Bionomics of *Anopheles Arabiensis* from Mamfene in KwaZulu-Natal (South Africa): An Area of High Malaria Transmission in the Province

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Research

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Abstract

Background: Although great strides have been made in controlling malaria, the disease is of significant public health importance. Historically, efforts to control the vector has concentrated on adult vector control targeting the female *Anopheles* mosquitoes. As there is now a focus on eliminating residual malaria from KwaZulu-Natal, new strategies are being investigated to increase the impact of malaria elimination strategies. Greater attention is now being given to larval control, as a complementary measure to indoor residual spraying. However, there is a large gap in knowledge of the bionomics of the larval stages of this mosquito vector of malaria in South Africa. In order to focus on both larval and adult mosquito control methods, larval development and the reproductive stages of the vector were investigated since these variables influences our ability to impact mosquito populations through larval control. This study was therefore conducted to determine the peak eruption times and the emergent sex ratios, as well as the peak egg oviposition time in order to attack the mosquito when it is at its most vulnerable and when control interventions will have the most impact.

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

Conclusions: The results of this study have provided information as to when interventions would be most effective in controlling mosquito populations and have provided information that highlights the value of larval control as a complementary measure to adult mosquito control. The most vulnerable stages of the female *Anopheles arabiensis* are when they have just emerged or when they have just oviposited. Vector control strategies should be designed to target these vulnerable stages at the breeding sites in order to have maximum impact.

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 2018 [1]. Africa bears the brunt of the disease burden and it is usually the low socio-economic populations that are most affected by the disease. Since malaria is totally preventable and curable, the disease has become one that is associated with poverty, occurring among those that do not have the means to protect themselves or the resources to seek treatment once infected. However, in the southern most parts of the continent, malaria has been quite effectively controlled using IRS or LLINs to reduce contact between the host and vector thus resulting in diminished transmission. Eight countries in southern Africa, including South Africa, are now

targeting malaria elimination as a result of having achieved low levels of transmission through the use of coordinated and sustained vector control measures [2]. However, the road to elimination is paved with many challenges and these would need to be overcome to achieve malaria elimination certification.

One of the main obstacles to achieving elimination is residual malaria transmission. Residual malaria is defined as the low levels of transmission that continues after full universal coverage with effective long-lasting insecticide impregnated nets and/or indoor residual spraying is achieved [3]. 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. Over the past century efforts to control the vector has concentrated on adult vector control more especially targeting the female *Anopheles* mosquitoes. These initiatives have focused on chemical methods of control that has resulted in the development of insecticide resistance to virtually all classes of insecticides registered for mosquito control.

Due to insecticide resistance and the advent of outdoor biting of mosquitoes, there is increasing attention being paid to larval source management and larval control. Traditionally, larval control has been limited to the control of active larval breeding sites using chemicals, toxins, bacteria, fungi and other biological control methods. However, the usefulness of these techniques is limited by the exorbitant labour costs and the cost of the chemicals but is recommended for use in areas where the breeding sites 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. Over time mosquitoes have adapted to outdoor feeding due to the continued use of IRS and feeding earlier through the use of LLINs [6]. Available information was collected decades ago [7, 8] and has changed over time [9]. Environmental changes have also occurred in terms of rainfall and temperature [10]. Changes in the ecological factors can affect malaria transmission by modifying the microclimate of the immature stages and adult mosquitoes [11]. 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 since these variables influence the ability to control mosquito populations through larval source management. This study was therefore conducted to determine the peak eruption times and the emergent sex ratios, as well as the peak egg oviposition time in order to better target the vulnerable stages of a mosquito when control interventions will have the most impact. Furthermore, the results from this study can feed into larval source management strategies as well as providing attractive toxic sugar baits for males and females when both sexes require energy the most.

Methodology

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_2 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 12 h light: 12 h 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 30 × 30 × 30 cm 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 autotimer switched lights off at 17h00 daily. 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. The eggs per mosquito were counted and 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 laying it took to refeed. Feeding was confirmed by observing blood in the abdomen.

Eruption Times and Emergent Sex ratios

The F_2 pupa that developed overnight were counted and used in this aspect of the study. The eruption trial used a 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 eruptions. 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 (Fig. 1). Periods when most eggs were laid also coincided to the time when most mosquitoes laid eggs (Table 1).

Table 1
Results of oviposition behaviour.

Time	No. females laying Eggs	No. Eggs Laid	Average per Female	% of total eggs laid
18:00–19:00	15	1160	77	8.53
19:00–20:00	20	1193	60	8.78
20:00–21:00	21	1444	69	10.62
21:00–22:00	28	1967	70	14.47
22:00–23:00	16	1187	74	8.73
23:00–24:00	17	980	58	7.21
00:00–01:00	15	1012	67	7.44
01:00–02:00	14	1188	85	8.74
02:00–03:00	18	1243	69	9.14
03:00–04:00	19	1641	86	12.07
04:00–05:00	9	455	51	3.35
05:00–06:00	4	124	31	0.91

Most eggs were also laid in the first half of the night (18h00-24h00) compared to the second half of the night (00h00–06h00). 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. Age and body size can impact on insemination success of *An. arabiensis* [12].

Time To Refeeding

All females that laid eggs during the course of 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.

Eruption 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 (Fig. 2). The last mosquito emerged from the pupal case just before dawn and no further eruptions were recorded after the sun had risen.

Based on the results, unbalanced sex-ratio was observed in the studied population. These eggs tend to give more females than males. 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-eruption			
		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]. Residual malaria is also influencing malaria burden in low transmission areas [3]. Killeen [3] also indicates that the control of the larval stages of the mosquito life cycle can play a significant role in curtailing transmission in areas with residual malaria. Current malaria control interventions are being affected by changing mosquito feeding and resting behaviour with mosquito feeding taking place earlier in the evening (before the host retires indoors) and outdoors (to avoid insecticides) [17]. The WHO World Malaria Report [1] shows that there have been major increases in the burden of disease in countries in southern Africa that have been targeting elimination.

Anecdotal evidence from Limpopo Province in South Africa shows that the increase in cases may be attributed to the sub-optimal vector control through low coverage rates with indoor residual spraying. Imported parasites from across the border may have been propagated by the local populations of malaria vectors [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 especially 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. Female mosquitoes are able to take a blood meal soon after emergence as well as immediately after egg-laying. In virgin females, such 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 a mechanism to promote outcrossing since females will mate with older mosquitoes and reduce the probability of siblings mating later when genital reorientation is complete. Masters [23] also recorded that most mosquitoes emerged in the early hours of the evening. Newly emerged males are not equipped to mate since the genitalia require up to three days to reorient in order to be able to inseminate the females they mate with [24] and *An. arabiensis* optimal mating occurs with 5–7-day-old males [25].

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 a high number of 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 Mamfene population reared under ideal conditions showed a clear female bias. Therefore, the population structure of these mosquitoes will require further study before a sterile Insect technique (SIT) programme can be implemented. It will require a sex distortion which is male biases 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]. However, 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] since vectors are in hibernation as larvae and are vulnerable to larval control measures. It was 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].

The use of baited traps near larval breeding sites could prove to be successful. 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 emerging females were to feed on toxic baits, 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 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 mosquito breeding sites, could be used as a supplementary measure to control females [16]. The study also supports the recommendation from investigations into the use of the 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 a large number of sterile males would dilute the influence of the smaller number of males reared in the wild [33]. However, according to Maharaj [26], there is a slight variation in sex ratio across seasons requiring that SIT programmes constantly monitor of wild versus sterile males to adjust the release rate over time.

Conclusion

The results of this study have provided information as to when interventions would be most effective in controlling mosquito populations. These intervals are 20h00–22h00 at night and 03h00–04h00 in the morning. The time when females have just oviposited or recently emerged would indicate the time when attractive toxic sugar baits would be effective as these females would be searching for a sugar source prior to seeking hosts. The smaller number of males that emerge cannot mate until they are at least three days old and these can also be controlled by baits and swarm space spraying [34]. Supplementary measures such as larviciding and the use of endectocides would have a further impact on transmission.

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.

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Figures

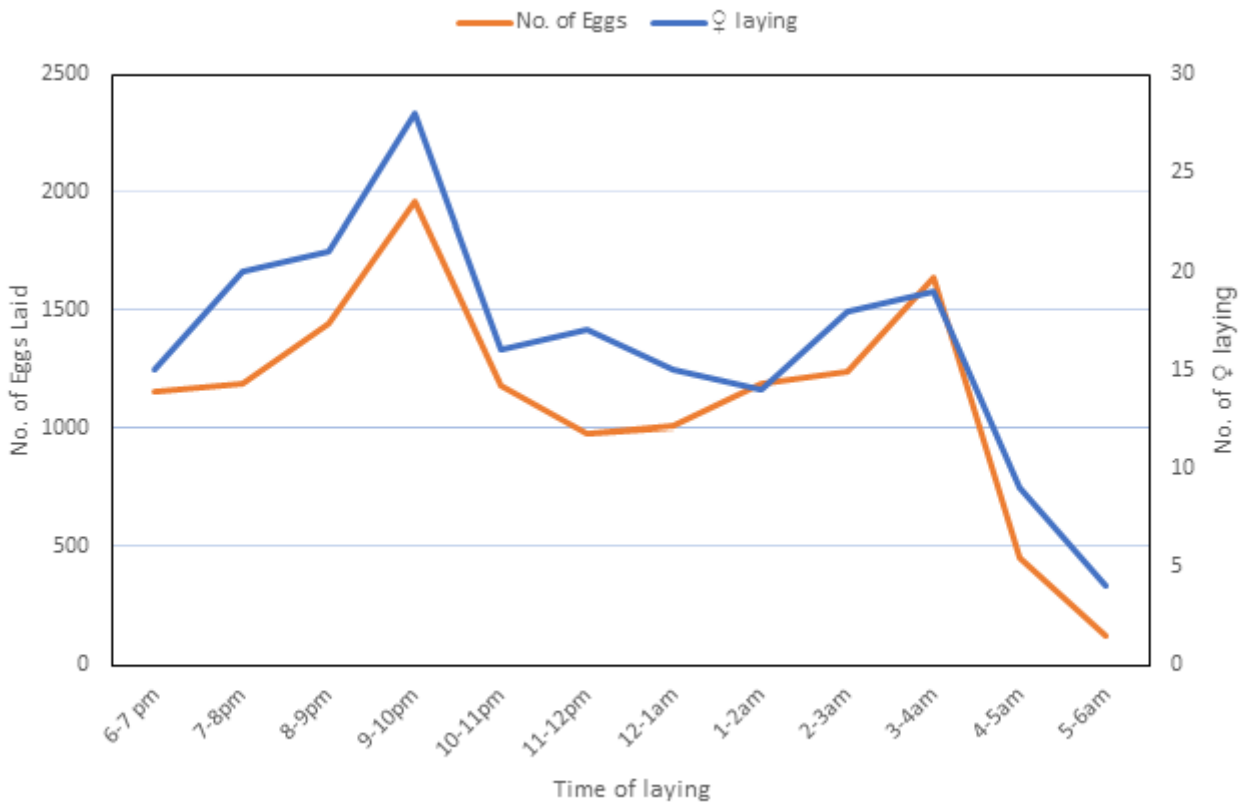

Figure 1

The numbers of females ovipositing, and eggs laid hourly.

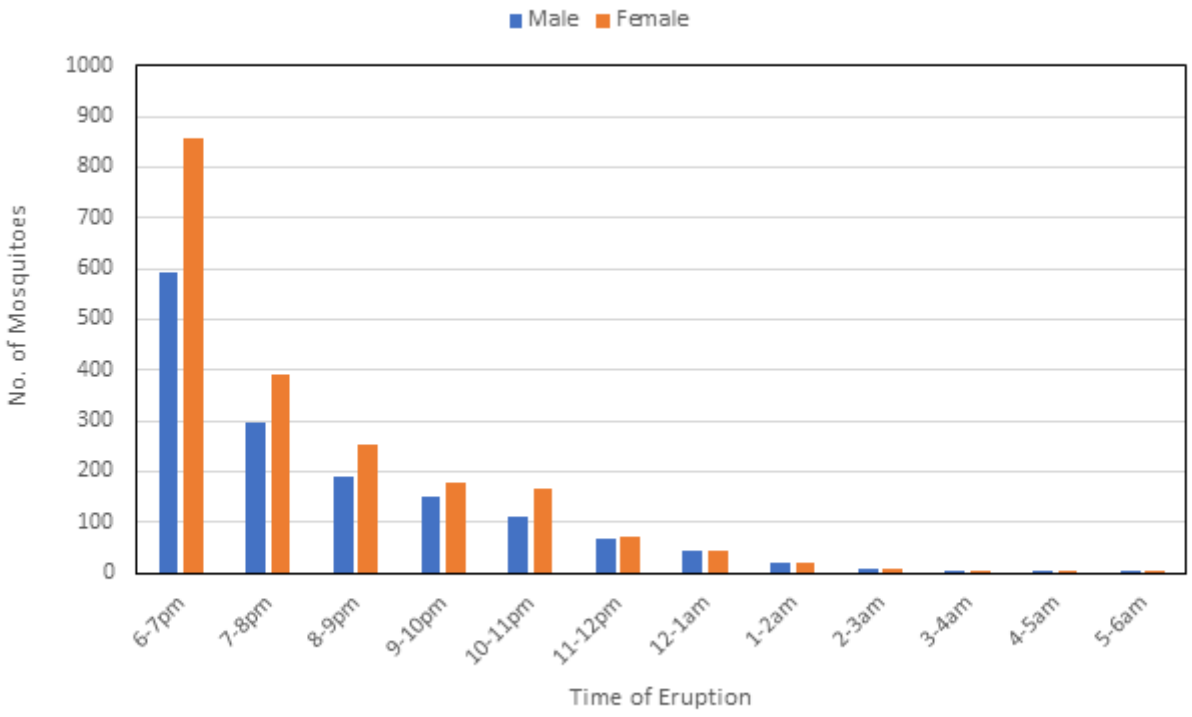

Figure 2

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