Charlwood JD. 2017. Some like it hot: a differential response to changing temperatures by the malaria vectors *Anopheles funestus* and *An. gambiae* s.l. PeerJ 5:e3099 https://doi.org/10.7717/peerj.3099

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

Jacques D Charlwood

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 6043 light-trap collections, 161, 466 in 7397 exit collections and 16, 995 in 1315 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.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016

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

J.D. Charlwood,

Mozambican-Danish Rural Malaria Initiative (MOZDAN), P.O. Box 8, Morrumbene, Mozambique.

National Institute of Health, Av. Eduardo Mondlane/Salvador Allende, P.O.
Box 264, Maputo, Mozambique

DBL Centre for Health, Research and Development, Fredriksberg, Denmark

Centro de Malária e Outras Doenças Tropicais, 96 Rua da Junqueira, Lisbon 1300, Portugal.

jdcharlwood@gmail.com

Running head

Temperature and African malaria vectors

Key words

Anopheles funestus, *Anopheles gambiae* s.l., temperature, rainfall, population dynamics

Introduction

In Sub-Saharan Africa, malaria transmission is almost entirely sustained by members of two mosquito vector species complexes: *Anopheles gambiae* and *A. funestus*. The larval ecology of these two vector groups differs.

Although immature forms of freshwater members of the *Anopheles gambia*e complex (*An. gambiae*, *An. coluzzi* and *An. arabiensis*) 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 & DeMeillon, 1968). These are often rain puddles. Puddles can rapidly dry, hence development of *An. gambiae* is a race against time, and egg to adult duration in this species complex can be remarkably short. Gillies & DeMeillion (1968) estimated that the minimum generation time for *An. gambiae* s.l. was 10 days (three or four of which were spent as adults) and that under cooler conditions it might extend to two or three weeks.

Immatures of *Anopheles funestus sensu stricto*, the other predominant African vector, are generally found in more permanent, shaded, water bodies with emergent vegetation. Time is less of a constraint for *An. funestus* and the duration of a generation is usually about 3 weeks to a month (Gillies and DeMeillon, 1968). Thus the mosquitoes are exposed to different micro-climates, a first order determinant of the distribution and abundance of species (Andrewartha and Birch, 1954).

More rain means more puddles and *Anopheles gambiae* s.l. tend to proliferate with rainfall. It is estimated that more than 350 mm of rain during the wettest five months of the year is required for *An. gambiae* s.l. to persist (Craig et al., 1999). Heavy rainfall can, however, cause high losses due to the flushing out of larvae (Paaijmans et al., 2007). In Kenya, both *Anopheles gambiae* and *An. arabiensis* were positively correlated with rainfall, but negatively correlated with humidity and temperature (the higher the temperature the faster the puddles dry up) (Kelly-Hope et al., 2013). In Mozambique, numbers of male *An. gambiae* s.l. from exit collections showed a peak three weeks after the rain (Charlwood, 2013). In both Kenya (Kelly-Hope et al., 2013) and Mozambique (Charlwood, 2013) *An. funestus* were, however, negatively correlated with rainfall. While *An. funestus* densities may be negatively associated with rainfall, populations may be less susceptible to 'flushing' and be less disturbed by heavy rainstorms (Charlwood & Bragança, 2012a).

Temperature is another major driving force in insect populations. Puddles exposed to the sun get hotter than larger bodies of water. The high water temperature and exposure to light in the favoured sites of *An. gambiae* s.l. also favour the blooming of phytoplankton and Gimnig et al., (2002) and Tuno et al., (2006) describe an association between the presence of a unicellular epizoic green algae, *Rhopalosolen* sp., and high larval densities.

Despite a proliferation of food *An. gambiae* s.l. larvae at higher temperatures may not be able to ingest as much prior to pupation as they do at cooler temperatures. Le Seur and Sharp (1991) concluded that the effect of temperature on *An. merus* (another member of the *An. gambiae* complex) was greatest in the pupal stage, as did Heuval den val (1963) for *Aedes aegypti*. The effect of temperature perhaps, being due to available energy reserves and temperature-related metabolism during metamorphosis (LeSeur and Sharp, 1991).

Diurnal fluctuations in temperature affect the development of many insects (Vangansbeke et al., 2015) including *Anopheles gambiae* s.l. (Lyons et al, 2013). 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).

In the laboratory the different species respond differently to different temperature regimes. Development rates were highest for *An. arabiensis* with peak survival at 32°C although rates were similar between 22°C and 32°C whilst an optimal temperature for larval development of *An. gambiae* was between 28-32°C but survival rate to adulthood was 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 and 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, had a single optimum temperature of 25°C for development with substantial declines in survival either side of this (Christiansen-Jucht, 2014).

Not surprisingly, given their larval habitat, both *An. gambiae* and *An. arabiensis*, also responded better to fluctuating temperatures than did *An. funestus* (Christiansen-Jucht et al., 2014). At temperatures above 35°C none of the species survived as larvae or pupae. 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 & DeMeillon 1968).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016

In their studies Jepson *et al.* (1947) concluded that temperature and food were the factors controlling *funestus* breeding under natural conditions and numbers of *An. funestus* in Kenya were positively correlated with temperature (Kelly-Hope et al., 2013). In Mozambique, temperature explained 60% of changes in the wing length of *An. funestus* and did not appear to affect adult survival (Charlwood & Bragança, 2012b).

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 more subtle 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).

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 *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 (10.5°C below the lower limit of 16.5°C for larval activity, (Jepson et al., 1947)) to a daytime maximum of 35°C in the hot season.

Methods

Description of study site

The approximately 5x4 km village of Furvela, (24°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. 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).

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 the house. 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.

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. 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, 2013). 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

All of the newly emerged population, equivalent to the unfed insects in the collection, is sampled every day, independent of temperature, whilst the proportion of the mature (gravid) population sampled depends on the duration of oogenesis following a blood meal.

Resting collection

Resting collections, using a torch and an aspirator, were performed, on an *ad hoc* basis, in houses 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 and De Meillon (1968) and Gillies and 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 et al. (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) at the edge of the village that recorded hourly information. 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 et al., 2011) 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.

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

Rainfall data

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016

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) x # rain days/7

The daily, weekly and monthly records of rainfall over the period 2000-2010 are available at supplemental file 1 (Rainfall data).

Analysis

Data were entered into, and analyzed with, Excel (supplementary files). Unfed mosquitoes from light-trap collections represent all age groups. Unfed mosquitoes from exit collection are, however, almost entirely newly emerged ones (Charlwood et al., 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. (supplemental file 2 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 to 24 Nov 2008 are shown in Fig 1. Diurnal variation in temperature differed between cool and hot seasons. Figure 2A shows the diurnal pattern recorded on the three coolest nights of the year (16-18th July) and 2B 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 3). 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

Of the 30 males and 407 females from an unselected sample of the *An. gambiae* complex identified by PCR from 2002 and 2004, 86%, and 83%, respectively, comprised *Anopheles gambiae*, the other species being *An. arabiensis* (13%) and *An. merus* (1%) (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).

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 *An. funestus*. Seventy-one females of the *An. funestus* group were identified by PCR (A.L Szalanski and J. Austin, unpublished data). All were *An. funestus*. Given that this is the endophilic member of the species group, and that it was endophilic behavior that we studied, we assume that this was the only member of the species group present in our collections.

301, 705 female *An. funestus* were collected in 6043 light-trap collections, 161, 466 in 7397 exit collections and 16, 995 in 1315 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 (supplemental file 3 – 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 4 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 (Charlwood et al., unpublished) 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 5090 light-trap, 4461 exit and 1315 resting collections were performed).

Numbers of mosquito were greatest in the warmer months. Figure 5 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 numbers of unfed and gravid insects by species from exit collections by ISO week in the years 2001-2006.

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 supplemental file 4 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 6A gives the relationship between numbers of *An. funestus* and *An. gambiae* s.l. caught in light-traps and air temperatures and 6B between mosquito numbers and soil temperature ($r^2 = 0.52$ for *An. funestus* and 0.77 for *An. gambiae* s.l.). Although both 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 Fig 7A & 7B. 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 7A). Numbers of both gravid and unfed *An. gambiae* complex females in exit collections continued to increase at all temperatures recorded (Fig 7B). 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 we do not know 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 ($p = 0.07$).

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 Figure 8. For both, species, or species group, a higher proportion of semi-gravid and gravid females compared to engorged females were collected during the cooler months of the year (May to August). In other words oogenesis was taking longer at the lower temperatures.

The proportion gravid to engorged *An. funestus* of 50% occured 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.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016

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. For most traits 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. Even in the straightforward analysis presented 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.*

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). 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 we presume 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,* 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 whilst for unfed (newly emerged) insects it was with outdoor air temperature. As pointed out by Pajimans et al., (2013) 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 and DeMeillon, 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 & Wilkes (1963). 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, 2013), 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.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016

Recently Pajimanns 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 (Paajimans et al, 2010). Thus the *An. gambiae* s.l. were well suited to the temperature regimes experienced in Furvela.

Unfortunately we do not know the species composition of the resting or exiting *An. gambiae* s.l. compared to those entering the house. Nevertheless, the proportion of gravid insects in resting catches varied in a similar fashion to that seen among the *An. funestus*. We also do not know if 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 kilometer stretch of coastline indicates that a climatic factor was responsible. Mean temperatures did not change nor did they affect the *An. gambiae* differently at different temperatures, so survival was not apparently affected by temperature changes. But what the factor might be we do not know.

Acknowledgements

I would like to thank Joao Pinto of the IHMT, Portugal for identifying the members of the *An. gambiae* complex in this study and for his perceptive comments on the manuscript. I also thank Corey LeClair of the LSHTM UK and Olivier Briët of the Swiss TPH for comments on the manuscript. Thanks to Nelson Cuamba and Ricardo Thompson for logistic support during the study and thanks to the members of the MOZDAN project and to the villagers of Furvela who let us collect mosquitoes from their houses

References

Andrewartha HG, Birch LC. 1954. The distribution and abundance of animals. University of Chicago Press.

Bayoh MN, Lindsay SW. 2003. Effect of temperature on the development of the aquatic stages of *Anopheles gambiae* sensu stricto (Diptera: Culicidae). *Bulletin of Entomological Research.* 93:375–381.

Beck-Johnson L, Nelson W, Paaijmans K, Read A, Thomas M, Bjørnstad O. 2013. The effect of temperature on anopheles mosquito population dynamics and the potential for malaria transmission. PLoS One 8(11):e79276.

Charlwood JD. 2011. Studies on the biology of male *Anopheles gambiae* Giles and *Anopheles funestus* Giles from southern Mozambique. *Journal of Vector Ecology 36: 382-394.*

Charlwood JD, Braganca M. 2012a. The effect of rainstorms on adult *Anopheles funestus* behavior and survival. *Journal of Vector Ecology* 2012a 37: 1-5

Charlwood JD, Bragança M. 2012b Some like it cool: The effect of temperature on the size of *Anopheles funestus* from southern Mozambique. *Journal of Medical Entomology* 49:1154-8.

Charlwood JD, Thompson R, Madsen H. 2003. Swarming and mating in *Anopheles funestus* from southern Mozambique. *Malaria Journal* 2003, **2**:3

Christiansen-Jucht CD, Parham PE, Saddler A, Koella JC, Basáñez M-G. 2014 Temperature during larval development and adult maintenance influences the survival of *Anopheles gambiae* s.s. *Parasites & Vectors*. 2014; 7:489. doi:10.1186/s13071-014-0489-3.

Christiansen-Jucht CD, Parham PE, Saddler A, Koella JC, Basáñez M-G. 2015. Larval and adult environmental temperatures influence the adult reproductive traits of *Anopheles gambiae* s.s. *Parasites & Vectors*. 2015; 8:456. doi:10.1186/s13071-015-1053-5.

Collins FH, Petrarca V, Mpofu S, Brandling-Bennett AD, Were JB, Rasmussen MO, Finnerty V. 1988. Comparison of DNA probe and cytogenetic methods for identifying field collected *Anopheles gambiae* complex mosquitoes. *American Journal of Tropical Medicine & Hygiene* 39, 545–550.

Craig MH, Snow RW, le Sueur D. 1999. A climate-based distribution model of malaria transmission in sub-Saharan Africa. *Parasitology Today* 15: 105–111.

Gillies MT, Coetzee M. 1987. A Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region)Publication no. 55. South African Institute for Medical Research, Johannesburg.

Gillies MT, De Mellion B. 1968. The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region), 2ndPublication no. 54 edn. South African Institute for Medical Research, Johannesburg.

Gillies MT, Wilkes TJ. 1963. Observations on nulliparous and parous rates in a population of *Anopheles funestus* in East Africa. Annals of Tropical Medicine and Parasitology. 57, 204-213.

Gimnig JE, Ombok M, Kamau L, Hawley WA, Gimnig JE, Ombok M, Kamau L, Hawley WA. 2001. Characteristics of Larval Anopheline (Diptera:Culicidae) Habitats in Western Kenya. *Journal of Medical Entomology* 38:282–288.

Haddow AJ. 1943. Measurements of temperature and light in artificial ponds with reference to the larval habitat of *Anopheles (Myzomyia) gambiae* Giles, and *A. (M.) funestus* Giles. *Bulletin of Entomological Research* 34:89–93.

Hancock PA, Godfray HCJ. 2007. Application of the lumped age-class technique to studying the dynamics of malaria-mosquito-human interactions. *Malar Journal* 6: 98.

Heuval MJ van den. 1963. The effect of rearing temperature on the wing length, thorax length, leg length and ovariole number of the adult mosquito, *Aedes acgyptii* (L.). *Transactions of the Royal Entomological Society of London*, 115, 197-216.

Holstein M. 1952. Biologie d'*Anopheles gambiae*. Monograph Series World Health Organization No 9, 176 pp.

Ishengoma DA, Mmbando BP, Segeja MD, Alifrangis M, Lemnge MM, Bygbjerg IC. 2013. Declining burden of malaria over two decades in a rural community of Muheza District,north-eastern Tanzania. Malaria Journal 12:338 DOI 10.1186/1475-2875-12-338.

Jepson WF, Moutia A, Courtis C. 1947. The malaria problem in Mauritius: the bionomics of Mauritian anophelines. *Bulletin of Entomological Research*, 38:177–208.

Kampango A, Bragança M, de Sousa B, Charlwood JD. 2013. Netting barriers to prevent mosquito entry into houses in southern Mozambique: a pilot study. *Malaria Journal* 12:99.

Koekemoer LL, Kamau L, Hunt RH, Coetzee M. 2002. A cocktail polymerase reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. *American Journal of Tropical Medicine and Hygiene,* 66, 804–811.

Kelly-Hope LA, Hemingway J, McKenzie FE. 2009. Environmental factors associated with the malaria vectors *Anopheles gambiae* and *Anopheles funestus* in Kenya. *Malaria Journal*. 8:268. doi:10.1186/1475-2875-8-268.

Kirby MJ, Lindsay SW. 2004. Responses of adult mosquitoes of two sibling species, *Anopheles arabiensis* and *An. gambiae* s.s. (Diptera: Culicidae), to high temperatures. *Bulletin of Entomological Research.* 94:441–448.

Le Sueur, Sharp BL. 1991. Temperature-dependent variation in *Anopheles merus* larval head capsule width and adult wing length: implications for anopheline taxonomy. *Medical and Veterinary Entomology* 5: 55-62.

Lyons CL, Coetzee M, Chown SL. 2013. Stable and fluctuating temperature effects on the development rate and survival of two malaria vectors, *Anopheles arabiensis* and *Anopheles funestus*. *Parasites & Vectors*. 6:104. doi:10.1186/1756-3305-6-104.

Meyrowitsch DW, Pedersen EM, Alifrangis M, Scheike TH, Malecela MN, Magesa SM, Derua YA, Rwegoshora RT, Michael E, Simonsen PE. 2011. Is the current decline in malaria burden in sub-Saharan Africa due to a decrease in vector population? *Malaria Journal*, 10:188

O'Loughlin SM, Magesa SM, Mbogo C. Mosha F. Midega J. Burt A. 2016. Genomic signatures of population decline in the malaria mosquito *Anopheles gambiae Malaria Journal* 15:182 DOI**:** 10.1186/s12936-016-1214-9

Paaijmans KP, Wandago MO, Githeko AK, Takken W. 2007. Unexpected high losses of *Anopheles gambiae* larvae due to rainfall. PLoS ONE 2: e1146. doi:10.1371/journal.pone.0001146.

Paaijmans KP, Blanford S, Bell AS, Blanford JI, Read AF, et al. 2010. Influence of climate on malaria transmission depends on daily temperature variation. Proceedings of the National Academy of Science USA 107: 15135–9.

Scott JA, BrogdonWG, Collins FH. 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene*, 49, 520–529.

Tuno N, Githeko AK, Nakayama T, Minakawa N, Takagi M, Yan G. 2006. The association between the phytoplankton, *Rhopalosolen* species (Chlorophyta; Chlorophyceae), and *Anopheles gambiae sensu lato* (Diptera:Culicidae) larval abundance in western Kenya. *Ecological Research* 21:47**6–482.**

Vangansbeke D, Audenaert J, Tung Nguyen D, Verhoeven R, Gobin B, Tirry L, De Clercq P. 2015 Diurnal Temperature Variations Affect Development of a Herbivorous Arthropod Pest and its Predators. PloS one 10 (4), e0124898

Table 1(on next page)

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

Table 2(on next page)

Correlation coefficients between mosquitoes and climate

Table 2. 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.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016

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(on next page)

Correlation between mosquitoes and rainfall

Table 3. 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.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016

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

Table 4(on next page)

Models and mosquito numbers

Table 4. Environmental regression models for the different categories of female mosquito collected, Furvela, 2001-1007

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

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 + 0.10869Soil temp – 0.734*Windspeed

Figure 1(on next page)

Annual temperature variation

Figure 1. Mean daily soil and air temperatures recorded by the Delta logger in Furvela village

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016

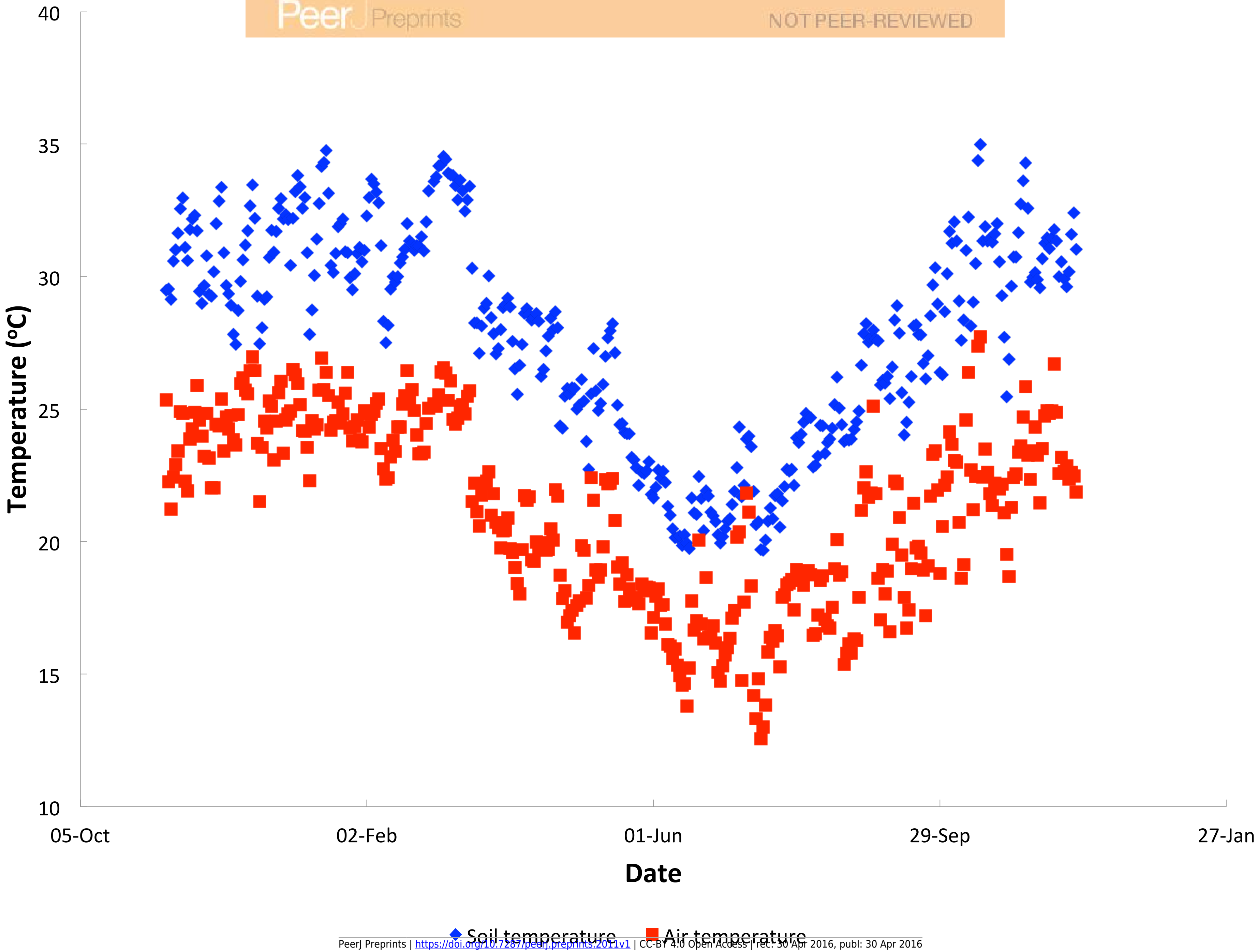
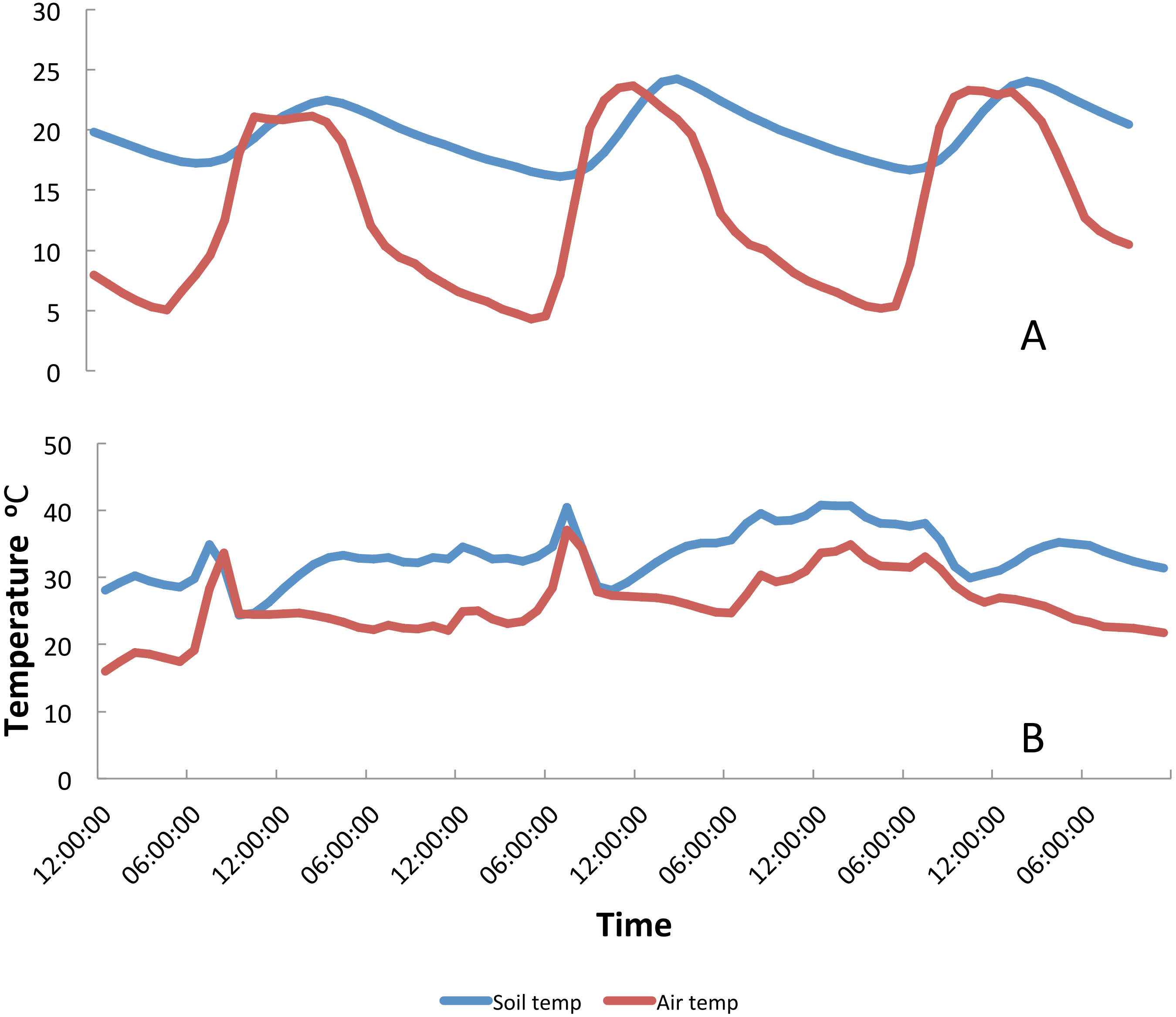

40
35
30
25
20
15
10
Temperature (ºC)
05-Oct
02-Feb
01-Jun
29-Sep
27-Jan
Date
◆ Soil temperature
■ Air temperature

Figure 2(on next page)

Daily variation in air and soil temperatures

Figure 2. Diurnal variation in soil and air temperature recorded in the village of Furvela during A) the cool season (16-18th July) and B) the hot season (14-16th October).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016



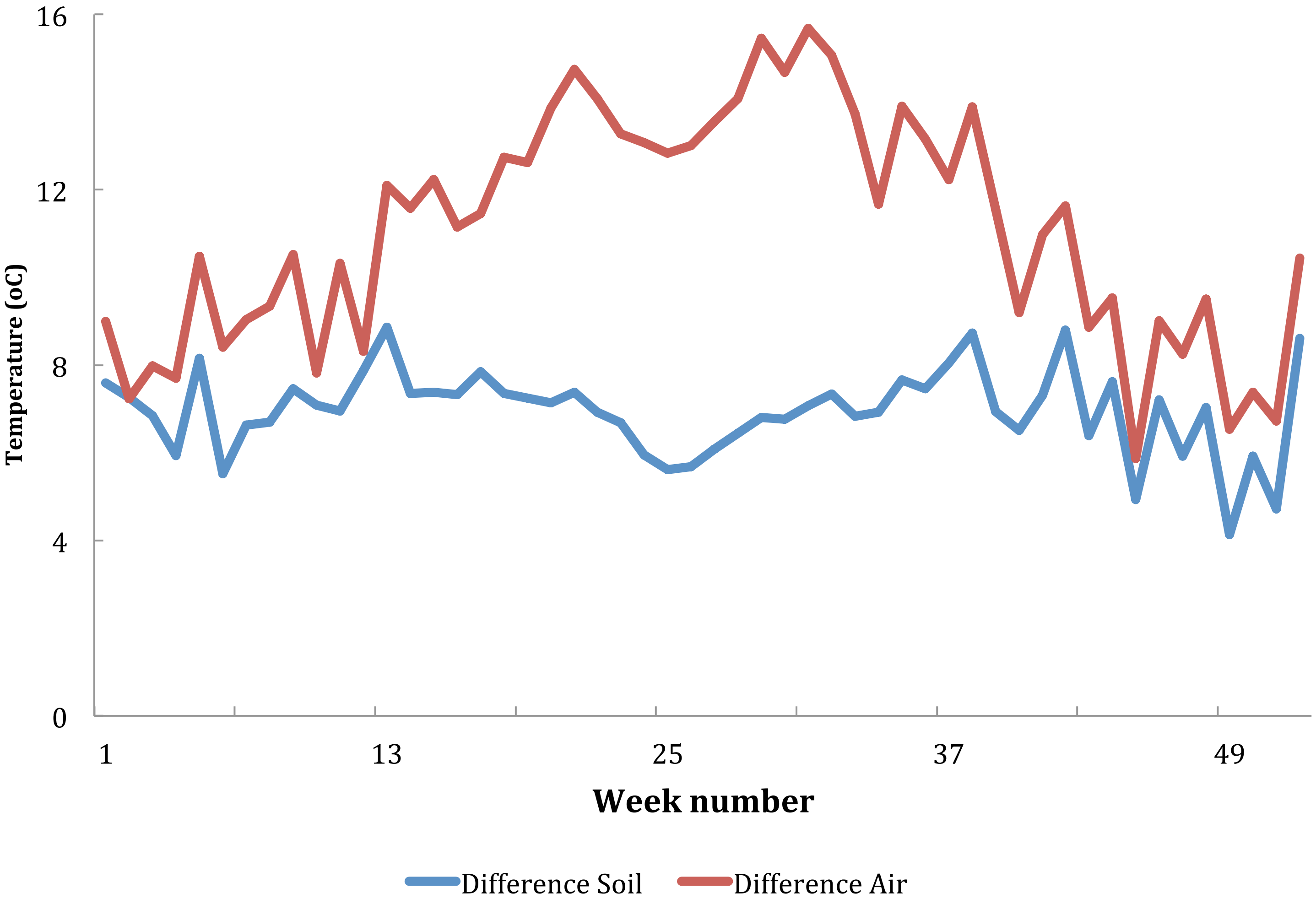
PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016

Figure 3(on next page)

Difference between maximum and minimum temperatures by ISO week

Figure 3. Difference between maximum and minimum air and soil temperature recorded from Furvela village, Mozambique, by ISO week number.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016



PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016

Figure 4(on next page)

Temperature, rainfall and number of mosquitoes collected in Furvela

Figure 4. Rainfall (measured in Maxixe), air temperature (measured in Vilanculos) and mean numbers of unfed Anopheles funestus (A) and Anopheles gambiae (B) collected from light-traps and in exit collections from Furvela village, 2001-2009.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016

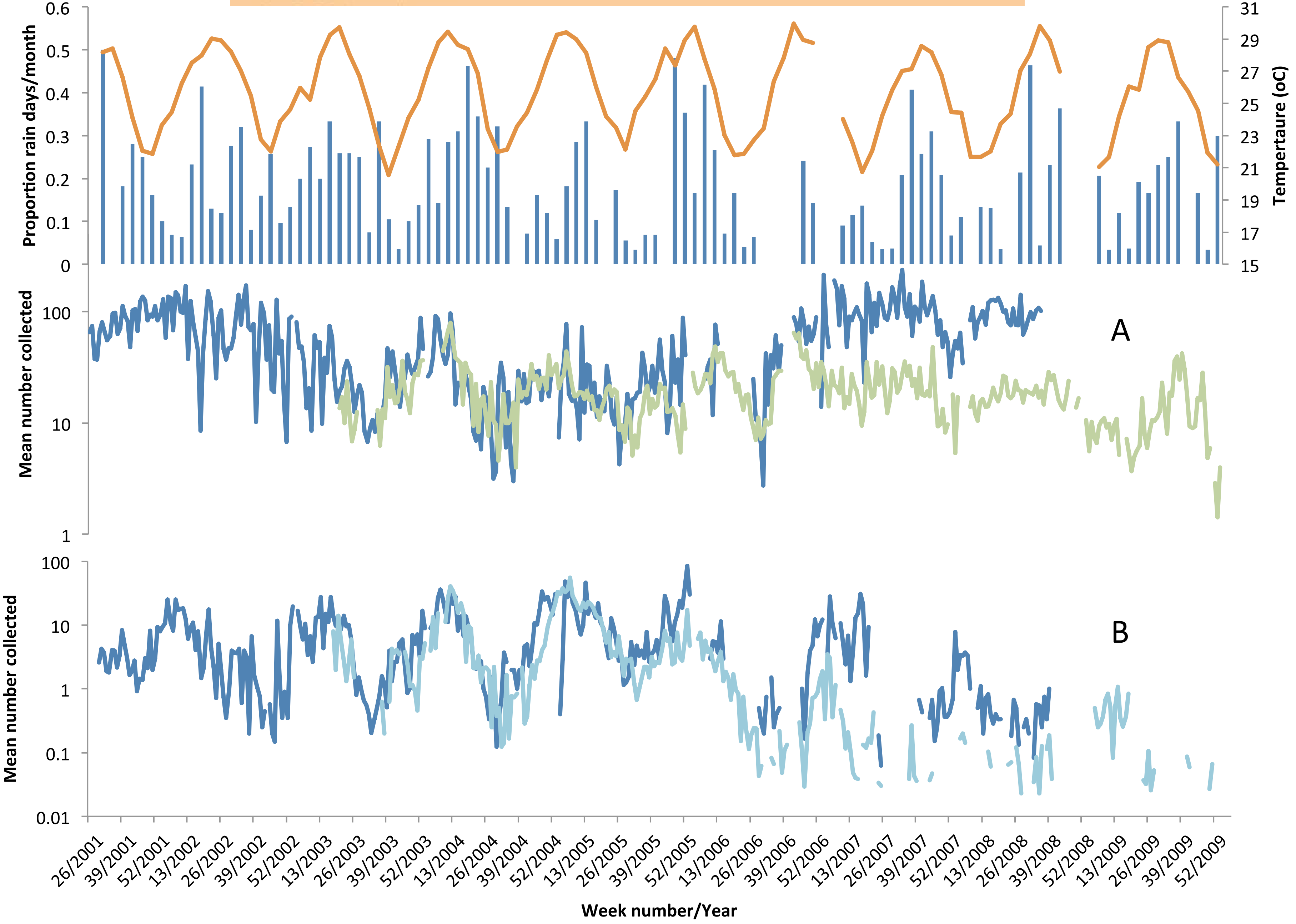

Proportion rain days/month
0.6
0.5
0.4
0.3
0.2
0.1
0
Tempertaure (oC)
31
29
27
25
23
21
19
17
15
Mean number collected
100
10
1
A
Mean number collected
100
10
1
0.1
0.01
B
26/2001
39/2001
52/2001
13/2002
26/2002
39/2002
52/2002
13/2003
26/2003
39/2003
52/2003
13/2004
26/2004
39/2004
52/2004
13/2005
26/2005
39/2005
52/2005
13/2006
26/2006
39/2006
52/2006
13/2007
26/2007
39/2007
52/2007
13/2008
26/2008
39/2008
52/2008
13/2009
26/2009
39/2009
52/2009
Week number/Year

Figure 5(on next page)

Weekly mean adjusted rain, soil temperature, air temperature, windspeed and mosquito numbers

Figure 5. Weekly mean adjusted rain, soil temperature (red), air temperature (blue), windspeed (green) and mean numbers of Anopheles funestus and An. gambiae s.l. collected in light-traps and. A) An. funestus B) An. gambiae s.l. in exit collection (orange = unfed insects; green = gravid insects).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016

Figure 6(on next page)

Relationship between numbers of mosquito collected in light-traps and A) soil and B) air temperature, Furvela, Mozambique

Figure 6. Mean number of Anopheles funestus and An. gambiae s.l. collected by A) soil temperature and B) air temperature Furvela, Mozambique.

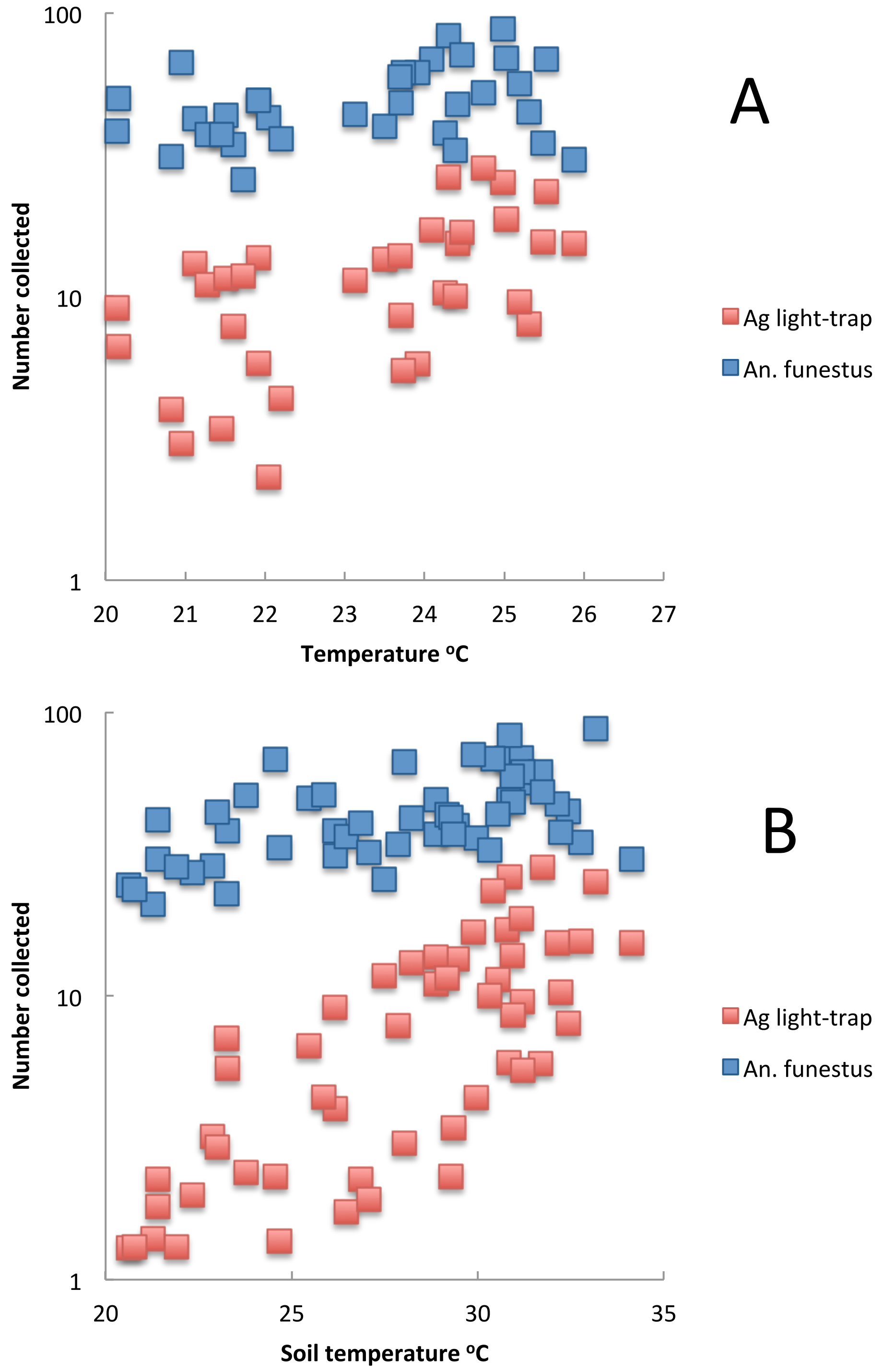
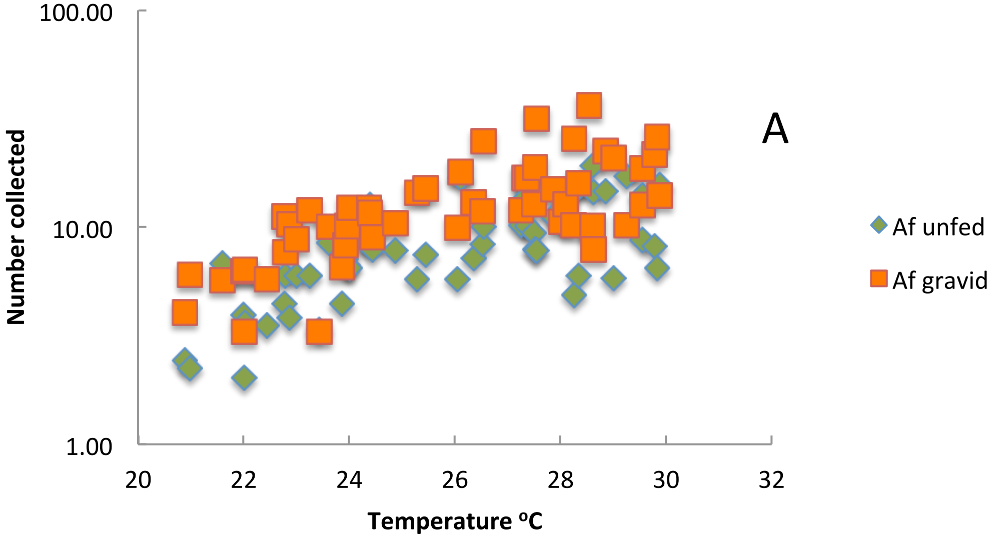

A
100
10
1
Number collected
20
21
22
23
24
25
26
27
Temperature °C
🟥 Ag light-trap
🟦 An. funestus
B
100
10
1
Number collected
20
25
30
35
Soil temperature °C
🟥 Ag light-trap
🟦 An. funestus

Figure 7(on next page)

Relationship between the mean number of unfed and gravid mosquitoes in exit collections and soil temperature in Furvela A) Anopheles funestus B) An. gambiae s.l.

Figure 7. The relationship between the mean number of A) Anopheles funestus and B) An. gambiae s.l. in exit collections and soil temperature Furvela, Mozambique.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016


A
100.00
10.00
1.00
Number collected
20
22
24
26
28
30
32
Temperature ºC
Af unfed
Af gravid

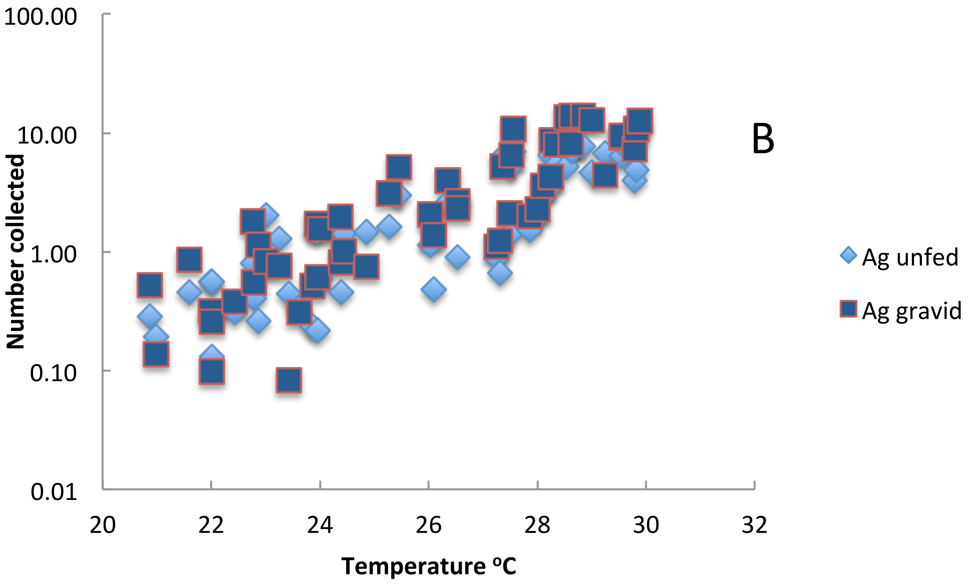
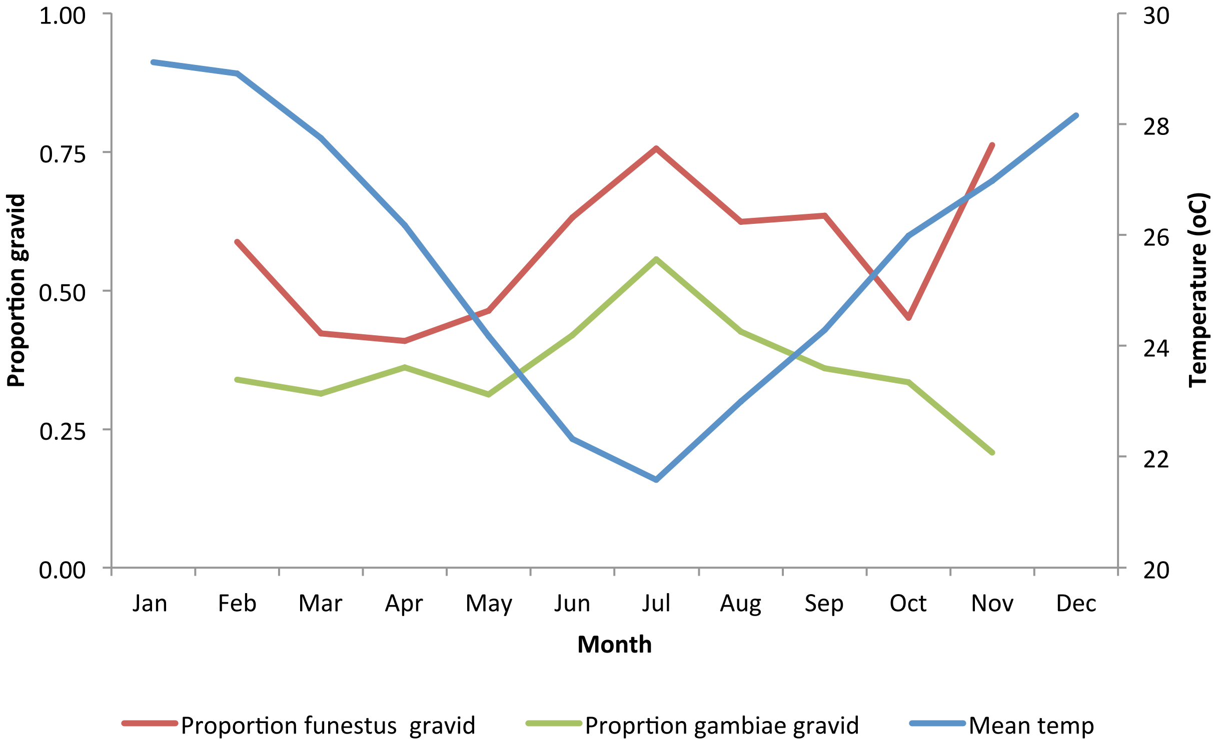

B
100.00
10.00
1.00
0.10
0.01
Number collected
20
22
24
26
28
30
32
Temperature ºC
Ag unfed
Ag gravid

Figure 8(on next page)

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

Figure 8. The proportion of female Anopheles funestus and An. gambiae s.l. indoor resting that were gravid at the time of collection and mean air temperatur, Furvela, Mozambique.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016



PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2011v1 | CC-BY 4.0 Open Access | rec: 30 Apr 2016, publ: 30 Apr 2016