METHODOLOGY

3.1 Sampling period

The study was conducted from March 2010 until June 2012 at two different selected sites which were Kota Gelanggi 5 and Lepar Utara 11 located in the central part of Pahang. A total of 36 trapping sessions were conducted and each trapping lasted for three consecutive nights (Table 3.1).

3.2 Description of study area

The distance sampling design method (Buckland *et al.*, 2001) was used to survey the small mammal. The study site consists of two types of habitat which are secondary forest and oil palm plantation. The main criteria applied for site selection process, the site must contain a forest reserve which is located adjacent to oil palm plantation and the oil palm plantation has different ages of oil palm trees. Two trapping sites (Kota Gelanggi 5 and Lepar Utara 11) were established. Both sites are located at least 10 kilometres apart. Line transect was used for this study and each transect consists of six plots with different characteristics. Three trapping sites were establish in logged forest which are located at different distances from the forest edge (adjacent, intermediate and interior) and three trapping sites were established in oil palm habitats covering young, mature, and old oil palm trees (Figure 3.2). Trapping was performed three times in each plot (Table 3.1). The general descriptions of all trapping sites are as follows:



Figure 3.1: Map showing location of the study sites (•) in Peninsular Malaysia located in Pahang state.

					\sum of trapping nights	
No. of trapping	Date	Location	GPS reading	Habitat type	Non-volant small mammals	Volant small mammals
			N 03°57.353'			
1	13-16 May 2010	Kota Gelanggi 5	E 102°35.853'	Old oil palm	300	120
			N 03°58.097'			
2	8-14 June 2010	Kota Gelanggi 5	E 102°35.954'	Adjacent	300	120
			N 03°56.735'			
3	8-11 July 2010	Kota Gelanggi 5	E 102°36.066'	Matured oil palm	300	120
			N 03°58.097'			
4	22-25 July 2010	Kota Gelanggi 5	E 102°35.954'	Adjacent	300	120
			N 03°54.709'			
5	12-15 August 2010	Kota Gelanggi 5	E 102°32.969'	Young oil palm	300	120
			N 03°59.405'			
6	27-30 August 2010	Kota Gelanggi 5	E 102°36.036'	Intermediate	300	120
			N 03°56.354'			
7	27-30 September 2010	Lepar utara 11	E 102°44.183'	Matured oil palm	300	120
			N 04°06.251'			
8	15-18 October 2010	Lepar utara 11	E102°47.189'	Interior	300	120
			N 03°59.541'			
9	28-31 October 2010	Lepar utara 11	E 102°42.576'	Old oil palm	300	120
			N 03°59.478'			
10	10-13 November 2010	Lepar utara 11	E 102°46.251'	Adjacent	300	120
			N 03°58.970'			
11	27-30 November 2010	Lepar utara 11	E102°42.894'	Young oil palm	300	120

 Table 3.1: The sampling periods and number of trapping night for both volant and non-volant small mammals in all six habitat types.

Table 3.1, continued.

					\sum of trapping nights	
No. of trapping	Date	Location	GPS reading	Habitat type	Non-volant small mammals	Volant small mammals
			N 0490 4611			
12	17-20 December 2010	Lepar utara 11	E 102°45.392'	Intermediate	300	120
		-	N 03°57.353'			
13	28-31 December 2010	Kota Gelanggi 5	E 102°35.853'	Old oil palm	300	120
			N 03°59.405'			
14	14-17 January 2011	Kota Gelanggi 5	E 102°36.036'	Intermediate	300	120
			N 04°01.282'			
15	26-29 January 2011	Kota Gelanggi 5	E 102°36.951'	Interior	300	120
			N 04°01.282'			
16	18-21 February 2011	Kota Gelanggi 5	E 102°36.951'	Interior	300	120
			N 03°54.709'			
17	4-7 March 2011	Kota Gelanggi 5	E 102°32.969'	Young oil palm	300	120
			N 03°56.735'			
18	18-21 March 2011	Kota Gelanggi 5	E 102°36.066'	Matured oil palm	300	120
			N 03°59.478'			
19	1-4 April 2011	Lepar utara 11	E 102°46.251'	Adjacent	300	120

Table	3.1,	continued.
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					\sum of trapping nights	
No. of trapping	Date	Location	GPS reading	Habitat type	Non-volant small mammals	Volant small mammals
			N 04°06.251'			
20	12-15 April 2011	Lepar utara 11	E102°47.189'	Interior	300	120
			N 04°0.461'			
21	14-17 May 2011	Lepar utara 11	E 102°45.392'	Intermediate	300	120
			N 04°0.461'			
22	18-21 May 2011	Lepar utara 11	E 102°45.392'	Intermediate	300	120
			N 03°59.478'			
23	18-21 June 2011	Lepar utara 11	E 102°46.251'	Adjacent	300	120
			N 03°56.354'			
24	22-25 June 2011	Lepar utara 11	E 102°44.183'	Matured oil palm	300	120
			N 04°06.251'			
25	9-12 July 2011	Lepar utara 11	E102°47.189'	Interior	300	120
			N 03°59.541'			
26	13-16 July 2011	Lepar utara 11	E 102°42.576'	Old oil palm	300	120
			N 03°58.970'	_		
27	5-8 August 2011	Lepar utara 11	E102°42.894'	Young oil palm	300	120
			N 03°59.541'			
28	9-12 August 2011	Lepar utara 11	E 102°42.576'	Old oil palm	300	120
			N 03°58.970'	-		
29	27-30 September 2011	Lepar utara 11	E102°42.894'	Young oil palm	300	120
	▲ 	-	N 03°56.354'			
30	2-5 October 2011	Lepar utara 11	E 102°44.183'	Matured oil palm	300	120

Table 3.1 ,	continued.
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					\sum of trapping nights	
No. of trapping	Date	Location	GPS reading	Habitat type	Non-volant small mammals	Volant small mammals
			N 03°59.405'			
31	22-25 October 2011	Kota Gelanggi 5	E 102°36.036'	Intermediate	300	120
			N 03°56.735'			
32	26-29 October 2011	Kota Gelanggi 5	E 102°36.066'	Matured oil palm	300	120
			N 04°01.282'			
33	15-18 November 2011	Kota Gelanggi 5	E 102°36.951'	Interior	300	120
			N 03°58.097'			
34	19-22 November 2011	Kota Gelanggi 5	E 102°35.954'	Adjacent	300	120
			N 03°54.709'			
35	14-17 December 2011	Kota Gelanggi 5	E 102°32.969'	Young oil palm	300	120
			N 03°57.353'			
36	18-21 December 2011	Kota Gelanggi 5	E 102°35.853'	Old oil palm	300	120
				Total	10800	4320

3.2.1 Secondary Forest

The Tekam Forest Reserve (TFR) in Jerantut, Pahang, covering 12400 ha, is located about 170 km from Kuala Lumpur, at latitude 4°15'N and 102°37'E. A prevalence of the genera *Dipterocarpus* and *Shorea* of the red meranti group dominates the floristic composition (Poore, 1968). This forest was logged between 11-27 years ago and was considered as secondary forest. Samsudin *et al.* (2010) found that in general the forests have not fully recovered in terms of stocking of commercial species with favouring higher dominance of non-dipterocarp species. Most of the plant species found in this area include Mahang (*Macaranga* spp)., Kelat (*Syzygium* spp.), Senduduk (*Melastoma* spp.) and shrubs.

There are Orang Asli (aborigine people) settlements at the entrance of Tekam Forest Reserve. These people used forest products for daily activities especially as food resources. According to the locals and circumstantial evidence, there are wild animals that have been seen in this forest such as Asian elephant (*Elephas maximus*), tigers (*Panthera tigris*), and wild boars (*Sus scrofa*).

3.2.2 Oil palm plantation.

The oil palm plantation is located in Kota Gelanggi 5 and Lepar Utara 11. The plantation is managed by Felda Agriculture Services Sdn Bhd (FASSB). The plantation area covers an area of 1575.92ha (Kota Gelanggi 5) and 3421.74ha (Lepar Utara 11) and is adjacent to the Tekam Forest Reserve area. The oil palm is monoculture plantation with different tree ages varies from 2 to 28 years old. Epiphytes are commonly seen on palm trees in this plantation area and the leguminous crops are typically established within oil palm plantations as natural cover crops to prevent soil erosion and desiccation. (Corley & Tinker, 2003).

3.2.3 Adjacent forest

Adjacent forest was located approximately one kilometre away from the forest edge or oil palm plantation. The forest was selectively logged around 35 years ago. This plot was connected with old logging roads. Taller trees ranges from 19 to 20 meters dominated the study plot. Dense vegetation was found in this study plot because of shrubs cover. This plot was categorised as having high degree of human disturbance because it has been intensely used by Orang Asli and local people.

3.2.4 Intermediate forest

Intermediate forest was located approximately two kilometres from forest edge. The plot was established in both trapping sites, Kota Gelanggi 5 and Lepar Utara 11. This site is characterised by a 20 meter tall canopy which were interconnected by branches and lianas. Clumps of bamboo were also observed in this study plot.

3.2.5 Interior forest

Interior forest trapping site was positioned approximately 3 km away from the forest edge. This site is characterised by a 20 m tall canopy. Interior forest have high number of tall and big trees. This site appeared to be less disturbed than adjacent and intermediate forests, probably due to the distance from the human residential areas. The plot was established in both Kota Gelanggi 5 and Lepar Utara 11.

3.2.6 Young oil palm plantation

The trapping site in this habitat was generally on flat to gently undulating terrain. The oil palm trees were spaced evenly about 9-10 m apart. The young oil palm plantation consisted of approximately 2 to 3 years old with heights ranging from 1.5 to 2 meter tall. Grasses and creepers densely covered the rest of the ground. Creepers are purposely cultivated throughout the plantation to control soil erosion and suppress weeds.

3.2.7 Mature oil palm plantation

The mature oil palm plantation consisted of approximately 8-10 years old oil palm trees with heights ranging from 10-12 meters. The canopy height of the oil palms was fairly uniform throughout the plantation. Old oil palm fronds that were cut off during harvesting to get access to the fruits were stacked between rows of oil palms.

3.2.8 Old oil palm plantation

The old oil palm plantation consisted of approximately 20-22 years old oil palm with height ranging from 23-25 meters tall. The leaves are pinnate, and reach between 2-5 meter long. The trapping site in this habitat was generally on flat to gently undulating terrain. Epiphytic plants can be easily seen in this area. One of the most abundant epiphytic plants in the oil palm plantation is the bird's nest fern (*Asplenium* spp.). This epiphytic fern play an important ecological role as they provide an important refuge for animals ranging from arthropods to small mammals especially bats.



Figure 3.2: Placement of trapping devices in the study areas in Kota Gelanggi 5 and Lepar Utara 11. P1= Adjacent forest, P2= Intermediate forest, P3= Interior forest, YOP= Young oil palm plantation, MOP= Mature oil palm plantation, OOP= Old oil palm plantation.

3.3 Sampling techniques

3.3.1 Non-volant small mammals

Locally made wired mesh traps, measuring 30 x 15 x 15 cm, were used to capture non-volant small mammals. One hundred traps were employed in each plot and all traps were set up 10 meters from each other. In the forest habitat, traps were placed along the trails, but always starting more than 10 m apart from the trail and positioned on trees, fallen logs or large vines. Trap lines inside the oil palm plantation habitat followed the inter-rows of the oil palm, and all traps were placed on the ground in the oil palm plantation habitats. Traps were baited with oil palm fruits as this bait was found to be the most efficient during the preliminary study. Traps were checked twice, in the morning between 0700 hours and 1000 hours and in the evening between 1700 hours and 1900 hours. Baits were replaced whenever necessary to maintain the freshness of the bait

3.3.2 Volant small mammals

Species differ in their susceptibility on the available capture techniques (Francis, 1989). Four units of four-bank harp traps (2 meter width and 3 meter high) were set up for three consecutive nights. Traps were set up on clear pathways approximately 1 meter above ground level, with trees and undergrowth on either side as well as above the traps. Harp trap targets insectivorous bats. The trap was set up across the study plot and each trap is set 40-60 meters apart. Vegetation above and surrounding the traps will funnel bats into the trap. In addition to harp traps, ten mist net (12 meters long ; 70 denier nylon material with four shelves, 36 millimeter mesh size) were also used to trap fruit bats. Traps and nets were operated from dusk (1830 hours) until midnight 2300 hours, unless it was rained. The traps and nets were checked every 30-minutes intervals to remove captured bat.

3.4 Handling of animals

3.4.1 Identification

Species identification was based on (Francis, 2008), (Kingston et al., 2006) and (Medway, 1983).

3.4.2 Non-volant small mammals

Cloth bags was used to keep captured animals. All captured non-volant small mammals were anaesthetized with chloroform to facilitate measuring and identification. The animals were carefully transferred from trap into a transparent plastic bag, in which chloroform was applied on a cotton wool. Standard body measurements were recorded for each individual. Captured animals were weighed to the nearest gram (g) using digital scale and each animal was sexed. The head-body length (abbreviated as HB) was measured from the anus to the front of the nose. The body was pushed flat and straight (but not stretched) on the ruler before taking the HB measurement. Tail (T) was measured from the anus to the tip of the fleshy or bony part of the tail, while hind foot (HF) was measured from the heel to the end of the longest toe. Ear length (EL) was measured from the bottom of the external opening of the ear to the tip (Figure 3.3). All individuals were released near capture site immediately after measurements were done, which normally took less than 15 minutes.

3.4.3 Volant small mammals

Captured bats were held individually in cloth bags before biometric data was taken. The forearm (FA) was measured from the outside of the elbow to the outside of the wrist in bent wing. Tail (T) (if present) was measured from the anus to the tip of the fleshy or bony part of the tail while hind foot (HF) was measured from the heel to the end of the longest toe. Ear length (EL) was measured from the bottom of the external opening of the ear to the tip (Figure 3.3). Individuals were further inspected to determine sex (male/female). All individuals were identified up to species level using morphological characteristics (Francis, 2008). All bats were released after the required information was recorded.



b)



Figure 3.3: The measurement of; (a) volant small mammal and (b) non-volant small mammal adapted from Francis (2008). (Legend: HB = head body length; E = ear length; FA = forearm length; HF = hind foot length; T = tail length). Measurements were taken to the closet one decimal point in millimetre.

3.5 Data analysis

Data collected on trapped small mammals were analyzed and compared across sites and species as mentioned in the study objectives. Small mammal composition was also compared among families.

3.5.1 Trapping efforts

The trapping efforts were determined to measure trapping success. The formula is given below:

Trapping efforts
$$= C X D$$

Where C = number of traps

D = number of trapping night

3.5.2 Percentage of trapping success

The trapping success is important in order to estimate the population of the small mammal in the selected habitats. The percentage of trapping success was determined in the percentage of the number of captures and the number of trapping efforts. The formula is given below:

$$\frac{\text{Percentage of trapping}}{\text{success}} = \frac{A}{D} \times 100$$

Where A = total number of individuals captured

D = number of trapping night

3.5.3 Species richness estimators and species rarefaction curve

Species richness is the most important criteria used to determine the conservation value of the ecosystem. A rarefied species accumulation curve was calculated for each site to determine if all species in the site had been sampled adequately (Kingston, 2009). The various estimators of species richness used are described in Appendix E. The species richness estimators and rarefaction curves and were generated using EstimateS package (Cowell, 2006) and EcoSim (Gotelli & Entsminger, 2001).

3.5.4 Species composition, relative abundance and conservation status.

The species relative abundance was calculated to determine the number of individual of small mammals that exist in different habitats. The relative abundance calculated was based on the number of individuals of particular species within specific plot to the total number of individual captured in that plot (Bayne & Hobson, 1998). The relative species abundance formula is given below:

Number of individual of particular species in

Relative species

a plot

abundance

The total number of individual of all species captured in that plot

The conservation status was based on Red List of Mammals for Peninsular Malaysia (DWNP, 2010) and the International Union for Conservation of Nature and Natural Resources (IUCN) status for each species (IUCN, 2013).

3.5.5 Diversity index

Alpha-diversity describes the variety of organisms occurring in a particular place or habitat (Swingland, 2001). To compare community diversities, the most common diversity indices of non-parametric indices, Shannon-Wiener (H') and Eveness (J) were used for all sites. The analysis was completed using R Statistical computing and the Vegan package (R development Core Team, 2009; Oksanen *et al.*, 2009). To display the differences of species composition between the study sites, a distance matrix was calculated using Jaccard coefficient index for species presence/absence.

Shannon-Wiener index, H'

The Shannon-Weiner index has been a popular diversity index in the ecological literature. H' represents the Shannon-Weiner diversity for each sample. The underlying function was devised to determine the amount of information in a code and is defined as:

$$H = -\sum_{i=1}^{\text{Sobs}} pi \log ep$$

Where pi = the proportion of individuals in the *i*th species

Sobs = observed species

Eveness J

Equitability or evenness refers to the pattern of distribution of the individuals between species. Its measure equitability compare to the observed Shannon-Weiner index against the distribution of individuals between the observes species which would maximise diversity. If H is the observed Shannon-Weiner index, the maximum value this could take is log(S) is the total number of species in the habitat. Therefore the index is:

$$J = H$$

$$log(S)$$

Where H = Shannon-Weiner index

S = number of species

3.5.6 Statistical analyses

Mann-Whitney U Test

Since the data of this study was not normally distributed, Mann-Whitney U test was used to test whether there is a significant difference between the forested habitat and oil palm plantation. The Mann-Whitney test is the non-parametric equivalent of the independent samples t-test. This test was completed using R Statistical computing and the Vegan package (R development Core Team, 2009; Oksanen *et al.*, 2009).

Kruskal-Wallis H Test

While the data of this study do not demonstrate normal distribution, the Kruskal-Wallis H test has been used. This analysis is a statistical method for comparing means of three or more groups of cases. The Kruskal-Wallis H Test is the non-parametric, equivalent of the one-way ANOVA. This test was completed using R Statistical computing and the Vegan package (R development Core Team, 2009; Oksanen *et al.*, 2009).