

LETTER

Solar radiation decreases parasitism in *Daphnia*

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Abstract

Climate change and variation in atmospheric ozone are influencing the intensity of ultraviolet radiation (UVR) reaching ecosystems. Changing UVR regimes, in turn, may alter epidemics of infectious disease. This possibility hinges on the sensitivity of epidemiologically relevant traits of host and parasite to UVR. We address this issue using a planktonic system (a zooplankton host, *Daphnia dentifera*, and its virulent fungal parasite, *Metschnikowia bicuspidata*). Controlled laboratory experiments, coupled with *in situ* field incubations of spores, revealed that quite low levels of UVR (as well as longer wavelength light) sharply reduced the infectivity of fungal spores but did not affect host susceptibility to infection. The parasite's sensitivity to solar radiation may underlie patterns in a lake survey: higher penetration of solar radiation into lakes correlated with smaller epidemics that started later in autumn (as incident sunlight declined). Thus, solar radiation, by diminishing infectivity of the parasite, may potentially reduce disease.

Keywords

Daphnia-Metschnikowia, epidemic size, host-parasite, solar radiation, ultraviolet.

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INTRODUCTION

Do ozone depletion and climate change increase disease in human and wildlife populations by changing ultraviolet radiation (UVR) intensity? Although UVR-absorbing ozone levels in the atmosphere are no longer declining, climate change will influence the recovery of the ozone layer through effects on factors influencing UVR reaching ecosystems (e.g. aerosols, clouds and surface reflectance: McKenzie *et al.* 2011). Furthermore, increasing concentrations of UVR-absorbing dissolved organic carbon (DOC) may reduce the amount of incident UVR that penetrates into inland waters. Such changes will modulate exposure of aquatic organisms – including waterborne pathogens – to UV (Williamson & Rose 2010). Changes in UVR could affect disease processes through several mechanisms. For instance, UVR can suppress the immune response of animals to some infections such as herpes simplex virus and human papillomavirus (Norval *et al.* 2011). In contrast, elevated UVR exposure correlates with decreases in diseases such as gastroenteritis (Lam 2007), invasive meningitis (Kinlin *et al.* 2009) and pneumonia (White *et al.* 2009). Thus, changes in UVR due to ozone recovery and climate change may elevate or diminish incidence of some diseases.

How can we know – mechanistically – if changes in UVR will catalyse or inhibit epidemics of infectious disease? In general, UVR can damage DNA, cell membranes and cellular processes, and it can suppress immune function (Norval *et al.* 2011). Thus, UVR could damage both hosts and parasites, but perhaps differentially. If we focus on such damage to epidemiologically relevant traits, theory suggests that UVR could increase disease spread by depressing the resistance of hosts to infection and the subsequent response of the immune system of hosts (Markkula *et al.* 2007). Inhibition of immune

responses can involve effects on short-term clearance of infection (Goettlich *et al.* 1994; Markkula *et al.* 2007), but also on longer term protective immunity to subsequent infections (Giannini 1986). In contrast, UVR could inhibit disease spread through deleterious effects on parasites themselves. UVR can depress infectivity of parasites (Connelly *et al.* 2007; King *et al.* 2008; Gomez-Couso *et al.* 2009), reduce development and production of infectious propagules (e.g. spores) within infected hosts (Ruelas *et al.* 2007; Martinaud *et al.* 2009), increase mortality of free-living parasites (van Dijk *et al.* 2009), and increase mortality of infected hosts (which then reduces the time during which infected hosts can contact susceptible hosts; Yamamoto *et al.* 2000). Thus, UVR can influence critical traits of both host and parasite that control disease spread – but the net effect of these various factors remains unknown.

To uncover links between UVR and disease, we must compare the relative sensitivity of both host and parasite to UVR. This kind of comparison remains surprisingly rare in UVR studies of disease, but the key concepts for environmental stressors in general have been delineated already (Lafferty & Holt 2003). Stressors could weaken hosts, boosting parasite epidemics, but stressors can also reduce fecundity or survivorship of hosts. The subsequent reduction in host densities should decrease disease transmission and therefore epidemics. In addition, if environmental stress kills parasites or reduces their infectivity, then disease should diminish as well (Lafferty & Holt 2003). We apply these broad, general concepts to the UVR stressor using a focal case study of disease in the plankton. In Midwestern (USA) lakes, the zooplankton grazer *Daphnia dentifera* becomes infected by the virulent fungal parasite *Metschnikowia bicuspidata* after consuming environmentally dispersed fungal spores (Ebert 2005). The epidemics begin during late summer and early autumn (Cáceres *et al.*

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2006; Hall *et al.* 2011), as incident UVR intensity declines from a summer peak. Thus, if either player is sensitive to UVR exposure, variation in UVR among lakes and/or variation from summer through autumn could matter greatly to epidemics.

Ultraviolet radiation could differentially affect the zooplankton host and fungal parasite. In general, *Daphnia* comprise one of the more UVR-sensitive zooplankton groups (Leech & Williamson 2000). If UVR exposure renders these hosts more susceptible to infection, UVR could promote disease spread and therefore boost epidemics. On the other hand, fungal spores typically remain suspended in the upper mixed portion of the water (i.e. the epilimnion, or the surface mixed layer: Cáceres *et al.* 2009; Hall *et al.* 2010) where UVR exposure is most intense. If UVR decreases spore infectivity or outright kills the fungus, UVR could decrease disease. In addition, UVR could delay the start of epidemics until incident radiation levels diminish following summer solstice. Because the epidemic start date correlates with the size of epidemics (Hall *et al.* 2011), outbreaks in lakes with higher UVR penetration (transparency) might start later and remain smaller. This epidemic size-timing issue matters for the host because larger epidemics more intensely depress densities of hosts (Hall *et al.* 2011).

Here, we present two major findings about UVR and disease. First, the host and fungus responded quite differently to UVR. Although the hosts were moderately sensitive to UVR, susceptibility of the host to parasites was insensitive to UVR levels that sharply reduced infectivity of fungal spores in laboratory-based exposure regimes. Furthermore, the fungus was damaged not only by shorter wavelength UV-B, but also by longer wavelength UV-A and photosynthetically active radiation (PAR, basically visible light) that actually help to repair UV-B induced damage in many organisms (Mitchell & Karentz 1993). Field-based exposure to UVR (and PAR) confirmed these findings: under natural conditions, UVR and/or PAR greatly diminished infectivity of fungal spores even at very low exposure levels. Second, field surveys showed why these effects on the fungus matter for epidemics. Lakes with higher UVR transparency had smaller epidemics: UVR correlated with a delayed start of epidemics and further reduction in their size once initiated.

METHODS

Five experiments assessed the relative sensitivities of the fungal parasite (*M. bicuspidata*) and zooplankton host (*D. dentifera*) to radiation produced in the laboratory (#1–#4) or by natural sunlight (#5). The first experiment (#1) simultaneously exposed the host and fungus to different levels of ‘UVR’ (mostly UV-B but also some damaging, shorter-wavelength UV-A), supplied with a constant level of photorepair radiation (‘PRR’: mostly longer wavelength UV-A and PAR, supplied by two cool-white fluorescent and two Q-Panel UV-A 340 lamps from below the dishes at the same level across all UVR treatments, see Williamson *et al.* 2001 for spectral composition of these lamps). This laboratory experiment also investigated the effect of spore density on infection prevalence. Then, three follow-up experiments (#2–#4) used 11 different UVR exposure levels to quantify the UVR sensitivity of either the host (#2) or parasite (#3–#4). Experiments #2 and #3 were run with PRR, while the other (#4) was not. A fifth, field-based experiment manipulated both UVR and PAR by exposing spores to ambient solar radiation at five different depths (i.e. along a gradient of UVR intensity) in two lakes. Finally, field surveys linked UVR transparency to the size and timing of fungal epidemics.

Laboratory experiments (#1–4)

Experiment 1: both host and parasite exposed to UVR and PRR

In the first experiment, we assessed the relative UVR and PRR sensitivity of the host (*D. dentifera*) and the parasite (*M. bicuspidata*) using a UV-lamp phototron (Williamson *et al.* 2001) and a subsequent infection assay. In a three-way factorial design, we manipulated exposure of a single clone of the host to UVR and PRR (two levels; ‘UVR-host’ treatment), exposure of fungal spores to UVR and PRR (three levels; ‘UVR-fungus’ treatment), and exposure of hosts to different fungal spore densities (two levels, 100 or 500 spores per mL; ‘spore density’ treatment). Specifically, in the UVR-host treatment, we exposed a total of 60 shallow quartz dishes of 10 6-day-old *D. dentifera* to either no UVR (dark controls) or low UVR (9.5 KJ/m²) from a UV-B lamp suspended above the dishes (see Williamson *et al.* 2001 for spectral composition of UV-B lamp). This lamp set up allowed us to manipulate UVR while keeping PRR constant; the full spectrum transmission of the quartz dishes ensured transmission of the radiation supplied. In the UVR-fungus treatment, 15 separate quartz dishes of fungal spores were simultaneously exposed to one of three UVR levels (from the UV-B lamp): no UVR (dark), very low UVR (3.7 KJ/m²), and low UVR (9.5 KJ/m²). In both the UVR-host and UVR-fungus treatments, exposure from the UV-B lamp (UVR_{290–400 nm}) was controlled by placing various combinations of wire mesh on top of a quartz lid over a quartz dish. Both the UVR-host and UVR-fungus exposures occurred in the presence of PRR. The PRR exposure was included because *Daphnia* can use some UV-A and PAR for photoenzymatic repair of UV-B damaged DNA (Siebeck & Bohm 1991; Williamson *et al.* 2001). The quartz dishes, each surrounded with a black collar to reduce stray light, were placed on a rotating wheel (2 rpm) and exposed to UVR and PRR for 12 h in a growth chamber (20 °C). Meanwhile, dark controls were kept in a covered cardboard box in the same chamber. (See Online Supplementary Information for more details).

To assess the response of hosts and parasites in Experiment #1, we used an infection assay. Five hosts were placed in 100 mL of 0.7 µm-filtered lake water in glass beakers (*N* = 108). The beakers were then inoculated with fungal spores at either a lower (100 spores per mL) or higher (500 spores per mL) initial concentration (spore density treatment). After exposure to spores for 24 h, hosts were transferred to spore-free, filtered lake water. Hosts were fed a high level (2.5 mg/L) of a chemostat-reared alga (*Ankistrodesmus falcatus*) daily, and culture water was changed every 3 days. After 10 days, we diagnosed hosts for infection using a dissecting microscope (at 40×; Green 1974).

Experiment #2: survival of hosts to UVR exposure

The next experiment further examined the sensitivity of host survivorship to a broader UVR range. As will be shown, Experiment #1 revealed little change in the susceptibility of hosts to fungal parasites when exposed to low amounts of UVR. However, previous work on other *Daphnia* species (Leech & Williamson 2000) suggests that this host should exhibit high survival at UVR levels that substantially reduced survival of the fungus (measured as infectivity). Thus, to further test UVR sensitivity of the host *Daphnia*, we conducted a survival experiment using a much broader range of UVR than in Experiment #1, one that bracketed this clone’s lethal exposure level (LE₅₀, the UVR exposure level from the UV-B lamp at which 50% of the test organisms survive – see Leech & Williamson 2000).

Four replicate dishes, each filled with 10, 6-day-old hosts and moderately hard synthetic freshwater (SFW), were exposed to one of 11 UVR levels (0–51 KJ/m² UVR_{290–400 nm} from UV-B lamp) with PRR in the phototron for 12 h. Taking into account the spectral differences between artificial and solar UV-B by using biological weighting functions, the highest UVR exposure treatment (PRR and 51 KJ/m² UVR_{290–400 nm} from UV-B lamp for 12 h) approximated 81% of natural solar UVR exposure in surface waters on a single sunny day during summer solstice at 40° N latitude (Williamson *et al.* 2001). Following the exposure, survival of exposed *D. dentifera* was monitored for 5 days and the LE₅₀ was estimated using linear regression following an arcsine square root transformation of per cent survival of the host data on Day 5.

Experiments #3 and #4: exposure of parasites to UVR

Based on results from the first experiment, two additional experiments more sharply characterised the response of the parasite to UVR. These experiments were conducted at different times, but were similarly designed except that one (Experiment #3) included PRR, while the other (Experiment #4) did not. Using the phototron methods described above (Experiment #1), four replicate dishes of spores were exposed to 11 levels of UVR ranging from 0 to 15 KJ/m² UVR_{290–400 nm} from the UV-B lamp. Following the exposure of spores to these radiation treatments, five 6-day-old, unexposed hosts were placed in 100 mL of SFW and inoculated with 500 spores/mL. After 24 h, we transferred hosts to new, spore-free SFW every three days, fed them nutritious algae daily (*Selenastrum capricornicum*, 2.5 mg/L), and later diagnosed them for infection as described above.

Statistical analysis of the laboratory experiments

We used two complementary approaches to analyse the laboratory-based data. In the first approach, we fitted logistic regression models using binomial errors in R (R Development Core Team 2011). These models quantified the effect of UVR and PRR exposure of spores, UVR and PRR exposure on *Daphnia*, spore level and all interactions on infection status (infected vs. not infected) using a GLM-based framework. To determine the effect of UVR exposure on *D. dentifera* survival, we used the same GLM approach, but survival rather than infection status was measured as the endpoint.

In the second approach, we waged a competition among models relating exposure level to UVR with infection prevalence (transmission rate) or survival (see details in the Online Supplementary Information). Briefly, we fitted three functional forms linking UVR (and PRR in Experiment #1) exposure to the focal traits. The ‘null’ model assumed that UVR had no effect. The ‘linear’ model assumed that UVR influenced transmission rate or survival via a single slope parameter. The ‘power’ model incorporated both the slope and an exponent parameter. This added exponent permitted more flexibility (i.e. susceptibility could decline more sharply with increasing UVR in the power model than in the linear model). We estimated these parameters using a binomial-based likelihood function. We then waged the competition using two information-theoretic (AIC) based statistics: AIC differences (Δ), and AIC weights (w). AIC Δ s of 4–7 indicate considerably less support for a model, while Δ s greater than 10 show essentially no support. AIC weights (w) indicate relative likelihood; values near one indicate strong performance, while those less than one reveal substantially less. Comparisons among competing models provide guides for interpretation. Support for either the ‘linear’ or ‘power’ models over the ‘null’ model quantified the

importance of UVR for the trait of interest. Furthermore, delineation between the ‘power’ and ‘linear’ model provided a sharper characterisation of the UVR-trait relationship.

Field experiment (#5)

We used a fifth, field-based experiment to determine the sensitivity of fungal spores to natural solar radiation including UV-B, UV-A and PAR. Energy spectra and therefore biological response can differ between simulated (phototron) and natural conditions (Williamson *et al.* 2001); thus, we needed to confirm our results with an analogous field experiment. Fungal spores were exposed to solar radiation in two highly transparent lakes in Sullivan County, Indiana, USA: Canvasback (Minnehaha Fish and Wildlife Area) and Gambill (Greene-Sullivan State Forest; the ‘lake’ treatment). Spores incubated in quartz tubes wrapped in plastic received one of three radiation treatments (‘radiation’ treatment): Aclar transmitted 100% of PAR (400–800 nm) and 99% of UVR (250–399 nm); Courtgard blocked UVR (transmitting only 4%) but allowed 97% of PAR to transmit; and black (dark) plastic blocked all UVR and PAR. Thus, the radiation treatments manipulated UVR and PAR. We then suspended the tubes at five depths (‘depth’ treatment) chosen to create a gradient of UVR (*ca.* 50, 20, 6, 3 and 1% of surface UVR_{320 nm}); all depths experienced nearly identical water temperatures. We incubated spores for 4 days in early August. Ambient solar data were contemporaneously measured using a UVR-PAR radiometer (see Online Supplementary Information). We then used field incubated spores in a laboratory-based infection assay with methods described above and 6-day-old, laboratory-reared hosts. The field data were analysed by fitting logistic regression models with binomial errors (R Development Core Team 2011). These GLM models estimated *lake*, *radiation* and *depth* effects and their interactions.

Field survey

Finally, we linked UVR penetration to fungal epidemics in natural populations. In 2009, we monitored epidemics weekly, mid-August – early December, in 18 lakes spread across a gradient of UVR penetration in southwestern Indiana (USA: Greene-Sullivan State Forest, Minnehaha and Hillenbrand Fish and Wildlife Areas). During each visit, we pooled three tows of a Wisconsin net (153 μ m mesh, 13 cm diameter) collected in locations separated by > 25 m. From this sample, we estimated infection prevalence after visually diagnosing 400 + live animals using a dissecting microscope. From these surveys, we estimated two metrics of epidemics: start date (ordinal day on which epidemic size reached 1% prevalence: only 16 lakes had epidemics) and maximum prevalence of infection. To characterise the UVR environment, integrated water samples (top 3 m) were collected around when epidemics would start (late July and early August). Filtered water samples (Whatman GF/F, 0.7 μ m) were analysed for dissolved absorbance (a_{4320} : m⁻¹; Shimadzu UV/Visible UV-1650 PC Spectrophotometer Kyoto, Japan; see Rose *et al.* (2009)). This absorption coefficient is generally inversely related to UVR penetration (Rose *et al.* 2009); here, it is used to index UVR penetration. (The relationship between a_{4320} and the extinction coefficient for 320 nm radiation, k_{4320} , is very strong among eight of our study lakes; $R^2 = 0.96$: $k_{4320} = 1.20 a_{4320} + 0.15$.) We then analysed the data using simple correlation, partial correlation and partial regression approaches. This latter method partitioned variation explained (%) in epidemic size to an effect of UVR absorption (a_{4320}) alone, to an effect

of start date of infection alone, and to variation shared jointly between them (see Online Supplementary Information for more details).

RESULTS

The first laboratory experiment (#1) revealed that fungal spores were far more sensitive than the host to UVR and PRR. Specifically, UVR and PRR exposure significantly decreased *M. bicuspidata* infectivity (GLM, *UVR-fungus* effect: $F_{2,105} = 62.64$, $P < 0.001$); meanwhile, UVR and PRR exposure did not affect susceptibility to infection in *Daphnia* (*UVR-host* effect: $F_{1,103} = 0.47$, $P = 0.50$; Fig. 1a). As expected, higher spore concentrations led to significantly higher infection (*spore density* effect: $F_{1,104} = 33.69$, $P < 0.001$; Fig. 1a). There was no significant interaction between any of the factors (*UVR-fungus* \times *spore density* effect: $F_{2,101} = 2.55$, $P = 0.08$; *UVR-fungus* \times *UVR-host*: $F_{2,99} = 0.18$, $P = 0.84$; *UVR-host* \times *spore density*: $F_{1,98} = 2.09$,

$P = 0.15$; *UVR-fungus* \times *UVR-host* \times *spore density* effect: $F_{2,96} = 1.40$, $P = 0.25$; see also the Online Supplementary Information, Table S1). The results from the model competition confirm these findings (see Online Supplementary Information and Tables S2 and S3 for details). The three-parameter 'power' model assuming no effect of host UVR and PRR exposure fit the data best ($\Delta = 0$, $w = 0.88$). This power model performed better than the two-parameter 'linear' model ($\Delta = 5.32$, $w = 0.06$) because it allowed a sharper drop in infection prevalence with increasing UVR and PRR exposure (Fig. 1a). The six-parameter 'power' model which accounted for exposure of hosts to UVR and PRR also received much less support ($\Delta = 5.68$, $w = 0.05$). The null models, which assumed no effect of exposure of fungal spores to UVR and PRR, performed terribly ($\Delta s > 107$, $w = 0$).

The host survival experiment (#2) supported these results. Although UVR exposure significantly decreased host survival ($F_{1,42} = 42.00$, $P < 0.001$), survival was not substantially reduced

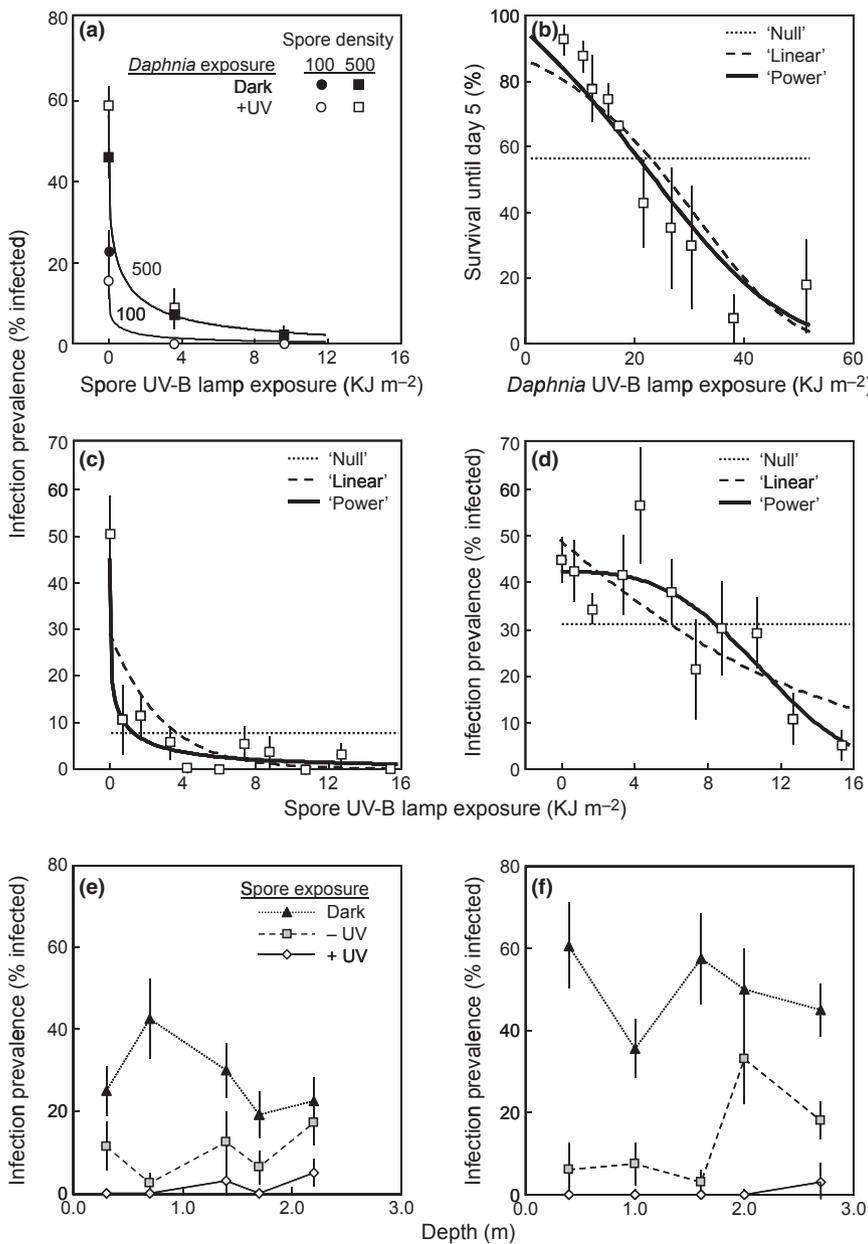


Figure 1 Response of both a virulent fungal parasite (*Metschnikowia*) and a *Daphnia* host to exposure to ultraviolet radiation (UVR) and photorepair radiation (PRR) in the lab and field. (a) *Experiment #1*: Infection prevalence after exposure to two fungal spore densities [100 (circles) and 500 (squares) spores per mL]. The fungus and/or host was exposed to UVR and PRR (open symbols) or not (closed symbols) in an orthogonal design. The curves are predictions of the best fitting mechanistic model fit to the data, the 'power' model. (b) *Experiment #2*: Survival of *Daphnia* after 5 days, spread over a much larger gradient of UVR and PRR. The three curves denote predictions of the competing survival models fit to the data (solid: 'power'; dashed: 'linear'; dotted: 'null'). (c) *Experiment #3*: When fungal spores alone were exposed to PRR and UVR, per spore infectivity dropped sharply with increasing UVR. (d) *Experiment #4*: When not exposed to PRR, infectivity of spores remained much higher (but still eventually dropped with UVR). Curves in both (c) and (d) are fits of competing, mechanistic models (solid: 'power'; dashed: 'linear'; dotted: 'null'). (e–f) *Experiment #5*: Infection prevalence as a function of depth, following exposure of spores to solar radiation under UV + PAR (+UV, white diamonds; solid line), PAR only (-UV, grey squares; dashed line), and dark conditions (black triangles; dotted line) in (e) Gambill and (f) Canvasback lakes. Deepest depths in both lakes receive 1% of surface 320 nm UV. Error bars are means \pm 1 SE.

until much higher UVR exposure than required to depress infectivity of the fungal parasite. *Daphnia* were less sensitive to UVR (and PRR) than were the spores. In fact, *Daphnia* survival remained high (above 70%) even when exposed to UVR and PRR conditions that caused substantial reductions in spore infectivity (Figs 1b and c; note difference in x-axis scales). The exposure level at which 50% of the *Daphnia* survived (LE_{50}) was 32 KJ/m², more than an order of magnitude greater than the 0.7 KJ/m² needed to suppress infectivity of the fungus. Thus, *Daphnia* hosts were less sensitive to UVR than were parasite spores. In the model competition, the 'power' model best described the UVR exposure-host survival relationship ($\Delta = 0$, $w = 0.995$). The 'linear' model ($\Delta = 10.7$, $w = 0.005$) and 'null' model ($\Delta = 148.3$, $w = 0$) performed poorly.

The third and fourth laboratory-based experiments confirmed the marked sensitivity of the parasite to UVR – and they also revealed an important role of PRR (Figs 1c and d). Infection significantly decreased with increasing exposure to UVR both with (experiment #3; $F_{1,42} = 12.01$, $P < 0.001$) and without PRR (experiment #4; $F_{1,42} = 22.97$, $P < 0.001$). The 'power' model captured the sharp decline in per spore infectivity in the +PRR experiment (#3: $\Delta = 0$, $w = 0.998$) more effectively than the 'linear' model ($\Delta = 12.7$, $w = 0.002$), whereas the 'null' model performed terribly ($\Delta = 50.0$, $w = 0$). Exposure of spores to UVR without PRR (experiment #4) also reduced infection prevalence – but at much higher UVR levels (almost 5 × as much, 12.7 KJ/m²; Fig. 1d). In this case, the 'power' model (#3: $\Delta = 0$, $w = 0.84$) still performed best and more flexibly captured the UVR-infectivity shape. However, the 'linear' model also captured this shape reasonably well ($\Delta = 3.3$, $w = 0.16$) because the drop in infectivity with UVR was so much less steep in experiment #3 than #4. A comparison of the estimated parameters of the models characterised this difference well (i.e. non-overlapping, profiled 95% confidence intervals around the parameters; a large negative slope and smaller exponent in the more linear experiment #3 allowed for a sharp decline with UVR; the very small slope but large exponent enabled the flatter drop of transmission with UVR until high levels of exposure in experiment #4: see Online Supplementary Information, Table S3).

The field experiment illustrated the sensitivity of spores to ambient UVR and PAR levels while accounting for some variation among the two study lakes (Figs 1e and f). Specifically, there was a significant radiation × lake interaction ($F_{2,111} = 3.60$, $P = 0.03$); light-induced decreases in infection were more pronounced in Canvasback. (At this point, we cannot explain why lake was involved in the interaction in this experimental spore incubation: both lakes have similarly high UVR penetration, and depths were chosen based on UVR penetration). When we analysed data from each lake separately, we found significant declines in infection prevalence from the –UVR to the +UVR treatments (Wald tests; Gambill: $P = 0.007$; Canvasback: $P = 0.02$). These results indicated that the negative effects of solar radiation became enhanced with UVR. Furthermore, the significant radiation × depth interaction ($F_{2,113} = 3.36$, $P = 0.04$) signalled that the negative effect of radiation on infection prevalence diminished with depth.

The field surveys revealed significant relationships between UVR and fungal epidemics. Epidemics started later in more UVR transparent lakes (lower a_{B20} ; Fig. 2a). Furthermore, lakes with lower UVR transparency had larger epidemics (Fig. 2b). Those larger epidemics started earlier in the season (Fig. 2c). Still, even when controlling for this start date–maximum prevalence relationship using partial correlation, epidemics grew larger in lakes with less UVR

penetration (partial $r = 0.6020$, $P = 0.016$). Furthermore, we see a positive relationship between a_{B20} and residuals from a linear regression fit between start date and maximum prevalence (Fig. 2d). Positive residuals imply a larger epidemic than predicted from the regression start date–epidemic size relationship, while negative residuals imply a smaller epidemic than predicted. Therefore, UVR transparent lakes had smaller epidemics than expected based on start date. UVR transparency (a_{B20}) alone explained 18.3% of variation in epidemic size, start date alone explained 16.3%, and the joint interaction between the two accounted for 33.3% (for a total of 68% explained by both factors combined).

DISCUSSION

Climate change and other types of environmental forcing are altering UVR regimes in a variety of habitats. In lakes, UVR transparency is dropping as DOC concentrations increase in many regions (Williamson & Rose 2010). Will changes in UVR exposure stimulate or inhibit epidemics of disease? Increases in UVR could stimulate disease by weakening the immune response of hosts (Giannini 1986; Goettsch *et al.* 1994). In our focal case study, however, we found no evidence for this effect. Exposure of the zooplankton host (*D. dentifera*) to low levels of UVR in the laboratory showed little influence on susceptibility to infection. This finding seems especially pertinent since this species of host typically migrates to deeper waters during day, possibly limiting its exposure to UVR (Hessen 1994; Rhode *et al.* 2001; Hylander *et al.* 2009; Williamson *et al.* 2011). In contrast, the fungal parasite (*M. bicuspidata*) showed sharp sensitivity to laboratory and natural solar sources of UVR, as seen for some other parasites [e.g. nematode larvae (van Dijk *et al.* 2009) and the two apicomplexan protozoans *Cryptosporidium parvum* (Connelly *et al.* 2007; King *et al.* 2008; Gomez-Couso *et al.* 2009) and *Isopora turdi* (Martinaud *et al.* 2009)]. This sharp drop in infectivity of fungal spores with even very low level exposure to UVR was readily captured by a 'power' model for transmission rate. Furthermore, the fungus showed similar sensitivity to natural levels of UVR present in the water column of two lakes.

These experiments also provided key insight into the mechanism behind UVR damage to the fungus. Fungal spores were damaged by both shorter wavelength UVR (UV-B and UV-A) but also by longer wavelength UV-A and PAR. In the lab experiments, infectivity of spores dropped dramatically when both UV-A and PAR were supplied in addition to UV-B and shorter wavelength UV-A (i.e. contrast Experiment #3 and #4). In the field, infectivity dropped for spores exposed to PAR only (as compared to dark controls) – even with UVR shielded. These results indicated that PAR further damaged spores. In contrast, longer wavelength UV-A and PAR help *Daphnia* repair damage from UV (Siebeck & Bohm 1991; Williamson *et al.* 2001). Thus, here the pronounced difference in the UVR sensitivity of host vs. parasite likely involved photoenzymatic repair. In photoenzymatic repair, UV-A and PAR catalyse action of an enzyme (photolyase) that repairs UV-B-damaged DNA. Like *Daphnia*, some parasites use this repair mechanism (Belosevic *et al.* 2001; Oguma *et al.* 2004; Ruelas *et al.* 2007). Yet *M. bicuspidata* apparently lacks the repair mechanism and became damaged by even UV-A and visible light. More generally, these results prompt a general hypothesis: differential use of this repair mechanism between host and parasite may drive infection prevalence and alter epidemic dynamics in contrasting UVR environments.

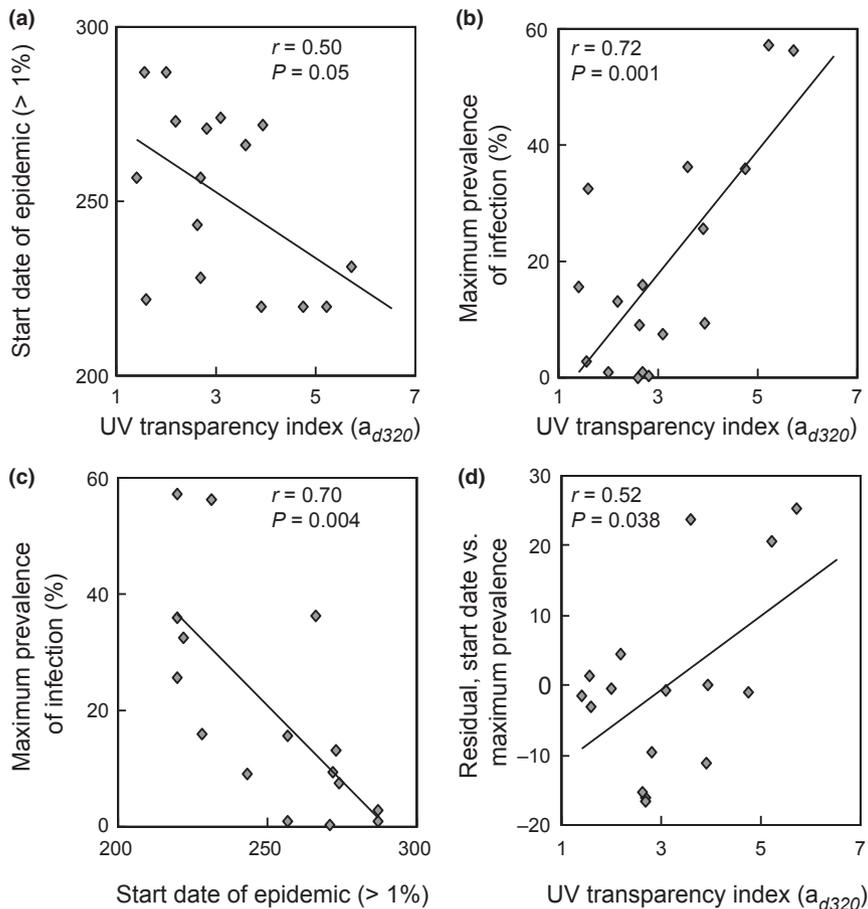


Figure 2 Relationships between an index of ultraviolet radiation (UVR) penetration (dissolved absorbance at 320 nm, expressed as the absorption coefficient, a_{d320}) and two metrics of fungal epidemics in *Daphnia* populations. Lower dissolved absorbance corresponds to higher UVR penetration. The other metrics involve start date of epidemic (i.e. ordinal date at which epidemics exceeded 1% prevalence) and maximum prevalence (%) of infection, an indicator of the size of the epidemics. Epidemics in lakes with lower UVR penetration (higher a_{d320}) (a) started earlier and (b) reached higher prevalence of infection. (c) Larger epidemics started earlier than smaller ones. (d) A positive correlation between the UVR index and residuals from the start date-max prevalence relationship. Probability values (P) accompany Pearson correlation statistics (r).

At the population level, the sharp sensitivity of the fungus to UVR – and even PAR – produced two interrelated predictions for epidemics. First, spores must remain suspended in the water column for successful transmission (i.e. they likely sink out of the hypolimnion, based on theoretical sinking calculations). Thus, UVR and PAR exposure could greatly reduce infectivity of spores before hosts eat them. As a result, lakes with higher UVR and PAR transparency should have smaller epidemics (since UVR and PAR reduce disease spread by destroying the fungus). Second, in lakes with higher UVR and PAR transparency, epidemics should start later during the transition from summer to autumn. During this seasonal transition, underwater radiation exposure levels drop from mid-summer highs due to a decline in both incident UVR as well as the transparency of the water (Morris & Hargreaves 1997; Williamson *et al.* 2007). Declining UVR and PAR exposure should, all else being equal, create more favourable habitat for fungal spores – particularly in lakes with initially higher UVR transparency. Indeed, our surveys showed that epidemics remained smaller in lakes that were more transparent to UVR and PAR (Online Supplementary Information Figs S1a and b). Furthermore, epidemics started later in lakes with higher UVR and PAR transparency. Epidemics that start later, in turn, typically remain smaller (results presented here; see also Hall *et al.* 2011 for similar results from Michigan lakes). Since the density of hosts can decline during large epidemics (Hall *et al.* 2011), these UVR- and PAR-based results may have important consequences for the population dynamics of hosts during epidemics.

In the long term, changes in concentrations of DOC due to climate change and other environmental forcing may also shape disease

prevalence among lakes. Since it absorbs UVR, DOC strongly drives UVR (and to a lesser extent PAR) transparency in lakes (Morris *et al.* 1995). In several geographic regions, DOC concentrations are rising (Findlay 2005; Evans *et al.* 2006) due to climate warming and reductions in acid precipitation (Monteith *et al.* 2007). Since DOC could protect spores from damaging solar radiation, increasing DOC levels could trigger the earlier start of larger epidemics that more extensively depress densities of hosts (all else being equal). This DOC-parasite link likely applies to other systems. For example, DOC levels modulate the damaging effects of UVR on an important protozoan parasite of humans (*Cryptosporidium parvum*; Connelly *et al.* 2007; King *et al.* 2008; Gomez-Couso *et al.* 2009). Thus, changes in DOC may alter disease in important, under-appreciated manners.

Penetration of UVR into the water column could also influence epidemics indirectly, via other players known to amplify or diminish disease. For example, increased UVR could enhance control of epidemics by vertebrate predation (Duffy & Hall 2008; Hall *et al.* 2010). UVR receptors can enhance foraging in some fish species (Leech & Johnsen 2003; Leech *et al.* 2009). As visual predators selectively prey on infected hosts, UVR-stimulated or transparency-stimulated increases in foraging could reduce disease prevalence (Johnson *et al.* 2006). Still, if UVR-sensitive fish predators or hosts avoid high UVR environments, they may exert less top-down control over epidemics. Second, the invertebrate predator *Chaoborus* may stay deeper in the water column or have lower survival in lakes with higher UVR transparency (Persaud & Yan 2003; Nagiller & Sommaruga 2009). UVR effects on *Chaoborus* matter because this predator can

enhance disease [by spreading spores (Cáceres *et al.* 2009) and/or inducing trait-mediated indirect effects that enhance susceptibility and spore yield (Duffy *et al.* 2011)]. Lakes with higher UVR penetration had fewer *Chaoborus* and smaller epidemics (Online Supplementary Information Figs S1d–f). Finally, UVR could alter the production and nutritional quality of algal resources of these zooplankton hosts (Xenopoulos *et al.* 2002, Hessen *et al.* 2008). As resource quality and quantity influence spore yield from infected hosts (Frost *et al.* 2008; Hall *et al.* 2009), UVR could influence epidemics via indirect connections with resources. All of these factors could contribute to the UVR transparency-epidemic patterns that we uncovered.

Our findings provide three important, broadly applicable insights into the ecology of infectious disease in a changing world. First, solar radiation can strongly shape disease epidemics. In this case study, UVR and PAR likely depressed epidemics through deleterious effects on the parasite. Since UVR penetration in lakes will likely decrease, epidemics may increase in the future – at least where UVR currently inhibits disease. Second, it is informative to simultaneously estimate UVR (and even PAR) sensitivity of epidemiologically relevant traits for both host and parasite (as recommended by Lafferty & Holt 2003 for environmental stressors in general). Here, that comparison revealed potent, mechanistic insight into why the fungal parasite responded so sensitively to UVR. How many other environmentally transmitted parasites are harmed by UV-B or by longer wavelength UV-A and PAR used by hosts to repair UVB-induced damage? Focus on this question could enhance predictive insight into UVR-disease links. Third, our field data suggest indirect effects of UVR on disease, mediated through community ecology. If other UVR-sensitive species amplify or decrease disease, it may become crucial to quantify these indirect pathways through which UVR influences epidemics.

AUTHOR CONTRIBUTIONS

EO, SH, CW, MD, CM and CC designed the research. EO, CM and SH performed the experiments, while SH and MD directed the field survey. EO, SH and CW wrote early drafts while all authors helped finalise the paper.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 Analysis of deviance results from GLM analyses of Experiments #1 and #5. (*indicates results significant at 0.05 level).

Table S2 Results from competition among hypotheses that model how transmission rate (host susceptibility and per spore infectivity) depends on exposure of fungal spores and/or host *Daphnia* to different levels of ultraviolet radiation (UV) and photorepair radiation (PRR).

Table S3 Maximum likelihood-based parameter estimates for the best fitting models described in Table S2.

Figure S1 Relationships between the UV transparency index, ad_{320} , and metrics of fungal epidemics [maximal prevalence and start time (ordinal date)] with two factors (a)–(c) light extinction coefficient, and (d)–(f) density of the invertebrate predator *Chaoborus*.

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