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Physiological age composition of female anopheline mosquitoes in an area endemic for malaria and filariasis

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Abstract- A total of 1350 mosquitoes were caught during the study. The monthly catches ranged from 210 to 360. Out of this, 1306 (96.7%) were dissected. Overall, parous rate was 75.0%, and ranged from 62.2% in August to 91.3% in December, 2012. The variability in parous rates between months was statistically significant (×2-test, $p < 0.05$). Relating monthly parous rates with the wet and dry seasons within the sampling months showed significant variability, with higher parity in the dry than wet season (×2-test, $p < 0.05$). The analysis of parity levels among female mosquitoes caught during the study showed that Parous-one (P1) was most abundant followed by parous-two. Parous-four (P4) was the least. The distribution of parity levels in relation to the sampling months indicated that the proportion of P3s increased in the dry season months while the P1s decreased. In conclusion, ascertaining the age distribution of vectors is very important as it is useful in monitoring the success or otherwise of large-scale vector control measures. It is therefore recommended that control measures against adult mosquitoes be initiated and sustained in the study area. Periodic verification of the success or otherwise of such control measures should be carried out using the physiological age determination method.

Index Terms- Anopheline mosquito, parous rate, biting rate, physiological age

I. Introduction

The study of the age structure of vector populations is of great importance in the epidemiology of vector-borne diseases and the assessment of all control measures. The most direct way of checking the efficiency of imagicidal measures is to ascertain the age composition of the vector population treated with insecticides and comparing that with the age composition of untreated population.

Different methods have been used to assess age of vectors. This includes assessing wear of the living scales (Zar, 1999), the changes in the size of the ampullae of the common oviduct (Banerjee *et al*., 1999), morphological changes in the ampullae of the tracheal system of the ovaries (Polovodova, 1941). Age determination has also been carried out using near-infrared Spectroscopy (Mayagaya*et al.*, 2009), red pigment in the wing veins of sharp shooter, *Homalocliscavitripennis*, and the ovarian oil injection technique (Hoc and Charlwood, 1990).

The number of previous blood meals taken by a mosquito can be deduced from the number of eggs batches laid. The number of times a female has laid eggs is determined by the number of follicular dilatations in the ovarioles (Githeko*et al*., 1993). This makes it possible to estimate the importance of each female mosquito in the transmission of malaria or filariasis. The older the female, the greater is her epidemiological significance, as each blood meal provides an additional chance of the vectors being infected by the host or of the hosts acquiring infection from a vector.

The genital apparatus of female mosquitoes experience changes in course of the gonotrophic cycles (Beklemisher, 1944). Important findings in this respect included age-related changes both in the ampullae of the oviduct (Polovodova, 1947), and in the tracheoles of the ovaries (Clements, 1992; Detinova, 1968), in the presence or otherwise of *Corpus luteum* in the distal part of the female oviduct (Beklemishev, 1944). These findings have enabled scientists to easily determine the number of egg batches laid by each female mosquito. This is predicated on the principle that after completion of a gonotrophic cycle, a normally developed follicle leaves a follicular dilatation in the distal end of the tube, indicating that the ovariole in question has produced a mature egg (Fox and Brust, 1994). However, not all follicular tubes behave similarly during each gonotrophic cycle (Hoc and Charlwood, 1990).

The usefulness of determining the physiological age of mosquitoes is in assessment of the effectiveness or otherwise of control measures. It becomes possible to evaluate imagicidal control measures. Determination of physiological age of mosquitoes enables scientists to estimate several epidemiological indices that help for our better understanding of the vector-borne disease for informed decision-making processes (Mayagaya*et al*., 2009).

It is more important to determine the physiological age (the number of gonotrophic cycles) of adult female mosquitoes than the number of days since their emergence. The chances of infection of the surviving females increase with each successive cycle. The number of gonotrophic cycles of each female is therefore very important epidemiologically. This study is therefore aimed at ascertaining the age composition of female anopheline mosquitoes in Calabar.

II. Materials and methods

A. Study location

The study location was Ekorinim area of Calabar, the Capital of Cross River State. The area is endemic for both malaria and bancroftian filariasis. The study was carried out between September and December, 2012.

B. Collection of mosquitos

The light trap catches were performed and in each trapping night, anopheline mosquitoes were collected with light traps in three houses. The method employed by Uttah et al. (2013a) was adopted. These houses were chosen to represent all parts of the study area. The light traps were used in rooms were only one occupant slept. The occupant was taught how to turn-on and turn-off the trap, and also given an un-impregnated bed-net for use around the bed while sleeping. The traps were turned on at 1800 hours and turned off at 0600 hours. They were emptied and the collected mosquitoes were kept in cool boxes until identified and possibly dissected.

C. Laboratory examination

Collected mosquitoes were brought to the laboratory to be sorted, identified and dissected. Anopheline mosquitoes were sexed and identified based on external morphology after Gillies and Coetze, (1987), Gillies and de Meillon (1968), and Edwards (1941).
The identified anopheline mosquitoes were knocked down with ether and kept in petri-dishes with moist cotton wool to prevent desiccation. After removal of legs and wings, the female anopheline mosquitoes were placed on a slide and the ovaries were extracted and quickly transferred to a drop of distilled water on the slide and examined under high magnification for tracheal skeins and classified as parous or nulliparous adapting the methods of Kardos and Bellamy (1961) with slight modification. All parous females were further dissected to reveal the follicular dilatations. The physiological age of each female mosquito was determined by counting the number of dilatations in the follicle (Hoc and Wilkes, 1995).

III. RESULTS

A total of 1350 mosquitoes were caught during the study. The monthly catches ranged from 210 to 360 (see Table 1). Out of this, 1306 (96.7%) were dissected.

Table 1. The total monthly cases and number of dissected anopheline mosquitoes during the study.

Catch days	**Total number of female mosquitoes caught**	**Total number of female mosquitoes dissected (%)**
August	360	348 (96.7)
September	300	288 (96.0)
October	276	270 (97.8)
November	210	204 (97.1)
December	204	196 (96.1)
Total	1350	1306 (96.7%)

Parity of female mosquitoes in relation to the month of the year is presented in Table 2. Overall, parous rate was 75.0%, and ranged from 62.2% in August to 91.3% in December, 2012. The variability in parous rates between months was statistically significant (×2-test, $p < 0.05$).

Table 2. Parity of female mosquitoes in relation to catch days

Catch days	**No of female Mosquitoes Dissected**	**No. of parous (%)**	**Percentage parous (%)**
August	348	216	62.1
September	288	204	70.8
October	270	208	77.0
November	204	173	84.8
December	196	179	91.3
Total	1306	980	75.0

Relating monthly parous rates with the wet and dry seasons (See Figure 1) within the sampling months showed significant variability, with higher parity in the dry than wet season (×2-test, $p < 0.05$).

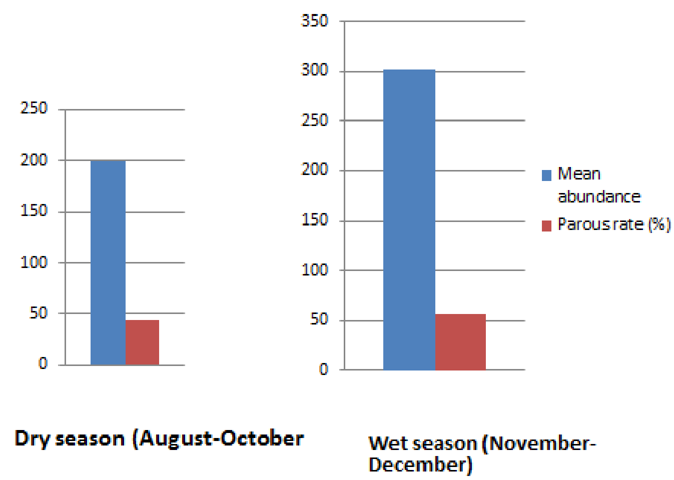

Figure 1. Seasonal differentials (Mean abundance and Parous rate) between the wet season months (August – October 2012) and the dry season months (November to December 2012).

Analysis of the age of anopheline mosquitoes

The analysis of parity levels among female mosquitoes caught during the study is presented in Table 3. Parous-one was most abundant followed by parous-two. Parous-four was the least.

Table 3. Parity levels among female mosquitoes caught during the study

Parity cycles	Number of anopheline mosquitoes	Percentage (%)
P_1	417	42.6
P_2	321	32.8
P_3	235	24.0
P_4	7	0.7
Total	980	100.0

The distribution of parity levels in relation to the sampling months is presented in Table 4. The proportion of P3s increased in the dry season months while the P1s decreased.

IV. DISCUSSION

The relative abundance of the adult female mosquitoes feeding on man is of great epidemiological importance. The intensive biting of adult female mosquitoes is indicative of high relative abundance. Secondly, the relatively high percentage of biting mosquitoes caught in the area indicates preponderance of breeding sites in the area, and this implicates the observed lapses in sanitary conditions in the area; aiding the preponderance of stagnant water bodies, empty cans, overhead water tanks, broken septic tanks, abandoned motor-tyres among others. Furthermore, it depicts absence or failure of mosquito control measures in the area.

Table 4: Distribution of parity levels in relation to the months of collection

Month	P1 (%)	P2 (%)	P3 (%)	P4 (%)	Total
August	108 (50.0)	67 (31.0)	39 (18.1)	2 (0.9)	216
September	96 (47.1)	74 (36.3)	31 (15.2)	3 (1.5)	204
October	102 (50.0)	67 (32.8)	37 (18.1)	2 (1.0)	208
November	54 (31.2)	59 (34.1)	60 (34.7)	0 (0.0)	173
December	57 (31.8)	54 (30.2)	68 (38.0)	0 (0.0)	179
Total	417 (42.6)	321(32.8)	235(24.0)	7 (0.7)	980

Comparing the relative abundance of biting female anopheline mosquitoes observed in this study with that of two similar studies in southern Nigeria shows similar seasonality trend in relative abundance both at Ogbakiri (Uttah et al., 2013a), and Umuowaibu, Okigwe (Uttah and Uttah, 2009). It also agrees with findings in other places (Gajanana et al., 1997; Mwangangi et al., 2009). This was expected as mosquito population density variations are closely linked to rainfall and temperature (Zyzak et al., 2002; Crowley 2003; Uttah et al., 2013a).

In another study in the study area, it was observed that the relatively higher malaria rates in the rainy season than in the dry season was due to higher infective biting by the anopheline mosquitoes during the rainy season. There was relationship between malaria occurrence and climatic factors such as rainfall, humidity and temperature (Uttah and Uttah 2013). A similar seasonal pattern of biting preponderance of female anopheline mosquitoes during the rainy season with consequent higher infection rates of malaria or filariasis in the same period has been reported from many regions of the world (Knight, 1980; Lindsay et al., 1991; Ramaiah and Das, 1992; Gyapong et al., 1996; Verhoef et al., 1999; Verhoef et al., 2001; Verhoef et al., 2002; Dekoninck et al., 2010). The relationship between the named climatic factors and malaria or filariasis has been corroborated in different regions of the world (Zyzak et al., 2002; Crowley 2003; Githeko and Ndegwa, 2001).

The need for blood by adult female mosquitoes to develop their eggs is one of the reasons that they have become successful vectors of tropical diseases. The age and the ability of vectors to survive are among the important factors in the epidemiology of vector-borne diseases. Results from this study indicate that there was high rate of parous females between August and December 2912 in the study area. This translates to high longevity of female anopheline mosquitoes in the area during the period of study. This is epidemiologically significant as any infected female anopheline mosquito would be involved in the transmission of malaria and filariasis, which are two important human tropical infections that are endemic in this area (Ejezie and Akpan, 1992), Parous rates in the respective months in this study were comparable to that reported at Ogbakiri which like Calabar is a coastal town in southern Nigeria (Uttah et al., 2013a). The seasonality pattern in parity observed in this study also agrees with that observed in Ogbakiri. This preponderance of older female mosquitoes during the dry season months could not be due to higher mortality during the rainy season because the higher humidity level during this season provides favorable conditions for survival (Uttah et al., 2013b). The explanation is a higher production of nulliparous mosquitoes during the rainy season due to the availability of more breeding sites. As the dry season gets underway, some of the breeding sites dry up completely while the permanent ones thin out both in expanse and productivity. This effectively translates to a drop in the recruitment of new nulliparous females into the female population thereby increasing the proportion of parous ones.

The emergence of Parous-four female anopheline mosquitoes in this study could be an indication that mosquitoes are living longer now that previously, possibly due to climate change. The danger of such trend is that the female anopheline mosquitoes would have longer longevity, more gonotrophic cycles, and more opportunities to bite and infect more people, making them better vectors than they were previously. This will further worsen the health status of people living in malaria or filariasis in endemic areas. The emergence of the parous-four further underscores the need for the health authorities to urgently commence imagicidal control measures against female anopheline mosquitoes to curb their continued biting menace and transmission of malaria and filariasis in Calabar. Filariasis control programme was undertaken in Cross River State some years ago, combining human treatment (with diethylcarbamizine (DEC) and /or Ivermectin) and vector control by different means. There has been no sustained effort at mosquito control since then. The high parity rate in the study area indicates absence of any effective on-going vector control measures. Imagicidal control measures should be pursued alongside use of Insecticide treated bed nets (ITN), since compliance with the latter is presently erratic among the people.

V. CONCLUSION

In conclusion, ascertaining the age distribution of vectors is very important as it is useful in monitoring the success or otherwise of large-scale vector control measures. This is important because it enables health authorities to have a clear picture of the outcome of every control effort targeted at the adult vectors. It is therefore recommended that control measures against adult mosquitoes be initiated and sustained in the study area. Periodic verification of the success or otherwise of such control measures should be carried out using the physiological age determination method.

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REFERENCES

[1] GBeklemishev, W. N. (1944). The Ecology of the Malaria Mosquito. Journal on Mosva accessed 2nd, July, 2012.

[2] Clements, A. N (1992) *"The Biology of Mosquitoes"* Journal on Development, Nutrition and Reproduction. Chapman and Hall, London. Vol **1:** 3 – 7

[3] Crowley, J.C. (2003). Determining seasonality of nuisance flies and evaluating stable fly pests on horses at an equine facility in North Central Florida [Ph.D. thesis], University of Florida, Gainesville, Fla, USA.

[4] Dekoninck, W., Pollet, M., and Grootaert, P. (2010). Composition and seasonal activity patterns of mosquito communities collected with malaise traps at Etang de Virelles Nature Reserve (Virelles, Hainaut), a migratory bird sanctuary and possible site for arbovirus transmission in Belgium," *European Mosquito Bulletin*, **28**: 213–224.

[5] Detinova, T.S. (1968) Age-Structure of insect Population of Medical Importance. *Annual Review of Entomology***13**: 427 – 50.

[6] Edwards, F.W. (1941). Mosquitoes of the Ethiopian Region III. culicidae adult and pupae Anopheles mosquitoes, *Publication of the Institute Medical Research*, vol. **23**: pp. 28-32.

[7] Ejezie, G.C. and I. F. Akpan, I.F. (1992). Human Ecology and Parasitic Infections 1: The effect of occupation on the prevalence of parasitic infections in Calabar*," Journal of Epidemiology, Microbiology and Immunology,* 36 (2): 161-168, 1992.

[8] Fox, A. S. and Brust, R. A. (1994).*How dilatation form in mosquito ovarioles Parasitology Today,***10:** 19 – 23.

[9] Gajanana, A., Rajendran, R., Samuel, P.P. et al., (1997). Japanese encephalitis in South Arcot district, Tamil Nadu, India: a threeyear longitudinal study of vector abundance and infection frequency," *Journal of Medical Entomology*, vol. **34**(6): 651–659.

[10] Githeko A.K. and Ndegwa, W. (2001). Predicting malaria epidemics in the Kenyan highlands using climate data: a tool for decision makers," *Global Change & Human Health*, vol. 2(1): 54–63.

[11] Gillies, M.T. and Coetzee, M. (1987). A supplement to the Anophelinae of Africa south of the Sahara (Afro- tropical Region)," *Publication of South African Institute of Medical Research,* No. 55, 1987.

[12] Gillies, M.T. and B. De Meillon. (1968). The Anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region)," Publications of the South African Institute for Medical Research, No .54, 1968.

[13] Githeko, A. K., Service, M. W, Atieli, F. K., Hill, S. M., Crampton, J. M. (1993). Field Testing an Enzyme-linked synthetic oligonvcleotide probe for identification of *Anopheles gambigess* and *A. arabiensis. Annals of Tropical Medicine and parasitology*87**:** 595 – 601.

[14] Gyapong, J.O., S. Adjei, S., and Sackey, S.O. (1996). Descriptive epidemiology of lymphatic lariasis in Ghana," *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 90(1): 26–30.

[15] Hoc, T. Q., and Charlwood, J. D. (1990). Age determination of Aedes contains using the ovarian oil injection technique. *Medical Veterinary Entomology* **4:** 227 – 233.

[16] Hoc, T. Q., and Wilkes, T. J. (1995). The ovarioles structure of Anopheles gambiae (Diptera: culicidae) and its use in determining physiological age. *Bulletin of Entomological Research*.**85:** 56-69.

[17] Kardos, E. H., and Bellamy, R. E. (1961).Distinguishing nilliparous from parous female culextarsalis by examination of the ovarian tracheoles. *Annals of Entomological Society of Americans.***54:**448-451.

[18] Knight, R. (1980). Current status of filarial infections in the Gambia," *Annals of Tropical Medicine and Parasitology*, vol. 74 (1): 63–68.

[19] Lindsay, S.W. Wilkins, H. A., Zieler, H.A., Daly, R.J., Petrarca, V., and P. Byass, P. (1991). Ability of Anopheles gambiae mosquitoes to transmit malaria during the dry and wet seasons in an area of irrigated rice cultivation in the Gambia," *Journal of Tropical Medicine and Hygiene*, 94(5): 313–324.

[20] Mayagaya , VS, Michel , K., Benedict , MQ, Killeen , GF, Wirtz , RA, Ferguson , HM, and Dowell FE (2009). Non-destructive Determination of Age and Species of *Anopheles gambiae* s.l . Using Near-infrared Spectroscopy. *Am. J. Trop. Med. Hyg*., 81(4), 2009, pp. 622–630

[21] Mwangangi, J.M., Muturi, E.J. and C. M. Mbogo, C.M. (2009). Seasonal mosquito larval abundance and composition in Kibwezi, lower eastern Kenya," *Journal of Vector Borne Diseases*, vol. 46 (1): 65-71.

[22] Polovadova, V. P. (1941). *Medical Parasitology* (Mosk.), **10,** 387 - 92

[23] Polovodova, V. P. (1947). "*Changes with age in the female genitalia of Anopheles and the age composition of mosquito population",* Moskva (thesis)

[24] Ramaiah K.D. and P. K. Das, P.K. (1992). Seasonality of adult Culex quinquefasciatus and transmission of bancroftian filariasis in Pondicherry, South India," *Acta Tropica*, 50 (4): 275–283.

[25] Uttah E.C. and Uttah, C. (2009). Human settlement and behavioural triggers of sustained endemic filariasis in Eastern Nigeria. Paper presented at the2009 Annual Conference of Parasitology and Public Health Society of Nigeria hosted at University of Sokoto, Sokoto.

[26] Uttah E.C. and Uttah, C. (2013). Influence of the Intra-annual and inter-annual climate variability on prevalence of malaria in Calabar, Nigeria. Paper 01/03 presented at the 2nd International Conference on Climate Change and Population, June 3-7th, 2013, organized by the Regional Institute for Population studies, University of Ghana, Legon.

[27] Uttah E.C. Ibe, D., and Wokem, GN (2013a). Filariasis Control in Coastal Nigeria: Predictive

[28] significance of baseline Entomological Indices of *Anopheles gambiae* s.l. (Diptera: Culicidae) *ISRN Entomology* Vol. 2013, Article ID 659468, 1-7.

[29] Uttah, E.C., Wokem, G.N., and Okonofua, C. (2013b). The Abundance and Biting Patterns of *Culex quinquefasciatus* Say (Culicidae) in the Coastal Region of Nigeria. *ISRN Zoology* Volume 2013, Article ID 640691, 7 pages http://dx.doi.org/10.1155/2013/640691.

[30] Verhoef, H., West, C.E., Ndeto, P., Burema, J., Beguin, Y., and Kok, F.J. (2001). Serum transferrin receptor concentration indicates increased erythropoiesis in Kenyan children with asymptomatic malaria. *American Journal of Clinical Nutrition* 74(6): 767–775.

[31] Verhoef, H., West, C.E., Veenemans, J., Beguin, Y., and Kok, F.J. (2002): Stunting may determine the severity of malaria-associated anemia in African children, *Pediatrics* 110(4): p. e48.

[32] Verhoef, H., Hodgins, E., Eggelte et al. (1999). Anti-malarial drug use among preschool children in an area of seasonal malaria transmission in Kenya," American Journal of Tropical Medicine and Hygiene, vol. 61, no. 5, pp. 770–775, 1999.

[33] Zar, J.H (1999) New Delhi: India Pearson Education Singapore. *Journal on Biostatistical Analysis* (4th ed.) Vol. 61: 721 – 5

[34] Zyzak, M., Loyless, T., Cope, S, Wooster, M., and Day, J.F. (2002). Seasonal abundance of *Culex nigripalpus* Theobald and *Culex salinarius Coquillett* in north Florida, USA," *Journal of Vector Ecology*, vol. **27** (1): 155–162.

[35] . O. Young, "Synthetic structure of industrial plastics (Book style with paper title and editor)," in *Plastics*, 2nd ed. vol. 3, J. Peters, Ed. New York: McGraw-Hill, 1964, pp. 15–64.

[36] W.-K. Chen, *Linear Networks and Systems* (Book style). Belmont, CA: Wadsworth, 1993, pp. 123–135.

[37] H. Poor, *An Introduction to Signal Detection and Estimation*. New York: Springer-Verlag, 1985, ch. 4.

[38] B. Smith, "An approach to graphs of linear forms (Unpublished work style)," unpublished.

[39] E. H. Miller, "A note on reflector arrays (Periodical style—Accepted for publication)," *IEEE Trans. Antennas Propagat.*, to be published.

[40] J. Wang, "Fundamentals of erbium-doped fiber amplifiers arrays (Periodical style—Submitted for publication)," *IEEE J. Quantum Electron.*, submitted for publication.

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