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Life-table analysis of *Anopheles* malaria vectors: generational mortality as tool in mosquito vector abundance and control studies

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Background & objectives: Vector control will for sometime remain a primary weapon in the war against vector borne diseases. Malaria is of paramount importance in this with its associated high morbidity and mortality especially in sub-Saharan Africa. This study on generational mortality associated factors in *Anopheles* mosquitoes life-table analysis was designed to investigate the fecundity, levels of mortality and mortality associated factors at the aquatic stages of anopheline malaria vectors.

Methods: Mortality associated factors were investigated at the eggs, I and II instar larval, III and IV instar larval and pupal stages of two anopheline species—*Anopheles pseudopunctipennis* (Theobald) and *An. gambiae* life-cycles in screen cages. Adult male and female mosquitoes were membrane filter-fed and algae in culture medium formed the bulk of food substances for the larval stage. Environmental temperature of culture media, pH and some associated physio-chemical factors were also determined.

Results: Results showed significant mortality rates at various aquatic stages. Infertility, cannibalism and environmental factors were the major factors responsible for mortality at the egg, larval and pupal stages respectively.

Interpretation & conclusion: The aquatic stages of *Anopheles* mosquito mortality factor K and the mortality factors at the various stages investigated k_1, k_2, k_3 and k_4 are discussed. Our recommendations include further studies on the possible genetic modification of predacious *An. pseudopunctipennis* larvae and/or its modification for the production of sterile/infertile eggs as possible alternatives in the reduction and control of anopheline malaria burden.

Key words *An. gambiae* – *An. pseudopunctipennis* – generational mortality – life-table analysis – Nigeria – vector abundance – vector control

Vector control is and will for sometime remain a primary weapon in malaria control as it is known that malaria could be eradicated from an area by greatly reducing the mosquito population. Before the development of resistance in the past, the use of chemical pesticides and insecticides against mosquito vector species was a major break through in the control of malaria in many endemic areas of the world including tropical Africa. This was before the development of resistance in the middle of last century[1,2].

Generally anti-vector measures in malaria control aim at a cost-effective reduction of the vector abundance and its transmission potentials below the critical level. Current available measures include those which target decrease in vector abundance, vector-human contact and vector survival rates. Also integrated control measures devoid of over amplification of a single method that may not effectively be implemented on a large-scale and community involvement in malaria control programmes have been recommended[3].

The reduction of mosquito breeding has been achieved in several areas through communities effort by the destruction of the aquatic stages in mosquitoes breeding cycle. These include the use of larvicides. Larviciding is of immense benefits in mosquito population reduction, but it is beyond most healthcare programmes on account of the skill, expertise, good judgement, equipments and transport required for its implementation. Most often mosquito breeding sites around villages (ponds, pools and streams) and water collection in drums, bowls and pots in and around houses are used as source of domestic water supplies[4].

Since vector abundance is a critical factor in the mosquito–malaria cycle, generational life-table studies is expected to be a useful tool in malaria early warning systems for the purpose of forecast, early warning, early detection of epidemics and intensity of disease prevalence in holoendemic areas.

The objective of the current work is to determine the types and rates of mortality that occur at the aquatic stages of mosquito life-cycles, the factors associated with such mortality and the relationship (physical/ physio-chemical) that exist between the breeding environment and the aquatic mosquitoes mortality rates. This is with a view to know possible cost-effective measures and stages of intervention in control of mosquito breeding sites.

Material & Methods

The study area for the research is the Esan/Etsako regions of Midwestern Nigeria, located between approximately latitude 5° 44' N and 7° 35'N; and longitude 5° 4' E and 6° 43' E and covering an estimated area of 20,000 km^2 with a projected population of 2.8 million by 2004 (at a 3.37% growth rate) from 1991 population census[5,6].

Mosquito cultures were set up in five different locations of the three ecological foci subdivided into areas of high human activities (AHHA) and areas of derived/ secondary vegetations (ADSV) chosen for the study.

Mosquito cultures were set up in a pilot study using four different containers—clay pots, metal cans, plastic wares and bamboo stumps – detailed description of the ecological zones are as presented in an earlier report by Okogun *et al*[4]. Mosquito larval species were identified and differentiated using published keys of Chandler[7-10]. Environmental temperature, rainfall and relative humidity were monitored during the study as presented in a separate report[11].

A total of 10 male and 10 female larvae of *An. pseudopunctipennis* and *An. gambiae* pooled from cultures above were transferred into a beaker containing some algae rich mosquito larval breeding water. The species were bred in separate screened cages to adult in the university insectary. Cultures were set up within the cages using same breeding materials above and nutrient-containing water where fed, mated and gravid female mosquitoes could easily have access in order to oviposit while preventing any interference.

The female adult mosquitoes were membrane-fed according to the method of Higgs and Beaty[12]. A mini membrane feeder was prepared according to the method of Mourya *et al*[13]. The males were observed to die out after sometime, probably after mating. After two weeks the number of adult mosquitoes increased and a base line count of eggs and larvae generated was made. Eggs laid were subsequently counted every 2–3 days and then transferred to experimental containers made of plastics, earthenware materials, bamboo stumps and metal cans kept in netted emergence cages measuring 60 × 45 × 45 cm to enable effective monitoring of the developmental stages, through the aquatic stages to the adult stages and also to prevent entry of other mosquitoes.

First and second instar larvae stages and, III and IV instar larval stages were grouped and counted. Pupal stages were extracted and counted in petri dishes using capillary tubes. They were all returned into the experimental containers after each count. The counts from all the locations of each focus were recorded and pooled together for that focus. The data from the

microhabitats and foci were recorded and the process repeated fortnightly—the estimated duration for the completion of each generation. A generation was defined by the method of McHugh[14] as the duration of a gonotrophic cycle from the emergence of one young adult to another. This was calculated to take 14 days in the study area. The counts were recorded for 24 generations (one year) for calculating a life budget and analysis. The key life-table components for eggs, larvae, and pupae were recorded. The differences in mortality (losses) due to life-table component were determined. The log number dying at a particular stage and the mortality factors k_1, k_2, k_3 and k_4 were determined. The k factor was calculated to represent the entire generation mortality rate by the method of Bayoh and Lindsay[15].

The life-table components investigated were mortality, fertility, pathogenicity, predation, cannibalism and environmental factors, egg losses were denoted as k_1. Fertility and failure to hatch were investigated by microscopic examination for wrinkled outer casing and used as a primary sign of infertility and immaturity (infertility due to female not mated). Pathogenicity and predation in eggs were investigated by microscopic and bacteriological examination of cultures. Microscopy revealed predating microbial and fungal organisms while broad spectrum isolates of mosquito cultures, were sub cultured and isolates bred out to obtain pure cultures of detected organisms that were inoculated into experimental medium containing mature, and fertile eggs for the detection of mortality. Culture and bacteria species isolation were done by the method of Stokes and Ridgway[16]. Resultant infertility (mortality) of mature eggs was seen as due to pathogenicity.

As reported by Burton and Burton[17] changes in temperature and pH of the medium were monitored to determine the effect of non-optimal temperatures on weakening of the egg shells and hatching of eggs.

Larval mortality was investigated for outright death or failure to moult into next larval and pupal stages. Predators were investigated for by visual and microscopic examination of culture media. Deposits recovered were separated into different petri dishes for easy examination. Larger invertebrates were also sought for.

The causes of cannibalism in I and II instar larvae were also studied. At low or lack of algae (preferred food substances) III and IV instar larval stages of *An. pseudopunctipennis* showed predatory activities on its species larvae and other mosquito larvae species in experimental cultures. Deaths due to low oxygen tension at high algae concentration was also observed. Biochemical analysis of the culture media was carried out and the number of larvae dying relative to biochemical changes observed. At the larval stages environmental (water) temperature was monitored. At temperature below 24^{o}C larvae were observed to have delayed moulting, such larvae were regarded lost in that generation.

Pupal mortality was investigated for outright death or failure to emerge as adult mosquitoes (denoted k_4). Predation at pupal stage was investigated by microscopic and macroscopic examinations of isolated predating organisms. Contents of the cultures were separated as for larva. Further investigations include assessment of damage or impairment of any of the organs especially the wing part of the pupa. Failure to emerge as adult was investigated for pathogenicity as was done for eggs and pathogenic organisms causing damage to pupa functional part was isolated. Effects of environmental factors on pupae were determined as for larva.

Results

Tables 1 and 2 show the life-table analysis of *An. pseudopunctipennis* and *An. gambiae* for 24 generations respectively. The mean eggs per generation for *An. pseudopunctipennis* was 2273 ± 224.9 and that for *An. gambiae* was 2227± 226.43.

The mean eggs infertility recorded for *An. pseudopunctipennis* was 1723 ± 213.99 and that for *An. gambiae* was 1618 ± 437. Eggs laid by non-mated

Table 1. Life-table analysis for *An. pseudopunctipennis* at Ekpoma for 24 generations (August 2001–July 2002)

Stage/age Int. x (x)	No. alive 1(x)	Factor causing dx (fdx)	No. dying at X (dx)	Mean mortality rate (kx)
Eggs		Infertility		k_1
Total	54546		41349	13.48
Mean	~ 2272.75		~1722.88	0.562
Standard deviation (SD)	± 224.90		± 213.99	± 0.24
Larvae instars I+II		Cannibalism		k_2
Total	13197		8620	11.15
Mean	~ 549.88		~359.17	0.47
Standard deviation (SD)	± 30.71		± 31.84	± 0.16
Larvae instars III+IV		Cannibalism		k_3
Total	4641		2771	9.66
Mean	~ 193.38		~115.46	0.403
Standard deviation (SD)	± 7.84		± 10.62	± 0.138
Pupae		Environmental factors		k_4
Total	1846		1192	11.06
Mean	~ 76.92		~ 49.67	0.461
Standard deviation (SD)	± 5.49		± 5.10	± 0.185
Adults				K
Total	664			46.7
Mean	~ 27.67			1.95
Standard deviation (SD)	± 2.33			0.37

Tale 2. Life-table analysis for *An. gambiae* at Ekpoma (August 2001–July 2002)

Stage/age Int. x (x)	No. alive 1(x)	Factor causing dx (fdx)	No. dying at X (dx)	Mean mortality rate (kx)
Eggs		Infertility		k_1
Total	54166		38823	13.44
Mean	~2226.92		~1617.63	0.56
Standard Deviation (SD)	± 266.43		± 437.00	± 0.283
Larvae instars I+II		Cannibalism		k_2
Total	13488		10691	12.3
Mean	562		~ 445.46	0.513
Standard deviation (SD)	± 48.28		± 86.72	± 0.14
Larvae instars III+IV		Cannibalism		k_3
Total	4157		2943	8.83
Mean	~ 173.21		~ 122.68	0.37
Standard deviation (SD)	± 20.33		± 25.78	± 0.14
Pupae		Environmental factors		k_4
Total	1807		1033	8.9
Mean	~ 72.30		~ 43.84	0.37
Standard deviation (SD)	± 54.12		± 5.78	± 0.15
Adults				K
Total	1045			43.47
Mean	~ 43.54			1.81
Standard deviation (SD)	± 12.78			± 0.34

$K_{1,2,3,4}$ = ? (1-n) log x-x_1, log x_1-x_2, log x_2-x_3, log x_3-x_4; K_n = ? ?log k_1-n + ? ?log k_2-n + ? log k_3-n + ? ?log k_4-n

females were largely responsible for infertility. The mean mortality rate at eggs stage (k_1) for *An. pseudopunctipennis* was 0.562 ± 0.24 and for *An. gambiae* 0.56 ± 0.28 non-optimal temperature and pH were the environmental factors responsible for eggs mortality.

Fig. 1 (a&b) shows the contribution of density to mortality at egg stage k_1 for *An. pseudopunctipennis* and *An. gambiae* respectively. Mean larval stages I and II harvested for *An. pseudopunctipennis* was 550 ± 30.71 and *An. gambiae* 562 ± 48.28. The mean larvae dieing at this stage x (dx) was 359 ± 31.84 and 446 ± 86.72 for *An. pseudopunctipennis* and *An. gambiae* respectively. Cannibalism was the key factor causing mortality at this stage. The mortality rates k_2 were 0.47± 0.16 and 0.513 ± 0.14 for *An. pseudopunctipennis* and *An. gambiae* respectively. Cannibalism and/or predation were the main factors responsible for mortality at III and IV instar larval stages. The III and IV instar larvae of *An. pseudopunctipennis* attacked and fed on themselves and other mosquito larvae in mixed cultures including *An. gambiae*.

Table 3 shows the pattern of predation exhibited by *An. pseudopunctipennis* in mixed experimental cultures. The biochemical indices of the culture media (water) showed a mean specific gravity of 1–1.5, pH range of 6.5–7, glucose (+), proteins (+), blood (++), Na^+ 0.11–1.7; K^+ 0.5–1.2 and Cl^- 18–26. Ketone was negative.

Environmental (water) temperature below 24°C was observed to delay IV instar larval moulting to pupa. Such larvae lost in the generation eventually moulted to pupae with favourable environmental temperatures.

Environmental factors including temperature and oxygen tension, and predation by various organisms were identified as causes of mortality at the pupal stage. Predators and pathogenic agents causing damage to pupa organs isolated include *Escherichia coli, Pseudomonas auruginosa, Klebsiella pnuemoniae, Staphylococcus aureus* and *Enterobacter* species. Other microbial isolates from the mosquito cultures include algae (+++), ciliates (+++) and flagellates (+).

It was observed that lower temperatures were more devastating on pupae than eggs and larvae. Apparently healthy pupae not affected by predators and pathogens that failed to develop to adults were monitored for temperature dependence as possible causes of mortality. Such pupa failing to develop to adult at lower temperatures eventually did as temperature increased to optimal levels. Pupa failing to mature to adult at low oxygen tension in high algae containing medium also eventually matured to adult on transfer to the medium with sparse algae concentration. This was likened to a high degree of eutrophy. Fig. 2 (a&b)

Table 3. Relative proportion of predation exhibited by *An. pseudopunctipennis* larvae on other mosquito larvae

Time/Algae	*Ae. aegypti*		*Culex (p) fatigans*		*An. gambiae*		*An. pseudopunctipennis*		Total
	+	–	+	–	+	–	+	–	
12 h	9	6	12	5	10	6	22	21	28
48 h	4	0	7	0	4	2	22	18	26
60 h	0	0	2	0	1	0	22	12	12
Total	9	6	12	5	10	6	22	11	

(+) Denote that substrate (algae) added to the media for larva/pupa species to serve as food for the species under consideration;
(–) Denote that no substrate (algae) added. Only the mosquito species served as food for the species under consideration.

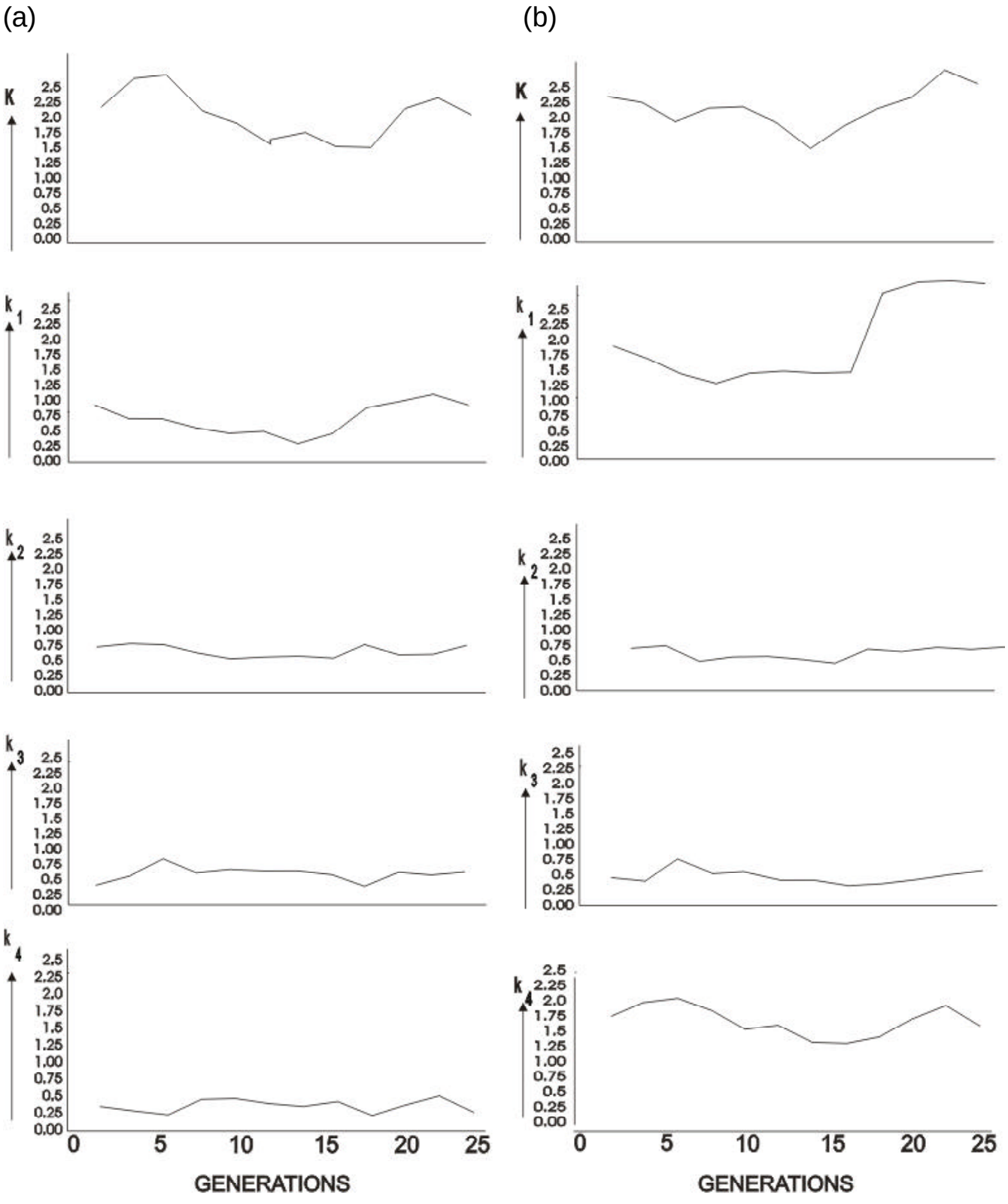

Fig. 1(a&b): Visual plot of K factor analysis for (a) *An. pseudopunctipennis* & (b) *An. gambiae* larvae populations at Ekpoma

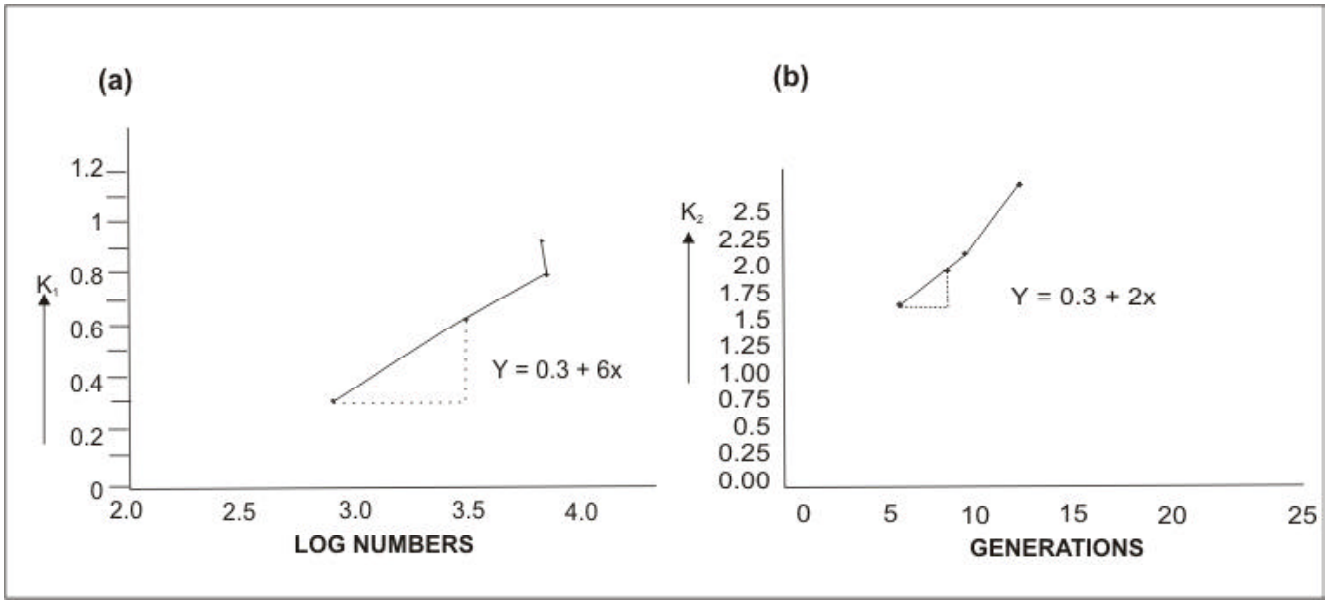

Fig. 2 (a&b): Contribution of density of mortality at early larval stages (K) for (a) *An. gambiae* & (b) *An. pseudopunctipennis* development
Note: The regression coefficient is less than unity

shows the visual plot of k factors analysis for *An. pseudopunctipennis* and *An. gambiae* populations recorded. Generally there was a higher harvest of the aquatic stages of mosquitoes and emergent adults in the wet months—March–October than in the dry months—November –February.

Discussion

About a quarter (25%) of eggs generated by each of the two *Anopheles* species investigated hatched to I and II instar larval stages with only 1.5% of this number becoming adults. This is a known characteristic of k factor reproducing organisms. The high fecundity of the *Anopheles* mosquitoes may be associated with its high mortality rates. The key factor responsible for mortality at the egg stage was infertility. A wide range of factors is responsible for eggs infertility ranging from immaturity and infertility due to non-insemination of the teneral female mosquitoes. The determination of the genetic factors responsible for fertility in female mosquitoes and male insemination in *Anopheles* mosquito species may be of importance in the species control at the egg stage. The process may be further enhanced by a detail study and application of method used in the determination of the genetic factors conferring capacity to feed on blood meals on female *An. gambiae* as determined by Area *et al*[18].

Abundance of water collection sites, pools, favourable environmental temperatures (tropical), is a permanent determinant of vectors abundance enhancing the vectorial capacity of *Anopheles* malaria vectors in the study area. Mosquito eggs once transferred out of water of a suitable pH did not hatch. Water of a near neutral pH 6.8–7.2 was found most optimal for the weakening of egg shells for the I stage larva to emerge.

At the larval stage the key factor responsible for mortality was cannibalism. Various attempts at controlling mosquito breeding by having predators' feeds on them have been reported in the past. These include tiger beetle *Cicindela octogutata* by Macfies[19], waterboatman *Anusops* spp[20], dragon fly nymphs of the *Chorixidae* family and tadpoles[21]. Others include larvivorus fish including *Gambusia, Poecilia* and *Tilapia* spp, *Ctenopharyngodon idella,* and parasites in-

cluding the nematode *Romanomermis culicivorax* and *R. xyangari*[22].

The predatory capacity of *An. pseudopunctipennis* is a control method worth investigating. Our finding in this report confirms earlier report by Cheng *et al*[23] that *An. punctipennis* larva a sibling of *An. pseudopunctipennis* is a predator of other mosquito larvae. The usefulness of this as a control method will, however, depend on the biological marking and genetic modification of the fertility form, as the resulting infertile eggs produced may reduce the malaria burden. The key factor responsible for mortality at the pupal stage is environmental factors; these include rainfall, still water, temperature, relative humidity, cool shade and absence of pathogenic organisms. Early rainfall and late rainfall tend to encourage breeding and the emergence of young adults, recurrent flooding as in mid-July/August torrential rains in the area reduce breeding and young adult emergence. Lower temperatures below 24^{o}C delayed pupal moulting.

This is a major determinant of the difference in generation cycle between tropical and temperate environments in *Anopheles* mosquitoes breeding, with a shorter generation cycle duration in tropical environments. Cool still water encourage breeding and high water current and flooding leads to *Anopheles* spp larval deaths by reduction of their oxygen tension and causing physical harm to the larva. Larvae bred in cool shaded environment were dark coloured and robust especially where provided with enough source of nutrients. Direct sunlight on the other hand had a bright, slim and in some cases lethal effect on *Anopheles* spp larvae development. The implication of a high mortality rate at various aquatic stages in *Anopheles* mosquitoes breeding is suggestive of favourable control methods for the eradication of mosquito species detrimental to man. Despite the compensatory mechanism of high fecundity of the female adult *Anopheles* mosquitoes (K reproductive strategy) complete control are within reach. Concerted efforts, integrated research and control methods are desired to make such possible control methods effective.

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