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

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7   KEYWORDS

8   zooplankton, UV, endospore, pathogen

9   ABSTRACT

10   Climate change is altering light regimes in lakes, which should impact disease outbreaks, since sunlight

11   can harm aquatic pathogens. However, some bacterial endospores are resistant to damage from light,

12   even surviving exposure to UV-C. We examined the sensitivity of *Pasteuria ramosa* endospores, an

13   aquatic parasite infecting *Daphnia* zooplankton, to biologically relevant wavelengths of light.

14   Laboratory exposure to increasing intensities of UV-B, UV-A, and visible light significantly decreased

15   *P. ramosa* infectivity, though there was no effect of spore exposure on parasitic castration of the host.

16   *P. ramosa* is more sensitive than its *Daphnia* host to damage by longer wavelength UV-A and visible

17   light; this may enable *Daphnia* to seek an optimal light environment in the water column where both

18   UV-B damage and parasitism are minimal. Studies of pathogen light sensitivity help us uncover

19   factors controlling epidemics in lakes, which is especially important given that water transparency is

20   decreasing in many lakes.

21   INTRODUCTION

22   Changing temperature and precipitation related to climate change are altering disease dynamics.

23   One factor that plays a role is declining water transparency, since ultraviolet light penetration into lakes

24   has germicidal effects (Williamson et al. 2017). Thus, by decreasing light penetration in lakes, climate

25   change has the potential to promote epidemics.

26   However, while we know that many microbes are harmed by exposure to light, we also know

27   some tolerate light remarkably well. Endospores, a resting stage found only in Gram positive bacteria

28   of the group Firmicutes, are highly resistant to disinfecting techniques (Nicholson *et al.*, 2000),

29   including high levels of UV-C radiation (Newcombe *et al.*, 2005). Surprisingly though, in the

30   endospore form, the highly studied *Bacillus* showed decreased survival across a wide range of

31   exposures, from UV-B to full sunlight (Xue and Nicholson, 1996). Other pathogens have shown a

32   similar sensitivity to longer wavelengths of light. For example, an aquatic pathogen - the fungus

33   *Metschnikowia* - was sensitive to solar radiation even in the absence of UV, and field surveys showed

34   larger epidemics in less transparent lakes (Overholt *et al.*, 2012).

35   Light can harm pathogens, and climate change is altering light regimes in lakes. Thus,

36   investigations into how aquatic pathogens and their hosts respond to light are needed to better

37   understand and predict disease dynamics. Here we test whether endospores of the virulent bacterial

38   pathogen *Pasteuria ramosa* are sensitive to biologically relevant wavelengths of light, and if light

39   exposure decreases its ability to lower fecundity in infected *Daphnia* hosts.

40   METHODS

41   We exposed *P. ramosa* to different environmentally realistic light conditions in the laboratory

42   and measured subsequent pathogen infectivity and host reproduction in *Daphnia dentifera*. Shallow

43   quartz dishes containing 25 mL aliquots of *P. ramosa* spores (2000 spores mL^{-1}) were placed on a

44   rotating wheel (2 rpm) for 12 h at 24°C in a UV-lamp phototron and exposed to different levels of

45   biologically relevant UV-B, and photorepair radiation (PRR, comprised of UV-A and visible light),

46   which stimulates repair of UV-damaged DNA (Williamson *et al.*, 2001). In experiment 1, we

47   examined the infectivity of *P. ramosa* under 10 intensities of PRR (8 replicates per treatment).

48   Experiment 2 used a two-way factorial design to measure the effects of light wavelength and intensity

49   on pathogen infectivity and host fecundity. Spores were exposed to either UVB and PRR or visible

50   light only at nine intensity levels. For the exposure, spores (2000 spores mL^{-1}) were aliquoted into five

51   replicates of each treatment (intensity x light type). (See supplement for additional details.)

52   Following exposure in the phototron, dishes were removed and a single, week-old *D. dentifera*

53   neonate was placed in each dish with the exposed *P. ramosa* spores. After three days, *D. dentifera*

54   were transferred to 50 mL beakers and filled with 30 mL of spore-less, filtered lake water. In both

55   experiments, offspring were removed during water changes. In experiment 2, neonates were quantified

56   during water changes and eggs in the brood chamber counted on day 25. After 25 days, individuals

57   were examined for infection.

58   We used a binomial logistic regression model to test the effects of increasing light intensity on

59   infectivity for each light treatment. A two-way ANOVA was used to test the effect of light treatment

60   and intensity on the number of neonates produced. Analyses were conducted using R version 3.4.4 (R

61   Core Development Team).

62   RESULTS

63   Light exposure greatly decreased parasite infectivity: In both experiments, the highest rates of

64   infection were in the dark (0% light) and decreased with increasing light exposure of the pathogen (Fig.

65   1). The fecundity of *Daphnia* also increased with increasing exposure of *P. ramosa* to light, but, for

66   hosts that became infected, there was no difference in the number of neonates produced (Fig. 2).

67   DISCUSSION

68   We found that endospores of *P. ramosa* are surprisingly susceptible to longer wavelength UV-

69   B, UV-A and even visible light. This influence of light on spores benefitted *Daphnia* via impacts on

70   infection: hosts that were exposed to spores that had been exposed to more light were less likely to

71   become infected and, therefore, produced more offspring. However, when just infected hosts were

72   considered, spore light exposure did not alter host reproduction.

73   Other aquatic pathogens are sensitive to longer wavelengths of light as well. For example,

74   *Cryptosporidium* cysts lost infectivity following exposure to UV (Connelly *et al.*, 2007; King *et al.*,

75   2008) and visible light (Connelly *et al.*, 2007). Natural sunlight caused additional sublethal effects on

76   the protein secretion required by *Cryptosporidium* for attachment to its host (King *et al.*, 2010). The

77   fungal parasite *Metschnikowia*, which can be found in the same lake systems as *P. ramosa*, was also

78   sensitive to both short wavelength UV-B as well as longer wavelength UV-A and visible light in both

79   laboratory and field studies (Overholt *et al.*, 2012). Another field study suggested that *Pasteuria* was

80   susceptible to solar radiation; however sensitivity to visible light was not specifically tested (C. L.

81   Shaw, unpublished data).

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82   In our study, *P. ramosa* exhibited decreased infectivity even under long wavelength UV-A and

83   visible light, in the absence of UV-B, though shorter wavelengths overall caused the greatest decrease

84   in infectivity. In contrast, these longer wavelengths benefit the host, *Daphnia*, by stimulating

85   photorepair of DNA damage (Sancar, 1994). Since the same wavelengths that damage the pathogen

86   can benefit the host, *Daphnia* may be able to find a refuge from disease and damaging UV-B at

87   intermediate depths in the water column where UV-A and visible light levels are high, but damaging

88   UV-B is less intense.

89   CONCLUSIONS

90   Lakes in many regions are experiencing lower intensity light regimes due to increased dissolved

91   organic matter inputs and/or eutrophication (Monteith *et al.*, 2007; Solomon *et al.*, 2015; Strock *et al.*,

92   2017; Williamson *et al.*, 2017; Williamson *et al.*, 2015). Our finding that the common bacterial

93   pathogen *P. ramosa* is sensitive to both UV and visible light suggests that decreases in lake

94   transparency through "browning" and/or "greening," may allow for increased *P. ramosa* prevalence.

95

96   SUPPLEMENTARY DATA

97   Supplementary data will be available online after publication.

98

99   ACKNOWLEDGMENTS

105   REFERENCES

106   Connelly, S. J., Wolyniak, E. A., Williamson, C. E. and Jellison, K. L. (2007) Artificial UV-B and

107   solar radiation reduce in vitro infectivity of the human pathogen *Cryptosporidium parvum*.

108   *Environ. Sci. Technol.,* **41,** 7101-7106.

109   King, B. J., Hoefel, D., Daminato, D. P., Fanok, S. and Monis, P. T. (2008) Solar UV reduces

110   *Cryptosporidium parvum* oocyst infectivity in environmental waters. *J. Appl. Microbiol.,* **104,**

111   1311-1323.

112   King, B. J., Hoefel, D., Wong, P. E. and Monis, P. T. (2010) Solar radiation induces non-nuclear

113   perturbations and a false start to regulated exocytosis in *Cryptosporidium parvum*. *Plos One,* **5**.

114   Monteith, D. T., Stoddard, J. L., Evans, C. D., De Wit, H. A., Forsius, M., Høgåsen, T., Wilander, A.,

115   Skjelkvåle, B. L., Jeffries, D. S., Vuorenmaa, J., Keller, B., Kopácek, J. and Vesely, J. (2007)

116   Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry.

117   *Nature,* **450,** 537-541.

118   Newcombe, D. A., Schuerger, A. C., Benardini, J. N., Dickinson, D., Tanner, R. and Venkateswaran,

119   K. (2005) Survival of spacecraft-associated microorganisms under simulated martian UV

120   irradiation. **71,** 8147-8156.

121   Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J. and Setlow, P. (2000) Resistance of

122   *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol. Mol.*

123   *Biol. Rev.,* **64,** 548-572.

124   Overholt, E. P., Hall, S. H., Williamson, C. E., Meikle, C. K., Duffy, M. A. and Cáceres, C. E. (2012)

125   Solar radiation decreases parasitism in *Daphnia*. *Ecol. Lett.,* **15,** 47-54.

126   Sancar, A. (1994) Mechanisms of DNA Excision-Repair. *Science,* **266,** 1954-1956.

127   Solomon, C. T., Jones, S. E., Weidel, B. C., Buffam, I., Fork, M. L., Karlsson, J., Larsen, S., Lennon, J.

128   T., Read, J. S., Sadro, S. and Saros, J. E. (2015) Ecosystem consequences of changing inputs of

129   terrestrial dissolved organic matter to lakes: Current knowledge and future challenges.

130   *Ecosystems,* **18,** 376-389.

131   Strock, K. E., Theodore, N., Gawley, W. G., Ellsworth, A. C. and Saros, J. E. (2017) Increasing

132   dissolved organic carbon concentrations in northern boreal lakes: Implications for lake water

133   transparency and thermal structure. *J. Geophys. Res.: Biogeosci.,* **122,** 1022-1035.

134   Williamson, C. E., Madronich, S., Lal, A., Zepp, R. E., Lucas, R. M., Overholt, E. P., Rose, K. C.,

135   Schladow, G. and Lee-Taylor, J. (2017) Climate change-induced increases in precipitation are

136   reducing the potential for solar ultraviolet radiation to inactivate pathogens in surface waters.

137   *Sci. Rep.,* **7**.

138   Williamson, C. E., Neale, P. J., Grad, G., De Lange, H. J. and Hargreaves, B. R. (2001) Beneficial and

139   detrimental effects of UV on aquatic organisms: Implications of spectral variation. *Ecol. Appl.,*

140   **11,** 1843-1857.

141   Williamson, C. E., Overholt, E. P., Pilla, R. M., Leach, T. H., Brentrup, J. A., Knoll, L. B., Mette, E.

142   M. and Moeller, R. E. (2015) Ecological consequences of long-term browning in lakes. *Sci.*

143   *Rep.,* **5,** 18666.

144   Xue, Y. M. and Nicholson, W. L. (1996) The two major spore DNA repair pathways, nucleotide

145   excision repair and spore photoproduct lyase, are sufficient for the resistance of Bacillus subtilis

146   spores to artificial UV-C and UV-B but not to solar radiation. *Appl. Environ. Microbiol.,* **62,**

147   2221-2227.

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148   Fig. 1. Proportion of *Daphnia* infected at each exposure level of the pathogen to visible light,

149   photorepair radiation, or UV-B + photorepair. Lines represent the fitted logistic regression model for

150   the visible light and photorepair radiation treatments. When UV was blocked, the proportion of *D.*

151   *dentifera* infected significantly decreased with increasing exposure of the pathogen (experiment 2

152   Visible Light, $p < 0.01$); the logistic model fit indicated that there was an 8.0% decreased probability of

153   infection for every 1% increase in light exposure. We also found a significant negative relationship

154   between light intensity and infectivity when the pathogen was exposed to increasing levels of

155   photorepair radiation (experiment 1 Photorepair Radiation, $p < 0.01$); the logistic model indicated that

156   for every 1% increase in PRR exposure the probability of *D. dentifera* infection decreased by 6.5%.

157   When exposed to the full spectrum of light (experiment 2 UV-B + Photorepair), there was also a

158   decline in infectivity; this model would not converge due to sharp cut off in proportion infected, so the

159   line does not represent the fitted model, but instead simply connects the points.

160   Fig. 2: Fecundity (total neonates plus eggs on day 25) of infected (triangles) and uninfected (circles)

161   *Daphnia* in experiment 2. Overall, *Daphnia* fecundity increased when spores were exposed to higher

162   intensities (intensity $p < 0.001$, light treatment $p = 0.03$), but, for hosts that became infected, there was

163   no difference in the number of neonates produced in the different treatments (intensity $p = 0.37$; light

164   treatment $p = 0.57$).   The lines represent linear regressions with shared 95% confidence intervals for

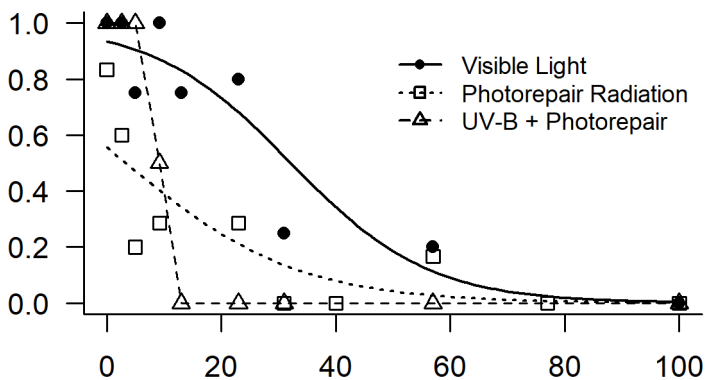165   infected and uninfected individuals.

SUPPLEMENTAL METHODS

D. dentifera were isolated from Bishop Lake (Livingston County, MI, USA) and maintained on a diet of *Ankistrodesmus falcatus* at 24°C on a 16:8 light: dark cycle throughout the experiment. A single clone of the host species was used to standardize the susceptibility of the host organism to the bacterial *P.ramosa* strains.

PRR was supplied by two 40-W UV-A bulbs (Q-Lab QUV UV-A, Q-Lab, Cleveland, OH) and two fluorescent, 40 W, cool white tube bulbs. Light intensity was manipulated using mesh screens as neutral density filters. A total of ten intensity treatments, with eight replicates each, were used in experiment 1 (0%, 2.6%, 5%, 9.2%, 23%, 31%, 40%, 57%, 77%, 100%).

In experiment 2, PRR and UV-B were crossed factorially. Covering the UV-B lamp (Spectronics XX15B, Rochester, New York) with acetate removed wavelengths less than 295 nm, allowing only biologically relevant wavelengths to be transmitted. The addition of light filters on individual dishes either transmitted UV (creating the "UV-B + photorepair" treatment) or blocked all UV (creating the "visible light" treatment). In the UV-B + photorepair treatment, a plastic filter (printed acetate, transmitting 91% photosynthetically active radiation (PAR) 400–700 nm, 87% UV-A 320–399 nm, and 70% UV-B 295–319 nm, in air) allowed full-spectrum exposure while keeping the starting intensity between UV-B + photorepair and visible light treatments similar. In the visible light treatment, Courtgard (CP Films, Inc., Martinsville, VA, USA) transmitted visible light, but blocked most UV (in air, transmits 87% of 400–700 nm PAR, 0% UV-B 295–319 nm and 6% of UV-A 320–399 nm). Similar to experiment 1, mesh screens acting as neutral density filters were again used to create nine intensity levels (0%, 2.6%, 5%, 9.2%, 13%, 23%, 31%, 57%, 100%).
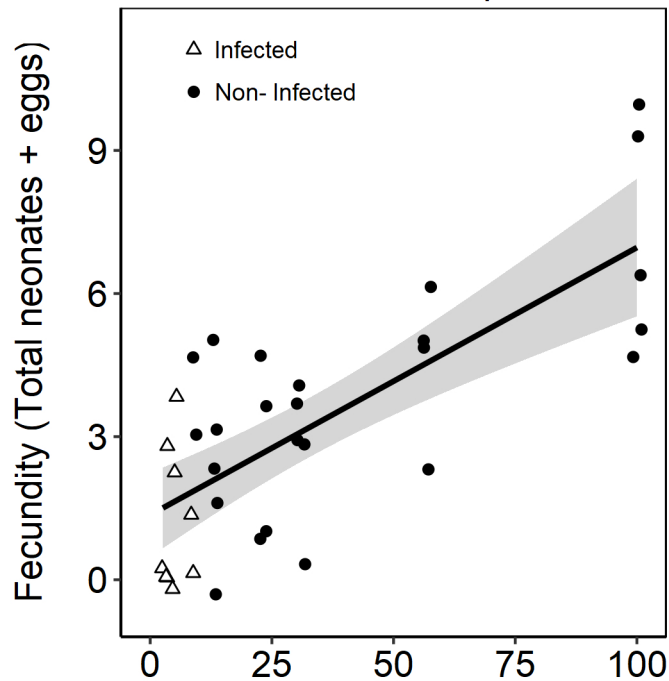
When *D. dentifera* were exposed to spores, all dishes were placed in an incubator at 24°C on a 16:8 light (cool white bulbs): dark cycle. Individual *D. dentifera* were fed *A. falcatus* (1 x 10$_3$ cells mL$_1$) daily for three days. After three days, individuals were moved to clean, spore-free water. Individuals were then fed daily, and the water was changed every three days.