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Abundance of immature *Anopheles* and culicines (Diptera: Culicidae) in different water body types in the urban environment of Malindi, Kenya

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ABSTRACT: In this study we 1) describe the abundance of *Anopheles* and culicine immatures in different water body types in urban Malindi, Kenya, 2) compare *Anopheles* immature density in relation to culicine immature density, and 3) identify characteristics that influence the likelihood of water bodies being co-colonized by *Anopheles* and culicines. Entomological and environmental cross-sectional surveys conducted in 2001 and 2002 were used in the analysis. A total of 889 *Anopheles* and 7,217 culicine immatures were found in diverse water body types in 2001 and 2002. Car-track pools (n=45) and unused swimming pools (n=25) comprised 61% (70 of 115) of all water bodies found and served as the main habitats for *Anopheles* immatures. Of the 38 water bodies found containing *Anopheles* immature mosquitoes, 63% (24 of 38) were car-track pools and unused swimming pools. Culicine immatures utilized several water body types as habitats. We found that *Anopheles* and culicine immatures had higher density when occurring individually compared to when they occurred simultaneously. We determined that season, permanency, and water body area size influenced the likelihood of water bodies being simultaneously positive for *Anopheles* and culicines. Though *Anopheles* immatures were found in diverse water body types, their numbers were low compared to culicine immatures. The low density of *Anopheles* immatures suggests that *Anopheles* larval control is an achievable goal in Malindi. ***Journal of Vector Ecology* 33 (1): 107-116. 2008.**

Keyword Index: Anopheles gambiae s.l. immatures, culicine immatures, urban environment, Malindi, Kenya.

INTRODUCTION

In urban environments of Sub-Saharan Africa, ecological characterization of larval habitats for Afro-tropical mosquitoes is necessary to understand the dynamics by which various vector species interact and thrive in urban areas. Importantly, *Anopheles gambiae* Giles s.l. and culicines, in particular *Culex quinquefasciatus* Say, have been shown to occur together in larval habitats in rural environments (Fillinger et al. 2004, Gimnig et al. 2001, Minakawa et al. 1999). In urban environments, lower densities of larval habitats are found relative to rural environments (Robert et al. 2003, Walker and Lynch 2007); this presumably fosters greater interactions between *An. gambiae* s.l. and other mosquito species, although it is not clear to what extent this occurs in areas with high human population density. Though *Anopheles* and culicines have different requirements for their immature survival (Service 1996), their interaction may have implications for the ecology of *Anopheles* mosquitoes in urban environments. Studies in North America with mosquito species known to favor urban environments (e.g., *Aedes albopictus* Skuse, *Aedes aegypti* (L.), and *Culex pipiens* L.) have demonstrated that competitive interactions exist among these mosquitoes when reared together under laboratory and particular natural conditions (Braks et al. 2003, Costanzo et al. 2005, Juliano et al. 2004). These studies suggest that competitive interactions among these mosquitoes may have ecological and/or medical significance.

Mean crowding, which measures the density and distribution (Lloyd 1967) of organisms, influences the availability of resources such as nutrition and space among different organisms. Thus, crowded habitats with one mosquito species are likely to prevent other mosquito species from colonizing that habitat. Furthermore, different habitat characteristics impact the extent to which these mosquitoes encounter one another; hence, influencing interspecific species interactions.

The differences in oviposition site selection by adult mosquitoes and immature mosquito behavior within selected water bodies are influenced by several factors such as pollution, permanency, substrate type, shade, habitat size, and distance to city center. For example, while *An. gambiae* s.l. immatures are generally thought to occur in clean sunlit temporary habitats with natural substrates (i.e., mud, sand, or rock) (Gillett 1972, Service 1996), several studies in rural and urban environments have found *An. gambiae* s.l. in polluted water bodies (Chinery 1995, Chinery 1984, Coene 1993, Sattler et al. 2005), permanent water bodies characterized as being structurally simple (i.e., absent of vegetation and/or debris or only present at the margins) (Carlson et al. 2004), and water bodies that are of artificial substrate type (i.e., concrete, rubber, or plastic) (Fillinger et al. 2004). Shade also influences *An. gambiae* immatures, where shaded water bodies are less likely to have *An. gambiae* than habitats that are not shaded (Jacob et al. 2005, Kaufman et al. 2006, Munga et al. 2006). Habitat size has also been shown to have varying effects on *An. gambiae* s.l. (Jacob et al. 2005). Finally, *An. gambiae* s.l. immature and adult density have also been shown to increase as distance from the city center increases (Coene 1993, Keating et al. 2004, Robert et al. 1993, Trape and Zoulani 1987). This observation has been attributed to decreased open space, increased pollution, different economic activities, and improved socioeconomic conditions as you approach urban centers. In contrast, though culicines such as *Cx. quinquefasciatus* generally have high abundance in latrines, soakage pits, septic tanks, and cess-pits in urban environments (Chavasse et al. 1995), these mosquitoes are generally known to be more opportunistic in selection of oviposition sites and have a ubiquitous distribution of immatures in various geographical areas and water body types (Service 1996).

The objective of this study was to add to the knowledge base of *Anopheles* in urban environments by providing further descriptive entomological information on the interaction between immature *Anopheles* and culicine mosquitoes and the physical factors that influence this interaction. We describe the distribution of *Anopheles* and culicine mosquitoes in urban Malindi and characterize the level of co-abundance and occurrence of these mosquitoes. We test the hypothesis that the density of *Anopheles* and culicine immatures occurring alone in water bodies of urban Malindi is different from the density of *Anopheles* and culicine immatures occurring simultaneously. Furthermore, we test the hypothesis that the water body characteristics of pollution, shade, water body area size, permanency, substrate type, season, and distance to city center influence the likelihood of water bodies being simultaneously positive for *Anopheles* and culicine immatures.

MATERIALS AND METHODS

Study site

Malindi, Kenya, has been described previously (Keating et al. 2003, Keating et al. 2004, Macintyre et al. 2002). Malindi is a coastal town situated at 3°14'S latitude and 40°04'E longitude, 108 km north of Mombasa. Conditions in Malindi are semi-arid with mean daily minimum and maximum temperatures of 22° C and 30° C, respectively, and the average relative humidity at 65%. The long rains occur April to June and the short rains occur October to November: the annual rainfall varies between 75 and 1,200 mm throughout the year.

Sample frame development and study design

The sampling and study design used in this study has been described elsewhere (Keating et al. 2003, Keating et al. 2004, Macintyre et al. 2002). ArcView 3.2® (Environmental Systems Research Institute, Redlands, CA) was used to overlay a series of 270 × 270 m grid cells on base-maps of Malindi. The grid cell size corresponded to a 9 × 9 pixel LANDSAT Thematic Mapper remote-sensing satellite image. The grid cells served as sampling units. The individual grid cells were stratified based on planning and drainage criteria and randomly selected for inclusion. Cross-sectional entomological and environmental surveys were conducted within the selected grid cells.

Entomological sampling and environmental attributes

In April and May of 2001 (a characteristically wet period) (Keating et al. 2003) and November and December of 2002 (an un-characteristically dry period) (Keating et al. 2004), all accessible water bodies found by our field team were sampled within selected grid cells using walking inspections. This was done to avoid the bias of only sampling water bodies where mosquitoes were thought to be found. Water bodies were defined as an accumulation of water in a natural or artificial container (e.g., tire, tree hole, bucket, etc.) or impoundment (large concentration of water, e.g., swamp, pond, fountain). Hand-held global positioning system (GPS) navigational units were used to record the geographic coordinates of water bodies. A 350 ml dipper was used to collect immature mosquitoes (1st – 4th larval instar and pupal stages) at random locations from within large water bodies (i.e., larger than 13 cm in diameter × 8 cm deep). Water bodies that could not accommodate the 350 ml dipper or contained a low volume of water were sampled using a suction meat baster or 1 ml transfer pipette. In both methods, more dips were taken for larger water bodies. Samples from each respective water body were pooled in Whirl Pak® plastic bags and transported to the laboratory where they were sorted into different instars of either anopheline or culicine. Anopheline larvae were counted and recorded. Since this study emphasized anophelines in an urban environment, no effort was put into quantifying the relative abundance of the different culicine species. All 3rd and 4th instars of anopheline were immediately preserved in 95% ethanol and later identified morphologically to species by using taxonomic keys (Gillies and Coetzee 1987). All 1st and 2nd instars of *Anopheles* were reared in plastic pans under semi-field conditions, and those larvae that survived to the 3rd instar were preserved and identified morphologically. The pupae of anophelines and culicines were kept in mosquito cages (60 cm × 60 cm × 60 cm), and the adult mosquitoes that emerged were identified morphologically using taxonomic keys. A subset of *An. gambiae* s.l. larvae was further identified to their sibling species using Polymerase Chain Reaction (PCR) techniques (Scott et al. 1993).

Water body features such as pollution, shade, permanency, substrate type, water body area size, and season were also recorded at each water body. In this study, polluted water bodies were defined as water bodies that had sewage, garbage, oils, and/or other debris at the time of sampling. Shaded water bodies were defined as water bodies that were completely or partially shaded by any type of nearby foliage and/or urban structures. Permanency was determined by previous experience with similar water body types and source and abundance of water for each water body: temporary water bodies were water bodies that had a high likelihood of drying out because they contained a relatively small volume of water and did not have a constant source of water. Water body substrate type was defined as water bodies contained within sand, mud, or rock/gravel (i.e., natural substrates) or water bodies contained within rubber, concrete, or plastic (i.e., artificial substrates). The water body area size dichotomization of less than or equal to 3 m^2 or greater than 3 m^2 was used based on the distribution of the data, as half of the water bodies were less than 3 m^2 in size, with very little variation in values. The distance to the city center for each water body was calculated using ArcView 3.2^* (Keating et al. 2004).

Data analysis

Abundance of Afro-tropical mosquitoes

Only water bodies that were positive for the presence of mosquitoes were included in the analysis (n = 66). The sum, mean density (m_x), and standard deviation (SD) were calculated for the number of sampling dips taken and the immature density for *Anopheles* and culicines for each water body type. The index of dispersion (variance-to-mean ratio: $I_D = s^2/ m_x$) was used to examine the departure from a random distribution of mosquito immatures among the water bodies. We tested the null hypothesis that the index of dispersion for *Anopheles* or culicine in the different water bodies was equal to one (random distribution) (Ludwig and Reynolds 1988). Lloyd's (1967) measure of intraspecific mean crowding (m^*_x) per water body and the interspecific mean crowding per water body (m^*_{xy}) were also calculated to describe the aggregation pattern of the *Anopheles* or culicine sub-family in the different water body types in Malindi (Lloyd 1967). In this study, intraspecific mean crowding measures the average number of mosquitoes one individual mosquito encounters of the same complex (as in the case with *An. gambiae* s.l.) or sub-family (as in the case with culicine) within a water body type (Bradshaw 1983, Costanzo et al. 2005). Intraspecific mean crowding is given by $m^*_x = \Sigma x_i (x_i - 1)/ \Sigma x_i$, where x_i is the mosquito density of a particular sub-family in the i^{th} water body. In this study, interspecific mean crowding measures the average number of mosquitoes one individual mosquito of a sub-family encounters of a different sub-family within a water body type (Bradshaw 1983, Costanzo et al. 2005). Interspecific mean crowding is given by $m^*_{yx} = \Sigma(x_i y_i)/\Sigma x_i$, where x_i is the mosquito density of a particular sub-family in the i^{th} water body and y_i is the number of total competitors for mosquito subfamily x.

Relative abundance

The objective of this analysis was to determine the influence of co-occurring mosquito sub-families on the density of an individual sub-family. We tested whether there was a difference in mosquito density between water bodies that are colonized with *Anopheles* (n = 14 water bodies) or culicines (n = 28 water bodies) individually, vs water bodies that are co-colonized with both *Anopheles* and culicines (n = 24 water bodies). The Mann-Whitney U test (Glantz 2002) was used to test this difference.

Co-occurrence of Anopheles *and culicine mosquitoes*

The objective of this analysis was to determine which water body characteristics are associated with water bodies that are co-colonized with both *Anopheles* and culicines. We tested whether water body characteristics of pollution, shade, permanency, substrate type, water body area size, season, and distance to city center influence the likelihood of water bodies being simultaneously positive for *Anopheles* and culicine immatures.

Logistic regression was performed with the presence or absence of simultaneously positive *Anopheles* immatures in the water bodies as the outcome variable, where one was water bodies simultaneously positive for *Anopheles* and culicine immatures and zero was water bodies either colonized by *Anopheles* or culicine individually. The explanatory variables were pollution, shade, permanency, substrate type, water body area size, season, and distance to city center. All analyses were performed using SPSS 11.5 and Microsoft Excel 2000.

RESULTS

Mosquitoes

In the 2001 wet season, 28 grid cells were sampled, of which 25 were found to harbor water bodies. In the 2002 dry season, 50 grid cells were sampled, of which 17 were found to harbor water bodies. The distribution of water bodies included in this analysis is illustrated in Figure 1. A total of 115 water bodies were identified and sampled for immature mosquitoes (86 water bodies in the 2001 wet season and 29 water bodies in the 2002 dry season), of which 66 (57.4%) were positive for the presence of mosquito immatures and 49 (42.6%) water bodies had no mosquitoes. Of the water bodies containing mosquito immatures, 14 (21.2%) had *Anopheles* only, 28 (42.4%) had culicines only,

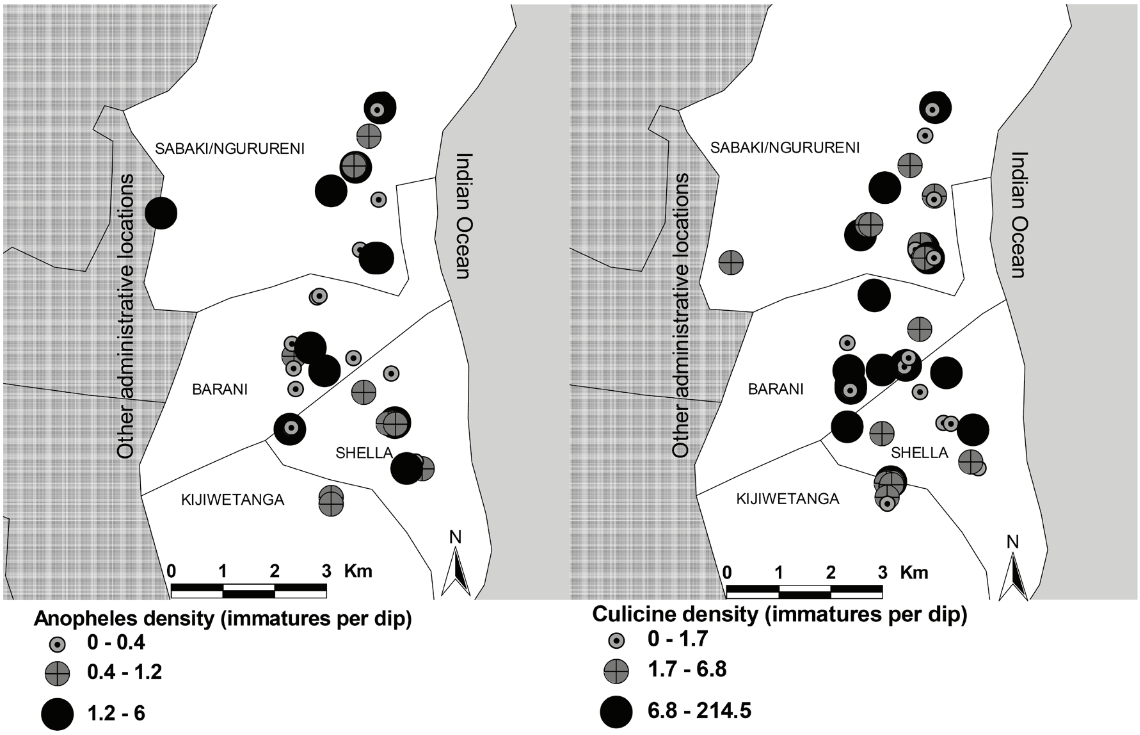

Figure 1. Density of immature mosquitoes in Malindi town.

and 24 (36.4%) had both *Anopheles* and culicines. Immature *Anopheles* mosquitoes were taxonomically identified to *An. gambiae* s.l. Of the 400 *An. gambiae* s.l immatures tested using PCR, 95.0% were *An. gambiae* Giles sensu stricto, 1.1% were *Anopheles arabiensis* Patton, 0.3% were *Anopheles merus* Dointz, and 3.6% were unidentifiable. Culicine mosquitoes were taxonomically identified to *Ae. aegypti*, *Cx. quinquefasciatus*, *Culex simpsoni* Theobald, *Culex decens* Theobald, and *Culex tigripes* DeGrandpré and DeCharmony. Though the proportions of the different culicine mosquito species were not recorded, since the emphasis of the study was anopheline immatures, *Cx. quinquefasciatus* was observed as being the most encountered culicine mosquito species. A total of 889 immature *Anopheles* and 7,217 immature culicine was collected in 2001 and 2002. Of these immature mosquitoes, eight *Anopheles* pupae and 558 culicine pupae were collected.

Proportion of water bodies

Table 1 summarizes the proportion of sampled water bodies positive for immature mosquitoes. The water bodies (n=115) identified in Malindi consisted of car-track pools, used swimming pools, water tanks, swamps, tires, ponds, drainage channels, septic tanks, and fish ponds. Other types of water bodies identified were a bathtub, a bucket, a ditch, a flower garden, a stream pool, a water trough, and a well. These water body types were only encountered once; therefore, they were pooled to make up the category of miscellaneous water body types (MWBT). Car-track pools (n = 45) and unused swimming pools (n = 25) were the most abundant water bodies found, making up 61% (70 of 115) of all water bodies (Table 1). Immature mosquitoes were found in 57% (66 of 115) of water bodies sampled in Malindi in 2001 and 2002. *Anopheles* immatures were found mostly in car-track pools and swimming pools, comprising 63% (24 of 38) of water bodies with *Anopheles*. Immature culicine mosquitoes were found in a wide array of water bodies. All sampled tires, ponds, drainage channels septic tanks, and MWBT were found to be colonized by either sub-family of immature mosquitoes. Septic tanks were the only water bodies exclusively colonized by culicine mosquitoes. Of the three fish ponds identified and sampled, neither *Anopheles* nor culicine immatures was found. More car-track pools, unused swimming pools, and swamps were colonized by *Anopheles* compared to culicines; in all other water body types with the exception of fish ponds, culicines colonized more water body types compared to *Anopheles*. Significant differences in the water body types that were positive for immature mosquitoes (i.e., anopheline and/or culicine) were found among different water body types ($\chi^2 = 71.671$, df = 27, p <0.001).

Abundance of mosquitoes in sampled water bodies

Table 2 summarizes the abundance of each mosquito sub-family in the water bodies. Unused swimming pools, car-track pools, MWBT and swamps had the highest density of immature *Anopheles*. All other water body types had very low densities of *Anopheles* immature mosquitoes. Drainage channels and septic tanks had very high densities of culicine immature mosquitoes. In each water body type, densities of culicine immature mosquitoes were much higher than densities of *Anopheles* mosquitoes. For *Anopheles* immatures, the index of dispersion (variance-to-mean ratio) was significantly greater than unity for all

Table 1. Proportion of water bodies with and without immature mosquitoes in urban Malindi, Kenya.

		Water body types									
Water bodies with immature mosquitoes [a]	Total	Car-track pool	Unused Swimming pool	MWBT*	Water tank	Swamp	Tire	Pond	Drainage channel	Septic tank	Fish pond
	N = 115	n = 45	n = 25	n = 8	n = 8	n = 7	n = 6	n = 5	n = 4	n = 4	n = 3
Anopheles positive [b]	0.33 (38)	0.31 (14)	0.40 (10)	0.63 (5)	0.25 (2)	0.43 (3)	0.17 (1)	0.40 (2)	0.25 (1)	0.00 (0)	0.00 (0)
Culicine positive [c]	0.45 (52)	0.24 (11)	0.28 (7)	0.88 (7)	0.75 (6)	0.29 (2)	1.00 (6)	1.00 (5)	1.00 (4)	1.00 (4)	0.00 (0)
Both sub-families present	0.21 (24)	0.16 (7)	0.24 (6)	0.50 (4)	0.13 (1)	0.29 (2)	0.17 (1)	0.40 (2)	0.25 (1)	0.00 (0)	0.00 (0)
Anopheles present only	0.12 (14)	0.16 (7)	0.16 (4)	0.13 (1)	0.13 (1)	0.14 (1)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
Culicine present only	0.24 (28)	0.09 (4)	0.04 (1)	0.38 (3)	0.63 (5)	0.00 (0)	0.83 (5)	0.60 (3)	0.75 (3)	1.00 (4)	0.00 (0)
Neither sub-family present	0.43 (49)	0.60 (27)	0.56 (14)	0.00 (0)	0.13 (1)	0.57 (4)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	1.00 (3)

*Miscellaneous water body types (MWBT) = water bodies where only one of that type were found in urban Malindi, Kenya (1 bathtub,1 bucket, 1 ditch, 1 flower garden, 1 fountain,1 stream pool, 1 water trough, 1 well).

[a] Values in parentheses are frequency of occurrence of mosquito species in all water bodies sampled of a given type.

[b] Represent the sum of water bodies with *Anopheles* occurring with or without culicines.

[c] Represent the sum of water bodies with culicines occurring with or without *Anopheles*.

Table 2. Abundance of immature mosquitoes in water body types that are positive for mosquitoes.

Water body type	Sum of sampling dips	Sub-family	Total	Mean density m_x ± SD	Per dip	Index of dispersion s^2/m_x	Intraspecific mean crowding m^*_x	Interspecific mean crowding Σm^*_{yx}
Car-track pools (n=18)	375	An	306	17.00 ± 18.63	0.82	20.41 [§]	35.27 (1.69)	24.88 (1.19)
		Cu	621	34.50 ± 52.52	1.66	185.05 [§]	208.27 (10.00)	12.26 (0.59)
Unused swimming pools (n=11)	313	An	358	32.54 ± 48.26	1.14	71.56 [§]	96.60 (3.40)	75.12 (2.64)
		Cu	407	37.00 ± 103.19	1.30	287.82 [§]	297.65 (10.46)	66.08 (2.32)
MWBT* (n=8)	142	An	133	16.62 ± 17.72	0.94	18.89 [§]	32.15 (1.81)	101.93 (5.74)
		Cu	612	76.50 ± 100.24	4.31	131.35 [‡]	190.43 (10.73)	22.15 (1.25)
Water tanks (n=7)	73	An	16	2.29 ± 5.22	0.22	11.92 [§]	11.50 (1.10)	9.13 (0.88)
		Cu	448	64.00 ± 56.44	6.14	49.78 [§]	105.67 (10.13)	0.33 (0.03)
Tires (n=6)	93	An	2	0.33 ± 0.82	0.02	2.00	1.00 (0.06)	60.00 (3.87)
		Cu	529	88.17 ± 92.75	5.69	95.58 [§]	168.48 (10.87)	0.23 (0.01)
Ponds (n=5)	65	An	7	1.40 ± 1.95	0.11	2.71 [†]	2.57 (0.20)	70.86 (5.45)
		Cu	296	59.20 ± 63.81	4.55	68.78 [§]	113.22 (8.71)	1.68 (0.13)
Drainage channels (n=4)	73	An	4	1.00 ± 2.00	0.05	4.00 [‡]	3.00 (0.16)	715.00 (39.18)
		Cu	2,528	632.00 ± 769.31	34.63	936.45 [§]	1,333.34 (73.06)	1.13 (0.06)
Septic tanks (n=4)	36	An	0	0.00 ± 0.00	0.00	0.00	0.00 (0.00)	0.00 (0.00)
		Cu	1,728	432.00 ± 133.65	48.00	41.35 [§]	462.01 (51.33)	0.00 (0.00)
Swamps (n=3)	121	An	63	21.00 ± 15.10	0.52	10.86 [‡]	27.24 (0.68)	18.19 (0.45)
		Cu	48	16.00 ± 17.69	0.40	19.56 [§]	28.04 (0.70)	23.88 (0.59)
Total (n=66)	1291	An	889	13.47 ± 24.96	0.69	51.75 [†]	58.03 (2.97)	59.43 (3.04)
		Cu	7,217	109.35 ± 245.75	5.59	595.67 [‡]	652.26 (33.35)	7.32 (0.37)

(n=66 water bodies positive mosquito immatures); An = *Anopheles*, Cu = culicine; per dip = mean immature per sampling dip.

*Miscellaneous water body types (MWBT) = water bodies where only one of that classification type were found in urban Malindi, Kenya (1 bathtub,1 bucket, 1 ditch, 1 flower garden, 1 fountain,1 stream pool, 1 water trough, 1 well).

Values in parentheses are mean crowdings by the average number of sampling dips for comparison between water body types.

[†] p<0.05, [‡] p<0.01, [§] p<0.001 (s^2/m_x is significantly greater than unity-aggregated distribution).

water bodies except tires and septic tanks. For culicines, the index of dispersion was significantly greater than unity for all water body types with culicine immatures.

Only eight *Anopheles* pupae were collected during the study, all during the 2001 wet season. The *Anopheles* pupae collected were found in car-track pools, swimming pools, a well, and a flower garden. For the combined survey years, 558 pupae were identified: 480 pupae in the 2001 wet season and 78 pupae in the 2002 dry season. Culicine pupae were collected from all water bodies with the exception of fish ponds. Drainage channels and septic tanks had the highest abundance of culicine pupae.

Intra- and interspecific mean crowding of *Anopheles* and culicines

For *Anopheles*, intraspecific mean crowding was greater than interspecific mean crowding in car-track pools, swimming pools, swamps, and water tanks (Table 2). For culicines, intraspecific mean crowding was greater than interspecific mean crowding in all water body types (Table 2). Intraspecific mean crowding for culicines was high in drainage channels and septic tanks; MWBT, swimming pools water tanks, car-track pools, and ponds followed, respectively. Intraspecific mean crowding for anophelines was highest for swimming pools, MWBT, car-track pools,

Table 3. Summary of multivariable logistic regression testing the likelihood that different water body characteristics influence water bodies simultaneously positive for *Anopheles* and culicine immatures* in urban Malindi, Kenya.

Water body characteristics	Adjusted Odds ratio (OR)	95.0% C.I.	
		Lower	Upper
Pollution [a]			
Polluted	0.85	0.19	3.85
Non-polluted	1		
Shade [b]			
Shaded	0.89	0.21	3.79
Non-shaded	1		
Permanency [c]			
Temporary	0.05 ‡	0.01	0.41
Non-temporary	1		
Substrate type [d]			
Artificial	0.51	0.09	2.76
Natural	1		
Water body area size			
> 3 m^2	12.09 ‡	2.06	70.74
≤ 3 m^2	1		
Season			
Dry season (2002)	0.05 †	0.01	0.50
Wet season (2001)	1		
Distance to city center [e]	1.00	1.00	1.00

* The outcome variable in this analysis is water bodies simultaneously positive for *Anopheles* and culicine immatures; n = 66 water bodies positive for mosquito immatures.
1 = Reference group.
[a] Polluted water bodies = water bodies that had sewage, garbage, oil and/or other debris.
[b] Shaded water bodies = water bodies that were partially or fully shaded by nearby foliage and/or other urban structures.
[c] Temporary water bodies = water bodies that had a high likelihood of drying out because they contained within a relatively small volume of water and did not have a constant source of water.
[d] Natural substrate type = water bodies contained within sand, mud, or rock/gravel; Artificial substrate type = water bodies contained within rubber, concrete, or plastic.
[e] Continuous variable.
† $p < 0.05$; ‡ $p < 0.01$.

and water tanks (Table 2). High values of interspecific mean crowding for *Anopheles* were seen in drainage channels, MWBT, ponds, tires, and swimming pools. Culicines had lower interspecific mean crowding values.

Co-abundance of immature *Anopheles* and culicine

Mann-Whitney U test revealed that the density of *Anopheles* immatures was less when they co-occurred with culicine immatures compared to when they occurred without culicine immatures (Z= -2.966, p=0.003); similar results were found for the density of culicine immatures in relation to *Anopheles* immatures (Z= -3.012, p=0.003).

Co-occurrence of immature *Anopheles* and culicine

Results of logistic regression are reported in Table 3. Water bodies that were greater than 3 m^2 were 12 times (OR = 12.09, 95% CI: 2.06 – 70.74) more likely to be simultaneously positive for both *Anopheles* and culicines immatures compared to water bodies less than 3 m$^{2.}$ Water bodies sampled during the 2002 dry season were less likely to have both sub-families co-occurring compared to the 2001 wet season (OR = 0.05, 95% CI: 0.01 – 0.50). Also, temporary pools (OR = 0.05, 95% CI: 0.01 – 0.41) were less likely than non-temporary pools to be simultaneously colonized by *Anopheles* and culicine immatures. Preliminary data analysis did show cross-correlation among some of the independent variables, however; diagnostic tests evaluating multi-collinearity suggested that this was not a major problem in the analysis (data not shown).

DISCUSSION

Since car-track pools and swimming pools contained all immature life stages, it is likely that these water body types serve as productive habitats for *Anopheles* immatures in Malindi. *Anopheles* were also found in water bodies, such as tires, drainage channels, and water tanks. This is contrary to where one would expect to find these mosquitoes, suggesting that *An. gambiae* s.l. mosquitoes are capable of colonizing urban water bodies fundamentally different than those observed in rural environments. It has been demonstrated that *An. gambiae* Giles s.s. females use multiple breeding sites for oviposition (Chen et al. 2006) to establish their progeny. In urban environments, this behavior may facilitate the establishment of *Anopheles* in diverse water body types. However, within atypical water body types, *Anopheles* densities were low and 4th instar larvae and pupae were absent. These water bodies may be nothing more than "ecological sinks" where there is an input of mosquito eggs that develop to early stage larvae, but due to other factors never undergo full development.

Anopheles and culicine immatures were found to have an aggregated distribution within the different water body types. This non-random distribution suggests that water body dynamics differ among habitats, suggesting that the interaction of factors such as nutrition, habitat dehydration, physical features, and social interactions influence the distribution and aggregation patterns of mosquito habitats. Due to the small sample size (n =115 water bodies), we did not account for the aggregated distribution of mosquitoes within the different water body types. For that reason, more detailed studies are needed in Malindi to further describe the ecology of *Anopheles* in urban environments.

We also observed lower intraspecific mean crowding for *Anopheles* immatures compared to culicines of the same water body type. This is consistent with other urban studies that show low densities of *Anopheles* immature densities but high culicine immature densities (Robert et al. 1998). The impact of crowded habitats has also been shown to influence some aspects of *An. gambiae* fitness. Male mosquitoes reared under less crowded conditions were 11 times more likely to mate first with con-specific females than those reared under high crowded conditions (Ng'habi et al. 2005). The relatively low crowding in Malindi may make *Anopheles* mosquitoes more fit than their rural siblings, although other factors are also known to adversely impact fitness (Trape and Zoulani 1987). The low crowding of mosquitoes would also tend to reduce intraspecific cannibalism, which has been observed in *Anopheles* mosquitoes (Koenraadt and Takken 2003, Schneider et al. 2000), although cannibalism by other species of mosquitoes may still occur (Haddow 1942, Jackson 1953, Jin et al. 2006). We also demonstrated that the density of *Anopheles* and culicines in the presence of their sub-family competitor was lower compared to when each sub-family was found alone. These results seem to correspond with studies in western Kenya, which show that female *Anopheles gambiae* s.l. laid fewer eggs where culicine eggs were present (Sumba et al. 2004).

Interestingly, while *Anopheles* immatures tend to exploit rain dependent habitats, culicines mostly occupy habitats resulting from drainage and sewerage systems, although our study shows that these mosquitoes can occur together. We found that season, water body area size, and permanency influence the likelihood of these water bodies to be simultaneously positive for *Anopheles* and culicines. These results are consistent with other mosquito studies (Costanzo et al. 2005, Sota et al. 1994). Though some of the independent variables tested in the logistic regression analysis were correlated, we found no presence of multicollinearity when we conducted diagnostic tests (data not shown); this suggests that this was not a major factor in influencing the regression results (Klienbaum et al. 1998). The close association of season, water body area size, and permanency with water stability (i.e., the probability of the occurrence of water in a habitat) (Minakawa et al. 2005, Sota et al. 1994) may also suggest that the co-occurrence and abundance of *Anopheles* and culicine are strongly regulated by the stability of the water source.

Though the low abundance of *Anopheles* pupae (n=8) in this study suggests that water bodies in urban Malindi are not very productive for *Anopheles*, the low sample size and the cross-sectional study design prevents us from determining this with certainty. However, urban spacing, pollution, and human activity have been suggested as the main factors limiting all life stages of *Anopheles* in urban environments (Coene 1993, Keating et al. 2004, Robert et al. 1993, Robert et al. 2003, Trape and Zoulani 1987); therefore it is likely that emergence rates of *Anopheles* in Malindi are significantly reduced by these factors as well. Nonetheless, emergence of *Anopheles* from urban water bodies in Malindi should be investigated to determine the extent of entomological risk that *Anopheles* mosquitoes pose to Malindi residents. Though data on adult mosquitoes are not presented here, this component will be reported in a separate paper.

Several limitations in this study include a lack of taxonomic detail for the culicine mosquitoes, the low abundance of water bodies sampled, the cross-sectional nature of the study design, failure to obtain permission to investigate private property when the owner was not home or refused to participate in the study, the lack of detail on the nutritional status of the various water body types, and the low abundance of *Anopheles* immatures, in particular pupal stages. Because of these limitations, this study should be interpreted cautiously. However, this study still provides some insight into the factors influencing co-occurrence and abundance of *Anopheles* and culicine mosquitoes, such as the productivity of swimming pools, the association between water bodies with *Anopheles* and culicine immatures, and the factors regulating their association. These results should be used to guide laboratory and field studies that further characterize the occurrence and abundance of *Anopheles* and culicines in urban areas.

Given the diversity and heterogeneity of larval habitats in urban environments, longitudinal investigations involving systematic entomological and environmental cross-sectional surveys over a larger temporal and geographical range would be useful to establish a baseline for survival, fitness, rate of development, and emergence of *Anopheles* immatures in different water body types in urban environments. For example, investigations into spatial, temporal, micro- and macro-climatic, landscape, trophic, hydrological, and eco-social (i.e., interaction of human social patterns with natural ecology) dynamics of water bodies in urban environments would serve to create criteria for selecting water bodies and geographical locations that pose the highest risk for harboring and propagating emerging mosquito populations with high vectorial capacity. Based on detailed longitudinal studies, appropriate approaches and stakeholders can be selected to address various problems. For instance, since swimming pools seem to be a significant source of potential malaria vectors, a city ordinance could be passed requiring owners to cover, drain, clean, or treat unused swimming pools to prevent mosquito proliferation. In contrast, car-track pools as habitats could be mitigated by municipal engineers through road construction and maintenance.

The co-occurrence of *Anopheles* and culicines is regulated by variables that are closely related to water stability. Yet, decreased density is observed when *Anopheles* and culicine immatures co-occur. This may suggest competitive interactions; however, several other factors must be considered. Nonetheless, the occurrence of *An. gambiae* s.l. in atypical habitats in urban environments, albeit in low density, does raise questions on the extent to which urban water bodies are producing fit adult *Anopheles* mosquitoes in urban Malindi. The degree to which *An. gambiae* s.l. are either physiologically adapting to the urban environment, behaviorally adapting to the urban environment, or are just finding suitable habitats is yet to be determined and beyond the focus of this study; however, urban mosquito control programs need to conduct routine and comprehensive entomological surveillance that examines all potential water bodies so that the adaptability of *An. gambiae* s.l. is not taken for granted.

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