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

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ABSTRACT

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: (1) UVR reduced the density of parasite predators, and (2) permanent sites fostered multi-season host larvae that fueled 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.

Key Words: Chytrid, habitat, UV, zooplankton, diversity, stage structure

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) [1-8]. Why? It remains challenging to answer this question because multiple correlated pathways drive disease [9-11]. Furthermore, these pathways may have contrasting effects, as some factors enhance disease while others diminish it. Thus, disease dynamics reflect tension between multiple driving factors 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 and across the ten different species of amphibian hosts inhabiting them [12-14]. Currently, however, the factors driving this pronounced variation in infection prevalence among sites 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*)[14-16]. Both species act as key drivers of 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 [14, 16, 17]. Zoospores are free-swimming propagules, which attach to and then replicate on the epidermis of amphibian hosts [18]. Infected hosts release new zoospores, which then infect other hosts. Hence, Bd dynamics depend sensitively on zoospore survival [19].

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; Fig. 1, Pathways 1A-C). In these mountainous regions, variation in UVR exposure starts with differences in underlying geology (e.g., bedrock, hydrology [20]) that governs pond depth and hydroperiod (permanent vs. ephemeral). Hydroperiod largely determines the type of habitat and vegetation surrounding ponds (e.g., moss in bogs vs. grass in knolls). These characteristics then 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 [21, 22]. Hence, hosts and parasites in different ponds experience dramatically different UVR exposures. Based on previous evidence [15, 23], 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; Fig. 1). First, UVR could constrain predators that consume infectious stages of parasites (Pathway 1B) [24, 25]. Zooplankton eat Bd zoospores [17, 26-28] and respond sensitively to UVR— especially in alpine habitats [reviewed by 21]. Therefore, high-UVR ponds could support fewer zooplankton that consume Bd zoospores. If zooplankton respond more sensitively to UVR than zoospores themselves, this indirect release from predation could overwhelm the direct mortality effect of UVR on zoospores (Pathway 1B, Fig 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, Fig 1). Here, habitat-diversity links could arise if hosts selectively oviposit based

on UVR exposure and/or other species [29-31]. In turn, selective oviposition (which determines the diversity of larval hosts found in a given pond) could drive variation in disease because hosts vary in disease competency [14, 16]. 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 [32]. Alternatively, an amplification effect could arise if higher diversity reflects higher frequencies of more competent (non-focal) hosts [33].

The second main pathway directly links variation in hydroperiod, stage structure of focal hosts, and parasite (zoospore) production (Pathway 2, Fig. 1). Here, hydroperiod could influence the distribution of key host stages that influence disease. Many amphibian species, including our two focal hosts, can have multi-season larvae. These multi-season larvae can delay metamorphosis. However, delayed metamorphosis requires a permanent water body since pond drying will catalyze larvae (which require ample water for respiration) to metamorphose. Importantly, these multi-season larvae often produce heavy Bd loads — an order of magnitude higher than single-season larvae [16, this study]. High production of zoospores by these life stages often explains Bd prevalence better than host density [2, 16, 19]. Here, strong links between hydroperiod and stage structure of focal hosts might predict infection prevalence better than any of the UVR-driven mechanisms.

We used an experiment, field observations, and a partition of variation based on partial regression analysis to evaluate the primary direct and indirect pathways driving infection prevalence in this system. All of these pathways involve 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. These results suggest that the direct effect of UVR on mortality was overwhelmed by

other factors. We explored additional direct and indirect effects with bivariate analyses and then synthesized them with a regression-based partition of variation in prevalence [34]. (Small sample sizes and co-linearity 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, hence it could not explain why disease was higher in permanent ponds with more 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., rivaling diversity effects). Together, these results highlight that indirect effects of habitat (and diversity) can outweigh direct environmental constraints on disease.

MATERIALS AND METHODS

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: 40º50'N, 3º57'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 [12-14, 16]. Again, we focused on two native hosts, the fire salamander and the midwife toad, because these hosts act as key drivers of disease in this system [14, 16]. All samples were collected on site and no animals were harmed during this study. Indiana University Animal Care and Use Committees and Consejería de Medio Ambiente de la Comunidad de Madrid approved sampling protocols and provided permits.

Determinants of UVR: The environmental component of Pathways 1A-C

Pathways 1A-C start with hydroperiod but all involve variation in penetration of ultraviolet radiation (UVR) into ponds (left hand side of Pathway 1, Fig. 1). To characterize UVR, we pooled water samples from three different locations in the pond bi-weekly throughout the 2011 breeding season. We filtered these samples (pre-combusted, Whatman GF/F, 0.7 μm) and estimated: (i) dissolved organic carbon (DOC; mg C^{-L}, using a Shimadzu TOC-5000 total Organic Carbon Analyzer) and (ii) the absorption coefficient, a_{d320} m^{-1} (using a spectrophotometer). DOC and a_{d320} are generally inversely related to UVR penetration [22, 35]. We then calculated a 'UVR index', which combines mean depth of habitat used by larvae, z (measured at 2-15 locations, depending on pond size) and a_{d320} (m^{-1}). We estimated the 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:

$$p = \frac{L(z)}{L_{in}} = 1 - \frac{\exp(-kz)}{kz} \qquad \text{(eq. 1)}$$

where k is the absorption coefficient (assumed here to equal a_{d320}). This UVR index essentially assesses the relative exposure experienced by a Bd zoospore suspended in the water column [based on: 36, 37]. 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.

Pathway 1A: UVR Directly Regulates Parasites

Experimental Evidence

149 We used an *in-situ* field experiment to examine the direct effect of natural solar radiation
150 (UV-B, UV-A, and photosynthetically active radiation [PAR] combined) on parasite survival
151 (Pathway 1A, Fig. 1A). Specifically, we exposed parasite zoospores to ambient solar radiation in
152 two highly transparent ponds [following 35]. We incubated zoospores [collected following 17]
153 on a standard growth substrate [following 38] in quartz vials (12 replicates per treatment). Vials
154 received either full exposure to radiation (Aclar sleeves, which transmits 100% of PAR [400-800
155 nm] and 99% of UVR [250-399 nm]) or no radiation (thick black polyethylene sleeves) [see 35].
156 To mimic exposure of zoospores to solar radiation, we suspended vials just below the surface for
157 48 hours. Both ponds experienced nearly identical water temperatures and PAR levels (see
158 supplementary material). At the end of the incubation, we looked for differences in parasite
159 levels (i.e., Bd zoospores) using qPCR [following 39]. We ran each sample in duplicate against
160 replicated standards of 0.1, 1, 10 and 100 genomic equivalents (GE) of zoospores and two
161 negative controls. We considered hosts infected if both duplicates amplified with a mean
162 genomic equivalent ≥ 0.1. From these samples, we calculated infection load (i.e., genomic
163 equivalents of zoospores per host). We tested for an effect of incubation site with ANOVA,
164 sequentially dropping non-significant terms [40]. Our results were qualitatively the same with
165 and without dropping non-significant terms.
166
167 *Field Survey*
168 Next, we looked for links between UVR (and hydroperiod) and disease using field patterns
169 from natural epidemics in eight permanent and six ephemeral ponds. Data on amphibian hosts
170 (infection prevalence, infection load, relative abundance, and frequency) come from a larger
171 survey conducted throughout the breeding seasons (after ice-melt in May through September) of

2009 – 2012. At each pond, we collected Bd samples (from epidermal swabs and tissue samples) at approximately the beginning and end of the season. (For ephemeral ponds, the end of the season depended on the hydroperiod of each pond). We estimated the average infection prevalence (proportion infected/total number sampled) from these samples of focal hosts. For each sample, we also recorded host species and stage to compare differences in mean infection load. 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 [40]. We assessed GLM model fit with the coefficient of discrimination, D (similar to an R^2 for logistic regression) [41].

Pathway 1B: UVR 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 1L of water from three different locations in the pond and then filtered the entire sample with mesh (153 μm). We preserved zooplankton samples with 70% ethanol for subsequent identification using a dissecting scope at 20 – 50X magnification [20]. The zooplankton sample from one ephemeral site was accidentally lost. Univariate relationships involving log-transformed zooplankton were tested using correlations (where the log-scale preserves normality assumptions). We examined whether community composition of zooplankton varied with UVR penetration (or hydroperiod) using constrained ordination methods [34]. We first log(X +1) transformed these data to help homogenize the variance. Then, we used the Hellinger distance transformation [following 42] prior to a redundancy analysis using 9,999 permutations to test for significance of the relationship (RDA; R package vegan).

Pathway 1C: UVR 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). To account for differences in host richness and relative abundance among sites, we 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 (index by Hellinger distance) using the RDA described for Pathway 1B.

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). These larval stages are easily differentiated (based on size and distinct color patterning). Infection load data (genomic equivalents per host) were overdispersed. Therefore, we fit zero inflated negative binomial models [43] to log transformed data (R package pscl). We tested the relationship between pond hydroperiod and presence of multi-season larvae of focal hosts using a Fisher's exact test.

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 [44]. The method separates fractions of variation attributable to each driver alone, independently (*a*-*c*), or to fractions shared due to correlation among drivers (*d*-*g*). The remaining fraction, the left-over variation unexplained (*h*), is also calculated. Estimates of independent and shared variation use adjusted R^2 values, which provide unbiased estimates [45].

Negative fractions indicate that shared partitions explain less variation than random normal variables [34]. Hence, we depict negative fractions of variation in the accompanying Venn diagram as zero overlap.

RESULTS

Determinants of UVR: The environmental components of Pathways 1A-C

Permanent and ephemeral ponds differed in two key factors that regulate exposure of aquatic organisms to UVR: larval depth and dissolved organic carbon (DOC). Larval hosts in permanent ponds occupied slightly deeper depths relative to hosts in more-shallow, temporary ponds (*t*-test; $t = 2.05$, df $= 9.69$, $p = 0.03$, n = 14, Fig. 2*a*). Thus, all else equal, hosts in permanent ponds should have lower UVR exposure. However, permanent sites had lower concentrations of DOC (*t*-test; $t = -2.57$, df $= 7.18$, $p = 0.04$, n = 14, Fig. 2*b*). DOC correlated strongly with the absorption coefficient (a_{d320} 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, since permanent sites (*slightly* deeper but lower DOC) had higher mean penetration of UVR compared to ephemeral sites (UVR index; *t*-test; $t = 2.15$, df $= 11.10$, $p = 0.05$, n = 14, Fig. 2*c*). Thus, higher levels of UVR penetrated into the water column in permanent relative to ephemeral sites.

Pathway 1A: UVR Directly Regulates Parasites

The field experiment confirmed that UVR harms zoospores, but epidemics grew larger in ponds with more, not less, UVR. In the field experiment, exposure to solar radiation significantly reduced zoospore levels. There was a main effect of solar radiation (ANOVA, radiation

241 treatment: $F_{1,40} = 4.91$, $p = 0.03$, Fig. 3*a*) but no difference between incubation ponds (pond: $F_{1,39}$
242 $= 2.82$, $p = 0.10$) or their interaction (radiation treatment x pond: $F_{1,38} = 0.55$, $p = 0.46$). These
243 experimental results support the hypothesis that UVR exposure could regulate Bd by directly
244 reducing parasite (zoospore) survival. Yet, sites with higher UVR exposure (permanent sites) had
245 higher — not lower — prevalence of infection (GLM: $\chi^2 = 39.12$, df = 1, $p < 0.001$, D = 0.357,
246 Fig. 3*b-c*). These field patterns contradict the experimental results that UVR directly regulates
247 parasites via mortality on zoospores. Instead, other factors might overwhelm the direct effects of
248 UVR on parasite survival.
249
250 **Pathway 1B: UVR Effect on the Parasite Predator (Zooplankton) Community**
251 The UVR-zooplankton-disease link of Pathway 1B was supported. As predicted, sites with
252 higher UVR had lower densities of these parasite predators (Pearson r = 0.611, p = 0.026, Fig.
253 4*a*). Sites with fewer zooplankton, then, had higher infection prevalence (GLM, $\chi^2 = 13.45$, df =
254 1, $p < 0.001$, D = 0.117, Fig. 4*d*). Zooplankton density, not zooplankton composition, drove
255 these effects. The community composition of zooplankton was fairly homogenous across focal
256 ponds. *Ceriodaphnia* spp. (mean frequency: 45%) and copepods (mean: 34%) dominated
257 zooplankton communities. Larger *Daphnia* spp. were present in only two sites. Composition did
258 not vary with UVR (RDA: $F_{1,11} = 1.65$, p = 0.16). Hence, the zooplankton effect involved
259 depression of density of these parasite predators with higher UVR.
260
261 **Pathway 1C: UVR Effect on the Composition and Diversity of Host Communities**
262 Only part of the UVR-host diversity-disease pathway (1C) was supported. UVR was not
263 related to host composition. Fire salamanders dominated host communities (mean frequency:

56%; maximum frequency: 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: the Iberian green frog, *Pelophylax perezi* (mean: 5%; max: 49%); the treefrog, *Hyla molleri* (mean: 5%; max: 60%); the Iberian frog, *Rana iberica* (mean: 5%; max: 87%); the native newt, *Triturus marmoratus* (mean: 2%; max: 17%), and the European toad, *Bufo spinosus* (mean: 0.04%; max: 6%). Hence, UVR *could* account for variation in community composition among ponds. 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, Fig. 4*b*), the frequency of focal hosts (r = 0.391, p = 0.167, Fig. 4*c*), or frequency of the second most abundant taxa, the introduced alpine newt (see electronic supplementary material; r = -0.419, p = 0.136, Fig. S1*a*).

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, $\chi^2 = 27.19$, df = 1, $p < 0.001$, D = 0.265, Fig. 4*e*). This diversity-disease pattern likely arose because higher diversity of host reflects lower frequencies of our focal hosts (r = -0.847, p = 0.0001, supplementary material Fig. S1*c*). Indeed, sites dominated by our focal hosts had higher infection prevalence (GLM, $\chi^2 = 28.34$, df = 1, $p < 0.001$, D = 0.269, Fig. 4*f*). Whereas, sites dominated by the introduced alpine newt had lower infection prevalence (GLM, $\chi^2 = 9.45$, df = 1, $p = 0.002$, D = 0.083, electronic supplementary material, Fig. S1*b*). Thus, we found evidence for potential dilution-like effects (but no amplification effects) unrelated to UVR.

Pathway 2: Hydroperiod, Stage Structure of Focal Hosts, and Parasite Production

287 Habitat structure, however, did connect with disease through multi-season larvae. Larger,
288 multi-season larvae produced higher levels of Bd zoospores than conspecific single-season
289 larvae (planned contrasts: $p < 0.001$; Fig. 5*a*) or multi-season larvae of newts and 'other' hosts
290 (both $p < 0.001$). Within focal hosts, multi-season larvae of rarer mid-wife toads produced more
291 zoospores than single season conspecific larvae or any stage of salamanders (p values < 0.001;
292 Fig. 5b). Similarly, for salamanders, multi-season larvae supported higher infection loads than
293 their single-season counterparts ($p = 0.019$). Multi-season larvae of our focal hosts were found in
294 all eight permanent ponds but in none of the six ephemeral ponds (which is very unlikely by
295 chance alone: Fisher's exact test: $p = 0.0003$; Fig. 5*c*). Thus, multi-season partially explain why
296 permanent sites have higher infection prevalence (*t*-test; $t = 2.27$, df $= 10.98$, $p = 0.04$, n = 14,
297 Fig. 5*d*), despite having more damaging UVR penetration (Fig. 2*c*).
298
299 **Synthesis of Indirect Effects Using Variation Partitioning**
300 The variation partition emphasizes a strong effect of diversity on disease, but it also indicates
301 important, joint effects of parasite predators and multi-stage larvae (Fig. 6). Infection prevalence
302 was well predicted by multiple linear regression with parasite predators (zooplankton), host
303 diversity, and multi-season larvae. Together, all factors explained 64% ($R^2_{adjusted} = 0.639$; Fig. 6)
304 of the variation in infection prevalence across these sites. These indirect effects together
305 overwhelmed the direct damaging effects of UVR on parasite survival. Independently neither
306 zooplankton [fraction *a*, 1.6% of variation] nor multi-season larvae [*c*, 4.1%] explained much
307 variation in prevalence. However, together these correlated drivers explained considerably more
308 [*f*, 28.2%]. Overall, they explained 33.9% of variation in prevalence [$a + c + f$] — rivaling that
309 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 nonsensical, but they indicate that these shared partitions explain less variation than random normal variables. Hence, these negative fractions are drawn graphically in the Venn diagram as regions with zero overlap [Fig. 6; 34]. 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 repeated the analysis replacing host diversity with the frequency of focal hosts or the frequency of introduced newts, the second most common taxa; each additional analysis yielded similar results (see Table S1, electronic supplementary material).

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 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 ultraviolet radiation (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 harbored multi-season larval that fueled disease (Pathway 2). Host diversity was unlinked to hydroperiod or UVR (Pathway 1C). Nonetheless, sites with higher diversity of hosts (and thus, 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 among the correlated pathways that modulate disease in this and other systems [46, 47]. In the meantime, these present results demonstrate the importance of creating mechanistic, food web based links between multiple habitat dimensions and disease [9-11].

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 by 48] and aquatic pathogens [see 35 and citations therein]. Could these contrasting results arise because UVR increased host susceptibility (as sometimes seen in other systems [49, 50])? More detailed experiments that account for both negative and beneficial effects of UVR (e.g., UV-A used for photorepair [51]) 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 behavioral and physiological responses that, combined with natural DOC 'sunscreen', drastically reduce the deleterious effects of UVR [52, 53]. 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 [10, 35].

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 [17, 26, 54]. The field patterns here suggest that smaller plankton (e.g., *Ceriodaphnia* and copepods) that dominated these alpine ponds may act as important predators. Bd zoospores [3–5 μ m; 18] fall well within the size range of food particles eaten by these plankton [55, 56]; 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 by 57]. This potential has sparked discussion about 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 [11]. 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 [11, 57, 58].

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 — harbored high infection loads that drove disease. In a comparable amphibian system in California, multi-season larvae with high infection

379 loads also serve as intraspecific reservoirs that maintain Bd infections [2]. Furthermore, this
380 result adds to mounting evidence that stage structure of hosts matters for disease more broadly
381 [59-62]. Here, as in other systems, larger hosts produce more parasites, which can increase
382 disease [63-65]. Thus, stage-specific differences in key epidemiological traits could inform
383 management strategies in various host-parasite systems. For example, across many sites, Bd has
384 reached an endemic state. Thanks to successful captive breeding programs, host re-introduction
385 plans now become feasible. The results here caution that the reintroduction of certain hosts with
386 extended larval stages could undermine post-epidemic reintroduction efforts if they produce
387 large numbers of parasites. Thus, management plans that do not consider the effects of host
388 stage-structure could catalyze reemerging epidemics.
389 The composition of host communities was linked to lower infection prevalence (potentially
390 through various mechanisms discussed below). Somewhat surprisingly, UVR did not shaped host
391 composition, as seen in other alpine-amphibian communities [52]. Perhaps other unmeasured
392 habitat characteristic structure the host communities focused on here. Regardless, sites with
393 higher host diversity had lower infection prevalence. This diversity-disease link could arise
394 through a potential dilution effect whereby highly competent and abundant species (our focal
395 host species) become less common in more diverse amphibian communities [32]. Future studies
396 combining experiments and field surveys (with more accurate density estimates of host species)
397 will help pinpoint the key species and their epidemiological traits that regulate Bd via dilution.
398 That information would enable a more mechanistic valuation of dilution in this host-parasite
399 system [66, 67].
400
401 **CONCLUSIONS**

Habitat-mediated indirect effects joined host diversity to shape infection prevalence via losses and gains of parasites. UVR reduced parasite survival by ~50%. Despite these direct effects, permanent, high UVR sites likely experienced net gains of parasites due to the 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 66, 67], it could not explain why Bd became more prevalent in permanent ponds having higher UVR penetration. More broadly, this work highlights the need for a more integrative approach to linking habitat variation (e.g., UVR) to disease.

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624 **FIGURE LEGENDS**

625 **Figure 1.** Hypothesized pathways connecting habitat to infection prevalence of Bd in
626 communities of amphibian hosts. Hydroperiod (ephemeral vs. permanent) is the ultimate driver
627 of disease in this alpine system. However, it influences disease via two pathways that modulate
628 gains and losses of parasite propagules (zoospores). *Pathway 1A-C*: Permanent ponds are deeper,
629 but have less dissolved organic carbon (DOC) and therefore higher exposure to damaging
630 ultraviolet radiation (UVR). UVR could directly damage zoospores (bottom, pathway 1A),
631 reduce zooplankton predators of zoospores (1B), or alter host composition (top, 1C). Dilution (—
632) or amplification (+) effects could arise from UVR-mediated changes in host community
633 composition. *Pathway 2*: Permanent ponds harbor multi-season larvae that produce high
634 densities of parasite zoospores. Positive (+) and negative (—) symbols denote the sign of
635 predicted relationships.

636 **Figure 2.** Environmental components linking habitat features of alpine ponds with changes
637 in ultraviolet radiation (UVR) —*Pathway 1:*(*A*) All else equal, permanent (Perm.) sites were
638 deeper than ephemeral (Ephem.) ones. (*B*) However, permanent sites had less dissolved organic
639 carbon (DOC). (*C*) Thus, UVR exposure was higher in deeper, permanent sites (large values of
640 "UVR index" indicate higher mean penetration of UVR in the water column [equ. 1]). Data are
641 means ± bootstrapped SE.

642 **Figure 3.** *Pathway 1A, UVR directly regulates parasites:* (A) *In situ*, exposure to solar
643 radiation (UVR + PAR) reduced survival of zoospores. However, (*B*) sites with higher UVR had
644 more disease. (*C*) Permanent sites have higher UVR exposure and prevalence (*E*: ephemeral; *P*:
645 permanent). Data are means ± bootstrapped SE.

646 **Figure 4.** Connections between habitat and disease via parasite predators (zooplankton;

647 *Pathway 1B*) and host communities (*Pathway 1C*). (*A-C*) *Habitat-composition links*: (*A*) Sites
648 with higher UVR index (i.e., higher mean levels of UVR) had lower density of zooplankton.
649 There was no relationship between UVR and (*B*) overall host diversity or (*C*) the frequency of
650 our focal hosts. (*D-F*) *Composition-disease links*: Infection prevalence was higher in ponds with
651 (*D*) lower zooplankton density, (*E*) lower host diversity, and (*F*) higher frequency of focal hosts.
652 **Figure 5**. Linking habitat, host stage structure, and disease (*Pathway 2*). (*A-B*) Infection
653 loads from host stages. *(A)* Infection loads were ~ an order of magnitude higher in multi-season
654 larvae of focal hosts (triangles) than in their single-season counterparts, newts (squares), or the
655 'other' host species (circles). (*B*) Infection loads were higher in rarer mid-wife toads than in
656 more dominant salamander hosts. Different letters indicate significant differences in planned *a*
657 *priori* contrasts. (*C*) Multi-season larvae of the focal hosts lived in all permanent but no
658 ephemeral sites. Data are means ± bootstrapped SE.
659 **Figure 6.** Variation partitioning of infection prevalence of Bd across 14 alpine ponds
660 (*Pathways 1* and *2*). The rectangle represents total variation in prevalence (100%). Together,
661 parasite predators (zooplankton, *Z*), multi-season larvae, MSL (*M*), and host diversity (*D*)
662 explained (64%, i.e., $R^2_{adjusted} = 0.639$) of the variation (filled in circles, accounting for negative
663 variation). This leaves the fraction *h*, 36.1%, as unexplained variation (white area). However,
664 zooplankton [fraction *a*, 1.6%] and MSL [*c*, 4.1%] explained only a small fraction of prevalence
665 themselves. Yet due to habitat-mediated correlation between them, they jointly explained a larger
666 fraction [*f*, 28.2%]. Hence, together, they explain 33.9% of variation [$a + c + f$]. That fraction
667 rivals the amount explained by diversity alone [*b*, 42.4%]. Additionally, diversity and MSL
668 shared variation [*e*, 13.8%], despite being uncorrelated themselves. Together, diversity and MSL
669 uniquely explained high variation in prevalence [$b + c + e$, 56.2%]. 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 text for explanation). Those regions of negative variation are drawn here as zero overlap.

Figure 1

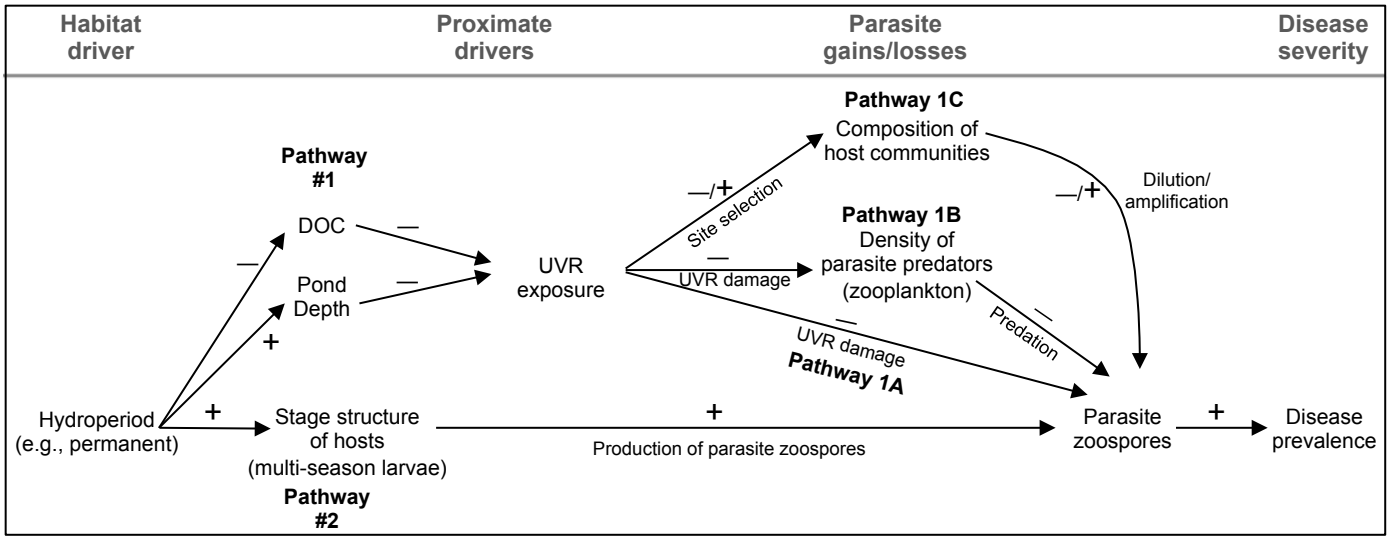

Habitat driver
Proximate drivers
Parasite gains/losses
Disease severity
Pathway 1C
Composition of host communities
Pathway #1
DOC
Pond Depth
UVR exposure
Site selection
Pathway 1B
Density of parasite predators (zooplankton)
UVR damage
Predation
Dilution/ amplification
UVR damage
Pathway 1A
Hydroperiod (e.g., permanent)
Stage structure of hosts (multi-season larvae)
Pathway #2
Production of parasite zoospores
Parasite zoospores
Disease prevalence

Figure 2

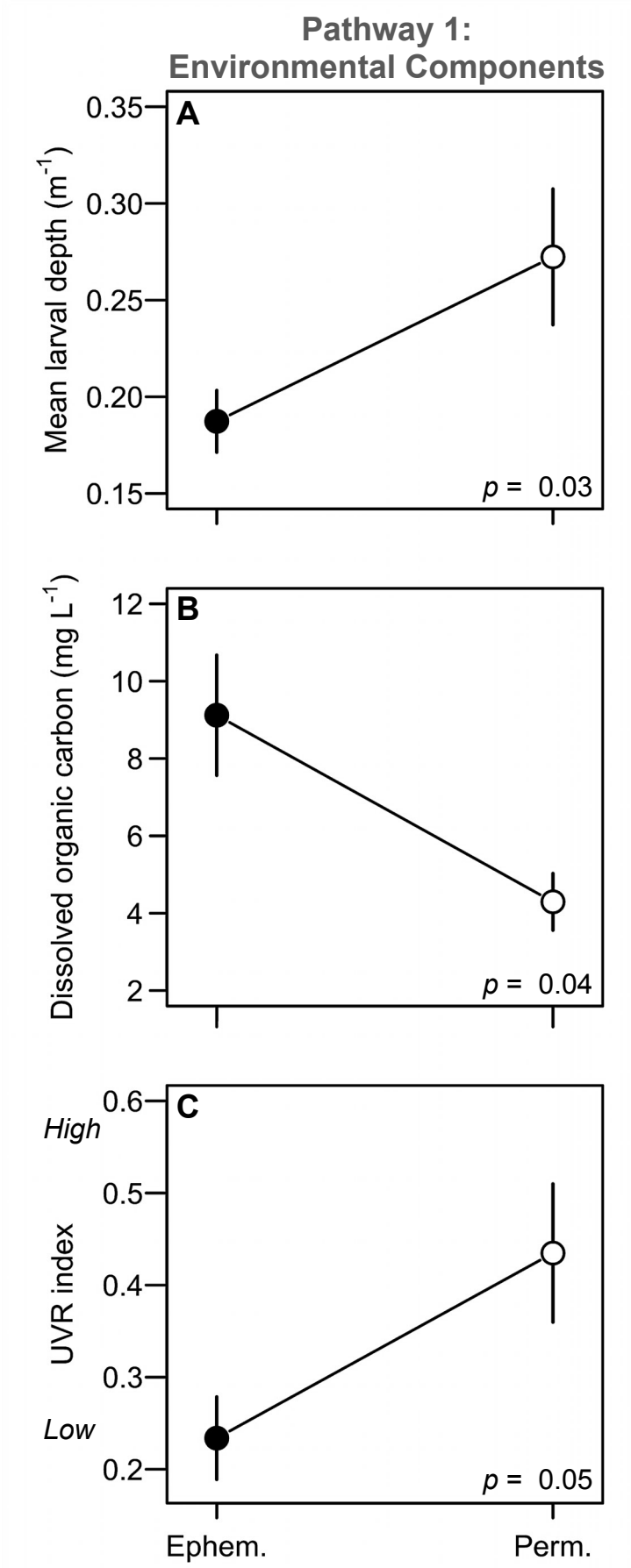

Pathway 1:
Environmental Components
A
Mean larval depth (m-1)
0.35
0.30
0.25
0.20
0.15
p = 0.03
B
Dissolved organic carbon (mg L-1)
12
10
8
6
4
2
p = 0.04
C
High
Low
UVR index
0.6
0.5
0.4
0.3
0.2
p = 0.05
Ephem.
Perm.

Figure 3

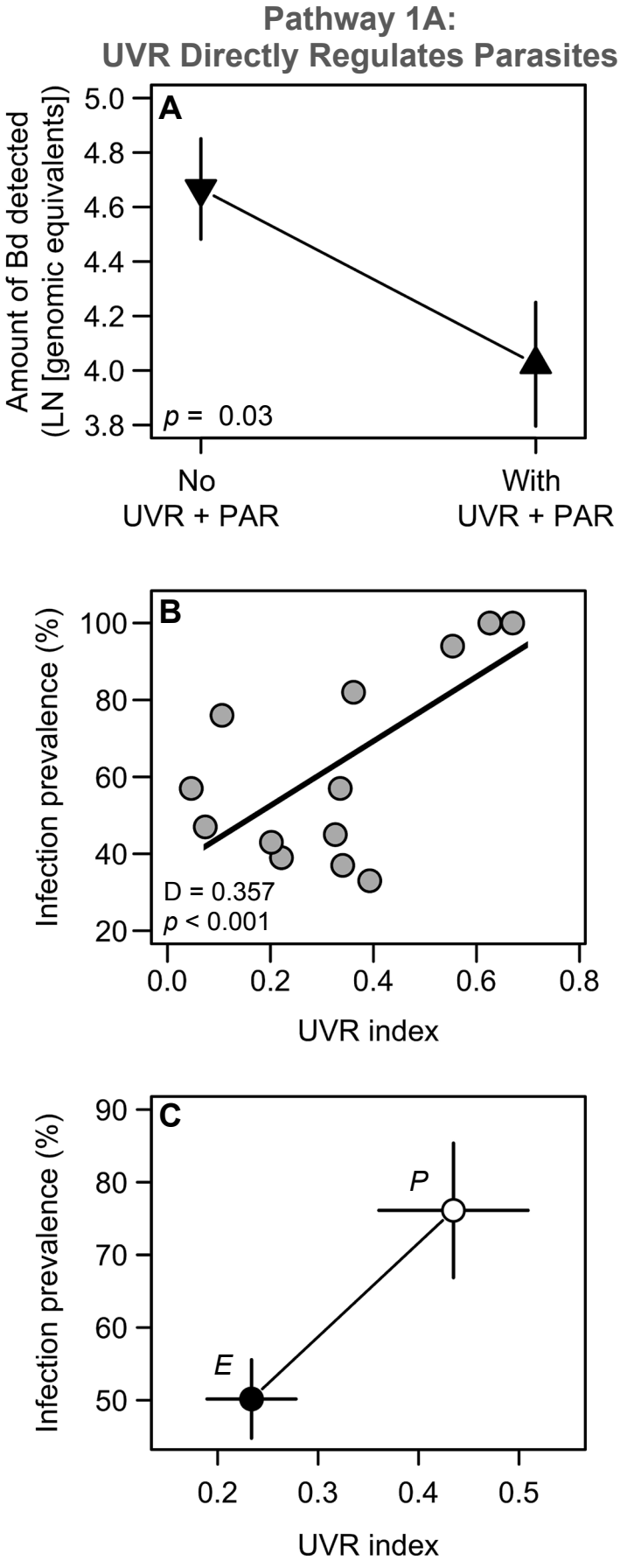

Pathway 1A:
UVR Directly Regulates Parasites
A
Amount of Bd detected
(LN [genomic equivalents])
5.0
4.8
4.6
4.4
4.2
4.0
3.8
p = 0.03
No
UVR + PAR
With
UVR + PAR
B
Infection prevalence (%)
100
80
60
40
20
D = 0.357
p < 0.001
0.0
0.2
0.4
0.6
0.8
UVR index
C
Infection prevalence (%)
90
80
70
60
50
P
E
0.2
0.3
0.4
0.5
UVR index

Figure 4

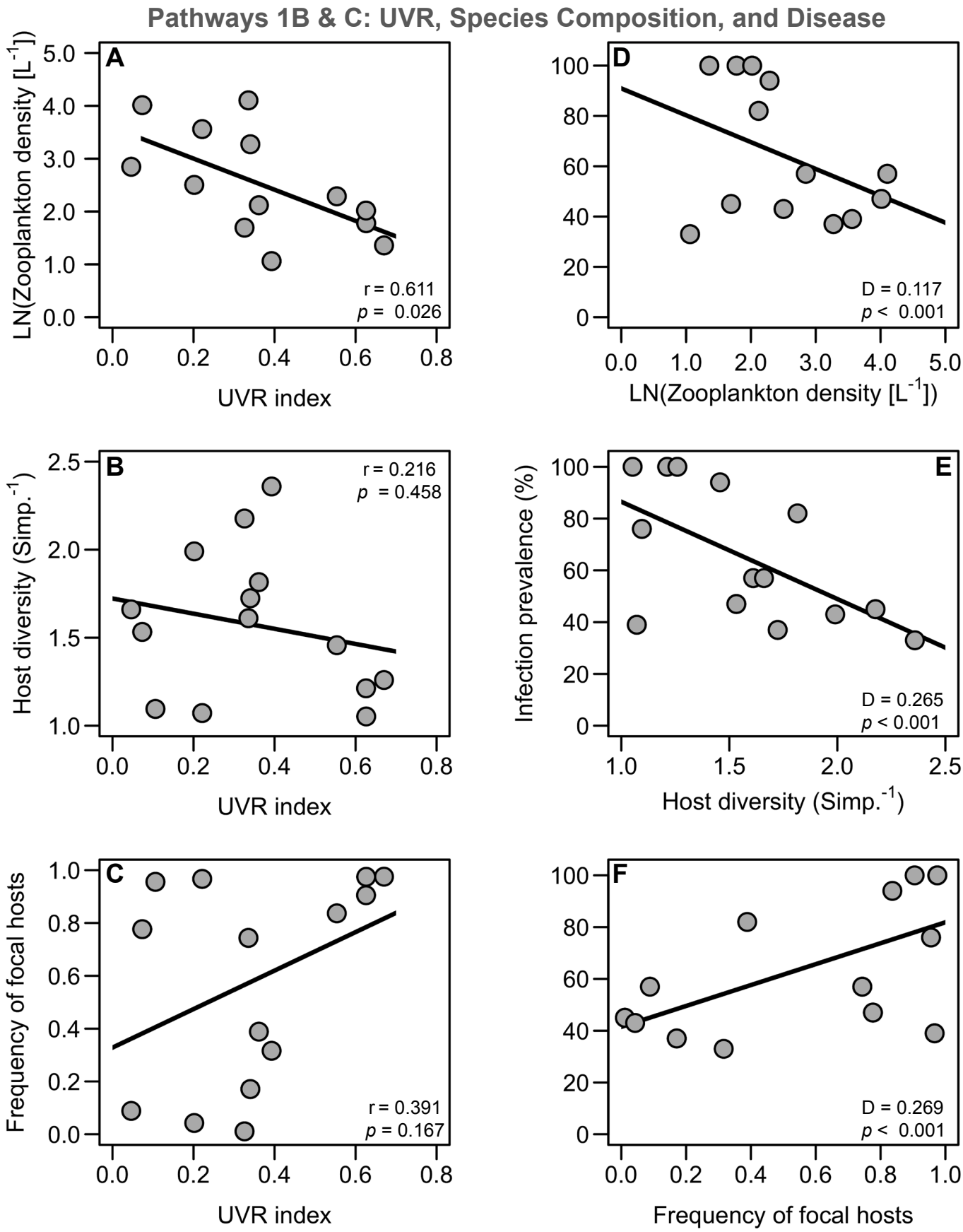
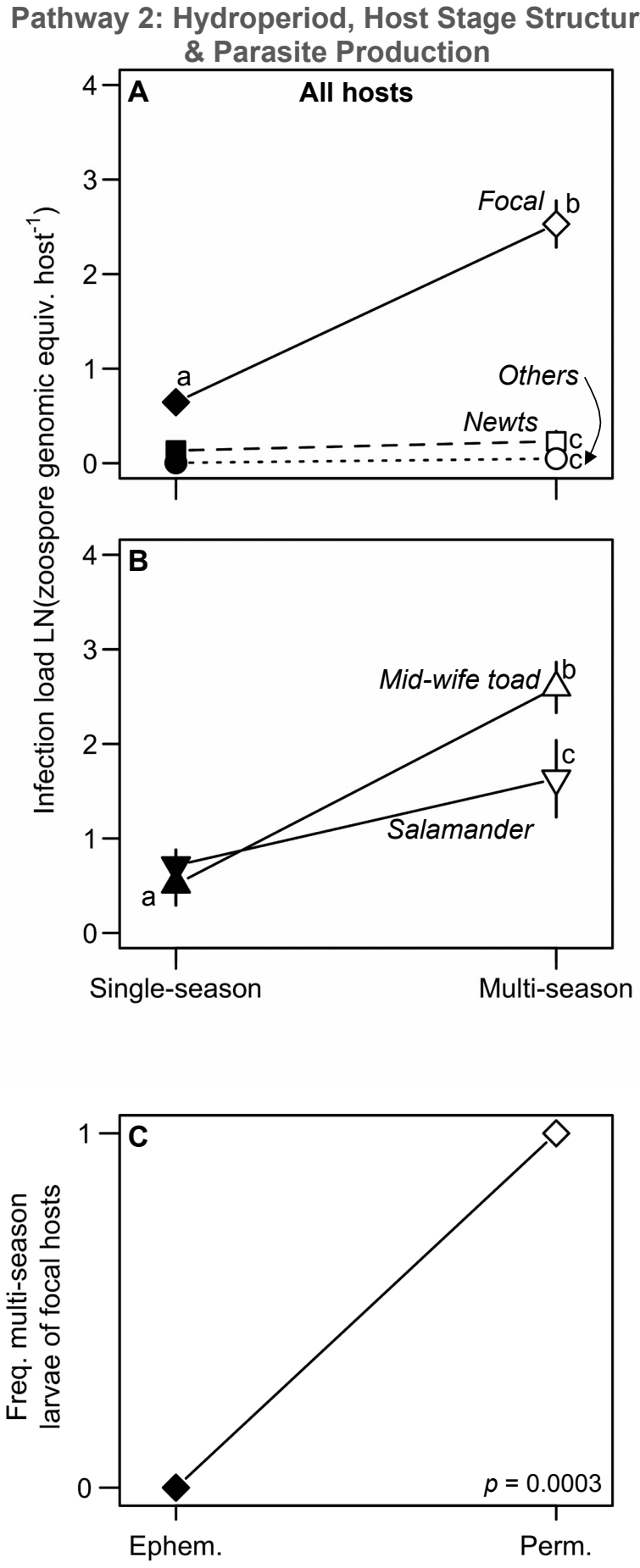
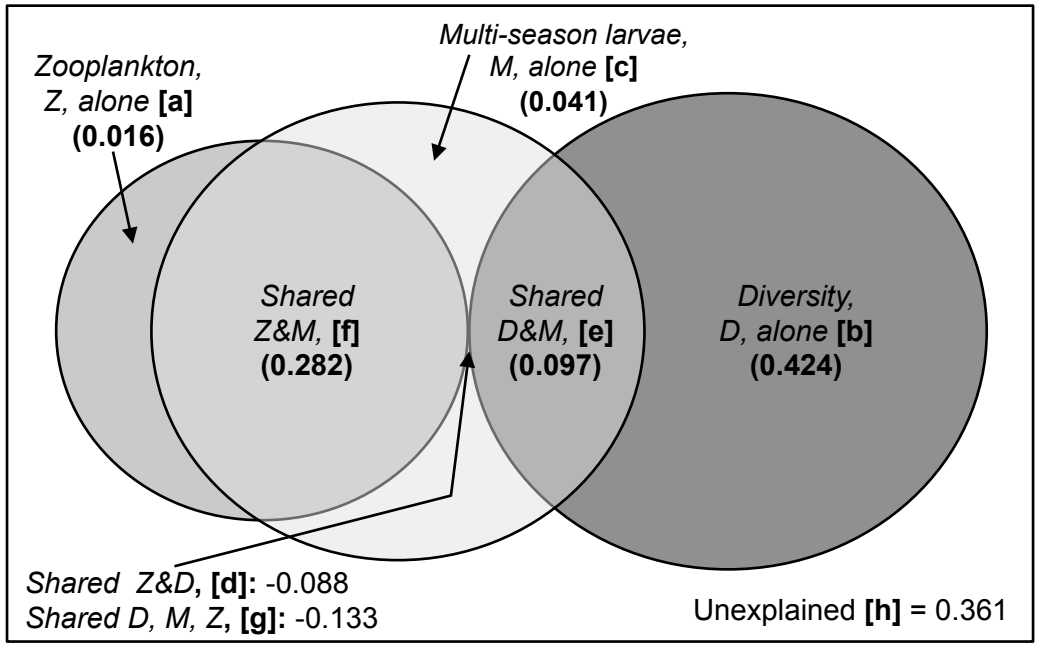

Pathways 1B & C: UVR, Species Composition, and Disease
A
LN(Zooplankton density [L-1])
UVR index
r = 0.611
p = 0.026
D
LN(Zooplankton density [L-1])
D = 0.117
p < 0.001
B
Host diversity (Simp.-1)
UVR index
r = 0.216
p = 0.458
E
Infection prevalence (%)
Host diversity (Simp.-1)
D = 0.265
p < 0.001
C
Frequency of focal hosts
UVR index
r = 0.391
p = 0.167
F
Frequency of focal hosts
D = 0.269
p < 0.001

Figure5


Pathway 2: Hydroperiod, Host Stage Structure, & Parasite Production
A
All hosts
Focal
b
a
Others
Newts
c
c
B
Mid-wife toad
b
c
Salamander
a
Infection load LN(zoospore genomic equiv. host-1)
0
1
2
3
4
Single-season
Multi-season
C
Freq. multi-season larvae of focal hosts
p = 0.0003
Ephem.
Perm.


Zooplankton, Z, alone **[a]** **(0.016)**
Multi-season larvae, M, alone **[c]** **(0.041)**
Shared Z&M, **[f]** **(0.282)**
Shared D&M, **[e]** **(0.097)**
Diversity, D, alone **[b]** **(0.424)**
Shared Z&D, **[d]:** -0.088
Shared D, M, Z, **[g]:** -0.133
Unexplained **[h]** = 0.361

ELECTRONIC SUPPLEMENTARY MATERIALS

In this supplement we present additional methods, and results from the *in situ* experiment, the field survey, and variation partitioning based on partial regression analysis. We also present two additional versions of the variation partitioning first substituting the frequency of focal hosts and then the frequency of the introduced alpine newt (Table S1).

ADDITIONAL METHODS AND RESULTS

Estimates of UVR exposure in the field experiment

During the incubation period, we measured PAR in the water column and water temperature to characterize differences between ponds. Accurately measuring UVR in the field is challenging, and due to logistical constraints, equipment was limited. Therefore, to provide an index of solar radiation, we measured photosynthetically active radiation (PAR) using a light meter (Li-Cor, Lincoln, Nebraska USA). Specifically, we measured (PAR) at three depths in each pond, once every hour during mid-day from 11:00 hours – 13:00 hours, then calculated the average solar radiation for each pond. There was no significant difference in mean PAR levels between the two incubation ponds (PAR *t*-test; $t = 0.72$, df $= 30$, $p = 0.48$, n = 33).

We also measured water temperature in each incubation pond for a portion of the assay. We measured water temperature every thirty minutes throughout from 9:30 – 14:00 hours (using a hand-held Horiba D55 meter, Southwest Scientific), and calculated the mean temperature. There was no significant difference in mean water temperature levels between the two incubation ponds (PAR *t*-test; $t = 0.85$, df $= 20.78$, $p = 0.40$, n = 23).

Pathway 1C: UVR Effect on the Composition and Diversity of Host Communities

There was no relationship between UVR and the frequency of the introduced alpine newt

(Pearson r = 0.419, p = 0.136 Fig. S1*a*). Sites dominated by the introduced alpine newt had lower infection prevalence (GLM, χ^2= 9.45, df = 1, p = 0.002, D = 0.083, Fig. S1*b*). Higher host diversity reflected lower frequencies of the focal hosts (r = -0.847, p = 0.0001, Fig S1*c*).

Synthesis: Variation Partitioning

In the text, we briefly describe a partition of variation using three potentially correlated explanatory variables. The partition using three variables requires an extension of the two variable method described previously (Legendre and Legendre 2012). Readers of the recipe below must understand the two variable case first, as we merely aim here to describe, in words, the strategy used in Legendre's varpart code for R (part of the vegan package); we borrow that code's strategy directly. Here, we partition variation in prevalence (*P*) as functions of host diversity (*D*), abundance of zooplankton predators (*Z*), and presence or absence of multi-season larvae (*M*). The partition involves three steps.

Step 1: Five simple or multiple regression analyses (all linear) are needed. The format below for the regression models is, e.g., *dependent variable* ~ sum of *independent variables*

Model 1 (M1), fractions *a*, *f*, *d*, *g*: $P \sim Z$ (A1.a)

Model 2 (M2), fractions *c*, *e*, *f*, *g*: $P \sim M$ (A1.b)

Model 3 (M3), fractions *b*, *d*, *e*, *g*: $P \sim D$ (A1.c)

Model 4 (M4), fractions *a*, *b*, *d*, *e*, *f*, *g*: $P \sim Z + D$ (A1.d)

Model 5 (M5), fractions *a*, *c*, *d*, *e*, *f*, *g*: $P \sim Z + M$ (A1.e)

Model 6 (M6), fractions *b*, *c*, *d*, *e*, *f*, *g*: $P \sim D + M$ (A1.f)

Model 7 (M7), all fractions *a* through *g*: $P \sim D + M + Z$ (A1.g)

where models M1 to M3 (equs. A1.a-c) are simple linear regression of each biological driver on infection prevalence; M4-M6 (equs. A1.d-f) are the various combinations of two of each driver; and M7 (equ. A1.g) is the three driver regression model. For each model, we calculate the adjusted R^2 (hereafter: R_a^2). (The fractions *a* through *g* encompassed by each regression model are defined below). The variation unexplained by the sum of the three drivers is then 1 - the R_a^2 value from M7, written in shorthand here and below as '1 - M7'. Then, to calculate the first three partitions, we must subtract the R_a^2 values of each two-driver model (M4, M5, and M6) from the first full, three driver model, M7, each in turn. These first three partitions characterize the fraction of variation in infection prevalence explained by each driver (*Z*, *D*, *M*) alone:

fraction *a* (*Z* alone): M7 - M6 (A2.a)

fraction *b* (*D* alone): M7 - M5 (A2.b)

fraction *c* (*M* alone): M7 - M4 (A2.c)

The fractions *d* through *f* involve variation shared between pairs of drivers, where *d* is the fraction shared between *D* and *Z*, *e* is that between *D* and *M*, and *f* is that between *M* and *Z*. To calculate them, the second step is needed; this intermediate step calculates the sum of one of the driver-alone fractions (*a*, *b*, or *c*) with one of the shared fractions (*d*, *e*, or *f*). Thus, these intermediate fractions involve subtracting R_a^2 values from the regressions models (equ. A1) in different ways:

fraction (*a* + *d*): M5 - M3 (A3.a)

fraction (*b* + *e*): M4 - M1 (A3.b)

fraction (*c* + *f*): M6 - M2. (A3.c)

These sums of variation then provide the core ingredients to isolate the remaining shared fractions in the third step (i.e., by subtracting particular combinations of equs. A1-3):

fraction d (shared by D and Z): $(a + d) - a$ (A4.a)

fraction e (shared by D and M): $(b + e) - b$ (A4.b)

fraction f (shared by M and Z): $(c + f) - c$ (A4.c)

fraction g (shared by D, M, and Z): M7 - $(a + d)$ - $(b + e)$ - $(c + f)$ (A4.d)

The seven partitions presented in the text (Fig. 6) were calculated using these methods (equ. A1-A4). The unexplained variation, h, is 1 - M7.

Below (Table S1), we present results comparing the model presented in the main text with two additional variants. First, we exchange host diversity with the frequency of focal hosts (*Salamandra salamandra* and *Alytes obstetricans*). Then, we exchange diversity with the frequency of the introduced alpine newt (*Ichthyosaura alpestris*). Two main points arise from this comparison. First, host diversity and the frequency of focal hosts alone explain similar amounts of variation in infection prevalence (0.424 and 0.363, respectively); newts alone explain a much smaller fraction (0.148). The similarity between the first and second models likely reflect the tight correlations between host diversity and the frequency of focal hosts (Fig. S1*c*). Second, while, the models vary in the weight given to each parameter alone (i.e., *a*,*b*,*c*), their overall joint contributions (i.e., zooplankton + multi-season larvae, [*a*+*c*+*f*] and community composition of hosts + multi-season larve, [*b*+*c*+*e*]) are similar across all models.

LITERATURE CITED IN SUPPLEMENTARY MATERIAL

1. Piotrowski J.S., Annis S.L., Longcore J.E. 2004 Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* 96(1), 9-15. (doi:10.2307/3761981).

2. Legendre P., L. Legendre. 1998 Numerical ecology. Second ed. Amsterdam, Elsevier.

SUPPLEMENTARY MATERIAL FIGURE LEGENDS

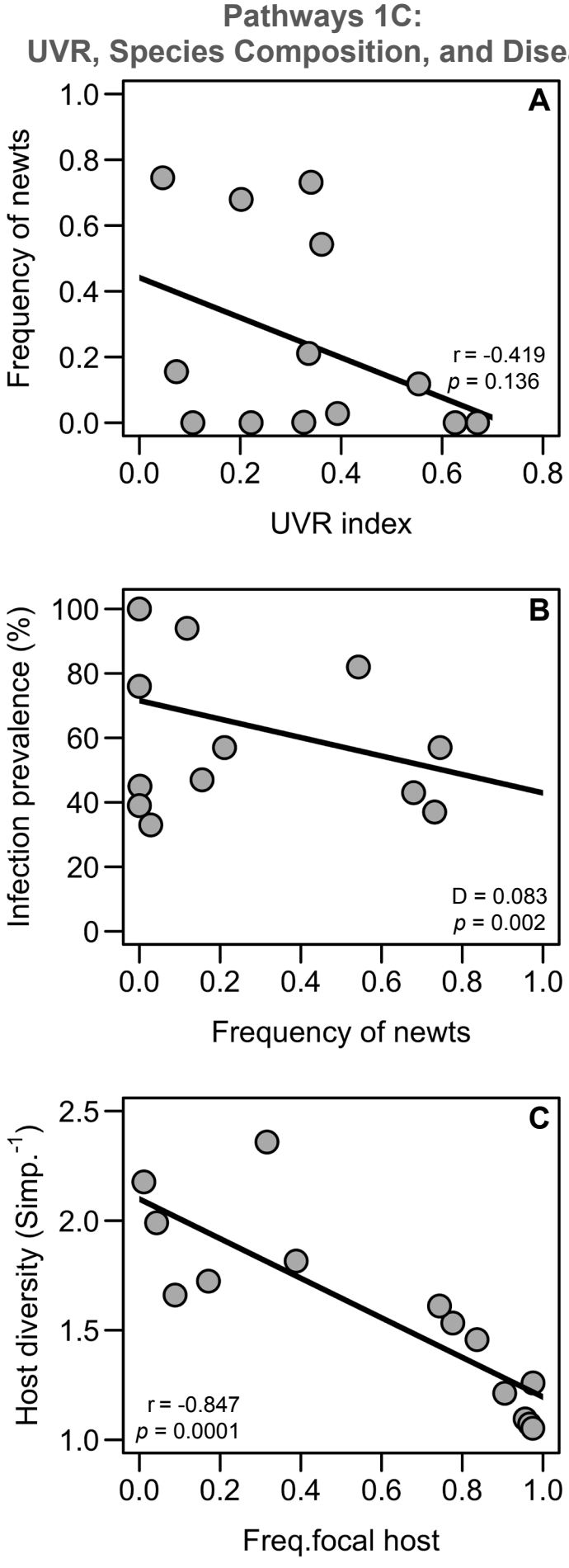
Figure S1. Habitat-disease connections via composition of host communities (*Pathway 1C*). (*A*) There was no relationship between UVR and the frequency of the introduced alpine newt. (*B*) Sites dominated by the introduced alpine newt had lower infection prevalence. (*C*) Relationship between host diversity and frequency of focal hosts. As pond communities became more dominated by focal hosts (fire salamanders and mid-wife toads), host diversity decreased. Each point is the mean of relative abundance surveys collected throughout the breeding season from 2009-2012.

Table S1. Three different versions of the multiple regression-based partition that explains variation in prevalence of Bd infection in focal hosts. The versions differ in the index used to characterize the host community, *D*. In model 1, Simpson's diversity characterizes it (as visualized in Fig. 6). In model 2, *D* is the closely correlated frequency of focal hosts. In model 3, *D* is frequency of alpine newts.

Parameter(s)	Fractions of variation	Model 1 Host diversity R_a^2	Model 2 Freq. focal hosts R_a^2	Model 3 Freq. alpine newt R_a^2
Full model	**[a] – [g]**	**0.639**	**0.684**	**0.364**
Zooplankton (*Z*) alone	[a]	0.016	-0.033*	-0.009*
Multi-season larvae (M) alone	[c]	0.041	0.102	0.327
Shared *Z* & *M*	[f]	0.282	0.211	0.027
Z & *M*, no *D*	[a+c+f]	0.339	0.314	0.345
Hosts (*D*) alone	[b]	0.424	0.363	0.148
Shared *D* & *M*	[e]	0.097	0.036	-0.188*
D & *M*, no *Z*	[b+c+e]	0.562	0.501	0.475
Shared *D* & *Z*	[d]	-0.087*	-0.038*	-0.063*
Shared *D*, *M*, & *Z*	[g]	-0.133*	-0.063*	0.122
Residuals	[h]	0.361	0.316	0.636

*Negative fractions indicate partitions that explain less variation than random normal variables. Hence, they are interpreted as zeros [2].

Figure S1


Pathways 1C:
UVR, Species Composition, and Disease
A
Frequency of newts
UVR index
r = -0.419
p = 0.136
B
Infection prevalence (%)
Frequency of newts
D = 0.083
p = 0.002
C
Host diversity (Simp.-1)
Freq.focal host
r = -0.847
p = 0.0001