

**DIVERSITY AND ECOLOGY OF IMMATURE BLACK
FLIES IN STREAMS OF TAMBUNAN, SABAH.**

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DEGREE OF MASTER OF SCIENCE**

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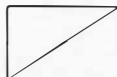
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ABSTRACT

A study on the diversity and ecology of black flies towards the rainfall variations in Sabah was conducted for 12 months from October 2015 until September 2016 at Tambunan district. The objectives of this study were, i) to determine the black flies species composition in selected streams at Tambunan., ii) to determine the relationship between seasonal changes and the abundance of black flies pupae, and iii) to determine the relationship of water quality with the abundance of black flies pupae. There were six study sites located in the Tambunan district, Sabah. The sampling point were chosen according to their accessibility for collection and the presence of water flow. The frequency of field sampling was once every fortnight. During every field sampling, black flies larvae and pupae were collected from all substrates. The physico-chemical parameter of water quality such as stream depth, width, temperature, pH, dissolved oxygen (DO), velocity, conductivity and total dissolved solids (TDS) were also measured at the time of each collection. Rainfall data information for Tambunan District was obtained from Department of Meteorology Malaysia (Sabah Branch and Batu Gajah Agriculture station, Tambunan). Results from this study recorded seven species of black flies from three sub genus: *Simulium*, *Gomphostilbia* and *Nevermania*. The black flies species recorded were *Simulium sabahense*, *Simulium keningauense*, *Simulium beludense*, *Simulium parahiyangum*, *Simulium sheilae*, *Simulium sp* and *Simulium aureohirtum*. A total of 9689 individual of black flies pupae were sampled for a period of 12 months. The Shannon Weiner diversity index (H') ranged between 0.06 to 1.65 which indicate low diversity of black flies in all the study sites. Sg Tambunan has the highest index value with 1.65 and the lowest diversity index value was recorded at Sg Lumondou, with the value of 0.06. The common species recorded in all sites were *Simulium beludense*, *Simulium sabahense*, *Simulium keningauense* followed by *Simulium sp*. Result shows that there were no significant differences in the mean number of black flies pupae sampled between wet and dry period (Kruskal Wallis test = 5, df = 23, $p=0.419$). The Spearman's rank correlation test shows that there was a weak, negative correlation between rainfall variation and the abundance of black flies pupae ($r_s = -0.414$, $p=0.044$). The Principal Analysis Component (PCA) analysis results indicated two principal components that have eigenvalue >1.0 and accounted 72.67% of the total variability of ecological factors among sampling sites. PC1 includes the stream depth, width, velocity, dissolved oxygen, conductivity and total dissolve solids, while the water pH was the only parameter in PC2. Spearman's correlation test shows that there was a significant relationship between of physico-chemical parameter such as stream depth, stream width, velocity stream temperature, DO, TDS and conductivity with the total individual of black flies pupae with p value <0.05 . Thus, it can be concluded that the rainfall variations and physico-chemical parameter have a significant relationship and influenced the abundance of black flies in Tambunan.

ABSTRAK

DIVERSITI DAN EKOLOGI LALAT HITAM PERINGKAT TIDAK MATANG DI SUNGAI TAMBUNAN, SABAH

Satu kajian mengenai kepelbagaian dan ekologi lalat hitam terhadap variasi hujan di Sabah telah dijalankan selama 12 bulan dari Oktober 2015 hingga September 2016 di daerah Tambunan. Objektif kajian ini adalah, i) untuk menentukan komposisi spesies lalat hitam di sungai terpilih di Tambunan, ii) untuk menentukan hubungan antara perubahan taburan hujan dan taburan pupa lalat hitam, dan iii) untuk menentukan hubungan kualiti air dengan taburan pupa lalat hitam. Terdapat enam tapak kajian yang terletak di daerah Tambunan, Sabah. Titik pensampelan di setiap sungai telah dipilih mengikut kebolehcapaian untuk pengumpulan larva dan pupa lalat hitam dan kehadiran aliran air. Kekerapan pensampelan lapangan adalah dua kali setiap bulan. Semasa setiap pensampelan lapangan, larva lalat hitam dan pupa dikumpulkan dari semua substrat. Parameter fiziko-kimia kualiti air seperti kedalaman sungai, lebar sungai, suhu, pH, oksigen terlarut (DO), halaju, kekonduksian dan jumlah pepejal terlarut (TDS) juga diukur pada setiap persampelan lalat hitam. Maklumat data hujan untuk Daerah Tambunan diperolehi dari Jabatan Meteorologi Malaysia (Cawangan Sabah dan stesen Pertanian Batu Gajah, Tambunan). Hasil daripada kajian ini mencatatkan tujuh spesies lalat hitam dari tiga sub genus: *Simulium*, *Gomphostilbia* dan *Nevermania*. Spesies lalat hitam yang direkodkan ialah *Simulium sabahense*, *Simulium keningauense*, *Simulium beludense*, *Simulium parahiyangum*, *Simulium sheilae*, *Simulium sp* dan *Simulium aureohirtum*. Sebanyak 9689 individu pupa lalat hitam telah diambil sampel untuk tempoh 12 bulan. Indeks kepelbagaian Shannon Weiner (H') berkisar antara 0.06 hingga 1.65 yang menunjukkan kepelbagaian lalat hitam di semua tempat kajian adalah rendah. Sg Tambunan mempunyai nilai indeks tertinggi dengan 1.65 dan nilai indeks kepelbagaian terendah dicatatkan di Sg Lumondou, dengan nilai 0.06. Spesies biasa yang dicatatkan di semua sungai adalah *Simulium beludense*, *Simulium sabahense*, *Simulium keningauense* diikuti oleh *Simulium sp*. Keputusan menunjukkan bahawa tidak terdapat perbezaan yang signifikan diantara bilangan purata pupa lalat hitam semasa tempoh basah dan kering (Kruskal Wallis test = 5, $df = 23$, $p = 0.419$). Selain itu, ujian korelasi peringkat Spearman menunjukkan bahawa terdapat korelasi negatif yang lemah antara variasi hujan dan kelimpahan pupa lalat hitam ($r_s = -0.414$, $p = 0.044$). Hasil analisis Komponen Analisis Utama (PCA) menunjukkan dua komponen utama yang mempunyai $eigenvalue > 1.0$ dan menyumbang 72.67% dari jumlah variabiliti faktor ekologi dari kawasan kajian. PC1 merangkumi kedalaman aliran, lebar, halaju, oksigen terlarut, kekonduksian dan jumlah pepejal yang larut, sementara pH air adalah satu-satunya parameter dalam PC2. Ujian Korelasi Spearman menunjukkan terdapat hubungan yang ketara diantara parameter kimia fizik seperti kedalaman aliran, lebar arus, suhu aliran halaju, DO, TDS dan kekonduksian dengan jumlah individu pupae lalat hitam dengan nilai $p < 0.05$. Oleh itu, dapat disimpulkan bahawa variasi curahan hujan dan parameter fisiko-kimia mempunyai hubungan yang signifikan dan mempengaruhi kelimpahan lalat hitam di Tambunan.

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LIST OF ABBREVIATIONS

TMB	-	Sg Tambunan
MLG	-	Sg Malungung
KNB	-	Sg Kinabaan
KRK	-	Sg Kerokot
MHU	-	Sg Mahua
LMD	-	Sg Lumondou
OCF	-	The Onchorcerciasis Control Programme
WHO	-	World Health Organization
MET	-	Malaysian Metrological Department
INWQS	-	Interim National Water Quality Standard
DO	-	Dissolved Oxygen
BOD	-	Biochemical Oxygen Demand
COD	-	Chemical Oxygen Demand
TSS	-	Total Suspended Solids
TDS	-	Total dissolved Solids
AN	-	Ammonia Nitrogen
sp	-	Species

LIST OF SYMBOLS

-	- Minus/ dash
+	- Plus/ positive/ present
%	- Percent
<	- Less Than
>	- More Than
=	- Equal to
°C	- Degree Celcius
SD	- Standard deviation
Sig.	- Significant
x	- Multiply
m	- Meter
mm	- Millimeter
m/sec	- Meter per second



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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Black flies (Diptera: Simuliidae), also known as “buffalo gnats” or “turkey gnats,” are very small, robust flies that are biting pests of wildlife, livestock, poultry, and humans (Catherine *et al.*, 2010). Recent revisions by Adler & Crosskey (2014) reported that, there are 2,154 species of black flies distributed worldwide. In some regions such as the Afrotropical and Neotropical region (Kettle, 1995), black flies have been reported to transmit parasites to the livestock that causes problems in terms of economic losses due to reduced beef and milk production, spread of disease and reduce the efficiency of agricultural and industrial workers (Takaoka *et al.*, 2011; Adler *et al.*, 2004). This is because, adult female black flies are blood-sucker and vectors of some parasites such as *Onchocerca spp* that cause the disease of Onchocerciasis or river blindness (Opoku, 2006). However, in Malaysia, there are no disease vector from black flies reported yet (Takaoka, 2003; Takaoka, 1994).

Black flies pass through four developmental stages wherein the immature stages inhabit and prefer running water areas to breed (Anbalagan *et al.*, 2017). Grillet and Barrera (1997) stated that black flies densities are maximum during rainy season and this finding are agreed by many researchers (Opoku, 2006; Srisuka *et al.*, 2017), but in some cases, the distribution of simuliid may decreased during rainy season due to fast water current (Srisuka *et al.*, 2015; Eyo *et al.*, 2014). Srisuka *et al.*, (2017) pointed out one of the important factors that affect the breeding habits and distribution of black flies is the seasonal changes (Zubaidah *et al.*, 2016a; Hamada *et al.*, 2002). Seasonal changes can comprised from the variation of rainfall precipitation, temperature anomalies and relative

humidity of certain places (Loo *et al.*, 2015). In Malaysia, seasonal changes is basically due to seasonal rainfall variation which occur during the Northeast and Southwest monsoon seasons (Zubaidah *et al.*, 2016b; Low *et al.*, 2012; Satari *et al.*, 2015).

Besides, most of the black flies studies that have been done in other countries focussed on seasonal variation of rainfall precipitation. This is due to the relationships of black flies associated with the streams flow during wet and dry period (Grillet & Barrera, 1997; Hamada & Grillet, 2001; Pramual & Wongpakam, 2010; Figueiro *et al.*, 2014). In relation to that, the focus of this study was to determine diversity and ecology of black flies in relation to the variability of rainfall in Tambunan district, Sabah.

This study was conducted in Tambunan district which is located at the Interior division of the state of Sabah (5.7213° N, 116.4108° E). Six streams were selected in Tambunan district namely, Sg Kerokot (KRK), Sg Mahua (MHU), Sg Tambunan (TMB), Sg Kinabaan (KNB), Sg Malungung (MLG) and Sg Lumondou (LMD).

1.2 PROBLEM STATEMENT

In Malaysia, there are numerous studies on aquatic insects that mostly focus on Ephemeroptera (mayflies), Plecoptera (stoneflies), and Tricoptera (caddisflies) or better known as EPT. In addition to EPT orders, Diptera orders are also widely reviewed but focusing on mosquitoes. However, at present, there are various studies conducted in other countries which focus on black flies such as morphotaxonomy (Takaoka & Choochote, 2004), phylogenetic (Pramual *et al.*, 2011) and ecology (Pramual and Kuvangkadilok, 2009). Currently, black flies studies in Malaysia have been focusing on the distribution and the taxonomy in Peninsular Malaysia, Sabah and Sarawak. However, studies on the distribution of black flies and its ecology in Sabah is very scarce and not well documented. Furthermore, the publications and reports on regional biodiversity, seasonal

abundance or distribution of blackflies specifically in Tambunan areas was also not well documented (Takaoka, 2001a, 2001b; Zubaidah *et al.*, 2016a; Suhaila *et al.*, 2014).

1.3 OBJECTIVES

The main objective of this study was to determine the diversity and seasonal distribution of black flies (Diptera: Simuliidae) at selected rivers in the Tambunan district, Sabah. The specific objectives were;

1. To determine the black flies species composition in selected streams at Tambunan.
2. To determine the relationship between seasonal changes and the abundance of black flies pupae.
3. To determine the relationship of water quality with the abundance of black flies pupae.

1.4 HYPOTHESIS

The hypothesis for this study were made based on objective two and three, and tested at a significance level of 0.05 are as follow;

H_0 = There is no significant relationship between seasonal changes and the abundance of black flies pupae.

H_1 = There is a significant relationship between seasonal changes and the abundance of black flies pupae.

H_0 = There is no significant relationship between the physico-chemical parameter with the abundance of black flies pupae.

H_1 = There is a significant relationship between the physico-chemical parameter with the abundance of black flies pupae.

1.5 JUSTIFICATION

This study is one of such a kind that will contribute to the understanding of aquatic diversity specifically for black flies. Furthermore, it was realized that there are not many entomologists in Malaysia that focus on this Diptera insects. Findings from this study are useful to determine the presence of black flies towards the seasonal changes, especially in Tambunan district. Since black flies are one of the indicators for water quality, the findings of this study can help organizations that are involved in maintaining and monitoring river water quality in Sabah to plan all activities that are involved in the conservation of the river, especially the Sabah Drainage and Irrigation Department. Therefore, this study is essential to provide initial information on the distribution of black flies in Tambunan district and its relationship with the seasonal changes.



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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of Black flies (Simuliidae)

Black flies belong to the Order Diptera, Family Simuliidae (Takaoka *et al.*, 2012; Werner & Pont, 2003). In most recent revision of the World Inventory of Simuliidae, there are 26 genera of black flies where most black flies species are placed into the genera *Simulium* (Adler & Crosskey, 2014). Simuliids are usually present in littoral habitat with high oxygen, complex vegetation structure and diverse food resources (Hershey *et al.*, 2010). Black flies are widely distributed in all zoogeographical region and occur from sea level to 4000 metres above sea level (Zubaidah *et al.*, 2016a; Figueiro *et al.*, 2008).

According to Anbalangan *et al.*, (2017), there are only seven countries under the ASEAN region that have recorded the black flies distribution, which are Myanmar with 8 species of black flies, Indonesia with 127 species, Malaysia with 82 species, Philippines with 85 species, Thailand with 107 species, Vietnam with 27 species and only one black flies species recorded in Laos. According to Kuvangkadilok and Takaoka (2000), the simuliids fauna of Malaysia is most similar with Thailand which is characterized by its richness and diversity at species group level. In Southeast Asia (Thailand, Malaysia, Philippines, and Indonesia) eight subgenera were recorded which include *Asiosimulium* Takaoka and Choochote, *Daviesellum* Takaoka and Adler, *Gomphostalbia* Enderlein, *Montisimulium* Rubtsov, *Morops* Enderlein, *Nevermania*, *Simulium* Latreille s. str, and *Wallacellum* Takaoka (Otsuka *et al.*, 2007). There are four subgenus that are recorded in Malaysia namely are *Daviesellum*, *Gomphostalbia*, *Nevermania* and *Simulium* (Low *et al.*, 2016; Takaoka *et al.*, 2012).

2.2 Life cycles of Simuliidae

Black flies undergo complete metamorphosis which means black flies goes through four distinct stages in their life cycle that consists of; eggs, larva, pupae and adults, as illustrated in Figure 2.1. The length of time to complete a life cycle normally takes about 4-30 days according to their species after the eggs are laid until it emerges into adults (Catherine *et al.*, 2010).

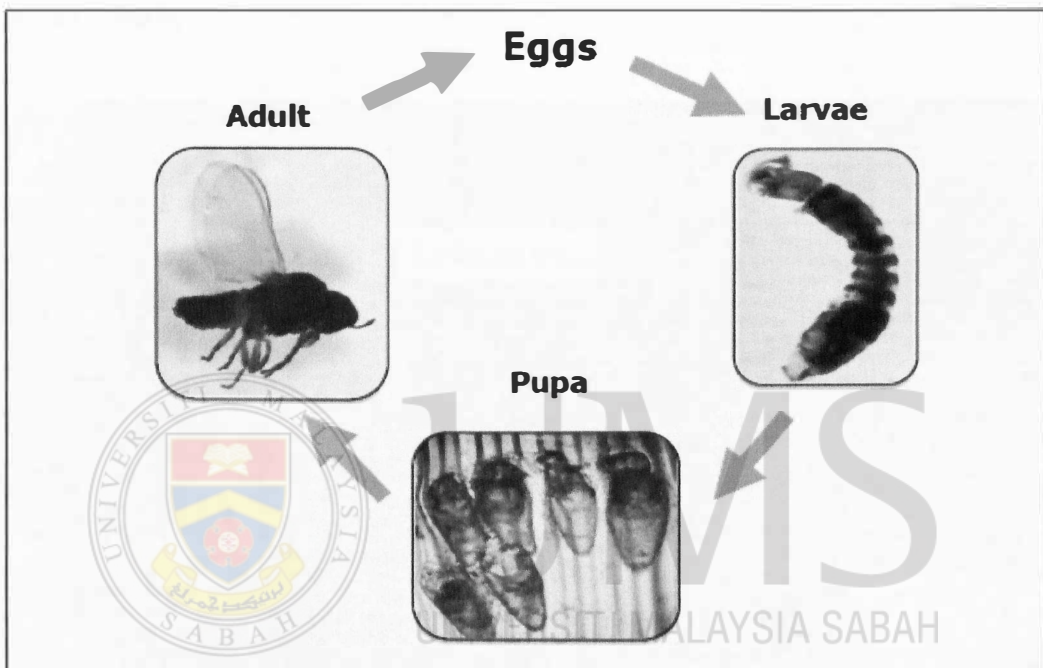


Figure 2.1 : General life cycle of black flies.

Source : Personal collection

2.2.1 Black flies Eggs

The eggs are commonly laid on any substrate in or on running water (Hart, 1986; Catherine *et al.*, 2010). Adult female of black flies lay glistening yellow eggs on vegetation, stones and other submerged object and sometimes it enters the water to deposit its eggs (Twinn & Peterson, 1955).