

# How do temperature, dissolved organic matter and nutrients influence the response of *Leptodiatomus ashlandi* to UV radiation in a subalpine lake?

SANDRA L. COOKE,\* CRAIG E. WILLIAMSON\*<sup>†</sup> AND JASMINE E. SAROS<sup>‡</sup>

\*Department of Earth and Environmental Sciences, Lehigh University, Bethlehem, PA, U.S.A.

<sup>‡</sup>Department of Biology and River Studies Center, University of Wisconsin – LaCrosse, La Crosse, WI, U.S.A.

## SUMMARY

1. Ultraviolet radiation (UV) is an important stressor for zooplankton in alpine lake ecosystems. Multiple environmental variables such as dissolved organic matter (DOM), temperature and nutrient availability may alter how UV affects zooplankton.
2. We conducted a week-long experiment manipulating UV, nutrients and DOM in enclosures suspended at the surface of cold and warm alpine lakes to determine the interactive effects of these variables on ovigerous *Leptodiatomus ashlandi* (Marsh, 1893), a calanoid copepod.
3. UV had a negative effect on nauplii and gravid females at the colder temperature and at low, ambient DOM levels, but had no effect at the warmer temperature or when DOM was added. At the warmer temperature, fewer nauplii were produced in the +nutrient compared to –nutrient treatment. Adult survival was not affected by UV or any other experimental variable.
4. These results demonstrate that the extent of the impact of UV radiation on zooplankton in alpine systems is altered by other environmental variables, and that these effects may not be apparent from experiments that look only at the survival of adult organisms that are better defended against UV.

*Keywords:* calanoid, dissolved organic matter, subalpine, temperature, ultraviolet radiation

## Introduction

Alpine lakes are among the most susceptible ecosystems to climatic and environmental change (Beniston *et al.*, 1996). Ultraviolet radiation (UV) is particularly important to these systems because of increased ambient UV at higher elevations and because of the high UV transparency of most mountain lakes (Sommaruga, 2001). Some climatic changes, such as increases or decreases in precipitation may alter inputs of

dissolved organic matter (DOM), including chromophoric dissolved organic matter (CDOM) to alpine lakes, and hence may alter the underwater UV environment. Other climatic changes may influence an organism's UV coping mechanism. For example, laboratory experiments have demonstrated the potential for changes in temperature to affect photoenzymatic repair (PER) of UV-induced DNA damage (Williamson *et al.*, 2002). Zooplankton pigmentation, behavioural avoidance (vertical migration) or other UV protection mechanisms can be influenced by food composition and availability (Winder, Spaak & Mooij, 2004; Moeller *et al.*, 2005), which may in turn be altered by nutrient deposition to mountain lakes (Wolfe, Van Gorp & Baron, 2003; Lafrancois *et al.*, 2004). Plankton ranging from bacteria to crustaceans

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Correspondence: Sandra L. Cooke, Illinois Natural History Survey, 1816 South Oak Street, Champaign, IL 61820, U.S.A.

E-mail: slcooke@uiuc.edu

<sup>†</sup>Present address: Craig E. Williamson, Department of Zoology, Miami University, 212 Pearson Hall, Oxford, OH 45056, U.S.A.

are affected by UV in high elevation lakes (Cabrera, Lopez & Tartarotti, 1997; Vinebrooke & Leavitt, 1998; Sommaruga, 2001), but the relative importance of other factors to an organism's UV response is not well known. The objective of this study is to determine how factors associated with environmental change in high elevation lakes – temperature, DOM and nutrients – may alter the response of zooplankton to elevated levels of UV often experienced at these habitats.

Some zooplankton species in alpine lakes, particularly copepods, are relatively UV tolerant due to protective pigmentation (Sommaruga & Garcia-Pichel, 1999; Tartarotti, Laurion & Sommaruga, 2001), while other species must rely on PER to repair their UV-induced DNA damage. Because PER is less effective at colder temperatures (Williamson *et al.*, 2002), this latter group of species may be particularly sensitive to seasonal temperature fluctuations and altered thermal regimes. While climatic change may increase mean temperatures over time and ultimately benefit a PER-dependent species, organisms that do not rely on PER may experience decreased UV tolerance with rising temperatures (Williamson *et al.*, 2002).

Concurrent to changes in temperature are changes in precipitation and biome boundaries. In high elevation lakes, inputs of allochthonous DOM may increase from the combination of treelines moving upland and precipitation events of increased frequency and intensity (Vinebrooke & Leavitt, 1998; Sommaruga *et al.*, 1999; France *et al.*, 2000). CDOM is the primary attenuator of UV in lakes across a range of latitudes and elevations (Morris *et al.*, 1995), although in highly transparent alpine lakes phytoplankton are an important contributor to UV attenuation (Laurion *et al.*, 2000; Hargreaves, 2003). In such systems, even small changes in CDOM concentrations or phytoplankton productivity can have a large effect on UV attenuation. CDOM, the DOM which causes UV attenuation, can also be an important nutritional source for zooplankton through stimulation of the microbial food web (Salonen & Hammar, 1986; De Lange, Morris & Williamson, 2003). Increases in CDOM may therefore benefit even UV-tolerant zooplankton species.

In addition to CDOM, nutrients from atmospheric deposition enter mountain lakes at rates that depend on catchment vegetation (Nydick *et al.*, 2003). *In situ* experiments have demonstrated that phytoplankton

in some mountain lakes are affected by interactions between UV, nutrients and temperature (Doyle, Saros & Williamson, 2005). Although zooplankton may not be directly affected by nutrient inputs, stimulation of the phytoplankton community could increase food availability or quality for zooplankton. This could potentially affect how the zooplankton respond to changes in other environmental variables.

In this study, we used microcosms incubated simultaneously at two different temperatures to examine the effects of UV, temperature, DOM and nutrients on the calanoid copepod *Leptodiatomus ashlandi* (Marsh, 1893). We tested the hypotheses that increases in DOM and temperature reduce the negative effect of UV on zooplankton. We also tested the hypothesis that nutrients reduce negative effects of UV and cold temperatures on zooplankton by increasing food availability, thereby increasing the ability of zooplankton to cope with these UV and temperature stresses.

## Methods

### Study area

The Beartooth Mountains are located in western Montana and Wyoming (45°15'N; 109°28'W). The source water and animals for our microcosm experiment were from Beartooth Lake, a semitransparent (1% attenuation depth of 320 nm UV in 2004 = 0.83 m), oligotrophic (chlorophyll concentration = 0.74 µg L<sup>-1</sup>) lake located at 2713 m elevation. The zooplankton assemblage is 85 % *Leptodiatomus ashlandi*, a calanoid copepod, with *Daphnia* spp. and a cyclopoid species composing the remaining 15%. Beartooth Lake has a dissolved organic carbon (DOC) concentration of 1.0 mg L<sup>-1</sup>. Approximately 0.9 km away and 50 m upland from the lake is a wetland with dark, humic-coloured water that drains into Beartooth Lake. The DOC concentration in this wetland is 10.3 mg L<sup>-1</sup> and the absorbance coefficient of CDOM at 320 nm ( $a_{\text{CDOM } 320}$ ) is 88.7 m<sup>-1</sup>. This wetland served as the DOM source for the experiment described below.

For the temperature component of our experiment, we took advantage of the natural thermal differences of two lakes located within several kilometres of Beartooth Lake. Beauty Lake, which served as our 'cold' incubation lake, is located at 2874 m elevation.

Our 'warm' incubation system was Meadow Pond #2, a small pond located at approximately 3080 m elevation, 44°56'N, 109°32'W. These lakes only served as incubation systems; the microcosms contained water and animals only from Beartooth Lake.

### Overview of experimental design

We conducted a microcosm experiment for which UV, DOM, nutrients and temperature were manipulated, with two levels of each variable, resulting in 16 treatments. The two levels of UV were attained by incubating the 1-L microcosms on PVC frames that were covered by transparent Aclar or Courtgard plastics. Aclar is a long-wave-pass plastic that in water transmits both photosynthetically active radiation (PAR) (100% 400–800 nm) and most UV (98% of UV-B 295–319 nm, 99% UV-A 320–399 nm, with a sharp wavelength cutoff and 50% transmittance at 212 nm). Courtgard is a long-wave-pass plastic that transmits PAR (95% 400–800 nm in water) but blocks most UV (transmits no UV-B 295–319 nm, and only 9% of UV-A 320–400 nm with a sharp wavelength cutoff and 50% transmittance at 400 nm). Microcosms either had ambient DOM (–DOM treatment) and nutrient levels (–nutrient treatment) or received an addition of filtered wetland water (+DOM treatment) and nitrogen and phosphorus (+nutrient treatment). These eight UV/DOM/nutrient combinations were incubated simultaneously at the surface of Beauty Lake, which had a relatively low mean temperature ('8 °C' treatment) and Meadow Pond #2, which had a higher mean temperature ('12 °C' treatment). Each of the 16 treatments had four replicates, resulting in a total of 64 microcosms. Additionally, the entire experiment was replicated without zooplankton so that grazing rates and phytoplankton abundances in the absence of grazing could be measured. The microcosms were 20 × 18 × 5 cm polyethylene bags (Bitran, Inmark, Inc., Austell, GA, U.S.A.) filled to a volume of 1 L and secured on 0.5 × 1 m PVC frames so that they floated flat on the lake surface.

### Field and laboratory methods

On 1 July 2004, the day before the experiment was deployed, *L. ashlandi* were collected from Beartooth Lake at 11:00 MT with a 30-cm diameter 243-µm mesh net towed from 7 m (the depth of the chlorophyll

maximum) to the surface. *Leptodiptomus ashlandi* were sorted into groups of six ovigerous females to be added to the microcosms the next day. Ovigerous individuals were chosen as the test organisms because we were interested in measuring hatching of the larval nauplii in addition to adult survival. The time between egg extrusion and hatching in freshwater copepods is generally 1–5 days (Williamson & Reid, 2001). Female clutch sizes at the start of the experiment were  $19.8 \pm 0.9$  eggs per female. We did not note if females were gravid at the start of the experiment. Zooplankton were kept at temperature in a dark cooler overnight. Beartooth Lake water to fill the microcosms was collected from 7 m, the depth of the chlorophyll maximum. This water was screened using a 153-µm mesh to remove most zooplankton but allow large phytoplankton colonies if they were present. DOM source water was collected from the wetland near Beartooth Lake at 9:00 MT on 1 July 2004. The DOM source water was filtered through a 1-µm prefilter and 0.2-µm filter (hydrophilic polymer filters; Cole-Parmer, Vernon Hills, IL, U.S.A.) using a canister filtration apparatus with polypropylene and ultra-high density polyethylene screen retainers (Cole-Parmer and Corning). The DOM source water comprised 30% of the +DOM treatment volume; thus, 30% of the –DOM treatment (Beartooth Lake water) was filtered using the same methods so that concentrations of organisms and particles greater than 0.2 µm would be the same initially in all treatments. The +DOM treatments had an initial DOC concentration of  $4.01 \pm 0.01$  mg L<sup>-1</sup> and an  $a_{\text{CDOM } 320}$  of 29.9 m<sup>-1</sup>; the –DOM treatments (Beartooth Lake water) had an initial DOC concentration of  $1.02 \pm 0.02$  mg L<sup>-1</sup> and an  $a_{\text{CDOM } 320}$  of 4.1 m<sup>-1</sup>. The pH values of the –DOM and +DOM treatments were compared to verify that the wetland water did not greatly increase the acidity. The –DOM pH was 7.30 and the +DOM pH was 7.12.

On 2 July 2004 the microcosms were deployed in Beauty Lake at 11:00 MT and in Meadow Pond #2 at 14:30 MT. Microcosms were filled to a volume of 1 L with the –DOM and +DOM Beartooth Lake water prepared the previous evening. The +nutrient treatments received 18 µmol L<sup>-1</sup> of N in the form of NaNO<sub>3</sub> and 5 µmol L<sup>-1</sup> of P in the form of NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O. These levels were used to follow the additions used in common culturing media. Total initial nutrient concentrations of Beartooth Lake were 3.66 µM of nitrogen and 0.08 µM of phosphorus. After the

microcosms were filled, six ovigerous *L. ashlandi* females were added to each microcosm. Also added to each microcosm was a dosimeter, a hollow quartz tube (UV transparent), approximately 5 cm in length (<1 cm diameter), filled with salmon testes DNA (Sigma-Aldrich, St Louis, MO, U.S.A.) suspended in sterile buffer solution (Jeffrey *et al.*, 1996) and hermetically sealed with silicone stoppers on each end. DNA dosimeters have been used in numerous studies to quantify DNA damage in the absence of photoprotection and repair processes (e.g. Mitchell & Karentz, 1993; Malloy *et al.*, 1997). The microcosms were placed inside pouches made of window mesh that reduced solar radiation transmittance by approximately 62%. The purpose of the mesh was to attain a radiation dose rate similar to that which would be received by organisms at subsurface depths. The depth at which the most biologically effective wavelength (320 nm) of UV was 60% of the subsurface in Beartooth Lake, the source of the zooplankton, is 0.07 m. The pouches were placed on the PVC frames between the Aclar and Courtgard acrylics above and nylon support netting below. Eight microcosms fit on each frame, and so the 64 microcosms were randomised within the four Aclar and four Courtgard frames. The frames were suspended at the surface near the centre of each lake. The experiment was taken down on 9 July 2004 at 13:40 MT at Beauty Lake and 17:30 MT at Meadow Pond #2. The rationale behind the 7-day incubation was that the experiment would be long enough to observe any differences in nauplii production and adult mortality but short enough that overgrazing and starvation would not occur. The copepods and nauplii were preserved in 70% ethanol and were enumerated using a Bogorov counting chamber. Reproductive state (gravid, ovigerous, both or neither) was noted. Three 50-mL subsamples from each microcosm were preserved in Lugol's solution, and the dominant phytoplankton taxa were enumerated after settling for at least 8 h in an Utermöhl chamber under an inverted microscope (Nikon TS-100, Nikon, U.S.A.).

Water samples from each microcosm were filtered through a GF/F (Whatman) filter. Absorbance of the filtrate was measured in a 1-cm quartz cuvette on the UV-1601 Scanning Spectrophotometer (Shimadzu, Columbia, MD, U.S.A.) to obtain dissolved absorption coefficients for 200–800 nm. The DOC concentration of each sample was measured using a Shimadzu TOC-5000 Analyzer. Initial nutrient concentrations were

measured for Beartooth Lake water using standard methods (American Public Health Association, 2000). Nitrate plus nitrite was measured by the cadmium reduction method, and soluble reactive phosphorus (SRP) by the ascorbic acid method. Particulate carbon and nitrogen collected on GF/F (Whatman) filters were measured using an elemental analyzer (Carlo Erba 1106, Milan, Italy). Particulate phosphorus was measured by persulphate digestion on material collected on 0.4- $\mu$ m polycarbonate filters. DNA damage was assessed by the concentration of cyclobutane pyrimidine dimers (CPDs) in the dosimeters, which were analysed using a radioimmunoassay (Mitchell, 1996).

Temperature was monitored continuously (15-min intervals) during the experiment using iButton thermocron DS1921 programmable temperature loggers (Maxim Dallas Direct, Dallas, TX, U.S.A.). Four temperature loggers were attached to two Aclar and two Courtgard frames in each lake. The temperature loggers were attached to the underside of the frames to maximise shading of the sensors. The cold incubation temperature had a mean  $\pm$  standard deviation of  $8.3 \pm 0.6$  °C, with a range of 6.5–10.0 °C. The warm incubation was  $11.7 \pm 2.0$  °C, with a range of 7.5–16.0 °C (Fig. 1). Solar radiation was also monitored continuously. An internally logging BIC radiometer (Biospherical Instruments, San Diego, CA, U.S.A.) was deployed on a nearby tower on Clay Butte (44°57'N, 109°37'W) at 3175-m elevation near the incubation lakes. The BIC is a medium-bandwidth instrument that records incident solar irradiance at 305, 320 and 380 nm, (8–10 nm full width at half-maximum) as well as PAR (400–700 nm).

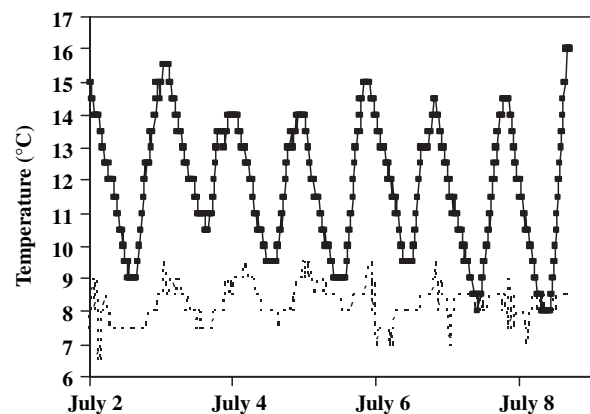


Fig. 1 Surface water temperatures during the experiment (2–9 July 2004) for Beauty Lake (dotted line) and Meadow Pond (solid line with dark squares).

### Statistics

We ran univariate four-way ANOVAs (two levels of UV  $\times$  two levels of DOM  $\times$  two levels of nutrients  $\times$  two levels of temperature) for the dependent variables of nauplii produced, surviving females and gravid females. Surviving nauplii were divided by the number of initial females (expressed as 'nauplii per female') to account for the loss of one or two females in some replicates during experiment deployment. Raw data were used for surviving females, as these initial losses did not affect overall survival results. Gravid females were expressed as a proportion of surviving females, and this proportion was arcsine transformed. Van der Waerden's proportion estimation formula and normal P-P plots were used to verify normality, and Levene's test verified homogeneous variance. One-way ANOVA tests were performed as a *post hoc* comparison of treatment means to help explain significant interactions. We used the software package SPSS 13.0 for Windows (Release 13.0.0, © 2004, SPSS, Inc., Chicago, IL, U.S.A.).

### Results

The DNA dosimeters in the warm incubation indicated that the Courtgard effectively removed damaging UV radiation, and that in the Aclar treatments the addition of DOM reduced 320 nm UV to 72% of that in the treatments without DOM additions (Table 1). In the warm treatment, DNA damage in +UV ranged from 112.19 to 885.75 CPDs per megabase of DNA and damage in the -UV ranged from 12.71 to 29.60 CPDs  $\text{mb}^{-1}$  (Fig. 2), demonstrating that the Courtgard and Aclar plastics were effective at manipulating damaging UV wavelengths. In the +UV treatment DNA damage ranged from 112.19 to 129.83 CPDs  $\text{mb}^{-1}$  in the +DOM and ranged from 512.59 to 885.75 CPDs  $\text{mb}^{-1}$  in the -DOM, demonstrating that CDOM reduced damaging UV exposure (Fig. 2). In addition, in the +UV -DOM treatments there was less DNA damage in the +nutrients compared to -nutrients ( $F_{1,6} = 11.74$ ,  $P = 0.014$ ). This is presumably due to higher phytoplankton growth: total phytoplankton cell counts averaged  $1094 \pm 280$  cells  $\text{mL}^{-1}$  in the +nutrient treatments versus  $108 \pm 11$  cells  $\text{mL}^{-1}$  in the -nutrient. The dosimeter data from the cold treatment had to be discarded due to a processing problem.

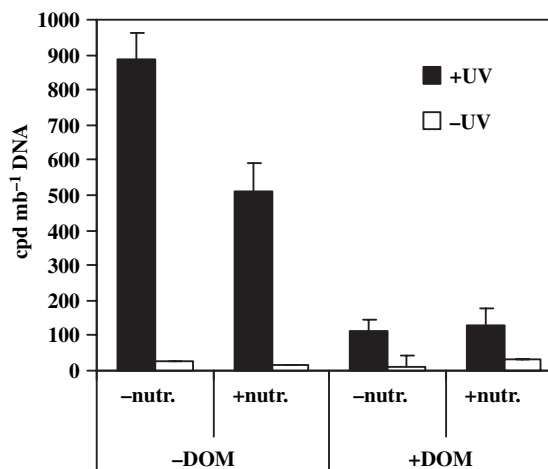


Fig. 2 DNA damage quantified by dosimeters (expressed as cyclobutane pyrimidine dimers per megabase of DNA) in the warm incubation. The dosimeter data in the cold incubation were lost.

Cumulative incident irradiance of 320-nm UV over the 7-day experiment was  $54.11 \text{ kJ m}^{-2}$ . This value reflects the sky conditions during the experiment, which were mostly clear and sunny with the exception of early afternoon thunderstorms on four of the 7 days (Fig. 3). After taking into account radiation lost due to surface albedo (approximately 5%), Aclar transmission (98%), mesh transmission (38%) and absorbance of DOC at 320 nm ( $a_{320}$ ) in each treatment (Table 1), cumulative 320 nm exposure ( $E_{320}$ ) received at the centre of the microcosms ranged from  $20.06$  to  $21.58 \text{ kJ m}^{-2}$  in the +DOM treatments and  $28.51$  to  $29.86 \text{ kJ m}^{-2}$  in the -DOM treatment (Table 1).

Two reproduction variables, nauplii production (nauplii per female) and females in the gravid state (proportion of surviving females that were gravid) were significantly affected by UV and DOM as main effects, a UV  $\times$  DOM interaction and a UV  $\times$  temperature interaction (Table 2). For nauplii, there was no effect of DOM in the absence of UV ( $F_{1,29} = 0.035$ ,  $P = 0.853$ ), but in the presence of UV there were more nauplii in the +DOM than in the -DOM ( $F_{1,28} = 23.763$ ,  $P = 0.000$ ; Fig. 4a). Likewise for gravid females, there was no effect of DOM in the absence of UV ( $F_{1,29} = 0.851$ ,  $P = 0.364$ ), but there was a higher proportion of gravid females in the +DOM than in the -DOM in the presence of UV ( $F_{1,28} = 21.135$ ,  $P = 0.000$ ; Fig. 4b). For nauplii, there was no effect of UV at the warmer temperatures ( $F_{1,30} =$

Treatment	[DOC] (mg L <sup>-1</sup> )	<i>a</i> <sub>CDOM 320</sub> (m <sup>-1</sup> )	DOC-specific abs. (m <sup>-1</sup> per mg L <sup>-1</sup> )	<i>E</i> <sub>320</sub> (kJ m <sup>-2</sup> )
Initial -DOM	1.02 ± 0.02	4.10 ± 0.01	4.02 ± 0.02	-
Initial +DOM	4.01 ± 0.01	29.90 ± 0.01	7.46 ± 0.01	-
8 °C ("cold")				
+UV -DOM -nutrients	1.61 ± 0.52	4.81 ± 0.81	5.09 ± 0.84	28.51
+UV -DOM +nutrients	1.20 ± 0.04	3.82 ± 0.69	3.25 ± 0.67	29.08
-UV -DOM -nutrients	1.35 ± 0.10	5.22 ± 0.74	3.95 ± 0.63	-
-UV -DOM +nutrients	1.24 ± 0.02	4.33 ± 0.36	3.51 ± 0.28	-
+UV +DOM -nutrients	4.05 ± 0.07	22.39 ± 0.47	5.53 ± 0.22	20.06
+UV +DOM +nutrients	3.55 ± 0.03	18.99 ± 0.26	5.35 ± 0.08	21.47
-UV +DOM -nutrients	4.17 ± 0.13	26.94 ± 0.78	6.47 ± 0.01	-
-UV +DOM +nutrients	3.98 ± 0.09	24.79 ± 0.63	6.23 ± 0.18	-
12 °C ("warm")				
+UV -DOM -nutrients	1.14 ± 0.03	2.50 ± 0.22	2.20 ± 0.24	29.86
+UV -DOM +nutrients	1.13 ± 0.01	3.62 ± 0.47	3.18 ± 0.39	29.20
-UV -DOM -nutrients	1.21 ± 0.04	3.35 ± 0.25	2.80 ± 0.30	-
-UV -DOM +nutrients	1.15 ± 0.02	2.95 ± 0.15	2.58 ± 0.18	-
+UV +DOM -nutrients	3.83 ± 0.13	20.61 ± 0.38	5.40 ± 0.26	20.79
+UV +DOM +nutrients	3.66 ± 0.03	18.74 ± 0.43	5.13 ± 0.16	21.58
-UV +DOM -nutrients	4.29 ± 0.12	25.16 ± 0.24	5.88 ± 0.22	-
-UV +DOM +nutrients	4.41 ± 0.40	23.70 ± 0.20	5.49 ± 0.42	-

The data are the mean ± standard error for all microcosms in each treatment. DOM, dissolved organic matter.

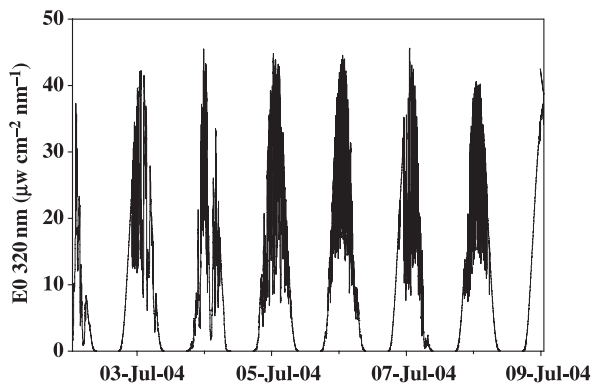


Fig. 3 Incident 320-nm irradiance ( $E_{0320 \text{ nm}}$ ) received over the course of the 7-day experiment.

2.724,  $P = 0.109$ ), but there were fewer nauplii in the +UV than in the -UV at the colder temperatures ( $F_{1,27} = 20.586$ ,  $P = 0.000$ ; Fig. 5a). Similarly for gravid females, UV had no effect at the warmer temperatures ( $F_{1,30} = 0.735$ ,  $P = 0.398$ ), but there were fewer gravid females in the +UV than in the -UV at the colder temperatures ( $F_{1,27} = 16.604$ ,  $P = 0.000$ ; Fig. 5b).

Nauplii production was also affected by a temperature × nutrient interaction (Table 2, Fig. 6). There was no effect of nutrients in the colder

Table 1 Final dissolved organic carbon (DOC) concentrations, absorbance of DOC at 320 nm ( $a_{320}$ ), DOC-specific absorbance and cumulative 320-nm irradiance ( $E_{320}$ )

Table 2 Results from four-way ANOVA tests on the effects of UV, DOM, temperature (temp.) and nutrients (nutr.) on *Leptodiatomus ashlandi* reproduction and survival

Source	Dependent variable	$F_{1,45}$	$P$
UV	Nauplii per female	30.332	0.000
	Gravid females	12.540	0.001
DOM	Nauplii per female	13.327	0.001
	Gravid females	6.600	0.014
UV × DOM	Nauplii per female	9.641	0.003
	Gravid females	14.245	0.000
UV × temp.	Nauplii per female	6.387	0.015
	Gravid females	4.625	0.037
temp. × nutr.	Nauplii per female	12.460	0.001

Only significant results ( $P < 0.05$ ) are shown. DOM, dissolved organic matter.

treatments ( $F_{1,27} = 1.559$ ,  $P = 0.223$ ), but there were fewer nauplii in the +nutrients than in the -nutrients at the warmer temperatures ( $F_{1,30} = 7.255$ ,  $P = 0.011$ ). Adult survival was not significantly affected by any variable, and had a mean of 76.2 % across all treatments.

Significant grazing rates were observed on *Dinobryon*, *Fragilaria* and *Cyclotella*, but not *Asterionella* (Fig. 7). Growth of these four taxa was highly stimulated by warmer temperatures and nutrients

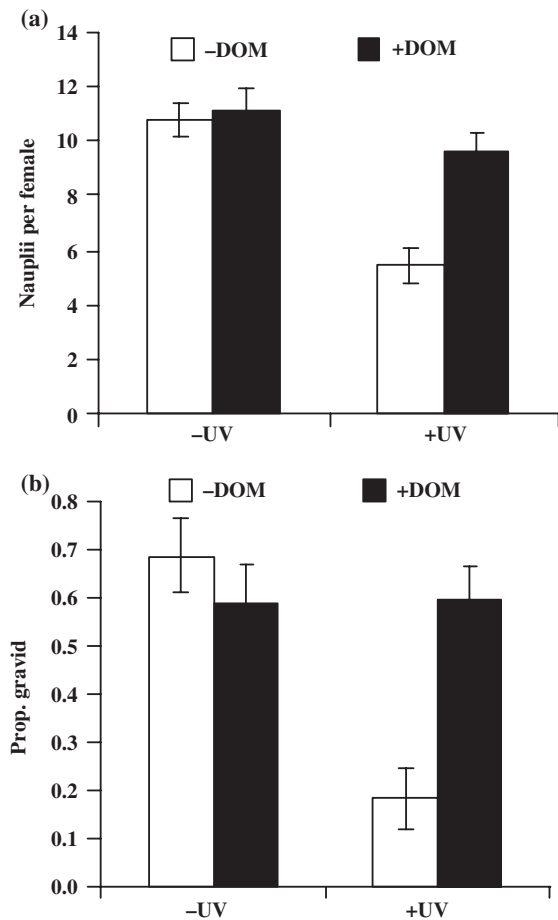


Fig. 4 Mean nauplii produced per female (a) and mean proportion of surviving females in the gravid state (b) in the +UV +DOM, +UV -DOM, -UV +DOM and -UV -DOM treatments. Error bars indicate standard error.

(C.E. Williamson, J.E. Saros, C. Salm, S.L. Cooke & S. Keseley, unpublished data). Phytoplankton responses, as well as the effects of UV and temperature on grazing rates will be presented in detail elsewhere (C.E. Williamson, J.E. Saros, C. Salm, S.L. Cooke & S. Keseley, unpublished data).

Dissolved organic carbon concentrations did not change significantly from initial levels. However, in the +DOM treatments, DOC-specific absorbance declined from initial levels ( $F_{1,31} = 34.547$ ,  $P = 0.000$ ). In the +DOM treatments, final DOC concentrations were lower in the +UV compared to -UV treatments (Table 1;  $F_{1,27} = 13.495$ ,  $P = 0.001$ ). In the +UV +DOM treatments, DOC concentrations were lower in the +nutrients compared to -nutrients ( $F_{1,12} = 10.836$ ,  $P = 0.006$ ).

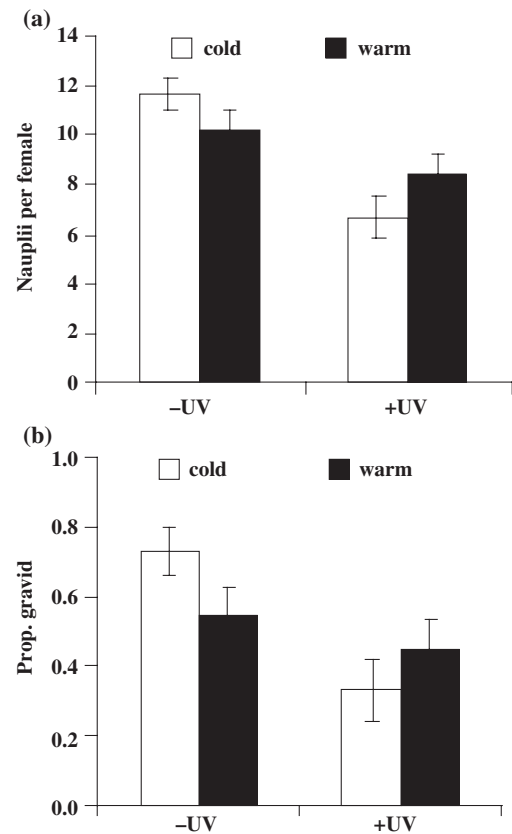


Fig. 5 Mean nauplii produced per female (a) and mean proportion of surviving females in the gravid state (b) in the warm +UV, warm -UV, cold +UV and cold -UV treatments. Error bars indicate standard error.

## Discussion

Previous work has shown that crustacean zooplankton in alpine lakes are generally well adapted to high intensities of solar radiation (Sommaruga, 2001). This relatively short-term experiment shows that while UV does not have any apparent effects on adult survival, effects on reproduction do occur, and are modified by DOM and temperature. The presence of DOM increased the survival of nauplii as well as the ability of females to develop to the gravid stage. UV was a more important stressor on *L. ashlandi* at colder temperatures than at warmer temperatures, as shown by the UV-temperature interactions on nauplii produced and gravid females. This is consistent with the enzyme kinetics hypothesis that DNA repair enzymes are slowed down at lower temperatures, and with previously observed laboratory experiments that demonstrated decreased UV tolerance in *Daphnia* at

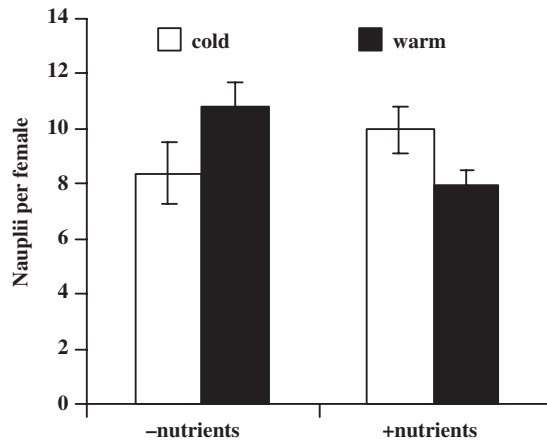


Fig. 6 Mean nauplii produced per female in the warm +nutrient, warm -nutrient, cold +nutrient and cold -nutrient treatments. Error bars indicate standard error.

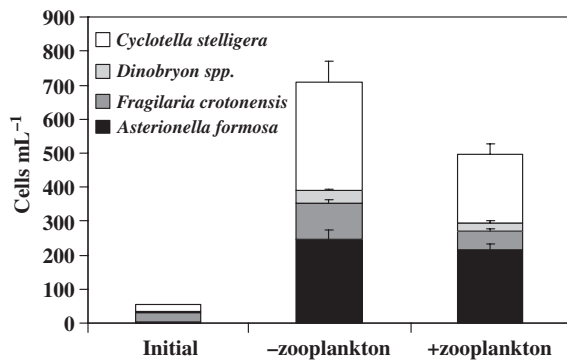


Fig. 7 Mean abundances of the four dominant phytoplankton taxa in the presence and absence of zooplankton. Error bars indicate standard error.

lower temperatures (Williamson *et al.*, 2002; MacFadyen *et al.*, 2004). These results indicate that multiple environmental factors can influence an organism's response to UV.

Other studies, both *in situ* and laboratory, have also shown that zooplankton survival in the presence of UV is higher when DOM is added (Rautio & Korhola, 2002; De los Ríos, 2005). The dosimeter data presented here show that DNA damage was also substantially lower in the +DOM treatments (Fig. 2). Correspondingly, DOM had no effect on *L. ashlandi* in the -UV treatments, but a positive effect on reproduction in the +UV treatments. These results indicate that optical properties of the DOM (i.e. UV attenuation) were important for the copepods (Table 1). Food stimulation by DOM may have occurred as well in the +UV

treatments, although we do not have data to support this. The higher DOC concentrations in the +DOM -nutrients compared to the +DOM +nutrients may be due to nutrients stimulating microorganisms that can metabolise the DOC (Table 1). DOM concentrations in high elevation lakes may gradually increase as watersheds previously above treeline become more vegetated (Vinebrooke & Leavitt, 1998; Sommaruga *et al.*, 1999; France *et al.*, 2000). DOM can also be a fluctuating factor on seasonal and interannual time scales (Pace & Cole, 2002). This is particularly true for high elevation and high latitude lakes, because DOM is excluded from the thick ice cover, creating a relatively high DOM concentration beneath the ice, which is then diluted during ice-out (Belzile, Gibson & Vincent, 2002).

Temperature and UV can affect zooplankton in ways other than simple DNA repair enzyme kinetics. UV can differentially affect other enzymatic processes of zooplankton at different temperatures (Borgeraas & Hessen, 2000). Indirect effects of UV and temperature on zooplankton may occur through the food web. Respiration of bacteria from northern Quebec freshwaters was inhibited in the presence of UV at 20 °C, but was higher at 10 °C and when UV was absent (Rae & Vincent, 1998). In a previous *in situ* experiment that examined phytoplankton from Beartooth Lake, UV depressed algal growth in the 6 °C treatment, but had variable effects in the 12 °C treatment (Doyle *et al.*, 2005). The interactions we observed on *L. ashlandi* are likely a result of both direct and indirect effects.

Interactive effects of UV and temperature have also been observed *in situ* for a calanoid copepod from a temperate lake (Persaud & Williamson, 2005), although the nature of these effects was different from what we observed. Persaud & Williamson (2005) found that in short-term exposure experiments in the field *Leptodiaptomus minutus* adult survival was higher in the +UV than in the -UV in the colder treatment, with no UV effect in the warmer treatment. In a separate longer term experiment, where incident UV exposure was reduced by a mesh, UV had a negative effect on *L. minutus* egg ratios in the warmer treatment (10.8 °C) but no effect in the cold (7.8 °C) (Persaud & Williamson, 2005). There may be several reasons for these apparent differences. These earlier experiments involved full zooplankton communities, and *L. minutus* is one of the more UV-tolerant zooplankton

species in that lake (Williamson *et al.*, 1994). Thus, *L. minutus* may have benefited from the negative impacts of competing species and subsequent release of food resources. Responses to UV and temperature are likely to be species specific, because species vary in their PER capabilities (Williamson *et al.*, 2002), temperature optimum and UV tolerances, and responses are also dependent on climatic and seasonal gradients.

We observed that at the warm temperature there were fewer nauplii in the +nutrients compared to -nutrients (Fig. 6). The reason for this is unclear, as all four dominant phytoplankton species were stimulated by nutrients and warmer temperatures (C.E. Williamson, J.E. Saros, C. Salm, S.L. Cooke & S. Keseley, unpublished data). The copepods may not have depended solely on the phytoplankton for their food source, and it is possible that the quality of non-algal food sources was altered by temperature and nutrients combinations. Temperature changes are likely to influence microbial community structure in mountain lakes (Rae & Vincent, 1998), as are nutrient additions (Vinebrooke & Leavitt, 1998).

Only *L. ashlandi*, the most prevalent species of the Beartooth Lake zooplankton community was examined in this study because of the desire to focus on one dependent variable with so many independent variables and to minimise over-grazing of phytoplankton. However, it is important to consider species interactions when predicting responses to global change, as different species vary in their environmental tolerances (Davis *et al.*, 1998). Responses of alpine zooplankton to UV vary both among species and within species (Tartarotti *et al.*, 1999). Acquisition of mycosporine-like amino acids (MAAs) is an important determinant of UV tolerance in some zooplankton (Tartarotti *et al.*, 2001; Moeller *et al.*, 2005). The *L. ashlandi* population in this study did not have substantial concentrations of MAAs in their tissues, compared to some *Boeckella* and *Hesperodiptomus* species (A.D. Persaud, R.E. Moeller, C.E. Williamson & C.W. Burns, unpublished data) but the availability of MAAs and other photoprotective compounds can vary seasonally in some lakes (Moeller *et al.*, 2005).

Time scale is also an important consideration for *in situ* UV experiments because of dose and dose rate effects. Zooplankton and phytoplankton may be both stimulated and suppressed by UV, depending on the sampling timepoint (Cabrera *et al.*, 1997). Persaud &

Williamson (2005) observed different results for *in situ* UV-temperature experiments when UV dose was kept mostly constant but UV dose rate was altered. Had our experiment been longer, we may have observed differences in adult survival and copepodid development. Nevertheless, the reproductive response of *L. ashlandi* in this short-term experiment demonstrates that multiple variables associated with climatic and environmental change in high elevation lakes can have interactive biological effects. The effects of UV, temperature, DOM and nutrients will vary with species, planktonic community structure, short- and long-term time scales and local climate. These variables should be considered in studies of both mountain and temperate lakes in order to better understand how planktonic communities may be affected by global change.

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