Microclimate and the vertical stratification of potential bridge vectors of mosquito-borne viruses captured by nets and ovitraps in a central Amazonian forest bordering Manaus, Brazil

Adam Hendy1, Danielle Valério², Nelson Ferreira Fé², Eduardo Hernandez-Acosta³, Claudia Mendonça², Eloane Andrade², Igor Pedrosa⁴, Edson Rodrigues Costa⁴, José Tenacho Andes Júnior², Flamarion Prado Assunção², Bárbara Aparecida Chaves², Vera Margarete Scarpassa⁵, Marcelo Gordo⁴, Michaela Buenemann⁶, Marcus Vinicius Guimarães de Lacerda²,⁷, Kathryn A. Hanley³,⁷ & Nikos Vasilakis¹

In the Americas, some mosquito-borne viruses such as Zika, chikungunya, and dengue circulate among humans in urban transmission cycles, while others, including yellow fever and Mayaro, circulate among monkeys in sylvatic cycles. The intersection of humans and wildlife at forest edges creates risk for zoonotic virus exchange. We built a scaffold tower at the edge of a treefall gap in rainforest bordering Manaus, Brazil, to identify vectors that may bridge transmission between humans and monkeys. We vertically sampled diurnally active, anthropophilic mosquitoes using handheld nets at 0, 5, and 9 m and container-breeding mosquitoes in ovitraps at 0, 5, 10, and 15 m. *Haemagogus janthinomys* and *Psorophora amazonica* were present in high relative abundance in nets at each height sampled, while anthropophilic species were uncommon in ovitraps. *Hg. janthinomys* was more abundant at elevated heights than at ground level, while *Ps. amazonica* abundance was not significantly stratified across heights. The presence of each species increased with increasing 7-day rainfall lagged at 1 week, and at 1 and 4 weeks prior to collection, respectively. In addition, *Hg. janthinomys* was most frequently collected at 29.9 °C, irrespective of height. These data provide insight into the potential role of each species as bridge vectors.

Arboreal mosquitoes transmit sylvatic arthropod-borne viruses (arboviruses), such as yellow fever virus (YFV, *Flaviviridae: Flavivirus*), among wildlife in forests in Africa and Latin America¹. YFV was first introduced to the Americas in the seventeenth century², transported by *Aedes aegypti* mosquitoes among shipments of enslaved people from West Africa³. Having arrived in the neotropics, the virus caused devastating urban outbreaks and eventually spilled back into a sylvatic cycle involving susceptible monkeys and *Haemagogus* and *Sabethes* species mosquitoes¹². Beginning in the 1940s, campaigns to eliminate *Ae. aegypti* throughout much of Latin America extinguished urban YFV transmission in the region¹³. Human disease is now a consequence of spillover from...
the sylvatic cycle mediated by mosquito bridge vectors that bite both humans and monkeys where they occur in close contact, such as at forest edges. The discontinuation of *Ae. aegypti* control programs in the 1970s has allowed this vector to re-establish across the neotropics. Despite this, YFV was largely confined to the Amazon basin until the early 2000s, when it resurfaced in several southeastern states including Minas Gerais, São Paulo, and Rio Grande do Sul. These outbreaks were followed by the largest modern epidemic of YFV in Brazil, which between 2016 and 2019, resulted in 2,166 confirmed human cases and 751 deaths caused by spillover from the sylvatic cycle. In addition, at least 1,567 YF infections were detected in monkeys during this period, of which 90.9% occurred in southeastern Brazil. While there was no clear evidence of *Aedes*-borne urban transmission among humans, the virus threatened large, immunologically naïve, urban populations in the states of Rio de Janeiro and São Paulo with the potential for devastating consequences.

In the presence of susceptible hosts and competent vectors, areas at risk of arbovirus spillover are also likely to be at risk of spillback from humans to wildlife. In Latin America, dengue (*DENV, Flaviviridae: Flavivirus*), chikungunya (*CHIKV, Togaviridae: Alphavirus*), and Zika (*ZIKV, Flaviviridae: Flavivirus*) viruses have all emerged or re-emerged in recent years, where they currently circulate in urban cycles maintained by *Ae. aegypti* and *Ae. Albopictus*. There is now concern that one or more of these viruses may spill back into neotropical forests in the region, which would make their elimination almost impossible and thereby create an enduring threat to human health.

Which mosquito species effectively bridge urban and sylvatic cycles is determined in part by their vertical stratification relative to humans living at ground level and sylvatic hosts which may prefer different heights within the forest canopy. We recently identified *Haenagogus janthinomys*, *Sabethes chloropterus*, and *Psorophora amazonica* as potential bridge vectors of mosquito-borne arboviruses in a rainforest reserve bordering the city of Manaus, Brazil. The two former species are major sylvatic vectors of YFV in Latin America and are commonly active in forest canopies while the latter species appears to be relatively scarce. All three species opportunistically feed on humans and are primarily active during the daytime. Twenty-four-hour BG-Sentinel trap collections made at 0, 5, 10, and 15 m in height revealed that *Hg. janthinomys* and *Sa. chloropterus* were significantly stratified and occurred most frequently above the ground, while *Ps. amazonica* was present at all heights sampled during collection periods and the occurrence of key taxa. *Haenagogus* species and *Sa. chloropterus* were associated with rainfall at 0 m and one week respectively, while *Ps. amazonica* was associated with rainfall at 15 m. In addition, analyses of relationships between microclimate and the occurrence of *Haenagogus* and *Sabethes* mosquitoes showed that detection of both genera significantly increased with increasing temperature and decreasing relative humidity at several heights sampled. These results provide additional evidence that *Hg. janthinomys* and other acrodendrophilic species increase in abundance at ground level at forest edges, where high temperatures and low humidity create conditions similar to those found in the forest canopy.

An important limitation of the above study was our reliance upon BG-Sentinel traps for sampling. These traps were designed for the collection of diurnally active urban *Aedes* vectors and although they should provide reliable estimates of the relative stratification of *Hg. janthinomys*, *Sa. chloropterus*, and *Ps. amazonica*, they do not permit inferences to be made about the absolute abundance of the anthropophilic fraction of these species. Indeed, based on these collections, both *Hg. janthinomys* and *Sa. chloropterus* appeared to be relatively scarce within the mosquito community, but all methods of sampling are biased towards the collection of certain species, or developmental and physiological stages of mosquito populations. In addition, spatial bias, caused by the type of habitat being sampled or the positioning of traps relative to the forest canopy and forest edges, may influence the composition of species collected, while temporal bias caused by the season or year of collection may also have an effect. Thus, robust inferences about highly diverse mosquito communities require a diversity of sampling approaches deployed across space and time.

To meet this need, we sampled diurnally active anthropophilic mosquitoes, the group most likely to effect spillover and spillback, using handheld nets, and ovipositing mosquitoes using artificial and natural containers. Sampling took place at a 10 m scaffold tower constructed at the edge of a treefall gap in the same rainforest reserve that we initially surveyed using BG-Sentinel traps. Focusing on potential arbovirus bridge vectors, we hypothesized that results from net collections would corroborate our initial conclusions regarding stratification and relationships with microclimate but would reveal different patterns in the relative abundance of key species.

Methods

**Study area.** The study was conducted at the Adolpho Ducke forest reserve (Ducke) bordering Manaus in Amazonas State, Brazil (Fig. 1). Locally circulating urban arboviruses include *DENV, CHIKV, and ZIKV*, while YFV is present in a sylvatic cycle. The reserve comprises 100 km² of primary rainforest which borders the city and is contiguous with the surrounding Amazon. Humans, wildlife, and both urban and sylvatic mosquitoes exist in close contact, creating conditions that are favorable to arbovirus spillover and spillback. Ducke is mainly accessed by researchers and students and is home to six monkey species. Several are common to the forest understory and edge, namely the golden-faced saki (*Pithecia chrysocephala*), the pied tamarin (*Saguinus bicolor*), and the tufted capuchin (*Sapajus apella*) which are known to be highly active during the day. The Guianan spider monkey (*Ateles paniscus*) and bearded saki (*Chiroptera chiroptera*) are also present. The rainy season generally lasts from November to May and the dry season lasts from June to October.

**Study site.** The study site, referred to as site B in our previous publication, was established at a large treefall gap 480 m from the forest edge in the northwestern corner of the reserve (02.92537° S, 059.96582° W). The
surrounding forest was populated by trees 20 – 30 m in height and mosquitoes present in the area included *Hg. janthinomys*, *Sa. chloropterus*, and *Ps. amazonica*.

**Adult mosquito collections.** A 10 m tall scaffold tower (Fig. 2a), with platforms constructed at 5 m and 9 m, was built next to a standing tree (*Geissospermum argenteum*) at the edge of the treefall gap to investigate the stratification of diurnally active, anthropophilic mosquito species (Supplementary Data S1). Mosquitoes were collected between 10:00 and 15:00 on each sampling day, a time period during which the species of interest tend to be active. Collections were made by three people using 30 cm diameter handheld nets who worked simultaneously at heights of 0, 5, and 9 m. Each mosquito was placed individually in a numbered 3.5 mL push cap tube (Sarstedt AG & Co., Nümbrecht, Germany) and the time of collection was recorded to the nearest minute. Collections were restricted to a maximum of one mosquito per minute due to the number of tubes available. Ten collectors were involved in sampling over the course of the study. The height at which a collector worked was rotated daily where possible to minimize potential effects of collector bias. Collections were generally made twice a week for a total of 36 days between 13 November 2019 and 9 April 2020, when sampling was terminated due to the COVID-19 pandemic. Mosquitoes were stored in the field on dry ice and were transferred to a −80 °C freezer at the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD) until identified.

Hygrochron iButton data loggers (Maxim Integrated, San Jose, California), placed in nylon mesh bags and attached to the tower at 0, 5, and 9 m, were used to record temperature (°C) and relative humidity (%) at 30-min intervals (on the hour and at 30 min past each hour) during each sampling period. Each individual mosquito was assigned to the temperature and humidity datapoint closest to its time of collection, with mosquitoes collected at 15 or 45 min past each hour assigned to the following datapoint. Precipitation data, obtained from...
the automated INMET meteorological station (Code: Manaus-A101, OMM: 81730) located within Manaus (3.103682° S, 60.015461° W), were used to calculate cumulative 7-day rainfall at lags of 1, 2, 3, and 4 weeks prior to each collection to test associations between past rainfall and the occurrence of adult mosquitoes.

Ovitraps. The stratification and trap preference of ovipositing mosquitoes was investigated by collecting mosquito eggs in three types of water-holding container (Figs. 2b, c, Supplementary Data S2). These were standard black plastic ovitraps (Gersonimo Strategic, El Paso, Texas), Brazil nut tree (Bertholletia excelsa) fruit pods, and 30 cm sections of bamboo with a lid and 2 cm diameter lateral hole. One of each container type was placed at each of four heights (0, 5, 9, and 15 m) in a factorial blocked design. The containers at 0, 5, and 9 m were placed on the tower, while those at 15 m were suspended on a rope hanging from a branch above the tower. Each container was lined with seed germination paper (Nasco, Fort Atkinson, Wisconsin) as an oviposition substrate, along with several decaying leaves and water from a nearby stream. Containers were left in the field for seven days before the water and germination paper were collected and replaced. Ten collections were made between 17 January and 9 April 2020.

Eggs were reared to adults at ambient temperatures (26 °C ± 1) and relative humidity (70–80%) in an insectary at FMT-HVD. Germination papers and water from each container were placed in rearing trays and topped-up with distilled water if necessary. If eggs did not hatch after three days, papers were dried for three days and then resubmerged, up to a maximum four times. Larvae were fed with fish food (Oscar Gold Ornamental Fish Food, Hai Feng, Taiwan) as required. Pupae were separated and placed in cups of distilled water in cages containing cotton wool soaked with 10% glucose solution. Adults were kept alive for 24 h after eclosion to allow male genitalia to rotate before being stored at -20 °C until identified. Any larvae remaining in trays one month after rearing were discarded.

Mosquito identifications. Mosquitoes were placed on a chill table (BioQuip, Rancho Dominguez, California, USA) and identified to species using a stereomicroscope and taxonomic keys as previously described5,15. Genus and species names follow the Walter Reed Biosystematics nomenclature36. Samples were stored at -80 °C for future arbovirus screening.

Statistical analyses. Where possible, analyses were performed that enabled comparisons with our previous work using BG-Sentinel traps15. Two approaches were used to investigate changes in the community composition of diurnally active, anthropophilic mosquitoes at each height. The first was the Morisita overlap index37 calculated using the PAST software package46. This is a commonly used measure of dispersion which provides a value between 0 (indicating no similarity between sample units) and 1 (complete similarity). The second approach was a principal components analysis of the relative frequency of each species followed by hierarchical clustering of communities. Contingency tables and Pearson’s chi-squared tests were used to compare the relative abundance of genera and species collected using nets and BG-Sentinel traps; rare genera were grouped and 1 count was added to each cell in the analysis to avoid zero values. Kruskal–Wallis tests followed by post-hoc Wilcoxon Each Pair tests were used to test the effect of height on the abundance of target species, and lag to first approach. Linear regressions were also used to test the effect of mosquito abundance, height, and mean daytime temperature on lag to first approach for Hg. janthinomys. Nominal logistic regressions were used to test the effect of date, height, mean daytime temperature, and 7-day cumulative rainfall at lags of 1, 2, 3, and 4 weeks on the occurrence (presence or absence) of target species. Nominal regressions were also used to investigate relationships between temperature, which was inversely correlated with relative humidity, and occurrence of these species.

For ovitraps, the Morisita overlap index was used to compare mosquito community composition between container types and among heights. Target species in ovitraps were insufficiently abundant for analysis of stratification in this study. All analyses were performed using JMP 1439 unless stated.

Ethics and permits. We note that our method of collection differed from a human landing collection in that we measured mosquito approach without permitting mosquito landing. No skin was deliberately exposed to attract mosquitoes, and mosquitoes were netted prior to landing on a collector. All collectors are listed among the coauthors and were fully aware of the nature of the research. They wore trousers and either a long-sleeved shirt or repellent to minimize the risk of being bitten and had been vaccinated against yellow fever. 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.

Results

Variation in microclimate. Microclimate at the tower varied across the daily sampling period with temperature highest and relative humidity lowest around midday and the early afternoon hours, although the time of peak temperature and nadir humidity varied by height (Fig. 3a, b). Mean temperature was highest at ground level at 11:30 (30.0 °C) when it was on average 0.2 °C hotter than at 9 m, whereas at 5 m and 9 m, it was highest at 13:30 (29.7 °C and 30.3 °C, respectively). The inverse was true for mean relative humidity, which was lowest at ground level at 11:30 (83.8%) and lowest at 5 m and 9 m at 13:30 (80.1% and 76.4%, respectively). Both variables showed substantial overlap in means and standard errors across the sampled heights during the morning hours, before diverging in the afternoon. For comparison, we extracted microclimate data from the corresponding sampling period in the BG-Sentinel trap study15, which revealed clear differences in temperature and humidity at each height sampled (Fig. 3c, d). BG-Sentinel traps were often hung beneath the forest canopy where it was considerably cooler and more humid than at the treefall gap, particularly at ground level.
Community composition of diurnally active, anthropophilic mosquitoes. A total of 2146 adult mosquitoes representing seven genera and 34 species were collected using nets (Fig. 4a), of which 99.8% (2142/2146) were female and 99.7% (2140/2146) were identified to species level. Mosquito abundance was similar at ground level and 9 m but was slightly lower at 5 m, while species richness was higher at ground level (28 species), than at 5 m (18 species) and 9 m (22 species). Psorophora was the most abundant genus (1231 mosquitoes, 57.4% of the total catch), followed by Haemagogus (32.3%), and Sabethes (6.6%). The genera Limatus (1.4%), Culex (1.2%), Wyeomyia (1.0%), and Onirion (< 0.1%), formed just 3.7% of the total catch. In agreement with our BG-Sentinel trap collections from the same area (Fig. 4b), Ps. amazonica (82.4% of identified Psorophora) and Hg. janthinomys (97.3% of identified Haemagogus) dominated collections within their respective genera, while Ps. albigena (16.8% of identified Psorophora) was also relatively abundant. Sabethes was the most diverse genus with 11 species collected, and Sa. chloropterus (32.4% of identified Sabethes) was the most abundant of these.

The Morisita overlap index for specimens identified to species level (n = 2140 mosquitoes) showed substantial overlap in community composition between the sampled heights. However, elevated (5 m and 9 m) communities were more similar to each other (0.966) than they were to the ground level community (0.945 and 0.896 for 5 m and 9 m comparisons with ground level, respectively). These results were consistent with clustering based on principal components generated using the summed numbers for each species per height (Fig. 4c) and agreed with the results of our BG-Sentinel trap collections (Fig. 4d).

Comparing nets with BG-sentinel traps. When comparing handheld net collections in this study with those made using BG-Sentinel traps in the same area of the reserve15, there was a significant difference in relative genus abundance, pooled across height (Pearson's chi-squared test, DF = 8, χ² = 423.9, P < 0.0001) (Fig. 4). The relative abundance of Haemagogus was higher in nets than in traps, while for Psorophora, the reverse was true. The relative abundance of Sabethes was similar using both methods. When comparing the methods for individual species stratified by height, there was a significant difference in the relative abundance of Hg. janthinomys (DF = 2, χ² = 22.7, P < 0.0001) which was collected in higher numbers at ground level compared with elevated heights in nets than in traps. There was also a significant difference in the relative abundance of Ps. amazonica (DF = 2, χ² = 102.1, P < 0.0001), which was collected in lower numbers at ground level compared with elevated heights in nets than in traps. Small sample sizes precluded analysis of Sabethes mosquitoes at species level.

Species abundance by height. As expected, Hg. janthinomys showed significant stratification in abundance across the sampled heights when analyzed using data summarized by date and height (Kruskal–Wallis test,
DF = 2, $\chi^2 = 10.05, P = 0.007$). Post-hoc Wilcoxon Each Pair tests revealed this species to be more abundant at 9 m than at 0 m ($P = 0.01$), but there was no difference in abundance between 0 and 5 m or 5 m and 9 m ($P > 0.09$ for both comparisons). In contrast, there was no significant stratification in abundance of $Ps. amazonica$ or $Ps. albigenu$ across sampled heights ($P > 0.12$ for both comparisons).

**Lag to first approach.** Lag to first approach was defined as the number of minutes that elapsed from the start of collections until the first mosquito of a given species was caught. If no mosquito of that species arrived during a particular collection, lag was set to 300 min (the full duration of collection). Lag to first approach differed significantly between heights for $Hg. janthinomys$ (Kruskal–Wallis, DF = 2, $\chi^2 = 6.4, P = 0.001$) in the afternoon, whereas comparisons between 0 and 5 m ($P = 0.085$) and 5 m and 9 m ($P = 0.051$) were not significant. Neither $Ps. amazonica$ or $Ps. albigenu$ showed significant stratification in the morning or afternoon sampling periods ($P > 0.08$ for all comparisons). *Sabethes* species were not included in analyses of abundance due to small sample sizes.

**Figure 4.** Relative mosquito species abundance (%) by height and collection method for net and BG-Sentinel trap collections. (a) shows the relative abundance of designated species collected using handheld nets, and (b) the relative abundance of designated species previously collected using BG-Sentinel traps$^{15}$. Number of mosquitoes (n) collected at each height listed at top of bar; number of individuals per species included in parentheses next to species name; *asterisk denotes species collected using both nets and BG-Sentinel traps; sp. = single species, spp. = potentially multiple species; genus and subgenus abbreviations follow Walter Reed Biosystematics Unit nomenclature$^{36}$. (c) and (d) each show a dendrogram of PC1 from a principal components analysis of relative species frequency at each height sampled for respective species stacks. Panels (b) and (d) are reproduced from our previous study$^{15}$ and reformatted for the current publication under a Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/).
faster at 9 m than at 0 m (Wilcoxon Each Pair, \( P = 0.01 \)), but did not differ between 0 m and 5 m or 5 m and 9 m \(( P > 0.1 \) for both comparisons). A linear regression showed that, across all heights, lag to first approach decreased significantly as \( Hg. \) janthinomys abundance increased \(( DF = 1, F = 52.1, \ P < 0.001 \)\), and the same pattern held when data from each height was analyzed separately \(( P < 0.005 \) for each analysis). As \( Hg. \) janthinomys abundance was higher at 9 m, this suggests that the difference among heights in lag to first approach is attributable to differences in abundance. When only considering collections in which least one \( Hg. \) janthinomys was caught, a multiple linear regression showed that lag to first approach did not differ by height but did differ by abundance \(( DF = 1, F = 16.7, \ P = 0.0001 \)\) and mean daytime temperature \(( DF = 1, F = 4.2, \ P = 0.04 \)\), with faster approaches occurring at higher abundance and higher temperature. Lag to first approach did not differ among heights for either \( Ps. \) amazonica or \( Ps. \) albigena. Again, small sample sizes precluded analysis of \( Sabethes \) at species level. The mean time (+/− 1 S.E.) to first approach for each species at each height is listed in Supplementary Table S4.

**Species occurrence.** Based on data summarized by date and height of collection, nominal logistic regressions revealed significant positive associations between several of the predictor variables and the occurrence of target species. Seven-day cumulative rainfall at a lag of 1 week \(( DF = 1, \chi^2 = 4.7, \ P = 0.03 \)\) was a significant positive predictor of \( Hg. \) janthinomys occurrence \(( \text{Fig. 5} \)\), while height also had a marginal effect but was not significant in this analysis \(( DF = 2, \chi^2 = 5.1, \ P = 0.08 \)\). Rainfall at a lag of 1 week was associated with an increase in \( Haemagogus \) occurrence in our earlier study15. Date of collection \(( DF = 1, \chi^2 = 27.8, \ P < 0.0001 \)\), rainfall at a lag of one week \(( DF = 1, \chi^2 = 8.9, \ P = 0.003 \)\), and rainfall at a lag of four weeks \(( DF = 1, \chi^2 = 4.6, \ P = 0.03 \)\) were all significant predictors of \( Ps. \) amazonica occurrence \(( \text{Fig. 5} \)\). Mean daytime temperature followed a similar pattern of increase with higher temperatures corresponding to higher occurrence. The mean time (+/− 1 S.E.) to first approach for each species at each height is listed in Supplementary Table S4.
but non-significant trend (DF = 1, \( \chi^2 = 3.3, P = 0.07 \)) and height had no significant effect on the occurrence of this species. The association between *Ps. amazonica* occurrence and rainfall at lags of one and four weeks corresponded with our previous results\(^{15}\). Rainfall at a lag of one week (DF = 1, \( \chi^2 = 9.3, P = 0.002 \)) was a significant predictor of *Ps. albigena* occurrence, while height (DF = 2, \( \chi^2 = 5.0, P = 0.08 \)) had a marginal but non-significant effect on this species which we had not previously analyzed\(^{15}\). Height was a significant predictor of *Sa. chloropterus* occurrence (DF = 2, \( \chi^2 = 14.5, P = 0.0007 \)), but there was no effect of 7-day cumulative rainfall (Fig. 5). We had previously shown that *Sa. chloropterus* occurrence significantly increased with rainfall at a lag of 3 weeks\(^{15}\).

**Relationships between temperature and occurrence.** BG-Sentinel trap collections had previously shown that *Haemagogus* and *Sabethes* species mosquitoes increased in occurrence with increasing temperature and decreasing relative humidity when analyzed at genus level\(^{15}\). In the current study, we found that these mosquitoes were mainly active between 26 °C and 33.9 °C when classified in ≈4 °C increments (Fig. 6), which were chosen to provide a balance between the range and sample size of each temperature class. Both *Ps. amazonica* and *Ps. albigena* were more tolerant of lower temperatures (22–25.9 °C), while *Ps. amazonica* was the most abundant of the species collected at temperatures higher than 33.9 °C.

When analyzing relationships between species occurrence and microclimate using data summarized at 15-min time intervals by date and height, a nominal logistic regression revealed a significant interaction in the impact of height and temperature on the occurrence of *Hg. janthinomys* (DF = 1, \( \chi^2 = 4.4, P = 0.036 \)). Subsequent nominal regressions analyzing the effect of temperature stratified by height showed a significant positive effect of temperature on occurrence at 0 m (DF = 1, \( \chi^2 = 13.9, P = 0.0002 \)) and 5 m (DF = 1, \( \chi^2 = 17.0, P = 0.0001 \)), but not at 9 m (DF = 1, \( \chi^2 = 2.0, P = 0.157 \)). Interestingly, the mean temperature for the intervals in which *Hg. janthinomys* was collected was 29.9 °C at all three heights. Nominal logistic regressions showed no effect of temperature on the occurrence of *Ps. amazonica* (DF = 1, \( \chi^2 = 0.3, P = 0.58 \)) but did reveal an effect on the occurrence of *Ps. albigena* (DF = 1, \( \chi^2 = 7.3, P = 0.007 \)), which preferred cooler than average conditions. There was no effect of temperature on the occurrence of either *Sa. chloropterus* (DF = 1, \( \chi^2 = 2.0, P = 0.15 \)) or *Sabethes* when analyzed at genus level (DF = 1, \( \chi^2 = 0.3, P = 0.59 \)), although sample sizes were small (n = 44 and n = 132, respectively).

**Community composition of mosquitoes in artificial and natural containers.** A total of 2070 adult mosquitoes (50.9% female) representing seven genera and 11 species were reared from ovitraps, of which 99.9%
were identified to species level (Fig. 7). In contrast to net collections, Culex was the most abundant genus (84.7% of the total catch), followed by Limatus (13.7%), Sabethes (1.3%), Wyomyia (0.2%), Haemagogus (0.05%), Orthopodomyia (0.05%), and Toxorhynchites (0.05%). Collections in standard and fruit pod ovitraps were dominated by Culex urichi which comprised 75.2% of the catch in these containers, while Limatus flavisetosus (11.5%) was also relatively abundant. Mosquitoes reared from bamboo represented just 6.1% of the overall catch but included Sa. quasicyaneus (22 mosquitoes), Sa. batesi (5), and Hg. janthinomys (1), which were absent from other container types.

Mosquito communities in standard and fruit pod ovitraps overlapped substantially when analyzed using the Morisita index at species level (Supplementary Table S4) but overlap decreased when comparing these traps with bamboo. When comparing heights for the combined standard and fruit pod ovitraps, there was substantial overlap at 0, 5, and 9 m, but this decreased slightly when comparing each height with 15 m. When comparing heights for individual container types (Supplementary Table S5), the standard ovitrap followed a similar pattern, but the fruit pod showed substantial overlap among all heights sampled. Bamboo communities were quite dissimilar when comparing 0 m with elevated heights, and those at 15 m were distinct from all other heights sampled, but this was based on a small number of specimens.

Discussion

The intersection of humans, mosquitoes, and monkeys in forests bordering urban environments creates risk for spillover and spillback of mosquito-borne viruses between urban and sylvatic cycles. We constructed a tower at the edge of a treefall gap to investigate relationships between microclimate and the vertical stratification of potential mosquito bridge vectors. Our results showed little difference in mean temperature and relative humidity across heights during the morning hours, but in the afternoon, it was hotter and less humid at 9 m than at ground level. This contrasted with the clear stratification of microclimate throughout the corresponding sampling period in our BG-Sentinel trap study15. Our findings at the tower mostly agreed with a study by Shuttleworth investigating the vertical stratification of microclimate at the Ducke reserve40. This showed that differences in temperature and humidity between heights gradually increased throughout the day and decreased at night, although temperature divergence began earlier in the morning than in our study. Others have also reported steep vertical gradients in temperature and humidity in the afternoon relative to the morning41,42. We observed higher mean temperatures at ground level than at 9 m during the morning collection hours and reasons for this may be explained by a breakdown in the vertical stratification of microclimate at forest edges, which has been shown to occur in temperate rainforest43.
We compared diurnally active, anthropophilic mosquito communities sampled with nets in this study with our previous BG-Sentinel trap collections\textsuperscript{15}. In the current study, \textit{Ps. amazonica}, \textit{Hg. janthinomys}, and \textit{Ps. albigena} were the most abundant species, while \textit{Sa. chloropterus} was poorly represented. In agreement with our earlier work\textsuperscript{15}, the Morisita index showed substantial overlap in species composition between the sampled heights but indicated that elevated mosquito communities were more like each other than they were the ground level community. This was also reflected in the results of the principal components analysis. Other studies\textsuperscript{44–46} have shown similar changes in community composition with increasing height above the forest floor and found that species richness and mosquito abundance are generally higher at ground level than at the heights sampled in this study. We also found species richness to be greatest at 0 m, although overall abundance was similar between 0 and 9 m. The latter finding may be explained by our sampling methods which limited collections to one mosquito per minute, potentially underestimating abundance at ground level. This limitation was applicable to \textit{Ps. amazonica} which was present in high relative abundance at ground level compared to elevated heights in BG-Sentinel traps but not in nets.

Our analyses of stratification showed that lag to first approach was closely negatively correlated with the abundance of our target species. The stratification in abundance of \textit{Hg. janthinomys} sampled using nets was less prominent than in the BG-Sentinel trap study (Fig. 4). However, it differed between the morning and afternoon sampling periods, corresponding with differences in the stratification of microclimate (Fig. 3a and b). This species was only more abundant at 9 m than at ground level after midday, when temperatures were higher at elevated heights. Whereas several studies have indicated that \textit{Hg. janthinomys} stratification shifts seasonally with changes in microclimate\textsuperscript{45,46}, there is little known about diurnal shifts in this regard. Unfortunately, we were unable to investigate seasonal changes in stratification or community composition since our collections were truncated by the COVID-19 pandemic. We found no evidence of stratification in the abundance of \textit{Ps. amazonica} or \textit{Ps. albigena} which were frequently caught at each height while \textit{Psorophora} species are known to be predominantly ground dwelling, they are often collected in the forest canopy\textsuperscript{46,47}. In our study, the high relative abundance of both \textit{Ps. amazonica} and \textit{Hg. janthinomys} at each height sampled indicates a clear potential route for arbovirus transmission between the ground and forest canopy.

In contrast with our previous study\textsuperscript{15}, the occurrence of \textit{Hg. janthinomys} was not significantly stratified by height. There was also no effect of height on the occurrence of \textit{Ps. amazonica} and \textit{Ps. albigena}. However, height did influence the occurrence of \textit{Sa. chloropterus} in keeping with its acroendophilic behavior\textsuperscript{15,47}. Several species showed significant associations with rainfall lag, an important predictor of mosquito emergence and vector-borne disease\textsuperscript{48,49}. On this occasion, \textit{Hg. janthinomys} occurrence increased with increasing rainfall at a lag of 1 week, an association that was previously reported for \textit{Haemagogus} mosquitoes at genus level\textsuperscript{15}. The occurrence of \textit{Ps. amazonica} increased at lags of 1 and 4 weeks, concurrent with our previous results\textsuperscript{15}. These findings support our earlier work and highlight reliable traits for modeling the emergence of both species. In addition, the occurrence of \textit{Ps. albigena} was associated with rainfall at a lag of 1 week which reflects the need for \textit{Psorophora} species to develop quickly in transient breeding sites\textsuperscript{50}. However, the association between \textit{Sa. chloropterus} occurrence and rainfall at a lag of 3 weeks that we previously reported\textsuperscript{15} was not detected on this occasion, despite the sample size being slightly larger than in the earlier study. Since \textit{Sa. chloropterus} develops slowly in large tree holes with small openings that minimize water loss\textsuperscript{51,52}, its emergence may be less dependent on rainfall in the weeks immediately prior to collection than other species.

In agreement with our BG-Sentinel trap study\textsuperscript{15}, we found that \textit{Haemagogus} and \textit{Sabethes} species were mainly active between \textasciitilde 26 °C and 34 °C (Fig. 6). In addition, \textit{Ps. amazonica} was more tolerant of more extreme temperatures while \textit{Ps. albigena} also tolerated cooler conditions. We previously suggested that relationships exist between the occurrence of \textit{Haemagogus} and \textit{Sabethes} mosquitoes and microclimate\textsuperscript{15} but had insufficient data to draw inference at species level. In this study, we showed that the mean temperature at time intervals in which \textit{Hg. janthinomys} was collected was 29.9 °C at each height. This was greater than the mean daily temperature at ground level (29.0 °C) and shows that this species prefers warmer conditions. We also found that \textit{Hg. janthinomys} occurrence increased with increasing temperature at 0 m and 5 m, but not at 9 m, where microclimate was more likely to be within its preferred range. Bates\textsuperscript{18} remarked that this species mainly occupied the lower canopy during his extensive investigations in Colombia, while Bates and Roca-Garcia\textsuperscript{19} showed that it was more tolerant than other local mosquitoes to laboratory temperatures of 30 °C. These findings agree with our evidence that \textit{Hg. janthinomys} abundance was not significantly stratified in the morning when temperatures were similar at each height. They may also explain the differences in relative abundance of \textit{Hg. janthinomys} collected at ground level in nets and in traps (Fig. 4). Whereas net collections were often made in direct sunlight, BG-Sentinel traps were mainly hung beneath the forest canopy where microclimate was cooler and more humid than in the current study\textsuperscript{15}. This was especially true at ground level where the mean temperature was never higher than 27.2 °C and \textit{Hg. janthinomys} was rarely caught. In addition to microclimate influencing stratification, a recent study of the sylvan malaria vector, \textit{Anopheles cruzii}, suggests that mosquitoes present at ground level may have a different genetic structure to those found in the forest canopy, and that anthropogenic habitat fragmentation may drive the vertical dispersal of particular genotypes\textsuperscript{53–55}.

In addition to collecting diurnally active, anthropophilic host-seeking mosquitoes, the tower also enabled us to conveniently sample certain container-breeding species. The composition of mosquitoes collected with ovitraps was strikingly different to those sampled with nets and BG-Sentinel traps. Despite rearing over 2000 adults from the three container types, the abundance of target species was too low to investigate vertical stratification. The predominant mosquito in standard and fruit pod ovitraps was \textit{Cx. urichii}, a diurnal species\textsuperscript{55} recently found in high abundance near forest edges at a rural settlement north of Manaus\textsuperscript{56}. However, there is little evidence to suggest that \textit{Cx. urichii} is attracted to humans\textsuperscript{55,57}. The bamboo community was quite distinct from the other ovitraps and was the only container type to yield \textit{Sabethes} mosquitoes. While bamboo ovitraps are useful for collecting certain \textit{Haemagogus} and \textit{Sabethes} species\textsuperscript{55,56}, Galindo et al.\textsuperscript{47,51,58} have shown that the abundance of
Hg. janthinomys and Sa. chloropterus sampled with this method is not always commensurate with the abundance of adult populations. We only reared a single Hg. janthinomys specimen from bamboo despite this species being common at our study site, although our methods differed slightly from others that have proven more successful for collecting this species. 

Our study primarily focused on the population of diurnally active, anthropophilic mosquitoes most likely to effect spillover and spillback in rainforest bordering Manaus. While we identified several potential routes of transmission for mosquito-borne viruses between the ground and forest canopy, there is scope to further investigate crepuscular and nocturnal species not detected during the current study.

Data availability
All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).

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**Author contributions**
NV, KAH, and MVGL conceived and designed the work; NV, KAH, MVGL, and MG supervised; AH DV, CM, ERC, JTAJ, FPA, EA, and IP collected mosquitoes and field data; NFF identified mosquitoes; AH, DV, and EHA curated data; KAH, AH, and EHA analyzed data; AH, KAH, and MB prepared the manuscript; KAH, NV, MB, BC, MG, VMS, and MVGL revised the manuscript. All authors reviewed and approved the manuscript.

**Competing interests**
The authors declare no competing interests.

**Additional information**

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**Correspondence** and requests for materials should be addressed to K.A.H. or N.V.

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