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Oviposition Site Selection and Habitat Characterization of Anopheles Mosquito in Abraka, Delta State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. The study was conceived by authors EOE and TIO. Author TIO provided relevant literature and guidance on field collection of mosquitoes and laboratory culture of Anopheles mosquitoes. Author JCN supervised field data collection and other aspects of the work, while author EOE wrote the first draft of the manuscript. Authors EOE and JCN finalized the manuscript. All authors read and approved the final version of the manuscript.

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ABSTRACT

Background: Malaria still remains a life-threatening disease worldwide causing between 190 and 311 million cases of malaria in 2008. Due to the ever increasing resistance to malaria drugs, source reduction has been recognized as a complementary approach to further reduce malaria transmission. Given that the availability of suitable habitats for the oviposition of anopheline mosquitoes increases their breeding and possibly malaria transmission. We proposed that characterizing the breeding site of Anopheles is of major importance for the transition from malaria control to elimination in our study area. However, information on the oviposition sites, characteristics and influencing factors of breeding sites of Anopheles mosquitoes is lacking. This study aimed to determine the preferential oviposition sites, breeding site characteristics and related environmental parameters.
Methods: A study was undertaken in Abraka, Delta State to determine the preferential oviposition

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sites of Anopheles mosquito and characterize such sites. Potential larval habitats that might harbor anopheline immatures were sampled between August and September. The larval sampling was done by the standard dipping method. The habitats were characterized based on substrate type, turbidity, habitat type, algal coverage, canopy coverage, surrounding vegetation, surface debris and distance to the nearest house. A two way analysis of variance (ANOVA) was employed to test for significant difference in the occurrence of anopheline and culicine larvae in the different aquatic habitats sampled. Correlation analysis was used to assess the relationship between the environmental variables and the occurrence of the anopheline larvae in the habitats sampled and also to assess the relationship among the environmental variables examined.

Results: A total of 80 aquatic habitats in 8 sites were sampled and 1117 anopheline and 370 culicine larvae were collected. Microscopic identification of the adult Anopheles mosquitoes yielded only *Anopheles gambiae*. There was no significant difference ($P>0.05$) in the occurrence of anopheline and culicine larvae in the different habitats sampled, but there was significant difference ($P<0.05$) for habitat type distribution for anophelines only and culicines only. Correlation analysis revealed that the occurrence of anopheline larvae was correlated with some of the environmental variables examined.

Conclusion: The results obtained indicate that *Anopheles gambiae* prefers open, sunlit and undisturbed habitats for oviposition and that abiotic factors play a vital role in larvae's habitat preference. Thus such factors should be considered when designing an integrated vector control programs.

Keywords: Oviposition site preference; anopheles mosquitoes; malaria; Nigeria.

1. INTRODUCTION

Malaria still remains a major life-threatening disease worldwide despite efforts made towards controlling it. Globally it occurs in tropical and subtropical regions. It is the leading cause of morbidity and mortality and also a threat to the socio-economic development of poor African countries. Accurate estimates of the epidemiology and burden of malaria are hard to pin-point, but the World Health Organization (WHO) estimated that between 190 and 311 million cases of malaria occurred in 2008 [1]. The emergence of highly drug-resistant parasites [2, 3] underscores the need for prevention, and suites of preventive interventions have produced marked declines in malaria infections and mortality in several sub-Saharan African settings [3-6]. In malarious countries like Nigeria, efficient intervention and preventive efforts must be guided by understanding the distribution of productive breeding sites.

The transmission of malaria parasite depends on the availability of competent malaria vectors such as the anopheline mosquitoes, although different species of anopheline mosquitoes are found in certain areas. Thus the rate of malaria transmission increases with the abundance of competent malaria vectors. In sub-Saharan Africa, the primary vectors of malaria parasites are the *Anopheles gambiae* senso stricto, *Anopheles arabiensis* and *Anopheles funestus* and they show highly anthropophilic tendencies [7]. Approximately 70% of the total sub-Saharan African populations live in areas infested with malaria vectors, although urban areas typically have low anopheline mosquito populations and less malaria as compared to rural areas [8]. It is generally thought that the abundance of clear sun-lit and shallow bodies of water makes rural population especially vulnerable to increased contact with anopheline mosquitoes. Likewise the absence of suitable habitats and increased water pollution generally inhibits the development of anopheline larvae in urban centers, resulting in few Anopheles mosquitoes [7]. Certain characteristics of sub-Saharan African urban environment such as high human population density, man-made depressions that retain water and additional habitat in form of domestic containers can also affect the overall propensity of an area to harbour Anopheles mosquito [7].

The oviposition of anopheline mosquitoes is influenced by physical parameters of the water and the characteristics of the water that serves as a habitat for the larvae of the anopheline mosquitoes. The presence of mosquito larvae in a collection of water is as a result of the oviposition behavior of gravid females [9]. Certain environmental factors affect the presence of anopheline mosquitoes in a habitat and there is evidence that species presence and abundance are tied to specific environmental variables [10,11]. Mosquito larvae are found in numerous habitats and each habitat produces

specific mosquito species and show a seasonal succession of species. The larvae of anopheline mosquito are found in certain habitats not all habitats, depending on the suitability of the habitat for their survival. The primary malaria vector in sub-Saharan African, *Anopheles gambiae* and *Anopheles arabiensis* are typical r-strategists, which colonize temporary habitats and rapidly increase in population size. It is well documented that the larvae of both species frequently occur in small temporary pools such as pits, tire tracks and animal foot prints [12-14]. A potentially important target of malaria vector control is the immature stages of anophelines mosquitoes [15-16]. Source reduction through modification of larval habitats has been an important tool for malaria eradication efforts in the United States, Israel and Italy [12,17]. Managing the availability of water for mosquitoes was also the key to successful malaria control programs in the Zambian copper belt [18] and in Dares Salaam in Tanzania [17]. In sub-Saharan countries, larval habitats of malaria vectors are widely distributed and these habitats are greatly reduced during the dry season as a result of low availability of aquatic habitats. Some anopheline mosquitoes are now beginning to develop adaptations to survive in habitats were they were previously not found.

The larval ecology of African malaria vectors has been a neglected area of research. Entomologists have been reluctant to study larval ecology [12]. One of the reasons for the lack of ecological studies on anopheline larvae is the difficulty associated with larval sampling from aquatic habitats in the field, especially when many larval habitats are not permanent [12]. Studies have been carried out on the breeding of mosquitoes in various habitats including test containers in western Nigeria - Lagos state, Ogun state and Oyo state. In Delta state not much work has been done on the breeding of mosquitoes especially the breeding sites of anopheline mosquitoes.

Having knowledge of the breeding habits and sites of the anopheline mosquitoes and their ecology will be helpful in designing novel strategies for malaria control in this area. Therefore it is essential to investigate the preferential oviposition sites of anopheline mosquitoes and the characteristics of such sites. These will provide vital information that can be used in the reduction of anopheline breeding sites in Abraka and its environ thus reducing malaria transmission.

Therefore, the main objectives of our study were to determine the preferential oviposition sites of anopheles mosquitoes in Abraka and to characterize these sites based on certain habitat variables with the view that our results can provide viable information for planning and implementing of anopheles larval control programs in study area.

2. MATERIALS AND METHODS

2.1 Study Area

Abraka is located in Ethiope East Government Area of Delta state on latitude 05°47'N and longitude 06°06'E. The annual rainfall in this area is 3978 mm while the mean annual temperature is 30.6°. The mean annual relative humidity is about 83% (Archive Department of Geography Weather Station- 1976-2005). Abraka has an estimated population of 100,000. Peak rainfall generally occurs between June and July. The Ethiope River which has its source at Umuaja flows through Abraka. The people in this area are farmers and traders and the major crops grown are cassava and oil palm.

2.2 Larval Sampling

Mosquito larvae were sampled from sites that might potentially harbour immature anopheline populations. Ten sites each were selected from eight strategic locations in Abraka. An aquatic habitat was first inspected visually for the presence or absence of mosquito larvae. An aquatic habitat was considered a positive site if at least one anopheles larvae could be found. According to the size of the site, a representative number dips were taken. Using standard dipper (350ml), a minimum of 6 and maximum of 60 dips were taken from each type of water body. All sites were shallow and collections were made between August and September 2008. Larvae sampling was done once every two weeks for the two months of collections. Sampling was always done between 7.00 am and 11.00 am in the morning and 4.00 pm and 6.00 pm in the evening.

2.3 Larval Habitat Characterization

Both anopheline positive and anopheline negative habitats were characterized with respect to certain environmental variables. The environmental variables recorded for each selected aquatic habitat during the larval sampling were substrate type, turbidity, habitat

type, canopy coverage, vegetation, algae coverage, surface debris coverage and distance to the nearest house. Substrate types were classified into mud, sand, clay and artificial substrate without soil (e.g. brick or concrete). Turbidity was measured by placing water samples in glass test tubes and holding against a white back ground and it was categorized in three levels: clear, low and high. The habitat types included water puddles, ditches, gutters, metal containers, plastic containers, tyres and concrete tanks. Canopy cover was defined as the amount of terrestrial vegetation and other objects above the habitat. Surrounding vegetation was recorded as either present or absent. Algal coverage was recorded as either present or absent. Surface debris coverage was recorded as either present (that is the presence of particles and materials e.g. polythene, decaying matter on the surface of the water) or absent. Distance to the nearest house was estimated visually as most of the aquatic habitats were close to houses.

2.4 Mosquito Culture and Identification

The larvae collected from positive sites were put in plastic containers and transported to the laboratory where they were sorted into either anopheline or culicine larvae. The absolute number of larvae gotten from the positive habitats was determined by removing all the larvae by hand with a plastic pipette. The larvae were counted and the number obtained recorded. Then the culicine larvae were discarded while the anopheline larvae were transferred to another plastic container with food for the larvae (low fat biscuit grinded with yeast). The larvae were reared to adults and the adults that emerged were collected. The adults were knocked off with chloroform vapour and then each was preserved individually in a 1.5 ml eppendorf tube that had silica gel and cotton wool. The adult anopheline mosquitoes were identified morphologically under a stereomicroscope using the taxonomic keys of Gilles and Coetzee [19].

2.5 Data Analysis

A two way analysis of variance (ANOVA) was employed to test for significant difference in the occurrence of anopheline and culicine larvae in the different aquatic habitats sampled and also to test for significant difference in the habitat type distribution for each species. The occurrence of a species is defined as the presence of a particular species (or sub-family) in a sample regardless of it density. Correlation analysis was used to assess the relationship between the environmental variables and the occurrence of the anopheline larvae in the habitats sampled and also to assess the relationship among the environmental variables examined.

3. RESULTS

3.1 Mosquito Larval Occurrence in the Aquatic Habitats Sampled

Of the 80 aquatic larval habitats sampled 33 were positive for both anopheline and culicine larvae and they occurred as follows; water puddle (25), concrete reservoir (4), gutter (1), ditch (1), tyre (1) and plastic container (1). The anopheline larvae were found in 30 of these habitats and 26 of them (32.5%) had only anopheline (Table 1). Culicine larvae were found in 7 of these habitats and 3 of them (3.75%) had only culicines while 4(5.0%) had both anophelines and culicines. 47 of the larval habitats (58.8%) encountered had no larvae in them (Table 1). The two-way analysis of variance (ANOVA) test indicated that there was no significant difference in the occurrence of the anopheline and culicine larvae in the different larval habitats (Table 1; $F=1.095$, degrees of freedom (df) = 3, $P > 0.05$). But there was a significant difference in the habitat type distribution for the habitats with anophelines only or culicines only (Table 1; habitats $F=2.832$, df=6, $P<0.05$).

3.2 Occurrence of Anopheline Larvae in 8 Different Sites Sampled

A total of 1117 Anopheles larvae were collected from 30 aquatic habitats positive for the anopheles larvae in seven of the eight sites sampled. The highest number of positive habitats was encountered in Otorho (8) which was used as the rural control while the least number of positive habitats was encountered in Ojeta (1). The highest number of larvae 375(33.5%) were collected from Ajanomi while the least number 6 (0.5%) were collected from Ojeta. The relative abundance of larvae in the positive sites was highest (93.75) for Ajanomi and least (6) for Ojeta. No larvae were found in the entire habitats sampled in Okpogoro (Table 2).

3.3 Characteristics of Aquatic Larval Habitats Sampled

The aquatic habitats sampled, both anopheline positive and anopheline negative habitats were

characterized based on the presence or absence of certain environmental variables. The environmental variables used were based on observations made in the field and information obtained at the time the larval habitats were identified and characterized. The environmental variables included substrate type, turbidity, habitat type, distance to the nearest house, algal coverage, surrounding vegetation, canopy coverage and surface debris coverage. Majority of the larval habitats sampled were water puddles (habitat type). The distance to the nearest house for most of the larval habitats was less than 5 meters (<5 m) as most of them were close to or in front of houses. The turbidity for each larval habitat varied. For example, all the concrete reservoirs examined were clear while some of the water puddles examined had high turbidity whereas others were either clear or had low turbidity. Some of the larval habitats characterized had algal coverage while others did not have. For example, some of the water puddles had algal coverage while others did not have. Also algal coverage was absent in all the concrete reservoirs and ditch examined (Table 3).

3.4 Correlation between the Environmental Variables and Occurrence of the Anopheles Larvae

Table 4 Present data on the association between the environmental variables and occurrence of the Anopheles larvae. The results of the correlation analysis showed that the occurrence of *Anopheles gambiae* adults was positively correlated with the clear or low turbidity, absence of surface debris, presence of surrounding vegetation and presence of artificial substrate but negatively correlated with high turbidity, presence of surface debris coverage, absence of surrounding vegetation and presence of soil substrate. For example, more anopheline larvae occur in habitats that are clear or have low turbidity because the amount of dissolved oxygen in habitats that are clear or have low turbidity is higher than in habitats with high turbidity. In this study, anopheline larvae were found both in habitats that were clear or had low turbidity and in habitats that had high turbidity. Although habitats that were clear or had low turbidity (16) were more than those that had high turbidity (14).

3.5 Correlation among the Environmental Variables

Several of the environmental variables were correlated while others were not correlated. There is a biological reason for many of them. For example high turbidity was negatively correlated with the absence of surface debris coverage because the absence of surface debris in a larval habitat reduces its turbidity. But presence of surface debris increases the turbidity of the larval habitat. Similarly, presence of algal coverage was positively correlated with clear or low turbidity possibly because algae may grow more in waters that are clear or have low turbidity reasons not fully understood. There was no correlation between presence of canopy coverage and surface debris (Data not shown).

Table 1. Habitat types and mosquito larval occurrence in aquatic habitats within the study area

	No of habitats (%)	Larval habitat types						
		Puddle	Gutter	Ditch	Tyre	Plastic container	Concrete reservoir	Metal container
Mosquito species								
Presence of anopheline larvae only	26(32.5)	21	1	1	0	0	3	0
Presence of culicine larvae only	3(3.75)	0	0	0	1	1	1	0
Presence of both anopheline and culicine larvae	4(5.0)	4	0	0	0	0	0	0
Absence of both anopheline and culicine	47(58.8)	44	1	0	0	1	0	1
Total	80(100)	69	2	1	1	2	4	1

Table 2. Occurrence of anopheline larvae in 8 different sites sampled

Sites	No. of habitats sampled	No. of habitats positive for anopheles larvae (%)	No. of larvae collected (%)	Relative abundance of larvae	Larval no. per habitat
Ajanomi	10	4 (40)	375 (33.6)	93.75	37.5
Ekrejeta	10	6 (60)	280 (26.1)	46.6	28.0
Monkey joint	10	4 (40)	83 (7.4)	20.75	8.3
Urhuoka	10	3 (30)	37 (3.3)	12.33	3.7
Ivie	10	4 (40)	71 (6.4)	17.75	7.1
Ojeta	10	1 (10)	6 (0.5)	6	0.6
Okpogoro	10	0	0 (0)	0	0
Otorho	10	8 (80)	265 (23.7)	33.13	26.5
Total	80	30 (37.5)	1117	37.23	13.96

Table 3. Characteristics of the larval habitats sampled in Abraka, Delta State

Variables	Puddle	Gutter	Ditch	Tyre	Plastic container	Concrete reservoir	Metal container
Substrate type	Mud, sand or clay	Muddy/ sandy	Clay	Rubber	Plastic	Concrete artificial substrate without soil	Metal
Turbidity	Low/high	Low/high	High	Low	Clear/low	Clear	Clear
Habitat type	Water puddle	Gutter	Ditch	Tyre	Plastic container	Concrete reservoir	Metal container
Distance to the nearest house	< 5m	<5m	< 5m	< 5m	< 5m	< 5m	<5m
Algal coverage	Present/absent	Absent	Absent	Absent	Present/ absent	Absent	Absent
Surrounding vegetation	Present/absent	Absent/ present	Present	Absent	Absent	Present/ absent	Absent
Canopy coverage	Present/absent	Absent	Absent	Absent	Present/ Absent	Absent	Absent
Surface debris coverage	Present/absent	Absent	Present	Absent	Absent	Present/ absent	Absent

Table 4.Correlation between environmental variables and occurrence of Anopheles larvae in the positive habitats sampled

Variables	No. of habitat positive for Anopheles larvae	No. of larvae collected
No of habitats positive for Anopheles larvae		
No. of larvae collected	0.651	
No. of habitats with high turbidity.	- 0.637	- 0.606
No. of habitats with clear or low turbidity.	0.637	0.606
No. of habitat with algal coverage.	0.346	0.305
No. of habitats without algal coverage	- 0.346	- 0.305
No. of habitat with canopy coverage	0.123	0.212
No. of habitats without canopy coverage	- 0.123	- 0212
No. of habitat with surface debris	- 0.795	- 0.460
No. of habitat without surface debris.	0.795	0.460
No. of habitats with surrounding vegetation.	0.678	0.413
No. of habitat without surrounding vegetation	- 0.678	- 0.413
No. of habitats with soil substrate.	- 0.823	- 0.663
No. of habitats with Artificial substrate.	0.823	0.663

4. DISCUSSION

Anopheles mosquitoes use different aquatic habitats for their oviposition and knowledge of where they prefer to oviposit is important for designing effective larval control programs targeted at modifying their habitats to reduce their numbers or completely destroying them to eradicate mosquitoes. The control of *Anopheles gambiae* through environmental control in several parts of the world has been successful. Source reduction through modification of larval habitats has been an important tool for malaria eradication efforts in the United States, Israel and Italy [12,17,20]. Also source reduction activities in Zambia seven decades ago reduced malaria incidence by 50% [18]. Eradication of introduced *Anopheles gambiae* from the northeast coast of Brazil and the Nile valley of Egypt [21], via antilarval measures provide additional examples where source reduction was successful.

This study identified the preferential oviposition sites (habitats) of Anopheles mosquitoes in Abraka, Delta State and characterized these larval habitats (sites).But the only anopheline species found in the samples was *Anopheles gambiae* other anophelines were not found. Seven larval habitats types were identified in this study and only 4 were positive for anopheline larvae. Majority of the habitats positive *Anopheles gambiae* were water puddles and the occurrence of the *Anopheles gambiae* larvae was highest in water puddles. This study showed that not all the water puddles were positive for anopheles larvae and it was observed during field sampling that water puddles that were constantly disturbed by the movement of vehicles did not harbor anopheline larvae. The possible reason for this could be because the development of the immature is indirectly and unintentionally disturbed by human interference, thus anophelines could not oviposit in such habitats. Furthermore, our results suggest that anophelines prefer unshaded, sunlit and undisturbed habitats for oviposition. Most of the water puddles were temporary, well sunlit and unshaded. The frequent occurrence of the *Anopheles gambiae* larvae in small, temporary and well sunlit habitats may be as a result of several factors. Three plausible explanations for this phenomenon have been suggested. Firstly, all *Anopheles gambiae* females preferentially select small, open, sunlit habitats for oviposition [22]. Secondly, larval predation is less prevalent in temporary habitats than in large permanent habitats [22]. Survivorship of Anopheles larvae can be higher in exposed habitats because higher temperature can be detrimental to the presence of many other aquatic arthropods including predators [23]. Thirdly, the *Anopheles gambiae* is a typical r-strategist (a species that is opportunistic and reproduces rapidly when density- independent limiting factors are not present), exploiting the increased resources of warmer, open habitats that tend to produce more algae (the main food source for the *Anopheles gambiae*) than do shaded habitats [14]. Warmer temperatures encountered in small and open habitats during day time hours shorten larvae to pupae development time and subsequent larval mortality due to dessication is reduced [22]. Also warm temperatures may allow more microorganisms to grow, which provide food sources for mosquito larvae [11]. It is assumed that the *Anopheles gambiae* may have evolved to exploit these favorable conditions by selecting small and open habitats for oviposition.

In several habitats sampled, anopheline and culicine larvae were found co-existing, especially in water puddles. However no significant difference was detected for the occurrence of the anopheline and culicine larvae in the different habitats sampled but a significant difference was detected for the habitat type distribution for each species. The possible reason for this variability could be because the number of each habitat type sampled varied. For instance majority of the habitats sampled were water puddles and anopheline larvae had a higher occurrence in water puddles than any other habitat sampled. The occurrence of anopheline larvae in larval habitats may be affected by the presence or absence of certain environmental variables, each of which may have a small effect. Robert et al. [24] found that the occurrence and abundance of anopheles arabiensis larvae in permanent habitats (Market garden wells) in Dakar, Senegal was determined by many physiochemical and biological variables. The environmental variables examined were not independent of each other. This is consistent with the results of Minakawa et al. [12] who found out that the environment variables they examined when characterizing the larval habitats were not independent of each other in their study area. Ecological studies that attempt to tie the Anopheles species presence or abundance to a single factor have usually been successful [9]. But rather than focusing on only one or two environmental factors, examining a broader array of habitat variables provides a better opportunity to describe the habitat in

greater detail with relation to species [9]. The occurrence of anopheline larvae was correlated with some environmental variables examined in the larval habitats. Some of the variables were positively correlated with the occurrence of the anopheline larvae while others were negatively correlated. High turbidity and presence of surface debris was negatively correlated with the occurrence of the anopheline larvae while low turbidity and absence of surface debris was positively correlated with the occurrence of the anopheline larvae. This result suggest that more anopheline larvae are likely to be found in habitats that are clear or have low turbidity and surface debris is absent than in habitats with high turbidity and surface debris present. But Awolola et al. [25], found *Anopheles gambiae* breeding in polluted water bodies with turbidities as high as 490FAU in a site in urban Lagos. This agreed with the findings of Keating et al. [7], who found out that during their sampling in Malindi Kenya, water bodies with polluted or floating debris were 14 times more likely to have anopheline larvae as compared to water bodies identified with no pollution or floating debris. Also absence of surrounding vegetation and presence of soil substrate was negatively correlated with the occurrence of anopheline larvae while presence of surrounding vegetation and artificial substrate was positively correlated with the occurrence of the anopheline larvae. The reasons why absence of surrounding vegetation and presence of soil substrate was negatively correlated with occurrence of the anopheline larvae and presence of surrounding vegetation and artificial substrate was positively correlated with the occurrence of the anopheline larvae is not fully understood. Distance to the nearest house was not considered in the correlation analysis because most of the larval habitats sampled were less than 5 metres from the nearest human habitation and all the larval habitats from which anopheline larvae were collected were close to houses. There were several instances when this distance was greater than 10 meters and in such cases no larvae was found in such habitats. This shows that anophelines are more likely to lay eggs in habitats close to house than those at a greater distance from houses. These observations are consistent with that Minakawa et al. [12] who found that *Anopheles gambiae* prefers laying eggs in habitats near house. They said that this may be as a result of the limited flight of the gravid females.

Based on observations made in the field, the habitat characteristics did not differ much for the habitats sampled. Analysis of water chemistry of the habitats sampled was not done due to logistic difficulties even though it is important in determining the occurrence of anopheles larvae in aquatic habitats. The purpose of this study was to determine the preferential oviposition sites of Anopheles mosquitoes but the only anopheline mosquito found in the sampled collected was *Anopheles gambiae*. Thus the result obtained demonstrated that *Anopheles gambiae* the only anopheline mosquito found in the samples collected prefers small, open, sunlit, temporary and undisturbed habitats for their oviposition.

5. CONCLUSION

In summary, this study has shown that *Anopheles gambiae* selects habitats for oviposition that are suitable for the development and survival of their larvae and that environmental variables affect oviposition site selection by *Anopheles gambiae*. Control programs targeted at the immature Anopheles species or reducing the amount of suitable habitats in proximity to vulnerable human populations should be devised to address the problem of malaria in this area. Also to better understand the relationship between anopheline larval occurrence and environmental variables, more variables should be examined including detailed analysis on the water chemistry of the larval habitats. Further studies considering larger number of habitats would be required to validate our findings.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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