DISTRIBUTION AND SPECIES DIVERSITY OF BLACK FLIES (DIPTERA: SIMULIIDAE) ASSOCIATED WITH HABITAT CHARACTERIZATION IN RANAU, SABAH.

ESTHERPENI STEPHEN

FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH
2018

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_____

ESTHERPENI STEPHEN
MS1421092T

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NAME	:	**ESTHERPENI STEPHEN**
MATRIC NO.	:	**MS1421092T**
TITLE	:	**DISTRIBUTION AND SPECIES DIVERSITY OF BLACK FLIES (DIPTERA: SIMULIIDAE) ASSOCIATED WITH HABITAT CHARACTERIZATION IN RANAU SABAH**
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1. SUPERVISOR
Dr. Maria Lourdes T. Lardizabal

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ABSTRACT

Black flies surveillance studies are still in its infancy in Sabah, as previous studies were limited to discovery of species and taxonomy. This study was conducted to investigate the distribution and species diversity of the black flies in Ranau District. The objectives of this study were (1) to study the distribution and species diversity of black flies in Ranau, (2) to determine the relationship between habitat characterization and distribution of black flies in Ranau and (3) to determine the relationship between micro climate changes on the species and black flies distribution in Ranau. Black flies were sampled every fortnight for a period of 12 months starting from October 2015 to September 2016. Seven study sites were assigned in Ranau District. Sampling of black fly larvae and pupae were manually collected directly from substrates underneath fast flowing water in the streams. A total of 4340 black flies adults were recorded from this study and were classified into subgenus *Gomphostilbia*, *Nevermania* and *Simulidae*. A total of 19 black flies species were identified and among the 19 species, two are newly recorded in Sabah namely; *S. alienigenum* and *S. nobile*. While the species *S. keningauense* and *S. sabahense* were the common species recorded in all the study sites. The Shannon Weiner's species diversity index, *H′* ranged from 0.45 to 1.68 which indicated low diversity of black flies in all the sites. The species diversity was higher in Kg. Kinarasan with a total of nine species recorded, with the index value, *H′* =1.68, while the lowest was in Nikgold area with only four species found and the index value, H'=0.46. The number of black fly pupae and larvae varied depending on the micro climate changes that occurred in Ranau, Sabah. The number of black fly individuals was higher during the rainy season (October – January, June – September 2016) and decreased during the dry season (Febuary – May 2016). The Principle Component Analysis (PCA) results revealed that water temperature, water depth and altitude were the main factors that influence the distribution of black flies species. The linear regression analysis shows that the black flies abundance were significantly associated with the PC-1 which is in lower altitude, wider, deeper, normal temperature, high conductivity, higher velocity and higher dissolve oxygen (F=53.3, df = 1, 4330, P<0.001). As a conclusion, there is a significant relationship between habitat characterization and distribution of black flies in Ranau with the Canonical Correspondence Analysis value was P= 0.002. In addition, the linear regression also shows there is a significant relationship between micro-climate changes on the species of black flies abundance in Ranau District (R^2=0.131, P<0.01).

ABSTRAK

TABURAN DAN KEPELBAGAIAN SPESIES LALAT HITAM (DIPTERA: SIMULIIDAE) BERKAITAN DENGAN CIRI-CIRI HABITAT DI RANAU, SABAH.

Kajian lalat hitam di Sabah masih berada di peringkat awal, kajian terdahulu hanya tertumpu kepada penemuan spesies baru dan taksonomi lalat hitam. Kajian ini dijalankan untuk mengenal pasti taburan dan kepelbagaian spesies lalat hitam di daerah Ranau,Sabah. Objektif kajian ini adalah (1) untuk menentukan taburan dan kepelbagaian spesies lalat hitam di Ranau, (2) untuk menentukan hubungan antara pencirian habitat dan taburan lalat hitam di Ranau dan (3) untuk mengkaji hubungan antara perubahan mikro- iklim ke atas taburan spesies lalat hitam di Ranau. Persampelan lalat hitam telah dilakukan untuk tempoh 12 bulan bermula dari Oktober 2015 hingga September 2016. Terdapat tujuh kawasan kajian yang telah dipilih di kawasan Daerah Ranau. Persampelan larva dan pupa lalat hitam adalah menggunakan kaedah pengutipan secara terus dari substrat yang berada di bawah air yang mengalir dengan deras. Sebanyak 4340 individu lalat hitam telah direkodkan daripada kajian ini dan telah dikelaskan kepada subgenus Gomphostilbia, Nevermania dan Simulidae. Sejumlah 19 spesies lalat hitam telah dikenal pasti dan antara 19 spesies tersebut, terdapat dua spesies lalat hitam yang baru direkodkan di Sabah iaitu; S. alienigenum dan S. nobile. Manakala spesies S. keningauense dan S. sabahense adalah, spesis yang dicatatkan di kesemua kawasan kajian di Ranau. Nilai indeks kepelbagaian spesies Shannon Weiner, H ' berada di antara julat 0.46-1.68. Kepelbagaian spesies adalah lebih tinggi di Kinarasan dengan jumlah, sembilan spesies direkodkan dan nilai indeks, H '= 1.68, manakala yang terendah adalah di kawasan Nikgold dengan hanya empat spesies ditemui dan nilai indeks, H' = 0.45. Spesimen pupa dan larva lalat hitam adalah bergantung kepada perubahan iklim setempat yang berlaku di Ranau, Sabah. Bilangan individu lalat hitam adalah lebih tinggi semasa musim hujan (Oktober - Disember, Jun-Sep 2016) dan menurun pada musim kering (Febuari - Mei 2016). Keputusan PCA mendedahkan bahawa altitud, suhu dan kedalaman air sungai adalah faktor utama yang mempengaruhi taburan spesies lalat hitam di Ranau. Analisis regresi menunjukkan bahawa terdapat hubungan yang signifikan antara pencirian habitat dan kekayaan spesis lalat hitam PC-1 dan menunjukkan skor yang lebih tinggi pada altitude rendah, kelebaran, kedalaman, suhu normal, konduktiviti yang tinggi, halaju tinggi dan kepekatan oksigen yang tinggi (F=53.3, df = 1, 4330, P<0.001). Kesimpulan daripada kajian ini hubungan yang signifikan diantara ciri-ciri habitat dan taburan lalat hitam di daerah Ranau dengan nilai analisis *Canonical Correspondence adalah P= 0.002. Analisis regresi menunjukkan perubahan cuaca setempat mempunyai hubungan yang signifikan terhadap jumlah kelimpahan lalat hitam di daerah Ranau (R^2=0.131, P<0.01).*

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LIST OF ABBREVIATION

CCA	**-**	Canonical Correspondence Analysis
PC	-	Principle Component
PCA	-	Principle Component Analysis
TDS	-	Total Dissolve Solid
RH	**-**	Relative Humidity
SO	**-**	Site Occurrence
FO	**-**	Frequency Occurrence

LIST OF SYMBOLS

°C	-	Degree Celsius
m.a.s.l	-	Meter Above Sea Level
Dmg	-	Dominance Index (Berger Parker)
DO	-	Dissolve Oxygen
H′	-	Shanon's Weiner Index
m	-	Meter
m^3/s	-	Cubic meter per second
%	**-**	Percentage
<	**-**	Less than
R^2	**-**	R Square
=	-	Equal to
± **S.D**	-	Standard Deviation
N	**-**	Number of Individuals

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CHAPTER 1

INTRODUCTION

1.0 OVERVIEW

Black flies are aquatic insects from the order Diptera and belong to the family Simuliidae. This group of insect is distributed worldwide (Crosskey, 1990). The black flies adults are one to five millimeter in length and usually can be seen black but occasionally yellow or yellowish-brown. These flies have prominent eyes, short mouthparts and segmented antennae. They also having a humped thorax, broad colorless wings with distinct venation and when at rest are held as closed blades of a pair of scissors which is additional morphological characteristics of these flies (Adler *et. al.* 2005). The mature larvae are poorly segmented, lightly colored, and distinguishable by a blackish head with a prominent pair of feeding brushes (Crosskey, 1990). Pupation takes place in several shapes such as slipper shape, shoe shape, woven shape and wall pocket shape. The pupa has respiratory gills projecting from the cocoon.

The adult black flies feed on plant nectar, but in most species, especially adult females require blood meal to obtain the protein necessary to mature their eggs (Youssefi *et. al*. 2008). They are particularly active during the morning and evening. Adult's black flies are strong fliers and are highly responsive to carbon dioxide and other host odors. The habitat of black flies can be found be in small streams to large rivers, depending on the habitat preferences by species (McCreadie & Adler, 2012). Black flies lay their eggs in running water, and the larvae attach themselves to substrates such as rocks, leaves, roots and litter.

Black flies larvae inhabit riffle habitat and play an important role in food chain and in processing nutrient cycle because they have a special feeding behavior such

as shredders, filter feeders, deposit collectors and aquatic insect's predators (Barman, 2014). The adults are blood feeders which may be vector to various diseases to animals and man (Adler *et. al*. 2005). For example, adult black flies have been reported to transmit *Onchocerciasis* or known as river blindness disease to human in South Africa, North America, and Brazil (Hill *et*. *al*. 2010).

Besides the *Onchocerciasis*, adult black flies also can cause Dermatitis diseases. The saliva secreted by black fly as they feed may cause allergic responses in the host. Previous study reported that *Simulium spp*. may transmit different microorganisms to human and animal hosts, for example the virus causing vesicular stomatitis and the avian protozoan *Leucocytozoon* (Youssefi *et. al.* 2008). The black flies bites are painful and due to the release of pharmacologically active substances such as histamine, leukotrienes, prostaglandins, platelet activating factor and eosiniphilic chemotactic factor (ECF) from IgE-sensitized basophils and mast cells after contact with antigens in the flies' saliva acute urticaria, which is one of the disorders due to hypersensitivity may occur (Youssefi *et. al.* 2008) . They also act as vectors for *filaroid helminthes Dirofilaria, Onchocerca, Mansonella*, and *Splendidofilaria* in humans, bears, cattle, ducks, goose and other livestock. However, in Malaysia and South East Country black flies have not been reported as disease vectors yet.

The Simuliidae family can be used as bio-indicator to the freshwater ecosystem, because the distribution and species diversity of black flies in certain streams or river can indicate the cleanliness level of the freshwater ecosystem (Feld *et*. *al*., 2002). This is because the fluctuation of black flies community can give early information about the freshwater's condition, as these group of insects usually respond to the changes in water quality due to the physical or chemical environment factor (Basoren & Kazanci, 2011).

Previous studies on black flies in Sabah were started as early as 1929 and only focused to describe the species taxonomy (Smart & Clifford, 1969). However, there is very limited information on the distribution and ecology of black flies in Sabah. Therefore, this study was conducted to focus more on the black flies distribution and its ecology. Throughout this study also, the relationship between the

habitat characterization on the distribution and species diversity of the black flies was also determined. In addition, findings from this study also determined the relationship between microclimate changes towards the black flies abundance in Ranau district.

This study was conducted in Ranau District, which is located in the West Coast of Sabah. A total of seven sampling sites which included streams and rivers were chosen. Nikgold site was located in a highland area with the altitude of 1427 meter above sea level, while Lohan is located in a lowland area (370 m.a.s.l). Libang, Poring, Kinarasan, Tungou and Ranau were located in the middle altitude which ranged between 528 meter to 754 m.a.s.l.

1.2 PROBLEM STATEMENT

Among the freshwater ecosystem macroinvertebrates in Malaysia, the larvae and pupae of black flies have received less attention. Earlier studies on black flies were only focused on the taxonomy and description of species but rarely investigated and described their ecology especially in Sabah. Studies on the black flies distribution and ecology have not been well documented in Sabah. Publication on the black flies taxonomy of recorded species in Sabah was focused on black flies specimens that were sampled from Mount Kinabalu, Tamparuli, Kota Belud, Tuaran and Keningau (Takaoka *et. al.* 2015; Takaoka, 2001b; Takaoka, 1996; Smart & Clifford 1969). In addition to that, this study was important because black flies would be a vector in future as reported from other countries. Thus, the study on black flies distribution and species diversity is timely necessary to document information and initiate database to facilitate the monitoring of black flies distribution in Sabah to gain knowledge regarding any potential vector in future.

1.3 OBJECTIVES

The objectives of this study are:

1. To study the black flies distribution and species diversity in Ranau District.
2. To determine the relationship between habitat characterization and distribution of black flies in Ranau District.
3. To determine the relationship between micro-climate changes on the species of black flies abundance in Ranau District.

1.4 JUSTIFICATION

This study was conducted to investigate whether the habitat characterization such as width, depth, canopy openness, and physicohemical parameters influenced the species diversity and distribution of black flies. In addition, the relationship between black flies abundance with micro-climate changes were determined. Results obtained from this study will provide information on the distribution and diversity of black flies species in Ranau District, which will contribute to the information on black flies ecology in Sabah.

1.5 HYPOTHESIS

Ho: There is a significant relationship between the habitat characterization and distribution of black flies.

H1: There is no significant relationship between the habitat characterization and distribution of black flies.

Ho: There is a significant relationship between the microclimate changes with the abundance of black flies.

H1: There is no significant relationship between the microclimate changes with the abundance of black flies.

CHAPTER 2

LITERATURE REVIEW

2.1 Aquatic Insects and Their Importance

Aquatic insects are the most diverse macroinvertebrates group of organism. According to Balaram (2005), there are more than 45000 black flies species that are known to inhabit the freshwater ecosystem. Freshwater ecosystem includes the streams, rivers or lakes. Aquatic insects usually spend a portion of their life cycle in the water (Voshell, 2002). The aquatic insects can be classified into aquatic or semi-aquatic. Semi- aquatic species are defined as insects that live in both land and in the water (Barman, 2014). The common orders of aquatic insects include Trichoptera, Ephemeroptera, Odonata, Coleoptera, Hemiptera and Diptera (Balaram, 2005).

The food source of aquatic insects is just as diverse as their habitat. Aquatic insects may feed on organic matter in water. Voshell (2002) categorized aquatic insects according to how they obtain their food in freshwater ecosystems. The categories are named according to their functional feeding groups. The scrapers' groups have mouthparts that are able to cut algae or other solid objects in the water. While the collectors feeding group usually, obtain pieces of decaying plant materials. The shredders' have mouthparts that are able to cut off pieces of soft vegetation. Most aquatic insects shred pieces of vegetation that have dropped off from plant parts that are decaying. Most of this material comes from trees and shrubs that grow on the river banks or streams (Voshell, 2002).

Aquatic insects inhabit a broad and diverse habitat based on their habitat preferences that is influenced by water temperature, turbidity, water discharge, water pH and altitude (Barman, 2014; Currie & Adler, 2008). Each group of aquatic insect have different requirement of microhabitats. Some aquatic insects are able to

live in temporary ponds that will dry during summer season. However, most aquatic insects that inhabit temporary habitats will remain as eggs, until the next rainy season which will fill up the ponds (Voshell, 2002).

Aquatic insects are well known to play an important role in the processing and nutrient cycle in lotic ecosystem because they have several specialized feeding groups. Barman (2014) stated that Ephemeroptera, Odonata, Plecoptera, Megaloptera and Tricoptera can be used as biomonitoring agents. These groups of aquatic insects are used as water quality indicator because they have different tolerances towards water pollution (Voshell, 2002). Ephemeroptera, Plecoptera and Tricoptera are considered the most sensitive groups as species from these groups are only found in good water quality ecosystems and unable to tolerate to polluted rivers or streams. While the Dipteran group are categorized as semi-tolerant species because some species are tolerant to polluted water, while some of the species can only breed in clean and unpolluted water (Barman, 2014).

2.2 Introduction to Black Flies (Simuliidae)

Black flies belong to the order Diptera and grouped in the family Simuliidae. The term Diptera or true flies were inspired by Aristotle, then also being used by Linnaeus in 1744 (Mitra *et al*., 2015). True flies are defined by two-winged flies (Smart & Clifford, 1969). Black flies are distributed worldwide and can be found in most continents and achipelagos, except for Antartica and Hawaii (Currie & Adler, 2008). According to Butler and Hogsette (1998), black flies are found in many parts of the United State America, Florida and Canada. While in South East Asia, black flies are distributed in Thailand, Japan, Malaysia, Philippines and Indonesia. According to Yashushi (2014), there are ten subgenera of black flies recorded from South East Asian countries. In Malaysia, there are 82 species of black flies recorded (Adler & Crosskey, 2016, Adler & Crosskey 2011, Takaoka, 2001a & 2001b) which includes 69 species from Peninsular (Adler & Crosskey 2011, Takaoka 2001a & 2001b) six species from Sarawak (Takaoka 2001a), and more than 27 species recently recorded in Sabah (Takaoka *et al*.,2015; Adler & Crosskey, 2011; Takaoka *et al.,*2011a, 2011b). To date there are 2,219 species of black flies from 2204 subgenera listed worldwide (Adler & Crosskey, 2015).

In temperate countries, black flies are also known as 'turkey gnats' or 'buffalo gnats', where the adult females feed on livestock blood, humans and wild mammals. The black flies are considered second most dangerous group as they can affect health of human and livestock by transmitting various types of pathogen such as the *Onchocercovolvulus*, *Leucocytozoon*, *Mansonella*, *Trypanosom* and *Dirfilaria* (Adler *et al.*, 2005). It was reported by Nakamura and Takahshi (1978) that the biting of black flies females can cause *Onchocerciasis* or river blindness disease. However, the disease was so far reported only in South Africa, North America, and Brazil (Hill *et al.*, 2010).

Black flies larvae act as colonizers on substrates. Colonization is a process whereby the black flies disperse in new breeding sites (Smock, 2006). The black flies larvae will apply silk to the substrates to attach themselves for filter feeding, locomotion and pupation (Lake *et al.*, 1998). However, according to Lake *et al.*, (1989), different black flies species will react differently to alteration of the substratum caused by the silk from previous colonizers. Previous studies reported that the black flies larvae densities are influenced by environmental parameters such as the velocity, turbidity, temperature as well as the riparian vegetation (Docile *et al.*, 2015; Kiel & Lautenschlager, 2001; Kiel *et al.*, 1998). In addition to environmental parameters, disturbance on the freshwater ecosystem such as agriculture activities and domestic sewage also affect the density of the black flies larvae (Docile *et al.*, 2015). Such disturbance will lead to modification of the physical, chemical and biological structure of the freshwater ecosystem, and this will affect the development of pre imaginal black flies.

2.3 Morphology of Black Flies

Black flies are identified and differentiated from other aquatic insects by looking into their larvae and pupae external morphology (Crosskey, 1990).

2.3.1 Adult Black Flies

Adult black flies are small insects with a total body length (head to abdomen) measurement between 5mm to 15 mm and have a thoracic region (Crosskey, 1990). It has a pair of compound eyes, short antennae, and a pair of wings (Figure 2.1).