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Oviposition Site Preference and Egg Hatchability of *Anopheles gambiae*: Effects of Land Cover Types

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ABSTRACT We studied the oviposition site preference and egg hatchability of *Anopheles gambiae* Giles with water collected from farmlands, forests, and natural wetlands. Water types significantly affected oviposition preference. Mosquitoes deposited significantly more eggs in rainwater in both the dry and wet seasons than waters from forests and wetlands, suggesting that *An. gambiae* prefers water with few impurities for oviposition. In the dry season, *An. gambiae* females also deposited significantly more eggs in waters from farmlands than those from forests and natural wetlands, but these differences were not statistically significant during the wet season. In both indoor and natural conditions, egg mortality in natural wetland habitats was significantly higher than in farmland habitats. The average water temperature in natural wetland habitats was significantly lower than farmland habitats in the natural conditions, but it remained the same under indoor experimental conditions, suggesting that factors other than water temperature play an important role in egg hatchability. Together with the findings from previous studies on the effects of land cover on larval survivorship, our results support the hypothesis that variations in habitat conditions induced by different land cover types contribute to the heterogeneous spatial distribution of *An. gambiae* larvae in the western Kenya highland.

KEY WORDS *Anopheles gambiae*, oviposition, land use, egg hatchability, malaria

LARVAE OF *Anopheles gambiae* Giles frequently occur in small and open aquatic habitats (Gillies and De Meillon 1968, Charlwood and Edoh 1996, Minakawa et al. 1999, Gimmig et al. 2001, Minakawa et al. 2002). In the lowland areas of western Kenya where malaria is endemic, the distribution of *An. gambiae* larval habitats exhibits a high spatial heterogeneity (Gimmig et al. 2001, Minakawa et al. 2002). In the western Kenya highlands where epidemic malaria transmission has frequently occurred for the past 15 yr, *An. gambiae* larvae are mainly distributed in temporary habitats such as cow footprints in the wetland margins without tall vegetation and in cultivated areas where forests had been recently cleared (Minakawa et al. 2004, 2005b). It has been suspected that land use change increases habitat availability for the malaria vector and consequently contributes to malaria epidemics in the east African highlands (Carter et al. 2000, Patz et al. 2000).

It has been suggested that the main reason that *An. gambiae* larvae are mostly found in open and temporary aquatic habitats is that the mortality of *An. gambiae* larvae is lower in these habitats than in large and shaded habitats (Minakawa et al. 2004, 2005a). Larval

predation is less prevalent in temporary habitats than in large, permanent habitats (Service 1977, Washburn 1995, Sunahara et al. 2002). *An. gambiae* exploits the increased resources in warmer, open habitats that tend to produce more algae, the main food source for *An. gambiae*, than shaded habitats (Merritt et al. 1992, Gimmig et al. 2002). The warmer temperatures encountered in open habitats also shorten larval-to-pupal developmental time and consequently reduce larval mortality associated with desiccation and predation (Gillies and De Meillon 1968, Gimmig et al. 2002, Bayoh and Lindsay 2004, Tuno et al. 2005). If mortality of embryos is also lower in small, open habitats, by selecting such habitats for oviposition, *An. gambiae* can maximize the growth and survivorship of offspring (Blaustein et al. 2004).

In this study, we tested two hypotheses related to the heterogeneous distribution of *An. gambiae* larvae in the field. The first hypothesis is that gravid mosquitoes select oviposition sites based on habitat conditions, and the second is that habitat conditions affect mortality of embryos (i.e., egg hatchability). In particular, we were interested in water conditions associated with different land cover types such as farmlands, forests, and natural wetlands. Elucidating the cues that govern oviposition behavior may provide a tool for behavioral manipulation of populations of the malaria vectors in the field and help to develop novel control techniques (Navarro et al. 2003).

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Materials and Methods

Mosquito Colony. A colony was established from 200 *An. gambiae* adults that were collected from 20 randomly selected houses in Iguhu (34° 45' E and 0° 10' N; 1,430–1,580 m above sea level), Kakamega District, western Kenya highland in February (dry period) 2003. The mosquito colony was maintained in an insectary of the Kenya Medical Research Institute, Kisumu, Kenya, with a minimum population size of 4,000 individuals per generation.

Study Site. The study was performed in a 4 by 4-km area in Iguhu. The average minimum and maximum temperatures during 1970–2000 were 13.8 and 28.0°C with the hottest season in January–February and the coolest season in July–August. The average annual rainfall is ≈1,950 mm. The Yala River transects the study site and most of the mosquito breeding takes place in aquatic habitats in farmlands and pastures in the valley (Minakawa et al. 2005b). The study area includes a mosaic of land use types. The hill is mostly maize plots dotted by patches of tea plantation, whereas several wetlands are located along the Yala River valley. A natural forest is located in the east side of the 4 by 4-km² study area, constituting ≈15% of the total area.

Oviposition Substrate Preference. Twenty *An. gambiae* mosquitoes that had fed on rabbit blood were kept in a cage (30 by 30 by 30 cm) and provided with four different substrates for oviposition 36 h after blood feeding. The mosquitoes were provided with 2% sugar solution after blood feeding. Four types of substrates were waters from randomly selected 20 aquatic habitats located in farmlands, and 10 field habitats in forests and wetlands in Iguhu, and rainwater. The waters were collected on the same day that the oviposition experiment was conducted. Rainwater, collected 1–4 d before the experiment, was used as control. Rainwater was stored in a black plastic container in the laboratory until the beginning of the experiment. Farmlands were characterized by the presence of agricultural crops such as maize, *Zea mays* L., or bare ground that had been prepared for planting crops. Aquatic habitats in the farmlands were mainly ditches and temporary pools forming in depressions created by anthropogenic activities. Wetlands were characterized by standing water and the presence of emergent aquatic plants such as *Cyperus papyrus* L. Forests were mainly composed of native tree species such as *Ficus lutea* Vahl and *Polyscias fulva* (Hiern). Forests and wetlands were the areas with the least anthropogenic modification.

Each (100 ml) of four different test waters was placed in a separate 300-ml transparent plastic cup (the mouth of the cup was 6.4 cm in diameter). A piece of white filter paper (9 cm in diameter) was placed tightly in an each cup by folding the paper edge upward, because the filter paper was larger than the mouth of the cup. In this way, all eggs were deposited on the filter paper. The four cups were placed randomly at the corners of the cage with 20 mosquitoes. The experiment was set up at 1,800 hours, and eggs

deposited in each oviposition substrate were counted under the microscope at 1,000 hours the next day. In total, 78 replicates were conducted during the period between 23 March and 12 April 2004 (dry period) and 48 replicates during the period between 11 August and 17 August 2004 (wet period). This experiment was conducted in a room with windows at the altitude of 1,500 m in Iguhu. There were no controls for lighting, humidity and temperature. A Hobo eight data logger (Onset computers, Bourne, MA) was used to measure the relative humidity and air temperature of the experimental room.

Egg Hatchability. Experiments were conducted indoor and outdoor to examine the hatchability of *An. gambiae* eggs. In the indoor experiment, we tested the effect of water collected from farmlands, forests, and natural wetlands on hatchability of *An. gambiae* eggs in the same room where oviposition preference was tested. Eggs used in this experiment were obtained from the insectary-reared mosquitoes. Twenty eggs (0–4 h old) were introduced into a 300-ml plastic cup that contained 100 ml of water from each of the three different land cover types (farmlands, forests, and wetlands) and rainwater. The eggs (80 eggs in total) were randomly selected from a batch of eggs oviposited by multiple females. A strip of white paper (2 cm in width and 15 cm in length) was attached to the inside wall of the cup at the water surface. We kept the upper half (1 cm) of the paper above water. Because the paper absorbed water, eggs did not adhere to the wall and dry up. All cups were placed in a washbasin containing water to keep their water temperatures constant. Larvae that hatched were counted twice a day at 0900 and 1600 hours and removed until no further larvae were seen. Egg hatchability was calculated as the proportion of eggs that hatched from the introduced 20 eggs. The experiment was replicated 30 times in February 2005.

A similar experiment for egg hatchability was conducted in field (outdoor). We added 20 eggs of *An. gambiae* to 100 ml of rainwater in a 300-ml plastic cup. The cups were placed in randomly selected aquatic habitats within each land cover type (farmlands, forests, and wetlands) in February 2005. Hatched larvae were counted twice a day at 0900 and 1600 hours and removed daily until no further instars were seen. A stowaway tidbit data logger (Onset Computers) was placed in an aquatic pool under each land cover type to monitor water temperature. The experiment had 30 replicates.

Statistical Analyses. We used two-way analysis of variance (ANOVA) to determine whether the different water sources and season had a significant effect on the preference for oviposition substrates. When the effects of the factors were significant, the differences in number of eggs deposited among the water sources and between seasons were tested using Tukey–Kramer honest significant difference (honestly significant difference, HSD) multiple comparison tests. One-way ANOVA was used to compare numbers of hatched eggs in water collected from the different water sources (indoor experiments) and under the

Table 1. Oviposition substrate preference for waters from farmland, natural wetland, and forest habitats

	Rainwater	Farmland	Forest	Wetland
Dry season				
Avg no. of eggs	186.9 ± 21.5a	145.3 ± 17.2a	48.2 ± 6.1b	70.6 ± 8.6b
Proportion of eggs in each substrate (%)	41.4	32.2	10.7	15.7
Wet season				
Avg no. of eggs	138.7 ± 15.5a	66.9 ± 10.0b	50.2 ± 7.6b	52.9 ± 6.9b
Proportion of eggs in each substrate (%)	44.9	21.7	16.3	17.1

Tukey–Kramer HSD test used for multiple comparisons of the eggs hatched; levels connected by the same letter are not significantly different. The number of replicates was 78 in the dry season experiment and 48 in the wet season experiment.

different land cover types (outdoor experiments). We also used one-way ANOVA to compare the mean number of days taken for eggs to hatch in the indoor and outdoor experiments. If the effect was significant, Tukey–Kramer HSD multiple comparison test was performed. ANOVA with repeated measures was performed to determine the effects of seasons on room temperature for the oviposition substrate preference experiment, and the effects of land covers on water temperature for the egg hatchability experiment.

Results

Oviposition Substrate Preference. Two-way ANOVA revealed that water source ($F = 33.06$; $df = 3, 472$; $P < 0.001$) and season ($F = 15.88$; $df = 3, 472$; $P < 0.001$) significantly influenced the number of eggs deposited. The interaction between water source and season was also significant ($F = 3.88$; $df = 3, 472$; $P = 0.009$). During the dry season, 41.4 and 32.2% of the eggs were deposited in rainwater and farmland water, respectively (Table 1). The results of Tukey–Kramer HSD tests indicated that the numbers of eggs deposited in rainwater and water from farmlands were significantly greater than those in water from forests and natural wetlands. In contrast, during the wet season, 44.9% of the total numbers of eggs were deposited in rainwater, and the number was significantly greater than those in water collected from farmlands, forests, or natural wetlands (Table 1). There were no significant differences in number of eggs among the three land cover types during the wet season.

Overall, the average number of eggs deposited per experiment during the wet season (112.8 ± 8.4 eggs,

mean ± SE) was significantly greater than during the dry season (77.2 ± 5.6). Average room temperatures during the dry season were significantly greater than during the wet season (23.4 versus 21.1°C). Similarly, average daily minimum and maximum room temperatures in the dry season were also higher than in the wet season (26.9 versus 24.8°C for maximum temperature and 20.7 versus 17.8°C for minimum temperature). However, average daily relative humidity during the dry season was significantly lower than during the wet season (63.8 versus 76.8%).

Egg Hatchability. In indoor environment, water source significantly affected egg hatchability in ($F = 6.94$; $df = 3, 119$; $P < 0.001$). In particular, significantly more eggs hatched in the water collected from farmlands and forests than natural wetlands (Table 2). The eggs placed in water from the forests took significantly longer period to hatch than in water from farmlands ($F = 2.98$; $df = 3, 119$; $P = 0.030$).

In the natural condition (outdoor experiment), the effect of land cover type on egg hatchability was marginally insignificant ($F = 3.04$; $df = 2, 89$; $P = 0.053$). The mean number of eggs hatched in farmland habitats was significantly more than that in habitats of natural wetland, but hatching rates were not significantly different between habitats in the farmlands and the forests (Table 2). The mean number of days taken for eggs to hatch was not significantly different among the land cover types ($F = 1.33$; $df = 2, 89$; $P = 0.27$; Table 2). Land cover type also had a significant effect on maximum water temperature ($F = 1,113.5$; $df = 2, 89$; $P < 0.001$) and mean water temperature ($F = 78.3$; $df = 2, 89$; $P < 0.001$) but not on the minimum water temperature ($F = 1.1$; $df = 2, 89$; $P = 0.23$). In par-

Table 2. Results of indoor and outdoor egg hatchability experiments

	Rainwater	Farmland	Forest	Wetland
Indoor exp				
Avg no. of eggs hatched	11.57 ± 0.64ab	13.06 ± 0.83a	14.43 ± 1.03a	9.30 ± 0.79b
Hatchability (%)	57.8	65.3	72.1	46.5
Mean no. of days taken for eggs to hatch	2.97 ± 0.13ab	2.64 ± 0.09b	3.07 ± 0.15a	2.73 ± 0.09ab
Outdoor exp				
Avg no. of eggs hatched		9.50 ± 1.04a	7.87 ± 0.45ab	6.70 ± 0.82b
Hatchability (%)		47.5	39.3	33.5
Mean no. of days taken for eggs to hatch		3.63 ± 0.31a	3.80 ± 0.16a	4.16 ± 0.22a
Avg max daily water temp (°C)		38.8 ± 0.26a	23.3 ± 0.34b	24.4 ± 0.24c
Avg min daily water temp (°C)		12.6 ± 1.22a	12.5 ± 0.59a	11.5 ± 0.53a
Mean daily water temp (°C)		23.0 ± 0.78a	17.7 ± 0.38b	17.2 ± 0.34b

Tukey–Kramer HSD test used for multiple comparisons of the eggs hatched; levels connected by the same letter are not significantly different. The number of replicates was 30 in both experiments.

ticular, maximum and mean water temperatures were significantly higher in farmland than in the other land types, and the lowest water temperature was observed in natural wetland habitats (Table 2).

Discussion

In this study, we demonstrated that water conditions significantly affected oviposition substrate preference of *An. gambiae* in western Kenya highland. Mosquitoes deposited significantly more eggs in rainwater in the both dry and wet seasons than waters from forests and natural wetlands, suggesting that *An. gambiae* prefers water with few impurities for oviposition. We also observed that in the dry season, *An. gambiae* females deposited significantly more eggs in waters from farmlands than those from forests and natural wetlands. When we collected water from ditches in farmlands in the dry season, the water was mostly clear, because farmers had drawn clear water from streams to ditches. Although more eggs also were found in water from the farmlands than from the forests and natural wetlands in the wet season, the difference was not statistically significant. The sample size was smaller in the study of the wet season than in that of the dry season, and the sample size was probably too small to detect the difference statistically.

Although the cleanliness of the water may be the explanation for the observed differences, it is not necessary the cause. A host of other physical or chemical factors could be involved. *An. gambiae* often develops in fresh, sunlit, transient pools, which are not organically polluted (Gillies and De Meillon 1968, Minakawa et al. 1999, Gimnig et al. 2001). Water from forests and natural wetlands may contain toxic substances from leaf litter, and some harmful microorganisms may be present in stagnant water. Leaf litter may produce phenolic compounds (tannins and phenolics) that are harmful for some mosquito species (Rey et al. 1998, 1999). Lignin-like compounds from some tree species also are known to be toxic to *Culex* and *Aedes* (David et al. 2000, 2001). These toxic substances may be present in natural wetlands and forests in our study area, and such habitats were less attractive for *An. gambiae* to deposit eggs. Microorganisms in larval habitats produce volatile compounds that either attract or repel gravid mosquitoes (Rejmankova et al. 2000, Knols et al. 2004). It is possible that stagnant water in forests and natural wetlands may contain such microorganisms and thus elicited low preference for oviposition, compared with rainwater.

We further showed that habitat conditions affected *An. gambiae* egg hatchability in the highland. In natural conditions, egg mortality in natural wetland habitats was significantly higher than in farmland habitats. Predation should not have contributed to the observed lower egg hatchability in natural wetland habitats, because the eggs and larvae in our experimental cups were isolated and thus protected from predators. Because maximum and mean water temperatures were significantly lower in natural wetland habitats, the low water temperatures may have contributed to

the high egg mortality. Previous laboratory studies demonstrated the importance of temperature on embryonic development in mosquitoes (Alto and Juliano 2001, de Carvalho et al. 2002, Bayoh and Lindsay 2003). However, under the indoor condition, egg mortality remained significantly higher in water from natural wetlands than that from farmlands despite of the same water temperature, suggesting that factors other than water temperature also play an important role in egg hatchability.

We have previously showed the relative abundance of *An. gambiae* larvae and proportion of larval habitats positive for *An. gambiae* larvae were significantly higher in farmland habitats than forest habitats in the highlands of western Kenya (Minakawa et al. 2005b). The results from this study suggest that small difference in oviposition substrate preference between waters from farmlands and forests cannot fully explain the positive association between larval abundance and farmlands. Other factors, such as amount of sunlight received by an aquatic habitat and the secondary effects of sunlight on algal or bacterial growth, may be more important determinants of larval survivorship in aquatic habitats. Tuno et al. (2005) demonstrated that the survivorship of *An. gambiae* larvae was reduced from 55 to 57% in habitats fully exposed to sunlight to 1 to 2% in habitats with full or partial forest canopy coverage. Together, our findings support the hypothesis that variations in habitat conditions induced by different land cover types contribute to the heterogeneous spatial distribution of *An. gambiae* larvae in western Kenya highlands. One important mechanism is through the effects of land covers on *An. gambiae* egg hatchability and larval survivorship.

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References Cited

- Alto, B. W., and S. A. Juliano. 2001. Temperature effects on the dynamics of *Aedes albopictus* (Diptera: Culicidae) populations in the laboratory. *J. Med. Entomol.* 38: 548–556.
- Bayoh, M. N., and S. W. Lindsay. 2003. Effect of temperature on the development of the aquatic stages of *Anopheles gambiae* sensu stricto (Diptera: Culicidae). *Bull. Entomol. Res.* 93: 174–179.
- Bayoh, M. N., and S. W. Lindsay. 2004. Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. *Med. Vet. Entomol.* 18: 174–179.
- Blaustein, L., M. Kiflawi, A. Eitam, M. Mangel, and J. E. Cohen. 2004. Oviposition habitat selection in response to risk of predation in temporary pool: mode of detection and consistency across experimental venue. *Oecologia* (Berl.) 138: 300–305.

- Carter, R., K. N. Mendis, and D. Roberts. 2000. Spatial targeting of the interventions against malaria. *Bull. World Health Organ.* 78: 1401-1411.
- Charlwood, J. D., and D. Edoh. 1996. Polymerase chain reaction used to describe larval habitat use by *Anopheles gambiae* complex (Diptera: Culicidae) in the environs of Ifakara, Tanzania. *J. Med. Entomol.* 33: 202-204.
- David, J. P., D. Rey, M. P. Pautou, and J. C. Meyran. 2000. Differential toxicity of leaf litter to dipteran larvae of mosquito development sites. *J. Invertebr. Pathol.* 75: 9-18.
- David, J. P., D. Rey, J. C. Meyran, and G. Marigo. 2001. Involvement of lignin like compounds of dietary alder leaf litter against mosquito larvae. *J. Chem. Ecol.* 27: 161-173.
- de Carvalho, S.C.G., A. de J. Martins Junior, J.B.P. Lima, and D. Valle. 2002. Temperature influence on embryonic development of *Anopheles albittarsis* and *Anopheles aquasalis*. *Mem. Inst. Oswaldo Cruz* 97: 1117-1120.
- Gillies, M. T., and B. De Meillon. 1968. The Anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region). Publication of the South Africa Institute for Medical Research, Johannesburg. No. 54.
- Gimnig, J. E., M. Ombok, L. Kamau, and W. A. Hawley. 2001. Characteristics of Anopheline (Diptera: Culicidae) Habitats in western Kenya. *J. Med. Entomol.* 38: 282-288.
- Gimnig, J. E., M. Ombok, S. Otieno, M. G. Kaufman, J. M. Vulule, and W.E.D. 2002. Density-dependent development of *Anopheles gambiae* (Diptera: Culicidae) larvae in artificial habitats. *J. Med. Entomol.* 39: 162-172.
- Knols, B.G.J., L. A. Sumba, T. O. Guda, A. L. Deng, A. Hassanali, and J. C. Beier. 2004. Mediation of oviposition site selection in the African malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) by semiochemicals of microbial origin. *Int. J. Trop. Insect Sci.* 24: 260-265.
- Merritt, R. W., R. H. Dadd, and E. D. Walker. 1992. Feeding behaviour, natural food and nutritional relationships of larval mosquitoes. *Annu. Rev. Entomol.* 37: 349-376.
- Minakawa, N., P. Seda, and G. Yan. 2002. Influence of host and larval habitat distribution on the abundance of African malaria vectors in western Kenya. *Am. J. Trop. Med. Hyg.* 67: 32-38.
- Minakawa, N., G. Sonye, and G. Yan. 2005a. Relationships between occurrence of *Anopheles gambiae* s.l. (Diptera: Culicidae) and size and stability of larval habitats. *J. Med. Entomol.* 42: 295-300.
- Minakawa, N., G. Sonye, M. Mogi, and G. Yan. 2004. Habitat characteristics of *Anopheles gambiae* s.s. larvae in a Kenyan highland. *Med. Vet. Entomol.* 18: 301-305.
- Minakawa, N., C. M. Mutero, J. I. Githure, J. C. Beier, and G. Yan. 1999. Spatial distribution and habitat characterization of anopheline mosquito larvae in Western Kenya. *Am. J. Trop. Med. Hyg.* 61: 1010-1016.
- Minakawa, N., S. Munga, F. K. Atieli, E. Mushinzimana, G. Zhou, A. K. Githeko, and G. Yan. 2005b. Spatial distribution of anopheline larval habitats in western Kenya highlands: effects of land cover types and topography. *Am. J. Trop. Med. Hyg.* 73: 157-165.
- Navarro, D.M.A. F., P.S.E. de Oliveira, R.P.J. Potting, A. C. Brito, and S.J.F. Fital. 2003. The potential attractant or repellent effects of different water types on oviposition in *Aedes aegypti* L. (Dipt., Culicidae). *J. Appl. Entomol.* 127: 46-50.
- Patz, J. A., T. K. Graczyk, N. Geller, and A. Y. Vittor. 2000. Effects of environmental change on emerging parasitic diseases. *Int. J. Parasitol.* 30: 1395-1405.
- Rejmankova, E., A. Harbin-Ireland, and M. Lege. 2000. Bacterial abundance in larval habitats of four species of *Anopheles* (Diptera: Culicidae) in Belize, Central America. *J. Vector Ecol.* 25: 229-238.
- Rey, D., A. Cuany, G. Marigo, J. M. Hougard, Y. Bissan, Y. Kone, M. P. Pautou, A. Long, and J. C. Meyran. 1998. Alder-mosquito interactions in the alpine hydrosystems: possible applications in dipteran pest control. *Acta Parasitol. Port.* 5: 40.
- Rey, D., M. P. Pautou, and J. C. Meyran. 1999. Histopathological effects of tannic acid on the midgut epithelium of some aquatic Diptera larvae. *J. Invertebr. Pathol.* 73: 173-181.
- Service, M. W. 1977. Mortalities of the immature stages of species B of the *Anopheles gambiae* complex in Kenya: comparison between rice fields and temporary pools, identification of predators, and effects of insecticidal spraying. *J. Med. Entomol.* 13: 535-545.
- Sunahara, T., K. Ishazaka, and M. Mogi. 2002. Habitat size: a factor determining the opportunity for encounter between mosquito larvae and aquatic predators. *J. Vector Ecol.* 27: 7-20.
- Tuno, N., W. Okeka, N. Minakawa, M. Takagi, and G. Yan. 2005. Survivorship of *Anopheles gambiae* sensu stricto (Diptera: Culicidae) larvae in western Kenya highland forest. *J. Med. Entomol.* 42: 270-277.
- Washburn, J. O. 1995. Regulatory factors affecting larval mosquito populations in container and pool habitats: implications for biological control. *J. Am. Mosq. Control Assoc.* 11: 279-283.

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