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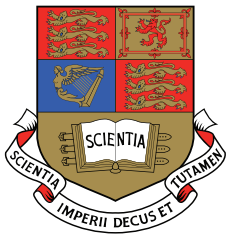

The impact of altered forest microclimate on the development rate of mosquito vectors

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Declaration

I carried out all fieldwork and data collection along with help of research assistants at the SAFE Project. I collated and processed the raw data and decided on the appropriate statistical analyses. I received input and suggestions by my supervisors throughout my project including the experimental design of the project, representation and interpretation of the results and writing of this thesis.

Elizabeth Psomas, 1st September 2015

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The impact of altered forest microclimate on the development rate of mosquito vectors

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ABSTRACT: Anthropogenic land-use changes such as deforestation have been associated with increased risk of mosquito-borne diseases. Ecosystem disturbances alter microclimate within the forest including temperature, a key determinant of mosquito development rate, adult body size and most significantly vector capacity. We compared the development rate of mosquito eggs under field microclimatic conditions across an environmental land-use gradient including primary forest (OG), twice-logged forest (LF) and oil palm plantations (OP). We measured adult body size and proportion surviving to adults as simple proxies of adult vectorial capacity. Mean maximum soil temperature and diurnal temperature range (DTR) increased across the land-use gradient from OG to OP. Greater DTRs recorded in OP increased the development time of mosquito vectors. Adult mosquito wing length at emergence significantly increased as development time increased. There was no significant difference between mean wing length and land-use areas, however significant differences were recorded between sampling sites within land-use areas. Microclimatic changes in temperature significantly impact the juvenile stages of mosquito development and therefore indirectly impacting the transmission of disease.

Keyword Index: Mosquito-borne diseases, development rate, vectorial capacity, microclimate, Diurnal temperature range (DTR).

INTRODUCTION

Human modified landscape changes such as deforestation, logging and conversion to agriculture have been associated with increased risk of mosquito-borne diseases (Norris et al. 2004, Hahn et al. 2014, Saxena et al. 2014, Srivastava et al. 2013). Land use changes such as open forest areas have been reported as favourable environments for mosquitoes, increasing the risk of spread of malaria vector habitats (Srivastava et al. 2013, Vittor et al. 2009). Creation of new malaria vector habitats may therefore play a critical role in malaria transmission in previously malaria free regions (Sexana et al. 2014, Srivastava et al. 2013, Norris 2004). In the absence of vaccines and suitable drugs for the majority of mosquito-borne diseases a better understanding of mosquito vector ecology is vital for implementing effective vector control strategies (Brady et al. 2015, Hossain et al. 2015).

Ecosystem disturbances can exert significant changes in microclimatic conditions (Hardwick et al. 2015) directly affecting the breeding, survivorship, development and disease transmission of mosquito vectors (Patz, et al. 2000, Yasuoka and Levins 2007, Parham et al. 2015). A decrease in canopy height and Leaf Area Index across a land-use gradient including primary forest, logged forest and oil palm results in a greater day-to-day variability in ecologically important climatic variables such as air temperature, relative humidity and soil temperature (Hardwick et al. 2015). Mosquitoes are ectotherms and therefore each life stage of a mosquito is temperature dependent. Mosquito vectors have four main lifecycle stages: egg, larva, pupa and adult. The first three stages form the juvenile stage, which is entirely aquatic and therefore intimately associated with the environment. Although the juvenile stage is not involved in mosquito-borne disease transmission, environmental conditions experienced during larval development determine development rate and survival of adult mosquitoes (Beck-Johnson et al. 2013, Couret et al. 2014).

Environmental variation in larval habitat quality including temperature has been shown to alter the biological competence of malaria vectors and therefore affect the control of vector-borne diseases (Moller-Jacobs et al. 2014, Paaijmans et al. 2008, Takken et al. 2013). Daily temperature fluctuations occur in natural habitats and affect numerous life-history traits including juvenile mosquito development and survival (Huang et al. 2006, Carrington et al. 2013, Paaijmans et al. 2009). Temperature fluctuations have been found to reduce development rates under warmer conditions and increase development rates under cooler conditions (Paaijmans et al. 2013). Carrington et al. (2013) demonstrated large diurnal

temperature ranges (DTR) at low temperature means (16^0C) significantly accelerated *Aedes aegypti* development compared to constant temperatures and small DTR. Changes in microclimate will therefore play an important role in determining the longevity of the juvenile stage and the proportion that develop into adults, thus affecting mosquito vectorial capacity and disease transmission.

Temperature is an important and key ecological determinant not only in the development of mosquito juvenile life stages but also during the *Plasmodium* extrinsic incubation period (EIP- parasite development time within the adult mosquito) (Couret and Benedict 2014, Beck-Johnson et al. 2013). Mean temperature differences between resting sites within transmission environments can lead to important variations in predicted EIPs (Cator et al. 2013). Temperature dynamics may also impact insect vector physiology and fitness by influencing vector immunity thus vector-parasite interactions (Parham et al. 2015, Murdock et al. 2012).

Many empirical studies have investigated the effects of temperature as a key determinant of mosquito development, by applying mean temperatures under controlled laboratory conditions (Murdock et al. 2014, Bayoh and Lindsey 2004, Phelan and Rotiberg 2013). Ectotherms do not experience mean temperature conditions in nature, but instead are exposed to daily temperature dynamics, typically ignored in model systems. Such models carried out under laboratory conditions overlook the environmental dynamics experienced by insect vectors under field conditions, which could potentially lead to an underestimation of disease transmission. Studies by Lambrechts et al. (2011), Carrington et al. (2013), Paaijmans et al. (2013) and Chen et al. (2015) demonstrated the importance of the influence of diurnal temperature ranges (DTRs) on ectotherm life-history traits. However, such studies fail to integrate other environmental microclimatic conditions experienced in nature, emphasizing the need to validate these models in the field. The majority of studies on mosquito vector ecology use laboratory line mosquitoes (Bayoh and Lindsey 2004, Christiansen-Jucht et al. 2014, Ciota et al. 2014), although some studies have reported to collect adult and immature mosquitoes from the field (Afrane et al. 2006, Carrington et al. 2013), they are still reared and maintained under laboratory conditions. Furthermore, there have been very few, if any, studies investigating mosquito development and survival rates under field microclimatic conditions.

In South East Asia the most common mosquito vectors include mosquitoes in the genera *Aedes, Anopheles, Culex and Mansonia* (Rattanarithikul et al. 2005). They are considered to be the most medically important mosquito vectors involved in the transmission

of Dengue Fever and Chikungunya (*Aedes*), Malaria (*Anopheles*), Japanese Encephalitis (*Culex*) and Filariasis (*Mansonia*) (Rattanarithikul et al. 2005, Yi et al. 2014). The unprecedented expansion of oil palm cultivation in Malaysia has led to significant microclimatic differences between primary forest and oil palm plantations (Hardwick et al. 2015, Luskin and Potts 2011). Oil palm plantations harbour warmer and drier microclimates compared to primary forest (Luskin and Potts 2011). Temperature and relative humidity have been reported to fluctuate much more on a daily basis in oil palm compared to primary forest (Foster et al. 2015).

In this study we aimed to investigate the development rate of mosquito vectors across an environmental land-use gradient including primary forest, twice-logged forest and oil palm plantations in the Sabah region of Borneo. Specific objectives were to 1) Determine the development rate and survival of mosquito eggs collected in the field. 2) Quantify simple proxies of adult vectorial capacity (proportion surviving to adult stage and body size) to determine how we may predict these changes in environment to indirectly impact the transmission of disease. We predicted that as canopy cover decreases across the land-use gradient from primary forest to oil palm, temperatures would increase exposing mosquitoes to different microclimatic conditions. Thus, we expected to observe an increase in the development rate and subsequently smaller adult wing size across the land-use gradient from primary forest to oil palm plantations.

MATERIALS AND METHODS

Study area

This study was conducted in the Maliau Basin Conservation Area ($4^0$49'N, $116^0$54'E) and the Kalabakan Forest Reserve ($4^0$42'N, $117^0$34'E) established as part of the Stability of Altered Forest Ecosystems (S.A.F.E Project) in Sabah, Malaysia (Ewers et al. 2011). Study sites were selected across a forest disturbance gradient: lowland dipterocarp primary rainforest (OG) in the Maliau Basin Conservation Area (MBCA), twice-logged dipterocarp forest (LF) and oil palm plantations located within the SAFE Project field site (Fig. 1).

MBCA is a Class 1 Protected Forest Reserve and has never been logged commercially, with the exception of a few sites (OG3), which were lightly logged in the 1970's and 1990's (Ewer et al. 2011). Survey points were selected in an area that has never been commercially logged (OG2). Logged forest survey points were selected in twice-logged forest fragment block D and E, of varying forest quality. Oil Palm survey points were selected in OP1, located 700m from the forest.

Figure 1. Field sites located along a forest disturbance gradient: (A) Primary forest (MBCA), (B) Twice-logged forest and (C) Oil palm plantation in Sabah, Borneo.

Experimental design

Modified oviposition traps were placed at the second order survey points, under SAFE Project leaf litter traps, between May- July 2015 (Fig. 2A). Second order survey points were already established across the landscape at fixed geometric intervals (178m apart) within a triangular fractal pattern (with sides of 564m and 1780m) (Ewers et al. 2011). Thirty-two survey points were sampled in this study with three replicates at each survey point, giving a total of one hundred and eight samples: 27 in primary forest, 54 in twice-logged forest and 27 in oil palm plantations. Primary forest (OG2) and oil palm plantation (OP1) sites each contain nine-second order survey points, with three replicates at each site this gives a total of 27 samples at each site. Sampling in the twice-logged forest fragments was restricted to 1 x100 ha fragment and 2 x 10 ha fragments, with the exception two second order survey points located in the matrix of fragment D. There are four second order survey points located in 100 ha forest fragment and two second order survey points located in each 10 ha forest fragment, with the addition of two second order survey points in D-matrix and three replicates at each site, this gives a total of 54 in twice-logged forest.

The oviposition trap consisted of a black plastic container (10 cm x 9 cm) with an overflow hole near the rim of the container to avoid overflow during heavy rain. Each container was filled with 300ml of standardized water and 6ml of stagnant water collected from active larval sites within each sampling site. Additionally, a small pinch of fish food (Tertafin) was added to each trap. Paper towels were placed around the entire rim of each container using an elastic band along with a thin paddle of wood (1 cm x 23 cm) to act as oviposition substrates.

After ~5 days, all traps were collected and all substrates were placed in individual plastic bags. Water in the traps was checked for any mosquito eggs or larvae, if present, they were placed in plastic containers filled with water from the traps to be bought back to the laboratory. Oviposition traps were collected in the morning (8am – 12pm). Eggs were counted on the oviposition substrates under a hand lens (x10 magnification). After counting, all unhatched eggs were left on the tissue substrate and placed along the inside of a plastic container (17cm x 7cm x 11.5cm) half submerged in 250 ml standardized water (Fig. 2B). Containers were kept in the laboratory for ~1 week to allow the unhatched eggs to embryonate before fully submerging the eggs into the water and adding a small pinch of fish food. Each container was then placed back out into the field in the same sample plot were they were collected (in a small hole dug in the ground, under the SAFE Project leaf litter traps) (Fig. 2C). Each sample was monitored daily in order to record the proportion that

emerge at each development stage (larva, pupa and adult) along with the number of transition days between each development stage. Once mosquitoes developed into pupae, the plastic containers were covered with muslin cloth, so that adult mosquitoes did not escape (Fig. 2D). Fish food (x 2 pellets) was dispensed daily on the water surface. Water depth was kept at a maximum 5 cm and topped up when needed.

Figure 2. (A) Oviposition traps set up beneath leaf litter canopy covers. (B) Mosquito eggs (*Aedes*) laid on paper towel oviposition substrate. (C) Plastic container with mosquito eggs fully submerged into the water, placed in the ground under leaf litter canopy. (D) Plastic container covered with muslin cloth under leaf litter canopy.

Identification of adult mosquitoes and wing size measurements

Once adults had emerged, containers were bought back to the laboratory for identification. The water from each container was drained and adult mosquitoes were then placed in the freezer for approximately 10 minutes. Adult mosquitoes were counted and individually transferred into labeled eppendorf tubes, and left to dry in a plastic bag of silica gel until mosquitoes could be identified.

Adult mosquito body size is associated with adult wing length (Nasci, 1990) therefore adult wing length (of each adult mosquito collected) was used as a simple proxy to measure adult vectorial capacity. Measurements of wing length were taken between the alular notch

and the posterior margin of the wing (excluding the fringe) (Fig. 3). Due to the absence of a graticule eyepiece in the dissection microscope available, a standard 30 cm ruler was used to take measurements under x1 magnification. The right wing of the adult mosquito was selected for measurements, however most specimens were in a very poor condition. In cases in which the wings had become detached from the thorax and the position of the wings could not be identified. In such cases the most intact wing was selected for measurement.

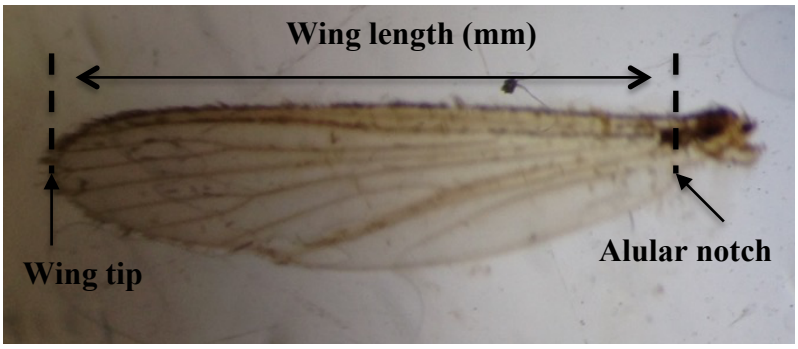

Figure 3. Positions of landmarks on adult mosquito wing indicating distance wing length measurements were taken.

Climate data collection

Ibutton data loggers were placed roughly 5cm below ground, in order to record soil temperature hourly, over a one-week sampling period. Ibutton data loggers were placed at the second order survey points under the SAFE Project leaf litter traps, the same site in which mosquito samples were monitored. Thermodata viewer software version 3.1.29 was used to download soil temperature data.

Statistical analysis

A Generalized linear model (GLM) was used to assess the response of wing length to development rate across all samples collected within twice-logged forest and oil palm. We performed an analysis of variance (ANOVA) followed by a post hoc Tukey's HSD test between each land-use type and individual sampling sites on wing length. Statistical analyses were performed in RStudio (version 0.98.1087 – © 2009-2014 RStudio, Inc.)

The mean soil diurnal temperature range (DTR) over one week and twenty-four hours was plotted using ggplot2 version 1.0.1 package in RStudio.

RESULTS

A total of 119 adult mosquitoes were collected from twice-logged forest (LF) and oil palm (OP). While we were unable to confirm identification, general observation, field notes and photographs taken, indicate that the majority of eggs collected in OP, LF-D, LF-E and OG belonged to the genus *Aedes* (Fig. 3). However, on daily monitoring of the samples it was evident from observation, mosquitoes belonging to other genera were also present including *Anopheles* (Fig. 4). This is to be expected based on the current knowledge of the mosquito-vectors present in Southeast Asia (Rattanarithikul et al. 2005), however mosquitoes could not be identified to species.

We were only able to collect a data on egg and larval development from primary forest (OG). A total of 157 eggs were collected in OG, 133 eggs were collected in LF-D and 256 eggs were collected in LF-E. No eggs were collected from OP1 or Fragment D-matrix samples, however immature larvae were collected in oviposition traps and monitored through to adult stage. A small number of samples (collected from LF sites) containing adult mosquitoes were damaged upon transportation or destroyed at the site (potentially by small forest mammals) therefore could not be included. Furthermore, samples monitored in LF fragment E did not all reach adult stage before samples were collected, but abundance and development stage were still recorded.

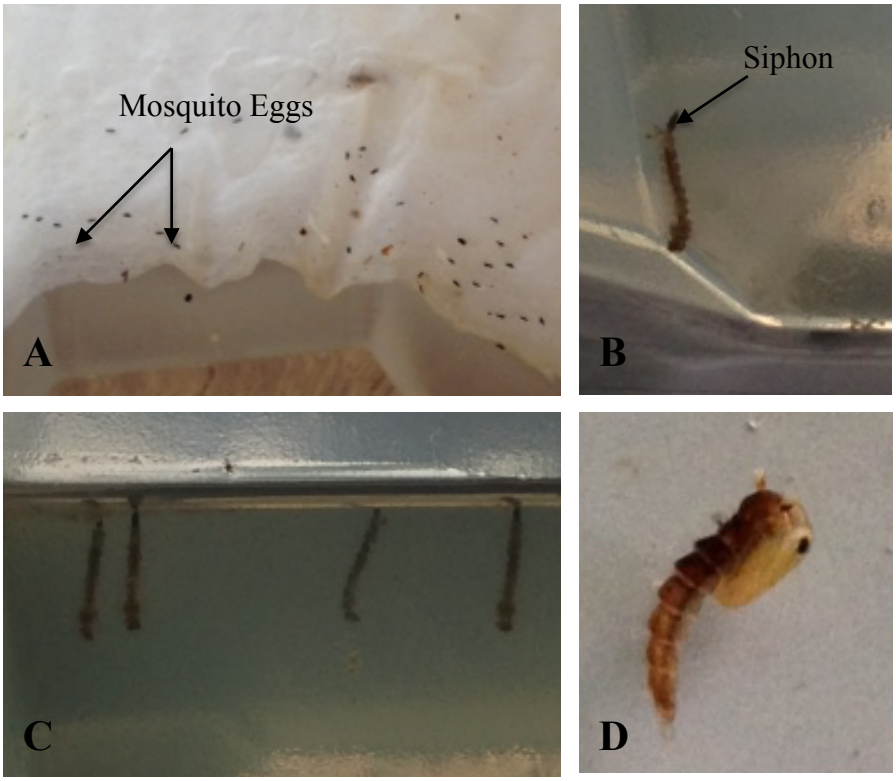

Figure 3. Development stages of *Aedes* mosquitoes collected in the field: (A) Mosquito eggs laid on paper towel oviposition substrate, (B & C) Fourth-instar larvae (D) and pupa.

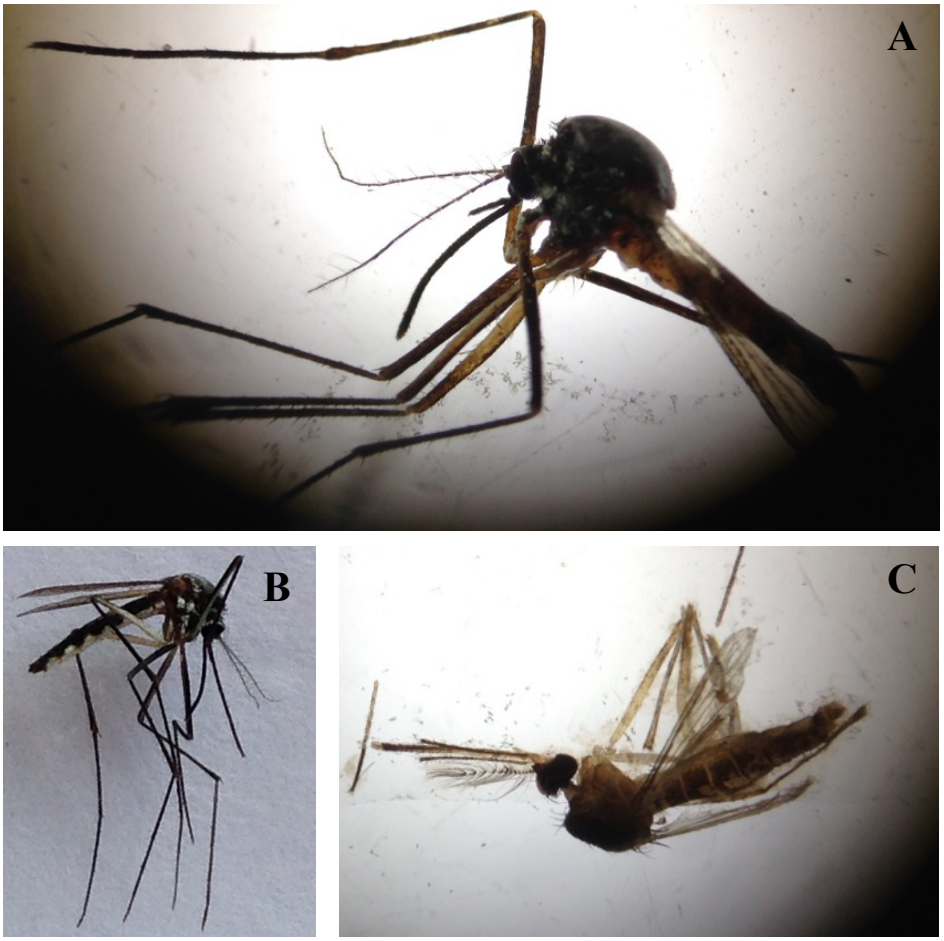

Figure 4. Adult mosquitoes developed from eggs collected in the field: (A and B) *Aedes* and (C) *Anopheles*.

Microclimate data

Temperature profiles have been shown to vary across environments (Hardwick et al. 2015, Foster et al. 2015). We compared both mean temperature and diurnal temperature range (DTR) across our sites. The mean diurnal cycle of soil temperature, over a sampling period of one week, varied considerably across the three land use types OG, LF and OP (Fig. 5A). The mean maximum soil temperature was largest in OP (30.6^0C) and smallest in OG (25.1^0C). Mean soil temperature increased across the land-use gradient from OG to OP. OP recorded the largest mean diurnal cycle (4.8^0C) followed by LF-D (3.6^0C), LF-D matrix (3.3^0C), LF-E (1.6^0C) and OG (1^0C). Interestingly, the mean diurnal cycle of LF-E was very similar to OG, with only a difference of 0.6^0C. Furthermore, the mean diurnal cycle of LF-D matrix was very similar to LF-D, with only a difference of 0.3^0C.

The soil diurnal temperature range (DTR) followed similar patterns across the five sampling sites (Fig. 5B). DTR increased across the land-use gradient from OG to OP. OP

recorded the largest DTR (6.5^{0}C), followed LF-D (4^{0}C), D-matrix (3^{0}C), LF-E (2.5^{0}C) and OG (1.5^{0}C). In OP, soil temperature reached its minimum value (25.6^{0}C) by 3am, before rapidly rising to its maximum value (32.1^{0}C) by 3pm. This was followed by a decrease in temperature at a slower rate throughout the afternoon and evening. LF fragment D and fragment E follow similar patterns in DTR. Both LF-D and LF-E reached minimum values (23.1^{0}C) by 2am, before reaching maximum values (27.1^{0}C and 25.6^{0}C) by 3pm and 4pm respectively. Soil temperature then dropped off, only slightly, falling down to a minimum value of 25.1^{0}C by 10pm in both LF-D and LF-E sites. LF fragment D-matrix reached its minimum value by 3am before rising to its maximum value (27.6^{0}C) by noon. This was followed by a steady decrease in temperature throughout the afternoon and evening. OG recorded the smallest DTR reaching its minimum value (23.6^{0}C) by 4am and maximum value (25.1^{0}C) by 3pm. Soil temperature remained constant at the maximum value throughout the afternoon, only cooling down by 1^{0}C to 24.6^{0}C by 10pm.

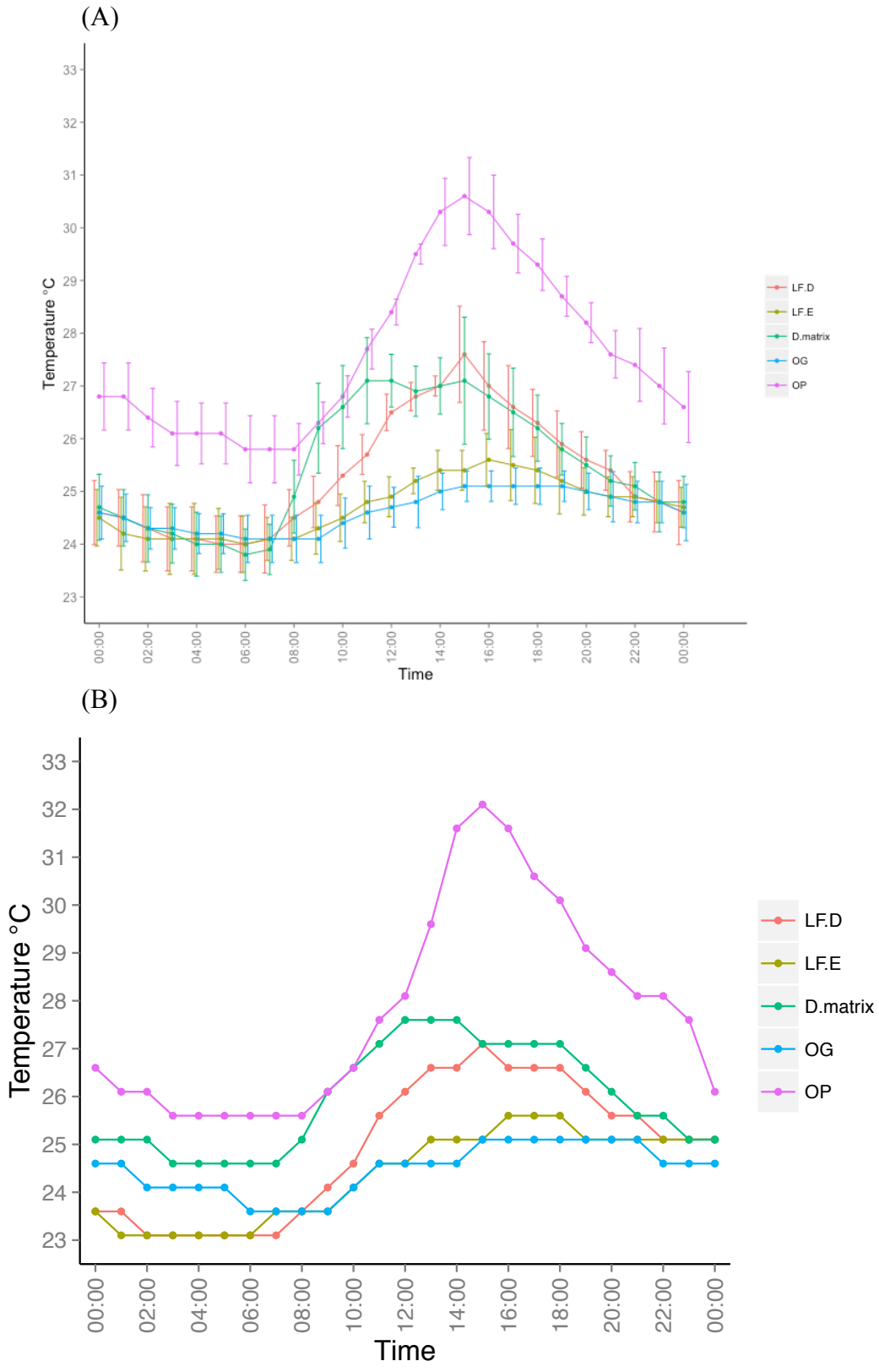

Figure 5. (A) Mean diurnal cycle of soil temperature over a sampling period of one week and (B) diurnal temperature range (DTR) of soil temperature over a period of 24 hours across the three land-use type: primary forest (OG), twice-logged forest (LF-D, LF-E and LF-D matrix), and oil palm (OP).

Mosquito development rate

The development time of mosquitoes is significantly associated with adult wing length (GLM, $F_{1, 7}$= 11.58, *P*= 0.009) (Fig. 6). Wing length increases as development time (number of days) increases. Development time varied across the sample sites between and within the land use areas (Fig. 7). OP recorded the shortest development time, however development was monitored from immature larvae to emergence as no eggs were collected. LF-E 100 ha (site 656) and LF-D 100 ha (site 643b) both recorded the longest development time. Average mosquito wing length did not differ significantly between LF-D, LF-E and OP (ANOVA, $F_{2, 71}$=1.814, *P*=0.171) (Fig. 8). However, there are significant differences between wing length and individual sampling sites within LF-D, LF-D matrix, LF-E and OP sites (ANOVA, $F_{8, 65}$=6.913, *P*= 0.00000151) (Fig. 9). Mean wing length in sample site 643b (LF-D 100 ha) is significantly bigger than mean wing length recorded in sample site 739 (OP) (*P*=0.039) and 654 (LF-E 10 ha) (*P*=0.018). Wing length in sample site 643a is significantly smaller than wing length recorded in sample site 656 (LF-E 100 ha) (*P*=0.041) and 655 (LF-E 10 ha) (*P*=0.008). Interestingly, mean wing length differed significantly between samples 643a and 643b (*P*=0.000004) both of which were exposed to the same microclimatic conditions in the same sampling site in LF-D 100 ha.

The mean survival of pupae and adults varied between OP and LF (Table 1). In OP, all pupae developed into adults, compared to LF, which recorded a decrease in the number of adults emerged from pupae. However, in LF, over half of all mosquitoes at each developmental stage progressed to the next development stage.

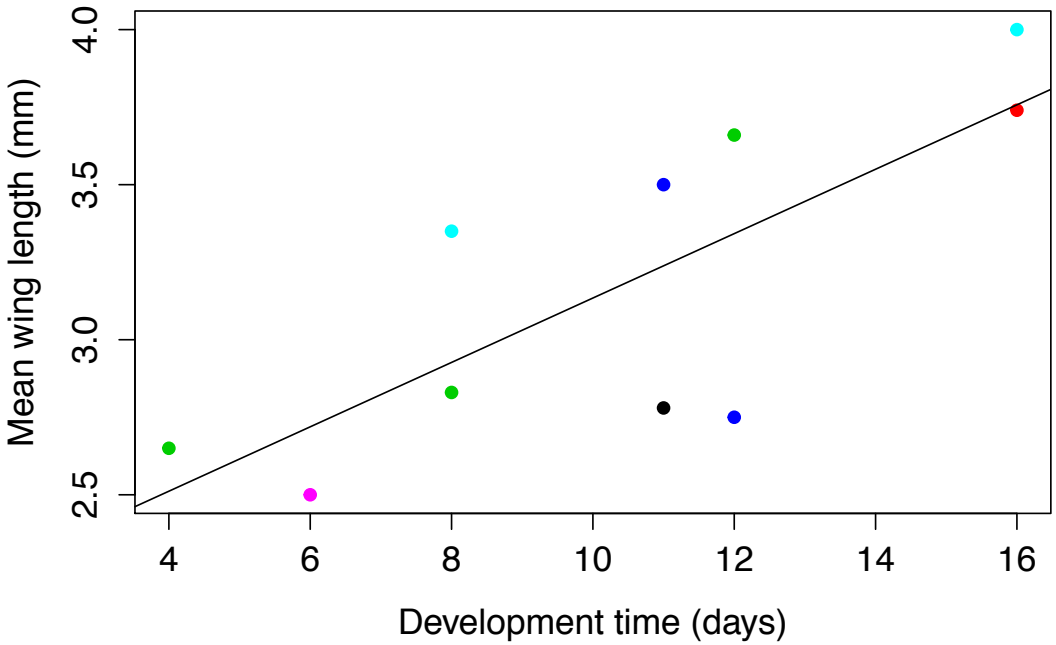

Figure 6. Generalized linear model (GLM) of average mosquito wing length at emergence in response to development time, recorded across the following sample sites: OP, LF-D (100 ha and 10 ha), LF-D matrix and LF-E (100 ha and 10 ha). OP (pink), LF-D matrix (green), LF-D 100 ha (black), LF-D 100 &10 ha (red), LF-E 100 (light blue) and LF-E 10 ha (dark blue).

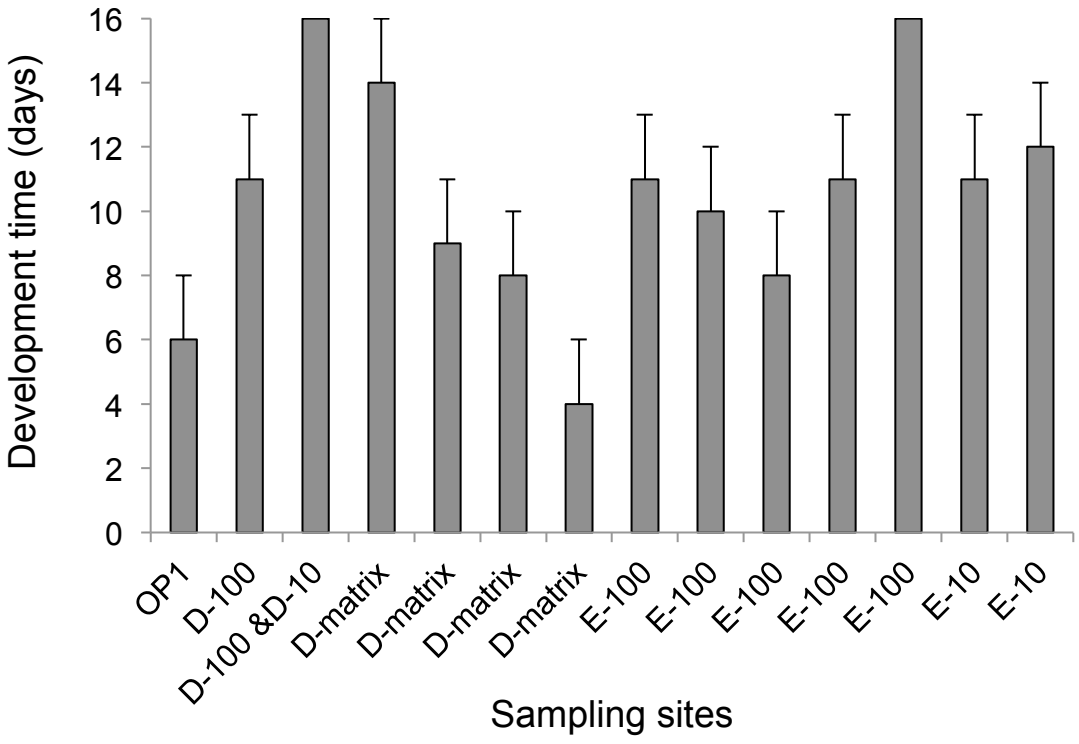

Figure 7. Development time of each sample monitored from hatch to emergence within OP, LF-D 100 & 10 ha, LF-D matrix and LF-E 100 & 10 ha. OP and LF-D matrix samples were monitored from immature larval stages to emergence.

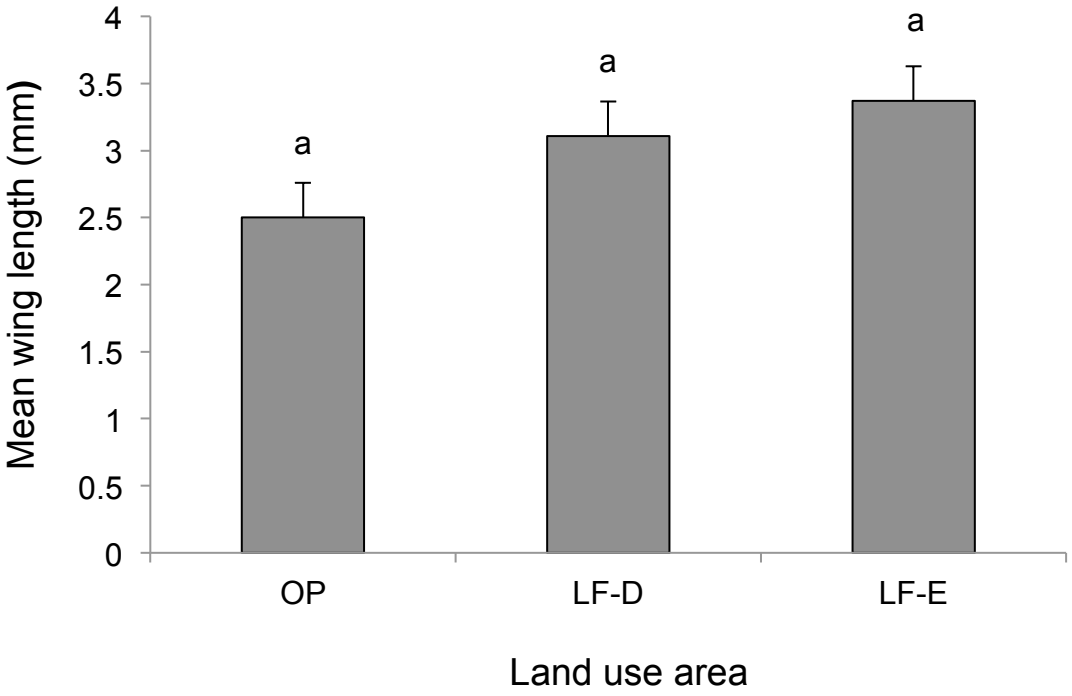

Figure 8. Mean wing length of all adult mosquitoes collected from each land use area. There are no significant pairwise differences between mean wing length and land use area (P>0.05).

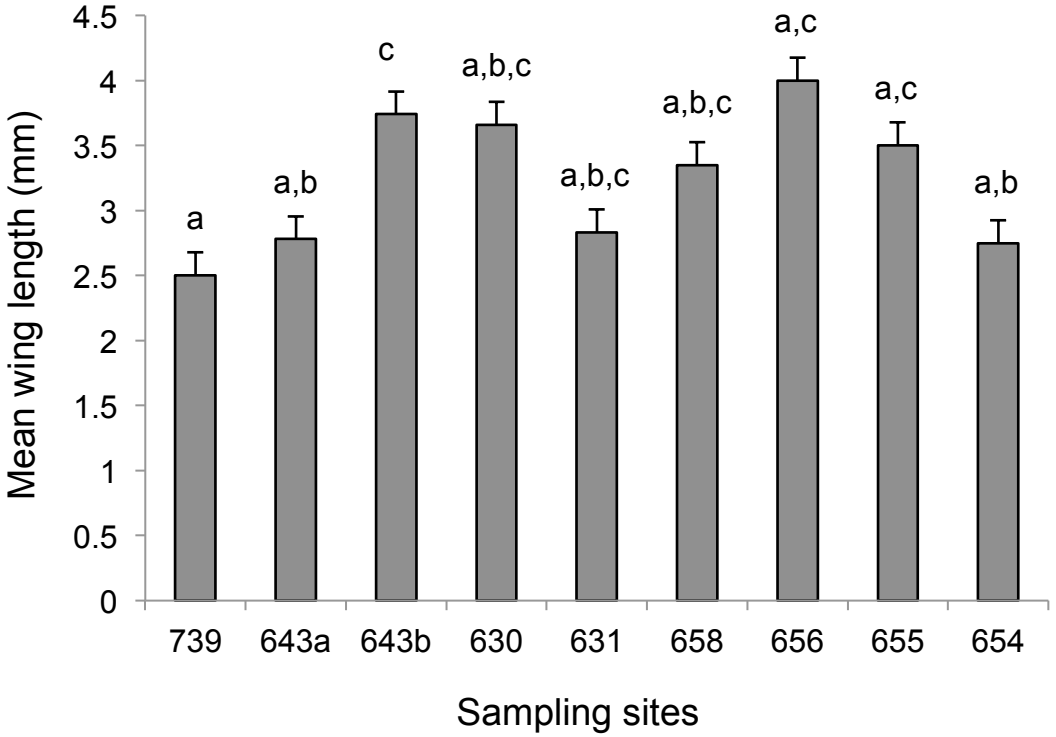

Figure 9. Mean wing length of all adult mosquitoes collected at individual sampling sites within OP (739), LF-D 100 & 10 ha (643a and 643b), LF-D matrix (630 and 631) and LF-E 100 & 10 ha (658, 656,655 and 654). The pairwise differences of sampling sites show a significant difference at P<0.05, P<0.01 and P<0.0001.

Table 1. The mean proportion (%) of mosquitoes emerging at each developmental stage across the three different land-use areas. (n=total number of containers per site).

Habitat	Number of Eggs	% Eggs→ Larvae	% Larvae→ Pupae	% Pupae→ Adults
Oil Palm (OP)	NA	NA	42.8 % (n=1)	100 % (n=1)
Primary forest (OG)	21.42 ±20.10 (n=7)	NA	NA	NA
Twice-logged forest (LF)	35.36 ±51.23 (n=11)	81.74 ±16.11% (n=11)	63.52 ±9.46% (n=11)	64.35 ±9.78% (n=11)

DISCUSSION

Microclimatic differences within tropical forest ecosystems, as a result of human modification, can have direct effects on the development rate of mosquito vectors. The results from this study demonstrate that greater diurnal temperature fluctuations (DTR) in soil temperature occur along a forest disturbance gradient from primary forest (OG) to oil palm (OP). In accordance with our predictions, mosquito development rate increased when exposed to greater soil DTRs and mean temperatures producing adult mosquitoes with smaller wing size at emergence.

Changes in climatic variables as a result of deforestation and logging can create more challenging environments, particularly for ectotherms (Colinet et al. 2015). Oil palm canopies are much lower and more open than primary forest creating a harsh microclimate (Luskin and Potts, 2011, Foster et al. 2015, Hardwick et al. 2015). Luskin and Potts (2011) reported oil palm plantations to be $+2.84^{0}$C hotter than primary forests, during diurnal hours. In the current study, mean maximum soil temperatures in OP reached 30.6^{0}C, $+5.5^{0}$C hotter than OG during diurnal hours.

Greater mean temperatures and DTRs recorded in OP yielded shorter development rates (although only recorded from larval stage) and smaller mean wing lengths at emergence. The association between greater DTRs and increased development rate is consistent with other findings (Huang et al. 2006, Carrington et al. 2013). This effect of temperature on ectotherms is reflected in the temperature-size rule (Angillettta et al. 2004). Higher temperatures increase the development rate both reducing age and producing adults with smaller wing size at emergence (Phelan and Rotiberg 2013). Adult mosquito wing length is directly related to body size (Nasci 1986). Body size has been suggested to influence adult feeding behaviour and survivorship, both of which are key determinants of transmission potential (Ameneshewa & Service, 1996, Takken et al. 1998). Small *Ae. aegypti* females express reduced levels of genes related to immunity, reproduction and metabolic pathways compared to larger individuals (Price et al. 2015). As a result, small adult females require more frequent blood meals after emergence in order to build sufficient energy reserves for survival before gonotrophic development can occur compared to larger adult females, and therefore may bite more often (Takken et al. 1998). Apoptopic genes have been shown to modulate *Ae. aegypti* susceptibility to Dengue virus, with smaller mosquitoes expressing increased levels of AeIAP1 (inhibitor of apoptosis) (Ocampo et al. 2013, Price et. 2015). In comparison, Alto et al. (2008) reported smaller sized *Ae. aegypti* females were more likely to become infected

and disseminate dengue virus than larger individuals. Westbrook et al. (2010) demonstrated cooler temperature prolonged larval development of *Ae. albopictus* yielding larger and more competent adult females increasing the dissemination of Chikungunya virus.

Epidemiological studies have shown large DTRs at cooler temperatures increase the incidence of both Dengue (Sharmin et al. 2015) and malaria (Zhao et al. 2014). No epidemiological data was collected in the current study, and therefore would require further investigation into how smaller adult females collected in oil palm affect the susceptibility and dissemination of mosquito-borne diseases. This may involve measuring the vector competence of adult females collected from the field. Further investigations may also consider collecting mosquito eggs from one habitat type and placing them in a different habitat to monitor development rates. Mosquitoes may show preference towards habitats along an environmental gradient for oviposition and therefore may observe an increase or decrease in the transmission of certain mosquito-borne diseases in certain land-use areas.

Increasing environmental temperatures during larval development has been shown to decrease larval survival and increase adult mortality (Christiansen-Jucht et al. 2014). In OP, all mosquitoes that survived to pupa stage developed into adults, however, fewer larvae survived and thus fewer adults were produced. Furthermore, no mosquito eggs were collected in OP only a small sample of larvae, suggestive of a low mosquito population. Natural stagnant pools of water were very sparse within OP and this is to be expected given the harsh dry environmental conditions created. Twice-logged forest recorded high egg abundance in addition to a high larval and adult survival rate. Temperature dynamics therefore has the potential to affect the abundance of adult mosquitoes at a specific site and thus disease transmission (Dodson et al. 2012).

Interestingly, two samples monitored in LF-D (100 ha) at the same sampling site, recorded considerably different development rates producing significantly different wing lengths at emergence. Although an increase in development rate (+5 days) in one of the samples significantly increased wing length at emergence, both samples were exposed to the same microclimatic conditions. In nature, larval environments are subject to multiple biological interactions such as nutrient availability and intraspecific larval competition combined with the effects of temperature dynamics. Environmental stresses such as crowding and nutrient reduction experienced during larval development have been shown to produce smaller mosquitoes at emergence (Mitchell-Foster et al. 2012, Price et al. 2015). Padmanabha et al. (2014) demonstrated development rate is sensitive to a combination of food and temperature interactions in larval habitats. The current study maintained food as a constant

variable throughout development, however egg and larval densities did vary between the two samples and may have led to larval resource competition. Prolonged development under low nutrient availability in cooler conditions increases female adult wing size at emergence (Padmanabha et al. 2014). The results in the current study may therefore be the result of a combined biological interaction between temperature and larval densities, however this would require further investigation. This may involve monitoring development rates under varying levels of nutrient availability when exposed to different field microclimatic conditions. Furthermore, it was evident from daily monitoring of samples, a mixed community of mosquito populations were present, consisting of both *Aedes* and *Anopheles* mosquitoes. Mosquitoes in the genera *Anopheles* and *Aedes* exhibit interspecies variation with regards to body size (Nasci 1986, Russell et al. 2011) and therefore may account for some of the differences in mean wing lengths recorded between the two samples.

There were a number of limitations encountered in the experimental design of this study. Transportation of live adult mosquitoes between sampling sites and the laboratory at camp proved to be a challenge, due to the rough terrain of the landscape. Thus the majority of samples were destroyed before they could be counted and stored for identification. Due to the absence of a dissection microscope in the laboratory, adult mosquitoes could not be identified immediately after collection. As a result, the majority of adult mosquitoes had disintegrated before they could be identified. Under field conditions it is to be expected that not all mosquitoes develop at the same rate. Once the muslin cloth was placed over the plastic container, counting the number of larvae and pupae still developing proved to be challenging without the risk of adult mosquitoes escaping. It was important to monitor the samples on a daily basis to record not only the proportion that develop at each stage but the transition days between each develop stage. However, there were numerous occasions when this was not possible for various reasons including transportation being unavailable and bad weather restricting access to the sites.

The intimate and highly dynamic association between mosquito vectors and the environment highlights the importance to carryout fieldwork to better understand vector ecology. Therefore various changes should be considered in order to improve the experimental design for future investigations. Modifications to the containers used to monitor development could be made by a) selecting larger containers so that once adults emerge it is easier to continue counting remaining larvae and pupae, b) create a two chamber container allowing adults to be separated once emerged. It was evident that whilst mosquitoes were developing in some samples, more eggs were laid and a second batch of mosquitoes

developed. Gravid female mosquitoes may therefore be attracted to active larval sites where other females have laid eggs. This water could then be used as an attractant in oviposition traps. Preventing the damage of live adult mosquitoes during transportation may prove to be a difficult obstacle to overcome, however, should not discourage future research in tropical forests.

CONCLUSION

The results from this investigation highlight the complex interactions between the direct influences of microclimate and the development rate of mosquito vectors. We found significant differences in soil DTRs across the environmental land-use gradient, which resulted in significant differences in both development rate and body size of mosquito vectors. This offers great potential for improving the understanding of how environmental variability at a microclimate level indirectly shapes the transmission of vector-borne diseases. Microclimate changes within forest disturbances should be considered in order to support effective vector control strategies concerning mosquito-borne diseases.

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Appendix

Table 1. Total abundance of mosquitoes emerging at each development stage.

		Proportion emerging			
Site	**Plot number**	**Egg abundance**	**Larva abundance**	**Pupa abundance**	**Adult abundance**
OP1	739	0	7	3	3
D-100	643	52	35	34	34
D-100 &D-10	643	81	40	26	21
D-matrix	630a	0	2	2	1
D-matrix	630b	0	3	3	3
D-matrix	631	0	17	11	9
D-100	643-2	0	27	23	Sample destroyed
D-matrix	630-2	0	31	20	19
E-100	659a	86	63	12	5
E-100	659b	33	14	7	1
E-100	658	10	11	10	10
E-100	657	17	5	5	3
E-100	656	24	12	5	2
E-10	655a	24	19	15	14
E-10	655b	21	15	Sample destroyed before pupa stage	
E-10	654	24	18	11	8
E-10	653	16	6	Sample destroyed before pupa stage	
OG2	728	38	Preliminary data		
OG2	723	17	Preliminary data		
OG2	724	61	Preliminary data		
OG2	721	12	Preliminary data		
OG2	727	3	Preliminary data		
OG2	725	6	Preliminary data		
OG2	726	2	Preliminary data		
OG2	720	0			
OG2	722	18	Preliminary data		

Table 2. Total number of transition days between each development stage.

		Transition days			
Site	**Plot number**	**Egg-larva**	**Larva-pupa**	**Pupae-adult**	**Total development time (days)**
OP1	739	0	3	3	6
D-100	643	5	4	2	11
D-100 &D-10	643	5	8	3	16
D-matrix	630a	0	11	3	14
D-matrix	630b	0	6	3	9
D-matrix	631	0	6	2	8
D-100	643-2	0	2	Sample destroyed	
D-matrix	630-2	0	2	2	4
E-100	659a	3	5	3	11
E-100	659b	3	5	2	10
E-100	658	3	4	1	8
E-100	657	3	6	2	11
E-100	656	3	9	4	16
E-10	655a	3	6	2	11
E-10	655b	3	Sample destroyed		
E-10	654	3	8	1	12
E-10	653	3	Sample destroyed		