

LETTER

## A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic

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### Abstract

Zoonotic pathogens are significant burdens on global public health. Because they are transmitted to humans from non-human animals, the transmission dynamics of zoonoses are necessarily influenced by the ecology of their animal hosts and vectors. The ‘dilution effect’ proposes that increased species diversity reduces disease risk, suggesting that conservation and public health initiatives can work synergistically to improve human health and wildlife biodiversity. However, the meta-analysis that we present here indicates a weak and highly heterogeneous relationship between host biodiversity and disease. Our results suggest that disease risk is more likely a local phenomenon that relies on the specific composition of reservoir hosts and vectors, and their ecology, rather than patterns of species biodiversity.

### Keywords

Dilution effect, hantavirus, Lyme disease, West Nile virus, wildlife biodiversity, zoonotic disease.

Ecology Letters (2013) 16: 679–686

### INTRODUCTION

Zoonotic pathogens – disease agents such as the SARS coronavirus, Lyme disease spirochete or West Nile virus, which are transmitted to humans from non-human animals – are significant burdens on global public health (Jones *et al.* 2008). Zoonotic transmission is inherently a multi-species phenomenon, often also involving vector intermediaries, making an understanding of underlying ecological processes essential for management and control. Indeed, a key question in disease ecology and epidemiology is how to mitigate or ameliorate pathogen transmission from wildlife reservoirs to human populations. The ‘dilution effect’ hypothesis, which suggests that disease risk will decrease as a result of increased species diversity, offers an intriguing possibility of harnessing conservation initiatives in order to reduce disease risk to human populations (Pongsiri *et al.* 2009; Keesing *et al.* 2010). Here, we use the inclusive definition of the dilution effect which describes the net effect of species diversity reducing disease risk by any of a variety of mechanisms (Keesing *et al.* 2006). For example, *Borrelia burgdorferi*, the bacterium that causes Lyme disease, is transmitted by ticks (*Ixodes* spp.), but persists in vertebrate hosts (e.g. white-footed mice, *Peromyscus leucopus*, *Sorex* shrews and other species), which differ in the degree to which they can infect ticks (i.e. reservoir competence) (LoGiudice *et al.* 2003; Brisson *et al.* 2008). The risk of human exposure to Lyme disease is therefore strongly influenced by the local abundance and interactions of tick hosts, *Borrelia* reservoir hosts, and *Ixodes* ticks. LoGiudice *et al.* (2003, 2008) have hypothesised that a higher diversity of hosts for ticks could reduce the risk of Lyme disease. Similarly, reservoir competence of mosquito-borne West Nile virus (WNV) varies widely among avian species (Komar *et al.* 2003), and higher avian biodiversity (species richness and evenness) has been postulated to reduce WNV risk (e.g. Ezenwa *et al.* 2006; Swaddle &

Calos 2008; Allan *et al.* 2009). Unlike WNV and *B. burgdorferi*, hantaviruses are generally associated with only a single species of rodent host, and transmission does not involve vectors, but a negative association between host diversity and hantavirus prevalence has nevertheless been proposed via mechanisms such as a higher biodiversity suppressing the encounter rate within a reservoir species, and therefore dampening intraspecific disease transmission (Clay *et al.* 2009; Dizney & Ruedas 2009; Carver *et al.* 2011).

If higher biodiversity reduces the risk of infectious diseases, then the dilution effect hypothesis has an obvious appeal as both conservation and public health agendas can be united in a common purpose: protect biodiversity, while simultaneously reducing deleterious health impacts of zoonotic and wildlife diseases (Pongsiri *et al.* 2009; Keesing *et al.* 2010). Indeed, a recent review concluded that ‘overall, despite many remaining questions, preserving intact ecosystems and their endemic biodiversity should generally reduce the prevalence of infectious diseases’ (Keesing *et al.* 2010).

However, emerging zoonoses involve multi-trophic level interactions that can be complex and variable and will often depend on the local idiosyncrasies of pathogen, host, vector and human ecology, which suggests that the dilution effect may not be a general phenomenon (e.g. LoGiudice *et al.* 2008; Ogden & Tsao 2009; Brooks & Zhang 2010; Salkeld & Lane 2010; Randolph & Dobson 2012; Wood & Lafferty 2012). In contrast to the predictions of the dilution effect, mammal biodiversity at a global scale is linked to increased likelihood of emerging zoonotic pathogens (Jones *et al.* 2008), healthy ecosystems may actually be richer in parasite diversity (Hudson *et al.* 2006; Wood *et al.* 2010), and disease dynamics may be determined by idiosyncratic species interactions (LoGiudice *et al.* 2003, 2008; Brisson *et al.* 2008; Salkeld & Lane 2010; Salkeld *et al.* 2010).

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These conflicting results about the relationship between biodiversity and disease risk suggest that consequences of conservation programmes for human health may be unpredictable and variable depending on the spatial scale and the ecological particulars of the system in question. If there is an argument to be made for redirecting scarce public health or conservation resources, it is critical to understand whether the relationship between biodiversity and disease risk is as generalisable as has been suggested. However, we are unaware of any formal assessment of the generality of the dilution effect.

Here, we analyse existing literature in a meta-analysis that examines the relationship between biodiversity and zoonotic disease risk. We investigate whether the inclusive definition of the dilution effect – that biodiversity is protective against disease risk – is generally supported and can be regarded as a useful tool in public health approaches to zoonoses. If there is no straightforward relationship between biodiversity and risk of zoonotic disease, then integrated approaches to disease control may require more detailed understanding of the transmission ecology of specific pathogen, vector and host species.

## METHODS

We aimed to collect and analyse all studies that evaluated links between host biodiversity and disease risk for disease agents that infect humans. There are other studies that ask related questions for non-human diseases, but our primary interest was the possible relationship between public health and wildlife biodiversity. Studies were only selected if they simultaneously provided field-based measures of disease risk (e.g. prevalence of infection in animal, human or vector populations; density of infected animals, humans or vectors) and measures of wildlife biodiversity [e.g. species richness (number of species present) or a diversity index (Shannon's index, Simpson's index, etc.)]. Studies most frequently involved multiple sites across which biodiversity varied, but we also included one study that examined disease prevalence in the presence and absence of a particular species (voles) over time (Carver *et al.* 2011), and one study where non-reservoirs were removed (Suzán *et al.* 2009). Where studies presented data on different subsets of biodiversity, we used the most complete representations of species richness, as this criterion can be applied consistently across all studies. For example, all bird species combined, rather than separate analyses of passerines or non-passerines (Ezenwa *et al.* 2006); or the 'whole model' rather than separate analyses of small mammals, large mammals or birds (LoGiudice *et al.* 2008). For studies that examined multiple measures of disease risk [e.g. seroprevalence and abundance of seropositive animals (Piudo *et al.* 2011)], we chose the disease risk measure most consistently used across the study system [e.g. seroprevalence was used in most other hantavirus studies, so seroprevalence was adopted from Piudo *et al.* (2011)].

We did not include studies where a proxy was used for biodiversity (e.g. forest fragmentation) or where disease risk was simply inferred (e.g. greater abundance of a particular reservoir host but without direct measures of disease). We did not incorporate studies that relied on county-level health data, because infection dynamics can operate at fine spatial scales and therefore data at coarse county or region levels may not be indicative of local pathogen transmission patterns, and because regional measures of biodiversity are sensitive to sampling effort, so that larger areas tend to report more

species that smaller areas, making it difficult to obtain an accurate measure of diversity (Loss *et al.* 2009; Hamer *et al.* 2011).

Studies were identified through a comprehensive search on Web of Science®, using search strings examining disease and biodiversity, with a particular focus on West Nile virus, hantaviruses and Lyme disease risk. These pathogens were chosen because they infect humans and are cited in recent reviews as good examples of disease systems linking changes in biodiversity to adverse impacts upon human health (e.g. Pongsiri *et al.* 2009; Keesing *et al.* 2010). Unpublished data on Sin Nombre virus seroprevalence in California small mammals were provided by California Department of Public Health (see Table 1). Also included are two studies of the tick-borne disease agent, *Anaplasma phagocytophilum* (Foley *et al.* 2009; unpublished data from Nieto *et al.* 2007), and unpublished data on plague (*Yersinia pestis*) in Colorado prairie dog populations from Stapp 2007 (Table 1).

An important finding of our meta-analysis is that the investigations of relationships between biodiversity and zoonotic disease risk is still in its infancy; a systematic literature search revealed only 13 published studies documenting relationships of wildlife biodiversity and zoonotic disease risk (following our criteria), and we were able to augment this with three unpublished data sets. Given the limited data available, conclusions regarding the biodiversity-disease relationship should be regarded with caution. Furthermore, our criteria for inclusion in the meta-analysis resulted in a small sample size and consequent low analytical power. Different selection criteria for the inclusion of studies may result in different conclusions.

Meta-analysis depends on a standardised measure of effect size (Rosenthal & DiMatteo 2001). This measure is essentially a correlation between a factor (e.g. biodiversity) and a response (e.g. infection prevalence). Most studies do not publish raw numbers but most (though not all) publish values of test statistics (e.g.  $F$ ,  $\chi^2$ ,  $t$ , or  $r^2$ ). For each study, we converted the test statistic for the relationships between biodiversity and disease risk to a standardised correlation-based effect size  $r$  (see Rosenthal & DiMatteo 2001). Where these results were not readily apparent in the published papers, we contacted authors and requested either the raw data or a derived effect size statistic, for example, via simplification of data to a contingency table.

The correlation measure of effect size,  $r$ , is heteroscedastic and is frequently transformed using Fisher's  $Z$  transformation to equalise variances (Rosenthal & DiMatteo 2001; Chaplin-Kramer *et al.* 2011). For effect-size measure  $r$ , Fisher's  $Z$  is calculated as follows:

$$Z = \frac{1}{2} \log \left[ \frac{1+r}{1-r} \right].$$

Studies where disease risk was lower in treatments of higher biodiversity were assigned a negative value.

We adopted a random-effects model, for which each study is taken to be a sample of a large population of possible studies (Cooper *et al.* 2009). The variance in random-effects specification includes both the error term common to the fixed-effects model but also a term that accounts for between-study heterogeneity. The null hypothesis that this between-study variance is zero can be evaluated with a Cochran's  $Q$ , which is distributed as chi-square with  $k - 1$  degrees of freedom. Confidence intervals on overall effect sizes are frequently constructed assuming a normal approximation. However, a number of authors have shown that normal confidence intervals substantially under-estimate the true uncertainty in overall

**Table 1** Details of studies included in meta-analysis: disease agent, study location, the components of the biodiversity–disease relationship (e.g. species richness, disease seroprevalence, Rosenthal's  $\tau$ , Fisher's  $Z$  and the source statistics used to generate  $r$  and  $Z$ . Sin Nombre, Choclo, Calabazo and Andes viruses are all hantaviruses

Pathogen, location (reference)	Biodiversity measure	Disease measure	$r$	$Z$	Source statistics	Notes
Sin Nombre virus, Montana, USA (Carver <i>et al.</i> 2011)	Presence or absence of voles from three long-term trapping grids	Sin nombre virus antibody seroprevalence in deer mice	-0.291, -0.213, -0.139	-0.22 (-0.30, -0.22, -0.14)	Linear regressions: Grid 10: $F_{1,50} = 4.634$ , $P = 0.036$ ; Grid 11: $F_{1,168} = 8.029$ , $P = 0.000$ ; Grid 12: $F_{1,168} = 3.309$ , $P = 0.071$	Used the average Fisher's $Z$ from the three grids
Sin Nombre virus, Oregon, USA (Dizney & Ruedas 2009)	Small mammal diversity – Simpson diversity index	Sin Nombre virus infection prevalence	-0.997	-3.25	Nonlinear regression: $n = 5$ , $r^2 = 0.9994$ , $P = 0.00001$	
Sin Nombre virus, Utah, USA (Clay <i>et al.</i> 2009)	Small mammal species diversity (Gini-Simpson index)	Seroprevalence of Sin Nombre virus antibodies in deer mice	-0.473	-0.51	Linear mixed model: $Z = -1.893$ , $n = 16$ , $P = 0.05$	
Sin Nombre virus, southwestern USA (Mills 2005)	Small mammal species diversity (Simpson's D index)	Seroprevalence of Sin Nombre virus antibodies in deer mice	-0.927	-1.64	Correlation: $n = 10$ , $r^2 = 0.86$ , $P = 0.0019$	
Sin Nombre virus, Channel Islands, California, USA (Orrock <i>et al.</i> 2011)	Small mammal richness	SNV seroprevalence	-0.03	-0.03	Correlation: $n = 8$ , $r = -0.03$ , $P = 0.94$	
Sin Nombre virus, California, USA (CDPH, unpublished data*)	Small mammal species diversity (Simpson's D index)	Sin Nombre virus antibodies in deer mice	0.129	0.13	Correlation: $n = 32$ , $r^2 = 0.017$ , $P = 0.48$	
Hantaviruses (Choclo & Calabazo), Panama (Suzán <i>et al.</i> 2009)	Comparison of plots with experimental removal of non-reservoirs and controls, at completion of experimental removal	Hantavirus antibody seroprevalence in reservoir hosts ( <i>Oligoryzomys fulvescens</i> & <i>Zygodontomys brevicauda</i> )	0.111	0.11	Chi-square: $\chi^2 = 1.44$ , $n = 116$ , $P = 0.23$	Statistics derived from presented data
Andes virus, Argentina (Piudo <i>et al.</i> 2011)	Species diversity ( $H'$ )	Andes virus antibody seroprevalence in <i>O. longicaudatus</i> (0 time-lag)	-0.15	-0.15	$n = 49$ , $r = -0.15$ , $P = 0.294$	
West Nile virus, Louisiana, USA (Eizenwa <i>et al.</i> 2006)	Bird species richness (passerine and non-passerine combined)	WNV infection prevalence in <i>Culex</i> spp.	-0.665	-0.78	Linear regression: $n = 6$ , $F_{1,4} = 3.007$ , $r^2 = 0.43$ , $P = 0.1579$	Statistics derived from presented data
West Nile virus, Illinois, USA (Loss <i>et al.</i> 2009)	Species richness (migrant & rare birds excluded, as were waterfowl, gulls, herons, raptors & shorebirds)	<i>Culex</i> infection rates	-0.188	-0.19	GLME: $n = 9$ , $t = -0.54$ , $P = 0.61$	
West Nile virus, Missouri, USA (Allan <i>et al.</i> 2009)	Bird diversity (Shannon's index)	WNV infection prevalence ( <i>Culex</i> species)	-0.7	-0.87	Pearson's correlation: $r = -0.7$ , $n = 16$ , $P < 0.05$	
Lyme disease spirochete ( <i>B. burgdorferi</i> ), New York State, USA (Prusinski <i>et al.</i> 2006)	Small mammal diversity (Shannon-Weiner diversity index (H'))	<i>Borrelia</i> infection rates	-0.080	-0.08	$n = 12$ , $r = -0.08$ , $P = 0.79$	

(continued)

Table 1. (continued)

Pathogen, location (reference)	Biodiversity measure	Disease measure	<i>r</i>	<i>Z</i>	Source statistics	Notes
Lyme disease spirochete ( <i>B. burgdorferi</i> ), north-east USA (LoGiudice <i>et al.</i> 2008)	Mammal/bird richness	Nymphal infection prevalence	0.280	0.29	Multiple last-squares regression (whole model): $R = 0.28$ , $n = 26$ , $P = 0.45$	Statistics derived from presented, using only transects with <i>Anaplasma</i> present
<i>A. phagocytophilum</i> , California (Foley <i>et al.</i> 2009)	Small mammal species richness	<i>Anaplasma</i> antibody seroprevalence	-0.168	-0.17	Linear regression: $n = 16$ , $F_{1, 14} = 0.408$ , $r^2 = -0.03$ , $P = 0.53$	Only using sites with <i>Anaplasma</i> present
<i>A. phagocytophilum</i> , California (NC Nieto, unpublished data, see Nieto <i>et al.</i> 2007)	Small mammal species richness	<i>Anaplasma</i> antibody seroprevalence	0.319	0.33	Linear regression: $n = 9$ , $r^2 = 0.10$ , $P = 0.102$	
Plague ( <i>Y. pestis</i> ), Colorado (Stapp, unpublished data, see Stapp 2007 for details on trapping)	Small mammal richness	Plague outbreaks on prairie dog colonies in following year	0.045	0.04	$t$ -test: $t = 0.15$ , $n = 16$ , $P = 0.88$	

\*Results from analysis of data contained in submitted trapping sheets for hantavirus seroprevalence carried out by California Department of Public Health, CA County Health agencies, and Vector and Mosquito Control Districts. Seroprevalence is restricted to seropositive *Peromyscus* species, and diversity is measured as Simpson's D to reflect existing published studies on hantaviruses.

effect size (e.g. Sidik & Jonkman 2002; Sánchez-Meca & Marín-Martínez 2008), suggesting that confidence intervals for a small sample of heterogeneous studies are better approximated using a  $t$ -distribution.

Because of the uneven reporting of test statistics, we also present the published  $P$ -values to interpret the statistical strength of the reported relationships between biodiversity and disease risk (Hedges & Olkin 1985).

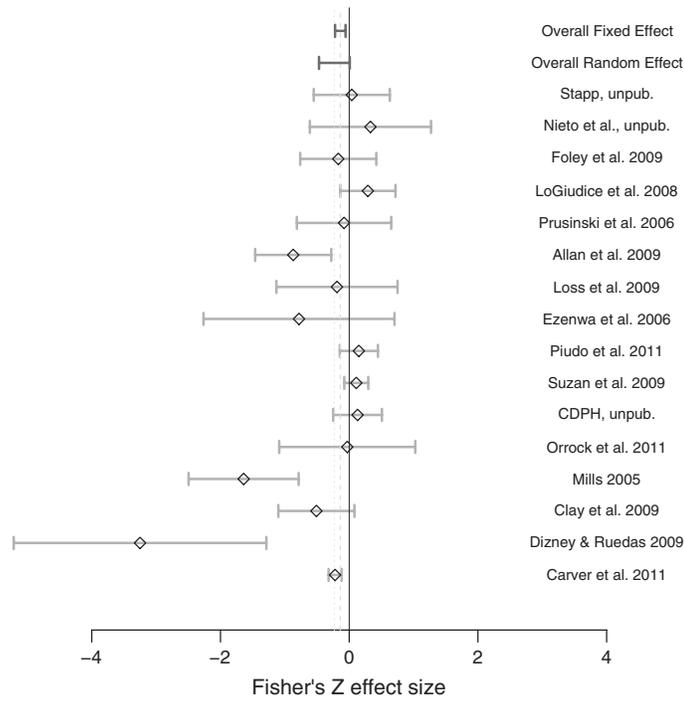
We used a funnel plot, which depicts standardised effect size against the study standard error, to search for any evidence of publication bias (Chaplin-Kramer *et al.* 2011). If there is no publication bias, effect sizes from studies with larger sample sizes will be near the average, whereas effect sizes from smaller sample sizes will spread on both sides of the average. That is, unbiased data should be shaped like a funnel, whereas biased data will generate an asymmetric funnel plot (Chaplin-Kramer *et al.* 2011). We also tested for the effect of potential publication bias by using the trim-and-fill method (Duval & Tweedie 2000a,b), implemented in the R package meta. This intuitively appealing method allows the imputation of possible missing effect-size estimates based on inferred publication bias. In the case of publication bias, confidence intervals are likely to be systematically under-estimated since the variance of the low-power samples (i.e. the bottom right of our funnel plot) is truncated by the missing samples. In trim-and-fill method, confidence intervals for the overall effect size are recalculated including imputed values. Confidence intervals for the trim-and-fill method are calculated using a normal approximation. A sample not subject to publication bias should show little change in the estimated confidence intervals from the original sample to the augmented sample. The trim-and-fill approach also allows rank-based estimates ( $R_0^+$  and  $L_0^+$ ) of the probable number of missing studies due to publication bias, rounded to the nearest integer as recommended (Duval & Tweedie 2000a,b). Because this is a question of publication bias, we calculate this value for both our complete sample (which includes three unpublished studies) and the sub-sample of only published papers ( $n = 13$ ).

## RESULTS

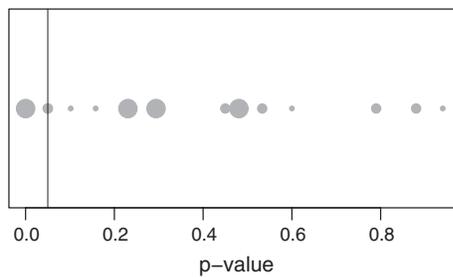
We analysed 16 biodiversity-disease relationships: eight studies on hantaviruses (including one unpublished data set), three studies on West Nile virus, two studies on Lyme disease risk, two studies on *A. phagocytophilum* (including one unpublished data set) and one study on plague (from an unpublished data set) (Table 1).

Across all studies, the mean Fisher's  $Z$  was  $-0.138$  (95% CI using  $t$ -distribution =  $-0.220$  to  $-0.057$ ;  $n = 16$ ) from the fixed-effects model. However, the test for heterogeneity in the error models of the individual studies was significant at the  $P < 0.0001$  level ( $Q = 66.66$ , d.f. = 15), indicating that the random effects model is more appropriate for the overall mean. The mean effect from the random effects model was  $-0.2301$  (95% CI using  $t$ -distribution =  $-0.470$ – $0.008$ ;  $n = 16$ ). The 95% confidence interval crosses zero, suggesting a non-significant relationship between measures of biodiversity and zoonotic disease risk (Fig. 1). The distribution of  $P$ -values was uniform (Fig. 2), suggesting that the relationship between biodiversity and disease risk fails to contradict the null hypothesis overall.

The funnel plot (Fig. 3) provides mixed evidence of publication bias but is nonetheless quite revealing. First, all the strongest negative effects (i.e. those supporting the dilution effect hypothesis) are

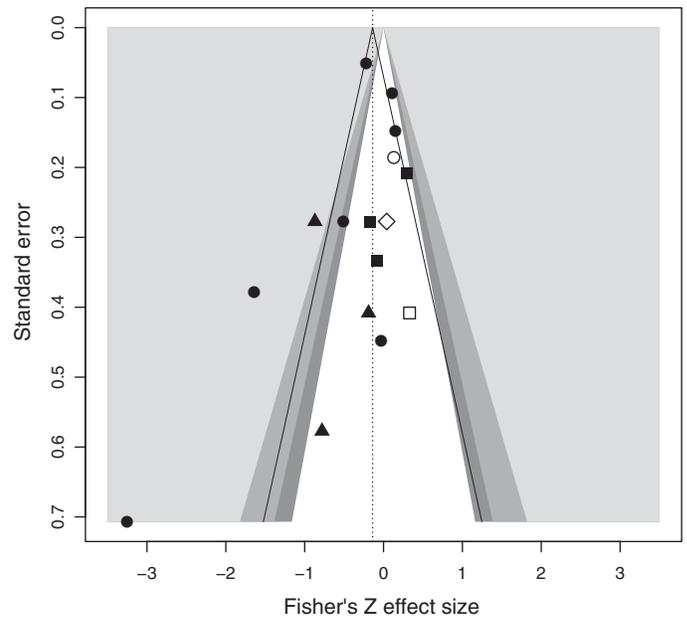


**Figure 1** Distribution of Fisher's *Z*-values (with 95% CI) for studies describing relationships between biodiversity and disease risk. Dotted line represents mean Fisher's *Z*-value from random effect model for all studies combined, and dashed line represents mean Fisher's *Z*-value from fixed-effect model for all studies combined.



**Figure 2** Distribution of *P*-values for studies describing relationships between biodiversity and disease risk. The size of the point is proportional to the sample size, and the vertical line indicates the conventional level of statistical significance,  $P = 0.05$ .

for studies with quite small sample sizes, and therefore large standard errors. Second, while the effect-sizes for the larger studies do indeed cluster near the overall mean effect size, this mean is quite modestly negative. Finally, there is a pronounced asymmetry to the funnel, with more highly negative results at the lowest sample sizes. This is suggestive that either (1) small studies that do not support the dilution effect hypothesis are not published or (2) that there is unmeasured heterogeneity in the studies. Spearman rank correlations between Fisher's *Z* and standard error were not statistically significant at the conventional  $P < 0.05$  level: excluding unpublished studies,  $\rho = 0.51$ ,  $P = 0.06$ ; across all studies,  $\rho = 0.46$ ,  $P = 0.11$ . This last point is suggestive of publication bias favouring negative relationships between biodiversity and disease risk.



**Figure 3** Relationship between effect size (Fisher's *Z*) and standard error for studies describing relationships between biodiversity and disease risk (dotted line shows mean Fisher's *Z* from random effect model). Points should be in the shape of an inverted funnel if there is no publishing bias. Gray shades (from darker to lighter) represent the confidence intervals around zero corresponding to  $0.1 > P > 0.05$ ,  $0.05 > P > 0.01$ , and  $P < 0.01$ . Circles = hantavirus, triangles = WNV, squares = tick-borne, diamond = plague. Open plotting symbols indicate the three unpublished studies.

The trim and fill analysis uses a normal approximation for the confidence interval of the estimated overall effect size (in contrast to the *t*-distribution CIs we report above): for the full sample, the fixed-effect model yielded a mean Fisher's *Z* of  $-0.138$  (95% CI using normal distribution =  $-0.213$  to  $-0.063$ ;  $n = 16$ ); for the random-effects model, the mean Fisher's *Z* was  $-0.230$  (95% CI using normal distribution =  $-0.450$  to  $-0.010$ ;  $n = 16$ ) with a significant test of heterogeneity in the error models of the individual studies ( $P < 0.0001$ ,  $Q = 66.66$ , d.f. = 15), indicating that the random effects model is more appropriate for the overall mean. For the sub-sample of published studies, the fixed-effect model yielded a mean Fisher's *Z* of  $-0.159$  (95% CI using normal distribution =  $-0.236$  to  $-0.081$ ;  $n = 13$ ); for the random-effects model, the mean Fisher's *Z* was  $-0.331$  (95% CI using normal distribution =  $-0.587$ – $0.075$ ;  $n = 13$ ) with a significant test of heterogeneity in the error models of the individual studies ( $P < 0.0001$ ,  $Q = 62.59$ , d.f. = 12), indicating that the random effects model is more appropriate for the overall mean.

Imputing the possible missing effect-size estimates using the trim and fill analyses calculates CI intervals that mirror the results found from our analyses of the *t*-distributions. For the full sample, the trim and fill imputation added one study to the sample. For the fixed-effect model, mean Fisher's *Z* was  $-0.129$  (95% CI using normal distribution =  $-0.204$  to  $-0.054$ ;  $n = 17$ ); and for the random effects model, mean Fisher's *Z* was  $-0.175$  (95% CI using normal distribution =  $-0.416$ – $0.066$ ;  $n = 17$ ). Once again, the test for heterogeneity in the error models of the individual studies was significant at the  $P < 0.0001$  level ( $Q = 86.21$ , d.f. = 16), indicating that the random effects model is more appropriate for the overall mean.

and suggests a non-significant overall effect of biodiversity on disease risk. The rank-based estimates of the probable number of missing studies due to publication bias are  $R_0^+ = 4$  and  $L_0^+ = 4$ , suggesting that four studies are missing from the meta-analysis due to publication bias (Duval & Tweedie 2000a,b). This represents 25% of the total sample of studies analysed here and 20% of the hypothetical sample. For the sub-sample of published studies, trim and fill analysis imputation added three studies to this sub-sample, and calculated a mean Fisher's  $Z$  for the fixed-effect model of  $-0.117$  (95% CI using normal distribution =  $-0.193$  to  $-0.040$ ;  $n = 16$ ); and for the random effects model, mean Fisher's  $Z$  was  $-0.105$  (95% CI using normal distribution =  $-0.388$ – $0.179$ ;  $n = 16$ ). Once again, the test for heterogeneity in the error models of the individual studies was significant at the  $P < 0.0001$  level ( $Q = 106.99$ , d.f. = 15), indicating that the random effects model is more appropriate for the overall mean and suggests a non-significant overall effect of biodiversity on disease risk. The rank-based estimates of the probable number of missing studies due to publication bias are  $R_0^+ = 7$  and  $L_0^+ = 5$ , suggesting that between five and seven studies are missing from the published sub-sample due to publication bias (Duval & Tweedie 2000a,b), representing 38–54% of the total sample of studies analysed here and 31–44% of the hypothetical sample.

Our results are not consistent with the claim that biodiversity provides a general service of reducing the risk of infectious disease. Considering just hantavirus study systems, the mean Fisher's  $Z$ -value was  $-0.1438$  ( $n = 8$ , 95%  $t$ -distribution CI =  $-0.231$ – $0.057$ ) from the fixed-effects model, with 3/8 studies reporting a statistically significant relationship at  $P < 0.05$  (Table 1). The hantavirus studies showed significant evidence for heterogeneity ( $Q = 52.44$ , d.f. = 7,  $P < 0.0001$ ). The mean and 95% CI from the random effects model was  $-0.325$  ( $-0.668$ – $0.019$ ). Mean Fisher's  $Z$ -value  $s$  for tick-borne disease agents (*A. phagocytophilum* and *B. burgdorferi* combined) was  $0.112$  (95%  $t$ -distribution CI =  $-0.186$ – $0.410$ ,  $n = 4$ ; fixed-effects model), with 0/4 studies reporting a statistically significant relationship at  $P < 0.05$  (Table 1). Mean Fisher's  $Z$ -values for West Nile virus was  $-0.672$  (95%  $t$ -distribution CI =  $-1.126$ – $0.272$ ,  $n = 3$ ), with 1/3 studies reporting a statistically significant relationship at  $P < 0.05$  (Table 1).

## DISCUSSION

Our meta-analysis of effect sizes and  $P$ -values from the published literature on human disease agents provides very weak support, at best, for the dilution effect, and by extension the assertion that the preservation of endemic biodiversity will reduce the prevalence of zoonotic diseases. Although the mean effect size is negative, the confidence interval around the mean crosses zero, so the null hypothesis of no effect cannot be rejected. Furthermore, there is strong evidence for heterogeneity in the effects, suggesting that different processes are acting on the different studies. We conclude that the relationship between biodiversity and zoonotic disease risk is probably idiosyncratic, and that understanding the ecological dynamics of specific disease systems is more important in predicting zoonotic disease risk.

A challenge in assessing the evidence in support of the dilution effect is the differences between disease systems and/or variable measures of disease risk. For example, we used two studies examining Lyme disease: LoGiudice *et al.* (2008) determined risk by

nymphal infection prevalence (NIP, i.e. proportion of ticks infected with *B. burgdorferi*), whereas Prusinski *et al.* (2006) measured *B. burgdorferi* infection prevalence in mammalian hosts. Lyme disease risk can also be measured as density of infected nymphs (DIN), and the implications of the dilution effect are starkly different depending on the risk measure adopted: for instance, a reduction in nymphal infection prevalence associated with increased host biodiversity may be negated if higher host biodiversity increases vector density (Schmidt & Ostfeld 2001; Salkeld & Lane 2010; Wood & Lafferty 2012), which is why several studies suggest that DIN is a more direct measure of risk to humans. Similarly, in Argentina, host species diversity and evenness was not correlated with Andes virus (a hantavirus) antibody seroprevalence in the reservoir host – the long-tailed colilargo, *Oligoryzomys longicaudatus* – but there was a correlation between species diversity and the abundance of antibody-positive *O. longicaudatus* (Piudo *et al.* 2011). We recommend reporting data on both prevalence and density of infection (in vectors and/or vertebrate hosts) so that public health agencies can interpret how biodiversity affects these key components of disease risk. Parallel issues arise when attempting to interpret measures of biodiversity: is species richness, evenness or some index the best indicator of community biodiversity?

We only included studies that explicitly measured biodiversity, because proxies may fail to accurately represent local community ecology (Wood & Lafferty 2012). When species richness was explicitly measured, there was little evidence of a significant relationship with Lyme disease risk (Prusinski *et al.* 2006; LoGiudice *et al.* 2008). Studies that do use substitute measures (e.g. forest fragmentation) provide conflicting results: a negative correlation between forest fragmentation and Lyme disease risk in New York & Connecticut (Allan *et al.* 2003; Brownstein *et al.* 2005); no relationship across New York, Connecticut and New Jersey (LoGiudice *et al.* 2008); and lower human incidence of Lyme disease in fragmented contexts in Connecticut (Brownstein *et al.* 2005).

Rather than biodiversity, it is more likely that the role of individual host species and their interactions with other hosts, vectors, and the pathogen that are more influential in determining local disease risk (Kilpatrick *et al.* 2006; LoGiudice *et al.* 2008; Salkeld & Lane 2010; Hamer *et al.* 2011). For example, the reservoir competence and abundance of particular bird species and the mosquito vector's feeding preferences may be better predictors of West Nile virus activity than avian species richness (Kilpatrick *et al.* 2006; Loss *et al.* 2009; Hamer *et al.* 2011). In Utah's Great Basin Desert, pinyon mice (*Peromyscus truei*) appear to reduce hantavirus antibody prevalence, whereas kangaroo rats (*Dipodomys ordii*) seem to increase hantavirus antibody prevalence (Clay *et al.* 2009), and in Montana, estimated Sin Nombre antibody prevalence in deer mice was reduced in the presence of voles (*Microtus* spp.), although vole abundance was not important (Carver *et al.* 2011).

At a larger spatial scale, some studies have demonstrated negative correlations between measures of diversity and disease, for example, human WNV incidence at the county level negatively correlated with data from breeding bird atlases or surveys (Ezenwa *et al.* 2006; Swaddle & Calos 2008; Allan *et al.* 2009). In contrast, mammal diversity at a global scale has been linked to increased likelihood of emerging zoonotic pathogens (Jones *et al.* 2008). We believe that these studies should be interpreted with caution given that vertebrate diversity, incidence of zoonoses, vector feeding ecology and infection rates are often recorded at different times or spatial scales

and rely on disparate sources of data. That is, disease transmission can operate at fine spatial scales and data at coarse county or region levels may not be indicative of local pathogen transmission patterns (Loss *et al.* 2009; Hamer *et al.* 2011). When data from larger aggregates are used to infer the properties of the elements of those aggregates, epidemiologists warn of an 'ecological fallacy,' and even though ecologists may bristle at the term, the effect is real and avoidable. We have attempted to avoid this fallacy by not including county-level studies.

We did not include studies that examine non-human pathogens in our meta-analysis because our interest was in the possible relationship between wildlife biodiversity and human health. However, we presume that impacts of biodiversity on non-human diseases will also depend on local ecology and the particular host-pathogen relationships (e.g. Hudson *et al.* 2006; Salkeld *et al.* 2008; Chasar *et al.* 2009; Wood *et al.* 2010). For example, a recent meta-analysis, found that relationships between primate parasite prevalence and mammal species richness were idiosyncratic (Young *et al.* 2013).

If we are to gain an understanding of causal mechanisms generating diversity-disease relationships, investigations into the relationship between biodiversity and zoonotic disease risk must collect data on biodiversity that is spatially and temporally related to the relevant measures of disease risk. For example, WNV infection rates in mosquitoes should be related to the bird community that fed the mosquito population. Investigations must acknowledge variation in vector feeding behaviour (host species preferences), vector and host transmission biology, and the influences of abiotic factors (e.g. temperature, rainfall) and habitat types upon disease dynamics (Randolph & Dobson 2012). Measures of biodiversity should incorporate additional species that may interact with reservoir species, for example, carnivores that might influence rodent host population biology and thereby influence hantavirus or *B. burgdorferi* transmission dynamics (Ostfeld & Holt 2004). A promising approach to understanding interactions between disease ecology and epidemiology is to focus on tractable manipulations such as removal of particular host or vector species (e.g. Tsao *et al.* 2004; Perkins *et al.* 2006; Suzán *et al.* 2009). Also, analyses must entertain and control for alternate hypotheses that affect both biodiversity and disease risk, such as rainfall gradients, urbanisation, vector abundance, human behaviour, latitude. Such study designs, incorporating ecologists, epidemiologists, medical entomologists and public health agencies may facilitate an understanding of exactly what metrics are required to understand the local disease risk, and the mechanisms that allow zoonotic disease to spill over into human cases.

Public health initiatives intending to ameliorate disease risk must rely on a comprehensive, empirically based understanding of local disease ecology and epidemiology. Our analyses suggest that the links between biodiversity and disease prevalence are variable and dependent on the disease system, local ecology and probably human social context. Broadly advocating for the preservation of biodiversity and natural ecosystems to reduce disease risk is an oversimplification of disease ecology and epidemiology. If the disease dynamics are not well understood, conservation of endemic biodiversity could, in fact, increase human disease risk (e.g. if density of reservoirs or vectors increases), and may simultaneously remove economic and logistical resources from more effective control measures (Randolph & Dobson 2012). Certainly, the links between measures of disease risk (e.g. infection rates in reservoir hosts or vectors) and actual human incidence (rates of infection in the local

human populations) need to be better established. Furthermore, how and where humans are exposed has important consequences for the biodiversity-disease buffer hypothesis, that is are the scales at which biodiversity and disease risk are investigated actually relevant to human exposure to disease?

It is possible that the hope for a win-win outcome – simultaneous protection of biodiversity and human health – has led to premature acceptance that the dilution effect is a general ecosystem service associated with biodiversity (Randolph & Dobson 2012). However, our meta-analysis suggests that the dilution effect is neither general nor strong. The ecology of infectious disease is often too complicated to expect that there will be simple relationships between disease risk and biodiversity. A focus on the local ecology and interactions of important hosts and vectors is more likely to reveal insights into how to reduce disease risk and humans.

## ACKNOWLEDGEMENTS

We thank many colleagues for helpful discussion on these issues, and for sharing information and data: B Allan, W Bagge, J Brownstein, J Brunner, S Carver, R Douglass, RJ Eisen, V Ezenwa, D Fish, J Foley, L Gaffikin, D George, G Glass, G Hamer, G Hickling, T Holsomback, F Keesing, A Kjemtrup, M Kilpatrick, RS Lane, K LoGiudice, NC Nieto, RS Ostfeld, L Piudo, M Pongsiri, W Reid, W Reisen, R Rosenthal, P Stapp, J Tsao, I Vilcins, three anonymous reviewers, CDPH's Vector-borne Disease Section and numerous Californian vector control or mosquito abatement districts, & SF Summit. Funding was provided by Environmental Venture Project awards from the Woods Institute for the Environment to JHJ and to R Dirzo, and from the Institute for Research in the Social Sciences Faculty Fellowship at Stanford University.

## AUTHORSHIP

DJS, JHJ & KAP designed the study, DJS & KAP collected and contributed data, DJS & JHJ analysed the data, and all authors wrote the manuscript.

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Editor, Kevin Lafferty

Manuscript received 20 September 2012

First decision made 16 October 2012

Second decision made 19 December 2012

Third decision made 7 January 2013

Manuscript accepted 13 February 2013