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Factors influencing differential larval habitat productivity of *Anopheles gambiae* complex mosquitoes in a western Kenyan village

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ABSTRACT

Background & objectives: The study was undertaken to characterize factors influencing differential productivity of *Anopheles gambiae* complex mosquitoes at larval habitats in a rural village in western Kenya.

Methods: Longitudinal larval sampling was done using an area sampler for 3 months. Emerged adults were identified to species level morphologically using taxonomic keys and to sub-species by polymerase chain reaction (PCR). Nutrient content was analyzed using persulphate oxidation method. Water pH was measured using an Orion pH/conductivity meter. Turbidity was measured using a Hach 2100A turbidity meter. Algal count density was estimated using a sedge-wick rafter cell.

Results: A total 3367 larvae were harvested. Out of 500 adults subjected to PCR analysis 358 (71.6%) were *Anopheles gambiae* s.s., 127 (25.4%) *An. arabiensis* while PCR amplification failed for 15 (3%) specimens. Rainwater pools were the most productive habitat type. There was a positive association between algal density and larval density ($p<0$). Total nitrogen, water pH and turbidity were positively correlated with larval density ($p<0.01$) and pH was negatively associated with larval density.

Conclusion: Results indicate water nutrient and algal content in larval habitats of *An. gambiae* play crucial, dual roles in the resource ecology of these mosquitoes. Overall, the findings of this study support the notion that anti-larval source reduction measures aimed at manipulating physicochemical variables in larval habitats to eliminate larval production have a chance of succeeding in an integrated vector control program.

Key words *Anopheles gambiae*; larval productivity, nutrients; rainwater pools

INTRODUCTION

Larvae of Afro-tropical malaria vectors exist in a variety of aquatic habitats but prefer small, confined, soil-lined puddles[1–6]. While some of these habitats are naturally derived, others are the result of human activities. Regardless of the mode of formation, each of these habitat types has unique ecological properties that are relevant to the fate of anti-larval biological control strategies targeted at reducing juvenile mosquito populations they support. Such properties are likely to make certain habitats more supportive to biological insect life than others. For example, ecologists have long recognized that habitat size has important inherent consequences on the organization, size and persistence of resident biological communities[7]. Smaller habitats are likely to contain fewer mosquito species, support smaller populations, and exhibit higher rates of extinction compared to larger habitats. Larvae are known to feed on bacteria and algae within surface microlayers[8,9]. Optimal amounts of these microbial fauna could influence larval productivity[10–12]. Consequently, a thorough understanding and appreciation of larval population dynamics requires a thorough appreciation of these factors and how they affect larval abundance.

Studies conducted in a western Kenya village showed that borrow pits were the most productive habitat type[5]. Apart from what we know from laboratory studies, reasons behind this differential productivity remain unknown. The predominant theme of the current research work was to estimate habitat-specific larval productivity of *Anopheles gambiae* s.l. (Diptera: Culicidae) in a western Kenya village on one hand and the factors behind deferential larval productivity of this mosquito species on the other. It is believed that there are certain intrinsic factors in larval habitats that make some habitats supportive to larval growth and development and not others. This creates a scenario in which productive larval habitats of *An. gambiae* s.s. (i.e. small, confined, soil-lined puddles formed by human and animal activity) are located in particular features among rural villages in malaria-endemic settings, largely due to human and domestic animal activities. It was hypothesized that: (a) productive habitats will be just a subset of all potential water-holding bodies; (b) the number of productive habitats can be estimated on an area-wide basis through rigorous sampling; and (c) their stability (capacity to hold water for a sufficient amount of time to

produce larval cohorts and adults) can be measured, their rate of turnover determined, and their productivity quantified. Knowing which habitats are the most productive, both temporally and spatially, can make targeted larval control measures highly effective resulting in rational use of already strained and limited resources.

MATERIAL & METHODS

Study area

The study was conducted in Kisian, a rural village 10 km west of the City of Kisumu in Nyanza Province in western Kenya, located at 0°-5' 60" N, 34° 40' 0" E. The village is located 10 km south of the Equator at an altitude of 1137 m above the mean sea level, and covers an area of 7.7 km^2.The long rainy season occurs in March–May followed with a short one in November–December. Malaria is holoendemic in the area with the hot and humid climate driving breeding of malaria vectors throughout the year. The principal mosquito vectors in this area are *An. gambiae* and *An. funestus* Giles with *An. arabiensis* playing a secondary role[13]. Potential breeding sites in the area include small streams that meander through the villages and empty into the Lake Victoria. The red laterite-based mineral material holds rainwater forming readily available breeding sites for mosquitoes.

Larval sampling and identification

Daily longitudinal larval sampling was done per habitat for 25 days per month for three successive months using an area sampler. This resulted into 75 sampling visits per habitat. The habitat types in the study area were: (i) drainage canals borrow pits, (ii) streambeds, rainwater pools, (iii) tyre tracks, (iv) ponds, and (v) swamps. The sampling period targeted the long rainy season months of March, April and May 2005. Sampling was done using an area sampler[5] consisting of a plastic cylinder 10 cm in diameter and 12 cm in height. At every habitat, the sampler was held firmly down into the mud until sampling was completed. Placement of the sampler was systematic (i.e. based on visual presence of larvae) and was not random relative to other locations in the habitat. Larvae enclosed in the area sampler were transferred (without replacement) by pipetting into a white collecting tray with clear water for categorization into different instar stages, followed by counting morphological identification and recording[14]. Sampling involved a quantitative system involving absolute area sampling on one hand and whole habitat census on the other. A more detailed description of this sampling method has been previously described[5]. Larval density was expressed in terms of numbers per unit area of the sampling device. Collected larvae and pupae were placed in capped specimen vials and transported to the Kenya Medical Research Institute-Centre for Global Health Research Laboratories in Kisumu where they were held in paper cups while in water to allow for emergence. Emerged adults were identified morphologically into anophelines according to the protocols of Gillies and deMeillon[1], and Gillies and Coetzee[2] and to sub-species by PCR[15].

Water chemistry analysis

The protocols of Kaufman *et al*[16] were used to collect water from larval habitats. Fifteen millilitres of water was collected from the habitats 1–2 cm below the surface micro-layer with a syringe and needle, and stored frozen in screw cap falcon tubes in a freezer until analysis. Phosphorus and nitrogen contents were determined in unfiltered samples using persulphate oxidation of all moieties of each respective element present in a water sample to phosphate nitrate according to the protocols of Menzel and Corwin[17], Murphy and Riley[18], Crumpton *et al*[19], and Bachman and Canfield[20]. The pH of water samples was measured using an Orion pH/conductivity meter. Turbidity was measured using a Hach 2100A turbidity meter.

Algal density estimation

Water samples containing algae were preserved in 10% formalin and kept at 4^{o}C. Algal density was estimated according to the protocols of Schoen[21] and Guillard[22]. Algal density was expressed in terms of number of algae per 15 ml of water.

Data analysis

The association between larval densities was ascertained by a repeated measures Poisson regression. Each test was done using the GENMOD procedure in SAS Version 8.0. Variables included in each model were algal density, total nitrogen, total phosphorus, pH and turbidity. Factors initially were screened by univariate analysis and those that were not statistically significant at a=0.05 were excluded from the model. The effects of these factors on larval density were tested using a two-way ANOVA. The non-parametric Spearman's correlation coefficient test was used to determine the extent to which larval densities are associated with physico-chemical parameters.

RESULTS

Table 1 summarizes the relative abundance of each habitat type from which sampling was done and yielded

Table 1. Larval habitat types in a western Kenya village

Habitat type	Number
Ponds	14 (7.65)
Rainwater pools	83 (45.36)
Tyre tracks	5 (2.73)
Borrow pits	35 (19.3)
Drainage canals	33 (18.03)
Streambeds	7 (3.83)
Swamps	6 (3.28)

Figures in parentheses indicate percentages.

3367 larvae. A total of 1800 larvae were successfully reared to emergence of adults; out of which 500 adults were subjected to PCR analysis. Of these, 358 (71.6%) were *An. gambiae* s.s., 127 (25.4%) were *An. arabiensis* while PCR amplification failed for 15 (3%) specimens, probably due to specimen processing challenges. The female to male sex ratio was interestingly varied at 1.24 females per male. Habitats were categorized based on their mode of creation and hydrology into seven main types as follows: borrow pits, drainage canals, tyre tracks, rainwater pools, streambeds, swamps and ponds (Table 1). Rainwater pools and streambeds were naturally occurring habitats, while the rest were man-made. Rainwater pools (45.36%) were the most abundant habitat type and tyre tracks the least abundant (2.73%).

Table 2 shows the relative contribution of each habitat type to larval and algal densities and test statistic for each habitat type. Streambeds had the highest algal density followed by drainage canals while ponds had the lowest densities. Larval density was significantly different across different habitat types with larvae being found across the whole range habitat types. Algal densities were also significantly different among the seven habitat types but did not significantly affect larval density. However, there was a positive association between algal density and larval density ($p<0.01$).

Table 2. Summary statistics for larval density in different mosquito breeding habitats

Habitat type	Larvae/78.5 cm^2	χ^2
Ponds	0.24 ± 0.09*	29.06
Rainwater pools	0.62 ± 1.00*	97.78
Tyre tracks	0.4 ± 0.3*	26.46
Borrow pits	0.47 ± 0.3*	63.18
Drainage canals	0.47 ± 0.49*	119.08
Streambeds	0.36 ± 0.24*	139.02
Swamps	0.24 ± 0.18	98.12

*Mean values are significant at $p< 0.05$.

Table 3. Habitat-wise summary statistics for physicochemical parameters

Parameter	Mean ± SD	χ^2
Rainwater pools		
Algae	304.5 ± 442.1*	108.62
N	9.70 ± 11.5	0.53
pH	7.36 ± 0.4	0.15
Turbidity	854.9 ± 1906.0*	2039.18
P	0.69 ± 0.7	28.47*
Ponds		
Algae	255*	281.89
N	0.65 ± 0.17	0.02
pH	7.5 ± 0.1	0.03
Turbidity	188.4 ± 177.4*	3843.63
P	0.40 ± 0.28	3.27
Tyre tracks		
Algae	420.5 ± 765.8*	145.71
N	1.73 ± 2.1	0.22
pH	7.16 ± 1.2	0.10
Turbidity	306.8± 535*	1741.33
P	0.30 ± 0.1	0.84
Borrow pits		
Algae	420.5 ± 765.8*	306.25
N	2.07 ± 2.3	0.55
pH	7.06 ± 1.40	0.16
Turbidity	647.1 ± 568.8*	1177.54
P	0.63 ± 0.3	3.28
Streambeds		
Algae	272 ± 694.7*	1401.17
N	1.30 ± 0.8	0
pH	7.40 ± 1.36	0.02
Turbidity	0.53 ± 0.38*	71.94
P	0.53 ± 0.38	0.67
Swamps		
Algae	53 ± 71.1	120.01
N	2.21 ± 2.02	1.72
pH	0.56 ± 0.36	0.08
Turbidity	7.8 ± 0.48	68.71
P	249.46 ± 274.03	2.01
Drainage canals		
Algae	389.45*	878.42
N	1.57 ± 1.56	1.24
pH	7.31 ± 1.2	0.08
Turbidity	636.13 ± 967.3*	1859.82
P	0.51 ± 0.34	111.09*

*Mean values are significant at $p< 0.05$; N — Nitrogen; P — Phosphorus.

Rainwater pool drainage had the highest water nitrogen concentration and ponds the lowest (Table 3). Drainage canals also had the highest phosphorus concentrations and streambeds the lowest. Overall, nutrient (N and P) concentrations were significantly different across all habitat types. Total nitrogen had a significant effect on larval den-

Table 4. Results of test statistics showing relationship between some key physicochemical co-variates and the larval densities of *An. gambiae* mosquitoes

Parameter	Test statistics	Correlation coefficient
Nitrogen	1.433*	0.897
Phosphorus	3.321	0.233
pH	–0.662*	0.721
Turbidity	0.421*	0.812
Algal count	0.331	0.021

*Values are significant at $p<0.05$.

sity ($p<0.01$) and was positively associated with larval density. Total phosphorus had no significant effect on larval productivity. Unlike nitrogen, however, a negative association was found between the concentration of this nutrient and larval density (Table 4). Overall mean water pH in habitats ranged from 7.06 to 7.8. Borrow pits water were the least alkaline water (7.06) and swamps the most alkaline (7.8). However, pH was not significantly different among the habitat types. Pond water was the most turbid (188.4) while streambed water was the least turbid (Table 4). Unlike pH, turbidity was significantly different among all the habitat types. Water pH and turbidity had significant effects on larval productivity and were negatively associated with larval density ($p<0.01$). However, pH was negatively associated with larval density and turbidity was positively associated with larval density ($p<0.01$).

DISCUSSION

The results of this study showed that rainwater pools were the most abundant and also the most productive habitat type. Total nitrogen, and water turbidity significantly affected larval production in mosquito habitats, the reverse was true for algal abundance, habitat size, total phosphorus and pH. Rainwater pools had significantly high concentrations of algae, phosphorus and dissolved solids suggesting that optimal presence of these biotic and abiotic factors may have played a role in their relative success in supporting *An. gambiae* larvae. The question as to how much of each of these factors is required to make them optimal to larvae of this species will only be answered through further investigations based under simulative laboratory conditions that involve precise manipulation of these nutrients. Rainwater pools are largely rain-fed and these were found to be the most frequent habitat type for the different mosquito larvae observed during the current study. That these were the most common and unstable habitat type suggests that larval population dynamics in the study area may take on a seasonal pattern with larval density peaks occurring during the rainy season. The findings of this study confirm those by other larval ecologists that have shown that *An. gambiae* complex mosquitoes prefer small temporary and shallow fresh water pools[3,4].

The role of habitat stability (the number of days that a habitat holds water) can best be seen in borrow pits and drainage canals, which were the second and third most productive habitat types, respectively. These habitats were most productive and also the most stable and could sustain larval production for several days after the rains had subsided. Burrow pits were found next to human dwellings, a fact that probably significantly reduces the flight distance between malaria causing mosquitoes and human dwellings. It may be possible that more larvae were collected from these habitats because gravid readily oviposited in them due to their closeness to human habitations. This then brings in the question on the roles of intrinsic factors within habitats which might support larval production against those influences that originate from outside habitats.

A majority of habitat types owed their existence either directly or indirectly to human activities and this included burrow pits, drainage canals, livestock hoof prints, and tyre tracks. Consequently, most of these habitats were highly clustered in dispersion pattern within the village landscape, corroborating the findings[5] from landscape analysis studies which established that 6 of 47 (0.09 km^2) cells superimposed over the village harbored 65% of all habits.

Algal abundance was an important factor in regulating adult production. This confirms previous findings[4] that showed algal densities significantly reduced in the presence of increasing numbers of larvae as measured by chlorophyll *a* in surface water samples and by counts of algae in sedimentation chambers, compared to situations in which larvae were absent. Algae as a group contain more essential nutrients, such as polyunsaturated fatty acids and sterols, necessary for mosquito development and adult emergence compared to bacteria. In addition, the absence of algae would limit bacterial growth since algal productivity and bacterial productivity usually tightly linked in freshwater systems[21].

The well being of plant life in mosquito habitats depends on total nitrogen concentrations for the purposes of synthesizing of compounds such as proteins and nucleic acids. Nitrogen is a necessary nutrient for the growth of aquatic plants and algae. Nitrogen- and phosphorus-based fertilizers such as urea act by attracting more gravid mosquitoes to lay eggs in the newly fertilized fields[23]. In the

current work, results indicated that the levels of nitrogen and phosphorus in different habitats studied were not affected by the presence of larvae although there was evidence for decreasing nitrogen levels with increasing larval densities suggesting that nitrogen may be a limiting resource in the larval environment. Earlier workers[24] have suggested that algal production and abundance of zooplankton and insects in small, turbid Kenyan ponds is limited by phosphorus (P) availability. Even when phosphorus was high, it may have been limiting because the ratio of nitrogen (N) to P was low. Thus, there exists evidence that either N or P or both are limiting to algal production, and therefore possibly for mosquito production. Residents in Kisian village rear livestock with most of the homes having some type of livestock—mainly cows, goats and sheeps. Livestock waste and runoff from perturbed areas may well-represent the most variable and important pulse source of N and P for the larval habitats. Cooper *et al*[25], for example, found that total P levels in Kenyan ponds were directly correlated with fecal input from animals and that total algal biomass was directly related to P levels. However, runoff from cattle waste is likely to have a lower N to P ration than direct input of the same waste[16] and thus the form and timing of animal waste inputs may be critical to nutrient dynamics in larval *An. gambiae* habitats. Anopheline larvae in general are strongly associated with algae in natural habitats. In some cases, the association appears to be one of larval refugia or attachment sites but studies have also shown the dominance of algae in larval diets and as a potential nutritional source[4,24–28].

CONCLUSION

The results of the study indicated that water nutrient content in larval habitats of *An. gambiae* probably have crucial, dual roles in the resource ecology of these mosquitoes. Because food limitation due to density-dependent processes likely occurs in larval habitats yielding adult females of variable sizes and nutrient reserves, this ultimately affects population dynamics and the vectorial capacity of this important mosquito species. Overall, the findings of this study support the notion that source reduction measures aimed at manipulating physicochemical variables in larval habitats to defeat larval production have a chance of succeeding in an integrated vector control program. The authors declare that they have no competing interests.

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