Some like it hot: a differential response to changing temperatures by the malaria vectors *Anopheles funestus* and *An. gambiae* s.l.

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ABSTRACT

Background. With the possible implications of global warming, the effect of temperature on the dynamics of malaria vectors in Africa has become a subject of increasing interest. Information from the field is, however, relatively sparse. We describe the effect of ambient temperature over a five-year period on the dynamics of *An. funestus* and *An. gambiae* s.l., collected from a single village in southern Mozambique where temperatures varied from a night-time minimum of 6 °C in the cool season to a daytime maximum of 35 °C in the hot season.

Results. Mean daily air temperatures varied from 34 °C to 20 °C and soil temperatures varied from 26 °C to 12 °C. Diurnal variation was greatest in the cooler months of the year and were greater in air temperatures than soil temperatures. During the study 301, 705 female *An. funestus* were collected in 6,043 light-trap collections, 161, 466 in 7,397 exit collections and 16, 995 in 1,315 resting collections. The equivalent numbers for *An. gambiae* s.l. are 72,475 in light-traps, 33, 868 in exit collections and 5,333 from indoor resting collections. Numbers of mosquito were greatest in the warmer months. Numbers of *An. gambiae* s.l. went through a one hundredfold change (from a mean of 0.14 mosquitoes a night to 14) whereas numbers of *An. funestus* merely doubled (from a mean of 20 to 40 a night). The highest environmental correlations and mosquito numbers were between mean air temperature ($r^2 = 0.52$ for *An. funestus* and 0.77 for *An. gambiae* s.l.). Numbers of mosquito collected were not related to rainfall with lags of up to four weeks. Numbers of both gravid and unfed *An. gambiae* complex females in exit collections continued to increase at all temperatures recorded but gravid females of *An. funestus* decreased at temperatures above 28 °C. Overall the numbers of gravid and unfed *An. funestus* collected in exit collections were not correlated ($p = 0.07$). For an unknown reason the number of *An. gambiae* s.l. fell below monitoring thresholds during the study.

Conclusions. Mean air temperature was the most important environmental parameter affecting both vectors in this part of Mozambique. Numbers of *An. gambiae* s.l. increased at all temperatures recorded whilst *An. funestus* appeared to be adversely affected by temperatures of 28 °C and above. These differences may influence the distribution of the vectors as the planet warms.

INTRODUCTION

Temperature is a major driving force in insect populations. In the laboratory the species that are almost entirely responsible for malaria transmission in Sub-Saharan Africa, the freshwater members of the *Anopheles gambiae* complex (*An. gambiae*, *An. coluzzi* and *An. arabiensis*) and *Anopheles funestus,* respond differently to different temperature regimes. Larval development rates for *An. arabiensis* peak within the temperature range of 22–32 °C with survival rates also highest at 32 °C, whilst an optimal temperature for larval development of *An. gambiae* is between 28 and 32 °C but survival rate to adulthood is highest between 22–26 °C (*Bayoh & Lindsay, 2003*, *Christiansen-Jucht et al., 2014*). This reflects the higher temperature tolerance of *An. arabiensis* compared to *An. gambiae* (*Kirby & Lindsay, 2004*) which itself may be responsible for the extended distribution of the former species into hotter and drier environments in Africa. *Anopheles funestus*, on the other hand, has a single optimum temperature of 25 °C for development with substantial declines in survival either side of this (*Christiansen-Jucht et al., 2014*). *Le Sueur & Sharp (1991)* concluded that the effect of temperature on *An. merus* (another member of the *An. gambiae* complex) was greatest during metamorphisis in the pupal stage, as did *Heuval van den (1963)* for *Aedes aegypti*. Temperature-related metabolism during metamorphosis was affected by available energy reserves (gathered during larval development) (*Le Sueur & Sharp, 1991*). At higher temperatures reserves are lower than at lower temperatures, hence emerging adults are smaller. In the laboratory none of the *An. gambiae* complex or *An funestus* survived as larvae or pupae at temperatures above 35 °C. In the field, however, larvae and pupae of *An. gambiae* s.l. (probably *An. arabiensis*) have been found in pools at temperatures of 40.5–41.8 °C (*Holstein, 1952*, quoted in *Gillies & De Mellion, 1968*).

The differential response to temperature in the laboratory reflects the different kinds of water in which larval development takes place in the field. Thus, although immature forms of the *An. gambiae* complex may occur in a great variety of water bodies, the most characteristic are the 'shallow open sun-lit pools with which every field worker in Africa is familiar' (*Gillies & De Mellion, 1968*). Immatures of *An. funestus* are generally found in more permanent, shaded, water bodies with emergent vegetation that are cooler than exposed puddles.

Diurnal fluctuations in temperature also affect the development of many insects (*Vangansbeke et al., 2015*). In addition to being hotter, temperature fluctuations in small pools are greater than those in larger, shaded, bodies of water. For example, although temperature minima in pools typically used by *An. gambiae* are similar to the minima in shaded ones, maxima may be 10 °C higher (*Haddow, 1943*) and, not surprisingly, given their larval habitat, both *An. gambiae* and *An. arabiensis*, also respond better to fluctuating temperatures than do *An. funestus* (*Lyons, Coetzee & Chown, 2013*; *Christiansen-Jucht et al., 2014*).

In contrast to the larvae, adults of both *An. gambiae* s.l. and *An. funestus*, experience similar microclimates due to their predominantly endophilic behaviour. Temperature influences the time it takes for egg development following a blood-meal but may also have subtler effects. For example, *An. funestus* delays returning to feed following oviposition at temperatures above 26.5 °C, but at lower temperatures females re-feed shortly after egg laying (*Gillies & Wilkes, 1963*). Ironically, the extra time spent in returning to feed at higher temperatures is compensated for by it taking two rather than three days for the mosquito to complete egg development, so that the duration of the complete gonotrophic cycle is three days at all temperatures (*Gillies & Wilkes, 1963*).

The effect of temperature on the larval stages is manifest in the emergent population (*Beck-Johnson et al., 2013*). Newly emerged insects can be distinguished from the mature population by their abdominal and gonotrophic state. Newly emerged males have un-rotated terminalia (*Charlwood, 2011*) whilst females have undeveloped ovaries and constitute the unfed portion of the resting or exiting population from houses (*Gillies & Wilkes, 1965*; *Charlwood, Thompson & Madsen, 2003*). The relative proportions of engorged to gravid females at different temperatures also provides information on the duration of egg development in mature insects.

Surprisingly, there remains a lack of comprehensive data on the effects of temperature and other environmental factors on mosquito population dynamics in the wild. Possible effects of temperature on mosquitoes in the field are most easily observed in areas with a wide variation in both daily and seasonal temperatures. Wild mosquito populations are, however, notoriously unpredictable and short-term, chaotic, fluctuations are common. Long-term observations can assist in reducing the 'noise' in such data. Here we describe the effect of ambient temperature, and other environmental parameters, over a five-year period, on the dynamics of newly emerged and mature *An. funestus* and *An. gambiae* s.l. Insects were collected from a single village in southern Mozambique where temperatures varied from a night-time minimum of 6 °C in the cool season (10.5 °C below the lower limit of 16.5 °C for larval activity, (*Jepson, Moutia & Courtis, 1947*)) to a daytime maximum of 35 °C in the hot season.

METHODS

Description of study site

The approximately 5 × 4 km village of Furvela, (23°43′S, 35°18′E), 475 km north of the capital Maputo, is bordered on two sides by the alluvial plain of two river systems (Fig. 1). The Furvela River valley to the north of the village in particular has a considerable amount of local irrigation for agriculture, which provides a large and relatively stable number of small canals. The Inhnanombe river to the east of the village consists largely of beds of the reed (*caniço*), used for housing, and sugar cane, used in the production of local alcohol; it does not flow as fast as the Furvela river. *Anopheles funestus* predominates on the Furvela River side of the village and *An. gambiae* s.l. on the Inhnanombe side (*Kampango et al., 2013*). Malaria is endemic in the village with 80% of 2–4 year olds being positive in a cross sectional survey undertaken in 2006 whilst children under one year of age comprised the majority of attendees at a clinic established by the project in 2001 (Files S1 and S2).

Figure 1 **Google Earth image of Furvela Village.** Furvela Village and its environ© Google Earth; Image 2016 ©CNES/ Astrium. Note the location of Linga Linga where information on the malaria and mosquitoes is also available (*Charlwood et al., 2013*; *Charlwood et al., 2015*).

Houses in the village are generally made with *caniço* walls and palm thatch roofs. Although most houses don't have windows the majority have a *ca.* 15 cm gap between the roof and walls at either end of the house. Doors and doorframes are also generally badly fitting; hence mosquitoes can easily enter these houses. Other styles of house include those with corrugated iron sheets for the roof and those made of concrete blocks (which do have windows). Houses are built either in family compounds of three to six houses or as relatively evenly spaced individual homes.

At the start of the study houses were mapped with handheld Global Positioning Units (Garmin etrex), numbered and the manner of construction and size noted (Files S3 and S4). A census was taken and residents were informed about the purpose of the study and consent concerning the possibility of future mosquito collections obtained. The initial study area was expanded in the second year of the study and mapping was also repeated in 2007.

Mosquito Collection
Light-traps

Host seeking mosquitoes were collected in CDC light-traps hung, inside bedrooms, approximately 1.5 m from the floor at the foot of the bed of people who themselves were sleeping under mosquito nets. A random list of houses was produced (using the random number generator in Microsoft Excel) and routine collections were made according to the

list. In addition, a number of houses known to have high densities of mosquitoes were used as sentinel sites. Collections were made in 764 houses on the Furvela river side of the village and 214 on the Inhnanombe side of the village. Eleven houses were used for sentinel collections; each being sampled for more than 100 nights.

Exit collection

From 2003 to 2007 mosquitoes were also collected exiting houses at dusk (*Charlwood, 2011*). The door of the house was left open and covered with a white mosquito-netting curtain. Mosquitoes were manually aspirated off the curtain as they attempted to leave. See: https://www.youtube.com/watch?v=SL8FeIuY1GM.

Most of the houses used for the exit collections were on the Furvela side of the village. The young men and women who did the work, and who lived in dispersed locations collected from their own or nearby houses. Altogether collections were made from 501 houses some of which also acted as sentinel sites.

Resting collection

Resting collections, using a torch and an aspirator, were performed on the Furvela side of the village, on an *ad hoc* basis on 163 days, from a total of 132 houses (mean number of 3.9 collections per house) where mosquito nets were not in use, and, initially, outdoors.

Mosquito processing

Collected *Anopheles* were separated into species or species group, according to the keys of *Gillies & De Mellion (1968)* and *Gillies & Coetzee (1987)* and sexed. Females were further separated into unfed, part-fed, engorged, semi-gravid and gravid categories. A number of the *An. gambiae* s.l. were identified to species by PCR. DNA extraction was performed individually following the protocols of *Collins et al. (1988)* and the species identified using the protocols of *Scott, Brogdonw & Collins (1993)*. A small number of *An. funestus* were also identified by PCR using the protocols of *Koekemoer et al. (2002)*.

Meteorology
Temperature, insolation and windspeed measurement
Daily variation estimates of soil and air temperature, insolation and windspeed were obtained with a Delta-T digital weather station (Delta-T Devices, Cambridge, UK) that recorded hourly information at the edge of the village. Soil temperatures approximate those that larvae are exposed to whilst air temperatures are those that more closely approximate those that adults may be exposed to. Unfed females exiting houses at dusk are newly emerged (*Charlwood, Thompson & Madsen, 2003*) and reflect the effect of temperature on the larvae whilst the ratio of unfed to gravid insects may reflect temperature effects on the adults. Unfortunately, the weather station did not operate throughout the study, nor did the humidity or rainfall meter work consistently. The longest hourly data sets were from 3rd May 2004 to 1 October 2005 and from 10 Nov 2007 to 24 Nov 2008. Hourly data from all years, including the later ones, was amalgamated into daily data and daily data amalgamated into ISO weeks. Mean values for the different ISO weeks from all the weather station files were determined and used in calculations (File S5).

Long term temperature data recorded at Vilanculos, a town ~200 km north of Furvela, were also obtained (long-term data available from http://www.tutiempo.net).

Rainfall data

Rainfall data was available from the town of Maxixe, 20 km to the south of Furvela. Since the distribution of rainfall is important (20 mm falling on seven consecutive days in a week is likely to have a different effect than 140 mm falling on a single day) a modified measure of rainfall was used to estimate effects:

- Modified weekly rain = (Rain (mm) * #rain days)/7
- Thus 140 mm on a single day is equivalent to $(140 * 1)/7 = 20$
- And 140 mm with rain every day is equivalent to $(140 * 7)/7 = 140$.

The daily, weekly and monthly records of rainfall over the period 2000–2010 are available at File S6 (Rainfall data).

Analysis

Data were entered into, and analyzed with, Excel (File S6). Unfed mosquitoes from light-trap collections represent all age groups. Unfed mosquitoes from exit collection are, however, almost entirely newly emerged ones (*Charlwood, 2011*), whilst gravid females have taken at least one blood meal sufficient to develop eggs, and will also include infectious ones. The weekly Williams mean ($\log_{10}(n+1)$) of these three groups of *An. funestus* and *An. gambiae* s.l. were compared to mean, maximum and minimum temperatures, temperature difference, insolation and wind speed, measured in Furvela, and modified rainfall measured in Maxixe.

The relationship between mosquito numbers and environmental factors was examined using bivariate correlations, and Pearson's correlation coefficient (2-tailed P value ≤ 0.05 significance). Least squares multiple linear regression (with climatic factors as independent variables) was also undertaken using the Excel add-in StatPlus. The most parsimonious model was determined by subtraction of least important variables.

Ethics

The study was conducted under the aegis of the joint Instituto Nacional de Saúde (INS)—DBL Centre for Health Research and Development project 'Turning houses into traps for mosquitoes', which obtained ethical clearance from the National Bioethics Committee of Mozambique on 2 April 2001 (ref: 056/CNBS/01). Householders were informed about the purpose of the collections. Verbal consent was obtained when collections were initiated.

RESULTS

Environmental variables

Mean temperatures recorded at Vilanculos were higher than those recorded in Furvela, but both followed a similar pattern (File S7 Temperature data). There was both a marked seasonality in temperatures and considerable variation from one day to the next. Mean soil and air temperatures from Furvela, derived from hourly measurements, 10 Nov 2007–24 Nov 2008 are shown in Fig. 2. Diurnal variation in temperature differed between cool and
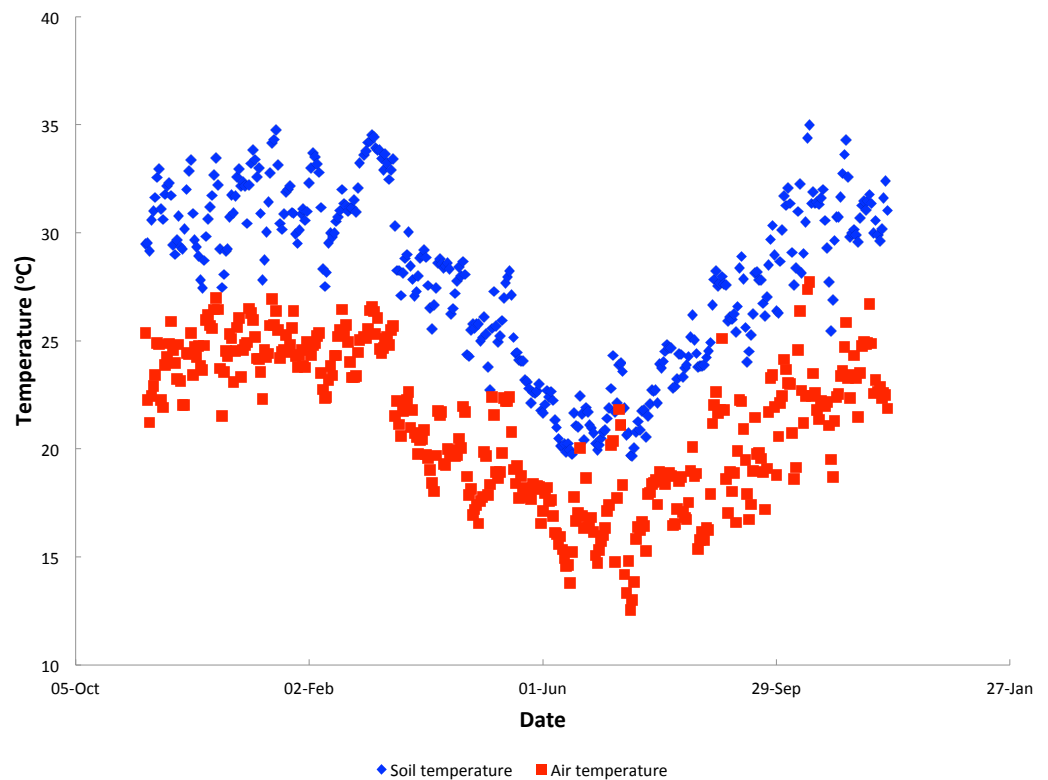
Figure 2   Annual temperature variation. Mean daily soil and air temperatures recorded by the Delta logger in Furvela village.

hot seasons. Figure 3A shows the diurnal pattern recorded on the three coolest nights of the year (16–18th July) and 3B the three warmest (14–16th October). Overall at the higher temperatures daily variation (difference between maximuim and minimum temperature) was less than it was at the cooler temperatures (Fig. 4). At a mean of 23.7 °C the variation in air temperatures was 5.9 degrees and at 18.1 °C was 15.7 degrees. Variation is soil temperature was lower than air temperatures being 4.1 degrees at 30.3 °C and 8.9 degrees at 28.2 °C.

Except for the mornings mean soil temperatures were consistently circa 5 °C warmer than air temperatures. Mean amounts of insolation showed a similar pattern to temperature.

Mosquito data

Twenty-six (86%) of 30 males and 331 (81.3%) of the 407 females from an unselected sample of the *An. gambiae* complex identified by PCR from 2002 and 2004 comprised *An. gambiae*, the other species being 4 male and 67 (16.5%) female *An. arabiensis* and nine (2.2%) female *An. merus* (Table 1). There was no statistical difference in the ratio of *An. gambiae* and *An. arabiensis* according to method of collection (light-trap, exit collection or resting collection) (File S8).

All of *An. funestus* examined morphologically had a single pale spot on the upper branch of the 5th vein and did not have a pale spot at the tip of the 6th vein and corresponded to
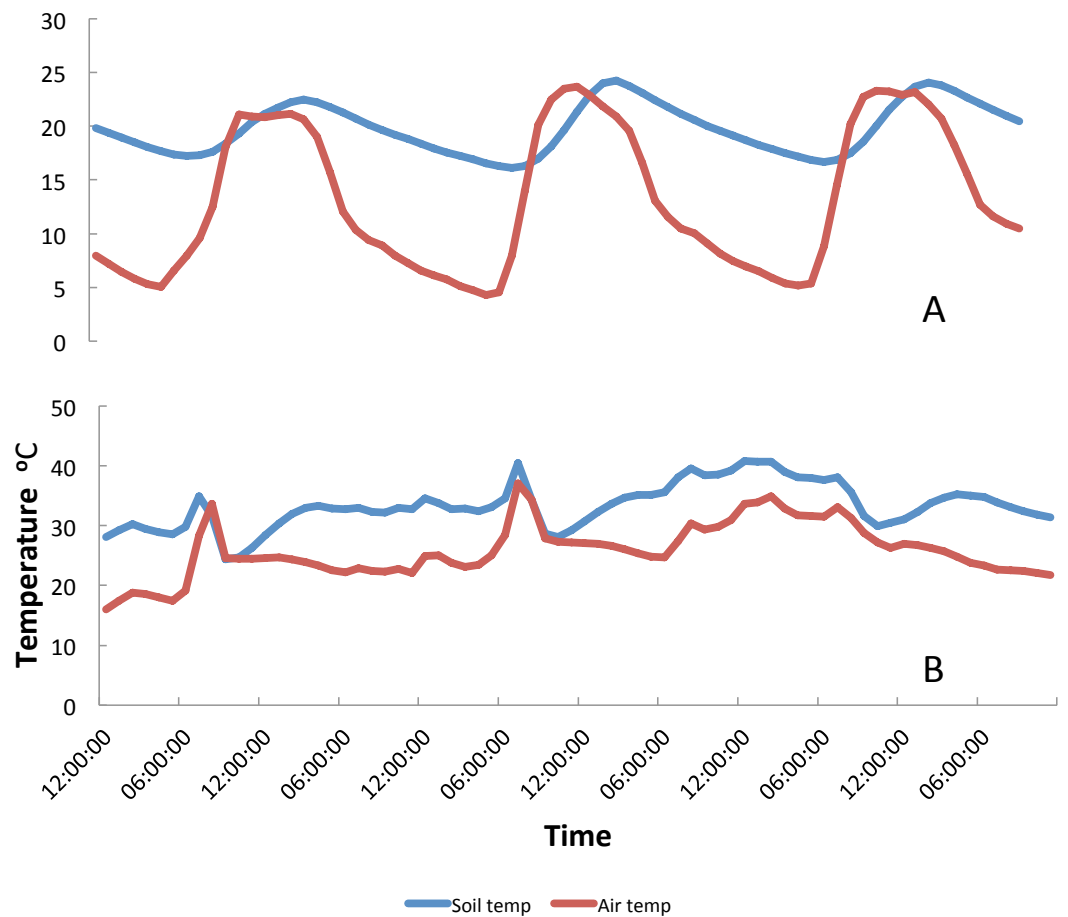
Figure 3 Daily variation in temperature during (A) the coolest months of the year and (B) the warmest months of the year, Furvela, Mozambique

Table 1 PCR identifications of members of the *An. gambiae* complex collected from light-traps, Furvela Village, Mozambique.

Year	2002		2003		2004		Total	
Species	N	%	N	%	N	%	N	%
A. arabiensis	16	27.1	35	23.3	20	8.8	71	16.2
A. gambiae	43	72.9	108	72.0	206	90.4	357	81.7
A. merus	0	0.0	7	4.7	2	0.9	9	2.1
Total per year	59	100.0	150	100.0	228	100.0	437	100.0

An. funestus. Seventy-one females of the *An. funestus* group were identified by PCR (Fil S9, courtesy of A. Szlanski). All were *An. funestus*. Given that this is the endophilic member of the species group, and that it was endophilic behavior that was studied, it is assumed that this was the only member of the species group present in our collections.

301, 705 female *An. funestus* were collected in 6,043 light-trap collections, 161, 466 in 7,397 exit collections and 16, 995 in 1,315 resting collections. The equivalent numbers for *An. gambiae* s.l. are 72, 475 in light-traps, 33, 868 in exit collections and 5, 333 from indoor
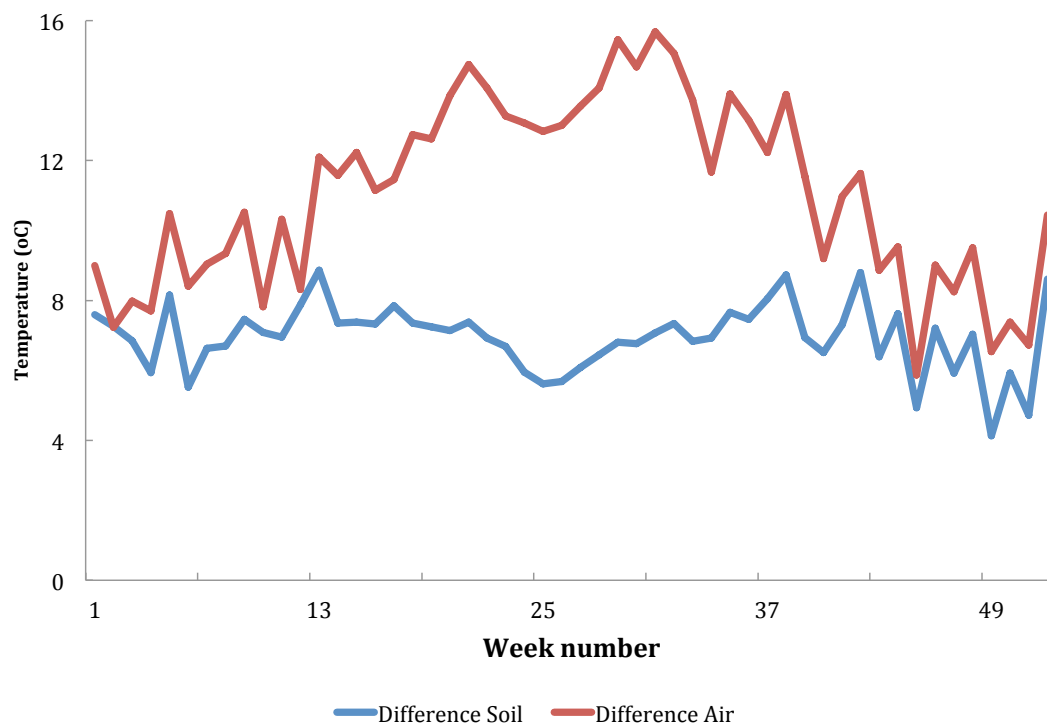
Figure 4 **Difference between maximum and minimum air and soil temperature recorded from Furvela village, Mozambique, by ISO week number.**

resting collections (File S10—Raw data). Outdoor resting collections failed to produce any mosquitoes. Other anopheline species collected in light traps included 5776 *Anopheles tenebrosus*, 725 *Anopheles letabensis*, 22 *Anopheles rufipes*, five *Anopheles squamosus*, and a single *Anopheles pharoensis*. A further 219 *An. tenebrosus* and five *An. rufipes* were collected exiting houses.

Figure 5 shows the weekly mean numbers collected per house per night of *An. funestus* and *An. gambiae* s.l. in light traps and exit collections over the course of the study in conjunction with temperatures recorded at Vilanculos and modified rainfall from Maxixe. Over the three years when both light trapping and exit collections were simultaneously undertaken (2003–2006) mean numbers of *An. funestus* per house, per method, were similar. In 2007 a cordon sanitaire of long lasting insecticide nets (LLIN's) was established around the Furvela River valley (JD Charlwood, 2007, unpublished data) and numbers in exit collections decreased relative to numbers in light-traps. Numbers of *An. gambiae* s.l. in exit and light-trap were also similar. Over the course of the project, however, *An. gambiae* s.l. disappeared from both light-trap and exit collection collections. Given the possible effect of the *cordon sanitaire* on numbers collected further analysis is confined to the years 2001–2006 (when 5,090 light-trap, 4,461 exit and 1,315 resting collections were performed).

Numbers of mosquito were greatest in the warmer months. Figure 6 shows the mean adjusted rainfall from Maxixe, mean soil and air temperature and wind speed from Furvela, mean numbers of unfed *An. funestus* and *An. gambiae* s.l. collected in light-traps and mean
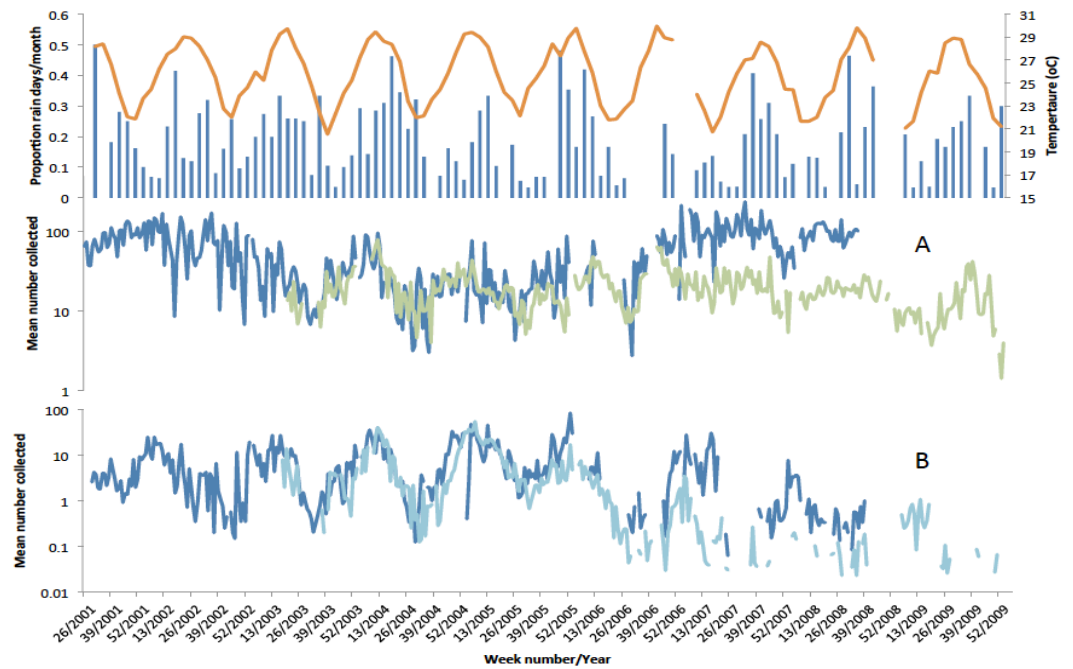
Figure 5 Temperature, rainfall and number of mosquitoes collected in Furvela. Upper graph—Rainfall (measured in Maxixe) (Blue histogram), air temperature (measured in Vilanculos) (orange line) (A) mean numbers of unfed Anopheles funestus form light-traps (blue line) and in exit collections (green line) (B) mean numbers of unfed Anopheles gambiae collected from light-traps (Dark blue line) and in exit collections (light blue line) from Furvela village, 2001–2009. Note the log scale.

numbers of unfed and gravid insects by species from exit collections by ISO week in the years 2001–2006. Unfed insects in exit collections comprised the newly emerged portion of the population. Gravid insects represent the gonotrophically active (mature) population. The proportion of that population that they represent depends on the duration of oogenesis following a blood meal. At warmer temperatures development takes two days and so the gravid sample represents half of the mature population whereas at cooler temperatures development may take three days and so the gravid sample will only represent one third of the mature population. The whole of the newly emerged population, on the other hand, is sampled every day.

The maximum Pearson correlation co-efficients between mosquito numbers and individual environmental parameters are shown in Table 2. Table 2 also gives the correlation co-efficients between the different mosquito groups. The highest environmental correlations and mosquito numbers were between temperature and all collections of *An. gambiae* s.l. (see File S11 for the other possible correlations). Adding a lag of up to four weeks to the light-trap data from either species did not improve the Pearson correlation co-efficient between rainfall and numbers (Table 3) Correlation co-efficients were always lower in comparisons involving *An. funestus*.

Figure 7A gives the relationship between numbers of *An. funestus* and *An. gambiae* s.l. caught in light-traps and air temperatures and Fig. 7B between mosquito numbers and soil temperature ($r^2 = 0.52$ for *An. funestus* and 0.77 for *An. gambiae* s.l.). Although both
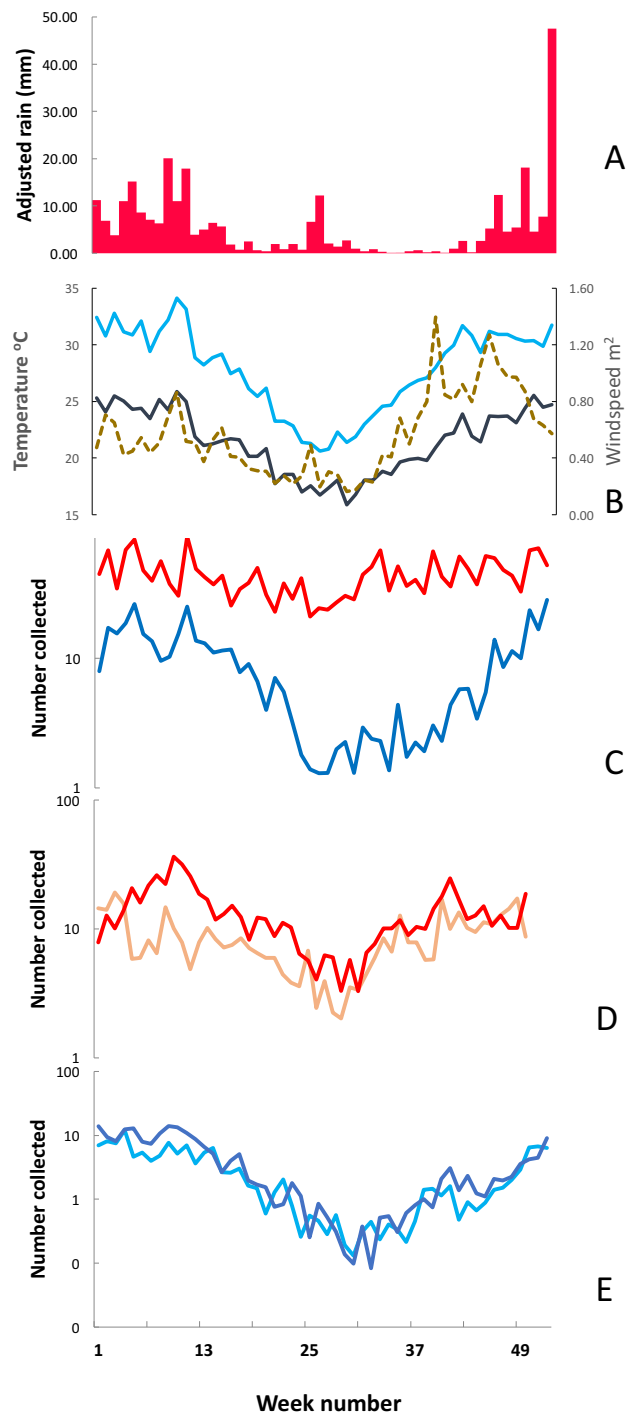
Figure 6 Weekly mean adjusted rain, soil temperature, air temperature, windspeed and mosquito numbers. Environmental parameters and mosquito numbers by ISO week, Furvela, Mozambique. (A) mean weekly adjusted rain in millimetres (measured in Maxixe), (B) Light blue line —mean soil temperature (in degrees Centigrade); Black solid line Mean air temperature (in degrees centigrade); dotted line —mean wind-speed (in metres per second), (C) Mean number of *Anopheles funestus* (red line) and *An. gambiae* s.l. (blue line) collected from light traps, (D) Mean number of unfed (pink line) and gravid (red line) *An. funestus* from exit collections,(note the log scale) (E) Mean number of unfed (light blue line) and gravid (dark blue line) *An. gambiae* s.l. from exit collections,(note the log scale).

Table 2 Correlation coefficients between mosquitoes and climate. Correlation coefficients between weekly mean numbers of mosquitoes according to the collection method and the highest correlation co-efficient by environmental variable, Furvela, Mozambique 2003–2006.

Species	Sample	Environmental variable	Correlation co-efficient	p
Anopheles funestus	Light-trap	Air temperature	0.5352	>0.0005
	Unfed exit	Min air temperature	0.71959	>0.0005
	Gravid exit	Max Solar	0.68915	>0.0005
	Unfed exit	Gravid exit	0.25169	0.071
Anopheles gambiae s.l.	Light-trap	Air temperature	0.75105	>0.0005
	Unfed exit	Air temperature	0.73002	>0.0005
	Gravid exit	Air temperature	0.74211	>0.0005
	Unfed exit	Gravid exit	0.86102	>0.0005
funestus/gambiae	Unfed exit	Unfed exit	0.58979	>0.0005
	Gravid exit	Gravid exit	0.44756	0.0009

Table 3 Correlation between mosquitoes and rainfall. Pearson correlation co-efficients between adjusted rainfall and numbers of *An. gambiae* s.l. and *An. funestus* collected in light traps at lags of 0–4 weeks.

Lag (weeks)	*An. gambiae* s.l.	*An. funestus*
0	0.671	0.245
1	0.435	0.285
2	0.517	0.275
3	0.358	0.150
4	0.379	0.248

species increased significantly as temperatures increased, numbers of *An. gambiae* s.l. went through a one hundredfold change (from a mean of 0.14 mosquitoes a night to 14) whereas numbers of *An. funestus* merely doubled (from a mean of 20 to 40 a night).

The number of unfed and gravid insects in exit collections by mean air temperature are shown in Figs. 8A and 8B. At temperatures below 28 °C the mean number of gravid *An. funestus* collected increased as the temperature increased; and at a faster rate than the rate of increase in immature insects but at temperatures above 28 °C the number decreased whilst numbers of newly emerged insects continued to increase (Fig. 8A). Numbers of both gravid and unfed *An. gambiae* complex females in exit collections continued to increase at all temperatures recorded (Fig. 8B). The proportion of the *An. gambiae* s.l. population that was gravid was more variable at lower temperatures. This was probably due to the small sample sizes at these temperatures. The proportion of *An. funestus* on the other hand was more variable at the higher temperatures but why this should be so remains unknown since the data comes from a time when large-scale interventions had not been applied. Overall the numbers of gravid and unfed *An. funestus* collected in exit collections were not correlated ($p = 0.07$).
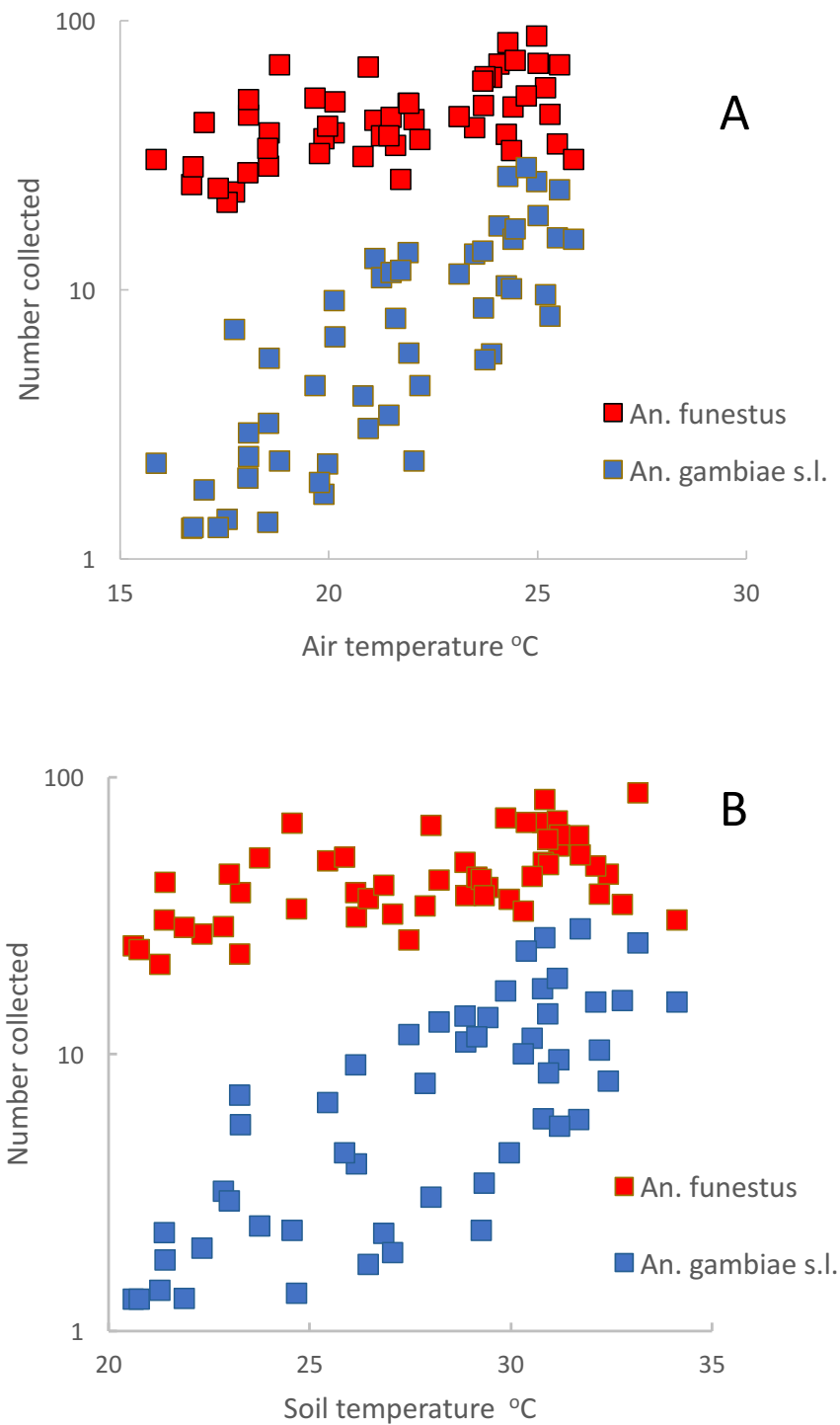
Figure 7 Mean numbers of *Anopheles funestus.* (red) and *An. gambiae* s.l. (blue) collected in light-traps from Furvela village, Mozambique (A) by air temperature and (B) by soil temperature (in degrees Centigrade).
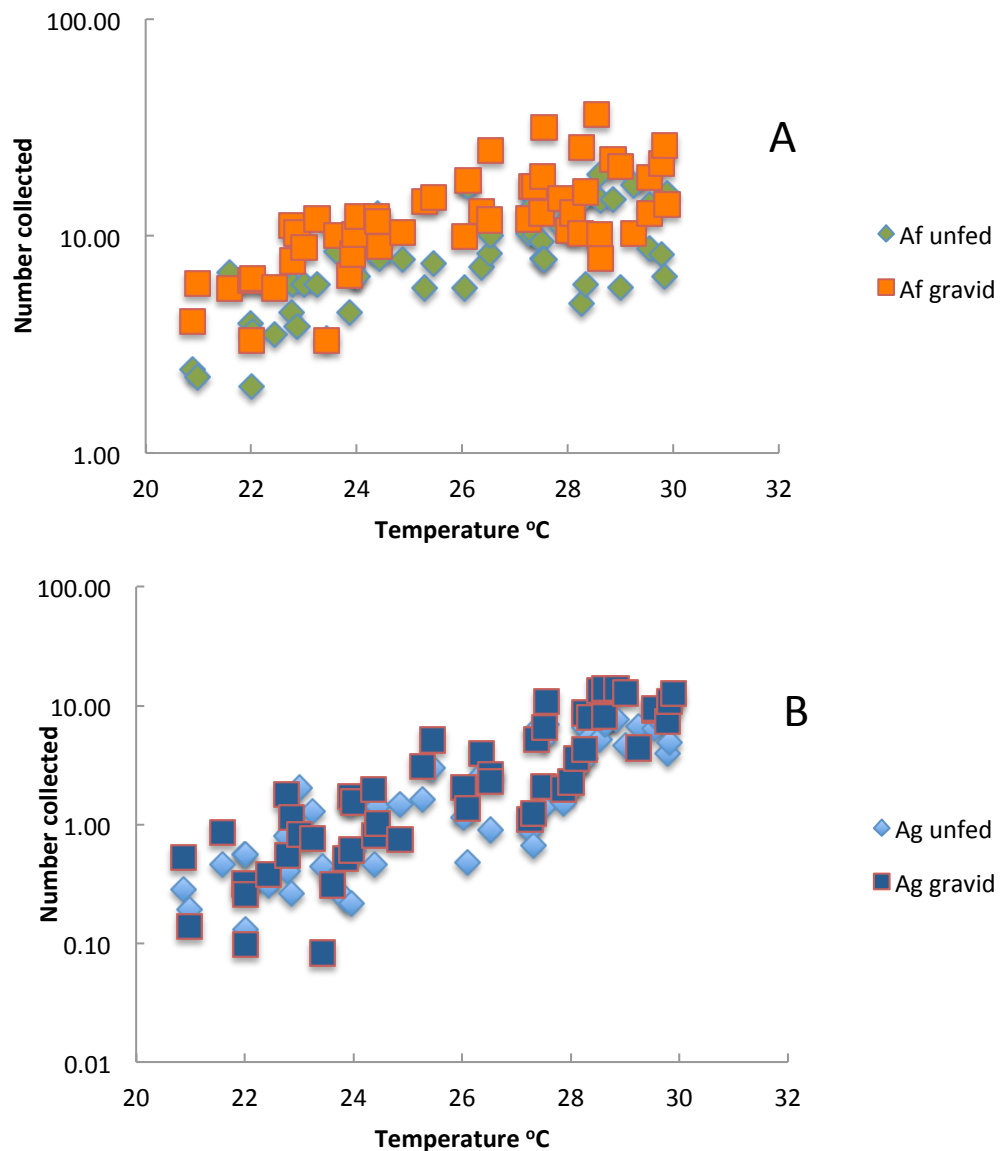
Figure 8 The relationship between the mean number of (A) *Anopheles funestus* and (B) *An. gambiae* s.l. in exit collections and soil temperature Furvela, Mozambique.

The best models for each species and each category of mosquito are given in Table 4. Adjusted rainfall was only included in one model. The models explained more of the variation in *An. gambiae* s.l. than they did for *An. funestus* although the environmental parameter used in the models, with the exception of the best model for *An. funestus* and *An. gambiae* s.l. in light-traps, were the same. The explanatory values (the adjusted R^2) were all higher for the *An. gambiae* s.l. than for the *An. funestus*.

The abdominal status of mature females collected from 1315 resting collections and mean monthly temperature is shown in Fig. 9. For both, species, or species group, a higher proportion of semi-gravid and gravid females compared to engorged females were collected

Species	Category	Parameters	R^2	p
An. funestus	Unfed exit	Air temp + windspeed	0.611	>0.00005
	Gravid exit	Soil temp + windspeed	0.563	>0.00005
	Unfed Light	Air temp + Rain	0.344	>0.00005
An. gambiae s.l.	Unfed exit	Air temp + Windspeed	0.752	>0.00005
	Gravid exit	Soil temp + Windspeed	0.822	>0.00005
	Unfed Light	Air temp + Windspeed	0.756	>0.00005

Notes.

The equations for the different models are listed below:

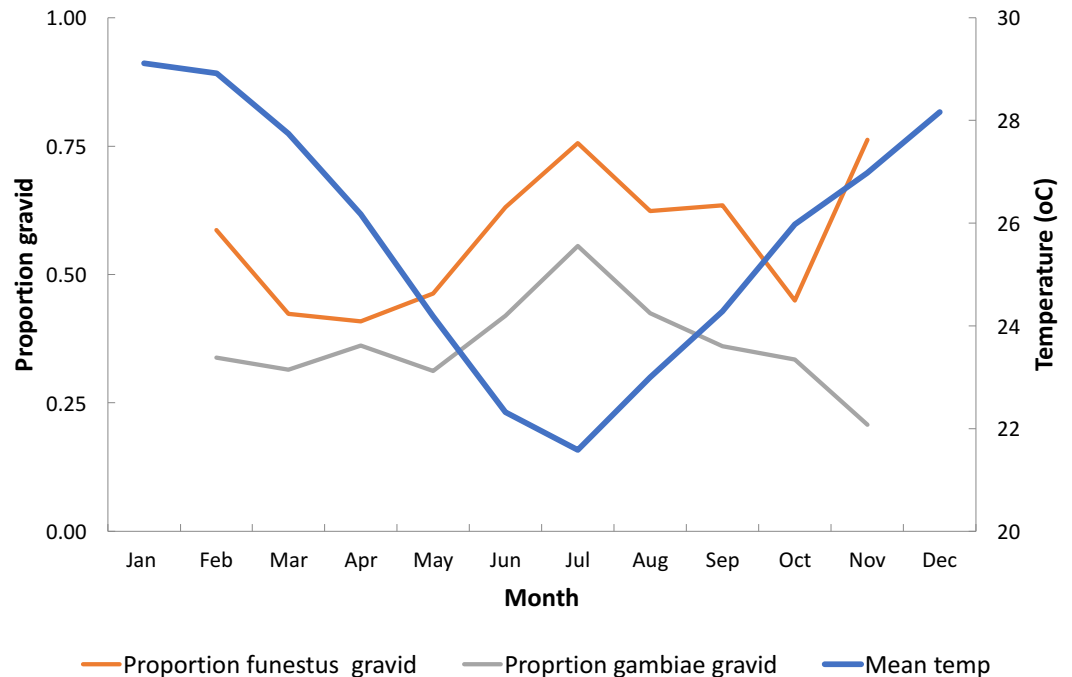Log *An. funestus* Light-trap $= +0.9178 - 0.445*$log rain $+ 0.0342*$Air temp.

Log *An. gambiae* s.l. Light-trap $= -1.419 + 0.117*$Air temp $- 0.392*$Windspeed.

Exit *An. funesus* unfed $= -0.100 + 0.044*$Air temp $+ 0.156*$Windspeed

Exit *An. funestus* gravid $= -0.099 + 0.0456*$Soil temp $- 0.111*$Windspeed.

Exit *An. gambiae* s.l. unfed $= -1.629 + 0.1097*$Air temp $- 0.446*$Windspeed

Exit *An. gambiae* s.l. gravid $= -2.05 + 0.10869$ Soil temp $- 0.734*$Windspeed.

Figure 9 Proportion of *Anopheles funestus* and *An. gambiae* s.l. indoor resting that were gravid at the time of collection and mean temperature, Furvela Mozambique.

during the cooler months of the year (May–August). In other words, oogenesis was taking longer at the lower temperatures.

The proportion gravid to engorged *An. funestus* of 50% occurred at 25 °C. Thus, at these temperatures, and above it took two days to mature the ovaries and below this three days post-feeding to mature them. The proportion gravid of *An. gambiae* s.l. from resting catches was always lower than that of the *An. funestus* and only reached 50% at the highest temperatures. At mean temperatures of 21.5 °C 76% of the *An. funestus* collected were

semi-gravid and gravid. This implies that it was taking not just three but four days to complete gonotrophic development.

DISCUSSION

With the possible implications of Global Warming, the effect of temperature and other environmental parameters on the dynamics of malaria vectors in Africa is an area of increasing interest. The temperature in Furvela fluctuates between the minimum and optimum temperature for mosquito development, hence over the linear part of the reaction norm. Temperature was the most important environmental parameter, of those measured, determining mosquito numbers in the village. Although a more in depth assessment would probably improve the interpretation of the results, even in the straightforward analysis presented here mean daily temperatures from either air or soil sensors explained 70% and 35% of the density changes observed in *An. gambiae* s.l. and *An. funestus* respectively (Table 2). As expected, given its rapid developmental time, rates of increase were substantially higher in the *An. gambiae* s.l. compared to the *An. funestus.* Sporozoite rates were also highest during the warmer months of the year (File S12). The combined effect on the entomological infection rate (EIR) and malaria morbidity in the village will be examined elsewhere.

The population dynamics of *An. funestus* on the peninsula of Linga Linga across the Morrumbene Bay (Fig. 1) is determined by rainfall (*Charlwood et al., 2013*). The absence of a relationship between rainfall and mosquito numbers, particularly the *An. gambiae* s.l., in Furvela is surprising. It may be due to the sandy soil in the village which means that rain is rapidly absorbed and the puddles are rare or absent. At the same time breeding conditions for the *An. funestus* in the valley remain reasonably constant due to the irrigation practices of the local farmers and even major storms have only a temporary effect on the dynamics of the mosquito (*Charlwood & Braganca, 2012*).

The ratio of gravid to unfed mosquitoes in exit collections depends on a number of factors, in particular house construction. The two sets of females enter the house at different times (unfed newly emerged insects entering at dawn to rest and, soon-to-be-gravid, host seeking females, to feed throughout the night). They use different cues (visual contrast and odour) and enter through different routes (open doors and eaves) (*Gillies, 1988*). Thus, houses that may allow access for one group are not necessarily suitable for the other. In addition to house effects, the proportion of egg development time spent inside houses (which is presumed to be 100%, at least for *An. funestus*), the survival rate per oviposition cycle and the duration of oogenesis, can all affect the unfed/gravid ratio. Should any of these factors change with temperature then the overall ratio will also change with temperature. The absence of change, as occurred with the *An. gambiae* s.l. implies that these factors remained constant, or compensated exactly, over the observed range of temperatures.

Ironically, the highest correlation between gravid insects in exit collections (for both species or species group) was with soil temperature (which mimics water temperature) whilst for unfed (newly emerged) insects it was with outdoor air temperature. As pointed out by *Paaijmans et al. (2010)* the micro-climate experienced by the mosquito inside houses may be quite different to that outside. Houses may be warmer in the cool season and cooler

in the hot season than temperatures recorded outside. Nevertheless, more sophisticated measurement would only improve models for *An. gambiae* by a maximum of 30% and for *An. funestus* by 47% (and would imply that other factors, such as humidity, were of lesser importance).

Unlike *An. funestus* both newly emerged and gravid *An. gambiae* s.l. increased in a similar fashion through the whole range of temperatures experienced in Furvela. There was no apparent effect of increasing temperatures on survival and the proportion of gravid to unfed insects remained more or less constant at all temperatures. The unfed/gravid ratio of the more common *An. funestus* did, however, change with temperature. As temperatures increase above 26.5 °C a higher proportion of gravid *An. funestus* is to be expected in exit collections since the duration of oogenesis is reduced from three to two days (*Gillies & De Mellion, 1968*). At the temperatures recorded in July it might take three or more days, as evidenced in the resting collections and formerly described by Gillies and Wilkes *Gillies and Wilkes (1963)* and *Gillies and Wilkes (1965)*. At the higher temperatures exit collections would therefore be expected to sample one half of the mature population (the other half being the semi-gravid insects that may move from one resting site to another, but in the absence of disturbance, do not leave the house) but at cooler temperatures only one third, or even less, of the population. At the higher temperatures, however, the proportion of gravid insects in the exit collections decreased, such that overall there was no significant relationship between the numbers of gravid and unfed insects in exit collections. This either means that that survival between emergence and maturity (i.e., becoming gravid) decreases at cooler temperatures or that post-teneral insects have a higher mortality at higher temperatures. Both sets of insects leave houses at the same time (*Charlwood, 2011*), hence sampling efficiency for the two groups should be the same.

A reduced survival among post-teneral adult *An. funestus* at the higher temperatures is possible as described from the laboratory (*Christiansen-Jucht et al., 2015*). High temperatures, independent of humidity, can have a lethal effect because as body temperature increases, metabolism and respiration increase up to a critical thermal limit, and there is a loss of integration between protein stability and metabolic processes that leads to death. *Anopheles gambiae* s.l. are larger than *An. funestus.* Volume to surface ratios differ and this may influence the ability of the adult insects to survive higher temperatures. Larger mosquitoes have a smaller surface to volume ratio and larger water reserves, which would allow them to offset the respiratory and cuticular water loss.

With one exception, windspeed was the only environmental parameter, other than temperature, included any model. Together they explained up to 82% of the *An. gambiae* changes and 61% of the *An. funestus*. The exception was *An. funestus* in light traps in which adjusted rain was included. Although still significant, this model had the lowest explanatory value (34%) of all the models.

Recently *Paaijmans et al. (2010)* have described that in addition to mean temperatures 'the key mosquito-related traits that combine to determine malaria transmission intensity (i.e., parasite infection, parasite growth and development, immature mosquito development and survival, length of the gonotrophic cycle, and adult survival) are all sensitive to daily variation in temperature'. Fluctuations in temperature (i.e., the difference between

maximum and minimum temperatures) were greatest in the cooler months. In the cool season the observed patterns in soil and air temperature were similar to shaded and open water as determined by *Haddow (1943)*. In the warmer months' fluctuations in temperature were less than at lower temperatures. Fluctuations around low mean temperatures can speed up rate processes, whereas fluctuations around high mean temperatures can slow them down (*Paaijmans et al., 2010*). Thus, the *An. gambiae* s.l. were well suited to the temperature regimes experienced in Furvela.

Unfortunately, the species composition of the resting or exiting *An. gambiae* s.l. compared to those entering the house is not known. Nevertheless, the proportion of gravid insects in resting catches varied in a similar fashion to that seen among the *An. funestus*. It is also possible that the different members of the *An. gambiae* complex behaved differently or disappeared from the study area at different rates. Nevertheless, all members of the complex did apparently disappear during the study so that, perhaps it was not just a specific species that was affected but was a complex wide problem. *Meyrowitsch et al. (2011)* were unable to determine the cause of the decline of *An. gambiae* s.l. in the Tanga region of Tanzania, three thousand kilometers to the north of Furvela. In the Kilifi area of Kenya population decline of *An. gambiae*, shown by a reduction in genetic diversity in the mosquito, was attributed to the introduction of LLIN's (*O'Loughlin et al., 2016*). The decline in Furvela started before the introduction of any control measures and although the introduction of LLIN's may have exacerbated the problem for the mosquito it may not have been the cause of the decline in the first place. The decline also paralleled that observed in malaria in the Rufiji basin (*Ishengoma et al., 2013*). That similar declines appeared to occur over a 3,000 km stretch of coastline indicates that a climatic factor was responsible.

CONCLUSION

Temperature is a major driving force in the dynamics of both *An. funestus* and *An. gambiae* s.l. in Furvela. Whilst gravid *An. funestus* appeared to be negatively affected by the highest temperatures encountered increasing temperatures were favorable for the *An. gambiae* s.l. which went through hundredfold changes in densities as a result. As average temperatures increase due to Global Warming areas where *An. gambiae* is the vector, if there remains sufficient water available for larval development, will be at heightened risk of malaria transmission.

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Competing Interests
The author declares there are no competing interests.

Author Contributions
- Jacques Derek Charlwood conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

Human Ethics
The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Ethical clearance from the National Bioethics Committee of Mozambique on 2 April 2001 (ref: 056/CNBS/01). Householders were informed about the purpose of the collections. Verbal consent was obtained when collections were initiated.

Data Availability
The following information was supplied regarding data availability:

The raw data has been supplied as a Supplemental Dataset.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.3099#supplemental-information.

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