

Temperature-dependent ultraviolet responses in zooplankton: Implications of climate change

Abstract—Climate warming and stratospheric ozone depletion increase temperature and ultraviolet (UV) in mid- to high-latitude ecosystems; however, little is known about the interactive effects of temperature and UV on organisms. We exposed *Daphnia catawba*, *Leptodiatomus minutus*, and *Asplanchna girodi* to UV-B at four different temperatures: 10, 15, 20, and 25°C. Elevated temperatures increased UV tolerance in *D. catawba* and *L. minutus*, species that depend heavily on photoenzymatic repair (PER), but decreased UV tolerance in *A. girodi*, a species that has less PER. Also, body size in *Daphnia* decreased with increasing UV dose. These results demonstrate that climate change can alter responses to UV through temperature-mediated effects in aquatic ecosystems, and these effects can be species-specific and dependent on PER ability.

The potential for climate warming to increase ambient ultraviolet (UV) exposure at mid- to high latitudes has been recognized and modeled (Williamson et al. 1996; Yan et al. 1996), but little is known about how temperature can alter organism-level responses to elevated UV. In temperate lakes, UV and epilimnetic temperatures vary seasonally in an asynchronous manner (Fig. 1A). For example, epilimnetic temperatures a month or two before summer solstice are often 8–10°C. During summer solstice, the period of peak solar UV, epilimnetic temperatures continue to rise. In the month or two after solstice they are 20–25°C or above. In this last period, there are frequently transitions in plankton communities that can be influenced by temperature–UV interactions in the more UV-transparent, low-dissolved organic carbon (DOC) lakes (Williamson et al. 1996). In particular, the temperature dependence of UV tolerance can be influenced to a great extent by the amount of photoenzymatic repair (PER) in a given species. PER is a light-dependent DNA repair process that is essential to UV tolerance in some species of zooplankton but weak or absent in others (Zagarese et al. 1997; Grad et al. 2001). PER in vitro tends to increase with increasing temperature because it is enzyme mediated (Langenbacher et al. 1997). Such an increase in PER in vivo could compensate in part for the increased photodamage, but this has not yet been explicitly investigated. According to this scenario, when PER is important, the lower temperatures experienced during periods of moderate to high environmental UV (high UV:temperature [UV:T] ratios, Fig. 1A) will slow repair mechanisms and increase net DNA damage. In contrast, the higher temperatures experienced under similar UV conditions later in the spring and summer (low UV:T, Fig. 1A) might enhance repair mechanisms and reduce net DNA damage. Thus, the effect of UV will vary seasonally as a function of ambient temperature, as well as incident UV, and be most severe during the late winter to early spring when the UV:T ratio is highest (Fig. 1A). This scenario is

supported by the strong negative seasonal correlation between the UV:T ratio and both cladocerans ($r_{\text{ratio}} = 0.68$, $P = 0.015$) and calanoid copepods ($r_{\text{ratio}} = 0.82$, $P < 0.010$), the two dominant zooplankton taxa in UV-transparent Lake Giles (Fig. 1B). Seasonal variations in temperature and food supply undoubtedly play a major role in these patterns. We are not arguing that UV:T ratios are primary. The correlations with temperature and zooplankton in these populations are, in fact, on the same order as those for the UV:T ratio ($r_{\text{temp}} = 0.62$, $P = 0.030$ for cladocerans and $r_{\text{temp}} = 0.84$ and $P < 0.001$ for calanoid copepods). The important point is that UV effects will vary with temperature, and seasonal changes in UV:T might play a role in explaining some of the seasonal patterns in abundance or body size that have been observed in nature and previously attributed to temperature alone.

An alternative scenario is possible for species in which PER is less important and temperature tolerance limits are exceeded during the warm summer months. If seasonal increases in temperature during summer months exceed the tolerance limits of zooplankton, as happens often when temperatures are 25°C or higher (Moore et al. 1996), UV tolerance will be lowest during the summer (low UV:T) because of a multiple stressor effect (Folt et al. 1999). This scenario is supported by the greater photodamage that has been observed at higher temperatures in both fish (Winkler and Fidhiany 1996) and copepods (Hairston 1979).

This study examines the relationship between PER and the temperature dependence of UV tolerance by exposing three species of zooplankton with different abilities to undergo PER to UV and by experimentally manipulating PER. The three zooplankters chosen for this study are *Daphnia catawba*, *Leptodiatomus minutus*, and *Asplanchna girodi*. Previous studies have shown that daphnids and calanoids have a significant amount of PER, whereas *Asplanchna* have little to no PER (Zagarese et al. 1997; Grad et al. 2001). Our experiments were carried out at temperatures of 10, 15, 20, and 25°C using an exposure method with a 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. 2001a).

Study organisms—We performed UV exposure experiments using *D. catawba*, *L. minutus*, and *A. girodi*. The organisms were cultured in the laboratory in either filtered lake water (*D. catawba* and *L. minutus*) or spring water (*Asplanchna*). All species were fed *Cryptomonas reflexa* cultures to a concentration of 5,000 cells ml⁻¹ and kept at 20°C with a 12:12 light:dark (LD) cycle. In order to acclimate all organisms before use, 1- to 2-d-old *D. catawba* and *L. minutus* were collected from the parental cultures and placed at the

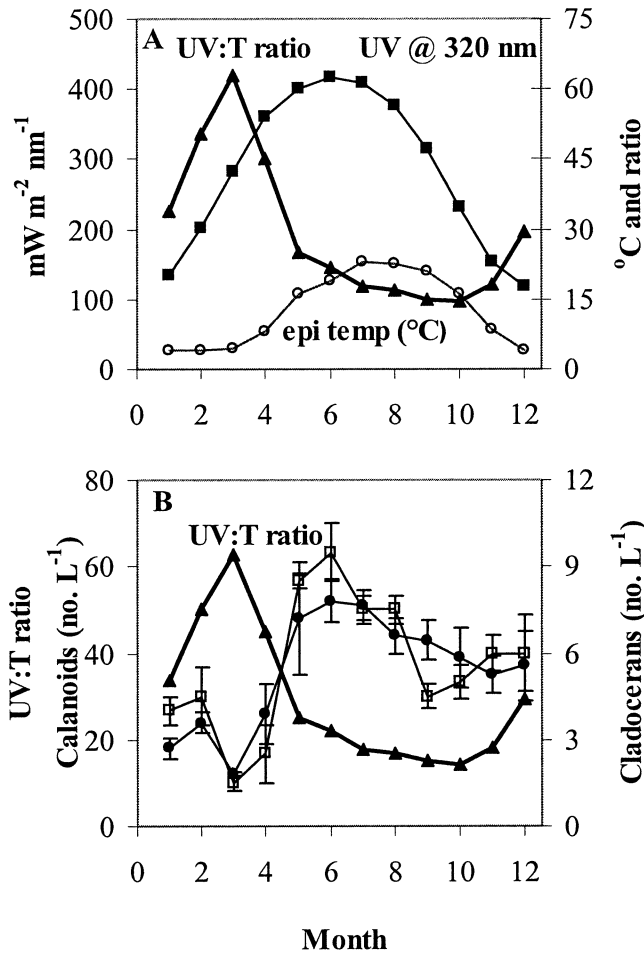


Fig. 1. (A) Seasonal variation in epilimnetic temperature, estimated incident UV irradiance at 320 nm, and the ratio of UV to epilimnetic temperature in Lake Giles, a low-DOC, high-UV U.S. lake in northeastern Pennsylvania. Seasonal temperature data were collected at biweekly to weekly intervals over a 4-yr period (1989–1993) in Lake Giles with a Yellow Springs Instruments submersible oxygen–temperature probe. Seasonal UV data were estimated with a radiative transfer model (RT 95, Biospherical Instruments) based on latitude and longitude of Lake Giles and assuming a column ozone value of 300 DU. (B) Monthly UV:T ratio and average (1989–1993, \pm SE) whole-water column density of the two dominant zooplankton taxa in Lake Giles (open squares are cladocerans; closed circles are calanoids). Zooplankton were collected with replicate tows of a 202- and 48- μ m mesh bongo net towed vertically through the whole-water column on a biweekly to monthly basis. Zooplankton were enumerated in a Bogorov chamber according to standard methods.

experimental temperature for 2 d. *Asplanchna* that were less than a day old were collected and placed at the experimental temperature overnight prior to UV exposure.

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 Plexiglas wheel fits. The wheel has 40 holes, each 5.1 cm diameter, over which custom flat-bottomed quartz dishes (30 ml capacity and \sim 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. Damaging UV-B radiation was provided from above by a UV-B Spectronics XX15B lamp (with two bulbs). The UV-B lamp was covered with cellulose acetate to eliminate the shortest wavelength UV-B and UV-C radiation that is not present in incident solar radiation. Lamps located in the box below the experimental organisms provided photorepair radiation (PRR, the wavelengths of light necessary to stimulate PER). These lamps consisted of two 40 W cool white fluorescent and two Q-panel 40 W UV-A 340 lamps (providing visible light, UV-A, and a small amount of UV-B). The box was ventilated with a high-rpm thermostatically controlled fan. The wheel rotated the experimental dishes horizontally at 2 rpm to provide uniform exposure among dishes and simulate the mixing in the surface waters of a lake. Black metal disks were placed below some dishes to remove exposure to PRR. The entire apparatus was placed inside a growth chamber with constant temperature. The details of this UV lamp phototron method approach are described fully in Williamson et al. (2001a).

Temperature experiments—At least 2 d prior to each experiment, the growth chamber was set to the experimental temperature (either 10, 15, 20, or 25°C). Containers of filtered water were placed in the growth chamber and allowed to come to temperature. Only one species was tested at a time. On the morning of the experiment, 10 acclimated individuals of a single species were placed in each quartz dish with the appropriate filtered water and a quartz lid. There were five replicate dishes per treatment, and four treatment exposure levels per experiment. Mesh screens of different sizes were placed on the dishes to provide the four UV exposure levels (medium mesh, 34 kJ m⁻²; fine mesh, 26 kJ m⁻²; medium and fine meshes, 15 kJ m⁻²; and medium, fine, and coarse meshes, 11 kJ m⁻²). Animals were exposed for a 12-h period. Exposure for 12 h with no mesh (55 kJ m⁻²) is approximately equal to a full day of solar radiation at the water surface near summer solstice (Williamson et al. 2001a). Half of the *Asplanchna* and *L. minutus* were exposed to both UV and PRR, whereas the other half were only exposed to UV. The *D. catawba* treatments were exposed to both UV and PRR, although several treatments at 20 and 10°C exposed the organisms to only UV. We have found in all our experiments with *Daphnia* that these organisms do not survive exposure to UV in the absence of PRR even at the lowest doses used in these experiments. Five dishes of dark controls were placed alongside the phototron in the growth chamber but were protected from light. After the 12-h exposure, all organisms were kept in the dark for the duration of the experiment. For further details of the experimental procedures refer to Williamson et al. (2001a).

Counts of survivors were done immediately after exposure and every day (*D. catawba* and *L. minutus*) or every 12 h (*Asplanchna*) after that. Experiments at 20 and 25°C were counted for a total of 5 d, whereas those at 10 and 15°C were counted for a maximum of 10 d. Counting ended at all temperatures if survival in the dark controls declined to 90% before the end of the allotted counting period. All organisms

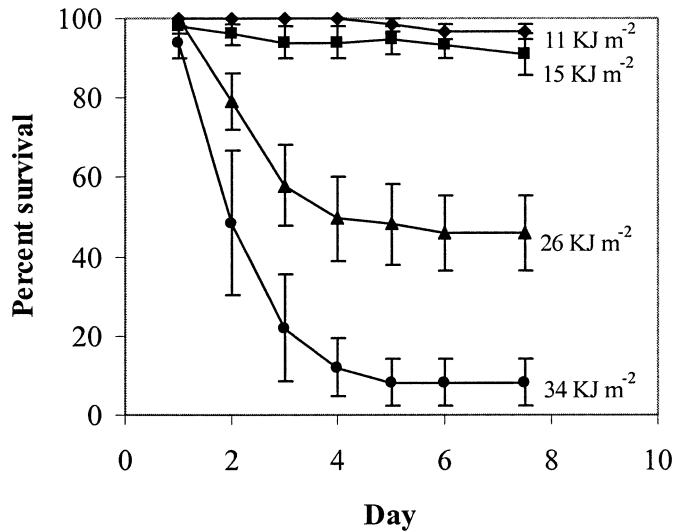


Fig. 2. Survival curves for *Asplanchna girodi* exposed in the UV-lamp phototron. Organisms were exposed to 11 kJ m⁻² (diamonds), 15 kJ m⁻² (squares), 26 kJ m⁻² (triangles), and 34 kJ m⁻² (circles) for 12 h at 15°C with photorepair radiation. The endpoint of this experiment was on day 7.5. Error bars are standard errors.

were fed after exposure and then as needed for the duration of the counting period. Algal densities in the dishes were kept at approximately 5,000 cells ml⁻¹. Any dead or newly born individuals were removed from the dishes at each count. Surviving *Daphnia* were followed until they reached maturity (had eggs in their brood pouch) and then were measured for length. Survival in all species was corrected for mortality in the dark 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 two-factor analyses of variance to determine whether temperature had significant effects on survival. If significant effects were observed, Tukey tests (Zar 1984) were done to determine where the differences were. The count used for this analysis was the last count in which the dark control survival was above or equal to 90%. Those data are summarized here, and one representative graph is shown (Fig. 2). Corrected daily percent survivals were also used to calculate the dose at which 50% survival occurred (LD₅₀). The LD₅₀ was estimated with a transformed logit function (Williamson et al. 1999) to provide a summary statistic to compare tolerance among experimental groups. The transformed logit function was selected because it behaves almost identically to the commonly used probit transformation, but it is particularly suitable for values near 0 and 100% survival.

In the absence of PRR, *D. catawba* and *L. minutus* did not survive at any of the UV exposure levels. In *Daphnia*, where survival following UV exposure is attributable almost exclusively to PER (Grad et al. 2001), UV tolerance increased with increasing temperature up to the highest temperature tested, 25°C (Fig. 3, $F_{3,64} = 98.81$, $P < 0.001$). A similar effect of temperature on tolerance was seen with *L. minutus* (Fig. 3, $F_{3,64} = 12.38$, $P < 0.001$). Tolerance was highest at the highest temperature tested. Unlike the daph-

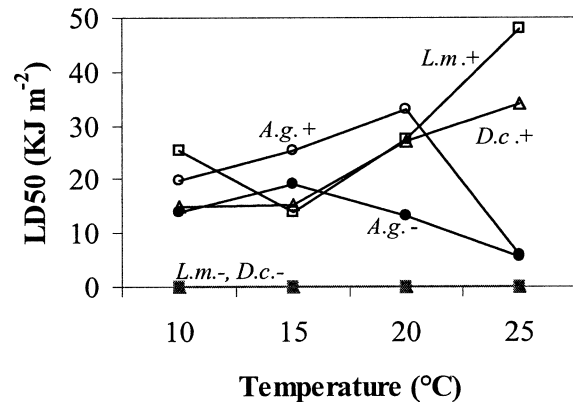


Fig. 3. Overall UV tolerance in laboratory experiments of *Daphnia catawba* (triangles), *Leptodiptomus minutus* (squares), and *Asplanchna girodi* (circles) as a function of temperature in the presence (open symbols) and absence (solid symbols) of photorepair radiation. UV tolerance is measured as an LD₅₀ (kJ m⁻²) at 280–400 nm and represents the cumulative UV dose that leads to 50% mortality at the experimental endpoint.

nids and copepods, *Asplanchna* did survive in the absence of PRR. This indicates that PER is not as important in this species (Grad et al. 2001) and that dark repair and photoprotection play an important role in the ability of *Asplanchna* to tolerate UV. UV tolerance in *A. girodi* peaked at a higher temperature in the presence of PRR (20°C) than in its absence (15°C) (Fig. 3). In addition, UV tolerance was substantially reduced at 25°C both in the presence and in the absence of PRR (Fig. 3, $F_{+PRR,3,64} = 4.087$, $P < 0.001$, $F_{-PRR,3,64} = 33.13$, $P < 0.001$), suggesting that 25°C exceeds the temperature tolerance limits of *Asplanchna* and actually increases sensitivity to UV exposure.

Body size at maturity also varied as a function of both UV dose and temperature in *Daphnia* (Fig. 4, $F_{4,57} = 7.25$, $P < 0.001$; $F_{3,57} = 3.87$, $P = 0.014$, respectively). The power of this statistical test was 0.94 relative to temperature and 0.99 relative to UV. The minimum detectable difference in body size at maturity was 0.052 mm relative to temperature and 0.079 mm relative to UV. Size decreased with increasing temperature between 15 and 25°C in the absence of UV (Fig. 4A, $F_{3,16} = 20.58$, $P < 0.001$). A similar decrease in body size was observed with increasing UV exposure for *Daphnia* at temperatures of 10, 15, and 20°C ($F_{temp,2,37} = 0.77$, $P = 0.47$; $F_{UV,3,37} = 21.28$, $P < 0.001$), with uniformly smaller body sizes at all UV exposure levels at 25°C (Fig. 4B, $F_{4,20} = 0.98$, $P = 0.44$).

Our results demonstrate the potential for organisms to exhibit very different responses to UV as temperature varies seasonally. It should be noted that the use of a single 12-h exposure in our experiments would suggest conservative results. Combined effects of UV and temperature might be more pronounced in nature. Species such as *D. catawba* and *L. minutus* that depend primarily on PER for their UV tolerance will likely exhibit reduced UV tolerance during periods of highest UV:T ratios in the late winter and early spring (Fig. 1A). Other species such as *Asplanchna* that depend to a lesser extent on PER for their UV tolerance but that are less tolerant of high temperatures might experience

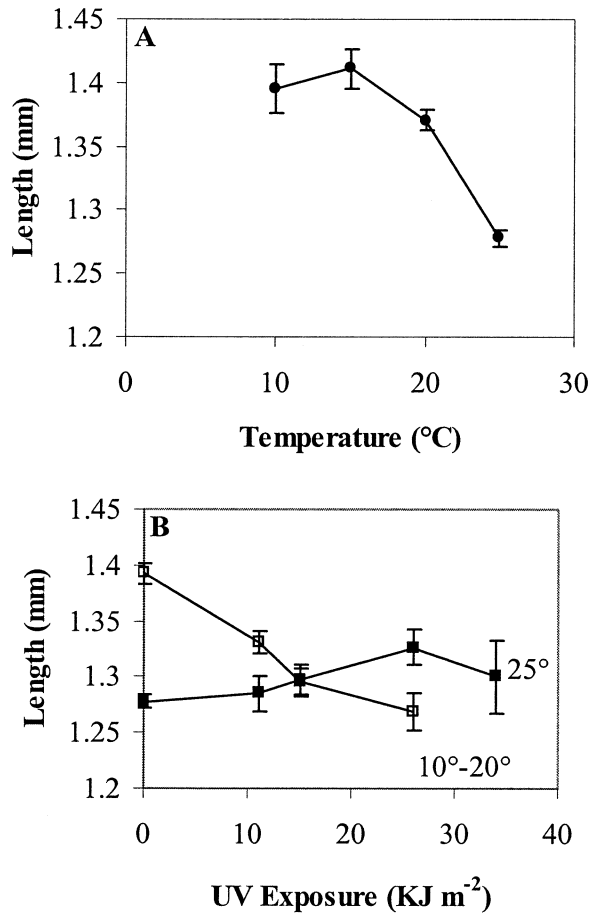


Fig. 4. (A) *Daphnia catawba* length at maturity as a function of temperature in the absence of UV (control treatments). (B) Length at maturity as a function of UV-B exposure at the four temperatures tested. The data for 10, 15, and 20°C (open squares) showed no significant differences among temperatures and were thus combined. Results are from laboratory experiments, error bars are standard errors.

greater UV stress during the warmer summer months when temperatures approach or exceed 25°C. We do not have data on *A. girodi* in our study lakes. However, this species might occur in the surface waters of lakes and exhibit a population peak around summer solstice, though it tends to migrate into deeper waters (4–8 m) during the day (Williamson 1993). Its congener, *A. priodonta* on the other hand, might be most abundant in the top meter or two of shallow lakes even during the day, but it is rare or absent in low-DOC systems (Williamson et al. 2001b).

One question that remains is whether interannual temperature variations in low-DOC lakes such as Giles are as intense as in higher DOC lakes. In order to address this question in our study lakes, we examined the mean temperatures in three of our study lakes that range in DOC from about 1 mg C L⁻¹ (Giles) to 4–5 mg C L⁻¹ (Lakes Lacawac and Waynewood). We used monitoring data from a 4-yr study carried out from 1989 to 1993 to examine the variability (standard deviations of mean) over this 4-yr period. Although this is a very limited data set, we found that the

interannual variability in temperature was comparable among the three lakes in both April (SD = 4.25–4.5°C) when temperatures were changing rapidly and in July (SD = 0.8–1.1°C) during peak solar radiation and summer stratification. Whether this relationship holds across lakes with a wider range of DOC concentrations, surface areas, and mixing regimes and with years of extreme weather conditions is unknown.

Numerous studies in both freshwater and marine systems have previously documented a decrease in body size of zooplankton at higher temperatures, as well as a generally positive relationship between zooplankton body size and fecundity (Moore et al. 1996). Our results suggest that UV also plays a role in these previously observed seasonal patterns in body size and fecundity. Data from our two study lakes indicates that *D. catawba* is significantly smaller in the low-DOC (1.1 mg L⁻¹) Lake Giles compared to the high-DOC (4.7 mg L⁻¹) Lake Lacawac (1.19 mm, SE = 0.02; 1.43 mm, SE = 0.03, respectively, $t_{0.05(2),98} = 7.63$, $P < 0.001$). However, it is important to note that although these differences are consistent with our findings, the difference in size between the two lakes could also be attributable to food quality and quantity, population differences, or a variety of other environmental and genetic factors.

Previous studies have clearly demonstrated the potential for climate change to increase underwater UV environments by decreasing the input of UV-absorbing dissolved organic carbon (Williamson et al. 1996; Yan et al. 1996; Vincent et al. 1998). Climate change is also altering the thermal regime of lakes (De Stasio et al. 1996) and hence the temperatures at which UV exposures occur. The timing of ice cover might be particularly important. For example, ice-out in Northern Hemisphere lakes is occurring up to 6.5 days earlier per 100 years (Magnuson et al. 2000), and the areal extent of Arctic sea ice cover has been decreasing substantially in recent decades (Vinnikov et al. 1999). The timing and extent of ice cover are important to UV exposure of planktonic organisms because of increased mixing following ice-out, as well as the greater UV transparency of water compared to ice (Perovich and Govoni 1991). Earlier ice-out could also alter the timing of the onset of vertical thermal stratification and the potential for the formation of temporary or more sustained thermoclines (Williamson et al. 1996). Stratification can trap less motile plankton species in the high-UV surface waters (Milot-Roy and Vincent 1994; Williamson et al. 1996) or lead to accelerated photobleaching and, hence, increased UV transparency of the surface waters of lakes (Morris and Hargreaves 1997). Ozone-driven changes in biologically effective UV are also showing their greatest seasonal increase during this same late-winter and early-spring period (Madronich 1994). Zooplankton populations often experience a seasonal minimum during this late-winter to early-spring period (Fig. 1B), because in large part of low temperatures and low food availability. This may increase sensitivity to UV damage during this period. Our phototron experiments on organisms ranging from phytoplankton and zooplankton to fish and amphibians demonstrate a strong variability in the importance of PER in UV tolerance among species and major taxa. The temperature depen-

dence of PER and the seasonal dynamics of underwater temperature environments are therefore key elements in our ability to predict the interactive effects of climate change and UV on aquatic ecosystems.

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