Short Research Communications

Life table analysis of *Anopheles gambiae* (Diptera: Culicidae) in relation to malaria transmission

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Malaria continues to be a major cause of morbidity and mortality in the tropics several years after the World Health Organization's roll back malaria (RBM) initiative. The disease is responsible for an estimated one million deaths per year, mainly in pregnant women and young children under the age of five, in Africa[1]. In Nigeria, malaria accounts for 40–50% outpatient hospital visits, and estimates of child mortality indicate that at least 30% of Nigerian children die of malaria each year[2].

Though vector control has been rightly recommended for reducing the burden of malaria[3], the success of the strategy in Nigeria has been hampered by inadequate knowledge of the biology of the anopheline vectors in the country. Again, most of the studies on the principal vector of the disease in Nigeria, i.e. *Anopheles gambiae* Giles (Diptera: Culicidae), are based on field ecology and behaviour of the species[4–7], with very little information available on the life cycle. It has been demonstrated that life table attributes of anopheline vectors play fundamental roles in the epidemiology of malaria; attributes such as fecundity, developmental and survival rates, longevity, etc. influence vector abundance and hence, disease transmission intensity[8].

To better understand baseline parameters of the life cycle of *An. gambiae*, a colony of the species was monitored under laboratory conditions, and a life table was constructed based on the developmental time of each instar and stage specific mortality.

Approximately, 120 partially engorged adult female *An. gambiae* mosquitoes, used as parental stock, were carefully collected from Ipata Market, Ilorin, Nigeria (Longitudes 4°30' and 4°45'E and latitudes 8°25' and 8°40'N). The mosquitoes were collected with the aid of oral aspirators[9], between 1800 and 2200 hrs. Collected mosquitoes were placed in 250 ml screened beakers and maintained in a humid box overnight and subsequently transported to the laboratory the following day. After confirming the identity of the mosquitoes, using standard keys[10], these were transferred to screened rearing cages (60 x 60 x 60 cm) and kept in the insectary. The mean ambient temperature and relative humidity of the insectary room were 27.33°C and 64.95%, respectively.

The mosquitoes were reared following standard techniques[11]. The adults were offered 10% glucose solution soaked in cotton pads daily, as a source of energy. Eggs were collected from the mosquitoes in an ovitrap by placing plastic cups (60 ml), containing well water and lined with filter papers, in the cage. The eggs were transferred from the filter papers with the aid of mounting needles into plastic bowls (5 cm height and 30 cm diam) where they were held for 24 to 72 h for hatching. Twenty-four hours after hatching, the larvae were transferred to a plastic tray (30 x 25 x 5 cm), at the rate of 50 larvae/tray for rearing. The larvae were fed with fish feed

(Tetramin®), sprinkled on the water surface. On every alternate day, the water from the culture tray was changed carefully until pupation. The pupae were separated from the larvae daily and placed in plastic bowls (5 cm height and 20 cm diam) half filled with well water. These plastic bowls with pupae were placed in adult-holding cages for emergence. Adults after emergence were offered 10% glucose solution soaked in cotton pads daily. As from two days after emergence, the females were offered an anaesthetized rabbit as a source of blood meal, and from Day 2 after blood meal, an ovitrap was placed in the adult-holding cages for the collection of eggs.

The colony was allowed to reach the third (F_3) generation prior to initiating life-table observations. Upon reaching the F_3 generation, four trays were set up with 50 first instar larvae (all larvae were within 12 h hatching period) per tray. The immature stages were reared as described for the regular colony. The trays were inspected every 12 h (i.e. 0700 and 1900 hrs) each day for dead larvae, and such were removed and noted. Also, the number of individuals transforming into the next larval instar stage was noted on daily basis, until pupation. Survival rate during the life stages was determined as the proportion of individuals at the beginning of a life stage that successfully entered the next stage while, instar duration was determined as the difference between mean age at the beginning of an instar stage and the one preceding it. Each emergent pupa was separated into a vial containing approximately 5 ml of water from their respective source. Each vial was identified 1 to 50 on a label carrying the date and time of pupation. After 72 h the vials were examined for pupal mortalities, and the number of dead pupae was noted. Mosquitoes that died in the process of emergence were also recorded as part of pupal mortality.

Upon adult emergence, the sex of the mosquito was determined and recorded along with the time of emergence. Thereafter, the adult mosquitoes were carefully pooled in adult rearing cages to a tune of approximately 50 females to 50 males, all which were within 24 h old post-emergence. The cages were examined daily for dead individuals which were removed and noted. For every dead mosquito, information regarding cage number, sex, and the date of death was recorded. A mosquito was ascertained dead if it did not respond to a prompting within a 10 min period. The adult mosquitoes were fed as described for the regular colony.

Fecundity was estimated based on the average numbers of eggs laid per female[12]. Egg-hatching rate was estimated from the number of eggs hatched per female. Duration of the pre-adult development periods was determined by observing each pan at 0800 and 1600 hrs daily, and all larval skins were removed, scored to instar, and counted. There were four replicates for each experiment described above and the whole study was repeated twice, resulting in the monitoring of about 1600 larvae from I instar stage to adulthood.

Standard life table analysis[13,14], was performed on the data. The mean pupation and eclosion times were estimated. Computation of stage-specific survivorship was according to the formula: $S_i = n_i/(x_{ni-1}) \times 100$; where, n_i is the number of larvae entering instar i, and x_{ni-1} is the number of larvae that entered the preceding instar.

Mean instar duration in days was determined using the formula: $D_i = T_i - (t_{i-1})$; where, T_i = Present mean age and t_{i-1} = The previous mean age at molting.

The percentage of total immature life spent in each instar was calculated as $L_i = t_i/t_n \times 100$, where, t_i = Duration of instar stage i, and t_n = Duration of all instar stages. The expectation of life of the adult mosquitoes was determined as the interval between imago emergence and death.

The mean duration and survival rates of different immature stages of *An. gambiae* are presented in Table 1. Embryony took 1.48 ± 0.3 days while egg-hatching rate was 63.18 ± 23.94%. Mean durations of specific larval stages ranged from 1.55 ± 0.21 days

Table 1. Mean duration and survival rates of immature life stages of *An. gambiae*

Attributes	Egg stage	Larval stage				Pupal stage
		L_1	L_2	L_3	L_4	
Egg-hatching rate	63.19 ± 23.91	–	–	–	–	–
Duration of immature stage (days)	1.48 ± 0.30	1.55 ± 0.21	2.72 ± 0.64	2.36 ± 0.33	2.84 ± 0.71	1.53 ± 0.36
Survival rate (%)	–	83.53 ± 8.81	91.36 ± 14.52	81.90 ± 6.33	87.44 ± 8.93	76.46 ± 21.35

in the first instar (L_1) to 2.84 ± 0.71 days in the IV instar (L_4). The pupal stage lasted for a mean period of 1.53 ± 0.36 days. On the average, it took 11.04 ± 2.25 days for *An. gambiae* to go from egg eclosion to adult emergence. Stage-survivorship rates were estimated to be over 80% in all larval stages, and 76.46 ± 21.35% for the pupal stage. The overall mean survival rate from eclosion to adult emergence was 84.14%.

The life table attributes of the adult stage are shown in Table 2. Mean fertility rate was 58.6 ± 11.36% and the mean fecundity was 78.33 ± 31.07 eggs per female, of which only 63.18 ± 23.94% successfully hatched into larvae. The male : female sex ratio was 1.01 : 1.24. The expectation of life for the mosquitoes after emergence was 17.19 ± 3.33 days for males and 23.10 ± 8.29 for females. Adult females began taking a blood meal approximately two days after emergence with the first batch of eggs laid five days post-adult emergence.

The life table attributes of *An. gambiae* obtained in this study were similar to those reported for the

Table 2. Mean attributes of adult stage of *An. gambiae*

Attributes	Male	Female	Aggregate
Sex ratio	1.01	1.24	–
Fertility rate (%)	–	58.6±11.36	–
Fecundity (eggs/female)	–	78.33±31.07	–
Life expectancy (days)	7.19±3.33	23.1±8.29	20.15±5.81

species in some studies[15], differed considerably from those of others[3]. Such differences have been attributed in part to variations in environmental conditions for larval development[16]. Egg-hatching rate of *An. gambiae* in this study was lower than reported for *An. nivipes* and *An. philippinensis* in Thailand[17]. Also, the larval developmental time of *An. gambiae*, from eclosion to adult emergence, of 11.04 days recorded in this study was shorter than the 12 to 14 days obtainable in the tropics generally[9]; and the mean survival rates of the life stages were generally high (>80%). Thus, the short larval developmental time and high survival rates recorded for *An. gambiae* in this study was indicative of rapid population growth, high density and potential risk for malaria transmission.

The adult female mosquitoes lived for an average of about 23 days. If the assertion that a minimum of 10 days was required for *An. gambiae* mosquitoes to take their first blood meal and get infective holds true[9], then a female mosquito will survive for an additional 13 days under laboratory conditions. With 2–3 days *An. gambiae*'s gonotrophic cycle in the tropics[9], an *An. gambiae* female mosquito could blood-feed six additional times in its life span, thus, further magnifying the malaria transmission chances, if the mosquito took an infectious blood meal soon after emergence.

The results of this study indicate that the reproductive performance of *An. gambiae* is very high, favouring rapid population growth and may thus, contribute to the success of the species for malaria

transmission. This portends great potentials for vector control in reducing the burden of malaria, as such interventions targeted at critical stages of the life cycle of the vector species will reduce the population density of the mosquito and hence, its vectorial capacity. The findings of this study will aid the development of anti-vector strategies for malaria control in Nigeria in particular and Africa in general.

Acknowledgement

References

1. Breman JG, Egan A, Keusch GT. The intolerable burden of malaria: a new look at the numbers. *Am J Trop Med Hyg* 2001; *64*(Suppl 1–2): iv–vii.

2. *Malaria Desk situation analysis, Nigeria 2000*. Federal Government Publication, Federal Ministry of Health, Govt. of Nigeria 2000; p. 1–27.

3. Okogun GRA. Life-table analysis of *Anopheles* malaria vectors: generational mortality as tool in mosquito vector abundance and control studies. *J Vector Borne Dis* 2005; *42:* 45–53.

4. Hannay PW. The mosquitoes of Zaria Province, northern Nigeria. *Bull Entomol Res* 1960; *51:* 145–71.

5. Mafiana CF, Anaeme L, Olatunde GO. Breeding sites of larval mosquitoes in Abeokuta, Nigeria. *Nigerian J Entomol* 1998; *15:* 136–43.

6. Manyi MM, Imandeh NG. The infection rates of mosquitoes with malaria and lymphatic filarial parasites in Makurdi, Benue state, Nigeria. *J Pest Dis Vector Management* 2008; *8:* 464–70.

7. Olayemi IK, Ande AT. Survivorship of *Anopheles gambiae* in relation to malaria transmission in Ilorin, Nigeria. *Online J Health Allied Sci* 2008; *7*(3): 1.

8. Reisen WK, Mahmood F. Horizontal life table characteristics of the malaria vector *Anopheles culicifacies* and *Anopheles stephensi* (Diptera: Culicidae). *J Med Entomol* 1980; *17:* 211–7.

9. *Manual on practical entomology in malaria. Part II. Methods and techniques*. Geneva: World Health Organization Offset Publication 13, 1975; p. 1–115.

10. Gillies MT, De Meillon B. *The anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region)*. Johannesburg: Hortors Printers 1968; p. 1–343.

11. Das S, Garyer L, Dimopoulos G. Protocol for mosquito rearing (*An. gambiae*). *J Visualized Exp* 2007; *5:* 221–5.

12. Roitberg BD, Gordon I. Does the *Anopheles* blood meal-fecundity curve, curve? *J Vector Ecol* 2004; *30*(1): 83–86.

13. Mahmood F. Life table attributes of *Anopheles albimanus* (Wiedemann) under controlled laboratory conditions. *J Vector Ecol* 1997; *22:* 103–8.

14. Edilo FE, Toure YT, Lanzaro GC, Dolo G, Taylor CE. Survivorship and distribution of immature *Anopheles gambiae* s.l. (Diptera: Culicidae) in Banambani village, Mali. *J Med Entomol* 2004; *41*(3): 333–9.

15. Menge DM, Guda T, Zhong D, Pai A, Zhou G, Beier J, Gouagna I, Yan G. Fitness consequence of *Anopheles gambiae* population hybridization. *Malar J* 2005; *4:* 44–9.

16. Grieco JP, Achee NL, Briceno I, King R, Andre R, Roberts D, Rejmankova E. Comparison of life table attributes from newly established colonies of *Anopheles albimanus* and *Anopheles vestitipennis* in northern Belize. *J Vector Ecol* 2003; *28:* 200–7.

17. Klein TA, Harrison BA, Inlao I, Boonyakanist P. Colonization of Thailand strains of *Anopheles nivipes* and *Anopheles philippinensis. Mosq News* 1982; *42:* 374–81.

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