

Primary Research Paper

## Beneficial and detrimental interactive effects of dissolved organic matter and ultraviolet radiation on zooplankton in a transparent lake

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### Abstract

While changes in dissolved organic matter (DOM) concentrations are expected to affect zooplankton species through attenuation of potentially damaging ultraviolet (UV) radiation, generation of potentially beneficial or harmful photoproducts, pH alteration, and microbial food web stimulation, the combined effects of such changes on zooplankton community structure have not been studied previously. Our purpose was to determine how an increase in allochthonous DOM and associated changes in pH in an initially transparent lake may affect zooplankton community structure, and how exposure to solar UV may alter these DOM and pH effects. We ran microcosm experiments manipulating UV, DOM, and pH near the surface of Lake Giles in northeastern Pennsylvania. We found that when DOM was added in the presence of ambient UV, *Daphnia* and copepod UV-mortality was reduced by approximately three and two times compared to UV exposure without extra DOM. When DOM was added in the absence of UV, adult *Daphnia* and copepods were reduced compared to no DOM addition in the absence of UV. *Daphnia* and cyclopoid egg production and rotifer abundance were generally higher in the presence of DOM, regardless of UV treatment. The lower abundance yet high egg production in the presence of DOM and absence of UV may be explained by higher abundance of egg-bearing adults compared to non-egg-bearers. We conclude that allochthonous DOM benefits some zooplankton in a high-UV environment, but may be detrimental under low-UV conditions. Overall, *Daphnia* abundance and egg production were higher than that of calanoid copepods in the DOM additions, indicating that in some lakes an increase in allochthonous DOM may lead to a zooplankton community shift favoring *Daphnia* over calanoid copepods.

### Introduction

Dissolved organic matter (DOM), a collection of plant- and microbial-derived substances, is an important regulator of lake ecosystems on a number of levels due to its biological, chemical, and optical effects (Williamson et al., 1999). The radiation-absorbing components of DOM, known as chromophoric dissolved organic matter (CDOM), are the primary attenuator of ultraviolet

(UV) radiation in temperate lakes in the northern and southern hemispheres (Morris et al., 1995; Hargreaves, 2003). Allochthonous DOM may confer acidity to a system over time, because of its organic acid components (Patrick et al., 1981). Additionally, DOM can stimulate planktonic food webs (De Lange et al., 2003). Planktonic organisms may differ in their sensitivity to UV, DOM, and pH dynamics, thus making community level responses to changes in these variables complex,

difficult to predict, and yet of great ecological interest. In this study our purpose was to determine if an increase in allochthonous DOM in a transparent lake would cause species differences in abundance and reproduction within a zooplankton community, and whether such differences were caused by the impact of DOM on UV exposure, pH, or the food web.

The biological effects of changes in allochthonous DOM are important because of the potential alteration of lake watersheds. In many regions, DOM in lakes is expected to decline as climate change-induced decreases in precipitation will lead to more DOM oxidation and less leaching and runoff (Schindler et al., 1996; Yan et al., 1996). In higher elevation lakes on the other hand, DOM may increase due to vegetation shifts causing treeline encroachment on alpine and high latitude biomes (Sommaruga et al., 1999). Anthropogenic disturbance of watersheds, such as logging and fire, may also increase lake DOM (Carignan et al., 2000; France et al., 2000). Our DOM-addition experiment is relevant to these latter scenarios of increases in allochthonous DOM.

We expect the beneficial and detrimental consequences associated with a change in DOM to have net effects that differ among zooplankton species, due to different feeding regimes and environmental tolerances. For example, *Daphnia* may indirectly benefit from DOM more than diaptomid copepods, as DOM photoproducts are substrates for bacterial metabolism (Wetzel et al., 1995; Moran & Zepp, 1997; Tranvik & Bertilsson, 2001), and *Daphnia* are more able to benefit nutritionally from bacteria and heterotrophic nanoflagellates, than are diaptomids (Sanders et al., 1996). Another effect that may favor *Daphnia* over diaptomids is the humic and fulvic acids contained in DOM. *Daphnia catawba* is acid-tolerant in laboratory bioassays (Locke, 1991) and is common in low pH lakes (Sprules, 1975; Keller & Pitblado, 1984), while Fischer et al. (2001) observed a decline in the diaptomid *Leptodiaptomus minutus* in response to experimental lake acidification. Finally, UV attenuation by DOM can also have differential effects on zooplankton. In Lake Giles, Pennsylvania, USA, calanoids and cyclopoids are generally more UV-tolerant than cladocerans (Leech & Williamson, 2000).

While previous work had demonstrated the importance of changes in nutrition, pH, and UV

attenuation to zooplankton, little is known about the net effect of DOM on species composition. Our purpose was to measure the overall effect of DOM on zooplankton abundance and reproduction by altering DOM, UV, and pH in small-scale enclosure experiments in the surface waters of a low-DOM, UV transparent lake. We hypothesized that because of differences in UV-sensitivity, acid-tolerance, and increases in the DOM-based microbial component of the food web, some species (e.g. *Daphnia catawba*) would have higher abundance and reproduction than others (e.g. *Leptodiaptomus minutus*) in the presence of additional DOM.

## Materials and methods

### Study site

Lake Giles is located on the Pocono Plateau of northeastern Pennsylvania (41° 23' N, 75° 06' W). It is a transparent (summer 1% attenuation depth of 320 nm UV in 2003 = 5 m), acidic (pH = 5.6), oligotrophic lake with a dissolved organic carbon (DOC – the carbon in DOM) concentration of approximately 2 mg l<sup>-1</sup> and a low acid-neutralizing capacity (usually –10 to 0 µeq l<sup>-1</sup>). It is noteworthy that the DOC concentration has slowly increased during the past few years and was previously less than 1 mg l<sup>-1</sup> with a UV-320 1% attenuation depth of up to 15 m (Williamson et al., 1999). Giles has a single small inlet stream that flows during wet periods through a boggy area of the watershed. The inlet stream is more than 95% shaded by hemlock and mixed oak forest. At about 100 m from its confluence with the lake the stream has a DOC concentration of approximately 6.5 mg l<sup>-1</sup>. Because of its high DOC concentration and location within the Lake Giles watershed, we used water from this inlet stream as an ecologically relevant DOM source for the experiment.

The zooplankton community in Lake Giles consists of relatively few species, most likely due to its acidic, oligotrophic status. The zooplankton community is dominated by *Daphnia catawba*, *Leptodiaptomus minutus*, *Aglaodiaptomus spatulocrenatus*, and *Cyclops scutifer*. The rotifers *Kellicottia longispina* and *Conochilus* spp. were present at large enough densities to be included in the experiment. Other species that were present but at

densities too low be included in this study are *Diaphanosoma* spp., *Bosmina* spp. and the rotifers *Keratella taurocephala*, *Polyarthra* spp., and *Gastropus* spp.

#### Overview of experimental design

UV, DOM, and pH were the three variables manipulated in this experiment, with two treatment levels of each variable. The enclosures were 3.8 l-sized polyethylene bags (Bitran, 50% transmittance at 234 nm) filled to a volume of 3.6 l with lake water. UV reaching the test organisms was controlled using two acrylics with sharp long wave pass cutoffs: OP-2 (50% transmittance at 410 nm, -UV treatment) and OP-4 (50% transmittance at 272 nm, +UV treatment) acrylics (CYRO Industries). Water collected from the bog inlet at approximately 100 m from the confluence with the lake was added to half of the bags (+DOM treatment); the other bags received no addition (-DOM treatment). The DOM additions lowered the pH to 4.5, about one unit below lake pH. To separate out the pH effects from other DOM effects sufficient 0.01 N NaOH (~12 ml per bag) was added to half of the DOM bags to raise the pH to the lake pH of about 5.6. The pH of the other half of the DOM treatments was kept at 4.5. The pH of half of the bags containing only lake water (-DOM treatment) was manipulated using 0.01 N H<sub>2</sub>SO<sub>4</sub> (~8 ml per bag) to bring the lake pH (5.6) down to the DOM treatment pH (4.5). Thus, there were eight treatment combinations: -UV-DOM, -UV+DOM, +UV-DOM, and +UV+DOM, each at two different pH levels (low pH = +DOM and -DOM + acid; normal pH = -DOM and +DOM + base), with four replicates of each treatment. The rigid OP-2 and OP-4 acrylics were cut into 0.5×1.0 m sections that were placed on top of PVC frames and secured over the bags with the acrylic edge extending 4 cm beyond the edge of the bag. Nylon netting tied underneath the frames supported 2 bags, so 16 frames were used to suspend the 32 bags.

#### Field and laboratory methods

The day before the experiment was set up, we collected surface water from Lake Giles and from the bog inlet stream. The lake was thermally stratified at approximately 7 m at the time of

collection. The inlet water was filtered through a 1 μm prefilter and 0.2 μm sterile filter (hydrophilic polymer filters; Cole-Parmer) using a canister filtration apparatus with polypropylene and ultra-high density polyethylene screen retainers (Cole-Parmer and Corning). Because the DOM treatment water was 50% filtered inlet water and 50% non-filtered lake water, we also filtered some lake water in the same manner to make the “-DOM” treatments 50% filtered and 50% non-filtered. This was to maintain equal concentrations of phytoplankton, protozoa, bacteria, and particulates among treatments. The “non-filtered” water was strained through a 36 μm nylon mesh to remove zooplankton.

After filtering, the pH of each water source was measured on stirred samples using an Orion SA 250 pH meter and Ross electrode with temperature compensation to 25 °C. Titrations were done with 0.01 N NaOH and 0.01 N H<sub>2</sub>SO<sub>4</sub> solutions to determine how much of each we needed to add to a 3.5 l bag of DOM treatment water and lake water, respectively, to adjust the pH. NaOH and H<sub>2</sub>SO<sub>4</sub> were chosen because we did not expect additional effects of the Na<sup>+</sup> and SO<sub>4</sub><sup>2-</sup> ions based on the known ionic composition of Lake Giles. The experiment was set up the next day, 22 May 2003 at 11:00. Bags were filled with measured amounts of filtered and non-filtered lake water and filtered inlet water, as appropriate. Aliquots of acid and base were added to the “-DOM+acid” and “+DOM+base” treatments. Four replicate initial water samples from each treatment were collected for laboratory analyses. Zooplankton were collected from integrated vertical tows of a 202 μm and a 363 μm net from 20 m to the surface, a depth range great enough to ensure that hypolimnetic cyclopoids would be included. To increase the low abundance of *Daphnia*, an additional 363 μm mesh net tow from 10 m to the surface was collected. The three tows were combined in 10 l of lake water. A gently mixed 150 ml aliquot of zooplankton was added to each bag. The initial densities within the 3.5 l bags were 23.3 ± 4.7 adult *Daphnia*, 16.9 ± 4.1 juvenile *Daphnia* and 51.9 ± 9.8 *Leptodiptomus* adults per liter. Average daytime whole water column densities during May and June in Lake Giles are on the order of 8–10 *Daphnia* and 40–45 adult *Leptodiptomus* per liter (Moeller et al., 1995). However, maximum

epilimnetic densities can be higher than this, because as much as 56% of the *Daphnia* population and 100% of the calanoid copepod community have been observed in the epilimnion during the day (Leech et al., 2005). Both species exhibit higher densities other times of year (Moeller et al., 1995). Additional 150 ml aliquots of zooplankton were collected for initial samples and were preserved with a sucrose formalin solution (10% of sample volume).

After the bags were filled with water and zooplankton they were sealed without air bubbles and placed in the PVC frames between the nylon netting below and acrylic sheet above (OP-2 or OP-4). The frames were suspended at 0.5 m depth near the center of Lake Giles for 8 days. On 30 May 2003 at 11:15 all zooplankton within each bag were collected with a 48  $\mu\text{m}$  mesh cup and preserved with sucrose formalin (10% of sample volume). Water samples from each bag were also collected for laboratory analyses. Initial and final zooplankton were enumerated in their entirety using a Bogorov counting chamber. The numbers of individuals surviving at the time of preservation and their eggs were the primary metrics for assessing the response of different species in the different treatments. Additionally, dry mass data for the same or similar species in this experiment were obtained from Dumont et al. (1975), Persson & Ekbohm (1980), Yan & Strus (1980), and Culver et al. (1985). These data were used to estimate each species' dry mass as a percentage of the zooplankton community mass. Unfortunately, there was one leaky bag both in the +UV+DOM+base treatment and in the -UV+DOM+base treatment, and thus these replicates were discarded.

Water column downwelling diffuse attenuation coefficients ( $K_d$ ) were calculated from data collected using a PUV 500 radiometer (Biospherical Instruments) on 22 May close to the time of experiment set up. The PUV 500 records downwelling solar irradiance at four UV wavebands centered at 305, 320, 340, 380 nm, and PAR (photosynthetically active radiation, 400–700 nm) and depth (0–40 m with better than 1 cm resolution).  $K_d$  is the slope of the linear regression between depth and the natural logarithm of irradiance expressed as a percentage of subsurface irradiance. A larger  $K_d$  indicates increased attenuation, or reduced transparency. Incident solar

irradiance data for the duration of the experiment were available from the GUV 521 radiometer (Biospherical Instruments) stationed nearby at Lacawac Sanctuary (41° 23' N, 75° 18' W). This instrument records 15 min averages at the same wavelengths as the PUV. Dissolved oxygen and temperature profiles of Lake Giles were collected 22 May using a YSI Model 58 Dissolved Oxygen Meter. Water temperature data were also available from the PUV 500.

Immediately after experiment set up and take-down, the pH of initial and final water samples was measured using an Orion SA 250 pH meter and Ross electrode with temperature compensation to 25 °C. The samples were then filtered onto GF/F (Whatman) filters which were frozen for later chlorophyll analysis. Absorbance of the filtrate was measured in a 10 cm quartz cuvette on the UV-1601 Scanning Spectrophotometer (Shimadzu) to obtain dissolved absorption coefficients ( $a_{\text{CDOM},\lambda}$ ) for 200–800 nm. Optical density ( $A_\lambda$ ) of the filtrate was measured in a 10 cm quartz cuvette on the UV-1601 Scanning Spectrophotometer (Shimadzu). To obtain dissolved absorption coefficients ( $a_{\text{CDOM},\lambda}$ ) for 200–800 nm ( $A_\lambda$ ) measurements were converted to  $a_{\text{CDOM},\lambda}$  using the following equation (Kirk, 1994):

$$a_{\text{CDOM},\lambda} = 2.303A_\lambda/l$$

where  $l$  is the pathlength in meters. The  $A_\lambda$  values for all samples were first corrected by subtracting  $A_\lambda$  of low-carbon deionized water, measured separately. The remainder of the filtrate was saved for analysis of DOC and biolability. The DOC concentration of each sample was measured using a Shimadzu TOC-5000 Analyzer.

As a rough assay to compare UV exposure effects, pH effects, and source effects (inlet or lake) on DOC biolability, the bioavailability of carbon, expressed as the rate of carbon consumption by microorganisms, was measured for the 12 water samples (four initial samples collected 22 May and eight final samples collected 30 May). Initial DOC concentrations were measured for all 12 water samples. Then, a small amount (5% of a 50 ml volume) of non-filtered Lake Giles water, presumably containing bacteria and other microorganisms, was added to GF/F pre-filtered, 0.2  $\mu\text{m}$ -sterile-filtered water samples from each treatment. Initial particulate carbon from this

non-filtered water was not measured but assumed to be negligible because of the small volume used and low particulate matter content of Lake Giles surface water. The samples were then stored in dark containers to prevent photodegradation at 20 °C for 10 weeks, the length of time needed to ensure a measurable effect in all treatments. After dark storage, the samples were GF/F filtered and their DOC concentrations were measured and compared to initial DOC concentrations. The difference between initial and final DOC was assumed to be due to processing of bioavailable carbon.

Chlorophyll *a* was used to assess food availability. The frozen filtered chlorophyll samples were extracted with a 5:1 (volume:volume) mixture of 90% aqueous acetone and methanol for 24 h. The fluorescence of the extract was measured on a Sequoia-Turner Model 112 Fluorometer. The instrument was calibrated using chlorophyll extracts in 90% acetone that were measured spectrophotometrically (Pechar, 1987). Chlorophyll concentrations were corrected for pheopigment using the acidification method (American Public Health Association, 1995).

Statistical analyses were performed using the software package SPSS 11.5. Raw count data were  $\log_{10}$  transformed as necessary to obtain normally distributed data as shown by Van der Waerden's proportion estimation formula and normal P-P plots. The Levene Test was used to test for homogeneity of variance. To test how the treatments affected species composition, we did a multivariate three-way ANOVA (UV  $\times$  DOM  $\times$  pH; two levels of each) for eight species groups and life stages: *D. catawba* adults and juveniles, *L. minutus* adults, *A. spatulocrenatus* adults, calanoid copepodids, total *C. scutifer* (adults and copepodids were combined because of low abundances), *K. longispina*, and *Conochilus* spp. If Roy's largest root criterion indicated a significant response, then tests of between-subjects effects were examined to determine which groups responded significantly to the different treatments. To test how the treatments affected reproduction, univariate three-way ANOVAs were conducted for *D. catawba* and copepod egg ratios (eggs per female) and nauplii. Univariate three-way ANOVAs were also used to test effects on UV, DOM, and pH on chlorophyll and biolability.

Tukey's test was used as a *post hoc* comparison of treatment means to help explain significant interactions.

#### *Environmental and experimental conditions*

At the start of the experiment surface temperature in the 4 m deep mixed layer was 14.0 °C, and was the same within all bags; dissolved oxygen ranged from 10.3 to 11.3 mg l<sup>-1</sup> in the mixed layer, and was saturated within the experimental bags at the end of the experiment. At the start of the experiment,  $K_{d320} = 1.20 \text{ m}^{-1}$ ,  $K_{d340} = 0.92 \text{ m}^{-1}$ , and  $K_{dPAR} = 0.33 \text{ m}^{-1}$  in the epilimnion. From measured PUV irradiance the 1% and 10% (of surface irradiance) attenuation depths for 320 nm UV were 4.09 m and 1.98 m, respectively. At 0.5 m, the depth at the center of the suspended bags, the wavelengths 320 nm, 340 nm, and PAR were attenuated to 54.9, 63.1, and 84.8%, of surface values, respectively. The cumulative 320 nm UV-B surface irradiance measured over the course of the 8-day experiment was 32.2 kJ m<sup>-2</sup> nm<sup>-1</sup>, which is equivalent to 3.0 "320-nm exposure days". A 320-nm exposure day is the amount of 320 nm (long UV-B) radiation received at the water surface at this location on a cloud-free and haze-free 24 h day at summer solstice under average ozone conditions for the region (332 Dobson units, average for 15–25 June from 1997–2001, Earth Probe TOMS, R. McPeters, URL = <http://www.toms.gsfc.nasa.gov/>). In other words, an exposure day is the maximum amount of 320 nm radiation that could theoretically be received in a 24 h day near summer solstice when sunlight intensity is at its highest in the Northern Hemisphere. The concept of 320-nm exposure day provides some context for interpreting cumulative irradiance values. The 320 nm wavelength has been recognized as biologically important when both wavelength-specific DNA damage and variations in sunlight photon flux density are considered (Williamson et al., 2001). The 320-nm exposure day was estimated by using the modeling program RTBasic (Biospherical Instruments). This program estimates incident solar radiation for a given date, time, and location. Other input parameters include ozone and cloud optical depth. Running this model at 15 min intervals on summer solstice yielded a 320 nm dose of 10.9 kJ m<sup>-2</sup> nm<sup>-1</sup> for the day. The measured cumulative 320-nm incident irradiance during our

experiment was divided by this number to obtain 320-nm exposure days.

In addition to cumulative 320-nm surface irradiance, we calculated cumulative 320-nm irradiance ( $E_{320}$ ) received at the center of each +UV bag at 0.5 m depth where the experiment was suspended. This was done using the  $K_{d320}$  obtained from PUV data, the percent transmission of the OP-4 acrylics and Bitran bags (91.4% and 83.8%, respectively; obtained from spectrophotometric scans conducted in water), and the CDOM absorption coefficients ( $a_{CDOM320}$ ) for all treatments in the +UV (Table 1). Because  $a_{CDOM320}$  declined in all treatments due to photobleaching, we simply averaged initial and final  $a_{CDOM320}$  values to calculate the overall effect of CDOM absorption on 320 nm attenuation. We also accounted for an estimated 5% loss of irradiance due to surface albedo.

## Results

### *Treatment conditions*

Despite attempts to obtain uniform pH levels within the “lake pH” and “DOM-altered

pH” groups the initial pHs were significantly different between the +DOM+base and -DOM treatments ( $F_{1,6} = 103.71$ ,  $p = 0.000$ ) and the -DOM+acid and +DOM treatments ( $F_{1,6} = 179.92$ ,  $p = 0.000$ ; Table 1). These differences were not significant by the end of the experiment. Except in the treatment with base added, the pH increased in all treatments during the experiment.

Initial DOC concentrations did not differ with pH and were approximately  $3 \text{ mg l}^{-1}$  higher in the +DOM treatments (Table 1). Although the initial DOC concentrations were not affected by pH, the acid and base additions raised  $a_{CDOM320}$  values for both the initial +DOM ( $F_{1,6} = 129.48$ ,  $p = 0.000$ ) and -DOM treatments ( $F_{1,6} = 42.88$ ,  $p = 0.000$ ; Table 1). By the end of the experiment, DOC concentrations and  $a_{CDOM320}$  values appeared to differ between the different pH treatments (e.g. +UV+DOM and +UV+DOM+base; Table 1), but these differences were not significant. In the +DOM treatments there was significantly less DOC in the presence of UV at the end of the experiment, indicating some combination of photodegradation and microbial degradation ( $F_{1,6} = 6.55$ ,  $p = 0.043$ ; Table 1). An unexpected result was that in the +DOM+base treatment

Table 1. Mean  $\pm$  standard error of pH, dissolved organic carbon (DOC) concentration, dissolved absorption coefficient ( $a_{CDOM320}$ ), chlorophyll a concentration, and DOC bioavailability from initial and final samples collected from experimental bags

Treatment	pH	DOC ( $\text{mg l}^{-1}$ )	$a_{CDOM 320}$ ( $\text{m}^{-1}$ )	chlorophyll $a$ ( $\mu\text{g l}^{-1}$ )	DOC bioavailability ( $\mu\text{g C l}^{-1} \text{d}^{-1}$ )
<i>Initial samples</i>					
-DOM	$5.55 \pm 0.01$	$1.87 \pm 0.04$	$1.40 \pm 0.01$	$0.19 \pm 0.04$	$2.8 \pm 1.6$
-DOM + acid	$4.35 \pm 0.00$	$1.88 \pm 0.05$	$1.69 \pm 0.04$	$0.12 \pm 0.02$	$3.1 \pm 3.7$
+DOM	$4.46 \pm 0.01$	$4.98 \pm 0.05$	$22.15 \pm 0.14$	$0.17 \pm 0.03$	$37.2 \pm 4.0$
+DOM + base	$5.96 \pm 0.03$	$4.97 \pm 0.02$	$23.80 \pm 0.05$	$0.22 \pm 0.04$	$35.6 \pm 2.1$
<i>Final samples</i>					
+UV-DOM	$5.81 \pm 0.01$	$1.68 \pm 0.07$	$1.19 \pm 0.07$	$0.42 \pm 0.03$	$2.9 \pm 2.0$
+UV-DOM + acid	$4.67 \pm 0.01$	$1.74 \pm 0.02$	$1.22 \pm 0.03$	$0.54 \pm 0.06$	$-0.7 \pm 1.1$
+UV+DOM	$4.96 \pm 0.02$	$3.62 \pm 0.12$	$13.61 \pm 0.78$	$0.40 \pm 0.07$	$14.5 \pm 1.8$
+UV+DOM + base	$5.79 \pm 0.35$	$4.68 \pm 0.34$	$15.19 \pm 0.24$	$0.48 \pm 0.07$	$30.1 \pm 5.0$
-UV-DOM	$5.86 \pm 0.04$	$1.60 \pm 0.07$	$1.46 \pm 0.09$	$0.69 \pm 0.08$	$-0.2 \pm 1.3$
-UV-DOM + acid	$4.68 \pm 0.01$	$1.82 \pm 0.04$	$1.36 \pm 0.12$	$0.87 \pm 0.08$	$0.6 \pm 0.7$
-UV+DOM	$4.86 \pm 0.01$	$3.95 \pm 0.05$	$17.54 \pm 0.28$	$0.67 \pm 0.10$	$13.0 \pm 3.4$
-UV+DOM + base	$5.84 \pm 0.29$	$3.89 \pm 0.09$	$18.37 \pm 1.00$	$1.10 \pm 0.53$	$8.7 \pm 5.6$

In all cases  $n = 4$  except for both base addition treatments where  $n = 3$ .

there was significantly less DOC in the absence of UV than in the presence of UV ( $F_{1,6} = 7.76$ ,  $p = 0.032$ ).

In the +UV treatments, cumulative  $E_{320}$  dose received at the center of the bags was 12.12 and 12.06  $\text{kJ m}^{-2}$  in the -DOM and -DOM+acid treatments, respectively, which is equivalent to 1.11 320 nm-exposure days.  $E_{320}$  in the +DOM and +DOM+base treatments was 6.78 and 6.41  $\text{kJ m}^{-2}$ , equivalent to 0.62 and 0.59 exposure days, meaning that added DOM reduced UV-320 by about 45%. The average daily dose of surface irradiance ( $E_{0320}$ ) was 4.01  $\text{kJ m}^{-2}$ , and the maximum daily dose was 5.53  $\text{kJ m}^{-2}$ , received on 29 May. The -DOM  $a_{\text{CDOM}320}$  values (mean 1.31  $\text{m}^{-1}$ ) were slightly higher than expected compared to the  $K_{d320}$  of 1.20  $\text{m}^{-1}$  (we would expect  $a_{\text{CDOM}320}$  to be lower because absorbance of water molecules and particles is not included and optical path is shorter for  $a_{\text{CDOM}320}$  than for  $K_{d320}$ ).

### Zooplankton

Zooplankton community composition differed significantly among the various treatments (Table 2, Fig. 1). The main effect of DOM was significantly positive for *D. catawba* adults, *L. minutus* adults, and *Conochilus* spp., but was not significant for any other groups compared in the MANOVA (Table 3). Additionally, *D. catawba* egg ratios were significantly higher in the +DOM than in the -DOM (Table 3, Fig. 2), as were *C. scutifer* egg ratios (Fig. 3). The main effect of pH was significant for calanoid adults,

cyclopoids, and *Conochilus* spp., but not other groups in the MANOVA (Table 3). These three groups had higher abundance at the high pH compared to low pH (Figs. 4 and 5). *A. spatulocrenatus* egg ratios, *C. scutifer* egg ratios, and nauplii were also significantly higher at the high pH (Table 3, Fig. 3), whereas *D. catawba* egg ratios were significantly higher at the low pH (Table 3, Fig. 2).

The UV×DOM interaction was significant for all groups compared in the MANOVA except rotifers, and was also significant for nauplii and *A. spatulocrenatus* egg ratios (Table 3). Tukey's HSD (Honestly Significant Difference) test, however, revealed few differences between treatment means. There was significantly higher abundance in the -UV-DOM compared to +UV-DOM for calanoid copepodids ( $q = 5.85$ , d.f. = 22,  $p < 0.01$ ) and nauplii ( $q = 6.46$ , d.f. = 22,  $p < 0.01$ ). For *D. catawba* adults, there was significantly higher abundance in the +UV +DOM compared to +UV-DOM ( $q = 5.86$ , d.f. = 22,  $p < 0.01$ ). However, the sense of the interactions for all groups appeared to be that abundance or egg ratios were higher in the +DOM when UV was present, but was lower in the +DOM when UV was absent (Figs. 2–5).

### Chlorophyll and biolability

Initial chlorophyll concentrations were low, and they more than doubled in all treatments by the end of the experiment (Table 1). Final chlorophyll concentrations were lower in the presence of UV than in the absence ( $F_{1,22} = 14.050$ ,  $p = 0.001$ ;

Table 2. Results from three-way MANOVA testing if UV, DOM, and pH significantly influenced zooplankton species composition as indicated by relative abundance of *D. catawba* adults, *D. catawba* juveniles, *L. minutus* adults, *A. spatulocrenatus* adults, calanoid copepodids, *C. scutifer*, *K. longispina*, and *Conochilus* spp.

Source	Roy's largest root	F	Hyp. d.f.	Error d.f.	p
UV	1.381	2.590	8	15	0.053
DOM	3.484	6.532	8	15	0.001 ***
pH	17.461	32.739	8	15	0.000 ***
UV×DOM	2.751	5.158	8	15	0.003 *
UV×pH	0.777	1.458	8	15	0.252
pH×DOM	0.879	1.648	8	15	0.193
UV×DOM×pH	1.141	2.140	8	15	0.097

\*\*\* =  $p < 0.001$ , \* =  $p < 0.01$ .

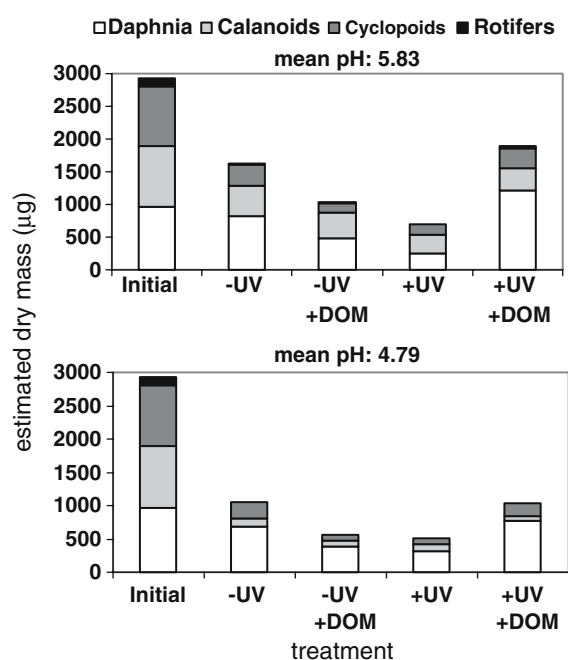


Figure 1. Stacked bars showing zooplankton community composition expressed as dry mass estimates at the start of the experiment (initial) and at the end for each treatment at lake pH of 5.83 (above) and DOM-altered pH of 4.79 (below).

Table 1). DOM and pH were not significantly correlated with changes in chlorophyll concentration.

The DOC bioavailability data indicate that the inlet DOM was more biolabile than Lake Giles DOM ( $F_{1,6} = 1387.69$ ,  $p = 0.000$ ; Table 1). For the +DOM treatment the biolability of DOM was lower in final *in situ* samples compared to initial samples collected at the beginning of the experiment ( $F_{1,6} = 165.33$ ,  $p = 0.000$ ). For the +DOM + base treatment, however, biolability was also significantly reduced in the -UV ( $F_{1,6} = 35.36$ ,  $p = 0.001$ ) but was not changed in the +UV treatment. Another significant difference in biolability was that the +DOM treatment was less biolabile than the +DOM + base treatment in the +UV ( $F_{1,6} = 18.85$ ,  $p = 0.005$ ). Negative bioavailability values resulted from low carbon levels near the detection limit of the TOC-5000 analyzer (Table 1).

## Discussion

A key result was that there was a significant interaction between UV and DOM for all

Table 3. Significant results of between-subjects effects from the MANOVA, and significant results from the univariate ANOVAs conducted for egg ratios and nauplii

Source	Species	$F_{1,22}$	$p$
UV	Nauplii	5.656	0.027 (-)
DOM	<i>D. catawba</i> adults	5.236	0.032 (+)
	<i>D. catawba</i> eggs per adult	13.081	0.002 (+)
	<i>L. minutus</i> adults	4.933	0.037 (+)
	<i>C. scutifer</i> eggs per female	6.185	0.021 (+)
	<i>Conochilus</i> spp.	11.111	0.003 (+)
pH	<i>D. catawba</i> eggs per adult	7.966	0.010 (+)
	<i>L. minutus</i> adults	200.036	0.000 (-)
	<i>A. spatulocrenatus</i> adults	60.026	0.000 (-)
	<i>A. spat.</i> eggs per female	39.368	0.000 (-)
	<i>C. scutifer</i> eggs per female	8.319	0.009 (-)
	Total <i>C. scutifer</i>	5.329	0.031 (-)
	Nauplii	12.383	0.002 (-)
UV×DOM	<i>Conochilus</i> spp.	22.283	0.000 (-)
	<i>D. catawba</i> adults	16.720	0.000
	<i>D. catawba</i> juveniles	12.383	0.002
	<i>L. minutus</i> adults	17.412	0.000
	<i>A. spatulocrenatus</i> adults	11.500	0.003
	<i>A. spat.</i> eggs per female	6.144	0.021
	Calanoid copepodids	12.422	0.002
	Total <i>C. scutifer</i>	15.700	0.001
	Nauplii	24.538	0.000

Non-significant results ( $p > 0.05$ ) are not shown. A (-) indicates that addition of the variable (low pH in the case of pH) had a negative effect and a (+) indicates the variable had a positive effect. Interactions are explained in the text.

zooplankton species except rotifers (Table 3), and that the nature of this interaction was quite similar in all cases. Abundance or egg production was highest in the presence of both UV and DOM and in the absence of both UV and DOM, whereas abundance was lower when only one of these factors was present (DOM alone or UV alone). Another important result was that the difference between abundance in the -DOM and +DOM treatments was greatest for *D. catawba* compared to copepods, and that *D. catawba* egg ratios were higher in the presence of DOM, regardless of UV treatment. These results have multiple implications related to DOM and zooplankton community dynamics and lake UV levels.

Community composition was significantly altered by the combined effects of UV and DOM

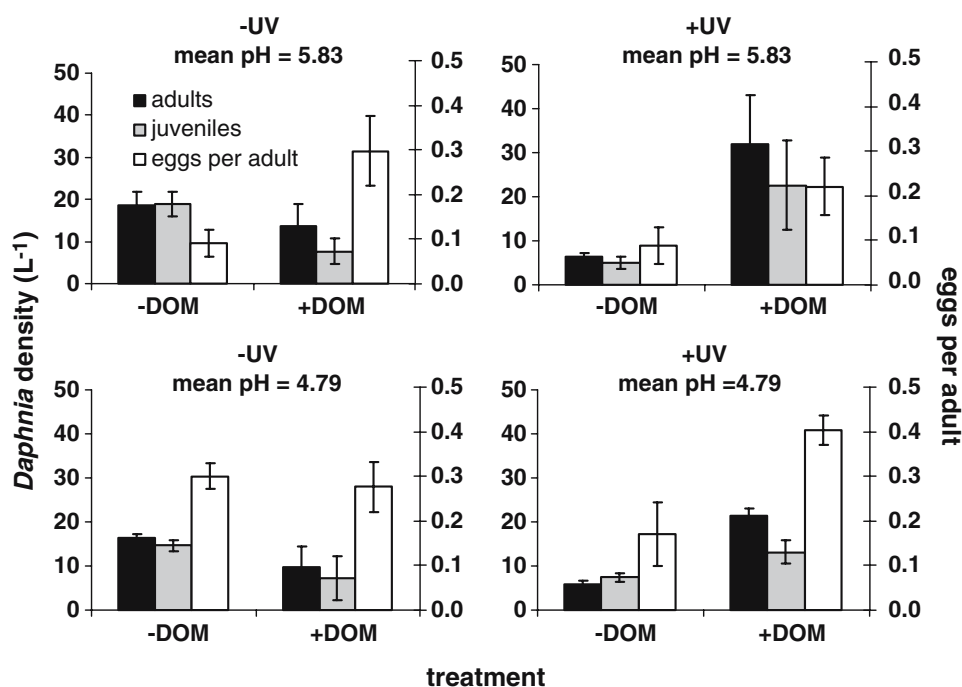


Figure 2. Mean abundance ( $\pm$  standard error) of *Daphnia* adults (black) and juveniles (gray) expressed as individuals  $l^{-1}$  and mean ( $\pm$ SE) eggs per adult (white). The mean initial densities were 23.3 adults  $l^{-1}$ , 16.9 juveniles  $l^{-1}$  and 1.5 eggs per adult.

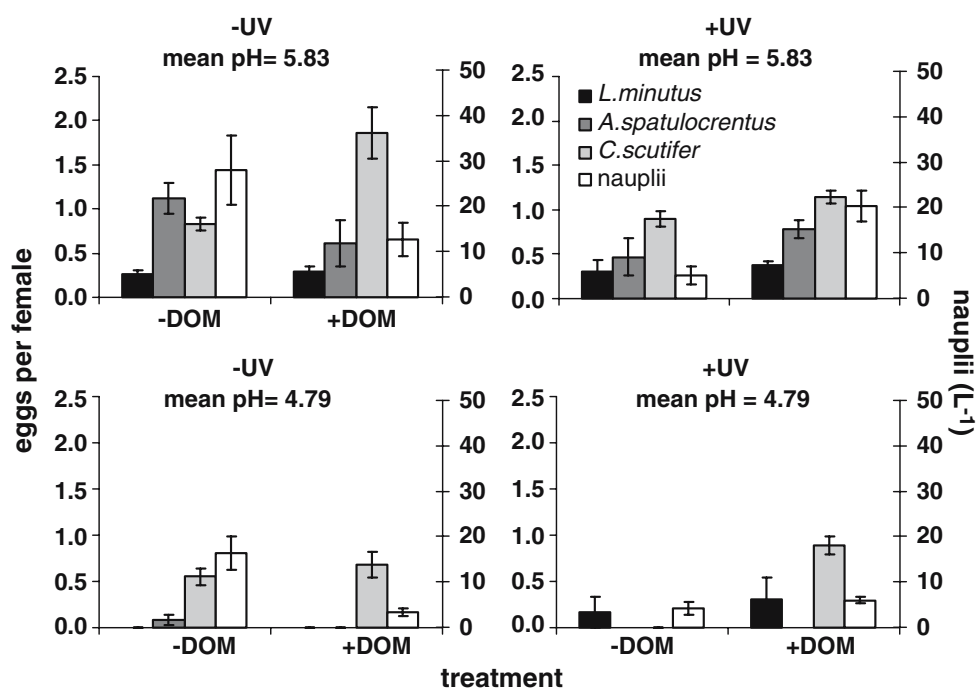


Figure 3. Copepod egg counts for each species expressed as mean ( $\pm$  SE) eggs per female. Nauplii are expressed as individuals  $l^{-1}$  and were not identified according to species. The mean initial egg ratios (eggs per female) were 0.26 for *L. minutus*, 0.48 for *A. spatulocrenatus*, and 0.29 for *C. scutifer* and the initial naupliar density was 8.1  $l^{-1}$ .

(Table 2) because rotifers responded differently than crustaceans. Tukey's HSD test did not yield many significant differences to help explain the UV×DOM interaction probably because it is a conservative test, and there were eight treatments and only four replicates. However, it is apparent that abundance of *D. catawba* and all copepods, including nauplii, was generally higher in the +DOM when UV was present, but was lower or about the same in the +DOM when UV was absent. For rotifers, on the other hand, abundance was generally higher in the +DOM, regardless of UV treatment. Community composition was also significantly altered by DOM as a main effect (Table 2), although the reason for this is difficult to interpret, even with use of *post hoc* tests. It is probably because of the Tukey test result that *D. catawba* adults had higher abundance in the +DOM compared to -DOM in the +UV, and perhaps also because *L. minutus* adults and *Conochilus* spp. had a significant main effect of DOM, whereas other species did not (Table 3).

Previous work has shown that allochthonous DOM is an important source of nutrition to zooplankton (Salonen & Hammar, 1986). Daniel et al. (2005) observed that crustacean zooplankton can survive in humic water in the dark for more than a year, but that survival was much higher in a full light treatment. This is consistent with our result that when DOM was present, abundance tended to be higher in the +UV compared to -UV. De Lange et al. (2003) observed in a lab experiment that *Daphnia* had higher egg production when fed bog DOM exposed to UV compared to DOM exposed only to PAR. We observed higher *D. catawba* abundance in the +UV+DOM compared to -UV+DOM, but egg production was higher in the +DOM in both UV treatments. Numerous studies have shown that UV greatly increases the biolability of humic substances (reviewed by Moran & Zepp, 1997; Tranvik & Bertilsson, 2001; De Lange et al., 2003). However, PAR and DOM quality also are important factors, and variation in the latter factor may account for different results observed among similar studies. Additionally, DOM quality may be different for each species, as it may stimulate different components of the microbial food web. Egg production can provide a rough indication of food limitation for zooplankton (Edmondson et al., 1962;

Williamson & Butler, 1987). The egg ratios in our study indicate that DOM may have been nutritionally beneficial for *D. catawba* and *C. scutifer*, whether UV-exposed or not; may have been somewhat beneficial for *A. spatulocrenatus* when exposed to UV; and probably had no nutritional effect on *L. minutus* (Figs. 2 and 3). Nauplii also appear to have benefited from DOM when UV was present (Fig. 3). Similarly, Vinebrooke & Leavitt (1998) found that nauplii were greater in DOM-addition enclosures after 1 month, while adult copepods and copepodids were unchanged in a study of littoral communities.

In addition to egg production, another variable we measured that indicates DOM quality is biolability. The rates of carbon processed by microbes in the lab indicate that the inlet DOM became less biolabile during the course of the field experiment, suggesting that labile carbon was incorporated into the food web (Table 1). Overall, the amount of biolabile inlet carbon remained high in the +UV, but decreased in the -UV, supporting the notion that UV increases (or at least maintains) the biolability of humic substances. But this UV treatment difference was due to the +UV+DOM+base treatment, which had approximately double the biolability of the +UV+DOM treatment. This suggests that the base addition may have increased DOM quality over time in the +UV, which would be an unintended side effect of the NaOH. Because there was no difference in biolability between +UV+DOM and -UV+DOM treatments, it is apparent that other wavelengths besides UV (i.e. PAR) are also important in generating biolabile DOM. Photobleaching, evident from the  $a_{CDOM\ 320}$  data, also occurred in both the +UV and -UV, a further indication that PAR influenced the DOM. This is consistent with research showing that photobleaching of DOM is affected more by UV-A and blue light than by UV-B (Osburn et al., 2001).

Aside from DOM quality, UV dose rate is another factor that will vary among field studies. In this study, the average daily dose of  $4.01\text{ kJ m}^{-2}$  and the maximum daily dose of  $5.53\text{ kJ m}^{-2}$  indicate that UV was transmitted at a fairly constant, low level dose, probably due to the cloudy weather during most of the experiment. We can speculate that UV could have been a greater stressor if

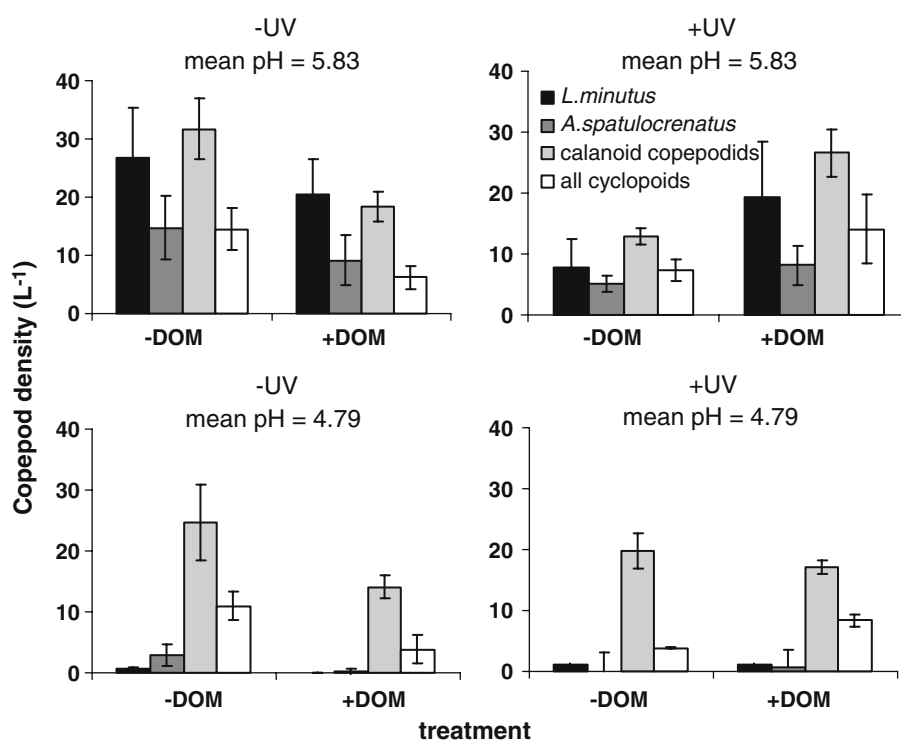


Figure 4. Mean  $\pm$  SE abundance of *L. minutus* adults (black), *A. spatulocrenatus* adults (dark gray), calanoid copepodids (light gray), and total cyclopoids (white) expressed as individuals  $l^{-1}$ . The mean initial densities for each were 51.9, 21.7, 54.5 and 42.1  $l^{-1}$ , respectively.

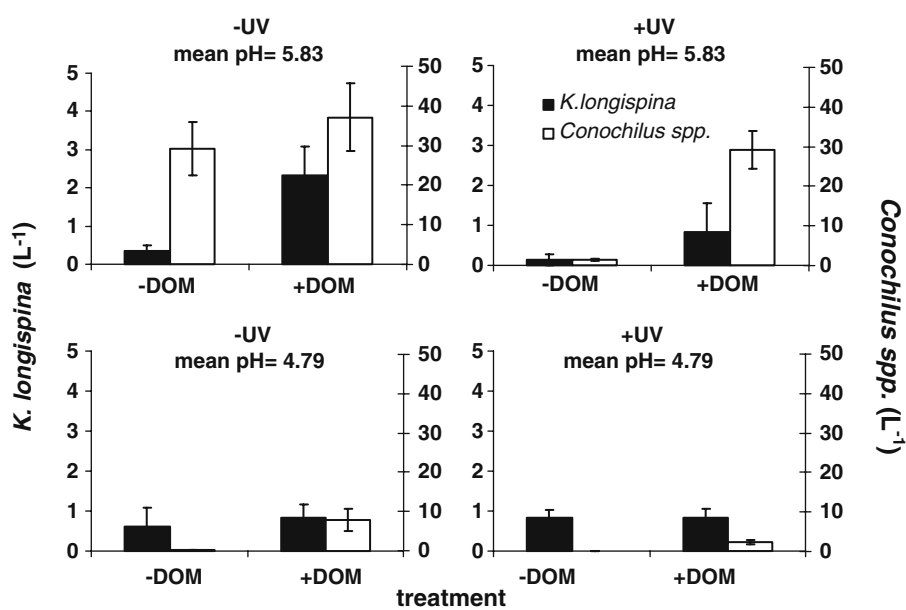


Figure 5. Mean  $\pm$  SE abundance of the rotifers *K. longispina* (black) and *Conochilus spp.* (white) expressed as individuals  $l^{-1}$ . The mean initial densities for each were 7.9 and 155  $l^{-1}$  respectively.

conditions had been different, and we might have obtained different results, especially with UV-sensitive *D. catawba* (Leech & Williamson, 2000). But despite the low UV levels, we observed direct UV effects on nauplii, photobleaching, and chlorophyll (Tables 1 and 3).

Community composition was also influenced by pH. This is not surprising given that *D. catawba* is reported to be more acid-tolerant than the copepods (Locke, 1991; Fischer et al., 2001). It is interesting, however, that calanoid copepodids were not as pH sensitive as their adult and nauplius counterparts (Table 3, Figs. 3 and 4). The DOM additions where pH was not adjusted represent the most “natural” scenario of a DOM increase in Lake Giles, which has a poor acid-neutralizing capacity. Acid-intolerant copepods would therefore be at a disadvantage compared to *D. catawba* if DOM were to increase due to climate change or other factors that might change watershed hydrology.

Some issues to consider in our study include various artifacts that often occur in microcosm experiments, such as possible food limitation. The closest indicator we have for food limitation is egg production, which declined over the course of the experiment for *D. catawba*, with about 5–30% of initial eggs per adult remaining, and for calanoids, with about 40–60% of initial eggs per female remaining. The average number of eggs per female remained about the same or increased for *C. scutifer*. Initial densities were higher than average natural lake densities. Thus, it is possible that *D. catawba* and calanoids may have been food limited. Chlorophyll *a* did increase several-fold by the end of the experiment, although this is not necessarily an indicator of food availability because food quality was not known. We do not know how other parts of the food web, such as bacteria, protozoans, and algae responded to manipulations of DOM, UV, and pH, but the net effect on zooplankton of these organisms and abiotic factors is evident from zooplankton abundance and reproduction.

Another issue that should be noted is the length of the experiment and absence of data at intermediate time points. At 14.5 °C and low food levels the duration of a *Daphnia* instar would be expected to be approximately 2–3 days, and egg development would be approximately 5 days (reviewed in Dod-

son & Frey, 2001). Development time from egg extrusion to hatching in copepods is approximately 1–5 days (reviewed in Williamson & Reid, 2001). Thus, over the course of the 8-day experiment we would expect some development of *Daphnia* eggs and juveniles into subsequent life stages and some hatching of copepod nauplii, in addition to mortality at all life stages. Over 8 days we would not expect much development into adult stages. The fact that *Daphnia* egg ratios declined substantially and neonates did not increase indicates that there may have been some stressful condition in the enclosures that caused mortality of egg bearing *Daphnia*. This condition may have been related to the high densities, or another stress with a relatively immediate (less than 8 days) impact. Because we only have initial and final sampling points we cannot determine if this decline was gradual or sudden. It is also unknown if other dynamics occurred during the course of the experiment, although we would not expect drastic fluctuations during an eight day springtime incubation.

In interpreting the results it is also important to consider the vertical distribution and migration patterns of these species. In Lake Giles *Daphnia* and diaptomid copepods undergo diel vertical migration (Fischer et al., 2006), and cyclopoids tend to avoid the epilimnion during both day and night (Leech et al., 2005). In addition to constraining the animals to higher UV levels and temperatures than they normally experience, they may have been subjected to suboptimal food levels, as summer phytoplankton in the epilimnion of Lake Giles can be photoinhibited (Moller, 1994).

## Conclusions

There are several implications of our conclusion that DOM affects zooplankton in different ways depending on UV level. Because of temperature differences, DOM “plumes” entering a lake from a shaded inlet like the one in Lake Giles might descend below the thermocline before significant sunlight exposure. Although a DOM increase in the surface waters of a lake may be advantageous for blocking UV and stimulating food web effects, less exposed DOM below the mixed layer or in a shaded littoral zone may be damaging for some

organisms. We observed species differences in response to our treatments; thus, for some organisms, such as rotifers, the benefits of DOM may not depend on UV levels, while for others UV is critical in determining whether DOM will be beneficial or harmful. These variable effects of DOM on abundance and reproduction of different species may not only alter species composition but may also alter the vertical distribution of some zooplankton.

This study provides evidence that an increase in DOM in a high UV environment may be beneficial to zooplankton, especially *Daphnia*, by mitigating the detrimental effects of UV exposure. However, DOM may have detrimental effects in a low UV environment, especially for calanoid copepods. Changes in pH, either associated with DOM or occurring independently of changes in DOM, appear to influence some zooplankton species. This collection of species differences in response to DOM, UV, and pH could produce a shift in zooplankton community structure favoring *Daphnia* and some rotifers while reducing calanoid copepods. Because of the ecological implications of these results and the dynamics of lake DOM inputs, further research into the food web links between DOM and zooplankton at different UV levels is warranted.

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