Limnol. Oceanogr., 49(6), 2004, 2011–2022

Effects of sunlight and hydroxyl radical on dissolved organic matter: Bacterial growth efficiency and production of carboxylic acids and other substrates

Michael J. Pullin,[1] *Stefan Bertilsson,*[2] *Jared V. Goldstone,*[3] *and Bettina M. Voelker*[4]
Ralph M. Parsons Laboratory, Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Abstract

This study examines the importance of several possible mechanisms causing sunlight-mediated changes in the amounts of bacterial utilization and biomass growth on dissolved organic matter (DOM) from allochthonous sources. Our results demonstrate that, while hydroxyl radical reactions with DOM can be an important process increasing its bioavailability, other photoreactions will cause most of the sunlight-induced increases unless hydroxyl production rates are high ($>\sim 7$ μmol L^{-1} d^{-1}). Low molecular weight carboxylic acids could not account for most of the observed sunlight and hydroxyl-induced increases in DOM bioavailability. Both sunlight and hydroxyl-mediated reactions significantly decreased the bacterial growth efficiency of DOM, indicating that photochemical reactions affect not only the fraction of the total DOM pool available to bacteria on ecologically relevant timescales but also the substrate quality and ultimately the environmental fate of this material. Extrapolation of these results to field conditions suggests that photochemical and biochemical mineralization could be an important sink of DOC and source of bioavailable carbon in the Plum Island estuary during the summer months.

Dissolved organic matter (DOM) is a heterogeneous mixture of natural organic compounds that is present in all natural waters. The sources of this carbon in freshwater systems include both in situ biological production (autochthonous sources) and detrital carbon from the surrounding terrestrial watershed (allochthonous sources). At one time, relatively labile autochthonous material was thought to be the dominant source of substrates for bacterial growth in these systems (Cole et al. 1982). More recently, it has become clear that relatively recalcitrant allochthonous (or humic) organic matter can also be a major source of energy and carbon for bacterial growth in many freshwaters (Tranvik 1988; Moran and Hodson 1990).

One important environmental factor that may change the utilization of DOM by bacteria is exposure to light. A variety of studies have shown that irradiation of DOM by natural and/or simulated sunlight can increase both the amount of DOM that is susceptible to bacterial utilization and the ability of this material to support bacterial growth (Miller and Moran 1997; Moran et al. 2000; Tranvik and Bertilsson 2001; Obernosterer and Benner 2004). This effect has, in part, been attributed to the photoproduction of biologically labile low molecular weight (LMW) organic compounds from DOM (Kieber et al. 1989; Bertilsson and Tranvik 1998). Identified photoproducts include LMW carboxylic acids, aldehydes, and ketones (Moran and Zepp 1997; Bertilsson and Tranvik 2000).

Photochemical reactions can also oxidize organic carbon to CO_2 (mineralization), removing it from the pool of potentially available carbon substrates (Miller and Zepp 1995; Miller and Moran 1997; Bertilsson and Tranvik 2000; Moran et al. 2000; Goldstone et al. 2002). The photoproduction rate of LMW carboxylic acids is reported to be 30–50% of that for CO_2 photoproduction. Because these organic acids are biologically labile, this reaction pathway represents a relatively important sink of DOC in natural surface waters (Bertilsson and Tranvik 1998, 2000). The photoproduction rates of LMW aldehydes and ketones are estimated to be comparable with those of CO (another photoproduct), or 10–20 times slower than for CO_2 (Miller and Moran 1997; Moran and Zepp 1997).

Exposure of DOM to solar radiation can also decrease the biological utilization of DOM in both freshwater and marine ecosystems (Benner and Biddanda 1998; Tranvik and Kokalj 1998; Obernosterer et al. 1999). This observation seems to be limited to systems dominated by autochthonous (phytoplankton-derived) inputs. Thus, the overall effect of photochemical processes on the ability of DOM to support bacterial growth is probably the net result of two underlying processes: photochemical increases in the lability of humic carbon and photochemical decreases in the lability of algal carbon (Tranvik and Bertilsson 2001).

One question not settled by previous studies is to what extent the observed increase in the bioavailability of alloch-

[1] Corresponding author's present address: Department of Chemistry, New Mexico Institute of Mining and Technology, Socorro, New Mexico 87801 (mpullin@nmt.edu).

[2] Present address: Department of Evolutionary Biology/Limnology, Uppsala University, SE-75236, Uppsala, Sweden.

[3] Present address: Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543.

[4] Present address: Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, Colorado 80401.

Acknowledgments

This research was funded by the National Science Foundation (Chemical Oceanography, grant OCE-9819089) and by postdoctoral fellowships to M.J.P. and S.B. through the National Science Foundation (Earth Sciences) and the Knut and Alice Wallenberg Foundation, respectively. The authors thank Phil Gschwend for the use of the HPLC equipment; Bob Chen, Chuck Hopkinson, and Pete Raymond for assistance in field sampling; Joe Vallino for background information on the Parker River; and Barbara Southworth for lab assistance and estimates of hydroxyl radical production rates.

thonous DOM is due exclusively to the photoproduction of the LMW organic compounds identified so far. In an effort to answer this question, Miller and Moran (1997) compared their observed photochemically induced increases in bacterial growth to previously reported rates of LMW substrate photoproduction. They concluded that there were either unknown labile photoproducts produced that were not accounted for in their mass balance or that other photochemical changes in DOM occurred that rendered it more susceptible to microbial utilization. However, their analysis did not take the photoproduction of LMW carboxylic acids into account. Later studies showed that these compounds may be the quantitatively dominant LMW photoproducts (Bertilsson and Tranvik 2000; Moran and Covert 2003). Furthermore, the combined uptake rate of only three carboxylic acids (formic, acetic, and malonic acid) was found to greatly exceed bacterial production, suggesting that these LMW substrates alone could account for the bulk of the observed increase in DOM bioavailability upon exposure to solar radiation (Bertilsson and Tranvik 1998). In contrast, Brinkmann et al. (2003) recently reported that the photochemical production of LMW carboxylic acids only accounted for 33% of the increased bacterial uptake (measured as DOC loss) after the ultraviolet (UV) irradiation of a bog-water sample.

The ability of DOM to support bacterial growth is not only governed by the overall amount of its utilization (generally defined as its bioavailability). The quality of this material, or its ability to support the production of new biomass, is also an important consideration. This is reflected in the bacterial growth efficiency (BGE), defined as the ratio of bacterial biomass production to total carbon utilization. This parameter describes the partitioning of utilized carbon substrates into new biomass and CO_2 and is important in determining the exchange of carbon and energy between DOM and aquatic food webs (del Giorgio and Cole 2000). Given that photochemical reactions are known to change the chemical structure and bacterial availability of DOM, it is certainly possible that photochemical processes could change also overall BGE on natural DOM.

Despite the potential importance of possible photochemical changes in this balance, only a few studies have examined the effect of sunlight exposure on BGE, with mixed results. In long-term incubations (51 d) of estuarine water initially treated with varying amounts of light (0–70 d), Moran et al. (2000) observed that increasing initial sunlight exposures increased bacterial respiration at a greater rate than the overall DOC utilization. This implies a decrease in the overall BGE due to the sunlight pretreatment (although the authors neither calculated nor discussed the BGE values for their experiments). Farjalla et al. (2001) examined bacterial utilization of dissolved organic carbon leached from aquatic macrophytes and found that exposure to UV light decreased the ability of the material to support bacterial growth whereas utilization of DOM was not affected, also suggesting a decrease in BGE. However, Reche et al. (1998) found that sunlight exposure caused an increase in BGE on DOM in lake-water incubations.

Although many studies have examined the overall effect of sunlight exposure on bacterial DOM utilization, the underlying photochemical mechanisms that cause these changes are not well understood. One possible mechanism is the reaction of DOM with photoproduced reactive oxygen species (ROS), such as the hydroxyl radical (HO·) (Goldstone et al. 2002; Scully et al. 2003*a*). Hydroxyl radical is produced photochemically in natural waters through at least three mechanisms: nitrite/nitrate photolysis, the photo-Fenton reaction (the reaction of photoproduced Fe(II) and H_2O_2 with each other), and the direct photolysis of DOM (Zepp et al. 1987; Mopper and Zhou 1990; Zepp et al. 1992; Voelker et al. 1997; Vaughn and Blough 1998; Southworth and Voelker 2003). Subsequently, HO· is rapidly consumed by reactions with organic and inorganic species (Brezonik and Fulkerson-Brekken 1998; Goldstone et al. 2002). In most freshwater systems, DOM is the major sink for photoproduced HO· (Brezonik and Fulkerson-Brekken 1998; Goldstone et al. 2002). In seawater, reactions of HO· with inorganic Br^- produce oxidized bromide radical species (Zafiriou et al. 1987) that appear to also react primarily with DOM (Song et al. 1996).

It has recently been shown that the reactions of hydroxyl radical (HO·) with isolated humic substances can produce LMW carboxylic acids, although at slower rates than needed to account for previously observed sunlight production rates (Goldstone et al. 2002). Additionally, this study reported that exposure to HO· did not change the ability of DOM to support bacterial growth. This suggests that the reaction of photoproduced hydroxyl radical with DOM is not an important mechanism affecting its bacterial utilization. However, because carbon utilization was not measured during the bioassays, changes in the magnitude or the efficiency of bacterial carbon utilization would have gone undetected in these experiments, possibly missing an effect of HO· on DOM bioavailability. Additionally, natural organic matter might react differently from the isolated humic substances investigated in the prior study (Goldstone et al. 2002), and DOM from different origins may also react differently.

The purpose of this study was to examine the importance of two processes involved in sunlight-mediated changes in the amount of bioavailable DOM and the resulting bacterial growth efficiency: the photoproduction of LMW carboxylic acids and the reaction of photoproduced HO· with DOM. This was accomplished by exposure of filter-sterilized natural water samples to either natural solar radiation or nonphotolytic HO·, followed by direct measurements of LMW acid production and utilization, uptake of DOC, and bacterial biomass accumulation in dilution cultures. The simultaneous assessment of both biomass and total carbon utilization allowed us to determine the effect of sunlight and HO· on the bacterial growth efficiency. In an effort to examine the effect of DOM source on these processes, experiments were conducted on water from two sites on the Parker River (coastal Massachusetts), one dominated by terrestrial DOM inputs and a second located at an upper-estuarine diatom bloom with an additional autochthonous DOM input. The implications of the results obtained in these laboratory experiments for the fate of organic carbon from the Parker River are also discussed.

Materials and methods

Field site and sampling—The Parker River is located on the coast of Massachusetts (USA). It drains a rural and suburban watershed (155 km^2), eventually traversing a salt marsh before emptying into Plum Island Sound. A small, spillover dam at a historic mill site separates the freshwater Parker River from its tidally influenced oligohaline zone. Further details regarding the watershed are given in several publications (Vallino and Hopkinson 1998; Holmes et al. 2000; Hullar 2000).

Whole-water samples were collected from two sites on the Parker River on 11 July 2001. Site 1 was just upstream of the above-noted dam, while Site 2 was approximately 50 m downstream of the dam. The sampling date and the location of Site 2 were chosen to correspond to a predictable diatom bloom that occurs annually in the Parker River (Holmes et al. 2000). The bloom was located in the river channel as a maximum in dissolved oxygen (~120% saturation) and by the obvious green color of the water. Later analysis demonstrated greatly elevated chlorophyll *a* (Chl *a*) concentrations (83.1 $\mu g\ L^{-1}$) relative to upstream Site 1 (10.0 $\mu g\ L^{-1}$). The salinity of the water at the location of the bloom was 0.90 ‰ during low tide. Samples (approximately 10 liters) were collected by hand in acid-washed fluorinated high-density polyethylene carboys (Nalgene) from shore (Site 1) and from a small boat (Site 2). The samples were kept at near ambient water temperature in coolers until the return to the laboratory (< 5 h). Each sample was then filtered into a second clean carboy using sterile 0.2 μm Maxi Capsule® membrane cartridge filters (Gelman Science) and stored in dark refrigeration until use (see dates below). To avoid carbon contamination, the filters were precleaned by recirculating 1.0 mol L^{-1} HCl through the filters and tubing for 30 min at approximately 1 liter min^{-1}, rinsing with 20 liters UV-treated deionized water (17.8 MΩ), and then discarding the first 1 liter of filtered sample. A new filter was used for each sample.

Pretreatment and incubations—Prior to incubation with bacteria, triplicate 500-ml aliquots of the 0.2 μm filtered samples from each sampling site were exposed to either sunlight or hydroxyl radical or left untreated as controls. The sunlight-treated samples were exposed to rooftop sunlight in 500-ml quartz round-bottom flasks (combusted, 450°C, 12 h). The flasks were filled, sealed with glass stoppers, wired closed, and submerged in a water bath to just cover the flasks, preventing the condensation of water vapor on their outer surfaces. The temperature of the water bath was fixed at 20°C by circulating refrigerated water through a coil of copper tubing at the bottom. The samples were exposed to 6 h of cloudless midday sun in Cambridge, Massachusetts, on 21 July 2001 and again for 8 h on the next day. Between exposure days and after irradiation, the samples were stored in dark refrigeration. The hydroxyl radical treatment (30 μmol L^{-1} total dose of HO·) was performed using the gamma radiolysis of water, as described in detail by Goldstone et al. (2002). Briefly, the samples were saturated with a 4 : 1 mixture of N_2O and O_2 by bubbling with a gas frit (30 min) and then exposing to the gamma radiation emitted by a ^{60}Co source (GammaCell 220) for 30 min. The rate of hydroxyl radical production by this method (1.0 μmol L^{-1} min^{-1}) was measured as described by Goldstone et al. (2002). As an untreated control, triplicates of each water sample were kept dark at room temperature (20°C) in the laboratory during the period of the sunlight irradiation. The pH of the samples (7.6 ± 0.1) was not affected by either treatment.

The triplicate samples of sunlight-treated, hydroxyl radical-treated, and untreated water from both sampling sites were inoculated with a natural bacterial assemblage. On the day the incubations were started (25 July 2001), we returned to Site 1 and collected a whole-water sample. Upon return to the lab (<2 h), the water was filtered through a combusted (450°C, 3 h) glass-fiber filter (Whatman GF/F, 0.7 μm nominal pore size) to remove bacteriovores, and then the filtrate was amended with sodium hydrogen phosphate and ammonium chloride. The resulting mixture of nutrients and bacteria was added 1 : 10 (v : v) to each treated sample and the controls. The final concentrations of the added nitrogen and phosphorus in the incubations were 100 μmol L^{-1}.

These cultures were incubated in darkness at 22°C and individually sampled both before and after addition of the bacterial inoculum and then after 3, 5, 7, 9, 11, 13, and 15 d. To sample the incubations for the analysis of DOC and LMW organic acids, 20 ml was removed from the incubation bottles and 0.2 μm filtered directly into precleaned 40-ml amber glass vials (TraceClean, I-Chem) using sterile polyethersulfone syringe filters (Nalgene 180-1320). For the determination of cell abundance, 5 ml of unfiltered water was also removed to sterile FACScan flow cytometry tubes (Becton Dickinson) and preserved by adding 0.2 μm filtered sodium tetraborate-buffered formaldehyde to a final concentration of 2%. All samples were refrigerated in darkness until analysis (within 1 month).

To avoid carbon contamination during sampling, we found that it was necessary to use glass and Teflon™ syringes and Teflon™ tubing for fluid handling and transfer and also to clean the syringes, tubing, and filters prior to use. The syringes and tubing were cleaned by soaking in 10% HNO_3 overnight and then rinsing with UV-treated deionized water and high-performance liquid chromotography (HPLC)-grade methanol (Mallinckrodt), followed by drying with 0.2 μm filtered TOC-grade air (BOC gases). For each sample, a new syringe filter was cleaned by filtering 5 ml 1.0 mol L^{-1} HCl followed by 10 ml UV-treated deionized water and then discarding the first 1 ml of filtrate. By measuring DOC (see below) before and after filtration with filters cleaned in this manner, we were able to confirm that no measurable amount of carbon was added to the filtrate by this method.

In addition to the incubation of untreated water samples as controls, we conducted an experiment to verify that the gamma radiolysis did not cause a slow or delayed abiotic loss of DOC or conversion of DOM to LMW acids over the timescale of our biological incubations. Such a delayed response may occur as a result of relatively long-lived organic radical species and could be misinterpreted as bacterial utilization. Water from Site 1 was filter-sterilized (0.2 μm) directly into 15 amber glass vials using a sterile, precleaned Nalgene cartridge filter. The vials were capped and saturated

with a 4 : 1 N_2O/O_2 gas mixture using two syringe needles passed through a silicone septum in the caps. The vials were exposed to 30 min of γ-radiation from the ^{60}Co source, with one set of three vials left unexposed. Three of the exposed vials and the three unexposed vials were immediately opened and analyzed for hydrogen peroxide, low molecular weight carboxylic acids, and DOC. The other nine exposed vials were left in the dark at room temperature and then opened in triplicates for immediate analysis of LMW acids and DOC after 2, 6, and 14 d. It is assumed that the γ-radiolysis treatment rendered the vials sterile until opened.

Analytical methods—DOC was measured using a Shimadzu TOC-5000 analyzer. Prior to analysis, 0.2 μm filtered samples (see above) and potassium hydrogen phthalate standards were acidified by adding 10.0 μl of 2.0 mol L^{-1} HCl per 1.0-ml sample, followed by sparging with TOC-grade air (BOC gases) for 5 min. A ThermoOrion 91-57 combination electrode and a 420A pH meter were standardized with NIST traceable buffers (VWR Scientific) and used to measure pH. Hydrogen peroxide was measured by chemiluminescence (Cooper et al. 2000; Southworth and Voelker 2003). Total dissolved iron was measured using hydroxylamine HCl and ferrozine on 0.2 μm filtered samples, according to Voelker et al. (1997). Chl *a* was measured spectrophotometrically on cells captured on Whatman GF/F glass-fiber filters (Carlsson et al. 1995). DOM absorbance spectra (200–800 nm) were obtained using a HP 8453 spectrometer (Agilent) in a 1.0-cm quartz cell with deionized water as a reference.

LMW carboxylic acids were measured by the ion-pairing reversed phased HPLC of their 2-nitrophenylhydrazide derivatives (Albert and Martens 1997), as described in detail by Goldstone et al. (2002). The method gives baseline resolution of glycolic, lactic, formic, acetic, levulinic, malonic, and oxalic acids in 35 min. Using 100 μl injections, the detection limit is 100 nmol L^{-1} and the peak area response is linear to at least 100 μmol L^{-1}.

Bacterial enumeration and biovolume estimation—Bacterial abundance was measured in the preserved samples (see above) using a FACScan® flow cytometer (Becton Dickinson) after staining with the nucleic acid stain SYTO 13 (50 μmol L^{-1} final concentration, Molecular Probes) del Georgio et al. 1996). Counting was performed at low flow (12 μl min^{-1}) with detector voltages set to 400 (side scatter) and 560 (green fluorescence). Fluorescent microspheres (Carboxy YG, 1.58-μm diameter, Polysciences) were added to all samples at a final concentration of 1.9×10^5 beads ml^{-1} for use as internal reference. Cells were separated from fluorescent beads in a log-log dot plot of side scatter and green fluorescence, and bacterial cell abundance was determined using the fluorescent beads as an internal standard. Samples were counted for 1 min or until a minimum of 2,000 beads had been detected.

Epifluorescence microscopy and image analysis were used to estimate bacterial cell size and to assure that predatory flagellates were absent from the cultures at the end of the incubations. Formaldehyde-fixed bacterial samples were stained with 4'6-diamidino-2-phenylindole (Porter and Feig 1980), and cells were visualized with an Axioskop 2 fluorescence microscope (Zeiss) equipped with an Atto-Arc variable light source. For each culture, duplicate images were acquired with a Magnafire cooled CCD camera (Optronics). Images were exported to the Scion Image 4.0.2 image analysis software and an edge-detection operator was used to define individual area, length, and width of >100 cells per individual culture. Cell volumes were then estimated by approximating the shape of bacterial cells as cylindrical with hemispherical end caps. Volumes were converted to bacterial carbon biomass for individual cells using the volume to dry weight relationship previously reported by Loferer-Krößbacher et al. (1998) and assuming that carbon comprises 50% of the bacterial dry weight.

Table 1. Effects of sunlight and HO· exposure on dissolved organic carbon (DOC) and LMW carboxylic acid concentrations for 0.2 μm filtered water from two sites on the Parker River, Massachusetts. LMW acids are presented as the sum of acetic, formic, malonic, and oxalic acid carbon. Reported values are the average of three separate treatments of the same water sample ($\pm 1\sigma$) and were determined prior to any bacterial addition or growth. (n/a = not applicable.)

Sample/ treatment	DOC (μmol L^{-1})	Loss of DOC due to treatment (μmol L^{-1})	Total of 4 LMW acids (μmol C L^{-1})
Site 1			
Control	703 ± 10	n/a	3.3 ± 1.4
Light	676 ± 15	27 ± 18	12.8 ± 2.0
HO·	674 ± 5.0	29 ± 11	32.3 ± 0.5
Site 2			
Control	759 ± 6.5	n/a	4.4 ± 0.8
Light	729 ± 7.0	30 ± 10	10.2 ± 1.6
HO·	722 ± 17	37 ± 18	22.6 ± 2.3

Results

Sampling Site 2 had a significantly (*t*-test, $p \leq 0.05$) higher concentration of DOC (759 ± 6.5 μmol L^{-1}) than Site 1 (703 ± 10 μmol L^{-1}), which was located only 50 m upstream (Table 1). Presumably, this was due to autochthonous inputs of organic carbon from the algal bloom present at the downstream Site 2. Both the light and HO· treatments decreased these DOC values (Table 1), causing the same relative loss of DOC in the water from both sites (~4%). The sunlight and hydroxyl radical treatments also caused the production of acetic, formic, malonic, and oxalic acids (Table 2). The production of these LMW organic acids was on the same order of magnitude as the abiotic loss of DOC during the sunlight and HO· exposure (Table 1). Levulinic, glycolic, and lactic acids are also measurable by our method but were not observed to be present above their detection limits during any of our experiments.

During the 15 d bacterial incubations, cell abundance increased by nearly two orders of magnitude in samples from both sites (Fig. 1). Generally, the cell counts stabilized within 7 d and, in most cases, remained constant until the end of the experiment. However, the untreated control incubations from Site 2 suffered a loss of cell abundance after day

Table 2. Effects of sunlight and HO· exposure on the individual concentrations of four LMW carboxylic acids for 0.2 μm filtered water from two sites on the Parker River, Massachusetts. The values are the average of three separate treatments of the same water sample ($\pm$ 1σ) and were determined prior to any bacterial addition or growth.

Sample/ treatment	Acetic acid (μmol L^{-1})	Formic acid (μmol L^{-1})	Malonic acid (μmol L^{-1})	Oxalic acid (μmol L^{-1})
Site 1				
Control	0.6 ± 0.5	0.8 ± 0.6	ND*	0.5 ± 0.3
Light	2.1 ± 0.4	3.2 ± 0.6	0.9 ± 0.3	1.3 ± 0.2
HO·	2.9 ± 0.7	11.1 ± 0.6	1.9 ± 0.4	4.8 ± 0.6
Site 2				
Control	0.9 ± 0.1	1.4 ± 0.4	ND	0.7 ± 0.1
Light	1.5 ± 0.3	4.6 ± 0.5	ND	1.3 ± 0.1
HO·	2.5 ± 0.2	8.5 ± 0.5	ND	4.5 ± 0.1

* ND = not detected, $<$ 0.1 μmol L^{-1}.

7 (Fig. 1b). This cell loss was likely a result of viral lysis because no protozoan grazers were observed in any of these cultures. This cell loss made it inappropriate to calculate average cell abundances for the control incubations after the first 7 d of incubation. As a result, the cell counts for each of the three control incubations are plotted separately after this point (Fig. 1b).

Significantly higher (*t*-test, $p \leq 0.05$) concentrations of cells were observed at the plateau in the light-treated incubations relative to the untreated controls (Fig. 1). The growth of cells in the HO·-treated incubations lagged behind the others during the beginning of the experiment, but eventually all abundances increased to similar levels as the untreated controls. There was no significant difference (*t*-test, $p \leq 0.05$) between the average cell abundances (at day 7) in the water samples from the two sites when they were exposed to the same pretreatment (light, HO·, or control).

In most cases, the time course of DOC loss during the incubations roughly corresponded to the increase of bacterial cell abundance, including the initial lag in the HO·-treated samples (Fig. 2). In a few cases, increases in DOC were observed near the end of the incubations, presumably due to a net release of DOC from bacteria due to cell lysis or excretion. The total utilization of carbon during bacterial growth was estimated as the difference between the highest and lowest observed DOC values for each of the incubations (Table 3).

The bacterial utilization of carbon (as loss of DOC) during the incubations was greater in both the light and HO·-treated water from both sampling sites relative to the untreated control incubations (Fig. 2). The increased carbon utilization due to the treatments was calculated as the difference in utilization between the treated samples and the controls (Table 3). The increased bacterial carbon utilization due to the treatments was approximately twice the abiotic conversion of DOC to CO_2 by the same treatments (Tables 1, 2). For water exposed to the same pretreatment, no difference in carbon utilization was observed between the two sites.

For each of the incubations, the concentration of net cell carbon at the plateau (Table 3) was determined from the

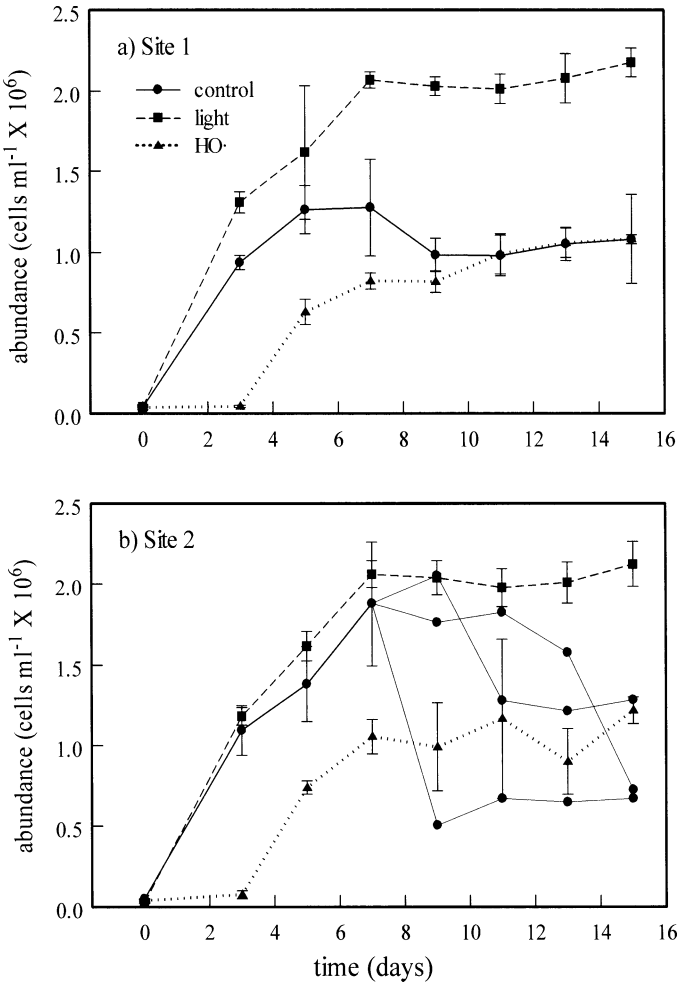

Fig. 1. Abundance of bacterial cells over time in dilution cultures prepared from Parker River water either kept in the dark (controls) or preexposed to solar radiation or HO·. (a) Results from Site 1 are presented as averages of triplicate cultures ($\pm$ standard deviation). (b) Results from Site 2 are presented in the same way except for data points after the first 7 d of incubation, where, due to lysis of cells, the abundances are plotted separately for individual cultures.

average carbon per cell for that incubation and the cell abundance at day 7. The cell carbon was estimated from measured cell volumes (see Methods for details). While this approach is subject to some uncertainty, the average cell volumes we observed did not significantly differ for the different treatments or sampling locations (*F*-test, $p \leq 0.05$; data not shown). Therefore, relative comparisons of net cell carbon between sites and treatments should be valid. The overall average carbon content per cell was 106 $\pm$ 13 fg (1 σ).

In the light-treated incubations, the increased carbon utilization corresponded to an increase in net cell carbon relative to controls (Table 3). However, the relative increase of cell carbon was not as large as the relative increase in DOC utilization. In the HO·-treated incubations, carbon utilization increased substantially while cell abundance (and therefore net cell carbon) was approximately the same as in the un-

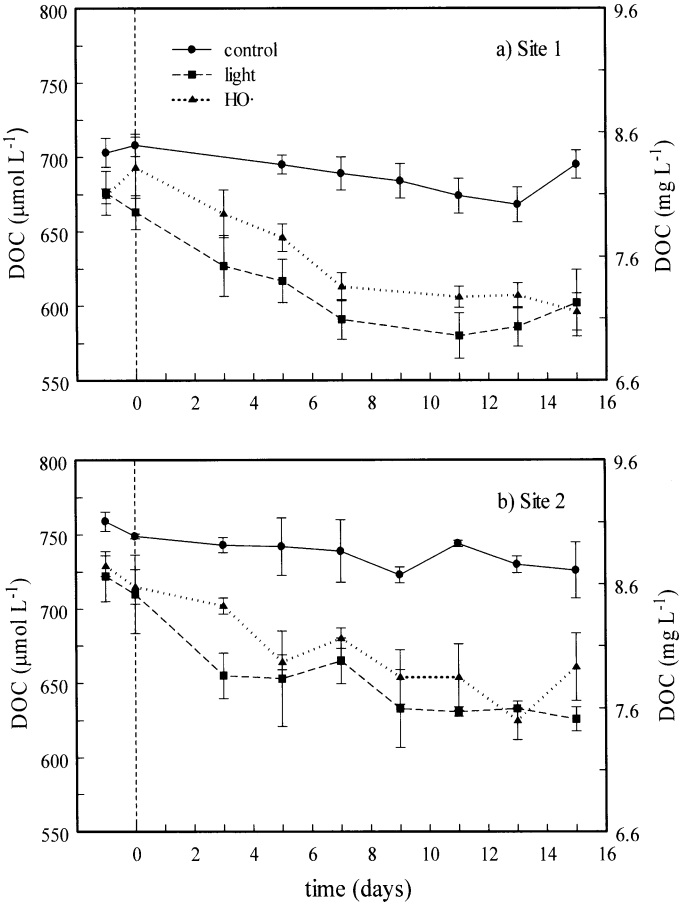

Fig. 2. The loss of dissolved organic carbon (<0.2 μm) over time in dilution cultures of Parker River water exposed to natural sunlight, hydroxyl radical, or no treatment (control) prior to inoculation. (a) Site 1 (b) Site 2. The data points are the average of three separate incubations ($\pm 1\ \sigma$). The values measured after treatment are given at $t = -1$, bacteria were added to start the incubation at $t = 0$ (vertical line).

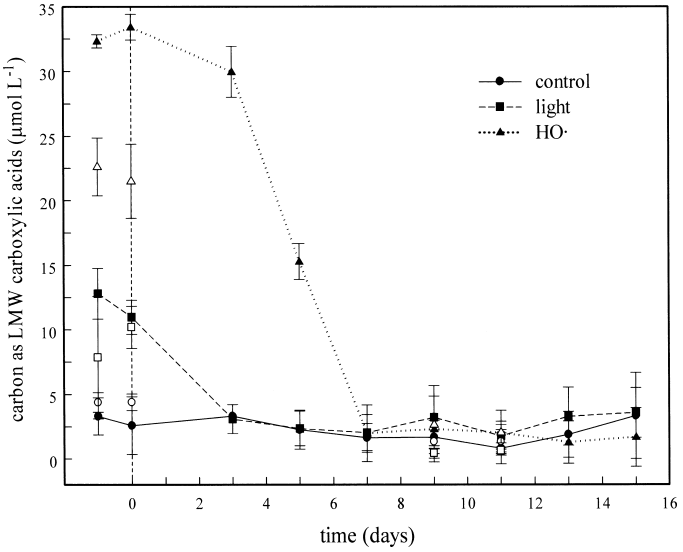

Fig. 3. The loss of low molecular weight carboxylic acids (sum of acetic, formic, malonic, and oxalic acid carbon) over time in dilution cultures of Parker River water exposed to natural sunlight, hydroxyl radical, or no treatment (control) prior to inoculation. The concentrations were measured at each sampling point for the Site 1 incubations (solid symbols) and at 0, 9, and 11 d for the Site 2 incubations (open symbols). The data points are the average of three separate incubations ($\pm 1\ \sigma$). The values measured after treatment are given at $t = -1$, bacteria were added to start the incubation at $t = 0$ (vertical line).

treated controls (Fig. 1). To further quantify this effect, we calculated the bacterial growth efficiency (BGE), defined as the relative ratio of net cell carbon to total carbon utilization (Heijnen and Roels 1981; Jahnke and Craven 1995). Both the light and HO· treatments decreased this value significantly (Table 3), indicating that much of the increased carbon utilization due to the treatments was metabolically converted to CO_2 rather than used to synthesize new cell carbon. There was no difference in the BGE between the incubations from the two field sites when the water samples had been exposed to the same pretreatment.

The bacteria utilized the LMW carboxylic acids produced by the light and HO· treatments during the incubations (Fig. 3; Table 4). As observed for the utilization of DOC, the time course of utilization of LMW acids roughly corresponded to the increases in bacterial abundances, including the time lag in the HO·-treated samples (Fig. 3). The utilization of the LMW acids (Table 4) was calculated as the difference be-

Table 3. Bacterial carbon utilization and growth in dilution cultures prepared from Parker River water. Filter-sterilized water from two sites was either kept dark (control) or exposed to natural sunlight or HO· prior to addition of bacteria and subsequent incubation. The reported values are averages of triplicate incubations ($\pm 1\sigma$). (n/a = not applicable.)

Sample/ treatment	Total carbon utilization (μmol L^{-1})	Increased carbon utilization due to treatment (μmol L^{-1})	Net cell carbon (μmol L^{-1})	Bacterial growth efficiency (BGE)
Site 1				
Control	40.5 ± 5.7	n/a	12.7 ± 0.2	31.7 ± 4.5%
Light	94.7 ± 2.9	54.2 ± 6.4	19.3 ± 2.2	20.4 ± 2.5%
HO·	91.8 ± 9.3	51.3 ± 10.9	10.6 ± 1.1	11.6 ± 2.0%
Site 2				
Control	46.5 ± 3.7	n/a	17.7 ± 5.3	38.2 ± 11.5%
Light	97.0 ± 10.1	50.4 ± 10.7	19.7 ± 1.8	20.4 ± 2.2%
HO·	99.8 ± 9.9	53.3 ± 10.6	13.4 ± 3.6	13.4 ± 3.0%

Table 4. The importance of four LMW carboxylic acids (sum of acetic, formic, malonic, and oxalic acid carbon) during bacterial utilization of dissolved organic carbon in dilution cultures prepared from Parker River water. Filter sterilized water from two sites was either kept dark (control) or exposed to natural sunlight or HO· prior to addition of bacteria and subsequent incubation. The reported values are averages of triplicate incubations ($\pm 1\sigma$). (n/a = not applicable.)

	Utilization of LMW acids		
Sample/ treatment	Total (μmol C L^{-1})	Relative to total carbon utilization	Relative to increased carbon utilization after treatment
Site 1			
Control	2.4 ± 1.4	5.9 ± 3.5%	n/a
Light	11.0 ± 1.2	11.7 ± 1.3%	20.4 ± 3.3%
HO·	30.1 ± 1.5	32.8 ± 3.7%	58.7 ± 12.8%
Site 2			
Control	3.5 ± 0.5	7.6 ± 1.3%	n/a
Light	9.8 ± 1.3	10.1 ± 1.7%	19.4 ± 4.9%
HO·	20.6 ± 1.7	20.7 ± 2.6%	38.7 ± 8.3%

tween the highest and lowest total acid concentration during the incubations.

The utilization of the LMW acids represented a significant fraction (6–30%) of the total carbon utilization during the incubations (Table 4). Additionally, the utilization of the elevated LMW acid concentrations present in the light- and HO·-treated samples represented an even larger fraction of the increased carbon utilization during the incubation of those samples (20–60%) relative to the controls (Table 4). In both water samples, the LMW acids were a quantitatively more important fraction of carbon utilization in the HO·-treated incubations than in the sunlight-treated incubations. However, it is important to note that the amounts of DOC utilized as LMW acids do not account for all of the increased utilization of carbon caused by either the light or the HO· treatments.

In addition to the incubations, we also conducted a separate control experiment to determine if relatively long-lived organic radicals produced during the HO· treatments could cause any fraction of the slow decrease in DOC we observed during the incubations (see Methods for details). While the HO· treatment did cause an immediate loss of DOC and production of LMW acids (as expected), the resulting concentrations of each were stable over the timescale of the incubations in the absence of any added bacteria (Fig. 4). The potential difficulty of keeping an analogous set of sunlight-treated samples sterile after exposure prevented us from conducting a similar control experiment for the light treatment. However, because we expect that much higher concentrations of radical species were produced during the γ-radiolysis procedure, we are confident that all of the DOC loss during both the light- and HO·-treated incubations was due to bacterial activity.

Discussion

Effects of sunlight on DOC and LMW acids—The observed loss of DOC upon exposure to sunlight (Table 1) is consistent with many previous studies, where photochemical conversion of DOM to inorganic carbon species (i.e., CO_2 and CO) has been demonstrated (e.g., Miller and Zepp 1995; Miller and Moran 1997; Bertilsson and Tranvik 2000). The observation of the production of acetic, formic, malonic, and oxalic acids by light exposure is also consistent with previous reports (Bertilsson and Tranvik 1998; Goldstone et al. 2002; Brinkmann et al. 2003). LMW acid production was found to be 34% and 47% of DOC loss for the light-exposed Site 1 and Site 2 samples, respectively. These ratios of LMW acid production to DOC mineralization are in agreement with previous studies of LMW acids (Bertilsson and Tranvik 2000). In contrast, the sunlight production rates of LMW aldehydes and ketones are estimated to be comparable with those of CO, or 10–20 times slower than dissolved inorganic carbon (DIC) (Miller and Moran 1997; Moran and Zepp 1997, and references therein). It should be noted that oxalic acid can undergo subsequent photoreactions that convert it to CO_2 (Bertilsson and Tranvik 1998), possibly leading to an underestimation of its photoformation rate.

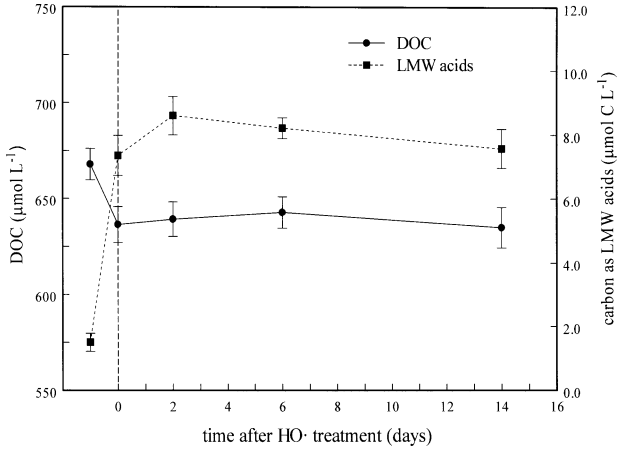

Fig. 4. The loss of DOC and the formation of LMW acids after the exposure of Parker River water (Site 1) to 30 μmol L^{-1} HO·. The *x*-axis is the time after the initial HO· exposure. Bacteria were not added to this control experiment. LMW acids are reported as the sum of acetic, formic, malonic, and oxalic acids, expressed as the concentration of carbon. The values are reported as the average of three replicate experiments ($\pm 1\ \sigma$). The values measured before the HO· treatment are given at $t = -1$ and the values at $t = 0$ (vertical line) were measured immediately after exposure.

Effects of sunlight and HO· on DOM bioavailability and bacterial growth efficiency—The enhanced accumulation of bacterial cells and the higher DOC utilization in the sunlight-treated incubations relative to controls indicate a net increase in the fraction of DOM that is susceptible to bacterial utilization. Only a small fraction of this increase (20%) can be explained by the photoproduction and subsequent utilization of LMW carboxylic acids (Table 4). Thus, our results demonstrate that either additional unidentified LMW substrates are formed or sunlight reactions modify the higher molecular weight DOM in a way that renders it more bioavailable to bacteria on the timescale of these incubations (7–10 d).

The 30 μmol L^{-1} HO· treatment also increased DOC loss

during the incubations relative to controls, indicating a net increase in DOM carbon utilization by the bacteria. Formation and utilization of the LMW acids accounted for a larger fraction of DOM utilization in the HO· treatment (Site 1, 59%; Site 2, 39%) than the sunlight treatment (20%). However, as in the light treatment, there was still significantly more DOC utilized by the bacteria than is accounted for by the utilization of the LMW acids. In the case of HO· reaction with DOM, the rates of formation of aldehydes and ketones are unknown and could possibly account for all of the remaining HO·-induced utilization. As with sunlight-mediated changes in DOM bioavailability, it is also possible that HO· produced other, as yet unidentified, LMW substrates or modified DOM to make it more easily utilized without generating identifiable LMW substrates.

The initial lag in the growth of the HO·-treated cultures (Fig. 1) was probably due to the formation of hydrogen peroxide or other short-lived toxic compounds during the γ-radiolysis process. Because such species could also be produced in sunlight (although this was not observed in the present study), our results suggest that caution may be warranted when using short-term bacterial growth experiments to examine light effects on DOM bioavailability.

Both sunlight and HO· treatments significantly decreased the overall bacterial growth efficiency during the bioassays (Table 3). Thus, while photochemical processes may increase the fraction of DOM that is susceptible to bacterial utilization, this increase in bioavailability may not correspond to increased growth of new bacterial biomass. For example, bacterial biomass in the HO·-treated incubations was similar to controls, despite much higher levels of DOC utilization (Table 3).

Decreasing BGE upon exposure to light or HO· can be partly explained by the highly oxidized nature of several of the quantitatively important LMW photoproducts identified here and elsewhere (Bertilsson and Tranvik 1998). In general, these oxidized molecules are expected to be utilized at a lower BGE compared with less oxygenated, more reduced substrates (Heijnen and Roels 1981; Vallino et al. 1996). For example, several of the major LMW organic photoproducts observed in the present study have previously been shown to be utilized at bacterial growth efficiencies below 20% (e.g., oxalic and formic acid; Heijnen and Roels 1981; Vallino et al. 1996). Additionally, a recent study of bacterial utilization of photochemically produced formic acid suggests that the bacterial growth efficiency on this compound may be very low in natural surface waters ($<$2%; Bertilsson and Tranvik 1998)

However, even the assumption that the BGE of all the LMW acids measured in these experiments is zero cannot fully explain the observed overall decrease in BGE. Hence, changes in the substrate quality of bioavailable DOM other than the LMW acids must also take place because of the two treatments. We cannot exclude the possibility that the bacterial populations that establish themselves in the cultures differed between treatments. If such differences in community composition also changed the functional features of the community (e.g., BGE), this could have influenced our results. This relationship between function and composition of bacterial communities is poorly studied so far, but methodological progress could make this a tractable problem in the near future (Gray and Head, 2001).

BGE values estimated from the O_2 consumption data of Moran et al. (2000) support our observations. Their overall BGE decreased with increasing sun exposure of DOC from the Satilla River Estuary. In contrast, another study of photochemical transformations of DOM observed that the direct solar irradiation of humic lakewater containing indigenous bacteria resulted in higher bacterial growth efficiencies compared with dark controls (Reche et al. 1998). These contrasting results may be partly explained by differences in the substrate quality of the original DOM used in the various studies. The observed growth efficiency on the original material used in the study by Reche et al. was very low (0.1–4%) compared both with DOM from the Parker River (27–32%; Table 3) and the typical range of values reported in the literature (20–70%) (Jahnke and Craven 1995). Hence, even if highly oxidized LMW organic substrates that are utilized at efficiencies below 10% were being produced in their experiments, they may still have enhanced the overall bacterial growth efficiency.

Interestingly, Reche et al. (1998) also found that the addition of N and P (together) to water from a humic lake increased bacterial growth efficiency on the bioavailable portion of this material. This implies that low concentrations of N and P relative to organic carbon substrates may decrease bacterial growth efficiency. It is unlikely that the shift in BGE in our experiments is related to a lack of inorganic nutrients because the ammonia and phosphorus concentrations were manipulated to be relatively high (100 μmol L^{-1} each). However, further research regarding the interactive effects of sunlight, DOM quality, and nutrients on growth efficiencies is needed.

Our observed decrease in the BGE on DOM upon light exposure has important implications for understanding the fate of dissolved organic matter in surface waters. It is well accepted that exposure of DOM to solar radiation can mineralize DOM and that this abiotic process represents an important sink for this material. Bacterial utilization in metabolic processes is another important sink for DOM, and earlier studies suggest that solar-driven DOM transformations could increase the magnitude of utilization, at least in waters dominated by terrestrial inputs. The present study indicates that sunlight-mediated increases in bacterial DOM utilization may not completely translate to increased bacterial biomass; some (or possibly all) of that increased uptake may be quickly respired. This would effectively increase the amount of DOM returned to CO_2 through solar-driven processes at the expense of bacterial biomass production. Furthermore, this effect on biomass production would not be limited to aquatic bacteria because they are, in turn, important sources of nutrients and energy for organisms at higher trophic levels in the food web.

Comparing the effects of HO· and light on DOM mineralization and bioavailability—We can use our results to estimate the HO· production rates required for HO· to be an important part of the sunlight-induced effects on DOM mineralization and bioavailability. In our samples, sunlight irradiation mineralized $\sim$14 μmol L^{-1} of C per day, and pro-

duced ~6 μmol L^{-1} per day of LMW acid carbon and ~21 μmol L^{-1} C per day of utilizable products other than LMW acids (average of the two water samples, Tables 1–4). HO· experiments mineralized ~1.1 mol C per mol HO·, and produced ~0.9 mol LMW acid carbon and ~0.8 mol non-LMW acid utilizable C per mol HO·. Assuming HO· production in the sunlight treatments was insignificant (see below), these ratios imply that, in waters with similar DOM concentration and quality as in our samples, an HO· production rate of ~7 μmol L^{-1} d^{-1} would be needed for HO· reactions to be a dominant source of LMW acids compared with other reactions occurring in sunlight. At this production rate, HO· would also be a major cause of mineralization and a minor source of utilizable products other than LMW acids.

HO· production rates of ~2–20 × 10^{-10} mol L^{-1} s^{-1} (4–40 μmol L^{-1} d^{-1}, approximating a summer day as 6 h of noontime sunlight) have been observed upon irradiation with sunlight or simulated sunlight of waters with sufficiently high Fe and low pH to make HO· production by Fenton's reaction likely (see Table 2 in White at al. 2003). Southworth and Voelker (2003, equation 6) estimated an upper limit on the noontime near-surface production rate of HO· from the photo-Fenton reaction of 0.8–4 × 10^{-10} [DOC] mol L^{-1} s^{-1}, corresponding to 2–9 μmol L^{-1} d^{-1} HO· for a DOC of 10 mg L^{-1} (830 μmol L^{-1}). This upper limit will be approached if enough iron(II) is present to react with all of the photoproduced H_2O_2 and if H_2O_2 photoformation rates correspond to the quantum yields discussed by Southworth and Voelker (2003). It is clear that HO· production from Fenton's reaction has the potential to cause significant effects on DOM transformations.

However, Fenton's reaction was probably not a significant source of HO· in our sunlight experiments. Although total iron concentrations were high (~5–9 μmol L^{-1}), iron photoreduction is expected to be slow at pH 7.6 (Southworth and Voelker 2003; Scully et al. 2003*b*), where our experiments took place. In comparison with light exposure, the HO· treatment produced proportionately more CO_2, more LMW acids, more formic acid compared with other LMW acids, and fewer unidentified substrates. This suggests that other reactions besides those occurring in the HO·-exposed samples played a major role during sunlight exposure.

Nevertheless, we cannot rule out that a portion of the DOM transformations we observed in sunlight is attributable to HO·. Little is known about the direct (non-Fenton) production of HO· from DOM, but it could be important. Apparent quantum yield values from one study (Vaughan and Blough 1998) suggest that the rate of HO· formation could be as high as 20% of the rate of hydrogen peroxide formation. White et al. report that up to 30% of the HO· formation rate they observed in high DOM river waters is not attributable to Fenton's reaction. HO· formation rates from nitrate are much lower (2 × 10^{-7} [NO_3^-] mol L^{-1} s^{-1}) and were not important in our experiments (Southworth and Voelker 2003).

The possible importance of HO· suggested by the present study (at least where its natural formation rate is relatively high) is surprising given the lack of significant effects observed in previous studies. Goldstone et al. (2002) observed much lower yields of DIC and LMW acid C (~0.3 mol and ~0.2 mol, respectively, per mol HO·) in similar γ-radiolysis experiments with DOM isolated from the Suwannee River. Because the average oxidation state of carbon in DOM is approximately 0 and that of CO_2 is 4, we would expect that, if HO· were the only oxidant participating in the mineralization process, the yield of DIC per mol HO· would be close to 0.25 (i.e., 4 HO· needed to oxidize DOM to CO_2) (Goldstone et al. 2002). Our much higher values (~1.1) suggest that the reaction of Parker River DOM with HO· involves additional oxidants that can further oxidize DOM to CO_2 or that HO· reactions with DOM are selective for more oxidized moieties (initial oxidation state >0). Additionally, Brinkmann et al. (2003) did not see a measurable effect of adding 1 mmol L^{-1} NO_3^- on photoproduction of DIC and LMW acids by simulated sunlight in a bog-water sample. The calculation of Brinkmann et al. of the HO· formation rate in those experiments implies yields of DIC and LMW acid C per HO· as low as, or lower than, those observed by Goldstone et al. (2002). However, because their calculation neglects the absorption of light by DOM in their samples as well as the wavelength dependence of the nitrate extinction coefficients and photon flux density, they may have significantly overestimated HO· formation rates. It is also possible that the differences between the present study and these others arise from differences in DOM origin and that isolated humic and fulvic acids have different properties than whole-water DOM.

Our results conflict with the recent results of Scully et al. (2003*a*), who attempted to study the effect of photoproduced reactive oxygen species on DOM bioavailability. They found that the use of a ROS scavenger (furfuryl alcohol) during sunlight irradiation increased subsequent bacterial growth (measured as cell abundance only) in incubations, relative to samples irradiated without the scavenger. This result was interpreted to mean that, in the absence of the scavenger, photoproduced ROS species reacted with bioavailable substrates produced by photochemical reactions, preventing their utilization by the bacteria. However, an alternative explanation is the production of bioavailable substrates from the reaction of the photoproduced ROS with the scavenger itself, which would have the same effect (and would not have been observed in their dark controls). In any case, the results presented here and in Goldstone et al. (2002) clearly show that reaction of the hydroxyl radical with DOM can cause the net production of bioavailable substrates such as LMW carboxylic acids. Additionally, at the concentration added (200 μmol L^{-1}), furfuryl alcohol could have scavenged the majority of the photoproduced hydroxyl radical in the experiments of Scully et al. but was not a major sink of singlet oxygen or other ROS.

Effects of autochthonous inputs on DOM photoreactivity—The present study was partly designed to examine the effect of DOM generated by an algal bloom on the photoreactivity and bioavailability of the total DOM pool. However, only minor differences in the results from Site 1 and Site 2 were observed (Tables 1 and 3). The most likely reason for this is that the amount of algal-derived carbon in the Site 2 sample was relatively small compared with the terrestrially derived bulk DOM already present at both sites.

Despite the elevated algal biomass below the dam (more than eightfold higher chlorophyll *a*), only 60 μmol L^{-1} (7.4%) more DOC was observed at Site 2 (Table 1) than at Site 1. While this increase is comparable with the total amount of DOM lost to photomineralization (3–5%) and bacterial uptake (6–14%), the DOM added at Site 2 would have to be much more bioavailable or would have to be changed dramatically by the treatments to have a significant effect on our bulk measurements (Table 3). The former scenario was probably not observed because heterotrophic bacterial growth is often tightly coupled to algal biomass (Chrost et al. 1989; Norrman et al. 1995). The most labile DOM fractions would have been rapidly utilized by indigenous bacteria, leaving behind more recalcitrant DOM (Bertilsson and Jones 2003). Previous researchers have found that irradiation of algal-derived DOM had a negative effect on its bacterial utilization, although only in waters where the magnitude of the autochthonous DOM dominated the total DOM pool (Tranvik and Bertilsson 2001).

Effect of sunlight on DOM in the Parker River estuary—To estimate the effect of sunlight on DOM while it travels through the Parker River estuary, we have to compare the dose of sunlight in our bottle experiments to the dose the DOM would see in the estuary. Apparent quantum-yield spectra for photoproduction of biologically labile photoproducts show that light with wavelengths shorter than 380 nm is responsible for the majority of the production of biologically labile DOM photoproducts in estuarine waters (Miller et al. 2002). To make a simple estimate, we will assume that all solar radiation capable of making bioavailable photoproducts is absorbed by DOM in our reaction vessel (volume-average path length ~8 cm) and therefore in the much deeper waters of the Parker River Estuary. According to our light-absorbance measurements (data not shown), the percentage of the incident light absorbed in 8 cm of water sample is 94, 82, and 65% at wavelengths of 320, 350, and 380 nm, respectively. Hence, our assumption that all of the relevant light is absorbed will underestimate photoproduct production in situ, although probably not more than twofold. Hullar (2000) measured UV light attenuation as a function of depth throughout the estuary and calculated a euphotic zone depth for UV radiation that generally did not exceed 33 cm. Thus, most of the incident UV will be absorbed by DOM in the estuary, as the depth of the water column normally exceeds several meters. Hullar also demonstrated that photochemical oxygen consumption rates normalized to absorbance at 350 nm were similar throughout the estuary, suggesting that photochemical reactivity of chromophoric DOM from various freshwater inputs is similar (another underlying assumption in our simple estimation).

The above assumptions imply that the surface-area normalized rates of the formation of DIC, LMW carboxylic acids, and other bioavailable carbon photoproducts, calculated to be 1.1×10^{-3}, 4.7×10^{-4}, and 1.6×10^{-3} mol C m^{-2} d^{-1} respectively, are the same in our reaction vessel and in the estuary. We obtained these rates from the average of our two water samples (Tables 1–4) using a sample volume of 0.5 liter and an estimated surface area for illumination of 64 cm^2. Because the total estuarine surface area amounts to 1.2×10^7 m^2 (J. Vallino pers. comm.), we estimate that on the order of 1.3×10^4, 5.6×10^3, and 1.9×10^4 mol d^{-1} of DOM carbon could be converted to DIC, LMW organic acids, and bioavailable C, respectively, in full summer sunshine.

This estimate can be compared with riverine inputs of organic carbon into the estuary. Total freshwater discharge into the estuary is expected to be 11 times the discharge measured by the United States Geological Survey gauging station at the Parker River dam (located between Site 1 and Site 2), based on watershed area ratios (Vallino and Hopkinson 1998). For June, July, and August, then, average monthly discharges are 7.6×10^5, 2.4×10^5, and 1.4×10^5 m^3 d^{-1}, respectively. DOC values in the various freshwater inputs (e.g., Ipswich River, Mill River) are similar to those measured in the Parker River, 400–1,300 μM. (DOC and discharge data obtained from: http://ecosystems.mbl.edu/pie/data.htm). Assuming an average DOC of 700 μmol L^{-1} leads to an input of riverine DOC of $\sim 5 \times 10^5$, 1.7×10^5, and 1×10^5 mol d^{-1} of carbon for June, July, and August, respectively. Therefore, during summer, we estimate that a significant fraction of the riverine DOC entering the estuary would be either mineralized to CO_2 or made bioavailable by sunlight (~8%, ~22%, and ~38% for June, July, and August, respectively, assuming full sunlight). However, on an annual basis, photochemical processing within the estuary will only affect a small fraction of the total input of carbon from the Parker River (~1.5%, assuming annual average sunlight is ~25% of full summer sunlight, and annual average freshwater discharge is 9.9×10^5 m^3 d^{-1}), and the majority of the photochemical processing will occur on the continental shelf. While these calculations are obviously approximate, they demonstrate the potential importance of these processes for the Parker River during summer, when irradiance is high and discharge is low.

References

ALBERT, D. B., AND C. S. MARTENS. 1997. Determination of low-molecular-weight organic acid concentrations in seawater and pore-samples via HPLC. Mar. Chem. **56:** 27–37.

BENNER, R., AND B. BIDDANDA. 1998. Photochemical transformations of surface and deep marine dissolved organic matter: Effects on bacterial growth. Limnol. Oceanogr. **43:** 1373–1378.

BERTILSSON, S., AND J.B. JONES. 2003. Supply of dissolved organic matter to aquatic ecosystems: Autochthonous sources, p. 3–25. *In* S. E. G. Findlay and R. L. Sinsabaugh [eds.], Aquatic ecosystems: Interactivity of dissolved organic matter. Academic Press.

———, AND L. J. TRANVIK. 1998. Photochemically produced carboxylic acids as substrates for freshwater bacterioplankton. Limnol. Oceanogr. **43:** 885–895.

———, AND ———. 2000. Photochemical transformation of dissolved organic matter in lakes. Limnol. Oceanogr. **45:** 753–762.

BREZONIK, P. L., AND J. FULKERSON-BREKKEN. 1998. Nitrate-induced photolysis in natural waters: Controls on concentrations of hydroxyl radical photo-intermediates by natural scavenging agents. Environ. Sci. Technol. **32:** 3004–3010.

BRINKMANN, T., P. HÖRSCH, D. SARTORIUS, AND F. H. FRIMMEL. 2003. Photoformation of low-molecular-weight organic acids

from brown water dissolved organic matter. Environ. Sci. Technol. **37:** 4190–4198.

CARLSSON, P., E. GRANELI, P. TESTER, AND L. BONI. 1995. Influence of riverine humic substances on bacteria, protozoa, phytoplankton and copepods in a coastal plankton community. Mar. Ecol. Prog. Ser. **127:** 213–221.

CHROST, R. J., U. MÜNSTER, H. RAI, D. ALBRECHT, P. K. WITZEL, AND J. OVERBECK. 1989. Photosynthetic production and exoenzyme degradation of organic matter in the euphotic zone of a eutrophic lake. J. Plank. Res. **11:** 223–242.

COLE, J. J., G. E. LIKENS, AND D. L. STRAYER. 1982. Photosynthetically produced dissolved organic carbon: An important carbon source for planktonic bacteria. Limnol. Oceanogr. **27:** 1080–1090.

COOPER, W. J., J. K. MOEGLING, R. J. KIEBER, AND J. J. KIDDLE. 2000. A chemiluminescence method for the analysis of H_2O_2 in natural waters. Mar. Chem. **70:** 191–200.

DEL GIORGIO, P., D. F. BIRD, Y. T. PRAIRIE, AND D. PLANAS. 1996. Flow cytometric determination of bacterial abundance in lake plankton with the green nucleic acid stain SYTO 13. Limnol. Oceanogr. **41:** 783–789.

———, AND J. J. COLE. 2000. Bacterial energetics and growth efficiency, p. 289–325. *In* D. L. Kirchman [ed.], Microbial ecology of the oceans. Wiley-Liss.

FARJALLA, V. F., A. M. ANESIO, S. BERTILSSON, AND W. GRANÉLI. 2001. Photochemical reactivity of aquatic macrophyte leachates: Abiotic transformations and bacterial response. Aquat. Microb. Ecol. **24:** 187–195.

GOLDSTONE, J. V., M. J. PULLIN, S. BERTILSSON, AND B. M. VOELKER. 2002. Reactions of hydroxyl radical with humic substances: Bleaching, mineralization, and production of bioavailable carbon substrates. Environ. Sci. Technol. **36:** 364–372.

GRAY, N. D., AND I. M. HEAD. 2001. Linking genetic identity and function in communities of uncultured bacteria. Environ. Microbiol. **3:** 481–492.

HEIJNEN, J. J., AND J. A. ROELS. 1981. A macroscopic model describing yield and maintenance relationships in aerobic fermentation processes. Biotechnol. Bioeng. **23:** 739–761.

HOLMES, R. M., B. J. PETERSON, L. A. DEEGAN, J. E. HUGHES, AND B. FRY. 2000. Nitrogen biogeochemistry in the oligohaline zone of a New England estuary. Ecology **81:** 416–432.

HULLAR, M. 2000. Regulation of dissolved organic carbon biogeochemistry by light: The impacts on bacterial productivity and community structure in aquatic systems. Ph.D. thesis, Harvard Univ.

JAHNKE, R. A., AND D. B. CRAVEN. 1995. Quantifying the role of heterotrophic bacteria in the carbon cycle: A need for respiration measurements. Limnol. Oceanogr. **40:** 436–441.

KIEBER, D. J., J. MCDANIEL, AND K. MOPPER. 1989. Photochemical source of biological substrates in seawater: Implications of carbon cycling. Nature **341:** 637–639.

LOFERER-KRÖßBACHER, M., J. KLIMA, AND R. PSENNER. 1998. Determination of bacterial cell dry mass by transmission electron microscopy and densitometric image analysis. Appl. Environ. Microbiol. **64:** 688–694.

MILLER, W. L., AND M. A. MORAN. 1997. Interaction of photochemical and microbial processes in the degradation of refractory dissolved organic matter from a coastal marine environment. Limnol. Oceanogr. **42:** 1317–1324.

———, ———, SHELDON, W. M., ZEPP, R. G., AND S. OPSAHL. 2002. Determination of apparent quantum yield spectra for the formation of biologically labile photoproducts. Limnol. Oceanogr. **47:** 343–352.

———, AND R. G. ZEPP. 1995. Photochemical production of dissolved inorganic carbon from terrestrial organic matter: Significance to the oceanic organic carbon cycle. Geophys. Res. Lett. **98:** 2289–2298.

MOPPER, K., AND X. ZHOU. 1990. Hydroxyl radical photoproduction in the sea and its potential impact on marine processes. Science **250:** 661–664.

MORAN, M. A., AND J. S. COVERT. 2003. Photochemically mediated linkages between dissolved organic matter and bacterioplankton, p. 243–262. *In* S. E. G. Findlay and R. L. Sinsabaugh [eds.], *Aquatic ecosystems: Interactivity of dissolved organic matter*. Academic Press.

———, AND R. E. HODSON. 1990. Bacterial production on humic and nonhumic components of dissolved organic carbon. Limnol. Oceanogr. **35:** 1744–1756.

———, W. M. SHELDON, AND R. G. ZEPP. 2000. Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. Limnol. Oceanogr. **45:** 1254–1264.

———, AND R. G. ZEPP. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnol. Oceanogr. **42:** 1307–1316.

NORRMAN, B., U. L. ZWEIFEL, C. S. HOPKINSON, AND B. FRY. 1995. Production and utilization of dissolved organic carbon during an experimental diatom bloom. Limnol. Oceanogr. **40:** 898–907.

OBERNOSTERER, I., AND R. BENNER. 2004. Competition between biological and photochemical processes in the mineralization of dissolved organic carbon. Limnol. Oceanogr. **49:** 117–124.

PORTER, K. G., AND Y. S. FEIG. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25:** 943–948.

———, B. REITNER, AND G. J. HERNDL. 1999. Contrasting effects of solar radiation on dissolved organic matter and its bioavailability to bacterioplankton. Limnol. Oceanogr. **44:** 1645–1654.

RECHE, I., M. L. PACE, AND J. J. COLE. 1998. Interactions of photobleaching and inorganic nutrients in determining bacterial growth on colored dissolved organic carbon. Microb. Ecol. **36:** 270–280.

SCULLY, N. M, W. J. COOPER, AND L. J. TRANVIK. 2003a. Photochemical effects on microbial activity in natural waters: The interaction of reactive oxygen species and dissolved organic matter. FEMS Microbiol. Ecol. **46:** 353–357.

SCULLY, N. M., L. J. TRANVIK, AND W. J. COOPER. 2003b. Photochemical effects on the interaction of enzymes and dissolved organic matter in natural waters. Limnol. Oceanogr. **48:** 1818–1824.

SONG, R. G., P. WESTERHOFF, R. A. MINEAR, AND G. L. AMY. 1996. Interaction between bromine and natural organic matter, p. 298–321. *In* R. A. Minear and G. L. Amy [eds.], Water disinfection and natural organic matter. American Chemical Society.

SOUTHWORTH, B. A., AND B. M. VOELKER. 2003. Hydroxyl radical production via the photo-Fenton reaction in the presence of fulvic acid. Environ. Sci. Technol. **37:** 1130–1136.

TRANVIK, L. J. 1988. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microb. Ecol. **16:** 311–322.

———, AND S. BERTILSSON. 2001. Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. Ecol. Lett. **4:** 458–463.

———, AND S. KOKALJ. 1998. Decreased biodegradability of algal DOC due to interactive effects of UV radiation and humic matter. Aquat. Microb. Ecol. **14:** 301–307.

VALLINO, J. J., AND C. S. HOPKINSON. 1998. Estimation of dispersion and characteristic mixing times in Plum Island Sound estuary. Estuarine Coastal and Shelf Science **46:** 333–350.

———, HOPKINSON, C. S., AND J. E. HOBBIE. 1996. Modeling bacterial utilization of dissolved organic matter: Optimization replaces Monod growth kinetics. Limnol. Oceanogr. **41:** 1591–1609.

VAUGHAN, P. P., AND N. V. BLOUGH. 1998. Photochemical formation of hydroxyl radical by constituents of natural waters. Environ. Sci. Technol. **32:** 2947–2953.

VOELKER, B. M., F. M. M. MOREL, AND B. SULZBERGER. 1997. Iron redox cycling in surface waters: Effects of humic substances and light. Environ. Sci. Technol. **31:** 1004–1011.

WHITE, E. M., P. P.VAUGHAN, AND R. G. ZEPP. 2003. Role of the photo-Fenton reaction in the production of hydroxyl radicals and photobleaching of colored dissolved organic matter in a coastal river of the southeastern United States. Aquat. Sci. **65:** 402–414.

ZAFIRIOU, O. C., M. B. TRUE, AND E. HAYON. 1987. Consequences of OH radical reaction in seawater: Formation and decay of Br^{2-} ion radical. *In* R. G. Zika and W. J. Cooper [eds.], Photochemistry of environmental aquatic systems. American Chemical Society.

ZEPP, R. G., B. C. FAUST, AND J. HOIGNE. 1992. Hydroxyl radical formation in aqueous reactions (pH 3–8) of iron(II) with hydrogen-peroxide—the photo-Fenton reaction. Environ. Sci. Technol. **26:** 313–319.

———, J. HOIGNE, AND H. BADER. 1987. Nitrate-induced photooxidation of trace organic-chemicals in water. Environ. Sci. Technol. **21:** 443–450.

Received: 17 October 2003
Accepted: 19 May 2004
Amended: 17 June 2004