UNIVERSITI PUTRA MALAYSIA

DIVERSITY AND TEMPORAL ACTIVITY PATTERNS OF DIPTERA ASSOCIATED WITH CAPTIVE WILD MAMMALS AND THE EFFICIENCY OF TRAPPING METHODS

JESSIE HO SI WAI

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JESSIE HO SI WAI

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "**Diversity and Temporal Activity Patterns of Diptera Associated with Captive Wild Mammals and the Efficiency of Trapping Methods**" by Jessie Ho Si Wai and in my opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

DR. REUBEN SHARMA
DVM. (UPM), MVSc (UPM), PhD (Cambridge), MSB, CBiol
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

DEDICATION

This project is dedicated to all the wild animals and the dipteran flies that have sacrificed their lives meaningfully.

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LIST OF ABBREVIATION

CDC	Centers for Disease Control and Prevention
UV	Ultraviolet light
CO_2	Carbon dioxide

ABSTRAK

Abstrak kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901- Projek Ilmiah Tahun Akhir

KEPELBAGAIAN DAN AKTIVITI TEMPORAL DIPTERA YANG MENGINFESTASI MAMALIA DALAM KURUNGAN DAN KECEKAPAN KAEDAH MEMERANGKAP

Oleh

JESSIE HO SI WAI

2015

Penyelia: Dr Reuben Sharma

Diptera gigit adalah artropod yang berkepentingan dalam bidang veterinar dan perubatan sebagai vektor kepada haiwan dan manusia. Kajian ini dijalankan untuk menentukan kepelbagaian, kelimpahan dan corak aktiviti Diptera yang menginfestasi mamalia liar dalam kurungan di zoo tempatan serta kecekapan kaedah memerangkap (Nzi, Malaise, Intercept, CDC-UV dan CDC-CO_2). Selama 1800 jam masa perangkap telah dijalankan dalam enam spesies mamalia liar yang berbeza (Lembu Ankole, Rusa Berbintik, Gajah Asia, Badak Putih, Kijang dan Tapir). Perangkap diletakkan 10m daripada satu sama lain dan akan diperiksa selang tiga jam dari pukul 0700 hingga 1900 dan dibiarkan semalaman untuk menentukan jumlah tangkapan malam. Lalat yang ditangkap kemudiannya dibunuh menggunakan etil asetat atau dengan pembekuan, dipasang-kering dan diperiksa dengan stereomikroskop. Pengenalpastian lalat dilakukan dengan menggunakan panduan kekunci taksonomi yang sudah diterbit. Enam famili Diptera gigit ditemui dalam kajian ini (Ceratopogonidae, Culicidae, Psychodidae, Sacrophagidae, Muscidae and Calliphoridae) yang terdiri daripada 17 genus. 20 spesies nyamuk (Culicidae) ditangkap terutamanya *Culex quinquefasciatus* dan *Aedes albopictus.* Agas (*Culicoides peregrinus*, *Culicoides gutiffer* dan *Culicoides actoni)* dan lalat pasir (*Sergentomyia spp.)* telah juga dijumpai. Majoriti (90%) lalat adalah daripada genus *Musca* spp. dikuti oleh *Stomoxys* spp., dan *Sacrophaga* spp. Perangkap Nzi telah mengumpul lebih banyak lalat secara signifikan (p <0.05) berbanding dengan perangkap Malaise dan perangkap Intercept. Berbanding perangkap yang diumpan dengan karbon dioksida, perangkap CDC-UV telah memerangkap 73 % lebih nyamuk. Terdapat corak aktiviti diurnal menaik untuk lalat besar, yang memuncak dari jam 1600-1900. Kebanyakan nyamuk adalah bersifat nokturnal dengan aktiviti puncak diperhatikan dari jam 1900-0700. Hubungkait kepelbagaian dan kelimpahan Diptera yang tinggi dengan mamalia liar dalam kurungan adalah membimbangkan kerana artropod ini boleh bertindak sebagai vektor untuk kebanyakan haemoparasit dan virus. Di samping itu, perkaitan antara haiwan liar, manusia dan vektor penyakit berkemungkinan akan menyebarkan penyakit zoonotik bawaan vektor yang menjadi perhatian kepada zoo.

Kata Kunci: Diptera gigit, mamalia liar dalam kurungan, aktiviti temporal, kaedah memerangkap

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project

DIVERSITY AND TEMPORAL ACTIVITY PATTERNS OF DIPTERA ASSOCIATED WITH CAPTIVE WILD MAMMALS, AND THE EFFICIENCY OF TRAPPING METHODS

By

JESSIE HO SI WAI

2015

Supervisor: Dr Reuben Sharma

Diptera are arthropods of veterinary and medical importance as a large number are efficient vectors of diseases for both animals and humans. This study was conducted to determine the diversity, abundance and activity patterns of Diptera associated with captive wild mammals at a local zoo, as well as the efficiency of five different fly traps (Nzi, Malaise, Intercept, CDC-UV, and CDC-CO_2). A total of 1800 trap hours were conducted in the enclosure vicinity of six different species of wild mammals (Ankole Cattle, Spotted Deer, Asian Elephant, White Rhinoceros, Barking Deer and Tapir). The traps were placed approximately 10m apart and checked at intervals of three hours and twelve hours respectively from 0700 – 1900hrs, and subsequently left overnight to determine the night catch. The captured flies were killed using ethyl acetate, dry mounted and examined under a stereomicroscope. Identification of the flies was done following published taxonomic keys. Six families of Diptera (Ceratopogonidae, Culicidae, Psychodidae, Muscidae, Sarcophagidae and Calliphoridae) comprising 17 genera, were encountered in this study. Twenty species of mosquitoes (Culicidae) were trapped, of which *Culex quinquefasciatus,* and *Aedes albopictus* were dominant. Biting midges (*Culicoides peregrinus*, *Culicoides gutiffer* and *Culicoides actoni*) and sand flies (*Sergentomyia* spp.) were also encountered. The majority (90%) of the filth flies were from the genus *Musca*, followed by *Stomoxys* and *Sacrophaga.* The Nzi traps collected a significantly ($p<0.05$) higher number of large flies compared to Malaise and Intercept traps. The CDC-UV traps caught 73% more mosquitoes compared to those baited with carbon dioxide. There was an ascending diurnal activity pattern for the large flies, which peaked from 1600-1900hrs. The mosquitoes on the contrary, were mostly nocturnal with peak activity observed from 1900-0700hrs. The high diversity and abundance of Diptera associated with captive wild mammals is of concern as these arthropods may act as vectors of many haemoparasites and viruses. In addition, the possibility of vector-borne zoonotic disease transmission warrants attention as the zoos provide a close interface between wild animals, humans and disease vectors.

Keywords: Diptera, captive wild mammals, temporal activity, trapping methods

1.0 INTRODUCTION

The order Diptera is one of the largest groups in the Insecta class, containing an estimated 240,000 species although only under half of these have been described. Diptera means ‘two-winged’ in Greek (di +petron). They are also known as true flies as they have a pair of functional forewings and the hind wings are small and modified into a club like organs known as the haltere which functions as a balancing organ during flight (Richard and David, 1997). The mesothorax is extraordinarily large due to the dependence on forewings for flying. Dipteran flies have mouthparts that are adapted for biting and piercing or sponging and lapping.

The order Diptera has been divided into three distinct suborders: Nematocera, Brachycera and Cyclorrhapha. Nematocera is a smaller fly with long or narrow wings and its antennae is composed of several segments. Among the families in this suborder that are of veterinary importance include the Ceratopogonidae (biting midges), Culicidae (mosquitoes), Simuliidae (black flies), and Psychodidae (sand flies, moth flies). Brachycera of veterinary importance include the Tabanidae (horse flies, deer flies, and clegs). Flies in this group are usually stout bodied, medium to large in size with very large eyes and a stout piercing proboscis. The Cyclorrhapa flies are thought to be more advanced members of Diptera and have three segmented aristate antennae. This sub-order includes a diverse group of fly families which are of veterinary importance. Of most concern is the Muscidae (house fly, stable fly, horn flies and others) followed by Glossinidae (tsetse flies), Hippoboscidae (louse flies), Calliphoridae (blow flies), Sacrophagidae (flesh flies) and Oestridae (sheep nose bot) (Williams, 2010).

Dipterans are of ecological, economical and medical importance. Flies that are ecologically associated with human are known as synanthropic flies which exploit food and habitat from human activities (Talib *et al*., 2014). They also act as disease transmitters acting as vectors for malaria, dengue, West Nile virus, yellow fever, encephalitis and other infectious diseases. The objectives of the present study are:

1. To determine the diversity and abundance of flies associated with captive wild mammals at a local zoo
2. To ascertain the temporal activity patterns of these flies
3. To compare the efficiency of five different fly trapping methods

The results from this study can be used to facilitate a more focused management program to be initiated in the zoo for the prevention and control of vector-borne diseases of both humans and animals.

2.0 LITERATURE REVIEW

2.1 Diptera as vectors of diseases in wild animals

Diseases in wild animals can be transmitted through close contact between host and pathogens or through vector such as arthropods transmitting pathogens to host. Diptera are arthropods of veterinary and medical importance as a large number are efficient vectors of disease for both animals and humans. There are a few reported cases of dipterans as vectors of borne diseases in wild animals especially those in captivity. *Culicoides* (biting midges) are known to transmit blue tongue virus in wild animals (Mellor *et al*., 2000). For instance, zebras imported from Namibia had an outbreak of *Culicoides* borne African horse sickness in Safari Park, Spain (Rodriquez *et al*., 1992). Simuliidae (black flies) have been closely associated with blood parasites *Leucocytozoon* spp. Hunter *et al*. (1997) reported leucocytozoonosis as the cause of mortality in fledging Great Horned Owls in Canada transmitted by black flies feeding on them. Trypanosomiasis, a parasitic protozoan affecting both animal and human, are known to be transmitted by biting Diptera such as tabanids, stable flies and tsetse fly. Five captive Sumatran Rhinoceros housed near a herd of buffalos in Peninsular Malaysia have been wiped out by trypanosomes. It is believed that the trypanosomes were transmitted from the buffalos to the Sumatran Rhinoceros through local tabanids (Chan *et al*., 2004). *Stomoxys calcitrans* is also a possible vector of *Trypanosome evansi*, found among camels in an affected area of the Canary Islands in Spain (Rodriguez *et al*., 2014). Tsetse flies act as transmitters of animal African trypanosomes not only in wild animals but also sleeping sickness in human (WHO, 2014). Besides that, Habronemiasis in horses caused by the nematode *Habronema microstoma* is vectored by *Musca domestica* and *Stomoxys calcitrans* (Yarmut *et al.,* 2008). In Zoo Negara Malaysia, Vellayan *et al*. (1984) reported a case of cutaneous myiasis in a sea lion caused by maggots of *Chrysomya bezziana.* Furthermore, the major vector for leishmaniasis is Psychodidae (sand fly) which has been reported to affect a few wild animals such as fennec fox (Conroy *et al*., 1970), red kangaroo in Australia Zoo (Rose *et al.*, 2004), wild canids in Brazil (Luppi *et al.*, 2008) and owl monkeys in Argentina (Acardi *et al.*, 2013).

Mosquitoes are the most notorious example of purveyors of vector borne diseases in the family Culicidae. Mosquitoes have been always a problem in zoos as they not only attack humans for a blood meal but also transmit various zoonotic viruses and parasites. In the history of zoos in United States, mosquito-borne West Nile Virus has infected and killed exotic animals in about 100 zoos from the year of 1999 to 2002 (Weiss, 2002). In Malaysia, mosquitoes act as a vector for diseases such as malaria, dengue, filariasis and Japanese encephalitis. The vectors that are responsible for these diseases are from the genera *Anopheles*, *Aedes*, *Mansonia* and *Culex* (Vythilingam *et al.*, 1992). The anopheline mosquitoes species are well known vectors of human and mammalian malaria parasites while for avian malaria, both the anopheline and culicine mosquitoes act as vectors (Paul *et al.*, 2003). Recently, dengue disease has increased the toll of death in Malaysia which is transmitted by *Aedes* mosquitoes, *Aedes albopictus* and *Aedes aegypti* (Lee, 2000). Human filariasis is caused by parasitic nematode *Brugia malayi* which is transmitted by *Mansonia* mosquitoes (Chang, 2000). Lymphatic filariasis caused by the nematode parasite

Wuchereria bancrofti is seen usually in the urban areas and is known to be transmitted by *Culex quinquefasciatus* (Vythilingam *et al*., 2005). *Aedes vexans* and *Culex quinquefasciatus* are said to be the efficient vectors for canine heartworm, *Dirofilaria immitis* (Loftin *et al.*, 1995) which has been found in a variety of zoo animals (Neiffer *et al*., 2002). Japanese encephalitis is a zoonotic disease that is transmitted by *Culex* mosquitoes to domestic and wild animals and birds (Yap *et al*., 2000).

2.2 The Zoo Environment

Zoos are public institutions for the display of exotic animals and wild animals. The word "zoo" is derived from the word zoological garden and was used from 1847 (Kisling, 2001). Zoo represents one of the very few environments in which arthropods, captive vertebrates, free roaming wildlife, humans and plants are found together. This provides a dynamic interface between wild animals, humans and vectors. Besides that, the aesthetic purpose to display animals in a more natural setting in the zoo has caused the zoo to be rich with foliage and water bodies that indirectly provide suitable breeding grounds for dipteran vectors. Many zoos are located in the areas that historically were hot beds for arthropod-borne diseases of humans (Alders & Wills, 2003). There are about 25 arthropod-borne diseases reported in zoo animals in the past 30 years (Alder *et al*., 2003). Translocation of rare animals into the zoo to attract public has always been common for a zoo. However, the translocation and reintroduction of animals into the zoos will not only represent a single species but a biological package containing a selection of viruses, bacteria, protozoa, helminths and arthropods (Woodford & Rossiter, 1993). The accidental importation of exotic arthropods on legally imported animals will put the zoo staff and the local animals in risk of arthropod borne diseases (Bram & George, 2000) and on the other hand, the local pre-existing arthropods acting as vectors of diseases will also put the imported animals in danger. The close proximity of the animals, vectors and humans allow for effective transmission of diseases. Therefore, zoo is an ideal place to study the transmission of zoonotic diseases harboured by both the captive and free ranging wild animals as well as to explore the relationships among vertebrates, arthropods, humans and pathogens.

2.3 Trapping methods

There are biological cues released by the host that attract certain dipterans towards them. Carbon dioxide is the main constituent of vertebrae breath which plays and important role in host seeking process for the mosquitoes (Spitzen *et al*., 2008). Large flies such as tabanids are attracted to the host by the odours, shape, movement, brightness and colour of the host (Sasaki, 2001). CDC light traps have been used for many years to sample mosquitoes populations, yet Vythilingam *et al*. (1992) found out that light traps on their own are not very attractive to mosquitoes. Therefore, CDC traps baited with carbon dioxide was then used with dry ice as a source of CO_2 (Vythilingam *et al.*, 1992). An alternative convenient method to supply CO_2 using yeast with sugar and water that converts sugars into CO_2 and ethyl alcohol was developed by Saitoh *et al*. (2004). There are a few collecting and trapping methods for adult flies. Nzi trap which is a product of research on tsetse flies has been known to be an attractive device for biting flies in Africa (Green, 1994). The blue-black colour from the Nzi trap may mimic natural forest edges where flies alight to rest and digest their blood meals (Mihok *et al*., 2006). Other large interception traps such as Malaise traps and Intercept traps can also be used to trap flies. Malaise trap was first invented by Dr. Rene Malaise in the year 1937. This trap is designed to intercept insects flying through understory and function to passively collect the insects. Insects fly through the forest understory enter the central sheet of the trap and fly upward until it fall into the collecting jars. The black and white malaise trap equipped with a 500ml container can be filled with 96% of alcohol (Grimbacher and Stork, 2006). A strategic placement at a favourable season of Malaise traps will give good results often up to 1000 specimens per trap per day. The majority of insects trapped are often active flyers such as those in Diptera and Hymenoptera orders (Gressitt *et al*., 1962). Intercept traps also known as SLAM traps have the same function of Malaise traps which is to intercept the flight path of the insects. The only difference is that Intercept traps are able to intercept the insect fly path from four different direction, making wind direction and exposure to sunlight less of a concern when considering trap placement. In addition, Intercept traps can be suspended in the air, float on the water with floating kits and stand on the ground.

3.0 MATERIALS AND METHODS

3.1 Study areas

The study was conducted in five different enclosures that housed six different species of wild mammals in a local zoo. The animals and numbers chosen for this study were Ankole cattle (5), Spotted deer (35), Asian Elephant (3), White Rhinoceros (3), Barking deer (7) and Tapir (1). Each site was chosen based on previous studies and the convenience to place the traps.

3.2 Trapping, sample collection and identification

Five different dipteran traps were used in this study: Nzi trap was designed in Africa and is made from phthalogen blue and black cotton and white polyster mosquito netting were used as standard traps (Mihok *et al*., 2006). Malaise trap, invented by Dr Rene Malaise in 1937, is to halt the insects in flight and then direct them up to the collecting head (Gressitt *et al.*, 1962). Intercept traps intercepts the flying insects that are directed to the collecting head. CDC-UV traps and CDC baited with CO_2 traps powered by 6 V batteries (John W Hock Co, Gainesville, FL) were positioned in an upright orientation 10-15cm from the ground. All the five traps were deployed simultaneously 10 m apart at the periphery of each animal enclosure for a period of three days with a total of 1800 trap hours. Traps were set for 24 hours throughout the day to determine the temporal activity of the dipteran flies. Nzi, Malaise and Intercept traps were checked every three hours intervals during the day from 0700-1900 hours and left overnight to determine the night catch. However, CDC traps were checked every twelve hours intervals from 0700-1900 hours. The captured flies were then killed by freezing or by ethyl acetate, dry mounted and examined under a stereomicroscope. In this study, only specimens from Diptera, which are of veterinary importance were identified. The biting midges were identified using the keys of Ratanaworabhan (1975), the mosquitoes using the keys of Jeffery *et al.* (2012), the filth flies using the keys of Emden (1965), and the sand flies using the keys of Lewis (1957).

3.3 Statistical analyses

The total number and species of Diptera trapped at the various mammals enclosures were calculated and tabulated into Microsoft ® Excel ® 2007. The temporal activity patterns of the Diptera and total numbers of the flies caught using the three different traps (Nzi, Malaise and Intercept traps) were calculated and tabulated into Microsoft ® Excel ® 2007 and graph was plotted from it. Statistical analysis was performed using SPSS ® 14 for Windows ® evaluation version. The efficacy of three dipteran traps (Nzi, Malaise and Intercept traps) were done using two-way analysis of variance (ANOVA) and the significant differences among traps were determined using the Tukey's method. However, for the efficacy of both the CDC dipteran traps, t-test was done using SPSS ® 14 for Windows ® evaluation version.

4.0 RESULTS

4.1 Diversity and abundance of the dipteran trapped

From the five traps deployed at the five study areas, a total of 2863 dipteran flies were caught comprising six families including the Ceratopogonidae, Culicidae, Psychodidae, Muscidae, Sacrophagidae and Calliphoridae (17 genera and 38 species) (Table 1). The most common large fly species trapped was *Musca* spp. comprising 90% of total flies while the most common mosquito trapped was *Culex quinquefasciatus* which made up 43% of the total mosquitoes captured. Among the 38 species of dipteran fly, there are 14 species of veterinary and medical importance which makes up 37% of the dipteran species trapped (Figure 1- 9). Comparing the five enclosures, traps placed close to the Ankole cattle captured the highest number of dipters (1296), followed by White Rhinoceros (618) and Spotted Deer (419). The highest number of dipteran species were caught at the White Rhinoceros enclosure (Table 2).

Table 1. Families and list of species of Diptera associated with wild mammals at a local zoo

Family	List of species
1. Ceratopogonidae	*Culicoides* *C. peregrinus, C. guttifer, C. actoni, Culicoides* spp. *Forcipomyia* *Forcipomyia* spp.
2. Culicidae	*Aedes* *Ae. caecus, Ae. vexans, Ae. albopictus* *Anopheles* *An. vagus, An. kochi* *Armigeres* *Ar. kesseli, Ar. subalbatus* *Culex* *Cx. fuscocephala, Cx. quinquefasciatus, Cx. vishnui,* *Cx. gelidus, Cx. cinctellus, Cx. tritaeniorhynchus,* *Cx. hutchinsoni, Cx. nigropunctatus, Cx. bitaeniorhynchus,* *Cx. fuscanus, Cx. (Culiciomyia)* sp., *Cx. (Lopho)* sp., *Cx.* (?) sp.
3. Psychodidae	*Sergentomyia* spp., *Phlebotomus* sp.
4. Muscidae	*Musca* spp., *Stomoxys* spp., *Ophyra* sp.
5. Calliphoridae	*Lucilia cuprina, Hemipyrellia ligurriens,* *Hemipyrellia tagalina,* Rhininae (sp), *Chrysomya megacephala, Chrysomya rufifacies,* *Phumosia* sp.
6. Sacrophagidae	*Sacrophaga* spp.

Table 2. Abundance, species richness and list of species of Diptera trapped at the various mammal enclosures.

Host	Abundance	Species richness	List of species
Elephant	379	16	*Ae (Aedimurphus) caecus, Ae. vexans, Ae* (Stegomyia) *albopictus, Cx (Cx) fuscocephala, Cx (Cx) quinquefasciatus, Cx (Cx) gelidus, Cx (?)* sp, *Cx (Lopho)* sp.,*C. peregrinus, C. guttifer, Musca* spp., *Stomoxys* spp., *Sacrophaga* spp., *Phumosia* sp., *Chrysomya megacephala, Ophyra* sp.
Spotted Deer	419	17	*Ae (Aedimurphus) caecus, Ae. vexans, Cx (Cx) quinquefasciatus, Culicoides peregrinus, Forcipomyia* sp., *Culicoides* spp., *Cx (Cx) fusocephala, Ar. subalbatus, Cx (Cx) vishnui, Ar. kesseli, Ae* (Stegomyia) *albopictus, Culicoides guttifer, Musca* spp., *Stomoxys* spp., *Lucilia cuprina,* Rhininae (sp), *Hemipyrellia tagalina*
Ankole	1296	13	*Cx (Cx) fuscocephala, Cx (Cx) quinquefasciatus,* Ceratopogonidae, *Sergentomyia* sp., *Culicoides* sp., *Ae* (Stegomyia) *albopictus, Musca* spp., *Stomoxys* spp., *Hemipyrellia ligurriens, Lucilia cuprina, Ophyra* sp., *Chrysomya rufifacies, Sarcophaga* sp.
Barking deer and Tapir	151	17	*Ar. subalbatus, Cx (Cx) quinquefasciatus, Cx (Culiciomyia) nigropunctatus, Cx gelidus, Culicoides* spp., *Phlebotomus* spp., *Sergentomyia* spp., *Ae (Aedimurphus) caecus, Cx (Cx) fuscocephala, Ae. vexans, Anopheles (Cellia) vagus, Ae. albopictus, Cx (Lophoceroamyia) cinctellus, Stomoxys* sp., *C. peregrinus, C. guttifer, Musca* spp.
Rhinoceros	618	30	*Cx (Cx) quinquefasciatus, Cx gelidus, Ae vexan, Cx tritaeniorhynchus, Musca* spp., *Forcipomyia* sp., *C. peregrinus, Culicoides* spp., *An. (Cellia) kochi, Ae (Aedimurphus) caecus, Cx (Cx) fuscocephala, Ae. albopictus, Ar. subalbatus, Cx vishnui, Lucilia cuprina, C. actoni, Cx (Oculeomyia) bitaeniorhynchus, Lutzia fuscanus, An. vagus,* Ceratopogonidae, *Sergentomyia* sp., *Phlebotomus* spp., *Ae (Stegomyia) albopictus, Ar. kesseli, Culicoides guttifer, Sergentomyia* sp., *Forcipomyia (Lasiohelea)* sp., *Stomoxys* spp., *Sacrophaga* spp., *Ophyra* sp.

Figure 1: *Culex gelidus*

Figure 2: *Culex quinquefasciatus*

Figure 3: *Culex vishnui*

Figure 4: *Culex fuscocephala*

Figure 5: *Culex tritaeniorhynchus*

Figure 6: *Aedes (Stegomyia) albopictus*

Figure 7: *Musca* sp.

Figure 8: *Stomoxys* sp.

Figure 9: *Culicoides* sp.

4.2 Temporal activity of the flies and efficiency of the traps

There were notable differences in the periodicity of the dipteran flies. The highest peak activity patterns of large flies were at 1600-1900 (Figure 10). Table 3 shows the temporal activity patterns of the Diptera. Significant differences ($p<0.05$) were detected at 1900-0700 hours, 0700-1300 hours and 1300-1900 hours. Mosquitoes on the other hand are mostly nocturnal where the highest catch for both the two dipteran traps were at 1900-0700 hours (Table 4). Comparing the three dipteran traps (Nzi, Intercept and Malaise), Nzi traps caught the highest catch at 1600-1900 hours and showed significantly ($p<0.05$) higher number of large flies compared to Intercept and Malaise traps (Table 5). For the two CDC dipteran traps, CDC traps with ultraviolet lights caught 73% more compared to those baited with carbon dioxide.

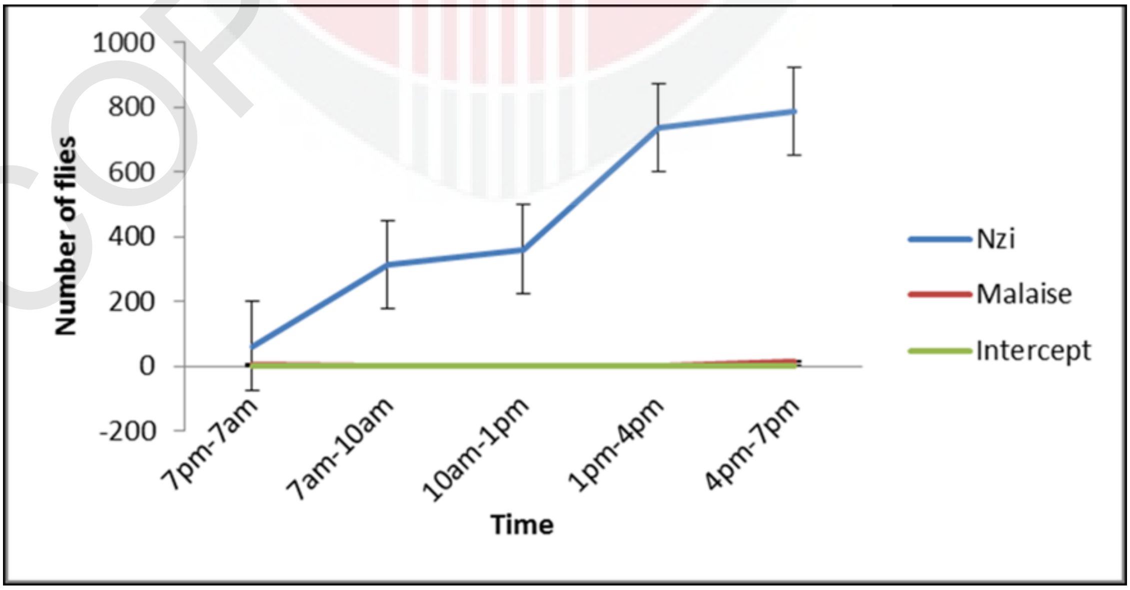

Figure 10: Temporal activity patterns of the Diptera and total numbers of flies caught using the different traps.

Table 3: Temporal activity patterns of the Diptera. Means within column with different superscript differ at $p<0.05$

Traps	Time (hrs)	No. trapped per day (mean ± SD)
CDC + UV	0700 – 1900	0
	1900 – 0700	142.1 ± 19.2[a]
CDC + CO_2	0700 – 1900	2.3 ± 4.0
	1900 – 0700	48.0 ± 30.3[b]

Table 4: Relative efficiency of two CDC dipteran traps. Means within column with different superscript differ at $p<0.05$

Trap time	No. trapped per day (mean ± SD)
7pm - 7am	10.1 ± 4.9[a]
7am - 10am	34.8 ± 17.6[b]
10am - 1pm	40.2 ± 21.4[b]
1pm - 4pm	81.8 ± 41.0[c]
4pm - 7pm	88.4 ± 43.2[c]

Table 5: Relative efficiency of three dipteran traps. Means within column with different superscript differ at $p < 0.05$

Trap	No. trapped per day (mean ± SD)
Nzi	151.6 ± 24.3[a]
Malaise	1.3 ± 0.9[b]
Intercept	0.3 ± 0.3[b]

5.0 DISCUSSION

5.1 Diversity and abundance of the dipteran trapped

This study is the first of its kind to monitor the diversity and abundance of dipteran vectors in Malaysian zoos, and has shown that captive wild mammals attract a high diversity and abundance of dipteran species. The most abundant genus collected in this study is *Musca* spp. and followed by *Stomoxys* spp. Both of these large flies were found from all the host species in this study. There are a few *Musca* spp. as blood feeder and they are able to scratch the skin surface of the animal for blood (Emden, 1965). Stomoxyini flies are hematophagous flies which attack most large mammals including the host from the families of Bovidae, Cervidae, Equidae, Canidae and Felidae (Moon, 2009). They are known as a vector for *Habronema microstoma*, *Trypansoma evansi* and retroviruses (Baldacchino *et al*., 2013). The family of Muscidae is a large and important group with several synanthropic species. They are usually important vectors of diseases causing organisms. *Ophyra* sp. under Muscidae family is also found in this study. Ahmed (2011) found that *Ophyra* sp. transmit diseases mechanically by contaminating food and spread diseases like polio, typhoid fever, dysentery and food poisoning. Out of the three major families of myiasis-producing flies, two are found in this study which is Calliphoridae and Sarcophagidae.

Culicoides (Biting midges) and Psychodidae (sand flies) are found in this study. Viennet *et al*. (2013) stated that *Culicoides* are transmitters of African horse sickness virus and bluetongue virus, both of which cause devastating disease that lead to economic losses. In Malaysia, the two important diseases that are transmitted by biting midges are leucocytozoonosis in poultry and bluetongue in sheep (Rahmat & Parameswaran, 1991). Sand flies on the other hand are species only important as vectors of leishmaniasis if they feed regularly on humans (Viroj, 2012).

The mosquitoes collected in the zoo during this study are typical species found in urban areas of Malaysia. The most abundant species of mosquitoes collected in Zoo Negara is *Culex quinquefasciatus* followed by *Aedes albopictus*. *Culex quinquefasciatus* is also known as tropical house mosquitoes and it is found as a vector of Japanese encephalitis (JE) (Sucharit *et al*., 1989), canine heartworm (Loftin *et al.*, 1995) and also lymphatic filariasis (Vythilingam *et al*., 2005). The principal vectors of JE in Malaysia are *Culex tritaeniohynchus* and *Culex gelidus* while elsewhere are *Culex vishnui* and *Culex fuscocephala* (Sucharit *et al*., 1989). All four of these *Culex* mosquitoes-borne diseases for JE are found in this study. *Aedes albopictus* which is one of the dengue vectors was also found in this study. Anopheline mosquitoes are known to be vectors for malaria disease. In this study, only two species of anopheles mosquitoes were collected, namely *Anopheles vagus* and *Anopheles kochi*.

The highest number of flies is found in the Ankole species while the lowest number of flies found is from the Barking Deer and Tapir enclosures. Fly numbers in a given locality vary with the availability of breeding places, sunshine hours, temperature and humidity (Keiding, 1986).

5.2 Temporal activity of the trapped and efficiency of the traps

The diurnal activity of dipteran flies was observed during different periods of time. The large flies are more active at 1600-1900 hours. Flies are most active at low air humilities and high temperature-above 20^{0}C (Keiding, 1986). Phasuk *et al.* (2013) showed that in hot season, *Stomoxys* flies peaked in the evening. In a study of traps performance by Gibson and Torr (1999), the Nzi trap typically caught more biting flies than other traps, which are often optimal for just a few groups while for large interception traps such as the Malaise, good trap performance for all the species is difficult to achieve. In this study, among the three traps (Nzi, Malaise and Intercept), the Nzi trap had the highest level of dipteran flies richness and abundance compared to the other two large interception traps. Nzi trap performance outweighs both the large interception traps due to the fly behaviour towards the visual cues. Flies are strongly attracted to the colour to blue and moderately attracted to black and red (Allan & Stoffolano, 1986).

Both the CDC -UV traps and CDC traps baited with carbon dioxide had the highest catch at the 1900- 0700 hours. *Culex* mosquitoes are active and feed mainly at night and peak period is between 2100 to 0200 hours (Yap *et al.*, 2000). Based on the study done by Vythilingam *et al*. (1995), most of the JE vectors were nocturnal where their peak biting time was between 1900 to 2100 hours. Lim (1979) found that *Aedes albopictus* has the highest landing and biting frequency at 1800 hours. A study done in zoos in South Carolina by Nelder (2007) showed that CDC-UV traps trapped more number and species of mosquitoes and biting midges. Similarly in this study, the CDC–UV trap captured more number of mosquitoes and biting midges compared with CDC-CO_2 trap. The CDC –UV trap captured the majority of nocturnal host seeking mosquitoes species at approximately 1.5 m above the ground. A reason for low collection numbers in the CDC-CO_2 trap might be the results of competition from abundance of potential host animals in the zoo.

6.0 CONCLUSIONS

From this study, there is an evidence of high diversity and abundance of Diptera associated with captive mammals in the zoo. 14 species of dipteran trapped from Zoo Negara are known vectors of zoonotic viruses and parasites. The large flies trapped are mostly diurnal whereas most mosquitoes are nocturnal. The temporal activity of the large flies showed that the peak activity is from 1600 hours to 1900 hours. Among the trapping method, Nzi trap was the most efficient in trapping the large flies while CDC -UV trap was best in trapping the mosquitoes. More zoos should be sampled throughout Malaysia to get a clearer picture on the diversity and abundance of dipteran vectors in zoos across the country. Further research for flies screening should be done to ascertain whether they harbour infective pathogens by using microscopy and polymerase chain reaction (PCR) from the salivary glands of the Diptera flies. Zoos should be alerted on the occurrence of these vectors in order to initiate proper control programs. For surveillance purpose, the captive animals should be periodically screened to determine their reservoir status for pathogenic organisms. The usage of Nzi trap is recommended to control the population of biting flies at the zoo. The CDC-UV traps are efficient at trapping various species, thus, it can be used as a supplementary means of mosquitoes control in the zoo.

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8.0 APPENDIX

Different types of traps

CDC- CO_2

CDC-UV

Malaise Trap

Intercept Trap

Nzi Trap