, aligning with the broader goals of protected areas (Mwase et al., 2007; Wijesundara & Wijesundara, 2014; Volenec & Dobson, 2019). These reserves serve as invaluable sources of scientific research, education, and recreation, effectively showcasing the multiple benefits derived from implementing conservation tools to preserve our planet's biodiversity. For example, a case study of a forest reserve in Japan reveals an annual value ranging from US\$ 1.427–1.482 billion, or approximately US\$ 17,016–17,671 per hectare highlighting the substantial economic and ecological importance of forests to the human well-being (Ninan & Inoue, 2013).

Panti Forest Reserve (PFR) in Kota Tinggi, Johor, Malaysia plays a vital role as a refuge for endangered species and contributes significantly to regional conservation efforts. Covering an area of approximately 13,410 hectares, PFR is a lowland dipterocarp rainforest located in the southern region of Peninsular Malaysia (Onn et al., 2010). It is part of the extensive Virgin Jungle Reserves in Johor, with a notable section spanning 802.10 hectares (Johor Forest Management Plan, n.d.). The reserve encompasses three prominent peaks: Gunung Panti (481 m), centrally located Gunung Panti Barat (513 m), and Gunung Muntahak (634 m) in the northwest region. The primary vegetation in PFR consists of lowland dipterocarp forests, which are connected to an expansive peat swamp forest formed by the convergence of Sungai Sedili Besar, Sungai Dohol, and their smaller tributaries (Onn et al., 2010). These peat swamps forests are particularly remarkable for their capacity to sequester carbon, storing it within the soil and plant biomass, thereby mitigating greenhouse gas emissions and combating global climate change (Furukawa et al., 2005; Tannaberger et al., 2020).

PFR, being home to a diverse range of flora and fauna, including several endemic and threatened species, is a significant focus of conservation efforts. During a forest expedition organized by the Johor Forestry Department in 2022, the expedition team documented more than 500 species of animals, plants, and insects, which included 17 dragonflies, seven butterflies, 50 species of ants, eight species of grasshoppers, 20 species of frogs, and 16 species of reptiles (The Star, 2022). Moreover, the reserve plays a crucial role in providing essential ecological services such as climate regulation, water purification, and pollinator habitats. These services contribute to the well-being of local communities and ensure the sustainability of the surrounding landscape.

Within the diverse ecosystems of PFR, freshwater ecosystems hold exceptional biodiversity and ecological significance. Despite covering a small fraction of Earth's surface, they make substantial contributions to overall biodiversity and play a crucial role in maintaining ecosystem functions (Dudgeon et al., 2007). These ecosystems are home to freshwater species of immense cultural importance and critical ecological functions, underscoring the need to prioritize their preservation through management and monitoring protocols. Freshwater ecosystems heavily rely on the essential functions performed by freshwater invertebrates in nutrient cycling, primary production, and energy transfer, which greatly influence the overall ecosystem dynamics (Crossman et al., 2018). Within stream ecosystems, different groups of invertebrates, such as scrapers, collectors, shredders, and predators, perform specific functions that contribute to the overall ecosystem balance. Scrapers are responsible for removing algae and organic materials from surfaces like rocks and plants, while collectors gather fine particles of organic material suspended or deposited on the streambed. Shredders play a crucial role in breaking down larger organic materials, such as leaves and twigs, into smaller particles. Predators actively hunt and consume other animals, completing the intricate web of interactions (Covich et al., 1999; Ramirez and Gutierrez-Fonseca, 2014). Freshwater invertebrates, with their pivotal roles in nutrient cycling, primary production, and energy transfer, serve as reliable bioindicators of environmental health due to their sensitivity to ecological disturbances (Buss et al., 2015). Notably, EPT taxa (Ephemeroptera-Plecoptera-Trichoptera), including mayflies, stoneflies, and caddisflies, have been identified as particularly sensitive to water pollution and habitat degradation, highlighting their potential as indicators of environmental quality (Barbour et al., 1999).

Nutrient concentrations, specifically total nitrogen, phosphorus, and organic carbon, significantly influence stream macroinvertebrate assemblages and functional group compositions (Covich et al., 1999; Maul et al., 2004; Friberg et al., 2010; Hamid et al., 2020). Variations in these nutrient levels can have substantial effects on the distribution, diversity, and functionality of macroinvertebrate communities in freshwater ecosystems (Dodds et al., 2013).

The study revealed that intensifying agricultural practices leads to an increase in nutrient enrichment, resulting in decreased abundances and richness of pollution-sensitive species, meanwhile, the opposite effect was observed on pollution-tolerant species (Zhang et al., 2018).

Despite their ecological significance, comprehensive information on the associations between nutrient concentrations and freshwater invertebrate biodiversity and community structure in protected areas, including forest reserves, remains limited. In addition, the management of protected areas often faces a lack of consistent access to up-to-date information regarding the current conditions and changes in nature and the various threats it faces (Hoffmann, 2021). Thus, understanding the complex relationships between nutrient dynamics and freshwater invertebrate communities is essential to inform conservation management efforts and maintain the integrity of these critical ecosystems.

This study aims to address the knowledge gap by assessing the status of freshwater invertebrate biodiversity and community structures within the Panti Forest Reserve (PFR), with a specific focus on two expedition trails: Pelepah Kiri and Gunung Muntahak. The objectives of the study include conducting a preliminary assessment of freshwater invertebrate biodiversity, along the stream from upstream to downstream, and investigating the influence of sediment characteristics, such as total nitrogen, phosphorus, moisture content, and total organic carbon, on macroinvertebrate family richness, diversity and abundance. This assessment will provide valuable insights into the diversity of freshwater

macroinvertebrates and the overall water quality within PFR. These findings will contribute to the development of more effective conservation management strategies, with a particular focus on freshwater protection, to ensure the long-term preservation and sustainable management of this vital ecosystem.

2. MATERIALS AND METHODS

This study was conducted in the Pantı Forest Reserve (PFR) (Figure 1), focusing on the Pelepah Kiri and Gunung Muntahak trails, during the period of August 21-24, 2022. The study area encompasses diverse landscapes, including pristine forest areas, oil palm plantations, and waterfalls. The presence of oil palm plantations highlights the potential impact of human activity on the surrounding ecosystem, while the waterfalls introduce a distinct element to the hydrological system that may influence the distribution and habitat of freshwater invertebrates and benthic sediments (Figure 2). A total of 11 stations were sampled, with each station featuring three replications representing the upstream, midstream, and downstream sections of the streams. Sampling in Pelepah Kiri was performed at six different locations, ranging from the base to the summit of the area. Similarly, in Gunung Muntahak, sampling was conducted along the stream, spanning from the base to the upper reaches at five specific points. The distance between sampling stations was at least 40 times the stream width, ensuring representation of different habitats and stream conditions and minimizing the likelihood of spatial autocorrelation (Barbour et al., 1999).

Stream macroinvertebrates were collected using a 1-mm mesh hand-net (pond net) through kick sampling, lasting approximately 3 minutes at each sampling point within the study plot. Various substrates at each study site were targeted, including sand and gravel bed materials, large woody debris and snags within the stream channel, stone and rocks (riprap), leaf packs, and coarse particulate organic matter (CPOM). After combining the three replicate samples into a single container, the container was filled with 100% ethanol for preservation. Once transported to the laboratory, the freshwater macroinvertebrates were sorted, identified down to the family level and their functional group (Dobson et al., 2012), and counted to determine their abundance. Additionally, sediment samples were collected and analysed for total nitrogen (APHA 4500), phosphorus (ICP-OES), moisture content (APHA240B), and total organic carbon (USEPA 9060).

All taxa sampled were included in the subsequent analyses. T-test (normal data) and Mann-Whitney (non-normal data) were used to compare family richness, abundance, diversity, functional feeding group abundance (i.e., collector, shredder, shredder, scraper) between two expedition trails (i.e., Gunung Muntahak and Pelepah Kiri). The Jaccard index was used to assess the similarity between stream macroinvertebrate community composition. Before analysis, invertebrate abundance data were (x+1) log-transformed and tested for normality with the Shapiro-Wilk Test. Principal Component Analysis (PCA) (using correlation matrix and abundance data) was used to compare stream macroinvertebrate invertebrate between two sampling location. All statistical analyses were performed in SPSS (version 25) except for PCA which was conducted in Minitab 18.

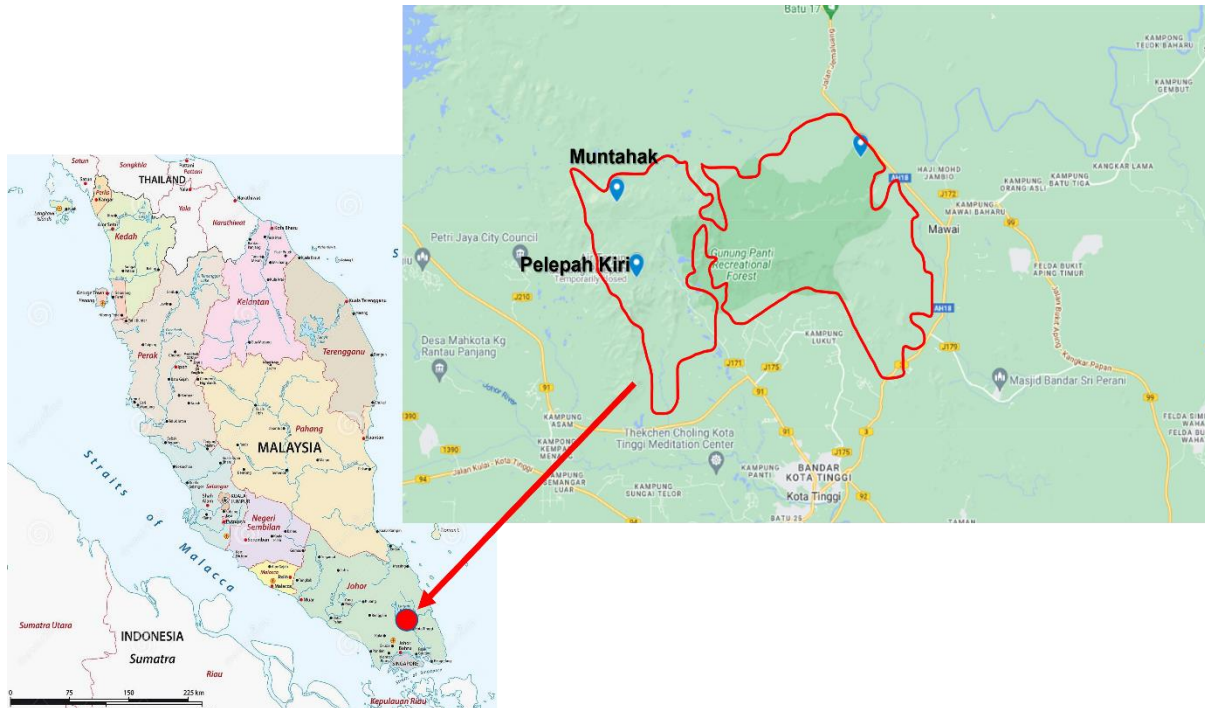


Figure 1. Map of Malaysia and Panti Forest Reserve (highlighted in red circle).



Figure 2. The diverse features of the study area, including a) the oil palm plantation located

at the entrance to Pelepah Kiri, b) the stream situated at the summit of Pelepah Kiri, c) the picturesque waterfall within the Pelepah Kiri area, and d) the meandering stream that runs along Gunung Muntahak.

3. RESULT AND DISCUSSION

Stream Invertebrate Community Structure

A total of 385 stream macroinvertebrates were identified across 11 sampling stations of two expeditions at Pelepah Kiri (six stations) and Gunung Muntahak (five stations) at PFR. The study recorded representatives from 28 families, with the most abundant being Baetidae (14.0%), Simuliidae (10.6%), Hydropsychidae (10.6%), Chironomidae (7.5%), and Heptageniidae (7.5%) (Figure 3 and 4).

Sensitive freshwater macroinvertebrates, such as Baetidae, Heptageniidae, and Hydropsychidae, are commonly recognized as reliable indicators of clean water conditions (Buss and Salles, 2007). These organisms were predominantly observed in association with stony substrates, pool litter, and riffle litter substrates, while their presence in fine sediment was relatively limited. Their preference for these coarse substrates highlights their ecological affinity for well-oxygenated habitats and stable substrates, which are characteristic of pristine or less impacted environments (Sartori & Brittain, 2015). Consequently, the absence or reduced abundance of these sensitive macroinvertebrates in fine sediment suggests their limited tolerance to degraded or polluted conditions. Their utilization as bioindicators contributes to the assessment of water quality and the conservation of freshwater ecosystems. Fine sediment, tend to have a higher water-holding capacity, leading to reduced water flow and restricted oxygen diffusion within the sediment. This limited water movement hampers the replenishment of oxygen in the sediment, further reducing oxygen availability for macroinvertebrates (dos Reis Oliveira et al., 2019).

Previous research has demonstrated that Simuliidae species exhibit a presence in streams characterized by intermediate urban pollution impacts, whereas they are notably absent in heavily impacted sites. These findings suggest that Simuliidae may serve as effective bioindicators for moderately impacted streams, showcasing their potential as indicators of environmental disturbance levels (Docile et al., 2015). Chironomidae, also known as midge flies, display different degrees of tolerance towards pollutants in freshwater ecosystems. They are frequently a prominent presence within benthic macroinvertebrate communities, with numerous species residing in sediment habitats and demonstrating a propensity for accumulating toxic and persistent pollutants (Popović et al., 2016). The presence and abundance of different stream macroinvertebrate taxa in freshwater ecosystems offer valuable indicators of water quality and ecosystem health. Any Changes in the composition or abundance of specific taxa can indicate shifts in water quality, habitat degradation, or the presence of pollutants.

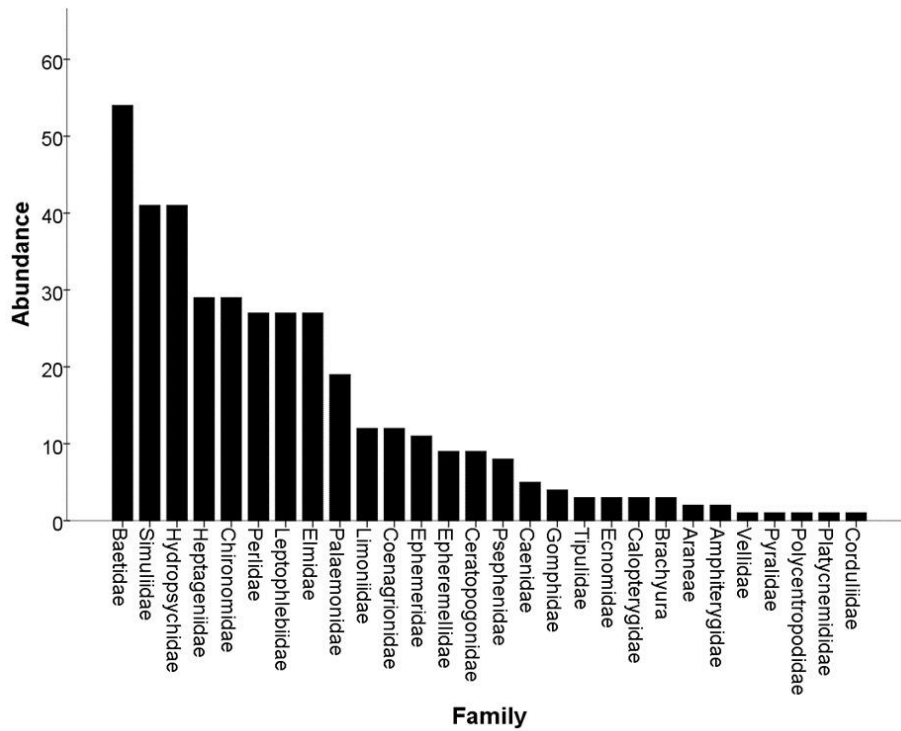


Figure 3. The abundance of freshwater invertebrates in PFR.

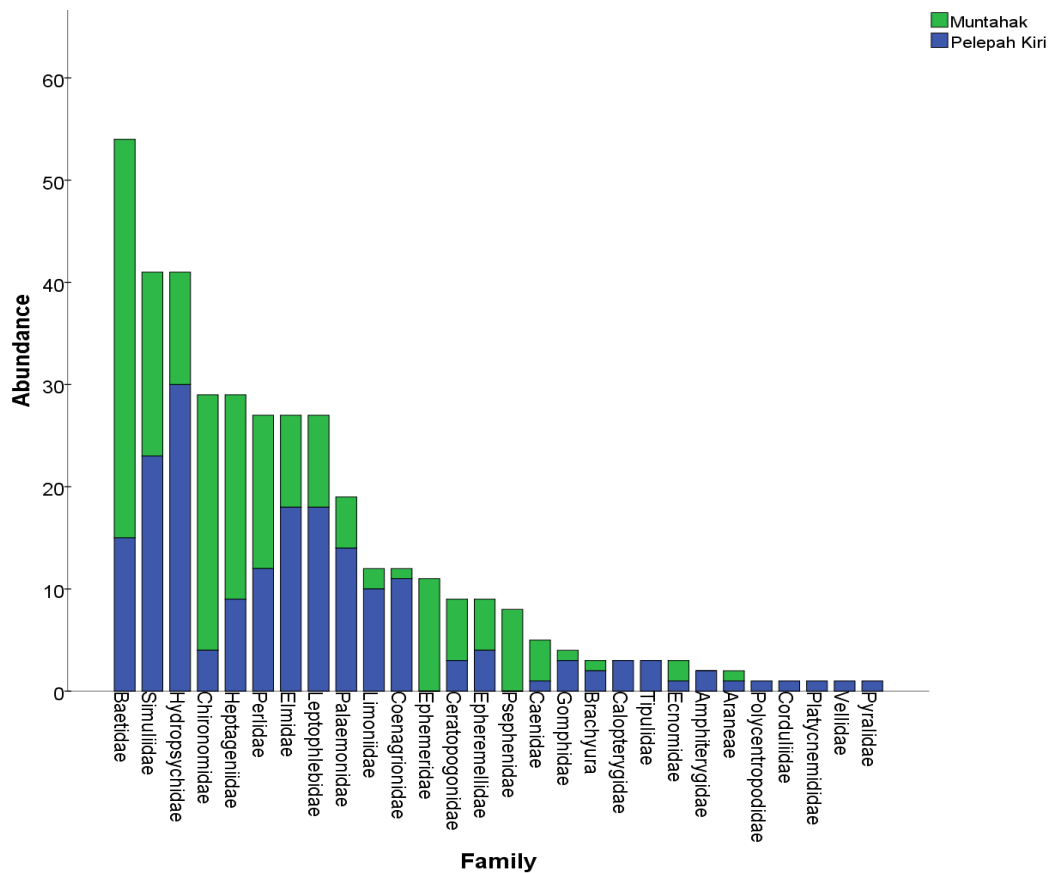


Figure 4. Abundance difference between Pelepah Kiri (blue) and Muntahak (green) in PFR.

In this study, the total abundance, family richness, and diversity ($t \leq 1.18$, $df = 8$, $p \leq 1.000$) of freshwater macroinvertebrate did not show a statistically significant difference between these two expedition trails (Figure 5). The analysis of the overall taxonomic composition (presence/absence data) revealed a Jaccard index similarity of 0.6429, indicating some overlap between the macroinvertebrate assemblages at the two sites. This suggests that both Pelepah Kiri and Gunung Muntahak have comparable overall macroinvertebrate populations.

Two families, Psephenidae and Ephemeridae, were absent in Pelepah Kiri, while six families, including Tipulidae, Calopterygidae, Platycnemididae, Corduliidae, Amphiterygidae, Polycentropodidae, Pyralidae, and Vellidae, were absent in Gunung Muntahak.

Significantly higher abundances of Psephenidae (U statistic= 3, $p = 0.03$), Chironomidae ($t = 3.904$, $df = 9$, $p = 0.004$), Baetidae ($t = 3.041$, $df = 9$, $p = 0.014$), and Ephemeridae (U statistic= 3, $p = 0.03$) were found in Gunung Muntahak, while Pelepah Kiri exhibited a significantly higher abundance of Coenagrionidae ($t = 2.676$, $df = 9$, $p = 0.025$) (Figure 6). Based on these findings, it can be inferred that the two expedition trails exhibit similarities in the overall macroinvertebrate composition, but certain families display distinct preferences for either Pelepah Kiri or Gunung Muntahak. These differences in family abundance suggest potential variations in habitat characteristics, resource availability, or local environmental conditions between the two sites.

A principal component analysis (PCA) was conducted on the freshwater macroinvertebrate assemblages from Pelepah Kiri and Muntahak locations. The analysis revealed that the first two principal components (PC1 and PC2) together accounted for 41.6% of the total variation in the data (Figure 7a). The largest positive association with PC1 was observed for Calopterygidae (0.32), whereas the largest negative association with PC1 was found for Chironomidae (-0.32). For PC2, the largest positive association was with Coenagrionidae (0.23), while the largest negative association was with Hydropsychidae (-0.38) (Figure 7b). The PCA also revealed differences in the macroinvertebrate assemblages between the two locations. The separation between the locations was evident in both PC1 and PC2, with little overlap between them. These findings suggest that the macroinvertebrate communities in the two locations may be influenced by distinct ecological conditions or environmental factors.

Predators were significantly more abundant in the Pelepah Kiri sampling area ($t = 2.503$, $df = 9$, $p = 0.034$), while collectors were significantly more abundant in the Gunung Muntahak sampling area ($t = 3.102$, $df = 9$, $p = 0.013$). Although not statistically significant, Pelepah Kiri exhibited higher shredder abundance, and Gunung Muntahak had a higher scraper abundance (Figure 8). The higher abundance of predators in Pelepah Kiri suggests that this sampling area probably provides suitable conditions and resources to support a thriving predator community. Predators, such as certain species of insects or fish, typically feed on other organisms, including other macroinvertebrates, thus indicating the presence of a food web structure that supports predator-prey interactions (Eriksson et al., 2012). The absence of predators in a freshwater ecosystem can also indicate potential ecological disturbances or degradation within the ecosystem (Premo & Tyler, 2013). It may suggest that the habitat has experienced significant changes or degradation, resulting in the loss or displacement of predator species. This can be a result of factors such as habitat destruction, pollution, or changes in the availability of resources (Ngai & Srivastava, 2006).

The observation of higher shredder abundances indicates potential differences in the availability of food resources and substrates between the two sampling areas. Shredders are macroinvertebrates that feed on coarse organic material, such as leaves or woody debris,

while scrapers feed on algae or biofilm attached to surfaces (Matoničkin et al., 2001). These differences in abundance reflect variations in resource availability and ecological conditions within the respective habitats. As they shred and consume these organic materials, they contribute to the decomposition process and facilitate the release of nutrients. The nutrients derived from the breakdown of leaves by shredders become available in the surrounding water, providing a valuable resource for other organisms, particularly collectors (Short & Maslin, 1977). Collectors are macroinvertebrates that feed on organic particles and detritus, including the smaller fragments produced by shredders. They play an important role in nutrient cycling and energy transfer within the ecosystem. According to the Matoničkin et al. (2001), the colonization sequence within the freshwater ecosystem started with shredders and collector-gatherers, followed by collector-filterers, and subsequently by predators and scrapers. The presence and abundance of different functional groups provide insights into the ecological processes occurring within the ecosystem, including energy flow, nutrient cycling, and trophic interactions.

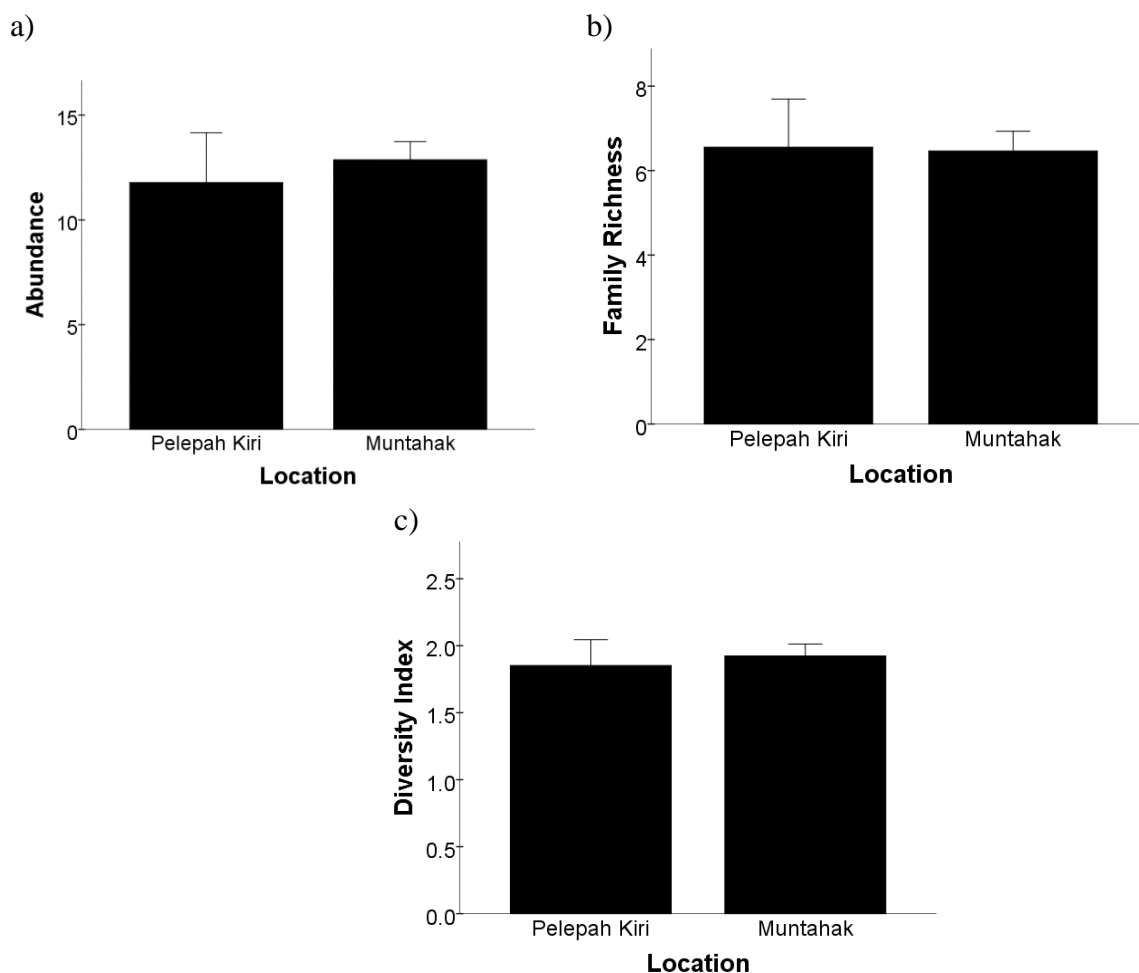


Figure 5. Mean ($\pm 1SE$) (a) Abundance, (b) Family richness, (c) Diversity index of aquatic macroinvertebrates of two different trails of Pelepah Kiri and Muntahak in PFR.

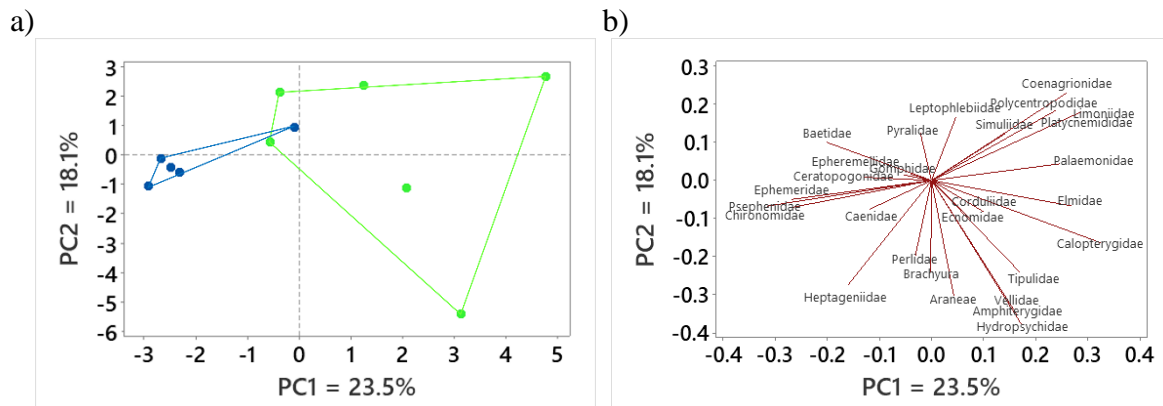


Figure 7. (a) Principal component analysis (PCA) of the freshwater invertebrates found between Pelepah Kiri (green) and Muntahak (blue) and its (b) loading plots.

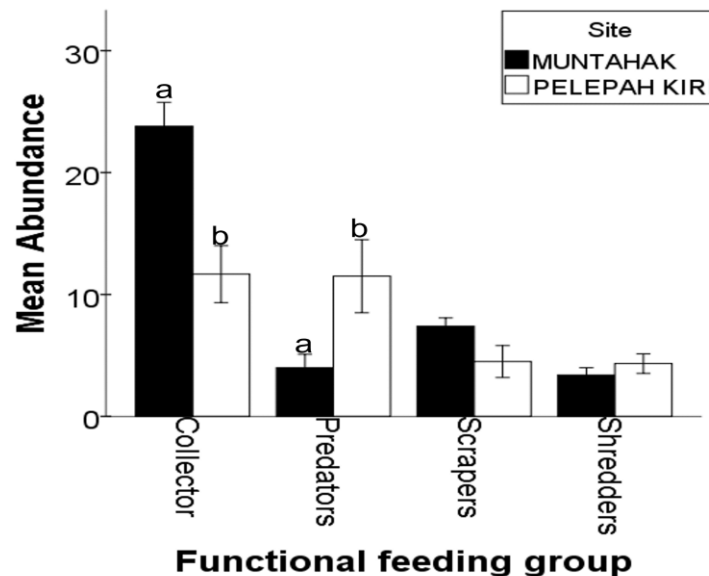


Figure 8. Mean (± 1 SE) abundance of different functional feeding group of freshwater invertebrates Pelepah Kiri (white bar) and Muntahak (black bar) in PFR.

The study findings reveal that Gunung Muntahak and Pelepah Kiri, the two expedition trails, displayed variations in moisture content, total organic carbon content, total nitrogen levels, and undetectable concentrations of phosphorus due to very low levels of loading.

The moisture content, which represents the amount of water present in the benthic sediment, ranged from 17.2% to 31.3% across the two trails. Pelepah Kiri displayed a higher moisture content compared to Gunung Muntahak suggesting potentially wetter conditions in that area. This difference in moisture content can influence the hydrological dynamics, including water availability and flow patterns, within the respective habitats.

The result indicates that the total organic carbon content in the benthic sediment ranged from 0% to 0.4% for both trails. Total organic carbon content is a measure of the amount of organic matter present in the sediment, which includes various forms of decaying

plant and animal materials (Sillanpää et al., 2015). The range of total organic carbon content suggests relatively low levels of organic matter within the sediment of the studied trails. A sediment organic carbon content of less than 1% may indicate various conditions and processes within the ecosystem. It could imply low organic input, limited vegetation cover, or active decomposition and mineralization of organic matter (Yang et al., 2014).

The results indicated that total nitrogen levels (mg/kg), total organic carbon percentage, and moisture content did not significantly affect family richness, abundance, diversity, or the abundance of shredders, collectors, and scrapers ($F_{1,9} \leq 3.14$, $p > 0.05$, adj. $R^2 \leq 17.61\%$). This implies that, within the studied system or context, the variations in total nitrogen levels, total organic carbon percentage, and moisture content did not have a strong influence on the metrics of family richness, abundance, diversity, or the specific functional groups of shredders, collectors, and scrapers. Ecological dynamics are complex and can be influenced by multiple interacting factors, making it challenging to isolate the effects of individual variables in this study without comprehensive sample collection and analysis.

The results of sediment nutrient analysis revealed varying total nitrogen levels, ranging from 1,330 to 3,180 mg/kg, across the two trails. Interestingly, phosphorus loading was undetected in both trails due to its extremely low concentration. The higher total nitrogen levels observed in Pelepah Kiri, in comparison to Gunung Muntahak, suggest the possibility of increased nutrient inputs or nitrogen enrichment in the Pelepah Kiri area. This disparity could be attributed to various factors, such as differences in land use practices or nearby influences. One potential contributing factor to the higher nitrogen levels in Pelepah Kiri is the presence of an adjacent oil palm plantation. Agricultural activities, particularly in oil palm plantations, often involve the application of fertilizers and the use of nitrogen-rich compounds, which can lead to increased nitrogen runoff into nearby water bodies. This agricultural nitrogen enrichment may result in elevated nitrogen levels within the sediment of Pelepah Kiri. It is important to note that the higher nitrogen levels observed in Pelepah Kiri may have implications for the ecological dynamics and water quality of the area. Elevated nutrient levels, particularly nitrogen, can influence primary productivity, algal blooms, and overall nutrient cycling in aquatic ecosystems. Both trails showed sediment contamination levels classified as moderately contaminated (ranging from 1000 to 2000 mg/kg), according to the total nitrogen pollution standards set by the US Environmental Protection Agency (Zhu et al., 2019).

According to Bode (1993) (Table 1), both trails examined were classified as streams with slight impacts on their biological metrics. The taxa richness of these streams ranged between 21 and 30, while EPT (Ephemeroptera, Plecoptera, Trichoptera) richness ranged between 6 and 10. Despite being slightly impacted, the biological metrics indicated good stream quality. Although the benthic-macroinvertebrate community in these streams was less diverse compared to non-impacted sites, it still contained important indicator species such as mayflies, caddisflies, and possibly some stoneflies. The dominant taxa in the community structure were typically caddisflies, elmids (riffle beetles), and chironomids. This suggests that these streams are affected by water-quality and habitat conditions, which have an impact on the benthic-macroinvertebrate community. Slightly impacted sites possibly receive some inputs from wastewater sources and/or agricultural/urban runoff, further influencing the water quality and overall condition of the streams.

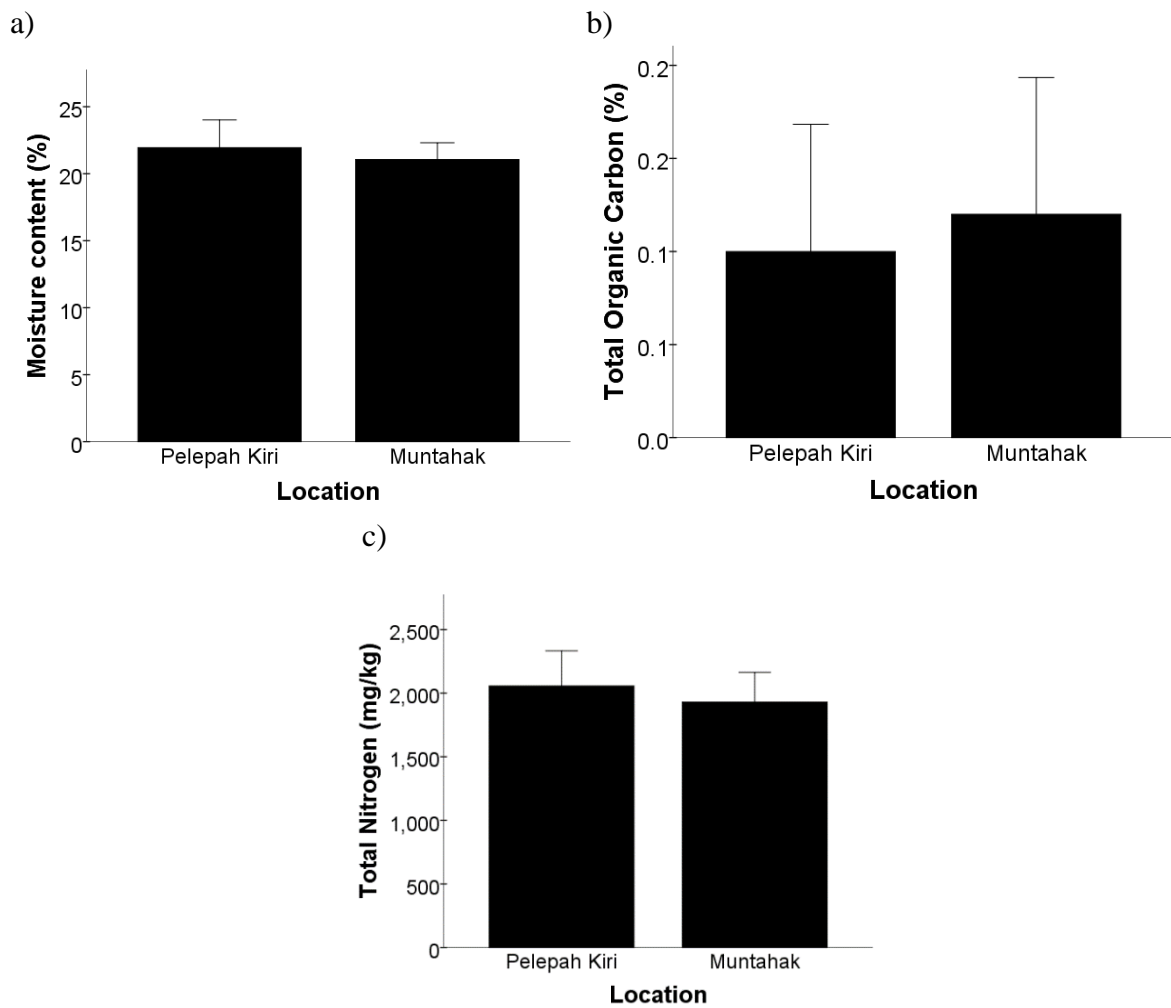


Figure 9 Mean (\pm 1SE) of the a) moisture content, b) Total Organic Carbon (TOC), c) Total Nitrogen of sediments in Panti Forest Reserve.

Table 1 Stream quality assessment criteria based on biological metrics (adapted from Bode, 1993).

Stream Quality Assessment	Taxa Richness	EPT Taxa Richness
Non-impacted	> 30	> 10
Slightly impacted	21 - 30	6 - 10
Moderately impacted	11 - 20	2 - 5
Severely impacted	0 - 10	0 - 1

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6. CONFLICT OF AUTHORS

There is no conflict of authors.

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