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LETTER

Does habitat disturbance increase infectious disease risk for primates?

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Abstract

Many studies have suggested that ecosystem conservation protects human and wildlife populations against infectious disease. We tested this hypothesis using data on primates and their parasites. First, we tested for relationships between species' resilience to human disturbance and their parasite richness, prevalence and immune defences, but found no associations. We then conducted a meta-analysis of the effects of disturbance on parasite prevalence, which revealed no overall effect, but a positive effect for one of four types of parasites (indirectly transmitted parasites). Finally, we conducted intraspecific analyses of malaria prevalence as a function of mammalian species richness in chimpanzees and gorillas, and an interspecific analysis of geographic overlap and parasite species richness, finding that higher levels of host richness favoured greater parasite risk. These results suggest that anthropogenic effects on disease transmission are complex, and highlight the need to define the conditions under which environmental change will increase or decrease disease transmission.

Keywords

Amplification effect, dilution effect, disease prevalence, disturbance, geographic range overlap, parasites, primates, species richness.

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INTRODUCTION

Recent reviews have argued that biodiversity reduces disease prevalence, via a hypothesis sometimes called the 'dilution effect' (e.g. Dobson *et al.* 2006; Keesing *et al.* 2010; Ostfeld & Keesing 2012). If widespread, a negative relationship between biodiversity and disease represents an exciting convergence of conservation and public health interests. Zoonotic diseases are a major public health threat, constituting approximately 60% of human infectious diseases (Taylor *et al.* 2001). Meanwhile, biodiversity loss is accelerating, often with concomitant reductions in ecosystem functioning (Barnosky *et al.* 2011; Mace *et al.* 2012). Efforts to market the 'ecosystem services' provided by intact ecosystems have ramped up to incentivize a wide array of conservation efforts; yet for many services – including disease protection – evidence for beneficial effects of biodiversity remains inconclusive (Cardinale *et al.* 2012).

The 'dilution effect' is often used broadly to refer to any scenario in which disease risk decreases as biodiversity increases. A more restrictive definition refers to a specific mechanism of disease reduction wherein less competent hosts have higher relative abundance in species rich communities and intercept pathogen transmission stages, thus reducing disease transmission (Keesing *et al.* 2006). Many studies further assume that disturbance will decrease biodiversity, leading to an indirect positive association between disturbance and infectious disease risk. Studies have described this phenomenon for Lyme disease (e.g. Allan *et al.* 2003; Ostfeld 2011), West Nile virus (e.g. Swaddle & Calos 2008) and hantavirus pulmonary syndrome (e.g. Clay *et al.* 2009); however, these remain the only well-studied examples, and they are contentious (e.g. Randolph & Dobson 2012; Wood & Lafferty 2013), with some suggestion of publication bias towards dilution effects (Salkeld *et al.* 2013).

For the dilution effect to operate broadly, two linked ecological phenomena must be true (Keesing *et al.* 2006). First, species' resilience to biodiversity loss must be linked with competence, such that the proportion of high-competence hosts is elevated in disturbed ecosystems. Second, a larger proportion of high-competence hosts must result in increased disease transmission. An alternate hypothesis, the amplification effect, predicts that increased biodiversity *increases* disease risk (Keesing *et al.* 2006), and can occur if the dilution assumptions are reversed, or if increased host richness and abundance in undisturbed ecosystems facilitate higher parasite richness and abundance (e.g. Hechinger & Lafferty 2005; Jones *et al.* 2008; Dunn *et al.* 2010). Because the dilution and amplification hypotheses lead to opposite predictions about the impact of biodiversity and disturbance on disease, it is critical to understand whether these relationships are strong and consistent across host–pathogen systems.

The first pre-condition of the dilution effect is posited to arise due to life history characteristics: species with 'slow' life history traits are hypothesised to be particularly vulnerable to disturbance due to slow reproductive rates (e.g. Cardillo *et al.* 2008) and to invest heavily in energetically expensive immune defences (Keesing *et al.* 2010; Previtali *et al.* 2012). This latter association could arise if natural selection favours stronger immune defences in 'slow-living' species with long life spans, slow development and low reproduc-

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tive rates. However, support for links between vulnerability to disturbance and life history traits remain equivocal (e.g. Isaac & Cowlishaw 2004; Wood *et al.* in review), and studies have failed to find consistent relationships between immune parameters and life history characteristics (Cooper *et al.* 2012a). Even for well-studied pathogens, the relationship among resilience, immune investment and competence remains unclear, and it is uncertain whether biodiversity loss or environmental disturbance disproportionately affect less competent hosts across a broad array of pathogens.

Here, we take a comparative and meta-analytic approach to investigate the links between disturbance, biodiversity, parasitism and immune defences. We focus on primates because they are important sources of zoonoses (e.g. Liu *et al.* 2010) and their parasites are well studied (Nunn & Altizer 2005). We used four approaches to investigate assumptions and predictions of the dilution (inclusively defined) and amplification hypotheses at a variety of biological scales (Fig. 1). For all four tests, results in the opposite direction of those predicted by the dilution effect are consistent with an amplification effect.

(1) We investigated correlations between host resilience to disturbance and parasite richness, parasite prevalence, and investment in immune defence. The restrictive definition of the dilution effect predicts that resilience is positively associated with parasite richness and prevalence, and negatively associated with immune investment.

(2) We conducted a meta-analysis of studies investigating the effects of disturbance on parasite prevalence and richness in primates. The dilution effect predicts that disturbance increases parasite prevalence and richness within species.

(3) We tested whether mammal richness and human density (a proxy for disturbance) predicts malaria prevalence using data on local prevalence from one study of chimpanzees and gorillas (Liu *et al.* 2010). The dilution effect predicts that lower mammal richness and higher human density increase prevalence.

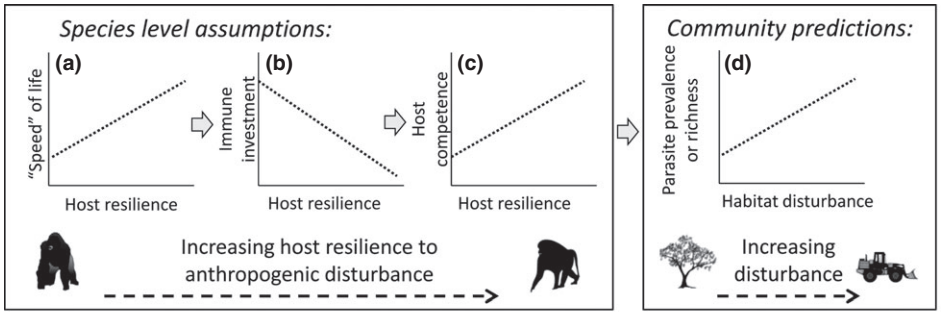
(4) We tested whether human density and geographic range overlap with other primate species predict parasite richness across primates. The dilution effect predicts that lower range overlap with other primates and higher human density increases parasite richness.

Notably, these analyses test effects of both disturbance and diversity on disease. These metrics are quite different; disturbed environments can actually have higher diversity than undisturbed environments (Sax & Gaines 2003), and may influence disease prevalence through other mechanisms (e.g. habitat structure). However, disturbance is often used as a proxy for diversity in dilution effect research and the presumed link between disturbance and disease – with dilution as the assumed mechanism – underlies the broader message in the dilution effect literature advocating conservation to protect public health (Keesing *et al.* 2010). Thus, we tested both metrics when data were available.

MATERIALS AND METHODS

Host competence and resilience: a comparative analysis

We used a comparative approach to identify whether host resilience to anthropogenic disturbance predicts host competence (parasite richness and prevalence) or investment in immune defence (white blood cell counts). This is the only analysis performed that tests specific predictions of the restrictively defined dilution effect (Keesing *et al.* 2006); it does so at the species level. We obtained measures of resilience to disturbance for different primate species from Isaac & Cowlishaw (2004), which quantified the average effect of three types of disturbance – hunting, agriculture and forestry. The authors compiled data from paired population surveys of primates in disturbed and undisturbed areas and calculated the effect size of disturbance as: (abundance in disturbed habitat)/(abundance



Tests conducted for each relationship

A. - *Isaac & Cowlishaw (2004)*

B. - *Comparative resilience: White blood cell count ~ Host resilience + body size*

C. - *Comparative resilience : Parasite species richness ~ Host resilience + body size + citation count*

- *Comparative resilience: Parasite prevalence ~ Host resilience + body size*

D. - *Meta-analysis: Parasite prevalence ~ Disturbance*

- *Meta-analysis: Parasite species richness ~ Disturbance*
- *Within species prevalence: P. prefacilfurm prevalence ~ Human density + mammal species richness + body size*
- *Across species prevalence: Parasite species richness ~ Human density + range overlap + body size + citation count*

Figure 1 Mechanistic assumptions underlying the dilution effect include that species that are more resilient to disturbance (and thus persist in disturbed, low-diversity environments) tend to (a) have faster life histories, (b) invest less in immune defence and (c) be more competent hosts (e.g. higher parasite richness or prevalence) as a result. Based on these patterns, the dilution effect thus predicts that disturbed, low-diversity communities should have an overall higher relative abundance of competent hosts and, through a variety of mechanisms, thus (d) higher prevalence or richness of parasites at the community level. Below we show each of these assumptions and predictions and describe which of the tests performed examine each of these relationships.

in undisturbed habitat). Increasing values reflect greater resilience to disturbance. Isaac & Cowlishaw (2004) found that species' resilience depended on threat type; therefore we maintained the breakdown by threat type in our analyses.

Parasite records were taken from the Global Mammal Parasite Database (GMPD; Nunn & Altizer 2005), a database of published records of parasites in wild primates. We calculated parasite richness for all primates with data on resilience to disturbance, including a total of 1077 unique host–parasite combinations, encompassing 72 primate species and 378 parasite species (202 helminths, 82 protozoa, 52 arthropods, 31 bacteria, 8 viruses and 3 fungi). We measured parasite richness for all parasites (micro- and macro-parasites), and for two subgroups – helminths and vector-borne parasites. Helminths were selected because they are relatively well studied and are commonly specialists on particular primates (Pedersen *et al.* 2005). Parasite specialisation reflects variation in competence among hosts, a key pre-condition for the dilution effect (Keesing *et al.* 2006). Vector-borne parasites were selected because they have been the focus of most previous studies of the dilution effect. We controlled for sampling effort using citation counts from PrimateLit (primatelit.library.wisc.edu). Sampling per individual was not equal across all studies, and studies with fewer samples-per-individual will likely underestimate prevalence, but we lack sufficient information to incorporate this into analyses. Body mass is known to correlate with resilience to disturbance and parasite richness (Isaac & Cowlishaw 2004; Cooper *et al.* 2012a); thus, we included body mass (mean adult female body mass; Smith & Jungers 1997) in our model. For each measure of richness, we fit the model:

$$\log(\text{richness}) \sim \log(\text{resilience} + 1) + \log(\text{body mass}) + \log(\text{citation count})$$

Variables were log transformed to improve model fit (after adding 1 to resilience to enable log transformation of zero values). The relationship between citation count and parasite richness (after log transformation) was linear.

For parasite prevalence, we grouped parasites at the genus level to increase sample size and conducted separate analyses of prevalence across primates for each parasite genus. We analysed primate–parasite pairs for which at least 10 primate species were adequately sampled for a given parasite, where 'adequate sampling' is defined as at least 10 total individuals sampled for presence of the parasite, combining data across studies. Twelve genera met this criterion: five helminths – *Ascaris, Enterobius, Oesophagostomum, Strongyloides* and *Trichuris;* four protozoa – *Chilomastix, Entamoeba, Plasmodium* and *Trypanosoma;* and three viruses – *Deltaretrovirus, Flavivirus* and *Lentivirus.* For each parasite, we computed mean prevalence (weighted by sample size) across studies as a measure of competence for each host, and fit the model:

$$\text{prevalence} \sim \log(\text{resilience} + 1) + \log(\text{body mass})$$

For immune investment, we used data from the International Species Information System (2002, Minnesota Zoological Garden, MN, USA). These samples were taken from healthy, well-fed zoo animals and are considered reference values for species' baseline immune function. We used total white blood cell count as a proxy for immune investment because it is energetically costly and is commonly used in comparative and field research (e.g. Nunn 2002). While reliance on captive animals for reference values has disadvantages, advantages lie in the precision of estimates and larger sample sizes. Sufficient data from wild animals are unavailable.

To control for phylogenetic autocorrelation, we used phylogenetic generalised least squares to incorporate expected dependence among species by representing the error term as a phylogenetic variance–covariance matrix (Pagel 1999). By estimating λ, a multiplier of the off-diagonal elements of the matrix, it is possible to assess the degree of phylogenetic signal in the model residuals. Values of λ range from 0 (phylogenetic independence) to 1 (perfect correlation between the phylogeny and the error structure of the data under a Brownian motion model of evolution). Analyses were run using the R package '*caper*' (Orme *et al.* 2012) with a dated consensus phylogeny from *10kTrees* (Version 3; Arnold *et al.* 2010).

Disturbance and parasitism: a meta-analysis

We conducted a meta-analysis to examine overall local effects of disturbance on parasite prevalence and richness within hosts. To locate appropriate papers we (1) examined all papers in the GMPD, (2) used Web of Science (Thomson Reuters), examining all papers including the keywords primate and parasite, and some combination of disturbance, fragmentation, hunting, logging, forestry and agriculture and (3) searched the references cited and citations of all papers identified using the first two techniques. We included studies if they presented data on both sample size and prevalence of any parasite in one or more species of primates across paired disturbed and undisturbed sites. Disturbance type and magnitude varied across studies and included fragmentation, logging/forestry, edge effects and increased human presence. Studies varied in their definition of fragments. When possible, we accepted authors' classifications of 'fragmented' vs. 'continuous' sites. When fragmentation was treated as a continuous variable, we used only large high-quality sites (> 110 ha) as controls, and small, low-quality sites (< 20 ha) as fragments. If multiple types of disturbance were compared with a single control for a given host–parasite combination, data for disturbed sites were pooled.

In total, we identified 14 observational studies that included 164 effect sizes (SI-1). Studies were conducted between 1996 and 2012. When possible, sample size was based on prevalence across the number of individuals sampled; however, some studies did not use individual identification and thus the unit of replication is by faecal specimen. Sample size ranged from 8–951 per study.

In coding each study, we included the following information: study identity, host species, the parasite species, transmission mode (direct or indirect) and parasite type (helminth, protozoan or other). The effect size was calculated as Hedge's *g* (Hedges 1981) in the programme Comprehensive Meta-analysis (CMA; Borenstein 2010). Analysis was repeated with the data grouped by study. We used a random-effects model because we predicted that individual studies would vary in the degree and type of disturbance, host/parasite identity and other unmeasured factors. We report effect sizes as correlation coefficients with 95% confidence intervals.

To examine the correlation between host resilience and effect size, we performed a meta-regression. For this analysis, we generated species-specific effect sizes, which we compared with species-level resilience data. Due to minimal overlap in species for which we had data on both resilience to a disturbance type and prevalence responses to disturbance, this was examined only for the response to the forestry disturbance type.

We also employed several methods to identify and quantify potential effects of publication bias. We used funnel plots to visu-

ally examine symmetry around the mean effect size and how this varied based on precision of each study, with a rank correlation metric (Begg & Mazumdar 1994) providing a way to quantify this symmetry. Finally, we used a trim-and-fill method, which aims to add hypothetically missing studies to the funnel plot and examine whether the addition of missing studies changes the overall result (Duval & Tweedie 2000).

For parasite richness, data were typically presented on a per-site (or per disturbance type) rather than per-individual basis. Thus, for this analysis, we simply counted the total number of parasites observed in all disturbed versus all control habitats per host species in each study, corrected for sample size. We then used a paired *t*-test to look for differences in parasite richness between disturbed and control sites.

Within-species effects of disturbance and species richness on prevalence

We used site-specific prevalence data on *Plasmodium* spp. (not including human *P. falciparum*) prevalence in chimpanzees (*Pan troglodytes*; $n = 32$) and gorillas (*Gorilla gorilla*; $n = 18$) from a single study (Liu *et al.* 2010). We only included sites at which at least 10 animals were sampled. Because data came from a single study, we avoided issues associated with comparing prevalence across studies, such as heterogeneous methods for sampling and detecting parasites.

To measure biodiversity, we used mammalian range data from the IUCN database to determine species richness of mammals at each location in our data set (IUCN 2010). As a proxy of disturbance, human population density was obtained from the Gridded Population of the World data set (Version 3, Year 2000; CIESIN & CIAT 2005). At high spatial resolutions (study grain < 1 km), human density tends to be negatively related to biodiversity, while at lower spatial resolutions the relationship becomes positive (Pautasso 2007). The grain of our human density data is approximately 4.6 km, suggesting that the spatial resolution is too low to reflect fine-scale losses of biodiversity that might be occurring in the region, but should still capture broad-scale impacts of human presence on the environment. Indeed, we find a positive correlation between mammal richness and human density in our data ($r = 0.42$, $P < 0.01$). Variance inflation factors were < 2, indicating acceptable levels of multicollinearity. GIS operations were performed in R (details in SI-2).

We examined the effects of mammalian richness and human density on *Plasmodium* spp. prevalence with the model:

$$\text{prevalence} \sim \text{mammal richness} + \log(\text{human density}) + \text{absolute latitude}$$

We included absolute latitude to control for climate. To more directly investigate specific climate variables, we repeated analyses using minimum annual temperature and average annual rainfall (SI-5). In addition, we ran a model in which only mammal richness was used as a predictor of prevalence (SI-6). We used the '*betareg*' package to perform beta regression in R (Cribari-Neto & Zeileis 2010). The beta distribution provides a flexible model for continuous variables defined on the interval (0, 1), and beta regression is well suited to modelling random variables that can be thought of as an unknown proportion of successes in a series of Bernoulli trials (Ferrari & Cribari-Neto 2004). We first analysed a combined data set for chimpanzees and gorillas, including host species as a dummy variable to account for possible differences between hosts. Next, we used the same model to analyse malaria prevalence in chimpanzees and gorillas separately. We calculated Moran's *I* using the '*ape*' package in R to assess spatial autocorrelation (Paradis *et al.* 2004).

Geographic range overlap and parasitism

To investigate the association between primate geographic range overlap and parasite richness, we obtained data on geographic range overlap from the IUCN mammalian range maps (IUCN 2010). From these maps, we calculated the average number of primate species that a given host overlaps with across its range, calculated as the sum of the areas of overlap with other primate species divided by the range area of the focal primate. As with analyses of malaria prevalence in apes, we included average human population density across the range as a proxy for habitat disturbance. We also included body mass, geographic range size and citation counts (a measure of sampling effort) as potentially confounding variables. Thus, we fit the following model in the R package '*caper*' while estimating λ (Orme *et al.* 2012):

$$\log(\text{richness} + 1) \sim \log(\text{mean overlap} + 1) + \log(\text{range size}) + \log(\text{body mass}) + \log(\text{human density}) + \log(\text{citations})$$

We repeated the analysis for total parasite richness (1700 unique host–parasite combinations), helminths (733 unique host–parasite combinations) and vector-borne parasites (521 unique host–parasite combinations). We had complete data and phylogenetic information for 125 host species. Variance inflation factors were < 2, indicating acceptable levels of multicollinearity.

RESULTS

Host competence and resilience: a comparative analysis

We found no evidence that resilience to disturbance predicts parasite species richness (Fig 2 and Table S1). This was true for all parasites combined, and for helminths and vector-borne parasites analysed separately (Table S1). Citation count was significant in all models. For parasite richness, body mass was significantly positively related to parasite richness, but only when considering resilience to forestry. Power analysis (with $\alpha = 0.05$) demonstrated that analyses had greater than 97% power to detect strong effects (correlation ≥ 0.35) and 59–85% power to detect medium effects (correlation ≥ 0.15), but weak power (only 22–37%) to detect small effects (correlation ≤ 0.05).

Among the twelve parasite genera meeting the sample size criterion, three showed significant relationships between parasite prevalence and host resilience to agriculture, but not to forestry or hunting (Table S2 and Fig. 3). For two parasite genera (*Ascaris* and *Oesophagostomum*), the relationship was positive with hosts resilient to agriculture having higher average prevalence. For one parasite genus (*Deltaretrovirus*), the relationship was negative. When combining results into a global meta-analysis to maximise power, we found no overall effect of host resilience on parasite prevalence for any type of disturbance or for types combined (Fig. 3). We found some evidence for positive relationships between parasite prevalence and host body mass (Table S2, Fig. 3) but no significant relationships between host resilience or body mass and white blood cell counts (Table S3).

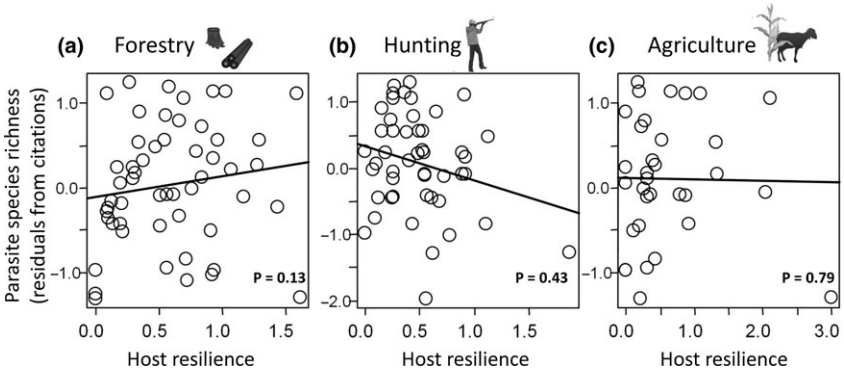


Figure 2 Relationships between parasite species richness (residuals from relationship of parasite species richness to citation counts) and host resilience to three different types of disturbance – forestry (a), hunting (b) and agriculture (c). All variables were log transformed prior to analysis using phylogeny-based comparative methods.

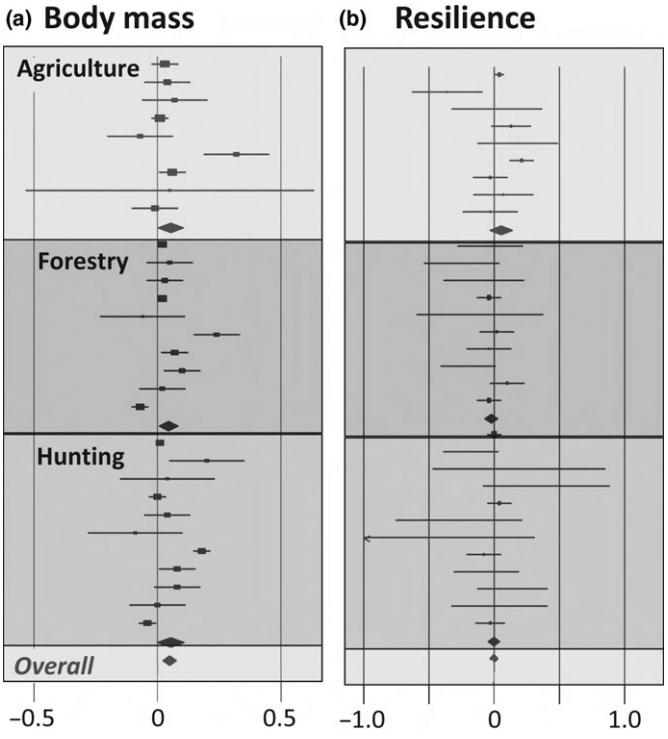


Figure 3 Forest plot of effect sizes and 95% confidence interval for each pathogen (rectangles and bars) of meta-analysis of effects (Z score) of body size (a) and host resilience to disturbance (b) on parasite prevalence (examined separately for each of three disturbance types and overall). The average fixed effect of all studies for each type of disturbance and overall is shown in diamond symbols.

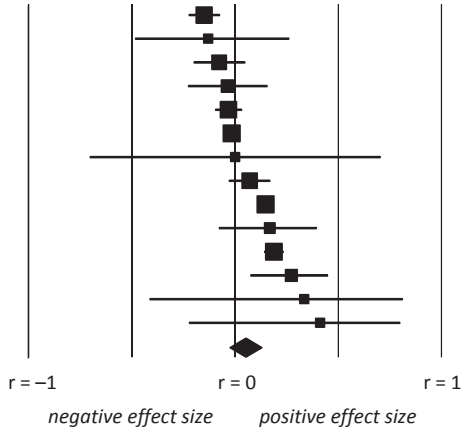


Figure 4 Forest plot of effect sizes and 95% confidence interval for each study (rectangles and bars). The average fixed effect of all studies is shown in diamond symbol. The size of the rectangle is proportional to the weight of the study (based on sample size).

Parasitism and disturbance meta-analysis

Fourteen studies met our criteria for inclusion in the meta-analysis. Six showed negative effects of disturbance on parasite prevalence, seven showed positive effects and one showed no effect. The meta-analysis revealed no overall effect (Fig. 4; $Z = 1.18$, $P = 0.24$), with a mean effect size of $r = 0.05$ (95% CI −0.03 to 0.12, $n = 14$). We found no evidence for publication bias based on funnel plot analysis (SI Fig. 1) or rank correlation between precision and effect size (Kendalls's $\tau = 0.12$, $P = 0.27$, one-tailed without continuity correction). Trim-and-fill analysis suggested that two studies were missing to the left of mean; imputing the two missing studies moved the 95% confidence interval for effect size slightly ($r = -0.12$; CI −0.38 to 0.13).

To understand drivers of heterogeneity in effect sizes, we broke results down by parasite group (helminths or protozoa) or mode of transmission (direct or indirect). Of these groups (Table S4), only indirectly transmitted parasites deviated from zero, with a significant, positive effect of disturbance on prevalence ($Z = 2.45$, $P < 0.001$; $r = 0.18$; 95% CI = 0.11–0.24, $n = 6$), which is consistent with dilution effect assumptions. Meta-regression revealed no relationship between effect size of disturbance and host resilience to forestry (slope = 0.02 ± 0.08, $P = 0.84$, Z = 0.20; nine species examined).

Paired t-tests on parasite species richness in disturbed and undisturbed communities were non-significant ($t = 1.11$, d.f. = 18, $P = 0.28$). From the 12 studies (19 host-study combinations), seven showed higher parasite richness in disturbed sites, six showed equal parasite richness across disturbance types and six showed lower parasite richness in disturbed sites.

Within-species effects of disturbance and species richness on prevalence

Analyses of *Plasmodium* spp. prevalence across geographic locations for chimpanzees and gorillas revealed different patterns for the two host species (Table 1). When data for chimpanzees and gorillas

Table 1 z-statistics and significance levels for regression coefficients in models with latitude as the only climate control variable

	P. troglodytes + *G. gorilla* (R^2 = 0.27)		*P. troglodytes* (R^2 = 0.44)		*G. gorilla* (R^2 = 0.06)	
	z-statistic	*P*-value	z-statistic	*P*-value	z-statistic	*P*-value
Mammal richness	3.72	<0.001	4.25	<0.001	0.36	0.72
Human density	−3.4	<0.001	−3.76	<0.001	−0.24	0.81
Absolute latitude	0.71	0.48	0.26	0.8	0.85	0.4
Host species (*G. gorilla*)	1.05	0.29	–	–	–	–

Table 2 Results of analyses investigating predictors of total, helminth and vector richness

	Total richness (R^2 = 0.54, λ = 0)		Helminth richness (R^2 = 0.38, λ = 0.36)		Vector richness (R^2 = 0.27, λ = 0.17)	
	t-statistic	*P*-value	*t*-statistic	*P*-value	*t*-statistic	*P*-value
Mean overlap	3.38	0.001	2.48	0.015	1.98	0.0505
Human density	−0.49	0.62	−1.09	0.28	−0.26	0.79
Geographic range size	1.51	0.13	−0.19	0.85	2.02	0.046
Female body mass	2.30	0.023	1.20	0.23	1.00	0.32
Citation counts	9.26	<0.0001	7.24	<0.0001	4.33	<0.0001

n = 125 species for each analysis.

were combined, we found an overall positive effect of mammalian species richness and a negative effect of human density on *Plasmodium* spp. prevalence, which is opposite to the prediction that biodiversity conservation protects hosts from infectious disease. Latitude and host species were non-significant. When analyses were conducted for chimpanzees and gorillas separately, we found that mammal richness and human density were significant only for chimpanzees, with a positive effect of mammal richness and a negative effect of human density (consistent with an amplification effect). Latitude was non-significant in all models. Results were similar when climate variables were used in place of latitude (Table S5), and mammal richness remained significantly positively associated with malaria prevalence when human density and latitude were dropped from the model (Table S6).

Geographic range overlap and parasitism

Mean geographic range overlap positively predicted parasite richness and helminth richness, with marginally significant results for vector-borne parasites (Table 2). Citation count was consistently significant as a measure of sampling effort, while body mass significantly predicted total richness and geographic range size predicted vector richness. Human density was not significant. Phylogenetic signal was low in all models (Table 2). Mean overlap covaried significantly with geographic range size (r = 0.38, P < 0.0001), but the variance inflation factor was less than two, indicating a lack of collinearity in the model.

DISCUSSION

Our results do not support the hypothesis that biodiversity conservation generally reduces the risk of infectious disease in primates. Overall, both within- and across-species analyses failed to support key assumptions and predictions of the dilution effect across a range of geographic scales (Fig. 1, Table S7). However, results varied across types of analyses and across species or groups within an analysis. Two analyses found strong support for amplification effects, while one of four tested subsets of the meta-analyses found support for the dilution effect.

Across-species comparisons

Our comparative analysis across primates revealed no significant relationships between host resilience and parasite richness or prevalence, suggesting that primates resilient to disturbance are not more susceptible to pathogens. While a previous study has shown that more threatened primates tend to have lower parasite richness (Altizer *et al.* 2007), this finding may reflect the fact that threatened hosts often have small, fragmented populations, which reduces parasite transmission and persistence. We also found no significant relationships between host resilience and white blood cell counts, which is consistent with other studies that find no correlation between white blood cell counts and parasite species richness (Cooper *et al.* 2012a) or 'speed of life' (Nunn 2002). However, it is possible that species with higher parasite risk invest more heavily in immune function (Moller *et al.* 1998), counterbalancing any negative relationship between resilience and prevalence. In addition, total white blood cell count is a coarse metric of immune investment, as is our metric of parasite richness (which does not distinguish between high and low intensity or prevalence of infections). Higher resolution metrics of immune function might reveal associations in future research.

Two additional caveats are associated with these analyses. First, it is possible that focussing solely on primates is too narrow for investigating the dilution effect. While primates show substantial variation in life history traits, this variation is limited relative to mammals more broadly. Primates might also have weaker relationships between key life history traits that influence resilience to disturbance and parasite species richness in comparison to some taxa (e.g. Cooper *et al.* 2012a). Thus, broader analysis may reveal patterns not found here. Second, our comparative analyses involving host resilience to disturbance did not account for geographic variation in parasitism, as data were pooled across sites for each primate. It is therefore possible that resilient hosts tended to be sampled in disturbed, low-diversity habitats while vulnerable hosts tended to be sampled more in intact, high-diversity environments, thus creating a sampling bias. If either the dilution or amplification effects are widespread, this bias should exaggerate the link between resilience and parasitism. As our findings revealed no effect, we believe our approach is conservative.

Our comparative analyses of parasite richness in relation to geographic range overlap found evidence for amplification effects. Specifically, parasite richness increases with greater geographic range overlap among hosts. Several factors might drive this pattern, including greater opportunities for host shifts among overlapping, closely related species or higher numbers of generalist parasites when more host species are available (Nunn *et al.* 2004; Cooper *et al.* 2012b). Results were independent of geographic range size, body mass and sampling effort.

Most tests of the dilution effect have focused on fine-grained data, with less effort focused at broader scales (e.g. across species). While effects may be expected across biological scales, we found no support for this possibility. It is conceivable that both amplification and dilution effects occur together, with parasitism generally increasing with host richness, and biodiversity loss modulating this baseline level of disease transmission. If the dilution effect holds, biodiversity loss would cause transmission to increase over this baseline level in disturbed environments. Our broad comparative and geographic range overlap analyses might have been unable to detect modulation of transmission rates, especially given the many other factors that affect parasite richness. However, our meta-analysis and malaria analyses should have greater power to detect effects. Yet, as discussed below, neither of these analyses showed strong evidence for the dilution effect, despite analysing differences in prevalence between disturbed and undisturbed habitats at finer spatial resolutions.

Within-species comparisons

In our comparison of *Plasmodium* prevalence among chimpanzees and gorillas, we found significant support for an amplification effect in chimpanzees, but not in gorillas. For chimpanzees, prevalence was higher at sites with higher mammal species richness, consistent with previous studies on effects of species richness on richness of zoonotic diseases (Jones *et al.* 2008) and all human pathogens (Dunn *et al.* 2010). We found no latitudinal effect on parasite prevalence, also consistent with findings on zoonotic pathogens (Jones *et al.* 2008). We found a negative relationship between human density and *Plasmodium* prevalence in chimpanzees, which is contrary to the general understanding that subtle increases in human activity often increase wildlife disease risk, particularly for great apes (Zommers *et al.* 2012). Possible mechanisms by which human presence might reduce malaria prevalence in chimpanzees include anthropogenically mediated reductions in malaria vector populations, reduced population density of chimpanzee hosts, and humans and domestic animals acting as diluting hosts for chimpanzee malaria vectors.

One methodological concern with this analysis is that our estimates of mammal richness are derived from range maps of current species occurrence based on best available records, rather than on field richness estimates. In addition, while the spatial scale of our analysis should be relevant to disease dynamics in chimpanzee and gorilla populations (~ 4.6 km^2 grain), the resolution might be too low to detect small-scale local extinctions from disturbance. Our meta-analysis (discussed below) complements this analysis by considering only very high-resolution data.

The meta-analysis revealed no overall relationship between parasite prevalence or richness and habitat disturbance. The lack of support for any overall relationships between disturbance and prevalence is surprising because many factors could cause an increase in prevalence under disturbance. For example, chronic stress from living in disturbed habitats could lead to higher parasite load (e.g. Chapman *et al.* 2006) and increased contact with humans should lead to increased transmission of shared parasites (Zommers *et al.* 2012). Fragmentation and/or logging might decrease home ranges, thereby increasing terrestriality or changing the diet of primates so as to increase exposure (Gillespie *et al.* 2009). However, both in the present study and in another review focused on the genus *Alouatta* (Kowalewski & Gillespie 2009), little support has been found for these effects.

Meta-analysis revealed a significant positive effect of disturbance on parasite prevalence for indirectly transmitted parasites. Although sample size was small ($n = 6$), this is consistent with a dilution effect, where one might expect indirectly transmitted parasites to be more susceptible to dilution because their complex life cycles provide opportunities for the dilution effect to act on multiple transmission stages. It is also in contrast to the amplification effect, which predicts that the complex host–parasite transmission pathways of indirectly transmitted parasites are among the most likely to be disrupted by habitat disturbance (Lafferty 2012). In light of our negative results for meta-analyses of other parasite types, and our finding of an amplification effect for indirectly transmitted malaria parasites in chimpanzees when broader spatial scales are considered, these results underscore the importance of considering both biology and scale when studying dilution or amplification effects.

Overview

Our findings are inconsistent with the hypothesis that biodiversity and habitat conservation have a general protective effect for primates against their parasites. Many of our findings are based on failure to reject the null hypothesis and are thus subject to concerns about statistical power. Also, as in much of the literature on this topic, our analyses are correlational and are limited in their ability to identify causal relationships. Yet, taken as a whole, our results strongly suggest that habitat disturbance or biodiversity loss is unlikely to have a broad, strong, positive effect on parasite risk in primate hosts. We suggest that comparative and meta-analytical approaches should play a greater role in future research by synthesising pathogen-specific studies to determine the generality of either the dilution or amplification effects and pinpointing when, where and in which host–parasite systems and disturbance regimes they occur most strongly.

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AUTHORSHIP

All authors contributed to conceptual study design. Data collection and analysis were performed by HY, RG and CN. HY wrote the first draft of the manuscript and all authors contributed substantially to revisions.

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