

Forest disturbance and vector transmitted diseases in the lowland tropical rainforest of central Panama

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Abstract

OBJECTIVE To explore possible changes in the community attributes of haematophagous insects as a function of forest disturbance. We compare the patterns of diversity and abundance, plus the behavioural responses of three epidemiologically distinct vector assemblages across sites depicting various levels of forest cover.

METHODS Over a 3-year period, we sampled mosquitoes, sandflies and biting-midges in forested habitats of central Panama. We placed CDC light traps in the forest canopy and in the understorey to gather blood-seeking females.

RESULTS We collected 168 405 adult haematophagous dipterans in total, including 26 genera and 86 species. Pristine forest settings were always more taxonomically diverse than the disturbed forest sites, confirming that disturbance has a negative impact on species richness. Species of Phlebotominae and *Culicoides* were mainly classified as climax (i.e. forest specialist) or disturbance-generalist, which tend to decrease in abundance along with rising levels of disturbance. In contrast, a significant portion of mosquito species, including primary and secondary disease vectors, was classified as colonists (i.e. disturbed-areas specialists), which tend to increase in numbers towards more disturbed forest habitats. At pristine forest, the most prevalent species of Phlebotominae and *Culicoides* partitioned the vertical niche by being active at the forest canopy or in the understorey; yet this pattern was less clear in disturbed habitats. Most mosquito species were not vertically stratified in their habitat preference.

CONCLUSION We posit that entomological risk and related pathogen exposure to humans is higher in pristine forest scenarios for *Culicoides* and *Phlebotominae* transmitted diseases, whereas forest disturbance poses a higher entomological risk for mosquito-borne infections. This suggests that the Dilution Effect Hypothesis (DEH) does not apply in tropical rainforests where highly abundant, yet unrecognised insect vectors and neglected zoonotic diseases occur. Comprehensive, community level entomological surveillance is, therefore, the key for predicting potential disease spill over in scenarios of pristine forest intermixed with anthropogenic habitats. We suggest that changes in forest quality should also be considered when assessing arthropod-borne disease transmission risk.

keywords tropical rainforest, Diptera, vector, diversity, disturbance, abundance, vertical stratification, disease emergence, Panama

Introduction

Anthropogenic actions are recognised as important drivers of environmental change in natural forest ecosystems, often producing negative effects on biodiversity and public

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health [1–3]. Human-induced deforestation has been shown to modify the natural transmission cycle of arthropod-borne zoonotic diseases (i.e. animal origin); forest fragmentation can push disease-causing pathogens to extinction or promote their adaptation and establishment into ecologically altered areas [4, 5]. Previous studies have linked epidemics of malaria, Chagas disease and several arboviroses (e.g. arthropod-borne viral diseases) to various degrees of forest degradation, supporting the effect of deforestation into facilitating disease expansion [6–8]. Nonetheless, the ecological mechanisms by which forest disturbance triggers disease spill over are still poorly understood, making it difficult to predict risky scenarios for future outbreaks in human-altered forest habitats [9].

Mosquitoes (Culicidae), sand flies (Psychodidae–Phlebotominae) and biting midges (Ceratopogonidae) are haematophagous (e.g. blood-feeding) insects in the Order Diptera, which play a key role in the biological transmission of pathogens to humans and wildlife in tropical forest environments. Mosquitoes transmit many important diseases worldwide, including malaria and arboviruses such as dengue, chikungunya, Zika, Yellow Fever and West Nile [10, 11]. Sand flies and biting midges are also involved in the transmission of zoonotic pathogens such as *Leishmania* and *Phlebovirus*, although their roles in the emergence of vector-borne pathogens are relatively less known than the role of mosquitoes [12–14]. Changes in the biodiversity, species composition and abundance of these dipteran assemblages in response to forest disturbance could impact vector–host interactions and/or vector demography, ultimately affecting the outcomes of disease emergence. For instance, deforestation can indirectly increase transmission intensity for certain pathogens, either by shifting vector species composition or increasing population sizes for most efficient vector species in disturbed habitats [2, 15–17]. Vector-specific behavioural responses (e.g. host preferences and vertical feeding or resting patterns) to forest disturbance are also likely to impact entomological risk and the potential for disease emergence [18–21].

In the last decades, rise in human malaria prevalence in the Brazilian Amazon was associated with deforestation favouring the population growth of the main local mosquito vector, *Nyssorhynchus darlingi* [16]. A study conducted by Navia-Gine *et al.* [21] in logged areas of eastern Panama suggests that mosquitoes feed upon hosts in proportion to their biomass, which could have implications for the role of livestocking patterns in vector-borne disease ecology. Biodiversity loss due to deforestation for cattle ranching and agricultural growth is presumed to intensify the interaction between *Didelphis marsupialis*, the main reservoir of *Trypanosoma cruzi* and the primary

vector, *Rhodnius pallescens*, facilitating the spill over of Chagas disease into disturbed areas of central Panama [18, 19]. To date, efforts to investigate the role of forest disturbance in shaping the ecology of insect vectors and related entomological risk have been limited to few species inside narrow taxonomic groups, hence their conclusions cannot be generalised to other vector assemblages.

Previous work by Loaiza *et al.* [22] in the lowland rainforest of central Panama hypothesised that mosquito diversity peaks in pristine forest habitats (i.e. old-growth forest), while the species spectra and relative abundance change across a gradient of disturbance. The abundance of colonist mosquito species (i.e. disturbed-areas specialists), including primary and secondary disease vectors, increased notably in disturbed forest habitat. Conservation of old-growth tropical rainforests was henceforth recommended as a strategy for preventing dangerous bites to humans in anthropic environments. Another study by Laporta *et al.* [20] showed that biodiversity effects such as the mosquito vector diffuse competition might support the role of forest conservation into buffering disease transmission or preventing pathogen invasion. Currently though, there is still a need to investigate how forest disturbance influences vector communities in other assemblages, as this might have an indirect impact on the entomological hazard and emergence of unknown arboviral and parasitic diseases [12, 23]. Further research is also needed to understand pathogen transmission dynamics through improved knowledge of the effects of forest disturbance on vector feeding and resting behaviours at different vertical strata and the ecological mechanisms that drive these changes.

Our goal was to explore possible changes in the community attributes of three ecologically and epidemiologically discrete groups of haematophagous dipterans in response to forest disturbance. We compare their diversity, species composition and abundance plus likely ecological roles and behavioural responses across sites depicting different levels of forest quality. If habitat disturbance is a proxy for changes in the community structure of haematophagous dipterans, this could have ramifications for disease emergence through variation of the species composition, abundance and behaviour impacting on entomological risk.

Methods

Analytical framework linking entomological risk with arthropod-borne disease emergence

We used the model of vectorial capacity proposed by Garrett–Jones [24], which is the formula attempting to

quantify the potential for a vector-borne disease outbreak to occur in a given space and time. Where ma is the successful biting (squared because two people need to be bit in order for transmission to occur), P is the extrinsic incubation period of a particular pathogen and n is the daily survival of the mosquito. We focus on m , which is the mosquito/man abundance ratio (Table 1).

Insect collection

Mosquitoes, sandflies and biting midges were sampled over a 3-year period (2010–2012) from locales at the Former Panama Canal Zone in central Panama. Adult specimens were gathered from three tropical-forested sites that varied in their levels of anthropogenic disturbance and original habitat quality. A pristine site, Barro Colorado Island (BCI), comprising old-growth forest type of habitat, with low levels of disturbance (e.g. >65% forest cover) and two disturbed forest sites, Achiote (ACH) and Las Pavas (PAV), encompassing patches of second-growth forests representative of intermediate (e.g. >35% and < 65% forest cover) and high levels of disturbance (<35%) were considered for insect sampling (Figure S1). A thorough description of the collecting procedure was published earlier [22, 25]. Adult specimens were identified using taxonomic keys [26–30]. A representative portion of the data sets can be obtained by request from the VectorMap portal (<http://vectormap.si.edu/>).

Modelling community metrics across the forest disturbance gradient

Species richness as Poisson distribution, total abundance as negative binomial distribution and evenness as Gaussian or log-normal distribution were the response variables in generalised linear models (GLMs). The explanatory variable was the gradient of forest disturbance (BCI = 1, ACH = 2, PAV = 3). Estimation of parameters of each distribution, for example, the lambda (λ) in Poisson or the mean and SD in Gaussian distribution, was done by maximum likelihood with optimisation algorithms using the package *bbmle* in the R v. 3.5.1 (R Development Core Team; www.r-project.org) [31] (see details in Table 1).

Ecological classification

In order to classify species into discrete ecological categories, a Multinomial Species Classification Method was applied to three independent data sets (e.g. Culicidae, Psychodidae and Ceratopogonidae) with the package *vegan* in R v. 3.5.1 [32]. The disturbance gradient was reclassified into a binomial variable with two categories

(Pristine Forest = BCI, Disturbed Forest = ACH, PAV). This method used a multinomial model based on species abundance in the two habitats (Pristine forest and Disturbed forest) [33]. The model classified species into one of four ecological categories: (i) Climax or forest specialist (species that occur more commonly in Pristine forest); (ii) Colonist or disturbed-areas specialists (species that occur more commonly in disturbed forest); (iii) disturbance-generalist (species that occur equally in both Pristine and disturbed forest); and (iv) Rare (species with low abundance that the model cannot classify with accuracy).

Hypothesis-testing

We expected our results to be in general agreement with that of Loaiza *et al.* [22], which is that ‘Species diversity overall and by dipteran assemblage decreases towards disturbed forest sites’ (Table 1). A GLM approach was used to test this expected result for each response variable, and as function of the disturbance gradient. All hypothesis testing relied in the following null hypothesis: $\beta_1 = 0$, which means there is no effect of disturbance on species richness, total abundance or evenness. Alternatively, this effect could be positive ($\beta_1 > 0$, more disturbance, more diversity) or negative ($\beta_1 < 0$, more disturbance, less diversity). Statistically non-significant results were considered when the probability of z -value was 1–0.05; otherwise, results were considered statistically significant.

We assumed that the observed proportion and abundance of climax species will be higher in pristine forest sites, whereas a higher proportion and abundance of colonist species will be observed in disturbed forest sites, again in accordance with prior findings by Loaiza *et al.* [22]. Pearson’s chi-squared test was applied to check if the proportions of climax, disturbance-generalist and colonist species were similar in mosquitoes, biting midges and sand flies across sites depicting different levels of habitat quality, having 0.05 as the significance threshold. We anticipated that the vertical distribution of haematophagous dipterans will follow an assemblage-specific tendency and change in response to disturbance. Species abundance of mosquitoes, biting midges and sand flies were sorted into a 2×3 contingency table with the disturbance gradient (e.g. from pristine forest habitats at BCI to more disturbed habitats at ACH and PAV) and vertical strata (ground, canopy) as explanatory dependency factors.

Results

Species accumulation curves were close to saturation at a sampling depth of 40 trapping events, indicating that we were able to capture the diversity of dipteran communities

Table 1 Methodological approaches including the description of the mathematical framework and statistical analyses implemented to investigate changes in the community of dipterans in response to habitat disturbance

Analysis	Description	Formula†	Assumption
Vectorial capacity‡	The model attempts to quantify the potential for a vector-borne disease outbreak to occur in a given space and time. The estimation is specific to a location, pathogen species and vector species, and it is affected by a wide array of variables. For instance, successful biting can be influenced by host abundance relative to vector abundance, the spatial distribution of hosts relative to the vector and the host's defensive behaviour. The extrinsic incubation period can be affected by environmental conditions such as temperature and length of daylight. Daily survival of the mosquito can be affected by the host's blood quality, ability of the mosquito to detoxify a bloodmeal, encounters with predators, encounters with insecticide and daily temperature shifts.	$\frac{ma^2 p^n}{-\log_e p}$ eq. 1	We argue about Loaiza <i>et al.</i> 's [22] proposal that the occurrence and relative abundance of colonist–vector mosquito species will increase in disturbed forest habitat, and, therefore, entomological risk and human exposure to disease-causing pathogens would theoretically be higher under this condition of habitat disturbance. Accordingly, the possibility of disease emergence or spill over by a given vector assemblage would also be higher in disturbed forest habitats. To test this empirical postulation, we use the same study scheme as that of Loaiza <i>et al.</i> [22] with blood-seeking females from three ecologically distinct dipteran groupings, which likely transmit parasitic and arboviral diseases in Neotropical rainforest environments.
Sampling efficacy§	The Coleman method was applied to estimate the mean richness, and its standard deviation following Coleman <i>et al.</i> [44]. This method is based on sampling sites/events without replacement.	N/A	Pristine habitats are expected to harbour a larger proportion of species than disturbed forest habitats, and this is irrespective of the insect assemblage being considered.
Species richness¶	Species richness was calculated as the number of taxonomic species in a given sampling site. For instance, if in sampling site #1, there were species 1, species 2 and species 3, then the species richness in this site equalled 3.	N/A	Species richness is expected to be higher in pristine habitats as compared to disturbed forest habitats, and this is irrespective of the insect assemblage being considered. Species richness overall and by dipteran assemblage decreases towards disturbed forest sites.
Species abundance	Total abundance was calculated as the number of specimens (individuals) in a given sampling site. If in site #1 there were sp1 = 10 individuals, sp2 = 12 individuals, sp3 = 7 individuals, then the total abundance equalled 29. Species abundance was calculated as the number of specimens per species, for example, sp1 = 10 individuals, and it was calculated as absolute abundance or natural log abundance.	N/A	Species abundance is expected to be higher in pristine habitats as compared to disturbed forest habitats, and this is irrespective of the insect assemblage being considered. Species abundance overall and by dipteran assemblage decreases towards disturbed forest sites.

Table 1 (Continued)

Analysis	Description	Formula†	Assumption
Pielou's evenness‡‡	Pielou's evenness was calculated to show how numerically equal was a given assemblage of species in a sampling site. For instance, comparing site #1 with site #2 (sp1 = 100, sp2 = 1, sp3 = 50), then site #2 was less even than site #1.	$J' = \frac{H'}{H'_{\max}}$	Evenness is expected to be higher in pristine habitats as compared to disturbed forest habitats, and this is irrespective of the insect assemblage being considered. Evenness overall and by dipteran assemblage decreases towards disturbed forest sites.
Shannon diversity‡‡	The Shannon index quantifies the entropy meaning that high entropy is related to high diversity (more species richness, more evenness).	$H' = -\sum_{i=1}^R p_i \ln p_i$	Diversity is expected to be higher in pristine habitats as compared to disturbed forest habitats, and this is irrespective of the insect assemblage being considered. Diversity overall and by dipteran assemblage decreases towards disturbed forest sites.

†Includes general equations for estimating each statistic and/or the respective modelling distribution equation.

‡We focus on a key component, namely m , as an important parameter of the Garret–Jones vectorial capacity formula (eq. 1). We further assume that the obtained results of expected changes in the abundance of vector species across a gradient of forest disturbance can represent variable levels of entomological risk and pathogen exposure for humans occupying a given scenario of habitat quality. Different from Loaiza *et al.* [22] though, we approach this subject using adult females rather than immature larvae.

§We assessed the property of our sampling effort by looking into the relationship between the proportion of sampled species and the number of trapping events at each sampling site. Species accumulation curves were used to estimate the expected species richness related to the number of sampling sites/events for the three dipteran assemblages separately.

¶Where λ is the estimation of the mean species richness in each sampling site and X_1 is the disturbance gradient.

Poisson($\lambda = \exp^{\beta_0 + \beta_1 X_1}$)

||Where NB stands for Negative Binomial with parameters μ , that is, the mean total abundance in each sampling site, and size, the dispersion parameter. X_1 is the disturbance gradient.

NB($\mu = \exp^{\beta_0 + \beta_1 X_1}$, size = $\frac{\text{mean}^2}{\text{var} - \text{mean}}$)

††Where mean is the mean evenness and SD is the standard deviation, whereas meanlog and SDlog are the analogous parameters for the log-normal generalised linear model. X_1 is the disturbance gradient.

Gaussian(mean = $\beta_0 + \beta_1 X_1$, SD)

log – normal(meanlog = $\beta_0 + \beta_1 \log(X_1)$, SDlog)

‡‡ H' is the Shannon diversity index and H'_{\max} is the maximum possible value of H' if every species was equally likely. J' ranges from 0 (less evenness, more dominance) to 1 (more evenness, less dominance), where p_i is the proportion of individuals belonging to a given species. N/A = It does not apply in this case.

in central Panama (Figure 1). We collected a total of 168 405 haematophagous dipterans, representing approximately 26 genera and 86 species (Tables S1–S3). Biting midges were the most abundant (66 850 - 39.8%) followed by sandflies (51 402 - 30.5%) and mosquitoes (50 153 - 29.7%); the number of observed species was higher in mosquitoes with 53 taxa followed by sandflies with 19 and biting midges with 14. Dominant species within each assemblage were *Psychodopygus panamensis* with 32 561 individuals (63.3%) and *Nyssomyia trapidoi* with 7949 (15.4%) in Psychodidae; *Culicoides heliconiae* with 22 998 individuals (34.4%) and *Culicoides batesi* with 21 376 (31.9%) in Ceratopogonidae; and *Culex coronator* with 15 023 individuals (29.9%) and *Culex*

declarator with 5441 (10.8%) in Culicidae respectively. *Psychodopygus panamensis*, *Nyssomyia trapidoi*, *Culicoides heliconiae* and *Culicoides batesi* were gathered mainly from pristine forest habitats, whereas *Culex coronator* and *Culex declarator* were more frequently collected from disturbed forest sites (Tables S1–S3).

Do patterns of species richness–diversity and dominance–evenness differ across discrete scenarios of forest disturbance?

Regardless of the dipteran assemblage being examined, pristine forest habitats were richer in species than disturbed forest sites, confirming that habitat disturbance

has a negative impact on species richness by triggering the loss of some taxa as the level of disruption increases (Figure 1). Species richness of Phlebotominae (−24%, $P < 0.001$), *Culicoides* (−22%, $P < 0.001$) and Culicidae (−10%, $P = 0.23$) were all lower in disturbed forest sites, but these outcomes are only statistically significant for Phlebotominae and *Culicoides* assemblages (Table 2; Figure 1). With increasing forest disturbance, evenness increases in Phlebotominae (+5%) and *Culicoides* (+4%), but decreases in Culicidae (−68%), whereas mosquito abundance (e.g. dominance) increases (+89%), while the proportion of Phlebotominae (−37%) and *Culicoides* (−69%) decreases, and all these comparisons are statistically significant ($P < 0.001$) (Table 2; Figure 1).

Does the proportion of climax, disturbance-generalist and colonist vary among dipteran assemblage as a function of disturbance?

Results from the Pearson's chi-square test indicate that Culicidae have a larger proportion of species classified as climax or colonist as compared to Phlebotominae and *Culicoides*, whereas these assemblages have more observed climax or disturbance-generalist species ($X^2 = 14.61$, $df = 6$, $P = 0.02352$) (Table 3). For mosquitoes, the proportion of species classified as colonist and their relative abundance increases when one moves from pristine sites to disturbed ones, whilst the opposite trend occurs for those classified as climax species (Figure 2; Figure S2). Except for *Lutzomyia gomezi*, which was the only species classified as a colonist, the rest of Phlebotominae species decrease in their relative abundance along the gradient of disturbance (Figure 2; Figure S3). *Culicoides* species decrease in their proportion in the same fashion as Phlebotominae, despite the ecological category in which they are classified into (Figure 2; Figure S3). These findings favour the interpretation that the species community structure of Phlebotominae and *Culicoides* act similarly, but in a different way to Culicidae, upon facing increasing levels of forest disturbance.

Do blood-seeking dipterans modify their vertical niche preference due to forest disturbance?

Species of Phlebotominae and *Culicoides* partition the vertical niche by being active at the understorey or in the forest canopy respectively. The demarcation of species between canopy and ground-level catches was highest in pristine habitats, with canopy favoured by *Culicoides*, ground by Phlebotominae and no clear vertical trend was detected for Culicidae. The three most abundant Phlebotominae species remain dominant at the understorey

regardless of sampling area, although their proportions tend to increase in the canopy of disturbed sites (Figure 3; Figure S4). Likewise, the three most abundant *Culicoides* species were largely prevalent in the canopy of pristine forest sites, but their numbers tend to increase slightly in the understorey of disturbed sites. Only *Aedeomyia squamipennis* was vertically stratified in pristine forest habitats, where it was more active in the forest canopy, and this acrodendrophilic behaviour persisted in disturbed forest sites (Figure 3; Figure S4).

Discussion

The role of forest disturbance in shaping the community of haematophagous dipterans

Our results partially support findings by Loaiza *et al.* [22] because the species richness of Culicidae, Psychodidae and Ceratopogonidae decreased along with rising levels of forest disturbance. Anticipated changes in mosquito species composition and relative abundance were not observed in Phlebotominae or *Culicoides* though; instead most members of these assemblages vanished almost entirely from disturbed forest scenarios. Differences in the community structure of these three dipteran assemblages in response to forest disturbance are likely due to variation in their ecological requirements as immature life stages, which might be influenced by habitat disturbance in different ways. For example, mosquito larvae develop in various types of aquatic habitats including polluted and unpolluted water, associated with microalgae, floating and emergent vegetation or in epiphyte plants [34]. Immature stages of Phlebotominae and *Culicoides*, instead, develop in damp soil with decomposing organic matter or within dark and humid places such as burrows and crevices associated with abundant leaf-litter [30, 35, 36]. Therefore, changes in soil composition and property owing to deforestation might impact larval breeding site availability more severely for Phlebotominae and *Culicoides* than for mosquitoes, hence increasing the long-term mortality rate, and allowing fewer species to cope with increasing levels of habitat constraints [37]. These conjectures generally agree with the outcomes of prior studies about the response of Phlebotominae and *Culicoides* assemblages to forest fragmentation [36, 38, 39].

Here, we provide further support for findings in Loaiza *et al.* [22] because colonist mosquito vectors were the most prevalent in disturbed habitats. These findings are comparable to those of Meyer Steiger *et al.* [40], who reported that most mosquito species in the Australian tropical forest were classified either as colonists of

grassland habitats or climax of pristine forest settings, while only two species were catalogued as disturbance-generalists out of 26 taxa. Meyer Steiger *et al.* [15] reported more mosquito species capable of transmitting pathogens to humans in grasslands than in pristine forest habitats, further showing that anthropogenic landscape changes could have implications for disease spill over. Alroy [1] further suggested that this bimodal trend in mosquito species diversity and abundance at the opposite extremes of the disturbance gradient is probably due to the invasion of colonist species adapted to open habitats, transiently increasing the size of the species pool in disturbed forest scenarios.

The ecological processes that increase the risk of vector-borne disease emergence

Community attributes of mosquitoes support increased entomological risk and potential for pathogen emergence in disturbed forest settings. For instance, zoonotic

and bridge vectors of several arboviruses including *Culex Mel. pedroi*, *Culex declarator*, *Culex coronator*, *Aedes serratus*, *Coquillettidia venezuelensis*, *Coquillettidia nigricans* and *Psorophora cingulata* increased significantly in abundance in disturbed forest sites, which means that the probability of an infectious bite to humans is higher under a scenario of low habitat quality. However, here we provide evidence for a different pattern in Phlebotominae and *Culicoides*, whose mainly climax or disturbance-generalist species dominated in pristine forest scenarios. Community attributes of Phlebotominae and *Culicoides* support increased entomological risk and potential for pathogen spill over in pristine forest settings. For instance, *Psychodopygus panamensis*, *Nyssomyia trapidoi* and *Lutzomyia sanguinaria*, major vectors of *Leishmania* parasites in Central America, as well as highly abundant species of biting midges, *Culicoides heliconiae*, *Culicoides foxi* and *Culicoides batesi* were predominant in pristine forest sites, which mean that the probability of an

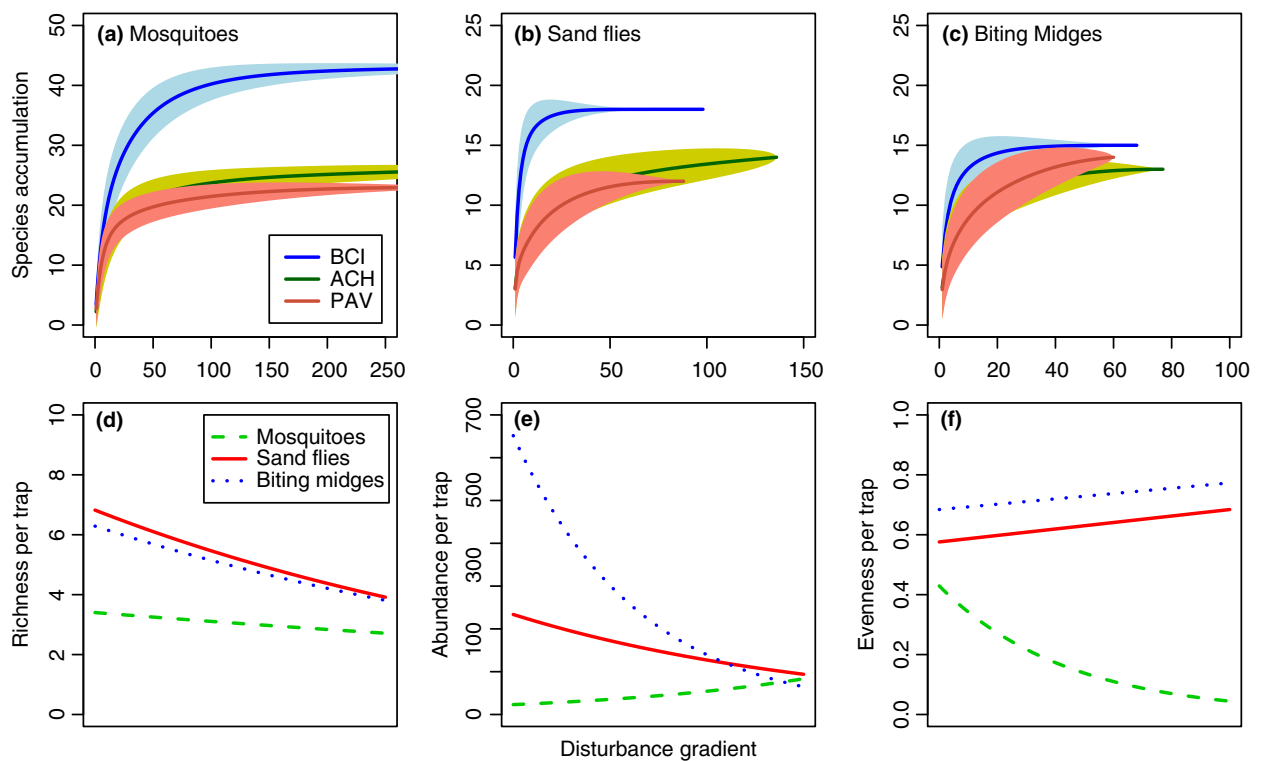


Figure 1 Species accumulation curves (a–c) and diversity–disturbance relationships (d–f). (a) Mosquito, (b) sand flies, and (c) biting midges species accumulation curves according to the sampling sites in BCI, ACH and PAV. Thick lines are the expected species richness and the buffer lines represent the 95%CI [44]. Richness (d), Abundance (e), and Evenness (f) per trap are modelled according to the disturbance gradient. Red solid lines represent sand flies. Blue dotted lines represent biting midges. Green dashed lines represent mosquitoes.

Table 2 Number of traps, species richness, total abundance, Pielou's evenness and Shannon diversity per dipteran assemblage and sampling area, in central Panama (2010–2012)

Arthropod assemblage	Site	Number of traps	Mean (SD) species richness per trap	Mean (SD) total abundance per trap	Mean (SD) Pielou's evenness per trap	Mean (SD) Shannon diversity per trap
Mosquitoes (Culicidae)	BCI	336	3.73 (4.37)	21.66 (45.53)	0.41 (0.41)	0.74 (0.81)
	ACH	336	2.39 (3.77)	49.13 (142.87)	0.22 (0.34)	0.43 (0.67)
	PAV	336	3.04 (4.97)	78.47 (147.69)	0.21 (0.34)	0.50 (0.79)
Sand flies (Psychodidae)	BCI	98	7.29 (5.58)	240.4 (235.52)	0.57 (0.16)	0.87 (0.51)
	ACH	136	4.5 (1.17)	142.03 (115.82)	0.65 (0.14)	0.95 (0.21)
	PAV	88	4.43 (1.06)	96.93 (48.87)	0.67 (0.12)	0.98 (0.19)
Biting midges (Ceratopogonidae)	BCI	68	6.6 (2.03)	697.01 (742.8)	0.68 (0.11)	1.21 (0.16)
	ACH	77	4.36 (1)	153.8 (80.54)	0.74 (0.10)	1.06 (0.11)
	PAV	60	4.15 (1.16)	74.95 (25.75)	0.77 (0.12)	1.04 (0.16)

Table 3 Number of species and relative frequencies per groupings and ecological role (Disturbance-generalist, colonist or disturbed-areas specialists, climax or forest specialist and rare), in central Panama (2010–2012)

Category	Species	Proportion
Mosquito – Culicidae		
Disturbance-generalist	7	0.132
Colonist	11	0.208
Climax	23	0.434
Rare	12	0.226
Sand flies – Phlebotominae		
Disturbance-generalist	8	0.444
Colonist	1	0.056
Climax	7	0.389
Rare	2	0.111
Biting midges – Culicoides		
Disturbance-generalist	8	0.533
Colonist	2	0.133
Climax	3	0.200
Rare	2	0.133

infectious bite to humans is higher under scenarios of high habitat quality.

Species of Phlebotominae showed vertical stratification with active search for hosts mainly at the ground level of pristine sites, but can also approach hosts at the forest canopy of disturbed sites. While a shift in their blood-seeking behaviour may contribute to the dispersion of parasites from canid and rodents into canopy species such as monkeys, sloth and birds, this is less likely to impact accidental pathogen transmission to humans. *Culicoides* species were more active in the forest canopy of pristine sites, where they could have contact with monkeys and canopy birds. However, they can also approach hosts at the understory of disturbed forest habitats, where humans can be exposed to their bites and associated disease-

causing pathogens. Nonetheless, the proportion of these *Culicoides* species as well as those in Phlebotominae was lower in disturbed forest sites resulting in reduced entomological risk.

The Dilution Effect Hypothesis

The Dilution Effect Hypothesis (DEH) suggests that high biodiversity in pristine natural ecosystems has a buffering effect on the emergence of zoonotic pathogens to humans [41]. This notion is anticipated because zoonotic pathogens can infect multiple host species in habitat with high biological diversity, while they have greater chances to thrive in generalist host species with fast life-history traits (e.g. r-strategist), including rapid development and explosive population growth, which are more prevalent in habitat with low biodiversity [6, 42]. According to the DEH, disease emergence is more likely to occur in ecologically altered areas where fewer host species exist, and more competent hosts are found, which in turn fosters the link between competent host and efficient vector species, thus increasing the odds for human infections. The question remains as to whether or not the assumption of the DEH applies broadly to arthropod-borne zoonotic diseases. If both host and vector communities respond analogously to habitat disturbance, they may correlate to determine risk of pathogen spill over in low biodiversity forest environments.

Our findings suggest that neglected tropical disease vectors such as Phlebotominae and *Culicoides* can increase disease risk in pristine forest habitats, meaning that the dilution of a zoonosis buffered by high biodiversity is context dependent. This may not simply be due to an inverse relationship between species diversity and forest disturbance, but rather to a higher risk of exposure to infectious bites for humans occupying pristine habitats,

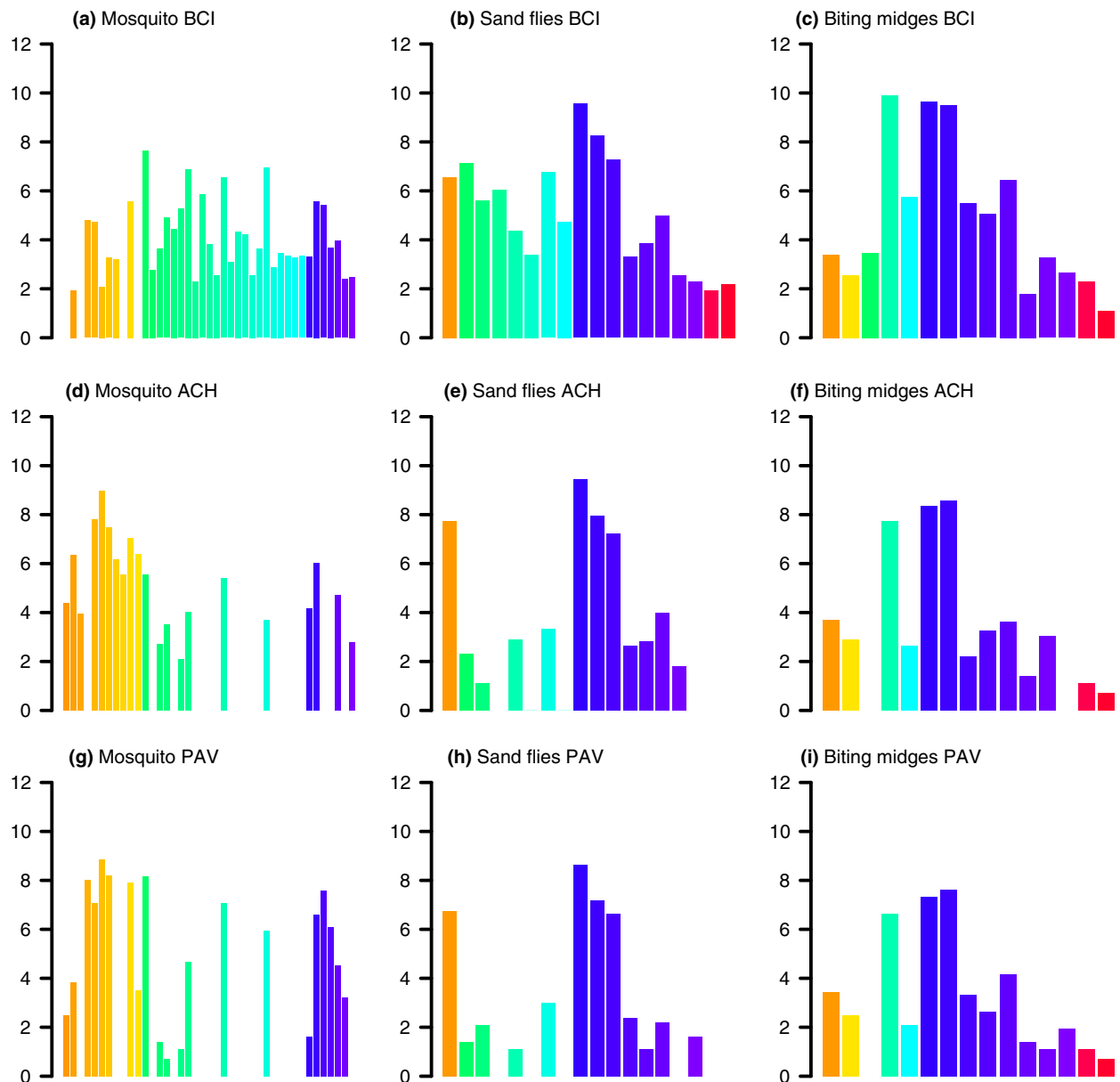


Figure 2 Barplots of log-abundance of species according to groupings and locations. (a, d, g) mosquito; (b, e, h) sand flies; and (c, f, i) biting midges in BCI, ACH, PAV respectively. Each bar represents the abundance (in a natural log scale) of a given species. Yellowish barplots represent Colonist Species (disturbance specialists as defined in [22]). Greenish barplots represent Climax Species (forest specialists as defined in [22]). Bluish barplots represent disturbance-tolerant species (generalists as defined in [22]). Reddish barplots represent rare species.

where major disease vectors prevail. We argue that entomological risk and related pathogen exposure to humans is higher in high biodiversity forest ecosystems for Phlebotominae and *Culicoides*-transmitted pathogens, whereas habitats with low biodiversity pose a higher

entomological risk in the case of mosquito-borne infections. We also argue that idiosyncratic findings show that the DEH was effectively applied to a narrow group of zoonotic diseases associated with rodents or tick vectors (e.g. hantaviruses and Lyme), leaving unsupported many

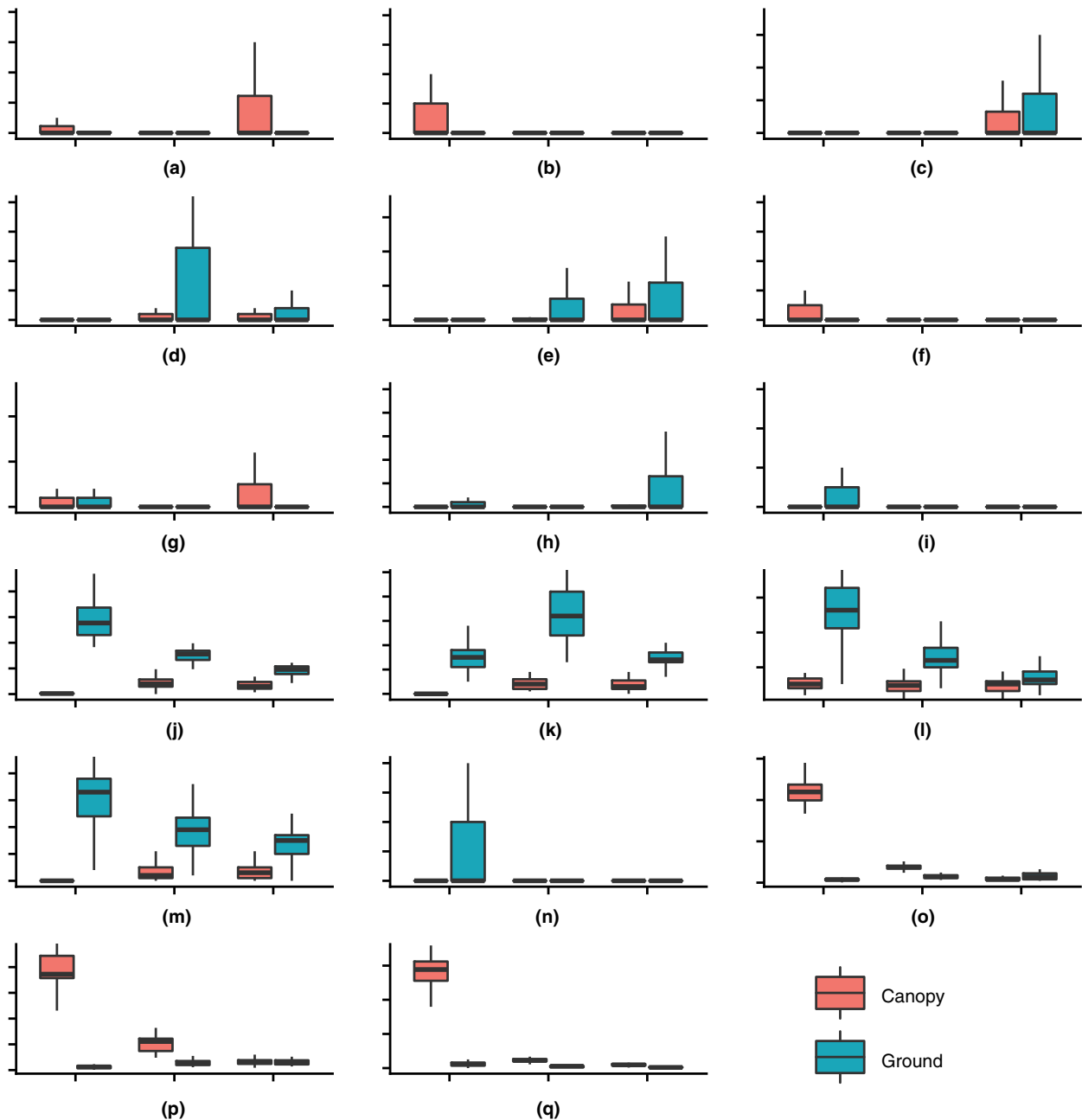


Figure 3 Boxplots of abundance (number of individuals) according to vertical strata and locations. X-axis represents BCI, ACH and PAV, in that order. Letters represent Dipteran species as follows: (a) *Aedeomyia squamipennis*, (b) *Nyssorbynchus triannulatus*, (c) *Coquillettidia nigricans*, (d) *Coquillettidia venezuelensis*, (e) *Culex coronator*, (f) *Culex interrogator*, (g) *Culex nigripalpus*, (h) *Culex pedroi*, (i) *Mansonia titillans*, (j) *Psychodopygus panamensis*, (k) *Lutzomyia gomezi*, (l) *Nyssomyia trapidoi*, (m) *Lutzomyia sanguinaria*, (n) *Nyssomyia ylephiletor*, (o) *Culicoides batesi*, (p) *Culicoides foxi* and (q) *Culicoides heliconiae*.

other neglected zoonotic diseases, especially those transmitted by Neotropical dipterans [12, 23, 43, 45]. To achieve an integrated surveillance system for disease-

causing pathogen in Panama, it will be necessary to know beforehand which arthropod species are present and could play a role in disease transmission. We add that

changes in forest quality should also be considered when assessing arthropod-borne disease risk (*but see the limitations of our work in Table S4*).

We propose two risky scenarios for disease emergence in relation to forest disturbance. Mosquito species richness was higher in pristine forest settings, but colonist species including primary and secondary disease vectors dominated in forest disturbed sites. This shows that mosquito diversity can contribute to the dilution effect of mosquito-borne diseases. Overall species richness and abundance of Phlebotominae and *Culicoides*, including mainly climax species and likely disease vectors, were higher in pristine forest habitats, thus showing an amplification effect on the entomological risk mediated by higher biodiversity. Our findings challenge the prediction power of the DEH in the tropical rainforest of Panama.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Map of Central Panama depicting the sampling locations of the study in the lowland tropical rainforest of the Former Panama Canal Zone: The Barro Colorado Island (BCI), Achioté (ACH), and Las Pavas (PAV).

Figure S2. Natural log-abundance of mosquito species according to forest disturbance (BCI = preserved, ACH = intermediate, PAV = degraded). Dark-blue bars represent disturbance-generalist species. Greenish bars represent forest specialist (climax species). Yellowish bars represent colonist (disturbance-tolerant) species.

Figure S3. Natural log-abundance of sand flies or biting midges species according to forest disturbance (BCI = preserved, ACH = intermediate, PAV = degraded). Yellowish bars represent colonist (disturbance-tolerant) species. Greenish bars represent forest

specialist (climax species). Blueish bars represent disturbance-generalist species. Red bars represent rare species.

Figure S4. Species abundance (number of individuals) of mosquito, sand flies or biting midges species according to forest disturbance and vertical strata. BCI = preserved, ACH = intermediate, PAV = degraded.

Table S1. Absolute and relative frequency of specimens of mosquitoes (Diptera: Culicidae) according to species and forest disturbance category, in central Panama (2010–2012).

Table S2. Absolute and relative frequency of specimens of sand flies (Diptera: Psychodidae) according to species and forest disturbance category, in central Panama (2010–2012).

Table S3. Absolute and relative frequency of specimens of biting midges (Diptera: Ceratopogonidae) according to species and forest disturbance category, in central Panama (2010–2012).

Table S4. Limitations of the study.

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