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Influence of biological and physicochemical characteristics of larval habitats on the body size of *Anopheles gambiae* mosquitoes (Diptera: Culicidae) along the Kenyan coast

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Abstract

Background & objectives—The number and productivity of larval habitats ultimately determine the density of adult mosquitoes. The biological and physicochemical conditions at the larval habitat affect larval development hence affecting the adult body size. The influence of biological and physicochemical characteristics on the body size of *Anopheles gambiae* was assessed in Jaribuni village, Kilifi district along the Kenyan Coast.

Methods—Ten cages measuring 1 × 1 × 1 m (1 m^3) with a netting material were placed in 10 different aquatic habitats, which were positive for anopheline mosquito larvae. Emergent mosquitoes were collected daily by aspiration and the wing lengths were determined by microscopy. In the habitats, physicochemical parameters were assessed: pH, surface debris, algae and emergent plants, turbidity, substrate, nitrate, ammonia, phosphate and chlorophyll *a* content.

Results—A total of 685 anopheline and culicine mosquitoes were collected from the emergent cages. Only female mosquitoes were considered in this study. Among the *Anopheles* spp, 202 were *An. gambiae s.s.*, eight *An. arabiensis*, two *An. funestus*, whereas the *Culex* spp was composed of 214 *Cx. quinquefasciatus*, 10 *Cx. tigripes*, eight *Cx. annulioris* and one *Cx. cumminsii*. The mean wing length of the female *An. gambiae s.s.* mosquitoes was 3.02 mm (n = 157), while that of *An. arabiensis* was 3.09 mm (n = 9). There were no associations between the wing lengths and the environmental and chemical parameters, except for a positive correlation between wing length of *An. gambiae* and chlorophyll *a* content (r = 0.622). The day on which the mosquitoes emerged was not significant for the anopheline (p = 0.324) or culicine mosquitoes (p = 0.374), because the mosquito emerged from the cages on a daily basis.

Interpretation & conclusion—In conclusion, there was variability in production of emergent mosquitoes from different habitats, which means that there should be targeted control on these habitats based on productivity.

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Keywords

Cage; emergence; mosquitoes; wing lengths

Introduction

The number and productivity of larval habitats ultimately determine the density of adult mosquitoes. Conditions of larval development also affect adult body size. Body size affects factors such as longevity, fecundity, and blood meal volume and all these factors may influence the fitness of the vector for malaria in parasite transmission. The body size of adult females influences the number of blood meals required to complete the first gonotrophic cycle and the number of eggs[1]. Smaller females take longer to achieve reproduction and produce fewer offsprings. For all suspension feeders, useful nutrients come through drinking if concentrations of dissolved materials are high enough. For mosquito larvae in aquatic habitats, the habitat water contains both nutrients and deterrents[2,3]. Higher concentration of dissolved organic material may occur adjacent to leaf and substrate surfaces or near decaying tissue, are associated with bacteria[4]. Various chemical properties of the larval habitat related to vegetation such as pH, and concentration of ammonia, nitrate and sulphate affect larval development and survival[5–7]. In this study, we investigated on the influence of biological and physicochemical parameters of the larval habitats on the productivity of mosquito larvae and its influence on the mosquito body size. The results of this study provide important information on vector productivity, which is useful in designation and implementation of an integrated control strategy for mosquito borne diseases along the Kenyan coast where malaria and Bancroftian filariasis are endemic.

Material & Methods

The study was done in the village Jaribuni in Kilifi district, Kenya from September to October 2001. This site has previously been described by Mbogo *et al*[8] and Mwangangi *et al*[9]. The criteria for selection were the presence of known aquatic habitats of anopheline mosquitoes, malaria vector species composition, and accessibility. Briefly, Jaribuni River traverses the site with numerous small pools of water and vegetation along both sides. The houses are mainly mud-walled and roofs are thatched with palm leaves. The vegetation consists mainly of shrubs and bushes. Inhabitants are mainly farmers, growing maize and cassava for subsistence and cashew nuts, mangoes and coconuts as cash crops. Domestic animals include cows, goats and sheeps.

Ten cages measuring 1 × 1 × 1 m (1 m^3) were placed in 10 stream pools covering only a small fraction of the main habitat. These cages were constructed from metal frames and covered with a fine netting material was placed over it to exclude any adult mosquito from oviposition. The cages were pressed in the substrate of the aquatic habitats and the netting material tucked in the soil to ensure that there is no movement of mosquito larvae in and out of the cage. These habitats were randomly selected, along Jaribuni River to monitor the body size of the emerging adult mosquitoes. These larval habitats were selected based on size and productivity of anopheline mosquito larvae, which was indicated by presence of *Anopheles* larvae by dipping technique before placing the cage. The placement of the cage within the main habitat was systemic (i.e., based on visual presence of larvae) and not random relative to other locations in the habitat.

The physicochemical parameters assessed in the larval habitat covered by the cages were nitrate, phosphate, conductivity, dissolved oxygen, temperature and chlorophyll *a* content. Conductivity, dissolved oxygen, temperature and pH were measured using field hand-held

machines (Corning®), while nitrate, phosphate and chlorophyll *a* content was measured using spectrophotometric technique[10]. The variables were measured immediately the cages were placed and the seventh day (end of first week). These parameters were assessed for their association with the wing length of the *An. gambiae s.s.* from the cages. At Day 0 (day of cage placement), the non-mosquito invertebrates within these habitats were qualitatively assessed and classified to family level.

On every day of visit, the cages were first inspected for the presence of emerged mosquitoes and those present were collected by a hand-held manual aspirator[11]. The mosquitoes in the cage were aspirated and placed in a paper cup. The mosquitoes were provided with 6% sucrose solution (w/v) placed in a cotton wool placed on the paper cups before transporting to the laboratory. In the laboratory the anopheline and culicine mosquitoes were identified morphologically[12,13]. The *An. gambiae s.l.* were further identified into sibling species by use of rDNA-PCR technique[14]. The wings were removed gently with forceps and mounted on microscope glass slide using DPX mountant. Wings were measured to the nearest 0.01mm with an ocular micrometre from the distal end of the alula to the wing tip, excluding the fringe scales.

The statistical analyses were done using SPSS software (Version 11 for windows, SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) was used to test whether the wing length of the emergent mosquitoes varied in different aquatic habitats and whether wing length varied according to the days of emergence.

Results

The initial sampling of the main habitat before placing the cages yielded 1,627 mosquito larvae of which 891 (54.76%) were early instars (I–II) and 291 (17.89%) late instars (III–IV) of *Anopheles* while 391 (24.03%) early instars and 54 (3.32%) late instars of culicine mosquitoes. In this study, a total of 686 mosquitoes emerged from the emergent cages. Of these 65% (n = 446) were females and 35 % (n = 240) were males. Among the female *Anopheles*, 202 were *An. gambiae s.s.*, nine *An. arabiensis*, two *An. funestus*, whereas the *Culex* included of 214 *Cx. quinquefasciatus*, 10 *Cx. tigripes*, eight *Cx. annulioris* and one *Cx. cumminsii* (Table 1). The number of emergent female mosquitoes from each of the ten cages was variable. Cage No. 4 had the most emergent mosquitoes (n = 118, 26.5%) whereas cage No. 5 had the least number of emergent mosquitoes (n = 11, 2.5%). There was significant variation in the female *Anopheles gambiae s.s.* productivity from the cages ($F_{(1,9)} = 2.719$, $p = 0.005$) but the day of production was not significant ($p = 0.155$), and the interaction between the cage and day was also not significant ($p = 0.430$). The production of female *Cx. quinquefasciatus* mosquitoes was significantly related to the day of emergence ($F_{(1,13)} = 1.409$, $p = 0.022$) but not the cage ($F_{(1,9)} = 1.78$, $p = 0.73$). Also, there was no interaction between the cage and the day of emergence ($p = 0.064$).

Table 2 shows the mean wing length of the *An. gambiae s.s.* and *An. arabiensis* emerging from each cage. The mean wing length of *An. gambiae s.s.* was 3.02 mm (n = 157), *An. arabiensis* was 3.09 mm (n = 9). The overall wing length of the 166 emergent *An. gambiae* mosquitoes was 3.02 mm (range 2.35 to 3.70 mm). The wing length of the emergent mosquitoes significantly varied from cage to cage ($F_{(1,9)} = 3.775$, $p < 0.001$).

The physicochemical parameters, measured in the emergent cages, were assessed for their association with the wing length of the *An. gambiae s.l.* from the cages. Two out of the 28 correlation coefficients (7.14%) were statistically significant indicating that there was a non-random association between some pairs of variables. The results showed that temperature was correlated with dissolved oxygen ($r = 0.588$) suggesting that temperature in the larval habitats

makes them more aerated. Moreover, chlorophyll *a* content was positively associated with the wing length of *Anopheles* (r = 0.622). The other micronutrients measured such as nitrate, ammonia, and phosphate, were not significantly associated with the wing length of *An. gambiae s.s.*

Discussion

The field observations demonstrate that adult mosquito production was very low, but was continuous in the habitats. The larval habitats enclosed by the cages had different mosquito larval instars, which resulted in emergence of mosquitoes on different days. Emergent anopheline mosquitoes produced were mainly *An. gambiae s.s.* which is a main malaria and filariasis vector in Kenya, and very few *An. funestus* emerged as adults from the cages. Earlier studies at the coast of Kenya indicated that this area has low vector densities[15,16], but this is the first study of the dynamics of mosquito emergence in this area. According to studies by Lyimo and Takken[1], *An. gambiae* females which produced eggs after only one blood meal had wing lengths larger than or equal to 3 mm. The mean wing length for the emergent *An. gambiae s.s.* was 3.02 mm (range 2.35–3.70) implying that most of these mosquitoes should be capable of producing eggs after the first blood meal.

Most of the physicochemical parameters measured were not associated with the body size of the emergent *An. gambiae* adults. However, chlorophyll *a* content was positively associated with the body size of *An. gambiae*. This finding was similar to Gimnig *et al*[17] in a study in western Kenya, who found that chlorophyll *a* content and algae were positively correlated with *An. gambiae* density and body size. This implies that gravid *An. gambiae* adults chose to oviposit in the aquatic habitats rich in chlorophyll *a* content in the habitat. Chlorophyll *a* content in a habitat is an indication of presence of phytoplankton, which indicates the habitat's dietary richness for the mosquito larvae.

This study further observed that there was significant variation in the female *An. gambiae s.s.* productivity from the cages. The differences could have been due to the composition of other non-mosquito invertebrates coupled with the physicochemical parameters in these stream pools. The non-mosquito invertebrates, which could have, either acted as predators or competitors of *An. gambiae s.s.* larvae consequently affecting adversely the numbers of emergent mosquitoes[18,19]. The non-mosquitoes invertebrates found in the stream pools along Jaribuni River included Gerrids, Hydrometrids, Notonectids, Naucorids, Dytiscids, Libullids, Coenagrionids and tadpoles. This calls for a need for ecological studies on the other non-mosquito invertebrates and their role in the regulation of malaria vectors along the coast using this experimental design of emergent cages. Further physical factors such as habitat permanence or degree of spatial heterogeneity and together with biotic factors such as predation are known to influence mosquito species assemblages[19]. Studies by Rejmankova *et al*[20,21], demonstrated that there was a strong association between larval distribution and the distribution of some habitat factors such as cyanobacterial mats and filamentous algae. Minakawa *et al*[22] in western Kenya did not detect any significant association between the occurrence of *An. gambiae* larvae and habitat variables.

This study observed that *An. gambiae s.s.* and *Cx. quinquefasciatus* co-existed in the larval habitats along Jaribuni River. *An. gambiae s.s.* usually breeds in sunlit, temporary pools of water whereas *Cx. quinquefasciatus* are usually found in more stable habitats, which have stayed for sometimes. It seems that *An. gambiae s.s.* have developed some evolutionary strategy to exploit stable habitats which in this study was indicated by the presence of chlorophyll *a*. Similarly *Cx. quinquefasciatus* within the rural villages share similar habitat with *Anopheles gambiae s.s.* which have been in existence for some period of time.

This is the first study along the Kenyan coast to use the emergent cages to study mosquito productivity and could be expanded in future to test mosquito productivity and survivorship from different habitat type in a wide geographic scale. Considering the success in the field utilisation of this experimental set up, there is need to further have a design that would consider the number of larvae and classification of other non-mosquito larvae within the cages at the first day so that an explanation of population regulation can be made. In this study, the samples involved were small especially for *An. arabiensis* and most of the samples were *An. gambiae s.s.* The variables tested were mostly negative in which further work is required to access these variables on a wider scale geographical scale to investigate site-to-site effect. In conclusion, there was variability in production of emergent mosquitoes from different habitats, which means that there should be targeted control on these habitats based on productivity.

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Table 1

Summary of the total number of emergent *Anopheles* and *Culex* females per cage collected at Jaribuni, Kenya

Species	Cage No.										Total
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
An. gambiae s.s.	19	14	26	70	7	25	20	7	14	0	202
An. arabiensis	0	0	4	5	0	0	0	0	0	0	9
An. funestus	0	0	0	0	0	2	0	0	0	0	2
Cx. quinquefasciatus	51	3	38	41	4	16	22	19	5	15	214
Cx. tigripes	1	0	1	1	0	2	2	0	2	1	10
Cx. annulioris	8	0	0	0	0	0	0	0	0	0	8
Cx. cumminsii	0	0	0	1	0	0	0	0	0	0	1
Total	79	17	69	118	11	45	44	26	21	16	446

Table 2

The mean wing length (in mm) for emergent *Anopheles gambiae s.s.* and *An. arabiensis* females

Cage No.	No. of emergent mosquitoes	Species			
		An. gambiae s.s.		*An. arabiensis*	
		Wings measured	Mean	Wings measured	Mean
1	20	5	3.21	0	—
2	15	10	3.17	0	—
3	28	21	3.08	4	3.23
4	75	60	3.03	5	2.98
5	7	5	3.11	0	—
6	24	17	3.10	0	—
7	20	18	2.87	0	—
8	7	7	2.77	0	—
9	14	14	2.90	0	—
10	0	0	—	0	—
Total	211	157	3.02	9	3.09

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