

Effects of sunlight on decomposition of estuarine dissolved organic C, N and P and bacterial metabolism

S. Leigh McCallister^{1,2,*}, James E. Bauer¹, Joann Kelly¹, Hugh W. Ducklow¹

¹School of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062, USA

²Present address: Département des sciences biologiques, Université du Québec à Montréal, CP 8888, Succ. Centre Ville, Montréal, Québec H3C 3P8, Canada

ABSTRACT: The effects of natural sunlight and microbial decomposition on DOC, DON, and DOP were investigated along the salinity gradient of a temperate coastal plain estuary. The impact of sunlight-irradiated DOM on bacterial properties (bacterial abundance, production, bacterial growth efficiency [BGE]) was also followed. Surface-water light levels resulted in no detectable abiotic production of NH_4^+ or PO_4^{3-} or loss of DOC. Bacterial decomposition of DOC was enhanced by 27 to 200% in irradiated relative to dark treatments. There was, however, no corresponding enhancement in DON and DOP remineralization. Significant differences in bacterial decomposition of light-exposed DOC were frequently observed following prolonged incubation (>7 d), suggesting that enhanced reactivity may result from photochemical modification of higher molecular weight organic matter. BGE in light relative to dark treatments was positively correlated ($r^2 = 0.38$, $p < 0.01$) with *in situ* NH_4^+ concentrations. In light treatments, significantly lower N and P remineralization in August 1999 corresponded with low *in situ* inorganic nutrient concentrations and bacterial growth efficiency (BGE) and with elevated bacterial DOC utilization. In contrast, enhanced DOC reactivity in April 2000 during nutrient-replete conditions corresponded with net immobilization of inorganic N and P by bacterial biomass production, but without a concomitant impact on BGE. These findings suggest that the combination of photochemical and microbial alteration of DOM may increase bacterial demand for inorganic nutrients, alter BGE, and influence the partitioning of C between bacterial biomass and respiration.

KEY WORDS: DOC · DON · DOP · BGE · Phototransformation · Bacterial bioassays

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INTRODUCTION

Dissolved organic matter (DOM) in estuaries is derived from diverse allochthonous and autochthonous sources, and its cycling is controlled by various biological, chemical and physical processes. Although bacteria have long been recognized as a primary control on DOM cycling (Pomeroy 1974, Azam et al. 1983), photochemical effects have been more recently found to both stimulate and inhibit DOM turnover (Benner & Biddanda 1998, Obernosterer et al. 2001, Tranvik & Bertilsson 2001). The majority of early photochemical-microbial studies were conducted in blackwater rivers,

streams, and lakes with light-limited primary production (De Haan 1993, Lindell et al. 1996, Bano et al. 1998), and stimulatory effects of light exposure were generally observed. More recent studies, however, have included systems other than high humic/high dissolved organic carbon (DOC) environments. For example, recent findings in subtropical lagoons and non-point sources to temperate rivers suggest that pre-exposure to light may have little impact on DOC bioavailability in certain natural waters (Ziegler & Benner 2000, Wiegner & Seitzinger 2001). Nonetheless, the restriction of most photochemical studies to high humic nearshore or inshore environments has resulted in '... little comparative

*Email: leigh@vims.edu

information available for estuarine DOM that is lower in vascular plant and soil influences and higher in contributions from younger (recently produced) or algal-derived organic matter' (Moran et al. 2000).

The effects of sunlight-exposure on DOM cycling in estuaries are difficult to quantify. Variations in initial bioreactivity, age, sources and structural character (e.g. aromaticity) of DOM may affect its biological and photochemical fate during seaward transit (Mopper & Kieber 2002, Moran & Covert 2003 and references therein). In general, the balance between photoinhibition and photostimulation of DOM cycling may hinge on the initial reactivity of various subcomponents of DOM (Moran & Covert 2003). Thus, prediction of sunlight effects on DOM bioavailability and fate on a system scale is limited by the ability to characterize and predict the bioreactivity of the majority of organic compounds comprising the bulk DOM pool (Hedges et al. 2000). This has sometimes been circumvented by the use of microbial activity (e.g. bacterial abundance, growth and production) as a proxy for integrating both positive and negative photochemical impacts on C flow (Lindell et al. 1995, Miller & Moran 1997, Benner & Biddanda 1998).

An important and potentially confounding factor in studies of DOM cycling is that measures of net bacterial production typically used in such studies account only for the portion of C assimilated into biomass and do not include changes in respiration (Jahnke & Craven 1995, del Giorgio & Cole 2000, Mopper & Kieber 2002). Bacterial growth efficiency (BGE), or the fraction of C incorporated into biomass relative to gross bacterial production (i.e. biomass production plus respiration), is generally less than 50% in most aquatic systems, and is subject to control by such factors as substrate quality, inorganic nutrient availability and cellular maintenance energy, all of which may vary independently as a result of photochemical impacts (del Giorgio & Cole 2000, Mopper & Kieber 2002). Thus, the factors controlling bacterial growth and production may not ultimately regulate total DOC utilization and its partitioning into cellular synthesis and maintenance pathways (Kirchman & Rich 1997). Nevertheless, to date, the effect of sunlight-exposed DOM on bacterial metabolism (e.g. respiration, BGE etc.) has been limited to relatively few studies (Reche et al. 1998, Mopper & Kieber 2002).

While the importance of photochemical processes on DOC cycling is well-documented, effects of sunlight on dissolved organic nitrogen (DON) and dissolved organic phosphorous (DOP) have not been examined to a comparable extent (e.g. Bertilsson et al. 1999, Tranvik et al. 2000, Wiegner & Seitzinger 2001). The present study was designed to explore the relationships between microbial and potential photochemical

decomposition of DOM, and the specific role of natural sunlight in DOC, DON and DOP bioavailability and bacterial metabolism in a temperate coastal plain estuary having significant allochthonous and autochthonous organic matter inputs. By further measuring BGE, alterations in total carbon utilization by bacteria were also evaluated (Jahnke & Craven 1995), thus allowing greater differentiation and quantification of the interactive effects of physico-chemical and biological processes on estuarine carbon flow.

MATERIALS AND METHODS

Experimental approach. Large-volume time series incubations were conducted at different times of the year using samples of varying salinity from the York River estuary, Virginia (Fig. 1A) that had either been

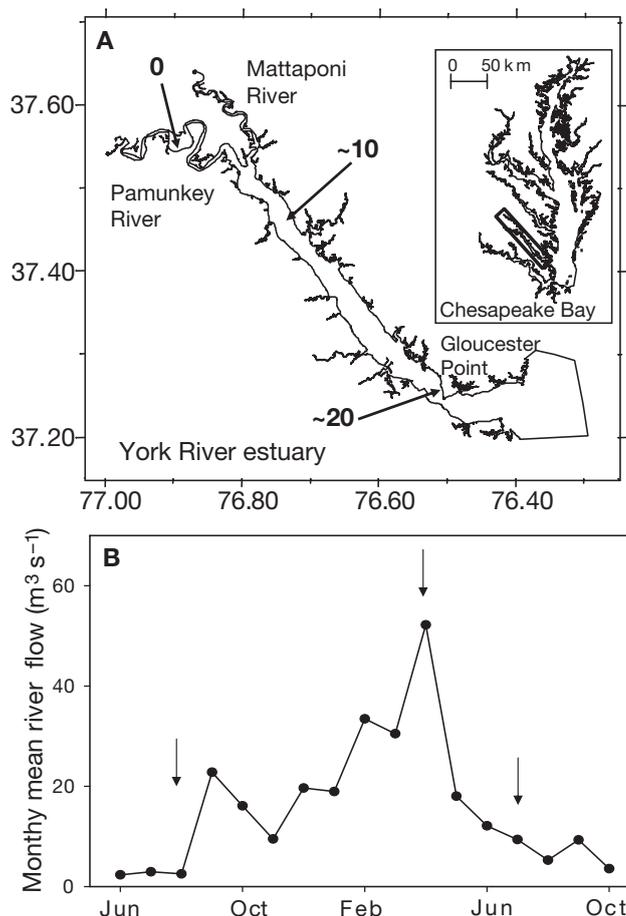


Fig. 1. (A) The York River estuary. Inset shows the York's location relative to the Chesapeake Bay proper. Sampling locations are designated by arrows and approximate salinities. (B) Monthly mean river flow statistics for the Pamunkey River near Hanover, Virginia, from June 1999 to October 2000. Arrows denote sampling periods in August 1999, April 2000 and July 2000

pre-exposed or not exposed to natural sunlight. Although sunlight exposure may have direct detrimental effects on bacterial growth and survival, this study was not designed to evaluate those potential impacts. Incubation times approximated water residence times in the estuary (~1 to 2 mo), and the bioavailability of DOC, DON, and DOP was monitored throughout the incubations.

Study site and sample collection. The York River estuary is a moderately stratified sub-estuary of the Chesapeake Bay and has distinct DOM sources and reactivity relative to other sub-estuaries in the Chesapeake system (Raymond & Bauer 2001, McCallister 2002). This is reflected in the changing stoichiometry of bulk DOM during estuarine transport and mixing, whereby freshwater DOM (C:N:P = 2150:92:1) is depleted in both N and P relative to the mouth of the York (C:N:P = 550:38:1; McCallister 2002).

Sampling periods bracketed extremes in monthly mean river flow and seasonal productivity (Fig. 1B). Surface water (~0.2 m depth) was collected in August 1999 (mean river flow $2.5 \text{ m}^3 \text{ s}^{-1}$), April 2000 ($52.2 \text{ m}^3 \text{ s}^{-1}$) and July 2000 ($9.4 \text{ m}^3 \text{ s}^{-1}$) from 3 sites: (1) the mouth of the estuary where it enters the Chesapeake Bay (salinity [S] ≈ 20); (2) the Pamunkey River (S ≈ 0); and (3) an intermediate site (S ≈ 10) which was near the estuarine chlorophyll maximum. The annual mean river flow for the Pamunkey River in 1999 ($13.9 \text{ m}^3 \text{ s}^{-1}$) represented the 10 yr minimum from 1990 to 2000 (available at: <http://waterdata.usgs.gov>).

Experimental set-up and treatments. Sample water for incubations was filtered sequentially through combusted (500°C , 4.5 h) GF/D ($2.7 \mu\text{m}$) and GF/F ($0.7 \mu\text{m}$)

filters and then through a $0.2 \mu\text{m}$ acid-soaked (10% HCl) Gelman capsule filter to remove POM and bacteria. Filtered water was stored at 4°C in the dark for no more than 2 d prior to the incubation. As a precautionary measure, samples were filtered an additional time through a $0.2 \mu\text{m}$ capsule just prior to the start of an experiment and examined to ensure that bacterial abundances were negligible.

Prior to sunlight pre-exposure, subsamples were collected for DOC, NH_4^+ and PO_4^{3-} . The starting bacteria-free filtrate was subsequently distributed between triplicate 1 l dark (foil-wrapped borosilicate bottles) and natural sunlight-exposed (quartz tubes) treatments. The quartz tubes received 9 h of sunlight exposure on a cloudless day, following which all controls and treatments were again sub-sampled for DOC, NH_4^+ and PO_4^{3-} to determine the abiotic effects of sunlight exposure. All sample and control tubes were maintained between 5 to 10°C throughout pre-exposure. Table 1 lists ultraviolet radiation measurements for the days of exposure.

DOM decomposition incubations (1 l) were initiated by addition of a natural bacterial ($0.7 \mu\text{m}$ filtrate) inoculum at a 100-fold dilution that was obtained from the same study sites. Incubations were maintained in the dark at room temperature (22°C) over both short (<7 d) and long (7 to 28 d) timescales, and subsamples were collected periodically to assess potential differences in the microbial response to sunlight-exposed DOM between these periods. All subsamples were frozen at -20°C until DOM and inorganic nutrient analyses.

Samples from the high-salinity site in August 1999 were compromised due to contamination. Bacterial

Table 1. Site and water column characteristics of York River estuary during sampling periods. C:N values were calculated from DOC/DON concentrations

Date	Streamflow ^a ($\text{m}^3 \text{ s}^{-1}$)	UV ^b (W m^{-2})	Salinity	Water temp. ($^\circ\text{C}$)	Chl <i>a</i> ($\mu\text{g l}^{-1}$)	NH_4^+ (μM)	PO_4^{3-} (μM)	DOC (μM)	DON (μM)	C:N
Aug 99	2.5	801	0	30	5.9	0.4	0.16	445	23.2	19.2
			10	29	26.9	0.9	0.97	410	22.4	18.3
Apr 00	52.2	872	0	13	4.5	2.7	0.34	438	20.6	21.3
			11	11	29.2	3.1	0.30	369	16.5	22.4
			22	11	7.0	0.6	0.08	250	15.2	16.5
Jul 00	9.4	910	0	28	5.7	0.5	0.29	452	24.6	18.4
			10	27	23.5	1.4	0.61	518	29.9	17.3
			20	27	17.9	4.2	0.19	239	18.7	12.7

^aData obtained from US Geological Survey (<http://waterdata.usgs.gov>). Pamunkey River freshwater flow reported for the York River estuary

^bUltraviolet (UV) irradiation data from Edgewater, Maryland, obtained from the Smithsonian Environmental Research Center in collaboration with the National Institute of Standards and Technology (<http://www.ebiks.com/solarnet/database.html>). Data are presented as the integrated daily average of incident solar UV-B and short-wavelength UV-A (290 to 324 nm)

production measurements from August 1999 were performed only at 36 h post-inoculation and are not presented as the time interval is inconsistent with subsequent samplings (36 h vs. 7 d).

Bacterial production and growth efficiency. Bacterial growth efficiency (BGE) is defined as the efficiency with which bacteria convert DOC into bacterial biomass (BB), expressed as $BGE = [\Delta BB / \Delta DOC] \times 100$, where ΔBB was estimated from time-dependent changes in bacterial abundance. Cell abundance was converted to BB using a cell-specific C content of 20 fg C cell⁻¹ (Lee & Fuhrman 1987). Changes in DOC concentration (ΔDOC) were measured directly (see next subsection).

Analytical methods. DOM and inorganic nutrients: Thawed subsamples were analyzed for DOC, total dissolved N and P (TDN and TDP, respectively), DIN ($NO_3^- + NO_2^- + NH_4^+$) and DIP (PO_4^{3-}). Phosphate was analyzed spectrophotometrically using a 5 cm path-length cell and the acidified molybdenum blue method (Koroleff 1983) having an analytical precision of $\pm 0.017 \mu M$ P. Inorganic nitrogen species were analyzed on a Technicon Autoanalyzer, and the analytical precision associated with these measurements was $0.1 \mu M$ for NO_3^- , NO_2^- and NH_4^+ . TDN and TDP (10 ml each) were analyzed by alkaline persulfate oxidation (Koroleff 1983), with an analytical precision of $\pm 1.2 \mu M$ and $0.2 \mu M$, respectively, and the organic fraction (DON and DOP) was determined by the difference between the total dissolved and inorganic nutrient fractions. DOC was determined by high temperature Pt-impregnated alumina combustion using a Shimadzu TOC-5000. Analytical precision was determined from multiple injections ($n = 3$ to 5) per sample and ranged from ± 3.1 to $6.8 \mu M$ for the range of concentrations found in the York.

Bacterial biomass and activity: Subsamples (10 ml) for bacterial abundance were preserved with $0.2 \mu m$ filtered 25% glutaraldehyde diluted to a final concentration of 2%. Bacterial abundance for August 1999 was determined by acridine orange epifluorescence microscopy (Hobbie et al. 1977) with a Zeiss Axiophot microscope. Duplicate slides were counted for each treatment at each sampling time, with a minimum of 350 cells counted per slide. Bacterial cell numbers for April and July 2000 samples were enumerated using a Becton-Dickinson FacsCalibur benchtop flow-cytometer and the nucleic acid stain Syto-13 following the methods of del Giorgio et al. (1996). Bacterial cells and microspheres were separated in a log-log cytogram of green fluorescence intensity and side scatter. Samples were run for 30 s or until a minimum of 20 000 events was counted. Bacterial cell numbers in the sample were calculated using microspheres as an internal standard. Previous work by del Giorgio et al.

(1996) has shown a strong agreement between cytometric and epifluorescence counts.

Bacterial productivity was measured by ³H-leucine (Kirchman et al. 1985) incorporation as modified by Smith & Azam (1992). A sample aliquot (1.7 ml) was added to a screw-top microcentrifuge tube, followed by addition of ³H-leucine at saturating concentrations (40 nM, Schultz et al. 2003). Procedural blanks consisted of simultaneous additions of sample water, ³H-leucine, and 100 μl 100% trichloroacetic acid (TCA). Triplicate live samples and a single blank were run for each assay. Tubes were incubated in the dark at *in situ* temperatures for approximately 1 h and then terminated by the addition of 100 μl of 100% TCA. Samples were radioassayed in a liquid scintillation counter (Wallac, Model 1409).

Data analysis. Data were imported into JMP 5.0.1 (SAS Institute) and an analysis of variance (2-way ANOVA) was used to examine statistical differences between sunlight-exposed and dark treatments and with salinity (with the exception of BGE, see 'Results', 4th subsection). Tukey's multiple comparison test was used to conduct pairwise comparisons between sites. Statistical differences in BGE were calculated only between sunlight-exposed and dark treatments (1-way ANOVA). Unless otherwise noted, all confidence intervals are expressed at the 95% ($p = 0.05$) level.

RESULTS

Water column characteristics

Water samples along the salinity continuum of the York River estuary were variable with respect to nutrient and DOM concentrations (Table 1) and sources. Chlorophyll *a* (chl *a*) was uniformly low at all sampling times at the freshwater site (~ 4 to $5 \mu g l^{-1}$), and maximal levels ($29 \mu g l^{-1}$) were observed at the mid-salinity site in April 2000, corresponding with peak annual streamwater discharge (Table 1). Low NH_4^+ and PO_4^{3-} (0.4 and $0.16 \mu M$, respectively) for the freshwater site coincided with minimum discharge in August 1999. Concentrations of DOC varied between ~ 240 and $520 \mu M$ and generally decreased seaward (Table 1). Elemental ratios of DOC:DON ranged from ~ 13 to 22 and were lowest at the mouth of the estuary in July 2000 (Table 1).

DOM remineralization

Dark and sunlight-exposed treatments from all York sites exhibited no significant post-exposure differences ($p > 0.05$) in DOC, NH_4^+ and PO_4^{3-} as a result of

sunlight-induced abiotic effects (Fig. 2A to C, respectively).

No differences within analytical error were detected in DON and DOP metabolism between light vs. dark treatments from all seasons and locations (e.g. Figs. 3B,C & 4B,C). Time course incubations of bacterial decomposition of DOM from the freshwater site in August 1999 (Fig. 3A) indicated that light and dark DOC losses were indistinguishable up to Day 7, then $\sim 8 \mu\text{M}$ additional DOC was consumed in the light

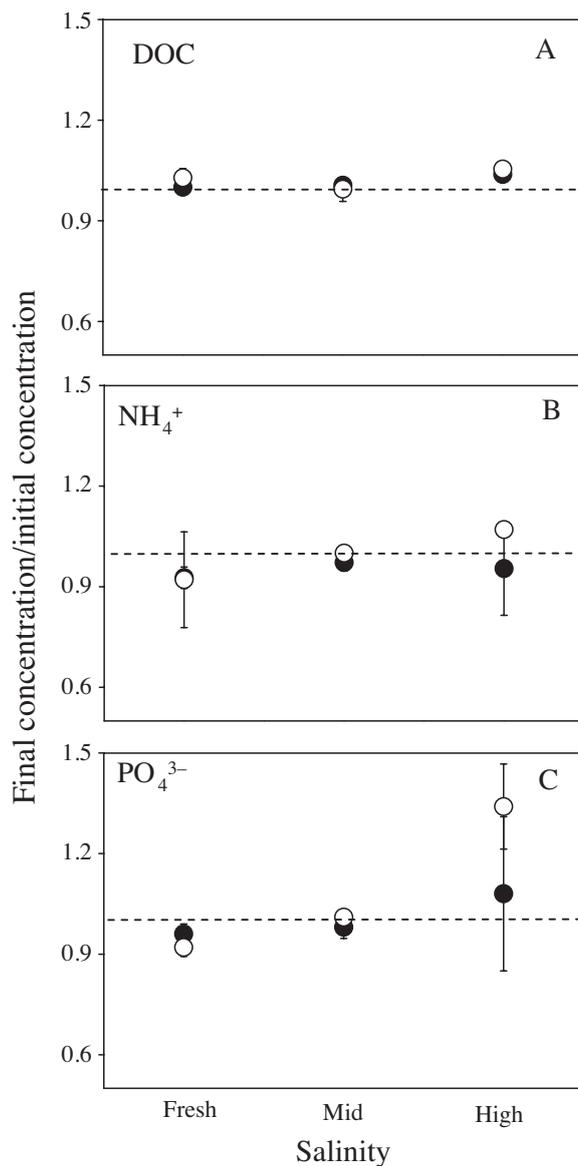


Fig. 2. Ratios of the concentrations of (A) DOC, (B) NH_4^+ , and (C) PO_4^{3-} in dark controls (●) and sunlight-exposed treatments (○) relative to the starting concentration before sunlight exposure (indicated by the dashed line). Data were pooled over all sampling periods. High salinity location contains only April and July 2000 data. Error bars denote ± 1 SD of the mean ($n = 6$ to 8)

treatment. DOC was utilized at mean rates of $0.7 (\pm 0.3) \mu\text{M d}^{-1}$ and $1.4 (\pm 0.3) \mu\text{M d}^{-1}$ over 14 d in light and dark treatments, respectively (Table 2). No significant differences in (NO_x) concentrations were observed between light and dark incubations (Fig. 3E). However, net production of NH_4^+ ($0.7 \mu\text{M}$) occurred in dark treatments (Fig. 3D), and a small ($0.02 \mu\text{M}$) but significant ($p < 0.05$) production of PO_4^{3-} (Fig. 3D,F). In comparison, NH_4^+ production decreased by $\sim 80\%$ ($p < 0.01$) in sunlight-exposed treatments (Fig. 3D). No net production of PO_4^{3-} was observed in the light treatments.

In April 2000 (Table 2, Fig. 4A), a 40% increase in DOC utilization was observed in light treatments at the mid-salinity site by the end of the experiment. Mean rates of DOC utilization for dark ($2.9 \mu\text{M d}^{-1}$) and light-exposed treatments ($3.9 \mu\text{M d}^{-1}$) in April were more than 2 times greater than their August counterparts. While there were no significant light vs. dark differences in DON and DOP over the time course, increases in both DON and DOP were observed during incubation (Fig. 4B,C). Concomitant with this increase in DON and DOP was a decline in all inorganic nutrients (Fig. 4D to F). Production of NH_4^+ and PO_4^{3-} in light relative to dark treatments was significantly reduced ($p < 0.05$, Fig. 4D,F).

In July 2000, rates of DOC utilization at the head of the estuary (1.4 and $4.0 \mu\text{M d}^{-1}$ for dark- and light-exposed treatments, respectively) were the highest freshwater rates across all sampling periods. In sunlight-exposed treatments both the rate ($4.0 \mu\text{M d}^{-1}$) and percentage (49%) of DOC utilization was more than 2 times greater than the dark treatment (Table 2). Initial concentrations of NH_4^+ ($0.5 \mu\text{M}$) were comparable to the August sampling time period ($0.4 \mu\text{M}$) (Table 1). There were no significant light vs. dark differences in DON and DOP over the time course; however, there was a significant decrease in NH_4^+ production in light-exposed relative to dark treatments (Table 3).

Bacterial production and growth

Estimates of bacterial net production rates, calculated from changes in bacterial abundances over 7 d, were significantly ($p < 0.05$) lower in light relative to dark treatments from the freshwater and intermediate salinity sampling locations in August 1999 (Table 3). Net production rates were not significantly different between light and dark treatments at any of the sampling locations in April 2000. In July 2000 there was a significant ($p < 0.05$) increase in net production rates in light-exposed treatments from the freshwater end-member.

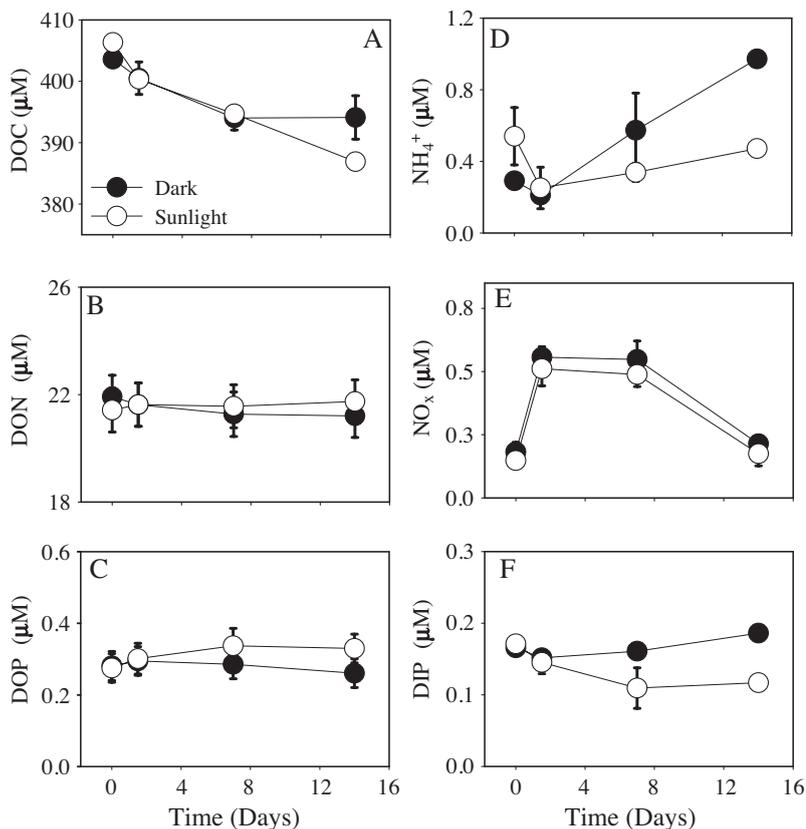


Fig. 3. Incubation time courses from August 1999 for the York freshwater end-member for sunlight-exposed and dark treatments for (A) DOC, (B) DON and (C) DOP concentrations. (D) to (F) show NH_4^+ , NO_x and PO_4^{3-} concentrations, respectively. Error bars denote ± 1 SD of the mean ($n = 2$)

Relative bacterial production estimates based on ^3H -leucine incorporation rates in light relative to dark treatments showed significant differences between the April and July 2000 sampling periods (Table 3). In July 2000, there was a 76 to 152% increase ($p < 0.01$) in integrated bacterial production in light treatments relative to dark over the initial 7 d incubation period (Table 3). In 2 of the 3 July experiments a corresponding increase in the cell-specific incorporation rate was observed with the increase in integrated production (Table 3). Although a 76% increase ($p < 0.0001$) in integrated production in light-exposed treatments was measured at the mouth of the York in July, there was no corresponding response in the specific incorporation rate (Table 3). In April, no significant differences were found in integrated production, nor were specific incorporation rates significantly altered at the freshwater and mid-salinity sites. However, a significant reduction in the specific incorporation rate was measured at the most saline site (Table 3).

Impact of sunlight exposure of DOM on BGE

Estimates of BGE calculated from increases in bacterial cell numbers are summarized in Table 3. We note that systematic differences in cell size resulting from changes in nutrient and C quality along the salinity gradient may result in variations in the C content per cell. Accordingly we compare BGE only between light and dark treatments where nutrient and DOC concentrations are comparable. Significantly lower bacterial cell abundance in light-exposed relative to dark treatments in August resulted in significant decreases in BGE of ~40 and 70% for the freshwater and mid-salinity location, respectively (Table 3). BGE in light relative to dark treatments was positively correlated ($r^2 = 0.38$, $p < 0.01$) with *in situ* NH_4^+ concentrations (Fig. 5). Concomitant with declines in BGE was a significant reduction in inorganic nutrient remineralization (Table 3). Similar findings of significant declines in BGE in light-exposed relative to dark treatments and concurrent decreases in the bacterial production of either NH_4^+ or PO_4^{3-} were found for the high-salinity location in April and the freshwater end-member in July (Table 3).

DISCUSSION

The present study examined the effects of sunlight-exposure on microbial DOM decomposition and bacterial metabolism in a temperate estuary. The integrated impact of photolytic processes is complex in estuaries due to the varying proportion of humic and algal sources, inorganic nutrients inputs, and DOM bioreactivity. For example, it has been hypothesized that the net effect of photochemically altered DOM on bacterial production and growth is related to the relative proportions of reactive algal-derived DOM and allochthonous humic DOM (Benner & Biddanda 1998, Tranvik & Bertilsson 2001, Mopper & Kieber 2002, Moran & Covert 2003). Thus, the impact of photochemistry on bacterial C processing may be complicated not only by multiple estuarine OM sources but also through additional metabolic constraints (inorganic nutrients, cellular maintenance costs, del Giorgio & Cole 2000) that regulate the balance between bacterial respiration and production (i.e. BGE). By following both DOM degradation and bacterial metabolic parameters (e.g. bacte-

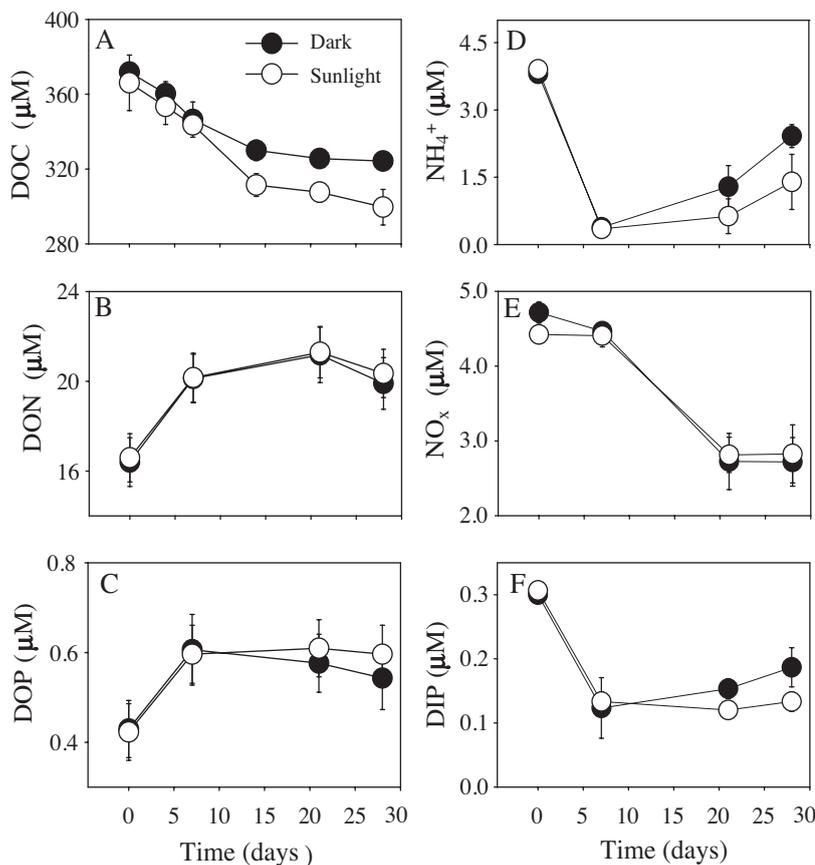


Fig. 4. Incubation time courses from April 2000 for the mid-salinity ($S = 11$) York River estuary sampling station for sunlight exposed and dark treatments for (A) DOC, (B) DON (C) DOP concentrations. Panels D–F show NH_4^+ , NO_x and PO_4^{3-} concentrations, respectively. Error bars denote ± 1 SD of the mean ($n = 3$)

rial abundance, production, BGE) we may better differentiate the potential positive and negative photochemical feedbacks on OM turnover and fate (production vs. respiration) by heterotrophic bacteria. Our findings for the York system suggest a scenario where sunlight in general increases DOC utilization, decreases inorganic nutrient remineralization and ultimately decreases the efficiency of C utilization (BGE).

Effects of sunlight on DOM utilization by estuarine bacteria

Between ~5 and 17% of the DOC was utilized in light-exposed incubations from along the York salinity gradient, representing up to a 2-fold increase in the bioavailable DOC pool relative to dark treatments (Table 2). The rate of DOC utilization in dark treatments declined relative to light treatments after 7 d (Figs. 3A & 5A). The lag time (7 d) prior to the divergence of DOC decomposition in light vs. dark treat-

ments supports previous findings by Miller & Moran (1997) attributing an increase in DOC bioavailability to photochemically induced structural modifications in humic or HMW DOC, rather than solely by direct photochemical cleavage of DOM to LMW compounds. Thus photo-sensitization of DOM may confer greater bioreactivity over timescales of days to weeks. However, this prolonged enhancement in DOM bioreactivity may not be detected by short-term (hours to days) bacterial production measurements.

During periods of lower river flow and longer water residence time (August 1999 and July 2000, Table 1), the increase in DOC utilization in light-exposed relative to dark treatments from the freshwater and intermediate salinity sites was significantly ($p < 0.05$) greater at the high-salinity site. DOC degradation at the high salinity site in light vs. dark treatments was greatest during the higher flow period of April when riverine/humic OM tends to be more homogeneously mixed along the estuary (McCallister 2002). Humics are condensed organic compounds, highly colored due to their high degree of aromaticity and strongly light adsorbing relative to their autochthonous counterparts (Miller 1998). The capacity of humic substances for light absorption drives their photochemical reactivity (Miller 1998). Thus, because our sampling period coincided with the 10 yr minimum in river discharge and correspondingly low riverine/humic OM input (McKnight & Aiken 1998), our estimates of the photochemical impact on DOC degradation (Table 2) are potentially underestimates for average flow years.

In previous studies, sunlight-induced condensation reactions were thought to be responsible for significant increases in the formation of refractory DOC and decreased bioavailability of algal-derived DOC (Keil & Kirchman 1994, Naganuma et al. 1996, Tranvik & Kokalj 1998). Despite the more than 6-fold differences in chl *a* concentration across sampling sites and times (Table 1), DOC degradation was an average of only 2 fold greater in sunlight-exposed treatments than non-irradiated treatments (see Table 2 for significance levels). Our findings therefore suggest that either riverine DOC was relatively undiluted by reactive algal sources of DOC or that the stimulatory effect of sunlight on DOC reactivity overwhelmed any inhibitory effects that may have occurred. Furthermore, chl *a* normalized to total DOC (chl *a*/DOC) at

Table 2. DOC losses in York River estuary microbial utilization assays. Numbers in parentheses are ± 1 SD of mean. Boldface italics denote significant differences ($p < 0.05$) in light vs. dark treatments using ANOVA. Total amount of DOC remineralized was calculated for the duration of the experiments as follows: August 1999 (14 d); April and July 2000 (28 d). The rate of DOC utilization was calculated over the first 14 d of all incubations

Date	Salinity	Treatment	DOC remineralized (μM)	Total DOC remineralized (%)	Rate of DOC utilization ($\mu\text{M d}^{-1}$)
Aug 99 ^a	S = 0	Light	19 (1.7)	4.8 (0.4)	1.4 (0.1)
		Dark	10 (2.2)	2.5 (0.5)	0.7 (0.2)
	S = 10	Light	26 (0.9)	6.5 (0.2)	1.9 (0.1)
		Dark	16 (0.7)	3.9 (1.4)	1.1 (0.1)
Apr 00	S = 0	Light	28 (20.1)	6.6 (4.3)	1.9 (1.2)
		Dark	15 (2.5)	3.5 (0.6)	1.1 (0.2)
	S = 11	Light	67 (6.9)	17.0 (3.5)	3.9 (0.6)
		Dark	48 (3.4)	12.8 (0.8)	2.9 (0.6)
	S = 22	Light	21 (4.4)	7.7 (1.7)	1.1 (0.04)
		Dark	7 (2.0)	2.5 (0.7)	0.4 (0.1)
Jul 00	S = 0	Light	49 (3.1)	11.0 (0.7)	4.0 (0.2)
		Dark	21 (1.4)	4.5 (0.4)	1.4 (0.2)
	S = 10	Light	35 (7.2)	6.7 (1.3)	2.2 (0.1)
		Dark	20 (15.1)	3.9 (2.9)	0.9 (0.2)
	S = 20	Light	14 (6.3)	5.3 (2.3)	0.8 (0.2)
		Dark	11 (2.4)	4.6 (1.0)	0.7 (0.2)

^aDOC data from high-salinity site is unavailable due to contamination

each sampling site was not correlated with bacterial production estimates in light vs. dark treatments ($p > 0.05$, data not shown). Chl *a*/DOC did, however, show a weak but significant negative correlation with DOC utilization in light-exposed relative to dark treatments ($r^2 = 0.31$, $p \leq 0.01$). Like other estuarine systems, the York is unique in that bacterial production is decoupled from algal sources (Schultz et al. 2003). Accordingly, the use of indirect parameters (e.g. bacterial production, chl *a* concentration) may not accurately reflect the role of photochemistry in C flow.

While rates of DOC decomposition were significantly greater in pre-irradiated vs. dark treatments (Table 2), no corresponding change in microbial utilization of the associated DON and DOP fractions was observed (Figs. 3B,C & 4B,C). These findings suggest that the reactivity (both photochemical and bacterial) of DON and DOP to in this

Table 3. Summary of bacterial net production rate, integrated bacterial production, BGE and inorganic nutrient production for York River estuary samples collected in August 1999, April and July 2000. Net production rate (Net prod. rate) was calculated from increases in cell abundance over time. Integrated production was calculated from estimates of ^3H -leucine incorporation integrated over 7 d (daily intervals) for April and July 2000 by means of trapezoidal integration. Specific incorporation rate (Sp. inc. rate) was calculated by measuring ^3H -leucine incorporation per cell. BGE was calculated from net cell production and DOC decline over a 7 d time interval. Significance was calculated by ANOVA ($p < 0.05$) and is denoted by (-) or (+) to indicate values for light-exposed treatments that are either smaller or greater than dark treatments. Boldface italics denote significant differences ($p < 0.05$) in light vs. dark treatments using ANOVA. Numbers in parentheses are ± 1 SD of mean. n.d.: not determined. n.s.: results that are not significant

Date	Salinity	Treatment	Net prod. rate (cells $\text{l}^{-1} \text{d}^{-1} \times 10^{-8}$)	Integrated Leu incorporation (nmol Leu l^{-1})	Sp. inc. rate (10^{-20} mol Leu cell $^{-1}$)	BGE (%)	Direction of significant changes in NH_4^+	Direction of significant changes in PO_4^{3-}
Aug 99	S = 0	Light	1.6 (0.04)	n.d.	n.d.	18.8 (3.9)	(-)	(-)
		Dark	2.3 (0.02)	n.d.	n.d.	32.8 (3.7)		
	S = 10	Light	2.1 (0.3)	n.d.	n.d.	19.8 (4.2)	(-)	n.s.
		Dark	2.8 (0.1)	n.d.	n.d.	65.1 (14.0)		
Apr 00	S = 0	Light	4.2 (0.9)	32.7 (34.7)	7.9 (4.1)	18.0 (11.1)	(+)	n.s.
		Dark	2.8 (2.1)	13.9 (10.5)	9.5 (2.5)	44.4 (18.6)		
	S = 11	Light	4.0 (1.4)	59.5 (13.8)	15.8 (4.1)	27.8 (24.5)	(-)	(-)
		Dark	3.5 (0.2)	86.7 (46.2)	16.7 (3.4)	22.1 (9.7)		
	S = 22	Light	1.8 (2.2)	10.1 (1.7)	5.3 (0.4)	24.2 (5.0)	n.s.	(-)
		Dark	3.4 (1.5)	10.5 (1.2)	10.2 (0.8)	67.9 (0.3)		
Jul 00	S = 0	Light	13.4 (4.3)	489.5 (80.2)	11.9 (4.4)	34.1 (14.8)	(-)	n.s.
		Dark	7.1 (2.4)	246.4 (17.2)	7.3 (0.6)	85.7 (2.3)		
	S = 10	Light	4.2 (0.3)	247.2 (32.4)	99.6 (8.4)	35.5 (8.8)	n.s.	(+)
		Dark	5.4 (2.9)	98.0 (21.6)	45.3 (12.9)	32.2 (10.2)		
	S = 20	Light	2.8 (1.5)	119.3 (3.3)	5.0 (0.4)	24.4 (15.1)	n.s.	n.s.
		Dark	1.6 (1.7)	67.6 (3.7)	4.1 (1.1)	23.0 (27.5)		

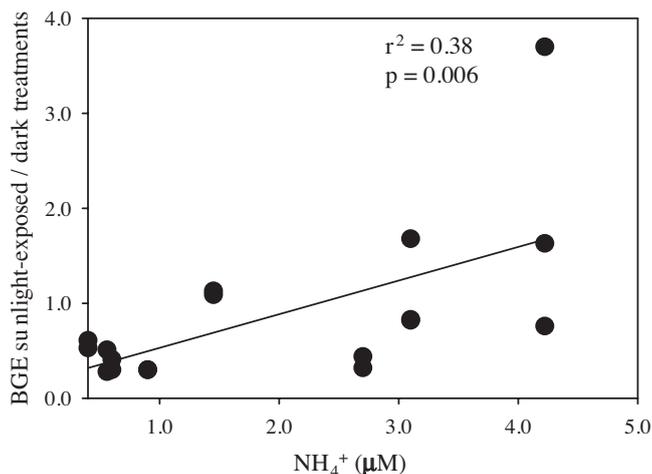


Fig. 5. Correlation between *in situ* NH_4^+ concentrations and BGE for all sampling periods (see Table 3 for mean BGE)

system may be uncoupled from that of DOC. Effects of sunlight on DON and DOP reactivity have been examined in a limited number of previous studies. For example, Bertilsson et al. (1999), in agreement with the present study, found that sunlight exposure increased the bioavailable pool of DOC from a boreal watershed, while DON remained unaffected. Wiegner & Seitzinger (2001) further determined that light-exposure of agricultural and forest run-off did not alter the microbial reactivity of DON. Thus, photochemical enhancement of DOC reactivity without a concomitant increase in DON and DOP reactivity may result in a greater bacterial demand for inorganic nutrients.

Impact of sunlight-exposed DOM on bacterial metabolism and inorganic nutrient demand

DOC decomposition was decoupled from bacterial production in light vs. dark incubations (Table 3; Tranvik & Bertilsson 2001). This variability may require a closer examination of the geochemical, microbial and photochemical factors (and their synergism) controlling OM turnover in aquatic, and especially, estuarine environments (Mopper & Kieber 2002). For example, although there were no significant changes in bacterial production in April 2000 ($S = 10$) in light-exposed treatments (Table 3), corresponding bacterial DOC decomposition showed increased reactivity (Table 3, Fig. 4A) after 7 d. Bacterial production and DOC utilization in light relative to dark treatments were independent ($r^2 = 0.08$, $p > 0.05$), further suggesting that impacts of photochemistry on bacterial growth and total C flow may be uncoupled.

The majority of previous studies assessing photochemical effects on bacterial DOC decomposition have not addressed the potential differential partitioning of DOC to anabolic (i.e. production) and catabolic pathways (i.e. respiration; Mopper & Kieber 2002). Variations in bacterial metabolic expenditures (i.e. as manifested in BGE) may significantly alter the ecological fate of photochemically altered DOM (i.e. retained within the system or exported to the atmosphere). The most important variables thus far identified as affecting BGE in aquatic systems include inorganic nutrients, C:N of substrate, cellular maintenance cost, oxidation state of DOM, and temperature (see review by del Giorgio & Cole 2000). However, at the cellular level there are 2 primary controls on BGE: (1) the quantity and quality of organic and inorganic substrates and (2) maintenance cost (del Giorgio & Cole 2000).

In the York River, the impact of sunlight-exposure on BGE was positively correlated with *in situ* NH_4^+ concentrations ($r^2 = 0.38$, $p < 0.01$, Fig. 5), suggesting that the net influence of photolytic reactions on C flow is partially dependent on NH_4^+ availability. Significant decreases in BGE in sunlight vs. dark treatments for August 1999 (Table 3) at both locations coincided with a period of low river flow and depleted stocks of NH_4^+ and PO_4^{3-} (Table 1). Furthermore, the increase in DOC reactivity was not balanced by a corresponding increase in DON or DOP degradation (Fig. 4B,C). In April 2000, inorganic nutrient concentrations were several times greater than August 2000 at the zero and mid-salinity sites (Table 1). As shown in Fig. 4, DOC was rapidly metabolized in April, and was paralleled by a corresponding uptake of inorganic nutrients. No significant difference in BGE was discernible between light and dark incubations at the zero and mid-salinity sites (Table 3), presumably because starting concentrations of inorganic nutrients were able to balance the increased availability of DOC in contrast to August 1999 incubations. However, at the high-salinity end-member, ambient inorganic nutrients were significantly lower in comparison to the mid- and zero-salinity sites (Table 1), and the 65% enhancement in DOC utilization (Table 2) in light vs. dark treatments was not paralleled by an analogous increase in bacterial biomass, but resulted in decreased growth efficiency (Table 3).

Previous findings have suggested that BGE may be controlled to a greater extent by inorganic nutrient availability than by DOC supply in riverine and coastal systems (Benner et al. 1995, Zweifel et al. 1993). One possible explanation for this relationship between BGE and nutrients is that the light-driven production of reactive DOC compounds necessary for biomass production must be stoichiometrically balanced by available

sources of N and P in order to meet cellular requirements. However, neither the potential photolytic subsidy of NH_4^+ and PO_4^{3-} (Fig. 2) nor enhanced reactivity of DON and DOP (Figs. 3B,C & 4C) provided this stoichiometric equivalent in the York. Consequently, the respiratory demands of bacteria may have increased in the absence of the requisite N and P to construct biomass. Under conditions of inorganic nutrient limitation, bacteria may shift their metabolism such that excess DOC is catabolized, thus increasing their respiration rate and decreasing biomass synthesis or BGE (Hessen 1992). The net effect of sunlight in this system may therefore have been the transient formation of 'excess' reactive DOC (Table 2), thus diminishing the role of bacteria as remineralizers of inorganic nutrients (Table 3).

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