ANALYSIS OF INTERMEDIATE HORSESHOE BAT'S (*Rhinolophus affinis*) DIET IN GUA SETIR, KELANTAN

by

BALQIS BINTI MOHD ZAHARI

A report submitted in fulfillment of the requirements for degree of Bachelor of Applied Science (Natural Resources Science) with Honours

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DECLARATION

I declare that this thesis entitled "Analysis of Intermediate Horseshoe Bat's (*Rhinolophus affinis*) Diet in Gua Setir, Kelantan" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ____________________________

Name : BALQIS BINTI MOHD ZAHARI

Date : ____________________________

APPROVAL

"We hereby declare that we have read this thesis and in our opinion this thesis is sufficient in terms of scope and quality for the award of the degree of Bachelor of Applied Science (Natural Resources Science) with Honors"

Signature : ________________________________

Name of Supervisor I : ________________________________

Date : ________________________________

Signature : ________________________________

Name of Supervisor II : ________________________________

Date : ________________________________

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Analysis of Intermediate Horseshoe Bat's (*Rhinolophus affinis*) Diet in Gua Setir, Kelantan

ABSTRACT

Southeast Asia is a critical area for biodiversity and bats comprising nearly a third of mammal's species in it. Gua Setir which located in Jeli, Kelantan is the suitable area to study the variety of bats since it is a limestone area and one of the main roosting areas for bats. This study provide the food habit of *Rhinolophus affinis* also known as Intermediate Horseshoe bat that captured in Gua Setir by using harp trap and mist net. The study was conducted to identify the order of insect in the Intermediate Horseshoe bat's diet based on the faecal samples and the analysed the stomach content of this species. There are 20 samples of insect parts found in the sample from six order of insect. The orders listed are Diptera, Coleoptera, Lepidoptera, Blattodea, Hemiptera and Hymenoptera. In the end of study, Diptera were there highest order found comprising the head and thorax parts of the insect. This study also list out the left-over body part of insect. The body parts commonly found in the samples are the wings part of insects, the legs segments of insect and the antennae of the insects. This study provides useful baseline information about the diet analysis of this insectivorous bats species.

Analisis diet kelawar “Intermediate Horseshoe” (*Rhinolphus affinis*) di Gua Setir, Kelantan

ABSTRAK

Asia Tenggara adalah kawasan kritikal untuk kepelbagaian biologi dan kelawar terdiri daripada hampir satu pertiga spesies mamalia di dalamnya. Gua Setir yang terletak di Jeli, Kelantan adalah kawasan yang sesuai untuk mengkaji kepelbagaian kelawar kerana ia adalah kawasan batu kapur dan salah satu kawasan utama untuk kelawar. Kajian ini menunjukkan tabiat makanan Rhinolophus affinis yang juga dikenali sebagai kelawar “Intermediate Horseshoe” yang ditangkap di Gua Setir dengan menggunakan perangkap harpa dan set jaring. Kajian ini dijalankan untuk mengenal pasti order serangga di dalam diet kelawar “Intermediate Horseshoe” berdasarkan sampel najis dan menganalisis kandungan perut spesies ini. Terdapat 20 sampel bahagian badan serangga yang terdapat di dalam sampel dari enam order serangga. Order yang disenaraikan ialah Diptera, Coleoptera, Lepidoptera, Blattodea, Hemiptera dan Hymenoptera. Pada akhir kajian, Diptera merupakan order tertinggi yang dijumpai merangkumi bahagian kepala dan toraks serangga. Kajian ini juga menyenaraikan bahagian tubuh serangga yang tersisa. Bahagian-bahagian badan yang biasa dijumpai di dalam sampel adalah bahagian sayap serangga, segmen-segmen kaki serangga dan antena serangga. Kajian ini menyediakan maklumat asas berguna tentang analisis diet spesies kelawar pemakan serangga ini.

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LIST OF SYMBOL AND ABBREVIATIONS

%	Percentage
UMK	Universiti Malaysia Kelantan
Hrs	Hours
mm	Millimeter
g	Gram
CBD	Convention on Biological Diversity

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Bats are classified under the order Chiroptera, where it has the natural significance in the ecology. In tropical forests, bats cover about 50% of mammal species and in worldwide its cover about 20% of mammal species (Lim et al., 2017). According to Davidson and Zubaid (2007), about 128 species of bats have been recorded in Malaysia. Total numbers species of bats in Peninsular Malaysia are 106 species and the others 92 species were located in Sabah and Sarawak. The number is growing due to the discovery of new species.

Bats can be classified in two suborders which are fruit bats known as Megachiroptera and insectivorous bats known as Microchiroptera. Usually fruit bats are bigger than insectivorous bats, but there are also several fruit bats in small sizes such as *Taphozous mauritianus*. The large eyes of fruit bats help them to control their movement visually in dusk, caves and in the forests. Different from insectivorous bats, most the fruit bats do not use echolocation (Jones & Teeling, 2006).

Microchiroptera is insect-eating bats or also known as insectivorous bats. There are about 105 species of insectivorous bats from the seven families that can be found in Borneo which are Rhinolophidae, Hipposideridae, Megadermatidae, Emballonuridae, Nycteridae, Molossidae, and Vespertilionidae (Anwarali, 2008).

Rhinolophus affinis or its common name is Intermediate Horseshoe bat is one of the insect-eating bats that classified in the family of Rhinolophidae (Horseshoe bats). Insectivorous bats show high species decent variety (Kingston et al., 2006). An insectivorous bat consumed about half of their body weight of insects per night. This is equal to six hundred mosquito-sized insects in just 60 minutes, and more than two thousand tons of insects every year were ate by a big groups of the bats (Kingston et al., 2006).

Insects are arthropods and can be differentiated from other arthropods by several distinct traits. The most common insects family that usually consumed by the Intermediate Horseshoe bat are Pyralidae, Geometridae, Melolonthidae and Noctuidae (Jiang et al., 2013). The most common part of the insects that have been founds in the faecal pallet are antennae, mandible, elytra, leg and wings (Norhayani, 2004).

1.2 Problem Statement

The subject related to the diet of insectivorous bats was limited in studies compared to the other mammals due to lack of knowledge and information spread about it. There was not enough research on bats especially on the specific diet of insectivorous bats in Malaysia. In this case, this study was conducted because there was less data regarding the Intermediate Horseshoe bat's diet in Gua Setir, Kelantan.

1.3 Objective

The main objective of this study is:

a) To identify the order of insect in the Intermediate Horseshoe bat's diet based on the faecal samples and stomach content analysis.

1.4 Scope of Study

This study focused on the faecal pallet release by the Intermediate Horseshoe bat or *Rhinolophus affinis* from the study area which is Gua Setir that located in Kelantan. The faecal pallet were analysed in the laboratory to identify the left-over body parts of the insect that consumed by this bats and classify it according to the lowest taxonomic level that it could be identified usually the order of the insect.

1.5 Significant of Study

Since there were lack of information of the diet insectivorous bats, this research would contribute to more information on Intermediate Horseshoe bat's diet. Furthermore, this study also can be a reference to provide more effective management and conservation of insects and bats in future. At the end of this study, the total individual of the Intermediate Horseshoe Bat (*Rhinolophus affinis*) and its morphometric data were collected and recorded. The order of insects found in the faeces pellets of the samples collected were listed out as the results of this study.

CHAPTER 2

LITERATURE REVIEW

2.1 The Bats of Southeast Asia

The Southeast Asia geopolitical region comprises of 11 countries which are Myanmar, Laos, Vietnam, Philippines, Cambodia, Indonesia, Brunei, Thailand, Malaysia and Timor-Leste. Southeast Asia is one of the richest areas of biodiversity in the world, but the rapidly changing in land-use in recent decades has placed much of its biodiversity in the risk of extinction (Sodhi et al, 2004). Critically threatened fauna in Southeast Asia are bats. The bats comprise almost 30% of the species of mammals in the region and its cover nearly half of all mammal species in the tropical rainforest ecosystem (Kingston et al., 2006). As Southeast Asia supports 30% of the world's fauna bats, this region has become an important area for international bat conservation. According to Simmons (2005), there are 320 species of bat are listed in Southeast Asia and there are further more species were described. More than 7% of the 342 species of bats in Southeast Asia have been described since 2000, and species discovery rates may increase each year (Kingston, 2013).

Southeast Asia is a habitat for more than 25% of the world's fauna bat, but rising in deforestation and land degradation rates have damaging the habitat of various threatened species of bats across the region. Researches on the ecological of bats in Southeast Asia are now lagging behind while the Neotropic countries, have stepped up efforts to assess the diversity response to the modification of anthropogenic habitats (Kingston, 2013). According to Hutson and Mickleburgh (2001), limestone caves in Southeast Asia, especially in Malaysia contain a various species of bats that can be found but the anthropogenic activities such as mining associated with limestone extraction in caves can affect the population and habitats of bats. Furthermore, bats not only play an important role in the ecological community, they also economically play a dominant role. Decreasing of bats population can give impact to the vast majority of Malaysian plant species because plant species are relying upon the bats to fertilize (Kingston et al., 2006).

2.2 Morphology of Bats

Bat is the only mammal capable of flying and the movement of this mammal are powered by the muscular and a pair of strong wing. The bats wing are made of skin stretched with a double membrane between the very long bones of four fingers and extending along the body from the forelimbs to the hind legs and from the hind legs to the tail. It has a small thumb with a claw that is free of membrane. The hind limbs is also small and can be rotated in such a way that the knees bend backward and not forward as in other mammals, this may be adjustment for flights. The bats rest by hanging the head down, holding the twigs or gaps by using the claw on the feet and it also take off into flight from this position (Norberg & Rayner, 1987). There are some body parts of bats that can be used to identify the morphology of bats. Figure 2.1 shows the anatomy of bats.

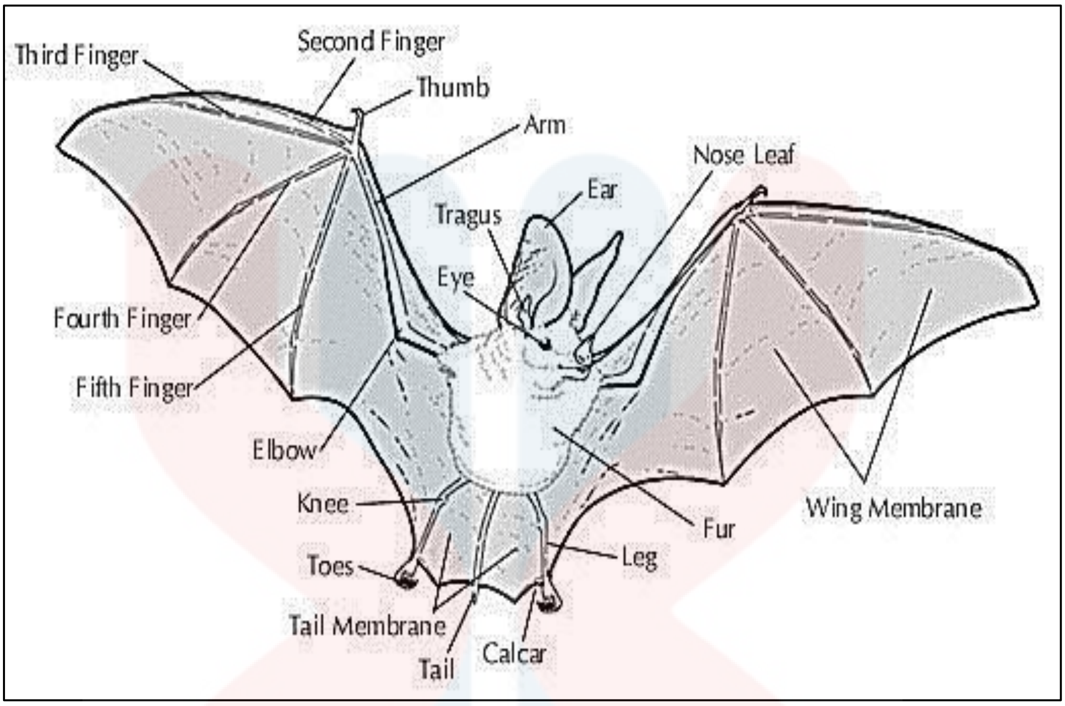


Figure 2.1: Anatomy of bats (Francis, 2008).

The basic measurements usually take for identifying bat species are started from head and body length (HB). HB is the part that measured from the anus until the front nose of the bats. Next is forearm length (FA), the FA is measured started from the outside elbow to the outside wrist in the curved wing. Besides, the tail measurement (TL) is also important, where the measurement is started from the anus until the tip of the fleshy or bony part of the tail. Then, the hind foot (HF) which is measured from the heel to the longest end of the foot. Last, the length of ear (E) that measured from the bottom of the external ear to the end tip of the ear. For *Rhinolophus affinis*, it has a special part include to be measured which is tragus (Francis, 2008). The tragus is a small pointed eminence of the external ear (refer to Figure 2.1).

2.3 Echolocation of Bats

Echolocation is the navigation technique used by bats to hunt down its prey (Norberg & Rayner, 1987). This process involves the pulses production sound of which compared the generated echoes that return back from the surrounding. Echolocation is a basic feature commonly used by all species from the suborder of Microchiroptera, while for the suborder of Megachiroptera, only one species is practicing this echolocation technique. Some kinds of bat can see almost clearly during the day. But, when the sun goes down, nocturnal bats rely on its ear more than their eye to navigate. Moreover, bat combined the sound, ears and echoes in order to create perceptual maps that allow the bats to see in the dark. So, there is not a problem for bats to hunt its prey in the complete darkness. Furthermore, bats can hear and produce sounds at frequencies of more than 100,000 waves per second (Fenton, 1994).

There are two categories of echolocation process which is low-frequency echolocation and high-frequency echolocation. When looking for prey, firstly the bat send out low-frequency of sound or short calls over a wide range. Once a bat receives low-intensity echoes from small potential prey, it will remit a high frequency sound directed towards it. When both high and low-frequency echoes return to the bats, it is able to ignore now-irrelevant and low-frequency ways that reflect from the trees or rocks that in the surrounding of its meal. Next, to gauge the distance of the object have been received, the bat analyses the time delay between, when it hears its sound and perceives the resulting echo. Directional information comes from comparing information that the bat receives in each ears (Arita & Fenton, 1997).

2.4 Importance of Insectivorous Bat in the Ecosystem

Bats are good as bioindicators to detect changes in the climate quality and the quality of habitats that affected by humans activity due to the extensive taxonomic and diversity function (Patterson et al., 2003). In facts, the largest diversity and a geographically dispersed groups of living mammals are bats. The bats form several large groups of non-human mammals, and may be one of the larger mammalian groups when it is measured in numbers of individual (Kunz, 2003; O'Shea & Bogan, 2003). Bats have its own important role in the ecological and economical, because the bat exists in various ecological niches, so bats provide an important role in evaluating ecosystem health.

The main consumers of nocturnal insects are the insectivorous bats species. The insect-eating bats usually consumed high numbers of insect which can reach almost 100% of its body mass per night (Kurta et al., 1989) and bats take long distances every night for the foraging activity. These bats have a major role in controlling the populations of nocturnal insect and conveying the nutrients in the landscape, especially on the corridor of the stream to the tree they roots (Pierson 1998).

Many bats fulfil essential ecosystem services. However, the decrease in bat population often reflects the characteristics of habitat degradation that have an impact on various taxonomies. Decreasing bat population showed that bat responses to environmental stress in terms of changes in habitat quality as well as climate change and direct exploitation. Bats are stable in terms of taxonomy and these small mammals can be monitored by various methodologies (Kunz & Parson, 2009). The use of bats as the bioindicator has been recognized. For example, based on the

Convention on Biological Diversity (CBD) in May 2008 in meeting target, the government of United Kingdom had adopted bats as biodiversity indicator into their environment for the sustainability of lifestyles.

2.5 Family Rhinolophidae (Horseshoe Bat)

Horseshoe bats are one of microbats that are small to medium-sized bats. It is known as Horseshoe bats because of the unique creatures of the noseleaf on their nose which look like horseshoe-shape as shown in Figure 2.2 (Francis, 2008).

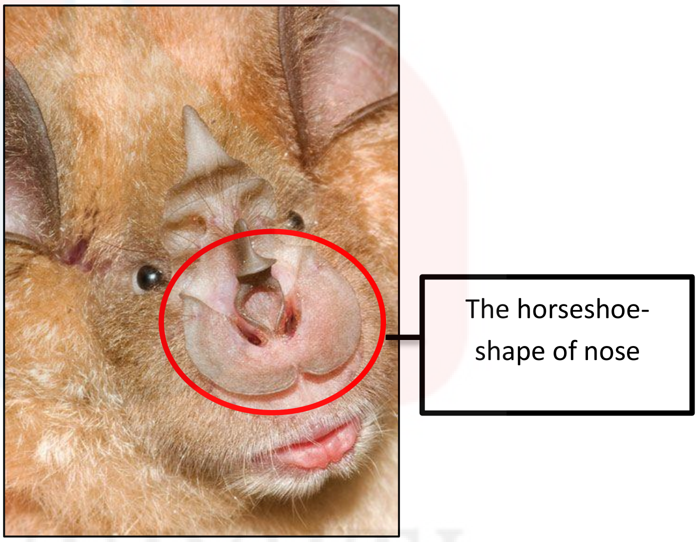


Figure 2.2: The Greater Horseshoe Bat (Francis, 2008).

These special creatures are the main key in the species identification of this family, and it is composed with several parts. The upper part nose area of these bats, there is posterior noseleaf that connected with triangular-shape of lancet. The lancet is points up between the eyes of these bats. In the middle area of the nose, there is a portion that flat, ridge-like structure, raised behind the nostrils and points out perpendicular from the head known as sella (Figure 2.3). The hairs found on the sella are usually less than hair in the noseleaf or the lancet (Francis, 2008). The connecting process and the shape of sella which joins to the posterior leaf vary between species and it is one of the useful diagnostic characters. This nasal structure helps to send echolocation calls which required to focusing on sounds. Figure 2.2 show the diagram of the *Rhinolophus* nose parts.

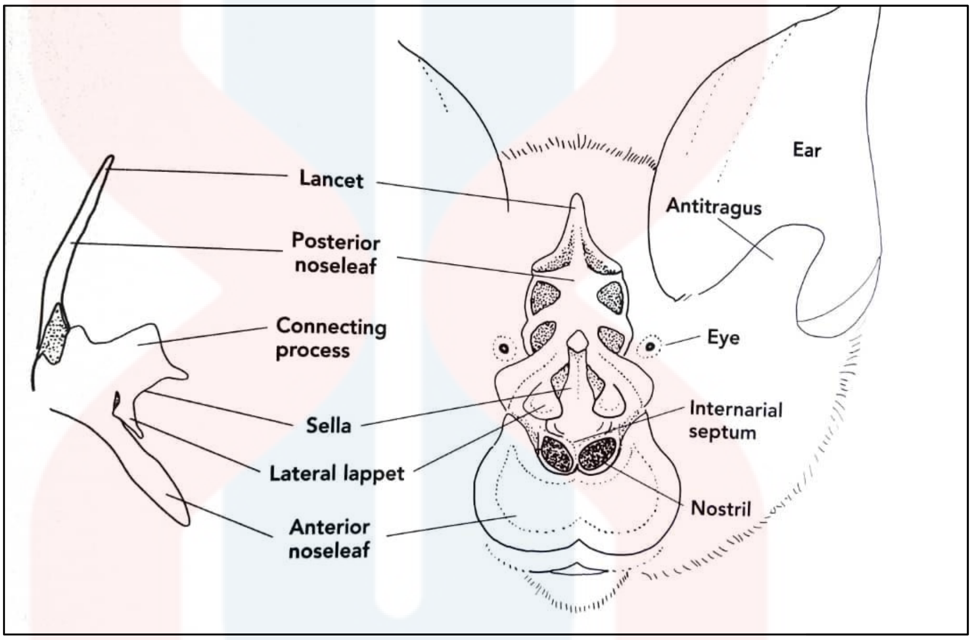


Figure 2.3: Diagrams of *Rhinolophus* noseleaf parts (Francis, 2008)

Horseshoe bats have a pair of small eyes and a pair of large ears with a prominent fold on the outside edge, the antitragus. The tail of this bats family usually short to medium which is about 30% to 37% of the total length, and is completely enclosed by the interfemoral membrane. There is only one genus of the Rhinolophidae which is *Rhinolophus*. Most of the species in this genus can be differentiated by combination of the body part size and the shape of their noseleaf, but there are few species that can only be differentiated by the skull characteristics because it has higher similarity percentage of the external character with the others species.

2.6 Intermediate Horseshoe Bat

Intermediate Horseshoe bat or *Rhinolophus affinis* is one of the species that categorized under the family if Rhinolophidae. According to Simmons (2005), *Rhinolophus affinis* is currently known from Nepal to southern China, India (including the Andaman Islands), the Lesser Sunda Islands, Southeast Asia, and Borneo. *Rhinolophus affinis* usually found roosting in the cave. This species is medium-size of body shape. Over its range, forearm length varies between 48 -54mm and body masses 12-20g (Francis, 2008). As the identification key for this species, the upper parts colour of the species is dark brown to reddish-brown while the under parts is a bit paler. The ear is moderately large around 19-24mm. The noseleaf relatively in a large size, covering muzzle and simple without extra lappets on sella. The sella is pandurate and slightly concave while the connecting process of this species is rounded. The lancet is straight sided; its tip is pointed (Csorba et al., 2003). The echolocation call is a long and continuous frequency signal, with a simple modulation frequency start and tail. Frequency call of this species in Peninsular Malaysia is 77-78 kHz. These species are usually found in medium-sized colonies of up to 400 individuals. In Thailand, *R. affinis* is a species that can be adapted in various forest types such as bamboo, dry leaves, flower leaves, green leaves and dipterocarp. It is also often found in farming areas, including orchards and rubber plantations, and is often observed roosted in limestone caves. While in Vietnam, *R. affinis* is found almost throughout the country, including mountainous areas. It usually roosts in limestone caves and inhabits tunnels. This species has also been recorded in various vegetation types from degraded to primary forests (Kingsada et al., 2011).

Figure 2.4: The Intermediate Horshoe Bat, *R. affinis.*
(3rd August 2018, Gua Setir).

2.7 Food Habits of Rhinolophidae

Almost all species from Rhinolophidae family prefer insects and others small arthropods as meals (Hill & Smith, 1984). Bats are one of the ports and serve as a resource for parasites such as trematodes, mites and fleas. These bats family are essential to ecosystem cycle to control the populations of insects and spiders. These bats prefer catching the insects during flight and sometime it hunts the insects and spiders from the surfaces of ground. Rhinolophidae usually forage near the surface of ground or near dense foliage, which enables the bats to recognize the non-flying insect. Rhinolophids are capable of extremely manoeuvrable flights, including the ability to hover. Bats capable of hovering can exploit sources of prey on the surface where most species of bats cannot exploit. Species in this family can use regular, well-defined foraging areas (Hill & Smith, 1984). Food items are thoroughly chewed by the high cusped molars and the stomach becomes distended after feeding. The gastrointestinal tract is typical of a small mammalian insectivore.

CHAPTER 3

MATERIALS AND METHOD

3.1 Study Site

This research was conducted at Gua Setir which is located at Jeli district in Kelantan. Gua Setir is a limestone hill that covers the length of 1300 meters. The highest peak of this hill is 100 meters above sea level with a width of 80 meters (Hamdan, 2013). Gua Setir was located near to Hutan Rizab Sejana, and Hutan Rizab Sungai Sator. Gua Maka and Kampung Pasir Dusun were also near to Gua Setir. Besides, there were several rivers near this limestone hill. The latitude of Gua Setir is 5°39'38.88" and longitude of this cave is 101°55'31.8" (Hamdan, 2013). Figure 3.1 shows the map of Gua Setir.

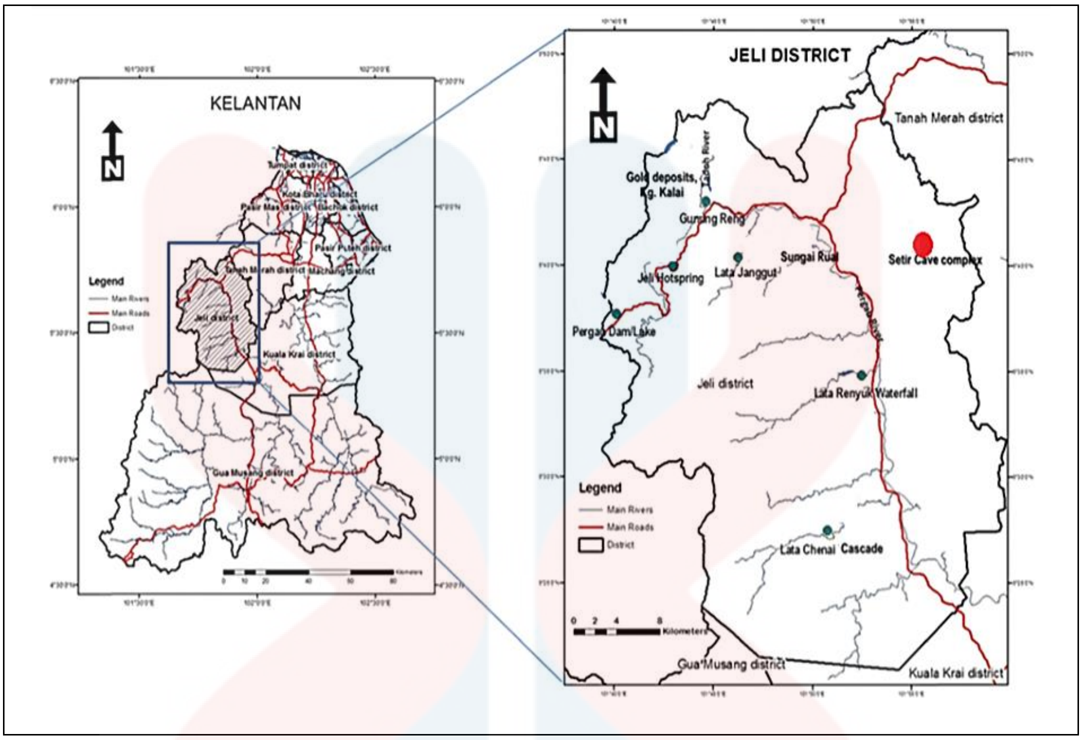


Figure 3.1: The Map of Gua Setir (ArcGIS, 2018).

3.2 Materials

The materials have been used in the research study were divided into two parts. First, the materials that have been used in the field work which used for sampling and preserving the collection of sample. Second, the materials that have been used in the laboratory for analyse the samples. Table 3.1 shows the list of main materials used.

Table 3.1: List of materials.

Equipment/Apparatus	Description
1. Mist net	It was used for capturing the bats
2. Harp trap	It was used for capturing the bats
3. Cloth bag	It was used to pressure the bats to collect their faeces
4. Airtight containers	It was used to store the samples
5. 70% of ethanol	It was used to prevent the faeces from contamination
6. 70% of glycerol	It was used to soft the faeces
7. Dissecting microscope	It was used to observed the left-over body parts of insect in the faeces
8. Nail varnish	It was used to tagged the bats samples

3.3 Methods

3.3.1 Collection of Bats

Bat trapping was conducted for two weeks by using mist net and harp trap at Gua Setir. Around eight mist nets have been mounted randomly in the area that usually passes through by bats at night and the other two harp traps have been set up near the entrance of the cave since the *R. affinis* roost in the cave. The mist nets were opened from 1800hrs until 0600hrs every day. About 24 individuals of *R. affinis* were captured for this study, 20 of it were released back to the nature and the other four were killed by using chloroform to collect sample of stomach content. There were only four individual have been dissect because of decreasing in the capturing rate due to raining season. Besides, strategic location was important to ensure the bats get into the trap. Every trap were checked and cleaned every two to three hour to make sure there was no bat injured or dead because of trapped too long (Zhang et al., 2005).

3.3.2 Bat Identification

Some species from Rhinolophidae family have the similarity colour of its fur, especially *Rhinolophus lepidus*, *Rhinolophus stheno* and *Rhinolophus accuminatus* which were also trapped in the mist net and harp trap. Visually there was little difference between these species. Therefore, only morphological measurements make it possible to accurately determine the species of bat. Firstly, bats were identified as juvenile or adult by inspecting the epiphyseal fusions on the forearms of bats (Jiang et al., 2013). Next, the weights of the bat were measured by using the electronic beam balance. Beside, an external vernier caliper and ruler was used to measure the external body part of bat such as total length (TL), head-body length (HB), tail length (TL), hind foot (HF), ear length (E), forearm length (FA) and Tragus (T). The gender of the bat has been identified by the existence of the mammary gland. The bats without the mammary gland were considered as male bats. Meanwhile, the bats with mammary glands were female and the mammary gland needs to be pinched to see whether it produce lactate or not (Kingston, Francis, Akbar, & Kunz, 2003). All the measurement has been recorded in the data sheet and the captured bats were identified by using the keys from the book, "*Bats of Krau Wildlife Reserve*" by Tigga Kingston, Lim Boo Liat and Zubaid Akbar (2006). After tagged with the nail varnish, the bat has been left for few hours in the cloth bag until it defecates.

3.3.3 Faeces Sampling

After the bats defecated, all the faeces left in the cloth bag were collected and placed in the air tight container. Next, all the bats were released back to the habitat accept for four individuals that were dissecting for its stomach samples. The faeces pellets collected were preserved by using 70% of ethanol for the laboratory analysis (Zhang et al., 2005).

3.3.4 Stomach Content Analyses

This analysis was depending on the abundance of the species, in this study only four individual of *R. affinis* (only adult and non-reproductive female) were captured and preserved for stomach content analyses (Aguirre et al., 2003). These four individuals were killed immediately before it's defecate by using 10% chloroform. Then, these bats were taken to the Microbiology Lab for dissecting process after finished fieldwork sampling in Gua Setir, Kelantan. In the laboratory, the removal of the mesentery were done carefully, the stomach was cut on regional basis that is cardia, fundus, corpus and pylorus (Enaibe & Ajayi, 2008). Stomachs were removed and stored in a 70% aqueous ethanol solution for analysis. For the analysis, the contents was removed from the preservation container, placed in a petri dish and analysed under a RaxVision dissecting microscope (Aguirre et al., 2003).

3.3.5 Diet Identification

The identification of insects found in the faecal samples done in the laboratory. The faeces sample from all individuals was placed in the petri dish. A few drops of glycerol and hot water were added to soften the samples and left about 24 hours. Then, it had been observed under RaxVision dissecting microscope (Appendix A) after being broken into fine pieces by using dissecting needles. All the left-over body parts were identified and classify under the lowest taxonomic level it can, usually until the insect order and family (Kunz & Whitaker, 1983). For this study the insect found in the faecal were identified until the order level of insect. The body parts of insect collected in the faeces pallet were calculated and sorted by distinguishing or diagnostic the characters (Whitaker, 1988). The identification of the insect parts found was done by referring to the book of "Insects: their natural history and diversity: with a photographic guide to insects of eastern North America." by Marshall (2006) and the journal by Pokhrel & Budha (2014) which entitle “Key to Identify Insects from Droppings of Some Insectivorous Bats of Nepal”.

3.4 Data Analysis

The findings data of diet of insectivorous bats were analysed by using traditional method that usually involves the visual estimating the percentage volume of insects found within the guano to the level of order (Whitaker et al., 2004). Although determining the ordinal percentage volumes through standard faecal analysis is the preliminary indicator of various prey can be consumed by predators. The diet actually can be differentiated at the level of the family, genus, or species but reliably identifying insects to such low taxonomic levels through visual faecal analysis is usually impossible. The amount of the insect order for all samples were calculated and list out in the graph.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 The Measurement and Identification of *R. affinis*

The identification of the *R. affinis* was guided by the keys identification of bats from the "*Bats of Krau Wildlife Reserve"* book writen by Tigga Kingstone, Lim Boo Liat and Zubaid Akhbar (2006). The basic measures were taken to identify the species. The measurement value should be accurate to ensure that the process of identifying the bat become easier. To ensure the measurements are accurate, a precaution step has been made by taking the measurement three times of each individual. Figure 4.1 below shows the *R. affinis* captured at Gua Setir, Jeli, Kelantan on August 2018.

Figure 4.1: *Rhinolophus affinis* (August 2018, Gua Setir)

According to Francis (2008), the range of forearm length of this species varies between 48-54mm and body masses 12-20g. As the identification key, the upper parts colour of this species seem to be dark brown to reddish-brown while the under parts is slightly paler. The ears are moderately large around 19-24mm. The noseleaf were in a large size, covering muzzle and simple without extra lappets on sella. The sella is pandurate and slightly concave while the connecting process of this species is rounded. The lancet is straight sided; its tip is pointed (Csorba et al., 2003). Table 4.1 shows the measurements body parts of the *R. affinis* that have been captured. Based on the table, there were 12 males and nine females were recorded. From 12 individuals of males' bats, seven of it was adults and five of it was juvenile. From nine individuals of female bats, six of it was adults and the others three of it was juveniles. Figure 4.2 show the number of gender and age group of bats individual samples captured at Gua Setir, Kelantan.

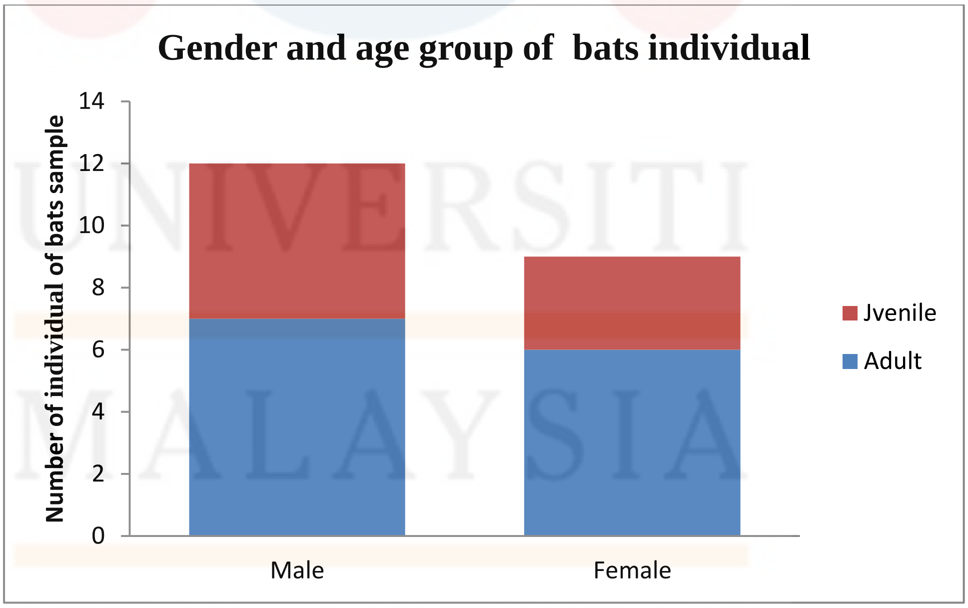


Figure 4.2: The number of gender and age group of bats individual sample

Table 4.1: The measurements body part of each individual of the *Rhinolophus affinis* that have been captured.

	W (g)	TL (mm)	HB (mm)	HF (mm)	EL (mm)	FA (mm)	Tail (mm)	Tragus (mm)	Gender	Age
Individual 1	14	83	54	5	17	49	29	7	F	Adult
Individual 2	12	72	50	5	15	52	22	8	M	Adult
Individual 3	12	77	56	5	17	49	21	9	M	Juvenile
Individual 4	13	85	60	4	16	49	25	8	M	Adult
Individual 5	13	83	60	5	17	49	23	8	F	Adult
Individual 6	12	77	52	6	16	50	25	9	M	Juvenile
Individual 7	14	83	60	5	16	51	23	9	F	Adult
Individual 8	15	85	61	6	17	51	24	7	F	Adult
Individual 9	12	73	52	4	15	49	21	8	M	Juvenile
Individual 10	13	72	52	4	15	51	20	8	M	Adult
Individual 11	14	83	56	5	17	50	27	7	F	Adult
Individual 12	14	82	55	5	15	50	27	7	M	Adult
Individual 13	14	82	56	5	16	52	26	9	F	Juvenile
Individual 14	12	72	52	4	15	49	20	8	M	Juvenile
Individual 15	15	85	60	6	17	50	25	7	F	Juvenile
Individual 16	13	73	52	5	16	50	21	7	M	Adult
Individual 17	14	83	56	5	15	52	27	8	M	Adult
Individual 18	14	82	56	4	15	52	26	9	M	Adult
Individual 19	14	72	51	6	15	52	21	9	F	Juvenile
Individual 20	14	82	60	6	16	49	22	8	F	Adult
Individual 21	12	72	50	4	15	49	22	7	M	Juvenile

*TL= Total Length, HB=Head body length, HF=Hind foot length, EL=Ear length, FA= Forearm length, F= Female, M=Male

4.2 Diet Analysis of Intermediate Horseshoe Bat (*Rhinolophus affinis*)

Some *R. affinis* individuals have been captured in the Gua Setir to get their faeces. Most of them were captured by using harp trap and only few individuals of this species were obtained from mist net. According to Francis (1989), the used of harp traps was believed to influence in the result of this survey. It is help in increasing the chances of capturing species that belongs to the family Hipposideridae, Rhinolophidae and Vespertilionidae, which were known as competent echolocators. These groups of bats can detect and avoid mist nets effectively, reflected by the ability to forage and maneuver in cluttered habitats (Neuweiler, 1990).

As a result, 21 faeces samples from 21 different bat individuals were collected and analysed under the microscope. In order to make sure the faeces were from different individuals, the *R. affinis* that have been captured were tagged with nail varnish before released it back to the nature. Table 4.2 shows the order of insect present in each of the samples. Based on the table, it shows that Diptera, Coleoptera, Blattodea and Lepidoptera were high abundance in the faeces sample. According to recent studies in Malaysia on insectivorous bat by Norhayani (2004), the family of Rhinilophidae were usually feed on insect from the order of Coleoptera and Diptera. The quantities of insect eaten by each individual of bats samples were different but mostly from the same order of insect. Foraging behaviour and diet are influenced by a complex array of intrinsic and extrinsic factors. Intrinsic factors, such as age (Sullivan, 1988; Weathers & Sullivan, 1991) and sex (Belovsky, 1978; Clutton-Brock, Guiness & Albon, 1982), as well as morphological characteristics, can have profound effects on feeding, both in terms of dietary requirements and foraging abilities. Different demands during certain times of the life cycle may alter the required complement of energy and nutrients, thereby influencing diet and foraging behaviour. For female mammals, energy and nutrient demands escalate during pregnancy and lactation (Gittleman & Thompson, 1988).

Table 4.2: The order of insect present in the samples

	Order of insects						
	Diptera	Coleoptera	Blattodea	Lepidoptera	Hemiptera	Hymenoptera	Unknown 1
Individual 1	√	-	√	√	-	-	-
Individual 2	√	-	√	√	-	√	-
Individual 3	√	√	√	√	-	-	-
Individual 4	√	√	-	√	-	-	-
Individual 5	-	-	-	-	-	-	-
Individual 6	-	-	-	-	-	-	-
Individual 7	√	√	√	√	-	-	√
Individual 8	√	√	√	√	-	-	-
Individual 9	√	√	√	√	-	-	-
Individual 10	√	√	√	√	-	-	√
Individual 11	√	√	√	√	-	-	-
Individual 12	√	-	-	-	-	-	-
Individual 13	√	√	√	√	-	-	-
Individual 14	√	√	-	√	-	-	√

Individual 15	√	√	√	√	√	-	√
Individual 16	√	√	√	√	-	-	√
Individual 17	√	√	√	√	-	-	-
Individual 18	√	√	√	√	-	-	√
Individual 19	√	√	√	√	-	-	√
Individual 20	√	√	√	√	-	-	-
Individual 21	√	√	√	√	-	-	√

The graph below (Figure 4.3) shows the differences consumption of insect order between male and female *R. affinis*. The male individuals of *R. affinis* consumed more insect from the order of Diptera, Coleoptera, Lepidoptera and Hymenoptera than female *R. affinis*. For the order of Blattodea, both gender consumed equally and for the order of Hemiptera only one individual of female *R. affinis* consumed it. According to previous study of insectivorous bat by Kunz (1974) and Mata et al. (2006), adult female consumed more quantities of insect than adult male. The male species forage and migrate in different area from female adult to avoid food competition (Kunz, 1974). This might be the reason why the adult male diet was higher than the female in based on the graph. The different foraging areas allow the diet of adult male bats more variation species of insects.

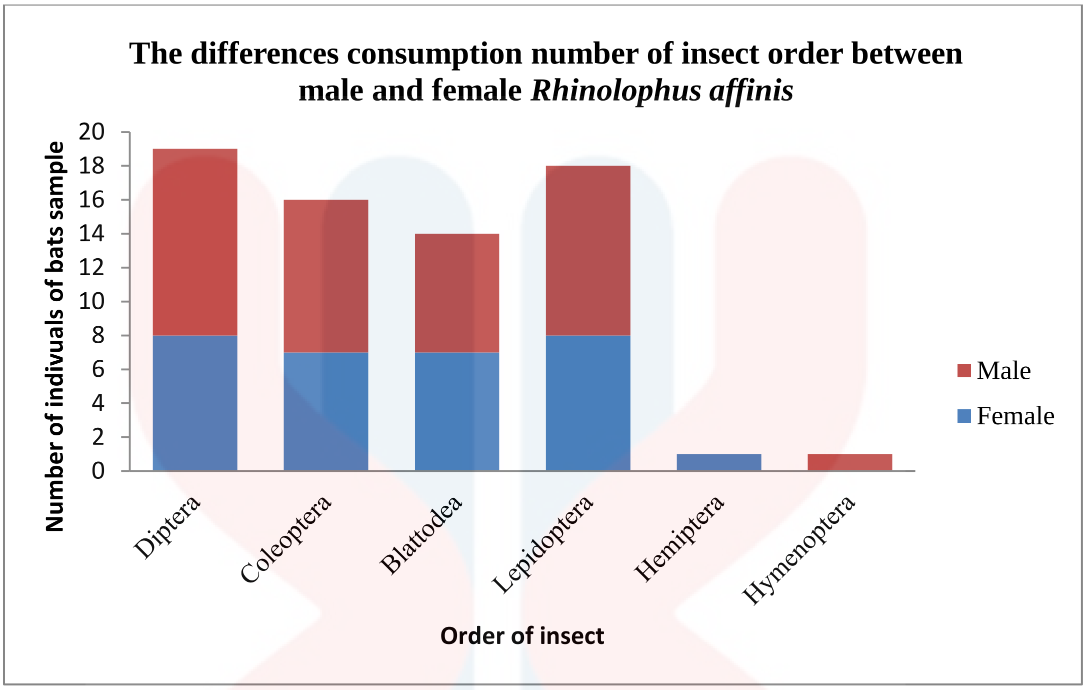


Figure 4.3: The differences consumption number of insect order between male and female *R. affinis*.

4.2.1 The Order of Insect Found in *Rhinolophus affinis*

There were six orders of insect have been identified from 137 samples of left-over insect part from the faeces. The orders of insect listed in the graph were Diptera, Lepidoptera, Blattodea, Coleoptera, Hemiptera and Hymenoptera. Based on the graph below (Figure 4.4), the result shows Diptera was the highest order of insect found in the faeces pallet which was 54 samples. Then followed by the order of Coleoptera with 36 samples, the order of Lepidoptera samples was 22 and the order of Blattodea with 15 samples. The lowest amounts of samples were from the order of Hemiptera and Hymenoptera which were only one sample each. Besides, there were also 8 samples from the unknown order which identified as unknown 1. The unknown 1 (Figure 4.4) is actually the compound eyes of insect with about 344 of facet. Since almost all order of insect share the similarity type of compound eye (Kimball, 2007), that make it hard to identify which order of insect it belong to.

Compound eyes are the main organ of vision for insects. The outer surface of each eye is formed by several small lenses called facets (Kimball, 2007). The number of facets varies from one in some insects to 20 000 on the other. Behind every facets there is a tube-like structure known as ommatidium (Horridge, 1977). Each of the ommatidium, or tube, gives a small segment of the image captured by the facets into the eye (Horridge, 1977).

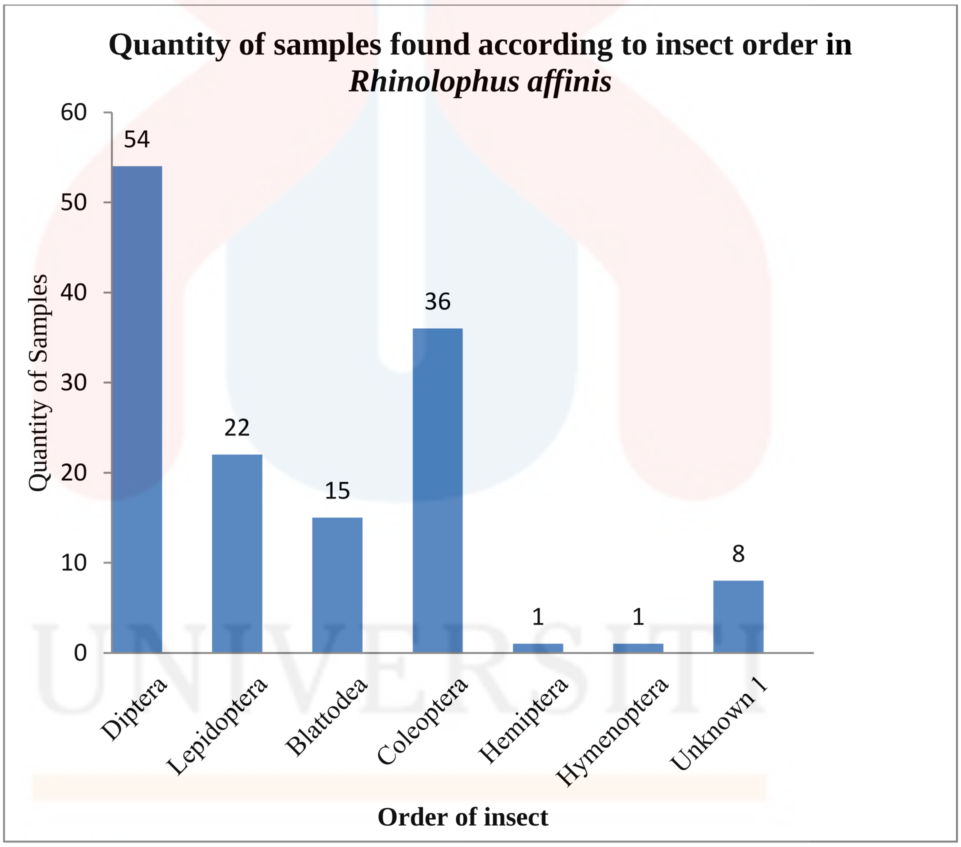


Figure 4.4: The quantity of samples according to insect order in *Rhinolophus affinis*.

In this study, the reason Diptera and Coleoptera were the mostly found in samples might be due to the geographical differences, economic activities such as rubber tree plantation near the study area. Wild plant such as bamboo species, durian and others were also found in the study. The study area is located near the rubber plantations area. Usually plantation ecosystem can be a place for pesticides or vector like beetle and mosquitoes to live (Phommexay et al., 2011). Insect are the one of pest for rubber plantations. The insects destroy the leaves of young plants by using their piercing and sucking mouth parts. Larval forms which are soil inhabitants destroy the roots (Tun et al., 2011). These pests can attack the nurseries and young plantations (Hill, 1983). These is one of the factors that play a significant role in insect abundances and consequently as food sources for bats.

Bat prefer to hunt for food near the roosting area because it is easier for the bats return to the cave or the roosting site after hunting and escape from the predators (Jayaraj et al., 2016). Most of insect from the order of Coleoptera are beetles which is form a diverse group of insect and can be found everywhere in the earth. Beetle can produce sound while in flight, feeding and sometime the beetle sound can be heard from a few feet away (Borror et al., 1981). This behaviour can be the attractive sound for bats to hunt for food and this is one of the reasons why the beetle or the insect from the order of Coleoptera were preferred to hunt by bats. Figures below show the left-over body parts of insect left from the order of Diptera (Figure 4.5), Coleoptera (Figure 4.6), Lepidoptera (Figure 4.7), Blattodea (Figure 4.8), Hemiptera (Figure 4.9), Hymenoptera (Figure 4.10) and unknown 1 (Figure 4.11).

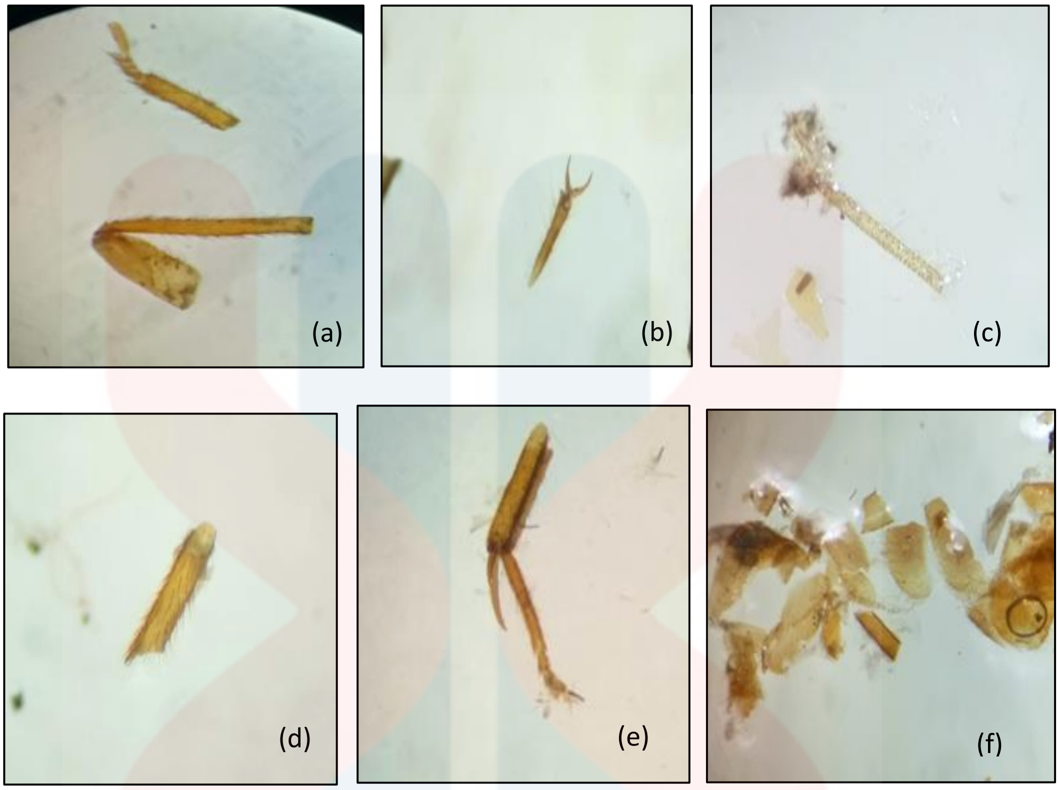
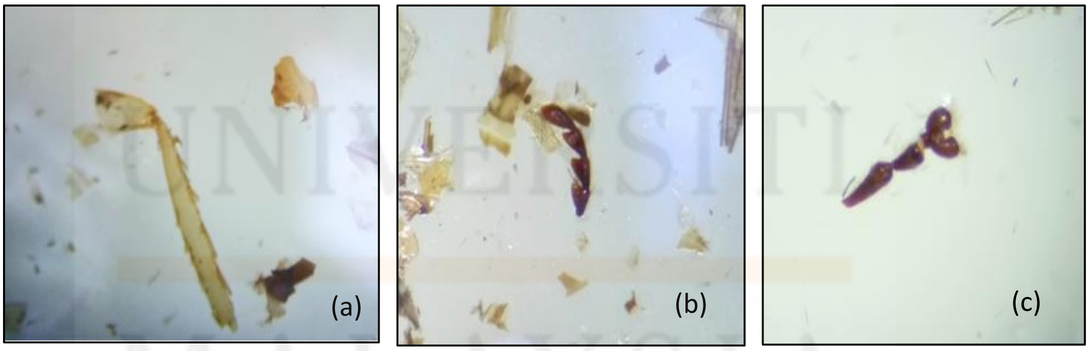


Figure 4.5: The order of Diptera: (a) The leg segments (femur and tibia) of the insect, (b) The tarsus part of insect, (c) and (d) The tibia part of insect, (c) The leg segment (tibia until tarsus) with a long tibia spur and (f) the skin part of insect.



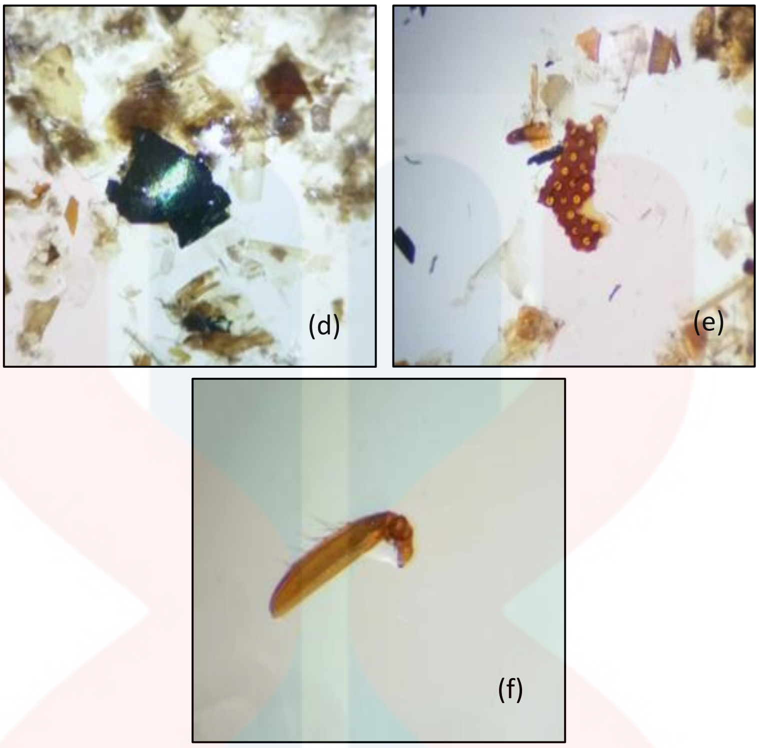


Figure 4.6: The order of Coleoptera: (a) The leg segment (from half of femur and tibia) of insect, (b) The serrate antennae, (c) The serrate antennae of beetles, (d) The hard skin of insect, (e) The skin part of beetle with bubble pattern and (f) The leg segment (from coxa until the femur).

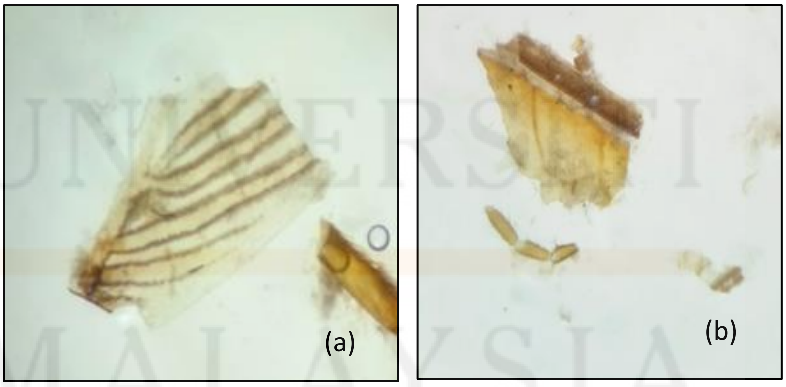


Figure 4.7: The order of Lepidoptera: (a) and (b) The wing segment of insect.

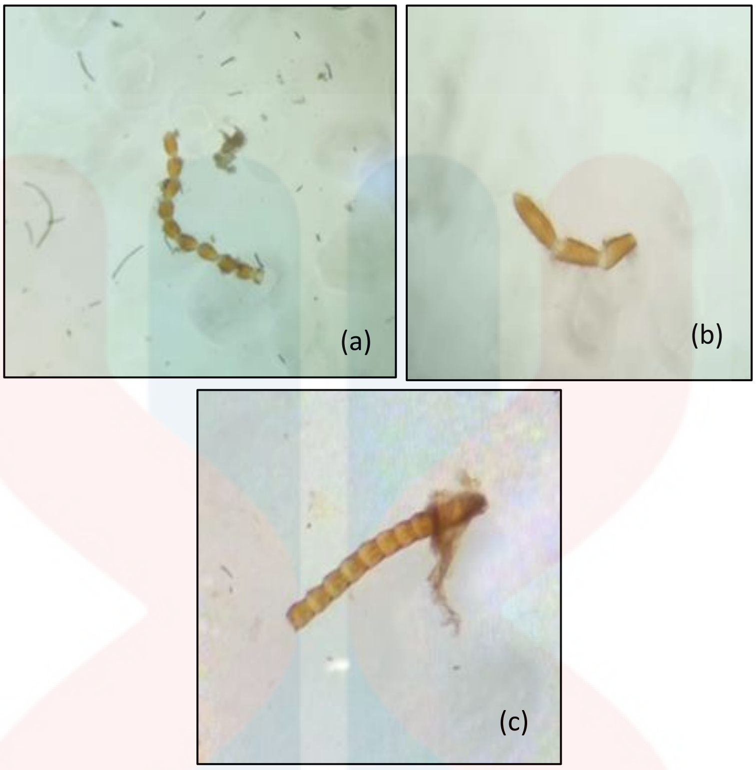


Figure 4.8: The order Blattodea: (a) Moniliform antennae, (b) The tip of moniliform antenna and (c) Filiform antenna.

Figure 4.9: The order of Hemiptera: The small and long antennae.

Figure 4.10: The order of Hymenoptera: The simple structure of ant leg

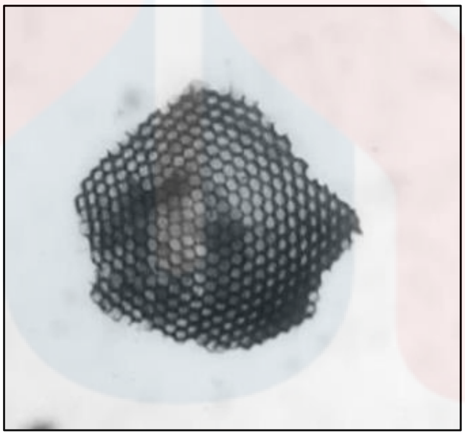


Figure 4.11: The Unknown 1: The facet on the eye of the insect

4.2.2 Type of Insect Parts Left in the Faeces Pallet of *Rhinolophus affinis*

Figure 4.12 show the percentage of insect part left in the sample faeces. The highest amount of items left was the thorax parts of insect which was 79%. Then follow by the head parts with 21%. The external anatomy of insects can be divided into three parts which were head, thorax and abdomen.

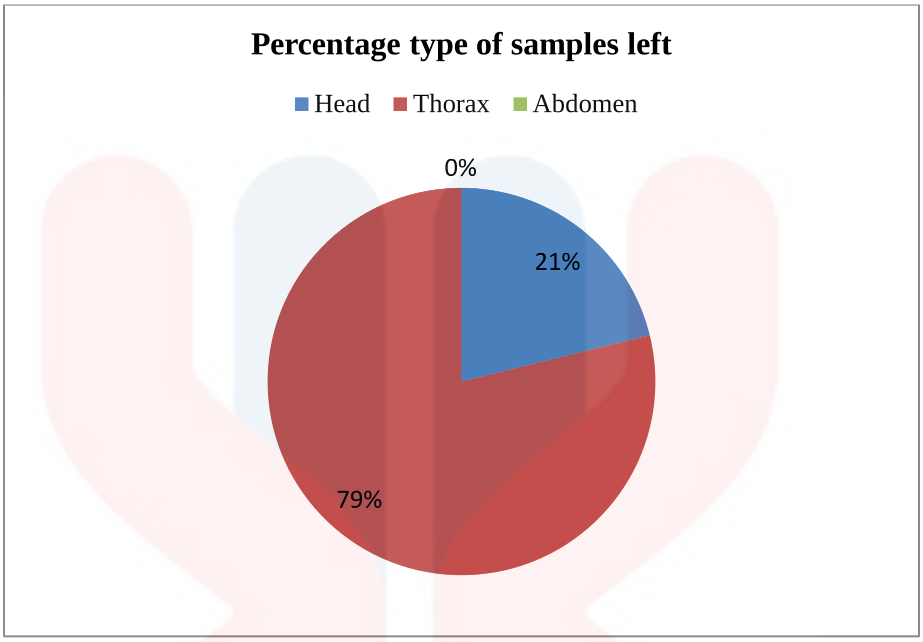


Figure 4.12: The percentage (%) type of insect parts left in the sample faeces

As shown in Figure 4.13, antennae and mouthparts were included in the head part of insect, while the wing and leg were included in the thorax part of insect and the rest is the abdomen part of insect.

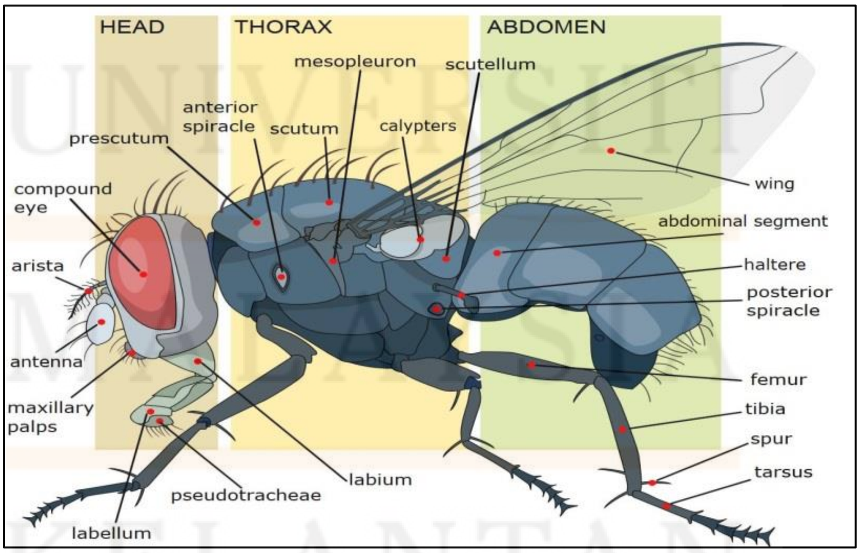


Figure 4.13: The anatomy of insect (Diptera) (Marshall, 2006)

From the 54 samples of Diptera, all of it was from thorax parts which were the legs, wings and some hard skin. Same goes to the order of Lepidoptera, 22 of the samples were thorax part which is bit of their wing part. Then the order of Coleoptera, 31 of the samples were the thorax parts and the other five of it were from the head parts which was their antennae. There were 15 sample of Blattodea from the head part of that insect which were also the antennae part of it. The one sample of Hemiptera was from the head part and one sample of Hymenoptera was from the thorax part. The abdomen parts of insect were hard to recognize because that part was usually the soft-bodies part. According to Buchler (1975), foods that consumed by the insectivorous bats passes through the entire digestive tract in 30 to 60 minutes. Insectivorous bats chew their food into tiny pieces, thereby exposing more easily digestible soft parts to the digestive processes. Chitin is resistant to the normal digestive system, resulting in parts of legs, antennae, and wings passing through the tract fully intact (Whitaker et al., 2004).

4.2.3 The Stomach Content Analysis of *Rhinolophus affinis*

There were only four individuals of the Intermediate Horseshoe Bat (*R. affinis*) killed to be dissected in the laboratory. From the analysis there were no result found in it. This might be happen because of this bat having not hunted the insects as their meals yet before being captured. This is because these individuals of bat were catches by using harp trap and the harp trap were installed in front of the cave entrance. Maybe this bat is trapped when they want to fly out from the cave to hunt for food. One of the reasons why there were only four of the individuals killed was the capturing rate of *R. affinis*. While in Gua Setir, a week before finishing the field sampling was a raining season. The capturing rate of bat was decreasing during that week. The raindrops make the bat less convenient to detect insects or any obstacles during flight as it may disrupt echolocation. According to Belwood & Fullard (1984), the insectivorous bats stop foraging and retreating into plants during heavy rain but continue to forage in the light rain. The raindrops may interfere with echolocation, making it not easier for bats to detect insect prey or obstacles. Perhaps bats avoid rain because the moistening of their body inflicts energy costs on flight by reducing lift and thrust production, or by adding thermoregulatory costs. Indeed, when 0.1 ml of water droplets evaporate from the surface of the body during a 1 min flight, 18 g bat need to invest 4W of thermoregulatory costs in order to maintain normal body temperature. This is about twice the flight cost that the same bat would encounter under dry conditions (Speakman et al., 2003).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

As conclusion, there are six orders of insect represented in the diet of *Rhinolophus affinis*. The order of Diptera was shown the most abundance of order with 54 samples that represented by this Intermediate Horseshoe bat. The lowest orders of insect found in this were Hemiptera and Hymenoptera. For this study, the objective to identify the order of insect in the Intermediate Horseshoe bat's diet based on the faecal samples was achieved the result succeeded in listing the order of insect in the *R. affinis* diet but to identify the order of insect in the Intermediate Horseshoe bat's diet based on stomach content analysis was unattainable due to the empty stomach of the sample taken. This study provides useful basic information about this species and it can be used to carry out further study on this species.

5.2 Recommendations

During this final year's project, there are too many constraints that need to be faced, including short periods of time to collect data samples and uncertain change of the weather especially during fieldwork. The sufficient times are needed to obtain enough result and to improve this study. Therefore, the time planning during this study is very important in order to have enough time. Furthermore, in order to enhance and proof the results of this study, the research on biodiversity of insect in this study area also can be done to collect the insect specimen that live in that particular area and compared it with the insect found in the bat faeces. Moreover, there are lacks a lot of information on bat flight of *R. affinis* species, especially in distance and time, but some species of bat, like the Mexican free-tailed bat, appear to fly almost all night long foraging for insects. Also, it could be flying as far as 30-50 miles each night. In addition, the study on flying distance of this species also can contribute to the study of the diet of this species. Besides further study on digestive system of this species since there is less study on this course.

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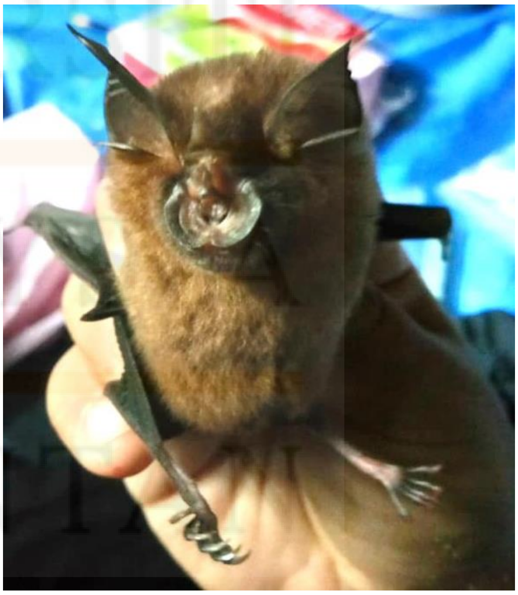
APPENDIX A

Figure of sampling work

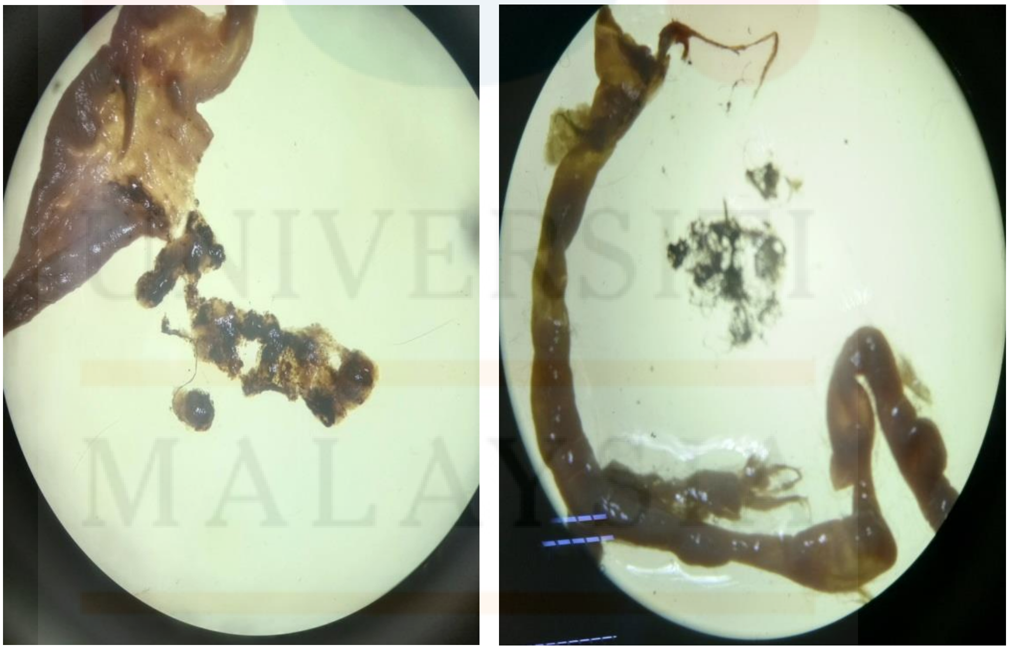
1. Process of removing bats from harp trap

2. Intermediate Horseshoe Bat, *Rhinolophus affinis*.



3. The dissecting microscope (RaxVision) used.

4. The stomach analysis of *Rhinolophus affinis*.



APPENDIX B

The table of Final Year Project (FYP I & FYP II)

Final Year Project I	
Research Activities	**Date**
Proposal writing	February 2018
Submission and proposal defences	May 2018
Completion of FYP I	July 2018
Final Year Project II	
Field Sampling	July-August 2018
Laboratory and Data Analysis	August-December 2018
Completion of chapter 4 and 5	December 2018
Submission of final report	December 2018
Presentation of FYP II	December 2018
Hardbound Submission	January 2019