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Effect of the larval habitat depth on the fitness of the malaria-vector mosquito, *Anopheles gambiae s. s.*

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This work was carried out with the aim to evaluate the impact of the lodging depth of *Anopheles gambiae* larvae on life features that characterize the population's fitness of this significant vector of human malaria. The study noted that depth is a significant factor that can considerably influence dynamics of the *A. gambiae* populations. Thus, on one hand depth increases the duration of larval stages from 8.16 days at 3 cm to 11.23 days at 25 cm depth, and on the other hand depth does not affect the survival of larvae. Depth reduces nymph mass from 2.1 mg at 0.5 cm to 1.4 mg at 25 cm depth. The size of adults is also influenced by this factor in both sexes. Among females, wings length passes from 3.6 mm at 1 cm to 2.8 mm at 25 cm depth, while width varies from 1 mm at 1 cm to 0.6 mm at 25 cm depth. For males, the wings length passes from 3.4 mm at 1 cm to 2.6 mm at 25 cm depth. The wings width passes from 0.97 mm at 1 cm to 0.79 mm at 25 cm depth. Depth acts even on the fecundity of females. Size of oviposition decreases from 117.61 eggs per female at 6 cm to 72.00 eggs per female at 25 cm and the hatching rate varies from 99.54% at 6 cm to 62.03% at 25 cm depth.

Key words: *Anopheles gambiae*, habitat depth, larvae, fecundity, fitness.

INTRODUCTION

Malaria is a plague in countries located in the intertropical zone. This disease is responsible for 207 million new clinical cases and 627 000 deaths per year (WHO, 2013). In Cameroon, health data indicates that this disease is responsible for 24% of death in health units, 52% of morbidity in children of age less than five years (MINSANTE, 2014). One of the most significant vectors of malaria in Africa is *Anopheles gambiae* (CDC, 2004). The fight against vectors should take into account processes controlling the population dynamics (Lyimo et al., 1992). Studies on the dynamic of mosquito populations should take into account mechanisms that affect life cycle, and thus growth rate of the population (Stearns, 1976). For some mosquitoes species, duration of larval development and size of adults, influence the dynamics of the population strongly (Lyimo et al., 1992). Peaks of *A. gambiae* pupating was observed to be coinciding with rainy seasons (Manga et al., 1992). Some ecological studies revealed that larvae of *A. gambiae* are generally present in temporary aquatic sites with very low depth and scarce in larval habitat of more or less depth (Manga et al., 1992; Timmermann and Briegel, 1993; Mwangangi et al., 2006).

Several factors such as temperature, water depth and

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larval density affect development, accumulation of reserves (Timmermann and Briegel, 1993), larval mortality, body size, fecundity (Lyimo et al., 1992) and adult longevity (Briegel, 1990 ; Kitthawee et al., 1992) of mosquitoes. Only *Aedes aegypti* larvae could dive, feed and successfully develop down to at least 14 cm (Timmermann and Briegel, 1993).

If the study considers the large adaptational character of insects, is it possible to find *Anopheles* larvae in the greater deep waters? Thus, this study have performed experiments with *A. gambiae* which is the most important vector of malaria in Africa whose larvae were reared in several levels of depth. The aim of this study is to evaluate the impact of the depth of water on development of aquatic stages of *A. gambiae* s.s. and on adult size and female fecundity which determine the dynamics of mosquito populations.

MATERIALS AND METHODS

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

Effects of depth on larval development

Larvae were reared in bottles of 10 cm diameter containing spring water. Eight different levels of depth (0.5 cm, 3 cm, 6 cm, 10 cm, 15 cm, 20 cm and 25 cm) were chosen for this work. One hundred larvae were introduced into each depth. Each larva potentially receives 0.01 mg of fish food (Tetramin) during the first four days and 0.02 mg for the following days in order to avoid pollution of the water. Each setting of the depths was repeated four times. Pupae were counted daily using a pipette in order to evaluate the larval phase duration and larval mortality. The larval phase duration was determined by the method of Dempster (1961), who defined it as the time of transformation of the 2/3 of the larvae into pupae. Pupae from each level depth were then introduced into a cage of 40 cm covered with mosquito net. After eclosion, dead pupae were counted.

Effects of depth on pupal biomass

One hundred pupae of the four replicates for each depth treatment were submerged into 70% alcohol for one day. These pupae were dried up from the alcohol and weighed on an electronic balance (Sartorius, dd = 0.1 mg).

Effects of depth on wing size of male and female adults

One wing of 30 adults from each depth is measured. Measurements were performed on the length (distance between insertion from the wing on the body with the silk fringe of the distal end) and the width (taken on the level of the median area of the wing) of adults wings according to Lyimo et al. (1992) method. A wing of each adult was removed using two needles and measured using a magnifying glass equipped with an ocular micrometer.

Effects of depth on female fecundity

Adults were feed with a saccharine solution to 10% *Ad libitum*. Three days after emergence, females were provided a blood meal on a rabbit. During the meal, females draught in each cage were counted in order to determine average egg number per female. Eggs were laid on a piece of filter paper soaked with spring water, which was set at the bottom of a Petri dish. Eggs from each cage were counted using a magnifying glass. One hundred eggs from each cage were introduced into another Petri dish containing spring water for hatching. The first-instar larvae were counted.

Statistical analysis

The ANOVA test was performed to compare variables of larval development duration, pupae mass or adult wing size at different depth, and the correlation test to see the effect of increasing depth on this parameters. The coefficient of correlation was also used to estimate relation variation between the wings length and width in both gender, the wings length of female and the oviposition (average egg number per female), then between the oviposition and the hatching rate. The different means of the larval and the pupae mortality, the oviposition and the hatching rate were compared by the Kruskall-Wallis test. The software statistical package for social science (SPSS) (Windows version 12.0) was used to perform the above statistical analyses.

RESULTS

Larval and pupae development duration

The duration of larval and pupae phases varied significantly according to the depth of breeding medium (Table 1). Duration of larval and pupae development also increased with depth. The duration of the larval phase was shortest at 3 cm depth with average duration of 10.16 days. It became longest at 25 cm depth with average duration of 13.23 days. There is higher positive correlation between aquatic stages duration and increasing depth (Table 4). Comparisons of the durations of the larval phase at various depth revealed that duration of larval development did not change significantly for mediums with depth under 10 cm (Table 4). On the other hand, at a depth of above 10 cm, the effect of depth on the duration of larval phase became perceptible and was accentuated for high depths (20 and 25 cm). The results indicated that the most favourable medium depth for larval development was around 3 cm. The depth of the medium did not significantly affect the survival of the larvae and the pupae. Larval and pupae mortality were low whatever the depth of the medium.

Pupal mass

Mass of the *A. gambiae* pupae varied very significantly with the depth of the breeding medium (Table 1). Pupal

Table 1. Variation of larvae and pupae duration, pupae mass, size of adult's wings of *A. gambiae* with habitat depth (ANOVA Test).

Parameters	0.5 cm	3 cm	6 cm	10 cm	15 cm	20 cm	25 cm	F	P<
Larval duration (days)	11.05±0.52a	10.16±0.45ab	10.64±0.28a	11.03±0.35b	12.37±0.84c	12.24±0.51d	13.23±0.65d	12. 28	0. 0001
Pupae mass	0.21±0.03 h	0.20±0.03 hg	0.20±0.03 h	0.18±0.04 g	0.16±0.03 k	0.15±0.04 k	0.17±0.04 e	24. 68	0. 0001
Length of male wings	3.13±0.09r	3.15 ± 0.17 rt	3.11±0.18 rt	3.11±0.12 rt	2.95±0.11 o	2.84±0.10 p	3.03 ±0.18 f	15. 27	0. 0001
Width of male wings	0.75±0.04 q	0.71±0.04 qj	0.74±0.08 qj	0.68±0.05 qj	0.66±0.04 jv	0.65±0.03 vw	0.70±0.07 e	15. 92	0. 0001
Length of female wings	3.25±0.11 n	3.30±0.18 nm	3.37±0.18 nm	3.30±0.16 nm	3.19±0.11 i	3.16±0.16i	3.23±0.17 yf	24. 64	0. 0001
Width of female wings	0.81±0.04 x	0.83±0.08 x	0.86±0.08 x	0.83±0.07 x	0.77±0.08 xl	0.74±0.06 l	0.79±0.07 z	24. 03	0. 0001

Same letters on same line: difference not significant.

mass decreased when the depth of the medium increased. The highest mass, 2.1 mg was obtained with depths of 0.5 and 1 cm while the lowest mass of 1.4 mg was at 25 cm depth. The comparison of the pupal mass at various depth revealed that mass did not vary significantly between the pupae for the mediums of depths lower than 10 cm, indicating that the depth acted on the pupal mass at 10 cm and above. The difference in the mass was highly significant between the pupae at ≤ 6 cm and those at ≥ 15 cm.

Wings size of male and female mosquitoes

The length of male and female wings of *A. gambiae* varied significantly with the depth of the breeding medium of larvae (Table 1). The length of male and female wings decreased with the medium depth. Although, the female wings were longer than the male's whatever the depth. The variations in the wing lengths were the same in both gender. Wings of the females and males resulting from 1 cm depth were the longest (3.6 mm for females and 3.4 mm for males) comparatively to those for adults in both gender from 25 cm depth (2.8 mm for females and 2.6 mm for males). Length of wings did not vary significantly between the females resulting from depth ranging in 0.5 to 10 cm (Table 1). Difference in length became significant between wings of females from depth higher than 10 cm and those of females from lower depth. The study had the same groups in males. Wing width showed the same trend in both gender. The differences in the width were highly significant with the various depths (Table 1). Width of wings decreased when the depth of the medium increased. The greatest widths (for females, 1 mm and among males, 0.9 mm) were recorded at 1 cm and the smallest ones (for females, 0.6 mm and 0.6 mm for males) were at 25 cm depth.

Within a gender, the wing lengths were separated into 2 significantly different groups from different ranges of the medium depth (Table 1). Among the females, those at depths ≤ 10 cm formed the first group. Within this group, width of wings did not differ significantly from one level of depth to the other. The second group consisted of the females from depth > 10 cm. In this group, the effect of depth was perceptible; the difference in width was significant from one level of depth to the other. In the males, the first group was those at 1 cm depth, whose wings were significantly larger than the rest from deeper medium. The second group included individuals coming from the mediums of depth lower than or equal to 6 cm. In this group, wing width did not differ significantly from one level of depth to the other. Finally, the third group formed by individuals proceeding mediums of depths higher than 6 cm, width of wings in general varied from one level of depth to another.

Fecundity of females

Average number of eggs per female significantly varied from one level of depth to another (Table 2). The number of eggs laid per female increased from lowest depth of breeding mediums to reach the maximum value, 117.61 eggs per female at 6 cm. Then, size of oviposition decreased when the depth increased; the lowest value, 72.00 eggs per female was recorded at 25 cm of depth (Table 2).

There was a significant correlation between female wing size (length or width) and average number of eggs per female, and between the wing length and width (Table 3). Hatching rate varied significantly with the depth of the medium (Table 2). This rate was high for eggs laid by females from lowest depth and decreased for the depths higher than 15 cm. The maximum rate of hatching, 99.54% was recorded at 6 cm depth and the minimal value, 62.03% at 25 cm of depth. A positive correlation existed between the size of

Table 2. Variation of oviposition and hatching rate of *A. gambiae* with habitat depth.(Kruskall-Wallis test).

Parameters	0.5 cm	3 cm	6 cm	10 cm	15 cm	20 cm	25 cm	H	P <
Oviposition	80.50±6.99 a	97.80±6.81 c	117.60±13.56 d	98.60±5.88 e	88.96±2.35 f	75.00±7.18 gh	71.92±5.83 h	185.17	0. 0001
Hatching rate	94.86±1.51 f	97.68±1.66 d	99.54±1.01 c	98.08±1.41j	88.75±1.35k	73.51±2.41 l	62.03±2.76 n	212. 57	0. 0001

Same letters on same line: difference not significant.

Table 3. Correlations of pearson.

Parameter	Wings length and wings width (female)	Wings length and wings width (male)	Wings length (female) and oviposition	Oviposition and hatching rate
r	0.972	0.953	0.301	0.673
-	P< 0.0001	P < 0.0001	P <0.0001	P <0.001

Table 4. Linear correlation.

Parameter	Increasing depth and aquatic stages duration	Increasing depth and pupae mass	Increasing depth and length of male wing	Increasing depth and length of female wing
r	0.814	-0.643	-0.616	-0.509
-	P< 0.0001	P < 0.0001	P < 0.0001	P <0.001

the oviposition and the hatching rate (Table 3).

DISCUSSION

This study showed that larvae of *A. gambiae* were sensitive to the depth environmental conditions during their growth. Thus, duration of the larval phase varies when the depth of the lodging changes. Larvae of *A. gambiae* present a short growth duration when the depth of the breeding medium is lower or equal to 10 cm. Beyond this level, increase of depth also involves an increase of larval phase duration. Duration of larval development that was obtained remain within the time limit found by Holstein (1954), Diop et al. (1998) and Foko et al. (2007) who found out that duration of larval phase ranges between 8 days and 12 days. On the other hand, Timmermann and Briegel (1993) noted that beyond 2 cm of depth, the duration of larval phase of *A. gambiae* becomes higher than 13 days. The effect of depth on the larval phase would be related to the energy expenditure caused by displacement of larvae. Indeed, in the natural habitats, larvae consume the food particles coming from the microbial decomposition of organic matter at the bottom of their habitats (Fish and Carpenter, 1982).

In the case of breeding in laboratory, it is the food which forms a deposit in the bottom of the boxes of breeding. These larvae must each time go up to the surface to breathe. Thus, energy expenditure related to displacement increases when the place of provision of food is far from the place of supply of oxygen. Under natural conditions, the authors noted that the larvae of *A. gambiae* met only in temporary lodgings of low depth (Ginning et al., 2001).

Timmermann and Briegel (1993), showed that development of *A. gambiae* larvae was only possible when the depth of medium is lower or equal to 5 cm. On the other hand, within the framework of the study, larval and pupae mortality were low whatever the depth. This shows that, *A. gambiae* is able to develop in mediums wherein depth is largely (5 times) higher than the limited depth found by these last authors. The fact that, in nature, *A. gambiae* is found only in very small deep aquatic sites could be then an adaptive preference selected by this mosquito's species.

Pupae is a stage after larval maturity, its mass

represents the biomass accumulated during the larval phase. Indeed, growth of the larvae is accompanied by an accumulation of reserves, in particular, proteins (Van Handel, 1986) and lipids (Timmermann and Briegel, 1993). Thus, pupal mass could reflect the quality of environmental conditions of larval growth. The study results showed that depth of breeding medium affected accumulation of biomass in the larvae of *A. gambiae.* Larvae in the conditions of lower depth medium could accumulate more reserves than those from deeper medium. The effect of depth on the accumulated biomass becomes perceptible as from 10 cm of depth. On the other hand, according to Timmermann and Briegel (1993), accumulation of reserves in larvae of *A. gambiae* grows with the depth, particularly in the accumulation of lipids. Depth of the breeding medium of larvae also affects size of adults.

Indeed individuals of bigger adult came from the mediums of depth lower than 10 cm. The highly-positive correlation between pupal mass and wing sizes of adults in both sexes supports this hypothesis. Adult body size is decided by the amount of biomass accumulated during the larval stage because pupae do not feed (Timmermann and Briegel, 1993).

Size of ovipositions and hatching rate of eggs were also affected by depth of breeding medium of *A. gambiae* larvae. Fecundity is high for females resulting from the mediums of depth lower or equal to 6 cm and low when the depth of medium increases. Effect of medium depth on fecundity would be rather indirect because of the positive correlation between fecundity and size of individual adults found in this study and others (Briegel, 1990; Karino et al., 2004). Indeed, bigger female mosquitoes take greater quantities of blood as Steinwaschen (1982) found in *A. aegypti*.

CONCLUSION

The depth of the lodging influences development of larvae of *A. gambiae S. S.* at the laboratory situation. The high depths lengthen the larval development time, reduce the size of adults and their reproduction, and then corrupt fitness of populations of this important malaria vector in sub-Saharan Africa. However, the depth of water is not a selection factor for the development of *A. gambiae S. S.* because it has no impact on the mortality of larvae of this vector.

RECOMMENDATION

During the collection of larvae of *A. gambiae S. S.* in the field study, in the case of lack of temporary lodging and then shallow, larvae of this vector must be checked also in the permanent lodging like lakes.

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Conflicts of interest

Authors have none to declare.

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