Variation in larval ecology and predictors of malaria risk across regions of Madagascar

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Abstract
Background: Deforestation and land use change is widespread in Madagascar, altering local ecosystems and creating opportunities for disease vectors, such as the Anopheles mosquito, to proliferate and more easily reach vulnerable, rural populations. Knowledge of risk factors associated with malaria infections is growing globally, but these associations remain understudied across Madagascar’s diverse ecosystems experiencing rapid ecological change. This study aims to uncover socioeconomic, demographic, and ecological risk factors for malaria infection across regions through analysis of a large, cross-sectional dataset.

Methods: Here, we assess (1) the ecological correlates of malaria vector breeding through larval surveys, and (2) the socioeconomic, demographic, and ecological risk factors for malaria infection in four ecological regions of rural Madagascar. We determine risk factors using multilevel models for each unique ecological region of included in the study.

Results: We find that regardless of the region, the presence of forms of aquatic agriculture around a community are predictive of the presence of malaria vectors. We determine that ecological and socioeconomic risk factors for malaria infection vary dramatically across study regions and range in their complexity.

Conclusions: Risk factors for malaria transmission differ dramatically across regions of Madagascar. These results may help stratifying current malaria control efforts in Madagascar beyond the scope of existing interventions.

Background
Agricultural expansion and associated changes in land use, such as deforestation for food production, can create new microhabitats and alter the distribution or density of species, including mosquitoes. These changes can increase the number of suitable larval habitats for mosquito species, including Anopheles mosquitoes, the vectors for human malaria (1-4){Tantely, 2016 #8;Myers, 2013 #116}. Changes in vector populations are mediated by local climate factors like temperature, rainfall, relative humidity, and the suitability of water habitats for mosquito larvae (5). For example, deforestation in the Brazilian Amazon has increased suitable breeding habitats and conditions for certain Anopheles
species, increasing vector density, while in the western Kenyan highlands, deforestation and changes to local ecology have lowered the survival time of certain *Anopheles* species (6, 7). In addition to ecological variables influencing the population dynamics, biting rates, and malaria transmission potential of *Anopheles* vectors (7-10), human behavior and resource access also play key roles in mediating malaria risk. Examples include bed net use, health and housing infrastructure, baseline health status, and treatment/prevention access (11, 12). These ecological and socioeconomic factors, and interactions among them, may also vary across geographic contexts. Thus, an individual’s risk for malaria infection results from a complex process involving socioeconomic and ecological factors acting at the individual, household, and community levels, and likely vary regionally.

Though knowledge of risk factors for malaria infection is growing globally, only a limited number of studies explore how malaria risk factors vary across Madagascar’s diverse, rapidly transforming landscapes. Nationally, large-scale anthropogenic deforestation and land-use change are widespread (13-16); 63% of Madagascar’s population of 26,262,368 lives in small farming communities in rural areas (17). However, the distribution of land-use change rates, known malaria risk factors, and malaria prevalence patterns vary regionally in Madagascar (18-21). Communities living in the central highlands, for example, experience unstable transmission and low prevalence coupled with a history of intense forest clearance and actively cultivated landscapes (16, 22). In Madagascar’s east and west coasts, deforestation rates are increasing. However, communities living in western dry forests experience more seasonal transmission while those living in eastern moist forests experience more consistent transmission throughout the year (23).

In rural Madagascar, humans live at the interface of their communities and their surrounding environments, spending substantial time in adjacent forests and agricultural fields. These interactions predicate many facets of population health, including nutrition, exposure to infections, and sanitation practices (24-26). However, little information exists on the extent that these exposures alter malaria risk across ecological contexts in Madagascar. Additionally, the natural and modified habitats surrounding human communities differ greatly between ecological regions of Madagascar (27). In eastern humid tropical forest, *Anopheles* mosquitoes are more abundant in agricultural land and
village environments than surrounding forests, indicating forest clearance as a possible driver of local malaria transmission (28). A cross-sectional study of communities in southeast Madagascar demonstrated that bed net use protected against malaria, while rural individuals in lower socioeconomic brackets, between 6-14 years of age, were at higher risk of infection (23, 29). However, they did not collect information on malaria vector distribution, nor did they analyze these patterns in other regions.

Additionally, *Anopheles* species have varying vectorial capacities and larval habitat preferences (30-32). However, little is known about the extent vector populations within and around local communities vary and how they respond to ecological change. For example, it has been hypothesized that as local ecology changes, opportunistic vectors move into places previously uninhabited, altering the profile of malaria risk across space (33). However, understanding how the distribution of malaria risk has or will differ due to land use change is difficult given insufficient data on current distributions of malaria vector communities in rural communities. To this end, we sought to characterize malaria risk factors and local vector ecology among rural communities in multiple distinct ecological regions of Madagascar.

A recent, large-scale cross-sectional survey showed marked heterogeneity in malaria prevalence between ecological regions and communities within regions in Madagascar (Rice et. al., 2020 In Prep.). In particular, the prevalence in some rural communities in southeast and southwest Madagascar was substantially higher than national reporting. Likewise, Kang et al (2018) estimated a 4-fold increase in the proportion of areas in Madagascar experiencing high transmission has occurred since 2011 (34). This motivates our study to identify risk factors associated with malaria infection in these areas to implement more efficient control methods.

The linkages between Madagascar’s unique ecologies, the distribution of malaria vectors, and malaria outcomes in human populations are unclear. As such, this study intends to identify (1) the ecological correlates of malaria vector larval presence through surveys of larval *Anopheles* mosquitoes within communities, and (2) the key socioeconomic, demographic, and ecological factors associated with malaria infection in four distinct ecological regions of rural Madagascar.
Methods

Site Selection and Population Description

Malaria outcome, socioeconomic, and demographic data were obtained from a cross-sectional study of 5,602 participants across 24 communities in four regions of Madagascar (Figure 1) (Rice et al 2020 In Prep., Golden, Rice et al 2020 Submitted). We conducted larval vector surveys and subsequent analyses in these four study regions. The Rice et al., 2020 study included data from a second sub-region of the east coast (their NE region) but malaria prevalence estimates and study design differed and thus this region is omitted here. Each region will be referred to by their geographical location throughout the paper, these are listed here along with their corresponding administrative districts: southeast, SE = Vatovavy Fitovinany: Mananjary district; southwest, SW = Atsimo Andrefana: Toliara II district; west coast, WC = Atsimo Andrefana: Morombe district; and high plateau, HP = Amoron’i Mania: Ambositra, Ambatofinandrahana, and Fandriana districts.

Sampling regions were selected to represent the different typologies of malaria transmission patterns in Madagascar (36,37). These zones correspond to Madagascar’s distinct ecological regions, with differing precipitation and vegetation patterns (38). The landscapes in these regions are a mosaic of original, natural vegetation and human modified areas where secondary forest, cultivated land, and pasture dominate. Ecotype descriptions were adapted from Goodman et al. 2018 and Moat & Smith 2007 (27, 35). The SE region falls under the ecotype humid forest, characterized by degraded humid forest, wooded grassland-brushland mosaic, lowland moist evergreen forest, secondary forest, and secondary grasslands. The SW region falls under the ecotype dry spiny forest and is characterized by dry spiny forest-thicket, degraded dry spiny forest-thicket, mangroves, secondary forest, and secondary grasslands. The WC region falls under the ecotype dry deciduous forest and is characterized as dry forest, dry spiny forest-thicket, mangroves, secondary forest, and secondary grasslands. Finally, the HP region ecotype is a wooded grassland-bushland mosaic, characterized by plateau grassland-bushland mosaic, secondary forest, and secondary grasslands. The SW and WC regions denoted in this study are both part of the Atsimo Andrefana region of Madagascar, but these regions are nearly 200km from each other and are characterized by distinct ecology thus providing us
motivation to consider them as separate.

In this study, communities are defined as clusters of households (the typical rural settlements in these areas, tanàna or tanàna kely) and the adjacent peri-domicile areas. Sites are defined as communities and the surrounding mosaic of cultivated land, uncultivated human-modified land, and undisturbed land. In all communities, sampling was performed by randomly selecting ~50 households with two selection criteria: 1) a child five years of age or younger; and 2) a woman of reproductive age (15-49 years) (see Golden, Rice et al 2020 Submitted for more details on enrollment). Six communities were sampled in each region, three more proximate to an urban area (defined as <20km) and three more distant (defined as >20km) from an urban area. 33-53 households (168-304 individuals) were sampled per site and 242-309 households (1,461-1,665 individuals) were sampled per region, though only complete cases were used for the analysis.

**Malaria outcome and associated socio-demographic variables**

Health and survey data were collected at the individual and household level (Golden, Rice et al., 2020 Submitted). In total, 10 of these variables were used (see Supplementary Table 1). Rapid diagnostic tests (RDTs) were used to diagnose malaria (SD Bioline Malaria Ag P.f/Pan RDT). False negativity and the contribution of infections by other species such as *Plasmodium vivax* are known limitations of RDT based surveys (36), however, we justify using RDT positivity as a reliable proxy for malaria infection as previous studies using these RDTs in Madagascar found high agreement when comparing between RDT results and molecular detection via PCR (over 87% sensitivity and specificity) (36-38). Regarding *Plasmodium* species, over 96% of malaria cases in Madagascar are due to *P. falciparum* (39, 40) and in previous molecular confirmation of RDT positive cases from the east coast of Madagascar, over 98% were *P. falciparum* infections (37). A total of 776 individuals were positive by RDT for malaria, varying from 6-381 individuals per region.

**Ecological variables and Anopheles larval sampling**

To complement the data described above, ecological surveys were conducted in the 24 study communities to determine ecological risk factors for malaria transmission between the months of May 2017 – August 2017. All potential and positive larval habitats were mapped with a Garmin Oregon
550t prior to larval collection. All habitats within a 25m radius of households or the community perimeter were geocoded and mosquito larvae were sampled from each. We also conducted transect mapping to identify larval habitats and species composition of the local ecology of each research site (all mosquito sampling methods described in detail in ESM2). The overlap between larval sampling and RDT testing in the SE and SW differed by up to five and up to three months, respectively. The peak transmission period in these regions has been found to be from January to May or June, so all larvae were still collected during the peak transmission season for each region (20). Thus, we would not expect the mismatched sampling times to bias our results.

All larvae were sorted by genus and instar prior to identification by morphological examination. *Anopheles gambiae* is a complex of species represented by *Anopheles gambiae sensu stricto*, *Anopheles arabiensis*, and *Anopheles merus* in Madagascar. These three species are indistinguishable by morphology, and necessitate molecular identification (42). As such, larval specimens identified by morphology will be collectively referred to as in *Anopheles gambiae* complex. All third/fourth instar larvae were identified morphologically to the lowest possible taxonomic level using the taxonomic key presented in Grjebine (1966) at Institut Pasteur Madagascar (43). Some *Anopheles* larvae were unidentifiable to species because they were damaged in the sampling process which removed features necessary to distinguish species.

Distance from the household waypoint to the edge of the nearest aquatic agriculture was calculated. For each household, we calculated the percentage area that was aquatic agriculture within a 1km buffer. Finally, we enumerated all possible mosquito breeding habitats within a 25m buffer around all households. All spatial variables were calculated using ArcMap and all satellite imagery was purchased from the AIRBUS Spot 6 and 7 Satellites at 1.5m spatial resolution (41).

**Statistical Analyses**

To assess the association between ecological correlates and mosquito larvae presence in habitats, we modelled five outcomes: (A) Presence/absence of *Anopheles* larvae, (B) Presence/absence of *Anopheles gambiae* complex larvae, (C) Presence/absence of *Anopheles mascarensis* larvae, (D) Presence/absence of *Anopheles coustani* larvae, and (E) Presence/absence of *Anopheles squamosus*
larvae. For model (A), data were restricted to habitats containing any genus of mosquito larvae. For models (B)-(E), habitats included in the model were only those with *Anopheles* mosquito larvae present, which was a subset of all habitats. Predictors included in model (A) were the Normalized Difference Vegetation Index (NDVI, proxy of green vegetation cover) at each habitat, habitat type (aquatic agriculture, ponds, containers), percent of forest cover in a 1km radius around the centroid of each site, soil moisture of each site, temperature of each site (mean monthly), precipitation at each site (mean monthly), and percent of aquatic agriculture in a 1km radius around the centroid of each site (ESM 1 lists variables/sources). Variables included in models (B)-(E) were identical to model (A), with indicator variables for the presence of each *Anopheles* species. We analyzed these data using logistic mixed-effects models with random effects at the site level.

To assess the ecological and socioeconomic correlates of malaria prevalence across the four study regions, we employed four multilevel logistic regression models, one for each region, with random effects for households nested within communities. Our outcome of interest was malaria infection status, as measured by individual RDT result. We employed separate models for each region due to the high variation in malaria prevalence (Rice et. al., 2020 *In Prep.*) and ecological setting (27).

For the SE and WC regions we began with the same full model, and for SW and HP we began with slightly modified models. The difference in models was due to household hamlet ownership in SW and HP having none or limited heterogeneity. We excluded incomplete cases from analyses (n=214). To test for bias induced by complete case analysis, we assessed correlations between remaining individuals and the outcome of interest. The correlation estimates from these data were within the confidence bounds of the correlation estimates from the full dataset, and thus we conclude no significant change in estimates after exclusion of individuals with missing data. All statistical analyses were conducted in R v3.5.1 with the lmerTest package (44, 45).

Results
A total of 4661 individuals were included in the study after exclusion of incomplete cases (Figure 1). 61.9% of individuals surveyed reported using bed nets at the time of the survey. Bed net coverage during sampling was highest in the SE (95.1%), then SW (80.2%), WC (75.2%), and HP (12.3%). Of
those using bed nets, 35.9% stated that their bed net had holes. 41.4% of non-bed net users were children under 18 years, and 52.7% were female.

From larval sampling, a total of 661 *Anopheles* larvae were identified, of which 448 were third/fourth instar and identifiable to the species/species complex level. *Anopheles* larvae were present in 16 of 24 study sites and in every region. The most common species of *Anopheles* identified was *Anopheles gambiae* complex (43.8% of all larvae), followed by *Anopheles coustani*, *Anopheles squamosus*, and *Anopheles mascarensis* (Figure 2). 70% of *Anopheles* species were collected from aquatic agriculture systems. *Anopheles coustani* was dominant in the SE, HP, and WC, while *Anopheles gambiae* was dominant in the Southwest. Aquatic agriculture covered 0% to 56.4% of the area in a 1km radius around a household. The average number of peridomicile habitats varied by region (from 0.24 to 2.82), with larger numbers on average existing in HP (2.82 habitats) and SE (2.71 habitats) compared to SW (0.24 habitats) and WC (0.37 habitats).

**Ecological Correlates of Mosquito Larvae Presence**

The variables in larval models B-E predicting presence/absence of *Anopheles gambiae* complex, *Anopheles mascarensis*, *Anopheles coustani*, and *Anopheles squamosus* larvae were not statistically significant. The only statistically significant variable (P<0.05) in larval model (A), which predicted presence/absence of *Anopheles* larvae in each habitat, was if the habitat was aquatic agriculture (Table 1). Since no other variable was statistically significant, risk factors including socioeconomic, demographic, and further ecological variables were explored as potential risk factors.

**Ecological and Socioeconomic Correlates to Malaria Prevalence**

**Southeast (Vatovavy Fitovinany, SE)**

Six variables were statistically significant (P<0.05) for SE Madagascar and were associated with an individual’s increased odds of having malaria (Table 2). These variables, from most influential in increasing the odds of malaria infection in an individual to the least influential, were: individuals living in more remote communities (>20km from an urban area); the presence of *Anopheles* larvae within communities; each of the other age groups, 5 to 15, 15 to 64, and 65+; if the individual lived in a household that owns a hamlet; and if the individual was a male. .
**Southwest (Atsimo Andrefana: Toliara II District, SW)**

One variable was statistically significant (P<0.05) for SW Madagascar and was associated with an individual’s increased odds of having malaria: presence of *Anopheles* larvae in a community.

**Central West Coast (Atsimo Andrefana: Morombe District, WC)**

Eight variables were statistically significant (P<0.05) for WC Madagascar, four of which were associated with an individual’s increased odds of having malaria and four of which was associated with an individual’s decreased odds of malaria (i.e. a protective effect). Variables associated with an increased odds of having malaria, from most to least influential, were: each of the age groups, 5 to 15, 15 to 64, and 65; and if the individual was male. Variables associated with a decreased odds of having malaria, from most influential to least influential, were: if the individual lives in a household that owns a hamlet; if the individual lives >20km from an urban area; if the individual lived in a household with a high school-educated head of household; and if the individual uses bed net.

**High Plateau (Amoron’i Mania, HP)**

The malaria prevalence in this region was 0.4% overall (range 0%-0.97% by site), and therefore limited heterogeneity exists in the outcome variable of interest. Estimates for this region are unstable (random effects variation: 3.16x10^{14}) and should be interpreted with caution. No variables included in this region’s model were statistically significant.

**Discussion**

To our knowledge, this is the first, most comprehensive study on risk factors for *Anopheles* larvae presence and malaria transmission across regions of Madagascar with distinct ecologies, climates, and transmission patterns. The goals of this study were two-fold: (1) to understand potential ecological drivers of *Anopheles* mosquito presence in peri-domicile habitats across rural Madagascar and (2) to identify socioeconomic, demographic, ecological, and geographic risk factors associated with malaria infection. We found that *Anopheles* larvae were more commonly found in peri-domicile aquatic agriculture as compared to other types of peri-domicile habitat, but that no other ecological variables predicted peri-domicile *Anopheles* larvae presence. We found differences in risk factors between study regions, with some regions demonstrating more complex suites of risk factors than
others. For example, *Anopheles* presence in peri-domicile habitat predicted an increased risk of malaria in the SE and SW but was not in the WC. Current vector control efforts in Madagascar target adult mosquitoes, however, our data suggest that locally managed control of peri-domicile larval sources could be an effective, novel intervention in SE and SW Madagascar.

This study has limitations, namely diagnosis by RDT as discussed above, and the scope of larval sampling. More accurate testing methods, like PCR of malaria parasites, may reduce bias introduced by RDT sensitivity/specificity. Additionally, most sampling of larvae was limited to habitats within 25m of households. Malaria prevalence in some communities with few peridomicile habitats was high compared to regional averages (17% in site SW.3, few peridomicile habitats but bordered by valley of rice fields ~0.5km away). *Anopheles gambiae* adults have been observed to have maximum flight distances of 0.2 to 6.4km (47). Larval sources more distant from households may be responsible for risk but little evidence exists (46). In support, in the SE, hamlet ownership increased malaria risk. We observed that household proximity to larval sources did not predict malaria risk in the WC region, which had the highest mean prevalence. As such, vector management may need to account for more distant larval sources.

*Anopheles* species composition varied by site, region, habitat preference, and relative abundance (Figure 2). Within the *Anopheles gambiae* species complex, *Anopheles gambiae* and *Anopheles arabiensis* oviposit in freshwater sites, while *Anopheles merus* targets brackish habitats (42). These three species are known as major malaria vectors in Madagascar (42). *Anopheles mascarensis* is a secondary major vector in Madagascar, and breeds in fresh and brackish habitats (48). Recent findings showed *Anopheles coustani* was a competent malaria vector in Madagascar for both *P. vivax* and *P. falciparum* (49). *Anopheles coustani* oviposit in freshwater and brackish habitats (30, 43). *Anopheles squamosus* is suspected to mainly transmit malaria in the HP region due to high abundance (48). The results of our modelling of larval habitat preference align with other studies in Madagascar. Marrama et al. (1995) showed rice fields had the highest percentage of mosquito larvae and provide half of all positive larval breeding habitats (50). Zohdy et al. (2016) demonstrated that *Anopheles* species are the dominant mosquitoes in agricultural and village settings, and are the
minority in forest settings (28).

According to the national Malaria Indicator Survey conducted between May and July 2016, countrywide prevalence of malaria infection in children under 5 is about 6%, and regional prevalence ranges from 1%-15% (51). Though our data are not nationally representative, our site prevalence estimates indicate high heterogeneity within regions, and that regional/national statistics should be interpreted cautiously. Our data demonstrate high variation between sites and alarmingly high prevalence in the WC and SE. In the WC, drivers of increased malaria risk were higher urbanicity, living in a household with a less-educated head, and not using a bed net. In the SE, drivers of increased malaria were the presence of vector larvae in peridomicile habitat and lower urbanicity. Bed net usage significantly reduced an individual’s risk for malaria in the WC. However, coverage in the WC was significantly lower than coverage in the SE and SW ($\chi^2$-test for independence, $p<0.001$). Our data suggest that interventions focused on improving bed net coverage in the WC, as well as education and behavior change campaigns, are high priority.

Different risk factors are associated with malaria infection in each region (Table 2, Figure 3). Each region varies in the site-, household-, and individual-level factors that are most important for predicting infection and determination of suitable control strategies. In the SE and the SW, the presence of *Anopheles* larvae was associated with an increased risk of malaria infection. The SW region is dry, with average annual rainfall below 400mm (52). This value is low compared to other study regions and accompanies a shift in dominant agriculture from flooded rice farming to dry-land farming. One out of the six SW sites had any form of aquatic agriculture, however, *Anopheles* larvae were found in three of the six surveyed sites. In sites without rice fields, larvae were found in small, man-made ponds. Additionally, in both sites, children under 5 had malaria infections, indicative of local transmission as children are less likely to travel outside the community (12). *Anopheles* larvae presence in these two sites was confined to a few small, easily identifiable locations that may be driving sustained transmission at the community level. In the SW region, application of larvicide to these few, identifiable *Anopheles* habitats may reduce malaria transmission burden significantly. Since water in these habitats is used for human consumption, a non-toxic larvicide is recommended.
The relationship between rurality and malaria risk also demonstrates regional heterogeneity. In the SE region, individuals living closer to urban areas were at lower risk of malaria infection. This may reflect a relationship between less remote sites and more distant *Anopheles* breeding habitats, more accessible healthcare, cash income from markets, and wage labor access which can support prevention or treatment (54). The opposite was found in the WC region. Here, the urban center is coastal and near a gradually widening river estuary area. Communities near this center sit closer to slow-moving water, some of which has been diverted to flooding rice fields. Increases in wetland areas around communities proximate to the coastal urban center may increase *Anopheles* breeding habitats near less remote sites. *Anopheles mascarensis* and *Anopheles coustani* have been found in brackish water, and both were present five of six sites in the WC. In the SW and HP, rurality had no significant effect on malaria prevalence. Of note, more remote and less remote sites generally had better road access in the SW and HP than in the SE and WC. As such, living in more remote versus less remote sites in the SW and HP may not be as different in ecological, occupational, and accessibility factors as in the SE and WC.

In the SE region, individuals were at higher risk of having malaria if they lived in a household owning a hamlet. Individuals in hamlets may be at higher risk of malaria infection because hamlets may be in locations with higher vector concentrations (i.e., near flooded rice paddies), have less protective household structures, or have fewer bed nets. Households owning hamlets may also be most dependent on seasonal agriculture, which may bring people into closer, more frequent contact with *Anopheles*-dense habitats. In contrast, households owning hamlets in the WC were protected against malaria infection, which may be explained by local ecology. In both regions, hamlets are located away from communities and other hamlets, but in the SE region, they are located in ecology rife with mosquito habitats. Hamlets in the WC are in drier habitats where mosquito density may be lower than the center of communities, often located near water sources. Our findings indicate the need for future studies on vector exposure risk for hamlet owners and agricultural activity in the SE and WC of Madagascar.
In the WC, heads of households with a high school education or higher imparted lower malaria infection risk for all household members. Other studies demonstrate that individuals living in households with more educated household leaders have improved awareness of family health (55). The observed association in the WC may indicate the need to increase awareness of symptoms and prevention mechanisms, such as bed net use.

In the SE and WC regions, individuals 5+ years old and males were more susceptible to malaria infection compared to women and children under five. Women and children under five may spend more time nearer to households where transmission is less severe. Furthermore, individuals not using a bed net were not at different risk than individuals using a bed net. However, bed net use in the SE was 95.1%, so sample size of those not using bed nets was limited. These relationships show that transmission is likely occurring outside households/community bounds. Bed nets remain effective in reducing malaria burden overall, but additional control measures may be needed in these areas to reduce transmission, such as better testing and treatment for high-risk individuals in the SE.

Contrarily, bed net use in the WC protected against malaria infection. This association demonstrate that transmission of malaria may occur in households and communities proper. Therefore, distribution of bed nets in the WC region to ensure universal coverage may be an effective control strategy.

The results of this study demonstrate the need for spatially targeted malaria control strategies in Madagascar. Each geographical context should be considered independently prior to employing interventions. Malaria stratification, the process of classifying regions based on malaria risk to aid in resource distribution, has existed since the 1940s (56). However, modern techniques like remote sensing, coupled with a growing case study literature, have amplified effectiveness of this stratification (57, 58). Though the national malaria control program of Madagascar stratifies by broad geographical zones, more granular stratification of control measures is needed. Our results help clarify risk factors to augment and tailor current malaria control programs according to socioeconomics, ecology, and geography. These data suggest the potential merits of sub-national, sub-regional stratification of malaria control efforts, specifically: (i) applying larvicide to peri-domicile habitats in the SE and SW, (ii) additional effort in distributing bed nets and prevention education in all
regions but most heavily in the WC, and (iii) targeting all family members, but most acutely males over the age of 5, for active case detection in the SE and WC.

Declarations

*Ethics approval and consent to participate*

All households were recruited and enrolled, and each individual consented or assented, following our IRB approved study (Protocol #16-0166, Committee on the Use of Human Subjects, Office of Human Research Administration at the Harvard T.H. Chan School of Public Health). The study was also reviewed and approved by the Malagasy Ministry of Health and the ethical review board at the Institut National de Santé Publique et Communautaire (INSPC). All point-of-care health results (anemia and malaria) were provided by our team of health professionals to the study subjects including access to free treatment and referrals to local clinics. All health results from laboratory analyses that were conducted outside of the study area will be returned to participants.

*Availability of data and materials*

Data and code for all models in this manuscript can be accessed here:

https://figshare.com/projects/Variation_in_larval_ecology_and_predictors_of_malaria_risk_across_regions_of_Madagascar/78159

*Competing interests*

We declare we have no competing interests

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*Authors' contributions*

NJA, CDG, BLR, MCC planned and designed the project. NJA, CDG, BLR, LMT, GNE, HJR performed data collection and analysis. RG, LMT oversaw mosquito larvae identification in Madagascar. NJA wrote an
initial draft of the manuscript. All authors contributed to manuscript revisions and interpretation of results.

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Abbreviations

SE = Southeast, Vatovavy Fitovinany: Mananjary district
SW = Southwest, Atsimo Andrefana: Toliara II district
WC = West coast, Atsimo Andrefana: Morombe district
HP = High plateau, Amoron’i Mania: Ambositra, Ambatofinandrahana, and Fandriana districts
RDT = Rapid diagnostic test

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Tables

Table 1

| Variable                        | Odds Ratio | Lower 95%CI |
|---------------------------------|------------|-------------|
| Habitat Level Variables         |            |             |
| Habitat Type (Ref = Containers) |            |             |
| Aquatic agriculture             | 6.36*      | 1.982       |
| Pond                            | 2.07       | 0.199       |
| NDVI                            | 1.70       | 0.066       |
| Site Level Variables            |            |             |
| Percent Forest Cover 2km Radius | 1.04       | 0.818       |
| Soil moisture                   | 6.75E-05   | 3.38E-27    |
| Log(Mean Monthly Temperature)   | 1.83       | 0.00047     |
| Log(Mean Monthly Precipitation) | 0.35       | 0.019       |
| Percent Aquatic Agriculture 1km Radius | 26.38     | 0.0044     |

Random Effects Variance (SD)

Site: 0.00 (0.00)

Note:

Table 2

| Site                      | Odds of an individual being RDT+ for P. falciparum |
|---------------------------|----------------------------------------------------|
| Vatovavy Fitovinany, SE   |                                                    |
| Anoph eles m              | 4.39** (2.92, 6.59)                                |
| Atsimo Andrefana (Southwest, SW) |                                  |
| Anoph eles m              | 12.70** (3.33, 48.43)                              |
| Atsimo Andrefana (W)      | 0.67 (0.3)                                         |
| Variable                                                                 | Coefficient | 95% CI          | p-value |
|-------------------------------------------------------------------------|-------------|-----------------|---------|
| Mosquitoes present in permanent habitats >20 km from urban center       | 0.69        | (0.29, 1.61)    | 0.47    |
| Household 1-unit increase in count of permanent larval habitats        | 0.95        | (0.87, 1.03)    | 0.91    |
| Distance from household to nearest aquatic agriculture (km)             | 2.51        | (0.24, 26.17)   | 2.05    |
| % of land used as aquatic agriculture 1 km around household             | 1.10        | (0.99, 1.24)    | 1.01    |
| Head of household high school education                                | 0.85        | (0.44, 1.64)    | 0.54**  |
| House | 1.27 | (0.82, 2.08) | 0.72 | (0.32, 1.64) | 1.09 | (0.7: |
|-------|------|-------------|------|-------------|------|------|
| House | 1.20* | (1.01, 2.04) | N/A | N/A | 0.13* | (0.0: |

| Individual |
|-------------|
| Curr | 0.74 | (0.37, 1.46) | 0.79 | (0.39, 1.61) | 0.66* | (0.4: |
| net | 1.00 | (0.68, 1.46) | 0.41 | (0.13, 1.26) | 0.74 | (0.5: |
| net | 1.58** | (4.70, 28.57) | 1.04E+10 | (1.55E-128, 6.91E+147) | 32.22* | (7.6l |
| Ages | 1.57** | (1.86, 10.94) | 2.19E+09 | (3.27E-129, 1.46E+147) | 15.46* | (3.7I |
| Ages | 1.63** | (5.30, 36.39) | 9.93E+09 | (1.49E-128, 6.62E+147) | 44.07* | (10. |
| ages | 1.17** | (1.27, 2.37) | 1.02 | (0.54, 1.91) | 1.67** | (1.2I |

| Obser | 1299 | 906 | 1252 |
|vation | | | |
| s | | | |
| Random | 0.82 (0.90) | 0.99 (0.99) |
| Effects | | |
| Variance (SD) | | |
| Site | | |
| House | 1.95E-13 (4.41E-7) | 1.06E-13 (3.26E-7) |

| Note: |

| Figures |

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Figure 1

A total of 4661 individuals were included in the study after exclusion of incomplete cases (Figure 1).
The most common species of Anopheles identified was Anopheles gambiae complex (43.8% of all larvae), followed by Anopheles coustani, Anopheles squamosus, and Anopheles mascarensis (Figure 2). Anopheles species composition varied by site, region, habitat preference, and relative abundance (Figure 2).
Different risk factors are associated with malaria infection in each region (Table 2, Figure 3).

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.
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