# 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.

1  **Some like it hot: A differential response to changing temperatures by the malaria vectors**
2  *Anopheles funestus* **and** *An. gambiae* **s.l.**
3

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18 **Running head**

20 Temperature and African malaria vectors

21 **Introduction**

23 Temperature is a major driving force in insect populations. In the laboratory the species

24 that are almost entirely responsible for malaria transmission in Sub-Saharan Africa, the

25 freshwater members of the *Anopheles gambiae* complex (*An. gambiae*, *An. coluzzi* and *An.*

26 *arabiensis*) and *Anopheles funestus,* respond differently to different temperature regimes.

27 Larval development rates for *An. arabiensis* peak within the temperature range of 22-32$^{\circ}$C

28    with survival rates also highest at 32ºC, whilst an optimal temperature for larval

29    development of *An. gambiae* is between 28-32ºC but survival rate to adulthood is highest

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

31    higher temperature tolerance of *An. arabiensis* compared to *An. gambiae* (Kirby and

32    Lindsay, 2004) which itself may be responsible for the extended distribution of the former

33    species into hotter and drier environments in Africa. *Anopheles funestus*, on the other hand,

34    has a single optimum temperature of 25ºC for development with substantial declines in

35    survival either side of this (Christiansen-Jucht, 2014). Le Seur and Sharp (1991) concluded

36    that the effect of temperature on *An. merus* (another member of the *An. gambiae* complex)

37    was greatest during metamorphisis in the pupal stage, as did Heuval den val (1963) for

38    *Aedes aegypti*. Temperature-related metabolism during metamorphosis was affected by

39    available energy reserves (gathered during larval development) (LeSeur and Sharp, 1991).

40    At higher temperatures reserves are lower than at lower temperatures, hence emerging

41    adults are smaller. In the laboratory none of the *An. gambiae* complex or *An funestus*

42    survived as larvae or pupae at temperatures above 35ºC.  In the field, however, larvae and

43    pupae of *An. gambiae* s.l. (probably *An. arabiensis*) have been found in pools at

44    temperatures of 40.5-41.8ºC (Holstein 1952, quoted in Gillies & DeMeillon 1968).

46    The differential response to temperature in the laboratory reflects the different kinds of

47    water in which larval development takes place in the field.  Thus, although immature forms

48    of the *An. gambia*e complex may occur in a great variety of water bodies, the most

49    characteristic are the 'shallow open sun-lit pools with which every field worker in Africa is

50    familiar' (Gillies & DeMeillon, 1968).  Immatures of *An. funestus* are generally found in

51    more permanent, shaded, water bodies with emergent vegetation that are cooler than

52    exposed puddles.

54    Diurnal fluctuations in temperature also affect the development of many insects

55    (Vangansbeke et al., 2015). In addition to being hotter, temperature fluctuations in small

56    pools are greater than those in larger, shaded, bodies of water. For example, although

57    temperature minima in pools typically used by *An. gambiae* are similar to the minima in

58    shaded ones, maxima may be $10^{\circ}$C higher (Haddow, 1943) and, not surprisingly, given their

59    larval habitat, both *An. gambiae* and *An. arabiensis*, also respond better to fluctuating

60    temperatures than do *An. funestus* (Lyons et al, 2013, Christiansen-Jucht et al., 2014).

61

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

63    microclimates due to their predominantly endophilic behaviour. Temperature influences

64    the time it takes for egg development following a blood-meal but may also have subtler

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

66    temperatures above $26.5^{\circ}$C, but at lower temperatures females re-feed shortly after egg

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

68    higher temperatures is compensated for by it taking two rather than three days for the

69    mosquito to complete egg development, so that the duration of the complete gonotrophic

70    cycle is three days at all temperatures (Gillies & Wilkes, 1963).

71

72    The effect of temperature on the larval stages is manifest in the emergent population.

73    Newly emerged insects can be distinguished from the mature population by their

74 abdominal and gonotrophic state. Newly emerged males have un-rotated terminalia

75 (Charlwood, 2011) whilst females have undeveloped ovaries and constitute the unfed

76 portion of the resting or exiting population from houses (Gillies and Wilkes, 1965,

77 Charlwood et al., 2003). The relative proportions of engorged to gravid females at different

78 temperatures also provides information on the duration of egg development in mature

79 insects.

80

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

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

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

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

85 however, notoriously unpredictable and short-term, chaotic, fluctuations are common.

86 Long-term observations can assist in reducing the 'noise' in such data. Here we describe

87 the effect of ambient temperature, and other environmental parameters, over a five-year

88 period, on the dynamics of newly emerged and mature *An. funestus* and *An. gambiae* s.l.,

89 collected from a single village in southern Mozambique where temperatures varied from a

90 night-time minimum of 6°C in the cool season (10.5°C below the lower limit of 16.5°C for

91 larval activity, (Jepson et al., 1947)) to a daytime maximum of 35°C in the hot season.

92

93 **Methods**
94

95 **Description of study site**
96
97 The approximately 5x4 km village of Furvela, (23°43' S, 35°18' E), 475 km north of the

98 capital Maputo, is bordered on two sides by the alluvial plain of two river systems (Fig 1).

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

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

101  small canals. The Inhnanombe river to the east of the village consists largely of beds of the

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

103  does not flow as fast as the Furvela river. *Anopheles funestus* predominates on the Furvela

104  River side of the village and *An. gambiae* s.l. on the Inhnanombe side (Kampango et al.

105  2013). Malaria is endemic in the village with 80% of 2-4 year olds being positive in a cross

106  sectional survey undertaken in 2006 and children under one year of age comprised the

107  majority of attendees at a clinic established by the project in 2001 (supplemental files 1 &

108  2).

109

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

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

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

113  hence mosquitoes can easily enter these houses. Other styles of house include those with

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

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

116  relatively evenly spaced individual homes.

117  At the start of the study houses were mapped with handheld Global Positioning Units

118  (Garmin etrex), numbered and the manner of construction and size noted (supplemental

119  files 3 & 4). A census was taken and residents were informed about the purpose of the

120  study and consent concerning the possibility of future mosquito collections obtained. The

121  initial study area was expanded in the second year of the study and mapping was also

122  repeated in 2007.

**Mosquito collection**

**Light-traps**

127  Host seeking mosquitoes were collected in CDC light-traps hung, inside bedrooms,

128  approximately 1.5 m from the floor at the foot of the bed of people who themselves were

129  sleeping under mosquito nets.  A random list of houses was produced (using the random

130  number generator in Microsoft Excel) and routine collections were made according to the

131  list. In addition, a number of houses known to have high densities of mosquitoes were used

132  as sentinel sites. Collections were made in 764 houses on the Furvela river side of the

133  village and 214 on the Inhnanombe side of the village. Eleven houses were used for sentinel

134  collections; each being sampled for more than 100 nights.

**Exit collection**

137  From 2003 to 2007 mosquitoes were also collected exiting houses at dusk (Charlwood,

138  2013). The door of the house was left open and covered with a white mosquito-netting

139  curtain. Mosquitoes were manually aspirated off the curtain as they attempted to leave. See

140  : https://www.youtube.com/watch?v=SL8FeIuY1GM

141  Most of the houses used for the exit collections were on the Furvela side of the village.  The

142  young men and women who did the work, and who lived in dispersed locations collected

143  from their own or nearby houses. Altogether collections were made from 501 houses some

144  of which also acted as sentinel sites.

145

146 **Resting collection**
147
148 Resting collections, using a torch and an aspirator, were performed on the Furvela side of

149 the village, on an *ad hoc* basis on 163 days, from a total of 132 houses (mean number of 3.9

150 collections per house) where mosquito nets were not in use, and, initially, outdoors.

151 **Mosquito processing**
152
153 Collected *Anopheles* were separated into species or species group, according to the keys of

154 Gillies and De Meillon (1968) and Gillies and Coetzee (1987) and sexed. Females were

155 further separated into unfed, part-fed, engorged, semi-gravid and gravid categories. A

156 number of the *An. gambiae* s.l. were identified to species by PCR. DNA extraction was

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

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

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

160 **Meteorology**
161

162 **Temperature, insolation and windspeed measurement**
163
164 Daily variation estimates of soil and air temperature, insolation and windspeed were

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

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

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

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

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

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

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

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

173 May 2004 to 1 October 2005 and from 10 Nov 2007 to 24 Nov 2008. Hourly data from all

174 years, including the later ones, was amalgamated into daily data and daily data

175 amalgamated into ISO weeks. Mean values for the different ISO weeks from all the weather

176 station files were determined and used in calculations (supplemental file 5)

177

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

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

180

181 **Rainfall data**
182
183 Rainfall data was available from the town of Maxixe, 20 km to the south of Furvela. Since

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

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

186 of rainfall was used to estimate effects:

187

188 Modified weekly rain = (Rain (mm) * # rain days)/7

189 Thus 140mm on a single day is equivalent to (140*1)/7 = 20

190 And 140mm with rain every day is equivalent to (140*7)/7 = 140

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

192 at supplemental file 6 (Rainfall data).

193

194 **Analysis**
195
196 Data were entered into, and analyzed with, Excel (supplementary files 6). Unfed

197 mosquitoes from light-trap collections represent all age groups. Unfed mosquitoes from

198 exit collection are, however, almost entirely newly emerged ones (Charlwood et al., 2011),

199 whilst gravid females have taken at least one blood meal sufficient to develop eggs, and will

200 also include infectious ones. The weekly Williams mean ($\log_{10}$ (n+1)) of these three groups

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

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

203 and modified rainfall measured in Maxixe.

204

205 The relationship between mosquito numbers and environmental factors was examined

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

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

208 independent variables) was also undertaken using the Excel add-in StatPlus. The most

209 parsimonious model was determined by subtraction of least important variables.

210

211 **Ethics**
212
213 The study was conducted under the aegis of the joint Instituto Nacional de Saúde (INS)–

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

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

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

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

218 **Results**
219
220 **Environmental variables**
221

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

223    both followed a similar pattern. (supplemental file 7 Temperature data) There was both a

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

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

226    2007 to 24 Nov 2008 are shown in Fig 2. Diurnal variation in temperature differed between

227    cool and hot seasons. Figure 3A shows the diurnal pattern recorded on the three coolest

228    nights of the year (16-18th July) and 3B the three warmest (14-16th October).  Overall at the

229    higher temperatures daily variation (difference between maximuim and minimum

230    temperature) was less than it was at the cooler temperatures (Fig 4).  At a mean of 23.7$^o$C

231    the variation in air temperatures was 5.9 degrees and at 18.1$^o$C was 15.7 degrees. Variation

232    is soil temperature was lower than air temperatures being 4.1 degrees at 30.3$^o$C and 8.9

233    degrees at 28.2$^o$C.

234

235    Except for the mornings mean soil temperatures were consistently circa 5$^o$C warmer than

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

237

238    **Mosquito data**

239

240    Twenty-six (86%) of 30 males and 331 (81.3%) of the 407 females from an unselected

241    sample of the *An. gambiae* complex identified by PCR from 2002 and 2004 comprised *An.*

242    *gambiae*, the other species being 4 male and 67 (16.5%) female *An. arabiensis* and nine

243    (2.2%) female *An. merus* (Table1). There was no statistical difference in the ratio of *An.*

244    *gambiae* and *An. arabiensis* according to method of collection (light-trap, exit collection or

245    resting collection) (supplemental file 8).

246

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

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

249   *funestus*. Seventy-one females of the *An. funestus* group were identified by PCR

250   (supplementary file 9, courtesy of A. Szlanski).  All were *An. funestus*. Given that this is the

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

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

253   collections.

254

255   301, 705 female *An. funestus* were collected in 6043 light-trap collections, 161, 466 in 7397

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

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

258   resting collections (supplemental file 10– Raw data). Outdoor resting collections failed to

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

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

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

262   *rufipes* were collected exiting houses.

263

264   Figure 5 shows the weekly mean numbers collected per house per night of *An. funestus* and

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

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

267   three years when both light trapping and exit collections were simultaneously undertaken

268   (2003-2006) mean numbers of *An. funestus* per house, per method, were similar. In 2007 a

269   cordon sanitaire of long lasting insecticide nets (LLIN's) was established around the

270  Furvela River valley (Charlwood et al., unpublished) and numbers in exit collections

271  decreased relative to numbers in light-traps. Numbers of *An. gambiae* s.l. in exit and light-

272  trap were also similar. Over the course of the project, however, *An. gambiae* s.l.

273  disappeared from both light-trap and exit collection collections.  Given the possible effect of

274  the *cordon sanitaire* on numbers collected further analysis is confined to the years 2001-

275  2006 (when 5090 light-trap, 4461 exit and 1315 resting collections were performed).

276

277  Numbers of mosquito were greatest in the warmer months. Figure 6 shows the mean

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

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

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

281  years 2001-2006. Unfed insects in exit collections comprised the newly emerged portion of

282  the population.  Gravid insects represent the gonotrophically active (mature) population.

283  The proportion of that population that they represent depends on the duration of

284  oogenesis following a blood meal.  At warmer temperatures development takes two days

285  and so the gravid sample represents half of the mature population whereas at cooler

286  temperatures development may take three days and so the gravid sample will only

287  represent one third of the mature population. The whole of the newly emerged population,

288  on the other hand, is sampled every day.

289

290

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

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

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

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

295    (see supplemental file 11 for the other possible correlations). Adding a lag of up to four

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

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

298    lower in comparisons involving *An. funestus*.

299

300    Figure 7A gives the relationship between numbers of *An. funestus* and *An. gambiae* s.l.

301    caught in light-traps and air temperatures and 7B between mosquito numbers and soil

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

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

304    through a one hundredfold change (from a mean of 0.14 mosquitoes a night to 14) whereas

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

306

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

308    shown in Fig 8A & 8B. At temperatures below 28°C the mean number of gravid *An. funestus*

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

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

311    numbers of newly emerged insects continued to increase (Fig 8A). Numbers of both gravid

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

313    temperatures recorded (Fig 8B).  The proportion of the *An. gambiae* s.l. population that was

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

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

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

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

318   the numbers of gravid and unfed *An. funestus* collected in exit collections were not

319   correlated (p = 0.07).

320

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

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

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

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

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

326   all higher for the *An. gambiae* s.l. than for the *An. funestus.*

327

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

329   monthly temperature is shown in Figure 9.  For both, species, or species group, a higher

330   proportion of semi-gravid and gravid females compared to engorged females were

331   collected during the cooler months of the year (May to August). In other words, oogenesis

332   was taking longer at the lower temperatures.

333

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

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

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

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

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

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

340    complete gonotrophic development.

341

342    **Discussion**
343

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

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

346    increasing interest. The temperature in Furvela fluctuates between the minimum and

347    optimum temperature for mosquito development, hence over the linear part of the reaction

348    norm. Temperature was the most important environmental parameter, of those measured,

349    determining mosquito numbers in the village.  Although a more in depth assessment would

350    probably improve the interpretation of the results, even in the straightforward analysis

351    presented here mean daily temperatures from either air or soil sensors explained 70% and

352    35% of the density changes observed in *An. gambiae* s.l. and *An. funestus* respectively

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

354    substantially higher in the *An. gambiae* s.l. compared to the *An. funestus.* Sporozoite rates

355    were also highest during the warmer months of the year (supplemental file 12). The

356    combined effect on the entomological infection rate (EIR) and malaria morbidity in the

357    village will be examined elsewhere.

358

359    The population dynamics of *An. funestus* on the peninsula of Linga Linga across the

360    Morrumbene Bay (Fig 1) is determined by rainfall (Charlwood et al., 2013).  The absence of

361    a relationship between rainfall and mosquito numbers, particularly the *An. gambiae* s.l., in

362    Furvela is surprising. It may be due to the sandy soil in the village which means that rain is

363    rapidly absorbed and the puddles are rare or absent. At the same time breeding conditions

364    for the *An. funestus* in the valley remain reasonably constant due to the irrigation practices

365    of the local farmers and even major storms have only a temporary effect on the dynamics of

366    the mosquito (Charlwood and Bragança, 2012).

367
368    The ratio of gravid to unfed mosquitoes in exit collections depends on a number of factors,

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

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

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

372    and enter through different routes (open doors and eaves) (Gillies, 1988). Thus, houses

373    that may allow access for one group are not necessarily suitable for the other.  In addition

374    to house effects, the proportion of egg development time spent inside houses (which we

375    presume to be 100%, at least for *An. funestus*), the survival rate per oviposition cycle and

376    the duration of oogenesis, can all affect the unfed/gravid ratio.  Should any of these factors

377    change with temperature then the overall ratio will also change with temperature. The

378    absence of change, as occurred with the *An. gambiae* s.l. implies that these factors remained

379    constant, or compensated exactly, over the observed range of temperatures.

380
381    Ironically, the highest correlation between gravid insects in exit collections (for both

382    species or species group) was with soil temperature (which mimics water temperature)

383    whilst for unfed (newly emerged) insects it was with outdoor air temperature. As pointed

384    out by Pajimans et al., (2010) the micro-climate experienced by the mosquito inside houses

385    may be quite different to that outside.  Houses may be warmer in the cool season and

386    cooler in the hot season than temperatures recorded outside. Nevertheless, more

387    sophisticated measurement would only improve models for *An. gambiae* by a maximum of

388    30% and for *An. funestus* by 47% (and would imply that other factors, such as humidity,

389    were of lesser importance).

390

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

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

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

394    unfed insects remained more or less constant at all temperatures.  The unfed/gravid ratio

395    of the more common *An. funestus* did, however, change with temperature. As temperatures

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

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

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

399    evidenced in the resting collections and formerly described by Gillies and Wilkes (1963,

400    1965). At the higher temperatures exit collections would therefore be expected to sample

401    one half of the mature population (the other half being the semi-gravid insects that may

402    move from one resting site to another, but in the absence of disturbance, do not leave the

403    house) but at cooler temperatures only one third, or even less, of the population. At the

404    higher temperatures, however, the proportion of gravid insects in the exit collections

405    decreased, such that overall there was no significant relationship between the numbers of

406    gravid and unfed insects in exit collections. This either means that that survival between

407    emergence and maturity (i.e. becoming gravid) decreases at cooler temperatures or that

408    post-teneral insects have a higher mortality at higher temperatures. Both sets of insects

409    leave houses at the same time (Charlwood, 2011), hence sampling efficiency for the two

410    groups should be the same.

411

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

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

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

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

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

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

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

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

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

421

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

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

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

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

426    value (34%) of all the models.

427

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

429    'the key mosquito-related traits that combine to determine malaria transmission intensity

430    (i.e., parasite infection, parasite growth and development, immature mosquito

431    development and survival, length of the gonotrophic cycle, and adult survival) are all

432  sensitive to daily variation in temperature'. Fluctuations in temperature (i.e. the difference

433  between maximum and minimum temperatures) were greatest in the cooler months. In the

434  cool season the observed patterns in soil and air temperature were similar to shaded and

435  open water as determined by Haddow (1943). In the warmer months' fluctuations in

436  temperature were less than at lower temperatures. Fluctuations around low mean

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

438  temperatures can slow them down (Paajimans et al, 2010). Thus, the *An. gambiae* s.l. were

439  well suited to the temperature regimes experienced in Furvela.

440

441  Unfortunately, we do not know the species composition of the resting or exiting *An.*

442  *gambiae* s.l. compared to those entering the house. Nevertheless, the proportion of gravid

443  insects in resting catches varied in a similar fashion to that seen among the *An. funestus*.

444  We also do not know if the different members of the *An. gambiae* complex behaved

445  differently or disappeared from the study area at different rates. Nevertheless, all members

446  of the complex did apparently disappear during the study so that perhaps it was not just a

447  specific species that was affected but was a complex wide problem.  Meyrowitsch et al.,

448  (2011) were unable to determine the cause of the decline of *An. gambiae* s.l.  in the Tanga

449  region of Tanzania, three thousand kilometers to the north of Furvela. In the Kilifi area of

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

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

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

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

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

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

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

457    **Conclusion**
458
459    Temperature is a major driving force in the dynamics of both *An. funestus* and *An. gambiae*

460    s.l. in Furvela. Whilst gravid *An. funestus* appeared to be negatively affected by the highest

461    temperatures encountered increasing temperatures were favorable for the *An. gambiae* s.l.

462    which went through hundredfold changes in densities as a result. As average temperatures

463    increase due to Global Warming areas where *An. gambiae* is the vector, if there remains

464    sufficient water available for larval development, will be at heightened risk of malaria

465    transmission.

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476    **References**
477
478    **Bayoh MN, Lindsay SW. 2003**. Effect of temperature on the development of the aquatic
479    stages of *Anopheles gambiae* sensu stricto (Diptera: Culicidae). *Bulletin of Entomological*
480    *Research.* 93:375–381.
481

482 **Beck-Johnson L, Nelson W, Paaijmans K, Read A, Thomas M, Bjørnstad O. 2013.** The
483 effect of temperature on anopheles mosquito population dynamics and the potential for
484 malaria transmission. PLoS One 8(11):e79276.
485

486 **Charlwood JD. 2011.** Studies on the biology of male *Anopheles gambiae* Giles and
487 *Anopheles funestus* Giles from southern Mozambique. *Journal of Vector Ecology 36: 382-394.*
488

489 **Charlwood JD, Braganca M. 2012.** The effect of rainstorms on adult *Anopheles funestus*
490 behavior and survival. *Journal of Vector Ecology* 2012a 37: 1-5
491

492 **Charlwood JD, Cuamba N, Tomás EVE. Briët OJT. 2013.** Living on the edge: a
493 longitudinal study of *Anopheles funestus* in an isolated area of Mozambique. *Malaria Journal*
494 12:208
495

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

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

501
502 **Christiansen-Jucht CD, Parham PE, Saddler A, Koella JC, Basáñez M-G. 2015.** Larval and
503 adult environmental temperatures influence the adult reproductive traits of *Anopheles*
504 *gambiae* s.s. *Parasites & Vectors.* 2015; 8:456. doi:10.1186/s13071-015-1053-5.
505
506 **Collins FH, Petrarca V, Mpofu S, Brandling-Bennett AD, Were JB, Rasmussen MO,**
507 **Finnerty V. 1988.** Comparison of DNA probe and cytogenetic methods for identifying field
508 collected *Anopheles gambiae* complex mosquitoes. *American Journal of Tropical Medicine &*
509 *Hygiene* 39, 545–550.
510
511 **Gillies MT. 1988**. Anopheline mosquitoes: vector behavior and bionomics. Chapter 16 In
512 WH Wersdorfer & I McGregor (eds) *Malaria, Principles and Practice of Malariology.*
513 Churchill Livingstone, Edinburgh
514
515 **Gillies MT, Coetzee M. 1987.** A Supplement to the Anophelinae of Africa South of the
516 Sahara (Afrotropical Region)Publication no. 55. South African Institute for Medical
517 Research, Johannesburg.
518
519 **Gillies MT, De Mellion B. 1968.** The Anophelinae of Africa South of the Sahara (Ethiopian
520 Zoogeographical Region), 2ndPublication no. 54 edn. South African Institute for Medical
521 Research, Johannesburg.
522

523  **Gillies MT, Wilkes TJ. 1963.** Observations on nulliparous and parous rates in a
524  population of *Anopheles funestus* in East Africa. Annals of Tropical Medicine and
525  Parasitology. 57: 204-213.
526
527  **Gillies MT, Wilkes TJ. 1965.** A study of age composition of population of *Anopheles*
528  *gambiae* Giles and *Anopheles funestus* Giles in North-eastern Tanzania. *Bulletin of*
529  *Entomological Research* 56: 129–135.
530
531  **Haddow AJ. 1943.** Measurements of temperature and light in artificial ponds with
532  reference to the larval habitat of *Anopheles (Myzomyia) gambiae* Giles, and *A. (M.) funestus*
533  Giles. *Bulletin of Entomological Research* 34:89–93.
534
535  **Heuval MJ van den. 1963.** The effect of rearing temperature on the wing length, thorax
536  length, leg length and ovariole number of the adult mosquito, *Aedes acgyptii* (L.).
537  *Transactions of the Royal Entomological Society of London*, 115, 197-216.
538
539  **Holstein M. 1952.** Biologie d'*Anopheles gambiae*. Monograph Series World Health
540  Organization No 9, 176 pp.
541
542  **Ishengoma DA, Mmbando BP, Segeja MD, Alifrangis M, Lemnge MM, Bygbjerg IC**. 2013.
543  Declining burden of malaria over two decades in a rural community of Muheza District,
544  north-eastern Tanzania. Malaria Journal 12:338 DOI 10.1186/1475-2875-12-338.
545
546  **Jepson WF, Moutia A, Courtis C. 1947.** The malaria problem in Mauritius: the bionomics
547  of Mauritian anophelines. *Bulletin of Entomological Research*, 38:177–208.
548
549  **Kampango A, Bragança M, de Sousa B, Charlwood JD. 2013.** Netting barriers to prevent
550  mosquito entry into houses in southern Mozambique: a pilot study. *Malaria Journal* 12:99.
551
552  **Koekemoer LL, Kamau L, Hunt RH, Coetzee M. 2002. A** cocktail polymerase reaction
553  assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. *American*
554  *Journal of Tropical Medicine and Hygiene,* 66, 804–811.
555
556  **Kirby MJ, Lindsay SW. 2004.** Responses of adult mosquitoes of two sibling species,
557  *Anopheles arabiensis* and *An. gambiae* s.s. (Diptera: Culicidae), to high temperatures.
558  *Bulletin of Entomological Research.* 94:441–448.
559
560  **Le Sueur, Sharp BL. 1991.** Temperature-dependent variation in *Anopheles merus* larval
561  head capsule width and adult wing length: implications for anopheline taxonomy. *Medical*
562  *and Veterinary Entomology* 5: 55-62.
563
564  **Lyons CL, Coetzee M, Chown SL. 2013.** Stable and fluctuating temperature effects on the
565  development rate and survival of two malaria vectors, *Anopheles arabiensis* and *Anopheles*
566  *funestus. Parasites & Vectors.* 6:104. doi:10.1186/1756-3305-6-104.

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

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

574 **Paaijmans KP, Blanford S, Bell AS, Blanford JI, Read AF, Thomas MB. 2010.** Influence of
575 climate on malaria transmission depends on daily temperature variation. *Proceedings of the*
576 *National Academy of Science USA* 107: 15135–9.
577
578 **Scott JA, BrogdonWG, Collins FH. 1993.** Identification of single specimens of the
579 *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical*
580 *Medicine and Hygiene*, 49, 520–529.
581
582 **Vangansbeke D, Audenaert J, Tung Nguyen D, Verhoeven R, Gobin B, Tirry L, De**
583 **Clercq P. 2015** Diurnal Temperature Variations Affect Development of a Herbivorous
584 Arthropod Pest and its Predators. *PloS one* 10 (4), e0124898
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589

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

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

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

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 | >0.00005 |
| | Gravid exit | Soil temp + windspeed | 0.563 | >0.00005 |
| | Unfed Light | Air temp + Rain | 0.344 | >0.00005 |
| *An. gambiae* s.l. | Unfed exit | Air temp + Windspeed | 0.752 | >0.00005 |
| | Gravid exit | Soil temp + Windspeed | 0.822 | >0.00005 |
| | Unfed Light | Air temp + Windspeed | 0.756 | >0.00005 |

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

Google Earth image of Furvela Village

Furvela village and its relation to Linga Linga.

To Morrumbene

416

*Morrumbene Bay*

Linga Linga

EN 1

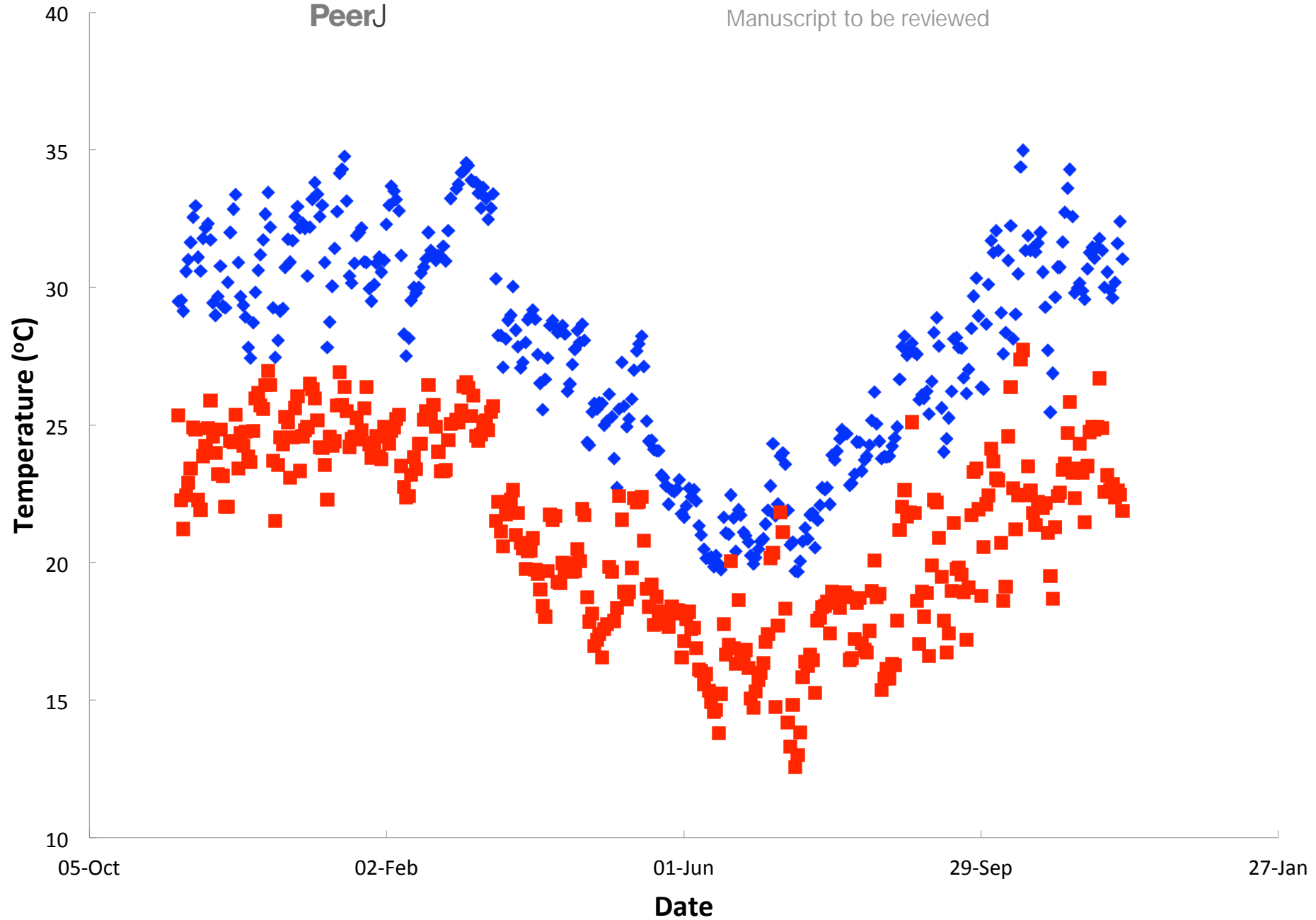
Furvela

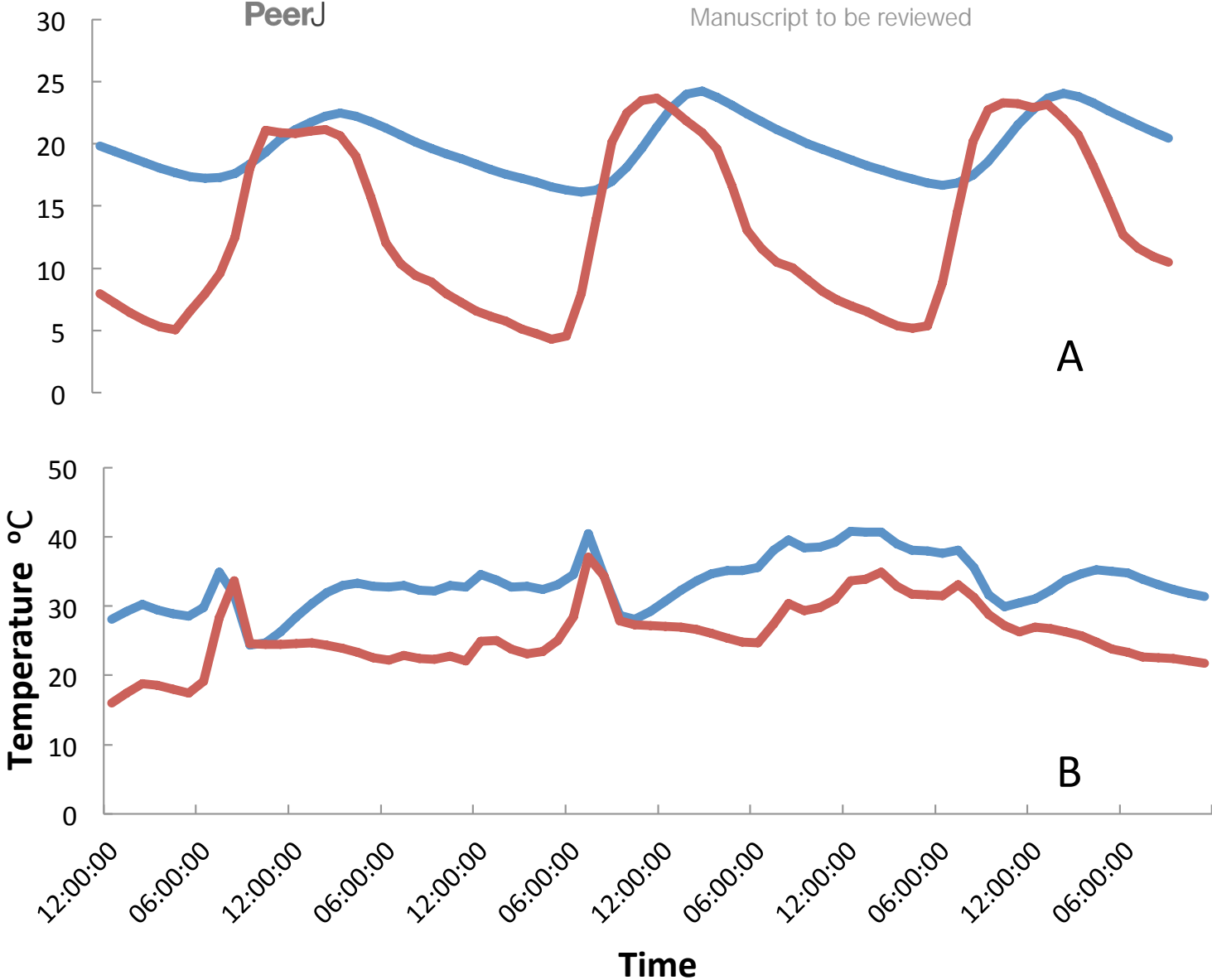
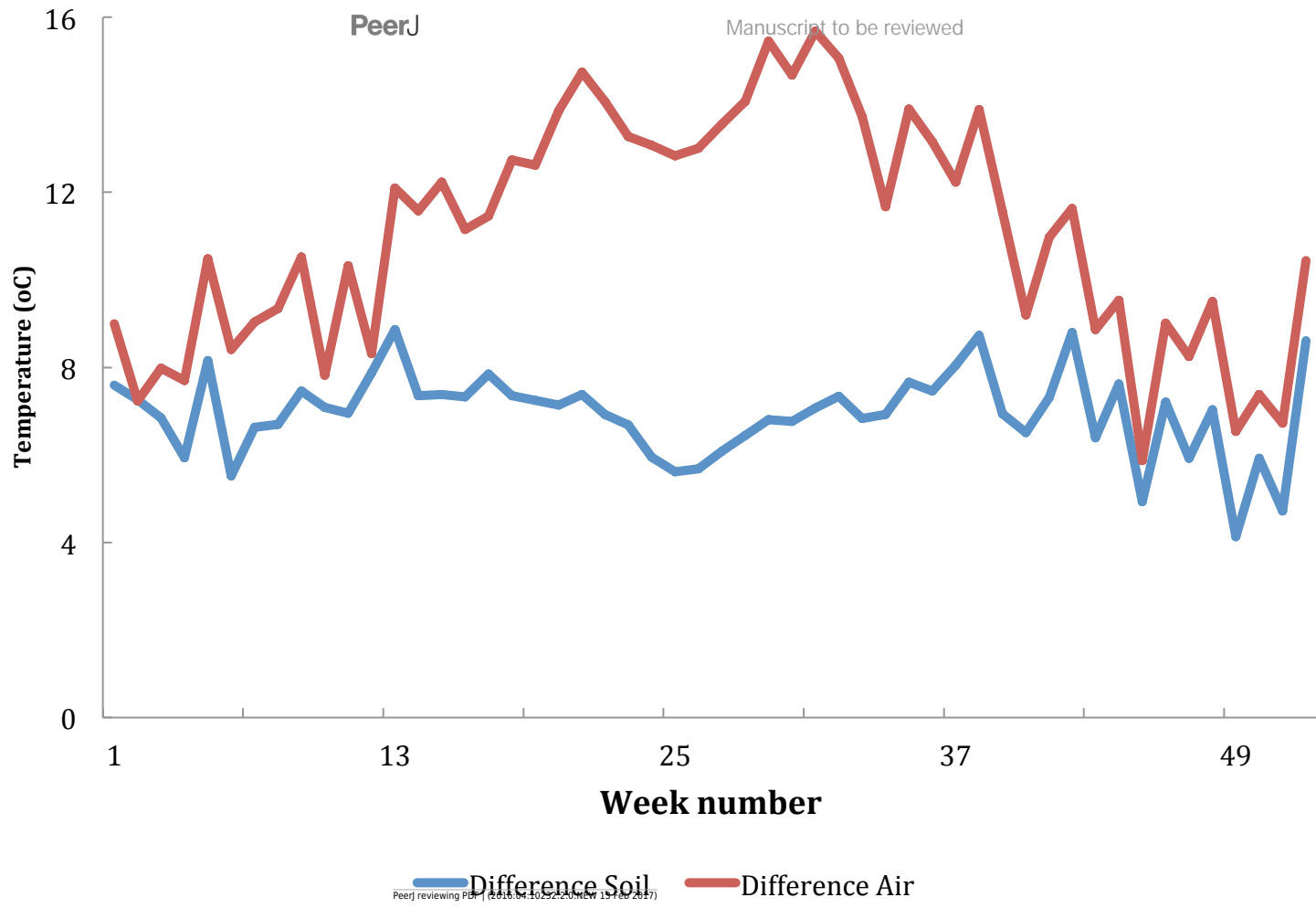
Beula

To Maxixe

N

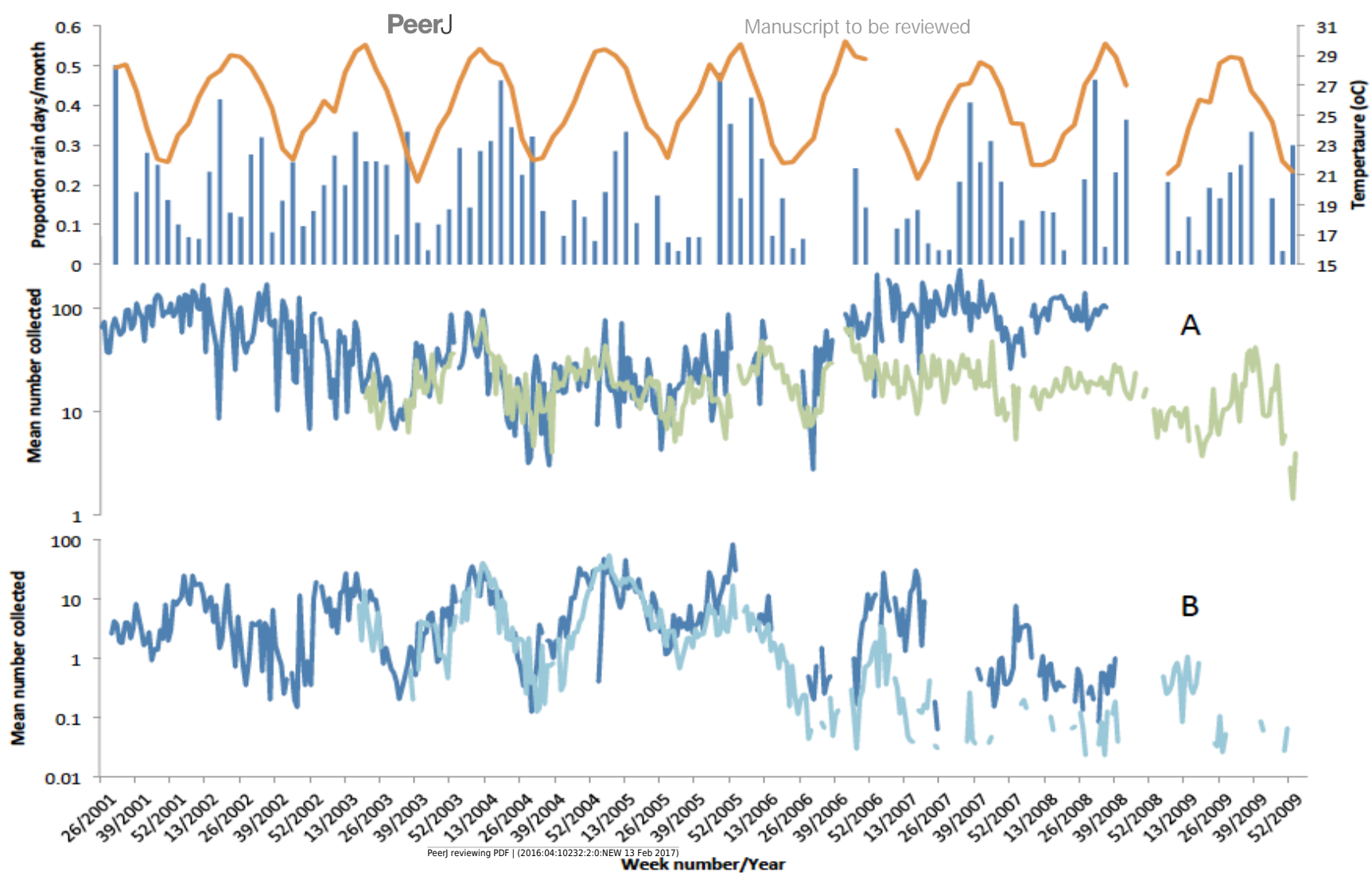
Annual temperature variation

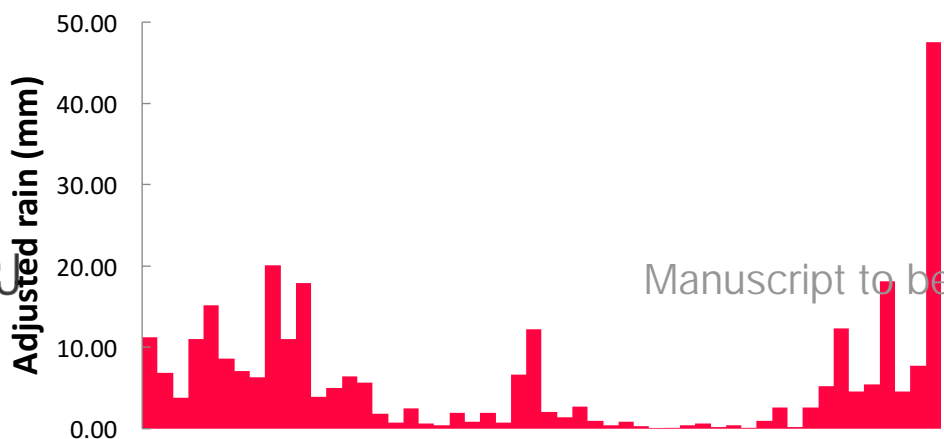
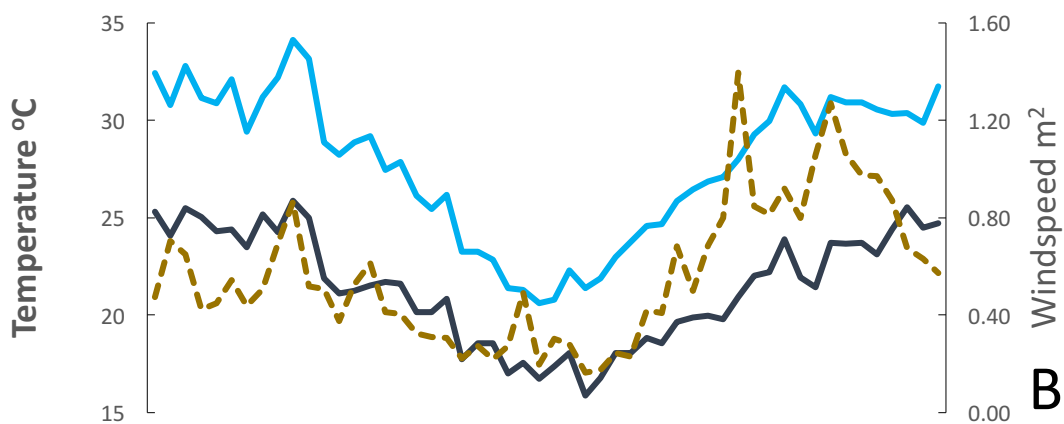
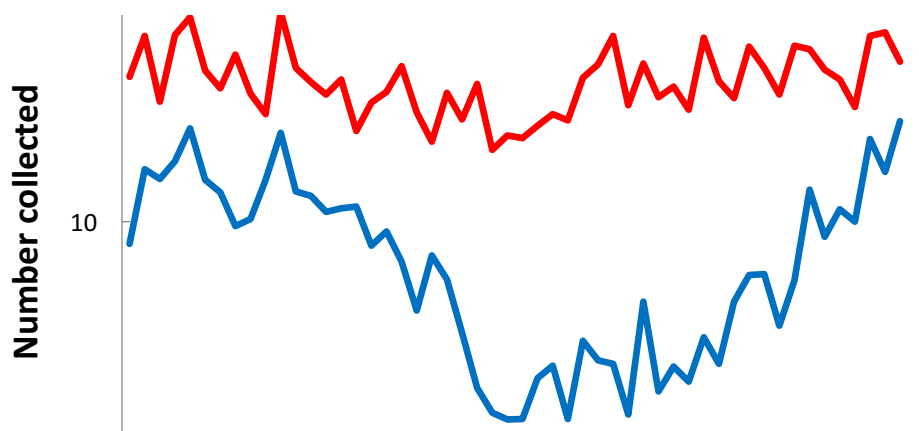
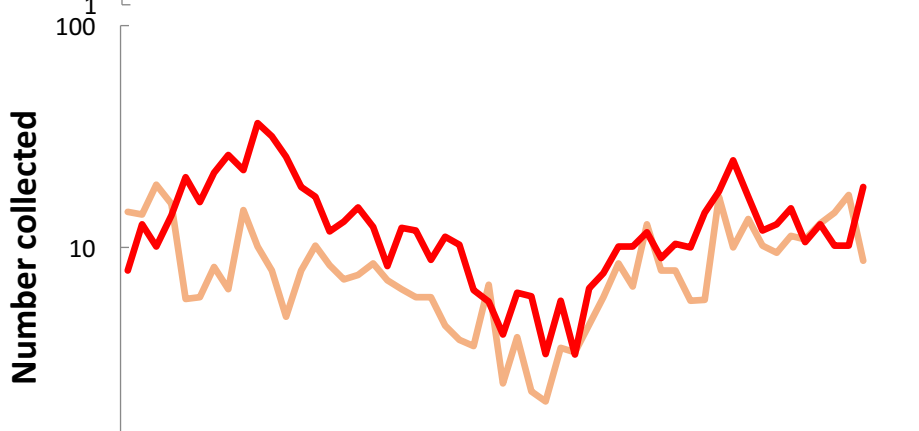
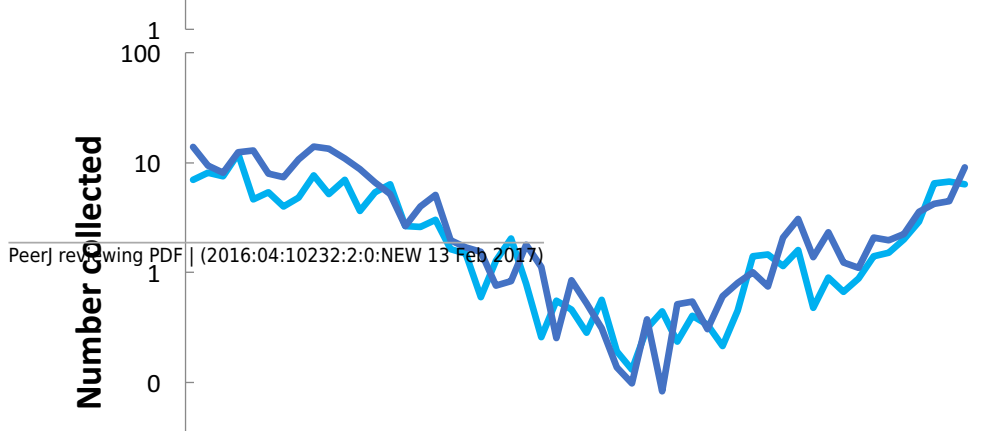
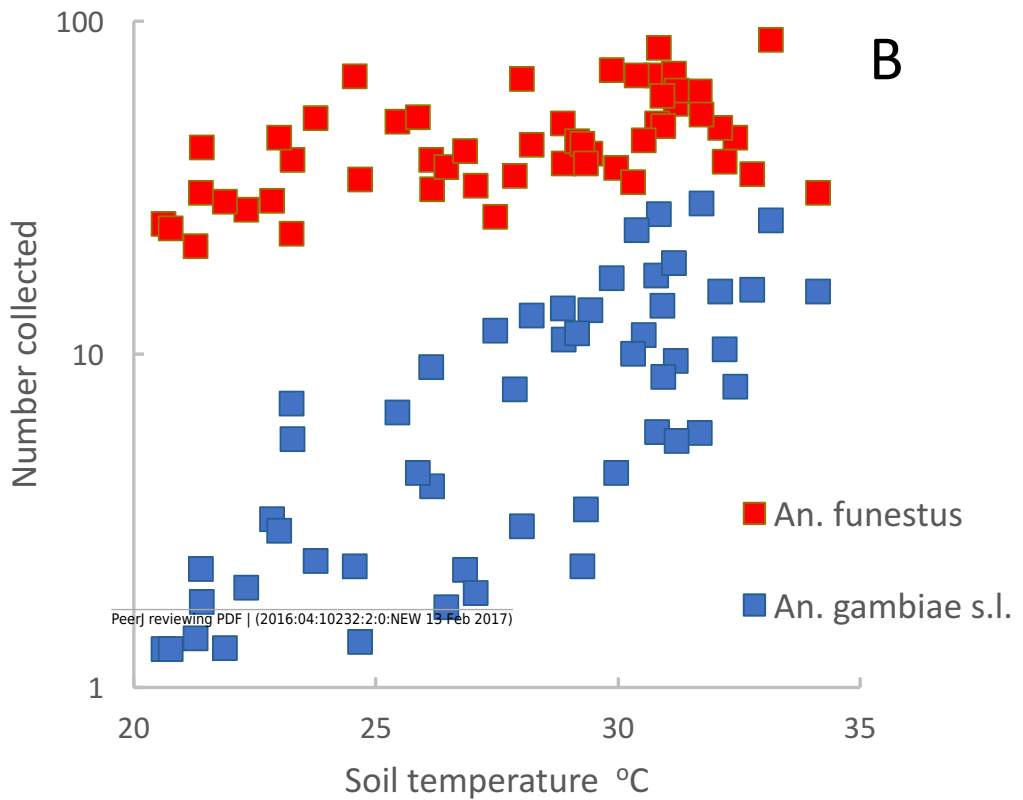
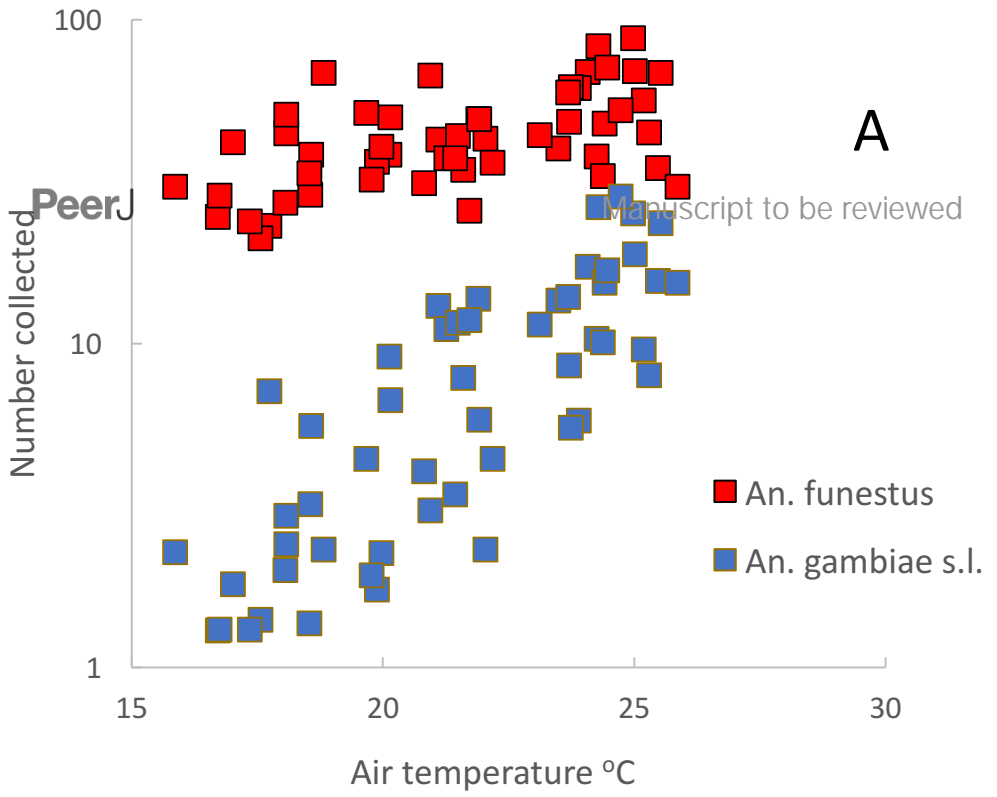
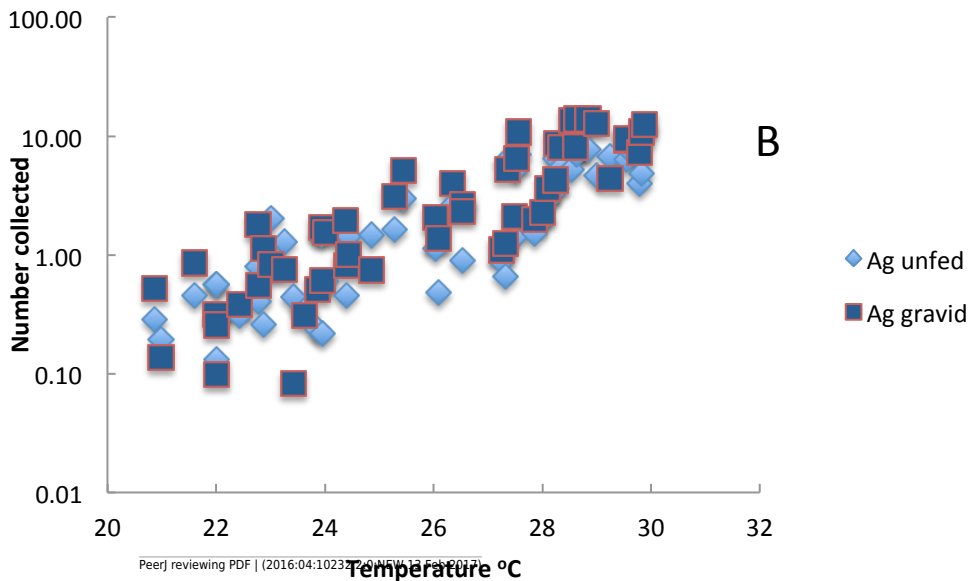
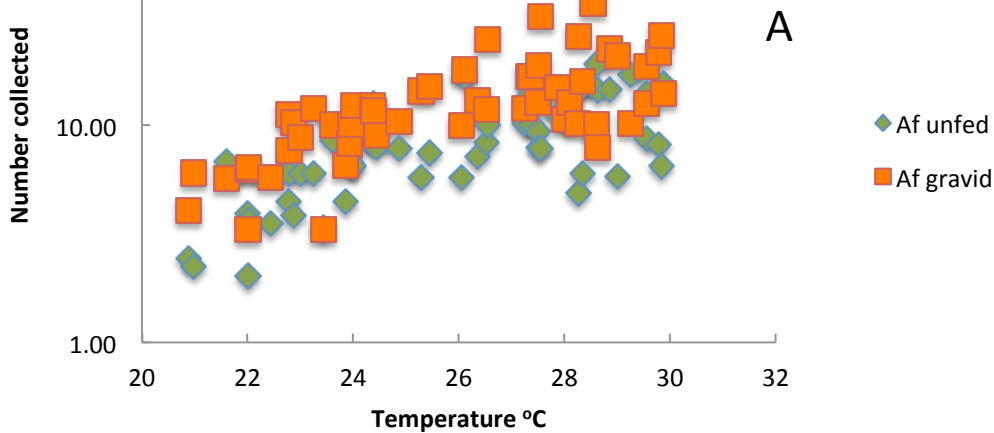
Mean daily soil and air temperatures recorded by the Delta logger in Furvela village

Daily variation in temperature during A) the coolest months of the year and B) the warmest months of the year, Furvela, Mozambique.

Soil temp    Air temp

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

Upper graph -Rainfall (measured in Maxixe) (Blue histogram), air temperature (measured in Vilanculos) (orange line)

(A) mean numbers of unfed Anopheles funestus form light-traps (blue line) and in exit collections (green line)

(B) mean numbers of unfed Anopheles gambiae collected from light-traps (Dark blue line) and in exit collections (light blue line) from Furvela village, 2001-2009. Note the log scale.

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

Environmental parameters and mosquito numbers by ISO week, Furvela, Mozambique. A) mean weekly adjusted rain in millimetres (measured in Maxixe), B) Light blue line – mean soil temperature (in degrees Centigrade); Black solid line Mean air temperature (in degrees centigrade); dotted line – mean wind-speed (in metres per second),C) Mean number of *Anopheles funestus* (red line) and *An. gambiae* s.l. (blue line) collected from light traps, D) Mean number of unfed (pink line) and gravid (red line) *An. funestus* from exit collections,(note the log scale) E) Mean number of unfed (light blue line) and gravid (dark blue line) *An. gambiae* s.l. from exit collections,(note the log scale).

Mean numbers of *Anopheles funestus* (red) and *An. gambiae* s.l. (blue) collected in light-traps from Furvela village, Mozambique A) by air temperature and B) by soil temperature (in degrees Centigrade)

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.

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.