

Zooplankton survival and reproduction responses to damaging UV radiation: A test of reciprocity and photoenzymatic repair

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Abstract

As stratospheric ozone concentrations are reduced, exposure of organisms to damaging ultraviolet radiation (UVR) reaching the earth increases. Many organisms can repair DNA damaged by UVR through photoenzymatic repair (PER) using the enzyme photolyase, in the presence of photorepair radiation (UV-A and visible light [PRR]). Biological weighting functions have been used to model UVR damage in organisms in an attempt to predict the short-term effects of ozone depletion. One assumption of these studies is that reciprocity—satisfied when the effect of a dose is independent of the dose rate—must hold. Here we exposed organisms to damaging UV-B radiation in both the presence and absence of PRR. This study explicitly tests whether reciprocity holds in two zooplankters with differing PER abilities: *Daphnia pulicaria*, and *Asplanchna girodi*. We found significant PER and a failure of reciprocity when comparing *Daphnia* and *Asplanchna* survival at different dose rates. However, in a higher dose experiment with *Asplanchna*, we found no significant PER, and reciprocity held. These experiments show a link between PER and reciprocity. The ability of *Daphnia* and *Asplanchna* to produce viable offspring after UVR exposure did not vary with dose rate but did vary with PRR. Reciprocity held in all cases where PRR was provided, but in the absence of PRR all offspring died. This study shows that overall dose, dose rate, the ability to undergo PER, and the presence of PRR are important factors to consider when studying the effects of UVR on organisms.

Ozone is the main atmospheric filter of solar ultraviolet radiation. As stratospheric ozone concentrations are reduced, the amount of ultraviolet-B radiation (UV-B, 280–320 nm) reaching the surface of the earth increases (Madronich 1994). Wavelengths below 305 nm have been shown to be most damaging to DNA (Mitchell and Karentz 1993), causing pyrimidine dimers at the molecular level, and affecting fitness at the organismal level.

Though water attenuates solar radiation, organisms in clear aquatic systems (with low dissolved organic compounds) can still receive damaging levels of UV radiation (UVR). A growing number of authors have explored the effects of UV-B on aquatic organisms, including rotifers (Cabrera et al. 1997), copepods (Tartarotti et al. 1999), *Daphnia* (Zagarese et al. 1994), shrimp and euphausiids (Damkaer and Dey 1983), fish (Gutiérrez-Rodríguez and Williamson 1999), as well as effects on planktonic communities (Williamson et al. 1994; Mostajir et al. 1999). In all cases, UVR was found to harm the organisms in question by decreasing their survival during at least one life stage.

Fewer studies have examined the effects of UV radiation on reproduction. Mostajir et al. (1999) reported that flagellates and bacterial populations increased in the presence of UVR, whereas Sawada and Enesco (1984) found that an in-

creased dose reduced the fecundity of the rotifer *Asplanchna brightwelli*. Work with cladocerans has shown that UV reduces the number of broods and number of progeny per brood in *Daphnia* (Jüttner 1989) and inhibits reproduction in *Chydorus* (Cabrera et al. 1997). On the other hand, work with copepods has yielded varied results. Williamson et al. (1994) found that reproduction in *Diaptomus* species was suppressed by UVR; Zagarese et al. (1997) found that UVR had no effect on the reproduction of *Boeckella gibbosa*; and Cabrera et al. (1997) found that UVR enhanced reproduction of *Boeckella gracilipes*.

Many organisms have the ability to repair the damage done to their DNA by UV radiation. One such mechanism is photoenzymatic repair (PER) in which the enzyme photolyase, in the presence of longer wavelength UV-A and visible light, reverses pyrimidine dimers (Sutherland 1981; Mitchell and Karentz 1993). Organisms ranging from blue-green algae to marsupials have demonstrated PER (in Mitchell and Karentz 1993); however, the rates of repair differ among species (Malloy et al. 1997) and within species among developmental stages.

Researchers have tried to model the damage caused to organisms by UV radiation in an attempt to predict the short-term effects of ozone depletion. Biological weighting functions (Cullen et al. 1992) and action spectra (Coohill 1989) have been used to model inhibition of photosynthesis in phytoplankton and higher plants, respectively. One assumption of these studies is that reciprocity must hold. Reciprocity is only satisfied when the effect of a total radiation exposure (dose) is independent of the time over which the exposure occurred (dose rate) (Luckiesh 1930) (Fig. 1). The assump-

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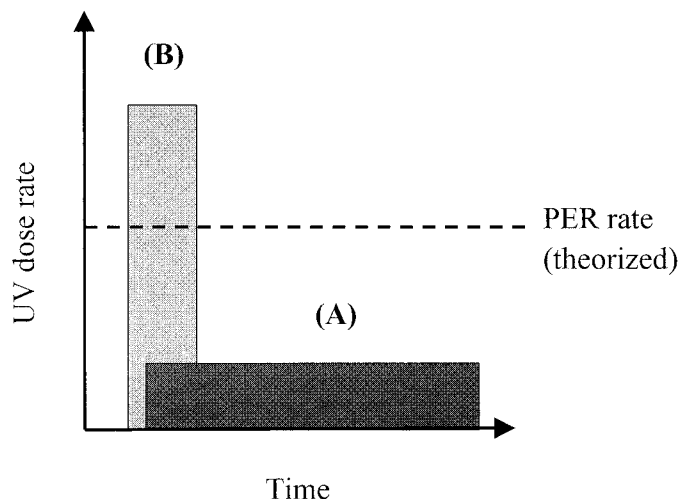


Fig. 1. Theoretical diagram of reciprocity. Doses (A) and (B) are equal (UV dose rate multiplied by time), and therefore (A) exposed organisms should have the same survival as (B) exposed organisms (reciprocity holds). For organisms with PER, it has been found that dose (A) (low UV dose rate times long time) will yield greater survival than dose (B) (high UV dose rate times short time). This would be true if cells could only repair UV damage done up to the theorized PER rate.

tion of reciprocity, either explicit or implicit, is common to many studies of UVR effects. Often, reciprocity is assumed to hold (Damkaer et al. 1981; Smith and Baker 1982) or its importance is acknowledged (Béland et al. 1999; Kouwenberg et al. 1999) though it is not directly tested.

Trocine et al. (1981) found that at a given UV-B dose, low dose rates over a long period of time produced the same degree of photosynthetic inhibition as higher dose rates over a shorter period of time in two of three species of seagrasses. Reciprocity held, and no significant PER was found in these two species. However, the third species, *Halodule wrightii*, showed significant PER and a failure of reciprocity. The apparent link between PER and reciprocity has been discussed at length (de Gruijl et al. 1986; Coohill 1989; Karentz and Lutze 1990; Cullen and Lesser 1991; Lesser et al. 1994; Cullen and Neale 1997) but has rarely been tested experimentally. In a study of the effects of UV-B dose and irradiance among benthic grazers, McNamara and Hill (1999) showed that reciprocity failed, discussed the importance of repair mechanisms, but never tested whether the organisms exhibited PER. Kouwenberg et al. (1999) found that reciprocity held in the absence of PER in Atlantic cod eggs, whereas Hunter et al. (1981) found that reciprocity failed in the presence of PER in larval Northern anchovy.

This study explicitly tests whether reciprocity holds in both survival and reproduction of two pelagic zooplankters with differing PER abilities: *Daphnia pulicaria* and *Asplanchna girodi*. Species of *Daphnia* have been found to undergo PER (Siebeck and Böhm 1991), but evidence against PER has been found for the rotifer *Asplanchna* (Sawada and Enesco 1984). It was expected that reciprocity should fail in *Daphnia* and hold in *Asplanchna* for both survival and reproduction data. These tests were carried out using an exposure method with a novel UV lamp phototron that permits

simultaneous exposure of test organisms to damaging shorter wavelengths of UV radiation in the presence and absence of longer wavelength radiation that stimulates PER (Williamson et al. in press).

Methods

Study organisms—The species used in these experiments were *Asplanchna girodi* and *Daphnia pulicaria*. The rotifers were cultured in the laboratory in filtered spring water and fed a lab culture of *Cryptomonas reflexa*. Only adult rotifers with full guts were used in experiments. *Daphnia pulicaria* were collected from Dutch Springs, Bethlehem, Pennsylvania, using a 202- μm tow net. Once the organisms were brought back to the lab, they were sorted and approximately 350 *Daphnia* were placed into each of two 700 ml dishes, in filtered Dutch Springs water. The *Daphnia* were fed *Cryptomonas reflexa* and kept at 20° C for 24 h prior to the experimental setup. All organisms, whether in culture or after exposure to UVR, were fed enough algae to maintain a concentration of 5,000 cells ml^{-1} .

Description of UV lamp phototron—The UV lamp phototron consists of a box with an opening centered in the top of it, into which a black plexiglass wheel fits. The wheel has 40 holes, each 5.1 cm diameter, over which custom flat-bottomed quartz dishes (30 ml capacity, and approximately 18 mm deep) are placed. Each dish is surrounded by a 2.5 cm high black collar of PVC to minimize exposure to stray radiation among dishes. Ten specimens are placed in each dish for exposure. The dishes are covered with quartz lids, and Schott® long wave pass cutoff filters (50 mm diameter, 50% transmittance at 305 ± 1 nm) were placed on the lids. Damaging UV-B radiation was provided from above the dishes by a Spectronics XX15B UV-B lamp (with two bulbs). We generally cover the UV-B lamp with cellulose acetate to eliminate the shortest wavelength UV-B and UV-C radiation that is not present in incident solar radiation (Williamson et al. in press). However, owing to the different exposure times used in these reciprocity experiments, we used Shott® glass filters here, which are more optically stable. Lamps located in the box below the experimental organisms provided photorepair radiation (PRR, visible, UV-A, and a small amount of UV-B from a combination of two 48 inch cool white bulbs and two 48 inch Q-Panel 340 bulbs). The box was ventilated with a high rpm thermostatically controlled fan. The wheel rotated the experimental dishes horizontally at 2 revolutions per minute to provide uniform exposure among dishes and simulate the mixing in the surface waters of a lake. Black metal disks were placed below the dishes to remove exposure to PRR. The entire apparatus was placed inside a growth chamber with constant temperature of 20° C. The details of this UV lamp phototron method approach are described in Williamson et al. (in press).

The spectral composition of the damaging radiation from above and repair radiation from below the wheel were measured at 1 nm resolution with a custom-made spectral radiometer. Measurements were made at 14 different positions of the rotating wheel for the UV-B lamp radiation (using the

Table 1. Corrected percent survival counts (and SE) at 48 h postexposure for each of four UV dose rates in the tolerance experiments.

Percentage full exposure	62%	48%	28%	20%
<i>Daphnia</i>				
+PRR	56 (4)	72 (5.8)	94 (4)	90 (5.5)
<i>Asplanchna</i>				
+PRR	0 (0)	8.5 (6.1)	21.1 (13.7)	42.2 (14.8)

Schott® filters) and eight different positions of the rotating wheel for the repair radiation. Values were integrated over these different positions to account for the rotation of the wheel in order to estimate spectral exposures. The spectral radiometer was custom made by Pat Neale at the Smithsonian Environmental Research Center. It consists of a scanning monochromator (model SP 300i, Acton Research) with a UV sensitive photomultiplier tube (PMT) (1P28) connected to a 3-m fiber-optic cable and a cosine-corrected flat diffuser collector. The response was calibrated using a 1,000 watt NIST traceable standard lamp.

The biologically effective exposure (BEE) for the damaging UV-B and repair radiation was calculated by multiplying the irradiance spectrum of the lamps by a biological weighting function (BWF) developed for *Daphnia* (Williamson et al. in press). The BWF adjusts for variations in the response (mortality in this case) to UV radiation at different wavelengths. This is necessary because short wavelength UVR is much more damaging per photon than is longer wavelength UVR.

Tolerance experiments—Organisms were exposed to different doses of UV radiation to determine which dose would yield a 50% survival rate after exposure. Fine to coarse mesh stainless steel screens were placed on top of each dish, within the PVC collars, to vary the intensity of UV exposure. It was possible to attain different levels of exposure by employing multiple screens simultaneously. Care was taken to use screens of different mesh sizes in order to minimize Moiré effects. The mesh treatments were as follows: (a) medium mesh, 62% transmittance; (b) fine mesh, 48% transmittance; (c) medium and fine mesh, 28% transmittance; and (d) medium, fine, and coarse mesh, 20% transmittance. Percentage transmittance is indicative of percentage of full exposure without any mesh screens.

Each treatment contained 10 dishes, five that received photorepair radiation (+PRR) and five that did not (−PRR). Each dish of 10 organisms was considered a replicate. Treatment dishes with quartz lids were placed on the phototron, Schott® filters were placed on the lids, then the appropriate mesh(es) were placed on the filters. Dishes were exposed to UV radiation for 6 h (*Daphnia*) and for 12 h (*Asplanchna*). Five control dishes were placed on a shelf in the growth chamber during exposure. The control organisms were placed in a plastic tray with a cardboard box covering them

Table 2. Duration (in h) of exposure for each of four dose rates (= percentage full exposure) in the reciprocity experiments. The bolded durations were the ones used to calculate all other durations for each experiment.

Percentage full exposure	62%	48%	28%	20%
<i>Daphnia</i>	6.00	7.75	13.28	18.60
<i>Asplanchna-2</i>	3.87	5.00	8.57	12.00
<i>Asplanchna-1</i>	4.13	5.33	9.17	12.83

to protect them from UV light. All organisms were fed *Cryptomonas* immediately after exposure, then again at 24 h postexposure. All dishes remained in the growth chamber in the dark during the counting phase. Percent survival in each dish was calculated at 48 h postexposure. Survival in treatment dishes was corrected for mortality in the control dishes using a modification of Abbott's formula (Williamson et al. 1999).

Reciprocity experiments—The reciprocity experiments were set up in much the same way as the tolerance experiments. However, to test reciprocity, the dosage of UV radiation (UV irradiance multiplied by exposure duration) must be kept constant. The tolerance experiments suggested that a standard dose of 6.00 h at 62% transmittance be used for *Daphnia*, and 12.00 h at 20% transmittance be used for *Asplanchna* (Table 1). These standard doses were used to calculate exposure durations at the other experimental percent transmittances for each species (Table 2). Table 1 does not contain any of the −PRR data because all of those organisms had died.

Data on the ability of *Asplanchna* to reproduce after exposure was easy to obtain due to the short generation time of this species. In order to gather similar information on *Daphnia*, all *Daphnia* used in the reciprocity experiments were egg bearing females with anywhere from two to eight eggs in their brood pouch.

Dishes were placed on the phototron wheel one dose rate treatment at a time, in such a way that they could all be removed at once (i.e., for the *Asplanchna* experiments, the 20% treatment was put on first, then 3.43 h later, the 28% treatment was put on, etc.). The first time this experiment was run with *Asplanchna*, the 62% transmittance dishes were accidentally left on the wheel for 4.13 h instead of 3.87 h. Exposure durations for the other percentage treatments were recalculated and the remaining dishes were removed after the appropriate time (Table 2, *Asplanchna-1*). Since the dose given these rotifers was somewhat higher than optimal, the *Asplanchna* experiment was repeated, and the originally calculated durations were used (Table 2, *Asplanchna-2*).

At the end of exposure, all dishes were placed in plastic trays on shelves in the growth chamber. Dishes were kept in the dark for the duration of the experiments. Counts were done using a dissecting microscope immediately after exposure, then daily for five consecutive days. Dishes were only exposed to direct light during counting, which would take approximately 30 min per treatment.

At each counting period, the number of living individuals

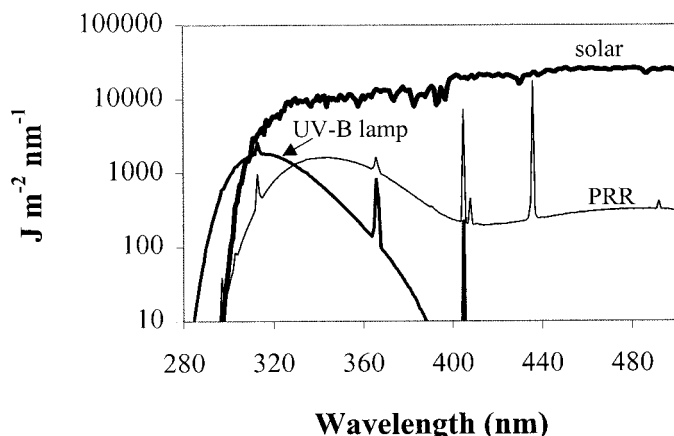


Fig. 2. Spectral composition of damaging radiation energy from a 12 h exposure to UV-B lamp with the use of 305 nm Schott® filters. Also shown are the solar energy spectrum for a 7-h period in the middle of a sunny day near summer solstice at 40°N latitude, and the 12 h photorepair radiation (PRR) energy spectrum.

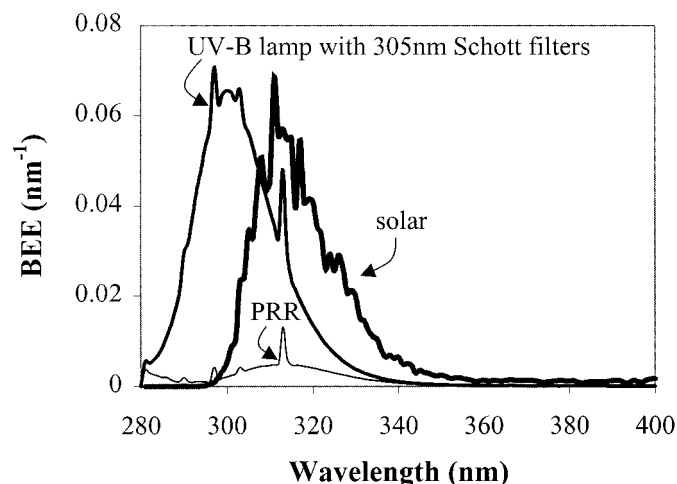


Fig. 3. Biologically effective exposure for the damaging UV-B lamp and photorepair radiation (PRR) compared to exposure to full sunlight (solar) for 7 h during midday (0930–1630 h) on a clear day near summer solstice at 41°N latitude.

in each dish was recorded. Any dead individuals were removed, as were all offspring. Offspring were placed into new dishes, and their survival was also recorded daily. All organisms were fed from a high density *Cryptomonas* culture and food concentrations were maintained at approximately 5,000 cells ml⁻¹.

Daily percent survival for each dish was calculated. Those numbers were then corrected for any mortality in the controls using the modified version of Abbott's formula (Williamson et al. 1999). Corrected daily percent survivals were then arcsine transformed (Zar 1984) and used in ANOVAs using repeated measures with several populations (RMANOVAs, Dunn and Clark 1987) to determine whether reciprocity held for the different dose rates. The RMANOVAs were also used to determine if *Asplanchna* and *Daphnia* exhibited significant PER during the experiment.

The total offspring number at the end of the experiment was divided by total female days (the sum of the surviving females on each day of the experiment) for each replicate. This weighted the cumulative offspring number for each treatment by the number of females remaining over time to give birth. Offspring per female days were then used in one-way ANOVAs to test for reciprocity in reproduction.

Results

The spectral composition of the UV-B lamp, the PRR lamps, and natural sunlight can be seen in Fig. 2. Some of the repair radiation is below 400 nm, meaning that the organisms exposed to +PRR treatments were also getting a higher dose of UVR than the -PRR organisms. However, the biologically effective exposure of the photorepair radiation was found to be quite low compared to both the UV-B lamp and solar radiation (Fig. 3).

Reciprocity did not hold for *Daphnia* as there were significant mesh treatment and interaction effects (Table 3). *Daphnia* also exhibited significant PER in all treatments (Table 3). The survival of the +PRR individuals was well above

that of the -PRR individuals in all cases (Fig. 4A–D). The fact that reciprocity did not hold for either the +PRR or -PRR treatments is also evident from the divergence of survival curves among treatments over time (Fig. 4E,F). A significant effect of day was seen throughout the RMANOVAs (Table 3). This effect simply indicates that there was significant mortality over time in all treatments. The control percent survivals were as follows: 100% on day 0.5 and day 1, 96% on day 2, 88% on day 3, 86% on day 4, and 84% on day 5.

In the first experiment, *Asplanchna* showed no significant effect of mesh treatment or interaction between mesh treatment and day, indicating that reciprocity does hold (Table 3, Fig. 5E,F). The analysis also showed that the rotifer does not exhibit significant PER (Table 3), although a significant interaction effect was calculated for 20% exposure. A trend for the -PRR individuals to have a lower survival than the +PRR individuals can be seen in Fig. 5A–D, however the differences are not significant. The control percent survivals for the first *Asplanchna* experiment were as follows: 90% on day 0.5, 86% on day 1, 78% on day 2, 72% on day 3, 56% on day 4, and 50% on day 5.

When the *Asplanchna* experiment was repeated, the rotifers showed a significant effect of PER in the 48% exposure treatment (Table 3, Fig. 6B), and significant interactions for the 62%, 48%, and 28% treatments (Table 3). Reciprocity held in the +PRR treatments, but not in the -PRR treatments (Table 3), where the 62% and 48% -PRR treatments survived consistently better than the 28% and 20% -PRR treatments (Fig. 6F). The control percent survivals for the second *Asplanchna* experiment were as follows: 100% on day 0.5, 94% on day 1, 90% on day 2 and day 3, 86% on day 4, and 76% on day 5.

Reciprocity failed when comparing offspring per female day to dose in *Daphnia* for the +PRR treatments ($F = 5.17$, $F_{0.05(2)3,16} = 4.08$). No offspring in the -PRR treatments survived until day 5 for either species. Figure 7 shows that embryo survival was greatest at the 20% exposure, and re-

Table 3. Repeated measures ANOVA results of corrected and arcsine transformed daily percent survival data for *Asplanchna* and *Daphnia*. Numbers in bold are significant at the $\alpha = 0.05$ level. Significant treatment effects indicate a failure of reciprocity (reciprocity group) or presence of significant photoenzymatic repair (PER group).

Test for:		<i>F</i> crit		<i>F</i> crit		<i>F</i> crit
Reciprocity	<i>F</i>	0.05(2),		0.05(2),	<i>F</i>	0.05(2),
PER	treatment	1, 8	<i>F</i> day	5, 40	interaction	5, 40
		0.05(2),		0.05(2),		0.05(2),
		3, 16		5, 80		5, 80
<i>Daphnia</i>						
Reciprocity						
+PRR/meshes	194.01	4.08	122.82	2.73	4.24	2.73
-PRR/meshes	88.21	4.08	409.18	2.73	24.88	2.73
PER						
62% +PRR/-PRR	223.41	7.57	141.89	2.9	22.73	2.9
48% +PRR/-PRR	455.61	7.57	158.59	2.9	45.59	2.9
28% +PRR/-PRR	47.37	7.57	61.29	2.9	5.53	2.9
20% +PRR/-PRR	38.27	7.57	67.97	2.9	16.49	2.9
<i>Asplanchna-1</i>						
Reciprocity						
+PRR/meshes	0.61	4.08	97.38	2.73	0.75	2.73
-PRR/meshes	1.36	4.08	448.59	2.73	0.50	2.73
PER						
62% +PRR/-PRR	3.65	7.57	78.17	2.9	1.78	2.9
48% +PRR/-PRR	2.56	7.57	74.55	2.9	1.30	2.9
28% +PRR/-PRR	2.95	7.57	163.71	2.9	2.72	2.9
20% +PRR/-PRR	3.99	7.57	91.08	2.9	3.60	2.9
<i>Asplanchna-2</i>						
Reciprocity						
+PRR/meshes	1.83	4.08	85.48	2.73	0.78	2.73
-PRR/meshes	13.98	4.08	318.02	2.73	1.87	2.73
PER						
62% +PRR/-PRR	0.69	7.57	65.01	2.9	4.65	2.9
48% +PRR/-PRR	9.19	7.57	84.97	2.9	7.52	2.9
28% +PRR/-PRR	5.16	7.57	71.37	2.9	5.45	2.9
20% +PRR/-PRR	2.44	7.57	141.61	2.9	2.01	2.9

duced at the 48% and 62% exposures. These differences were found to be significant when a post hoc Tukey Test was performed. Reciprocity held for offspring per female days in both *Asplanchna* experiments ($F = 0.024$, $F = 0.263$, $F_{0.05(2)3,16} = 4.08$, respectively). The control *Asplanchna* reproduced significantly more than the treatments (Fig. 7). The *Asplanchna* numbers include some second generation individuals.

Discussion

These experiments clearly show that when significant PER is exhibited by any organism, reciprocity may fail when survival data are used. Past studies have shown that in species that can undergo PER using UV-A, visible light, and the enzyme photolyase to repair damage done to their DNA, the manner in which the UV dose is administered is a crucial element in determining survival. If organisms are exposed to a small amount of UV irradiance for a long period of time in the presence of PRR, cellular damage will be minimized as repair keeps up with damage. Therefore survival will be

high. However, if organisms are exposed to a large amount of UV irradiance for a short period of time in the presence of PRR, the damage done by the high irradiance may be too great for the cells to repair, resulting in lower survival (Fig. 1, theorized line). This relationship was observed in a species of seagrass (Trocine et al. 1981) and in a marine diatom (Cullen and Lesser 1991). It was found that for equal doses of UV-B, a relatively short exposure to high UV-B irradiance is more damaging to photosynthesis than a longer exposure to lower irradiance.

Short durations of high UV dose rates were not more lethal than long durations of low UV dose rates in *Daphnia* or *Asplanchna*. In the cases where reciprocity failed, the 20% exposure, long duration treatments had the lowest survival, whereas the 48% exposure, shorter duration treatments had a higher survival. This is the opposite of what has been previously seen in plants. Other studies have also shown evidence that failed to fall into the pattern observed in plants. Kouwenberg et al. (1999) found that the mean survival of Atlantic cod eggs exposed to a high dose rate for a short period of time was higher than those exposed to a low

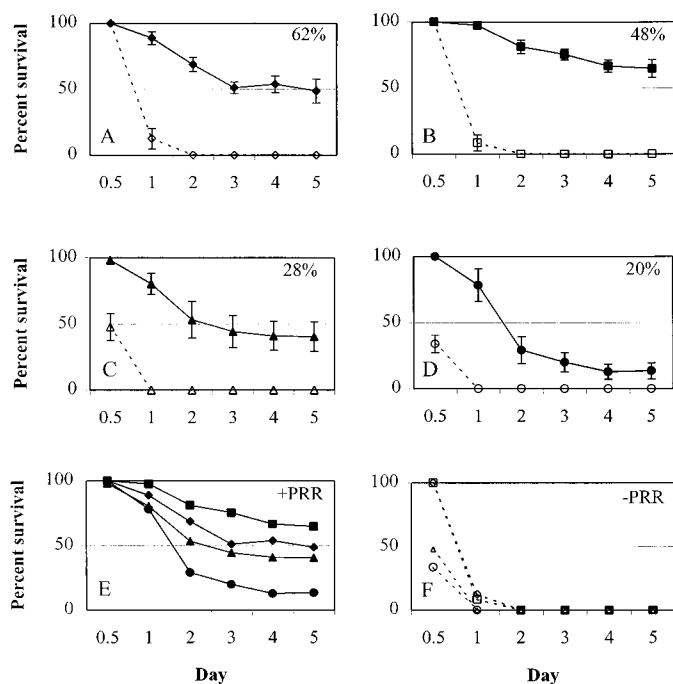


Fig. 4. Reciprocity curves for *Daphnia pulicaria* showing daily corrected survival. Treatments are +PRR (solid lines and markers) and -PRR (dotted lines and open markers). Top four panels have equal doses but differing dose rates. Percent exposures are as follows: (A) 62% diamonds, (B) 48% squares, (C) 28% triangles, (D) 20% circles. See Table 2 for exposure durations. Bottom two panels allow for easy comparison of all dose rates for (E) +PRR and (F) -PRR curves. Error bars indicate ± 1 SE.

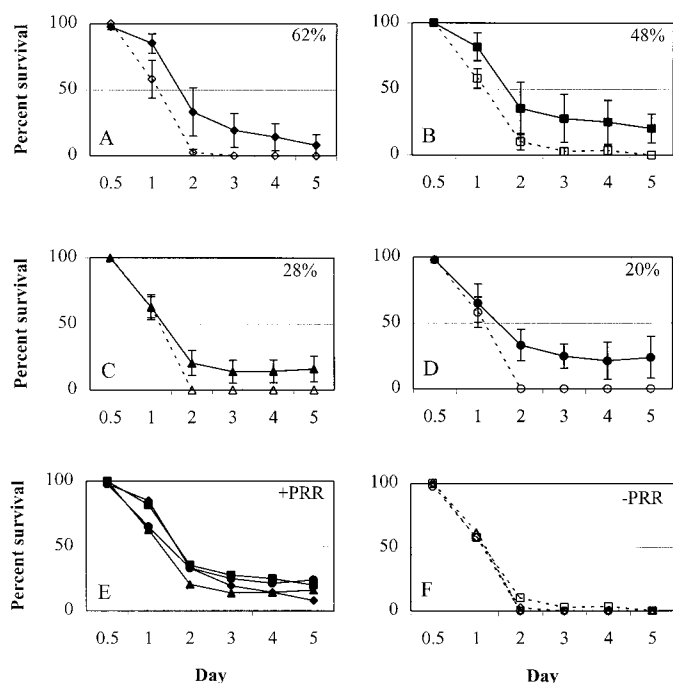


Fig. 5. Reciprocity curves for *Asplanchna girodi* (experiment 1). Explanation as per Fig. 4.

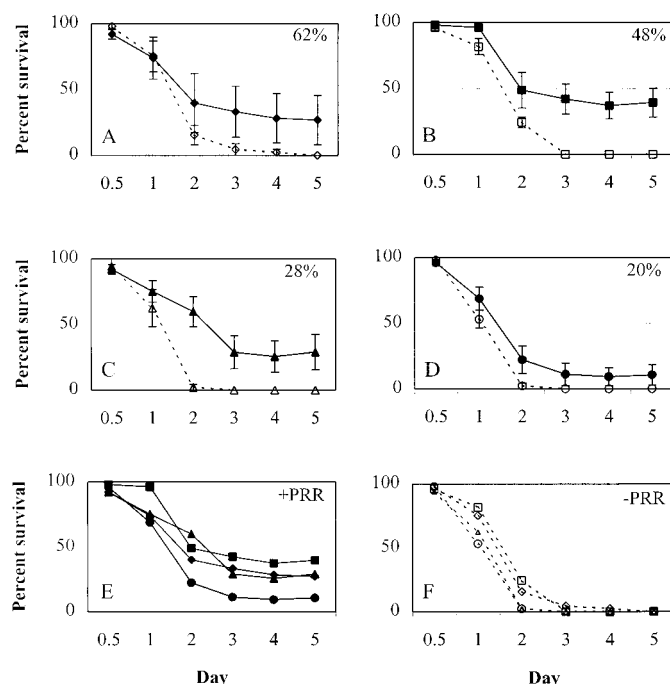


Fig. 6. Reciprocity curves for *Asplanchna girodi* (experiment 2). Explanation as per Fig. 4.

dose rate for a long period of time. When the overall dose of the experiments was doubled, they found that the high dose rate, short exposure survived better than the low dose rate, long exposure, which survived better than the medium dose rate, medium exposure. The same lack of pattern between percent survival and dose rate was seen by McNamara and Hill (1999) when observing the survival of mayflies and chironomids. It is possible that the theorized PER rate (Fig. 1) is nonlinear in animals, which do not require light for photosynthesis as do plants.

When the cumulative offspring produced by the +PRR treatment adults 5 d after exposure were compared, reciprocity failed in *Daphnia* but held in both *Asplanchna* ex-

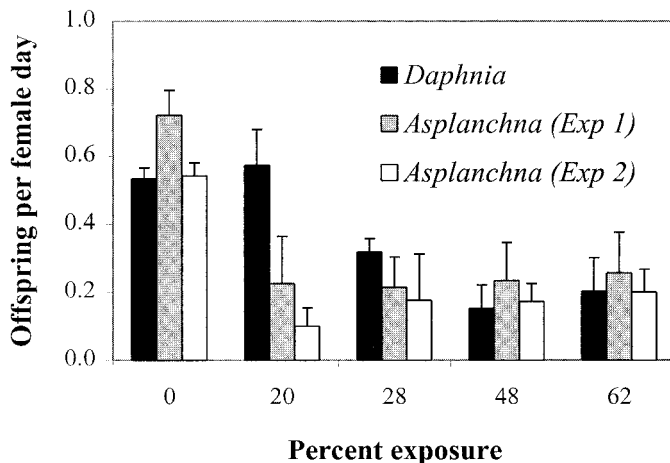


Fig. 7. Average offspring per female day at 5 d after exposure to UV for both species. Error bars indicate $+1$ SE.

periments. The ability of *Daphnia* eggs in the brood pouch to survive after being exposed to UVR did vary with dose rate, as did adult survival. However, it was interesting to note that in *Daphnia* there was a significant trend for greater offspring survival at a low dose rate, a trend that was expected in adult survival, but not observed. The production and survival of juvenile *Asplanchna* did not vary with dose rate for either *Asplanchna* experiment. Since only the +PRR treatment adults produced offspring that survived until the end of the experiment, and since reciprocity held for both +PRR adult experiments, production and survival of *Asplanchna* juveniles did in fact mirror the adult survival data. There clearly was an effect of UV exposure in both species, as the control reproduction greatly exceeded that of the exposure treatments. There was also a significant effect of PRR in both species, since only the offspring whose parents were exposed to PRR survived to day five. By providing only a small amount of PRR during the experiment, having done that only once for the +PRR treatments, and keeping all treatments in the dark afterward, we have shown that a minimal amount of PRR can have significant effects (and differing effects) in zooplankton. The fact that photorepair radiation is crucial to both survival and the ability to produce live young is apparent in the data gathered for *Daphnia*. Even though the +PRR daphnids received a higher dose than the -PRR daphnids, survival and reproductive success were greater in the presence of repair radiation. It seems that in *Asplanchna* photorepair radiation is much more important to reproductive processes than to survival processes. Though only one significant example of PER could be found in the survival data, there was a consistent trend throughout for reduced reproduction and eventual mortality of juveniles produced in the absence of PRR. These data seem to indicate differences in the effectiveness of repair systems for survival and reproductive processes.

As stated by Cullen and Neale (1997), when reciprocity fails, plots of effect versus cumulative exposure lose meaning and attempts to determine wavelength dependence are compromised. Biological weighting functions (BWF) or action spectra (AS) can be defined for a species, but their usefulness may be limited if reciprocity does not hold (Coohill 1989). It is not enough to assume that reciprocity should hold; the theory must be tested under the range of conditions used to determine the BWF or AS. Researchers must be able to model time as a variable when extrapolating laboratory exposure versus survival data to natural conditions before such data can be used to determine the short-term effects of increased solar irradiance. What these experiments demonstrate is that overall dose, dose rate, and ability to undergo PER are all important factors to consider when studying the effects of UV radiation on organisms. In cases where reciprocity does not hold, as in *Daphnia*, effects of different dose rates on survival and reproduction may not yield similar trends even when the total UV dose is the same. In addition to the important similarities and differences in survival versus reproductive responses demonstrated here, other sublethal responses as well as interactive stressors will need to be studied if we are to understand how natural populations will respond to changing UV environments.

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