



*J. Plankton Res.* (2020) 00(00): 1–4. doi:10.1093/plankt/fbz070

## BRIEF COMMUNICATION

# Light exposure decreases infectivity of the *Daphnia* parasite *Pasteuria ramosa*

ERIN P. OVERHOLT<sup>1,\*</sup>, MEGHAN A. DUFFY<sup>2</sup>, MATTHEW P. MEEKS<sup>1</sup>, TAYLOR H. LEACH<sup>1</sup> AND CRAIG E. WILLIAMSON<sup>1</sup>

<sup>1</sup>DEPARTMENT OF BIOLOGY, MIAMI UNIVERSITY, 501 E. HIGH STREET, OH 45056, USA AND <sup>2</sup>DEPARTMENT OF ECOLOGY AND EVOLUTIONARY BIOLOGY, UNIVERSITY OF MICHIGAN, 500 S. STATE STREET, MI 48109, USA

\*CORRESPONDING AUTHOR: overhoep@miamioh.edu

Received October 1, 2019; editorial decision November 30, 2019; accepted November 30, 2019

Corresponding editor: John Dolan

Climate change is altering light regimes in lakes, which should impact disease outbreaks, since sunlight can harm aquatic pathogens. However, some bacterial endospores are resistant to damage from light, even surviving exposure to UV-C. We examined the sensitivity of *Pasteuria ramosa* endospores, an aquatic parasite infecting *Daphnia* zooplankton, to biologically relevant wavelengths of light. Laboratory exposure to increasing intensities of UV-B, UV-A, and visible light significantly decreased *P. ramosa* infectivity, though there was no effect of spore exposure on parasitic castration of infected hosts. *P. ramosa* is more sensitive than its *Daphnia* host to damage by longer wavelength UV-A and visible light; this may enable *Daphnia* to seek an optimal light environment in the water column, where both UV-B damage and parasitism are minimal. Studies of pathogen light sensitivity help us to uncover factors controlling epidemics in lakes, which is especially important given that water transparency is decreasing in many lakes.

KEYWORDS: zooplankton; UV; endospore; pathogen

### INTRODUCTION

Changing temperature and precipitation related to climate change are altering disease dynamics. One factor that plays a role is declining water transparency, since ultraviolet light penetration into lakes has germicidal effects (Williamson *et al.*, 2017). Thus, by decreasing light penetration in lakes, climate change has the potential to promote epidemics.

However, while we know that many microbes are harmed by exposure to light, we also know some tolerate light remarkably well. Endospores, a resting stage found only in Gram positive bacteria of the group Firmicutes, are highly resistant to disinfecting techniques (Nicholson *et al.*, 2000), including high levels of UV-C radiation (Newcombe *et al.*, 2005). Surprisingly though, in the endospore form, the highly studied *Bacillus* showed

decreased survival across a wide range of exposures, from UV-B to full sunlight (Xue and Nicholson, 1996). Other pathogens have shown a similar sensitivity to longer wavelengths of light. For example, an aquatic pathogen—the fungus *Metschnikowia*—was sensitive to solar radiation even in the absence of UV, and field surveys showed larger epidemics in less transparent lakes (Overholt *et al.*, 2012).

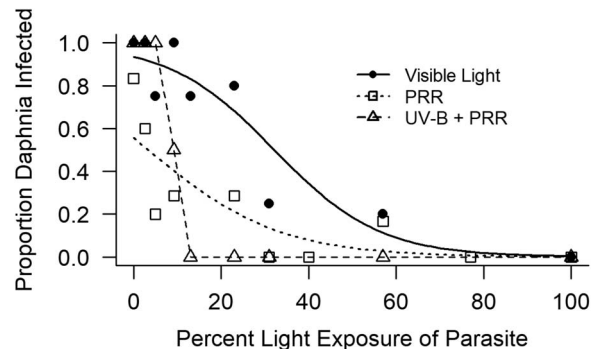
Light can harm pathogens, and climate change is altering light regimes in lakes. Thus, investigations into how aquatic pathogens and their hosts respond to light are needed to better understand and predict disease dynamics. Here, we test whether endospores of the virulent bacterial pathogen *Pasteuria ramosa* are sensitive to biologically relevant wavelengths of light, and if light exposure decreases its ability to lower fecundity in infected *Daphnia* hosts.

## METHOD

We exposed *P. ramosa* to different environmentally realistic light conditions in the laboratory and measured subsequent pathogen infectivity and host reproduction in *Daphnia dentifera*. Shallow quartz dishes containing 25 mL aliquots of *P. ramosa* spores (2000 spores mL<sup>-1</sup>) were placed on a rotating wheel (2 rpm) for 12 h at 24°C in a UV-lamp phototron (Williamson *et al.*, 2001) and exposed to different levels of biologically relevant UV-B, UV-A and visible light. In experiment 1, we examined the infectivity of *P. ramosa* under 10 intensities of photorepair radiation (PRR, comprised of UV-A and visible light), which stimulates repair of UV-damaged DNA (eight replicates per treatment). Experiment 2 used a two-way factorial design to measure the effects of light wavelength and intensity on pathogen infectivity and host fecundity. Spores were exposed to either UV-B and PRR or visible light only at nine intensity levels (five replicates per treatment) (see supplement for additional details).

Following exposure in the phototron in both experiments, dishes were removed and a single, week-old *D. dentifera* neonate was placed in each dish with the exposed *P. ramosa* spores. After 3 days, *D. dentifera* were transferred to 30 mL of spore-free, filtered lake water. In both experiments, offspring were removed during water changes. In experiment 2, neonates were quantified during water changes and eggs in the brood chamber counted on day 25. After 25 days, individuals were examined for infection. Animals that died before infections were detected were not included in the analysis.

We used a binomial logistic regression model to test the effects of increasing light intensity on infectivity for each light treatment. A two-way ANOVA was used to test the effect of light treatment and intensity on the number of neonates produced. Analyses were conducted using R version 3.4.4 (R Core Development Team).



**Fig. 1.** Proportion of *Daphnia* infected at each exposure level of the pathogen to visible light, photorepair radiation (PRR, UV-A and visible light) or UV-B + PRR. Lines represent the fitted logistic regression model for the visible light and PRR treatments. When UV was blocked, the proportion of *D. dentifera* infected significantly decreased with increasing exposure of the pathogen (experiment 2: visible light,  $P < 0.01$ ); the logistic model fit indicated that there was an 8.0% decreased probability of infection for every 1% increase in light exposure. We also found a significant negative relationship between light intensity and infectivity when the pathogen was exposed to increasing levels of PRR (experiment 1: PRR,  $P < 0.01$ ); the logistic model indicated that for every 1% increase in PRR exposure, the probability of *D. dentifera* infection decreased by 6.5%. When exposed to the full spectrum of light (experiment 2: UV-B + PRR), there was also a decline in infectivity; this model would not converge due to the sharp cut off in proportion infected, so the line does not represent the fitted model, but instead simply connects the points.

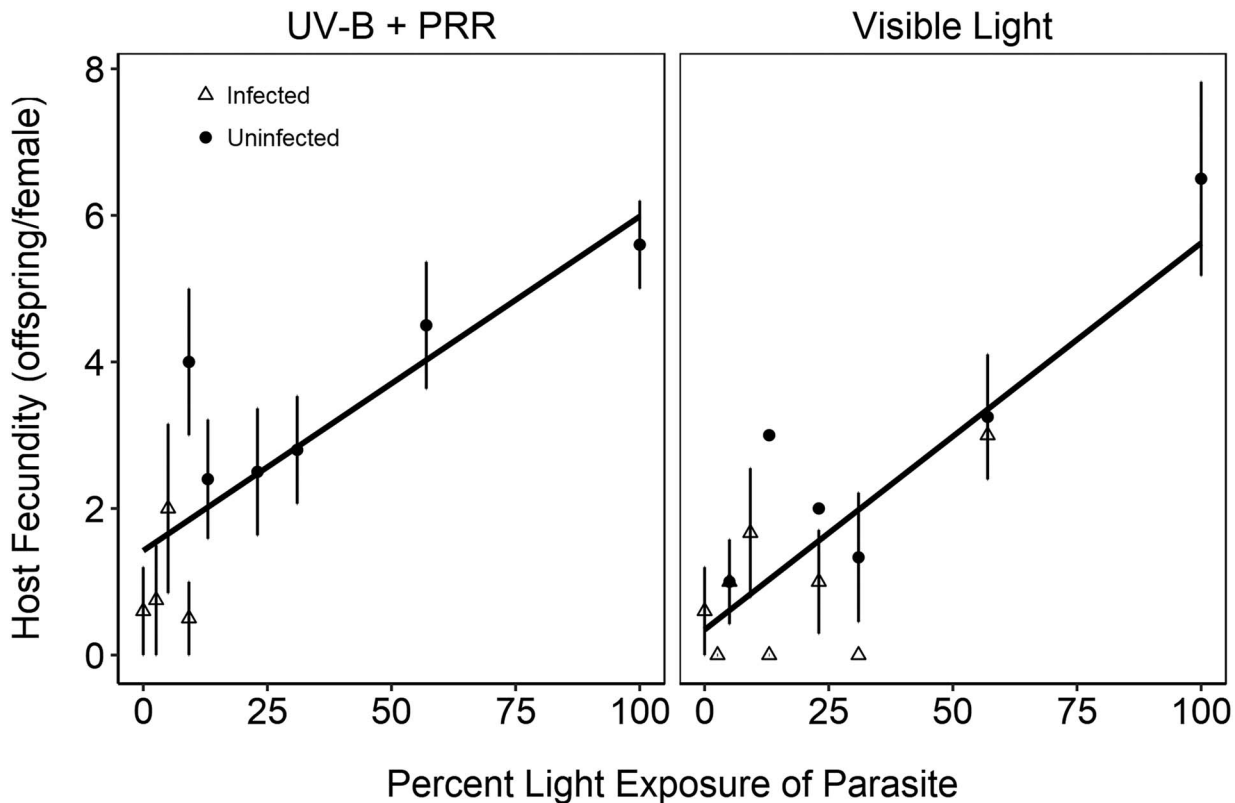
## RESULTS

Light exposure greatly decreased parasite infectivity: in all three wavelength treatments, the highest rates of infection were in the dark (0% light) and decreased with increasing light exposure of the pathogen (Fig. 1). The fecundity of *Daphnia* also increased with increasing exposure of *P. ramosa* to light, but, for hosts that became infected, there were no detectable changes in the number of neonates produced over the small range of exposures where infections occurred (Fig. 2).

## DISCUSSION

We found that endospores of *P. ramosa* are surprisingly susceptible to longer wavelength UV-B, UV-A and even visible light. This influence of light on spores benefitted *Daphnia* by decreasing infection: hosts that were exposed to spores that had been exposed to more light were less likely to become infected and, therefore, produced more offspring. When only infected hosts were considered, spore light exposure did not alter host reproduction. However, the small range of exposure levels at which infected hosts were observed provides little power to detect an effect.

Other aquatic pathogens are sensitive to longer wavelengths of light as well. For example, *Cryptosporidium* cysts lost infectivity following exposure to UV (Connelly *et al.*,



**Fig. 2.** Fecundity (neonates plus eggs on day 25 per female) of infected (triangles) and uninfected (circles) *Daphnia* in experiment 2 under conditions of UV-B + PRR or visible light. Overall, *Daphnia* fecundity increased when spores were exposed to higher intensities (intensity  $P < 0.001$ , light treatment  $P = 0.04$ ), but, for hosts that became infected, there was no difference in the number of neonates produced in the different treatments (intensity  $P = 0.12$ ; light treatment  $P = 0.41$ ), though the narrow range of light exposures in which there were infected hosts led to low power to detect an effect. Error bars represent means  $\pm 1$  SE.

2007; King *et al.*, 2008) and visible light (Connelly *et al.*, 2007). Natural sunlight caused additional sublethal effects on the protein secretion required by *Cryptosporidium* for attachment to its host (King *et al.*, 2010). The fungal parasite *Metschnikowia*, which can be found in the same lake systems as *P. ramosa*, was also sensitive to both short wavelength UV-B, longer wavelength UV-A and visible light in both laboratory and field studies (Overholt *et al.*, 2012). Another field study suggested that *Pasteuria* was susceptible to solar radiation; however, sensitivity to visible light was not specifically tested (Shaw, 2019). By decreasing the infectivity of spores, light exposure may also affect reproduction through incomplete parasitic castration, which can result from low spore dose (Ebert *et al.*, 2004).

In our study, *P. ramosa* exhibited decreased infectivity even under long wavelength UV-A and visible light, in the absence of UV-B, though shorter wavelengths overall caused the greatest decrease in infectivity. In contrast, these longer wavelengths benefit the host, *Daphnia*, by stimulating photorepair of DNA damage (Macfadyen *et al.*, 2004). Since the same wavelengths that damage

the pathogen can benefit the host, *Daphnia* may be able to find a refuge from disease and damaging UV-B at intermediate depths in the water column where UV-A and visible light levels are high, but damaging UV-B is less intense. However, many cues influence *Daphnia* position in the water column, and avoidance of visual predators in the surface waters may actually increase infection risk in some systems (Decaestecker *et al.*, 2002). Future studies on the relative positions of *Daphnia* and different natural enemies in the water column would help to uncover how they balance different risks.

## CONCLUSION

Lakes in many regions are experiencing lower intensity light regimes due to increased dissolved organic matter inputs and/or eutrophication (Monteith *et al.*, 2007; Solomon *et al.*, 2015; Williamson *et al.*, 2015; Strock *et al.*, 2017). Our finding that the common bacterial pathogen *P. ramosa* is sensitive to both UV and visible light suggests that decreases in lake transparency through “browning” and/or “greening,” may allow for increased *P. ramosa*

prevalence. Climate change induced increases in thermal stratification and decreases in transparency in lakes may also alter the exposure of both parasites and hosts to sunlight (Williamson *et al.*, 2019).

## SUPPLEMENTARY DATA

Supplementary data are available at Journal of Plankton Research online.

## ACKNOWLEDGEMENTS

We thank Rebecca Healy for preliminary *Pasteuria* work and three anonymous reviewers whose comments improved the manuscript.

## FUNDING

This work was supported by the US National Science Foundation (grant number NSF-DEB 1305836 to M.A.D. and NSF-DEB 1754276 to C.E.W.) and Miami University Eminent Scholar Funds to C.E.W. and E.P.O.

## REFERENCES

- Connelly, S. J., Wolyniak, E. A., Williamson, C. E. and Jellison, K. L. (2007) Artificial UV-B and solar radiation reduce in vitro infectivity of the human pathogen *Cryptosporidium parvum*. *Environ. Sci. Technol.*, **41**, 7101–7106.
- Ebert, D., Carius, H. J., Little, T. and Decaestecker, E. (2004) The evolution of virulence when parasites cause host castration and gigantism. *Am. Nat.*, **164**, S19–S32.
- Decaestecker, E. L., De Meester, L. and Ebert, D. 2002. In deep trouble: habitat selection constrained by multiple enemies in zooplankton. *Proc. Natl. Acad. Sci. U.S.A.*, **99**, 5481–5485.
- King, B. J., Hoefel, D., Daminato, D. P., Fanok, S. and Monis, P. T. (2008) Solar UV reduces *Cryptosporidium parvum* oocyst infectivity in environmental waters. *J. Appl. Microbiol.*, **104**, 1311–1323.
- King, B. J., Hoefel, D., Wong, P. E. and Monis, P. T. (2010) Solar radiation induces non-nuclear perturbations and a false start to regulated exocytosis in *Cryptosporidium parvum*. *Plos One*, **5**, e11773.
- Macfadyen, E. J., Williamson, C. E., Grad, G., Lowery, M., Jeffrey, W. H. and Mitchell, D. L. (2004) Molecular response to climate change: temperature dependence of UV-induced DNA damage and repair in the freshwater crustacean *Daphnia pulex*. *Global Change Biol.*, **10**, 408–416.
- Monteith, D. T., Stoddard, J. L., Evans, C. D., De Wit, H. A., Forsius, M., Högåsen, T., Wilander, A., Skjelkvåle, B. L. *et al.* (2007) Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, **450**, 537–541.
- Newcombe, D. A., Schuerger, A. C., Benardini, J. N., Dickinson, D., Tanner, R. and Venkateswaran, K. (2005) Survival of spacecraft-associated microorganisms under simulated martian UV irradiation. *Appl. Environ. Microbiol.*, **71**, 8147–8156.
- Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J. and Setlow, P. (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol. Mol. Biol. Rev.*, **64**, 548–572.
- Overholt, E. P., Hall, S. H., Williamson, C. E., Meikle, C. K., Duffy, M. A. and Cáceres, C. E. (2012) Solar radiation decreases parasitism in *Daphnia*. *Ecol. Lett.*, **15**, 47–54.
- Shaw, C. L. (2019) Drivers of epidemic timing and size in a natural aquatic system. *Doctoral dissertation, University of Michigan, Ann Arbor, MI, USA*. Retrieved from <https://deepblue.lib.umich.edu/handle/2027.42/151685>.
- Solomon, C. T., Jones, S. E., Weidel, B. C., Buffam, I., Fork, M. L., Karlsson, J., Larsen, S., Lennon, J. T. *et al.* (2015) Ecosystem consequences of changing inputs of terrestrial dissolved organic matter to lakes: current knowledge and future challenges. *Ecosystems*, **18**, 376–389.
- Strock, K. E., Theodore, N., Gawley, W. G., Ellsworth, A. C. and Saros, J. E. (2017) Increasing dissolved organic carbon concentrations in northern boreal lakes: implications for lake water transparency and thermal structure. *J. Geophys. Res.: Biogeosci.*, **122**, 1022–1035.
- Williamson, C. E., Madronich, S., Lal, A., Zepp, R. E., Lucas, R. M., Overholt, E. P., Rose, K. C., Schladow, G. *et al.* (2017) Climate change-induced increases in precipitation are reducing the potential for solar ultraviolet radiation to inactivate pathogens in surface waters. *Sci. Rep.*, **7**, 13033.
- Williamson, C. E., Neale, P. J., Grad, G., De Lange, H. J. and Hargreaves, B. R. (2001) Beneficial and detrimental effects of UV on aquatic organisms: implications of spectral variation. *Ecol. Appl.*, **11**, 1843–1857.
- Williamson, C. E., Neale, P. J., Hylander, S., Rose, K. C., Figueroa, F. L., Robinson, S. A., Hader, D. P., Wangberg, S. A. *et al.* (2019) The interactive effects of stratospheric ozone depletion, UV radiation, and climate change on aquatic ecosystems. *Photochem. Photobiol. Sci.*, **18**, 717–746.
- Williamson, C. E., Overholt, E. P., Pilla, R. M., Leach, T. H., Brenttrup, J. A., Knoll, L. B., Mette, E. M. and Moeller, R. E. (2015) Ecological consequences of long-term browning in lakes. *Sci. Rep.*, **5**, 18666.
- Xue, Y. M. and Nicholson, W. L. (1996) The two major spore DNA repair pathways, nucleotide excision repair and spore photoproduct lyase, are sufficient for the resistance of *Bacillus subtilis* spores to artificial UV-C and UV-B but not to solar radiation. *Appl. Environ. Microbiol.*, **62**, 2221–2227.