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Single-cell response of bacterial groups to light and other environmental factors in the Delaware Bay, USA

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ABSTRACT: We examined the incorporation of leucine, protein, and a mixture of 15 amino acids by selected phylogenetic groups in light and dark incubations of coastal waters of the Delaware Bay and Mid-Atlantic Bight. In experiments conducted over 3 yr, the single-cell activity of different groups of bacteria varied with molecular weight of the substrate, photosynthetically active radiation (PAR), and other environmental conditions. The fraction of cells active in using leucine and incorporating protein differed among groups and between summer and fall. About 30% of all cells incorporated the amino acid mixture, while only 10% incorporated protein. PAR availability affected single-cell activity in 20% of all experiments, and PAR conditions prior to sampling correlated with light effects on single-cell activity, varying with the compound (amino acid mixture, leucine or protein). The bacterial group most consistently affected by PAR was the SAR11 clade; 25% more SAR11 bacteria used leucine in the light than the total community. The study illustrates the complex effects of light on single-cell activity of bacterial groups and helps to explain the variability in the impact of light on dissolved organic carbon fluxes.

KEY WORDS: Microautoradiography · Bacterial activity · Light · DOM incorporation

INTRODUCTION

The number of bacteria actively using dissolved organic material (DOM), as assessed by single-cell assays, varies greatly within aquatic habitats for reasons that are not completely understood (Smith & del Giorgio 2003, del Giorgio & Gasol 2008). Some of this variation is related to changes in the phylogenetic composition of bacterial communities. The abundances of even broad phylogenetic groupings, such as those recognized by commonly used fluorescence *in situ* hybridization (FISH) probes, vary in response to different environmental properties, including DOM availability (Amann et al. 1990b, Amann & Fuchs 2008). There is evidence that these broad groups of bacteria also differ in their use of organic material (Cottrell & Kirchman 2000, Longnecker et al. 2006, Elifantz et al. 2007). For example, more cells in the *Alphaproteobacteria* class use low molecular weight compounds like amino acids or glucose than high molecular weight compounds such as protein or polysaccharides (Cottrell & Kirchman 2000, Elifantz et al. 2005, Alonso-Saez et al. 2009). Within the *Alphaproteobacteria*, more than half of the ubiquitous SAR11 clade incorporated amino acids and dimethylsulfoniopropionate (DMSP) in the North Atlantic (Malmstrom et al. 2004). However, the fractions of SAR11 that use polysaccharides and protein are smaller than those that use low molecular weight material such as leucine and other amino acids (Elifantz et al. 2005, Malmstrom et al. 2005). The single-cell activity of *Gammaproteobacteria* using different compounds does not vary overall in western Arctic waters (Elifantz et al. 2007). In contrast, the fraction of *Gammaproteobacteria* that uses glucose is smaller than those that use other compounds in both the Mediterranean Sea and the Delaware Estuary (Elifantz et al. 2005, Alonso-Saez & Gasol 2007).

The use of DOM by these broad bacterial groups may shift in response to many environmental factors, some

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of which vary seasonally (Fuhrman et al. 2006, Henriques et al. 2006). In Blanes Bay in the northwest Mediterranean Sea, more *Gammaproteobacteria* incorporated amino acids in winter than in summer (Alonso-Saez & Gasol 2007). Active fractions of *Gammaproteobacteria* and the *Sphingobacteria-Flavobacteria* group increased with higher concentrations of glucose during a spring phytoplankton bloom in the North Sea (Alonso & Pernthaler 2006). Mortality due to grazing or viral lysis also impacts bacterial activity, and there is some evidence of differing responses by different bacterial groups as well as a greater response in oligotrophic systems (Longnecker et al. 2010). In the California Current System, the fraction of cells in the whole bacterial community using leucine responded to changes in temperature and salinity but there were no differences in the responses of the bacterial groups studied, including *Sphingobacteria-Flavobacteria* and the *Alpha-*, *Beta-*, and *Gammaproteobacteria* (Longnecker et al. 2006). The environmental factors driving variation in single-cell activity of specific bacterial groups are not well characterized.

The ability of some heterotrophic bacteria to use light as an additional source of energy may drive variation in growth and abundance (Kolber et al. 2000, Béjá et al. 2001). Exposure to photosynthetically active radiation (PAR) can stimulate the total community uptake of compounds such as leucine and methionine (Moran et al. 2001, Church et al. 2004, Mary et al. 2008), but the effect may not always be present or the same (Michelou et al. 2007). Some of the light effect may be due to proteorhodopsin, a potential light-harvesting mechanism found in many bacterial taxa (Béjá et al. 2000, de la Torre et al. 2003, Morris et al. 2010). A cultured *Flavobacteria* strain containing proteorhodopsin grew faster in the light than in the dark (Gómez-Consarnau et al. 2007), and cultured *Vibrio* survived starvation better with proteorhodopsin than without it (Gómez-Consarnau et al. 2010). However, other cultured proteorhodopsin-bearing strains have not responded to PAR in experiments conducted so far (Giovannoni et al. 2005, Stingl et al. 2007). In the environment, stimulation of bacterial activity by light exposure may be offset by light inhibition. Alonso-Saez et al. (2006) observed higher sensitivity to UV light in the *Alphaproteobacteria*, while other groups such as the *Gammaproteobacteria* and *Bacteroidetes* were more resistant to photo-damage. More work is necessary to determine the effects of light on different bacterial groups.

The goal of this study was to identify the variation in single-cell activity of different bacterial groups in the lower Delaware Bay in response to environmental properties. We hypothesized that substrate use by bacterial groups varies with molecular weight of the substrate, related to PAR exposure. We tested the incorporation of leucine (an index for bacterial biomass production), protein, and a mixture of 15 amino acids in both light and dark by selected phylogenetic groups in summer and fall. Over the 3 yr of the study, single-cell activity varied among the compounds and groups, and was both stimulated and inhibited by light, depending on the compound and the bacterial group.

MATERIALS AND METHODS

Surface waters were sampled monthly at the mouth of the Delaware Bay (site 'Bay') and 18.5 km offshore (site 'FB') from 2006 to 2009 (www.ocean.udel.edu/cms/dkirchman/MOPE). Abundance of all prokaryotes was measured using epifluorescence microscopy with 4′-6-diamidino-2-phenylindole (DAPI) staining (Porter & Feig 1980, Cottrell et al. 2006). Concentrations of chlorophyll *a*, dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were estimated by standard methods (Parsons et al. 1984, Benner & Strom 1993, Sharp et al. 1995). Surface PAR was measured using a Biospherical light meter.

Bulk bacterial production. Bacterial production was estimated by the microcentrifuge method from incorporation of ^{3}H-leucine (20 nM, final concentration) in 30 min incubations at *in situ* temperatures in the dark (Smith & Azam 1992, Kirchman 2001). Bulk growth rates were calculated by dividing bacterial production (mg C m^{-3} d^{-1}) by biomass (mg C m^{-3}), assuming conversion factors of 20 fg C $cell^{-1}$ and 1.5 kg C per mole of leucine (Lee & Fuhrman 1987, Kirchman et al. 2009). Additionally, bulk incorporation of ^{3}H-leucine in the light was measured using 6 h incubations in clear plastic bags (Whirlpak, Nasco) exposed to PAR from a metal halide lamp (Hydrofarm). Light intensity from the lamp was 1×10^{16} quanta s^{-1} cm^{-2}. Mean surface PAR at the time of sampling was 8×10^{16} quanta s^{-1} cm^{-2}. The dark incubations were the same except that the bags were covered in double-layered black plastic. These conditions were also used for single-cell assays described below.

Abundance of bacterial groups. The abundance of selected bacterial taxa was estimated using catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH; Pernthaler et al. 2002). Water samples were fixed overnight at 4°C with paraformaldehyde (PFA, 2% final concentration). Samples were filtered through 0.22 µm polycarbonate filters (Millipore) with a 0.45 µm nitrocellulose support filter (Millipore), rinsed with 0.22 µm filtered deionized water, and stored at −20°C until further processing. Probes for *Alphaproteobacteria* (Alf968), *Gammaproteobacteria* (Gam42a), SAR11 clade (SAR11-441r), all bacteria

(EUB338) and a negative control were used (Amann et al. 1990a, Manz et al. 1992, Karner & Fuhrman 1997, Glöckner et al. 1999, Morris et al. 2002). Cells were embedded on the filter using 0.1% agarose then treated with lysozyme to increase permeability. Filter pieces were hybridized with horseradish peroxidase-labeled oligonucleotide probes, and subsequently stained with cyanine-3 (Cy3)-labeled tyramides (TSA kit, Perkin Elmer). Following this staining, the filter pieces were either processed for microautoradiography (see below) or transferred onto slides and stained with DAPI (0.5 ng μl^{-1}) in a 4:1 mixture of Citifluor (Ted Pella) and Vectashield (Vector Labs) anti-fading mountants.

Single-cell assay of bacterial activity. The incorporation of selected compounds by specific bacterial groups was examined using microautoradiography combined with CARD-FISH (Micro-FISH) on samples taken in the summer and fall (see Fig. 1). Water samples for Micro-FISH were incubated with 20 nM ^{3}H-leucine (Perkin Elmer), a mixture of 15 tritiated amino acids including leucine (0.5 nM, American Radiolabeled Chemicals), or 0.4 µg ml^{-1} ^{3}H-protein. ^{3}H-protein was extracted from a *Vibrio alginolyticus* culture fed with ^{3}H-leucine (Nagata et al. 1998). Leucine was added at saturating concentrations to examine bacterial biomass production, while the incorporation of tracer concentrations of amino acids and protein provided a measure of DOM use. Incubations in clear polycarbonate bottles with leucine or the amino acid mixture lasted 2 h and protein incubations lasted 6 h at *in situ* temperatures in the dark or exposed to PAR as described above. After incubation, samples were fixed with PFA (2% final concentration) overnight at 4°C and filtered as for FISH. PFA was added to killed controls prior to the addition of labeled compounds.

Microautoradiography was carried out as described by Cottrell & Kirchman (2003). Slides were dipped in film emulsion (Amersham Hypercoat EM-1), then filter pieces were placed cell-side down onto the emulsion. A time series of exposure in the emulsion before developing was used to select the shortest time required to identify a maximum number of active cells. The exposure time for leucine samples was 24 h, for amino acids 3 to 4 d, and for protein samples 6 d. At the end of the exposure time, slides were developed and fixed (Dektol developer and fixer, Kodak). After drying overnight, the filter pieces were carefully peeled away and the cells stained with DAPI in the 4:1 mountant as described above.

Cells were counted using a semi-automated microscope system, described previously by Cottrell & Kirchman (2003). Ten fields of view were counted for each sample, with a constant exposure time of 100 ms for silver grain images. DAPI image exposure times ranged from 75 to 100 ms, and Cy3 image exposure times were set by the negative control and ranged from 300 to 500 ms. The proportion of the total community that was active was calculated as the number of DAPI-stained objects with associated silver grain clusters divided by the total number of DAPI-stained objects. Similarly, the number of probe- and DAPI-stained objects with silver grains was divided by the total number of probe- and DAPI-positive cells to calculate the percentage of a group that actively took up a given compound. Non-specific probe binding was below 5% for all samples. All percentage data were arcsine-transformed for statistical analyses.

RESULTS

We examined the influence of environmental changes on variation in abundance and single-cell activity of different bacterial groups in the lower Delaware Bay over 3 yr. Environmental properties at the 2 sites varied over time (Table 1). PAR and the concentrations of DOC and DON did not differ between seasons. Bulk ^{3}H-leucine incorporation and temperature peaked in the fall, whereas total bacterial abundance was highest in late summer. Bacterial abundance averaged $4.76 \pm 0.83 \times 10^6$ cells ml^{-1} over all samples (Fig. 1). Chlorophyll *a* concentrations correlated positively with bacterial abundance ($r = 0.70$, $N = 78$, $p < 0.05$) and temperature ($r = 0.78$, $N = 78$, $p < 0.05$). ^{3}H-leucine incorporation correlated with temperature ($r = 0.74$, $N = 70$, $p < 0.05$) (Fig. 1) and bacterial abundance ($r = 0.53$, $N = 70$, $p < 0.05$). As the environmental properties from the 2 sites did not differ significantly, the results from both stations were combined for analyses.

Table 1. Environmental properties and bulk bacterial characteristics during sampling in the Delaware Bay. Mean ± SE of 6 samples in the summer and 8 samples in the fall

Property	Summer	Fall	Mean
Environmental properties			
Water temperature (°C)	17 ± 1.7	19 ± 1.5	18 ± 1.1
Chl *a* concentration (µg l^{-1})	5.2 ± 0.02	9.9 ± 0.02	7.9 ± 0.11
PAR (quanta cm^{-2} $s^{-1} \times 10^{17}$)	0.81 ± 0.17	0.71 ± 0.15	0.75 ± 0.11
Dissolved organic carbon (µmol l^{-1})	98 ± 0.01	101 ± 0.1	99 ± 0.2
Dissolved organic nitrogen (µmol l^{-1})	8.9 ± 0.04	13 ± 0.02	11 ± 0.14
Bacterial characteristics			
Abundance (cells $ml^{-1} \times 10^6$)	8.1 ± 0.65	3.7 ± 0.12	5.6 ± 0.22
Leucine incorporation (pmol l^{-1} h^{-1})	460 ± 0.5	260 ± 0.3	360 ± 0.5

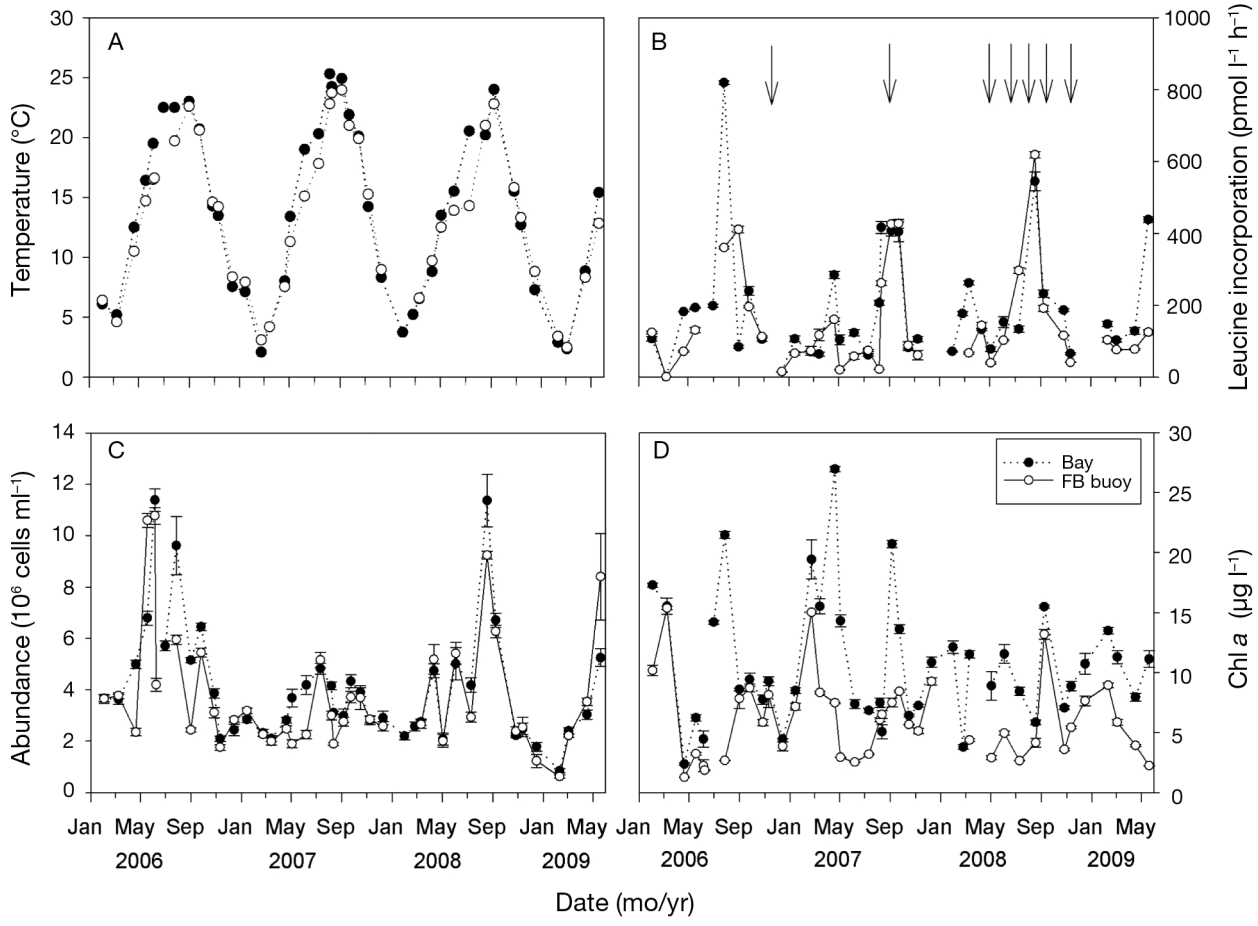

Fig. 1. (A) Temperature, (B) bulk ^{3}H-leucine incorporation, (C) bacterial abundance, and (D) concentrations of chlorophyll *a* in Delaware Bay (Bay) and coastal waters (FB buoy). Arrows indicate times of Micro-FISH sampling. Error bars are 1 SE

Abundance of bacterial groups

We analyzed the relative abundance of selected bacterial groups using CARD-FISH. *Gammaproteobacteria* and *Alphaproteobacteria* were equally abundant, each accounting for around 25% of the community. SAR11 abundance averaged 15% (coefficient of variation [CV] = 0.78) of the total microbial abundance. Relative *Gammaproteobacteria* abundance varied more than the other groups (CV = 0.98) and positively correlated with bulk ^{3}H-leucine incorporation, temperature, and concentrations of DOC and DON (Table 2). Alphaproteobacterial abundance (CV = 0.58) did not vary significantly with environmental conditions, but SAR11 abundance was negatively correlated with bacterial abundance, temperature and chlorophyll *a* concentration (Table 2). *Sphingobacteria-Flavobacteria* averaged 9.8% relative abundance in initial samples from both stations in the summer and fall of 2007 (n = 7, data not shown). Due to the low abundance of the group, the abundance of the active cells was near the detection limit for Micro-FISH. As a result of this methodological limitation *Sphingobacteria-Flavobacteria* were not examined further.

Table 2. Pearson correlation coefficients between relative abundances of *Gammaproteobacteria* (*Gamma-*), *Alphaproteobacteria* (*Alpha-*) and SAR11, and environmental variables. N is the number of dates examined over 3 yr. $^{*}p < 0.05$, $^{**}p < 0.01$

Property	*Gamma-*	*Alpha-*	SAR11
Temperature	0.31*	0.001	−0.37**
Chl *a* concentration	0.08	−0.12	−0.36**
Bacterial abundance	0.15	−0.07	−0.32*
Bacterial production	0.38**	0.09	−0.12
Dissolved organic carbon	0.25*	0.22	0.22
Dissolved organic nitrogen	0.26*	0.18	0.20
N	64	71	63

Table 3. Fraction of groups incorporating the indicated compounds in light and dark incubations conducted over 3 yr. Mean % ± SE of 11 to 14 samples of total community or 16 to 30 samples for bacterial groups. AA mix is the mixture of 15 amino acids

Bacterial group	Leucine light	Leucine dark	AA mix light	AA mix dark	Protein light	Protein dark
All cells	28 ± 3	32 ± 4	28 ± 4	33 ± 5	7 ± 1	11 ± 3
Bacteria[a]	25 ± 2	30 ± 3	27 ± 7	26 ± 4	8 ± 1	13 ± 4
Gammaproteobacteria	36 ± 5	34 ± 5	21 ± 6	25 ± 4	11 ± 3	10 ± 3
Alphaproteobacteria	59 ± 6	60 ± 3	36 ± 11	33 ± 9	10 ± 3	13 ± 4
SAR11	51 ± 6	46 ± 6	20 ± 6	21 ± 5	4 ± 2	7 ± 2

[a]Cells identified with EUB338 CARD-FISH probe

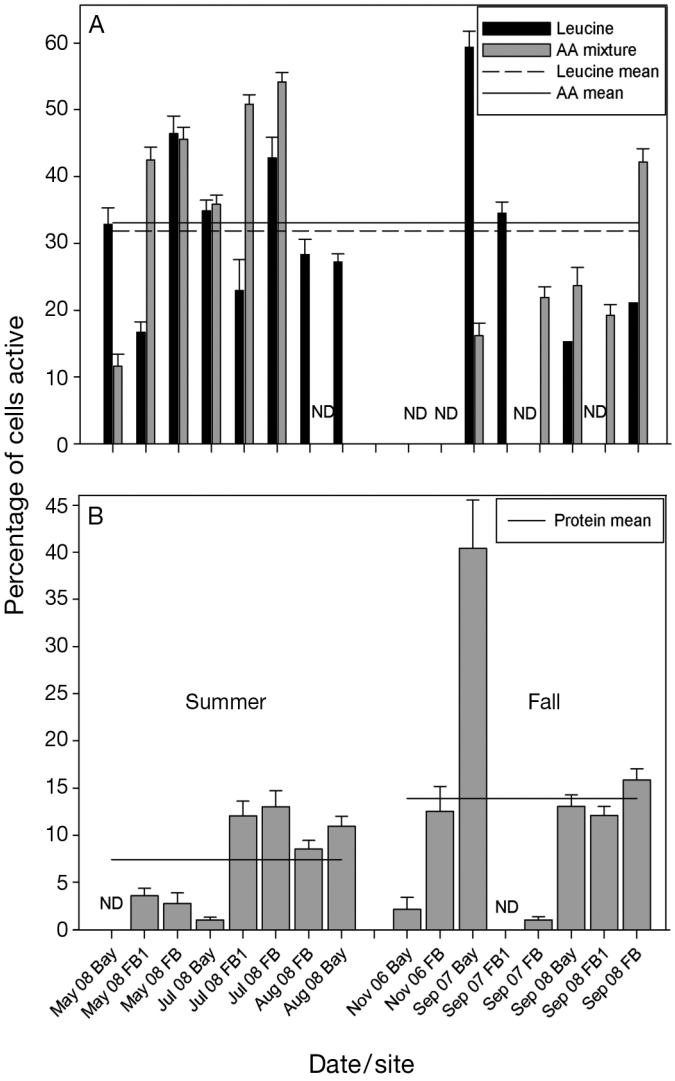

Fig. 2. Single-cell incorporation of (A) leucine and an amino acid (AA) mixture and (B) protein by the bacterial community in the dark. FB1 samples were taken at site FB (see 'Materials and methods' for details of sites) during the night (no night samples were taken in Nov 06 or Aug 08). Horizontal lines indicate mean fractions incorporating a compound. Error bars are 1 SE. ND = no data

Single-cell incorporation of DOM

Incorporation of DOM by bacteria differed among the substrates examined, and varied with environmental properties. About 31% of total DAPI-stained cells were active in dark incubations (incorporating leucine at saturating concentrations), and a similar fraction (33%) incorporated the amino acid mixture (Table 3, Fig. 2). In contrast, only 11% of cells on average incorporated protein. The percentage of cells taking up leucine decreased with total bacterial abundance (Table 4). The fractions of the bacterial community incorporating leucine and amino acids negatively correlated with chlorophyll *a* concentration (r = −0.47 and −0.33, respectively), while the fraction using protein positively correlated with chlorophyll *a* concentration (r = 0.42, Table 4). The proportion of the bacterial community using protein also positively correlated with temperature and bacterial abundance (Table 4).

Incorporation of DOM in the dark varied within groups and among the different groups (Table 3). Fewer EUB338-positive cells incorporated protein (13%) than were active in incorporating leucine or the amino acid mixture (both about 30%). Greater proportions of *Alpha-* and *Gammaproteobacteria* actively incorporated leucine than protein, but for both groups the fraction incorporating the amino acid

Table 4. Pearson correlation coefficients of environmental variables and the fraction of the total community incorporating a given compound. N is number of samples taken over 3 yr. AA mix is the mixture of 15 amino acids. $^{*}p < 0.05$, $^{**}p < 0.01$

Compound	Temp.	Secchi depth	Chl *a* conc.	Bacterial abundance	N
Leucine	−0.33	0.42*	−0.47**	−0.41*	31
AA mix	−0.28	0.18	−0.33*	−0.24	37
Protein	0.40*	−0.17	0.42**	0.45**	39

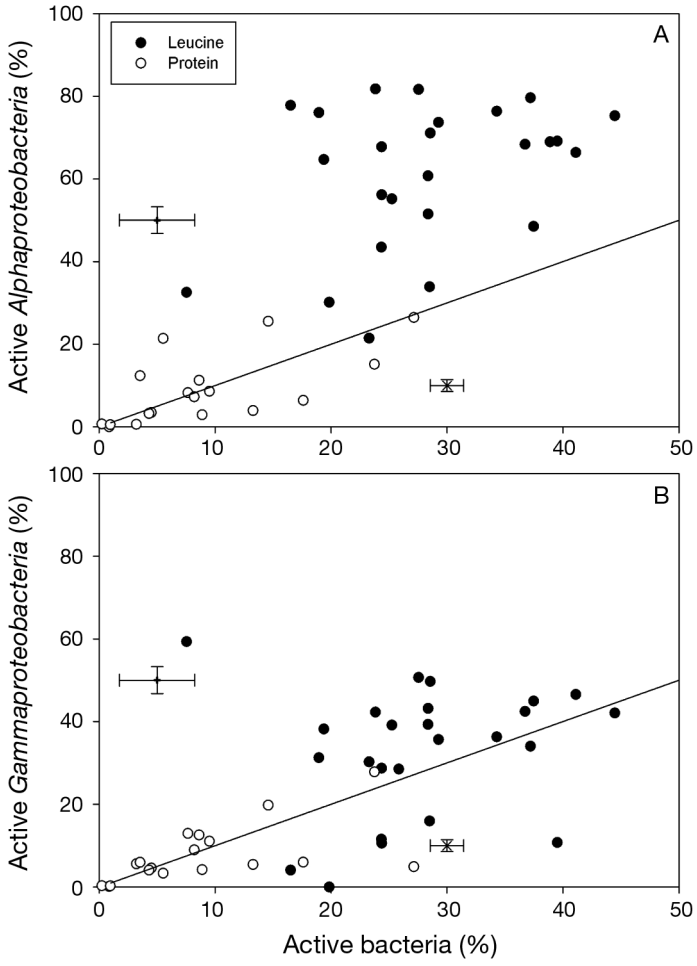

Fig. 3. Incorporation of leucine and protein by *Alpha-* and *Gammaproteobacteria* compared to the total bacterial community (EUB338-labeled cells) in the dark in experiments conducted from 2006 to 2008. Line is the 1:1 line. The mean standard errors are indicated for leucine (+) and protein (×)

mixture did not differ significantly from the leucine-active fraction or the fraction incorporating protein (Table 3, see Figs. S2–4 in the supplement at www.int-res.com/articles/suppl/a062p267_supp.pdf). In dark experiments, more *Alphaproteobacteria* incorporated leucine than *Gammaproteobacteria* and the total community; *Gammaproteobacteria* and the total community did not differ (Fig. 3). The fraction of SAR11 incorporating leucine in the dark did not differ from the other groups. Although alphaproteobacterial abundance did not vary significantly with environmental conditions (Table 2), the fraction of the *Alphaproteobacteria* that used all compounds negatively correlated with bacterial production ($r = -0.25$, $N = 69$, $p = 0.038$) and the concentration of DOC ($r = -0.24$, $N = 67$, $p = 0.045$).

We compared bacterial activity between summer (late May to August) and fall (September to November) in 16 dark incubations (Fig. 2, see Fig. S1 in the supplement at www.int-res.com/articles/suppl/a062p267_supp.pdf for light incubations). Environmental conditions did not differ significantly among years, and seasons from different years were combined for analysis. Bulk growth rates did not differ between summer and fall, averaging 0.11 ± 0.02 d^{-1}, and the fraction of cells that used leucine was also the same in both seasons (Table 1). The total fraction of cells incorporating protein was 2-fold higher in the fall than in the summer, while the incorporation of the amino acid mixture did not differ between the 2 seasons. A larger proportion of *Gammaproteobacteria* actively used leucine in the summer (45%) than in the fall (23%). More *Alphaproteobacteria* and SAR11 incorporated amino acids in the summer (50 and 28%, respectively) than in the fall (23 and 16%, respectively). Likewise, more SAR11 used protein in the summer (10%) than in the fall (2%).

Effects of light exposure

Occasionally, PAR had significant effects on total ^{3}H-leucine incorporation and on single-cell activity. Overall, bulk ^{3}H-leucine incorporation did not differ between light and dark in 6 h experiments conducted over 2 yr, but in some individual cases light did have an effect (Fig. 4). Single-cell activity of the total community using the 3 compounds was affected by PAR in 6 out of 35 experiments; 3 were stimulated by light while 3 had higher activity in the dark (Table 5). In

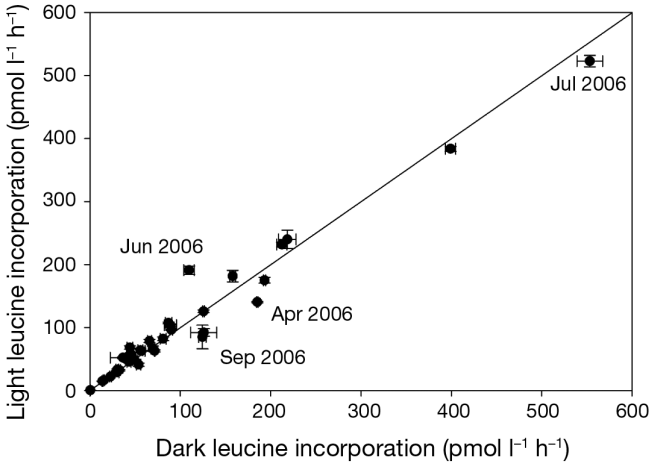

Fig. 4. Bulk ^{3}H-leucine incorporation in light vs. dark incubations in experiments conducted from 2006 to 2008. Line is the 1:1 line. Error bars are 1 SE. Sample dates for selected cases of the greatest light effect are noted

Table 5. Response to photosynthetically active radiation (PAR) by the total community and bacterial groups. No. is the number of experiments with a significant difference within the group tested (ANOVA, $p < 0.05$) between dark and light incubations. The total number of experiments conducted over 3 yr was 11 to 13 for total samples and 9 to 15 for groups. The percent stimulation or inhibition by PAR was calculated as $(Activity_{Light} - Activity_{Dark})/Activity_{Dark}$, and means of the affected samples are reported here. These means were all greater than the mean variance in single-cell activity within a sample, as determined from replicate counts (32%, n = 23)

Bacterial group	Compound	Stimulated No.	Stimulated %	Inhibited No.	Inhibited %	No. unaffected
Total	Amino acids	1	149	2	−68	8
Total	Leucine	2	113	0	–	9
Total	Protein	0	–	1	−67	12
Bacteria[a]	Amino acids	1	251	1	−97	6
Bacteria	Leucine	1	276	0	–	14
Bacteria	Protein	0	–	4	−72	5
Alphaproteobacteria	Amino acids	2	218	2	−84	4
Alphaproteobacteria	Leucine	0	–	0	–	15
Alphaproteobacteria	Protein	0	–	2	−77	7
Gammaproteobacteria	Amino acids	0	–	2	−91	6
Gammaproteobacteria	Leucine	3	271	1	−100	11
Gammaproteobacteria	Protein	2	171	1	−95	6
SAR11	Amino acids	0	–	0	–	8
SAR11	Leucine	1	353	0	–	14
SAR11	Protein	1	100	2	−95	6

[a]Cells identified with EUB338 CARD-FISH probe

the 2 leucine assays with significant differences, only stimulation of single-cell activity by light was observed. In 1 experiment, protein was taken up by significantly more cells in the dark than in the light. For amino acid incorporation, both stimulation and inhibition by PAR occurred (Table 5). The response by EUB338-labeled cells matched that of the total cells for leucine and the amino acid mixture, and the incorporation of protein by EUB338-positive cells was even more strongly inhibited by light with roughly half of the experiments inhibited. Bacteria responded to light with a change in single-cell incorporation in about 25% of protein and amino acid mixture experiments, while only 11% of leucine experiments were affected, consistent with the low number of cases in which bulk leucine incorporation was affected by PAR.

Single-cell activity of bacterial groups responded to light in a similar proportion (20%) of cases as single-cell activity of the total community (Table 5). There was a similar number of cases of light stimulation and light inhibition, but the magnitude of response was approximately 2-fold greater in cases of light stimulation (mean 211% stimulation and 85% inhibition). *Alpha-* and *Gammaproteobacteria* did not differ in their response to light (Table 5), except that *Alphaproteobacteria* was the only group for which light stimulated amino acid incorporation. *Gammaproteobacteria* and SAR11 both responded to light with changes in the fractions using leucine and protein, but both stimulation and inhibition by light occurred (Table 5). About 25% more SAR11 cells than bacterial cells (EUB338-positive) used leucine when exposed to light (ANOVA $p < 0.05$, Fig. 5). In light incubations, more *Gammaproteobacteria* incorporated protein than SAR11 cells, though the difference was only about 7% (Table 3).

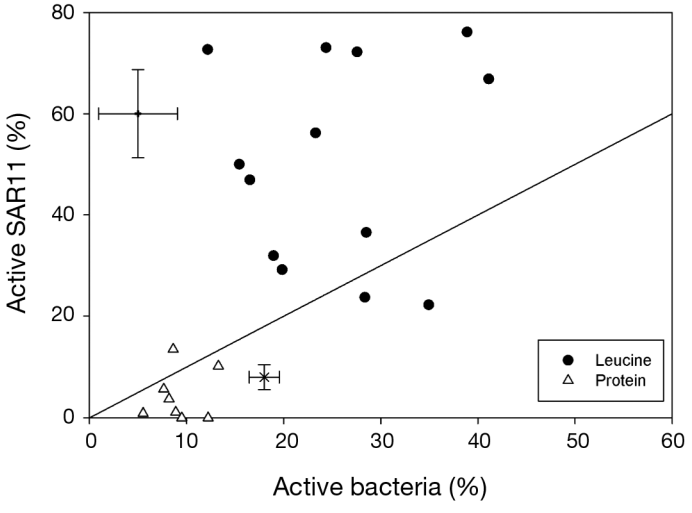

Fig. 5. Incorporation of leucine and protein by SAR11 bacteria compared to the total community in the light in experiments conducted from 2006 to 2008. Line is the 1:1 line. The mean standard errors are indicated for leucine (+) and protein (×)

Light conditions prior to sampling may affect the bacterial response to PAR (Alonso-Saez et al. 2006). To explore this effect, we examined the relationship between light conditions prior to sampling and the single-cell responses in our assays (Table 6). The fraction of the total bacterial community that used the amino acid mixture correlated negatively with the mean PAR during the 3 d preceding sampling ($r = -0.66$, $p < 0.05$, $n = 11$), but there was no relationship for leucine or protein single-cell activity. Light-affected single-cell incorporation of leucine negatively correlated with PAR at sampling and the integrated PAR during the sampling day (Table 6). In contrast, the magnitude of the light effect on incorporation of the amino acid mixture correlated negatively with the mean and peak PAR during the 3 d prior to sampling. Light-affected protein incorporation did not correlate with light conditions prior to sampling.

Table 6. Correlation between the significant light effect on single-cell activity for a given compound (Light – Dark) and light environment. The number of samples was 11 to 13 taken over 3 yr. AA: amino acid. *$p < 0.05$

	Leucine	AA mixture	Protein
Date[a]	0.32	0.04	0.23
PAR at sampling	−0.66*	−0.24	−0.39
Mean PAR[b]	−0.01	−0.66*	0.26
Peak PAR[c]	−0.06	−0.69*	0.02
Sampling time[d]	−0.57	−0.06	−0.05

[a]Time of year
[b]Mean from 08:00–16:00 h during the 3 d preceding sampling
[c]Peak during 08:00–16:00 h averaged over the 3 d preceding sampling
[d]Time of day

DISCUSSION

The abundance of broad phylogenetic groups of bacteria differs among locations and varies with environmental properties (Eilers et al. 2001, Kirchman et al. 2005, Longnecker et al. 2006). Causes for this variation in abundance and activity, including DOM incorporation, are still uncertain. The aim of this study was to assess the abundance of broad bacterial groups and their use of DOM in relation to light and other environmental factors. Temperate estuaries like the Delaware Bay are useful model systems because they change greatly over the seasons (Pennock & Sharp 1986). Our results add to data showing differences in substrate use by broad phylogenetic groups in marine environments, including both seasonal changes and the use of compounds with different molecular weight (Cottrell & Kirchman 2000, 2003, Elifantz et al. 2007). We found variation in abundance and single-cell activity with environmental conditions, including light exposure.

Bacterial group activity differs among compounds of different molecular weight. The fraction of the total bacterial community using low molecular weight amino acids was similar to the fraction using leucine, but only about a third of all leucine-active cells incorporated the high molecular weight protein. High fractions of *Alphaproteobacteria* have been seen to use low molecular weight substrates (Cottrell & Kirchman 2000, Longnecker et al. 2006, Alonso-Saez & Gasol 2007). Our results agree with these findings, as more cells of *Alphaproteobacteria* used leucine than the high molecular weight protein. Members of the *Bacteroidetes*, predominantly *Sphingobacteria* and *Flavobacteria* in marine systems, use high molecular weight material (Cottrell & Kirchman 2000, Kirchman et al. 2005, Malmstrom et al. 2007). The low abundance of *Sphingobacteria-Flavobacteria* in these samples may explain in part the low fraction of cells using protein.

Some studies have tracked the single-cell activity of bacterial groups over seasons (Alderkamp et al. 2006, Alonso-Saez et al. 2007, 2008), but the factors resulting in changes in the abundance of broad bacterial groups remain largely unknown (Cottrell & Kirchman 2003, Alderkamp et al. 2006, Longnecker et al. 2006). The abundance and activity of *Alpha-* and *Gammaproteobacteria* appear to be controlled by different environmental pressures. In our study, the abundance of total *Alphaproteobacteria* did not vary with environmental properties, suggesting a role for top-down factors. The number of leucine-active *Alphaproteobacteria* increased when grazing and viral abundance were reduced in experiments using oligotrophic waters (Longnecker et al. 2010). In contrast, the abundance of *Gammaproteobacteria* did vary significantly with several properties during our study. Other studies have also observed that gammaproteobacterial abundance changes with environmental conditions, including phytoplankton blooms and grazer abundance (Alderkamp et al. 2006, Teira et al. 2008, Longnecker et al. 2010). This group is thought to be specialized to DOM-rich conditions (Kirchman et al. 2005, Alonso-Saez et al. 2007). Whereas *Gammaproteobacteria* are a small fraction of the total community in other marine systems (Alonso-Saez et al. 2007), we found high gammaproteobacterial abundance in the estuary and coastal waters we examined. In our study, the correlation between gammaproteobacterial abundance and total bacterial production, as well as concentrations of DOC and DON, supports the hypothesis that this group is specialized for highly productive conditions (Rehnstam et al. 1993, Eilers et al. 2000).

The SAR11 clade is ubiquitous in many marine environments, but the fraction of the group active in DOM incorporation varies (Rappé et al. 2002, Alonso & Pernthaler 2006, Straza et al. 2010). In the North Atlantic, SAR11 cells have been found to contribute to about half of the leucine and glucose incorporation by the total community, but they are not dominant in the assimilation of protein (Malmstrom et al. 2005). Alonso & Pernthaler (2006) observed a higher proportion of *Roseobacter* cells using glucose than SAR11 cells, consistent with other studies demonstrating variation in glucose use by different types of SAR11 bacteria (Schwalbach et al. 2010). Previous studies have also shown that SAR11 abundance varies seasonally in the Mid-Atlantic Bight and the northwest Mediterranean Sea (Alonso-Saez & Gasol 2007, Campbell et al. 2009). The data presented here provide further evidence that SAR11 cells are active in marine waters and respond to environmental changes. The abundance of SAR11 varied, and the fraction using amino acids and protein

changed between summer and fall. Overall, leucine incorporation by SAR11 cells was stimulated by PAR, in agreement with other results suggesting that SAR11 bacteria in natural communities can use light to supplement energy requirements (Campbell et al. 2009, Lami et al. 2009).

The use of sunlight for energy has been identified as a factor potentially influencing heterotrophic bacterial growth (Kolber et al. 2000, Béjà et al. 2001). The discoveries of aerobic anoxygenic phototrophic bacteria and the presence of rhodopsin genes in many divisions of bacteria necessitate reconsideration of bacterial responses to light and the subsequent impact on DOM cycling (Kolber et al. 2000, Béjà et al. 2001). The light responses of a few bacterial groups have been analyzed, including the incorporation of leucine by *Prochlorococcus* and *Synechococcus* in the light (Church et al. 2004, Michelou et al. 2007). Expression of the proteorhodopsin gene was upregulated in *Flavobacteria* and the SAR11 clade during microcosm experiments with Delaware coastal waters exposed to PAR, though continuous light did not stimulate growth (Lami et al. 2009). The mechanisms by which light affects bacteria may vary among groups, and include stimulation of both ATP production and membrane transport (Morris et al. 2010). However, light can also have negative effects, which vary among bacterial groups. Alonso-Saez et al. (2006) found that single-cell activity of *Alphaproteobacteria* was more affected by prior exposure to UV light than was the activity of *Gammaproteobacteria* cells. One of the effects of UV irradiation may be a switch from lysogenic to lytic state in bacteria infected by viruses (Weinbauer & Suttle 1996). In addition to UV, our results suggest that even PAR can have negative effects on single-cell activity of various bacterial groups, as observed in a few of the light exposure experiments and in the negative correlations between light availability and bulk leucine incorporation. The magnitude of a light effect on bacterial activity, as seen by leucine incorporation, was diminished with greater light exposure prior to our sampling, indicating a potential delayed effect from over-exposure to light (Alonso-Saez et al. 2006).

PAR had a direct effect on bacterial activity in about 20% of our experiments. Rather than a uniform stimulation, the result of light exposure differed between groups and among substrate types, and depended upon natural light conditions prior to sampling. This variability may be due to the abundance and activity of SAR11 bacteria, a large fraction of which have proteorhodopsin genes (Giovannoni et al. 2005, Campbell et al. 2009). SAR11 bacteria, like other members of the *Alphaproteobacteria*, use low molecular weight compounds more than high molecular weight compounds (Malmstrom et al. 2005, Straza et al. 2010). Light use by SAR11 bacteria helps to explain why the magnitude of activity stimulation by light was much greater than inhibition by light and why the use of high molecular weight DOM (protein) did not change with previous light conditions.

Some of the variability in light effects observed in this study and by others (Alonso-Saez et al. 2006, Michelou et al. 2007) may be due in part to methodological limitations. As a result of long doubling times, the bacterial community may take several hours or more to respond to changes. We had to keep these whole-water sample incubations short to avoid 'bottle effects' and limitation of labeled substrate, as well as indirect effects of light through production of DOM by photoautotrophs and grazers. Determining the number of active bacteria by microautoradiography may not detect some effects of light. Also, the bacteria analyzed in this study were broad phylogenetic groups, and even the clade of SAR11 is very diverse (Carlson et al. 2009). The integrated response of a broad group may have masked differences in response by sub-groups, as we observed with *Alphaproteobacteria* and SAR11.

CONCLUSIONS

In this study, we describe the impact of environmental conditions on the single-cell activity of bacteria in a temperate estuary and coastal waters. Environmental changes resulted in characteristic differences in the use of organic compounds by broad phylogenetic groups of bacteria. Like other environmental factors, light has a complex effect on bacterial communities. The broad groups tested here responded differently to light, but more work is needed, including exploring the responses of bacterial groups at finer phylogenetic levels. Characterizing the role of light in the regulation of bacterial activity is an important component of understanding the contribution of bacterial groups to biogeochemical processes.

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