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FAMILY COMPOSITION, DIVERSITY AND DISTRIBUTION OF AQUATIC INSECTS IN STREAMS OF TAMAN NEGARA SUNGAI RELAU, MERAPOH, PAHANG, PENINSULAR MALAYSIA

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ABSTRACT

A study was conducted in the streams of Taman Negara Sungai Relau with the objective of determining the family composition, diversity, and distribution of aquatic insect communities among different streams within Taman Negara Sungai Relau, Merapoh, Pahang. Little is known about the aquatic insects at Taman Negara Sungai Relau to date; hence, this study was initiated to fill that gap. Sampling was carried out using the disturbance-removal technique, whereby a bottom kick net was used to dislodge the aquatic insects from the substrates. Following collection, the samples were cleaned in trays before being sorted and screened using a 1 mm sieve. Overall, 1,035 individuals belonging to 46 families across seven orders were collected. Among the identified orders, Ephemeroptera (36.20%) was the most abundant in terms of the number of individuals, followed by Coleoptera (19.98%), Odonata (15.06%), and Plecoptera (13.42%). The most abundant families found in the study were Perlidae, Leptophlebiidae, Heptageniidae, and Dytiscidae. The highest Shannon diversity index recorded in this study was in Sungai Kelam (2.48) and the upstream section of Sungai Tanum (2.45). The upstream section of Sungai Tanum and Sungai Kelam also had the highest Simpson, Margalef, Menhinik, and Equitability indices compared to the other three streams. The Evenness index was found to be highest in the upstream section of Sungai Tanum (0.70) and Sungai Cheruai (0.65). Inter-site similarities were high, indicating that many streams share similar aquatic insect communities. Cluster analysis did not distinctly separate the sites, as shown by the high similarity values. The high family richness, diversity, and evenness denote that the water quality of the stream is good and the surrounding ecosystem is healthy. Thus, based on this study, all streams were found to be in good water quality condition.

Introduction

Taman Negara (previously known as King George IV National Park) is located in the central region of Peninsular Malaysia and spans three states: Terengganu, Kelantan, and Pahang (Azlizam *et al*., 2018). The largest portion lies within the state of Pahang. The forest is reputed to be one of the oldest tropical equatorial forests, comparable to the Amazon and Congo forests, covering an area of 4,343 km² of tropical rainforest (Azlizam *et al*., 2018). It serves as a habitat for a diverse range of flora and fauna. Taman Negara is open to the public and offers various facilities, including chalets, recreational amenities, and nature trails. The park has attracted interest from both local and international nature lovers and conservationists, including researchers who seek to explore and study the rich biodiversity of this pristine environment.

There are at least four official entrances to Taman Negara, two of which are in Pahang: Kuala Tahan and Merapoh. The Merapoh entrance is known as Taman Negara Sungai Relau (TNSR). Several studies have been conducted at TNSR, Merapoh, Pahang, including ecotourism surveys (Azlizam *et al*., 2018), floristic studies (Parris & Edwards, 1987; Yong, 2012; Nizam *et al*., 2018), herpetofaunal inventories (Das *et al*., 2004), wildlife research (Kawanishi *et al*., 2003), and water quality analyses (Nadarajah *et al*., 2012). Recently, Quah *et al*. (2025) reported a new location for a water snake, *Kualatahan pahangensis*, from Sungai Cheruai, a tributary of Sungai Relau in Merapoh. However, a study on the assemblages of aquatic insects has yet to be conducted at TNSR, Merapoh, Pahang.

Numerous streams and rivers flow through TNSR, Merapoh, Pahang. Chiras (2001) once elucidated that no two rivers are identical, as each possesses its own unique set of ecosystems that differ both physically and biologically. Furthermore, the wide variety of microhabitats, substrates, and surrounding environmental conditions within these streams can influence the composition and diversity of aquatic insects (Maneechan & Prommi, 2015).

Given that the area within TNSR remains largely pristine, it is expected to harbour a high diversity of aquatic insects. Aquatic insects play crucial roles in aquatic ecosystems, serving as food sources in the food web, decomposing organic materials, and acting as bioindicators of water quality (Maneechan & Prommi, 2015). They are easily observed in a wide range of aquatic ecosystems, including lakes, streams, saline pools, acid peat swamps, groundwater, coastal waters, and estuaries (Yule & Yong, 2014). Jung *et al*. (2007) highlighted that tropical Asian streams, which vary in microhabitats, support a diverse array of aquatic insects.

In Malaysia, numerous studies have documented the high diversity and abundance of aquatic insects across different locations (Suhaila *et al*., 2014; Mohamad *et al*., 2015; Shafie *et al*., 2017; Suhaila & Che Salmah, 2017; Wahizatul *et al*., 2018; Suhaila *et al*., 2018). The warm and wet climate of Malaysia supports the life cycles of aquatic insects (Mohd Rasdi *et al*., 2012), suggesting a strong possibility that the streams in the study area may contain a high abundance and diversity of aquatic insects. Therefore, this study was conducted to determine the family composition, diversity, and distribution of aquatic insect communities among different streams within Taman Negara Sungai Relau, Merapoh, Pahang. The results obtained will provide baseline information on the current status of aquatic insects in the streams of TNSR, Merapoh, Pahang, serving as a reference for future research.

Materials and Methods

Site Description

Sampling was conducted at TNSR, Merapoh, Pahang, located in the western part of Taman Negara Pahang (Figure 1). The site is approximately 100 km from Kuala Lipis and 30 km from Gua Musang (Nizam *et al*., 2006). Five sampling locations were selected and sampling took place over five days. Detailed information for each sampling site is provided in Table 1.

Aquatic Insect Sampling

Sampling occurred from September 12th to 16th, 2019, using the disturbance-removal technique during daytime hours between 09:00 hours and 17:00 hours. This technique was adapted from a previous study (Wahizatul & Hoon, 2016), employing a bottom kick net to dislodge aquatic insects from substrates. Additionally, hand-lifting and rubbing of boulders and rocks were performed to remove insects clinging to the surfaces.

Three separate sampling sites were used at each stream, with three subsamples collected at each site to ensure a representative sample of the population. The collected samples were washed in trays for sorting and screening through a

1 mm sieve. The sorted aquatic insects were then transferred to urine bottles filled with 70% ethanol for preservation. All samples were stored in labeled vials for further analysis. Identification of aquatic insects to the family level was performed in the imaging laboratory using a dissecting microscope. The taxonomic keys used for identification were based on McCafferty and Wang (2000), Yule and Yong (2004), Ephler (2006), Melo and Scheibler (2011), Dobson (2013), and the pictorial guide by Che Salmah *et al*. (2014).

Data Analysis

The diversity of aquatic insects in Taman Negara Sungai Relau was calculated using several ecological indices: The Shannon index, Simpson index (D), Margalef index, Menhinick index, Evenness index (J'), and Equitability index, as suggested by Magurran (2004). The values of these indices were compared using one-way ANOVA in IBM SPSS Statistics version 22 to determine statistical differences among the five study sites.

An interpolation and extrapolation graph for family richness of aquatic insects was plotted using the iNterpolation and EXTrapolation (iNEXT) online software (Chao *et al*., 2016). Size-based rarefaction was applied based on order q=0, illustrating family diversity collected from the study areas against the abundance of aquatic insects, with an unconditional 95% confidence interval plotted based on the bootstrap method. Rarefactions were used to compare family richness among streams with different sample sizes (Colwell, 2009). This analysis was computed using *EcoSim* 700. Additionally, cluster analysis was conducted to examine the family composition and distribution of aquatic insects across the five streams, employing the Unweighted Pair-Group with Arithmetic Average (UPGMA) method with Bray-Curti's similarity.

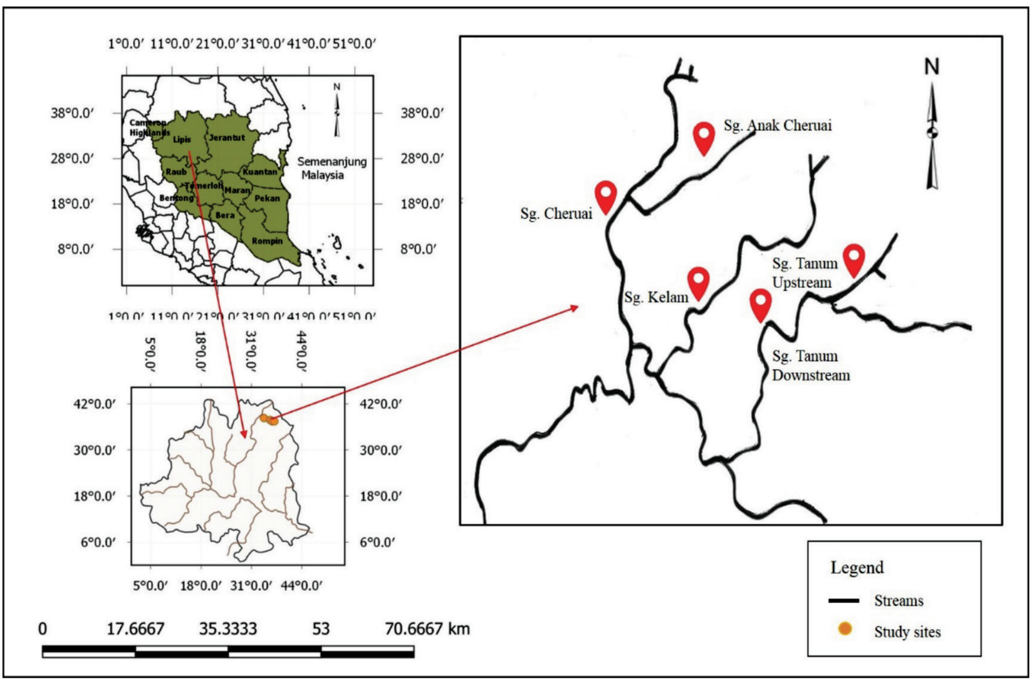

Figure 1: Map of the five study sites in Taman Negara Sungai Relau, Merapoh, Pahang

Table 1: Locality and site description of the five sampling sites within Sungai Relau, Merapoh, Pahang

Streams	Location (ºN, ºE)	Description of Surrounding Environment	Description of the Stream and Substrates
Sungai Cheruai	N 04º 39’55.4”, E102º 04’59.8”	Riparian vegetation on both site of the riverbank. 50% of riparian canopy shaded on the stream.	A mean width of 6.78 ± 1.35 m and depth in the average of 54.17 ± 1.50 cm stream has a slow flow of average 0.39 ± 0.22 m/s. Habitat dominated by cobbles, gravels, sand, leave, and woody debris.
Sungai Anak Cheruai	N 04º 39’38.5”, E102º 05’ 17.9”	Riparian vegetation on both site of the riverbank. 30% of riparian canopy shaded on the stream.	The average width and depth of the stream are 5.50 ± 0.03 m and 43.73 ± 16.07 cm, respectively. The water velocity is rather slow in the mean of 0.23 ± 0.16 m/s. Habitat dominated by gravels, sand, leave litters, and woody debris.
Sungai Kelam	N 04º 39’18.3”, E 102º 06’ 54.8”	Forest canopy shaded about 70% of the streams. Grasses and mosses cover the bank of the stream.	This stream has an average of 4.22 ± 0.59 m width and 40.50 ± 10.17 cm depth. The mean flow of the water is 0.32 ± 0.09 m/s. Habitat dominated by boulders, cobbles, gravel, sand, woody debris, and leaves litters.
Sungai Tanum (Downstream)	N 04º 38’53.8”, E 102º 07’ 30.8”	Forest canopy shaded about 50% of the stream. Riparian vegetation on both side of the stream.	The average width and depth of the river are 21.40 ± 1.95 m and 25.0 ± 3.33 cm, respectively. The water velocity is in the average of 0.48 ± 0.06 m/s. The substrates are predominantly cobbles, pebbles, sand, woody debris, and leaves litters.
Sungai Tanum (Upstream)	N 04º 38’ 53.9”, E 102º 08’ 11.9”	Both side of the riverbank consist of riparian vegetation. The canopy shaded about 40% of the stream.	The mean width of the river is 19.38 ± 0.18 m, depth in average of 59.13 ± 24.47 cm. The water flow recorded in average 0.49 ± 0.07 m/s, the fastest among the four streams. The habitat are predominantly boulders, cobbles, pebbles, sand, and leave litters.

Results

A total of 1,035 individual aquatic insects were recorded, representing 46 families and seven orders: Ephemeroptera, Plecoptera, Diptera, Odonata, Hemiptera, Trichoptera, and Coleoptera (Table 2). The family Perlidae had the highest abundance, with 139 individuals, followed by Leptophlebiidae (132 individuals) and Heptageniidae (119 individuals). These families were present at all sampling sites. Families such as Gomphidae, Corduliidae, Euphaeidae, Stenopsychidae, Leptoceridae, Hydropsychidae, and Elmidae were found in moderate numbers across all streams. Some families, however, were restricted to specific streams and habitats. Families such as Ephemerellidae, Isonychidae, Coenagrionidae, Libellulidae, Nepidae, Veliidae, Gerridae, Polycentropodidae, Helicopsychidae, Lepidostomatidae, and Hydroscaphidae were recorded in very low numbers, with only a single individual noted in Table 2.

Among the sampling sites, Sungai Cheruai had the highest number of aquatic insects (339 individuals), followed by Sungai Kelam (228 individuals) and Sungai Anak Cheruai (207 individuals). Sungai Tanum upstream had the lowest number of aquatic insects, with only 124 individuals. In terms of diversity, Sungai Cheruai recorded the highest number of families (30), followed by Sungai Kelam (29) and both Sungai Anak Cheruai and Sungai Tanum downstream, each with 24 families. The lowest number of aquatic insect families was recorded at Sungai Tanum upstream, with 23 families.

Table 2: The composition and abundance of aquatic insects in selected streams of Taman Negara Sungai Relau, Merapoh

Taxa/Site	Sg. Cheruai	Sg. Anak Cheruai	Sg. Kelam	Sg. Tanum Downstream	Sg. Tanum Upstream	Total
Ephemeroptera						
Baetidae	23	0	24	22	6	75
Ephemerellidae	0	0	0	0	1	1
Leptophlebiidae	35	15	26	38	18	132
Isonychidae	1	0	0	0	0	1
Heptageniidae	27	41	20	9	16	119
Neoephemeridae	2	0	0	0	0	2
Caenidae	0	9	24	3	9	45
Plecoptera						
Perlidae	65	24	16	10	24	139
Diptera						
Tipuliidae	6	6	0	2	3	17
Tabanidae	0	0	1	2	0	3
Ceratopogonidae	1	0	0	3	0	4
Athericidae	4	3	3	0	1	11
Chironomidae	0	2	1	9	2	14
Odonata						
Gomphidae	40	8	19	5	11	83
Corduliidae	7	6	5	1	2	21
Chlorocyphidae	2	4	1	1	0	8
Calopterygidae	1	0	2	2	0	5
Megapodagrionidae	0	1	1	1	0	3
Coenagrionidae	0	0	1	0	0	1
Euphaeidae	11	5	8	1	5	30
Platystictidae	1	1	2	0	0	4
Libellulidae	1	0	0	0	0	1

Hemiptera						
Naucoridae	0	0	0	0	1	2
Apheloceridae	2	2	2	0	0	6
Nepidae	0	1	0	0	0	1
Corixidae	0	0	0	3	0	3
Helotrephidae	0	0	0	9	0	9
Veliidae	0	0	0	0	1	1
Gerridae	1	0	0	0	0	1
Trichoptera						
Stenopsychidae	1	1	17	1	3	23
Leptoceridae	2	13	16	1	1	33
Hydrophychidae	4	3	14	1	2	24
Polycentrapodidae	1	0	0	0	0	1
Calamoceratidae	0	1	1	0	0	2
Helicopsychidae	1	0	0	0	0	1
Lepidostomatidae	0	0	0	1	0	1
Philopotamidae	0	0	1	0	1	2
Coleoptera						
Hydrophilidae	19	0	1	8	4	32
Hydroscaphidae	1	0	0	0	0	1
Scirtidae	11	7	4	0	4	26
Elmidae	17	12	5	3	4	41
Dytiscidae	49	40	3	1	0	93
Dryopidae	1	1	1	0	0	3
Psephenidae	0	0	2	0	4	6
Gyrinidae	2	1	0	0	0	3
Noteridae	0	0	1	0	1	2
Total number of individuals	**339**	**207**	**228**	**137**	**124**	**1,035**
Number of families	**30**	**24**	**29**	**24**	**23**	**46**

The dominant taxa varied among the streams. Sungai Cheruai was dominated by the family Perlidae, with 65 individuals recorded while in Sungai Anak Cheruai, the family Heptageniidae was more abundant, comprising 41 individuals. Interestingly, Sungai Kelam, Sungai Tanum downstream, and Sungai Tanum upstream were dominated by the family Leptophlebiidae, with 26, 38, and 18 individuals recorded, respectively. Among the aquatic insects documented in this study, 10 families were identified at all five sites. In the order Ephemeroptera, two families—Leptophlebiidae and Heptageniidae—were present at every site. The order Plecoptera was represented by a single family, Perlidae, which was found at all study sites. The order Odonata included three families present at all sites: Gomphidae, Cordulidae, and Euphaeidae. The order Trichoptera was also represented by three families found across all sites: Stenopsychidae, Leptoceridae, and Hydrophilidae.

Only one family from the order Coleoptera, Elmidae was found at all study sites. Several families were unique to specific locations, including Ephemerellidae, Isonychidae, and Neoephemeridae (Order Ephemeroptera); Coenagrionidae and Libellulidae (Order Odonata); all families of the order Hemiptera except for Apheloceridae; Polycentropodidae, Helicopsychidae, and Lepidostomatidae (Order Trichoptera); and Hydrophilidae from the order Coleoptera. Based on Figure 2, Ephemeroptera accounted for 36% of the aquatic insects, making it the most common order found in the streams of TNSR, Merapoh, followed by Coleoptera (20%), Odonata (15.07%), and Plecoptera (14%). Hemiptera was the least common order, constituting only 2% of the total aquatic insects collected at TNSR, Merapoh.

Six ecological indices were employed to assess the diversity, richness, and evenness of the aquatic insect composition at TNSR, Merapoh. Table 3 indicates that most indices exhibited high values with minimal variation among sites. The highest Shannon diversity index value was recorded at Sungai Kelam (2.48 ± 0.08) while the lowest was at Sungai Cheruai (1.88 ± 0.18).

For the Simpson index, Sungai Tanum had the highest value (0.89 ± 0.00), whereas Sungai Cheruai recorded the lowest (0.79 ± 0.04). The highest Margalef richness index value was observed at Sungai Kelam (4.02 ± 0.12), with the lowest at Sungai Cheruai (2.78 ± 0.32). The Menhinik index was highest at Sungai Tanum (2.14 ± 0.00) and lowest at Sungai Cheruai (1.67 ± 0.09). For evenness indices, the Evenness index was highest at Sungai Tanum while the lowest values were recorded at Sungai Anak Cheruai (0.59 ± 0.05) and Sungai Tanum (0.59 ± 0.05). The Equitability index was also highest at Sungai Tanum (0.87 ± 0.03), with the lowest at Sungai Cheruai (0.80 ± 0.03). One-way ANOVA indicated no significant difference ($p \leq 0.05$) among the ecological indices between the streams.

Figure 3 (A) illustrates the sample-size rarefaction curve (with 95% confidence intervals) of aquatic insect communities at the study sites based on different abundances (number of individuals). The collected samples showed that Sungai Cheruai was the most diverse and highly abundant (339 individuals, 30 families). However, after rarefying to the smallest sample size (124 individuals), the diversity of the aquatic insects found at all study sites showed no significant difference, as indicated by the overlapping of the 95% confidence intervals of all curves.

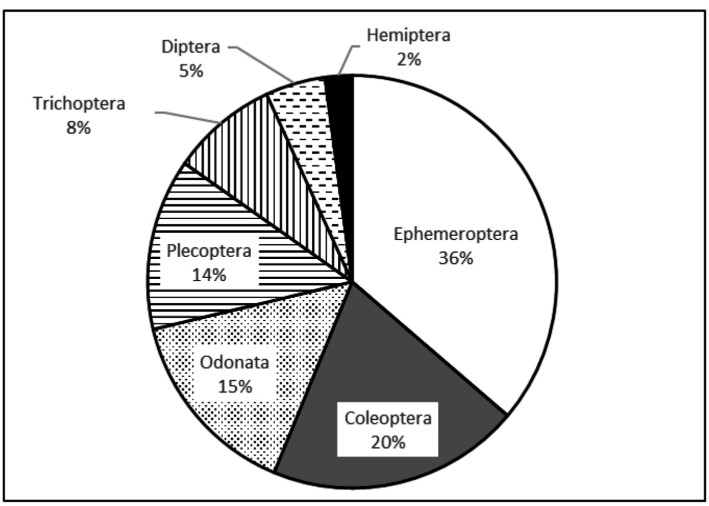

Figure 2: The composition of aquatic insects per order at the sampling sites of Taman Negara Sungai Relau, Merapoh

Table 3: The results of ecological indices (mean ± SE) in selected streams of Taman Negara Sungai Relau, Merapoh

Streams	Ecological Indices					
	Family Diversity		Family Richness		Family Evenness	
	Shannon	Simpson	Margalef	Menhinik	Evenness	Equitability
Sungai Cheruai	1.88 ± 0.18	0.79 ± 0.04	2.78 ± 0.32	1.67 ± 0.09	0.65 ± 0.09	0.81 ± 0.05
Sungai Anak Cheruai	2.32 ± 0.17	0.86 ± 0.03	3.58 ± 0.43	1.74 ± 0.26	0.59 ± 0.05	0.81 ± 0.04
Sungai Kelam	2.48 ± 0.08	0.89 ± 0.01	4.02 ± 0.12	1.89 ± 0.13	0.60 ± 0.05	0.83 ± 0.03
Sungai Tanum (D)	2.17 ± 0.02	0.84 ± 0.00	3.33 ± 0.06	1.84 ± 0.09	0.59 ± 0.05	0.80 ± 0.03
Sungai Tanum (U)	2.45 ± 0.01	0.89 ± 0.00	3.79 ± 0.32	2.14 ± 0.29	0.70 ± 0.02	0.87 ± 0.01
F-value	2.29	1.65	2.26	1.31	0.24	0.27

Furthermore, the extrapolation of the curves (represented by dotted lines) suggests that none of the five study streams have reached an asymptote, indicating that more abundant aquatic insects can be found with additional sampling effort. The sample coverage, as illustrated in Figure 3 (B), varied from 93.5% to 96.8%. According to the observed richness, Sungai Cheruai had the highest sample coverage (96.8%) of aquatic insect communities, followed by Sungai Anak Cheruai (96.6%). The least coverage was observed in Sungai Tanum downstream (93.5%) and Sungai Tanum upstream (94.4%). When extrapolated by doubling the sample size of all streams, Sungai Tanum downstream showed a 3.3% increment in sample coverage from 93.5% to 96.8%, indicating an addition of 24 families, with seven families expected to be discovered at this particular stream if the sample size increases (i.e., if more sampling is conducted). Sungai Cheruai had the least increment in sample coverage (1.9%), compared to the other four streams when extending the sample size to 678 individuals.

This indicates that Sungai Cheruai can also be expected to have a higher family diversity of aquatic insects (> 30 families) with more sampling effort. Individual rarefaction analysis was used to compare the abundance of aquatic insect communities between pairs of streams and the results showed that only the Sungai Anak Cheruai-Sungai Kelam pair was significantly different, as indicated by the non-overlapping of the 95% Confidence Intervals (CIs) of the two curves (Figure 4). The other pairs were not significantly different, as their 95% CIs were overlapping (results not shown).

Figure 5 illustrates the paired rarefaction curves (with 95% confidence intervals) of Shannon index values of aquatic insect communities across the study sites at different sample sizes. When rarefaction analysis was conducted to compare the Shannon index values at the lowest abundance of aquatic insects collected at the sampling sites, only four pairs of streams—namely Sungai Cheruai-Sungai Kelam, Sungai Anak Cheruai-Sungai Kelam, Sungai Tanum-Sungai Kelam, and Sungai Tanum upstream-Sungai Tanum downstream—showed significant differences (Figure 5). The other pairs of streams were not significantly different (results not shown).

Cluster analysis was performed to examine the similarity among the study sites based on the abundance of aquatic insects. The resultant cophenetic correlation index was 0.52, splitting

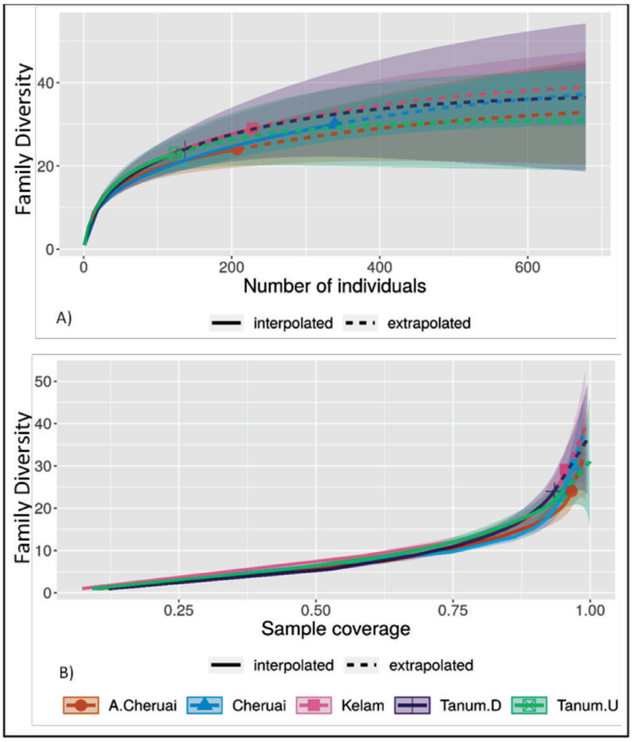

Figure 3: (A) Sample-based rarefaction (solid-line) and extrapolation (dashed-line) comparing the family richness of the aquatic insects in the streams of Taman Negara Sungai Relau, Merapoh represented by the respective shape of 95% confidence interval: and (B) coverage-based rarefaction and extrapolation curves of the study areas

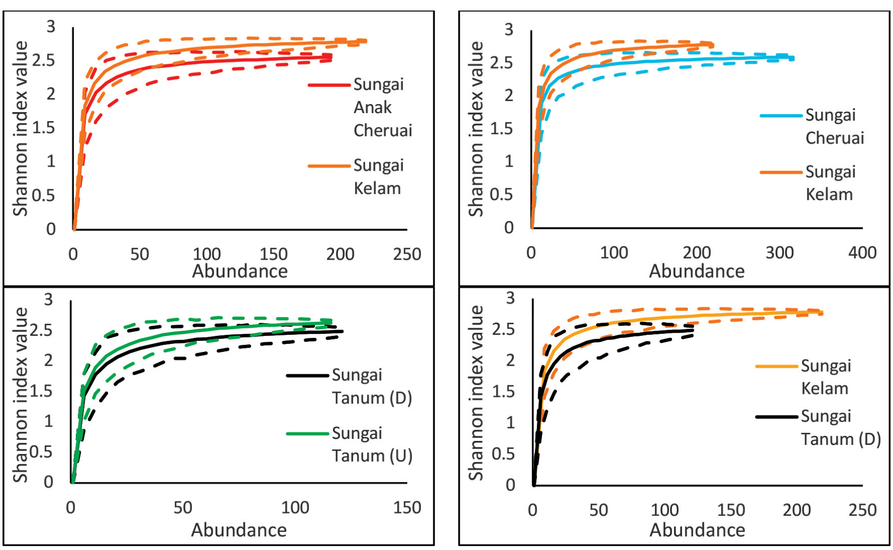

Figure 4: The comparison in pair of rarefaction curve on the Shannon index of the study sites. For clarity, 95% confident interval was plotted and represented by dashed line

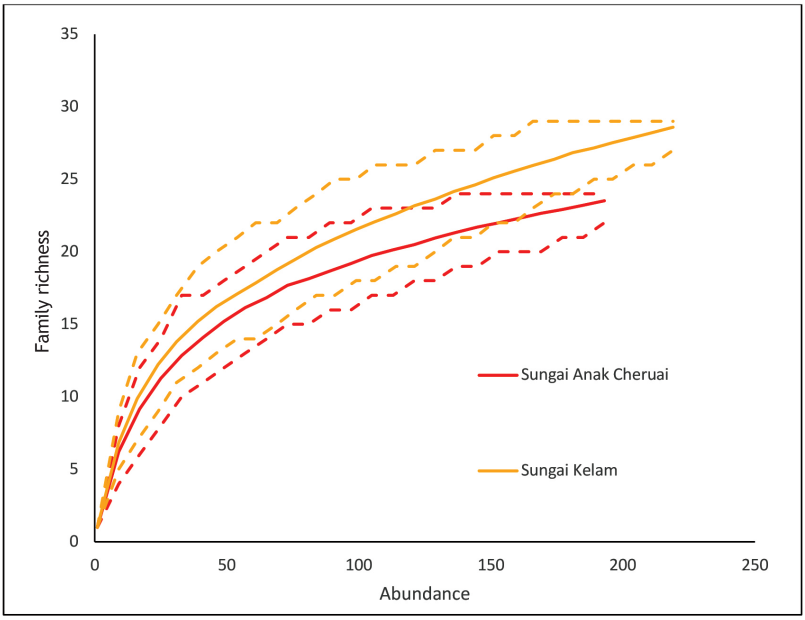

Figure 5: The rarefaction curves based on individual of aquatic insects were plotted for every study site. 95% of confident interval were represented in dashed line

the sites into two distinct groups of different similarity values (Figure 6). Sungai Tanum downstream-Sungai Tanum upstream showed the least similarity among the five sites in this study. Meanwhile, the other three sites showed closely similar communities, with Sungai Cheruai being the most similar to Sungai Anak Cheruai in terms of aquatic insect community composition.

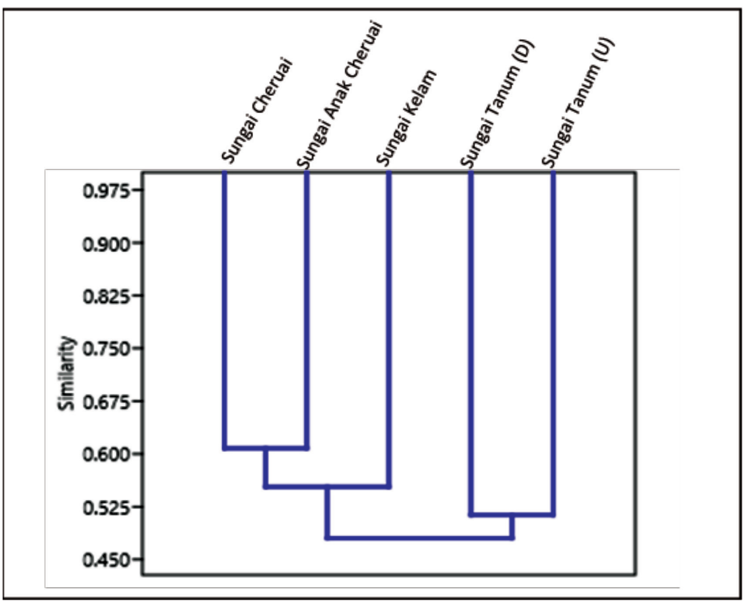

Figure 6: Dendrogram derived from UPGMA clustering analysis with Bray-Curti's distance

Discussion

The aquatic insect community is integral to the functioning of freshwater ecosystems, serving various ecological roles influenced by their diversity and abundance. In this study, a total of 1,035 individuals representing 46 families across seven orders of aquatic insects were recorded, highlighting the rich biodiversity within the surveyed streams. This high diversity is critical, as different families perform specific ecological functions.

For instance, certain families such as Perlidae, Leptophlebiidae, and Heptageniidae were among the most abundant, indicating that they are likely key players in nutrient cycling and food web dynamics within these streams (Suter & Cormier, 2014; Basumatary, 2023). A notable observation within the results of this study was that specific families such as Gomphidae and Hydropsychidae were present across all sampling sites but with moderate counts, highlighting the importance of certain taxa in maintaining ecological balance, even when not highly abundant.

Conversely, the presence of some elusive families with low individual numbers such as Ephemerellidae and Isonychidae, suggests their localised ecological niche or vulnerability to environmental pressures (Abo-Elmaged, 2014). The importance of individual families extends beyond mere presence; many studies have demonstrated that functional redundancy among taxa can be low, meaning the loss of certain families can have substantial impacts on ecosystem services (Suter & Cormier, 2014; Dijkstra *et al*., 2014).

Among the sampling sites, Sungai Cheruai was found to have the highest species richness, with 30 families recorded. This suggests that a greater variety of habitats and food resources may be available in that stream, which in turn supports a higher diversity of aquatic insects. Miserendino *et al*. (2001) emphasised that habitat heterogeneity plays a crucial role in the assemblages and richness of aquatic insects. The correlation between habitat quality and insect diversity is well-documented, as varied environmental conditions can support a wider range of species (Coram & Jarzembowski, 2021; Keinath *et al*., 2023).

Interestingly, Sungai Tanum upstream showed the lowest number of individuals and families, possibly suggesting incomplete sampling despite good water quality and low habitat degradation. Further investigation would be necessary to confirm whether other environmental factors or sampling limitations influenced the observed patterns.

Additionally, information on the distribution patterns of these families can provide insights into the overall ecological health of the streams. Families of the order Ephemeroptera, Plecoptera, and Trichoptera (collectively termed EPT) are widely used as bioindicators since they are pollutant-sensitive; their occurrence generally indicates good water quality while their absence may indicate ecological stress from pollution (Hamid & Rawi, 2017; Rahim & Hamid, 2023).

The reported diversity and abundance in various streams corroborate findings from other regions, indicating that certain aquatic insects serve as crucial indicators of environmental conditions and species interactions within freshwater ecosystems (Imoobe & Ohiozebau, 2010; Popoola *et al*., 2019). Overall, this synthesis emphasises the importance of maintaining aquatic insect diversity and abundance for ecological health. These organisms not only contribute to the ecological fabric of freshwater systems but also serve as bioindicators for water quality monitoring, thus, underscoring their significance for conservation and resource management efforts.

The results of this study demonstrate notable variations in the dominant aquatic insect taxa among different streams in the Taman Negara Sungai Relau (TNSR), Merapoh. Notably, Sungai Cheruai was characterised by a significant presence of the family Perlidae, highlighting its association with high-quality water conditions, as these taxa are often indicative of good water quality due to their ecological requirements (Belmar *et al*., 2012).

In contrast, Sungai Anak Cheruai showed dominance of the family Heptageniidae while Leptophlebiidae prevailed in Sungai Kelam, Sungai Tanum downstream, and Sungai Tanum upstream. This shifting dominance among families illustrates the influence of local environmental factors on the distribution of aquatic insects, corroborating findings that show habitat conditions can significantly affect macroinvertebrate assemblages (Hoang & Bae, 2006; Souza *et al*., 2015). Overall, the composition of aquatic insects reveals key insights into the habitat's ecological status. The presence of families from the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) across all sites further supports the conclusion that these streams are relatively healthy ecosystems. Interestingly, no families from the orders Diptera and Hemiptera were found at any site, which may signal specific ecological constraints or environmental stressors in those locations (Carvalho & Uieda, 2009).

The dominance of Ephemeroptera, comprising over 36% of the recorded aquatic insect community, alongside a significant representation of Coleoptera and Odonata, suggests that these streams might foster relatively diverse and stable ecosystems. The sparse representation of Hemiptera, accounting for only 2%, indicates that further investigations into the factors influencing their diversity in these freshwaters could provide vital management insights (Heino *et al*., 2004). Further monitoring of these taxa could prove essential for conservation efforts aimed at maintaining biodiversity and ecological integrity in TNSR streams.

The application of six ecological indices in assessing the diversity, richness, and evenness of aquatic insect communities at Taman Negara Sungai Relau (TNSR), Merapoh has provided informative results that showcase the ecological health of these streams. The Shannon diversity index indicated Sungai Kelam as the most diverse site, with a value of 2.48 ± 0.08 while Sungai Cheruai exhibited the lowest diversity (1.88 ± 0.18). This pattern suggests that Sungai Kelam may offer more favourable habitat conditions that promote higher species richness. Conversely, the Simpson index reflected a greater degree of dominance in Sungai Tanum, with a high value of 0.89, suggesting lower species evenness, contrasting with Sungai Cheruai, which recorded a lower dominance (0.79).

These variations highlight that the differences in community composition and abundance across the sampled streams could be influenced by habitat heterogeneity and environmental conditions or possibly signify the artefact of sampling effort. Moreover, the richness indices, represented by the Margalef and Menhinik indices also supported these observations, with Sungai Kelam and Sungai Tanum showing significantly higher values compared to Sungai Cheruai.

Notably, the evenness index revealed a pronounced lack of uniform species distribution, particularly at Sungai Anak Cheruai and Sungai Tanum, each having an evenness index of 0.59. This inconsistency in evenness might suggest competitive exclusion or other ecological pressures influencing the community structure. Despite these dependent factors, one-way ANOVA results indicated no significant differences among the indices across the different streams ($p \leq 0.05$), which may suggest a relatively homogeneous ecological response across the sites, possibly shaped by similar environmental factors affecting all streams within TNSR. Such insights can guide conservation efforts, emphasising areas that may require more focused ecological monitoring to sustain aquatic insect diversity and health across different streams (Deacon *et al*., 2018; Alkhayat *et al*., 2024).

The results presented in Figure 3 (A) and (B) illustrate significant findings regarding the diversity and abundance of aquatic insect communities across different riverine environments in Taman Negara Sungai Relau (TNSR), Merapoh. The sample-size rarefaction curves indicate that Sungai Cheruai harbours the most diverse and abundant stream, yielding 339 individuals representing 30 families. However, the overlapping 95% confidence intervals across all study sites, when rarefied to the smallest sample size (124 individuals), suggest that the overall diversity did not significantly differ among the streams, indicating a level of ecological similarity despite variations in individual abundance.

The extrapolation of the curves reinforces the notion that additional sampling in these streams could unveil even greater insect diversity, as none of the sites have reached an asymptote. Such findings align with previous studies indicating that biodiversity increases with greater sampling effort (Rochlin *et al*., 2011; Nnoli *et al*., 2019). The coverage values represented in Figure 3 (B) demonstrate a robust sampling strategy, with coverage ranging from 93.5% to 96.8% across sites, indicating substantial representation of the aquatic insect communities.

Notably, Sungai Cheruai exhibited the highest coverage at 96.8% while Sungai Tanum downstream had the lowest at 93.5%. The notable increment in coverage when doubling the sample size in Sungai Tanum downstream, a projected increase of 3.3%, suggests the potential to discover additional families, thereby enhancing the perceived biodiversity of this stream.

Conversely, the low increment in coverage for Sungai Cheruai (1.9%) implies that this site may already reflect a substantially complete picture of its aquatic biodiversity. Such findings emphasise the importance of strategic sampling in effectively assessing the ecological nuances of aquatic insect communities, thereby informing conservation practices that prioritise biodiversity monitoring within these critical freshwater systems (Rochlin *et al*., 2011; Hasriyanty *et al*., 2022).

The rarefaction analysis of aquatic insect communities across the sampled streams revealed significant differences in abundance and diversity, providing key insights into the ecological dynamics within these environments. A notable finding was that only the pair of Sungai Anak Cheruai and Sungai Kelam exhibited non-overlapping 95% confidence intervals, suggesting a distinct ecological variance between these two sites and indicating that environmental factors may play a significant role in shaping community composition (Souza *et al*., 2024).

The remaining stream pairs showed overlapping confidence intervals, indicating no significant differences; these results align with previous studies that suggest ecological similarities in closely situated or hydrologically connected habitats (Maneechan & Prommi, 2015), as demonstrated at TNSR, Merapoh. Such patterns highlight the importance of habitat-specific conditions, which can enhance or restrict aquatic insect diversity and abundance.

Figure 5 further illustrates the Shannon index values, underscoring the insignificant variations in community diversity among the streams. Significant differences were observed in the Shannon index when comparing specific pairs, including Sungai Cheruai-Sungai Kelam, Sungai Anak Cheruai-Sungai Kelam, Sungai Tanum-Sungai Kelam, and Sungai Tanum upstream-Sungai Tanum downstream. These findings confirm the relevance of the Shannon index as a viable measure of biodiversity that reflects both species richness and evenness in these freshwater ecosystems (Collins & Simberloff, 2007).

Moreover, the evident variation in Shannon index values shows that while some of the streams may share similar total richness, there is uneven species distribution, a factor that could be attributed to substrate composition, water quality, and the physical structure of habitats (Sharma *et al*., 2008; Priyanka & Prasad, 2016). This observation is relevant to informing future ecological surveys and conservation efforts, emphasising the need for special management regimes to maintain the diverse aquatic insect assemblages that support the overall health of freshwater ecosystems.

The cluster analysis performed to assess the similarity of aquatic insect communities among the study sites indicated distinct groupings based on community composition. With a cophenetic correlation index of 0.52, the analysis effectively stratified the sites into two groups, demonstrating considerable differences in aquatic insect assemblages at TNSR, Merapoh. Surprisingly, despite being on the same river stretch, Sungai Tanum downstream and Sungai Tanum upstream exhibited the least similarity, suggesting that environmental factors such as differences in substrate types may influence community structures at these locations differently.

This observation aligns with findings that highlight habitat variations as critical determinants of biodiversity within freshwater ecosystems (Tubić *et al*., 2024). The clustering of Sungai Cheruai and Sungai Anak Cheruai, which are closely similar in their aquatic insect communities, further emphasises the role of specific ecological and environmental factors in shaping community dynamics beyond their shared drainage system. Such findings shed light on the ecological balance present in these streams and underscore the importance of inter-stream relationships within closely located aquatic habitats.

The ability to identify distinct community structures through cluster analysis is vital, as it can inform conservation strategies that target the preservation of unique ecosystems and the management of biodiversity (Ruaro & Gubiani, 2013). This approach not only provides insights into community composition but also serves as a tool for evaluating the health of aquatic ecosystems, indicating areas that may be more susceptible to environmental stressors and thus require prioritised conservation efforts (Ferreira *et al*., 2016).

Conclusions

The comprehensive analyses of the aquatic insect communities across the sampled streams have highlighted significant patterns of diversity, abundance, and community structure influenced by local environmental conditions. The use of rarefaction analysis, cluster analysis, and ecological indices has provided robust insights into the interrelationships among the various streams in Taman Negara Sungai Relau, Merapoh. Notably, variations in community composition were observed, particularly between Sungai Anak Cheruai and Sungai Kelam, which could result from distinct ecological characteristics while communities at other sites exhibited closer similarities. This indicates that specific habitat conditions such as water quality, availability of resources, and structural complexity of the environment play critical roles in determining the diversity of aquatic insect communities at TNSR.

Furthermore, the overarching trends suggest that the aquatic insect assemblages are resilient yet sensitive to ecological changes, evidencing the necessity for continuous monitoring to maintain biodiversity, especially in sensitive areas. The significant differences in metrics such as the Shannon diversity index among specific stream pairs illustrate that while some streams may share similar characteristics, others are distinctly diverse, revealing the potential for hidden biodiversity yet to be uncovered. Conservation strategies must be informed by these findings to ensure habitat protection and enhance ecosystem management efforts. This work not only contributes to the understanding of aquatic insect diversity but also underscores the value of these organisms as biological indicators of ecosystem health in freshwater environments.

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Conflict of Interest Statement

The authors agree that this research was conducted in the absence of any self-benefits, commercial, or financial conflicts and declare absence of conflicting interests with the funders.

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