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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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.

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2 ***Anopheles funestus* and *An. gambiae* s.l.**

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12 **Running head**

13 Temperature and African malaria vectors

14 **Key words**

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

Introduction

17   In Sub-Saharan Africa, malaria transmission is almost entirely sustained by members of two

18   mosquito vector species complexes: *Anopheles gambiae* and *A. funestus*. The larval ecology

19   of these two vector groups differs.

20   Although immature forms of freshwater members of the *Anopheles gambiae* complex (*An.*

21   *gambiae*, *An. coluzzi* and *An. arabiensis*) may occur in a great variety of water bodies, the

22   most characteristic are the 'shallow open sun-lit pools with which every field worker in

23   Africa is familiar' (Gillies & DeMeillon, 1968). These are often rain puddles. Puddles can

24   rapidly dry, hence development of *An. gambiae* is a race against time, and egg to adult

25   duration in this species complex can be remarkably short. Gillies & DeMeillion (1968)

26   estimated that the minimum generation time for *An. gambiae* s.l. was 10 days (three or four

27   of which were spent as adults) and that under cooler conditions it might extend to two or

28   three weeks.

29   Immatures of *Anopheles funestus sensu stricto*, the other predominant African vector, are

30   generally found in more permanent, shaded, water bodies with emergent vegetation. Time

31   is less of a constraint for *An. funestus* and the duration of a generation is usually about 3

32   weeks to a month (Gillies and DeMeillon, 1968). Thus the mosquitoes are exposed to

33   different micro-climates, a first order determinant of the distribution and abundance of

34   species (Andrewartha and Birch, 1954).

35   More rain means more puddles and *Anopheles gambiae* s.l. tend to proliferate with rainfall.

36   It is estimated that more than 350 mm of rain during the wettest five months of the year is

37   required for *An. gambiae* s.l. to persist (Craig et al., 1999). Heavy rainfall can, however, cause

38   high losses due to the flushing out of larvae (Paaijmans et al., 2007). In Kenya, both

39   *Anopheles gambiae* and *An. arabiensis* were positively correlated with rainfall, but negatively

40   correlated with humidity and temperature (the higher the temperature the faster the

41   puddles dry up) (Kelly-Hope et al., 2013). In Mozambique, numbers of male *An. gambiae* s.l.

42   from exit collections showed a peak three weeks after the rain (Charlwood, 2013). In both

43   Kenya (Kelly-Hope et al., 2013) and Mozambique (Charlwood, 2013) *An. funestus* were,

44   however, negatively correlated with rainfall. While *An. funestus* densities may be negatively

45   associated with rainfall, populations may be less susceptible to 'flushing' and be less

46   disturbed by heavy rainstorms (Charlwood & Bragança, 2012a).

47   Temperature is another major driving force in insect populations. Puddles exposed to the

48   sun get hotter than larger bodies of water. The high water temperature and exposure to light

49   in the favoured sites of *An. gambiae* s.l. also favour the blooming of phytoplankton and

50   Gimnig et al., (2002) and Tuno et al., (2006) describe an association between the presence

51   of a unicellular epizoic green algae, *Rhopalosolen* sp., and high larval densities.

52   Despite a proliferation of food *An. gambiae* s.l. larvae at higher temperatures may not be

53   able to ingest as much prior to pupation as they do at cooler temperatures. Le Seur and

54   Sharp (1991) concluded that the effect of temperature on *An. merus* (another member of the

55   *An. gambiae* complex) was greatest in the pupal stage, as did Heuval den val (1963) for

56   *Aedes aegypti*. The effect of temperature perhaps, being due to available energy reserves

57   and temperature-related metabolism during metamorphosis (LeSeur and Sharp, 1991).

58   Diurnal fluctuations in temperature affect the development of many insects (Vangansbeke et

59   al., 2015) including *Anopheles gambiae* s.l. (Lyons et al, 2013). In addition to being hotter,

60   temperature fluctuations in small pools are greater than those in larger, shaded, bodies of

61   water. For example, although temperature minima in pools typically used by *An. gambiae*

62   are similar to the minima in shaded ones, maxima may be 10ºC higher (Haddow, 1943).

63   In the laboratory the different species respond differently to different temperature regimes.

64   Development rates were highest for *An. arabiensis* with peak survival at 32ºC although rates

65   were similar between 22ºC and 32ºC whilst an optimal temperature for larval development

66   of *An. gambiae* was between 28-32ºC but survival rate to adulthood was highest between

67   22-26ºC (Bayoh & Lindsay, 2003, Christiansen-Jucht et al, 2014). This reflects the higher

68   temperature tolerance of *An. arabiensis* compared to *An. gambiae* (Kirby and Lindsay, 2004)

69   which itself may be responsible for the extended distribution of the former species into

70   hotter and drier environments in Africa. *Anopheles funestus*, on the other hand, had a single

71   optimum temperature of 25ºC for development with substantial declines in survival either

72   side of this (Christiansen-Jucht, 2014).

73   Not surprisingly, given their larval habitat, both *An. gambiae* and *An. arabiensis*, also

74   responded better to fluctuating temperatures than did *An. funestus* (Christiansen-Jucht et

75   al., 2014). At temperatures above 35ºC none of the species survived as larvae or pupae. In

76   the field, however, larvae and pupae of *An. gambiae* s.l. (probably *An. arabiensis*) have been

77   found in pools at temperatures of 40.5-41.8ºC (Holstein 1952, quoted in Gillies & DeMeillon

78   1968).

79   In their studies Jepson *et al.* (1947) concluded that temperature and food were the factors

80   controlling *funestus* breeding under natural conditions and numbers of *An. funestus* in

81   Kenya were positively correlated with temperature (Kelly-Hope et al., 2013). In

82   Mozambique, temperature explained 60% of changes in the wing length of *An. funestus* and

83   did not appear to affect adult survival (Charlwood & Bragança, 2012b).

84   In contrast to the larvae, adults of both *An. gambiae* s.l. and *An. funestus*, experience similar

85   microclimates due to their predominantly endophilic behaviour. Temperature influences

86   the time it takes for egg development following a blood-meal but may also have more subtle

87   effects. For example, *An. funestus* delays returning to feed following oviposition at

88   temperatures above 26.5°C, but at lower temperatures females re-feed shortly after egg

89   laying (Gillies & Wilkes, 1963). Ironically, the extra time spent in returning to feed at higher

90   temperatures is compensated for by it taking two rather than three days for the mosquito to

91   complete egg development, so that the duration of the complete gonotrophic cycle is three

92   days at all temperatures (Gillies & Wilkes, 1963).

93   Surprisingly, there remains a lack of comprehensive data on the effects of temperature and

94   other environmental factors on mosquito population dynamics in the wild. Possible effects

95   of temperature on mosquitoes in the field are most easily observed in areas with a wide

96   variation in both daily and seasonal temperatures. Wild mosquito populations are, however,

97   notoriously unpredictable and short-term, chaotic, fluctuations are common. Long-term

98   observations can assist in reducing the 'noise' in such data. Here we describe the effect of

99   ambient temperature, and other environmental parameters, over a five-year period, on the

100 dynamics of *An. funestus* and *An. gambiae* s.l., collected from a single village in southern

101 Mozambique where temperatures varied from a night-time minimum of 6°C in the cool

102 season (10.5°C below the lower limit of 16.5°C for larval activity, (Jepson et al., 1947)) to a

103 daytime maximum of 35°C in the hot season.

Methods

104

Description of study site

105

106 The approximately 5x4 km village of Furvela, (24°43' S, 35°18' E), 475 km north of the

107 capital Maputo, is bordered on two sides by the alluvial plain of two river systems. The

108 Furvela River valley to the north of the village in particular has a considerable amount of

109 local irrigation for agriculture, which provides a large and relatively stable number of small

110 canals. The Inhnanombe river to the east of the village consists largely of beds of the reed

111 (*caniço*), used for housing, and sugar cane, used in the production of local alcohol; it does

112 not flow as fast as the Furvela river. *Anopheles funestus* predominates on the Furvela River

113 side of the village and *An. gambiae* s.l. on the Inhnanombe side (Kampango et al. 2013).

114

115 Houses in the village are generally made with *caniço* walls and palm thatch roofs. Although

116 most houses don't have windows the majority have a *ca.* 15 cm gap between the roof and

117 walls at either end of the house. Doors and doorframes are also generally badly fitting;

118 hence mosquitoes can easily enter the house. Other styles of house include those with

119 corrugated iron sheets for the roof and those made of concrete blocks (which do have

120 windows). Houses are built either in family compounds of three to six houses or as

121 relatively evenly spaced individual homes.

Mosquito collection

122

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

145   performed individually following the protocols of Collins *et al.* (1988) and the species

146   identified using the protocols of Scott et al. (1993). A small number of *An. funestus* were

147   also identified by PCR using the protocols of Koekemoer et al. (2002).

148   **Meteorology**

149   **Temperature, insolation and windspeed measurement**

150   Daily variation estimates of soil and air temperature, insolation and windspeed were

151   obtained with a Delta-T digital weather station (Delta-T Devices, Cambridge, UK) at the

152   edge of the village that recorded hourly information. Soil temperatures approximate those

153   that larvae are exposed to whilst air temperatures are those that more closely approximate

154   those that adults may be exposed to. Unfed females exiting houses at dusk are newly

155   emerged (Charlwood et al., 2011) and reflect the effect of temperature on the larvae whilst

156   the ratio of unfed to gravid insects may reflect temperature effects on the adults.

157   Unfortunately the weather station did not operate throughout the study, nor did the

158   humidity or rainfall meter work consistently. The longest hourly data sets were from 3rd May

159   2004 to 1 October 2005 and from 10 Nov 2007 to 24 Nov 2008. Hourly data from all years,

160   including the later ones, was amalgamated into daily data and daily data amalgamated into

161   ISO weeks. Mean values for the different ISO weeks from all the weather station files were

162   determined and used in calculations.

163   Long term temperature data recorded at Vilanculos, a town ~200 km north of Furvela, were

164   also obtained (long-term data available from www.tutiempo.net).

165   **Rainfall data**

166   Rainfall data was available from the town of Maxixe, 20 km to the south of Furvela. Since the

167   distribution of rainfall is important (20 mm falling on seven consecutive days in a week is

168   likely to have a different effect than 140 mm falling on a single day) a modified measure of

169   rainfall was used to estimate effects:

170   Modified weekly rain = Rain (mm) x # rain days/7

171   The daily, weekly and monthly records of rainfall over the period 2000-2010 are available at

172   supplemental file 1 (Rainfall data).

173   **Analysis**

174   Data were entered into, and analyzed with, Excel (supplementary files). Unfed mosquitoes

175   from light-trap collections represent all age groups. Unfed mosquitoes from exit collection

176   are, however, almost entirely newly emerged ones (Charlwood et al., 2011), whilst gravid

177   females have taken at least one blood meal sufficient to develop eggs, and will also include

178   infectious ones. The weekly Williams mean ($\log_{10}$ (n+1)) of these three groups of *An.*

179   *funestus* and *An. gambiae* s.l. were compared to mean, maximum and minimum

180   temperatures, temperature difference, insolation and wind speed, measured in Furvela, and

181   modified rainfall measured in Maxixe.

182   The relationship between mosquito numbers and environmental factors was examined

183   using bivariate correlations, and Pearson's correlation coefficient (2-tailed P value ≤ 0.05

184   significance). Least squares multiple linear regression (with climatic factors as independent

185   variables) was also undertaken using the Excel add-in StatPlus. The most parsimonious

186   model was determined by subtraction of least important variables.

Ethics

188 The study was conducted under the aegis of the joint Instituto Nacional de Saúde (INS)–DBL

189 Centre for Health Research and Development project 'Turning houses into traps for

190 mosquitoes', which obtained ethical clearance from the National Bioethics Committee of

191 Mozambique on 2 April 2001 (ref: 056/CNBS/01). Householders were informed about the

192 purpose of the collections. Verbal consent was obtained when collections were initiated.

Results

Environmental variables

195 Mean temperatures recorded at Vilanculos were higher than those recorded in Furvela, but

196 both followed a similar pattern. (supplemental file 2 Temperature data) There was both a

197 marked seasonality in temperatures and considerable variation from one day to the next.

198 Mean soil and air temperatures from Furvela, derived from hourly measurements, 10 Nov

199 2007 to 24 Nov 2008 are shown in Fig 1. Diurnal variation in temperature differed between

200 cool and hot seasons. Figure 2A shows the diurnal pattern recorded on the three coolest

201 nights of the year (16-18th July) and 2B the three warmest (14-16th October). Overall at the

202 higher temperatures daily variation (difference between maximuim and minimum

203 temperature) was less than it was at the cooler temperatures (Fig 3). At a mean of 23.7℃

204 the variation in air temperatures was 5.9 degrees and at 18.1℃ was 15.7 degrees. Variation

205 is soil temperature was lower than air temperatures being 4.1 degrees at 30.3℃ and 8.9

206 degrees at 28.2℃.

207   Except for the mornings mean soil temperatures were consistently circa 5°C warmer than

208   air temperatures. Mean amounts of insolation showed a similar pattern to temperature.

209   **Mosquito data**

210   Of the 30 males and 407 females from an unselected sample of the *An. gambiae* complex

211   identified by PCR from 2002 and 2004, 86%, and 83%, respectively, comprised *Anopheles*

212   *gambiae*, the other species being *An. arabiensis* (13%) and *An. merus* (1%) (Table 1). There

213   was no statistical difference in the ratio of *An. gambiae* and *An. arabiensis* according to

214   method of collection (light-trap, exit collection or resting collection).

215   All of *An. funestus* examined morphologically had a single pale spot on the upper branch of

216   the 5th vein and did not have a pale spot at the tip of the 6th vein and corresponded to *An.*

217   *funestus*. Seventy-one females of the *An. funestus* group were identified by PCR (A.L

218   Szalanski and J. Austin, unpublished data). All were *An. funestus*. Given that this is the

219   endophilic member of the species group, and that it was endophilic behavior that we

220   studied, we assume that this was the only member of the species group present in our

221   collections.

222   301, 705 female *An. funestus* were collected in 6043 light-trap collections, 161, 466 in 7397

223   exit collections and 16, 995 in 1315 resting collections. The equivalent numbers for *An.*

224   *gambiae* s.l. are 72, 475 in light-traps, 33, 868 in exit collections and 5, 333 from indoor

225   resting collections (supplemental file 3 – Raw data). Outdoor resting collections failed to

226   produce any mosquitoes. Other anopheline species collected in light traps included 5776

227   *Anopheles tenebrosus*, 725 *Anopheles letabensis*, 22 *Anopheles rufipes*, five *Anopheles*

228 *squamosus*, and a single *Anopheles pharoensis*. A further 219 *An. tenebrosus* and five *An.*

229 *rufipes* were collected exiting houses.

230 Figure 4 shows the weekly mean numbers collected per house per night of *An. funestus* and

231 *An. gambiae* s.l. in light traps and exit collections over the course of the study in conjunction

232 with temperatures recorded at Vilanculos and modified rainfall from Maxixe. Over the three

233 years when both light trapping and exit collections were simultaneously undertaken (2003-

234 2006) mean numbers of *An. funestus* per house, per method, were similar. In 2007 a cordon

235 sanitaire of long lasting insecticide nets (LLIN's) was established around the Furvela River

236 valley (Charlwood et al., unpublished) and numbers in exit collections decreased relative to

237 numbers in light-traps. Numbers of *An. gambiae* s.l. in exit and light-trap were also similar.

238 Over the course of the project, however, *An. gambiae* s.l. disappeared from both light-trap

239 and exit collection collections. Given the possible effect of the *cordon sanitaire* on numbers

240 collected further analysis is confined to the years 2001-2006 (when 5090 light-trap, 4461

241 exit and 1315 resting collections were performed).

242 Numbers of mosquito were greatest in the warmer months. Figure 5 shows the mean

243 adjusted rainfall from Maxixe, mean soil and air temperature and wind speed from Furvela,

244 mean numbers of unfed *An. funestus* and *An. gambiae* s.l. collected in light-traps and mean

245 numbers of unfed and gravid insects by species from exit collections by ISO week in the

246 years 2001-2006.

247 The maximum Pearson correlation co-efficients between mosquito numbers and individual

248 environmental parameters are shown in Table 2. Table 2 also gives the correlation co-

249 efficients between the different mosquito groups. The highest environmental correlations

250 and mosquito numbers were between temperature and all collections of *An. gambiae* s.l..

251 (see supplemental file 4 for the other possible correlations). Adding a lag of up to four

252 weeks to the light-trap data from either species did not improve the Pearson correlation co-

253 efficient between rainfall and numbers (Table 3) Correlation co-efficients were always

254 lower in comparisons involving *An. funestus*.

255 Figure 6A gives the relationship between numbers of *An. funestus* and *An. gambiae* s.l.

256 caught in light-traps and air temperatures and 6B between mosquito numbers and soil

257 temperature (r^2 = 0.52 for *An. funestus* and 0.77 for *An. gambiae* s.l.). Although both species

258 increased significantly as temperatures increased, numbers of *An. gambiae* s.l. went through

259 a one hundredfold change (from a mean of 0.14 mosquitoes a night to 14) whereas numbers

260 of *An. funestus* merely doubled (from a mean of 20 to 40 a night).

261 The number of unfed and gravid insects in exit collections by mean air temperature are

262 shown in Fig 7A & 7B. At temperatures below 28°C the mean number of gravid *An. funestus*

263 collected increased as the temperature increased; and at a faster rate than the rate of

264 increase in immature insects but at temperatures above 28°C the number decreased whilst

265 numbers of newly emerged insects continued to increase (Fig 7A). Numbers of both gravid

266 and unfed *An. gambiae* complex females in exit collections continued to increase at all

267 temperatures recorded (Fig 7B). The proportion of the *An. gambiae* s.l. population that was

268 gravid was more variable at lower temperatures. This was probably due to the small sample

269 sizes at these temperatures. The proportion of *An. funestus* on the other hand was more

270 variable at the higher temperatures but why this should be so we do not know since the

271 data comes from a time when large-scale interventions had not been applied. Overall the

272 numbers of gravid and unfed *An. funestus* collected in exit collections were not correlated (p

273 = 0.07).

274 The best models for each species and each category of mosquito are given in Table 4.

275 Adjusted rainfall was only included in one model. The models explained more of the

276 variation in *An. gambiae* s.l. than they did for *An. funestus* although the environmental

277 parameter used in the models, with the exception of the best model for *An. funestus* and *An.*

278 *gambiae* s.l. in light-traps, were the same. The explanatory values (the adjusted R^2) were all

279 higher for the *An. gambiae* s.l. than for the *An. funestus.*

280 The abdominal status of mature females collected from 1315 resting collections and mean

281 monthly temperature is shown in Figure 8. For both, species, or species group, a higher

282 proportion of semi-gravid and gravid females compared to engorged females were collected

283 during the cooler months of the year (May to August). In other words oogenesis was taking

284 longer at the lower temperatures.

285 The proportion gravid to engorged *An. funestus* of 50% occured at 25°C. Thus, at these

286 temperatures, and above it took two days to mature the ovaries and below this three days

287 post-feeding to mature them. The proportion gravid of *An. gambiae* s.l. from resting catches

288 was always lower than that of the *An. funestus* and only reached 50% at the highest

289 temperatures. At mean temperatures of 21.5°C 76% of the *An. funestus* collected were semi-

290 gravid and gravid. This implies that it was taking not just three but four days to complete

291 gonotrophic development.

292 **Discussion**

293 With the possible implications of Global Warming, the effect of temperature and other

294 environmental parameters on the dynamics of malaria vectors in Africa is an area of

295 increasing interest. For most traits the temperature in Furvela fluctuates between the

296 minimum and optimum temperature for mosquito development, hence over the linear part

297 of the reaction norm. Temperature was the most important environmental parameter, of

298 those measured, determining mosquito numbers in the village. Even in the straightforward

299 analysis presented mean daily temperatures from either air or soil sensors explained 70%

300 and 35% of the density changes observed in *An. gambiae* s.l. and *An. funestus* respectively

301 (Table 2). As expected, given its rapid developmental time, rates of increase were

302 substantially higher in the *An. gambiae* s.l. compared to the *An. funestus.*

303 The ratio of gravid to unfed mosquitoes in exit collections depends on a number of factors,

304 in particular house construction. The two sets of females enter the house at different times

305 (unfed newly emerged insects entering at dawn to rest and, soon-to-be-gravid, host seeking

306 females, to feed throughout the night). They use different cues (visual contrast and odour)

307 and enter through different routes (open doors and eaves). Thus, houses that may allow

308 access for one group are not necessarily suitable for the other. In addition to house effects,

309 the proportion of egg development time spent inside houses (which we presume to be

310 100%, at least for *An. funestus*), the survival rate per oviposition cycle and the duration of

311 oogenesis, can all affect the unfed/gravid ratio. Should any of these factors change with

312 temperature then the overall ratio will also change with temperature. The absence of

313   change, as occurred with the *An. gambiae,* implies that these factors remained constant, or

314   compensated exactly, over the observed range of temperatures.

315   Ironically, the highest correlation between gravid insects in exit collections (for both species

316   or species group) was with soil temperature whilst for unfed (newly emerged) insects it was

317   with outdoor air temperature. As pointed out by Pajimans et al., (2013) the micro-climate

318   experienced by the mosquito inside houses may be quite different to that outside. Houses

319   may be warmer in the cool season and cooler in the hot season than temperatures recorded

320   outside. Nevertheless, more sophisticated measurement would only improve models for *An.*

321   *gambiae* by a maximum of 30% and for *An. funestus* by 47% (and would imply that other

322   factors, such as humidity, were of lesser importance).

323   Unlike *An. funestus* both newly emerged and gravid *An. gambiae* s.l. increased in a similar

324   fashion through the whole range of temperatures experienced in Furvela. There was no

325   apparent effect of increasing temperatures on survival and the proportion of gravid to unfed

326   insects remained more or less constant at all temperatures. The unfed/gravid ratio of the

327   more common *An. funestus* did, however, change with temperature. As temperatures

328   increase above 26.5°C a higher proportion of gravid *An. funestus* is to be expected in exit

329   collections since the duration of oogenesis is reduced from three to two days (Gillies and

330   DeMeillon, 1968). At the temperatures recorded in July it might take three or more days, as

331   evidenced in the resting collections and formerly described by Gillies & Wilkes (1963). At

332   the higher temperatures exit collections would therefore be expected to sample one half of

333   the mature population (the other half being the semi-gravid insects that may move from one

334   resting site to another, but in the absence of disturbance, do not leave the house) but at

335   cooler temperatures only one third, or even less, of the population. At the higher

336   temperatures, however, the proportion of gravid insects in the exit collections decreased,

337   such that overall there was no significant relationship between the numbers of gravid and

338   unfed insects in exit collections. This either means that that survival between emergence

339   and maturity (i.e. becoming gravid) decreases at cooler temperatures or that post-teneral

340   insects have a higher mortality at higher temperatures. Both sets of insects leave houses at

341   the same time (Charlwood, 2013), hence sampling efficiency for the two groups should be

342   the same.

343   A reduced survival among post-teneral adult *An. funestus* at the higher temperatures is

344   possible as described from the laboratory (Christiansen-Jucht et al., 2015). High

345   temperatures, independent of humidity, can have a lethal effect because as body

346   temperature increases, metabolism and respiration increase up to a critical thermal limit,

347   and there is a loss of integration between protein stability and metabolic processes that

348   leads to death. *Anopheles gambiae* s.l. are larger than *An. funestus.* Volume to surface ratios

349   differ and this may influence the ability of the adult insects to survive higher temperatures.

350   Larger mosquitoes have a smaller surface to volume ratio and larger water reserves, which

351   would allow them to offset the respiratory and cuticular water loss.

352   With one exception, windspeed was the only environmental parameter, other than

353   temperature, included any model. Together they explained up to 82% of the *An. gambiae*

354   changes and 61% of the *An. funestus*. The exception was *An. funestus* in light traps in which

355   adjusted rain was included. Although still significant this model had the lowest explanatory

356   value (34%) of all the models.

357   Recently Pajimanns et al., (2010) have described that in addition to mean temperatures 'the

358   key mosquito-related traits that combine to determine malaria transmission intensity (i.e.,

359   parasite infection, parasite growth and development, immature mosquito development and

360   survival, length of the gonotrophic cycle, and adult survival) are all sensitive to daily

361   variation in temperature'. Fluctuations in temperature (i.e. the difference between

362   maximum and minimum temperatures) were greatest in the cooler months. In the cool

363   season the observed patterns in soil and air temperature were similar to shaded and open

364   water as determined by Haddow (1943). In the warmer months fluctuations in temperature

365   were less than at lower temperatures. Fluctuations around low mean temperatures can

366   speed up rate processes, whereas fluctuations around high mean temperatures can slow

367   them down (Paajimans et al, 2010). Thus the *An. gambiae* s.l. were well suited to the

368   temperature regimes experienced in Furvela.

369   Unfortunately we do not know the species composition of the resting or exiting *An. gambiae*

370   s.l. compared to those entering the house. Nevertheless, the proportion of gravid insects in

371   resting catches varied in a similar fashion to that seen among the *An. funestus*. We also do

372   not know if the different members of the *An. gambiae* complex behaved differently or

373   disappeared from the study area at different rates. Nevertheless, all members of the

374   complex did apparently disappear during the study so that perhaps it was not just a specific

375   species that was affected but was a complex wide problem. Meyrowitsch et al., (2011) were

376   unable to determine the cause of the decline of *An. gambiae* s.l. in the Tanga region of

377   Tanzania, three thousand kilometers to the north of Furvela. In the Kilifi area of Kenya

378   population decline of *An. gambiae*, shown by a reduction in genetic diversity in the

379   mosquito, was attributed to the introduction of LLIN's (O'Loughlin et al, 2016) The decline

380   in Furvela started before the introduction of any control measures and although the

381   introduction of LLIN's may have exacerbated the problem for the mosquito it may not have

382   been the cause of the decline in the first place. The decline also paralleled that observed in

383   malaria in the Rufiji basin (Ishengoma et al., 2013). That similar declines appeared to occur

384   over a 3,000 kilometer stretch of coastline indicates that a climatic factor was responsible.

385   Mean temperatures did not change nor did they affect the *An. gambiae* differently at

386   different temperatures, so survival was not apparently affected by temperature changes. But

387   what the factor might be we do not know.

388   **Acknowledgements**

389   I would like to thank Joao Pinto of the IHMT, Portugal for identifying the members of the *An.*

390   *gambiae* complex in this study and for his perceptive comments on the manuscript. I also

391   thank Corey LeClair of the LSHTM UK and Olivier Briët of the Swiss TPH for comments on

392   the manuscript. Thanks to Nelson Cuamba and Ricardo Thompson for logistic support

393   during the study and thanks to the members of the MOZDAN project and to the villagers of

394   Furvela who let us collect mosquitoes from their houses

395

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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

3
4

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.

2

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

5
6

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.

2

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

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	

4
5   The equations for the different models are listed below:
6
7   Log *An. funestus* Light-trap = +0.9178 – 0.445*log rain+ 0.0342*Air temp
8   Log *An. gambiae* s.l. Light-trap = -1.419 + 0.117*Air temp – 0.392*Windspeed
9
10 Exit *An. funesus* unfed = -0.100+0.044*Air temp + 0.156*Windspeed
11 Exit *An. funestus* gravid = -0.099 + 0.0456*Soil temp – 0.111*Windspeed
12
13 Exit *An. gambiae* s.l. unfed = -1.629 + 0.1097*Air temp – 0.446*Windspeed
14 Exit *An. gambiae* s.l. gravid = -2.05 + 0.10869Soil temp – 0.734*Windspeed
15
16
17

Annual temperature variation

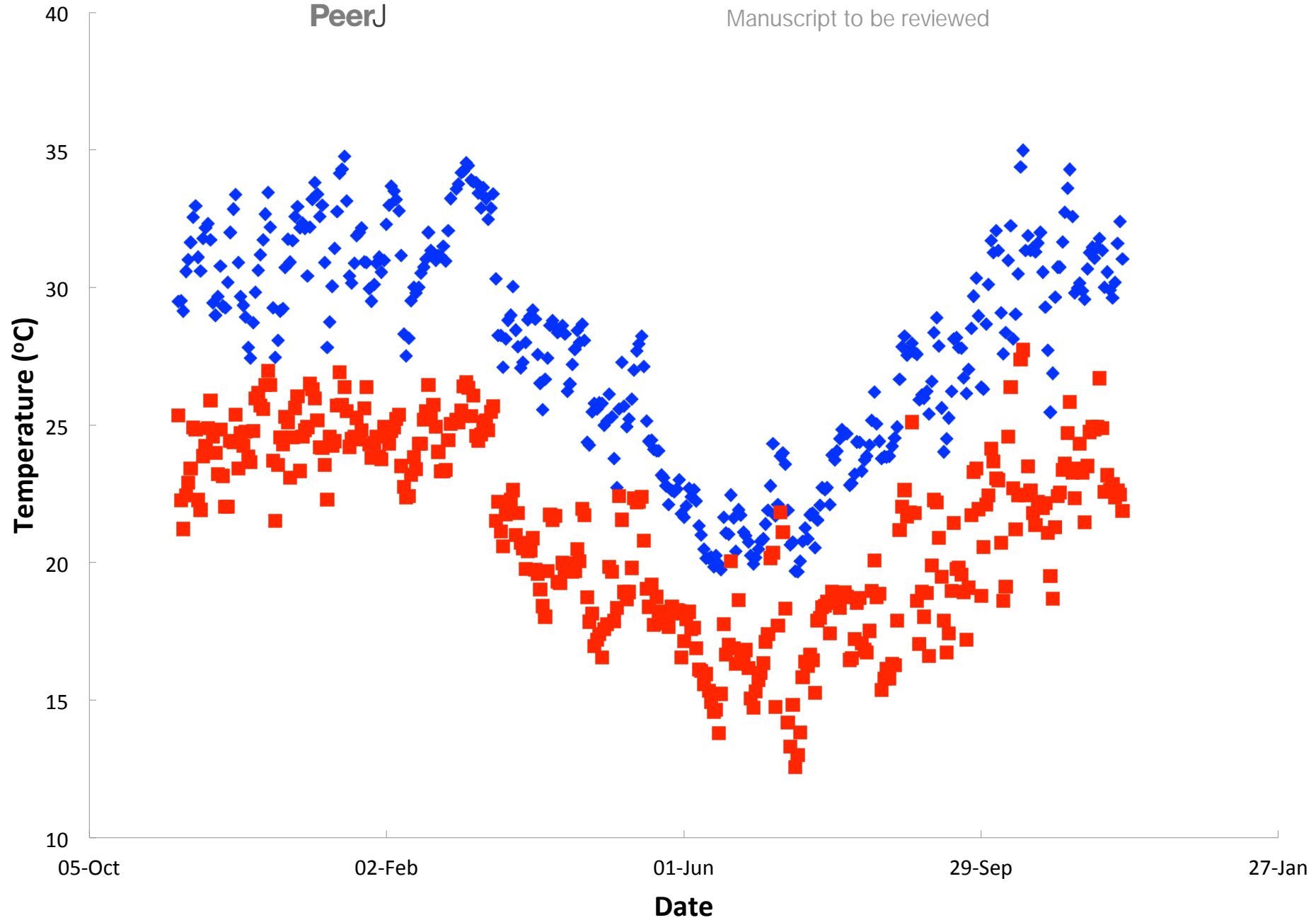
Figure 1. Mean daily soil and air temperatures recorded by the Delta logger in Furvela village

Daily variation in air and soil temperatures

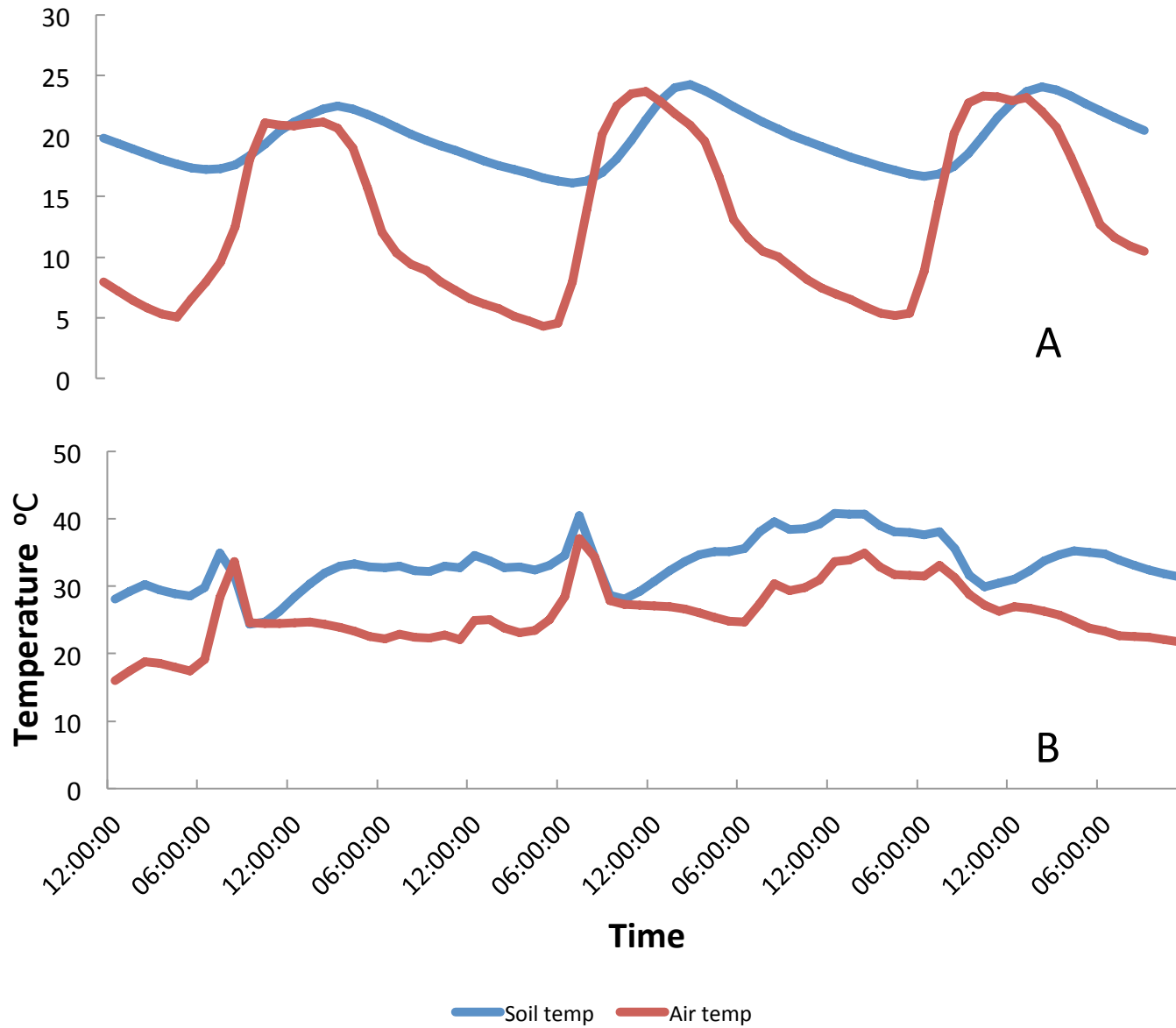
Figure 2. Diurnal variation in soil and air temperature recorded in the village of Furvela during A) the cool season (16-18th July) and B) the hot season (14-16th October).

Difference between maximum and minimum temperatures by ISO week

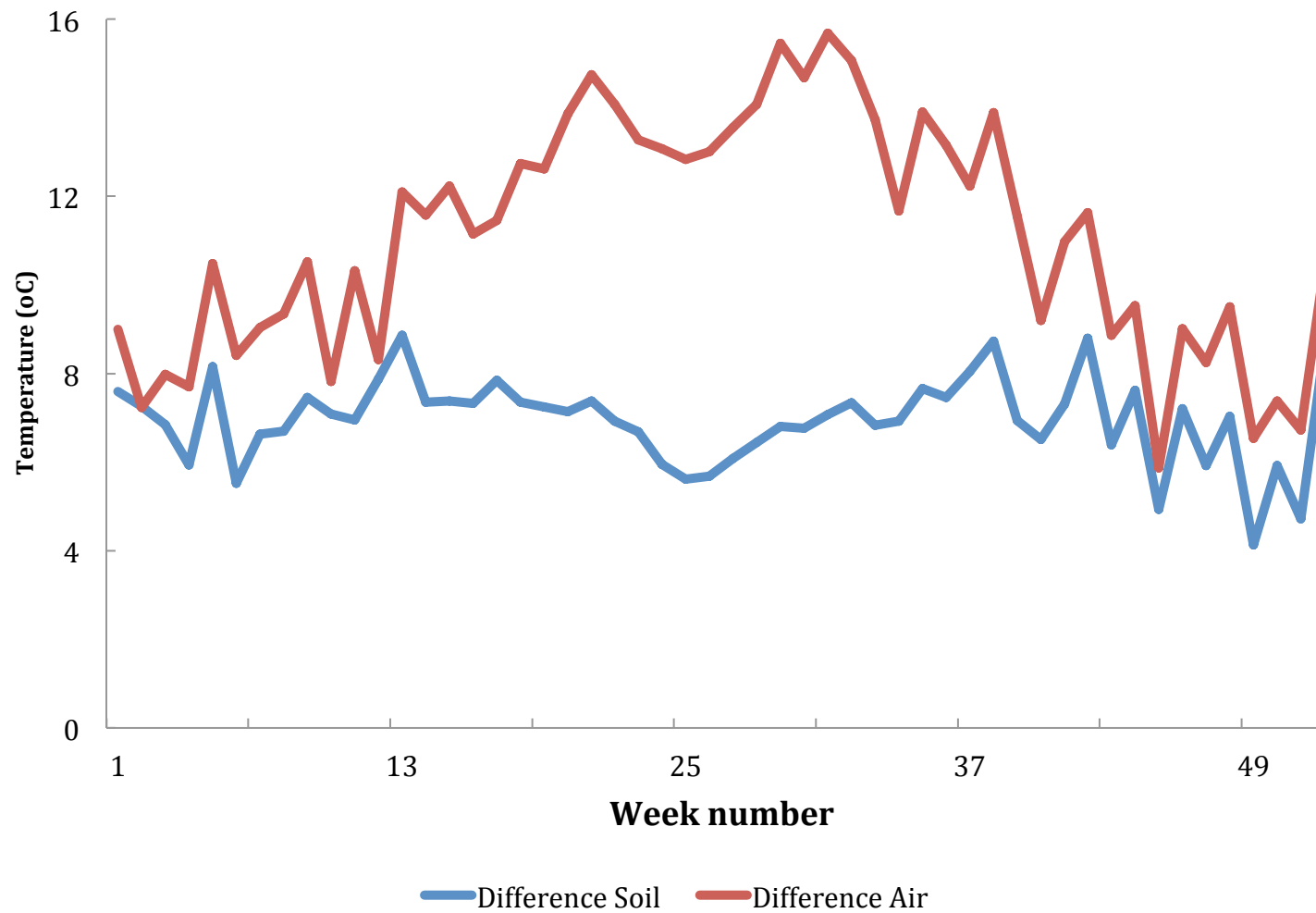
Figure 3. Difference between maximum and minimum air and soil temperature recorded from Furvela village, Mozambique, by ISO week number.

Temperature, rainfall and number of mosquitoes collected in Furvela

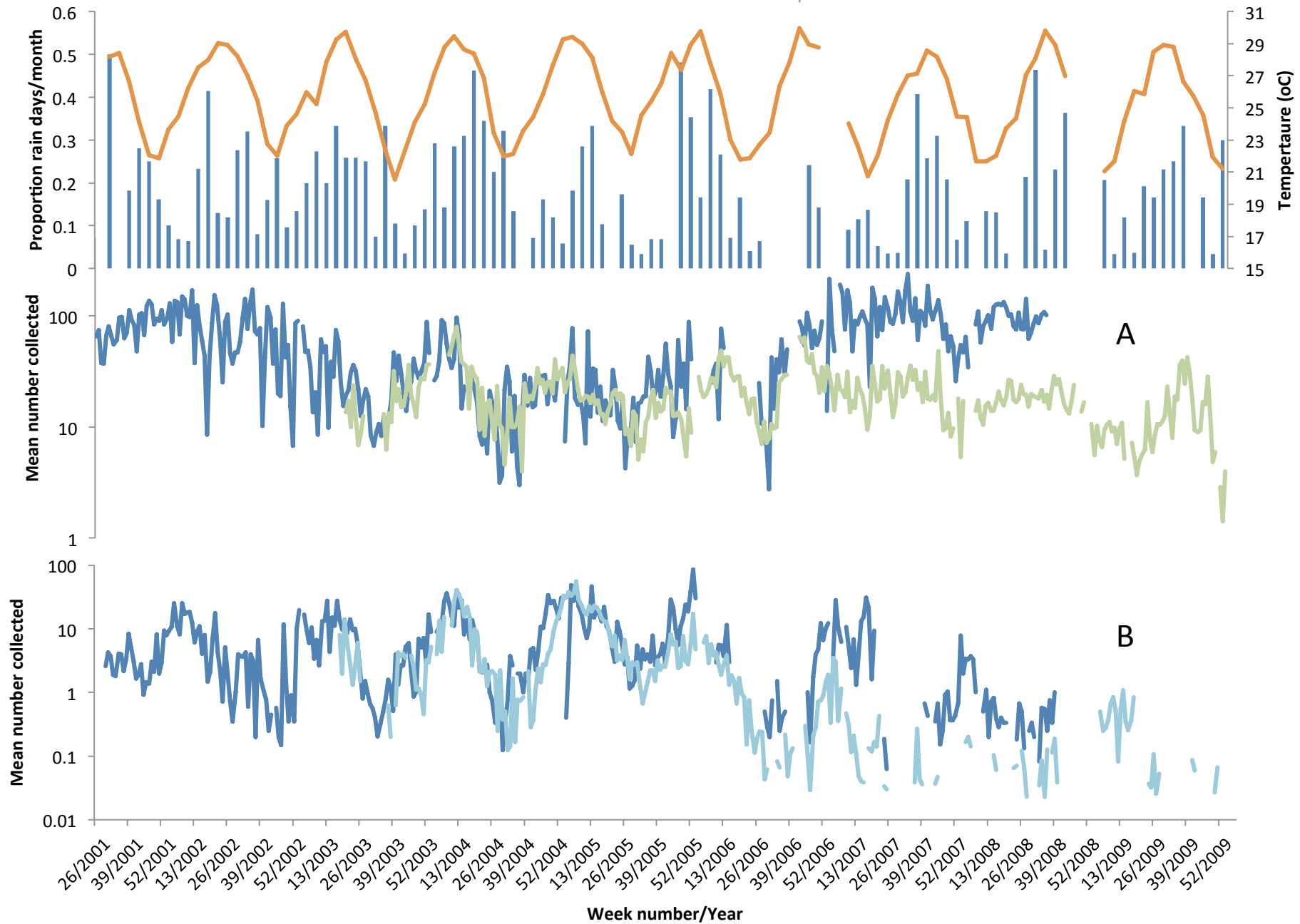
Figure 4. Rainfall (measured in Maxixe), air temperature (measured in Vilanculos) and mean numbers of unfed Anopheles funestus (A) and Anopheles gambiae (B) collected from light-traps and in exit collections from Furvela village, 2001-2009.

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

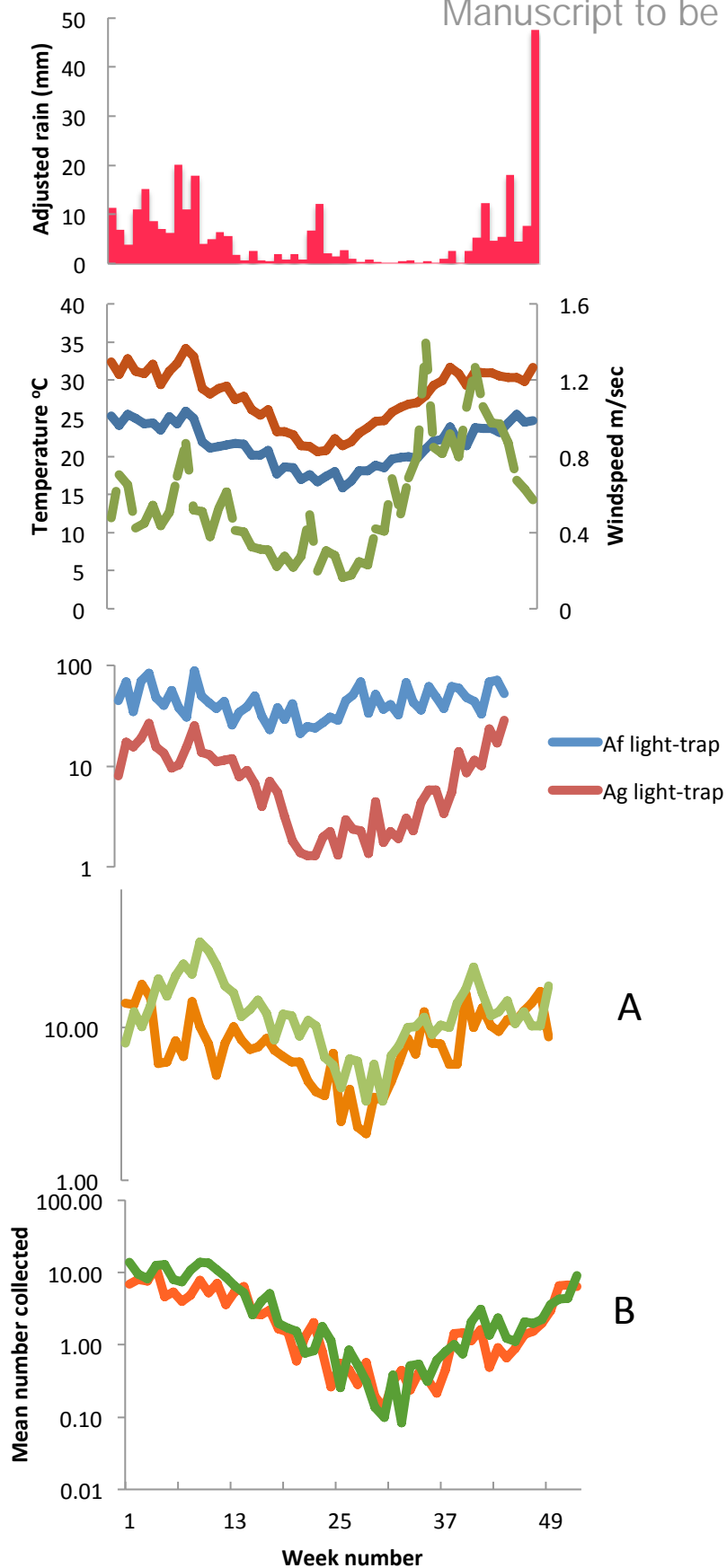
Figure 5. Weekly mean adjusted rain, soil temperature (red), air temperature (blue), windspeed (green) and mean numbers of Anopheles funestus and An. gambiae s.l. collected in light-traps and. A) An. funestus B) An. gambiae s.l. in exit collection (orange = unfed insects; green = gravid insects).

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

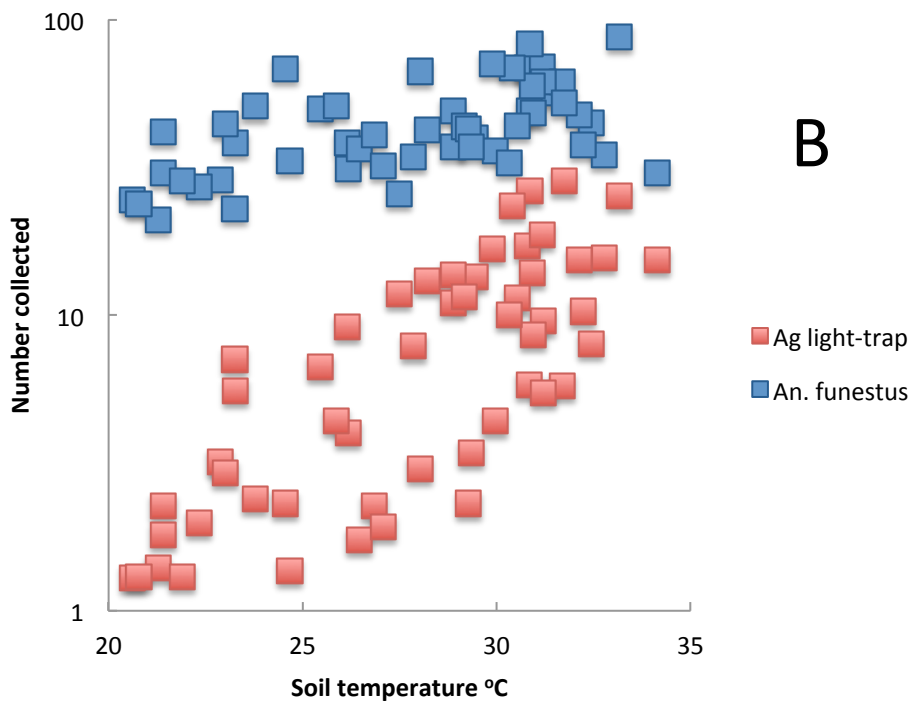
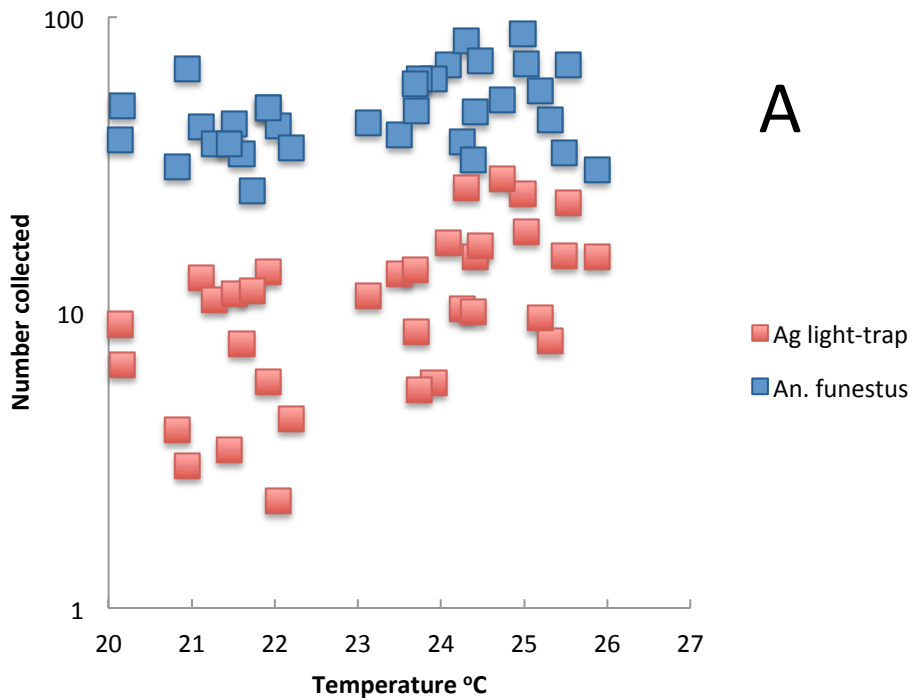
Ag light-trap
An. funestus

Number collected

Temperature °C

B

Ag light-trap
An. funestus

Number collected

Soil temperature °C

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.

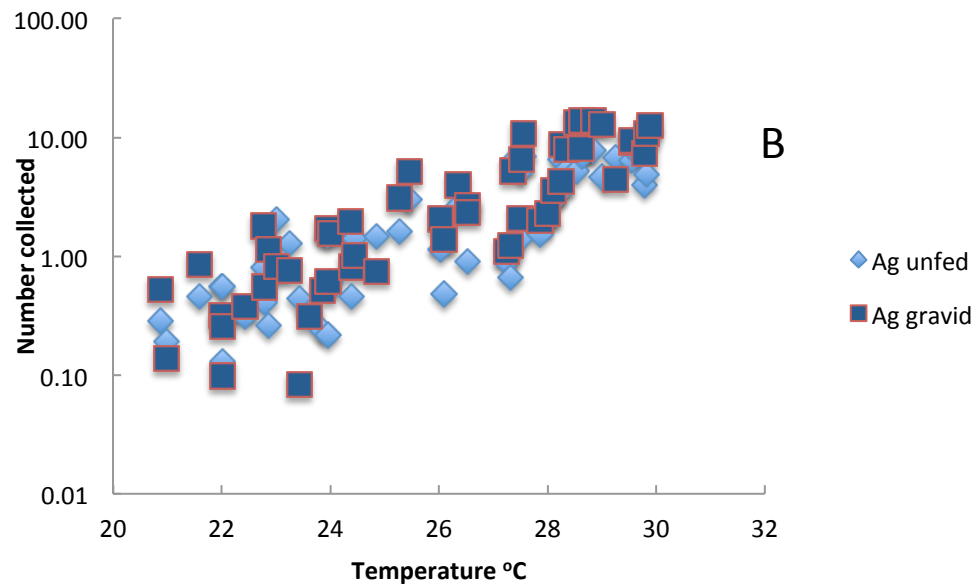
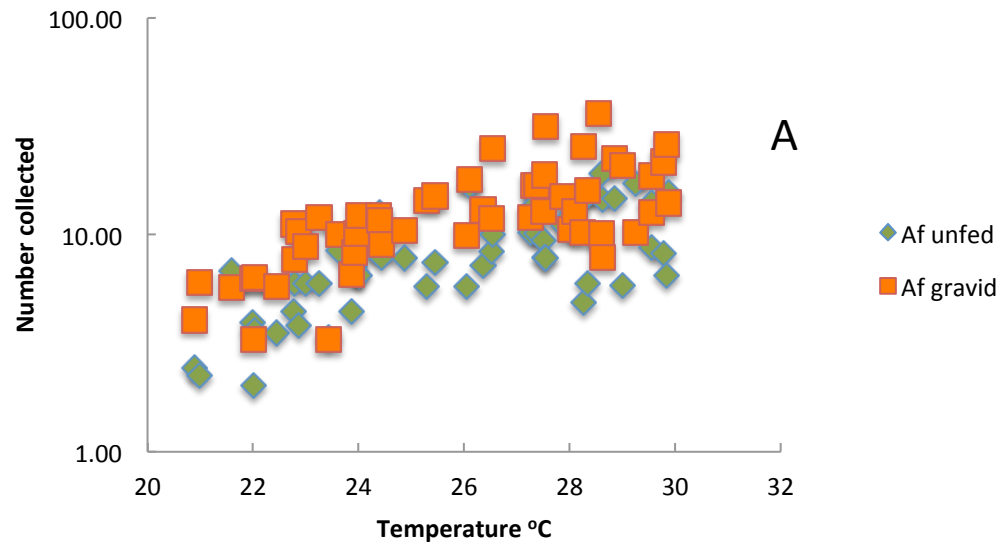
Figure 7. The relationship between the mean number of A) Anopheles funestus and B) An. gambiae s.l. in exit collections and soil temperature Furvela, Mozambique.

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.