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Spatial distribution, environmental and physicochemical characterization of *Anopheles* breeding sites in the Mount Cameroon region

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In Cameroon, malaria stands as one of the major public health problems. The warm tropical climate of Cameroon and the dense forest and grassland vegetation provide conditions that are favourable for reproduction and survival of the malaria vector, and the consequent increase in the prevalence of malaria. Urban malaria is seriously on the rise. Urban farming, poor or inexistent drainage facilities, chaotic location of dwellings and factories in most towns, influence the mosquito population and affect malaria transmission intensity[1]. Important malaria vectors found in Cameroon are unequally distributed, and it is known that the occurrence of *Anopheles* species varies according to macro and micro environmental differences exhibited by different bioecological areas[1–3]. The spatial distribution of *Anopheles* vectors has been shown to greatly affect malaria transmission intensity[4,5]. The distribution and abundance of mosquito larvae actually reflect the oviposition preferences of adult females and the ability of immature stages to tolerate the conditions that prevail in aquatic habitats[6]. Therefore, characterizing *Anopheles* breeding sites in order to determine their influence on *Anopheles* distribution and densities could be very useful to understand the variations observed in malaria transmission intensity, so that more efficient vector control strategies could be planned. However, no substantial information exists on the influence of breeding sites distribution, environmental and physicochemical characteristics on malaria vectors distribution and densities in the Mount Cameroon region in particular and in Cameroon as a whole.

This paper reports on the study carried out in the Mount Cameroon region to determine which water bodies constitute breeding sites for *Anopheles* and what are the possible associations between *Anopheles* larvae and the environmental and physicochemical characteristics of the breeding sites. The overall goal of this work is to find out which breeding site characteristics could best explain the distribution and abundance of *Anopheles* larvae in the Mount Cameroon region.

Mount Cameroon rises from the Atlantic Ocean at the Golf of Guinea and culminates at 4100 m above sea level in Buea. It is the highest mountain in West Africa and an active volcano. In this forested area of Cameroon, the equatorial climate is modified by the double influence of the Atlantic Ocean and the mountain. Temperatures are lower than in the other areas of the southern part of the country—the mean annual temperature varies between 18 and 35ºC, with the minimum temperatures in December and August, and the maximum temperatures in February and March. The terrain is fairly hilly with about 20 streams of prime importance that empty into the ocean. From Mutengene, the terrain gradually elevates to an alti-

tude of 800–1200 m in Buea town, the highest altitude occupied by populations. At the lower altitudes, the extensive plantations of banana, palms, rubber and the agglomerations have almost entirely replaced the primary forest. More than one million people live around Mount Cameroon, mostly attracted by soil fertility and jobs. The habitats are highly diversified and the levels and types of anthropophilic activities vary from one area to another. The present study was carried out during the rainy season, from August to November 2005 in six localities that were selected based on malaria transmission history[5] in this region, on anthropophilic activities and environmental specificities, climatic and topographic features and altitude. These were: Mutengene (4°05'N, 9°18'E; 100 m a.s.l.), Meanja camp (4°15' N, 9°23' E; 300 m a.s.l.), Bolifamba (4°08' N, 9°18'E, 501 m a.s.l.), Bomaka (4°10'N, 9°18'E, 548 m a.s.l.), Muea (4°10'N, 9°18' E, 549 m a.s.l.), and Likoko (4°19'N, 9°21'E; 723 m a.s.l.).

A thorough search for potential breeding sites was carried out in each locality. All breeding sites found were inspected for the presence or absence of anopheline larvae, and described based on the turbidity, exposure to sunlight, type of bottom, the vegetation, predators (larvivorous fishes, dragonfly larvae, water scorpions, water bugs, etc.). Mosquito were counted in productive breeding sites. Breeding sites were classified as temporary or permanent, based on whether they were subjected to periodic flooding and drying within the study period (temporary) or able to continuously hold water throughout that same period (permanent)[1,2]. Their distances to the nearest inhabited house (DNIH) were also determined. The same team did the breeding sites classification to maintain consistency. The selection of breeding sites for physicochemical analyses was done in such a way as to reflect the diversity of breeding places present in the region. To avoid sample clustering, collections from commonly encountered types of breeding sites were spaced throughout each locality. Larvae were collected from productive breeding sites, which were defined as those that contained at least one larva for at least one field trip during the study period. Breeding sites that yielded only species other than *Anopheles* were not further considered in the study. Larval densities were estimated by counting the number of *Anopheles* larvae returning to the surface to breathe, within a 100 cm^2 light wooden frame dropped into the larval environment. The count took place at least 1 min after dropping the frame in water and was made as quickly as possible. The larval density was thereafter obtained by multiplying the number of larvae counted by the area of the water body sampled. This was expressed as the number of larvae per cm^2 of water sampled. The larvae collected from the field were transported to the laboratory for culture and morphological identification.

Some larvae were transferred into plastic cups containing 500 ml clean tap water and kept in cages for adults to emerge. They were fed with crushed biscuits. The 1 m^3 cages covered with netting materials were left at room temperature until the adult mosquitoes emerged. The adults were identified morphologically to species level under a dissecting microscope, using the morphological keys of the Afro-Tropical Region. Some larvae were preserved in 75% ethanol[7] and subsequently identified morphologically to species level under a light microscope at x100 magnification, using the morphological keys of the Afro-Tropical Region.

In each locality, water samples were collected from at least four breeding sites for physicochemical analyses. Water samples were transferred to the laboratory in tightly closed plastic bottles and kept at 2–8°C in a refrigerator. They were analyzed within a maximum of 10 days post-collection.

Physicochemical analysis of water from *Anopheles* breeding sites was done in the laboratory of soil, water and plant extract of the Institute for Agronomic Research and Development (IARD) in Ekona, using standard methods[8]. Temperature, exposure to sunlight, pH, calcium, potassium, sodium, chloride, carbonate, bicarbonate, ammonium, nitrate, phosphate, magnesium, sulfate ions and conductivity were the physicochemical parameters analysed.

SPSS software, version (version 15.0 for SPSS Inc., Chicago, US) was used for Statistical analyses. Physicochemical parameters were distinguished from environmental parameters and analyzed separately. Principal component analysis was used to screen variables so that only those of the physicochemical variables that explain most of the variations observed among the *Anopheles* densities in the various localities were selected and further used. These analyses were repeated with environmental variables. Linear regression analysis was subsequently carried out, including selected variables only, to assess the relationships existing between *Anopheles* density variation and the independent variables. Breeding sites were subsequently categorized as temporary or permanent breeding sites and compared with respect to physicochemical characteristics, and possible association with *An. gambiae* larval densities assessed.

Table 1. Distribution of *Anopheles* breeding sites, percentage of productive breeding sites per locality in the Mount Cameroon region during the study period

Locality	Distribution of potential breeding sites		% of productive breeding sites
	Temporary Productive (Unproductive)	Permanent Productive (Unproductive)	
Mutengene	12 (4)	0 (4)	60
Meanja	8 (5)	1 (1)	60
Bolifamba	18 (9)	0 (4)	58.06
Bomaka	97 (13)	0 (3)	85.84
Muea	95 (7)	0 (3)	90.47
Likoko	0 (1)	1 (1)	33.33
Total	230 (39)	2 (16)	80.83

The number of *Anopheles* breeding sites identified during the study, their types and the percentages of productive breeding sites per locality are presented in Table 1. Out of 287 water bodies found, 232 (80.83%) contained *Anopheles* larvae. Muea and Bomaka recorded the highest percentages of productive breeding sites (Table 1) and 93.73% of the breeding sites were temporary water bodies while only 6.27% were permanent water bodies. Temporary breeding sites included roadside ditches, rain pools, shallow drainages, footprints, hoof prints, artificial holes, and building foundations. Permanent breeding sites found during the study period included streams and fishponds.

Most temporary breeding sites were very shallow, with muddy bottom and high turbidity (77.45%). Most of these (85.71%) were found within 20 m from the nearest inhabited house and 21.43% of temporary breeding sites were found between 20 and 50 m from the nearest inhabited houses and none were found beyond a distance of 50 m. Some stagnant rain pools harboured green spirogyra. Tadpoles, *Chironomus*, dragonfly larvae and water bugs, were also found in some temporary breeding sites and most often, when they were found, very few *An. gambiae* or no *Anopheles* larvae were present. All permanent breeding sites were found at distances >50 m. The waters were rather clearer, with sandy and muddy bottoms. The presence of vegetation on the water surface (*Pistia* spp, *Mimosa* spp, *Nymphea* spp, floating algae and green spirogyra) and around the pool (guava trees, palm trees and flowers) was remarkable and provided shade to the water surface. The fishponds and rivers contained various species of fishes.

There were significant differences in potassium, bicarbonates, nitrates, sulfate ions concentrations and conductivity ($p<0.05$) between temporary and permanent breeding sites. Both types of breeding sites registered pH values around neutral and the mean temperatures were relatively lower in permanent breeding sites than in temporary breeding sites.

Anopheles species in permanent breeding sites were: *An. gambiae*, *An. funestus, An. moucheti*, *An. sergentii, An. hargreavesi* and *An. hancocki* in Likoko, and *An. gambiae, An. marshallii, An. concolor* in Meanja River. Temporary breeding sites harboured *An. gambiae* species only. Fig. 1 presents *An. gambiae* larval density in temporary breeding sites in the various localities. It was the only species identified in

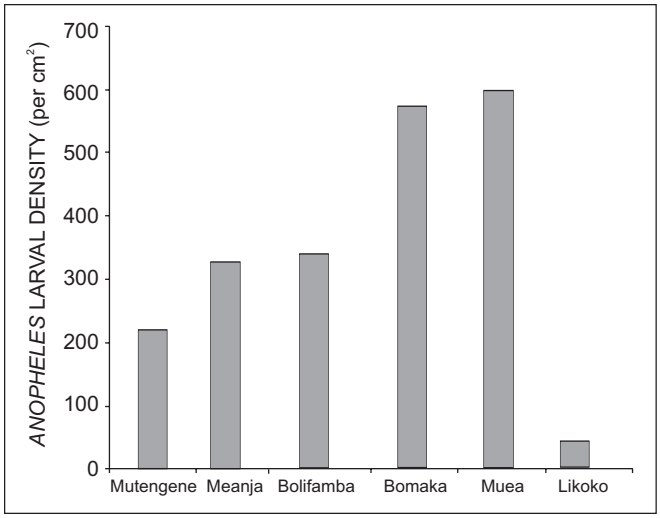

Fig. 1: Mean *Anopheles* larval densities per locality

Mutengene, Bolifamba, Bomaka, and Muea. Muea and Bomaka recorded the highest larval densities. Likoko fishponds and Meanja River that are permanent breeding sites, exhibited the greatest diversity in species composition, and the larval densities were lower compared to the densities observed in temporary breeding sites. Likoko located at the highest altitude surveyed, 723 m a.s.l., recorded the lowest larval density.

Principal component analysis detected potassium, sodium, conductivity and chloride as the physicochemical parameters that best explain *Anopheles* densities variations among the localities, but only the association between larval density and potassium was statistically significant ($p = 0.003$). The distance to the nearest inhabitated house (DNIH), the type of breeding sites and the presence of predators were detected as important environmental predictors of *Anopheles* larvae abundance in different localities (Table 2).

The results suggest that the distribution and the types of *Anopheles* breeding sites as obtained in this study is in conformity with the results presented by other authors from various sub-Saharan countries[1,2]. There was a marked dominance of temporary breeding sites over permanent breeding sites, probably because the study was carried during the rainy season when most of the pools are filled with water[9]. This distribution

Table 2. Results of test statistics showing relationship between some key environmental and physicochemical parameters and the *Anopheles* larval densities

Parameters	Test statistics	p-value	Correlation coefficient
Physicochemical			
Potassium	5.291	0.003*	0.921
Sodium	0.257	0.810	0.128
Conductivity	0.967	0.388	0.435
Chloride	–0.973	0.386	–0.437
Environmental			
Distance to the nearest breeding site	–2.905	0.012*	–0.723
Type of breeding site	2.257	0.030*	0.593
Predators	2.967	0. 028*	–0.529

*Correlation is significant ($p < 0.05$).

could also be influenced by the topography of the area. Most of the breeding sites were found in localities where the terrain is fairly flat and could allow water to stand, whereas the relief in Likoko (723 m a.s.l.) is hilly and prevents water from standing.

Eight *Anopheles* species were identified from the larvae collected. *An. gambiae* was the only species collected from temporary breeding sites. *An. funestus*, *An. hancocki An. moucheti*, *An. sergentii*, *An. hargreavesi*, *An. marshallii* and *An. concolor*, along with *An. gambiae* were collected from permanent breeding sites. Temporary breeding sites contained higher concentrations of potassium ions than permanent ones. Significantly higher concentrations of potassium were also recorded in *An. albimanus* positive sites in the wet season in Mexico[10]. Potassium quickly gets dissolved in water. The high concentrations observed could result from the dissolution in water and transportation of the potassium present in domestic wastes, agricultural products such as fertilizers, sewage effluent or leachates from dumping sites. Such habitats with important quantities of additional nutrients are generally tolerated by other genera than *Anopheles*[11,12], though *An. gambiae* seem to adapt well to such conditions.

DNIH showed a negative association with *Anopheles* densities (Table 2). The farther the breeding site to a home, the lower the *Anopheles* densities recorded. The proximity of *An. gambiae* breeding sites to houses in the Mount Cameroon region was remarkable. This is probably a way for adult female *Anopheles* to increase the chances of the emerging adults to interact with human hosts and feed to guarantee their survival. Similar findings were obtained in Kenya[9,13]. Increase efforts to reduce man-made temporary breeding sites in close proximity to houses can therefore be very efficient as a vector control strategy, especially against the most efficient vector, *An. gambiae*, that preferably breed in such water bodies in the Mount Cameroon region

Permanent breeding sites were found to harbour many species while temporary breeding sites exclusively harboured *An. gambiae*. This is coherent with the known preference of most *Anopheles* species to breed in natural permanent waters, while only few species are abundant in temporary breeding sites[14].

Tadpoles, water bugs, dragonfly and *Chironomus* larvae are suspected to be potential larvae predators. When they were found in water, larvae were generally absent. It could be very useful to check the predation efficiency of these species as *Anopheles* larvae predators so that they could be used to control vectors in permanent breeding sites in the Mount Cameroon region.

Data obtained here will serve as a baseline for future studies which will be carried out in the Mount Cameroon region to analyse the impact of any malaria control measures on the breeding sites distribution and *Anopheles* larvae densities and species.

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