Monitoring of Larval Habitats and Mosquito Densities in the Sudan Savanna of Mali: Implications for Malaria Vector Control

Nafomon Sogoba, Seydou Doumbia, Penelope Vounatsou, Ibrahima Baber, Moussa Keita, Mamoudou Maiga, Sékou F. Traoré, Abdoulaye Touré, Guimogo Dolo, Thomas Smith, and José M. C. Ribeiro*
Malaria Research and Training Center, Faculty of Medicine, University of Bamako, Bamako, Mali; Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland; Swiss Tropical Institute, Basel, Switzerland

Abstract. In Mali, anopheline mosquito populations increase sharply during the rainy season, but are barely detectable in the dry season. This study attempted to identify the dry season mosquito breeding population in and near the village of Bancoumana, Mali, and in a fishing hamlet 5 km from this village and adjacent to the Niger River. In Bancoumana, most larval habitats were human made, and dried out in January–February. In contrast, in the fishing hamlet, productive larval habitats were numerous and found mainly during the dry season (January–May) as the natural result of drying riverbeds. Adult mosquitoes were abundant during the dry season in the fishermen hamlet and rare in Bancoumana. To the extent that the fishermen hamlet mosquito population seeds Bancoumana with the advent of the rainy season, vector control in this small hamlet may be a cost-effective way to ameliorate malaria transmission in the 40-times larger village.

INTRODUCTION

Vector control is one of the major elements of the World Health Organization (WHO) global malaria control strategy in 2005 that primarily focused on indoor residual spraying and the use of insecticide-treated nets. However, these control measures have drawbacks, including insecticide resistance and difficulties in achieving high coverage. Larval control through source reduction and routine application of larvicides was a key intervention in eradicating malaria in many parts of the world, but this control has been largely neglected in recent decades in sub-Saharan Africa, partly because of the perceived difficulty of identifying larval habitats in rural areas. Larval control can be effective where larval habitats occur seasonally or are relatively limited and well defined.

In areas of Sudan savanna with seasonal malaria transmission, larval habitats of the Anopheles gambiae complex are considerably reduced during the dry season. Adult vector densities are thus also very low in the dry season, but increase sharply at the onset of the rainy season. Permanent breeding sites during the dry season may serve to seed the additional habitats formed during the rainy seasons. Therefore, dry season larval control might prevent this sharp increase, and thus play an important role in integrated vector control strategies. However, it has also been suggested that adult mosquitoes survive the dry season by estivating in yet undetermined locations.

We report the mapping, characterization, and monitoring of larval habitats for the presence of anopheline larvae and the monitoring of the distribution of adult anopheline mosquitoes in a rural savanna area of Mali. We consider whether analyses of the factors influencing the fluctuations of adult and larval abundance, and in particular the dry season ecology, provide a basis for a selective larval control strategy.

* Address correspondence to José M. C. Ribeiro, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 12735 Twinbrook Parkway, Rockville, MD 20892-8132. E-mail: jribeiro@niaid.nih.gov

MATERIALS AND METHODS

Description of the study site. The study was carried out in the village of Bancoumana, which is located at 60 km southwest of Bamako (12°20'N, 8°20'W) and in a fishing hamlet 5 km from this village adjacent to the Niger River (Bozokin) (Figure 1).

The total population of Bancoumana is approximately 8,000 inhabitants, predominantly of the Malinké ethnicity living in 340 compounds. The main economic activity is agriculture. The fishing hamlet has approximately 300 inhabitants of Bozo ethnicity living in 10 compounds. The land between the village and the river is used for growing rain-fed rice during the rainy season (usually June to October) and for growing other crops (onions, tomatoes) during the dry season (November to May).

There is intense malaria transmission during the rainy season and for the next two months. The major vectors are An. gambiae (approximately 95.5%) and An. arabiensis (approximately 4.5%). The mean monthly entomologic inoculation rate was 2.8 infectious bites per person with marked seasonal variations (Bagayoko M, 2000. Application des Systèmes d’Information Géographiques à l’Étude Micro-Épidémiologique de la Transmission du Paludisme à Bancoumana (Arrondissement de Sibi, Cercle de Kati). Thèse de Doctorat de Spécialité de l’ISFRA. Bamako, Mali: University of Bamako). The prevalence of Plasmodium falciparum infection in children less than five years of age varies from approximately 30–50% during the dry season to 75% during the rainy season (Doumbia S, 2002. Determinants of Semi-Immune State in an Area of Seasonal Malaria Transmission in Bancoumana, Mali. Doctoral Thesis. New Orleans: Tulane University).

Identification and characterization of potential anopheline breeding sites. From June 2004 to December 2005, we performed a monthly active search to identify and geo-locate all larval habitats in both Bancoumana and the fishing hamlet. The search was extended to a perimeter 2 km around the two study sites and also included the Niger River riverbed. The search was carried out by three entomologists assisted by two local guides who had good knowledge of the area. Villagers
were questioned about their awareness of open water bodies around the villages, particularly during the dry season.

A unique identification number was assigned to each water body according to its location (block), type (ponds, brick pit, puddles, and tire prints), and the order in which it was identified. Geographic coordinates for all identified water bodies were recorded using a global positioning system (GPS) (GeoXM; Trimble Navigation Ltd., Sunnyvale, CA) with a spatial margin of error of 2–5 meters. All surface waters were mapped and sampled with a WHO standard mosquito dipper to determine the presence or absence of immature mosquitoes. During each monthly survey, investigators recorded information on characteristics of the water bodies (type of larval habitat, the presence of vegetation and other co-occurring arthropods, exposure to sunlight, water turbidity and transparency, and the color of the bottom), and productivity (presence or absence of anopheline larvae).

Anopheles gambiae s.l. mosquito larvae were morphologically identified and separated from other species by experienced entomologists. A polymerase chain reaction method was used to identify molecular forms (M and S) on a random sample of anopheline larvae selected from each monthly collection.

Monitoring adult mosquito density. In both Bancoumana and the fishing hamlet, standard indoor pyrethrum spray catches were used to collect adult mosquitoes during the dry season (December 2004 and May 2005). Collections were performed during the last two weeks (16th–27th) of each month.

In Bancoumana, we updated the existing geo-referenced base map established with GeoExplorer 3° GPS receivers (Trimble Navigation Ltd.) with an accuracy of 1–3 m (Bagayoko M, 2000. Thèse de Doctorat de Spécialité de l’ISFRA). This map includes landmarks and all housing compounds and larval habitats. A unique identification number was assigned to each compound. Adult mosquito collections were conducted in 180 houses sampled to represent the two types of housing (thatch roof versus metal roof) and located in 180 different compounds randomly selected from the list of the 340 compounds of the village. The identification number assigned to each selected compound was marked on the door of the house using a permanent marker. In the fishing hamlet, mosquito collection was performed in all 10 housing compounds that composed the hamlet.

Adult An. gambiae s.l. and An. funestus from both sites were identified morphologically. The total number of An. gambiae s.l. and An. funestus, the identification number, the type of the house, and the number of people sleeping in them were recorded onto appropriate data sheets. In the laboratory, a sample (at least 120 specimens) of An. gambiae s.l. was further identified to species (An. gambiae s.s. and An. arabiensis) and sub-species (molecular forms M and S).

Data analysis. Statistical analysis was carried out with STATA version 9.0 (Stata Corporation, College Station, TX). Logistic regression models were used to determine the key factors influencing anopheline larvae presence in larval habitats. The key factors included in the models were type of water bodies, their size and depth, turbidity and transparency of the water, bottom color, presence and abundance of vegetation, and the co-occurrence of other arthropods. The chi-square test was used to compare the proportion of the different type of water bodies positive for anopheline larvae.

Ethics. This study did not involve human subjects. The inherent ethical considerations with the execution of this research were related to pyrethrum spray catches. No house was sprayed without the approval of its owner. The insecticide used was a pyrethroid marketed under the name of Premium Killer® (NIRA BVBA, Antwerp, Belgium). This product has a weak persistence, has no human toxicity under normal conditions of use, and is intended for use as an indoor spray. The treated house is reusable a few minutes after spraying. The study was reviewed and approved by the ethical committee of the Faculty of Medicine and Pharmacy of the University of Bamako, Bamako, Mali.

RESULTS

Characteristics of water bodies. Bancoumana. From June 2004 to December 2005, 63 major water bodies were identified in and around the village of Bancoumana. Overall, these belonged to four major types (Figure 2) comprising brick pits (74.6%), tire prints (14.3%), puddles (9.5%), and ponds (1.6%). There were temporal variations in the number of water bodies in the village of Bancoumana (Figure 3). During the rainy season (June–September), tire prints formed a slightly increased proportion (16.4%). There were innumerable small water bodies created by human footprints and cattle hoof prints; however, these usually did not persist for more than two weeks and were not counted. At the end of the rainy season (October–November), brick pits accounted for up to 83.3% (n = 8) of the water bodies. Ten weeks after the rainy season ended (January and February corresponding to the dry cold season), we did not find any additional water bodies. Figure 4 shows the frequency distribution of the different type of water bodies positive and negative for anopheline larvae during the dry season (December 2004–May 2005). In March 2005, subsequent to 60 mm of rain, 26 of the 63 water bodies (41.3%) were replenished. These comprised brick pits (65.4%), ponds (19.2%), tire or foot prints (11.5%), and rain puddles (3.8%).

There was no significant difference ($\chi^2 = 7.5, P = 0.058$) between brick pits (59.0%), tire prints (62.9), puddles (80.0%) and ponds (42.9%) for anopheline larvae. The high-
The highest proportion of anopheline-positive water bodies was observed in August 2005 (92.7%, n = 55) and the lowest was observed in November 2005 (37.5%, n = 8). In December 2004, the only potential breeding site was a single brick pit but this was negative for anopheline larvae. Among the replenished water bodies after the rainfall of March 2005, 66.7% of the brick pits (n = 21) and the tire prints (n = 3) were positive for anopheline larvae. In April, three additional ponds were found between Bancoumana and the fishing hamlet, but all were negative for anopheline larvae. Accordingly, in the immediate surroundings of Bancoumana, only four potential larval habitats were found, although we could not find anopheline larvae in them. In May, at the onset of the rainy season, 45 water bodies were found, mainly composed of brick pits (73.3%), but no anopheline larvae were found in any of them.

**Fishing hamlet.** During the period when all larval habitats were almost dried out in the village of Bancoumana (dry season), numerous water puddles (Figure 2) created in the riverbed by the drying river were found highly positive for anopheline larvae. Unlike the larval habitats observed in Bancoumana, all water bodies found in the fishing hamlet were naturally made and most often full of larvae. No vegetation was found in these larval habitats but other co-occurring arthropods were often present, and the water was always very clear.

**Key environmental factors associated with anopheline larvae in water bodies.** Bancoumana. Table 1 shows the results of the bivariate regression between the presence/absence of anopheline larvae and the environmental variables in the village of Bancoumana. Water turbidity and transparency, other co-occurring arthropods, and vegetation presence and abundance were significantly associated with the presence/absence of anopheline larvae in the water bodies. Water bodies with vegetation (odds ratio [OR] = 5.1, 95% confidence interval [CI] = 3.4–7.5), other co-occurring arthropods (OR = 3.0, 95% CI = 1.9–4.6), and a brownish bottom (OR = 2.4, 95% CI = 1.5–3.6) were much more likely to contain anopheline larvae than when vegetation and other co-occurring arthropods were absent, and when the bottom was a different color. Compared with opaque but non-turbid water, both turbidity and transparency (OR = 0.5, 95% CI = 0.4–0.8) decreased the chance of finding anopheline larvae. The multivariate logistic regression analysis indicated that only larval habitats with other co-occurring arthropods (OR = 3.0, 95% CI = 1.8–4.9) and vegetation (OR = 8.7, 95% CI = 4.7–16.3) were much more likely to contain anopheline larvae than all other larval habitats; vegetation abundance was negatively associated with larvae.

**Fishing hamlet.** The water bodies were exclusively natural puddles with clear water, not vegetated, and highly positive for anopheline larvae. No further analysis to assess associa-
tions between anopheline larvae presence and the environmental variables was performed.

Monitoring adult mosquito density during the dry season. Bancoumana. During the dry season (December 2004 to May 2005) in Bancoumana, mosquitoes were nearly undetectable in human dwellings (Figure 5). Overall, only 175 mosquitoes were collected in 1,078 spray collections (mean mosquito density = 0.16, 95% CI = 0.11–0.21). The few mosquitoes collected in Bancoumana were clustered at the side of the village facing the fishing hamlet (Figure 6).

Fishing hamlet. During the dry season (December 2004 to May 2005) in the fishing hamlet, mosquito density was relatively high throughout the study period (Figure 5), and peaked in December when the mosquito density in Bancoumana was very low. The mean mosquito density was 8.16 per house (95% CI = 7.4–9.0). Overall, 506 mosquitoes were collected in only 62 spray collections compared with 175 in 1,078 collections in Bancoumana.

Estimates of larval *An. gambiae* molecular form frequencies in the two villages. A comparison between the molecular forms frequencies of anopheline larvae collected in the riverbed and in the rain-fed larval habitats of the main village was done after the heavy rain in March 2005. The mosquito population was identical in the fishing hamlet and Bancoumana village with a predominance of the M form, 79.0% (n = 286) and 79.4% (n = 34), respectively.

**DISCUSSION**

We mapped and characterized larval habitats in two ecologic settings: Bancoumana, where no permanent water is present, and a fishing hamlet lying adjacent to the Niger River. Our study focused on *An. gambiae* s.l., which is the main vector for malaria transmission and accounts for 98% of

**FIGURE 3.** Temporal variation of watered major larval habitats in the village of Bancoumana, Mali: June–September (rainy season), October–November (end of the rainy season), December–February (cold dry season), March–May (hot dry season). This figure appears in color at www.ajtmh.org.

**FIGURE 4.** Frequency of different larval habitats positive and negative for anopheline larvae during the dry season in the village of Bancoumana, Mali.
the mosquitoes versus only 2% for An. funestus. Anopheles funestus is mostly observed towards the end of the rainy season (October–November). In Bancoumana, nearly all larval habitats were human-made and rain-dependent, attesting to the human-dependent ecology of Afrotropical Anopheles. By 10–12 weeks after the end of the rainy season, most water bodies have dried and few mosquito larvae can be found. As a result, the number of adult mosquitoes collected in the houses became very small. The study confirmed previous reports of undetectable transmission in Bancoumana during the dry season.

Although the dry season in the study area typically lasts from November through April–May, a short rainfall lasting a few days often occurs in March or April. Because this period corresponds to the maturation of mangoes, this phenomenon is called “mango-rain”. After such rainfall in March 2005, larval habitats, mainly composed of brick pits, were replenished with water and became positive for anopheline larvae. This shows the rain dependency of overwhelmingly human-made larval habitats and indicates that in Bancoumana mosquitoes probably laid their eggs quickly at the onset of the rainy season. The near absence of watered larval habitat in January and February, when no rain was observed, supports the hypothesis. However, this refilling of most larval habitats does not translate into high anopheline larvae productivity in the subsequent months of April and May 2005, presumably because of higher temperature (> 40°C), and low relative humidity (minimum = 26%, maximum = 62%) occurring in these months. Studies in Kenya showed a reduction from 55–57% in the survivorship of An. gambiae s.l. larvae in open larval habitats associated with an increase of 3–3.4°C in their average daily water temperature compared with full forest-canopy coverage (forest habitats) and partial canopy coverage (forest edge habitats) larval habitats.

The greater dependency of An. gambiae s.l. on humid conditions has also been described. However, at the same period (dry season) in the fishing hamlet adjacent to the receding Niger River riverbed, there were numerous small natural puddles that were highly productive for anopheline larvae. As a result, the mosquito density was higher in the hamlet during the dry season than in Bancoumana. The quick re-colonization of the larval habitats shortly after a rainfall in Bancoumana suggests that mosquitoes that emerged from the riverbed are an important seed of the rain-fed water bodies of Bancoumana. The distance of 3–5 km that separates the river and the village is well within the flight range of An. gambiae. We did not find any potential obstacles to the flight of mosquitoes between Bancoumana and the fishing hamlet. Moreover, the different molecular forms of An. gambiae larvae after the first rain after the dry season had near identical frequencies in the two sites. It thus appears that the vectors in the two villages are from a common population. If the small fishing village was targeted for larval and adult mosquito control during the dry season (February and March), it could have a substantial impact on malaria transmission in surrounding areas such as the main village of Bancoumana.

The high anopheline larvae productivity of the larval habitats created by the receding riverbed parallels the ecology of An. culicifacies in Sri Lanka more than the usual situation of An. gambiae in Africa. However, in various areas with seasonal malaria transmission in Africa, it has proved possible to identify local reservoirs of transmission during the dry season. Identifying sources of mosquito larvae during the dry season may provide a basis for selective larval control, which

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* CI = confidence interval.
may impact on subsequent malaria transmission in the rainy season.

In this area of seasonal malaria transmission, most productive larval habitats are human-made and rain-dependent, drying out within 10–12 weeks after the rainy season ends. Not very far away, numerous highly productive anopheline larvae may be found in favorable ecologic conditions (e.g., along the receding riverbed), which may sustain malaria transmission at a low level during the dry season and may serve as inoculums in surrounding areas. This scenario is similar to those in other areas of seasonal malaria transmission and provides an opportunity for a mosquito control strategy targeting dry season larval control and environmental management.

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Authors' addresses: Nafomon Sogoba, Malaria Research and Training Center, Faculty of Medicine, University of Bamako, Bamako BP 1805, Mali, E-mails: ndoumbi@mrtcbko.org, baber@mrtcbko.org, moussa@mrtcbko.org, maigam@mrtcbko.org, cheick@mrtcbko.org, and guimogo@mrtcbko.org. Thomas Smith, Swiss Tropical Institute, Socinstrasse 57, PO Box 4051 Basel, Switzerland, E-mail: Thomas.A.Smith@unibas.ch. José M. C. Ribeiro, Laboratory of Malaria and Vector Research, 12735 Twinbrook Parkway, Room 2E-32, Twinbrook III Building, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892-8132, E-mail: jribeiro@niaid.nih.gov

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