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Stimulation of metazooplankton by photochemically modified dissolved organic matter

Cesar Daniel,[1] *Wilhelm Granéli, and Emma S. Kritzberg*
Department of Ecology/Limnology, Lund University, SE-223 62 Lund, Sweden

Alexandre M. Anesio
Institute of Biological Sciences, Edward Llwyd Building, University of Wales, Aberystwyth SY23 3DA, United Kingdom

Abstract

We examined the response of bacteria and proto- and metazooplankton to photomodified dissolved organic matter (DOM). Sterile filtered water from a eutrophic and a humic lake, that was either exposed to artificial ultraviolet (UV) radiation or kept dark, was added to semicontinuous laboratory microcosms that lasted for 7 weeks. Bacterial production responded positively to photochemical modification of DOM regardless of lake type. Final heterotrophic biomass (bacteria+proto+metazooplankton) was 47 ± 5 and 37 ± 5 μg carbon (C) L^{-1} in microcosms with UV-exposed and unaltered eutrophic water DOM and 15 ± 4 and 11 ± 2 μg C L^{-1} in microcosms with UV-exposed and unaltered humic water DOM, respectively. For the eutrophic water, there were no significant differences in proto- or metazooplankton biomasses between microcosms receiving UV-exposed or nonexposed DOM. Differences between eutrophic water microcosms were not significant when flagellates, ciliates, cladocerans, and copepods were examined separately. In microcosms with UV-exposed humic water, biomasses of heterotrophic flagellates, rotifers, nauplii, and cladocerans were higher than in those with nonexposed DOM. Higher final metazooplankton biomass following addition of UV-exposed humic water indicates that photochemically modified DOM can be effectively transferred through the microbial loop.

In many lakes, a large fraction of dissolved organic matter (DOM) is composed of recalcitrant humic substances (MacKnight and Aiken 1998). Ultraviolet (UV) radiation (UV-R = 280–400 nm) promotes the breakdown of humic substances into low-molecular-weight compounds that are more easily assimilated by heterotrophic bacteria (Kieber et al. 1989; Mopper et al. 1991; Lindell et al. 1995). It has repeatedly been shown, often using predator-free dilution cultures, that exposure of humic substances of terrestrial or littoral origin to solar radiation can increase the bacterial carrying capacity (i.e., the number or biomass of bacteria that a specific amount of humic substances can support; Lindell et al. 1995; Wetzel et al. 1995).

A reduction of bacterial growth can possibly result from photochemical transformation of labile bacterial substrates into refractory DOM (Keil and Kirchman 1994; Tranvik and Kokalj 1998). Indeed, exposure of phytoplankton-derived DOM often decreases bacterial carrying capacity (reviewed by Moran and Covert 2003). Studies conducted on marine surface water with a relatively high contribution of freshly produced DOM have shown that previous exposure to solar radiation can strongly reduce bacterial production (Mopper et al. 1991; Benner and Biddanda 1998; Obernosterer et al. 1999).

Less is known of whether changes in bacterial growth, caused by photomodified DOM, will propagate to any noticeable extent up through the plankton food web. Although most of the carbon entering the microbial loop is respired (Biddanda et al. 1994), the gain in bacterial biomass is expected to stimulate protozoan growth and ultimately reach higher trophic levels, such as larger zooplankton and fish. In this study we address the question of whether photochemical changes in DOM can affect secondary production above the bacterial level.

There are several aspects of our experimental system that make our approach different from those of earlier studies investigating the bioavailability of UV-exposed DOM: (1) the inclusion of heterotrophic organisms above the bacterial level (i.e., flagellates, ciliates, rotifers, cladocerans, and copepods), (2) the use of an extended experimental period (several weeks, as opposed to most experiments, which last only a few days), and (3) the use of semicontinuous microcosms receiving either unexposed lake water or lake water exposed to artificial UV-R (most studies have used batch cultures). We also explore the relation between depth-integrated UV-R absorbed in situ, for each lake, and the UV-R provided in our experiment.

We used in this study two types of water, that obtained from a humic (brown-colored) forest lake with low phytoplankton biomass and that obtained from a eutrophic–hypertrophic lake with relatively lower humic content. We expected both bacterial production and metazooplankton biomass to increase in humic microcosms with additions of UV-exposed DOM, relative to microcosms receiving unexposed DOM. For the eutrophic water, we expected a decreased bacterial production and metazooplankton biomass in microcosms with additions of UV-exposed DOM, relative to microcosms receiving unexposed DOM.

[1] Corresponding author (Cesar.Daniel@limnol.lu.se).

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Table 1. Descriptive data on the lakes used in the study. Ranges are based on summer months for 1969–1982 for Lake Vomb (Almestrand and Lundqvist 1983) and 1971–1993 for Lake Skärshult (Bergvall 1992; Bengtsson 1993).

	Lake Vomb (eutrophic)	Lake Skärshult (humic)
Lake area (km^2)	12.8	0.3
Mean depth (m)	5.5	3.8
Retention time (yr)	0.6	0.7
Tot-N (mg L^{-1})	1.8–3.3	0.5–0.8
Tot-P (μg L^{-1})	69–242	4–15
Chlorophyll *a* (μg L^{-1})	20–87	4–8
Water color (mg Pt L^{-1})	15–50	50–125
DOC (mg L^{-1}), this study	7.4	11.6

Materials and methods

Description of the lakes—We performed the experiment with water from two lakes with contrasting phosphorus and humic contents, both located in Southern Sweden. Lake Vomb is a calcareous lake situated in an area in which agriculture is intensive. The total phosphorus (P) concentration recorded in summer months ranges between 69 and 242 μg L^{-1} and indicates high trophic state, whereas water color is comparatively low (Table 1). Hydrological retention time is moderately short in both lakes, but Lake Skärshult receives higher inputs of humic organic matter from coniferous forests and wetland vegetation in the drainage area. In Lake Skärshult, water color is typically high, and the total P concentration in summer ranges from 4 to 15 μg L^{-1}, indicating oligotrophic conditions (Table 1). Throughout this essay we will use the denominations eutrophic for Lake Vomb and humic for Lake Skärshult.

In March 2001, we observed chlorophyll *a* concentrations of 58 and 0.3 μg L^{-1}, respectively, in Lakes Vomb and Skärshult (Daniel et al. 2005) at the same sites we used for water collection in this study. These levels are in good agreement with literature records for different years, despite large seasonal variations in Lake Vomb (Almestrand and Lundqvist 1983; Bertilsson and Tranvik 2000). Both lakes have a reduced littoral zone, and macrophyte production is assumed to be of minor importance compared to inputs of DOM from phytoplankton and DOM of allochthonous origin. Characteristics of the lakes indicate that in Lake Vomb, the autochthonous DOM fraction (relative to the allochthonous fraction) is proportionally higher than in Lake Skärshult.

Experiment set-up 1: Semicontinuous microcosms—Collection of water was carried out on 29 and 30 March 2003 by pumping 350 liters of lake water into plastic containers; thin ice cover was observed in Lake Skärshult but not in Lake Vomb. Following transport to the laboratory, 230 liters of water from each lake were stored unfiltered in darkness at 4°C until the semicontinuous microcosms were ended. Also, 120 liters (350 − 230 liters) were kept at 22°C in dark plastic containers to induce zooplankton hatching. As oxygen levels (monitored once a week) did not drop below 5 mg O_2 L^{-1} while zooplankton hatching occurred, aeration was presumed to be unnecessary during the experiment. After 1 week, copepods, cladocerans, and rotifers were observed, and after 2 weeks, the 120 liters from each lake were transferred to polycarbonate bottles (6 × 20 liters) to start the semicontinuous microcosms.

Every third day, the water stored cold was filtered and submitted to UV or dark exposure to be added semicontinuously to the microcosms. Membrane filters (0.2 μm; Gelman), previously rinsed in Milli-Q water, were used to obtain bacteria-free lake water medium (12 liters, 2 liters microcosm replicate^{-1}) for the additions. Thus, only the volumes added were filtered each time, and this procedure took place 16 times during the 48-d period. Immediately after filtration, equal volumes (6 liters) of lake water were distributed to acid (10% HCl)–washed 210-mL quartz tubes with glass stoppers or to 1-liter glass bottles. The glass bottles were kept at 22°C in darkness, whereas the quartz tubes were arranged horizontally on a bench and were exposed to artificial UV-R. Eight UVA-340 (Q-Panel) fluorescent tubes were placed 25 cm above the quartz tubes. Radiation was measured using a Radiometer/Photometer Model IL 1400A (International Light) connected to broad-band sensors with cutoff filters (UVA: 320–400 nm; UVB: 280–320 nm). The intensities of UVA and UVB radiation obtained were 20.3 W m^{-2} and 0.62 W m^{-2}, respectively. The amount of energy (97% UVA, 3% UVB) reaching the quartz tubes was 960 J L^{-1} h^{-1}, and the exposure period lasted 12.75 h. Water temperature in the quartz tubes during exposure was 22°C. UV-exposed and nonexposed (control) water was kept in darkness for 24 h before being added to the microcosms. This was done in order to minimize any potential negative effects to bacteria by natural oxidants produced in waters exposed to UV-R, such as hydrogen peroxide (Scully et al. 1996). Two liters (10% of the microcosm volume) were removed from all microcosms and the same volume of 0.2 μm–filtered and UV-exposed/unexposed water of the respective lake type added separately for the treatments within humic and eutrophic water types. When removed, the water was gently passed through a plankton net with 10-μm mesh size. We adopted this procedure to avoid a situation in which the proto- and metazooplankton were flushed out of the microcosms. A total of 32 liters for each 20-liter microcosm were exchanged during the 48-d semicontinuous microcosm experiment. Water residence time in the microcosms was thus 30 d.

Experimental set-up 2: Bacterial regrowth cultures—In addition to the semicontinuous microcosms, 1-week-long batch cultures were run to investigate the effect of photomodified DOM on bacteria, in the absence of bacterivores and other zooplankton. Water from each lake (2 liters) was filtered through prerinsed 0.2-μm membrane filters (Gelman). Six acid-washed quartz tubes (210 mL) were filled with filtered water, three of which were exposed to UV-R following the procedure described above and three of each were kept in darkness at 22°C. A predator-free bacterial inoculum was obtained by filtering lake water through GF/C glass-fiber filters. The inoculum (10% of the volume) was added to the quartz tubes 24 h after the UV exposure/dark incubation was finished. Thereafter, the tubes were wrapped

in aluminum foil and kept at 22°C on a horizontal shaker. Aliquots of 10 mL were removed at 24-h intervals during 5 d for determination of bacterial abundance.

Dissolved organic carbon (DOC) and abundance and production of bacteria—Following water collection and transport to the laboratory, DOC concentrations were analyzed through Pt-catalyzed high-temperature combustion using a Shimadzu TOC-5000 total carbon analyzer equipped with autosampler. Lake water was 0.2-μm filtered and the inorganic carbon was eliminated from the samples by bubbling with CO_2–free air after addition of HCl. For determination of bacterial abundance, samples were collected from batch cultures (daily, during 5 d) and microcosms (days 0 and 48) and were preserved with formaldehyde. Samples were analyzed after SYTO 13 (Molecular Probes) staining, using a FACSort (Becton Dickinson) flow cytometer, as described in del Giorgio et al. (1996). For microcosms, bacterial biomass was calculated assuming a fixed value of 0.05 pg C bacterium^{-1} (Vrede et al. 2002). Bacterial production was measured at days 0, 15, 32, and 45, always 6 h after the microcosms received addition of filtered water (either UV-exposed or unexposed), using the leucine incorporation method (Smith and Azam 1992). Aliquots of 1.7 mL were added from each microcosm into two Eppendorf tubes. The isotope (4,5-^{3}H L-leucine) was added to both tubes to a final concentration of 100 nmol L^{-1} (specific activity, 11.5 Ci mmol^{-1}). The control was killed with trichloroacetic acid (TCA) (5% final concentration), and the incubation was terminated after 1 h by adding TCA to the second tube. Subsequently, the bacteria were concentrated to a pellet by centrifugation at 14,000 $\times$ g for 10 min, rinsed with 5% TCA, and then with 80% ethanol. The bacteria were resuspended in scintillation cocktail (Ecoscint A, Kimberly Research) and the incorporated leucine measured in a Beckman LS 6500 scintillation counter.

Zooplankton—Sampling for zooplankton was done in the 20-liter microcosms before the first addition of UV-exposed or dark incubated lake water and on day 48 (3 d after the last addition). Abundance and biomass of heterotrophic flagellates (HF) were determined from unscreened samples preserved with formaldehyde (2% final concentration) and stored at 4°C. An epifluorescence microscope (Nikon Labophot-2) equipped with measuring scale and UV filter was used. HF were stained with DAPI on 0.8-μm pore–sized polycarbonate membrane filters, and their abundance was estimated after counting a minimum of 100 individuals (resulting in an accuracy of $\pm$20%), whereas cell volumes were calculated from length and width of 20 HF. For determination of abundance and biomass of large ciliates, rotifers, and microcrustaceans, 10 liters from each microcosm replicate was screened through a 45-μm mesh–sized plankton net, and samples were preserved with formaldehyde. A dissecting microscope was used to quantify microcrustaceans, whereas an inverted microscope was used for quantification of nauplii and rotifers. Triplicate 100-mL aliquots of prescreened (45 μm) water were preserved with acid Lugol's solution for estimation of abundance and biomass of small ciliates, using the inverted microscope. Conversion factors to obtain biomasses of HF, small and large ciliates, rotifers, and microcrustaceans in terms of carbon are given in Daniel et al. (2005).

Calculations of UV energy—Energy absorbed in the quartz tubes during laboratory exposure was estimated from simultaneous exposure of 0.2-μm filtered water in quartz tubes and in open cylindrical glass dishes (12 cm in diameter, 4.5 cm in height). The glass dishes were filled with a water column corresponding to the mean radiation path in the quartz tubes (2.8 cm). The radiation intensities were measured at the depths of 0.1 cm and 2.8 cm using the radiometer and sensors specified above, and the amount of energy absorbed could be determined from the difference between the measurements. Exposures were carried out using similar periods and UV intensities to those in the procedure explained above. Absorbances at 254 nm were measured before and after exposure using 1-cm HCl–washed quartz cuvettes and a Beckman DU 650 spectrophotometer. The decrease in absorbances observed for both eutrophic and humic waters was 13%, and reductions in absorbances from exposures using glass dishes or quartz tubes were the same, regardless of water type. Thus, similar relations between the energy absorbed and the decrease in absorbance were assumed for the dishes and the quartz tubes. The amount of energy absorbed in the tubes could then be obtained by applying the following equations:

$$\mathrm{AbsE_{UVA}(kJ\ L^{-1}\ d^{-1})} = \Delta_{254} \times \mathrm{C_{UVA}} \quad \text{and}$$

$$\mathrm{AbsE_{UVB}(kJ\ L^{-1}\ d^{-1})} = \Delta_{254} \times \mathrm{C_{UVB}}$$

where Δ_{254} is the decrease in absorbance at 254 nm occurring in the quartz tubes following UV exposure and C_{UVA} and C_{UVB} are the conversion factors obtained from exposure of water using the glass dishes (amount of energy absorbed divided by decrease in absorbance at 254 nm). For the eutrophic water, the C_{UVA} and C_{UVB} values obtained were 6.4 and 0.47, respectively. Values of C_{UVA} and C_{UVB} obtained for the humic water were 7.5 and 0.53, respectively. The amounts of absorbed UV-radiation energy provided to eutrophic and humic microcosms were calculated from the UV doses absorbed in quartz tubes using each water type, considering the exchanged fraction (10%) per unit time (once every third day). Microcosm UV-R doses (kJ L^{-1} d^{-1}) were then compared with the doses expected in the epilimnion of each lake during summer (Granéli et al. 1998). The mixing depths considered for the lakes were, respectively, 5 m (eutrophic) and 2.5 m (humic).

Statistics—Differences between treatments were analyzed separately within eutrophic and humic microcosms. Biomasses and abundances were compared between treatments for each zooplankton group using a *t*-test for independent samples. A *t*-test was also performed for each time at which bacterial production was measured. Repeated-measures analysis of variance (rm-ANOVA) was used to compare the treatments in terms of bacterial abundance in the regrowth cultures. Log transformation of abundances and biomasses for the humic water and of bacterial production for both humic and eutrophic microcosms was required to meet the

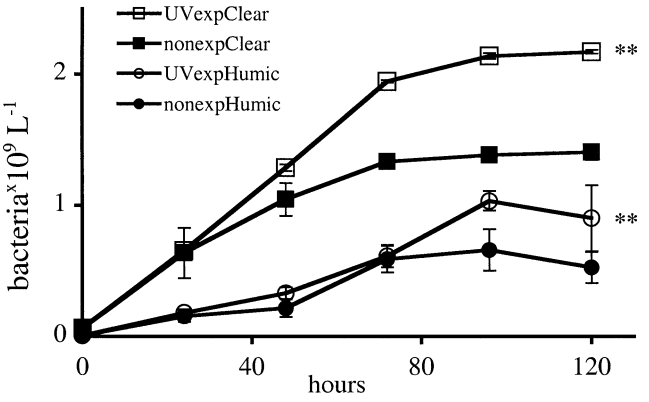

Fig. 1. Bacterial numbers in batch cultures started on water from each lake (eutrophic and humic) that was either nonexposed or UV-exposed. Statistically significant differences between treatments within the water types are indicated (* $p < 0.05$; ** $p < 0.01$).

assumption of homogeneity of variances. Statistical analyses were performed in SPSS (version 10.0 for Macintosh) after testing for homoscedasticity (Levene's test).

Results

Bacterial batch cultures—The maximum carrying capacity of bacteria (number L^{-1}) was reached after 72 h, regardless of treatment (Fig. 1). Visual inspection of samples under the epifluorescence microscope revealed no presence of flagellates in the first 96 h of the experiment. Exposure to UV-R stimulated bacteria in the regrowth batch cultures (rm-ANOVA, $p < 0.01$) in both lake types (i.e., humic and eutrophic). The bacterial carrying capacity in the exposed eutrophic water was higher than in the exposed humic water ($p < 0.05$).

Bacteria in semicontinuous microcosms—Estimated UV-R doses in the eutrophic and humic microcosms resulting from additions of UV-exposed water were 0.10 and 0.19 kJ L^{-1} d^{-1}, respectively. Initial bacterial biomasses in eutrophic and humic microcosms were, respectively, 60 ± 1 and 5.4 ± 3.1 μg C L^{-1}. Final bacterial biomasses obtained for eutrophic and humic microcosms receiving UV-exposed DOM were 20 ± 0.5 and 7.4 ± 0.2 μg C L^{-1}, higher than in their respective controls: 15 ± 1.5 and 5.8 ± 0.5 μg C L^{-1} ($p < 0.05$). Bacterial production varied between 0.8 and 4.2 μg C L^{-1} h^{-1} and 0.2 and 0.6 μg C L^{-1} h^{-1} in the eutrophic and humic microcosms, respectively (Fig. 2). Bacterial production was higher in the treatment receiving UV-exposed water for both lake types (*t*-test, $p < 0.05$). Stimulation of bacterial production due to DOM photomodification in eutrophic and humic microcosms ranged from 50% to 114% and from 12% to 90%, respectively, relative to the controls. Initial production values were the highest recorded for the humic microcosms (0.6 μg C L^{-1} h^{-1}), whereas the highest obtained for eutrophic microcosms receiving UV-exposed DOM were 4.2 μg C L^{-1} h^{-1} and in the eutrophic control were 2.3 μg C L^{-1} h^{-1} ($p < 0.05$).

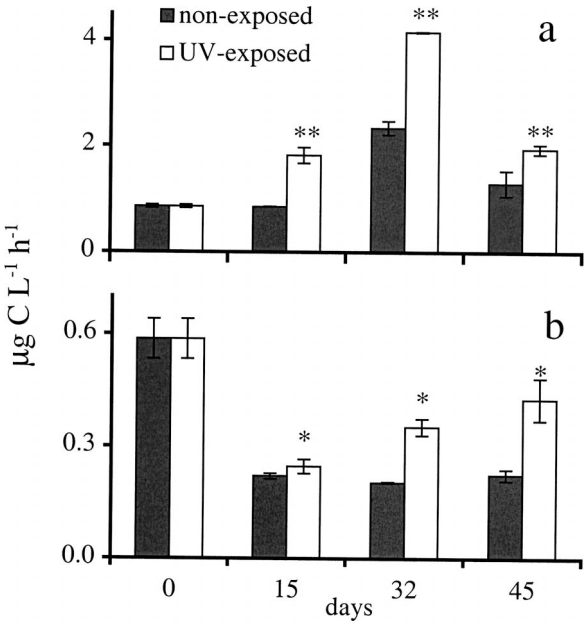

Fig. 2. Bacterial production in (a) eutrophic and (b) humic microcosms receiving either nonexposed or UV-exposed DOM. Statistically significant differences between treatments within the water types are indicated (* $p < 0.05$; ** $p < 0.01$).

Initial community structures—Bacteria constituted a large fraction of the initial heterotrophic biomass in eutrophic microcosms (41% of total 146 μg C L^{-1}). Cyclopoid copepods dominated over cladocerans (*Chydorus*) and contributed, with 27%, to the initial heterotrophic biomass, whereas the contributions of small ciliates and HF were 11% and 15%, respectively. In eutrophic microcosms, large ciliates (0%) and rotifers (1%) were quantitatively unimportant in the initial community.

Initial heterotrophic biomass in humic microcosms was 58 μg C L^{-1}, of which 9% was bacterial carbon. Cyclopoid copepods and cladocerans (*Bosmina*) contributed, with 10% and 14%, respectively, to the initial biomass. In contrast to rotifers (1%), the initial occurrence of large ciliates (65%) in the humic water community was substantial.

Zooplankton stimulation by photomodified DOM—For the eutrophic water, final abundances of HF and small ciliates (Table 2) were not affected by the treatment ($p > 0.05$), whereas the abundance of copepods was statistically higher in the microcosms receiving UV-exposed water ($p < 0.05$). At the end of the experiment, HF constituted the most important zooplankton group, making up 30% of total heterotrophic biomass in both treatments. The contribution of cyclopoid copepods (initially 27%) decreased by the end of the experiment (12%), and differences in final biomass between treatments were not significant ($p > 0.05$) (Fig. 3). As in the beginning of the experiment, copepods dominated over other metazoan groups in both treatments. Differences in final biomasses of proto- and metazooplankton between treatments were not statistically significant (Table 3), regardless of the stimulation of bacterial production in the microcosms receiving UV-exposed water. Final biomasses of total het-

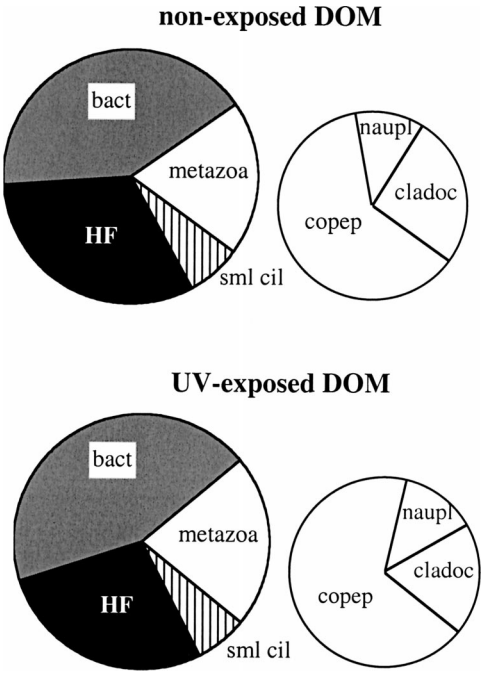

Fig. 3. Biomass distributions in eutrophic water microcosms receiving either nonexposed DOM or UV-exposed DOM after 48 d of experiment.

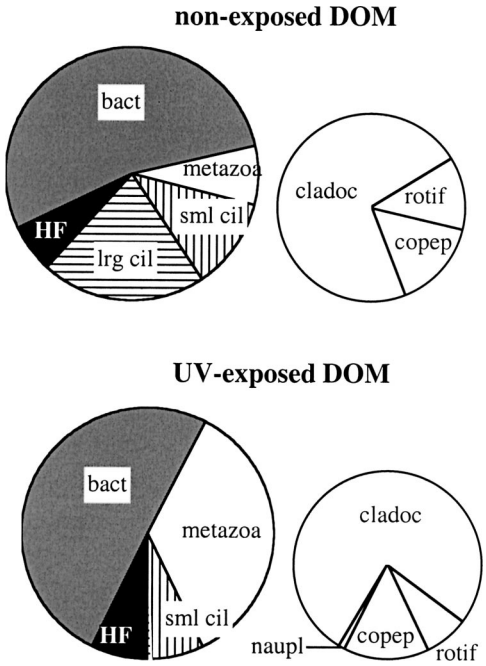

Fig. 4. Biomass distributions in humic water microcosms receiving nonexposed DOM and UV-exposed DOM after 48 d of experiment.

erotrophic plankton in the eutrophic microcosms receiving UV-exposed and unaltered DOM (47 ± 5 and 37 ± 5 μg C L^{-1}, respectively) were not statistically different ($p > 0.05$).

For the humic water, nauplii disappeared completely in the control microcosms, whereas the abundance of large ciliates increased compared to microcosms with UV-exposed water ($p < 0.05$). The abundance of adult copepods was significantly higher ($p < 0.05$) in microcosms receiving UV-exposed humic water (Table 2). In the microcosms with additions of UV-exposed humic DOM, *Bosmina* showed the highest final biomass among zooplankton, whereas large ciliates had the highest final biomass in the humic control microcosms (Fig. 4). The biomass of large ciliates (>45 μm) was lower in the microcosms with UV-exposed humic water ($p < 0.05$), following the pattern observed for the abundance. Significantly higher biomasses of HF, rotifers, and cladocerans were observed in the treatment with UV-exposed humic water ($p < 0.05$; Fig. 4). Although the mean biomass of copepods in this treatment was five times (520%) higher compared to that in the humic control, this difference was not statistically significant because of a high variation among replicates (Table 3). Final biomasses of total heterotrophic plankton were 11 ± 2 and 15 ± 4 μg C L^{-1} in the humic microcosms receiving unaltered and UV-exposed DOM, respectively, but differences between treatments were not statistically significant.

Discussion

Bacterial responses to DOM photomodification—We observed increased production and biomass of bacteria in semicontinuous microcosms receiving UV-exposed DOM, re-

Table 2. Mean (±standard deviation) final numbers of bacteria and zooplankton in microcosms. Stars indicate significantly higher means according to *t*-test comparing treatments within lake type.

	Eutrophic water (Vomb)		Humic water (Skärshult)	
	Nonexposed	UV-exposed	Nonexposed	UV-exposed
Bacteria$\times 10^5$ mL^{-1}	3.0±0.3	4.1±0.1**	1.2±0.1	1.5±0.1**
HF 10^5 L^{-1}	6.3±0.6	5.6±0.9	2.1±0.6	4.1±1.1*
Small ciliates$\times 10^3 L^{-1}$	42±11	50±12	8.9±3.1	8.1±0.9
Large ciliates$\times 10^3$ L^{-1}	Absent		6.0±3.2*	0.1±0.2
Rotifers L^{-1}	Absent		3.5±1	30±8**
Cladocerans L^{-1}	0.9±1.0	0.8±0.9	1.2±0.6	8.6±7.3
Nauplii L^{-1}	1.3±0.7	2.0±0.5	Absent	0.5±0.1
Copepods L^{-1}	0.3±0.1	0.8±0.2*	0.02±0.03	0.38±0.05**

* $p < 0.05$. ** $p < 0.01$.

Table 3. Mean (±standard deviation) final biomasses (μg C L^{-1}) of bacteria and zooplankton in microcosms. Stars indicate significantly higher means according to *t*-test comparing treatments within lake type.

	Eutrophic water (Vomb)		Humic water (Skärshult)	
	Nonexposed	UV-exposed	Nonexposed	UV-exposed
Bacteria	15±1.5	21±0.5**	5.8±0.5	7.4±0.2**
HF	12±1.0	13±2.1	0.7±0.1	1.1±0.2*
Small ciliates	2.6±0.8	3.2±0.4	1.3±0.6	1.0±0.4
Large ciliates	—	—	2.3±1.2**	0.1±0.1
Rotifers	—	—	0.1±0.0	0.4±0.1**
Cladocerans	1.9±1.9	1.9±2.3	0.6±0.3	4.0±3.2*
Nauplii	0.9±0.3	1.3±0.3	Absent	0.1±0.0
Copepods	4.5±2.5	6.9±1.4	0.1±0.2	0.8±0.8
Protozooplankton	14±1.1	16±1.5	4.2±1.6**	2.1±0.5
Metazooplankton	7.3±2.6	10±3.0	0.8±0.5	5.2±4.2**
Total plankton	37±4.9	47±5.1	11±1.7	15±4.2

* $p < 0.05$; ** $p < 0.01$.

gardless of lake type (i.e., humic or eutrophic). We expected bacteria to respond negatively to photomodification of DOM in the eutrophic lake (Obernosterner et al. 1999; Tranvik and Bertilsson 2001). The unexpected positive bacterial response in UV-exposed eutrophic water may be explained by only low amounts of algal-derived DOM when the lake water was collected, in spite of spring chlorophyll *a* levels being typically high (*see Lake description*). Stable carbon isotope signatures of DOM in Lakes Vomb and Skärshult support the argument that photomodification of the terrestrial component of DOM played a major role in the stimulation of bacteria. In spite of contrasting humic content and trophic state (i.e., humic and eutrophic), $DO^{13}C$ signature is close to −27‰ for both lakes (Kritzberg unpubl. data), which is a typical value of terrestrial organic matter (Meili et al. 1993; Lajtha and Marshall 1994).

Zooplankton and food web responses—We expected that photochemical transformation of DOM from the eutrophic lake would result in decreased metazooplankton biomass. In spite of bacterial stimulation due to UV exposure of eutrophic water, there was no significant effect on summed metazooplankton biomass or biomasses of cladocerans and copepods separated. Thus, our hypothesis that photochemical modification of DOM decreases zooplankton biomass was rejected for the eutrophic water. An explanation to the negligible metazooplankton response in the eutrophic water may be that the community was dominated by cyclopoid copepods (*see Life history*).

For the humic water, the difference in community structure between microcosms was substantial, as addition of nonexposed DOM resulted in significantly higher biomass of protozooplankton. Metazooplankton dominated the community in microcosms receiving photomodified DOM, whereas the contribution of protozoans to total heterotrophic biomass was comparatively small. Thus, UV exposure of humic water contributed to a substantial increase in metazooplankton production, confirming our hypothesis that photomodification of DOM results in higher summed metazooplankton biomass. Absence of nauplii in microcosms receiving nonexposed humic DOM may have resulted from fewer copepods reaching adulthood (sexual maturity), as indicated by the significantly lower copepod abundance. Higher food availability in microcosms receiving photomodified humic DOM appeared to allow the copepod population to complete the reproductive cycle. Total heterotrophic biomass (bacteria+proto+metazooplankton) was not different between treatments, in spite of the metazooplankton biomass being significantly lower in the microcosms receiving nonexposed humic water. Reduced difference in total heterotrophic biomass between treatments was due to a high contribution of large ciliates in microcosms receiving nonexposed humic water.

Life history considerations—The gain in bacterial production due to UV exposure of eutrophic water did not significantly affect copepod biomass. In humic microcosms, there was a high variability among replicates in the response (not significant) of copepod biomass to additions of UV-exposed DOM, whereas the increases in biomasses of cladocerans and rotifers were statistically significant. Nonsignificant copepod responses to UV exposure of eutrophic and humic waters could be explained by differences in feeding mode, as cyclopoid copepods typically feed on larger prey than filter-feeding cladocerans and rotifers. Many cyclopoid copepods are unable to feed efficiently on bacteria and nanoplankton, whereas cladocerans and rotifers are capable of efficiently filtering bacteria and HF smaller than 10 μm (Stoecker and Capuzzo 1990; Arndt 1993; Brandl 1998 and references therein). In comparison to cyclopoid copepods, organisms feeding directly on bacteria seem more susceptible to respond to an increased bacterial production (on photomodified DOM), as less carbon is lost following passage through several consumer levels. In addition, the longer generation times of cyclopoid copepods may result in lower potential rates of biomass change (slower response), in comparison to some cladocerans and rotifers.

When interpreting our data it is also important to consider the extent to which the zooplankton production depends on feeding on the heterotrophic biomass, which may vary with

developmental stage, taxon, and season. The direct phytoplankton–zooplankton, as well as the indirect (the bacterial loop) phytoplankton exudates–zooplankton trophic pathways, were shut down in our dark microcosms to allow a more straightforward evaluation of how photochemical changes in DOM affect carbon transfer upward in the food web. Bacterial production and biomass of zooplankton decrease substantially in complete darkness, but especially in humic lake water, metazooplankton can thrive in the absence of phytoplankton for extended time periods (>1 yr; Daniel et al. 2005), fuelled by terrestrially derived DOM.

Microcosm versus in situ UV-R doses—Our artificial UV-R doses have to be compared to in situ conditions for temperate latitudes, where the lakes used in this study are situated. In the lake, all the UV-R is likely to be absorbed in the top few decimeters (humic lake) or the top few meters (eutrophic lake), and because of efficient mixing, photomodified DOM will be more or less homogeneously distributed within the epilimnion. Typical mixed depths (epilimnion depths) during summer in eutrophic and humic lakes are 5 and 2.5 m, respectively (Lindell et al. 1996). Using these epilimnion depths, mean summertime absorbed UV-R doses would be 0.1 and 0.2 kJ L^{-1} d^{-1} for the eutrophic and humic lakes, respectively, while corresponding absorbed UV-R doses in our microcosms were one order of magnitude larger (1.1 kJ L^{-1} d^{-1} for eutrophic and 4.2 kJ L^{-1} d^{-1} for humic).

The overall (visible+UV) effect of photochemical modification of DOM on zooplankton can be substantially larger than that of UV-R isolated. Shorter wavelengths of the visible light are involved in abiotic transformation of DOM, and the resulting labile photoproducts possibly stimulate bacterial growth (Moran and Zepp 1997; Granéli et al. 1998). Since visible light was not used in our study, the increase in biomass obtained for metazooplankton should be larger than what we have shown for the humic water. Thus, our experimental set-up does not seem unrealistic with respect to photochemical effects, in spite of increased UV-R doses used for the microcosms, in comparison to in situ conditions.

We have shown that metazooplankton growth can be stimulated by photomodification of DOM. Stimulation may depend on a strong direct trophic coupling to bacteria and HF, which exists for rotifers and cladocerans, but which, because of feeding mode, is absent for copepods. Response of zooplankton at the community structure level to photomodification of DOM may affect developmental stages of fish feeding on specific components of the plankton community, such as larvae or juveniles. Our findings are in line with those of an earlier investigation, in which the transfer of carbon to *Daphnia* via the microbial loop was affected positively by exposure of recalcitrant DOC (de Lange et al. 2003). Indirect effects of solar UV-R on plankton communities, mediated through natural DOM photochemistry, deserve more attention. Research should be devoted to studies that go beyond the bacteria level, and efforts should be made to transfer results from laboratory experiments to in situ conditions, taking into account UV-R attenuation with depth.

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