

VECTOR ABUNDANCE AND MALARIA TRANSMISSION IN RICE-GROWING VILLAGES IN MALI

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Abstract. Anophelism without malaria has long been recognized. In large irrigation projects, such as that around Niono, Mali, villages in irrigated areas sometimes have more anopheline vectors of malaria than adjacent nonirrigated villages, but overall malaria prevalence is substantially less. One hypothesized explanation for this is high anopheline densities lead to smaller adults, who do not live so long and hence are less efficient at transmitting the disease. We analyzed serial collections from 18 villages in an irrigated area of Mali, measuring correlations between mosquito densities and survival rates, zoophilic rates, and vectorial capacity over the villages and times. Adult density was inversely related to anthropophily and adult survival and its relationship with vectorial capacity was positive at low mosquito densities, flat at intermediate densities, and negative at high densities. This may partly explain why malaria prevalence is low in irrigated villages with high *Anopheles* density.

INTRODUCTION

Dams and projects for irrigation of rice have dramatic consequences for the health of people living in the areas around them. Along with their economic benefits, such projects may also bring sharply increased incidences of disease, including malaria, schistosomiasis, and filariasis. Although irrigated regions typically do produce more mosquitoes, these need not transmit more disease.¹ Surveys of published reports indicate that no change, or even decreases, in malaria transmission occur as often as do increases (see references in Table 1). Our goal in this project has been to better understand the relationship between rice irrigation and malaria in a focal irrigation project of Mali, the *Office du Niger*, more specifically, why it is that malaria transmission is (generally) lower there than in nearby nonirrigated villages. This might assist the management of current irrigated areas and the design of new ones.

Several hypotheses have been suggested to explain why irrigation might lead to lower rates of malaria transmission. These include the following: 1) Irrigation projects lead to greater wealth, which in turn leads to better health care and an increase in personal protection against mosquitoes. 2) The large number of mosquitoes from irrigated fields cause so much nuisance that it induces people to use their bed nets more than they would if numbers were smaller. 3) Irrigated fields may favor certain species/forms of vectors that are less effective. 4) High *Anopheles gambiae* densities in the larval stages lead to adults that are less efficient at malaria transmission. Following Dolo and others,⁷ we will call this the “competition hypothesis.” Of course, these are not mutually exclusive, and all might contribute.

Two large, intensive studies of the relationship between irrigation and disease have been conducted by the Malaria Research and Training Center (MRTC) at the University of Bamako in Mali. The first of these included three irrigated and three nonirrigated villages, described in Refs. 7, 8, and 19. They observed that malaria was quite seasonal in the nonirrigated villages, being concentrated in the wet season. Anophelines were rare there during the dry season, so there was little malaria then. In irrigated villages, however, especially those where a second crop of rice was grown, mosqui-

toes and malaria were observed through much more of the year. Nonetheless, the total incidence of malaria transmission in these irrigated villages was much smaller than in nonirrigated ones. For example, the average entomological infection rate (*EIR*) was 8.7 infective bites per month for the nonirrigated villages but only 2.4 infective bites per month for the irrigated villages. This paralleled the numbers of clinically diagnosed cases of malaria in children at the same times.⁸

In an effort to understand this surprising relation, Dolo and others^{7,19} considered the several hypotheses listed above and ultimately favored the competition hypothesis, hypothesis no. 4. They could rule out the species/form composition hypothesis, no. 3, because the composition was substantially the same in both regions. Bed nets were used almost universally in both regions, as determined from surveys, ruling out that explanation, no. 2. Generally, the same medical care was available throughout the region, at the time of this study, suggesting that differences in health care, no. 1, was unlikely to account for the differences in malaria transmission; though actual use of services could be affected by social conventions and distance to the health care facility. This left the competition hypothesis, no. 4, as the remaining possibility. It did receive some support in their study; for example, they observed that during the season of peak density, adult survivorship was less than at other times. Also, the sporozoite rates and vectorial capacity were lower when densities were high. These are all expected from the competition hypothesis but of course do not prove it. Our goal in this study was to examine further the claims of the competition hypothesis in the same irrigated area. Specifically, we analyzed data that had been collected in a follow-up study from a larger number of irrigated villages than in the Dolo and others⁷ original study (3) and over a different period of time, 1999–2001. We measured correlations between mosquito densities and survival rates, zoophilic rates, and vectorial capacity (*C*) over the villages and times of this expanded study. The question was, would vectorial capacity quantitatively follow mosquito densities in these additional locations and times in the same manner that Dolo and others^{7,19} had hypothesized it should?

TABLE 1

Studies comparing vectorial capacity (C), EIR , or human prevalence that compare irrigated and nonirrigated areas or the same area before and after irrigation

| Effect | Source | Parameters used |
|-------------------|--------|----------------------------|
| No difference | | |
| Burkina Faso | 2 | EIR |
| Cameroon | 3 | Prevalence |
| Cameroon | 4 | Prevalence |
| Ivory Coast | 5 | EIR |
| Senegal | 6 | EIR , prevalence |
| Less transmission | | |
| Mali | 7 | EIR |
| Mali | 8 | Prevalence |
| Tanzania | 9 | EIR (but increased C) |
| Burkina Faso | 10 | Prevalence |
| The Gambia | 11 | Prevalence |
| More transmission | | |
| Burkina Faso | 12 | EIR |
| Burundi | 13 | C , prevalence |
| Cameroon | 14 | Prevalence |
| Guinea-Bissau | 15 | Prevalence |
| Kenya | 16 | EIR |
| Madagascar | 17 | Prevalence |
| Sierra Leone | 18 | Prevalence |

EIR , entomological inoculation rate.

METHODS

Study area. The district of Niono is located in the Sahelian area of Mali, 350 km from Bamako. Detailed descriptions of the area, climate, and irrigation patterns and malaria epidemiology have been published in Refs. 7, 8, 20, 21, and 22, so only a summary will be presented here. The year can be divided into three seasons: the rainy season (July to September/October), the "cold" dry season (November to February), and the hot dry season, extending from March to May/June. The pattern of irrigation and rice cultivation is in step with the seasons. In general, flooding for irrigation begins in June/July, and the rice is harvested in October/November. Not all stages of rice growth produce anophelines equally. The peak production of anophelines occurs in August/September, when the rice has been transplanted but is not yet dense enough to shade the water. Malaria transmission typically occurs shortly after the peak mosquito production and is low peaks the fields are fallow during January to June. In some of the irrigated areas and in some years, a second rice crop is also grown, beginning in March and harvested in June. In such areas, there are anophelines and malaria transmission through much of the year, with less marked seasonality.⁷

A Landsat 7 ETM+ false color image of the Niono area is shown in Figure 1. This was obtained during October 1999. The color scheme is red for ETM+ band 5, green for ETM+ band 4, and blue for ETM+ band 3. In their original study of malaria transmission in Niono, Dolo¹ included three nonirrigated villages (Toumakoro, Dokoboukou, and Kalanampala) and three irrigated villages (Niessoumana, Tenegué, and Tissana). The nonirrigated villages are shown in blue. The study reported here did not include any nonirrigated villages but did include the three irrigated villages studied in Ref. 1 plus 15 additional ones. There were thus 18 villages throughout the irrigated area: 8 in the Niono subzone (Nango, Tigabougou, Niessoumana, Tissana, Tenegué, Koyan Coura, Kolodougou Coura, and Mourdian), 5 in the N'Débougou

subzone (Siengo, Nara, Toukoun Coura, Sounkalokan, and Sarango), and 5 in the Molodo subzone (Sokourani, Molodo, Niono Koroni, Coccodi, and Hamdalaye). The selection criteria included that the villages be at least 2 km apart to decrease the likelihood of capturing mosquitoes coming from the ricefields of a neighboring village, accessibility and the willingness of villagers to cooperate with the mosquito collection efforts. The fact that villages were located in different subzones increased the variability in the numbers of mosquitoes captured, as each subzone was irrigated independently and had been subjected to different levels of rehabilitation.

Malaria transmission. Malaria in this region is transmitted by mosquitoes in the genus *Anopheles*, predominantly *Anopheles gambiae* s.l. and the *Anopheles funestus* group. Dolo and others⁷ found that 99.6% of all *An. gambiae* s.l. were *An. gambiae* s.s., and of those, 98.6% were of the Mopti chromosomal form, or "M" molecular form. (In Mali, there is nearly a 1:1 association between the two, see Ref. 23.) Irrigation typically provides breeding sites for these species, with consequent increases in mosquito densities. But clearly, the simple number of mosquitoes is not the same as their ability to transmit malaria.

A useful and widely adopted estimate of a vector population's ability to transmit malaria is the vectorial capacity, C . It may be described as follows²⁴: Let the number of vectors per human be m and the number of bites per mosquito per night on humans be a , then a human is bit ma times per day, on average. Assuming an exponential survival rate with daily survival p , then a proportion p^n of these vectors survives the incubation period (sporozoite cycle) of the parasite, so that it could then transmit the pathogen. The vectors are then expected to survive another $1/\log p$ days and bite other persons a times per day, on average.

Combining this, a reasonable measure of transmission is the vectorial capacity, defined to be:

$$C = ma^2p^n / -\log p$$

These terms and how they were measured are summarized in Table 2.

Entomological studies. Eight entomological surveys were conducted between April 1999 and January 2001, during the middle and end of the rainy season (August and October, respectively), the off-season crop (April), and the harvesting period (January). Each survey consisted of 2-day visits to each of the study villages. Included in the survey were two types of entomological surveys: day collections and night captures. For the day collections, a team of three people estimated indoor resting density (N_i) using the pyrethrum spray catch (PSC) method in 30 randomly chosen houses (15 per visit) between 3 and 5 PM. This consisted of covering all exposed surfaces with white sheets, spraying the rooms and collecting all fallen specimens. Anopheline mosquitoes of interest were identified to species (*An. gambiae* s.l. or members of the *An. funestus* group).

Night captures were conducted at two houses in each village, at least 200 m apart, between 6 PM and 6 AM with a personnel change at midnight. At each house, a collector was posted indoors and another outdoors with a flashlight and a mouth aspirator.

Collected females were classified by abdominal status (un-

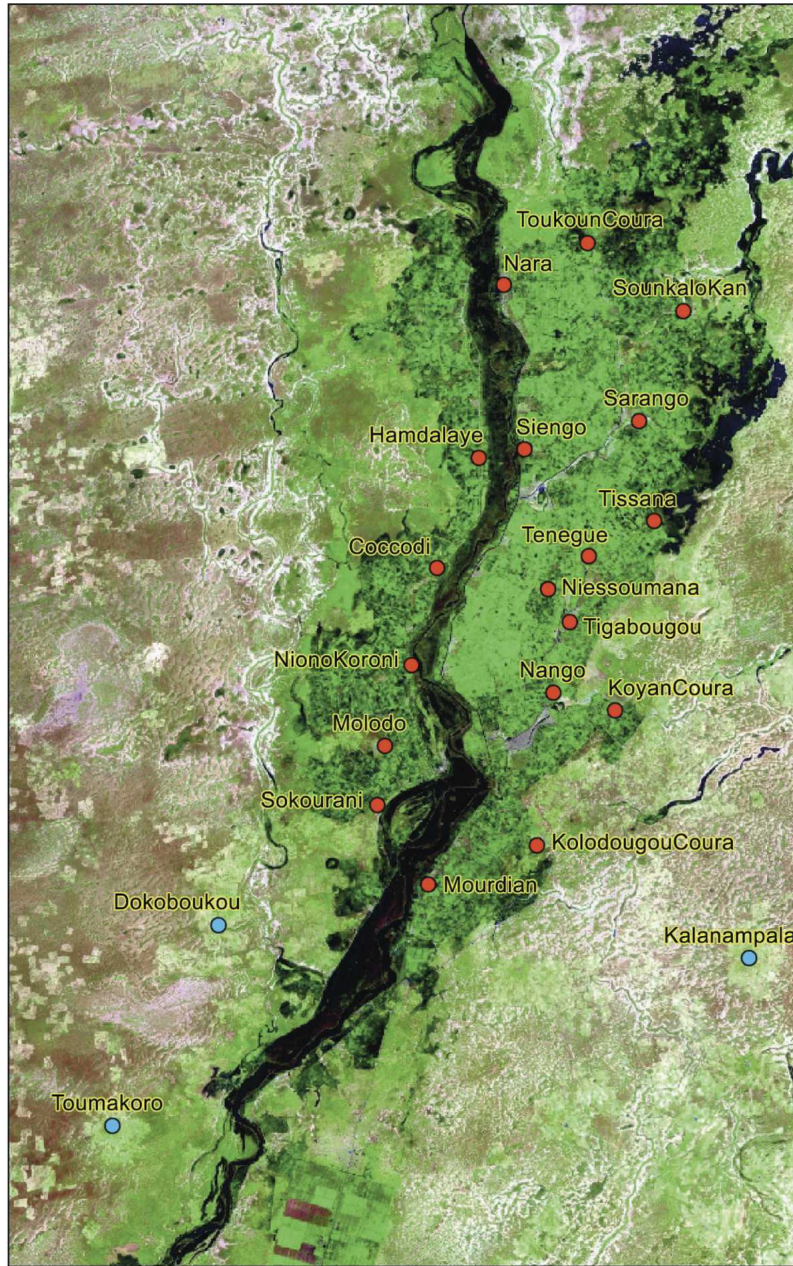


FIGURE 1. Map of study area.

TABLE 2
Variables used in the calculation of vectorial capacity*

| Variable | Definition | Estimation method |
|----------|---|--|
| N_i | Indoor resting density | Sum of mosquitoes captured by PSC per house |
| N_f | Number of recently fed female mosquitoes | Sum of fed and semigravid mosquitoes captured by PSC per house |
| N_s | Number of human sleepers in rooms where PSC was carried out | Number of humans in rooms surveyed |
| ma | Human biting rate (bites per human per day) | N_f / N_s |
| P | Proportion of parous females (parity rate) | Detinova ²⁶ |
| p | Probability of daily survival | g th root of parity rate |
| A | Proportion of bites on humans (anthropophilic rate) | From ELISA |
| a | Number of bites per mosquito per night ("man-biting habit") | A/g |
| C | Vectorial Capacity | $ma \times a \times p^n / -\ln(p)$ |

* The values of n (length of sporozoite cycle) and g (length of gonotrophic cycle) are taken from the literature for this area. Their values are 12 and 2 days, respectively. PSC, pyrethrum spray catch.

fed, fed, semigravid, and gravid) in the field when possible. At high density, specimens were conserved in Carnoy's fixer (3 parts ethanol:1 part glacial acetic acid), and classification was conducted in the laboratory. The number of human occupants during the previous night was recorded for each surveyed house. To estimate the proportion of the blood-fed and semi-gravid *An. gambiae* s.l. and *An. funestus* that had fed on humans (anthropophilic rate), a blood aliquot was extracted, conserved in Carnoy's, and analyzed with enzyme-linked immunosorbent assay (ELISA).²⁵ Parity rates were estimated from the night catches, using the method of Ref. 26, observed the day after capture. Much of this has been compiled in the Ph.D. thesis of Mahammadou Touré²¹; further details may be found there.

Statistical methods. We used population-averaged panel data regression models (XTGEE models, STATA 8.0, Stata Corporation) for all analyses.²⁷ These groups of models are extensions of generalized linear models (GLMs) that are useful to model count data, as the user can specify a negative binomial distribution for the response variable (e.g., mosquito counts). In addition, they allow the specification of a variable over which observations are not independent (either in space or over time), called the clustering variable. We used village as the clustering variable to correct for the correlation among mosquito samples taken in houses within the same village. In this way, we could relate the density measure done at the house level to the response variables measured at the village level (parity and anthropophily).

We considered the different surveys as independent, given the large temporal separation between the samples, and assumed no spatial dependency among the villages sampled, given the minimum separation of 2 km and the fact that cultivation patterns are driven mainly by irrigation patterns, which are not expected to vary consistently with distance.

We first fit a regression model with total number of mosquitoes N_t as the response variable and species, season, and year as predictors. In the second set of models, we tested the effect of human-biting rates ma and survey on parity and an-

thropophilic rates. A negative relationship between ma and either parity or anthropophily would decrease the rate at which vectorial capacity increases with density and could even cause C to decrease at very high densities. Finally, we used a second-degree polynomial multiple regression model to examine the relationship between ma and vectorial capacity estimated per village. We compared linear and quadratic models to see if a nonlinear density term significantly improved the fit.

RESULTS

Spatial and seasonal patterns of anopheline indoor resting density. The geometric mean of the indoor resting density, N_t , was 19.1 *An. gambiae* per house per night, and 3.6 *An. funestus* per house per night. *An. gambiae* was more abundant than *An. funestus* in all surveys but one, October 2000. N_t of the two species significantly differed with season. *An. gambiae* was proportionately more abundant than *An. funestus* in August and proportionately less in January and October. This is consistent with the observation that *An. gambiae* breeds principally in irrigated fields, abundant during the rainy seasons, whereas *An. funestus* typically breeds in more permanent water sources, hence its predominance when irrigation is absent. N_t also varied significantly over seasons and years.

The highest *An. gambiae* N_t was in the middle of the main cropping season (August) in both years, followed by the off-season crop (April). In April 2000, N_t was significantly lower than in April 1999, coincident with a shortage of irrigation due to work on channel maintenance. *An. funestus* N_t also showed significant differences among seasons and years, with the highest abundance in October and April of both years. Given these differences between the 2 years of the study, we will hereafter refer to surveys ($N = 8$) instead of years/seasons. We performed all subsequent analyses for each vector species separately.

Figure 2 shows the number of indoor resting *An. gambiae*

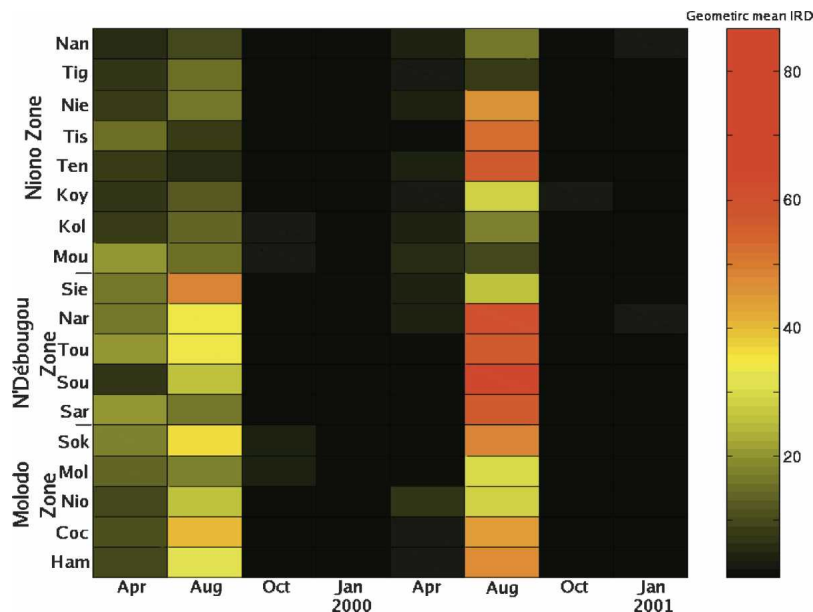


FIGURE 2. Geometric mean of indoor resting density N_t of *An. gambiae* in irrigated villages samples from 1999 to 2000.

females, N_f , for each village and collection. Each column represents one collection, and each row shows the values per village. The indoor resting density of *An. gambiae* is coded in color, ranging from a low of 0 (black) to a high of 80 (red). This large variability was found even within the same survey/village (e.g., in August 1999, the numbers captured in one house in Toukoun Coura was 0, whereas in another house it was 2,487). Even with this large within-village variability, the differences in mosquito numbers were statistically significant for both *An. gambiae* ($\chi^2 = 155.40$, $df = 17$, $P < 0.001$) and *An. funestus* ($\chi^2 = 409.32$, $df = 17$, $P < 0.001$).

Table 3 summarizes the mean values of the malaria parameters obtained in each of the surveys. Human biting rate (ma) and anthropophilic rates (A) showed a significant negative relationship, as illustrated in Figure 3a and in Table 4, part a. There was also a statistically significant but weaker relationship between density and daily survivorship estimated through parity rates (Figure 3b and Table 4, part b). To con-

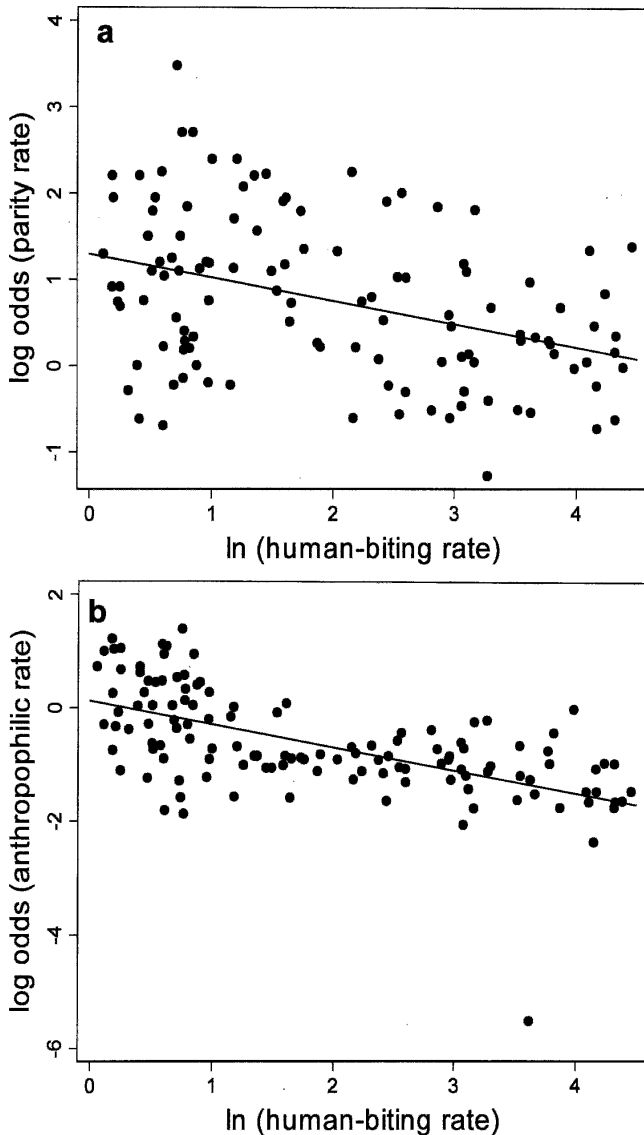


FIGURE 3. Relation between (a) human biting rate (ma) and daily survivorship (p) and (b) human biting rate (ma) and percent feeding on humans (A).

TABLE 3

Survey summaries of the variables used in the calculation of vectorial capacity

| Variable | 1999 | | | 2000 | | | | 2001 Jan |
|----------|-------|--------|------|------|-------|--------|------|-------------|
| | Apr | Aug | Oct | Jan | Apr | Aug | Oct | |
| N_t | 70.39 | 337.72 | 7.01 | 1.60 | 17.34 | 356.78 | 5.12 | 6.13 |
| N_f | 40.91 | 75.52 | 4.59 | 1.72 | 10.36 | 129.04 | 3.48 | 3.55 |
| N_s | 3.65 | 3.79 | 3.84 | 3.90 | 3.65 | 3.54 | 3.67 | 3.95 |
| ma | 11.21 | 19.92 | 1.20 | 0.44 | 2.84 | 36.43 | 0.95 | 0.90 |
| A | 0.30 | 0.29 | 0.43 | 0.64 | 0.36 | 0.16 | 0.36 | 0.55 |
| a | 0.15 | 0.14 | 0.22 | 0.32 | 0.18 | 0.08 | 0.18 | 0.28 |
| P | 0.62 | 0.52 | 0.77 | 0.93 | 0.87 | 0.58 | 0.55 | 0.80 |
| C | 0.39 | 0.16 | 0.45 | 2.53 | 3.08 | 0.41 | 0.02 | 0.60 |

ontrol for the effect of the time of the year in these relationships, we included survey as a covariate in the previous models. Inclusion of survey improved the R^2 to 0.33 for parity rates and to 0.49 for anthropophilic rates. Human biting rates still had a significant effect on anthropophily when the effect of survey was included, though not on parity rates.

Vectorial capacity and adult density. We used a second-degree polynomial multiple regression to compare linear and quadratic regression models of the relationship between ma and C (Figure 4). We found that adding the squared term improved the fit and that this effect was statistically significant.

DISCUSSION

The main finding of this study has been that vectorial capacity varies with adult anopheline density in a nonlinear manner, shown in Figure 4. At lower densities, C increases with density, it levels off at intermediate densities, and decreases at higher densities. In principle, this might go a long way toward explaining the varied response of malaria transmission to irrigation projects that are summarized in Table 1.

TABLE 4
Combined regression table

| | Estimate | SE | z Value | Pr (< z) |
|---|----------|-------|---------|------------|
| (a) Proportion of parous females (log odds P) and human biting rates (ln ma) | | | | |
| Intercept | 1.104 | 0.089 | 12.44 | <0.001 |
| ln (ma) | -0.18 | 0.009 | -20.65 | <0.01 |
| Wald χ^2 (df=1) | 426.45 | | | |
| Prob > χ^2 | <0.001 | | | |
| Adjusted R^2 | 0.104 | | | |
| (b) Proportion of bites on humans (log odds A) and human biting rates (ln ma) | | | | |
| Intercept | -0.134 | 0.086 | -1.55 | <0.122 |
| ln (ma) | -0.28 | 0.007 | -37.65 | <0.001 |
| Wald χ^2 (df=1) | 1417.17 | | | |
| Prob > χ^2 | <0.0001 | | | |
| Adjusted R^2 | 0.248 | | | |
| (c) Vectorial capacity (ln C) and human biting rates (ln ma) | | | | |
| Intercept | 1.8 | 0.24 | -7.51 | <0.001 |
| ln (ma) | 0.76 | 0.076 | 9.92 | <0.001 |
| (ln (ma)) ² | -0.103 | 0.015 | -6.8 | <0.001 |
| Wald χ^2 (df=2) | 170.48 | | | |
| Prob > χ^2 | <0.0001 | | | |
| Adjusted R^2 | 0.004 | | | |

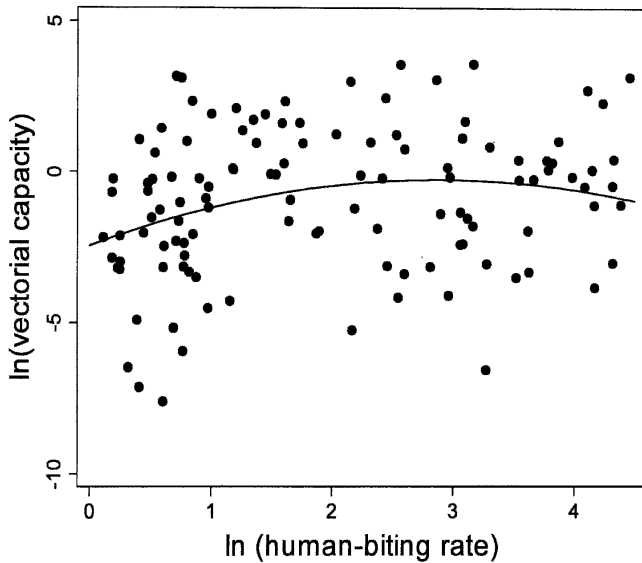


FIGURE 4. Relation between vectorial capacity (C) and human biting rate (ma).

It should be recognized, however, that there is a great deal of variation about the curve in Figure 1. Although the relationship is statistically significant, one could question whether it is sufficient to explain the fairly large differences in malaria that were observed. A core part of the competition hypothesis is that body size decreases with larval density and that smaller adult mosquitoes survive less well. Support from this comes from Refs. 28, 29, and 30. We have begun experiments to test this, and preliminary results do suggest statistically significant, but again small, effects in support of the competition hypothesis. A full evaluation will require that all of the observed mathematical relationships be combined into a model and their respective roles compared.

It appears that the strongest relationship between density and vectorial capacity comes from a , the man-biting habit, or number of bites per human per night (Figure 3a). Although it is not hard to imagine reasons why this might occur, the most obvious is certainly that when densities are high, people protect themselves, and the mosquitoes are forced to alternate hosts. Human night catches have been made in this area, and during the rainy season the number of bites per person may exceed 550 bites/night by *An. gambiae*.^{7,19} Dolo and others⁷ report near-universal use of bed nets, based on surveys, but a more detailed study might be warranted.

We used adult mosquitoes from indoor PSC catches as the measure of mosquito density. This might not be correct for testing the competition hypothesis. There is ample reason to believe that competition in *An. gambiae* occurs in the immature stages.^{31,32,33} But what is important for the competition hypothesis is the numbers relative to available resources. We are aware of no good way to assess accurately how many resources are available or to measure how good they are for *An. gambiae*. What makes one patch of water suitable and another not? Human experts can often do a pretty good job of guessing where larvae will be found, but they are very frequently in error. Chemical composition of the water and its physical appearance both seem important. It would be helpful to have better means for doing this, both for studies of this

sort and for determining species composition (Edillo FE and others, unpublished data).

Related to this issue of measuring suitability of habitats is the contentious issue of density-dependence among immature *An. gambiae*. Surely there is density-dependent survival in laboratory and pseudonatural circumstances,³⁰ but more work needs to be done to demonstrate the same in nature. Larval densities in such studies (e.g., Refs. 30 and 31) are typically much greater than those commonly observed in natural water (DiukWasser MA and others, unpublished data). Service³³ has reviewed much of this work, citing a variety of ways the question has been addressed and referencing several dozen studies that found evidence for or against density dependence. He could conclude only that "... it may be difficult to prove the existence, or otherwise, of density-dependent population regulation" (p. 757).

Finally, vectorial capacity is essentially a measure of *transmissibility*—the potential to transmit malaria. It is not the same as malaria transmission itself (more accurately estimated by EIR) and certainly not the same as numbers of clinical malaria human cases. Dolo and others⁷ found that the seasonal changes in the sporozoite index in the irrigated zone correspond well to the seasonal changes in anthropophily and parity ratios. Seydou Doumbia and co-workers at MRTC are currently compiling data about EIR in the serial studies of the villages used in this study. It is likely that they will bear directly on the observations reported here.

As to the medical relevance of malaria transmission indices, Sissoko and others⁸ compared the EIR to diagnosed cases of malaria in the three irrigated and three nonirrigated villages that were studied by Dolo and others.⁷ They observed a high correlation between the two in nonirrigated villages, though it was less so in the irrigated ones.

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