Preliminary Study on the Distribution and Diversity of Diptera at Tuba Island Reserve Forest, Langkawi, Malaysia

N A Nizam¹, C N I I Mohd Najib¹, N N Md. Yusof¹, N B Mohammad Naser¹ and S K Mohd Hatta¹,²*  
¹Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Malaysia  
²Sustainable Crop Protection Research Group, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia  
*Email: sitikhairiyah@uitm.edu.my

Abstract. Insects, the ecologically important organisms, contribute most to the world’s biodiversity. A study on distribution to diversity of order Diptera was conducted in a selected area of Tuba Island Forest Reserve, Langkawi from 17th September to 21st September 2020 using Malaise traps. Three study sites which were forest fringe, middle forest and inner forest were chosen. A total of 5450 individuals of Diptera belonging to 18 families and 27 morphospecies were collected in this study. Diptera samples were highly abundant in the forest fringe with Mycetophilidae as the most abundant family recorded, while the least abundant family was Drosophilidae. The Shannon-Weiner Diversity Index (H’) showed that the forest fringe had the highest diversity value (H’= 2.05), followed by the inner forest (H’= 0.67) and middle forest (H’= 0.45). The highest Evenness Index (E’) value was recorded from the inner forest with E’=0.66 while the highest value for Margalef Richness Index (R’) was from the forest fringe which was 1.91. The Kruskal-Wallis H test conducted indicates that there was a significant difference in the distribution of Dipteran across the three locations of the forest with P<0.05. Overall, this study suggested that the diversity of order Diptera was higher in the forest fringe compared to the middle and the inner forest. This study is important as it is helpful for future study of Diptera species in Tuba Island as well as for conservation measures in the island ecosystem. 

Keywords: diversity, abundance, flies, mosquitoes, island forest

1. Introduction  
In simple words, biodiversity is defined as the complete number of all biotic variations from genetic levels to ecosystems [1]. Globally, insects make a major contribution to the emergence of our great biodiversity where they are estimated to have 5.5 million species [2]. Insects classified under Class Insecta belong to the phylum of Arthropoda and they are usually recognized by the hardened parts of integument in most species [3]. The four major orders of these diverse species are Coleoptera (beetles), Lepidoptera (moths and butterflies), Hymenoptera (sawflies, ants, bees and wasps) and Diptera (true flies) [4].
Among all the families of insects, order Diptera consists of two-winged or actual flies, and it has been one of the world’s most widely known and important insects [5]. Diptera has been divided into two main suborders which are Nematocera, commonly known as mosquitoes and other flies with long antenna in general while suborder Brachycera is classified as flies with short antenna [6]. Flies and mosquitoes are widely scattered almost everywhere due to their incredibly broad range of larval habitats. They also play vital roles in the ecological relations of the earth such as controlling pests, flowers’ pollinators, decomposers and disease’s vectors [7]. According to Brundage [8], true flies are indeed the key insects that are forensically important as they are usually the first organism to colonize the corpse.

Several aspects including habitat diversity, island age, climatic condition, extreme isolation and status of knowledge influence the number of species on islands [9]. Species richness, phylogenetic diversity and phylogenetic composition are also determined by the role of the island area and the distance from the mainland [10]. The study on the composition of the insects on the island helps to understand more about the species interaction and how to conserve them in preventing the extinction of the species. As for now, there is an absence of insect studies conducted in Tuba Island, Langkawi. Due to that, there is definitely insufficient statistics and data related to the abundance and diversity of insects, especially Diptera. The data from this study will be a helpful reference for future study of Diptera species in Tuba Island as this island is inhabited by human. This study can also help authorities to come up with any conservation action or protection measures for the insects such as enforcing the laws by the government and conservation campaigns by non-government organisations.

Langkawi Island which is located on the north-western coast of Peninsular Malaysia is an archipelago of 99 islands [11]. In 2007, Langkawi Island was declared a Geopark by the United Nations Educational, Scientific and Cultural Organisation (UNESCO). Labelled as the forgotten Island of Langkawi, Tuba Island is one of the inhabited islands in Langkawi Archipelago which is also rich in flora and fauna. It is located five kilometres southwest of Kuah Jetty, the main ferry terminal in Langkawi. With a 20-minute boat ride from Kuah Jetty, this less-travelled Island can be reached [12]. About more than 2000 people lived in the community where most of the work was as fishermen. Although the locals viewed themselves as a fishing society, they are still engaging with animal rearing and some agriculture activities such as fruits, vegetables, and rice farming [13].

The objective of the study is to quantify the number of Diptera at the selected forest of Tuba Island. The study is also to evaluate the relationship between order Diptera’s diversity with the variations in the environmental gradient from the forest fringe to the inner forest of the island. It is crucial to understand the distribution and diversity of functionally distinct insect communities within forest landscapes to conserve and promote biodiversity in forest ecosystems [14]. In addition, this study can also help the Langkawi Management to manage and conserve the valuable species of Diptera.
2. **Materials and Methods**

2.1. **Study site**

This study was conducted at Tuba Island Forest Reserve in Tuba, Langkawi, Malaysia from 17th to 21st September 2020. The forest of Tuba Island with the size of 504 hectares is listed under the UNESCO Global Geopark as Dayang Bunting Marble Geoforest Park [15]. It is a combination of two reserved forests, together with Dayang Bunting Island reserved forest. In terms of geography, Tuba Island is a mountainous island with very few lowlands.

2.2. **Sampling methods**

Samplings were carried out by using a Malaise trap attached with a collecting bottle that was half-filled with 70% alcohol to preserve the specimen. Three units of Malaise traps were placed at three different locations. Trap 1 represented the forest fringe; Trap 2 represented the middle forest and Trap 3 represented the inner forest. The distance that separated each Malaise trap was 100 metres (Figure 1). The traps then were left unattended for five days.

![Figure 1. The placement of malaise traps on the census line.](image)

All samples from the collecting bottles were sorted out according to their order with the aid of a stereomicroscope. The collected insects were preserved, pinned and labelled to prevent any defect. Then, the specimens have been identified at family level by referring to a reference book titled Borror and DeLong’s Introduction to the Study of Insects based on their characteristics [16].

2.3. **Data analysis**

The programme, called PAST (PAleontological STatistics) was used to analyze the data to determine the sp. abundance, sp. diversity, sp. richness, the similarity and equality of sp., the index of overlapping species as well as common species and also rare species in the study sites. Following this, Kruskal-Wallis test was used to compare the diversity of Diptera families across the three different locations of the forest.
3. Results And Discussion

3.1. Overall abundance of Diptera at selected area in Tuba Island

A total of 5450 specimens from order Diptera and 27 morphospecies were collected at Tuba Island, Langkawi (Figure 2). In this study, 18 families of Diptera were identified including two unknowns’ families due to certain limitations. The families recognized in this study were Anisopodidae, Calliphoridae, Cecidomyiidae, Chironomyiidae, Culicidae, Drosophilidae, Muscidae, Mycetophilidae, Phoridae, Sarcophagidae, Sciaridae, Simuliidae, Syrphidae, Tabanidae, Tephritidae, and Tipulidae. The most abundant family found at Tuba Island with 2736 individuals was family Mycetophilidae with a percentage of 50.20% from overall Dipteran collected followed by family Sciaridae 20.22% and Phoridae recorded the third largest family at the study site with 7.36% and 401 individuals. Anisopodidae and Drosophilidae ranked as the least families recorded from this study with the percentage of 0.20% and 0.18% respectively.

Figure 2. A pie chart showing the percentage held by each family of Diptera at the study site.

According to Jaschhof and Hippa [17], these findings are similar to the conventional fact that Manota sp. which are under the family of Mycetophilidae is one of the main forest residents even though the adults are quite inconspicuous to their habitats. This result was also supported by a study from Kitching...
et al. [18] showing that Sciaridae and Phoridae were the common largest families found in a study conducted in the forests. A study by Pape et al. [19] had identified a total of 233 genera and 4,500 species of Mycetophilidae throughout all biogeographic areas. The adults of Mycetophilidae can be found in the undergrowth of woods, shady locations beside streams, tunnels, and are especially widespread in tree root holes and overhanging stream banks [20]. The larvae mostly feed on mycelia and sporophores penetrating hyphae, however many species’ biology remains unknown [20].

The least family of Diptera which were Anisopodidae and Drosopholidae recorded a total of 11 and 10 individuals respectively. The family of Anisopodidae is considered small with only seven genera described [21]. The results from this study can be supported by Oboňa and Dvořák [22] saying that adults of Anisopodidae can be commonly found in forests, even though numerous species can also be discovered in semi-open areas such as bushes, gardens, and orchards. Saprophagous, which is the larvae of Anisopodidae can be spotted in fermented grounds, rotten vegetables, decomposed leaves and roots, and tree holes [23]. Previous study also recorded less number of Anisopodid on smaller, “oceanic” islands compared to major islands [24].

Meanwhile, Drosophilidae, the least number of Dipteran recorded in this study were commonly known as pomace flies or small fruit flies. Over 4000 species of Drosophilidae have been recognized all over the earth [21]. Drosophila Fallén with 1157 described species is the largest genus of Drosophilidae where the highest number were occurring in tropical regions. A few hundred other undiscovered species are expected to remain to be found in tropical areas [25, 26]. Fermenting, rotting fruit or fruit juice are usually associated with this Drosophilidae species [27]. In fact, the genus Drosophila shows a vast trophic ecosystem which includes species that evolve in decaying plants, decaying fruit, tree sap, fungi and feed on other invertebrates. Some of the larvae live by attaching to the exoskeleton of crab and ingesting the semi-liquid excretions released by them [28].

### 3.2. Abundance of Diptera at different sites

This study shows that the highest number of individuals of Diptera were collected in Trap 1 with a total of 2549 individuals followed by Trap 2 with 2488 individuals and Trap 3 with 413 individuals. Mycetophilidae, the largest family in this study, recorded the highest number of individuals in Trap 2 which was from the middle forest with a total of 2224 individuals compared to the forest fringe and inner forest (Table 1).

| Families  | Morphospecies | Trap | Total |
|-----------|---------------|------|-------|
| Mycetophilidae |                |      |       |
| Anisopodidae |                |      |       |
| Drosopholidae |                |      |       |
| Drosophilidae |                |      |       |

**Table 1.** Number of individual Dipteran families in each study site. Trap 1 represents the Forest fringe, Trap 2 representing the middle forest and Trap 3 representing the inner forest.
| Family            | 1 | 2   | 3   |
|-------------------|---|-----|-----|
| Culicidae         | 2 | 140(2) | 0 | 91(1) | 231 |
| Sciariidae        | 3 | 991(3) | 63(1) | 48(1) | 1102 |
| Mycetophilidae    | 2 | 350(2) | 2224(2) | 162(2) | 2736 |
| Chironomidae      | 1 | 128(1) | 0 | 0 | 128 |
| Simuliidae        | 1 | 83(1) | 0 | 0 | 83 |
| Cecidomyiidae     | 1 | 113(1) | 165(1) | 23(1) | 301 |
| Sarcophagidae     | 1 | 19(1) | 0 | 0 | 19 |
| Anisopodidae      | 1 | 11(1) | 0 | 0 | 11 |
| Tipulidae         | 2 | 18(1) | 0 | 3(1) | 21 |
| Tabanidae         | 3 | 32(2) | 2(1) | 0 | 34 |
| Tephritidae       | 1 | 30(1) | 16(1) | 0 | 46 |
| Syrphidae         | 1 | 86(1) | 4(1) | 32(1) | 122 |
| Phoridae          | 2 | 345(2) | 12(1) | 44(1) | 401 |
| Calliphoridae     | 1 | 32(1) | 0 | 0 | 32 |
| Muscidae          | 2 | 152(2) | 0 | 0 | 152 |
| Drosophilidae     | 1 | 0 | 0 | 10(1) | 10 |
| Unknown 1         | 1 | 19(1) | 0 | 0 | 19 |
| Unknown 2         | 1 | 0 | 2(1) | 0 | 2 |

|                | 2549 | 2488 | 413 | 5450 |
|-----------------|------|------|-----|------|
| Percentage (%)   | 46.77 | 45.6 | 7.57 | 100  |
| Total Family     | 18 | 16 | 8 | 8 |
| Morphospecies    | 27 | 23 | 9 | 9 |

*The value in the bracket is the total number of morphospecies among Dipteran families in the study sites.

Trap 1 that recorded the greatest number of Diptera among all was located at the forest fringe, which is the outer layer of the forest with fewer trees and more sunlight available compared to the inner forest. In this case, the forest’s environmental condition that determines the diversity and abundance of Diptera includes the amount of sunlight and type of flora that they are inhibited and attracted by. The forest fringe which is characterised by many shrubs, small trees, epiphytes, and herbaceous species, received
lateral sunlight until the ground [29]. Based on studies by Shean et al. [30] and Sharanowski et al. [31], Diptera was shown to favour the sun over the shade. Furthermore, the presence of sunlight also determines the temperature and conditions of surroundings. The high temperature usually stimulates the activity of insects. According to Bansode et al. [32], the most important factors influencing the development of arthropods include the temperature and humidity where insects’ development is more rapid under higher temperatures and high moisture conditions.

The least number of Diptera in Trap 3 were influenced by the densely forested area with fewer shrubs, herbs and flowering vegetation that act as food resources to the families of Diptera. Woody shrubs usually thrive more in the forest edge, open fields, along the riverbank, and in open shrubs [33]. This result supports a previous study done by M H et al. [34] where a smaller number of Diptera found at the inner forest compared to the forest fringe. It is also predicted that during the short sampling period, families of Diptera roaming around were not numerous. The insect’s sampling has also been done during the monsoon season, where several insect species have been reported to decrease in number during the rainy mid-season, displaying an abundance that is lower than can be seen during the dry season. The insect productivity in tropical locations is triggered by macroclimatic and microclimatic changes such as temperature, rainfall, and variations in the availability of food supplies [35].

The duration of the study conducted also greatly influenced the number of Diptera individuals collected. This study was done in a short time on-site where during the sampling activity, Malaise traps were only set up for five days in the forest. In comparison with research done by Mohd Hatta et al. [36] in a selected forest in Langkawi, the Malaise trap was set up for six months while the killing bottle was replaced every two months. The temporal differences shown here are the main effect on why the amount of Diptera collected was quite low compared to other studies on Diptera diversity.

3.3. Diversity, evenness and richness of Diptera
Species diversity is determined by two important aspects which are the evenness and richness of species in the selected area or habitat of study [37]. The term species richness can be described best as the variety of different species in an environment or ecosystem [38]. As for the evenness, Moore [39] claimed that species evenness considers the number of species in a community as well as their relative abundance. The Shannon-Weiner Diversity Index (H’), Evenness Index (E’) and Margalef Index (R’) were used in this study to analyze the data.
Table 2. Diversity index analysis of Diptera in selected forest of Tuba Island.

| Study site | Shannon-Weiner Diversity Index (H') | Evenness Index (E') | Margalef Index (R') |
|------------|-------------------------------------|---------------------|---------------------|
| Trap 1     | 2.05                                | 0.48                | 1.91                |
| Trap 2     | 0.45                                | 0.19                | 0.89                |
| Trap 3     | 1.67                                | 0.66                | 1.16                |

Based on Table 2, the Shannon-Weiner Diversity Index reading for Trap 1 had the highest value of diversity index with $H'=2.05$ followed by Trap 3 with $H'=1.67$. Trap 2 recorded the lowest Shannon-Weiner Diversity Index value with $H'=0.45$. For the Evenness Index (E'), Trap 3 showed the highest with 0.66 followed by Trap 1 with 0.48 while Trap 2 showed the lowest with 0.19. For the Margalef Index (R'), Trap 1 showed the highest value again with $R'=1.91$ while Trap 2 and 3 recorded values of 0.89 and 1.16, respectively. The values of $H'$ are certainly affected by the value of $E'$ and $R'$, where the higher the number of values in $E'$ and $R'$, the higher the value of $H'$ will be.

From the results, even though Trap 1 has the highest diversity of Diptera, the species are less evenly distributed. According to Smith and Wilson [40], the distribution of individuals of species becomes more uniform and evenly distributed when the Evenness index (E') attains 1.00. Individuals in Trap 2 were less uniform with the lowest E' value due to some species dominating the area such as Mycetophilidae with the highest count compared to species with low numbers. Both E' and R' values recorded the lowest value making Trap 2 which is the middle forest low in diversity and abundance of Diptera. Still, the highest number of R' indicates that Trap 1, which is the forest fringe, is rich in Dipteran species. This can be supported by the fact 16 families of Diptera can be found in the location.

Kruskal-Wallis test was performed to find out the comparison of the significant difference among the distribution of Dipteran family across all the three traps. In this study, there was a significant difference in the distribution of Diptera across all traps (Kruskal Wallis, $\chi^2= 17.75$, df=2, $P < 0.05$). After that, the post hoc test through pairwise comparison showed the significance comes from all traps. The difference comes from all traps where Trap 1 and Trap 2 come out with $P=0.028$, while Trap 1 and 3 showed a value of $P=0.033$, as did Trap 2 and 3 with $P<0.000$. The distribution of the family of Diptera across all traps is significant and determines that the family’s distribution differs between each location.

4. Conclusion

In conclusion, the study reported a total of 5450 individuals of Diptera where forest fringe is highly abundant and diverse in this species compared to the middle and inner of this forest. Weather and climate changes are the examples that mainly affected the results obtained from this study. Environmental
conditions such as the availability of sunlight, accessibility to nectar and food supplies, and dwelling factors also contribute to the diversity of Diptera. Besides, the duration of the study such as insects sampling and laboratory works influenced the results too.

Diptera is known for multiple important roles, so there is no doubt that they play a vital role in the cycle of the ecosystem. Humans should put more effort into protecting Dipteran diversity and stop their species from any extinction threat. The data from this study can help Langkawi Management to conserve the valuable species on this island. Lastly, a future study on the diversity and abundance of Diptera can be done more widely and deeply to enhance the vision and comprehension of this species biodiversity, especially at this location.

Acknowledgments
The authors would like to thank Mr Adnan and Mr Fazlan for assisting in collecting the samples. Special thanks also go to Jabatan Perhutanan Negeri Kedah for giving permission to conduct this study. This research has been funded by the Universiti Teknologi MARA under grant number 600-RMC/LESTARI SDG-T 5/3 (168/2019).

References
[1] Purvis A and Hector A 2000 Getting the measure of biodiversity Nature 405 212-219
[2] Stork N E 2018 How many species of insects and other terrestrial arthropods are there on earth? Annual Review of Entomology 63 (1) 31-45
[3] Gibb T J and Oseto C 2020 Chapter 4 - Classification of insects and mites Insect Collection and Identification (Second Edition) ed T J Gibb and C Oseto (Academic Press) pp 129-145
[4] Jarzembowski E A 2021 Insects Encyclopedia of Geology (Second Edition) ed D Alderton and S A Elias (Oxford: Academic Press) pp 266-272
[5] Sarwar M 2020 Typical flies: natural history, lifestyle and diversity of Diptera Life Cycle and Development of Diptera ed M Sarwar (IntechOpen)
[6] Gerhardt R R and Hribar L J 2019 Chapter 11 - flies (Diptera) Medical and Veterinary Entomology (Third Edition) ed G R Mullen and L A Durden (Academic Press) pp 171-190
[7] Skevington J H and Dang P T 2002 Exploring the diversity of flies (Diptera) Biodiversity 3 (4) 3-27
[8] Brundage A 2020 Diptera development: a forensic science perspective Life Cycle and Development of Diptera ed M Sarwar (IntechOpen)
[9] Gillespie R G and Roderick G K 2002 Arthropods on islands: colonization, speciation, and conservation Annual Review of Entomology 47 (1) 595-632
[10] Portillo J T d M, Ouchi-Melo L S, Crivellari L B, de Oliveira T A L, Sawaya R J and Duarte L d S 2019 Area and distance from mainland affect in different ways richness and phylogenetic diversity of snakes in Atlantic Forest coastal islands Ecology and Evolution 9 (7) 3909-3917
[11] Lagkawi UNESCO Global Geopark 2021 Lembaga Pembangunan Langkawi (accessed August 5, 2021) Available from: https://www.lada.gov.my/langkawi-unesco-global-geopark/
[12] Naturally Langkawi 2019 Langkawi Development Authority (accessed August 7, 2021) Available from: https://naturallylangkawi.my/
[13] Berger M M 2015 An ecological study of the fishermen of Pulau Tuba, Langkawi, Malaysia M.Sc. Thesis University of Oslo Norway 21 pp Biodiversity 3(4) 3-27
[14] Diekötter T, Billeter R and Crist T 2008 Effects of landscape connectivity on the spatial distribution of insect diversity in agricultural mosaic landscapes Basic and Applied Ecology 9
[15] Sumber Hutan 2021 Jabatan Perhutanan Negeri Kedah (accessed July 30, 2021) Available from: http://www.kedforestry.gov.my/ms/sumber-hutan.html

[16] Charles A T, Norman F J and Donald J B 2005 Borror and DeLong's Introduction to the Study of Insects (Belmont, CA: Thompson Brooks/Cole)

[17] Jaschhof M and Hippa H 2005 The genus Manota in Costa Rica (Diptera: Mycetophilidae) Zootaxa 1011 1-54

[18] Kitching R L, Buckel D, Creagh A C, Hurley K and Symonds C 2004 The biodiversity of Diptera in old world rain forest surveys: a comparative faunistic analysis Journal of Biogeography 31 (7) 1185-1200

[19] Pape T, Blagoderov V and Mostovski M 2011 Order Diptera Linnaeus, 1758. In: Zhang, Z.-Q. (Ed.) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness Zootaxa 3148 222

[20] Sevecik J 2010 Czech and Slovak Diptera associated with fungi (Slezské zemské Muzeum)

[21] Pirani G and Amorim D D S 2016 Going beyond the tip of the Drosophilidae iceberg: New Cladochaeta Coquillett, 1900 (Diptera: Drosophilidae) from Brazil Zootaxa 4139 (3) 301–344

[22] Mignaut T, Senterre B, Müller J, Lejoly J and Parmentier I 2010 Shrubby and forest fringe communities of the inselberg - rainforest ecotone in Atlantic Central Africa Plant Ecology and Evolution 143 128-137

[30] Shean B S, Messinger L and Papworth M 1993 Observations of differential decomposition on sun exposed v. shaded pig carrion in coastal Washington State J. Forensic Sci. 38 (4) 938-949

[31] Sharanowski B, Walker E and Anderson G 2008 Insect succession and decomposition patterns on shaded and sunlit carrion in Saskatchewan in three different seasons Forensic science international 179 219-240
H 2015 Species diversity and abundance of Hymenoptera; Ichneumonidae at selected forest in Langkawi Islands, Malaysia Adv. Environ. Biol. 9 (19) 1-4

[37] Zhang H, John R, Peng Z, Yuan J, Chu C, Du G and Zhou S 2012 The relationship between species richness and evenness in plant communities along a successional gradient: a study from sub-alpine meadows of the Eastern Qinghai-Tibetan Plateau, China PLoS One 7 (11) e49024

[38] Kaper H and Rousseau C 2019 Mathematics of planet earth Notices of the American Mathematical Society 66 1

[39] Moore J C 2013 Diversity, taxonomic versus functional Encyclopedia of Biodiversity vol 7, ed S A Levin (Amsterdam: Elsevier Academic Press) pp 648–656

[40] Smith B and Wilson J B 1996 A consumer’s guide to evenness indices Oikos 76 (1) 70-82