Emergence, feeding and oviposition behaviour of Anopheles arabiensis: a vector from Mamfene in KwaZulu-Natal (South Africa)

Rajendra Maharaj (Rajendra.Maharaj@mrc.ac.za)
South African Medical Research Council
Vishan Lakan
South African Medical Research Council
Kiash Maharaj
University of KwaZulu-Natal

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Abstract

Background

Malaria is a disease of public health importance. To eliminate malaria from KwaZulu-Natal, new strategies are needed to increase the impact of existing interventions. Greater attention is being given to controlling the larval stages, as a complementary measure to indoor residual spraying. However, there is limited information available on bionomics of this South Africa mosquito vector. To fill ithe knowledge gaps in vector bionomics, this study interrogated the emergence, feeding and oviposition behaviour of *Anopheles arabiensis*.

Methods

To observe the number of eggs laid per F_1 *An. arabiensis* female, 300 fully gravid mosquitoes were individually placed into breeding tubes which were observed hourly from 18h00 to 06h00 and number of eggs laid by each female was recorded. Egg hatch rate was measured. Immediately after oviposition, the female mosquitoes were offered a blood meal to determine the duration post laying it took to refeed. To observe emergence times and sex ratios, a total of 4000 F_1 pupa were placed in large containers which were observed hourly from 18h00 to 06h00. The newly erupted mosquitoes were collected and placed in bug-dorm cages. A sample of the newly erupted female mosquitoes were removed from the cages and placed individually into a test container and offered a blood meal. The female was then observed for a probing behaviour indicating its willingness to feed.

Results

Oviposition studies revealed two peaks corresponding with late evening and just before dawn. Most eggs were laid in the first half of the night (18h00 – midnight). Most mosquitoes erupted just after sunset and the sex ratios showed twice as many females as males emerged from pupae. Females readily took a bloodmeal after oviposition or just after emerging. Hatch rate to viable first instar larvae was 74.5%.

Conclusions

This study provided information on egg laying, hatch rates, emergence times, feeding behavior and sex ratios. The sex ratio of emerging mosquitoes is biased to females. The feeding behaviour suggests that the use of control interventions close to known vector breeding sites would be a tool to eliminate many males and females seeking sustenance soon after emergence or oviposition.

Background

Although the incidence and mortality due to malaria are decreasing remarkably, malaria remains a disease of major public health importance. The latest available statistics report that there has been 405 000 deaths and 219 million cases of malaria world-wide during 2021 [1]. However, in the southern most parts of the continent, malaria has been quite effectively controlled using indoor residual spraying (IRS) or long lating insecticide-treated nets (LLINs to reduce contact between the host and vector thus resulting in diminished transmission. South Africa is now targeting malaria elimination as a result of having achieved low levels of transmission using coordinated and sustained vector control measures [2]. However, there are gaps in our knowledge of the vectors that need to be addressed before elimination can be attained.

Killeen [3] proposed that there should be a greater emphasis on larval control as a tool to eliminating residual malaria transmission. In South Africa, the province of KwaZulu-Natal has been in elimination for the past decade [4] but residual transmission is sufficient to make malaria elimination unattainable without additional vector control tools and technologies. Currently, the only malaria control intervention is indoor residual spray. Limited larval control is being conducted in malaria foci areas where there is active transmission. Moreover, they are only applied to the sites that are few, fixed and findable [5]. There is thus a need to understand the interface between the larval and adult stages in order to identify any opportunities that can be exploited for control purposes.

In South Africa, the biology of the immature stages and the females feeding behaviour is poorly understood. The developmental attributes of the various life stages of *Anopheles arabiensis* were studied with a focus on larval, pupal and adult survivorship and included egg hatch rates and sex ratios [26]. It was found that these life table characteristics were influenced by seasonal temperature and humidity and hence demonstrated the need for malaria control interventions that spanned the transmission season from October to May [26]. Over time mosquitoes have adapted to outdoor feeding due to the continued use of IRS [6]. Available information on mosquito biology was collected decades ago [7,8] and has changed over time [9], influenced by environmental changes [10]. Changes in the ecological factors can affect malaria transmission by modifying the microclimate of the immature stages and adult mosquitoes [11]. Changes in biology and physiology can also impact mosquito development [12]. In order to focus on both larval and adult mosquito control methods, there needs to be a greater understanding of larval development and the reproductive stages of the vector.

This study was therefore conducted to determine the peak emergence times and the emergent sex ratios, as well as the peak egg oviposition time in order to better understand the overlap between the aquatic larvae (through egg hatch rates), pupae (adult emergence times) and adult feeding and oviposition behaviour.

Methodology

Study Area

The mosquitoes used in this study were collected from Mamfene in KwaZulu-Natal, South Africa. In this area, the main vector is *Anopheles arabiensis* and the most common parasite is *Plasmodium falciparum*. Indoor residual spraying is currently the only intervention in place since the malaria control policy of the country does not include the use of bed nets. Presently, the only other control measure being considered is the use of larvicides, limited to areas where cases have been notified. Since this province is targeting elimination, the distribution of malaria is well defined and active breeding sites are well charcterised and geo-located. Also, *An. arabiensis* has been identified as an opportunistic feeder capable of feeding on domestic livestock and humans.

Collection of Mosquitoes

Laboratory reared *An. arabiensis* from Mamfene, in KwaZulu-Natal, South Africa was used for the observations in this study. The mosquitoes used were the F_1 progeny of wild-caught females. Field collected females readily laid eggs under insectary conditions maintained at constant temperature of 27°C and relative humidity of 70% with a photoperiod of 12h light: 12h dark with one hour simulated crepuscular period. During the study, the mosquitoes were maintained at a constant temperature of 25°C (the average summer temperature in KwaZulu-Natal).

Oviposition Behaviour

The variables under investigation were the time at which the eggs were laid, the number of eggs laid, the egg hatch rate and the time it took the female to find a blood meal.At the start of the trial, adult female mosquitoes were collected from Mamfene in KwaZulu-Natal, South Africa. All F_1 mosquitoes emerging on day 1 were pooled together in cages to give the mosquitoes an opportunity to mate. Three hundred unfed three-day old *Anopheles arabiensis* female mosquitoes were blood fed two days prior to the trial. Thereafter they were kept in 30x30x30cm BugDorm Insect Rearing Cage (BugDorm, USA) under controlled environment with optimum environmental conditions of 27°C and a humidity of 70% which represents the average summer conditions in KwaZulu-Natal. To observe the number of eggs laid per female, 300 fully gravid blood fed female mosquitoes were individually placed into a 60 ml test tube prepared with a water saturated piece of cotton wool beneath a 3 cm diameter disc of filter paper to serve as an egg laying surface. The test tubes were left in a controlled environment for observation. The test tubes were observed on an hourly basis from 18h00 to 06h00 and the number of eggs laid by each female was then recorded. Counting of the eggs was done by viewing the eggs per mosquito under a stereo microscope and counted using a 6-key economy benchtop counter.

Egg Hatch Rate

Eggs from individual females were collected, counted and placed in individual containers containing distilled water. Hatched first instar larvae were removed and counted daily, up to four days after the first egg hatched. This life history trait was measured in percentage as the number of first instar larvae by the total number of collected eggs.

Refeeding rates

The female mosquitoes that had just laid eggs were then offered a blood meal to determine the duration post-oviposition it took to refeed. Feeding was confirmed by observing blood in the abdomen.

Emergence Times and Emergent Sex ratios

The F_1 pupa that developed overnight were counted and used in this aspect of the study. The emergence study used four thousand 3-day old *An. arabiensis* pupae. The pupae were put into 5 litre test containers filled with 2 litres of de-ionized water. The test containers were kept in a controlled environment with optimum environmental conditions of 25-27°C and a humidity of 70%. The environmental conditions are maintained using an industrial humidifier and air control system designed specifically for the insectary. The insectary has an autotimer that gradually switches the lights on or off to mirror the dusk to dawn effect in nature. The autotimer switched the lights off from 17h00 and switched them on from 05h00 daily. Researchers used head lamps on trial day to view the emergence. The test containers were observed on an hourly basis from 18h00 to 06h00 and the newly erupted mosquitoes were collected hourly using a mouth aspirator. The collected mosquitoes were then placed in bug-dorm cages. The male and females were placed in separate cages and counted. A sample of the newly erupted female mosquitoes were removed from the cages and placed individually into a test container. A guinea pig with its fur shaved on the ventral side, was offered as a blood source. The female was then observed for a probing behaviour indicating its willingness to feed off the blood source and the observations of them feeding was recorded.

Results

An analysis of the 12-hour period over which time mosquitoes were observed to have oviposited showed that there are two peaks that corresponds with late evening and again just before dawn (Figure 1).

Periods when most eggs were laid also coincided with the time when most mosquitoes laid eggs (Table 1). Most eggs were also laid in the first half of the night (18h00-24h00) compared to the second half of the night ($z = 29.9$, P-value <0.00001, $p < 0.05$). 58.34% of eggs were laid in the first half as compared to 41.66 oviposited in the second half (Table 1). Of the 300 mosquitoes that were blood fed and observed only 196 laid eggs. 104 females did not lay eggs which may be an indication of poor blood feeding or egg retention.

Table 1 Results of oviposition behaviour.

Time	No. females laying Eggs	No. Eggs Laid	Eggs laid(Mean ± SD)	% of total eggs laid
18:00 – 19:00	15	1160	77 ± 29.87	8.53
19:00 – 20:00	20	1193	60 ± 21.15	8.78
20:00 – 21:00	21	1444	69 ± 1.39	10.62
21:00 – 22:00	28	1967	70 ± 4.71	14.47
22:00 – 23:00	16	1187	74 ± 17.14	8.73
23:00 – 24:00	17	980	58 ± 13.01	7.21
00:00 – 01:00	15	1012	67 ± 5.93	7.44
01:00 – 02:00	14	1188	85 ± 21.64	8.74
02:00 – 03:00	18	1243	69 ± 16.24	9.14
03:00 – 04:00	19	1641	86 ± 24.08	12.07
04:00 – 05:00	9	455	51 ± 16.93	3.35
05:00 – 06:00	4	124	31 ± 9.06	0.91

Time to refeeding

All females that laid eggs during the night (196) were given access to a blood source and all mosquitoes thus exposed fed on the blood source within an hour of their oviposition. This demonstrated the capacity of females to take a blood meal immediately after egg oviposition to mature the eggs that would contribute to the population of the next generation.

Egg Hatch Rates

Of the 13 594 eggs laid during this study, the hatch rate to viable first instar larvae were 74.5% resulting in 9 991 offspring with each female producing 52 ± 21.28 larvae (range 14 – 97).

Emergence times and ratios

When calculating the absolute number of mosquitoes erupting each hour for the 12-hour observation period, it was found that the majority mosquitoes erupted just after sunset (Figure 2). The last mosquito emerged from the pupal case just before dawn and no further emergence were recorded after the lights came on.

Based on the results, unbalanced sex-ratio was observed in the studied population. These eggs tend to produce more females than males ($z = 2.81$, P-value = 0.00248, $p < 0.05$). Immediately after dusk, almost 25% more females emerged than males and this trend continued until just after midnight. After this period, the number of males and females emerging was similar.

Blood feeding

It was found that newly emerged females took a blood meal. From Table 2 it can be seen that females readily took a blood meal in the first hour after emergence with a minimum 70% of the exposed females taking blood. Most feeding was completed two hours after exposure, with an average of 2 hours 15 minutes, but this was not the case in the 19h00-20h00 cohort when a single mosquito only took a blood meal in the fourth hour after emergence.

Table 2 Summary of the feeding behaviour newly emerged females.

Observation period	Sample Size (n)	Proportion (%) feeding post-emergence			
		Hour 1	Hour 2	Hour 3	Hour 4
18:00 – 19:00	50	82	100		
19:00 – 20:00	50	72	80	98	100
20:00 – 21:00	50	88	100		
21:00 – 22:00	50	82	88	100	
22:00 – 23:00	50	100			
23:00 – 24:00	50	92	100		
00:00 – 01:00	40*	90	100		
01:00 – 02:00	20*	85	100		

*All the females that erupted.

Discussion

Increasing evidence of a change in biting and resting behaviour of the main malaria vectors has been mounting up in recent years as a result of selective pressure by the widespread and long-term use of LLINs and IRS [13,14,15]. Mosquito behaviour is quite variable, with changes in mosquito behaviour posing great challenges to malaria elimination efforts [16, 17]. Residual malaria is also influencing malaria burden in low transmission areas [3].

Anecdotal evidence from Limpopo Province in South Africa shows that the increase in cases may be attributed to the sub-optimal vector control [18]. Since adult control methods have been proven to be insufficient to control the disease, larval source management and especially larviciding is increasingly being recommended as an additional strategy in integrated vector control programmes mainly in elimination settings [19]. Mosquito behaviour is quite diverse with distinct preferences in terms of when egg oviposition and adult emergence from the pupal stage occurs [20,21]. It was shown that there are definite periods during the night when oviposition is favoured. Adult emergence from the pupal cases also occurs at a preferred time in early evening just after sunset. It was demonstrated that female mosquitoes can take a blood meal soon after emergence as well as immediately after egg-laying. Newly emerged mosquitoes took a bloodmeal in the laboratory where conditions were carefully controlled and this behaviour would need to be corroborated by field studies. In virgin females, a blood meal is essential for the development of metabolic reserves prior to mating [22]. Proportionally 25–30% more females are produced which may be due to differential mortality of males and females since climatic variables influence the lifespan of the sexes [8]. Masters [23] also recorded that most mosquitoes emerged in the early hours of the evening. Newly emerged males are not equipped to mate until genital reorient is complete [24] and *An. arabiensis* optimal mating occurs with 5–7-day-old males [25]. Since males only swarm for mating purposes, virgin females will enter the swarm only when they are ready to mate.

In order to target the aquatic stages of the mosquito life cycle, a comprehensive knowledge of the larval bionomics is essential. An understanding of mosquito oviposition behavior is necessary for the development of surveillance and control opportunities directed against specific disease vectors [27]. In this study, vector oviposition and emergence coincide with the bedtime of people living in rural areas as Pates and Curtis [28] found and that the traditional vector control interventions are ineffective as a result of this human behavior. Most people in rural areas go to bed just after sunset (between 20h00 and 22h00) and get up just before sunrise (between 04h00 and 05h00). With vector females laying eggs during these times and with the emergence of many females, there are a large number of vectors that are able to take a blood meal. According to the study by Milali et al [29] this coincides with the peak biting times of *Anopheles arabiensis* of 21h00 -22h00 and 03h00-04h00.

Approximately 75% of all eggs laid produce viable first instar larvae and this is in keeping with the observations of the same mosquito populations made by Maharaj [26]. This study complements the study of adult life table characteristics detailed by Maharaj [8,26]. Impoinvil *et al* [30] found that mosquito eggs held at 22 and 27°C had the highest overall mean hatching count. The temperature at which the eggs in this study was maintained fell within this range so the egg hatch rate was at its optimal. The sex ratio of the emerging mosquitoes from the Mamfene population reared under ideal conditions showed a clear female bias. This has implications for the sterile insect technique (SIT) studies planned by the control programmes for this area in KwaZulu-Natal. The population structure of these mosquitoes will require further study before such a programme can be implemented. It will require a sex distortion which is male biased for SIT to succeed [31].

The results of this study can help in streamlining vector control interventions targeting malaria elimination. When temperatures are high and the development of mosquitoes is rapid [26], larviciding can become a costly and labour intensive exercise since the breeding sites are many during summer and the generation time is short [8,26]. The study has shown that the egg hatch rates are quite high under optimum conditions, and therefore the use of larval control measures would prevent the rapid build-up of the populations under optimum environmental conditions. There are many proponents for winter larviciding [7] which is the application of chemicals to the breeding sites found during the dry winter season since vectors are in hibernation as larvae and are vulnerable to larval control measures. Previous studies have shown that winter larviciding delays the onset of transmission since the mosquito populations emerging from winter hibernation is low [7,26] and hibernating females have undergone gonotrophic dissociation [26].

This study has shown that inseminated females that have just oviposited are able to take a blood meal. Immediately upon emergence, both the males and females need to replenish their energy, the males for swarming and the females for mating and host detection. If females emerging from known breeding sites were to feed on toxic baits or insecticide treated animals, strategically placed around the geo-located active breeding sites, the population available for blood feeding and transmission would be lowered [16]. However, as this study has shown, newly emerged females as well as those having just oviposited can, under specific laboratory conditions, also take a blood meal without the benefit of a sugar supplement. Since the females feed opportunistically, livestock treated with endectocides, and placed strategically close to geo-located permanent mosquito breeding sites, could be used divert females from feeding on humans [16]. The study also supports the recommendation from investigations into the use of the sterile insect technique (SIT) that more sterile males be introduced into an area to outcompete non-sterile males [32]. This study has demonstrated that even under optimum conditions fewer males than females are produced and hence the release of large numbers of sterile males would be successful in reducing competition from wild, unsterilized males [33]. Furthermore, male control can be achieved by baits and swarm space spraying [34] However, according to Maharaj [26], there is a slight variation in sex ratio across seasons requiring that prior to the implementation of a SIT program, constant monitoring of the wild male population to adjust the release rate over time may be required.

This study adds to the sparse body of literature available on vector bionomics in South African mosquito populations but is limited by the laboratory conditions under which the study was conducted. Certain aspects would need to be evaluated under field conditions, especially the time to blood-feeding by newly emerged females. Nevertheless, the study has provided data on oviposition, egg hatch rates, sex ratios and adult emergence. The value of baited traps, space spraying and animal barriers need to be investigated in their own right under field conditions in low transmission settings such as KwaZulu-Natal.

Conclusion

The results of this study have provided information on egg laying, hatch rates, emergence times, feeding behavior and sex ratios. The sex ratio is biased to females and may be due to differential mortality rates. The feeding behaviour suggests that the use of interventions such as the attractive toxic sugar baits and insecticide treated animals close to known vector breeding sites would be an effective tool to eliminate large numbers of males and females seeking sustenance soon after emerging or ovipositing. Whether the female is attracted to the energy source or blood, it will be exposed to a chemical that can kill. Also, the high larval eclosion rate suggests that targeting larvae for control would serve as an effective population reduction mechanism. The smaller number of males that emerge could be targeted using baits and swarm space spraying. Supplementary measures to kill larvae and prevent adult females from feeding on human hosts should be investigated for their impact on transmission by decreasing the adult population from breeding sites. The results obtained in the laboratory should be evaluated under field conditions where possible..

Abbreviations

IRS: Indoor residual spraying

LLINs: Long-lasting insecticide treated net

SIT: Sterile insect technique

WHO: World Health Organisation

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author's contribution

RM developed the concept and the study design. VL and KM conducted the laboratory studies, RM and VL analysed and interpreted the data. RM, VL and KM contributed significantly to the write-up of the results.

Authors' Information

[1]Office of Malaria Research, South African Medical Research Council, South Africa

[2]School of Life Sciences, University of KwaZulu-Natal, South Africa

[3]School of Engineering, University of KwaZulu-Natal, South Africa

VL: vlakan@mrc.ac.za

KM: 217022911@stu.ukzn.ac.za

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References

1. World Health Organisation. World Malaria Report. World Health Organisation; 2021, Geneva.
2. Malaria Elimination 8. https://malariaelimination8.org. Accessed 10 March 2019.
3. Killeen GF. Characterizing, controlling and eliminating residual malaria transmission. Malar J. 2014; 13:330. https://doi.org/10.1186/1475-2875-13-330.
4. Raman J, Morris N, Frean J, Brooke B, Blumberg L, Kruger P, Mabusa A, Raswiswi E, Shandukani B, Misani E, Groepe MA, Moonasar D. Reviewing South Africa's malaria elimination strategy (2012-2018): progress, challenges and priorities. Malar J. 2016 27;15(1):438. doi: 10.1186/s12936-016-1497.
5. World Health Organisation. Guidelines for malaria vector control. Geneva: World Health Organisation. 2019. www.who.int/malaria/publications/atoz/9789241550499/en/
6. Sherrard-Smith E, Skarp JE, Beale AD, Fornadel C, Laura C. Norris LC, Moore SJ, Mihreteab S, Charlwood JD, Bhatt S, Winskill P, Griffin JT and Churcher TS. Mosquito feeding behavior and how it influences residual malaria transmission across Africa. Proceedings of the National Academy of Sciences. 2019; 116(30):201820646: doi:10.1073/pnas.1820646116.

7. Le Sueur D. The ecology, over-wintering and population dynamics of the pre-imaginal stages of the *Anopheles gambiae Giles* complex (*Diptera: Culicidae*) in northern Natal, South Africa. PhD Thesis; 1991. University of Natal, South Africa.
8. Maharaj R. Effects of temperature and humidity on adults of the *Anopheles gambiae* complex (*Diptera: Culicidae*) in South Africa: implications for malaria transmission and control. PhD Thesis; 1995. University of Natal, South Africa.
9. Coetzee M, Kruger P, Hunt R, Durrheim D, Urbach J, Hansford C. Malaria in South Africa: 110 years of learning to control the disease. South African Medical Journal. 2013; 103(10), 770-778. doi:10.7196/SAMJ.7446
10. Ryan SJ, Lippi CA, Zermoglio F. Shifting transmission risk for malaria in Africa with climate change: a framework for planning and intervention. Malar J. 2020; 19:170. https://doi.org/10.1186/s12936-020-03224-6.
11. Minakawa N, Munga S, Atieli F, Mushinzimana E, Zhou G, Githeko A, et al. Spatial distribution of anopheline larval habitats in Western Kenyan highlands: effects of land cover types and topography. Am J Trop Med Hyg. 2005;73:157–65.
12. Okal MN, Lindh JM, Torr SJ, Masinde E, Orindi B, Lindsay SB, and Fillinger U. Analysing the oviposition behaviour of malaria mosquitoes: design considerations for improving two-choice egg count experiments. Malaria Journal. 2015; 14: 250. doi: 10.1186/s12936-015-0768-https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4474426/#
13. Sangoro O, Kelly AH, Mtali S, Moore SJ. Feasibility of repellent use in a context of increasing outdoor transmission: a qualitative study in rural Tanzania. Malar J. 2014; 13:347. doi: 10.1186/1475-2875-13-347.
14. Ferreira CP, Lyra SP, Azevedo F, Greenhalgh D, Massad E. Modelling the impact of the long-term use of insecticide-treated bed nets on *Anopheles* mosquito biting time**.** Malar J. 2017;16(1):373. doi: 10.1186/s12936-017-2014-6.
15. Kisinza WN, Nkya TE, Kabula B, Overgaard HJ, Massue DJ, Mageni Z, Greer G, Kaspar N, Mohamed M, Reithinger R, Moore S, Lorenz LM, Magesa S. Multiple insecticide resistance in *Anopheles gambiae* from Tanzania: a major concern for malaria vector control. Malar J. 2017;16(1):439. doi: 10.1186/s12936-017-2087-2.
16. Kiware SS, Chitnis N, Tatarsky A, Wu S, Castellanos HMS, Gosling R, Smith D, Marshall JM. Attacking the mosquito on multiple fronts: insights from the Vector Control Optimization Model (VCOM) for malaria elimination. PLoS One. 2017;12(12):e0187680. doi: 10.1371/journal.pone.0187680. eCollection 2017.
17. Mmbando AS, Ngowo H, Limwagu A, Kilalangongono M, Kifungo K, Okumu FO. Eave ribbons treated with the spatial repellent, transfluthrin, can effectively protect against indoor-biting and outdoor-biting malaria mosquitoes Malar J. 2018;17(1):368. doi: 10.1186/s12936-018-2520-1.
18. Maharaj R, Moonasar D, Baltazar C, Kunene S, Morris N. Sustaining control: lessons from the Lubombo spatial development initiative in southern Africa. Malar J. 2016;15(1):409. doi:

10.1186/s12936-016-1453-9.

19. van den Berg H, van Vugt M, Kabaghe AN, Nkalapa M, Kaotcha R, Truwah Z, Malenga T, Kadama A, Banda S, Tizifa T, Gowelo S, Mburu MM, Phiri KS, Takken W, McCann RS. Community-based malaria control in southern Malawi: a description of experimental interventions of community workshops, house improvement and larval source management. Malar J. 2018;17(1):266. doi: 10.1186/s12936-018-2415-1.
20. Ramsey JM, Salinas E, Lopez JR, del Angel-Cabañas G, Martinez L, Bown DN. Laboratory oviposition, fecundity and egg hatching ability of colonized *Anopheles albimanus* from southwestern Mexico. J Am Mosq Control Assoc. 1988;4(4):509-15.
21. Chadee DD, Beier JC, Mohammed RT. Laboratory studies of diel oviposition, fecundity, survival, and gonotrophic cycles in *Anopheles homunculus.* J Am Mosq Control Assoc. 1998;14(2):153-8.
22. Takken W, Klowden MJ, Chambers GM. Effect of body size on host seeking and blood meal utilization in *Anopheles gambiae sensu stricto (Diptera: Culicidae*): the disadvantage of being small. Journal of Medical Entomology. 1998;35 (5), 639-645
23. Masters CO. A study of the proportion of male and female mosquitoes immediately after emergence. The Ohio Journal of Science, 1949; 49(5). https://kb.osu.edu/bitstream/handle/1811/3714/1/V49N05
24. Kaindoa EW, Ngowo HS, Limwagu A, Mkandawile G, Kihonda J, Masalu JP, Bwanary H, Diabate A, Okumu FO. New evidence of mating swarms of the malaria vector, *Anopheles arabiensis* in Tanzania. Wellcome Open Res. 2017; 22;2:88. doi: 10.12688/wellcomeopenres.12458.1. eCollection 2017.
25. Verhoek BA, Takken W. Age effects on the insemination rate of *Anopheles gambiae s.l.* in the laboratory. Entomologia Experimentalis et Applicata 1994;72 (2), 167-172
26. Maharaj R. Life table characteristics of *Anopheles arabiensis* (Diptera: Culicidae) under simulated seasonal conditions. J Med Entomol. 2003; 40(6):737-42.
27. Day JF. Mosquito Oviposition Behavior and Vector Control. 2016;18;7(4). pii: E65.
28. Pates H, Curtis C. Mosquito behavior and vector control. Annu Rev Entomol. 2005; 50:53–70.
29. Milali MP, Sikulu-Lord MT, Govella NJ. Bites before and after bedtime can carry a high risk of human malaria infection. Malar J. 2017;16(1):91. doi: 10.1186/s12936-017-1740-0.
30. Impoinvil DE, Cardenas GA, Gihture JI, Mbogo CM, Beier JC. Constant temperature and time period effects on *Anopheles gambiae* egg hatching. J Am Mosq Control Assoc. 2007; 23(2):124-30.
31. Bernardini F, Kriezis A, Galizi R, Nolan T, Crisanti A. Introgression of a synthetic sex ratio distortion system from Anopheles gambiae into Anopheles arabiensis [published correction appears in Sci Rep. 2019 May 22;9(1):7915]. *Sci Rep*. 2019;9(1):5158. Published 2019 Mar 26. doi:10.1038/s41598-019-41646-8
32. Chung HN, Rodriguez SD, Gonzales KK, Vulcan J, Cordova JJ, Mitra S, Adams CG, Moses-Gonzales N, Tam N, Cluck JW, Attardo GM, Hansen IA. Toward Implementation of Mosquito Sterile Insect Technique: The Effect of Storage Conditions on Survival of Male *Aedes aegypti* Mosquitoes (Diptera: Culicidae) During Transport. J Insect Sci. 2018;18(6). doi: 10.1093/jisesa/iey103.

33. Le Goff G, Damiens D, Ruttee AH, Payet L, Lebon C, Dehecq JS, Gouagna LC. Field evaluation of seasonal trends in relative population sizes and dispersal pattern of *Aedes albopictus* males in support of the design of a sterile male release strategy. Parasit Vectors. 2019;12(1):81. doi: 10.1186/s13071-019-3329-7
34. Diabate A, Tripet F. Targeting male mosquito mating behaviour for malaria control. Parasit Vectors. 2015;8:347. doi: 10.1186/s13071-015-0961-8.

Figures

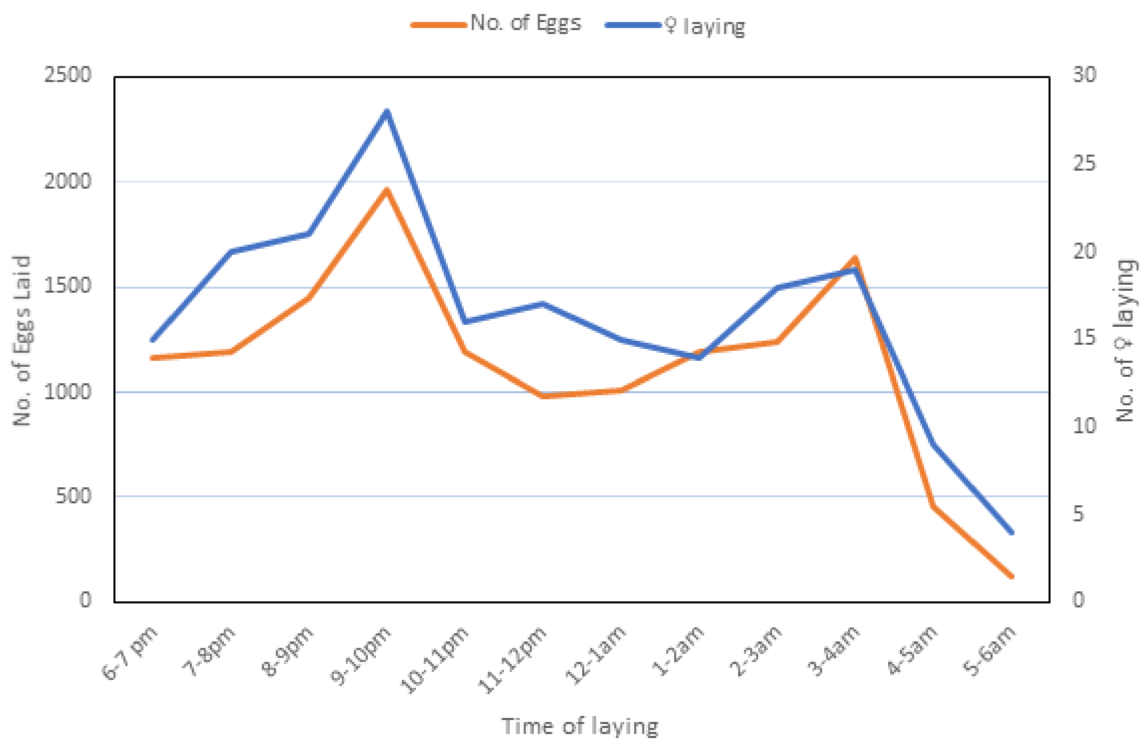

Figure 1

The numbers of females ovipositing, and eggs laid hourly.

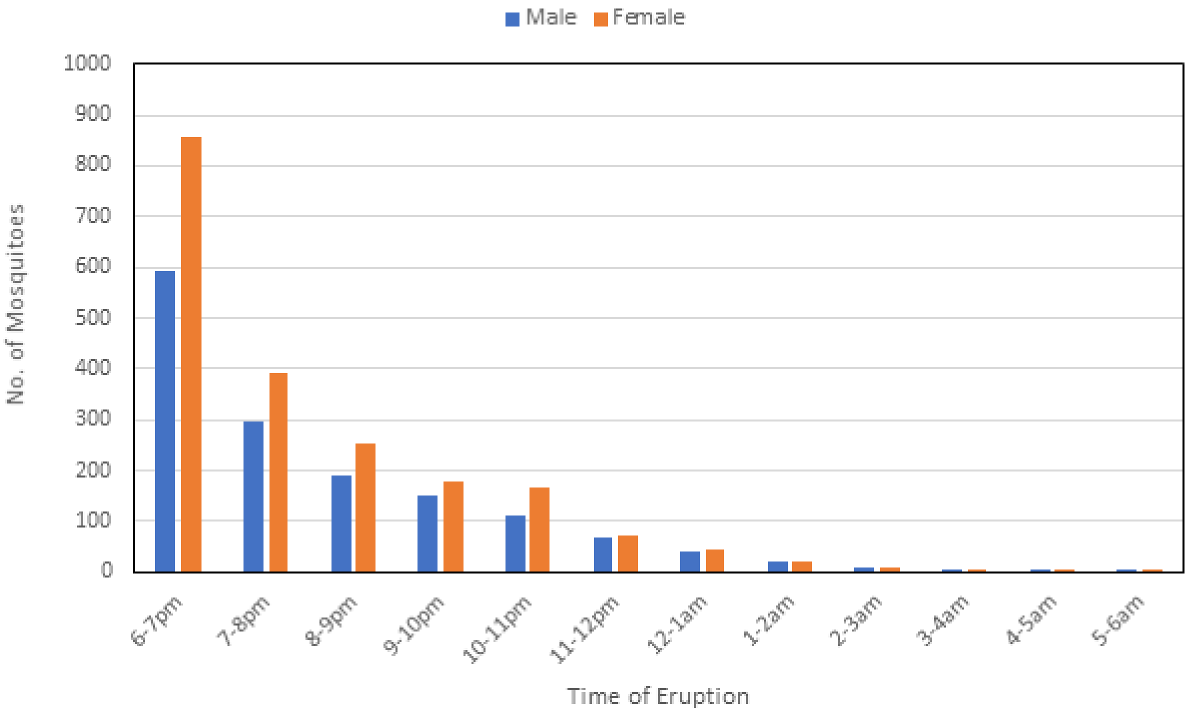

Figure 2

Adult emergence according to sex over the 12-hour observation period.