

SHORT COMMUNICATION

Solar ultraviolet photodegradation of DOC may stimulate freshwater food webs

HENDRIKA J. DE LANGE^{1,*}, DONALD P. MORRIS AND CRAIG E. WILLIAMSON

DEPARTMENT OF EARTH AND ENVIRONMENTAL SCIENCES, 31 WILLIAMS DRIVE, LEHIGH UNIVERSITY, BETHLEHEM, PA 18015-3188, USA

¹PRESENT ADDRESS: AQUATIC ECOLOGY AND WATER QUALITY MANAGEMENT GROUP, DEPARTMENT OF ENVIRONMENTAL SCIENCES, WAGENINGEN UNIVERSITY, PO BOX 8080, 6700 DD WAGENINGEN, THE NETHERLANDS

*CORRESPONDING AUTHOR: marieke.delange@wur.nl

The UV component in solar radiation increased the availability of DOC for bacterial growth, and led to an increase in bacterial and bacterivore abundance in laboratory plankton cultures. UV radiation may thus stimulate ecosystem productivity by increasing dissolved organic carbon lability and facilitating the transfer of carbon to higher trophic levels via the microbial loop.

Dissolved organic carbon (DOC) plays a central role in many aquatic ecosystem processes [e.g. (Williamson *et al.*, 1999)] and is the primary substrate for bacterial growth in ecosystems. Bacteria use both autochthonous organic carbon sources [e.g. (Cole *et al.*, 1982)] and allochthonous organic carbon sources [e.g. (Tranvik, 1992; Reche *et al.*, 1998)]. DOC is also an important factor in regulating transmission of solar radiation, especially UV radiation (UVR = 280–400 nm) (Scully and Lean, 1994; Morris *et al.*, 1995). UVR can have detrimental effects on aquatic organisms ranging from bacteria and phytoplankton (Herndl *et al.*, 1993; Karentz *et al.*, 1994) to zooplankton (Williamson *et al.*, 1994; Zagarese *et al.*, 1994) and fish (Siebeck *et al.*, 1994).

Many of the optical properties of natural waters may be modified by exposure to UVR. A study in North American lakes showed that photochemical degradation of DOC caused a decrease in UV absorbance. Effects of photochemical degradation differed between lakes and were related to differences in the source and composition of DOC, previous photochemical degradation and differences in DOC processing *in situ* (Morris and Hargreaves, 1997). A seasonal study in two Swedish lakes showed that DOC became less photoreactive after extensive exposure

to solar radiation in summer (Lindell *et al.*, 2000). A study in 38 Swedish lakes showed that DOC from oligotrophic humic lakes was more easily photomineralized than DOC from eutrophic lakes with high algal production (Bertilsson and Tranvik, 2000).

Bioassays have demonstrated stimulation of bacterial growth after photodegradation of DOC from humic-rich systems (Lindell *et al.*, 1995; Wetzel *et al.*, 1995; Jørgensen *et al.*, 1998). Other studies showed that photodegradation of algae-derived DOC did not stimulate bacterial growth (Benner and Biddanda, 1998; Obernosterer *et al.*, 1999; Pausz and Herndl, 1999). The importance of photochemical transformations of DOC for bacterial activity seems to be related to the initial availability for bacteria (Ziegler and Benner, 2000).

Here we test the hypothesis that photodegraded DOC stimulates bacterial production, and in turn bacterial grazers in the microbial loop are also stimulated.

We performed three experiments with water from Lake Giles, Lake Lacawac and a *Sphagnum* bog adjacent to Lake Lacawac (Table I). Lake water was sampled from the metalimnion. Bog water was sampled from a lysimeter, diluted 1:10 with low-carbon deionized water, and aerated before being used in the experiments. The water

Table I: Characteristics of the two study lakes

Variable	Giles	Lacawac
Latitude	41°22'34"N	41°22'57"N
Longitude	75°05'33"W	75°17'35"W
z_{\max} (m)	24	13
z_{avg} (m)	10	5
Lake area (ha)	48	21
Watershed area (ha)	183	70
Hydraulic retention (years)	5.6	3.3
Chlorophyll <i>a</i> ($\mu\text{g l}^{-1}$)	1.5	2.3
DOC (mg C l^{-1})	1.09	4.80
pH	5.35	6.03
Alkalinity ($\mu\text{eq l}^{-1}$)	-4.1	30

Chemical data are mean summer epilimnetic values measured over a 4 year period. Data from Moeller *et al.* (Moeller *et al.*, 1995). The *Sphagnum* bog surrounds Lake Lacawac for ~40% and is the main source of DOC for Lake Lacawac.

was filter sterilized (Sterivex 0.22 μm ; Millipore) into eight quartz bottles (volume 2 l, diameter 10.8 cm) and eight glass bottles (volume 2 l, diameter 11 cm), without headspace. Samples of the sterile filtered water were taken for measurements of DOC concentration, absorbance and a biolability assay. These samples are referred to as START.

The bottles were placed in racks and covered with acrylic sheets (CYRO Industries, Orange, CT, USA). The quartz bottles were covered with OP-4 (sharp cut-off with 50% transmittance at 272 nm; +UVR treatment). The glass bottles were covered with OP-2 (sharp cut-off with 50% transmittance at 410 nm; -UVR treatment). The racks were suspended at the lake surface, with ~1 cm of

water covering the bottles. The experiment with water from Lake Giles was incubated in Lake Giles; the experiments with water from the bog and Lake Lacawac were incubated in Lake Lacawac. The incubation was ended when the cumulative UVB-305 nm ambient exposure above the water surface had reached ~5 kJ m^{-2} (Table II). Samples were taken from each bottle for measurements of DOC and absorbance. These samples are referred to as -UVR (visible radiation only) and +UVR (full solar radiation). The photodegraded water from the replicate bottles within each treatment was then combined and used in the biolability experiment and the plankton experiment.

DOC concentrations were measured by a high temperature combustion method (Shimadzu TOC-5000) according to Sharp *et al.* (Sharp *et al.*, 1993). Absorbance of filtered samples from 200 to 800 nm was determined by spectrophotometry (Shimadzu UV-1601 spectrophotometer) using 10 cm quartz cuvettes and a blank consisting of low-carbon deionized water. Absorption coefficients (a_d) and DOC specific absorbance ($a_d:[\text{DOC}]$) were calculated similarly to Morris and Hargreaves (Morris and Hargreaves, 1997).

The availability of DOC for bacterial growth (biolability) was measured using a bioreactor [cf. (Kaplan and Newbold, 1995)]. The bioreactor had a volume of 450 ml, was kept in the dark at 20°C and supplied continuously with GF/F-filtered water in an upflow mode at 1 ml min^{-1} . One bioreactor was exclusively dedicated to Lake Lacawac and a second to Lake Giles. The biolability was measured with initial water (START) and after incubation water (-UVR and +UVR). Inflow and outflow samples for DOC were taken after 32 h of running water through the bioreactor. The difference in DOC concentration between inflow and outflow was defined as the biolabile fraction.

Table II: Total cumulative incident energy exposure at several UVR wavelengths as well as PAR (400–700 nm) during each experiment (1999), measured with a GUV-521 (Biospherical Instruments Inc.) atmospheric sensor deployed at the Lacawac Sanctuary (~0.5 km away from Lake Lacawac and ~15 km from Lake Giles)

Experiment	Start	End	305 nm (kJ m^{-2})	320 nm (kJ m^{-2})	340 nm (kJ m^{-2})	380 nm (kJ m^{-2})	Sum PAR (E m^{-2})
Lacawac	June 9	June 17	5.32	44.6	93.6	128	309
Giles	June 28	July 6	6.56	46.5	94.1	131	313
Bog	July 9	July 16	4.86	39.5	81.1	115	281

Irradiance was measured simultaneously at 305, 320, 340 and 380 nm (8–10 nm full width at half-maximum response), as well as broadband PAR (400–700 nm).

Plankton cultures were started by mixing 150 ml of photodegraded water (–UVR or +UVR) with 150 ml of lake water that had been sampled from 2 m depth and screened through a 48 μm mesh. This retained the natural bacteria, protozoa and phytoplankton composition (<48 μm). Lake Giles plankton was used in the Giles experiment, Lake Lacawac plankton in the Lacawac and bog experiment. These cultures were diluted daily with photodegraded (–UVR or +UVR) water; after 4 days and every second day thereafter, 250 ml were sampled from the cultures and used in the *Daphnia* experiment. Ten replicate cultures were set up for each treatment, with an average volume of 550 ml, and an average daily dilution rate of 30%. The cultures were exposed to natural solar radiation at an intensity of $\sim 50\%$ of ambient. Temperature was kept constant and at the same value as the lake from which plankton was sampled (20°C for the Lacawac experiment, 18°C for the Giles experiment and 23°C for the bog experiment). At day 5 (halfway) and at day 11 (end), samples were taken for bacterial counts (fixed with formalin) and plankton composition (fixed with Lugol's solution).

Bacterial abundance was determined by epifluorescence microscopy of DAPI-stained samples collected on 0.2 μm black Millipore filters. At least 10 fields of view and 400 cells were counted at 1250 \times magnification [cf. (Porter and Feig, 1980)].

Protozoa and phytoplankton were filtered on a 0.8 μm polycarbonate filter (Osmonics), inverted onto coated slides (0.5% gelatin with 0.05% chrome alum), quick frozen (cryogenic aerosol spray) and stripped away [filter–transfer–freeze technique adapted from Hewes and Holm-Hansen (Hewes and Holm-Hansen, 1983)]. Slides were mounted with Aqua-Polymount (Polysciences) and counted at 400 \times magnification by light microscopy. Composite samples of the 10 replicates per treatment were examined for the Giles experiment, showing no difference between treatments. In the Lacawac and bog experiment, the composite samples showed differences, therefore each replicate sample was analyzed. Only these results are presented. Cells were identified to species level where possible. All species were classified as autotrophic, mixotrophic or heterotrophic, according to judgment and presence of chloroplasts. Each species was measured and biovolume was calculated (Wetzel and Likens, 1991).

A clone of *Daphnia pulicaria* Forbes (isolated from Dutch Springs Reservoir, Bethlehem, PA), adapted to lake water, was used in the *Daphnia* reproduction experiments. One (Lacawac experiment) or two (Giles and bog experiments) mature female *Daphnia* were placed in each of 10 replicate 250 ml bottles for both radiation treatments. Each replicate plankton culture was used to feed one replicate *Daphnia* bottle. The bottles were rotated continuously (1

r.p.m.) on a plankton wheel in the dark at 20°C. Every 48 h, individuals were transferred to clean bottles with fresh food from the plankton cultures. Survival, number of eggs and number of juveniles born were recorded, and offspring removed. After 8 days, the experiment ended by measuring the length of the individuals and counting the number of eggs in the broodpouch.

The +UVR treatment led to a significantly lower DOC concentration than the –UVR treatment for both bog water and Lacawac water experiments (Figure 1A). In each experiment, the –UVR treatment had no effect on DOC absorptivity compared with START, but the +UVR treatment caused a significant reduction in DOC absorptivity (Figure 1B). Both –UVR and +UVR treatments generally resulted in an increase in DOC biolability compared with START, with the exception being the Lacawac –UVR treatment (Figure 2). For the Lacawac and bog experiments, biolability was higher in the +UVR than in the –UVR treatment.

Bacterial abundance was significantly higher in the +UVR than in the –UVR treatment in both Lacawac and

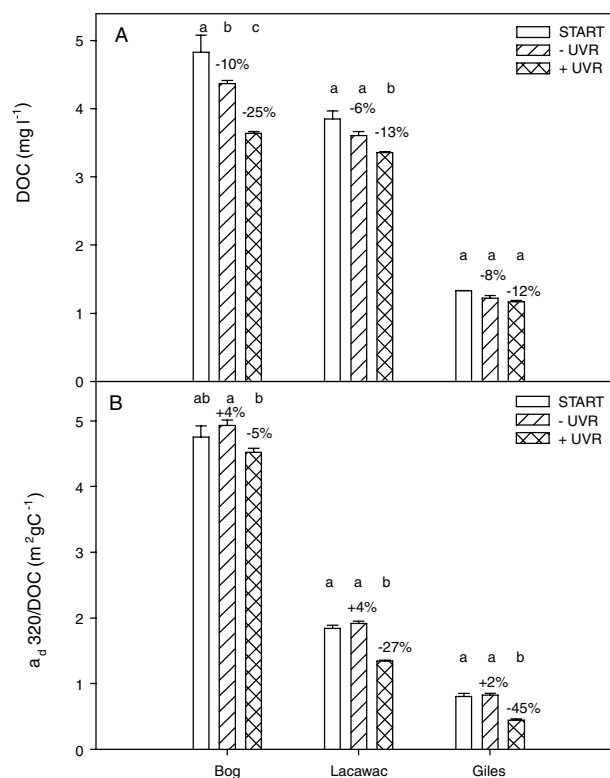


Fig. 1. DOC before (START) and after exposure to –UVR or +UVR treatment. **(A)** DOC concentration; **(B)** DOC specific absorption coefficient at 320 nm. Error bars represent standard errors (SEs). Changes are given as a percentage of START. Lower case letters indicate homogeneous groups (ANOVA followed by Tukey–Kramer test, $P < 0.05$, number of replicates = 8).

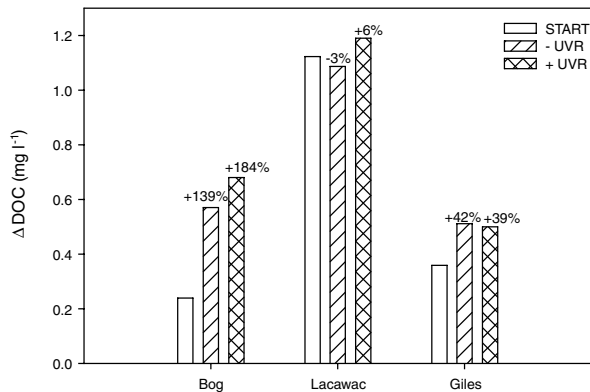


Fig. 2. Decrease in DOC in bioreactor (biolability = inflow – outflow). The change in biolability in –UVR and +UVR treatments is given as a percentage of START.

bog experiments. In the Giles experiment, the bacterial abundance was significantly lower in the +UVR treatment at the end of the experiment (Figure 3).

Plankton biovolume increased in the +UVR treatment in the bog experiment due mainly to an increase in

heterotrophs. In the Lacawac experiment, the higher biovolume was mainly due to an increase in mixotrophs (Figure 4). The species that showed significant differences between –UVR and +UVR were mostly (assumed to be) mixotrophic chrysophytes, like *Pseudopedinella* sp. and *Uroglena* sp. (Table III). In the bog experiment, small ciliates were also more abundant in the +UVR treatment.

Total offspring produced during the *Daphnia* experiment was statistically indistinguishable among treatments in all three experiments. The number of eggs in the broodpouch at the end of the experiment was higher for the +UVR than the –UVR treatment in both bog and Lacawac experiments, but statistically significant only for the bog experiment. In the Giles experiment, none of the *Daphnia* had eggs at the end of the experiment (Table IV).

Our results confirm prior studies showing that UVR is more effective than photosynthetically active radiation (PAR) in photodegradation of DOC (Morris and Hargreaves, 1997). The UVR photochemical reactions of DOC resulted in a reduced molar absorptivity. The bioreactor experiments showed that for both Lacawac and bog experiments, the +UVR treatment resulted in the largest increase in biolability.

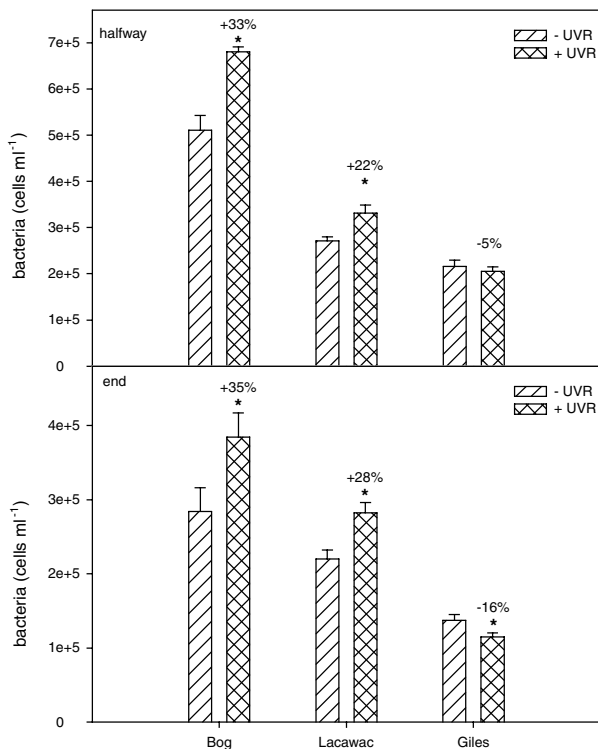


Fig. 3. Bacterial concentration in the plankton cultures halfway and at the end of the experiment. Error bars represent SEs. The difference between –UVR and +UVR is given as a percentage of –UVR; asterisks indicate a significant difference (*t*-test, *P* < 0.05, number of replicates = 10).

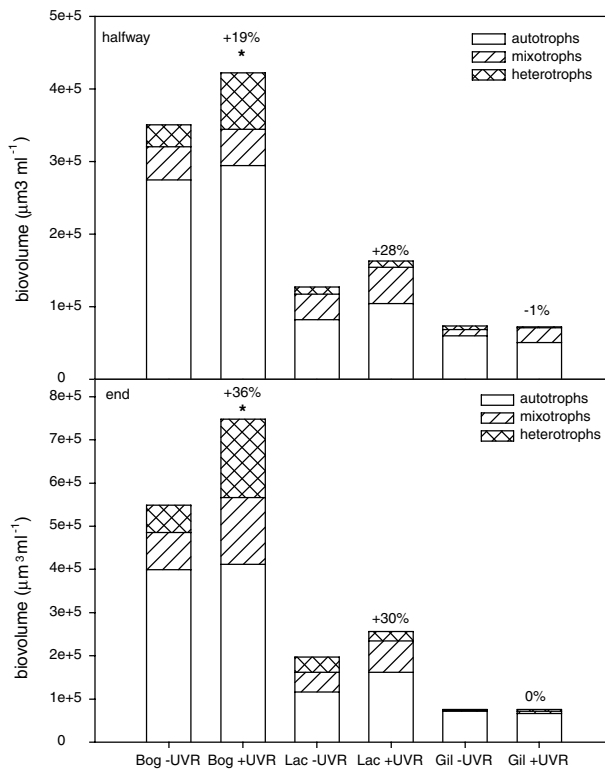


Fig. 4. Plankton concentration and trophic status halfway and at the end of the experiment. Giles counts were made on composite samples; Lacawac and Bog on 10 replicate samples. The difference between –UVR and +UVR is given as a percentage of –UVR; asterisks indicate a significant difference (*t*-test, *P* < 0.05, number of replicates = 10).

Table III: Species with a significant difference in abundance

Species	Lacawac half	Lacawac end	Bog half	Bog end
Small <i>Pseudopedinella</i> sp. (m)	+122%	+155%	+96%	+291%
<i>Pseudopedinella</i> sp. (m)	+112%		+362%	
<i>Dinobryon</i> sp. (m)		+68%		+231%
<i>Uroglena</i> sp. (m)		+618%		+322%
<i>Bitrichia</i> sp. (a)				+268%
Round chrysophyte (a)	+39%			
Small flagellate (a)		+49%		
<i>Monosiga</i> sp. (h)	-54%	-57%		
Round ciliate (h)			+191%	+134%
Total plankton biovolume	+28% n.s.	+30% n.s.	+19%	+36%

t-test, $P < 0.05$, number of replicates = 10.

Differences are calculated as the percentage change in +UVR relative to -UVR.

(a), (m) or (h) indicates autotrophic, mixotrophic or heterotrophic, respectively; n.s., no significant difference.

Table IV: Results of the *D. pulicaria* experiment (\pm SE)

Experiment	Treatment	Survival (%)	Total offspring (no. female ⁻¹)	Eggs at end (no. female ⁻¹)	Length at end (mm)
Lacawac	Food addition	100	12.7 \pm 1.3	13.9 \pm 0.3	2.67 \pm 0.10
	-UVR	100	4.5 \pm 1.4	0.8 \pm 0.4	2.32 \pm 0.09
	+UVR	100	4.3 \pm 1.4	1.2 \pm 0.3	2.35 \pm 0.09
Giles	Food addition	60	8.1 \pm 1.5	7.6 \pm 1.3	2.38 \pm 0.03
	-UVR	50	2.3 \pm 0.7	0	2.03 \pm 0.04
	+UVR	40	1.4 \pm 0.4	0	2.02 \pm 0.05
Bog	Food addition	90	9.2 \pm 1.4	8.9 \pm 1.4	2.40 \pm 0.03
	-UVR	60	2.4 \pm 0.6	2.4 \pm 0.6*	2.15 \pm 0.03
	+UVR	55	2.9 \pm 0.9	4.8 \pm 0.6*	2.15 \pm 0.02

The food addition treatment is filtered lake water with *Cryptomonas* (2000 cells ml⁻¹) to control for *Daphnia* viability. This food concentration was above the incipient limiting level (Sanders *et al.*, 1996). Asterisks indicate a significant difference between -UVR and +UVR (*t*-test, $P < 0.05$, number of replicates = 10).

The responses in the plankton cultures were the second level in our experiments. DOC degraded by UVR increased the abundance of bacteria, mixotrophic and heterotrophic organisms, but not of autotrophic organisms. This demonstrates that UVR may facilitate the transfer of carbon to higher trophic levels in the food web via the microbial loop.

The *Daphnia* experiment was the third level in our experiments. This experiment showed only one significant difference (Table IV): in the bog experiment, the number of eggs in the broodpouch was significantly higher in the +UVR treatment. The experiment was designed in three

levels (DOC \rightarrow plankton \rightarrow *Daphnia*), with UVR exposure at the DOC level. Information is lost at every step between these levels and only the strongest effects will be sustained at the zooplankton level. Our results show that UVR can have an indirect stimulating effect on planktonic organisms by making recalcitrant DOC more available for microorganisms. It must be stressed that the plankton were cultured under laboratory (artificial) conditions. The responses of these planktonic communities are not likely to directly reflect responses under natural conditions, but they do demonstrate potentially important changes in community structure due to UVR exposure.

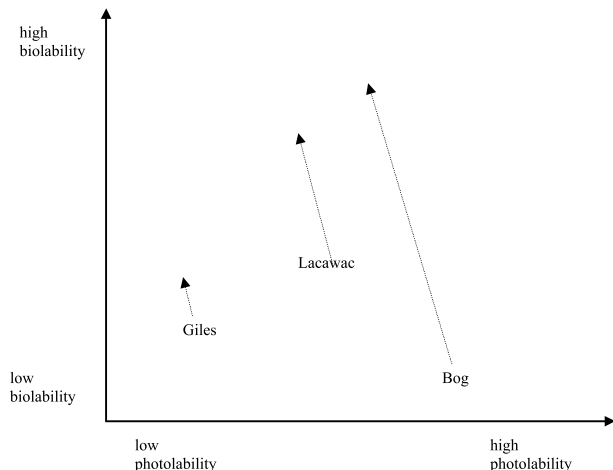


Fig. 5. Photolability and biolability of DOC from the different experiments. Scales are relative; the length and direction of the arrow indicate the effect of full solar radiation on biolability and photolability.

Direct effects of UVR on bacterial communities are negative, although in freshwater systems the negative effects are usually limited to the water nearest the surface (Rae and Vincent, 1998). The actual effect of UVR on organisms is, therefore, the result of the balance between direct negative effects and indirect stimulating effects such as those demonstrated here.

Photodegradation processes generally decrease the photolability and increase the biolability (visualized in Figure 5). Humic systems, such as bog water, will show the strongest responses to UVR. UVR photodegradation of DOC may be a stimulus to the ecosystem.

The concurrent effect of photodegradation is that it reduces UV absorptivity of DOC, thus increasing the penetration of UVR in a system. This may have inhibitory effects on organisms. The overall net effect in a system depends on the initial UV transparency, positioning of the organisms in the water column, and other factors such as mixing processes, relative exposure to UV-B, UV-A and PAR, temperature and nutrient conditions. Research into the interaction between UVR and other environmental factors is still limited, but deserves our full attention.

ACKNOWLEDGEMENTS

We thank the Lacawac Sanctuary and the Blooming Grove Hunting and Fishing Club for access to their lakes. This research was supported by NSF grant DEB-9629639.

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Received on September 25, 2001; accepted on September 20, 2002

