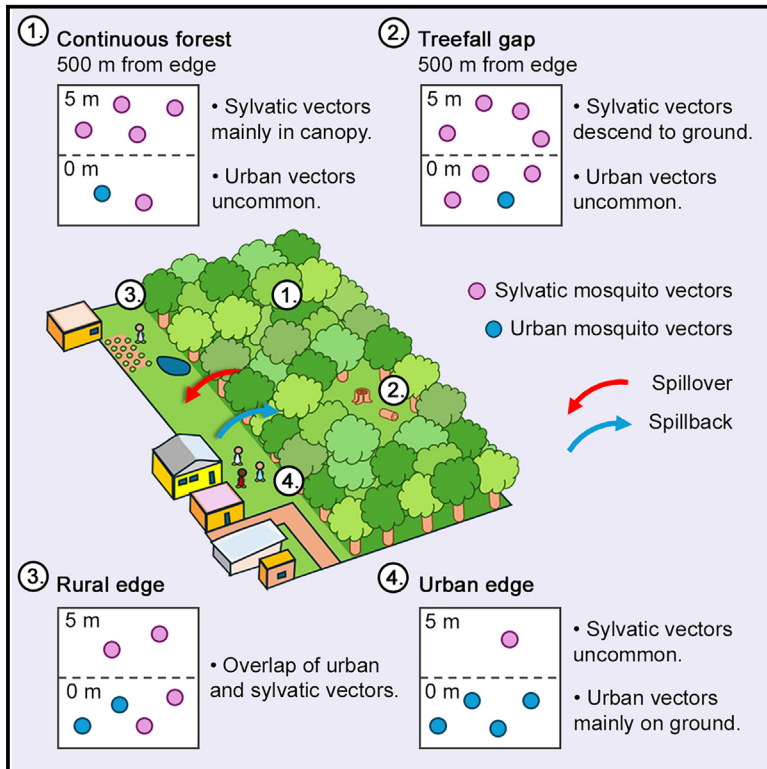


Forest edge landscape context affects mosquito community composition and risk of pathogen emergence

Graphical abstract



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In brief

Public health; Environmental science; Ecology; Entomology; Virology

Highlights

- Landscape context of forest edges shapes mosquito communities
- Mosquito diversity peaks and urban and sylvatic vectors overlap in rural edges
- Urban edges are less diverse and less suitable for sylvatic vectors
- Some canopy-dwelling species come to ground level more frequently at edges



Article

Forest edge landscape context affects mosquito community composition and risk of pathogen emergence

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SUMMARY

Forest edges, where humans, mosquitoes, and wildlife interact, may serve as a nexus for zoonotic arbovirus exchange. Although often treated as uniform interfaces, the landscape context of edge habitats can greatly impact ecological interactions. Here, we investigated how the landscape context of forest edges shapes mosquito community structure in an Amazon rainforest reserve near the city of Manaus, Brazil, using hand-nets to sample mosquitoes at three distinct forest edge types. Sampling sites were situated at edges bordering urban land cover, rural land cover, and natural treefall gaps, while sites in continuous forest served as controls. Community composition differed substantially among edge types, with rural edges supporting the highest species diversity. Rural edges also provided suitable habitat for forest specialists, including key sylvatic vectors, of which *Haemagogus janthinomys* was the most abundant species sampled overall. Our findings emphasize the importance of landscape context in assessing pathogen emergence risk at forest edges.

INTRODUCTION

Spillover of arthropod-borne viruses (arboviruses) from enzootic foci, facilitated by bridge vectors that feed on both wildlife and humans, can result in isolated human infections or in local epidemics driven by competent urban vectors.¹ Subsequent human-mediated translocations can lead to larger outbreaks with potential global reach.² The introduction of pathogens into new geographic regions creates a risk of spillover into sylvatic cycles which, once established, pose a long-term threat to human health through spillover infections.^{1,2} All of the arboviruses of greatest public health importance, including yellow fever (YFV), dengue (DENV), Zika (ZIKV) (all *Flaviviridae: Orthoflavivirus*), and chikungunya (CHIKV) (*Togaviridae: Alphavirus*),^{3,4} originated

via spillover from ancestral sylvatic cycles in Africa and Southeast Asia involving monkeys and canopy-dwelling mosquitoes^{1,5} and subsequently established urban transmission cycles in humans sustained by *Aedes* species mosquitoes. YFV was translocated from Africa in the 1700s via the slave trade and established an endemic sylvatic cycle in the Americas involving neotropical monkeys and sylvatic *Haemagogus* and *Sabethes* species mosquitoes.^{6,7}

Forest edges, where humans, mosquitoes, and wildlife overlap may serve as a nexus for zoonotic arbovirus exchange.⁸ Studies have investigated shifts in mosquito communities^{9–12} and wildlife^{13–15} from interior forest to forest edge, and then to human-modified landscapes. Despite suggestions that mosquito diversity should peak in edge habitats,¹⁰ where urban



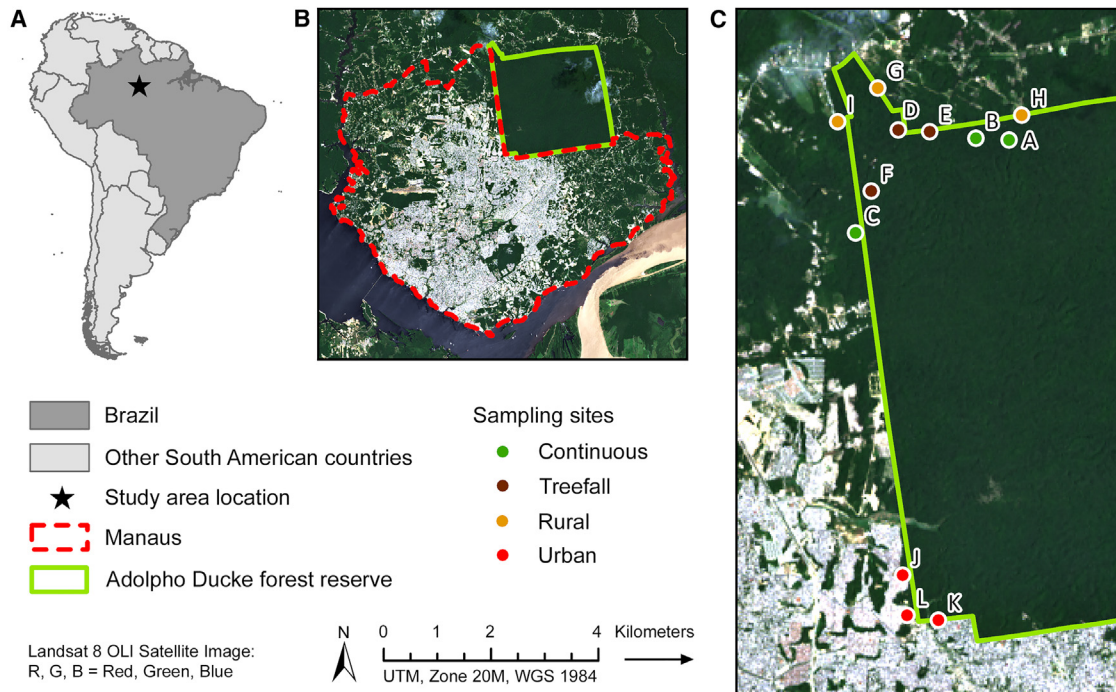


Figure 1. Reference map of the study area

Panels show (A) the location of Manaus in Brazil, (B) the Adolpho Ducke forest reserve on the edge of Manaus, and (C) the location of sampling sites in each category.

and sylvatic species overlap, empirical studies have not consistently found highest diversity at edges.^{9,10,16,17} However, mosquito species composition and potential routes of spillover and spillback have been shown to change rapidly within a few hundred meters of the edge.^{17,18} Several studies have reported higher diversity of wildlife inside forest or at forest edges than in disturbed habitat^{13–15} and have shown that land cover bordering edges may influence wildlife composition.¹⁴ Notably, forest edges influence monkey distributions^{19,20} and risk of interaction with known vectors.¹⁸ Howler monkeys (*Alouatta* spp.), major reservoirs of YFV, are mainly found in the mid to upper forest canopy²⁰ and display varying edge associations probably linked to the availability of food.^{19–21} Tamarins (*Saguinus* spp.), squirrel monkeys (*Saimiri* spp.), and capuchins (*Sapajus* spp.) are found in the lower canopy and understory, and often in edge habitats,^{15,20,22} sometimes venturing into human-modified landscapes^{15,23–25} where they may encounter high densities of dominant vector species, including *Aedes aegypti* and *Aedes albopictus*.^{10,26}

Although the likely importance of forest edges for spillover is well recognized, they are often treated as uniform interfaces, regardless of their landscape context. However, forest edges with high habitat contrast, such as those bordering urban land cover, may experience strong edge effects²⁷ including a loss of large trees²⁸ providing large fruits preferred by larger monkeys,²⁹ as well as oviposition sites for tree-hole breeding *Haemagogus* and *Sabethes* mosquitoes.³⁰ Conversely, forest edges with lower habitat contrast, such as those bordering rural or agricultural land cover, may experience weaker edge effects,²⁷ al-

lowing habitat to remain suitable for forest interior species³¹ including known arbovirus vectors.

Our recent studies of mosquito communities in rainforest fragments bordering Manaus in the Brazilian Amazon^{17,18,32,33} revealed a high relative abundance of *Ae. albopictus* near urban edges.^{17,18} The mean Normalized Difference Built-up Index (NDBI), a remote sensing index used to map urbanized areas, was higher in a 100 m radius around sites where this species was present compared to where it was absent when sampling with BG-Sentinel traps,¹⁷ while the opposite trend was observed for *Sabethes* mosquitoes. The presence of *Haemagogus* mosquitoes was not associated with mean NDBI but these were rarely sampled at anthropogenic edges using traps.¹⁷ However, *Haemagogus janthinomys*, a typically canopy-dwelling species and a major neotropical vector of YFV, was frequently encountered at natural edges formed by treefall gaps when sampling at ground level using hand-nets.³³

In this study, we compared communities of diurnally active, anthropophilic mosquitoes sampled at ground level and on 5 m platforms, along with environmental variables at forest edges bordering urban versus rural land cover in Manaus (Figure 1). We also compared these anthropogenic edges with natural edges formed by treefall gaps as well as with sites in continuous forest. We hypothesized that differences in habitat contrast and environmental variables associated with landscape context would shape differences in mosquito communities. We predicted that diversity would be highest at rural edges owing to overlap of both urban and forest species and that forest edges would alter the vertical stratification of typically canopy-dwelling

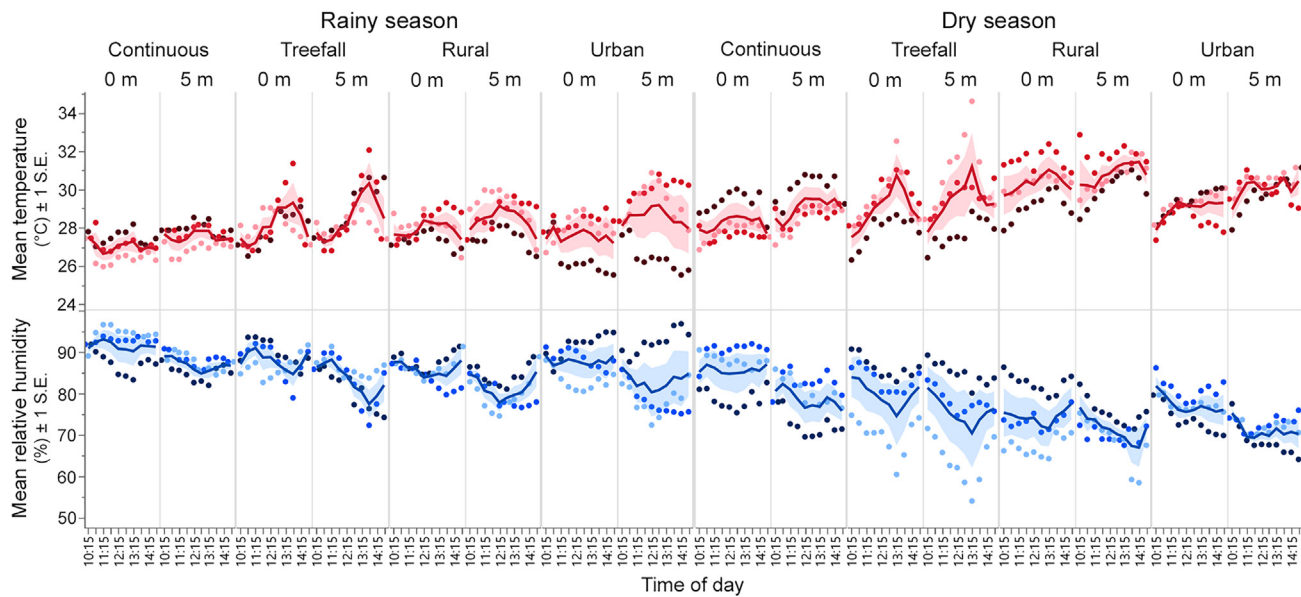


Figure 2. Variation in microclimate by season, edge type, and height during the daily sampling period

Colored dots represent the mean temperature (°C) and mean relative humidity (%) for the three sites sampled in each edge type at each designated timepoint. Solid lines represent the mean of the three site values and shaded areas show ± 1 standard error (S.E.).

vectors, including *Haemagogus* and *Sabethes* species. Both changes would have significant implications for the risk of spillover and spillback.

RESULTS

Mean NDBI varied most between urban edge sites

NDBI has theoretical values ranging between -1 and $+1$, with higher values representing more densely built-up areas. The mean NDBI values within a 100 m buffer surrounding each sampling site ranged from -0.223 to -0.532 at urban edges, -0.582 to -0.629 at rural edges, -0.666 to -0.700 at treefall gaps, and -0.696 to -0.728 in continuous forest. Despite the presence of built-up areas at forest edges, the substantial amount of forest cover within the 100 m buffers resulted in relatively low NDBI values across all edge types.

Forest edges, irrespective of landscape context, were hotter and drier than continuous forest

The fluctuation in temperature and humidity across the daily sampling period (10:00–15:00) differed between forest edges and continuous forest and differed among forest edges depending upon landscape context and season (Figure 2). Mean temperature and relative humidity were significantly correlated with all other microclimate variables (Spearman's rank correlation, $p < 0.0001$). Both variables remained stable throughout the day in continuous forest but fluctuated considerably at treefall gaps, which were hottest and driest during the early afternoon hours. The magnitude of fluctuations in microclimate at rural and urban edges was intermediate between continuous forest and treefall gaps. As expected, mean temperature was significantly higher at forest edges than in continuous forest (two-tailed

unequal variances t-test, $DF = 169.2$, $t = 3.87$, $p = 0.0002$) and was significantly higher during the dry season than in the rainy season ($DF = 277.7$, $t = -5.57$, $p < 0.0001$). Conversely, mean relative humidity was lower at forest edges (Wilcoxon Rank Sum, $DF = 1$, $\chi^2 = 14.5$, $p = 0.0001$) and lower during the dry season ($DF = 1$, $\chi^2 = 47.3$, $p < 0.0001$). When data from both 0 m and 5 m heights were combined, there were no significant differences in the mean temperature (one-way ANOVA, $DF = 2$, $F = 1.42$, $p = 0.24$) or relative humidity (Kruskal-Wallis, $DF = 2$, $\chi^2 = 5.92$, $p = 0.052$) among the three edge habitats. The same was true when analyzed separately by height ($p > 0.1$ for all comparisons). Across all edge habitats, conditions were slightly hotter (two-tailed equal variances t-test, $DF = 217$, $t = 1.93$, $p = 0.06$) and less humid (Wilcoxon Rank Sum, $DF = 1$, $\chi^2 = 11.1$, $p = 0.0009$) at 5 m above the ground than at ground level.

Overview of mosquito collections

Mosquito sampling was conducted over 71 rainy season days and 75 dry season days. Sampling effort was relatively evenly distributed across categories, with 36 days spent in continuous forest, 37 days at treefall gaps, 35 days at rural edges, and 38 days at urban edges. Collections yielded 4,425 adult mosquitoes (97.5% female, 13 genera, and 69 identified species) including 1,503 in continuous forest, 1,682 at treefall gaps, 629 at rural edges, and 611 at urban edges (Figure 3, Dataset³⁴). Of these, 2,524 were sampled at ground level and 1,901 at 5 m, while 2,854 were sampled during the rainy season and 1,571 during the dry season. The most abundant genera were *Haemagogus* (30.2%), *Psorophora* (21.6%), *Sabethes* (16.2%), *Limatus* (12.3%), and *Wyeomyia* (9.6%), while *Aedes* mosquitoes formed 5.1% of the total catch. The most abundant species were *Hg. janthinomys* (27.0%), *Psorophora amazonica* (19.2%),

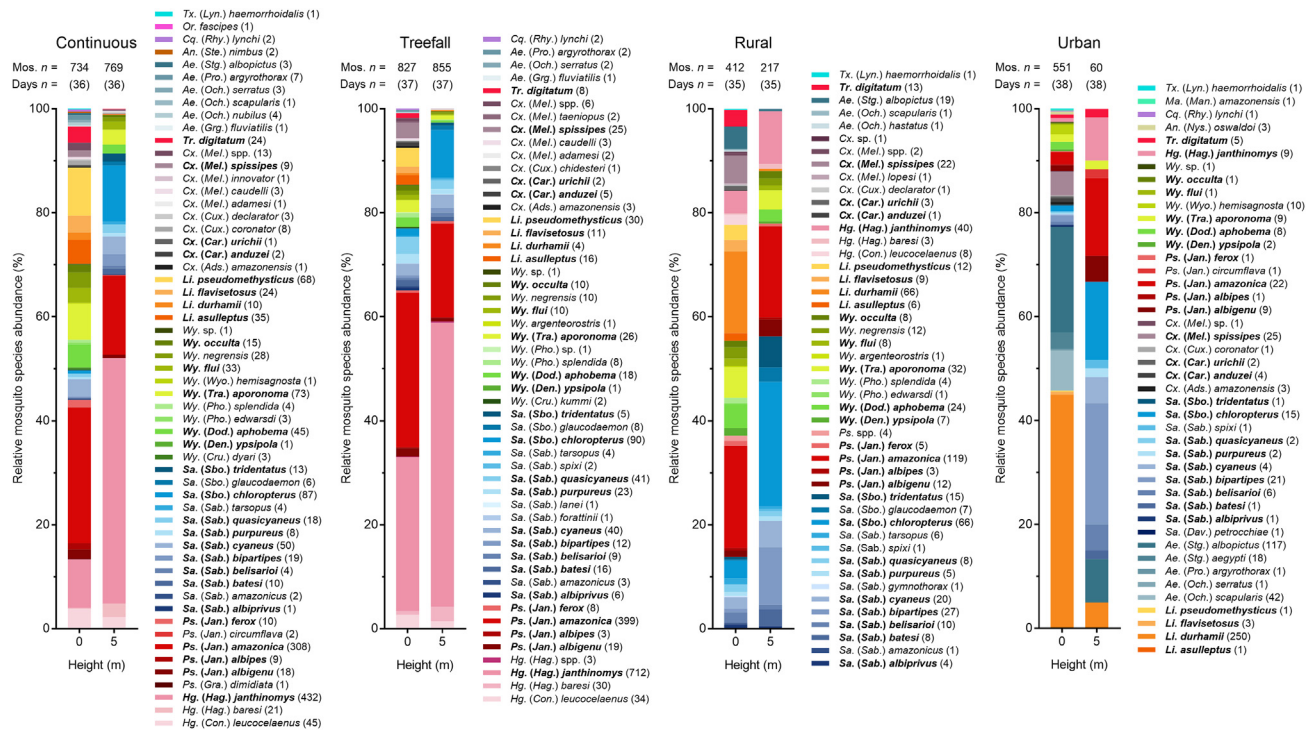


Figure 3. Relative mosquito species abundance by edge type and height of collection

Stacks ordered by genus abundance and then alphabetically by subgenus and species. Number of mosquitoes (Mos. $n =$) and number of sampling days (Days $n =$) at each edge type and at each height shown above bar. Number of individuals per taxon included in parentheses next to corresponding name; sp. = single species, spp. = potentially multiple species. Abbreviated names are given in full in the dataset. Species collected across all four categories highlighted in bold text.

Limatus durhamii (7.5%), *Sabethes chloropterus* (5.8%), *Wyeomyia aporonoma* (3.2%), *Ae. albopictus* (3.1%), *Sa. cyaneus* (2.6%), and *Li. pseudomethysticus* (2.5%).

Landscape context and height together shaped the mosquito community composition in different edge habitats

Haemagogus janthinomys and *Ps. amazonica* dominated collections in continuous forest and at treefall gaps, where *Sabethes* species, including *Sa. chloropterus*, were also common (Figure 3). These taxa were particularly abundant at 5 m, while at ground level, *Wyeomyia* and *Limatus* also formed a high proportion of the catch. The relative abundance of *Wyeomyia* and *Limatus* at ground level was more than twice as high in continuous forest than at treefall gaps. The opposite was true for *Sabethes* species, while the ground level relative abundance of *Hg. janthinomys* was 3.5 times higher at treefall gaps than in continuous forest. At rural edges, the relative abundance of mosquitoes at ground level was more evenly distributed among genera, with *Sabethes*, *Psorophora*, *Wyeomyia*, and *Limatus* being well represented. At urban edges, *Limatus* and *Aedes* dominated collections. *Psorophora amazonica*, *Li. durhamii*, and *Ae. albopictus* were the dominant species within their genera at rural and urban edges. In these settings, *Sabethes* species formed more than 50% of the mosquitoes sampled on platforms.

The Morisita index revealed high overlap between sites in continuous forest and at treefall gaps, but lower overlap between

sites at rural edges and at urban edges (Table S1). Based on species combined at both ground level and 5 m (Table 1), mosquito communities in continuous forest were similar to those at treefall gaps but differed greatly from communities at the urban edge. Communities at rural edges were moderately similar to all other edge types. When this analysis was broken down by height (Table 2), ground level community composition followed the pattern described above. At 5 m above the ground, however, continuous forest and treefall gap communities were almost indistinguishable; rural edges and urban edges showed substantial overlap with each other, and both showed moderate overlap with interior forest communities. There was little change between rainy and dry season in mosquito community composition at ground level or 5 m, although urban edges were excluded from the latter comparison due to small sample size (Table S1).

To conduct a principal components analysis of relative species abundance at each edge type for both heights combined, we first checked pairwise correlations of each species and removed one species from each pair that was highly significantly ($p \leq 0.01$) correlated. Principal components (PC)1 and PC2 captured 40.7% and 38.1% of the variation in data, respectively (Figure 4). PC1 represented the relative abundance of *Hg. janthinomys*, *Hg. leucocelaenus*, *Sa. bipartipes*, and *Sa. belisarioi*, along with rarer species including *Culex caudelli* and *Cx. adamesi*, while PC2 represented the relative abundance of a group of *Sabethes* species containing *Sa. chloropterus*, *Sa. batesi*, and *Sa. albiprivus*, along with *Wy. argenteostris* and *Ae.*

Table 1. Morisita overlap index for comparisons by edge type

	Cont.	Treefall	Rural	Urban
Cont.	1	–	–	–
Treefall	0.939	1	–	–
Rural	0.686	0.524	1	–
Urban	0.107	0.083	0.445	1

Cont. = Continuous forest.

hastatus (Table S2). In a principal components analysis of relative species abundance at each edge type and height, PC1, PC2, and PC3 captured 23.1%, 22.4%, and 16.8% of the variation in data, respectively. PC1 represented the relative abundance of several *Sabethes* species including *Sa. cyaneus*, *Sa. purpureus*, and *Sa. chloropterus*, along with *Wy. hemisagnosta*, *Ae. aegypti*, and *Ae. scapularis* (Table S3). PC2 represented the relative abundance of *Wy. aporonoma*, *Ps. amazonica*, *Hg. leucocelaenus*, and *Trichoprosopon digitatum* among other predominantly rarer species, while PC3 represented the relative abundance of *Wy. ypsipola* and *Hg. janthinomys* among the highest loading species. We did not perform principal components analysis by season since we detected little seasonal change in mosquito community composition using the Morisita index.

Results of hierarchical clustering of the principal components generally agreed with Morisita comparisons. Hierarchical clustering of PC1 and PC2 by edge type showed that continuous forest and treefall gap communities were very similar, but differed considerably from rural edge communities, which in turn differed from urban edge communities (Figure 4). For the edge type and height comparison, hierarchical clustering of PC1, PC2, and PC3 showed that continuous forest and treefall gap communities were more similar to each other than to the cluster of rural edge and 5 m urban edge communities, while 0 m urban edge communities were distinct from both clusters. Continuous forest and treefall gap communities 5 m above the ground were very similar and more closely resembled ground level treefall gap communities than ground level continuous forest communities.

Landscape context shaped species diversity at forest edges

A total of 55 identified species were sampled in continuous forest, 48 at treefall gaps, 44 at rural edges, and 42 at urban edges. Urban edges exhibited the greatest variation in species richness, ranging from 14 to 33 species per site. During the rainy season, rarefaction curves among edge types were rather similar, but rural edges showed the highest richness relative to sample size at both sampling heights (Figure 5). However, richness in both continuous forest and treefall gaps exceeded that at rural edges due to the higher number of individuals sampled, while richness at urban edges was lower than the other three edge types. During the dry season, rarefaction and extrapolation curves were more divergent, particularly at 0 m, where richness was highest at rural edges and in continuous forest. Species richness was higher at treefall gaps than in continuous forest when sampling at 5 m, while sample coverage was low at rural edges.

Species evenness was significantly higher in the dry season than the rainy season (DF = 1, F = 11.3, $p = 0.002$) (Table S4),

irrespective of edge type or height. In contrast, the impact of edge type varied by height (DF for interaction effect = 3, F = 7.7, $p = 0.0005$) (Table 3). At ground level, evenness in continuous forest was comparable to that at rural edges and both were higher than either treefall gaps or urban edges, which were similar to each other (DF = 3, F = 9.86, $p = 0.0003$). At 5 m, evenness at rural edges was significantly higher than continuous forest or treefall gaps, which did not differ from each other. Urban edges were excluded from this analysis due to small sample size.

Mosquito abundance decreased but key vector species persisted through dry season months

Comparisons of rainy season vs. dry season abundance at each edge type for the eight most abundant species revealed significant differences only for *Hg. janthinomys* and *Sa. chloropterus* at treefall gaps (Wilcoxon Rank Sum, DF = 1, $\chi^2 = 12.5$, $p = 0.0004$ and DF = 1, $\chi^2 = 3.84$, $p = 0.0499$, respectively) and *Li. durhamii* at rural edges (DF = 1, $\chi^2 = 9.43$, $p = 0.002$). In all cases, the median number of mosquitoes was significantly higher during the rainy season (Figure 6; Table S5). The contrast between seasons was most pronounced for *Hg. janthinomys* sampled at treefall gaps, although *Ps. amazonica* exhibited a tendency toward higher rainy season abundance in continuous forest, at treefall gaps, and at rural edges. While the overall mosquito abundance tended to be higher during the rainy season, several species, including *Hg. janthinomys* and *Ae. albopictus*, maintained appreciable numbers throughout the dry season. There was little difference between the number of *Sa. chloropterus* sampled in rainy season vs. dry season months in continuous forest (DF = 1, $\chi^2 = 0.04$, $p = 0.83$) and at rural edges (DF = 1, $\chi^2 = 0.7$, $p = 0.4$). Even at treefall gaps, where the difference was significant, *Sa. chloropterus* still persisted in relatively high numbers.

Occurrence and abundance of key vectors showed species-specific associations with edge type and other environmental variables

Nominal logistic regression (Table 4) showed that edge type effects on occurrence (presence/absence) of the same eight species were almost universal but partitioning by edge differed among species. During the rainy season, predominantly sylvatic species (all but *Li. durhamii* and *Ae. albopictus*) were prevalent in continuous forest and at treefall gaps, while *Hg. janthinomys*, *Ps. amazonica*, *Sa. chloropterus*, and *Wy. aporonoma* were also common at rural edges (Table S6). Of the urban species, *Ae. albopictus* was more prevalent at urban edges than at rural edges, *Li. durhamii* occurred evenly between the two, but neither were common inside the forest. These patterns held true during the dry season, albeit at slightly lower levels for several species. Notably, there was little difference in occurrence across edge types between rainy and dry seasons for *Hg. janthinomys*, *Sa. cyaneus*, and *Ae. albopictus*.

Height and other environmental variables were included in both nominal logistic regression and standard least squares (Table S7) models to assess their impact on species occurrence and abundance, respectively. During the rainy season, these models consistently indicated that *Hg. janthinomys* and

Table 2. Morisita overlap index for comparisons by edge type and height

	Cont. 0	Treefall 0	Rural 0	Urban 0	Cont. 5	Treefall 5	Rural 5	Urban 5
Cont. 0	1	–	–	–	–	–	–	–
Treefall 0	0.816	1	–	–	–	–	–	–
Rural 0	0.790	0.609	1	–	–	–	–	–
Urban 0	0.098	0.065	0.540	1	–	–	–	–
Cont. 5	0.499	0.855	0.345	0.037	1	–	–	–
Treefall 5	0.461	0.837	0.309	0.033	0.987	1	–	–
Rural 5	0.570	0.588	0.539	0.072	0.563	0.489	1	–
Urban 5	0.473	0.490	0.539	0.261	0.446	0.390	0.833	1

Cont. = Continuous forest, 0 = 0 m, 5 = 5 m.

Sa. chloropterus were more common on 5 m platforms than at ground level, while the remaining species, except for *Ps. amazonica*, and *Sa. cyaneus*, which exhibited no height preference, were more common at ground level. Focusing primarily on highly significant variables or on all significant variables where occurrence and abundance models concurred, *Hg. janthinomys* was positively associated with mean temperature, while *Ps. amazonica* exhibited a negative correlation with the same variable. Both *Sabethes* species were negatively associated with mean weather, suggesting they are more active under clearer skies. An increase in 7-day cumulative rainfall lagged at 1 week was linked to a marginal decrease in *Hg. janthinomys* occurrence, as well as *Sa. cyaneus* occurrence and abundance, and an increase in *Li. durhamii* occurrence. Increasing rainfall lagged at 3 or 4 weeks was associated with an increase in occurrence or abundance of *Ps. amazonica*.

Supplemental analysis using a generalized linear model revealed interaction effects between edge type and height for occurrence of *Hg. janthinomys* and *Sa. chloropterus* sampled during the dry season (Table S8). Simple effects tests for *Hg. janthinomys* showed a significant effect of edge type at ground level (Kruskal-Wallis Rank Sum, $DF = 3$, $\chi^2 = 10.2$, $p = 0.02$), where occurrence was highest at treefall gaps, and at 5 m ($DF = 3$, $\chi^2 = 9.58$, $p = 0.02$), where occurrence was highest at treefall gaps and in continuous forest. For *Sa. chloropterus*, there was no significant effect of edge type at ground level ($DF = 3$, $\chi^2 = 2.51$, $p = 0.47$), but a marginal effect at 5 m ($DF = 3$, $\chi^2 = 7.35$, $p = 0.06$), where occurrence was lower at urban edges than other edge types. The influence of these variables on species occurrence otherwise remained fairly consistent between seasons, although *Ps. amazonica* was marginally more common at ground level during the dry season. Furthermore, relative humidity had a greater impact on occurrence in the dry season, particularly for *Sa. chloropterus* and *Wy. aporonoma*, which exhibited positive associations with this variable. Mean weather was positively correlated with *Ps. amazonica* occurrence during the dry season but not during the rainy season, indicating its increased presence during harsher conditions. The same variable retained a negative association with *Sa. chloropterus*, confirming its heightened activity under clearer skies. Cumulative rainfall showed stronger positive associations with *Ps. amazonica*, reliant on ground water for breeding, and negative associations with *Li. pseudomethysticus*, during the dry season.

Landscape context influenced changes in the vertical stratification of *Sabethes* subgenera

The two main *Sabethes* subgenera, *Sabethes* ($N = 354$) and *Sabethoides* ($N = 257$), differed in their vertical stratification. Contingency table analyses revealed a marginally lower overall occurrence of the subgenus *Sabethes* at 0 m compared to 5 m (Pearson's chi-square, $DF = 1$, $\chi^2 = 3.72$, $p = 0.054$), and a substantially lower occurrence of *Sabethoides* at 0 m compared to 5 m ($DF = 1$, $\chi^2 = 131$, $p < 0.0001$) (Table S9). When analyzed by edge type, the occurrence of both subgenera was lower at 0 m relative to 5 m within continuous forest ($p < 0.0001$ for both comparisons). At treefall gaps and rural edges, there was no significant difference in *Sabethes* occurrence between heights ($p > 0.05$ for both comparisons), although *Sabethoides* occurrence remained significantly lower at 0 m compared to 5 m ($p < 0.0001$ for both comparisons). Despite this, the ratio of *Sabethoides* occurring at 0 m and 5 m was 1:3.4 at rural edges compared to 1:16.4 in continuous forest. At urban edges, there was no notable difference in occurrence of either subgenus between heights ($p > 0.2$ for both comparisons), although *Sabethoides* mosquitoes were uncommon.

DISCUSSION

Landscape context may determine the role of forest edges in facilitating or retarding spillover and spillback of arboviruses. Our study shows that mosquito communities at edges bordering rural land cover are especially diverse and provide suitable refuge for known urban and sylvatic vectors. In contrast, those bordering urban land cover exhibit a reduced diversity and are less suitable for sylvatic species. However, urban edges intersect with the distribution of *Ae. albopictus* providing a pathway for its spread into forests.¹⁸ Crucially, both anthropogenic and natural forest edges impact the vertical stratification of certain canopy-dwelling species, bringing them into contact with novel hosts.

In our study, mosquito communities were similar at treefall gaps and in continuous forest, while composition at rural edges was intermediate between interior forest and urban edges. Our findings are consistent with studies of plant communities showing that increased habitat contrast at forest edges negatively impacts suitability for specialist forest species.^{27,35} In agreement, we sampled more forest mosquitoes at rural edges

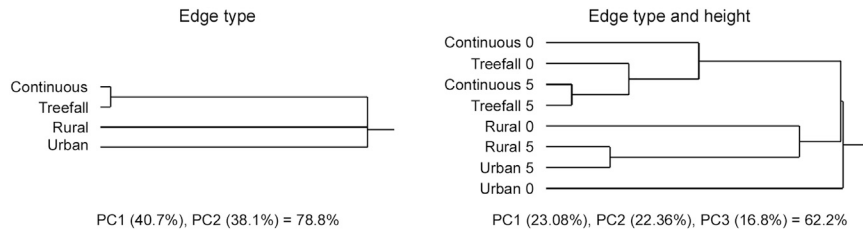


Figure 4. Mosquito community structure

Dendrograms show hierarchical clustering of principal component PC1 and PC2 from the analysis of relative species abundance by edge type, and PC1, PC2, and PC3 from the analysis by edge type and height (0 = 0 m, 5 = 5 m).

than at urban edges, which were dominated by ground dwelling *Limatus* and *Aedes* species. A loss of large trees,²⁸ positively associated with urbanization,³⁶ is likely to contribute to a decline in forest specialists, particularly those relying upon tree holes for breeding.³⁷ Our findings also revealed that ground level mosquito communities at treefall gaps were similar to elevated interior forest communities, whereas ground level continuous forest communities were more distinct. This suggests that canopy disruption impacts the vertical distribution of certain canopy-dwelling mosquito species, potentially bringing them into contact with terrestrial mammals associated with treefall gaps, including agoutis,³⁸ which have shown evidence of exposure to YFV.³⁹ Additionally, forest disturbance affects the vertical stratification of other canopy-dwelling wildlife,^{40,41} although further studies are needed to determine the impact of edges on the distribution of vertebrates potentially involved in arbovirus transmission.

Species diversity was also shaped by landscape context. Mean species evenness was highest at rural edges at both sampling heights and in both seasons. At ground level, evenness in continuous forest was comparable to that of rural edges, whereas at 5 m evenness in continuous forest was lower than that of rural edges. In continuous forest and treefall gaps, evenness decreased 5 m above the ground due to the dominance of *Hg. janthinomys*. These findings offer mixed support for the hypothesis of higher biodiversity and mixing of species from adjacent habitats at forest edges^{42,43}: this pattern was apparent

at rural edges but not at urban edges, which consistently showed the lowest diversity. An important codicil to this claim is that the diversity of anthropophilic mosquitoes was higher at rural edges than at urban edges. Rural edges also appear to enhance permeability for sylvatic vectors⁸ and inevitably their pathogens. Studies failing to detect higher diversity at forest edges have often restricted sampling to within a few hundred meters of the boundary,^{9–11,44} yet we have only detected substantially lower diversity when sampling beyond 500 m into the forest.^{17,18} We also found that species evenness was slightly, but significantly lower during the rainy season compared to the dry season. While higher diversity is generally associated with rainy months,¹¹ our findings may reflect an increased dominance of *Hg. janthinomys*, *Ps. amazonica*, and *Li. durhamii* during this period. Rarefaction estimates of species richness revealed less pronounced differences between forest edges, although these were based on the richness per edge type, which masked the heterogeneity of the urban edge sites.

The heterogeneity of sampling sites at anthropogenic edges, influenced by neighboring habitat, can affect arthropod composition.⁴⁵ Given that both male and female mosquitoes consume nectar, variations in the distribution of flowers across sampling sites or seasons could potentially impact mosquito distributions.⁴⁴ Flowering in Amazonian plants is generally synchronized and peaks in November at the end of the dry season,⁴⁶ though flowering may be earlier and more intense at forest edges.^{47–49} While we did not directly measure floral diversity or abundance,

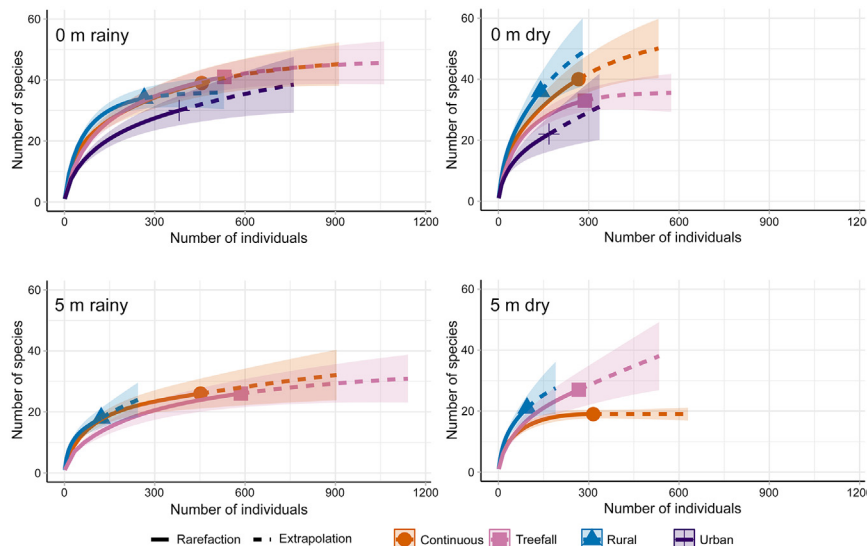


Figure 5. Species richness rarefaction and extrapolation curves

Panels show richness for data grouped by edge type (based on $N = 3$ biologically independent sampling sites per edge type) for each height and season. Shaded areas surrounding rarefaction and extrapolation lines represent 95% confidence intervals. Urban edges were excluded at 5 m due to small sample size.

Table 3. Species richness, diversity, and evenness by edge type and height, pooled across season

Edge type	N =	Richness		Diversity (H')		Evenness	
		0 m	5 m	0 m	5 m	0 m	5 m
Continuous	3	33.0 (1.15)	21.0 (0.58)	2.76 (0.01)	1.95 (0.14)	0.79 (0.01) ^a	0.64 (0.05) ^b
Treefall	3	31.7 (2.40)	21.3 (2.19)	2.29 (0.18)	1.65 (0.11)	0.66 (0.04) ^b	0.54 (0.02) ^b
Rural	3	30.0 (0.58)	15.3 (4.67)	2.81 (0.09)	2.14 (0.27)	0.83 (0.03) ^a	0.83 (0.03) ^a
Urban	3	19.7 (6.17)	–	1.75 (0.05)	–	0.62 (0.04) ^b	–

^{a,b}Superscript letters indicate the results of post-hoc comparisons within the column; values that do not share a letter are significantly different. Note that comparisons were conducted within and not across height classes.

H' = Shannon-Wiener diversity index.

Arithmetic means (± 1 standard error) calculated per sampling site ($N = 3$). Urban edges were excluded at 5 m due to small sample size (60 mosquitoes).

urban edge sites varied most in both mean NDBI and mosquito community composition (Table S1).

It is well-established that forest edges significantly alter microclimate,¹⁷ and our findings reflect this, with anthropogenic and natural edges exhibiting hotter and drier conditions compared to continuous forest. Landscape context also affected fluctuations in microclimate across the daily sampling hours, most notably at treefall gaps where temperature peaked, and relative humidity reached its lowest point in the early afternoon hours, as documented in our previous work.³³ We saw greater variation in microclimate between sites at urban edges (clustered in the southwest of the reserve) during the rainy season compared to the dry season, while the opposite was true at rural edges, treefall gaps, and in continuous forest (clustered in the northwest). Geographic orientation of sites, and season, are among the factors known to affect microclimate at the edges of forest fragments, with north-facing edges exhibiting higher temperatures and lower humidity than south-facing edges in the southern hemisphere.⁵⁰ These differences in microclimate may influence mosquito development rate,⁵¹ pathogen extrinsic incubation period,⁵² and other factors affecting vector competence.⁵³

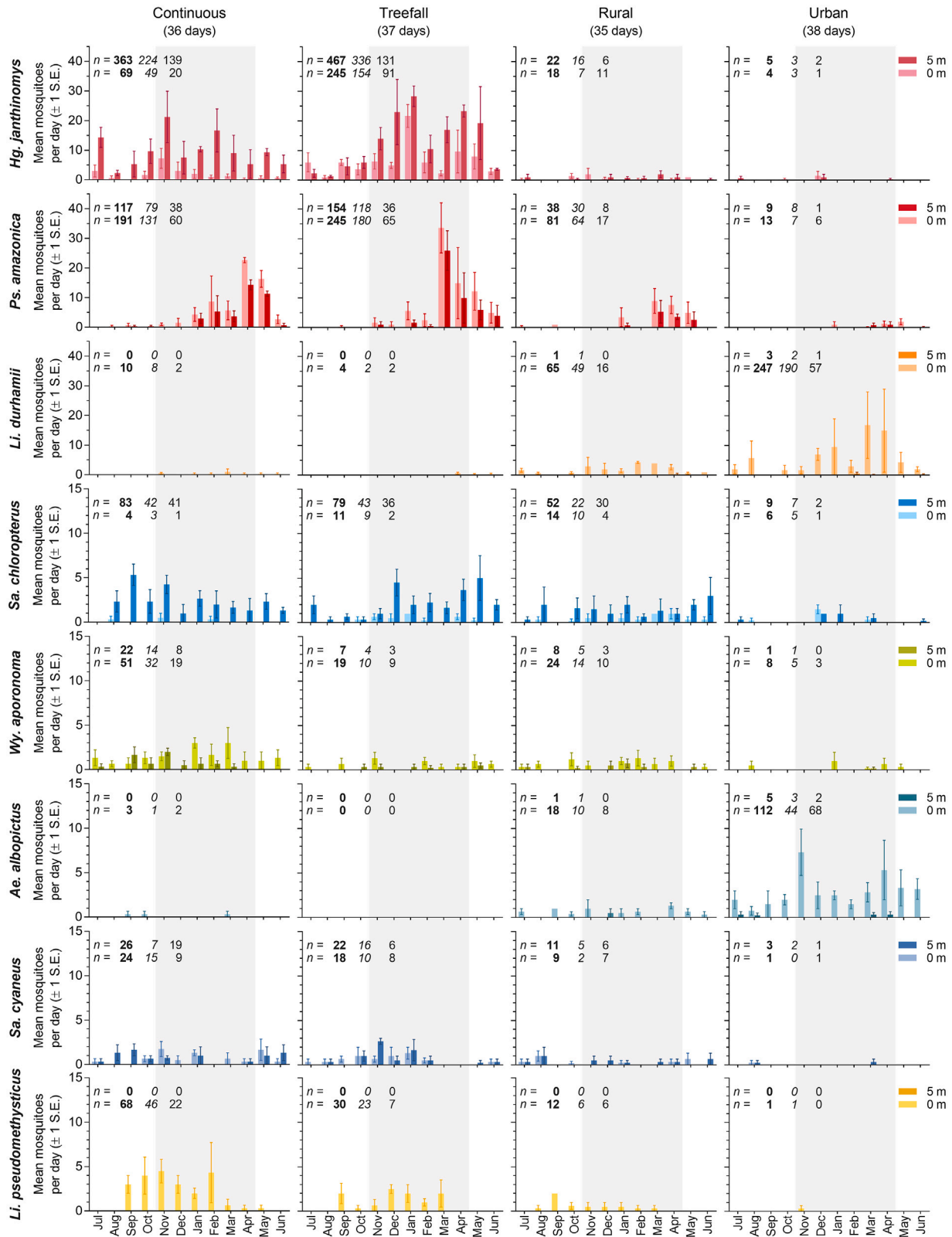
The means by which sylvatic mosquito-borne viruses are maintained throughout dry seasons has long intrigued researchers. Studies conducted in Panama during the 1950s, in tropical deciduous forest/rainforest, showed reductions in *Haemagogus* and *Sabethes* abundance to very low levels during the dry season.⁵⁴ Our analysis revealed modest effects of season on mosquito abundance, with a tendency for higher numbers during the rainy season. However, our finding that key species persisted at appreciable levels during dry season months has important epidemiological implications. While transovarial transmission is often proposed as a mechanism for sustaining virus circulation,⁵⁵ we demonstrate that adult mosquito populations may play a crucial role in the Amazon, particularly in the forest canopy.

Environmental factors associated with the occurrence and abundance of key vector species can be used to characterize suitable habitat and refine risk models for pathogen emergence. We previously detected positive associations between *Haemagogus* mosquitoes, and both temperature and 7-day cumulative rainfall lagged at 1 week.^{32,33} On this occasion, we confirmed the positive association between *Hg. janthinomys* and mean temperature but detected a marginal negative association with rainfall lagged at 1 week during the rainy season. Relationships

between precipitation and tree hole breeding mosquitoes are likely to be complex. While rainfall is essential for eggs to hatch, too much water can flush out mosquitoes and diminish populations.⁵⁶ This species was seldom encountered at anthropogenic edges. It was mostly active inside the forest and above the ground but descended to ground level in abundance at treefall gaps, making *Hg. janthinomys* a potential bridge vector in these settings. Research is now required to assess the vector competence of this species for DENV, ZIKV, CHIKV, and Mayaro virus (MAYV, *Togaviridae: Alphavirus*).^{57,58}

We have sampled *Ps. amazonica* in high relative abundance throughout our studies.^{17,18,32,33} It was mainly captured in continuous forest and at treefall gaps but was also the most abundant mosquito at rural edges. Again, its relationship with precipitation is likely to be complex, as indicated by associations with rainfall that deviated from our previous results.³² Species of the subgenus *Janthinosoma* lay desiccation resistant eggs in pools of ground water.^{59,60} At the Ducke reserve, these only form after sustained heavy rainfall saturates the forest floor. However, once established, *Psorophora* species can develop through multiple generations very quickly.⁶¹ We detected a negative association with temperature in the rainy season adding to evidence that *Ps. amazonica* tolerates cool conditions.³³ There was a limited effect of height on the occurrence and abundance of *Ps. amazonica*, which has potential to interact with humans and with wildlife at ground level and in the canopy. Despite these traits and its aggressive biting behavior, we know nothing about its vector status. However, other closely related *Janthinosoma* species harbor medically important orthoflaviviruses including Ilhéus and West Nile viruses.⁵⁹

Sabethes mosquitoes, recognized as important secondary vectors of YFV,⁶² were common in both continuous forest and at treefall gaps, particularly under favorable weather conditions. Notably, *Sa. chloropterus* was frequently captured at rural edges, further emphasizing the affinity of certain *Sabethes* species for edge habitats.^{17,37,63} However, the contrasting vertical distributions of *Sa. (Sabethoides) chloropterus* and *Sa. (Sabethes) cyaneus* has important implications for pathogen transmission. In line with Galindo et al.,⁶⁴ our findings revealed a strong preference for elevated heights by *Sa. chloropterus*, while *Sa. cyaneus* only exhibited a slight, insignificant, preference. Our subgenus analysis confirmed these observations and demonstrated a relative increase in *Sabethoides* mosquitoes at ground level at rural edges compared to continuous forest. *Sabethes*



(legend on next page)

species possess a strong bridge vector potential and should be targeted for arbovirus surveillance, including with BG-Sentinel traps.^{17,32} These mosquitoes display an intriguing preference for biting noses,⁶⁵ a behavior that could be exploited in the development of attractants.

The Asian tiger mosquito, *Ae. albopictus*, has gained attention for its potential role as a bridge vector,^{66,67} which is unsurprising considering its presence at forest edges, global distribution, and critical vector status.⁵ After *Li. durhamii*, *Ae. albopictus* was the most abundant species found at urban edges. Neither species was common at 5 m indicating that their potential involvement in zoonotic arbovirus transmission might be limited to ground level. Other studies investigating *Ae. albopictus* activity have also shown that this species is mainly active near the ground.^{68,69} However, our understanding of the vertical stratification of *Ae. albopictus* at forest edges remains limited, and further research that considers the broader landscape context is needed. Whereas *Ae. albopictus* is widely recognized as an important global vector,^{1,5} evidence of a role for *Li. durhamii* in arbovirus transmission is scarce.⁷⁰

We found that mosquito diversity peaks at rural forest edges, where urban and sylvatic species overlap. In the presence of suitable hosts, there is potentially a high risk of spillover and spillback in each edge setting compared to continuous forest, although the likely pathways of transmission differ. The landscape context of forest edges must be considered when assessing pathogen emergence risk, along with relative human population densities, and interactions between humans and forest environments. A synergistic approach integrating in-depth field studies with big data analysis will be crucial to understanding the nuances of human-mosquito-wildlife interactions and developing risk models that accurately reflect the dynamics of these complex ecological systems.

Limitations of the study

While our study provides valuable insights into mosquito dynamics at forest edges, there were several limitations. The small number of sampling sites at each edge type may restrict the generalizability of findings, yet the tantalizing heterogeneity of mosquito communities among urban edge sites deserves further investigation. In addition, our sampling strategy focused on peak activity times of major sylvatic vectors³³ which may have underestimated the abundance of species like *Ae. albopictus*, if exhibiting different activity patterns.⁷¹ Simultaneous ground and platform sampling could underestimate vertical mosquito movement, but rotating sampling introduces logistical challenges. Furthermore, a single-year study may not capture the full range of mosquito community dynamics. Sustained, multi-year investigations to assess the influence of annual environmental variations are needed. Lastly, our study focused on anthropophilic, day-biting mosquitoes. Whether our findings can be generalized to mosquitoes with other host preferences

or diel cycles, including ornithophilic and nocturnal species, warrants further investigation.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Nikos Vasilakis (nivasila@utmb.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data generated or analyzed during this study have been deposited in the Mendeley Data repository and are publicly available as of the date of publication. Accession numbers are listed in the [key resources table](#).
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request

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AUTHOR CONTRIBUTIONS

Conceptualization, A.H. and K.A.H.; Data curation, A.H., I.P., A.G., T.N.F.S., and C.R.M.; Formal analysis, A.H., V.S., and K.A.H.; Funding acquisition, M.V.G.d.L., N.V., and K.A.H.; Investigation, A.H., N.F.F., I.P., A.G., T.N.F.S., C.R.M., J.T.A.J., F.P.A., and E.R.C.; Methodology, A.H., J.T.A.J., F.P.A., E.R.C., M.P.G.M., and K.A.H.; Supervision, M.G., M.V.G.d.L., M.P.G.M., N.V., and K.A.H.; Visualization, A.H., V.S., M.B., and K.A.H.; Writing—original draft, A.H. and K.A.H.; Writing—review and editing, A.H., V.S., M.G., V.M.S., M.B., M.V.G.d.L., M.P.G.M., N.V., and K.A.H. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- [KEY RESOURCES TABLE](#)
- [METHOD DETAILS](#)
 - Study area
 - Forest edge type, site selection, characterization
 - Sampling platforms
 - Mosquito collections
 - Microclimate, weather, rainfall
 - Mosquito identifications

Figure 6. Mean number of mosquitoes sampled per day ± 1 standard error (S.E.) by edge type, height, and month of collection for the eight most abundant species

Collections were made from July 2021 – June 2022. Gray shaded areas show rainy season months (November 2021 – April 2022). Note that species 1–3 are plotted on a different y axis to species 4–8. *n* = shows the total (bold font), rainy season (italic font) and dry season (regular font) number of mosquitoes sampled at 0 m (bottom row) and 5 m (top row) for each species.

Table 4. Nominal logistic regression (p value and (χ^2)) testing associations between occurrence of the eight most abundant species overall and environmental variables in rainy and dry seasons

Species [$n =$] ^a	Season	Edge type	Height ^b	Mean temperature	Mean relative humidity	Mean weather ^c	7-day cumulative rainfall lag			
							1 week	2 weeks	3 weeks	4 weeks
<i>Hg. janthinomys</i> [78]	Rainy	<0.0001 (97.0)	0.03 (4.6) ^{pos}	0.03 (4.6) ^{pos}	–	–	0.07 (3.3) ^{neg}	–	–	–
<i>Ps. amazonica</i> [64]	Rainy	0.001 (16.2)	–	0.003 (8.8) ^{neg}	–	–	–	–	0.0008 (11.2) ^{pos}	–
<i>Li. durhamii</i> [37]	Rainy	<0.0001 (30.4)	<0.0001 (39.9) ^{neg}	0.04 (4.1) ^{neg}	0.04 (4.4) ^{pos}	–	0.005 (7.7) ^{pos}	–	–	–
<i>Sa. chloropterus</i> [66]	Rainy	0.0003 (18.6)	<0.0001 (17.4) ^{pos}	–	–	0.002 (9.7) ^{neg}	–	–	–	–
<i>Wy. aporonoma</i> [48]	Rainy	0.0008 (16.6)	0.02 (5.3) ^{neg}	–	–	0.048 (3.9) ^{neg}	–	–	–	–
<i>Ae. albopictus</i> [29]	Rainy	<0.0001 (59.1)	<0.0001 (31.9) ^{neg}	–	–	–	–	–	–	–
<i>Sa. cyaneus</i> [36]	Rainy	0.002 (15.0)	–	–	–	0.005 (8.0) ^{neg}	0.05 (3.9) ^{neg}	–	–	–
<i>Li. pseudomethysticus</i> [29]	Rainy	<0.0001 (23.9)	<0.0001 (57.9) ^{neg}	–	–	0.02 (5.4) ^{neg}	–	0.01 (6.2) ^{neg}	–	–
<i>Hg. janthinomys</i> [74]	Dry	<0.0001 (89.8)	0.06 (3.5) ^{pos}	–	–	–	–	–	–	–
<i>Ps. amazonica</i> [34]	Dry	<0.0001 (23.1)	0.05 (4.0) ^{neg}	–	–	0.0003 (12.9) ^{pos}	–	0.002 (9.3) ^{pos}	0.01 (6.1) ^{pos}	<0.0001 (19.7) ^{pos}
<i>Li. durhamii</i> [28]	Dry	<0.0001 (29.7)	<0.0001 (34.7) ^{neg}	–	0.02 (5.9) ^{pos}	–	–	–	–	–
<i>Sa. chloropterus</i> [45]	Dry	0.002 (14.4)	<0.0001 (47.7) ^{pos}	–	<0.0001 (15.7) ^{pos}	0.002 (9.4) ^{neg}	–	–	–	0.08 (3.1) ^{pos}
<i>Wy. aporonoma</i> [35]	Dry	–	–	0.005 (8.1) ^{neg}	<0.0001 (34.7) ^{pos}	0.08 (3.0) ^{pos}	–	–	–	–
<i>Ae. albopictus</i> [28]	Dry	0.0001 (20.6)	0.0008 (11.2) ^{neg}	–	–	–	–	–	–	–
<i>Sa. cyaneus</i> [38]	Dry	0.0001 (20.8)	–	–	–	–	–	0.05 (3.8) ^{neg}	–	–
<i>Li. pseudomethysticus</i> [14]	Dry	0.02 (10.2)	<0.0001 (15.8) ^{neg}	0.01 (6.7) ^{pos}	0.02 (5.5) ^{pos}	–	0.04 (4.1) ^{neg}	0.005 (7.8) ^{neg}	0.004 (8.4) ^{neg}	–

Positive and negative associations are indicated by ^{pos} or ^{neg} after the χ^2 value. p values in bold font represent highly significant results ($p < 0.01$). $N = 3$ biologically independent sampling sites per edge type.

^aNumber of person days during which a species occurred (71 rainy and 75 dry season days, 2 x people sampling) shown in square brackets.

^b^{pos} or ^{neg} indicates whether a species was more common on 5 m platforms or at ground level, respectively.

^c^{pos} or ^{neg} indicates a positive (decrease in occurrence) or negative (increase in occurrence) association with mean weather value, respectively.

- Ethics and permits
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Mosquito and environmental data (complete dataset)	Mendeley data	https://doi.org/10.17632/nyjmc2htd.1
Software and algorithms		
ArcGIS Pro 3.1	ESRI	https://www.esri.com/
ENVI 6.0	NV5 Geospatial	https://www.nv5geospatialsoftware.com/
iNEXT package	Hsieh et al. ⁷²	https://doi.org/10.1111/2041-210X.12613
JMP Pro 17	JMP Statistical Discovery	https://www.jmp.com/en_us/home.html
PAST version 4.14	Hammer et al. ⁷³	https://www.nhm.uio.no/english/research/resources/past/
R version 4.2.2	R Foundation	https://www.r-project.org/

METHOD DETAILS

Study area

The study was carried out at the Adolpho Ducke forest reserve⁷⁴ (Ducke) near Manaus, a city of more than two million people situated at the confluence of the Negro and Solimões rivers in the Brazilian Amazon. Figure 1 shows a reference map created using ArcGIS Pro 3.1 (ESRI, Redlands, California) of the study area based on Landsat 8 Operational Land Imager (OLI) surface reflectance imagery obtained from the USGS Earth Explorer data portal.⁷⁵ The Manaus polygon was derived from a GISMAPS neighborhoods shapefile,⁷⁶ while the Ducke reserve boundary was provided by the Instituto Nacional de Pesquisas da Amazônia (INPA). Ducke is 100 km² of *terra firme* rainforest home to multiple mammal species including six species of monkeys which differ in their ecology and behavior.⁷⁷ The reserve forms an abrupt border with the city along its southwestern edge, where people live in close contact with wildlife and sylvatic mosquitoes.¹⁷ The remaining border of Ducke abuts rural areas, where edges may be similarly abrupt, or where the transition between primary and secondary vegetation and nearby habitations is more gradual. Rural areas are mostly characterized by villages and other smallholdings where produce includes cupuaçu (*Theobroma grandiflorum*), açai (*Euterpe oleracea*), and andiroba (*Carapa guianensis*),⁷⁸ and animals such as dogs and chickens are commonly kept. Anecdotally, in both urban and rural areas, residents occasionally enter the forest for gathering fruit, hunting, or bathing in streams.¹⁷ DENV, ZIKV, and CHIKV circulate in urban cycles in Manaus,⁷⁹ while YFV⁸⁰ and MAYV⁸¹ circulate in nearby forests. Mosquito abundance is highest during the rainy season, which usually lasts from November until May, and decreases over the drier period from June until October.⁷⁴

Forest edge type, site selection, characterization

Three biologically independent sampling sites, located by direct surveillance, were situated in each of three types of forest edge: edges bordering urban land cover, edges bordering rural land cover, and internal edges formed by natural treefall gaps. A further three sites situated in areas of continuous forest served as controls (Figure S1). Thus, the study comprised 12 sites in total. Urban and rural edge sites were established where deemed safe, accessible, and in agreement with local communities. Urban edge sites were close to build-up residential areas within 10 m of the nearest house. Rural edge sites were in more sparsely populated village and agricultural areas at least 40 m from the nearest house. Treefall gap and continuous forest sites were 500 m from the forest edge and were different from those sampled in previous studies.^{32,33} Treefall gap sites exhibited noticeable openings in the canopy caused by one or more fallen trees (>10 m in height) allowing for a moderate to significant amount of sunlight to reach the forest floor. Continuous forest sites were heavily shaded by intact canopy allowing very little sunlight to reach the forest floor. All sampling sites were situated at least 500 m apart to minimize spatial autocorrelation.

The amount of built-up land surrounding each sampling site was characterized using remote sensing data and methods. Landsat 8 OLI surface reflectance imagery was obtained from USGS Earth Explorer,^{17,75} representing a relatively cloud-free day over the study area on 30 July 2017. The imagery was then subset to the Manaus study area and classified using Support Vector Machine in ENVI 6.0 (Harris Geospatial, Boulder, California) into five land cover classes at 30 m spatial resolution: urban and built-up land, forest land, grassland and agricultural land, barren land, and water.¹⁷ NDBI values were subsequently derived in ENVI for urban and built-up land pixels.⁸² Finally, ArcGIS Pro was used to calculate the mean NDBI value in a 100 m buffer surrounding each sampling site.

Sampling platforms

A 5-m-high timber platform was constructed between two nearby trees within two meters of the forest edge at edge sites, or beneath intact canopy at continuous forest sites. We chose this height as we previously found significant changes in mosquito communities between ground level (0 m) and 5 m when sampling at a treefall gap inside the same forest.³³ We also saw significant breakpoints in temperature and relative humidity between these heights when sampling beneath the forest canopy.³²

Mosquito collections

Mosquitoes were sampled using hand-nets over 12 months beginning in the early dry season on 6 July 2021 and ending on 30 June 2022. When sampling a site, two collectors worked simultaneously, one at ground level and one on the platform, to collect all approaching mosquitoes between 10:00 and 15:00, when the vector species of primary interest tend to be active.^{71,83} These were aspirated and separated into 50 mL Falcon tubes at 30-min intervals. Tubes containing live mosquitoes were placed in a Styrofoam box in the shade to prevent desiccation until they were transferred to a -80°C freezer at the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD) at the end of each day. The height at which a collector worked was generally alternated daily to reduce bias caused by individual differences in mosquito attraction and collection ability. We aimed to sample one site per day, three days per week, rotating between sites in a 12 x 12 Latin square design.

Microclimate, weather, rainfall

Environmental variables were recorded to investigate their associations with edge type and/or the abundance and occurrence (presence/absence) of key mosquito taxa. Hygrochron iButton data loggers (Maxim Integrated, San Jose, California) were used to record temperature ($^{\circ}\text{C}$) and relative humidity (%) at each height at 15 and 45 min past each hour (i.e., the midpoint of each 30-min interval) throughout the daily sampling period. iButtons were placed in nylon mesh bags; one was hung from vegetation close to the collector at ground level while the other was hung above the platform. Data were additionally used to calculate the daily minimum, maximum, mean, and range of both temperature and relative humidity variables. Weather was manually recorded at 30-min intervals in dry conditions as 1 = clear skies, 2 = scattered cloud, 3 = overcast, and in wet conditions as 4 = light rain, or 5 = heavy rain. Collections were suspended in stormy conditions. The daily mean weather was then calculated, with values closer to 1 indicating favorable weather and values closer to 5 indicating inclement weather. Precipitation data, obtained from an automated meteorological station⁸⁴ (INMET code: Manaus-A101, OMM: 81730, 3.103682° S, 60.015461° W), were used to calculate 7-day cumulative rainfall lagged at 1, 2, 3, and 4 weeks prior to each sampling day. In this study, we defined the rainy season as November until April when cumulative monthly rainfall consistently exceeded 250 mm⁸⁴; this is a slightly shorter period than the standard regional rainy season of November until May.

Mosquito identifications

Mosquitoes were placed on a chill table (BioQuip, Rancho Dominguez, California, USA) and morphologically identified by Mr. Nelson Ferreira Fé,⁸⁵ who was unaware of the site of origin of the specimens, using a stereomicroscope and taxonomic keys as previously described.³² Genus and species names and respective abbreviations follow Wilkerson et al.⁶⁰ Samples were stored at -80°C for future arbovirus screening.

Ethics and permits

Mosquito collections at the Ducke reserve were approved by local environmental authorities (SISBIO license 57003-6) and the study did not involve endangered or protected species. When collecting with hand-nets, skin was not deliberately exposed to attract mosquitoes and mosquito landing was not permitted. Collectors are listed among the co-authors and were fully aware of the nature of the research. They wore trousers, a long-sleeved shirt and/or repellent to minimize the risk of being bitten and had been vaccinated against yellow fever.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were performed with JMP Pro 17⁸⁶ and significance was defined as $p < 0.05$ unless stated. Details of statistical tests are described below and summarized in the results, figures, and figure captions, including the exact value of N and what it represents, and measures of central tendency and dispersion.

To investigate how microclimate varied across forest edges, by season, and by height of collection, Spearman's rank correlation was first used to identify significantly associated variables, of which, mean temperature and mean relative humidity were chosen for further analysis. To compare microclimate between two groups: forest edges ($N = 9$) vs. continuous forest ($N = 3$), rainy season vs. dry season, and 0 m vs. 5 m, a two-tailed t-test for normally distributed data and a Wilcoxon Rank-Sum test for non-normal data were used. To compare microclimate across the three edge habitats for both combined and height specific data, a one-way ANOVA (normal data) and a Kruskal-Wallis test (non-normal data) were used.

Measurements of mosquito community similarity and diversity were based on specimens identified to the rank of species. For these analyses, data were grouped by 1) edge type, 2) edge type and height, or 3) edge type and season (at 0 m and 5 m separately). The Morisita overlap index, based on species count data, was calculated using the PAST version 4.14 software package⁷³ to

compare mosquito community composition between sampling sites for each edge type, and for data grouped as described above. To compare the similarity of communities based on relative species abundance data, Spearman's rank correlation was first used to identify highly significantly correlated species. Where significance was $p \leq 0.01$, the least abundant of the two species was excluded. The resulting datasets were then used for principal components analysis followed by hierarchical clustering of the principal components.

To estimate species richness and examine whether sampling was adequate to capture total richness, iNEXT⁷² (R version 4.2.2) was used to generate rarefaction curves by edge type for each height and season sampled. Species evenness was calculated for each site as Shannon-Wiener diversity index (H') divided by the natural logarithm of species richness.¹⁰ Multiple regression was used to test the association between evenness and edge type, height, and season, as well as the pairwise interactions among each of the three independent variables. When significant interaction effects were detected, simple effect tests (ANOVA) were used to identify the effect of one variable with the other held constant, and post-hoc Student's t-tests were used to identify significant differences among levels of the target variable. Data from 5 m at the urban edge was excluded from this analysis due to small sample sizes that may skew values of evenness.

A Wilcoxon Rank-Sum test (non-normal data) was used to compare differences in mosquito abundance between rainy season vs. dry season at each edge type for each of the eight most abundant species sampled overall. Data from both heights were combined, and comparisons with fewer than 60 mosquitoes (an arbitrary cutoff to reduce random sampling effects) were excluded.

Nominal logistic regression was used to test associations between the occurrence of each of the eight most abundant species with environmental variables chosen based on our field observations and previous work.^{17,32} These were: edge type, mean weather, mean temperature, mean relative humidity, height, and 7-day cumulative rainfall lagged at 1, 2, 3, and 4 weeks. Variables were removed sequentially from the model until all remaining variables contributed significantly or only one variable was left. Due to the high number of variables, we chose an alpha value of 0.01 as highly significant for this analysis, while $0.01 > p < 0.1$ was considered marginally significant, and tested associations in each season separately. We supplemented this analysis by testing the effect of edge type and height on species occurrence using a generalized linear model with a normal distribution and an identity link function, based on the % positive sampling days at ground level and platform sites (thus $N = 6$ in total) for each edge type. If the interaction effect was significant, we conducted a Kruskal-Wallis Rank-Sum test to analyze simple effects of edge type for each height separately. We additionally used a standard least squares analysis to test rainy season associations between environmental variables and abundance of the eight species. For this analysis, sampling sites were nested into edge type, which was not included as a variable, although edge types with significantly lower abundance were excluded. Variables included in the model were otherwise the same as described above. We did not test dry season associations with species abundance due to data being heavily zero inflated.

Prompted by our field observations, we used contingency tables and a Pearson's chi-square test for large frequencies to further explore relationships between *Sabethes* mosquitoes grouped at subgenus level (*Sabethes* or *Sabethoides*), height, and edge type using 30-min occurrence data.³⁴