Photochemistry and Photobiology, 2006, 82: 547–550

Sublethal Exposure to UV Radiation Affects Respiration Rates of the Freshwater Cladoceran *Daphnia catawba*

Janet M. Fischer*[1], Peter A. Fields[1], Peter G. Pryzbylkowski[1], Jaclyn L. Nicolai[1] and Patrick J. Neale[2]

[1]Department of Biology, Franklin & Marshall College, Lancaster, PA
[2]Smithsonian Environmental Research Center, Edgewater, MD

Received 30 August 2005; accepted 15 November 2005; published online 16 November 2005 DOI: 10.1562/2005-08-30-RA-664

ABSTRACT

We examined the effects of UV radiation (UVR) on metabolic rates of the freshwater cladoceran *Daphnia catawba*. We exposed *D. catawba* to UVB for 12 h in a lamp phototron at levels of 2.08 and 4.16 kJ m^{-2} both with and without concomitant exposure to UVA and visible photorepair radiation (PRR). We also included a group that received PRR only and a dark control group. Respiration rates were measured for 6 h following exposure. Respiration rates increased by 31.8% relative to the dark control at the lowest level of UVB stress (2.08 kJ m^{-2} UVB with PRR), whereas respiration was inhibited by 70.3% at the highest stress level (4.16 kJ m^{-2} UVB without PRR). Survival rates in the group that received PRR only and the group exposed to 2.08 kJ m^{-2} and PRR were not significantly different from that in the control group; however, the survival rate was reduced for all other UVR exposures. We hypothesize that enhanced respiration rates reflect energetic costs related to repair of cellular components damaged by sublethal levels of UVR. Increases in respiration rate of the magnitude we found in our experiment could significantly reduce energetic reserves available for growth and reproduction, especially in cases where these costs are incurred repeatedly during a series of days with high levels of UVR.

INTRODUCTION

The detrimental effects of UV radiation (UVR) on survival of aquatic organisms have been documented for a wide range of taxa and ecosystems (1,2). However, UVR effects factors other than the mortality rate for aquatic biota (3) and many studies have revealed subtler responses to UVR in the field. For example, yellow perch spawn deeper in systems with high UVR exposure (4) and the freshwater cladoceran *Daphnia catawba* adopt a deeper daytime vertical distribution in the presence of UVR (5–7). In addition to studies that have shown behavioral changes associated with UVR exposure, several recent studies have documented a metabolic response to UV exposure (8,9). However, the nature of the metabolic response to UVR is inconsistent among studies. Respiration rates of juvenile rainbow trout increase with UV exposure (8), whereas maximum routine respiration rates and metabolic scope of vendace and whitefish larvae decrease in response to UVR (9). In our own preliminary studies, we observed elevated respiration rates in *D. catawba* at low UVR levels but decreases in respiration rates at higher exposure levels.

Metabolic responses to UVR may be mediated by a number of factors, including the need to repair cellular components damaged by UVR exposure. UV radiation can cause damage directly, through absorption of high-energy photons by proteins and DNA (10), and indirectly through the creation of reactive oxygen species (ROS) that can cause widespread damage to proteins, lipids and nucleic acids (3,11,12). Much of the damage to DNA is in the form of cyclobutane pyrimidine dimers and, thus, can be repaired by photolyase without energetic cost to the cell. However, ATP is consumed by alternate repair pathways for DNA and other classes of macromolecules, which may require varying levels of degradation and synthesis. At low levels of UVR exposure, activation of these repair pathways would be expected to increase the metabolic rate. However, if damage due to UVR is severe, it is possible that cells will lack (or have lost) the resources to perform efficient repair, the metabolic rate will be depressed and cell death will ultimately ensue.

The purpose of this study was to examine the effects of UVR on metabolic rates of the freshwater cladoceran *D. catawba*. *D. catawba* are a critical link between phytoplankton and fish in the foodwebs of many lakes. Most research has focused on the effects of UVR on the survival of *D. catawba* and, consequently, UVR exposure levels have been relatively high (*e.g.* >26 kJ m^{-2} UVB) (13). In this study, we extend these previous studies by exposing *D. catawba* to lower levels of UVR and monitoring respiration and survival rates. We hypothesized that low doses of UVR, although sublethal, may impose energetic costs that could ultimately influence growth and reproduction in the field.

MATERIALS AND METHODS

Organisms. D. catawba were collected from Lake Giles, PA, on 15 November 2004 using a 0.5 m diameter, 60 μm plankton net towed from 18 m to the surface. Adult females were isolated into 1.5 μm filtered Lake Giles water, fed an excess amount of *Cryptomonas ozolini* and placed in a dark 19°C incubator overnight. The following morning, we exposed *D. catawba* to UVR on a lamp phototron in the laboratory (13).

Lamp phototron. Housed within a light- and temperature-controlled environmental chamber at 19°C, the phototron had a 30 cm diameter horizontal, opaque polymethyl methacrylate wheel that rotated at 2 rpm to provide uniform exposure. The wheel had forty 5.1 cm diameter holes arranged in two rows of 20 around the outside of the wheel. For each group, we stocked nine or ten *D. catawba* into five replicate 30 mL quartz dishes filled with 1.5 μm filtered Lake Giles water. Mean *D. catawba* length (1.67

*Corresponding author email: jfischer@fandm.edu (Janet M. Fischer)
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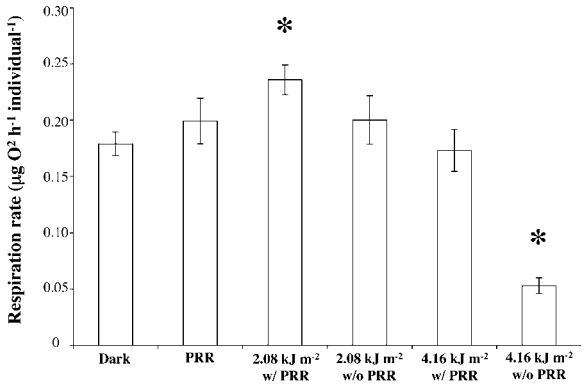

Figure 1. Mean respiration rates for *D. catawba* over 6 h following a 12 h UVR exposure on the lamp phototron. Treatment labels indicate total unweighted UVB exposure and the presence or absence of PRR. Respiration rates were linear during this period ($R^2 > 0.95$ for all replicates) and were adjusted for bacterial respiration using *Daphnia*-free controls. Error bars indicate standard error for each treatment. Asterisks indicate treatments that differed significantly from the dark control.

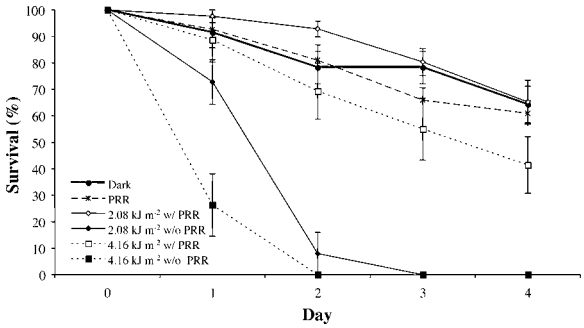

Figure 2. Mean survival rate of *D. catawba* over 4 days following a 12 h exposure on the lamp phototron. Treatment labels indicate total unweighted UVB exposure and the presence or absence of PRR. Error bars indicate standard error.

± 0.15 mm SD) did not differ significantly among exposure groups ($F_{5,12} = 1.83$; $P = .18$). Each quartz dish was covered with a circular quartz lid, placed inside a 25 mm high opaque collar and set on top of a hole in the wheel. The wheel sat on top of a light-tight polystyrene plastic box with a circular opening immediately below the wheel. *D. catawba* were exposed to UVB with or without concomitant exposure to UVA and visible light. The combination of UVA and visible light is termed photorepair radiation (PRR) because it promotes activity of the DNA repair enzyme photolyase, known to be present in *D. catawba* (13). One Spectronics XX15B lamp was suspended 24 cm above the wheel to provide UVB exposure from above. The lamp was covered with cellulose acetate (opaque to UV wavelengths <290 nm) to remove low levels of UVC radiation emitted from the lamp. The UVB exposure level was manipulated by placing stainless steel mesh screens on top of the opaque collars housing quartz dishes. PRR was provided upward through holes in the wheel from two 40 W cool white fluorescent lights and two 40 W Q-panel UVA340 bulbs located 32 cm below the wheel. For groups exposed to PRR, holes were covered with Mylar® to remove wavelengths <320 nm emitted from the Q-panel 340 bulbs. For groups that were note exposed to PRR, the holes were covered with black metal discs. In the dark control group, black metal discs were placed on the bottom and top of the dishes.

Exposures. Spectral irradiance was measured using a specially designed scanning spectroradiometer with a planar (cosine corrected) diffuser mounted on a fiber optic cable. The system and methods to determine UVB and PRR exposure in each dish are described in more detail by Williamson *et al.* (13). Screen density was chosen to obtain two levels of total (unweighted) exposure (2.08 and 4.16 kJ m^{-2}) from the UVB lamp over a 12 h period. The Mylar®-shielded UVA340 and cool white lamps provided negligible additional UVB. To assess biological effectiveness, spectral irradiance was weighted with a biological weighting function (BWF) for UVR effects on survival of *Daphnia pulicaria* (13). Weighted exposure to full (unscreened) irradiance in the phototron over a period of 12 h is similar to a full day's weighted exposure to surface irradiance at 40° latitude on a sunny day at summer solstice (13). The two levels of UVB exposure used in our experiment (2.08 and 4.16 kJ m^{-2}) represent approximately 4% and 8%, respectively, of this full exposure. As an additional indicator of biological effectiveness, irradiance weighted with the CIE biological weighting function for erythema (on the international UV index scale of mW m^{-2}/25 [14]) was 0.2 and 0.4 UV index units, respectively.

Respirometry. After the 12 h exposure period, two *D. catawba* from each quartz dish were transferred into a 10 mL SGE Gas-Tight syringe containing 1.5 µm filtered Lake Giles water. We measured the oxygen concentration at 2 h intervals for 6 h by injecting 0.6 mL from each syringe into a Strathkelvin respirometer system consisting of a 70 µL MC100 Microcell measurement chamber connected to a model 1302 oxygen electrode and a model 782 oxygen meter. The measurement chamber was connected to a recirculating water bath set at 19 ± 0.1°C. Between measurements, all syringes were housed in a dark incubator at 19°C. After correcting for the decrease in water volume in the syringes at each measurement, we calculated the cumulative amount of oxygen consumed at the 2, 4 and 6 h time points. We used the slope of this linear relationship to estimate respiration rates of *D. catawba* in the syringes. All slopes were significant at an α level of .05 and had R^2 values >0.95. Final respiration values for each syringe containing *D. catawba* were corrected for bacterial respiration by subtracting the mean oxygen consumption in 5 replicate *D. catawba*–free syringes. We did not include three replicates from the group exposed to 2.08 kJ m^{-2} UVB without PRR and two replicates from the group exposed to 4.16 kJ m^{-2} UVB without PRR in our analysis because one or both *D. catawba* died during the 6 h period that respiration was measured in these syringes. We used analysis of variance (ANOVA) to compare mean respiration rates in our six groups. We also compared mean respiration rates in each UVB exposure group with that for the dark control group by using planned contrasts.

Survival. In addition to measuring respiration, we also assessed survival of *D. catawba* in all exposure groups. After the phototron exposure, we transferred six to eight *D. catawba* per replicate from quartz dishes to 25 mL plastic Petri dishes containing 1.5 µm filtered Lake Giles water. Each dish was covered with a thin film of clear polyethylene to prevent *D. catawba* from being caught in the surface tension and individuals were fed *C. ozolini* in excess. *D. catawba* were placed in a dark 19°C incubator and survival in each dish was assessed for 4 days. Dead organisms were removed daily. We used multivariate repeated-measures ANOVA to assess differences in survival in our six groups and compared survival rates in each UV treatment group with that for the dark control by using planned contrasts. We normalized the data using the arcsin square root transformation and used Pillai's trace to test for time and time-by-treatment effects.

RESULTS

Our results indicated that respiration rates of *D. catawba* were affected by UVR exposure and that the nature of the response depended on the quality and quantity of UVR (Fig. 1). Mean respiration rates varied significantly among exposure groups ($F_{5,19} = 11.52$; $P = .0001$). Specifically, there was a significant increase (31.8%) in mean respiration rate in the group exposed to 2.08 kJ m^{-2} UVB with PRR, compared with the dark control group ($F_{1,8} = 6.99$; $P = .016$). In contrast, mean respiration rate was significantly reduced in the group exposed to 4.16 kJ m^{-2} UVB without PRR, compared with the dark control group ($F_{1,5} = 25.59$, $P = .0001$). Respiration rates in all other exposure groups were not significantly different from that in the dark control group (PRR *vs* dark control, $F_{1,8} = 2.89$ [$P = .11$]; 2.08 kJ m^{-2} UVB without PRR *vs* dark control, $F_{1,5} = 0.55$ [$P = .47$]; and 4.16 kJ m^{-2} UVB with PRR *vs* dark control, $F_{1,8} = 0.07$ [$P = .79$]).

Repeated-measures ANOVA revealed that survival also was affected significantly by UVR exposure (treatment, $F_{5,23} = 35.30$ [$P = .0001$]; time, $F_{4,92} = 285.96$ [$P = .0001$]; time by treatment,

$F_{20,92} = 2.56$ [$P = .0013$]) (Fig. 2). We considered receipt of PRR alone and exposure to 2.08 kJ m^{-2} UVB with PRR to be sublethal, because there were no significant main effects of treatment or time-by-treatment interactions in contrasts of these exposure groups with the dark control group (Table 1). Conversely, both exposures without PRR were lethal, with all *D. catawba* in these exposure groups dying within two days (for the group exposed to 4.16 kJ m^{-2} UVB without PRR) or three days (for the group exposed to 2.08 kJ m^{-2} UVB without PRR) of exposure (Fig. 2). Repeated-measures ANOVA contrasts with the dark control indicated significant main effects of treatment and time-by-treatment interactions for both of these exposure groups (Table 1). Survival in the group exposed to 4.16 kJ m^{-2} UVB with PRR was not affected as dramatically as survival in the group exposed to 4.16 kJ m^{-2} without PRR, but survival in the latter group was lower than survival in the dark control group. The marginally significant time-by-treatment interaction suggests that the survival trajectories in this exposure group and the dark control group differed (Table 1). The divergence between these exposure groups was most notable on day 4, when mean survival was 41.4% ± 10.7% in the group exposed to 4.16 kJ m^{-2} UVB with PRR and 64.2% ± 6.9% in the dark control group.

Table 1. Repeated measures ANOVA statistics for contrasts of *Daphnia* percentage survival in each UVR treatment *vs* the dark control.

Contrast	Treatment		Time by treatment	
	$F_{1,23}$	P	$F_{4,92}$	P
PRR *vs* dark	0.06	0.80	1.32	0.30
2.08 kJ m^{-2} UVB w/PRR *vs* dark	0.98	0.33	1.05	0.41
2.08 kJ m^{-2} UVB w/o PRR *vs* dark	62.45	0.0001	35.50	0.0001
4.16 kJ m^{-2} UVB w/PRR *vs* dark	2.75	0.11	2.70	0.06
4.16 kJ m^{-2} UVB w/o PRR *vs* dark	74.33	0.0001	27.31	0.0001

DISCUSSION

Metabolic responses to UVR

In our study, respiration rates of *D. catawba* were stimulated at the lowest level of UVB stress (2.08 kJ m^{-2} UVB with PRR), whereas respiration was inhibited at the highest level of stress (4.16 kJ m^{-2} UVB without PRR). In addition to the experimental results presented here, we also observed this general pattern during preliminary experiments performed to refine the experimental protocol (J. Nicolai and P. Pryzbylkowski, unpublished data). Although others have attributed elevated respiration rates during UVR exposure to changes in behavior (8), it is unlikely that this mechanism is driving the pattern in our experiment, because of the time lag between exposure and respiration measurements. Previous studies indicate that *Daphnia magna* swimming behavior shifts rapidly in response to changing light conditions (15); therefore, it seems unlikely that energetically costly changes in behavior would be maintained while respiratory measurements were performed during the 6 h period after UVR exposure. Furthermore, it was previously shown that UVA wavelengths have the strongest effects on *D. magna* swimming behavior (15) but we did not observe enhanced respiration rates in the *D. catawba* groups exposed to PRR, which were also exposed to UVA wavelengths.

Because changes in behavior seem to be unlikely explanations of the metabolic increases we observed, we hypothesize that enhanced respiration rates reflect, in part, energetic costs related to repair of cellular components damaged during exposure to sublethal levels of UVR. For example, because respiration was measured in the dark, excision repair was the only mechanism available to restore damaged DNA (16). Nucleotide excision repair in particular, which removes bases on either side of the lesion and synthesizes a new strand to replace the excised portion (17), is an ATP-dependent process. Similarly, proteins damaged through oxidation by photoinduced ROS must be repaired or must be degraded *via* the ubiquitin-proteasome pathway and replaced by newly synthesized protein. It is likely that the synthesis of protective antioxidant enzymes, such as catalase and superoxide dismutase, would also be upregulated (11). Membrane lipids, which undergo peroxidation under photooxidative stress, would also need to be repaired or replaced. Depending on the amount and extent of damage caused by UVR exposure, these repair and replacement mechanisms could require a significant energetic investment, which ultimately would lead to increased oxygen consumption.

Respiration rates were impaired for *D. catawba* exposed to the most severe UVR dose in our study (4.16 kJ m^{-2} UVB without PRR). We hypothesize that this response may reflect a combination of excessive damage to nuclear DNA, thus limiting transcription; damage to components of the repair pathways described above; and broadly distributed damage to proteins, lipids and nucleic acids with important structural or metabolic roles in the cell. Such widespread damage could depress metabolism and preclude repair; thus, a reduction in respiration rate may be a general precursor to UVR-induced mortality. It is important to point out, however, that we did not observe respiratory impairment in groups exposed to lethal UVR doses. For instance, although all *D. catawba* exposed to 2.08 kJ m^{-2} UVB without PRR died by the third day of the experiment, their respiration rates were not significantly different from the mean rate for the control group. We suspect that impaired respiration rates would have been observed in this group if the respiration rate had been measured closer to the mortality event. It is also likely that the lack of a respiratory response in this exposure group reflects a complex balance between increased metabolism due to repair processes and decreased metabolism due to damage. Inhibition of gut function is one possible mechanism for decreased metabolism in *Daphnia* after lethal exposure to UVR (18).

Ecological implications

Although UVR exposure from the lamp phototron differs from natural UVR exposure in many ways (such as the spectral composition of UVR), the general finding that *D. catawba* incurs a metabolic cost after sublethal levels of UVR exposure has important ecological implications. Increases in respiration rate of the magnitude (31.8%) we found in our experiment could dramatically reduce energetic reserves available for growth and reproduction, especially when these costs are repeatedly incurred over a series days of high UVR levels. It is important to note that our approach probably underestimated the total metabolic cost of UVR exposure, because we focused only on overnight respiration rates immediately following exposure and ignored metabolic costs incurred during exposure or during subsequent days.

Our results indicate that UVR may affect freshwater zooplankton across a broader range of conditions than was suggested by survival experiments alone. In highly transparent lakes, UVR may be lethal only near the surface but sublethal effects could extend much deeper in the water column. Previous studies have

demonstrated that the vertical distribution of *Daphnia* is strongly correlated to the fitness distribution as measured by the individual growth rate of juvenile *Daphnia* (19). Most studies have focused on how vertical gradients in factors, such as temperature, food quality and quantity and predators, affect vertical migration in zooplankton (19,20). However, our findings reinforce those from several other recent studies suggesting that UVR may also play a role in determining daytime vertical distribution of *Daphnia* in some lakes (5–7). Specifically, our results point to a physiological mechanism through which UVR could have a detrimental effect on *D. catawba* at relatively low levels of exposure.

To our knowledge, ours is the first study to address the metabolic costs of sublethal UVR exposure in *D. catawba*. The ability to measure energetic consequences of UVR exposure in short-term experiments may be a powerful tool for investigating the effects of UVR on aquatic organisms. Possible future applications of this tool include experiments that more fully characterize the shape of the exposure response curve for *D. catawba*, experiments that compare the response curve for *D. catawba* to that for other zooplankton species, polychromatic experiments to construct a biological weighting function and predict responses under *in situ* conditions (13) and experiments that use a modified protocol to measure respiratory responses to UVR in the field.

Acknowledgements—We thank Mark Olson, Philip Salko and Craig Williamson for technical assistance and advice. This work was supported by funds from Franklin & Marshall College, NSF grant DEB-0210972 and Howard Hughes Medical Institute grant 52002643.

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