Changes in Abundance, Diversity and Community Composition of Mosquitoes Based on Different Land Use in Sabah, Malaysia

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A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science of Imperial College London and Diploma of Imperial College London

September 2011

Abstract

Land use changes, such as deforestation, urbanisation and agriculture can affect mosquito abundance, diversity and community composition. The expansion of oil palm plantation sites in South-east Asia is the main cause for deforestation which could result in a higher prevalence of mosquito-borne diseases, such as dengue fever. This study focuses on how different land use areas (old growth, secondary forest, oil palm and housing) affect mosquito diversity, abundance and community composition. Modified ovitraps with oviposition substrates were placed in each land use area to collect mosquito eggs and larvae. Water temperatures, amount of shade and leaf number were recorded for each site. Results showed that there was a higher diversity and abundance in the old growth and secondary forest areas, but this decreased in oil palm and housing areas. A high abundance of *Aedes* eggs were associated with increased shade and leaf litter. The dengue vectors *Aedes aegypti* and *Aedes albopictus* were found within the housing areas, but not in other land use areas. Very few *Aedes* eggs hatched successfully in the housing area. This study highlighted that the diversity of mosquito decreased from old growth to housing areas, but the most medically important mosquitoes were only found after urbanisation.

Contents

1. Introduction

1.1. Tropical forest modification

Tropical forest ecosystems provide at least two-thirds of the Earth's terrestrial biodiversity, but habitat loss through forest degradation and forest modification is a serious threat to this biodiversity (Gardner *et al.* 2009). Due to the dramatic loss of tropical rainforests, there has been an increase in the number of studies focusing on how forest modification affects biodiversity (Schulze *et al.* 2004). Many studies have shown that forest modification and clearing has a negative effect on biodiversity (Schulze *et al*. 2004; Fitzherbert *et al.* 2008; Sodhi *et al.* 2010). There is, however, little data on how tropical forest modification will affect mosquito populations.

South-east Asia includes four hotspots containing high biodiversity and a large number of endemic species (Myers *et al.* 2000), but also has one of the highest rates of deforestation of any tropical region (Sodhi *et al.* 2004; Sodhi *et al.* 2010). The expansion of oil palm (*Elaesis guineensis*) cultivation is a great threat to biodiversity and a major driver of deforestation (Koh & Wilcove 2007; Fitzherbert *et al.* 2008; Brühl & Eltz 2010). It is predicted that South-east Asia could lose up to three quarters of its original forest and 42% of its biodiversity by 2100 (Sodhi *et al.* 2004).

Malaysia and Indonesia are the top producers of palm oil, providing 87% of global production (Koh *et al.* 2011). Since 1990, the tropical forests in Malaysia have decreased by 1.2 million ha and have been converted into farmlands or agro-forests (Adachi *et al.* 2011). The oil palm area has increased rapidly in Malaysia, from 1.8 million ha in 1990 to 4.2 million ha in 2005 (FAO 2010), while 1.1 million ha of forest were lost (Fitzherbert *et al.* 2008). Malaysia is one of fourteen major deforestation countries, losing 250,000 ha or more annually, which is a major concern in terms of biodiversity loss (McMorrow & Talip 2001).

Many oil palm plantations have been created by planting on pre-existing crop land (e.g. rubber) but between 55-59% of oil palm expansion resulted in deforestation between 1990-2005 (Koh & Wilcove 2008; Koh *et al.* 2011). As less than 10% of South-east Asia's forests

are protected (IUCN 1994), it is likely that habitat degradation and deforestation will continue (Sodhi *et al.* 2010).

1.2. Land use changes and mosquito abundance

There are many studies focussing on the medical importance of mosquitoes, but far fewer have focussed on the effect of human activities on mosquito populations (Dorvillé 1996). Human alteration of habitats can play an important part in changing the ecological balance within which mosquitoes breed, develop and transmit diseases (Patz *et al.* 2000; Norris 2004). Land use changes include deforestation, agricultural development, water control systems (water management), and urbanisation (Gratz 1999; Norris 2004), which can affect mosquito abundance, biodiversity, human biting behaviour, and vector competence (Patz *et al.* 2000).

Water control systems, such as reservoirs, irrigation canals and dams can shift mosquito vector populations. They can provide new vector breeding sites where water was previously limited (e.g. irrigation canals) or by damming water, which is associated with higher malaria prevalence (Alemayehu *et al.* 1998). In urban areas sewage management, runoff, sedimentation and artificial containers can provide many more mosquito breeding sites (Norris 2004). Artificial containers, such as tires, bottles, buckets water butts, cups can provide a large number of mosquito breeding sites and must be removed to reduce a disease outbreak (Norris 2004; Rattanarithikul *et al.* 2005a).

Deforestation has long been associated with the resurgence of malaria in Africa (e.g. Manga, Toto & Carnevale 1995), Asia (e.g. Bunnag *et al.* 1979) and Latin America (e.g. Vittor *et al.* 2006). The forest floor in primary forests tends to be shaded and covered with thick organic leaf litter and acidic water environments whereas cleared lands tend to have sunlit neutral water environments. Cleared lands are also prone to the formation of puddles (Patz *et al.* 2000). Different mosquito species vary in their habitat requirements, and this gives a succession of vector species (Patz *et al.* 2000; Norris 2004). Deforestation also increases the number of humans in the area due to employment for road building, logging or in agriculture (Norris 2004). Areas of high disease transmission can be quite localised, for example Manga,

Toto & Carnevale (1995) showed that the number of daily infective bites from *Anopheles gambiae* were much higher in deforested areas than forested areas only 3km away.

Agricultural development increases the number of humans working in an area, but can alter environmental conditions so that they favour mosquitoes (e.g. deforestation and water management) (Norris 2004). Agriculture can cause sedimentation, which can slow or block streams and decrease the water depth (Dian & Changxing 2001). This provides a larger number of mosquito habitats and increases the water temperature for vector development (Norris 2004). Changing landscapes can also significantly affect the microclimate of a habitat, such as temperature, runoff, evapotranspiration. These factors are key in determining the abundance, survivorship and diversity of mosquito vectors (Patz & Olsen 2006).

There are also examples of when ecological changes reduced the risk of malaria transmission. Chang *et al.* (1997) showed that the development of oil palm plantations in Sarawak reduced the number of four *Anopheles* species within that area. Mosquito samples were collected during different stages of land development including; forested areas, clearing, burning and cultivation and maintenance. A decreased prevalence of malaria was shown over the four-year sampling period. There was, however, an increase in the dengue vector *Aedes aegypti*.

There have been few studies identifying the mosquito species in the primary forest, logged forests and oil plantations in Malaysia. Many of these studies focus on the mosquito vectors present, with few identifying all mosquito species. It is important to study mosquito species succession so that we can have a better understanding of how land use affects mosquito populations and the prevalence of diseases. This is so that we can provide a better land use strategy for the reduction of vector mosquitoes.

1.3. Project aims and objectives

This study aims to investigate what impact forest modification has on the abundance, diversity and ecology of mosquito populations in Sabah, Malaysia by using modified ovitraps. The main objectives are to:

1. Investigate the differences in the diversity and composition of mosquito populations across different habitat types (secondary forest, old growth, oil palm and housing areas)
2. Investigate the effect of environmental variables on mosquito abundance and lifecycle in different habitat types (including percentage of mosquito eggs hatched)
3. Investigate how the medically important mosquitoes are impacted by land use change

2. Literature Review

2.1. Mosquito biology

Mosquitoes belong to the order Diptera (True flies), in the family Culicidae. There are 3 subfamilies; Anophelinae, Culicinae and Toxorhynchitinae (Snow 1990). Only Anophelinae and Culicinae contain mosquitoes that are able to spread diseases (Knight & Stone 1977). Culicidae contain around 3,500 species, which have been grouped into 42 genera and 135 subgenera (Knight & Stone 1977; Crans 2004; Rueda 2008). They are found across almost the entire globe, but the majority are found in the tropics and subtropics. The warmer climates in tropical areas allow them to be active all year round, with the ideal conditions being hot and humid with moderate rainfall. In hot climates they are able to be more active, and the rainfall gives them aquatic sites for larval and pupal stages (Gillett 1971).

Mosquitoes are amongst the anthropophagic insects because they feed on human blood. Figure 2.1 shows the major characteristics of a mosquito, including how to differentiate between male and female mosquitoes. They are significant pests to not only humans, but also domestic animals, with potentially fatal outcomes (Snow 1990). Since discovering the link between mosquitoes and transmitting viruses, it has been a major priority to prevent the spread of diseases and to control mosquito populations (Medlock, Snow & Leach 2006). Although control methods are used, mosquito-borne diseases still thrive in many countries and cause millions of deaths (Tren & Bate 2001).

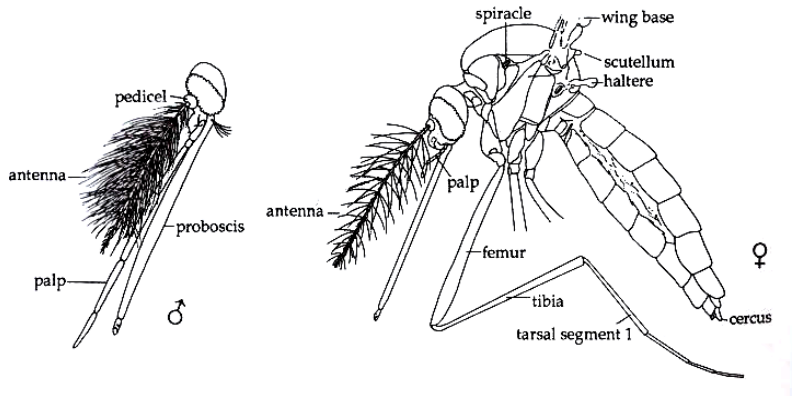

Figure 2.1: Characteristics of adult mosquitoes, including differences between males and females (Snow 1990)

Mosquitoes are capable of breeding in a variety of environments. Many mosquitoes are generalists and choose a variety of oviposition sites, whereas others are specialists and choose unique habitats for laying eggs. The specialist mosquitoes tend to disappear after land use

changes (e.g. deforestation), but generalists are able to survive in a wide range of habitats (Rattanarithikul *et al.* 2005a). There are several types of oviposition sites, which can be categorised into ground water sites or container sites. Ground water sites include rivers, lakes, ground pools and many more. Container sites include artificial containers (such as tires, bottles, cups, jugs) or natural containers (such as fallen leaves, tree holes, tree stumps, plant axils). Mosquitoes are able to breed in permanent water, semi-permanent water or temporary pools (Rattanarithikul *et al.* 2005a).

All mosquitoes undergo complete metamorphosis within their lifecycle, which has four stages of development (egg, larva, pupa and adult) and each stage results in a cast of the exuviae (Cranston *et al.* 1987). The lifecycle starts by laying eggs on the surface of the water, either singly (*Anopheles, Aedes, Orthopodomyia* and *Culiseta* subgenus *Culicella*), or in batches (*Culex, Uranataenia, Coquillettidia,* and *Culiseta* subgenus *Culiseta*) (Figure 2.2) (Snow 1990). Some mosquitoes are able to lay their eggs singly into moist soil (e.g. *Aedes* or *Ochlerotatus*). After the eggs hatch, they pass through four larval instars. During this time they feed on detritus, algae, and biofilms. A few non-vector mosquito larvae can be predaceous (Norris 2004).

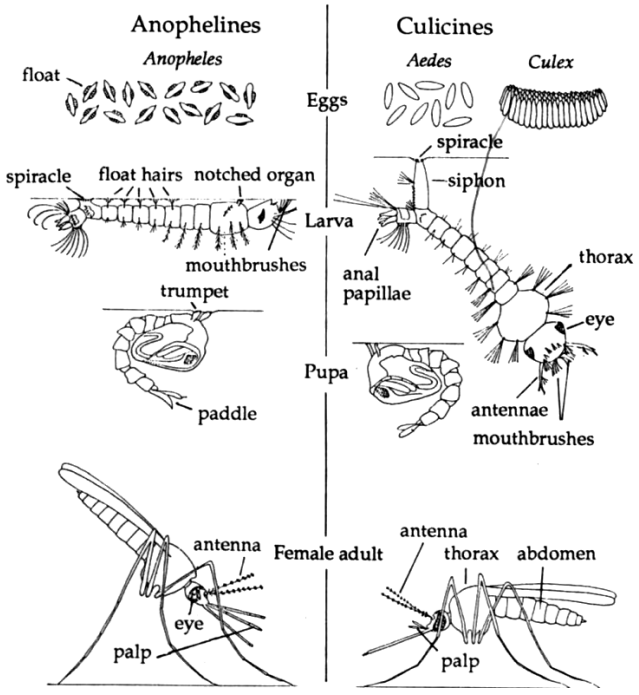

Figure 2.2: Differences between Anopheline and Culicine lifecycles (Snow, 1990)

Mosquito larvae are identified by their well-developed head bearing mouth-brushes, anal papillae on their anal segment, and the presence of spiracles (*Anopheles*) or a respiratory

siphon (remaining genera) in their 8th abdominal segment (Figure 2.2) (Snow 1990). The factors affecting larval distribution include; terrain, elevation, shade, water condition (fresh, brackish, polluted etc.), water movement, water temperature, aquatic vegetation (e.g. algae), types of water source, water permanency, and competition (other invertebrates such as Odonata) (Rattanarithikul *et al.* 2005a). The larvae take a few days to several weeks to develop, depending on nutrient levels, temperature, competition and water condition (Becker *et al.* 2010).

Adult mosquitoes feed on juices from flowers and fruits, but females of most species require a blood meal to obtain enough nutrients for the development and maturation of eggs, known as anautogenous (Clements 1992). Female mosquitoes have been found to have a complex oviposition behaviour, which strongly influences where eggs are laid. Studies have suggested that females may be able to detect the level of predation or potential competition from other mosquitoes in aquatic habitats, which affects where eggs are laid (Williams *et al.* 2008). After the female mosquito has laid her eggs, the cycle repeats.

2.2. Medical importance of mosquitoes

The mosquito genera of medical importance are *Aedes, Anopheles, Culex, Haemagogus, Mansonia*, and *Sabethes* due to their blood sucking behaviour on humans (Service 2004). *Psorophora* and *Coquillettidia* are of lesser medical importance Mosquitoes are able to transmit many diseases such as malaria (protozoan), filariasis (nematode), dengue chikungunya, Japanese B-encephalitis and yellow fever (viruses) (Becker *et al.* 2010). These diseases are found widespread in the tropics and subtropics. The mosquito-borne diseases currently in Malaysia include malaria, dengue, urban filariasis, rural filariasis, Japanese B-encephalitis and chikungunya (Yap *et al.* 1994; Lam *et al.* 2001; Benitez *et al.* 2009) and it is predicted that the spread of these diseases may worsen with climate change (Benitez *et al.* 2009).

2.2.1. Malaria

Malaria is a mosquito-borne virus, which has caused an estimated 247 million malaria cases among 3.3 billion people in 2005, resulting in nearly a million deaths (WHO 2008). Malaria is not present in all continents and occurs mainly in Africa, but also occurs in South-east

Asia, Central America and South America. Malaria is caused by the protozoan *Plasmodium*, which is transmitted by mosquitoes of the genus *Anopheles* (de Castro *et al.* 2006).

Malaria has decreased in Malaysia due to mosquito control methods, with number of cases as high as 58,958 in 1994 but has decreased to 5,456 in 2007 (VBDCP 2009). Control methods include indoor residual spraying (IRS), insecticide treated bednets (ITN), anti-malarial drugs, larviciding, environmental management measures and personal protection methods (Becker *et al.* 2010). The primary vectors for spreading malaria in Sabah are *Anopheles donaldi*, *Anopheles balabacensis* and *Anopheles maculatus. Anopheles balabacensis* used to be the predominant vector but has now been largely displaced by *An. donaldi* (Vythilingam *et al.* 2005). In the coastal areas, *Anopheles sundaicus* and *Anopheles flavirostris* were found to be secondary vectors (Vythilingam *et al.* 2005).

2.2.2. Dengue

Dengue fever (DF) is a mosquito-borne disease endemic to tropical and subtropical areas, found mainly in urban and suburban areas (Chen *et al.* 2005; Guzman & Istúriz 2010). DF is spread by the mosquito genus *Aedes*. *Aedes aegypti* and *Aedes albopictus* are the main transmitters of DF and are closely associated with humans, water and domestic environments (Guzman & Istúriz 2010). The disease occurs in more than 100 countries in the Asia-Pacific region, Americas, Middle-east and Africa (Guzman & Istúriz 2010). There are four known serotypes worldwide (DENV1, DENV2, DENV3 & DENV4), and have all been known to cause DF and dengue haemorrhagic fever (DHF) in Malaysia (Abubaker & Shafee 2002). The number of DF cases has been increasing in the past 50 years, and as there has been an absence of an effective vaccine, mosquito control is the only known option to interrupt the transmission of the disease (Lee & Rohani 2005; Benitez *et al.* 2009). There were less than 1,000 cases in 1973, but this increased to 46,000 in 2007 (Benitez *et al.* 2009).

DF is spread throughout Malaysia, and is thought to have followed the spread of *Ae. aegypti*, that replaced *Ae. albopictus* as the main carrier of the viruses (Abubaker & Shafee 2002). The distribution of *Ae. aegypti* and *Ae. albopictus* overlap in Malaysia and both spread DF and DHF (Chen *et al.* 2006b; Rozilawati, Zairi & Adanan 2007). There are new developments that may control DF and DHF in the future. Malaysia is currently working with Oxitec Ltd to release genetically modified mosquitoes to prevent breeding of *Ae. aegypti*. There are also developments for producing a vaccine by 2012 (Benitez *et al.* 2009).

2.3. Mosquitoes in Malaysia, including medically important vectors

In Malaysia, there are 434 species of mosquitoes, representing 20 different genera (Rahman, Che'Rus & Ahmad 1997). Many studies have indicated that *Aedes aegypti*, *Ae. albopictus* and *Culex quinquefasciatus* are the most abundant urban mosquitoes found in Malaysia (Yap & Thiruvengadam 1979; Chen *et al.* 2006a; Rozilawati, Zairi & Adanan 2007; Rohani *et al.* 2008). *Aedes, Anopheles, Culex* and *Mansonia* are the four main genera containing medically important mosquitoes in Malaysia. *Aedes* species are of concern because they transmit DF and DHF (Lam 1993), *Anopheles* species transmit malaria and filariasis (Rattanarithikul *et al.* 2006a), *Culex* species can transmit Japanese B-encephalitis and filariasis (Rattanarithikul *et al.* 2005b) and *Mansonia* species can transmit filariasis (Table 2.1) (Rattanarithikul *et al.* 2006b).

Table 2.1: Medically important mosquito species found in Malaysia (Service 2004; Rattanarithikul *et al.* 2005a,b; Rattanarithikul *et al.* 2006a,b)

Mosquito species	Larval habitat	Disease spread
Aedes aegypti	Clean and clear stagnant water. In artificial containers or natural habitats	Dengue, Chikungunya virus
Aedes albopictus	Clean and clear stagnant water. In artificial containers or natural habitats	Dengue, Chikungunya virus
Anopheles balabacensis	Small pools of muddy water within the forest	Malaria
Anopheles campestris	Still fresh water (marshes, drains, rice fields)	Malaria, Malayan filariasis
Anopheles donaldi	Stagnant pools at the edge of forest	Malaria
Anopheles maculatus	Slow flowing clean water exposed to sunlight	Malaria
Anopheles peditaeniatus	Swamps and grassy ponds	Bancroftian & Malayan filariasis
Culex gelidus	Freshwater ground pools, rivers, marshes and containers	Bancroftian & Malayan filariasis. Chikungunya virus
Culex quinquefasciatus	Stagnant water	Bancroftian filariasis
Mansonia uniformis	Open ponds and swamps with floating vegetation	Bancroftian & Malayan filariasis. Chikungunya virus

2.4. Mosquito trapping techniques

2.4.1. Sampling the egg population

There are few techniques for collecting all types of egg samples from different genera. There are different oviposition strategies for mosquitoes such as, *Aedes* and *Ochlerotatus* lay their eggs singly on substrates subject to flooding, *Culex* lay egg-rafts onto the water surface and *Mansonia* attach their eggs to submerged vegetation (Silver 2008). Eggs can be directly collected from natural oviposition sites, egg extractions from soil or from artificial oviposition sites. Ovitraps are a commonly used method for collecting mosquito eggs. They are easy to construct and use, even when the presence of gravid females is low (Silver 2008).

Disadvantages of ovitraps are that they can't be used to determine absolute population density and are labour intensive (Silver 2008).

A common ovitrap used for collecting *Ae. aegypti* larvae is a 'CDC ovitrap' (Figure 2.3). These traps consist of a dark water filled container and a thin paddle of wood, used as an oviposition substrate (Lenhart *et al.* 2005). The oviposition substrates are slightly taller than the container, one inch wide and usually made out of hard-board. Eggs of *Ae. aegypti* and *Ae. albopictus* are usually deposited just above the water line on the paddle. It has been shown that rough oviposition substrates are preferred over smooth (O'Gower 1963; Wong *et al.* 2011). Yap *et al.* (1995) showed that *Ae. aegypti* preferred ovitraps painted black, red or blue over white, yellow, green or clear glass. Different materials have been used as a container, including glass (Yap *et al.* 1995), tin cans (Chan, Ho & Chan 1971) and plastic (Goettel, Toohey & Pillai 1980). Plastic cups are increasingly replacing glass jars as the preferred container material (Silver 2008). It has also been found that adding 10% hay infusion or rabbit chow infusions to the ovitraps can act as attractants for mosquitoes (Reiter, Amador & Colon 1991; Lee & Kokas 2004). Rainwater may cause ovitraps to overflow, but this can be prevented by drilling an overflow hole near the top of the trap (Silver 2008).

Figure 2.3: traditional CDC ovitrap

2.4.2. Sampling the larval population

The larval and pupal mosquito population can be found in a range of habitats (mentioned in section 2.1), which can be collected by using dippers or aquatic nets. Dippers can vary in

sizes, including soup ladles holding around 100-150ml water (Silver 2008) or a larger 500ml dipper (Forattini *et al.* 1993). The larvae are collected by either skimming the dipper through the water at an angle or lowering the dipper slowly into the water (Silver 2008). Dippers can be used to sample mosquito populations. The larvae from dips are usually transferred to small plastic tubs (30ml) or 'Whirl-pak' bags to transport them back to the laboratory (Silver 2008). Aquatic nets work in a similar way to dippers, but can catch a higher number of larvae and detecting smaller mosquito populations (Silver 2008).

2.4.3. Sampling the adult population

Mosquitoes can be collected by catching the emerging adults, resting adults, or by using attractant or non-attractant traps. Emerging traps (e.g. floating traps) can collect mosquitoes in inaccessible areas such as deep wells. They can collect other aquatic invertebrates using the same trap but catch rate can depend on the colour of the trap (Silver 2008). Sampling the resting adult population will collect blood-engorged females, unlike attractant traps. Collecting these adults will identify host preference and can be found resting in man-made shelters (Silver 2008).

Attractant traps, such as light or carbon dioxide traps collect host seeking female mosquitoes. Certain mosquito species are attracted to light, which are sucked through the trap by a battery powered fan (Silver 2008). Hii *et al.* 1986 showed that the light traps catch unfed and blood engorged females. Carbon dioxide traps can also attract host-seeking females. This is done by putting dry ice in the trap and letting the trap run overnight (Silver 2008). There are a few traps that use light and carbon dioxide as an attractant, for example the CDC miniature light trap. These traps catch a wider range of mosquito species than light traps or carbon dioxide traps alone. Fermenting yeast is an alternative to dry ice, especially when dry ice is not readily available (e.g. tropical areas) (Oli, Jeffery & Vythilingam 2005).

Non-attractant traps are less biased than attractant traps as they sample a larger number of species more or less equally, such as malaise traps or suction traps. Non-attractant traps have a lower mosquito catch rate, but can collect male mosquitoes unlike attractant traps (Silver 2008). These traps can collect a variety of flying invertebrates, and can be labour intensive. Another disadvantage for using non-attractant traps is that the mosquito wing scales can also be washed off when they are collected in alcohol. A few mosquito keys need the wing scale patterns to identify mosquitoes to species.

3. Materials and Methods

3.1. Study site and SAFE Project

The study was conducted in the Benta Wawasan oil palm plantation (N4.64612, E117.44934) and Maliau Basin Conservation Centre (N4.74471, E116.95711) in Borneo (Sabah, Malaysia) with the Stability of Altered Forest Ecosystems (SAFE) Project (Figure 3.1). Data was collected at Maliau Basin Conservation Centre, oil palm plantations at Benta Wawasan, small villages for oil palm workers at Benta Wawasan and survey sites set up by the SAFE Project.

Figure 3.1: Location of SAFE Project sites within Sabah, Malaysia (Ewers *et al.* in press)

Maliau Basin (hereafter referred to as old growth, OG) was designated as a Class 1 Protection Forest Reserve by the Sabah State Assembly in 1997 due to its conservation importance (Jones 2000). The old growth sites have never been logged commercially, with the exception of a few sites being selectively logged in the 1970s and 1990s to build the Maliau Field Centre (Old growth, OG3) (Figure 3.2). Forest quality is still very high in OG3 so it is still classed as unaffected by logging. The oil palm plantation (OP) consists of monocultures of *E. guineensis* (Figure 3.2). OP1 and OP2 were planted in 2006, and are 500m and 700m from the forest respectively. OP3 was planted in 2000 and is 1km from the forest.

The SAFE Project is carrying out a large-scale forest fragmentation experiment in the lowland tropical areas of Borneo (Sabah, Malaysia), which will take advantage of the planned logging and conversion to oil palm plantations. This is to understand the impact of forest modification on the biodiversity and the provision of ecosystem services (Ewers *et al.* In press). A hierarchical sampling design has been established around a triangular fractal design, consisting of Logged Forest (LF), Oil Palm Plantations (OP) Old Growth (OG) and six replicate blocks (A-F) (Figure 3.2) (Ewers *et al.* In press.).

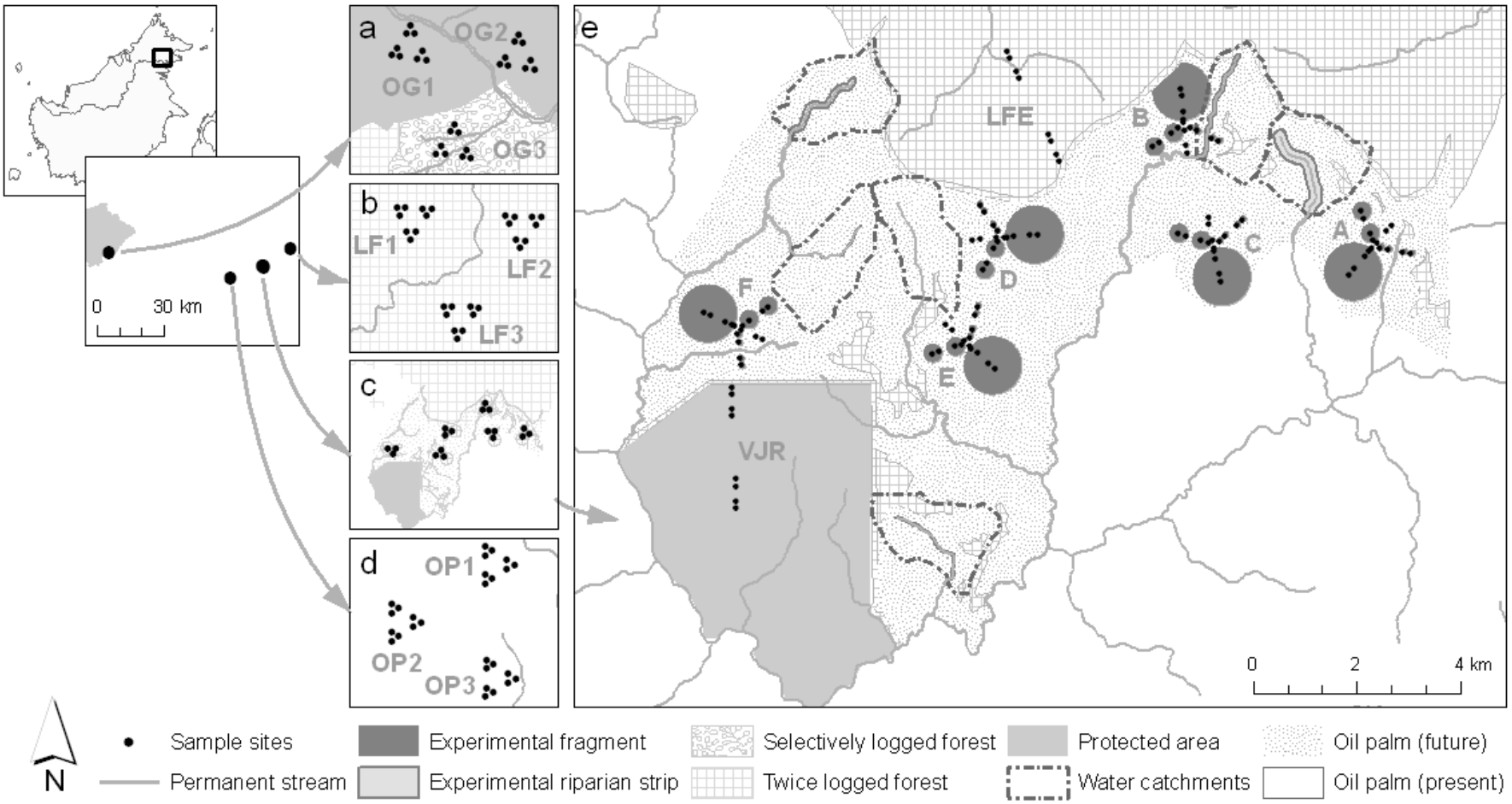

Figure 3.2: Map of the SAFE Project in Sabah, Malaysia. (a) Old growth sites (b) Twice-logged forest (c) Twice-logged forest and location of blocks A-F (due to be fragmented in December 2011) (d) Oil palm plantation sites. Second order sampling points (□) at OG2, OG3, OP1 and OP3 were used, as well as blocks C, D and E (Ewers *et al.* In press).

The design involves nested triangles of survey points at different spatial scales. The first order points are the vertices of equilateral triangles, with sides of 56m. The first order points are then arranged so that the centre of the first order triangles forms the vertices and points of the second order equilateral triangles, with sides of 178m. The second order points are then nested within the third and fourth order fractals with sides of 564m and 1780m respectively (Figure 3.2) (Ewers *et al.* In press). The experimental design of the SAFE Project consists of six replicate blocks (A-F) to create fragments of various sizes (due to be logged and fragmented in December 2011). Each block will contain four plots of 4 x 1ha fragments, 2 x 10ha fragments, 1 x 100ha fragment and in pre-fragmentation continuous forest that will be converted to oil palm (matrix plot) (Figure 3.2). The survey points of each fragment will be monitored before and after logging to see how forest modification alters the structure and functioning of tropical ecosystems (Ewers *et al.* In press.).

Eighty-four of the SAFE Project second order sampling points were used within this survey. Eighteen Old Growth (OG2-3), eighteen Oil Palm (OP1, OP3), sixteen Block C, sixteen Block D, sixteen Block E second order points were surveyed within the SAFE Project experimental area. Blocks C-E will become fragments C-E once logging commences in 2011. Blocks C-E were chosen to provide a variation in forest cover and quality (Table 3.1). In addition to the SAFE Project sampling points, the oil palm villages for the oil palm workers were sampled. A total of two housing estates were sampled, both in the Benta Wawasan oil plantation sites (Sabah, Malaysia). These were located midway between the oil palm sites OP2 and OP3 (Figure 3.2), which provided twenty-two sampling points. Eight ovitraps were placed 30m apart in the first estate (N4.64397, E117.44671), and 14 traps were placed in the larger estate located 1km away from the first housing estate (N4.63529, E117.44426).

Table 3.1**:** The land use intensity gradient for each site. Sites are ordered from least to most disturbed (Ewers *et al.* In press).

Block	Habitat	Logging	Forest Cover*	Forest Quality**
OG2	Forest	Never	100.00%	4.88 (4-5)
OG3	Forest	Low intensity	100.00%	4.22 (3-5)
D	Forest	Continuous	35.00%	2.06 (1-3)
E	Forest	Continuous	21.00%	1.94 (1-4)
C	Forest	Continuous	16.00%	2.06 (1-4)
OP1	Oil Palm	Cleared	15.00%	N/a
OP3	Oil Palm	Cleared	15.00%	N/a
H	Housing estate	Cleared	N/a	N/a

*Forest cover: the average amount of forest cover in a 3km radius around the 2nd order points

**Forest quality: average forest quality at each 2nd order point. Forest quality ranges from 1-5. (1) very poor quality, no trees, open canopy with ginger/vines or low scrub, (2) poor, open canopy, occasional small tree over ginger/vine layer, (3) OK, small trees fairly abundant , canopy partially closed, (4) good, Lots of trees, canopy closed, some large trees, (5) Very good, closed canopy with large trees and no evidence of logging

3.2. Methodology

3.2.1. Trapping technique

Mosquitoes were caught at the sampling sites by a modified "CDC ovitrap". The modified ovitrap consisted of a black plastic container (1000ml) with an overflow hole 15mm from the top of the trap and a thin paddle of wood (3cm x 20cm) as an oviposition substrate. The modified ovitraps were much larger than the traditional CDC ovitrap, to attract a higher diversity of mosquitoes. The containers were placed at the second order points, under the SAFE Project leaf litter traps. This was to reduce the number of leaves falling into the traps. The ovitraps could not be placed under leaf litter traps in the oil palm villages. They were placed 1m high and nailed to the side of the houses, to stop children and dogs tampering with them. Containers were filled with 500ml water (from a flowing stream), and water was passed through a fine sieve to remove any tadpoles or invertebrates. Fish food pellets (2g) were added to each container as a mosquito attractant. The oviposition substrates were labelled on its posterior with the tub location, using a permanent marker. The GPS co-ordinates of each site were collected from using a hand-held GPS reader (Garmin) within an accuracy of 8 metres.

After one week, the ovitraps were collected. The oviposition substrates were carefully removed from the containers and placed in individual plastic bags. Soil temperature (T-shaped waterproof thermometer, ETI), water depth, number of leaves in traps and water surface temperature (Infrared-thermometer, TFI) were recorded at each site. Soil temperature and water surface temperature readings were taken three times, and then averaged. Shade was recorded in the morning as; none, partial or full at each site, and was based on the canopy cover above.

Samples were also taken from potential mosquito habitats within each area, such as tree holes, ground pools or water butts. These samples were collected by using a dipper (200ml), or a pipette (usually for tree-holes). Soil temperature, water surface temperature, shade and water depth readings were also taken for these habitats. The dimensions of the mosquito habitat (width and length) were taken to work out the water volume. These mosquitoes were transported back to the laboratory the same way as the larvae from the ovitraps.

Excess water was carefully checked for mosquitoes, and then removed. The remaining water (100-300ml) was then carried back to the laboratory in plastic bags placed in the used ovitrap (to give plenty of air and to minimise shock). Ovitraps were always collected in the morning (7am-12pm) to reduce heat shock. The oviposition substrates were placed within a freezer to preserve and prevent further hatching.

3.2.2. Larval rearing and identification of adult mosquitoes

Water collected from the field was transferred to clear plastic containers (500ml), including extra fish food pellets. Total larval number and larval instar were recorded within 24 hours of ovitrap collection. Mosquito nets were placed over the top of the plastic tub to prevent any adult mosquitoes escaping. Water depths were kept at a minimum of 6cm, and were topped up by using sieved stream water.

Once mosquitoes reached the pupal stage, they were removed from the plastic tub and placed in a plastic tube (30ml) using a pipette. The plastic tube contained a small volume of water (approximately 7mm in depth) and was labelled with the trap location, date and a unique mosquito number. The tube was covered using cotton wool to prevent the mosquito escaping but allowing respiration.

Once the adult emerged (1-3 days), the water from the plastic tube was drained into the cotton wool and the tube placed in a freezer (-15ºC) for approximately 10 minutes. The adult mosquito was then transferred to a labelled Eppendorf tube (2ml) with a hole in the top, and left to dry in a bag of silica gel for 2-4 weeks until the mosquito could be identified. Ideally ethyl-acetate is used to kill the adult mosquito, but this was not available for the project. The genus was identified using a dissection microscope and dichotomous key (White, Harbach & Sandlant 2004). The mosquitoes were then sorted into morpho-groups based on the visible characteristics in the key (e.g. femora and tibiae spotted and ringed with pale scales). Some characteristics were not visible using the dissection microscope and these were to be later identified in the Natural History Museum. The morpho-groups were named by using the abbreviation of the genus (e.g. *Culex=Cx*) and then adding a number to sort it into a group (e.g. *Culex* group 1= *Cx1*).

3.2.3. Identification of mosquito larvae

There were rare occasions when mosquitoes failed to reach adults, because they were unable to emerge from their pupal case completely. There were also rare occasions when only male mosquitoes were collected from a trap. Larval identifications increased the chances of identifying these mosquitoes and could confirm the genus of adult mosquitoes. The larvae had to be removed at 4^{th} instar stage, before they pupated, and placed in hot water (60ºC) for a few minutes to kill them quickly. They were then placed in 70% denatured ethyl alcohol. The larvae were identified to genus using a key (White, Harbach & Sandlant 2004). Larvae were not sorted into morpho-groups because the key only identified larvae to genus. Five larvae from each trap were also identified, even when the adults had hatched to confirm that the right genus had been identified. Larvae were not identified if samples had less than five mosquito larvae.

3.2.4. Oviposition substrate and egg counts

The eggs were counted on the oviposition substrate by using a dissection microscope. The egg type and description were recorded for all of the oviposition substrates. Eggs were recorded as hatched or whole to calculate the percentage of eggs hatched. Photographs were taken of the different egg types (Appendix, Section 9.3)

3.3. Analyses

All analyses were carried out using R version 2.9.0 (R Development Core Team 2009). In addition, the R package 'Vegan' was used for ordination (Detrended correspondence analysis).

3.3.1. Diversity and composition of mosquitoes across different habitat types

The community composition of mosquitoes was analysed by using the function 'decorana' in the R package 'Vegan'. Decorana performs detrended correspondence analyses (DCA) in R. DCA is a multivariate ordination method for analysing biological communities. The DCA finds the main gradients producing variation in a species data matrix. DCA is an improved version of correspondence analysis (CA) because there is an option to down-weight rare species, the axes are rescaled to equal variances of species scores and the axes are detrended to avoid single long gradients (Oksanen 2011).

A DCA was performed for larvae and female mosquitoes first. Male mosquitoes were not included because there was not an adequate male mosquito key for Malaysian mosquitoes and male mosquitoes cannot be easily identified to genus. Mosquitoes from tree-holes were included in the first DCA, but this indicated that the tree-holes (morpho-group *Ae9*) had a very different species composition to the other. The tree-hole outliers distorted the ordination diagram. As the tree-hole sites were not a main collection site, they were excluded from further analyses. Species found in the water butts near the housing area were still used in the female and larval mosquito analysis (morpho-groups *Ae1 & Ae10*). An ANCOVA was then used to test the first axis scores against the environmental variables (land use area (OG, SF, OP, H), shade, number of leaves, altitude and the number of days the traps were left out.

A DCA was also produced for only female mosquitoes, using the same method. The DCA indicated that the mosquitoes found in the housing area (including those found in the water butts) had a very different assemblage structure to the other sites. The mosquitoes from H were then removed so that the DCA could continue. All first axis scores against environmental data were tested for normal distribution first. The models were plotted to see how well the model fitted the data.

3.3.2. Effect of environmental variables on mosquito abundance & lifecycle

The larval and egg counts were used to test the mosquito abundance against environmental variables, including land use area. Interactions were tested between the environmental variables before fitting a model. A generalised linear model (GLM), with quasipoisson errors, was used to test larval and egg abundance. A GLM with poisson errors showed that the data was over-dispersed.

The percentage of eggs hatching and sex ratio were analysed by using a GLM with quasibinomial errors. The environmental variables were added to the models to see if these had an influence on the percentage of eggs hatched. A GLM with binomial errors showed that the data was over-dispersed. All models were plotted to see how well the model fitted the data.

3.3.3. Analysis of the medically important mosquitoes

The genera containing medically important mosquitoes (*Culex, Aedes* & *Anopheles*) were analysed. This was done by analysing the adult counts of the genera separately against land use area. A GLM was used with quasipoisson errors. The model was plotted to see how well the model fitted the data.

4. Results

A total of 847 mosquitoes were captured and reared through to adults over the eight-week trapping period. They consisted of 446 females and 452 males. The female mosquitoes represented 16 morpho-groups and 6 genera (Table 4.1). A total of 159 larvae were identified and represented 4 morpho-groups from 4 genera (Table 4.2).

Table 4.1: Morpho-groups of the reared and identified female mosquitoes. A description of each morpho-group and a corresponding photograph is given in Appendix (Section 9.2)

Morpho-group	Genus*	Areas found	Container type	No. individuals
Cx1	*Culex*	OG, C, D, E	Modified CDC trap	180
Ae1	*Aedes*	H	Modified CDC trap, water butts	47
Ae2	*Aedes*	OG, C, E	Modified CDC trap	17
Ae3	*Aedes*	OP	Modified CDC trap	9
Ae4	*Aedes*	OP	Modified CDC trap	7
Ae5	*Aedes*	C, D, E	Modified CDC trap	65
Ae6	*Aedes*	OG	Modified CDC trap	1
Ae7	*Aedes*	OG	Modified CDC trap	48
Ae8	*Aedes*	OG	Modified CDC trap	9
Ae9	*Aedes*	C, D	Treeholes	9
Ae10	*Aedes*	H	Water butts	7
He1	*Heizmannia*	OG	Modified CDC trap	1
Ze1	*Zeugnomyia*	D	Modified CDC trap	1
An1	*Anopheles*	OG, D, OP	Modified CDC trap	16
An2	*Anopheles*	D, OP	Modified CDC trap	6
Ar1	*Armigeres*	OG, C, D, E	Modified CDC trap	23
Totals				446

*Predicted Genus. Genus could not be confirmed by a mosquito expert during the project timeframe

Table 4.2: Genera of identified mosquito larvae. A description of each morpho-group is given in Appendix (Section 9.2)

Morpho-group	Genus*	Areas found	Container type	No. individuals
Cx	*Culex*	OG, C, D, E, OP	Modified CDC trap	46
AeCo	*Aedes* Complex**	OG, C, D, E, OP, H	Modified CDC trap, water butts, treeholes	96
An	*Anopheles*	C, D, OP	Modified CDC trap	8
Ar	*Armigeres*	C, D, E, OG	Modified CDC trap	9
Totals				159

*Predicted Genus. Genus could not be confirmed by a mosquito expert during the project timeframe
**Mosquitoes in the *Aedes* Complex couldn't be identified to genus as larvae of *Aedes, Ayurakitia, Ochlerotatus* and *Verrallina* are extremely difficult to identify to genus (Rattanarithikul *et al.* 2005a). For a full description of morpho-groups, see Appendix (Section 9.2).

4.1. Diversity and composition of mosquitoes across different habitat types

Figure 4.1a shows the ordination of female and larval abundance on DCA axes 1 and 2, including mosquitoes from water butts. The eigenvalues of the first 4 axes were 0.79, 0.63, 0.54 and 0.41 respectively, showing that the first two axes were by far the most important. Blocks C-E were combined for this analysis and described as secondary forest sites (SF) as they had the same land use type. The DCA illustrated that OG, H and the combination of OP and SF (blocks C-E) were separated along the first axis (Figure 4.1a). Blocks C-E and OP were not separated along the first axis, but were separated along the second axis with the exceptions of a few outliers (Figure 4.1a). Blocks C-E and OG were very varied along the first and second axis, whereas H and OP were not (Figure 4.1a). The DCA also showed that the mosquitoes in ovitraps in area H had a similar assemblage to those in the water butts (area H). Figure 4.1b shows the ordination of female and larval abundance on DCA axes 1 and 2 without the mosquitoes from water butts. The eigenvalues of the first 4 axes were 0.7, 0.63, 0.44 and 0.5 respectively. The results were very similar to those in Figure 4.1a.

A DCA of only female mosquitoes showed that in H were very different to the other forest types, which resulted in DCA1=1 for H (Figure 4.1d). The eigenvalues of the first axes were 1, 0.85, 0.71 and 0.58 respectively. Due to the high variation and the mosquitoes in site H being vastly different to the other sites, this had to be removed to allow the DCA to continue. Once the female mosquitoes in site H had been removed, it formed a very pattern of variation by using DCA (Figure 4.1e). Figure 4.1e showed that all sites are separated on the first axis, with the exception of one OP outlier (-2.63,-1.49). The eigenvalues of DCA axes 1-4 were 0.85, 0.71, 0.64 and 0.54.

The DCA comparing the female mosquitoes and larvae in blocks C, D and E showed variation between blocks (Figure 4.1c). The eigenvalues of the first 4 axes were 0.67, 0.49, 0.44 and 0.4 respectively. There appears to be a slight separation in blocks along the first axis, especially blocks C and E, but there were many similarities in species composition within the blocks (Figure 4.1c). Similar results were seen in the DCA with only female mosquitoes (Figure 4.1f). The eigenvalues for axes 1-4 were 0.84, 0.72, 0.48 and 0.48. Block D appeared to have a wide range over the first axis, and block C had a wide range over the second axis (Figure 4.1f).

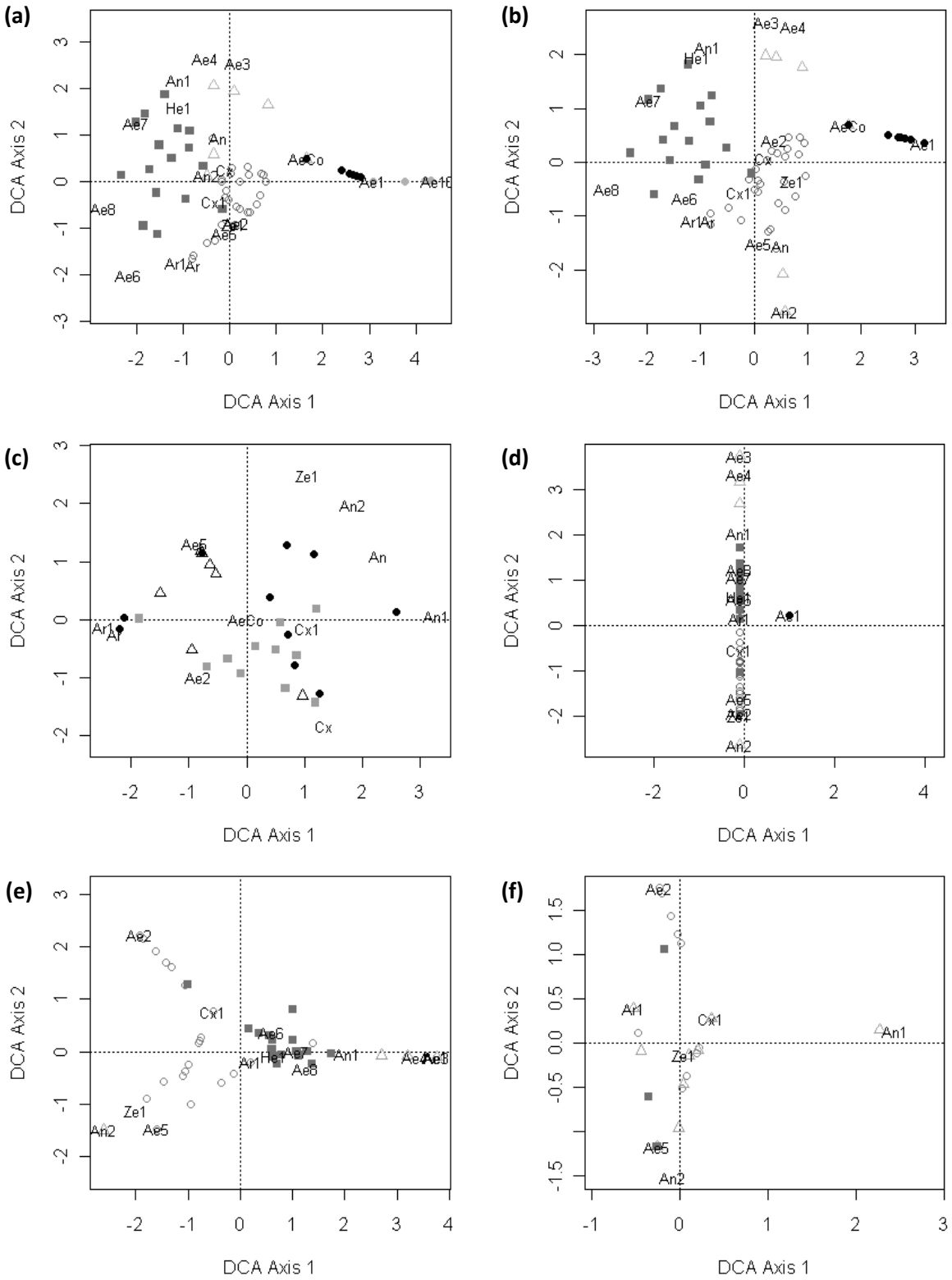

Figure 4.1: Two-dimensional Detrended correspondance analysis (DCA) plot showing the major axes of variation for (a) present female mosquitoes and larvae including water butts from area H. Each point is coded to represent the habitat type (■= old growth, ○= Secondary forest (SF) (blocks C-E), △= Oil palm, ●= Houses, ●= Houses with water butts (b) female mosquitoes and larvae, excluding water butts (c) female mosquitoes and larvae within blocks C, D and E (■= block C, ●= block D, △= block E) (d) female mosquitoes (■= old growth, ○= Secondary forest (SF) (blocks C-E), △= Oil palm, ●= Houses (e) female mosquitoes once area H has been removed (f) female mosquitoes within blocks C, D, and E (■= block C, ●= block D, △= block E). Rare species were downweighted. Species names correspond to those in tables 4.1 and 4.2.

All first and second axis scores were tested against environmental variables (land use area (OG, SF, OP, H), shade, number of leaves, altitude and the number of days the traps were left out). Results from the female mosquitoes and larvae showed that the land use type H significantly increased the DCA1 score (ANOVA, $F_{3,98}$=129.44, p<0.0001) (Figure 4.2a). It was also shown that oil palm increased the DCA2 score, whereas secondary forest decreased the score (ANOVA, $F_{3,98}$=6.892, p<0.0001). Results from the female mosquitoes alone showed that land use area affected the DCA1 axis score with the area SF significantly decreasing the score (ANOVA, $F_{2,99}$=16.809, p=0.001).

First and second axis scores for the different types of secondary forest (blocks C-E) were tested against the environmental variables. Results from plotting the female mosquito and larval second axis scores against environmental variables showed that there were differences in the secondary forest areas with E having a higher DCA2 score (ANOVA, $F_{2,25}$=4.32, p=0.024). Results from the female mosquitoes alone showed that each block was significantly different from each other, with blocks D and E decreasing the second axis and block C increasing the score (ANOVA, $F_{2,24}$=6.23, p=0.007) (Figure 4.2b).

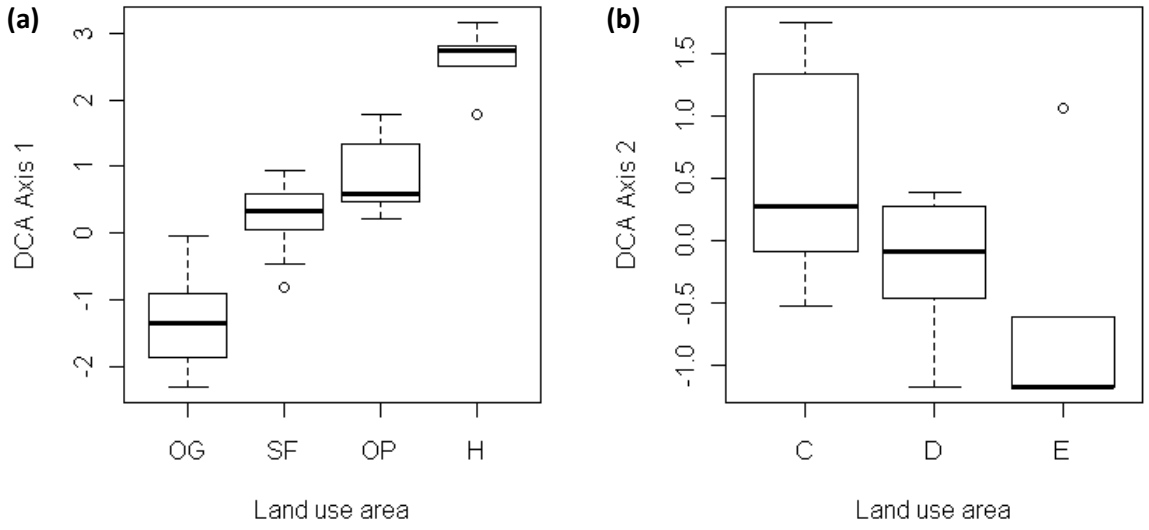

Figure 4.2: (a) First axis scores from Detrended Correspondance Analysis of female mosquito and larval data, plotted against land use area. SF includes blocks C-E (b) Second axis scores from Detrended Correspondance Analysis of female mosquitoes, plotted against blocks C, D and E.

4.2. Effect of environmental variables on mosquito abundance and lifecycle

Analyses showed that the mean water surface temperature was significantly higher in the oil palm plantation sites and housing areas (ANOVA, $F_{2,99}$=122.11, p<0.0001), this was also seen in mean soil temperature (ANOVA, $F_{2,99}$= 197.96, p<0.0001). Mean water depth showed that the old growth sites had a significantly increased depth (ANOVA, $F_{3,98}$=80.39, p=0.008), whereas oil palm (ANOVA, $F_{3,98}$=80.39, p=0.037) and the housing area decreased the depth (ANOVA, $F_{3,98}$=80.39, p<0.0001) (Table 4.3).

Table 4.3: Means of the environmental variables based on land use area

Land use Area	Mean water surface temperature (°C) ±SE	Mean soil temperature (°C) ±SE	Mean water depth (cm)
OG	24.51±0.12	24.23±0.09	6.14±0.12
C	23.65±0.19	24.28±0.1	5.66±0.09
D	24.26±0.42	24.68±0.2	5.63±0.18
E	24.29±0.25	24.58±0.14	5.64±0.12
SF	24.07±0.18	24.51±0.09	5.64±0.08
OP	26.44±0.39	26.29±0.2	5.24±0.21
H	29.35±0.35	27.46±0.12	3.03±0.22

Interactions showed that water surface temperature was strongly correlated with soil temperature (linear model, r=0.85 F_1=562.1, p<0.0001). Water surface temperature was strongly correlated with depth (linear model, r=0.74, F_1=285.9, p<0.0001). There was a significant relationship between shade and water surface temperature (ANOVA, $F_{2,99}$=16.04, p<0.0001). All interactions are illustrated in Figure 4.3 (a-c). Due to the interaction, depth, soil temperature and water surface temperature were removed. Midges appeared to have a significant influence on total number of mosquito larvae, but as the number of midges present was extremely low this could not be used in the model (Figure 4.3d). The explanatory variables used in each of the following models include land use area, shade, days, altitude and number of leaves.

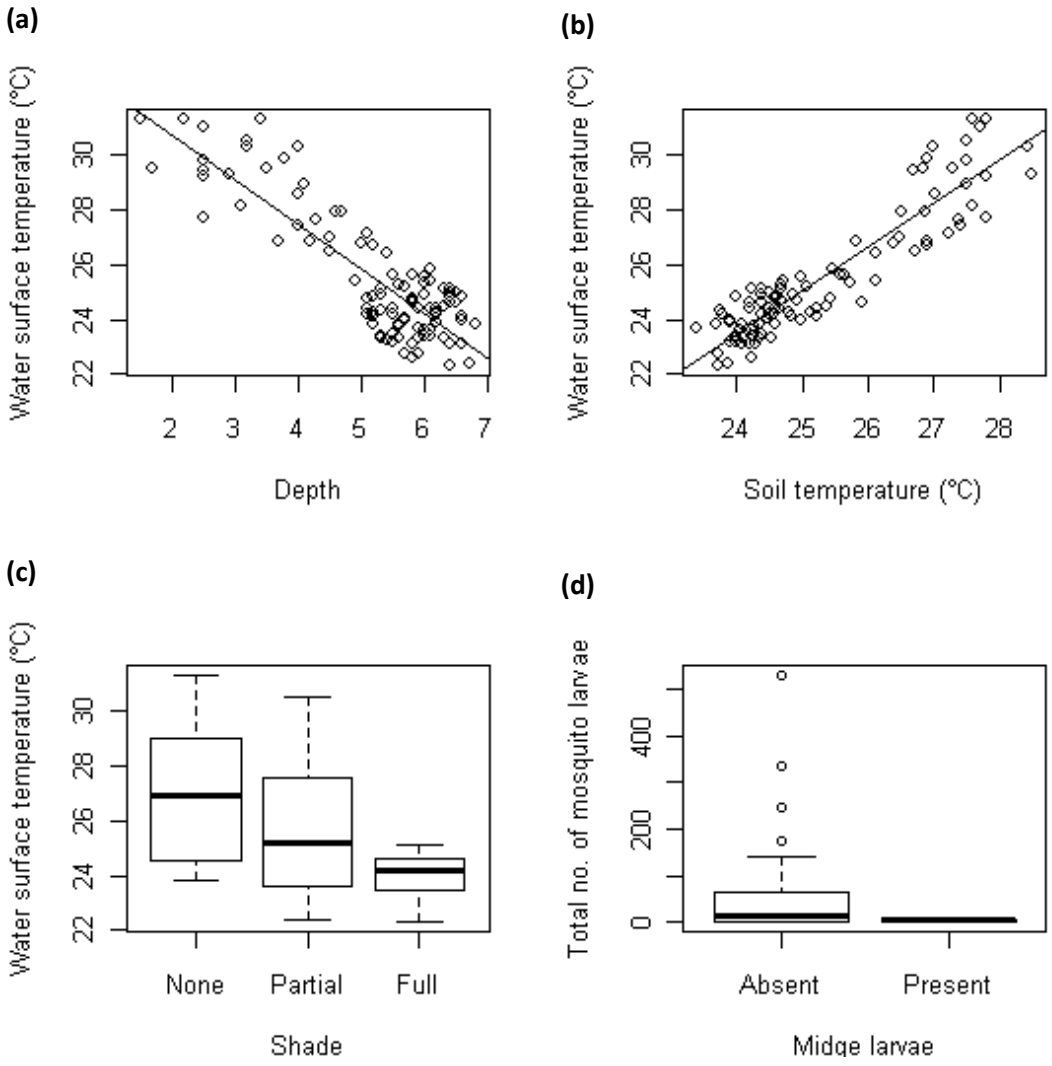

Figure 4.3: (a) Interaction between water surface temperature and water depth (b) Interaction between water surface temperature and soil temperature (c) Interaction between water surface temperature and shade (d) Midge larvae against number of mosquito larvae

4.2.1. Larval and egg abundance in comparison to environmental variables

There were a total of 4212 larvae and 3155 counted from 106 modified ovitraps, with a high abundance in the OG area. Mosquito larvae counted ranged from first instar to pupae. For a full list of larval counts, site location and environmental variables see Appendix (Section 9.1).

Shade was used over water surface temperature because it was less variable and was based on the canopy cover. Time of day did not significantly change the amount of shade present at each site (ANOVA, $F_{2,99}$=1.03, p=0.36). Area C and D were combined in the larval abundance model because they were not significantly different ($F_{1,94}$=0.005, p=0.947), and Area C, D and E were combined in the egg abundance model for the same reason ($F_{2,93}$=0.055, p=0.947). Table 4.4 shows the results from the minimum adequate model. Figure 4.4 shows total larval abundance and total egg abundance plotted against different land use areas, shade and if leaves were present or not. Leaves present or not showed a better graphical representation than number of leaves in traps (mainly zeros or ones).

Table 4.4: Results for larval and egg abundance from generalised linear model with quasipoisson errors. *** indicates p<0.001, ** p<0.01, * p<0.05 and n.s. indicates not significant. The intercept is the intercept for areas C,D and full shade for larval abundance and C, D, E and full shade for egg abundance. The interaction between shade and leaves was only significant for larval abundance, so was excluded from the egg abundance minimum adequate model

Coefficients:	Larval Abundance					Egg Abundance				
	Estimate	Std. Error	t_{94}	P		Estimate	Std. Error	t_{96}	P	
(Intercept)	3.155	0.297	10.614	<0.0001	***	2.471	0.32	7.731	<0.0001	***
Area E	-1.304	0.413	-3.16	0.002	**	n/a	n/a	n/a	n/a	
Area H	-2.759	0.794	-3.477	0.0007	***	0.739	0.27	2.733	0.007	**
Area OG	0.8327	0.293	2.846	0.005	**	1.758	0.332	5.288	<0.0001	***
Area OP	-2.188	0.743	-2.945	0.004	**	-2.032	0.674	-3.02	0.003	**
No. of leaves	0.849	0.141	6.017	<0.0001	***	0.849	0.141	6.017	<0.0001	***
Partial shade	1.153	0.363	3.18	0.002	**	0.526	0.34	1.548	n.s	
No shade	0.869	0.601	1.446	n.s.		1.118	0.419	2.669	0.009	**
Partial shade:Leaves	-0.936	0.435	-2.152	0.034	*	n/a	n/a	n/a	n/a	
No shade:Leaves	-0.448	1.308	-0.343	n.s.		n/a	n/a	n/a	n/a	
Altitude	-	-	-	n.s.		-	-	-	n.s.	
Days	-	-	-	n.s.		-	-	-	n.s.	

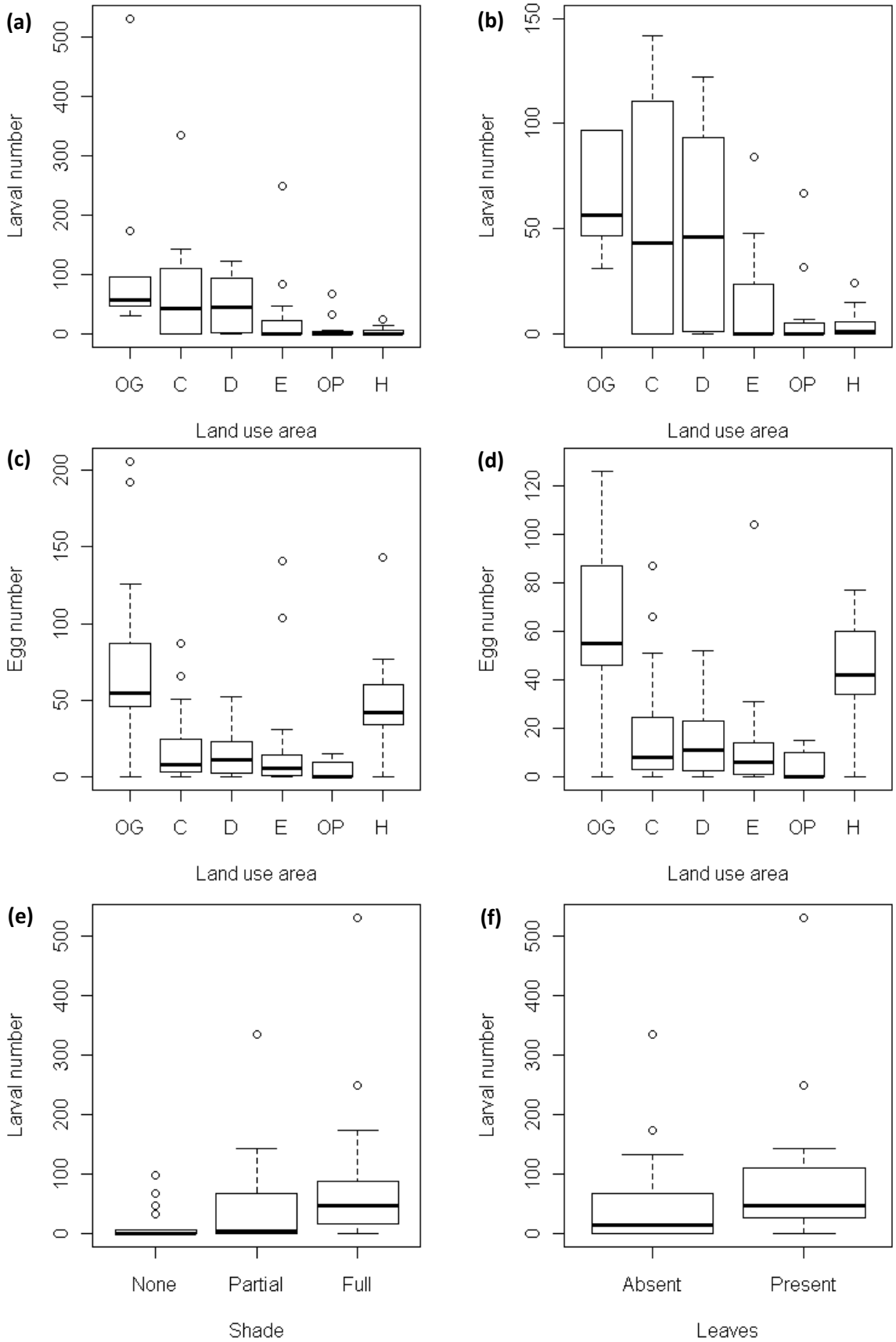

Figure 4.4: (a) Larval number (per site) plotted against land use area, with outliers (b) without outliers (c) Egg number (per site) plotted against land use area, with outliers (d) without outliers (e) Larval number (per site) plotted against shade. Egg number showed a similar result (f) Larval number (per site) plotted against leaves. Present number of leaves ranges from 1 to 4

4.2.2. Percentage of eggs hatched

The egg numbers were recorded as hatched (total of 1401) or whole (total of 1754) to work out the percentage of hatched eggs. The environmental variables used were the same as the previous section (4.2.1). Area C, D and OP were combined because they were not significantly different (ANOVA, $F_{2,71}$= 0.943, p=0.335). Table 4.5 shows the results from the minimum adequate model. Figure 4.5 shows percentage of eggs hatched plotted against area. Photographs of two different egg types are in the Appendix (Section 9.3).

Table 4.5: Results for percentage of eggs hatched from generalised linear model with quasipoisson errors. *** indicates p<0.001, ** p<0.01, * p<0.05 and n.s. indicates not significant. The intercept is the intercept for areas C,D and OP.

Coefficients:				
	Estimate	Std. Error	t_{76}	P
(Intercept)	1.027	0.293	3.504	0.0007 ***
Area E	-1.677	0.474	-3.534	0.0007 ***
Area H	-3.342	0.482	-6.927	<0.0001***
Area OG	-0.743	0.346	-2.147	0.035 *
No. of leaves	-	-	-	n.s.
Shade	-	-	-	n.s.
Altitude	-	-	-	n.s.
Days	-	-	-	n.s.

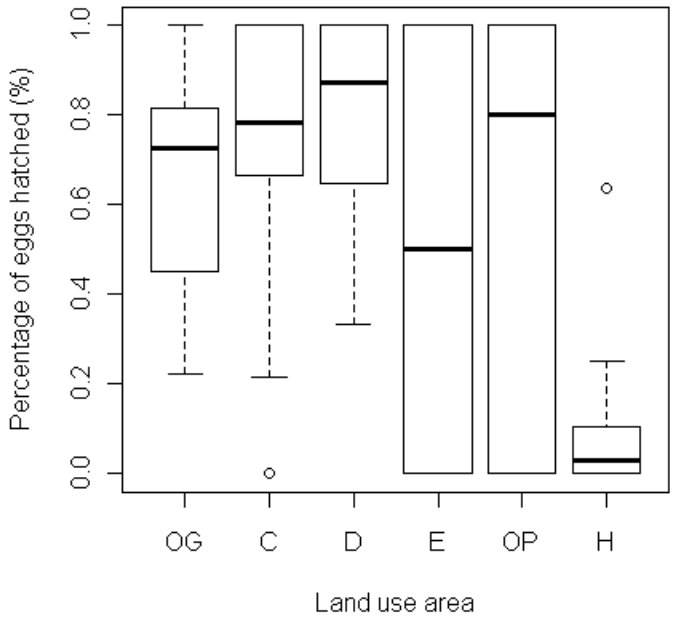

Figure 4.5: Percentage of eggs hatched against area

4.2.3. Sex ratio

There were a total of 413 females and 425 males collected from the ovitraps. The GLM showed that there was not a significant difference in sex ratio between the different land use areas (Table 4.6)

Table 4.6: Results for sex ratio from generalised linear model with quasipoisson errors. n.s. indicates not significant. The intercept is the intercept for Area C

Coefficients:				
	Estimate	Std. Error	t_{76}	P
(Intercept)	-0.011	0.162	3.504	0.941 (n.s)
Area D	0.23	0.227	-3.534	0.316 (n.s)
Area E	-0.065	0.262	-0	0.805 (n.s)
Area H	0.081	0.319	0.24	0.801 (n.s)
Area OG	0.12	0.21	0.522	0.604 (n.s)
Area OP	0.146	0.352	0.413	0.681 (n.s)

4.3. Analysis of the medically important mosquitoes

The only mosquito that could be identified as a potential disease-transmitting mosquito was *Ae1*. This morpho-group was only present in the housing area (H), and the identification key suggested that this mosquito was *Ae. albopictus*. The mean abundance of *Ae1* larvae in the housing tubs was 4.44±1.58 (n=16). The identification key used also suggested that *Ae10* was *Ae. aegypti*. A mosquito taxonomist expert, within the project timeframe, could not confirm these identifications. Table 4.7 summarises the medically important genera found within the different land use areas.

Table 4.7: Morpho-groups and medically important genera found within different land use areas

Land use Area	Morpho-groups present	Medically important genera present
OG	*Cx1, Ae2, Ae6, Ae7, Ae8, He1, Ar1, An1*	*Culex, Aedes, Anopheles*
C	*Cx1, Ae2, Ae5, Ar1*	*Culex, Aedes,*
D	*Cx1, Ae5, Ze1 Ar1, An1, An2*	*Culex, Aedes, Anopheles*
E	*Cx1, Ae2, Ae5, Ar1*	*Culex, Aedes,*
SF*	*Cx1, Ae2, Ae5, Ze1, Ar1, An1, An2*	*Culex, Aedes, Anopheles*
OP	*Ae3, Ae4, An1, An2*	*Aedes, Anopheles*
H	*Ae1*	*Aedes*

*SF: secondary forest, blocks C, D and E combined

Aedes was the only genus found in all land use areas. The OG area had a significantly higher number of *Aedes* than the other land use areas (Generalised linear model with quasipoisson

errors, t_3=2.894, p=0.005). *Culex* mosquitoes were not found within areas H and OP. There was no significant difference between the number of *Culex* mosquitoes found in SF and OG (Generalised linear model with quasipoisson errors, t_1=1.191, p=0.24) (Figure 4.6). *Anopheles* was not analysed because only a total of 7 sites had this genus (only 1 site in SF).

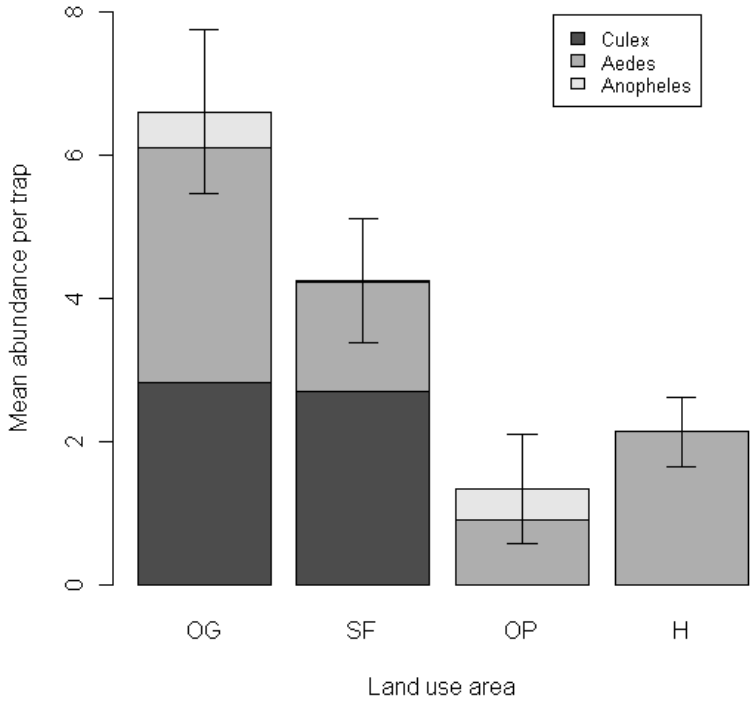

Figure 4.6: Mean abundance of mosquitoes per trap, separated by genus. Error bars show ± 1SE of the mean abundance across all genera

5. Discussion

Oil palm is increasing in South-east Asia, but there are few studies focusing on the mosquito populations present in different land use areas. This project studied how different land use areas affect mosquito populations and the proliferation of diseases. Mosquitoes have been suggested as bio-indicators of the level of forest degradation. This was suggested in South-eastern Brazil, with the four indicators *Kerteszia, Aedes scapularis,* the Mansoniini and *Haemagogus* (Dorvillé 1996). Perhaps mosquitoes could be used as bio-indicators of forest degradation in South-east Asia.

5.1. Diversity and composition of mosquitoes across different habitat types

The morpho-groups varied significantly across the different land use areas shown in the DCA (Figure 4.1, 4.2). This was shown for female mosquitoes and larvae and female mosquitoes alone. The mosquito composition in the housing area was vastly different to the other sites. This suggests that there is a different diversity and composition of mosquitoes across different land use area types. These results are consistent to those seen in published papers. Johnson, Gómez & Pinedo-Vasquez (2008) showed that different land use categories in Peru (urban, peri-urban and rural) resulted in significantly different mosquito diversity. This was also seen in the diversity of mosquitoes in Africa when villages were compared to irrigated and non-irrigated agroecosystems (Muturi *et al.* 2006). The different land use types had different effects on the mosquito diversity and abundance.

The differences in mosquito diversity and composition may be related to the number of breeding sites within each land use area. Old growth and secondary forest sites were cooler, had an increased number of tree-holes and more ground pools than the oil palm plantation sites (Table 4.3). An observation made in the oil palm plantation site was that there were few natural breeding sites left. There were differences in the diversity of mosquitoes in the secondary forest sites (blocks C, D and E). This may be based on which breeding sites are found in these forests. Forest cover ranged from 16% in block C to 35% in block D. Block C may have more breeding sites due to the forest being more open, and thus forming more puddles. Further work could look into the differences in secondary forest blocks, based on the same factors used in this project.

A suitable bio-indicator could not be found in this study, because mosquitoes could not be identified to species. Perhaps mosquitoes in the genus *Armigeres* (morpho-group *Ar1*) could be a potential bio-indicator of forest quality. *Ar1* was most abundant in the old growth area (forest quality= 4.22-4.88, forest cover=100%), fairly abundant in block D (forest quality=2.06, forest cover=35%) but least abundant in blocks C (forest quality=2.06, forest cover= 16%) and E (forest quality=1.94, forest cover= 21%). Further work would have to be done to look into whether the genus *Armigeres* is a good forest quality indicator by continuing to collect mosquitoes in Sabah and comparing to forest quality.

The DCA showed that *Ae1* and *Ae10* were strongly associated with the housing area. *Ae1* was identified as *Ae. albopictus*, and *Ae10* as *Ae. aegypti*. As *Ae1* was the only mosquito species present in this area, and was not found in any other area, it would imply that in the housing area mosquito diversity decreases but the percentage of medically important species increases. The old growth and secondary forest areas contained genera that were not of medical importance (E.g. *Zeugnomyia*). This suggests a higher diversity of mosquitoes, but a lower percentage of medically important species.

Chang *et al.* (1997) showed that species composition shifted during forest clearance resulting in a higher abundance of dengue vectors. There are few studies focussing on the impact of oil palm plantations on mosquito diversity and composition. As oil palm plantations are increasing in South-east Asia, it is important to continue monitoring how land use change is affecting mosquito composition. Identifying the mosquitoes within different land use areas would not only provide data on how the community composition and abundance changes, it would also identify the medically important species. Knowing the species present could help implement control strategies, for example, identifying that *Ae. aegypti* is present would mean targeting water storage containers and other artificial containers.

5.2. Effect of environmental variables on mosquito abundance and lifecycle

5.2.1. Larval and egg abundance in comparison to environmental variables

Mosquito larval and egg abundance varied across different land use types (Figure 4.4). The larval abundance was highest in old growth sites, followed by the secondary forest blocks C and D. Secondary forest block E, oil palm plantation sites and housing areas showed a much lower abundance of mosquito larvae. Similar results were seen when Chang *et al.* (1997) sampled mosquitoes (*Anopheles*) from the forest before development, forest clearing, burning

and cultivation to maintenance and found an 82% reduction in the number of adult mosquitoes caught. Egg abundance showed similar results to larval abundance, apart from a high abundance of eggs in the housing area. The total egg number only identified eggs of *Aedes/Ochlerotatus*, as egg rafts and *Anopheles* eggs were difficult to sample. It is likely that the eggs were from the genus *Aedes*, based on the morpho-groups identified in Table 4.1 and 4.2.

This study showed that the number of leaves and amount of shade in traps affected how many larvae and eggs were present in each ovitrap (Table 4.4). There were more eggs and larvae found in ovitraps with a higher number of leaves (Figure 4.4). Leaf litter in mosquito habitats provides essential organic carbon for growth of mosquito larvae (Strand *et al.* 1999). It has been shown, in previous experiments, that there is no difference between mosquito productivity in tree holes with leaf litter and those without, indicating there are other factors that influence mosquito growth (Walker *et al.* 1991).

The elevated level of nitrogen and protein dissolved from leaf litter increases microbial growth, which has a higher nutritional value than leaf letter alone (Lounibos, Nishimura & Escher 1993; Strand *et al.* 1999). Many mosquito larvae feed upon leaf litter colonised by fungi and bacteria. Experiments have shown leaf litter is essential for the development of the tree-hole mosquito, *Aedes triseriatus*, and perhaps other *Aedes* mosquitoes (Carpenter 1982; Fish & Carpenter 1982; Reiskind, Greene & Lounibos 2009). Despite the leaf litter being controlled by the leaf litter traps, leaves still landed in the ovitraps. This may have helped increase egg and larval abundance in the forest, but it is possible that a combination of factors, such as shade and land use type, affected abundance. There was also an interaction between partial shade and the number of leaves (Table 4.4). This may be because the areas with partial shade were the secondary forest blocks (C-E), so there were probably a higher number of leaves in the ovitraps.

Full and partial shade increased the number of larvae found within the ovitraps (Figure 4.4). Studies have shown that, if shade is completely removed from mosquito breeding sites, it can reduce mosquito population numbers (Vythilingam *et al.* 2005). In an experiment measuring *Aedes aegypti* abundance in discarded tyres, the shaded tyres contained three times as many larvae as sunlit tyres (Beier *et al.* 1983). Gingrich *et al.* (2006) showed increased mosquito abundance in ponds with higher shade.

The oviposition preference theory predicts that a gravid female will oviposit in a breeding site that will maximise the offspring fitness (Jaenike 1978). This suggests that adult mosquitoes will oviposit in sites with high shade, high level of detritus and a low level of competition. Evidence for this theory ranges from excellent to poor (Liu, Scheirs & Heckel 2010). This theory may suggest why there was a higher abundance of eggs in ovitraps with high shade and leaves.

Due to the correlation between shade, water temperature and soil temperature, this study also showed that areas with no shade had extremely high temperatures (Figure 4.3) and this reduced the number of mosquito larvae present (Figure 4.4). If the water temperature rises, the larvae develop faster (Rueda *et al.* 1990; Clements 1992). However, a temperature above 34°C will generally decrease the survival rate of vectors and their parasites (Rueda *et al.* 1990).

The housing area had a very low abundance of larvae in the ovitraps, but very high egg abundance (Figure 4.4). The housing area had significantly higher water temperatures and lower depths than the other sites (Table 4.3). As water surface temperatures were only taken in the morning during this study and the depths were low within the housing area, perhaps the water temperatures reached higher than 34°C in the oil palm and the housing area. This may have reduced the number of mosquito larvae present in these sites, or prevented eggs from hatching. The high abundance of eggs suggests that mosquitoes are laying their eggs, but they are not hatching through to larvae (Section 5.2.2).

An observation made from the egg counts is that a large number of eggs were laid inside the grooves of the substrate. O'Gower (1963) showed that a rough oviposition substrate is preferred over a smooth surface (Wong *et al.* 2011). The water butts surrounding the houses had a high abundance of *Ae. albopictus* and *Ae. aegypti* so perhaps these habitats were preferred over the ovitraps.

Due to project limitations, intensive entomological monitoring could not be conducted. This could have given an insight into seasonal influence on larval and egg numbers. This study was conducted during the dry season, but the wet season may produce a higher number of breeding sites, but could cause flushing, thus killing the immature stages (Rohani *et al.* 2010). It is also unknown what affect the presence or absence of midge larvae (Chironomidae) had on the abundance of mosquito larvae. They may provide competition to mosquito larvae as both Diptera families feed on detritus (Gillett 1971; Armitage, Cranston & Pinder 1995).

Photographs showed that different egg types were observed (Appendix, Section 9.3), but these were too difficult to identify to species.

Identification of these hatched eggs could show which species breed in tree holes, and which ones are medically important across the different land use areas. The dark coloured oviposition substrates were not ideal in the study as the dark coloured eggs were harder to find. Future improvements would be to use a light coloured oviposition substrate. The water chemical properties were also unknown in each ovitrap (e.g. pH, Nitrite/Nitrate, ammonia, etc.). Water samples were taken at each site, but due to limitations these could not be tested.

5.2.2. Eggs hatching

This study showed that despite the high abundance of eggs within the housing area, only a small percentage of these eggs hatched (Figure 4.5). The old growth site, block C and block D had a relatively high number of eggs hatching in comparison. Block E and the oil palm plantation site had a very varied number of eggs hatching.

The poor hatching rate in the housing area suggests that the ovitrap was not suitable for the mosquitoes. The eggs were very spread out on the oviposition substrate, so it is likely that the mosquitoes lay their eggs but the water evaporated before they could hatch. Perhaps these eggs would hatch if they were submerged. Eggs of the genera *Aedes* and *Ochlerotatus* can withstand desiccation, which can remain dry for months and still hatch when soaked in water (Service 2004). Juliano *et al.* (2002) found that there was high egg mortality in *Ae. aegypti* and *Ae. albopictus* when temperatures were high and relative humidity was low. The water butt depth, with mosquitoes present, in the housing area ranged from 15cm-88cm. These containers had a wider diameter (~50cm) and would take longer to decrease in depth than the ovitraps. It may also be likely that the temperatures are too high for the eggs to hatch. The ovitrap had a smaller volume of water than the water butts, and would heat up much quicker.

The percentage of eggs hatching in the old growth area, block C and block D were much higher than the other sites. This is perhaps because there is a higher rainfall and a slower rate of evaporation, which increased the depth in these sites. This would result in a larger number of eggs being submerged. The percentage of eggs hatching was not affected by any environmental factors, suggesting that another factor was influencing the hatching rate. Oil palm plantation sites and block E had a very varied number of eggs hatching. It is likely that the eggs didn’t hatch in the oil palm plantation sites due to the same reasons as the housing

area, but as water surface temperature was slightly lower perhaps a higher percentage hatched. It is unknown why block E had a varied hatch rate, ranging from 0 to 100%. The mean water surface temperature and depth were very similar within the secondary blocks, so there must be another factor preventing the eggs from hatching.

5.2.3. Sex ratio

This study showed that land use area did not alter the sex ratio of mosquitoes (Table 4.6). Overall results suggest a 1:1 sex ratio, but it is difficult to work out the sex ratio as many different mosquito species were breeding within the same ovitrap. Perhaps different mosquitoes display different sex ratios. It has been observed that *Ae. aegypti* generally lays about five males to three females and there is a genetic variability among different strains (Hickey & Craig 1966). Some strains showed 50% females to 50% males, but others showed 30% females to 70% males. A significant female bias has never been reported (Lounbios & Escher 2008). The mosquitoes from the housing region suggested a ratio of 42% females to 58% males for *Ae. albopictus*. This may be because male eggs hatch one day earlier than female and male eggs are more sensitive than females to hatching stimuli (Gillett 1971; Lounibos & Escher 2008). This could mean the male eggs hatched before the water depth decreased in the housing area. The other sex ratios could not be analysed due to the different morpho-groups present and these could not be identified to species.

5.3. Analysis of the main disease transmitting mosquitoes

This study showed that there was a high abundance of the genera *Culex* (n=180), and *Aedes* (n=219). *Aedes albopictus* and *Ae. aegypti* were found breeding within the housing regions. These mosquitoes are known to spread DF, DHF and the chikungunya virus (Lam *et al.* 2001; Guzman & Istúriz 2010). Although the number of dengue cases within the housing regions could not be confirmed, it is likely that dengue could be spread. These mosquitoes were not collected in any other land use area. *Aedes albopictus* and *Ae. aegypti* are usually closely associated with humans (Chen *et al.* 2005; Guzman & Istúriz 2010), which explains why these species were not found in the old growth or secondary forest sites.

Chang *et al.* (1997) showed that land use area decreased the malaria vectors, but increased the dengue vectors. In this study, *Aedes albopictus* was not found in the ovitraps within the

oil palm plantations. This may be because there is little shade within the oil palm, increased temperatures and few oviposition sites. In this study, the housing areas were a distance of 440-1000m from the nearest oil palm plantation site. Perhaps housing areas near the oil palm provide suitable breeding habitats (e.g. large water containers) for *Aedes albopictus*, and these mosquitoes fly within a 1km range of the breeding site to the oil palm workers. Mosquitoes can fly up to 5km, but usually fly within a 1km radius (Le Menach et al. 2005).

In this study, many water butts were not covered in the first housing area, resulting in a high abundance of dengue vectors. The abundance of dengue vectors could be decreased by eliminating standing water. This is done by unclogging roof gutters, getting rid of old tyres (sometimes used as decoration in the oil palm plantation), emptying and turning unused containers upside down and covering water storage containers to prevent access of gravid females (USAID 2003). Temephos and *Bacillus thuringiensis israelensis* (Bti) may be used to treat the water, but these methods may be too expensive for the housing estates (Loke *et al.* 2010).

There was a low abundance of *Anopheles* found within this study. As *Anopheles* mosquitoes are usually found in small stagnant pools, or still freshwater (Table 2.1), it is possible that they are breeding elsewhere. A different collection technique, for example a miniature light trap or a human landing catch, may have identified a higher abundance of *Anopheles.* Rohani *et al.* (2008) used human landing catches to determine the species composition of villages in the Ranau District, Sabah. Species collected included *Ae. albopictus*, *An. balabacensis, An. donaldi, An. maculatus, An. peditaeniatus, Cx. gelidus, Cx. quinquefasciatus* and *Ma. uniformis*, which are mentioned as medically important mosquitoes in Table 2.1.

Due to project limitations, it is unknown which *Culex* species were present. The morpho-group (*Cx1*) may be made up of many different species. It is also unknown which *Aedes* species were present in the old growth and secondary forest sites, but as morpho-group *Ae1* was *Ae. albopictus* and *Ae10* was *Ae. aegypti*, it is unlikely that the other *Aedes* species were dengue vectors.

5.4. Further work

This study focussed on mosquito abundance, diversity and community composition based on the mosquitoes found in ovitraps. A larger number of SAFE Project blocks could be used to

provide more information on whether secondary forests have different community compositions (blocks A-F). Different collection methods may give different results, for example those mentioned in Section 2.4. A miniature light trap with carbon dioxide could collect mosquitoes of medical importance as they are attracted by both light and carbon dioxide. This would identify the vectors within the old growth, secondary forest and oil palm sites. Miniature light traps were the original method to be used in this study, but arrived outside of the project timeframe. These traps could be used in future studies. The mosquitoes from this study could also be identified to species to give an improved community composition.

Very few morpho-groups overlapped within the different land use areas. A further question is, how quickly does the mosquito composition change when the forest is cleared? As *Ae. albopictus* was not identified in the forested area, when does this mosquito start breeding (during or after land use changes)? Further work could improve the land use strategy so that medically important mosquitoes can be reduced.

The data collected in this study took place before fragmentation of the secondary forest. If data collection was to take place during the land use change (logging in December 2011), it could identify how quickly the mosquito diversity, composition and abundance changes in relation to humans present, number of breeding sites available and environmental variables. A longer-term study could also take the different seasons into account to determine if there is a difference between the dry and wet season.

It has also been suggested that mosquitoes could act as bio-indicators of forest quality. Further work could look into whether mosquitoes could provide information on forest quality. This study indicates there are different compositions of mosquitoes in different land use areas, but this could not be done in this project because mosquitoes were not identified to subgenus or species.

6. Conclusions

It is likely that forest modification will continue in South-east Asia, so it is important that mosquito vectors are identified and controlled to reduce disease outbreaks. Dengue fever and dengue haemorrhagic fever is increasing in South-east Asia with no current vaccine or treatment. This project also provided baseline data for the SAFE Project. This study highlighted that there was a different mosquito composition in each land use area (old growth, secondary forest sites, oil palm and housing areas). The loss of old growth and secondary forest causes a decline in mosquito diversity and abundance, but an increase in the medically important mosquitoes (*Ae. albopictus* and *Ae. aegypti*). Increased shade and leaf litter were associated with high egg and larval abundance. This study also found that few *Aedes* eggs hatched successfully in the housing area. The high abundance of dengue vectors could be reduced by simple control techniques, such as covering water storage containers.

7. Acknowledgements

I would like to thank my supervisor, Rob Ewers for all his help and support during the project. I am also grateful for the advice and help from those in the field, including Tom Fayle and all the SAFE Project research assistants. A special thanks to Ed Turner, Jon Knight and Tilly Collins for their help and support during the project. Thanks to the Natural History Museum for their advice on mosquito collection and for offering to help with the identification. For permission to conduct this research, I would like to thank SEARPP, SaBC and Maliau Basin Conservation Committee. Thanks to Glen Reynolds and Rory Walsh for their help with the permit application process, and to my Malaysian collaborator, Suzan Benedick. I am also grateful to the Sime Darby Foundation for funding the SAFE Project and to the SAFE Project for allowing me to use their sites. Finally I would like to thank all my family and friends for their support during this project.

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9. Appendices

9.1. Site Data, including environmental data

Table 9.1: Larval, egg counts and environmental variables within each site.

Area	Site	SAFE site*	Altitude	Soil Temp	Water temp	Depth	Shade	Leaves	Midges	Days	Larval no.	Eggs (hatched)	Eggs (whole)
OG	OG1	734	371	23.9	23.9	6.2	Full	1	Absent	8	87	1	0
OG	OG2	732	421	23.8	24.2	6.2	Full	2	Absent	8	52	48	18
OG	OG3	733	366	24.2	24.6	5.8	Full	0	Absent	8	80	20	29
OG	OG4	735	463	24	24.8	6.4	Full	0	Absent	8	50	0	0
OG	OG5	729	535	24.4	24.6	6.5	Full	0	Absent	8	57	41	0
OG	OG6	731	550	24.7	25.1	5.3	Partial	1	Absent	8	97	109	97
OG	OG7	736	505	24.4	24.9	6	Full	0	Absent	8	31	33	13
OG	OG8	737	517	24.5	25	6.5	Full	0	Absent	8	56	22	5
OG	OG9	730	458	24.4	25.1	6.4	Full	0	Absent	8	52	55	32
OG	OG10	728	378	23.4	23.7	5.9	Full	0	Absent	8	47	41	7
OG	OG11	723	351	23.9	24	6.6	Full	2	Absent	8	531	87	105
OG	OG12	724	359	23.7	23.8	6.8	Full	0	Absent	8	34	34	69
OG	OG13	722	454	24.4	24.2	5.1	Full	0	Absent	8	173	49	13
OG	OG14	720	528	24.2	24.4	5.9	Full	0	Absent	8	77	12	42
OG	OG15	726	445	24.5	24.4	6.2	Full	0	Absent	8	97	47	5
OG	OG16	725	323	24.7	24.7	5.8	Full	0	Absent	8	97	52	15
OG	OG17	727	302	24.6	24.8	6.6	Full	0	Absent	8	39	37	89
OG	OG18	721	352	24.5	25	6.4	Full	1	Absent	8	41	44	12
C	C1	619	335	24.93	24.67	5.8	Partial	0	Absent	8	0	0	3
C	C2	618	409	24.50	24.17	5.5	Full	1	Absent	8	14	8	0
C	C3	617	506	24.40	23.47	5.5	Full	1	Absent	8	39	18	5
C	C4	616	512	24.13	23.30	5.3	Partial	0	Absent	8	70	8	0
C	C5	612	461	24.03	23.23	5.4	Partial	0	Absent	8	133	7	2

C	C6	613	462	23.73	22.77	5.9	Partial	1	Absent	8	142	6	0
C	C7	614	455	24.30	23.13	5.8	Partial	0	Absent	8	335	11	4
C	C8	615	442	23.73	22.30	6.4	Full	1	Absent	8	47	23	3
C	C9	620	427	23.90	23.20	5.5	Full	0	Absent	8	0	0	0
C	C10	621	417	24.00	23.40	5.3	Partial	1	Absent	8	0	0	5
C	C11	622	403	24.87	24.87	5.3	Partial	0	Absent	8	91	58	29
C	C12	623	376	24.83	24.10	5.2	Partial	0	Absent	8	0	0	0
C	C13	624	466	24.70	25.23	5.6	Partial	0	Absent	8	3	3	0
C	C14	625	489	24.27	23.73	5.6	Partial	0	Absent	8	47	11	40
C	C15	626	466	24.00	23.30	6.3	Partial	1	Absent	8	130	59	7
C	C16	627	463	24.23	23.60	6.1	Partial	0	Absent	8	0	0	0
C	D1	635	499	24.27	23.40	6	Partial	0	Absent	8	92	48	4
C	D2	634	480	24.80	24.23	5.2	None	0	Absent	8	98	23	0
C	D3	633	460	24.23	23.37	6.1	Full	1	Absent	8	122	16	7
C	D4	632	465	24.10	23.83	5.6	Full	1	Absent	8	0	0	0
C	D5	628	492	24.23	22.60	5.8	Full	0	Absent	8	16	7	7
C	D6	629	407	24.10	23.13	6.6	Partial	0	Absent	8	66	5	2
C	D7	630	465	24.20	23.10	6.4	Full	1	Absent	8	0	0	0
C	D8	631	457	24.37	23.30	5.6	Full	0	Absent	8	3	3	6
C	D9	636	406	24.07	23.60	6	Partial	0	Absent	8	77	7	0
C	D10	637	411	24.63	24.80	5.2	Full	0	Absent	8	0	0	0
C	D11	638	372	26.83	29.47	3.5	None	0	Absent	8	0	0	0
C	D12	639	376	25.13	24.23	6.2	Full	0	Absent	8	20	23	5
C	D13	640	496	24.37	23.83	5.6	Full	1	Absent	8	26	13	0
C	D14	641	408	24.47	24.07	5.7	Full	1	Absent	8	93	3	2
C	D15	642	343	24.60	24.37	5.5	Full	0	Absent	8	94	21	0
C	D16	643	335	26.40	26.80	5	Partial	0	Absent	8	102	50	0
E	E1	651	470	23.87	22.37	6.7	Partial	1	Absent	7	18	7	0
E	E2	650	284	23.73	22.73	5.7	Partial	0	Absent	7	17	5	0
E	E3	649	414	25.57	25.63	5.8	Partial	0	Absent	7	84	3	0

E	E4	648	436	25.43	24.73	5.8	None	0	Absent	7	0	4	4
E	E5	644	389	24.67	24.40	5.1	Partial	0	Absent	8	0	0	0
E	E6	645	389	24.60	23.83	5.2	None	1	Absent	8	0	0	2
E	E7	646	438	24.00	23.20	5.5	Full	0	Absent	8	0	15	2
E	E8	647	481	24.23	25.13	6.3	Partial	0	Absent	8	8	8	3
E	E9	652	380	23.77	24.30	5.3	Partial	0	Absent	7	0	0	1
E	E10	653	347	24.57	24.20	5.5	Full	0	Absent	7	0	0	0
E	E11	654	425	24.53	23.30	5.9	Full	4	Absent	7	248	27	77
E	E12	655	366	24.97	23.97	5.7	None	0	Absent	7	48	38	103
E	E13	656	379	25.07	25.20	5.7	Partial	0	Absent	8	0	0	31
E	E14	657	370	24.6	24.73	5.1	Partial	0	Absent	8	0	0	1
E	E15	658	409	24.97	25.55	6	Partial	1	Absent	8	0	0	0
E	E16	659	425	24.73	25.37	4.9	Partial	1	Absent	8	29	10	0
OP	OP1	741	381	26.87	27.90	4.6	None	0	Absent	7	0	0	0
OP	OP2	738	392	25.37	24.50	6.3	None	0	Absent	7	67	10	0
OP	OP3	740	383	25.23	24.10	6.6	None	0	Absent	7	0	0	0
OP	OP4	746	353	25.90	24.60	6.4	None	0	Absent	7	0	0	0
OP	OP5	742	328	25.23	24.37	6.1	None	1	Absent	7	32	12	3
OP	OP6	745	350	26.47	26.97	4.5	None	0	Absent	7	0	0	0
OP	OP7	739	396	27.23	27.13	5.1	None	0	Absent	7	1	0	0
OP	OP8	743	386	26.50	27.90	4.7	None	0	Absent	7	0	0	0
OP	OP9	744	396	25.83	26.83	4.2	None	0	Absent	7	0	0	0
OP	OP10	761	327	26.73	26.50	4.5	None	0	Absent	7	0	0	0
OP	OP11	758	308	27.03	28.57	4	None	0	Absent	7	0	0	0
OP	OP12	762	321	25.47	25.80	6.1	Partial	0	Absent	7	7	8	2
OP	OP13	756	283	26.13	25.43	6.1	Partial	0	Absent	7	0	0	0
OP	OP14	757	298	25.63	25.63	5.5	None	0	Absent	7	5	5	0
OP	OP15	764	278	27.37	27.63	4.3	None	0	Absent	7	0	0	15
OP	OP16	763	281	26.13	26.43	5.4	None	0	Absent	7	6	6	0
OP	OP17	759	269	28.43	30.27	4	None	0	Absent	7	3	0	7

OP	OP18	760	292	25.73	25.33	6	Partial	0	Absent	7	1	0	11
H	H3		392	26.7	29.40	2.50	Partial	0	Absent	7	1	1	33
H	H5		402	26.9	26.73	5.20	Partial	0	Absent	7	15	16	48
H	H6		389	27.3	29.50	1.70	Partial	0	Absent	7	0	0	38
H	H8		392	27.4	27.43	4.00	Partial	0	Absent	7	3	3	35
H	H9		279	28.5	29.30	2.90	Partial	0	Absent	7	24	23	13
H	H10		275	26.9	29.83	3.80	Partial	0	Absent	7	0	0	0
H	H11		265	26.9	26.83	3.70	Partial	0	Present	7	0	0	51
H	H12		268	27	30.27	3.20	Partial	0	Absent	7	0	0	28
H	H13		269	27.5	28.90	4.10	Partial	0	Absent	7	0	0	60
H	H14		262	27.6	28.13	3.10	Partial	0	Absent	7	1	1	42
H	H15		271	27.8	27.73	2.50	Partial	0	Absent	7	13	11	66
H	H16		269	27.8	29.20	2.50	Partial	0	Absent	7	0	0	28
H	H17		273	27.5	29.77	2.50	Partial	0	Absent	7	0	0	29
H	H18		281	27.7	31.00	2.50	None	0	Absent	7	0	0	57
H	H19		276	27.6	31.27	2.20	None	0	Present	7	6	6	137
H	H20		287	27.5	30.47	3.20	Partial	0	Absent	7	7	8	33
H	H21		286	27.8	31.30	3.40	None	0	Present	7	6	6	52
H	H22		283	27.8	31.27	1.50	None	0	Present	7	4	5	60

*Site number given by the SAFE Project

9.2. Identifications

Table 9.2: Morphological characteristics of the different female morpho-groups

Morpho-group	Predicted Genus	Morphological characteristics	Photograph of mosquito from morpho-group
Cx1	*Culex*	Prespiracular setae absent, tarsi with pulvilli, dorsocentral setae on mid-later areas of scutum, base of hindcoxa above base of mesomeron, scutellum tri-lobed with setae in three distinct groups, proboscis without pale ring, 4 lower mesepimeral setae	
Ae1	*Aedes*	Postspiracular setae present, prespiracular setae absent, tarsi without pulvilli, Scutal scale pattern with a single mid line, dorsocentral setae, wings not spotted with pale and dark scaling, erect scales restricted to occiput, striped legs	
Ae2	*Aedes*	Postspiracular setae present, prespiracular setae absent, tarsi without pulvilli. Prealar area with scales, no acrostichal setae, erect scales restricted to occiput, no defined scutal scale pattern,	
Ae3	*Aedes*	Postspiracular setae present, prespiracular setae absent, tarsi without pulvilli, no defined scutal pattern, no acrostichal setae, proboscis mostly black with median pale pales, wings without dark and pale scales	
Ae4	*Aedes*	Postspiracular setae present, prespiracular setae absent, tarsi without pulvilli. No erect scales restricted to occiput, femora and tibiae not spotted and ringed with pale scales, acrostichal setae present. proboscis mostly black with median pale pales	
Ae5	*Aedes*	Postspiracular setae present, prespiracular setae absent, tarsi without pulvilli, wings not spotted with pale and dark scaling, femora and tibiae not spotted or ringed with pale scales, no defined scutal pattern, dull brown colour, proboscis black	
Ae6	*Aedes*	Postspiracular setae present, prespiracular setae absent, tarsi without pulvilli, no defined scutal scale pattern, subspiracular scales absent, femora and tibiae not spotted and ringed with pale scales, acrostichal setae, three lines of silver scales down the side of the thorax	

Ae7	*Aedes*	Postspiracular setae present, prespiracular setae absent, tarsi without pulvilli, no defined scutal scale pattern, two lines of silver scales down the side of the thorax	
Ae8	*Aedes*	Postspiracular setae present, prespiracular setae absent, tarsi without pulvilli, no defined scutal scale pattern, one line of white scales along the thorax	
Ae9	*Aedes*	Postspiracular setae present, prespiracular setae absent, tarsi without pulvilli, no defined scutal scale pattern, two areas of silver scales on the side of the thorax	
Ae10	*Aedes*	Postspiracular setae present, prespiracular setae absent, tarsi without pulvilli, clypeus with white scales, wings without pale or dark scales, scutal patter lyre shaped	
Ar1	*Armigeres*	Compound eyes separated ventrally by two long rows of scales. Postspiracular setae present, prespiracular setae absent, lower mesepimeral setae present, base of hindcoxa slightly above base of mesomeron	
He1	*Heizmannia*	Prespiracular setae absent, base of hind coxa slightly above base of mesomeron, prealar setae present, blue/silver scales on head (behind compound eyes)	
Ze1	*Zeugnomyia*	Scales on paratergite, prealar setae present, base of hindcoxa slightly above base of mesomeron, silver scales behind spiracle	
An1	*Anopheles*	Maxillary palps as long as the proboscis, Pale and dark scales on wings, alula of wings without setae or hairs, veins M1+2 and M3+4 straight, wings with at least 4 areas on leading margin, hindtarsomere V entirely white-scaled	
An2	*Anopheles*	Maxillary palps as long as the proboscis, Pale and dark scales on wings, wings without setae or hairs, veins M1+2 and M3+4 straight, wing veins with light and dark scaled areas, pale fringe spot at end of vein R2	

Table 9.3: Morphological characteristics of the different larval morpho-groups

Morpho-group	Genus	Description
AeCo	*Aedes Complex*	Respiratory siphon present, Seta 3-C dorsal position, antennae not broad and flattened, siphon tube with one pair of setae, no seta 2-C, pectin spines present
Cx	*Culex*	Respiratory siphon present, seta 12 on abdominal segment 1, 3 or more setae on siphon tube
Ar	*Armigeres*	Respiratory siphon present, Seta 3-C dorsal position, no pectin spines on siphon,
An	*Anopheles*	Respiratory siphon absent

9.3. Photographs

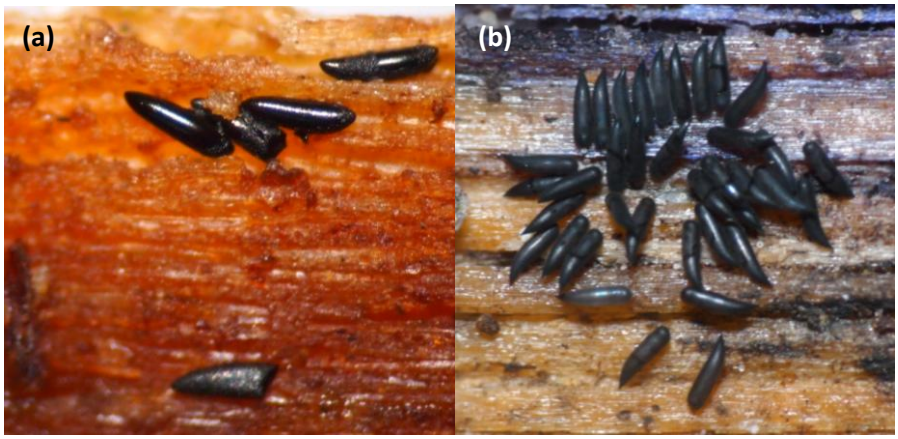

Figure 9.1: Eggs from (a) the housing area (b) the old growth area

Figure 9.2: The different land use areas (a) Oil palm plantation (Photograph used with permission of C. Gray) (b) secondary forest (c) old growth