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Linking Deforestation to Malaria in the Amazon: Characterization of the Breeding Habitat of the Principal Malaria Vector, *Anopheles darlingi*

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Abstract

This study examined the larval breeding habitat of a major South American malaria vector, *Anopheles darlingi*, in areas with varying degrees of ecologic alteration in the Peruvian Amazon. Water bodies were repeatedly sampled across 112 km of transects along the Iquitos-Nauta road in ecologically varied areas. Field data and satellite imagery were used to determine the landscape composition surrounding each site. Seventeen species of *Anopheles* larvae were collected. *Anopheles darlingi* larvae were present in 87 of 844 sites (10.3%). Sites with *A. darlingi* larvae had an average of 24.1% forest cover, compared with 41.0% for sites without *A. darlingi* (*P* < 0.0001). Multivariate analysis identified seasonality, algae, water body size, presence of human populations, and the amount of forest and secondary growth as significant determinants of *A. darlingi* presence. We conclude that deforestation and associated ecologic alterations are conducive to *A. darlingi* larval presence, and thereby increase malaria risk.

INTRODUCTION

Deforestation in the Amazon rainforest has been linked to the rise in malaria prevalence.[1–4] We examined this relationship in the Peruvian Amazon in an area with varying degrees of deforestation. Our study had three components: 1) a field survey of *Anopheles* larval breeding sites, 2) adult *Anopheles* collection,[5] and 3) a malaria prevalence survey of villagers in the study area. These data were examined in relation to satellite-derived vegetation data. In this study, we describe the ecologic conditions favorable for *Anopheles darlingi* breeding, and the association of larvae with various vegetation types, including forest, secondary forest, and grass or crop land.

In the Northern Amazonian region of Peru, swidden-fallow agriculture has been identified as one of the primary drivers of deforestation.[6–9] Although cattle ranching and logging do occur, they do not play as large a role in deforestation as seen in other regions such as the

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Brazilian Amazon. 10,11 The largest city in this region, Iquitos, experienced significant population growth during the latter half of the 20th century (pop. 305,514 in 1996), the result of new industries such as oil exploration and the coca trade. 12 This growth was accompanied by rural expansion and increased pressure on forested land. Gomez and Ortiz 12 estimated 4,257 hectares of forest cleared per year during the peak deforestation period between 1983 and 1995.

In the midst of this development, a dramatic rise in falciparum and vivax malaria occurred in the region. After eradication efforts in the 1960s and until the early 1990s, malaria prevalence was low (2.1/1,000 in 1992 13). However, in 1997 malaria prevalence reached 343/1,000, 45% of which was a result of *Plasmodium falciparum*. This epidemic was associated with the re-introduction of South America's most significant malaria vector, *A. darlingi*.13 *Anopheles darlingi* was thought to have been eradicated in the 1960s, but was collected again in the early 1990s and is now common throughout the region. 14,15 As de Oliveira-Ferreira and others 16 and Tadei and others 17 have shown through enzyme-linked immunosorbent assay (ELISA) assays for the detection of *P. falciparum* and *P. vivax* circumsporozoite antigens, *A. darlingi* is the most important malaria vector in South America. Flores-Mendoza and others 18 further confirmed that *A. darlingi* is the most important vector in eastern Peru.

Available data on larval ecology indicate that *A. darlingi* is typically a riverine species, 19–21 but *A. darlingi* larvae have also been found in irrigation canals, rice fields, flooded cane fields, and pastures. 22 In Belize, Manguin and others 21 determined that 75% of the breeding sites testing positive for *A. darlingi* larvae were located along river or stream margins, followed by lake margins, small lagoons, and ground pools. They also found that *A. darlingi* larval presence was positively associated with shade and submersed vegetation. In the Upper Orinoco in Venezuela, Rejmankova and others 23 surveyed 46 bodies of water, including lagoons (ponds created from receding river floodplains), forest pools, and forest streams. *Anopheles darlingi* was collected at only one location—a lagoon with submersed macrophytes.

We postulate that deforestation in the Amazon changes the epidemiology of malaria by contributing to a change in *Anopheles* species composition and abundance, which results from the change in breeding site availability. In addition to changes in shade, vegetation, and hydrology resulting from deforestation, settlers often create artificial bodies of water such as fish farms and wells, and introduce new animal species. 24 The construction of roads through tropical rainforests also changes the surrounding ecology through erosion, blockage of streams, and by allowing previously inaccessible areas to become colonized by cattle ranchers, loggers, and farmers, thus exacerbating deforestation. 6,7 In this study, we examine the associations between *A. darlingi* larval presence and ecologic factors, in addition to studying the influence of landscape at larger scales.

MATERIALS AND METHODS

Geography of study area

The study area is located in the northern Peruvian Amazon, spanning the Iquitos-Nauta road (3°S, 73°W) (Figure 1). The road extends 95 km, 72 km of which had been paved by August 2001 (compared with 48 km in March 1999). Further specifics have been described previously.5

Site selection and specifications

Fifty-six sites were selected for *Anopheles* collection.5 Sites were stratified by vegetation type and human population density to obtain an ecologically representative sample (guided

by a 2000 Landsat Thematic Mapper [TM] satellite image and a population census conducted before the start of larval collections). Sixteen sites were in village areas, 12 in lowly populated, deforested areas, 12 in secondary forest, and 16 in primary forest. We then created transects extending 500 m due north and south of the adult *Anopheles* collection site for a total of 1 km. If time permitted, the ends of this transect were extended due west at both ends for 1 km (Figure 1). These transects formed the basis of the larval collection effort. Because of the differing density of vegetation and water bodies, the total length of transects surrounding the 56 sites varied (ranging from 1 to 4 km, for a total of 112 km of transects). This was taken into account in the statistical analysis.

A Landsat TM image from March 12, 2001 was obtained, and 10 landscape categories were identified by unsupervised classification. The 10 landscape categories were collapsed into eight, because three forest categories were indistinguishable using ground-truth data. The eight categories were: "Forest" – closed-canopy and tall forest; "Varillal" – forest growing on sandy soils that support vegetation of lesser height and sparser canopy; "Secondary growth" – successive vegetation after deforestation (~15 years prior); "Shrub" – younger successive vegetation ~5 years after deforestation; "Grass/crop land" – cleared land that may or may not be cultivated and maintained; "Bare surface" – compacted soil, asphalt, concrete structures, and tin roofs; and "Deep water" and "Shallow water" are bodies of water at least 20×20 m in size and not obscured by forest canopy with the former containing little sedimentation and the latter is either shallow or contains large amounts of sediment.

Different grid sizes were examined (200×200 m, 1×1 km, 2×2 km, 5×5 km, and 7×7 km) because the scale that influences *A. darlingi* behavior was unknown. The limit of 7×7 km was chosen because the maximum flight range reported for *A. darlingi* is 7 km. [25] The percentage of each landscape class within the grids was calculated. In addition, the distance to the nearest edge of each of these features was determined. Human population density was ascertained by calculating the number of people within a 200 m, 500 m, 1 km, and 1.5 km radius from the collection site using geo-referenced houses from the accompanying census data obtained by our team. Further information regarding the satellite image processing has been described previously.[5]

Collection method

Transects surrounding the 56 sites were sampled every 3 weeks from September 1, 2000 through August 30, 2001, for a maximum of 16 repetitions for each transect. On a given day, 8 larvae collectors would walk along transects of different sites, spending 8 hours (one work day) per person per site (8 sites per day). All 56 sites were sampled within 1 week and defined as one collection cycle. Upon encountering any body of water (Figure 2), the site was geo-referenced using global positioning system (GPS) and basic ecologic observations were made: type of body of water, water color, water turbidity, current, artificial/natural, permanent/temporary, surrounding vegetation type, circumference or cross section, depth at 1m from the bank, pH (using pH strips), temperature,% shade, soil type, presence of leaf litter, filamentous algae, emergent grasses, aguaje palm (*Mauritia flexuosa*), number of dips, *Anopheles* larvae encountered at the edge, and *Anopheles* larvae encountered at 1 m from edge or center. The circumference (or cross section) was used to determine the number of dips the collector was required to take, using a standard 0.5 L dipper (Bioquip Co., Gardena, CA). At larger bodies of water, 55 dips were taken at various points along the edge, in addition to 30 dips one meter from the edge. At the smallest streams and puddles, 15 dips were taken at the edge and 10 in the center. [26] The collector then placed the Anopheline larvae alive in Whirlpak bags (Bioquip Co.). The Whirlpak bags were transported in a styrofoam box to the laboratory in Iquitos at the end of the day. Each body of water was assigned a unique identifier, consisting of the site number, the transect number, and its exact position along this transect in meters.

Larvae were kept alive in the laboratory until they matured to the fourth instar stage for identification, because earlier stages do not display all of the structures necessary for identification. Larvae from each body of water of each site were kept in separate containers at all times. Some of the fourth instar larvae were allowed to develop into pupae and emerge, at which time they were identified as adults with additional confirmation using the exuviae. A subset of larval specimens was cleared using a 10% solution of NaOH followed by ethanol, and then mounted in balsam for archiving purposes. The remaining larvae were cleared, identified directly, and stored in ethanol. Emergent adult Anophelines and their exuviae were also mounted and identified. Keys by Faran 27 and Consoli and Lourenco-de-Oliveira 28 were used for larval and adult identification.

Statistical analyses

Means reported in Table 1 were computed by collapsing time such that a site was defined as being positive if *A. darlingi* had ever been found at the site. The *P* values reported for these tables were computed using all the data, adjusted for spatial clustering of cases (sites positive for *A. darlingi*). Descriptive analysis for *A. darlingi* breeding site characteristics (means and odds ratios [OR]) are reported with confidence intervals (CI) adjusted for temporal correlation.

A multivariate model was fit to identify variables related to *A. darlingi* presence. The outcome was defined as the number of times *A. darlingi* was detected in a body of water (collection site) during a data collection cycle. Four collection cycles were defined that roughly correspond to seasons: T_1 = Days 1–50 corresponding to March 1–April 19; T_2 = Days 51–99 corresponding to April 20–June 7; T_3 = Days 100–149 corresponding to June 8–July 27; T_4 = Days 150–199 corresponding to July 28–September 15. Sites were visited an average of 1.5 times per collection cycle. Both spatial and temporal correlations were examined using a univariate conditional model of season (collection cycle) and space-time covariance structures that included: spatial semivariograms, spatially weighted errors, transect cluster random effects, autoregressive (AR), unstructured (UN), and a space-time structure. With the exception of AR, UN, and transect cluster random effects, model convergence and computation times were problematic even though Gaussian adaptive quadrature was used in estimation. The AR and UN covariance performed better than any of the spatial covariance structures and no significant spatial correlation remained after modeling temporal correlation using these structures (not the case when controlling for space, i.e., temporal correlation remained after controlling for space). Generalized estimating equations (GEE) were used to fit the model and the quasi-likelihood information criterion (QIC) statistic was used to assess goodness of fit, which is the preferred GoF statistic in GEE models. 29,30

To identify the best set of variables that predict *A. darlingi*, a forward stepwise procedure was used, which involved three steps. First, collinearity and backward selection was performed within five categories of variables of potentially high correlation: "Ecological characteristics," defined as type of body of water and the presence of leaf litter, algae, grasses, and aguaje palm; "Physical characteristics," defined as water color, turbidity, current, soil type, depth, cross section or circumference of the body of water, and the percentage of the water body covered by shade; "Demographic characteristics," defined as population density within 200 m, 500 m, 1 km, and 1.5 km radii, and a binary variable for the presence/absence of people living within these ranges around the site; "Distance," which included the proximity to Iquitos (km) and to specific types of land cover (m); and "Land use and land cover," defined as the percent of specific types of land cover at five scales (200 m, 1 km, 2 km, 5 km, and 7 km). Within each category, collinearity was assessed by examining the correlation of variables (correlations above 0.5 were considered highly collinear, above 0.3 as moderately collinear, and below 0.3 not collinear). Collinear

variables with lower QIC (based on a univariate model) were chosen for inclusion. Once collinear variables were removed, backward selection was performed on the entire set of non-collinear variables in each category. The procedure was slightly modified for the land use category: backward selection was first performed for each scale, then collinearity was assessed across scales, and finally backward selection was again performed on the set of non-collinear variables. The second and third steps of our model selection involved assessment of collinearity of variables across all categories and adding each category to the full model according to their final QIC. For example, demographic variables (people living within 500 m for a collection site) resulted in the lowest QIC with land use variables (forest, secondary growth, and bare surface) having the second lowest. Collinearity among these variables was assessed first and then backward selection was applied. The model proceeded forward by adding the category with the next lowest QIC (physical characteristics), assessing collinearity among the three categories of variables, and performing backward elimination. The GEE was used throughout and an unstructured covariance was assumed as it was shown to have a better fit than the AR structure according to the QIC statistic. In addition, seasonality variables (T1–T4) were included in each model selection step as these variables help control for temporal correlations. Climate variables (precipitation, temperature, and wind velocity) were considered, but were collinear with the four seasonality time points and were not included. All statistical analyses were conducted in SAS (version 9.1, Cary, NC).

RESULTS

Sample and site characteristics

There were 1,224 bodies of water that were sampled over 12 months starting in September 2000. Each body of water was visited an average of 4.5 times (5,524 total visits). However, larval maintenance difficulties resulting in high larval mortality before March 2001 constrained the analysis to samples obtained from March 1 through September 15, 2001. During this period, 844 bodies of water were sampled, with an average of 3.5 visits (2,950 total visits).

Fifty-two percent of the bodies of water sampled were classified as streams, compared with 48% classified as ponds, of which a subset of 39 was fish farms (4.5% of total). The majority of the bodies of water had leaf litter (68%), no visible mats of algae (82%), and clay soil (72%). The mean depth measured at 1 m from the edge, or at the center for small bodies of water, was 27.2 cm. Ponds were small, with an average circumference less than 25 m (62%); however, ponds classified as fish farms were large, with an average circumference over 100 m (81% of fish farms). Fifty-seven percent of the streams sampled had a cross section between 0.5 and 1.5 m; 23% had a cross section of > 2 m. Human population density was low. Only 31% of breeding sites had people living within a 200 m radius, with a mean of 27.4 people (SE = 2.27).

Anopheles prevalence

Between March 1, 2001 and August 31, 2001, 46,553 *Anopheles* larvae were collected, 22,816 *Anopheles* were speciated as larvae, and 1,616 were speciated after they emerged as adults in the laboratory. Forty-seven percent of the larvae were not speciated because of larval mortality caused by fungal infections before reaching the fourth larval instar. Seventeen anopheline species were identified. Only 651 (2.7%) of all larvae identified were *A. darlingi*. *Anopheles darlingi* was the sixth most commonly found *Anopheles* larvae, after *A. triannulatus*, *A. rangeli*, *A. mediopunctatus*, *A. nimbus*, and *A. punctimacula*. Of the 844 individual bodies of water sampled during the latter half of the study period, 87 (10.3%) of the bodies of water tested positive for *A. darlingi* at least once. Fourteen bodies of water

tested positive twice; 7 tested positive 3 times; 4 tested positive 4 times; and 2 tested positive 6 times. Other *Anopheles* species collected included *A. benarrochi, A. oswaldoi, A. nuneztovari, A. argyritarsis, A. mattogrossensis, A. eiseni, A. neomaculipalpus, A. kompi, A. thomasi, A. squamifemur*, and *A. gilesi*.

Landscape features and *A. darlingi* breeding sites

Anopheles darlingi larvae were found most frequently in sites with little forest remaining (< 20% in a 1 × 1 km grid), and least frequently in sites that were surrounded predominantly by forest. For sites with little forest remaining, 17.1% were positive at least once for *A. darlingi*, compared with 10.0% of sites with a moderate amount of forest (20–60%) and 2.3% of primarily forested sites (> %).

Table 1 illustrates scale differences in the distribution of land cover variables according to the presence and absence of *A. darlingi* larvae. This table shows that positive breeding sites were surrounded by less forest and more shrub and crop land than breeding sites where *A. darlingi* was absent. This trend was seen consistently across scales. However, shallow and deep water were more abundant around sites with *A. darlingi* larvae only at the largest scales, and bare surface differences were not significant.

A similar analysis compared the distances from breeding sites with and without *A. darlingi* to the edge of the land cover categories. Results demonstrate that *A. darlingi* breeding sites are situated further from forest, but closer to ecologically altered landscapes: *A. darlingi* positive sites were farther from the edge of varillal (257.2 m versus 179.6 m, $P < 0.05$) and forest (83.8 m versus 50.9 m, $P < 0.05$); and *A. darlingi* positive sites were closer to shrub (46.2 m versus 139.3 m, $P < 0.05$) and grass/crop land (82.1 m versus 177.1 m, $P < 0.05$).

Human population and *A. darlingi* breeding sites

The number of people living within radii of 200, 500, 1,000, and 1,500 of sampled locations were analyzed. Results indicate that human population density was not significantly related to the presence of *A. darlingi*; only the presence of 1 inhabitant in a 500 m radius was significant ($P < 0.01$). Eighty-three percent of positive breeding sites had 1 person living within a 500 m radius, while 51.8% of negative breeding sites had 1 person living within a 500 m radius ($P < 0.01$). The mean number of inhabitants within this radius was larger in sites where *A. darlingi* was present (45.0) than *A. darlingi* negative sites (36.2), but the difference was not statistically significant.

A. darlingi breeding site characteristics

Univariate analysis identified site characteristics favorable to *A. darlingi* breeding: the type of body of water (fish farm OR = 12.4, 95% CI: 7.3–21.3); no current (OR = 2.6, 1.4–4.9) or slow current (OR = 2.5, 1.4–4.6); presence of algal mats (OR = 3.1, 2.2–4.5), emergent grasses (OR = 3.4, 2.3–5.0), and aguaje palm (OR = 5.9, 3.8–9.1). Compared with sites classified as forest, the odds of finding *A. darlingi* were significantly higher in shrub (OR = 3.1, 1.5–6.5), farm (OR = 3.3, 1.6–6.9), and village sites (OR = 2.7, 1.3–5.8). Water depth of > 45 cm, compared with < 10 cm, as measured at 1 m from the bank was positively associated with *A. darlingi* larval presence (OR = 2.6, 1.4–4.8). Sites with < 70% of the body of water covered in shade had almost twice the likelihood of *A. darlingi* larval presence (OR = 1.9, 1.3–2.7), compared with sites with 70% shade cover. For streams and ponds, the larger the size, the higher the likelihood that *A. darlingi* was collected and identified. However, this relationship was significant only for ponds.

Model

Anopheles darlingi presence was best predicted by the model shown in Table 2 (QIC = 425.9). Spatial and temporal correlation was detected before model selection: Ripley's K identified moderate spatial clustering among cases during collection cycle 2, 3, and 4 within 4 km of a particular breeding site, whereas Dat's method indicated significant temporal autocorrelation (A = 3, E(A) = 4.8, P = 0.037). However, no significant spatial correlation existed after controlling for temporal correlation. The QIC indicated good fit of the data in our model.

Time of collection (seasonality) was highly significant, with peak larval presence occurring during the months of June and July. Collection cycles 3 and 4 were not significantly different from each other (P = 0.20), but were significantly higher than cycles 1 and 2 (P < 0.0001). Bodies of water with algal mats had a 2.6 times higher odds of having *A. darlingi* than bodies of water without algal mats (P < 0.0001). Bodies of water with a larger circumference, characteristic primarily of fish farms, were positively correlated with *A. darlingi* presence. Deeper bodies of water 1 m from the edge was positively associated with *A. darlingi*, but the relationship was borderline significant (P = 0.07). The presence of people within a 500 m radius was also strongly correlated with *A. darlingi* presence. Distances to the nearest edge of particular types of land use were standardized before model selection (i.e., transformed to the standard normal scale). Thus, a one standard deviation increase in distance to secondary growth reduces the odds of *A. darlingi* larval presence by 0.80 (P = 0.21). Finally, the model indicates that more forest cover in a 1 km grid surrounding a site has lower odds of *A. darlingi* than sites with less forest cover (P = 0.045), whereas sites with more secondary growth have higher odds compared to sites with less secondary growth. More specifically, a 1% increase in forest cover or secondary growth (1 × 1 km scale) is associated with a 0.98 decrease or 1.06 increase, respectively, in the odds of *A. darlingi* larvae being present.

The predicted probability of *A. darlingi* larval presence by the percent of forest cover and secondary growth is shown in Figure 3. The likelihood of *A. darlingi* presence decreases from ~2% in areas with very little forest cover to less than 1% in areas with more than 50% forest cover. During the peak *A. darlingi* period, the likelihood decreases from ~6–2%. More striking is the relationship between secondary growth and *A. darlingi*: areas with little secondary growth (less than 10%) have a 1–2% probability of having *A. darlingi*, whereas areas with over 40% secondary growth have a 12–20% probability of detecting *A. darlingi*. This increase is even more rapid during the peak *A. darlingi* period.

DISCUSSION

In this study we show that various landscapes and ecologic features associated with deforestation are positively associated with *A. darlingi* larval breeding sites. This was born out in the univariate and multivariate analyses, which showed positive associations with the amount of secondary growth, forest cover, and the breeding site characteristics consistent with these ecologic habitats, even after controlling for the effects of human presence and spatial clustering. This suggests that *A. darlingi* may be especially attracted to conditions resulting from human settlement that is typical for non-nomadic farmers in the Amazon basin. This process consists of settlement and subsequent deforestation of land neighboring the village area for farming, followed by land abandonment after they become infertile and deforestation of adjacent land. [31]

The role of algae in promoting *A. darlingi* breeding may be a result of its biochemical properties and function as an indicator of some underlying process. In Mexico, Bond [32] studied the effect of filamentous algae on *A. pseudopunctopennis'* habitat selection and diet,

and found that the females oviposited nearly exclusively in containers with the algae, and almost never in containers with water alone. Analyzing the gut contents of the larvae, they found that 47% consisted of algae, with the remainder being unspecified organic debris. Thus, algae served as an important source of food, and the mosquitoes were able to select breeding sites containing algae. Rejmankova and others 33 tested oviposition by *A. albimanus* and *A. vestipennis* by exposing them to volatile compounds extracted from cyanobacterial mats and tall dense macrophytes. Each species oviposited at a higher rate when exposed to volatile compounds from their natural habitat. It is likely that *A. darlingi* also uses olfactory cues from algae and decaying leaf litter as a means to select appropriate breeding sites. Furthermore, Dhar and others 34 reported that certain trees, such as neem (*Azadirachta indica*) and reetha (*Sapindus mukorossi*), exert an inhibitory effect on *A. stephansi* and *A. culifacies* oviposition. It is therefore conceivable that certain plants in the Amazon rainforest would also have such inhibitory properties, and influence *Anopheles* breeding preference or avoidance.

Human presence did affect the likelihood of *A. darlingi* larval presence. This may be explained by the availability of humans as bait for the adult mosquitoes, and human alteration of the surrounding ecology, which creates conditions favorable to larval breeding. This study suggests that the altered conditions most responsible for *A. darlingi* larval breeding are the creation of large ponds with algae and the creation of secondary growth.

One of the challenges encountered was the interpretation of the effect of scale. We showed that forest, varillal, secondary growth, shrub, and grass/crop land were consistently associated with *A. darlingi* larval presence across different scales. Although the maximum area of influence considered was ~3.5 km from the breeding site (7×7 km grid), it may extend further. The area of influence most relevant to *A. darlingi* breeding may also differ according to the landscape category. Indeed, before collinearity adjustments, our model identified forest cover at 1, 2, 5, and 7 km scales to be key indicators associated with *A. darlingi*, showing that the area influencing *A. darlingi* breeding behavior appears to be relatively large.

Furthermore, the effect of water (as seen on the satellite image) was not fully captured. The resolution of the satellite image was not sufficient to analyze the roles of shallow (or sediment) and deep water. Virtually none of the streams or ponds sampled in the study appeared in the image. Prior to backward selection of the final model, both shallow (2 km scale) and deep water (5 km scale) were identified as potentially important, but neither remained in the model. Improved classification of water through a study of hydrology and topography of the region may be very instructive in predicting where ponds and streams are likely to form, which could then be correlated with land use and used to predict likely sites for *A. darlingi* breeding.

Related to water and hydrology is the absence of climate variables in our models because of their correlation with collection cycle variables. Although some studies have demonstrated a link between mosquito presence, rainfall, and temperature, our data show that *A. darlingi* larval presence is moderately related to rainfall (higher presence during transition months from the rainy to the dry season) and weakly associated with temperature. We did not observe associations of *A. darlingi* presence with wind speed or humidity. In addition to climate, water temperature and pH were collected during the latter half of the study period, but were recorded for just 7% and 21% of the collections, respectively. Although *A. darlingi* larval presence was associated with a lower temperature and pH, more research is required for our study region.

In the model, large ponds were strongly associated with *A. darlingi* larvae. Many of these large ponds were classified as fish farms in the field, often based on reports by the owners or inhabitants. However, this classification was not always consistent. It is unclear whether *A. darlingi* larval presence was greater in these large bodies of water because of properties inherent to fish farms, or other factors associated with size. Some of the fish most frequently cultured; e.g., boquichico (*Prochilodus nigricans*), gamitana (*Colossoma macropomum*), paco (*Piaractus brachypomus*), sábalo (*Brycon* sp.)35,36 are known to be insectivorous, but the presence of aquatic macrophytes may diminish their ability to prey on *Anopheles* larvae. 35 According to Oregon State University's Pond Dynamics/Aquaculture Collaborative Research Support Program, 37 the practice of aquaculture along the Iquitos-Nauta road commenced around 1991, at which point 22 hectares of pond surface were identified. By 2002, pond surface increased to 200 hectares in this region. As these figures continue to increase, reduction of aquatic macrophytes and algal mats, and the introduction of larvivorous fish may prove beneficial in reducing *A. darlingi* breeding and malaria risk.

In conclusion, this study reveals the close association between the breeding behavior of the dominant malaria vector in the region and human alteration of the natural habitat. Possible environmental interventions to reduce malaria risk in this region, while protecting rainforest ecology and respecting the needs of the farming population, include education pertaining to fish farming practices (e.g., maintaining the pond free of aquatic vegetation) and policies that promote sustainable agriculture and reduce land abandonment. Although secondary growth is favored for forest regeneration, encouraging agricultural methods that do not deplete the fragile soil, while keeping the land clear of the type of water body favored by *A. darlingi* (large ponds, and the presence of leaf litter, algae, and emergent grasses) may curb the cycle of deforestation, abandonment, and proliferation of secondary growth, thereby reducing the number of *A. darlingi* breeding sites.

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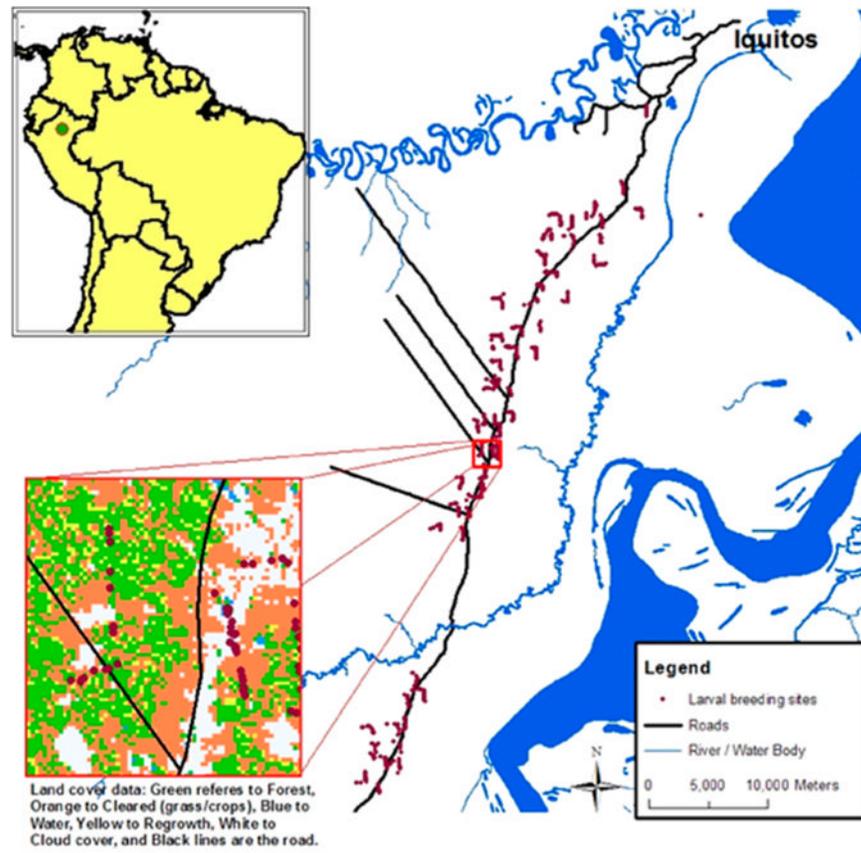
Figure 1.
Map of the study area. This figure appears in color at www.ajtmh.org.

Figure 2.
(**A**) Bodies of water in forest, (**B**) secondary growth, (**C**) grass/crop land, and (**D**) fish farm (aguaje palm depicted center-left). This figure appears in color at www.ajtmh.org
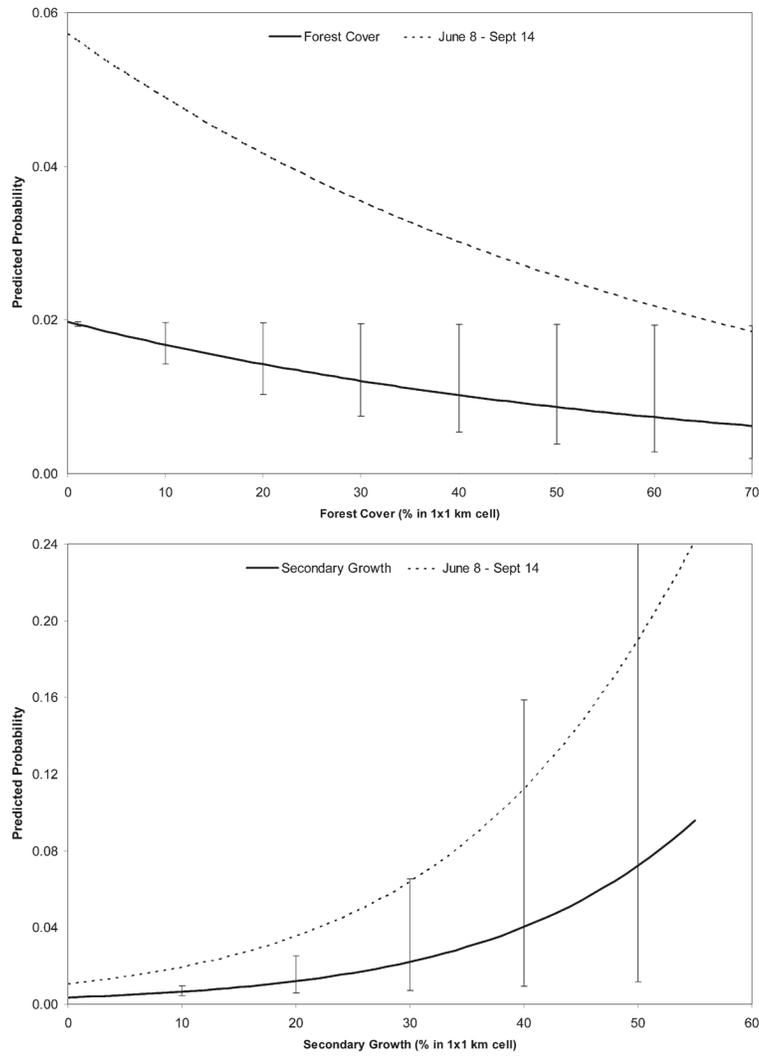
Figure 3.
Predicted probability (adjusted variable plot) of *A. darlingi* larvae presence by percent
Forest Cover and Secondary Growth on a 1×1 km grid surrounding a site. Land use is
graphed according to its approximate range in the data: Forest cover ranges from 0.75% to
93%; Secondary growth ranges from 1% to 47%.

Table 1

Comparison of mean percentages of each landscape category by the presence or absence of *A. darlingi* larvae

Scale	0.2 km		1 km		2 km		5 km		7 km	
Land cover	Present	absent	Present	absent	Present	absent	Present	absent	Present	absent
Varilla[a]	1.5*	3.5	1.7†	3.8	2.5†	4.1	3.3†	4.3	3.8†	4.6
Forest[b]	16.8‡	38.5	24.1‡	41.0	33.3‡	46.0	41.1‡	50.6	44.2‡	52.8
Secondary Growth[c]	21.2	17.5	19.7*	17.2	20.4†	17.8	20.5†	17.4	19.7†	17.0
Shrub[d]	35.0‡	19.7	32.4‡	21.2	27.2‡	18.6	23.4‡	17.4	22.2‡	16.7
Grass/crop land[e]	24.3	18.7	20.8†	15.5	15.6†	12.5	10.8†	9.6	9.3†	8.2
Bare surface[f]	0.8	1.5	0.9	1.0	0.8	0.6	0.4	0.4	0.4	0.3
Shallow water[g]	0.3	0.5	0.2	0.2	0.1	0.1	0.2*	0.1	0.2†	0.1
Deep water[h]	0.2	0.2	0.3	0.2	0.2	0.2	0.3†	0.2	0.4†	0.2

*
$P < 0.05$;

†
$P < 0.01$;

‡
$P < 0.0001$.

[a] Forests with poor drainage, sandy soils, and a relatively sparse canopy.

[b] Tall forest, dense canopy, with little evidence of human exploitation.

[c] Tall, older secondary forest (deforested ~15 years before the study) with a canopy height of 5–15 m.

[d] Younger secondary growth (deforested ~5 years before the study) with a height of 2–5 m.

[e] Grass or low vegetation (< 2 m).

[f] Compacted soil, asphalt, tin roofs.

[g] Bodies of water that are at least 20 × 20 m (for satellite detection), deep, and with low amounts of sedimentation.

[h] Bodies of water that are at least 20 × 20 m, shallow, and/or heavy sedimentation.

Table 2

Multivariate model of *Anopheles darlingi* presence in the Peruvian Amazon

Variable category and description	Odds ratio	95% CI (LCL–UCL)	*P* value
Collection cycle			
Cycle 1: March 1–April 19	Reference		
Cycle 2: April 20–June 7	3.34	1.37–8.12	0.0079
Cycle 3: June 8–July 27	17.89	7.56–42.34	< 0.0001
Cycle 4: July 28–September 15	13.50	5.75–31.67	< 0.0001
Ecological Characteristics of the site			
Filamentous algae present	2.64	1.69–4.11	< 0.0001
Physical characteristics of the site			
Circumference, pond, well, or fish farm (m)			
1 = 0–10 m	Reference		
2 = 11–25 m	1.18	0.57–2.43	0.6561
3 = 26–50 m	1.81	0.79–4.16	0.1623
4 = 51–100 m	5.55	2.69–11.45	< 0.0001
5 = > 100 m	6.73	3.77–12.03	< 0.0001
Depth (m)[†]	1.18	0.99–1.41	0.0683
Demographic characteristics surrounding site			
People live within 500 m buffer	2.55	1.28–5.09	0.0077
Distance measures			
Distance to secondary growth[†]	0.80	0.57–1.13	0.2094
Land use and land cover surrounding site			
% Forest 1 × 1 km	0.98	0.97–1.00	0.045
% Secondary growth 1 × 1 km	1.06	1.02–1.10	0.001
QIC (goodness of fit)	425.94		

[*] CI = confidence interval; LCL = lower confidence limit; UCL = upper confidence limit.

[†] Depth and Distance are standardized to have mean 0 and standard deviation of 1 by subtracting the mean and dividing by the standard deviation for each observation. Means and (SD) are: secondary growth 59.7 (68.3); depth 26.7 (28.9).

Model is fit using generalized estimating equations (GEE) with an unstructured covariance. The log-odds value for the intercept is −7.74, which can be used to estimate predicted values.