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Joint effects of habitat, zooplankton, host stage structure and diversity on amphibian chytrid

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Why does the severity of parasite infection differ dramatically across habitats? This question remains challenging to answer because multiple correlated pathways drive disease. Here, we examined habitat–disease links through direct effects on parasites and indirect effects on parasite predators (zooplankton), host diversity and key life stages of hosts. We used a case study of amphibian hosts and the chytrid fungus, *Batrachochytrium dendrobatidis*, in a set of permanent and ephemeral alpine ponds. A field experiment showed that ultraviolet radiation (UVR) killed the free-living infectious stage of the parasite. Yet, permanent ponds with more UVR exposure had higher infection prevalence. Two habitat-related indirect effects worked together to counteract parasite losses from UVR: (i) UVR reduced the density of parasite predators and (ii) permanent sites fostered multi-season host larvae that fuelled parasite production. Host diversity was unlinked to hydroperiod or UVR but counteracted parasite gains; sites with higher diversity of host species had lower prevalence of infection. Thus, while habitat structure explained considerable variation in infection prevalence through two indirect pathways, it could not account for everything. This study demonstrates the importance of creating mechanistic, food web-based links between multiple habitat dimensions and disease.

1. Introduction

Parasite infection differs dramatically across habitats. In some cases, parasites exert strong negative effects on host populations. Yet, severe epidemics occur infrequently and in a relatively small subset of habitats [1]. For example, epidemics of the virulent amphibian chytrid, *Batrachochytrium dendrobatidis* (hereafter, Bd) erupt catastrophically in some habitats and locations (e.g. geothermal ponds, undisturbed forests) but not others (e.g. non-geothermal ponds, disturbed forests) [1–6]. Why? It remains challenging to answer this question because multiple correlated pathways drive disease [7–9]. Furthermore, these pathways may have contrasting effects; some factors enhance disease while others diminish it. Thus, disease dynamics reflect tension between multiple driving factors potentially linked via habitat.

Here, we disentangle multiple pathways governing variation in Bd infection in amphibian hosts. In a set of alpine ponds, prevalence and severity of Bd infections differ dramatically across sites [10–12]. Currently, however, the factors driving this pronounced variation in infection prevalence remain unknown. We focus on infection prevalence in two native hosts that are highly susceptible to Bd (fire salamander: *Salamandra salamandra* and the midwife toad: *Alytes obstetricans*) [12–14]. Both species drive disease in this system [13,15]. To explain variation in infection prevalence, we examine direct and indirect factors that connect to Bd epidemics via gains and losses of zoospores [12,14,15]. Zoospores are
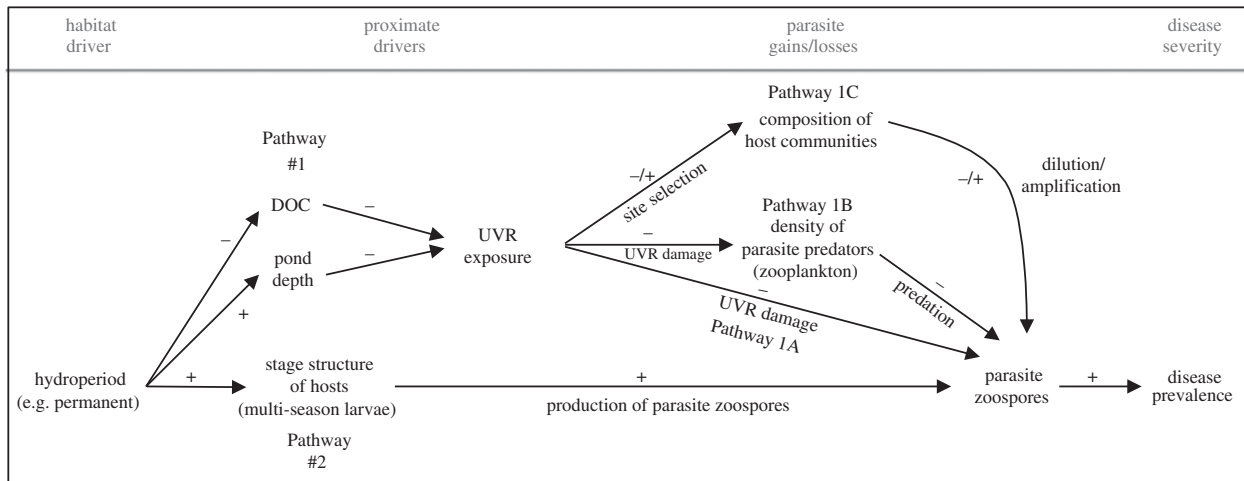
Figure 1. Hypothesized pathways connecting habitat to infection prevalence of Bd in amphibian hosts. Hydroperiod (ephemeral versus permanent) influences disease via two pathways governing parasite propagules (zoospores). *Pathway 1A – C*: permanent ponds are deeper, but have less dissolved organic carbon (DOC) and therefore higher exposure to ultraviolet radiation (UVR). UVR could directly damage zoospores (bottom, pathway 1A), reduce zooplankton predators of zoospores (1B) or alter host composition (top, 1C). Dilution (−) or amplification (+) could arise from UVR-mediated changes in host composition. *Pathway 2*: permanent ponds harbour multi-season larvae that produce many parasite zoospores. Positive (+) and negative (−) symbols denote sign of relationships.

free-swimming propagules, which attach to and then replicate on the epidermis of amphibian hosts [16]. Infected hosts release new zoospores, which then infect other hosts. Hence, Bd dynamics depend sensitively on zoospore survival [17].

The first main pathway governing Bd epidemics involves direct and indirect effects of ultraviolet radiation (UVR). UVR exposure may either directly damage Bd zoospores or alter the distribution of key species that influence disease (via multiple food web interactions; figure 1, Pathways 1A–C). In these mountainous regions, variation in UVR exposure starts with differences in underlying geology (e.g. bedrock, hydrology) that governs pond depth and hydroperiod (permanent versus ephemeral) [18]. Hydroperiod largely determines the type of habitat and vegetation surrounding ponds (e.g. moss in bogs versus grass in knolls). These characteristics influence the quality and quantity of dissolved organic carbon (DOC) in ponds. DOC acts as a natural aquatic 'sunscreen' that strongly regulates exposure of aquatic organisms to UVR. Together, variation in depth and DOC govern attenuation of UVR in the water column [19,20]. Hence, hosts and parasites in different ponds experience dramatically different UVR exposures. Based on previous evidence [13,21], solar radiation should damage Bd zoospores, thereby depressing infection prevalence via direct, damaging effects of UVR (Pathway 1A).

Variation in UVR could also indirectly alter disease by modulating the distribution of other key species (e.g. predators and hosts) that influence disease (Pathway 1B,C; figure 1). First, UVR could constrain predators that consume infectious stages of parasites (Pathway 1B) [22,23]. Zooplankton eat Bd zoospores [15,24–26] and respond sensitively to UVR—especially in alpine habitats (reviewed in [19]). Therefore, high-UVR ponds could support fewer zooplankton that consume Bd zoospores. If zooplankton respond more sensitively to UVR than zoospores, this indirect release from predation could overwhelm the direct mortality effect of UVR on zoospores (Pathway 1B, figure 1). In other words, epidemics could become larger in ponds with more UVR due to the loss of key parasite predators that are sensitive to UVR. Second, habitat variation could influence the abundance of other host species that also govern disease (Pathway 1C, figure 1). Here,

habitat–diversity links could arise if hosts selectively oviposit based on UVR exposure and/or other species [27–29]. Consequentially, selective oviposition (which influences the diversity of larval hosts found in a given pond) could drive variation in disease because hosts vary in disease competency [12,14]. These other species, then, could produce a dilution effect (i.e. reduced disease with higher diversity) if highly competent focal hosts are less common in more diverse communities [30]. Alternatively, an amplification effect could arise if higher diversity reflects higher frequencies of more competent (non-focal) hosts [31].

The second main pathway directly links variation in hydroperiod, stage structure of focal hosts and parasite (zoospore) production (Pathway 2, figure 1). Hydroperiod could influence the distribution of key host stages that influence disease. Many amphibian species, including focal hosts, can have both single-season and multi-season larvae. Delayed metamorphosis requires a permanent water body; pond drying will catalyse larvae to metamorphose. Thus, ephemeral ponds (i.e. those that completely dry up each year) have only single-season larvae whereas permanent sites have both single- and multi-season larvae. Importantly, these larger multi-season larvae often produce heavy Bd loads—an order of magnitude higher than smaller single-season larvae ([14] and this study). High production of zoospores by these life stages often explains Bd prevalence better than host density [2,14,17]. Here, strong links between hydroperiod and stage structure of focal hosts might predict infection prevalence better than UVR-driven mechanisms.

We used an experiment, field observations and a partition of variation to evaluate the primary direct and indirect pathways driving infection prevalence in this system. Each pathway involves gains and losses of zoospores. An *in situ* experiment revealed that incident UVR exposure increased mortality of zoospores. Yet ponds with more UVR penetration (permanent ponds with low DOC) had higher prevalence of disease. Thus, the direct effect of UVR on zoospore mortality was overwhelmed by other factors. We explored additional direct and indirect effects and synthesized them with a regression-based partition of variation in prevalence [32].

(Small sample sizes and colinearity problems prevented a path analysis.) This partition supported the dilution pattern; host diversity alone explained 42% of the variation in disease prevalence. However, diversity was unrelated to either hydroperiod or UVR. Instead, the combined effects of parasite predators (zooplankton) and multi-season larvae—both strongly regulated by UVR and hydroperiod, respectively—explained 33.9% of the variation in infection prevalence (i.e. rivalling diversity effects). Together, these results highlight that indirect effects of habitat (and diversity) can outweigh direct environmental constraints on disease.

2. Material and methods

(a) Study system

We examined our different habitat–disease hypotheses using a field survey of amphibian communities in the Peñalara Massif (Guadarrama Mountains National Park, central Spain: 40°50′ N, 3°57′ W). Ten different species of amphibian hosts occur in these sites (see Results for frequencies of each species). However, the outcome of infection varies markedly among host species and stage [10–12,14]. Again, we focused on two native hosts, the fire salamander and the midwife toad, because these hosts drive disease in this system [12,14].

(b) Determinants of ultraviolet radiation: the environmental component of Pathways 1A–C

Pathways 1A–C start with hydroperiod but all involve penetration of UVR (UVR) into ponds (left-hand side of Pathway 1, figure 1). To characterize UVR, we pooled water samples collected from three locations in the pond at bi-weekly intervals throughout the 2011 breeding season. We filtered these samples (pre-combusted, Whatman GF/F, 0.7 μm) and estimated: (i) DOC (mg C^{-1}, Shimadzu TOC-5000 Analyzer) and (ii) the absorption coefficient, a_{d320} m^{-1} (using a spectrophotometer). DOC and a_{d320} are inversely related to UVR penetration [20,33]. We then calculated mean exposure in the water column, p, by integrating UVR penetration from surface, L_{in}, to depth (z), $L(z)$, using Lambert–Beer's law

$$p = \frac{L(z)}{L_{in}} = 1 - \frac{\exp(-kz)}{kz}, \qquad (2.1)$$

where k is the absorption coefficient (assumed to equal a_{d320} m^{-1}). This 'UVR index' essentially assesses the relative exposure experienced by a Bd zoospore suspended in the water column (based on: [34,35]). This metric strongly correlates with UVR reaching depth z, $L(z)$ (Pearson $r = 0.993$, $p < 0.0001$). We compared variation in depth, DOC and the UVR index between ephemeral and permanent sites using unpaired, two-tailed t-tests. We tested the directional hypothesis that larvae occupy deeper depths in permanent ponds with one-tailed t-tests and Welch's heteroscedasticity correction.

(c) Pathway 1A: ultraviolet radiation directly regulates parasites

(i) Experimental evidence

We used an *in situ* field experiment to examine the direct effect of natural solar radiation (UV-B, UV-A and photosynthetically active radiation (PAR) combined) on parasite survival (Pathway 1A, figure 1*a*). Specifically, we exposed parasite zoospores to ambient solar radiation in two highly transparent ponds (following [33]). We incubated zoospores (collected following [15]) on a standard growth substrate (following [36]) in quartz vials (12 replicates). Vials received either full exposure to radiation (Aclar sleeves transmit 100% of PAR (400–800 nm) and 99% of UVR (250–399 nm)) or no radiation (thick black polyethylene sleeves) (see [33]). To mimic exposure of zoospores to solar radiation, we suspended vials just below the surface for 48 h. Both ponds experienced nearly identical water temperatures and PAR levels (see the electronic supplementary material). After the incubation, we looked for differences in parasite levels (i.e. Bd zoospores) using qPCR (following [37]) (see the electronic supplementary material). From these samples, we calculated infection load (i.e. genomic equivalents of zoospores per host). We tested for an effect of incubation site with ANOVA, sequentially dropping non-significant terms [32]. Our results were qualitatively the same with and without dropping non-significant terms.

(ii) Field survey

Next, we looked for links between UVR (and hydroperiod) and disease using field patterns from natural epidemics in eight permanent and six ephemeral ponds. Data on amphibian hosts (infection prevalence, infection load, relative abundance, stage and frequency) come from a larger survey conducted throughout the breeding seasons (after ice-melt in May through September) of 2009–2012. (For ephemeral ponds, the end of the season depended on the hydroperiod of each pond.) Here, we sampled each pond at the beginning and end of the season (and some sites up to six times throughout the season for other hypotheses; Bosch *et al*. 2009–2012, unpublished data). At each visit, we collected Bd samples (from epidermal swabs and toe clip samples following [14,37], see the electronic supplementary material). We estimated the average infection prevalence (proportion infected/total number sampled) of focal hosts. We fit a linear relationship between UVR and Bd prevalence (i.e. averaged over 2009–2012) among sites using a generalized linear model (GLM) with binomial errors [38]. We assessed fit with the coefficient of discrimination, D (similar to R^2) [39].

(d) Pathway 1B: ultraviolet radiation effect on the parasite predator (zooplankton) community

To characterize zooplankton communities, we collected plankton samples bi-weekly throughout the 2011 breeding season. From each sampling date at each pond, we collected 1 l of water from three different locations in the pond and filtered the entire sample with mesh (153 μm, identified at 20–50× magnification [18]. The sample from one ephemeral site was accidentally lost. Univariate relationships involving log-transformed zooplankton were tested using correlations (to preserve normality assumptions). We examined whether community composition of zooplankton varied with UVR penetration (or hydroperiod) using constrained ordination methods [32]. We first log($X + 1$) transformed these data to homogenize variance. Then, we used the Hellinger transformation (following [40]) prior to redundancy analysis (RDA) with tests of significance using 9999 permutations (R package vegan).

(e) Pathway 1C: ultraviolet radiation effect on the composition and diversity of host communities

We estimated frequencies of each taxon in the amphibian community using abundance data from the larger multi-year survey (2009–2012; see the electronic supplementary material). We then calculated the mean inverse Simpson's diversity index (where larger numbers denote higher diversity) for each site. We tested relationships between UVR and diversity indices using correlations. We also tested for links between UVR and community composition (Hellinger distance) using the RDA described for Pathway 1B.

(f) Pathway 2: hydroperiod, stage structure of focal hosts and parasite production

We estimated differences in infection load among host stages from the larger multi-year survey (2009–2012; see the electronic supplementary material). These larval stages are easily differentiated (based on size and distinct colour patterning). Infection load data (genomic equivalents per host) were overdispersed. Therefore, we fit zero inflated negative binomial models [41] to log-transformed data (R package pscl). We tested the relationship between pond hydroperiod and the presence of multi-season larvae of focal hosts using a Fisher's exact test.

(g) Synthesis of indirect effects using variation partitioning

To identify the relative contributions of our three main indirect effects (parasite predators, host diversity and multi-season larvae), we used a partition of variation based on partial regression analysis (following [32]). The method separates fractions of variation attributable to each driver alone, independently ($a-c$), to fractions shared due to correlation among drivers ($d-g$) and the remaining fraction, unexplained variation (h). Estimates of independent and shared variation use adjusted R^2 values. Negative fractions indicate that shared partitions explain less variation than random normal variables. Hence, we depict negative fractions of variation in the accompanying Venn diagram as zero overlap [32].

3. Results

(a) Determinants of ultraviolet radiation: the environmental components of Pathways 1A–C

Permanent and ephemeral ponds differed in two key regulators of UVR: larval depth and DOC. Larval hosts in permanent ponds occupied slightly deeper depths relative to hosts in more-shallow, temporary ponds (t-test; $t = 2.05$, d.f. $= 9.69$, $p = 0.03$, $n = 14$; figure 2a). Thus, all else equal, hosts in permanent ponds should have lower UVR exposure. However, permanent sites had lower concentrations of DOC (t-test; $t = -2.57$, d.f. $= 7.18$, $p = 0.04$, $n = 14$; figure 2b). DOC correlated strongly with the absorption coefficient ($a_{d320}\,\mathrm{m}^{-1}$) used to calculate the UVR index (Pearson $r = 0.77$, $p < 0.0001$). Together, DOC and a_{d320} overwhelmed larval depth as drivers of mean UVR penetration, because permanent sites (slightly deeper but lower DOC) had higher mean penetration of UVR compared with ephemeral sites (UVR index; t-test; $t = 2.15$, d.f. $= 11.10$, $p = 0.05$, $n = 14$; figure 2c). Thus, permanent sites had higher levels of UVR exposure relative to ephemeral sites.

(b) Pathway 1A: ultraviolet radiation directly regulates parasites

The field experiment confirmed that UVR harms zoospores, yet epidemics grew larger in ponds with higher, not lower, UVR. In the experiment, exposure to solar radiation reduced zoospore levels via a main effect of solar radiation (ANOVA, radiation treatment: $F_{1,40} = 4.91$, $p = 0.03$; figure 3a) and no difference between incubation ponds (pond: $F_{1,39} = 2.82$, $p = 0.10$) or their interaction (radiation treatment × pond: $F_{1,38} = 0.55$, $p = 0.46$). These results suggest that UVR exposure could regulate Bd by directly reducing zoospore survival. However, sites with higher UVR exposure (permanent sites) had higher prevalence of infection (GLM; $\chi^2 = 39.12$, d.f. $= 1$, $p < 0.001$,
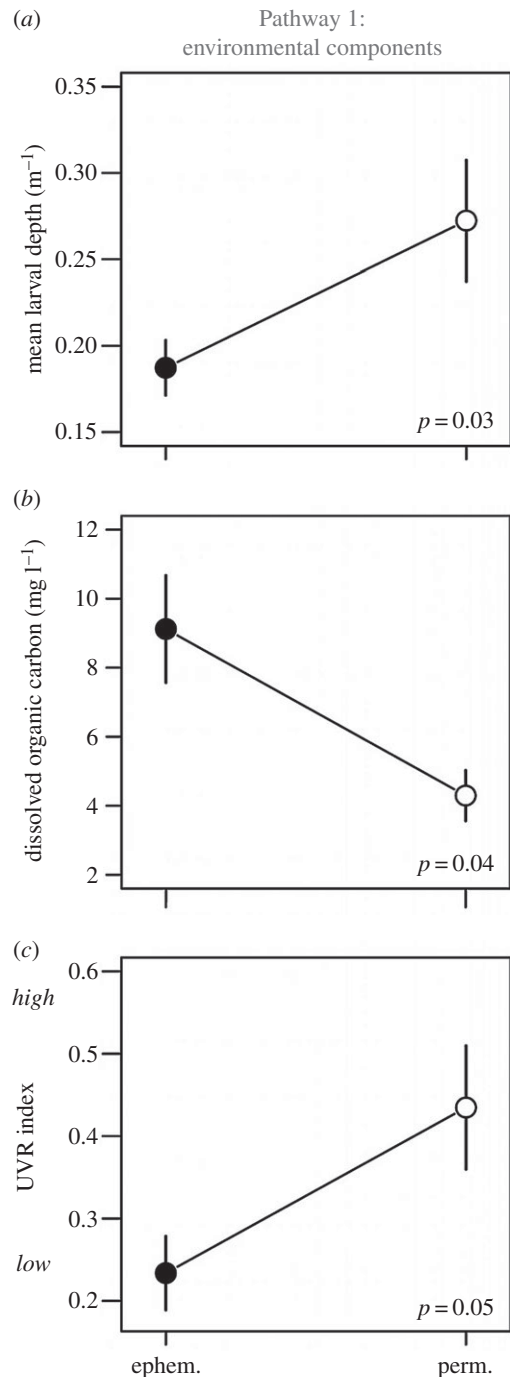

Figure 2. Environmental links to ultraviolet radiation (UVR) in alpine ponds—*Pathway 1*: (a) all else equal, permanent (perm.) sites were deeper than ephemeral (ephem.) ones. (b) However, permanent sites had less dissolved organic carbon (DOC). (c) Thus, UVR exposure was higher in deeper, permanent sites (a larger 'UVR index' indicates higher mean UVR penetration, equation (2.1)). Data are means ± bootstrapped s.e.

$D = 0.357$; figure 3b,c). These field patterns contradict the direct effects suggested by the experimental result and suggest that other factors must overwhelm the direct effects of UVR on parasite survival.

(c) Pathway 1B: ultraviolet radiation effect on the parasite predator (zooplankton) community

The UVR–zooplankton–disease link of Pathway 1B was supported. As predicted, sites with higher UVR had lower densities of these parasite predators (Pearson $r = 0.611$, $p = 0.026$;
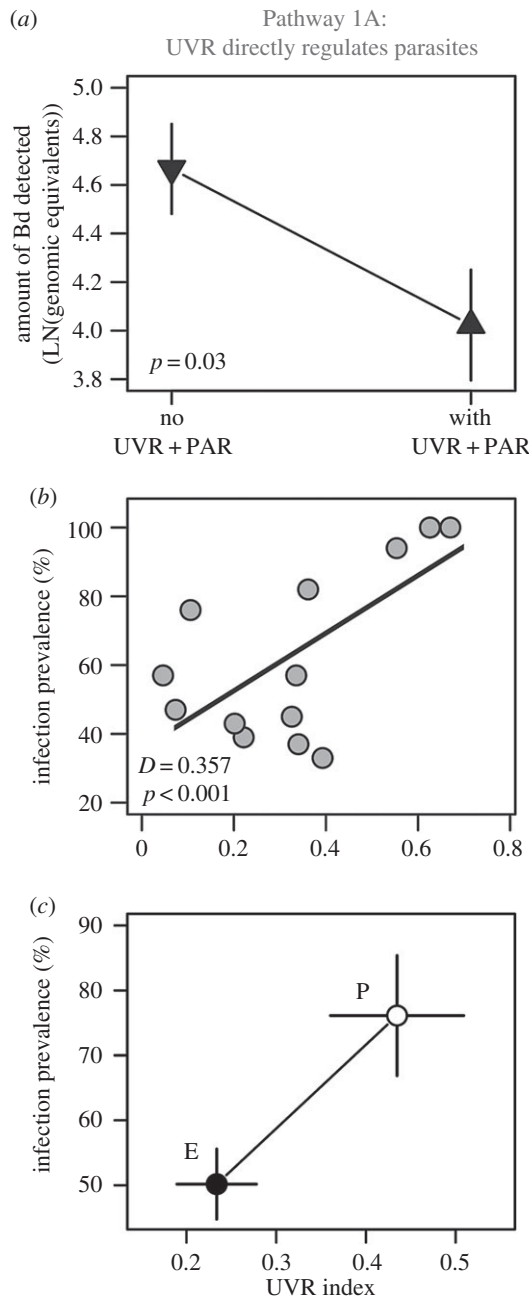
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Figure 3. *Pathway 1A, UVR directly regulates parasites: (a) in situ, exposure to solar radiation (UVR + PAR) reduced survival of zoospores. However, (b) sites with higher UVR had more disease. (c) Permanent sites have higher UVR exposure and prevalence (E: ephemeral; P: permanent). Data are means ± bootstrapped s.e.*

figure 4a). Sites with fewer zooplankton, then, had higher infection prevalence (GLM, $\chi^2 = 13.45$, d.f. = 1, $p < 0.001$, $D = 0.117$, figure 4d). Zooplankton density, not composition, drove these effects. The community composition of zooplankton was fairly homogeneous across focal ponds. *Ceriodaphnia* spp. (mean frequency: 45%) and copepods (34%) dominated zooplankton communities. Larger *Daphnia* spp. appeared in only two sites. Composition did not vary with UVR (RDA: $F_{1,11} = 1.65$, $p = 0.16$). Hence, higher UVR could depress the density of these parasite predators, thereby increasing disease.

(d) Pathway 1C: ultraviolet radiation effect on the composition and diversity of host communities

Only part of the UVR–host diversity–disease pathway (1C) was supported. UVR was not related to host composition.

Fire salamanders dominated host communities (mean frequency: 56%; maximum: 100%). The second focal host, the midwife toad (mean: 2%; max: 33%) was rarer. The introduced alpine newt, *Ichthyosaura alpestris*, was the second most common host (mean: 23%; max: 94%). All 'other' taxa were considerably less common (see the electronic supplementary material). However, overall host composition did not vary along the UVR gradient (RDA: $F_{1,12} = 1.42$, $p = 0.21$). Not surprisingly then, no strong relationships arose between the UVR index and overall host diversity (Pearson $r = 0.216$, $p = 0.458$; figure 4b), the frequency of focal hosts ($r = 0.391$, $p = 0.167$; figure 4c) or frequency of the second most abundant taxa, the introduced alpine newt (see the electronic supplementary material, figure S1a; $r = -0.419$, $p = 0.136$).

However, strong host composition–disease links did emerge (in the second part of Pathway 1C). Consistent with the dilution effect, sites with high host diversity had lower infection prevalence (GLM, $\chi^2 = 27.19$, d.f. = 1, $p < 0.001$, $D = 0.265$; figure 4e). This diversity–disease pattern probably arose because higher diversity of hosts reflects lower frequencies of our focal hosts ($r = -0.847$, $p = 0.0001$; electronic supplementary material, figure S1c). Indeed, sites dominated by our focal hosts had higher infection prevalence (GLM, $\chi^2 = 28.34$, d.f. = 1, $p < 0.001$, $D = 0.269$; figure 4f) whereas, sites dominated by the introduced alpine newt had lower infection prevalence (GLM, $\chi^2 = 9.45$, d.f. = 1, $p = 0.002$, $D = 0.083$; electronic supplementary material, figure S1b). Thus, we found evidence for potential dilution-like effects (but no amplification effects) unrelated to UVR.

(e) Pathway 2: hydroperiod, stage structure of focal hosts and parasite production

Habitat structure, however, did connect with disease through multi-season larvae. Larger, multi-season larvae produced higher levels of Bd zoospores than conspecific single-season larvae (planned contrasts: $p < 0.001$; figure 5a) or multi-season larvae of newts and 'other' hosts (both $p < 0.001$). Within focal hosts, multi-season larvae of rarer midwife toads produced more zoospores than single-season conspecific larvae or any stage of salamanders (p-values < 0.001; figure 5b). Similarly, for salamanders, multi-season larvae supported higher infection loads than their single-season counterparts ($p = 0.019$). Multi-season larvae of our focal hosts were found in all eight permanent ponds but in none of the six ephemeral ponds (which is very unlikely by chance alone: Fisher's exact test: $p = 0.0003$; figure 5c). Thus, the presence of multi-season larvae partially explains why permanent sites have higher infection prevalence, despite having more damaging UVR penetration (t-test; $t = 2.27$, d.f. = 10.98, $p = 0.04$, $n = 14$; figure 2c).

(f) Synthesis of indirect effects using variation partitioning

The variation partition emphasizes a strong effect of diversity on disease, but it also indicates important, joint effects of parasite predators and multi-stage larvae (figure 6). Infection prevalence was well predicted by multiple linear regression with parasite predators (zooplankton), host diversity and multi-season larvae. Together, all factors explained 64% ($R^2_{\mathrm{adjusted}} = 0.639$; figure 6) of the variation in infection

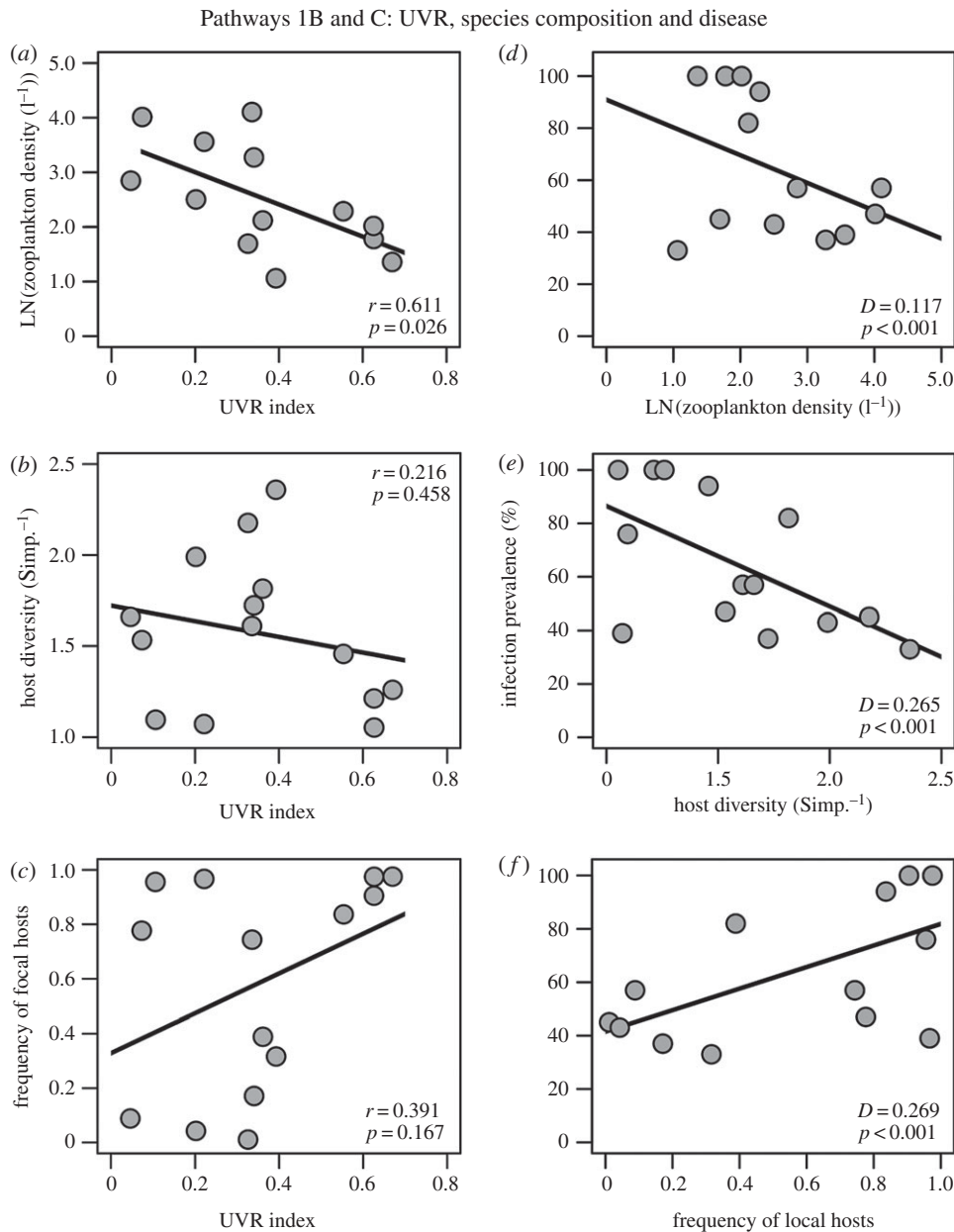Pathways 1B and C: UVR, species composition and disease

Figure 4. Connections between habitat and disease via parasite predators (zooplankton; *Pathway 1B*) and host communities (*Pathway 1C*). (*a*–*c*) *Habitat–composition links*: (*a*) sites with higher UVR index (i.e. higher mean levels of UVR) had lower density of zooplankton. There was no relationship between UVR and (*b*) overall host diversity or (*c*) the frequency of our focal hosts. (*d*–*f*) *Composition–disease links*: infection prevalence was higher in ponds with (*d*) lower zooplankton density, (*e*) lower host diversity and (*f*) higher frequency of focal hosts.

prevalence. These indirect effects together overwhelmed the direct damaging effects of UVR on parasite survival. Independently neither zooplankton (fraction *a*, 1.6% of variation) nor multi-season larvae (*c*, 4.1%) explained much variation in prevalence. However, together they explained considerably more (*f*, 28.2%), reaching 33.9% of variation in prevalence overall (*a* + *c* + *f*)—rivalling that explained by host diversity alone (*b*, 42.4%). Additionally, host diversity and multi-season larvae jointly explained even more variation (*e*, 9.74%), despite being uncorrelated themselves. Together, host diversity and multi-season larvae uniquely explained much variation in prevalence (*b* + *c* + *e*, 56.2%). When accounting for the full partition of variation, we found negative variation explained by diversity and zooplankton together (*d*, −8.75%) and the joint, three-way intersection (*g*, −13.33%). Again, these negative fractions seem non-sensical, but they indicate that these shared

partitions explain less variation than random normal variables. Hence, these negative fractions are drawn as regions with zero overlap (figure 6; [32]). The essential point here, together, predators of parasites and host stage structure, linked together via UVR and hydroperiod, explain a similar amount of variation in prevalence as host diversity alone. For completeness, we replaced host diversity with the frequency of focal hosts or of introduced newts; each analysis yielded similar results (see the electronic supplementary material, table S1).

4. Discussion

We examined whether variation in a key habitat characteristic (hydroperiod) could explain differences in infection prevalence of Bd across natural populations. We tracked factors
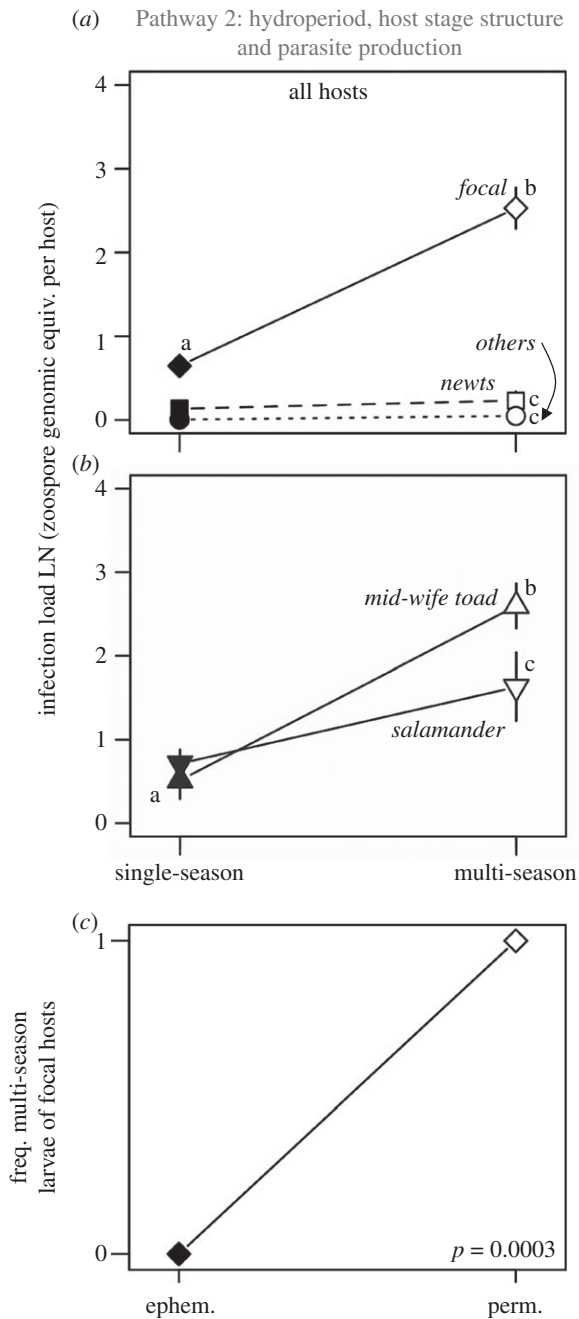
(a) Pathway 2: hydroperiod, host stage structure and parasite production

Figure 5. Linking habitat, host stage structure and disease (*Pathway 2*). (*a,b*) Infection loads from host stages. (*a*) Infection loads were approximately an order of magnitude higher in multi-season larvae of focal hosts (triangles) than in their single-season counterparts, newts (squares) or the 'other' host species (circles). (*b*) Infection loads were higher in rarer midwife toads than in more dominant salamander hosts. Different letters indicate significant differences in planned *a priori* contrasts. (*c*) Multi-season larvae of the focal hosts lived in all permanent but no ephemeral sites. Data are means ± bootstrapped s.e.

governing gains and losses of parasite zoospores through two main pathways, all originating with hydroperiod. One suite of habitat-based pathways (Pathway 1A–C) started proximately with variation in penetration of UVR (UVR) into pond water. An *in situ* experiment revealed that incident UVR exposure killed the infectious stage of the parasite (Pathway 1A). In the field, however, sites with higher UVR exposure had higher infection prevalence; thus, any direct effects of UVR on zoospores must become overwhelmed by other factors. Indeed, other direct and indirect pathways better predicted prevalence.

Permanent, high-UVR sites had lower density of predators of zoospores (zooplankton, Pathway 1B) and harboured multi-season larvae that fuelled disease (Pathway 2). Host diversity was unlinked to hydroperiod or UVR (Pathway 1C). Nonetheless, sites with higher diversity of hosts (hence, lower frequencies of focal hosts) had lower prevalence of infection. Thus, while habitat structure explained considerable variation in infection prevalence via pathways involving zooplankton and multi-season larvae, it could not explain everything. Clearly, a multi-pathway approach was needed here: focus on any one pathway alone would have prompted incorrect, incomplete or potentially misleading conclusions. Armed with additional data, path analysis might further delineate the correlated pathways that modulate disease in this and other systems [42,43]. In the meantime, these present results demonstrate the importance of creating mechanistic, food web-based links between multiple habitat dimensions and disease [7–9].

Infection reached higher prevalence in ponds with more UVR, despite that UVR reduced survival of the free-living stage of the parasite (i.e. Bd zoospores) by approximately 50%. Additionally, UVR potently regulates a wide-array of terrestrial (reviewed in [44]) and aquatic pathogens (see [33] and citations therein]. Could these contrasting results arise because UVR increased host susceptibility (as sometimes seen in other systems [45,46])? More detailed experiments that account for both negative and beneficial effects of UVR (e.g. UV-A used for photorepair [47]) across a wide range of host species are needed to address this question. Currently, the only study to address this question (to our knowledge) indicates that natural UV-B exposure increased survival of Bd infected toads [13]. Further, in other alpine systems amphibians exhibit behavioural and physiological responses that, combined with natural DOC 'sunscreen', drastically reduce the deleterious effects of UVR [48,49]. Together, these results (though admittedly limited) do not suggest that UVR exposure increased host susceptibility. Instead, our results indicate that the net effect of UVR on disease depends on both direct and indirect effects mediated through community ecology [8,33].

Variation in UVR penetration indirectly influenced disease prevalence by constraining predators that consume parasites. Sites with higher UVR had lower zooplankton densities and higher infection prevalence. Lower density of zooplankton matters because they can consume Bd zoospores; therefore, these parasite predators potentially reduce disease risk for hosts [15,24,50]. The field patterns suggest that smaller plankton (e.g. *Ceriodaphnia* and copepods) that dominated these alpine ponds may act as important predators. Bd zoospores (3–5 μm; [16]) fall within the size range of food particles eaten by these plankton [51,52]; yet, confirmation with experiments (as done with *Daphnia*) remains important. Nonetheless, this study contributes more broadly to growing evidence that predators play a key role in regulating disease by consuming parasites (reviewed in [23]). This potential has sparked discussions of using predators of parasites such as zooplankton as 'biocontrols'. However, any intentional introduction of predators could be undermined by environmental (e.g. UV) or food web constraints [9]. Here, for example, introducing zooplankton in these alpine sites could be undermined by strong UVR constraints. Such environmental constraints and food web effects associated with predators of parasites should be taken into account in disease management plans attempting to use them [9,23,53].
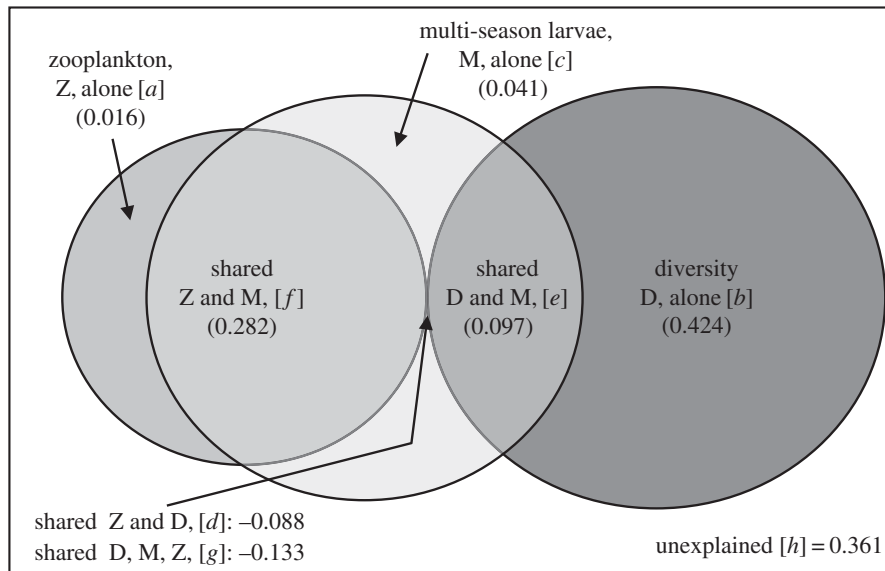
Figure 6. Variation partitioning of infection prevalence of Bd across 14 alpine ponds (*Pathways 1* and *2*). The rectangle represents total variation in prevalence (100%). Together, parasite predators (zooplankton, Z), multi-season larvae, MSL (M), and host diversity (D) explained (64%, i.e. $R^2_{adjusted} = 0.639$) of the variation (filled in circles, accounting for negative variation). Fraction *h*, 36.1%, is unexplained variation (white). See the text for details. The full partition includes negative variation explained by diversity and zooplankton together [*d*, −4.7%] and the joint, three-way intersection [*g*, −17.38%] (see the text). Regions of negative variation depicted as zero overlap.

Hydroperiod also influenced epidemic size because permanent ponds supported multi-season larvae, key producers of parasite propagules. More specifically, multi-season larvae of the focal hosts—not the introduced newt or 'other' hosts—harboured high infection loads that drove disease. In a comparable amphibian system in California, multi-season larvae with high infection loads also serve as intraspecific reservoirs that maintain Bd infections [2]. Furthermore, this result adds to mounting evidence that stage structure of hosts matters for disease more broadly [54–57]. Here, as in other systems, larger hosts produce more parasites, which can increase disease [58–60]. Thus, stage-specific differences in key epidemiological traits could inform management strategies in various host–parasite systems. For example, across many sites, Bd has reached an endemic state. Thanks to captive breeding programmes, host re-introduction plans now become feasible. However, reintroduction of certain hosts with extended larval stages that produce large numbers of parasites could undermine post-epidemic reintroduction efforts. Thus, management plans that ignore stage structure could catalyse re-emerging epidemics.

The composition of host communities was linked to lower infection prevalence (potentially through various mechanisms discussed below). UVR did not shape host composition, as seen in other alpine-amphibian communities [48]. Perhaps, other unmeasured habitat characteristics structure the host communities here. Regardless, sites with higher host diversity had lower infection prevalence. This diversity–disease link could arise through a potential dilution effect whereby highly competent and abundant species (our focal host species) become less common in more diverse amphibian communities [30]. Future studies combining experiments and field surveys (with more accurate density estimates of hosts) will help pinpoint the key species and their epidemiological traits that regulate Bd via dilution. That information would enable a more mechanistic valuation of dilution in this host–parasite system [61,62].

5. Conclusion

Habitat-mediated indirect effects joined host diversity to shape infection prevalence via losses and gains of parasites. Solar radiation reduced parasite survival by approximately 50%. Despite these direct effects, permanent, high-UVR sites experienced a net gain of parasites probably via reduction of UV-sensitive predators and high parasite production from multi-season larvae. Therefore, indirect pathways created double jeopardy for hosts in permanent ponds with higher UVR. Host diversity may sometimes counter these gains of parasites: more diverse sites had lower infection prevalence. However, diversity was unconnected to UVR penetration. Thus, while host diversity may regulate Bd (as seen in [61,62]), it could not explain why Bd became more prevalent in permanent ponds having higher UVR penetration. More broadly, this work highlights the need for more integrative links between habitat variation (e.g. UVR) and disease.

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