Niche-based host extinction increases prevalence of an environmentally acquired pathogen

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Understanding the ecology of environmentally acquired and multi-host pathogens affecting humans and wildlife has been elusive in part because fluctuations in the abundance of host and pathogen species may feed back onto pathogen transmission. Complexity of pathogen-host dynamics emerges from processes driving local extinction of the pathogen, its hosts and non-hosts. While the extinction of species may entail losses in pathogen–host interactions and decrease the proportion of hosts infected by a pathogen (prevalence), some studies suggest the opposite pattern. Niche-based extinction, based on the species tolerance to environmental conditions, may increase prevalence of infection because the pathogen and its hosts persist, while other species go extinct. Hence, understanding prevalence of infection requires disentangling random- and niche-based extinction processes occurring simultaneously. To contribute to this exercise, we analysed the prevalence of an environmentally acquired, multi-host pathogen, Mycobacterium ulcerans (MU), in a unique dataset of 16 communities of freshwater animals, surveyed during 12 months in Akonolinga, Cameroon in equatorial Africa. Two different ecosystems were identified: rivers (lotic) and swamps and flooded areas (lentic). Increased prevalence of MU infection was correlated with niche-based extinction of aquatic host invertebrates and vertebrates in the lentic ecosystems, whereas decreased prevalence was associated with random disassembly of the lotic ecosystems. This finding suggests that random and niche-based extinction of host taxa are key to assessing the effect of local extinction of species on the ecology of environmentally acquired and multi-host pathogens.

Anthropogenic changes of the environment contribute to local extinction of species (Dirzo et al. 2014, Sandom et al. 2014). Thus, environmental management aimed at preserving ecosystem health and human welfare requires understanding the ecological consequences of local extinction of species (Balvanera et al. 2006). Notably, local extinction of species may influence the emergence of infectious diseases shared between humans and wildlife, and this is of concern because zoonotic diseases represent more than 60% of emerging human pathogens (Woolhouse et al. 2005). Generally, the processes driving local extinction of host species in a community affect prevalence of these zoonotic pathogens (i.e. the proportion of individuals infected with a pathogen; Ostfeld and LoGiudice 2003, Lafferty 2012, Lacroix et al. 2013).

Theory of community ecology recognizes two distinct processes driving local extinction of species. First, niche-based extinction occurs when the species which go locally extinct are the less tolerant to environmental conditions (abiotic and biotic) affecting their ability to gather resources, survive, and reproduce (Leibold 1995, Chase and Leibold 2003). Second, local extinction of species may occur by chance alone (i.e. random extinction due to dispersal, chance of colonization, and demographic stochasticity affecting abundance of the species; Connor and Simberloff 1979, Tokeshi 1999, Hubbell 2001). Within the framework of community ecology, a seminal study on Lyme disease provides insights on how prevalence of a vector-transmitted pathogen is affected by niche-based and random extinction of hosts and non-hosts in a community (Ostfeld and LoGiudice 2003). Lyme disease in humans, in the US, is caused by the spirochete bacterium Borrelia burgdorferi, which is hosted by the white-footed mouse Peromyscus leucopus and transmitted to humans by the tick Ixodes scapularis. In their
study on *B. burgdorferi*, Ostfeld and LoGiudice (2003) found that while random extinction of host species resulted in a decrease in the prevalence of nymphal ticks infected with *B. burgdorferi*, niche-based extinction favouring persistence of hosts resulted in increased prevalence. The authors suggested that prevalence increased under niche-based extinction due to an increase in the abundance of hosts and the loss of biotic interactions hindering infection of susceptible individuals (e.g. ticks feeding on non-competent hosts; Keesing et al. 2006, Ostfeld et al. 2008). However, local extinction of species occurs by both, random- and niche-based processes simultaneously (Takai 1999, Hubbell 2001, Thompson and Townsend 2006, Chase and Myers 2011). Thus, understanding the effects of species extinctions on the incidence of zoonotic disease in humans is elusive. Furthermore, while the effects of local extinction of species on prevalence have been investigated on parasites or pathogens such as plant viruses, trematodes in frogs, and *B. burgdorferi* in white-footed mice (Ostfeld and LoGiudice 2003, Johnson et al. 2013, Lacroix et al. 2013), it is unknown whether environmentally acquired pathogenic bacteria with a free-living stage, respond likewise. Some environmentally acquired pathogenic bacteria have a wider ecological niche than their hosts because they may persist in both the environment and the hosts. Thus niche-based extinction must increase prevalence if these bacteria survive while the populations of hosts decrease; whereas random extinction of taxa should decrease prevalence because the bacterium and its hosts may go locally extinct.

One possible way to disentangle the contribution of niche-based and random extinction processes is to use a multi-scale metacommunity framework (Leibold et al. 2004, Presley et al. 2010, Ulrich and Gotelli 2013). In this framework, a metacommunity is a set of communities interconnected by species’ dispersal; and the extent of random processes influencing the membership of taxa in each community is assessed by comparing the observed presence and absence of taxa with the patterns generated by random structures of a null model (Gotelli 2000, Chase and Myers 2011). Significant deviation from random distribution of species, would suggest niche-based extinction (Presley et al. 2010, Ulrich and Gotelli 2013).

Within this approach we investigated the effects of niche-based and random extinction of host species on prevalence of the pathogenic bacterium *Mycobacterium ulcerans* (MU), in 16 interconnected communities of aquatic invertebrates and vertebrates, surveyed monthly during one year in Akonolinga, Cameroon, central Africa. *Mycobacterium ulcerans* is the causative agent of Buruli ulcer, a human skin disease. It is present on a wide diversity of substrates such as mud, organic detritus, and as biofilms on aquatic plants and is carried by a large diversity of aquatic invertebrates, fish, amphibians, reptiles, and mammals (Wansbrough-Jones and Phillips 2006, Merritt et al. 2010, McIntosh et al. 2014). In this study we considered that all animal species (hereafter considered as hosts) could potentially acquire MU from the aquatic environment and each taxon had a particular potential to harbour MU (see host abundance in Methods).

**Methods**

We performed our study in two main steps. First, we examined the structure of the metacommunity of aquatic invertebrates and vertebrates (109 taxa overall; see Garchitorena et al. 2014 for details) and identified two main subsets representing two distinct types of aquatic ecosystems: lotic systems formed by flowing water, mainly tributaries of the Nyong and Mfoumou rivers; and lentic systems consisting of stagnant water, swamps and flooded areas within the Nyong catchment area. We classified ecosystems into these two types based on a previous study reporting different dynamics in MU presence in Ghana (Benbow et al. 2013). The difference between the lotic and the lentic systems was corroborated with a non-metric multidimensional scaling analysis (NMDS) of the abundance of taxa (Legendre and Legendre 1998). Thus these two subsets of the metacommunity were analysed separately to assess whether niche-based or random processes determine species diversity.

Second, we tested the correlation between the loss of host species and prevalence of infection with MU by performing stepwise selection of generalized linear models (GLM) based on Akaike’s information criterion, AIC (Burnham and Anderson 2002). As a response variable, we analysed prevalence of infection with MU in the five most abundant orders of invertebrates (hereafter MU prevalence). These orders of invertebrates were present during the year, across all communities sampled, and are known to have high prevalence of infection with MU (Garchitorena et al. 2014). Each pair of communities in the lotic and the lentic systems were analysed as a pseudo-experiment to test the correlation between the loss of host taxa and MU prevalence. For each pair of communities (hereafter a region) we estimated the loss of taxa and connectivity between the two communities of each pair, and the regional MU prevalence estimated in the two communities combined. According to our hypothesis, we predicted that niche-based processes of extinction must increase regional MU prevalence, whereas random extinction of host taxa should decrease prevalence.

**Study sites and sampling**

Monthly sampling of aquatic invertebrates and vertebrates was performed in 16 locations (biotic communities) in Akonolinga, central Cameroon, between June 2012 and May 2013. Distributed within a region of approximately 3600 km², these included seven lentic communities, in swamps and flooded areas, and nine lotic communities, formed by streams and rivers. Sampling of invertebrates and vertebrates in all communities was performed monthly for five consecutive days, from 8 a.m. to 4 p.m., and the same method was carried out by the same researchers throughout the study (Garchitorena et al. 2014). At four sites in each community, five sweeps were performed with a metal dip net (32 × 32 cm, 1-mm mesh) within 1 m² at depths of 0 to 1 m below water level. All biological material collected was passed through a 3-layer filter (32 × 32 cm grid; 20, 5- and 1-mm mesh, respectively) with abundant water. The material in the first two layers included visible aquatic organisms that were identified, classified, and stored separately in tubes with 70%
ethanol. The material captured by the finest filter was a
mixture of plant debris and small invertebrates (> 1 mm), which
was preserved in 95% ethanol, and taxa were identified with
a binocular microscope at the laboratory. Classifying aquatic
organisms at the family level or higher, we identified 109
distinct taxa (Garchitorena et al. 2014). Taxonomic classi-
fication was performed with keys provided in the Guide to
the Freshwater Invertebrates of Southern Africa series and
other relevant literature (Durand and Lévêque 1980, 1981,
Scholth and Holm 1985, Fay 2001, Day and de-Moor
2002a, b, Day et al. 2003, de-Moor et al. 2003, 2004, Stals
and de-Moor 2007, Moisan 2010).

Detection of M. ulcerans with qPCR

For each community and month sampled, the presence of
MU was tested in pools containing up to 2 g of animals
belonging to the same taxonomic group (Garchitorena et al.
2014). In all pools, MU was detected in 17 distinct taxo-
nomic groups. However, not all of these groups were present
during all months of the study. This may add variation in MU
presence due to differences across host taxa in their potential
to harbour the bacillus (Portaels et al. 2001, Marsollier et al.
2004). To ensure consistency in the sensitivity of detecting
MU we focused on the orders of macroinvertebrates that
were consistently present throughout the year (Coleoptera,
Diptera, Ephemeroptera, Hemiptera and Odonata (Supple-
mentary material Appendix 1–3). Across time and sampled
communities, variation in the abundance of these five higher
taxa correlated with the abundance of all taxa ($R^2 = 0.94$,
Supplementary material Appendix 2). Quantitative poly-
merase chain reaction (qPCR) was used to detect two specific
markers of MU in each sample: oligonucleotide primer and
TaqMan probe sequences of IS2404 and the ketoreductase B
domain of the mycolactone polyketide synthase (mls) gene
from the plasmid pMUM001 (Garchitorena et al. 2014). The
sample was considered positive only if both markers
were detected, with threshold cycle (Ct) values strictly < 35
cycles. The qPCR is the most sensitive technique available
for the study of MU in the environment and detects very
low numbers of DNA sequence copies (Fyfe et al. 2007).
We eliminated false positives and false negatives, by assessing
inhibition of the PCR. For each 96-well plate, we included
known DNA concentrations of MU at the amplification
stage. The DNA concentrations were diluted over 5 logs
(from $10^6$ to $10^2$ cfu ml$^{-1}$) and served as positive controls
for the PCR mix. Furthermore, DNA extracts were purified
with a high-throughput silica-membrane-based purifica-
tion method, combining bind–wash–elute procedure with
vacuum recovery (QIAquick 96 PCR Purification Kit). This
purification technique allows recovery of up to 90% of DNA
while removing most inhibitors, such as insect exoskeletons
and many other products.

Richness and local extinction of taxa

Biotic interactions may increase with richness of host taxa
and thus influence MU prevalence (Benbow et al. 2013). We
considered this possibility in our analysis by testing the rela-
tionships between MU prevalence and the richness of host
taxa in each region (i.e. each pair of communities).

Accounting for the effects of regional richness, we analy-
sed the relationships between regional MU prevalence and
the local extinction of host taxa within each region. Nested-
ness between the communities of a region was examined as
a surrogate of local extinction of taxa within each region. To
examine nestedness separately from turnover of host taxa, we
estimated nestedness from the decomposition of the dissimi-
larity between the pair of communities, $\beta$-diversity (Koleff
et al. 2003). First, we considered strict nestedness, estimated
by decomposition of the Sørensen index (Baselga 2010). Sec-
ond, we considered a gradient of diversity loss ($\beta_{\text{gradient}}$
that was derived from the Bray–Curtis dissimilarity (Baselga
2010, 2013). While strict nestedness captured local extinct-
tion of taxa, $\beta_{\text{gradient}}$ captured decreases in the abundance
of each host taxon (Supplementary material Appendix 4). In
principle, including both predictors, nestedness and $\beta_{\text{gradient}}$
in generalized linear models allowed considering changes in
MU prevalence associated with changes in host abundance
independently from host richness.

Host abundance

Higher MU prevalence may be due to increments in host abundance (Mihaljevic et al. 2014). Thus the abundance of
individual taxa with potential to harbour MU was consid-
ered in our analysis. A previous study suggested that not all
host species exhibit the same prevalence, and this may be due
to ecological or taxonomic associations between MU and the
host taxa (Garchitorena et al. 2014). To account for this pos-
sibility, the abundance of each taxon at each community was
multiplied by the prevalence reported for that taxon ($p_i$),
and included as a predictor in the GLMs. The reported prev-
lence ($p$) of each taxon ($i$) was obtained from a larger survey
in the region (Garchitorena et al. 2014) and weighted by the
reported sample size such that $p_w = p \left( \frac{N_i}{\sum_i N_i} \right)$, because
the number of trials ($N_i$) presented by Garchitorena et al.
(2014) was not the same for all host taxa.

Deforestation index

Deforestation is known to affect incidence of Buruli ulcer
and may thus influence MU infection prevalence as well
(Brou et al. 2008, Wagner et al. 2008). Indeed land cover
variables influence MU prevalence (Carolan et al. 2014)
and, among a wide spectrum of landscape variables, pre-
vious studies showed that deforestation explains the most
variation in MU prevalence in French Guiana, South
America (Morris 2014). Therefore, as a source of variation
that may confound our analysis we considered the effects of
deforestation on MU infection prevalence. Deforestation
was estimated by using information from the Global Forest
Watch survey (<www.globalforestwatch.org>), which cap-
tures whether deforestation occurred in a 30 $\times$ 30 m cell
from the year 2000 to 2011. As a surrogate for deforesta-
tion we counted the number of cells that were deforested
within circular areas of different radius (1, 2.5, 5, 10, 20
and 40 km) around each surveyed community. Among these
measurements we selected the estimate with the highest
correlation coefficient (Supplementary material Appendix
5) and used it as predictor in the GLM (i.e. deforestation around 10-km radius). For analysis we used a regional estimate, the total deforested cells counted in each pair of local communities.

**Probabilistic dispersal**

Stochastic extinction and colonization of species in communities may occur due to probabilistic dispersal of species between neighbour communities (Hubbell 2001, Chase and Myers 2011). In this case, nearest communities are expected to be more similar than distant ones. Thus, we considered the connectivity between communities as a surrogate for probabilistic dispersal. The effect of community connectivity on prevalence of MU infection was accounted for with two surrogates: Euclidean connectivity and downstream connectivity. Euclidean connectivity assumes that host and non-host species can disperse freely between communities across the landscape. This assumption is sensible as many host and non-host taxa can fly and disperse away from the hydrologic system. Downstream connectivity assumes that species disperse within the hydrologic system in the Akonolinga area. The distances of the shortest paths between communities were estimated on a geometric network weighted by the Strahler’s scores (Strahler 1957), representing downstream drift in the hydrologic network of Akonolinga (Supplementary material Appendix 6). For analyses, our estimates of connectivity were transformed as the inverse of distances (d) between communities (Euclidean and Strahler): \[ \sqrt{\frac{1 - d}{d_{\text{max}}}}. \]

from no connectivity (0) to full connectivity (1). While the main results did not change in quality using either of the connectivity surrogates, some effects were different when considering (Strahler) downstream drift or Euclidean connectivity; hence we present both GLMs.

**Data analysis**

**Metacommunity analysis**

Lotic systems were analysed separately from lentic systems because they involve different processes of community assembly. We used the quantitative framework proposed by Ulrich and Gotelli (2013) to test for non-randomness in the assemblage of the lentic and the lotic communities separately, by a combination of metrics to decompose matrix-wide patterns into those of individual pairs of species and sites. These metrics were estimated with the software Turnover <www.keib.umk.pl/> and its interpretation was based on benchmark studies (Ulrich and Gotelli 2013). We performed metacommunity analyses in two matrices (lotic systems and lentic systems) filled with 0 or 1, representing the absence or presence of each taxon (Supplementary material Appendix 7), and ordered by reciprocal averaging, minimizing embedded absences (i.e. maximizing coherence; Leibold and Mikkelsen 2002). Statistical inference of each metric was assessed by a Z test, comparing the observed with the expected estimates, derived from 1000 random assemblages generated by randomization of rows and columns with 100 000 swaps of the independent swap algorithm. The independent swap algorithm performs randomization of the fixed-fixed null model, which performs better than other null models, to control for type I errors (Ulrich and Gotelli 2013).

**GLM on regional prevalence of MU infection**

We performed two sets of GLM. First, GLM was used to test the relationships between regional MU prevalence (i.e. the prevalence of MU infection in each pair of communities, combined) and nestedness and β-gradient, while accounting for the effects of connectivity between communities, regional richness of taxa, host abundance and deforestation, and whether the ecosystems were lotic or lentic. Note that lentic communities were paired with lentic communities and lotic communities were paired with lotic communities, to ensure comparisons of similar types of aquatic ecosystems (Benbow et al. 2013). Second, once we corroborated that the relationship between richness of host taxa and MU prevalence was contingent on the environment (it was different between lotic and lentic systems), we repeated the GLM selection, separately for the lotic (n = 21) and the lentic (n = 36) systems.

We performed stepwise model selection based on the AIC while allowing the predictors to interact and improve the fit of the model to the data. The GLMs considered all two-way interactions between predictors, and assumed a binomial distribution of the response variable (regional MU prevalence). Predictors were standardized to their means, (ln x)−ln(i=1NXi/N), and normality of residual deviance was assessed by Kolgomorov–Smirnov’ tests. Because multicolinearity may be due to correlation between predictor variables, we ensured that the variance inflation factors of each principal term in the models were < 3 (Heiberger and Holland 2004). We assured that the results were not biased due to pseudoreplication and the contribution of a particular local community by removing each community from the data in turn, and repeating the analysis (Supplementary material Appendix 8:2). For all GLMs, we present parameter-estimates (b) ± standard errors, and a Z test rejecting the null hypotheses that b = 0. All analyses were performed in R software (<www.r-project.org/>).

**Results**

The first ordination axis of the NMDS analysis supported that lotic and lentic communities of invertebrates and vertebrates were significantly different (t = 3.65, DF = 12.03, p = 0.003). The first axis explained 75.6% of the data, the second axis 11.9%, and the third axis 4.2%. Furthermore, deforestation was higher in lentic communities than in lotic ones, and the lotic had lower species richness and higher abundance of host taxa than the lentic (Supplementary material Appendix 9). Metacommunity analysis of the lentic communities suggested niche structuring by spatial turnover linked to environmental gradients (Cturn = 0.02 (Z = 2.1, p = 0.035)), while no structure different than random was found in the lotic communities. In the lotic communities no metric of the metacommunity structure was significantly different than the random model except embedded
absences = 0.15 (Z = –2.7, p = 0.007), suggesting fewer embedded absences than expected. All metrics of metacommunity structure are presented in the Supplementary material Appendix 10.

Stepwise GLM supported that the relationship between regional richness of host taxa and regional MU prevalence was contingent on the environment. Prevalence decreased in taxon-poor regions of the lotic systems and increased in taxon-poor regions of the lentic systems (Fig. 1, Table 1). These relationships were consistent after accounting for the effects of connectivity and regional host abundance on regional MU prevalence (Table 1). Furthermore, GLM of the lotic system supported that regional MU prevalence was negatively related to regional host abundance (b = –0.3 ± 0.1, Z = –2.9, p = 0.0004). In the GLM of the lotic systems, richness was also included in the final GLM, after AIC model selection, but its effect was not statistically significant (b = 1.6 ± 1.0, Z = 1.5, p = 0.125; normality test of residual deviance: D = 0.28, p = 0.125). Nevertheless, regional host abundance was negatively correlated with richness (r_{Spearman} = –0.34, S = 10432, p = 0.04), thus potential collinearity between these two predictors should not be disregarded (see Supplementary material Appendix 11 for starting GLMs).

The GLM of the lentic systems showed positive relationships between regional MU prevalence, host abundance and deforestation (Table 2). Regional MU prevalence was positively related to the interaction between regional species richness and nestedness when connectivity was considered Euclidean (Table 2). This interaction suggested that MU prevalence increased with nestedness in taxon-rich regions. However, when downstream drift was considered as a measurement of connectivity, increases in MU prevalence were associated with two interactions: deforestation in taxon-rich regions (richness × deforestation), and the loss of species with reduced abundance of taxa (nestedness × β_{gradient}).

### Discussion

The order of host species extinction drives epidemiological patterns of multi-host pathogens. To this extent, simultaneous occurrence of niche-based and random host species extinction impedes our understanding of these threatening pathogens. Our metacommunity analyses suggest that stochastic processes dominate diversity in lotic communities whereas niche-based processes dominate diversity in lentic communities. Turnover of taxa, represented by the metric Crnun is recognized as evidence of niche-based processes driving community assembly (Ulrich and Gotelli 2013). These niche-based processes may include both affinities of species for non-overlapping habitats and inter-species interactions affecting birth and death rates of species (Chase and Myers 2011). Disentangling the effects of non-overlapping habitats and inter-species interactions affecting prevalence of infection with pathogens is a fundamental and novel subject of research in disease ecology (Lafferty 2012, Suzán et al. 2015). In our study we focused on the local extinction of host taxa by niche-based processes without being able to separate these mechanisms (non-overlapping habitats from biotic interactions). However, our study gives insights on the effects of niche-based extinction of host taxa on prevalence of infection of an environmentally acquired bacterium, *Mycobacterium ulcerans*.

According to Ostfeld and LoGuidice (2003), niche-based processes driving extinction of taxa and persistence of hosts must increase prevalence, whereas random extinction must decrease prevalence. Indeed, we found that lentic systems dominated by niche-based processes entail different correlations between MU prevalence and richness of host taxa, compared with lotic systems dominated by random processes (Fig. 1). These results are consistent with a study in Ghana showing a positive relationship between presence of MU and diversity (measured by Shannon’s index) in the lotic systems, but not in the lentic systems (Benbow et al. 2013). We corroborated a positive relationship between taxon richness and prevalence in the lotic systems and found a negative relationship between taxon richness and MU prevalence in the lentic systems. As predicted, the relationship between MU prevalence and taxon richness was contingent of the environment, such that niche-based extinction of host species in the lentic system correlated with increases of MU prevalence whereas random extinction of host taxa in the lotic systems correlated with decreases of prevalence. Nevertheless, one important factor to consider is the abundance of hosts in the community (Begon 2008). In this regard, GLM analyses of the lotic systems supported that both host abundance and taxon richness were informative predictors of MU prevalence. Host abundance was negatively correlated with MU prevalence but richness was not significantly related to MU prevalence. This negative relationship is counterintuitive and may have at least four non-mutually exclusive explanations. First the physical force of streams may wash away MU cells while increasing the abundance of hosts that are more resilient.

![Figure 1. The relationship between regional richness of taxa and prevalence of infection with Mycobacterium ulcerans (MU) is substantially different between lotic (open circles, dashed line, and red polygon) and lentic systems (closed circles, solid line and blue polygon). The relationship was positive in lotic environments and negative in lentic environments. Respectively, circles and polygons represent the MU infection prevalence and standard errors, predicted by the general linear model.](Image)
to this physical force. Second, it is possible that the most suitable environment for the hosts is not the most suitable for MU (Garchitorena et al. 2015). Notably, the relationship between host abundance and MU prevalence is positive in the lentic system, and is explained by the combined effects of deforestation. In lentic systems, MU prevalence increases in deforested regions with high abundance of hosts (Table 2). Likewise, positive relationships for MU prevalence and host abundance were found in a second endemic region in Cameroon where abiotic conditions were optimal for MU (Garchitorena et al. 2015). Third, the negative relationship between host abundance and taxon richness may be hindered by the relationships between MU prevalence, taxon richness and host abundance (Mihaljevic et al. 2014). The biological correlation between predictors of MU prevalence, host abundance and richness, limits separating their effects in the GLM. In this case, prevalence may decrease in taxon-poor habitats with high abundance of hosts, because the likelihood of extinction is high in these habitats and MU and other taxa may go extinct locally, regardless of the abundance of hosts (Fig. 1). Finally, it is possible that MU prevalence in the most persistent orders of invertebrates decreases because the abundant hosts have high prevalence but are not good at transmitting MU into the ecosystem, (e.g. into the food web; Garchitorena et al. 2015). Together, these mechanisms may act simultaneously such that prevalence of MU decreases in taxon-poor habitats of the lotic system due to both abiotic and biotic factors affecting the ecological networks in which MU circulates.

In lentic systems, MU prevalence increases in taxon-poor regions having few host and non-host taxa (Fig. 1). We also found that the local extinction of taxa (nestedness) in taxon-rich regions increases MU prevalence substantially, represented by the significant interaction between nestedness and host richness (Table 2). Furthermore, when we consider the downstream connectivity between communities (GLM Strahler, Table 2), our GLM analysis suggests that both the local extinction of taxa (nestedness) and reductions in abundance ($\beta_{\text{gradient}}$) correlate with an increase in MU prevalence. This is represented by the significant interaction between

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**Table 1.** Final GLMs of AIC stepwise model selection on the regional prevalence to *Mycobacterium ulcerans* (response variable) across all communities, considering two types of connectivity between the communities: Strahler (downstream connectivity) and Euclidean. 57 regions were defined by all the possible pairwise combinations of seven lentic communities, and all possible pairwise combinations of nine lotic communities. GLM Strahler (AIC = 310.5, residual deviance = 61.9, null deviance = 91.7) was selected from the starting model with principal terms (AIC = 320.2, residual deviance = 71.6). GLM Euclidean (AIC = 310.1, residual deviance = 71.5) was selected from the starting model including only principal terms (AIC = 320.1, residual deviance = 71.5). The relationship between MU prevalence and host richness was contingent on whether the environment is lotic or lentic (interaction $R \times L$). Normal distribution of final model residuals was corroborated for GLM Strahler ($D = 0.19, p = 0.240$) and GLM Euclidean ($D = 0.14, p = 0.633$). Significant statistics are in bold characters.

<table>
<thead>
<tr>
<th></th>
<th>GLM Strahler</th>
<th>GLM Euclidean</th>
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<tbody>
<tr>
<td></td>
<td>$b \pm SE$</td>
<td>$Z(p)$</td>
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<tr>
<td>Richness ($R$)</td>
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<tr>
<td>Host abundance</td>
<td>$-0.4 \pm 0.1$</td>
<td>$-3.8 (&lt;0.001)$</td>
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<tr>
<td>Connectivity ($C$)</td>
<td>$-0.5 \pm 0.4$</td>
<td>$-1.2 (0.249)$</td>
</tr>
<tr>
<td>Lotic</td>
<td>$-0.4 \pm 0.1$</td>
<td>$-3.3 (0.001)$</td>
</tr>
<tr>
<td>$\beta_{\text{gradient}}$</td>
<td>$-2.5 \pm 1.3$</td>
<td>$-1.9 (0.061)$</td>
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<tr>
<td>$C \times \beta_{\text{gradient}}$</td>
<td>$3.4 \pm 1.7$</td>
<td>$2.0 (0.047)$</td>
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<tr>
<td>$R \times \text{lotic}$</td>
<td>$2.7 \pm 1.4$</td>
<td>$1.9 (0.052)$</td>
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**Table 2.** Final GLMs of AIC stepwise model selection on the regional prevalence to *Mycobacterium ulcerans* (response variable) in lentic communities, considering two types of connectivity between the communities: Strahler (downstream connectivity) and Euclidean. 21 regions were defined by all the possible combinations given by pairing the seven lentic communities. GLM Strahler (AIC = 119.9, residual deviance = 40.2, null deviance = 3.1) was selected from the starting model with principal terms (AIC = 138.8, residual deviance = 71.6). GLM Euclidean (AIC = 126.9, residual deviance = 40.2, null deviance = 16.1) was selected from the starting model including only principal terms (AIC = 138.9, residual deviance = 32.1). Estimated parameters (b), standard errors (SE) and a Z-test rejecting that $b=0$, are presented. Normal distribution of final model residuals was corroborated for GLM Strahler ($D = 0.29, p = 0.365$) and GLM Euclidean ($D = 0.33, p = 0.196$). Significant statistics are in bold characters.

<table>
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<td>$Z(p)$</td>
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<td>$-2.1 (0.032)$</td>
</tr>
<tr>
<td>$H \times D$</td>
<td>$16.9 \pm 4.0$</td>
<td>$4.2 (&lt;=0.001)$</td>
</tr>
<tr>
<td>$N \times \beta_{\text{gradient}}$</td>
<td>$79.9 \pm 21.1$</td>
<td>$3.8 (&lt;=0.001)$</td>
</tr>
<tr>
<td>$R \times \beta_{\text{gradient}}$</td>
<td>$-44.1 \pm 11.4$</td>
<td>$-3.9 (&lt;=0.001)$</td>
</tr>
<tr>
<td>$R \times D$</td>
<td>$21.5 \pm 11.0$</td>
<td>$2.0 (0.051)$</td>
</tr>
</tbody>
</table>
nestedness and $\beta_{\text{gradient}}$ (Table 2). In this regard, the dilution-effect theory suggests an increase in prevalence due to the loss of some interspecific interactions that hinder infection of susceptible individuals and/or recovery of infected individuals (Mitchell et al. 2002, Keesing et al. 2006). Thus, the nestedness $\times \beta_{\text{gradient}}$ interaction suggests that a dilution effect may also occur in MU in the lentic ecosystems. Nevertheless, the identities of these key interspecific interactions are not well known and conclusions may be speculative. One plausible explanation is that some key species may act as amplifiers (e.g. Naucoridae and Belostomatidae water bugs) and boost prevalence while others may act as dilutors (e.g. gastropods) and reduce prevalence (Marsollier et al. 2003, 2004). While this evidence comes from experiments in laboratories, and may not capture the complex ecology of MU, other studies reveal that MU prevalence is associated with the identity of host species in the community (Benbow et al. 2013, Garchitorena et al. 2015) and the occurrence of some functional groups, such as filter feeders and scavengers, in the ecosystems (Roche et al. 2013, Morris 2014). In this regard our study focuses on the prevalence of MU in the five most persistent and abundant taxa, which also have high prevalence reported with field data (Garchitorena et al. 2014). Species that persist and have high colonizing potential have either a high reproduction rate, a long life span, or both (Pimm et al. 1988, Sæther et al. 2004, Sol et al. 2012). Whether these life history traits favour susceptibility to infection with MU remains as a question of further research (Joseph et al. 2013) and we suggest that investigating the symbiotic relationships between host species and MU infection will be a fundamental step toward mitigating Buruli ulcer in humans.

Overall, our study underscores the importance of considering niche-based and random processes driving diversity, when investigating the relationships between diversity and prevalence of infection with a pathogen. Once we account for these effects, our results are consistent with previous studies on the tick-borne pathogen causing Lyme disease, helminths on frogs, and aphid-borne viruses on plants (Ostfeld and LoGiudice 2003, Johnson et al. 2013, Lacroix et al. 2013). While these previous studies demonstrated the importance of niche-based extinction on prevalence of strict parasites, our study expands this view to an environmentally-acquired pathogen, which is not a strict parasite and seems ubiquitous in the environment. This suggests that the effects of niche-based extinction of hosts on pathogen prevalence may be generalizable to other categories of infectious diseases as shown here for the saprophyte $M. ulcerans$. On these grounds, prevalence of pathogen infection in humans is likely to increase worldwide amid the current extinction of host and non-host taxa due to anthropogenic environmental changes. Thus, the potential emergence of zoonotic disease and environmentally persistent microbes associated with the local extinction of species should not to be disregarded in the policies of environmental management, human health, and well-being.

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Supplementary material (available online as Appendix oik-2700 at <www.oikosjournal.org/appendix/oik-02700>), Appendix 1–11.