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Adaptative Responses of *Anopheles gambiae* in Crowding Larvae Conditions in Laboratory

[1]H.G. Tsila, [1]J. Messi and [2]G.A. Foko Dadji
[1]Laboratory of Zoology Faculty of Science, P.O. Box, 812 Yaoundé, Cameroon
[2]Laboratory of Zoology, Higher Teacher's College, University of Yaounde I, Cameroon

Corresponding Author: Tsila Henri Gabriel, Laboratory of Zoology, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon Tel: 237 96482371/237 22112237

ABSTRACT

Anopheles gambiae, an important Malaria vector in Africa is generally found in temporal and reduced pools which may result in crowding larvae conditions. This crowding could affect population's evolution of this species in natural conditions. The aim of this study is to investigate adaptive adjustments of this species to the excess larval concentration. In this case, four groups of 100 larvae each one were reared in different levels of density. Then we determine larval duration, larval mortality. We have measured length and width of adults in order to value variations of the size of the body with larval density. The size of oviposition and the hatching rate was also determined by counting of eggs and larvae of the first stage after hatching. We noticed that when the larval density is low (less than 3 larvae per mL), the main traits of life which could affect population's evolution (larval development, mortality, size of body, fecundity) are better. But when larval concentration increases (more than 5 larvae per mL), period of larval development and mortality increase, size of body and fecundity decrease. If larval density gets as high as 8 larvae per mL, mortality is very high especially during the two first days of larval development, the duration of larval phase drops significantly and body size increases significantly.

Key words: *Anopheles, gambiae*, density, larvae, mortality, crowding, adults, laboratory, fecundity, size, adaptative

INTRODUCTION

Anopheles gambiae is the main vector of Malaria in the inter-tropical area of Africa (CDC, 2004) in general and in Cameroun in particular where it is observed everywhere (Carnevale and Mouchet, 1990). Malaria accounts for much of the disease burden in Cameroon, claiming about 30-35% of the total deaths each year and 40-45% of morbidity cases. Although, over 90% of the population is at risk of this disease (Antonio-Nkondjio *et al*., 2008). Malaria control may be achieved by three complementary methods: drugs, vaccine and vector control. For efficiency of the last method, well knowledge of vector ecology is necessary. The fight against vectors should take into account processes controlling the population dynamics (Lyimo *et al*., 1992). Selection of an ovipositional site is a critical element in the life history of female mosquitoes because it ultimately influences the fitness of progeny, distribution of larvae and larval population dynamics (Spencer *et al*., 2002; Huang *et al*., 2006). The location of larvae in a habitat is due to selection of oviposition site by gravid females (Edillo *et al*., 2006). On the ecological side, *An. gambiae* has a preference for temporary water collections and sometimes very restricted puddle pools of the prints

of shoes. This fact can induce crowding of larvae in natural environment. One ecological factor known to have a great influence on the life-history of adult Anophelines, Culicines and Aedes mosquitoes is the density at which larvae develop (Ng'habi *et al*., 2005). And according to Timmermann and Briegel (1993), great larval densities are generally responsible for intra or interspecific competition which affects the population's evolution. In the absence of predators and pathogens, the number of larvae in a particular habitat and the amount of food available to them determines the number of adults that emerge from a habitat, their survival and body size (Ng'habi *et al*., 2005). The growth of populations of mosquitoes is strongly correlated with certain parameters such as duration of larval development and the size of adults (Lyimo *et al*., 1992).

Demographic boom of our cities induce occupation of suburban areas that could create new breeding sites. In urban environment Anopheles sp. Mosquitoes adapt to new breeding sites created by urbanization and hence their ecology might differ from rural settings (Sattler *et al*., 2005). The vast geographical distribution of *An. gambiae* can be related to the great adaptability of this vector to the varied living conditions (Danis and Mouchet, 1991). The objective of this study is to highlight the mechanisms of adjustment of *An. gambiae* population according to its larval density.

MATERIALS AND METHODS

In the present study, larvae came from a *An. gambiae* strain from Yaounde (Cameroon) colonized continously in the laboratory for more than 5 years. This work was carried out at the Biotechnology Centre of University of Yaoundé I, (Yaounde, Cameroon) from 2006 to 2007. These experiments proceeded under temperature ranging between 26° and 30°, RH within 70-80% and a photoperiod of L/D: 12/12.

Larval development: The larvae were reared in vat of 8.5×3.5×3. 5 cm. Six levels of density were retained within this framework. They are 0.5, 1.5, 3.5, 8 and 15 larvae per mL. Larvae were fed daily by the Tétramin (01 mg per larva during the first four days and 0.02 mg for the following days) according to Lyimo *et al*. (1992). Each experiment was repeated four times. At the end of the larval development, nymphs were pipetted and counted on daily intervals in order to evaluate the larval phase duration and larval mortality. The duration of larval development was determined according to Dempster (1961) method where duration of larval phase is considered as the time of transformation of 2/3 of the larvae into nymphs. Nymphs from each level of density are then introduced into a cage covered with mosquito net. After hatching, dead nymphs are counted.

Biomass accumulation of larvae: One hundred pupae from each level of depth were pipetted and introduced into 70% alcohol for one day. The pupae were later removed from alcohol and weighed using an electronic balance, Sartorius (dd = 0.1 mg).

Fecundity of *An. gambiae*: Adults are nourished with a saccharine solution of 10% *ad libitum*. Three days after emergence, females take a blood meal on a rabbit. During the blood meal, females draught in each cage were counted. Egg laying occurred in Petri dishes lined with filter paper soaked in spring water. Eggs from each cage were counted using a magnifying glass. Then, with the number of females draught in the cage we can determine average size of eggs per female. One hundred eggs from each cage were introduced into another Petri dish containing spring water for hatching. Larvae of first stage obtained were then counted.

Size of the adults of *An. gambiae*: Measurements were performed on the length (distance separating insertion from the wing on the body with the silk fringe of the distal end) and the width (taken at the level of the median area of the wing) of adult wings according to Lyimo *et al.* (1992). A wing of each adult was removed using two needles and measured using a magnifying glass equipped with an ocular micrometer.

Statistical analyses: The ANOVA test was performed to compare variables of larval development duration, pupae mass or wing size at different levels of density. The different means of the larval and the pupae mortality, the oviposition and the hatching rate were compared by the Chi-square test. The software SPSS (Windows version 12.0) was used to perform the above statistical analysis.

RESULTS

Larval development: The duration of larval development varied very significantly ($F = 64, 14$; $df = 5$; $p<0.0001$) from one level of density to the other. A minimal duration of 202.75 h (approximately 8.5 days) was recorded for a density of 0.5 larvae per mL and a maximum duration of 339.74 h (14.2 days) when the density reaches 8 larvae per mL. Above this density, the duration of the larval phase drops. The statistical analysis revealed that the duration of the larval phase does not differ significantly between densities 0.5 and 1.5 larvae per mL. It is the same for densities 3, 5 and 8 larvae per mL. Density level of 15 larvae per mL had the same duration of development as those with 3 larvae mL^{-1} (Table 1).

The variations in density was also accompanied by very significant variations in larval mortality. However, the larval density did not affect the mortality of nymphs ($\chi^2 = 7, 68$; $df = 5$; $p = 0,17$). Larval mortality increased with increase in density of larvae in the medium, ranging from 0.45% for a density of 0.5 larvae per mL to 92.70% when density reaches 15 larvae per mL. This mortality was especially observed during the first two days of experimentation.

Mass of pupae: Mass of pupae varied very significantly ($F = 16,63$; $df = 5$; $p < 0,0001$) with the density of larvae. This mass decreases as the larval density increases. A maximum mass of 0.0023 g was recorded for a density of 0.5 larvae per mL and a minimal mass of 0. 0009 g for a density of 8 larvae per mL. The mass goes up to 0.0015 g when the density passes to 15 larvae per mL. A comparison of the masses of pupae at various levels of density showed that the mass does not vary significantly between density levels of 0.5, 1.5 and 3 larvae per mL, between density levels of 5 and 8 larvae per mL. Also, the mass of pupae was the same for density levels of 15 and 3 larvae per mL (Table 1).

Table 1: Variation of larvae duration, pupae mass, size of adult's wings, oviposition and hatching rate of *An. gambiae* with density (number of larvae mL^{-1})

	Size (larvae mL^{-1})					
Characteristics	0.5	1.5	3	5	8	15
Larval duration	219.19±19.77a	219.19±19.77a	320.11±9.08b	335.23±6.71b	339.73± 14.85b	302.27±5.04c
Pupae mass	0.23±0.05a	0.20±0.03ab	0.16±0.01bc	0.12±0.03c	0.10±0.02d	0.14±0.01cd
Length of male wings	3.17±0.21ab	3.20±0.17ab	3.13±0.12ab	3.06±0.21ac	2.99±0.13c	3.18±0.18ab
Width of female wings	0.77±0.09ab	0.77±0.08ab	0.75±0.06ac	0.71±0.09ac	0.68±0.05ac	0.76±0.04cd
Length of female wings	3.59±0.14a	3.59±0.15a	3.32±0.08bce	3.26±0.20bcd	3.13±0.20bd	3.46±0.24ae
Width of female wings	0.93±0.04a	0.93±0.06ab	0.84±0.03bce	0.81±0.10bcd	0.75±0.07bd	0.88±0.09ae
Oviposition	104.60±5.13a	98.50±6.80b	74.70±7.67c	38.00±6.04d	39.20±5.34d	40.00±5.15d
Hatching rate	95.33±1.51a	84.56±3.34b	82.63±2.50ce	74.84±3.10d	60.91±4.09e	69.74±4.74f

Same letters: Difference not significant

Size of the adult wings: In general, size of body decreased with increase in larval density for both gender. It was however observed that the length and the width of the wings rather increased when the larval density got higher than 8 larvae per mL for both sexes. The wings of females resulting from media with 0.5 and 1.5 larvae per mL are longer and broader (3.60 mm for the length and 0.93 mm for the width) whereas those from medium with 8 larvae per mL have the shortest (3.13 mm) and least broad (0.75 mm) wings. The length and the width of the wing do not differ significantly between the females from media of 0.5 and 1.5 larvae per mL, 3 and 5 larvae per mL and finally 15 larvae per mL (Table 1).

For the males, the maximum length (3.20 mm) and the maximum width (0.79 mm) were recorded for mosquitoes coming from the media of 1.5 larvae per mL with the minimal length (3.00 mm) and the minimal width (0.68 mm) obtained for mosquitoes from the medium with 8 larvae per mL. The males from media with densities of 0.5, 1.5, 3 and 15 larvae per mL had virtually the same size while males from media with densities of 5 and 8 larvae per mL had a similar size (Table 1).

Fecundity of females: The size of oviposition varied very significantly (χ^2 = 150,89; df = 5; p<0,0001) with the larval density. In general, number of eggs per laying decreased with increase density of larvae in the medium. Thus, the size of oviposition was maximal (approximately 110 eggs) for the female from the medium of 0.5 larvae per mL and minimal (approximately 40 eggs) for the female from the medium of 5 larvae per mL (Table1).

The rate of hatching also varied very significantly according to the larval density of the medium (χ^2 = 163,59; df = 5; p<0.0001). This rate was highest for eggs laid by females from the medium with 1.5 larvae per mL with a value of 95.33% and it is lowest (60.95%) for eggs laid by the females from a medium of 8 larvae per mL (Table 1).

DISCUSSION

The density of the larvae of *An. gambiae* influences considerably their development. When the density of the larvae is low (less than 3 larvae per mL), the duration of the larval phase is approximately 8 days (first nymphs appearing after 7 days) which corresponds to the normal duration of development of the larvae of this species of mosquitoes (Holstein, 1954; Diop and Molez, 1998; Aurelie *et al*., 2007). But when the density becomes high (more than 3 larvae per mL) we observe a prolongation in duration of the larval stages. Similar results have been reported in the same species by Gimning *et al*. (2002). They have recorded an average of an additional 1-2 days. But for our experiment the duration of the larval phase is prolonged for approximately 6 days when the larval density increases to the level of 8 larvae per mL. This represents a very significant delay which can significantly affect the dynamics of adult populations. Indeed, if we consider the very temporary character (less than 10 days) of the *An. gambiae* larval lodgings, most of larvae do not complete their development. This situation would be very frequent at the beginning and at the end of the rainy seasons because rains are very sporadic and the water puddle pools quickly dry up. The long duration of the larval phase in the case of crowding larval conditions would relate to a phenomenon of growth inhibition. Indeed, Moore and Fisher (1969), Kuno and Moore (1975), Reisen and Emory (1977) and Suleman (1982) found a factor called GRF (Growth Delaying Factor) responsible for increasing of the duration of the larval phase of several species of Culicidae such as *Aedes aegypti, Anopheles stephensis* and *Culex quiquefasciatus* under the crowding conditions

of larvae. According to the same authors, this factor is also responsible for the high mortality recorded under the same conditions. Within the framework of our work, mortality becomes significant when the density becomes higher than 3 larvae per mL. For densities higher than 8 larvae per mL, GRF concentration would be very weak because most larvae die two days after the culture is set up and the duration of the larval phase decreases significantly. The very high mortality would probably be related to competition by interference (for space) from consecutive of larval overload because larvae receive sufficient food. This result is contrary to that of Suleman (1982) who noticed that for *Culex quinquefasciatus*, when the quantity of food is sufficient, the larval density does not have any effect on the larval development.

Growth of mosquito larvae as that of any other organism is accompanied by the accumulation of reserves especially of proteins and lipids (Van Handel, 1986; Timmermann and Briegel, 1993). So pupae proceeding to larvae which accumulates more reserves also has a higher mass. Mass of the pupae can thus constitute an indicator of food availability during the larval development. Within the framework of our work, we noticed that the greatest mass of pupae was recorded for the media with densities lower than 3 larvae per mL. But for density between 3 and 8 larvae per mL, accumulation of the reserves is disturbed. Low teneral energy reserves typically are associated with crowded larval conditions (Gary *et al*., 2009). This disturbance of reserves accumulation is undoubtedly a consequence of intraspecific competition by exploitation induced by the phenomenon of interference. Although, theoretically, all the larvae receive the same quantity of food, there always exists a problem of space because during provision of food, it is not evenly distributed in the medium; the surface of water and the bottom of the vat are the principal places of food supply (after a certain time, food forms a deposit). This fact considerably limits the permanent access to food for all the larvae. Then we observe a great variation in the mass between pupae. These facts were observed for *Culex quinquefasciatus* by Suleman (1982).

The density of larvae also affects the size of adults. Indeed big adults come from the media of very low densities as 0.5 and 1.5 larvae per mL. This shows that the conditions of the larval development also influences the size of adult *An. gambiae*. Size of adult depends on the accumulation of biomass during the larval growth because the pupae does not feed. The same reports were made by Timmermann and Briegel (1993), Takken *et al*. (1998) and Gimning *et al*. (2002). The increase in the size of adults when the larval density becomes higher than 8 larvae per mL could be explained by the high mortality of the larvae during the first few days of the experimentation. This fact contributes to reduce competition and consequently the GRF production, a factor which also disrupts the accumulation of reserves during the larval phase.

Overcrowding affect the fecundity of females of mosquitoes. The size of oviposition and the rate of hatching of eggs are also affected by the density of larvae. Fecundity is high for the females resulting from the medium with density of 0.5 larvae per mL and low when the larval density of the medium increases. The effect of the density on fecundity would be rather indirect because we note like Briegel (1990) and Karino *et al*. (2004) a high correlation between fecundity and the size of adults. Indeed, the big females take in great quantities of blood and Steinwascher (1982) found for *Ae. aegypti* a positive correlation between the absorptive quantity of blood and number of mature eggs in the ovaries.

Crowding larvae conditions do not affect only larval development but even adult life traits that can regulate populations. High mortality occurs two days after hatching and reduce density. This fact improve some life traits in particularly size of adults and their fecundity. Besides geographic location, knowledge of ecological features breeding sites of mosquito is a potential key element for implementing efficient and effective larvae control measures.

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REFERENCES

Antonio-Nkondjio, C., J. Atangana, C. Ndo, P. Awono-Ambene, E. Fondjo, D. Fontenille and F. Simard, 2008. Malaria transmission and rice cultivation in Lagdo, Northern Cameroon. Trop. Med. Hyg., 102: 352-359.

Aurelie, F.D.G., M. Jean and T.J. Lebel, 2007. Influence of water type and commercial diets on the production of *Anopheles gambiae* giles, under laboratory conditions. Pak. J. Biol. Sci., 10: 280-286.

Briegel, H., 1990. Metabolic relationship between female body size, reserves and fecundity of *Aedes aegypti*. J. insect Physiol., 36: 165-172.

CDC, 2004. The Impact of Malaria, has Leading Causes of Death Worldwide. CDC, USA.

Carnevale, P. and J. Mouchet, 1990. La lutte anti-vectorielle et lutte anti-paludique. Med. Trop., 50: 392-398.

Danis, M. and J. Mouchet, 1991. Paludisme. Universite Francophone, Paris.

Dempster, J.P., 1961. The analysis dated obtained by regular sampling of animal insect population. J. Anim. Ecol., 30: 429-432.

Diop, O.F. and J.F. Molez, 1998. Installation in insectarium of a stock of *Anopheles arabiensis* (Diptera: Culicidea). Bull. Soc. Pathol., 91: 340-343.

Edillo, F.E., F. Tripet, Y.T. Toure, G.C. Lanzaro, G. Dolo and C.E. Taylor, 2006. Water quality and immatures of M and S forms of *Anopheles gambiae* s.s. and *Anopheles arabiensis* in a Malian village. Malaria J., 5: 35-35.

Gary, R.E. Jr., J.W. Cannon III and W.A. Foster, 2009. Effect of sugar on male *Anopheles gambiae* mating performance, as modified by temperature, space and body size. Parasites Vectors, 2: 19-19.

Gimning, J.E., M. Ombok, S. Otieno, M.G. Kaufman, J.M. Vulule and E.D. Walker, 2002. Density-dependent development of *Anopheles gambiae* (Diptera: Culicidea) larvae in artificial habitats. J. Med. Entomol., 39: 162-172.

Holstein, M.H., 1954. Biology of *Anopheles gambiae*: Research in Western Africa. WHO, Geneva, Swiss.

Huang, J., E. Walker, J. Vulule and J. Miller, 2006. The influence of darkness and visual contrast on oviposition by *Anopheles gambiae* in moist and dry substrates. Physiol. Entomol., 32: 34-40.

Karino, K., N. Seki and M. Chiba, 2004. Larval nutritional environment determines adult size in Japanese horned beetles *A. dichotoma*. Ecol. Res., 19: 663-668.

Kuno, G. and C.G. Moore, 1975. Production of larval growth retardant in axenic cultures of *Aedes aegypti*. Mosquito News, 35: 199-201.

Lyimo, E.O., W. Takken and J.C. Koella, 1992. Effect of rearing temperature and larval density one larval survival, age at pupaison and adult size of *Anopheles gambiae*. Entomol. Exp. Appl., 63: 265-271.

Moore, C.G. and B.R. Fisher, 1969. Competition in mosquito. Density and species ratio effects on growth, mortality, fecundity and production of growth retardant. Ann. Entomol. Soc. Am., 62: 1325-1331.

Ng'habi, K.R., B. John, G. Nkwengulila, B.G. Knols, G.F. Killeen and H.M. Ferguson, 2005. Effect of larval crowding on mating competitiveness of *Anopheles gambiae* mosquitoes. Malaria J., 4: 49-49.

Reisen, W.K. and R.W. Emory, 1977. The effects of larval intraspecific competition on imaginal densities in *Anopheles stephensi* (Diptera: Culicidea): A laboratory evolution. Can. Entomol., 109: 1481-1484.

Sattler, M.A., D. Mtasiwa, M. Kiama, Z. Premji, M. Tanner, G.F. Killeen and C. Lengeler, 2005. Habitat characterization and spatial distribution of *Anopheles sp.* mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. Malaria J., 4: 4-4.

Spencer, M., L. Blaustein and J.E. Cohen, 2002. Oviposition al habitat selection by mosquitoes (*Culiseta longiareolata*) and consequences for population size. Ecology, 83: 669-679.

Steinwascher, K., 1982. Relationship between pupal mass, adult survivorship and fecundity for *Aedes aegypti*. Environ. Entomol., 11: 150-153.

Suleman, M., 1982. The effects of intraspecific competition for food and space on the larval development of *Culex quinquefascitaus*. Mosquito News, 42: 150-153.

Takken, W., M.J. Klowden and G.M. Chamber, 1998. Effect of body size on host seeking and blood meal utilization in *Anopheles gambiae* ss (Diptera: Culicidea): The disadvantage of being small. J. Med. Entomol., 39: 639-645.

Timmermann, S.E. and H. Briegel, 1993. Water depth and larval density affect development and accumulation of reserves in laboratory populations of mosquitoes. Bull. Soc. Vect. Ecol., 18: 174-187.

Van Handel, E., 1986. Growth of three mosquitoes on two larval diets measured by protein accumulation. J. Am. Mosquito Control Assoc., 2: 289-291.