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Impact of light on marine bacterioplankton community structure

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ABSTRACT: Reports of widespread proteorhodopsin and bacteriochlorophyll *a* genes suggest that light may facilitate the growth of many marine bacteria. We tested this hypothesis by conducting 3 light manipulation experiments with bacterial communities obtained off the Southern California coast. In each experiment, duplicate 20 l mesocosms were subjected to either 14:10 h light:dark or continual darkness for 5 to 10 d. Automated Ribosomal Intergenic Spacer Analysis (ARISA), a whole-community fingerprinting technique, was used to determine how light affects bacterial community structure. Light removal resulted in only minor changes in ARISA profiles (mean decrease in similarity between treatments: 6 ± 1% via Sorenson's index [only considers presence/absence of taxa], 15 ± 7% via Pearson's coefficient [considers relative abundance of taxa]) at the conclusion of the experiments. Oligotrophic communities responded nearly twice as strongly to light removal compared to mesotrophic communities. Phototrophs such as cyanobacteria exhibited consistent, sharp declines in dark treatments, indicating whatever heterotrophic abilities they possess were not sufficient to sustain them when faced with natural removal processes such as grazing or viral lysis. Members of the broad SAR86, SAR11, SAR116, CFB and *Roseobacter* phylogenetic groups exhibited minor, mixed responses to light removal, suggesting that only a few select members may rely on phototrophy to a measurable extent. Our results indicate that the majority of non-cyanobacterial bacteria in the ocean do not depend heavily on light to maintain themselves over 5 or 10 d periods in the presence of natural removal processes, and hint that proteorhodopsin and bacteriochlorophyll *a* genes may have alternative, undetermined ecological benefits besides phototrophy.

KEY WORDS: Marine bacterioplankton · ARISA · Proteorhodopsin · Anoxygenic photosynthesis · Bacteriochlorophyll

INTRODUCTION

Recent reports of abundant aerobic, anoxygenic phototrophic bacteria (AAPB) that contain bacteriochlorophyll *a* (Bchl *a*) (Kolber et al. 2000) and the discovery of proteorhodopsin (PR) (Beja et al. 2000) suggest that non-chlorophyll *a* dependent phototrophy may be more common in marine systems than previously realized, and raise new questions about the importance of light to microbial communities. Only recently has the distribution and abundance of bacterial phototrophy genes become more apparent, due largely to increased environmental metagenomic surveys. AAPB genes which encode the pigment binding subunit of the photosynthetic reaction center (*pufLM* genes) have been found in freshwater, estuarine and oceanic DNA samples collected from around the world (Beja et al. 2002, Allgaier et al. 2003, Karr et al. 2003, Koblizek et al. 2003, Oz et al. 2005, Schwalbach & Fuhrman 2005), suggesting that AAPB are widespread. Recently it has also been shown that AAPB can comprise a significant proportion of the bacterial abundance in select locations (Kolber et al. 2001, Oz et al. 2005), most notably in nearshore and estuarine environments (Shiba et al. 1979, Schwalbach & Fuhrman 2005).

Similar genomic surveys for PR genes have also found such genes at numerous locations and depths studied to date, including Monterey Bay, California,

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Hawaii Ocean Time Series (HOTS) station, Palmer Station in Antarctica, and the Mediterranean and Red Seas (Beja et al. 2000, 2001, Sabehi et al. 2003, de la Torre et al. 2003). In addition, Venter et al. (2004) reported nearly 800 new PR genes from the Sargasso Sea, including homologs potentially belonging to members of the *Cytophaga-Flavobacteria-Bacteroides* (CFB) group, following a whole-genome shotgun sequencing effort in the Sargasso Sea. The phylogenetic association of newly discovered photoheterotrophs suggests that some belong to major marine bacterioplankton groups such as the alpha- and gamma-*Proteobacteria*. Given that certain *Proteobacteria* clades such as the SAR11, *Roseobacter* and SAR86 clades combined can account for upwards of 50% (Giovannoni & Rappe 2000) of open ocean 16S rRNA clone libraries, these initial reports suggest that non-chlorophyll *a* dependent phototrophy may conceivably be readily found amongst the most common open ocean marine prokaryotes.

Initial investigations into the ecological benefits of aerobic, anoxygenic phototrophy have been conducted using a variety of approaches. Kolber et al. (2001) used AAPB isolates from the Coastal North Atlantic Ocean, North Eastern and Equatorial Pacific Ocean, Southern Ocean and Mediterranean Sea to investigate the role of AAPB in the ocean carbon cycle. One isolate, NAP1, was reported to fix CO_2 in amounts sufficient to support up to 20% of a cell's energy demands (Kolber et al. 2001), suggesting that mixotrophy may be a common life-history strategy among oceanic AAPB. However, NAP1 is an *Erythrobacter* relative, which are thought to be relatively rare in the open ocean. Hence, additional studies are needed to determine how representative *Erythrobacter* physiology is of more common marine bacteria which contain *puf* genes. Additional cultivation studies by Allgaier et al. (2003) yielded numerous new *Roseobacter* isolates from the North Sea, which were subsequently screened for the presence of *pufLM* genes and Bchl *a* pigment production. Surprisingly, many of the *Roseobacter* isolates associated with dinoflagellates contained functional Bchl *a* genes; however, none of the planktonic *Roseobacter* isolates screened, which presumably live in lower DOM environments than dinoflagellate symbionts, possessed *pufLM* genes. The lack of phototrophy in the planktonic *Roseobacter* isolates is consistent with reports demonstrating that AAPB are more abundant in meso- and eutrophic environments compared to oligotrophic environments (Goericke 2002, Schwalbach & Fuhrman 2005). However, questions remain regarding where in the ocean an anoxygenic phototrophic life-history strategy would be most advantageous to marine prokaryotes.

To date there have been few direct investigations into the ecological benefit of PR, due in part to the difficulty in cultivating bacteria containing the gene. Initial *in vitro* studies suggest that PRs may facilitate ATP production by functioning as a light driven proton pump that is finely tuned to ambient light wavelengths (Beja et al. 2000, Man et al. 2003). Initial field tests of this hypothesis determined that instantaneous growth rates of open ocean bacteria at the HOTS station ALOHA, as measured by leucine incorporation, were 48 to 114% higher in light incubations compared to dark controls (Church et al. 2004). However, it is quite possible that these results are due to cyanobacteria such as *Prochlorococcus*, which are known to take up leucine (Zubkov et al. 2003, 2004) and are highly abundant at Stn ALOHA (Campbell & Vaulot 1993). Thus, it is currently unknown which bacteria are responsible for the results observed by Church et al. (2004) or if PR genes generate additional energy for marine bacteria *in situ*. Furthermore, recent studies indicate that some PRs may function in a sensory capacity rather than as an energy source (Wang et al. 2003). In order to better understand the significance of newly discovered and widely distributed non-chlorophyll *a* dependent phototrophy genes, it is important to learn to what extent, if any, marine bacteria rely directly on sunlight as an energy source. This prompted us to investigate changes in marine bacterioplankton community structure and bacterial abundances following the removal of light.

MATERIALS AND METHODS

Light manipulation experiments. Seawater was collected at the San Pedro Ocean Time Series (SPOTS) station ~15 km southwest of Los Angeles (33° 33′ N, 118° 24′ W) for phototroph experiments P1 and P2 in July and September 2002 at 13 and 20 m depth, respectively. Water for phototroph experiment P3 was collected in April 2003 from 5 m depth at the California Cooperative Oceanic Fisheries Investigations (CalCOFI) station 87.110 (31° 19.4′ N, 123° 44.6′ W), ~570 km west of Ensenada, Mexico. Sampling depths for Expts P1 and P2 corresponded with 16°C isotherms because facilities were available for incubation at that temperature. All sites were characterized by moderately low chl *a* concentrations: 0.75, 0.66 and 0.07 µg chl *a* l^{-1} with *in situ* light levels of approximately 350, 200 and 750 µE m^{-2} s^{-1} (Expts P1, P2 and P3, respectively) at the time of sample collection.

At each site, seawater was collected using Niskin bottles and subsequently transferred to duplicate 20 l polycarbonate carboys that had been rinsed once with 1.2 N HCl and 3 times with small amounts of collected seawater prior to being filled.

Light levels were manipulated in all experiments by wrapping each 'dark treatment' carboy in 3 heavy-

duty black plastic bags, while 'light treatment' carboys remained exposed to light. Following collection and light manipulation, SPOTS mesocosms were stored in unlit coolers during transport to the laboratory (3 h maximum) and upon arrival were incubated in a walk-in cooler at 16°C. Light treatments in Expts P1 and P2 experienced approximately 75 µE m^{-2} s^{-1} of light from fluorescent bulbs (F40 PL/AQ wide spectrum Plant & Aquarium, General Electric). Following water collection in Expt P3, CalCOFI mesocosms were placed in a flow-through deck incubator that was maintained at ambient seawater temperature (15°C). The deck incubator was covered with a single layer of shade cloth and light treatment carboys experienced a maximum of approximately 400 µE m^{-2} s^{-1} of natural sunlight throughout the experiment. In all experiments, carboys exposed to light experienced a 14:10 h light:dark cycle (on-off in Expts P1 and P2, natural diel variation in Expt P3), while plastic-wrapped, dark treatments were incubated in complete darkness (<0.1 µE m^{-2} s^{-1}) for the duration of the experiment. Mesocosms were sampled regularly for total bacteria counts and bacterial community fingerprint analysis via Automated Ribosomal Intergenic Spacer Analysis (ARISA).

Bacteria enumeration. Samples for bacteria counts were collected at each time point, preserved with formalin (1% final vol:vol) and stored at 4°C in the dark until the time of slide preparation. All bacteria in duplicate 5 ml samples of preserved seawater were stained using 40 µl of 0.1 mg ml^{-1} 4′,6-diamidino-2-phenylindole (DAPI) solution (Sigma Chemical) (Porter & Feig 1980) and enumerated manually using an Olympus BX60 microscope.

Community structure analysis. ***DNA collection:*** Bacterial community DNA from light manipulation experiments was collected at each time point by filtering 1 l of water from each mesocosm through a 47 mm 0.22 µm Durapore GV (Millipore) filter. Following filtration, the filters were transferred to individual polyethylene whirl-pak bags and stored at −80°C until the DNA could be extracted. Community DNA was extracted using hot sodium dodecyl sulfate to lyse the cells followed by phenol:chloroform:isoamyl alcohol extractions and ethanol precipitation as described by Fuhrman et al. (1988).

Community fingerprinting via ARISA: The internal transcribed spacer (ITS) region between the 16S and 23S ribosomal genes was amplified using a method slightly modified from the original description (Borneman & Triplett 1997). From all samples, 2 ng of extracted community DNA (quantified via Pico Green fluorescence; Molecular Probes) were amplified in 50 µl reactions that contained the following (final concentrations): 1X PCR Buffer (Promega); 2.5 mM $MgCl_2$ (Promega); 250 µmol of each dNTP (Nucleotide Mix; Promega); 2.5U *Taq* DNA Polymerase (Promega); 2 ng $µl^{-1}$ BSA (Sigma 7030); and 40 pmol of each primer. ARISA primers were the 16S rDNA universal 1392 (*Escherichia coli* numbering) forward primer (5′-GYACACACCGCCCGT-3′) (melting temperature, T_m: 60.3°C) and a fluorescently labeled 23S bacterial specific 130 (*E. coli* numbering) reverse primer ((TET)-5′-GGGTTBCCCCATTCRG-3′) (T_m: 59.7°C) (Borneman & Triplett 1997). Note that these primers will amplify bacteria as well as eukaryotic plastids. Eukaryotic plastid DNA was present because we did not pre-filter the water prior to DNA collection. All PCR reactions were amplified in a PerkinElmer Cetus GeneAmp 9600 thermocycler beginning with an initial 3 min at 95°C followed by 30 cycles of the following: denaturing for 45 s at 95°C, annealing for 45 s at 55°C, and extending for 1.5 min at 72°C, followed by a 5 min final extension at 72°C. Following amplification, ARISA PCR products were cleaned and concentrated using Qiagen MinElute PCR purification kit (#28004). PCR products were quantified using a Pico Green nucleic acid quantification kit (Molecular Probes) and diluted with deionized water to a final concentration of 5 ng $µl^{-1}$ prior to fragment analysis. ARISA PCR products were run in duplicate on an ABI 377XL automated sequencer for 5 h, each lane containing either 1.5 µl of ABI Genescan 2500 basepair (bp) Tamra labeled size standard or 0.5 µl of Bioventure 1500 bp ROX labeled size standard.

ARISA fingerprint analysis: Raw ARISA fingerprints were analyzed using Genescan software (Applied Biosystems). ARISA fingerprints were manually binned to minimize spurious and non-replicated peaks in electropherograms and the percent area (integrated fluorescence) of each operational taxonomic unit (OTU) was calculated for all ARISA fingerprints. Resulting fingerprints within an experiment were compared using several analyses. Initially, they were compared as a whole to each other using a Sorenson's index (Sorenson 1948) and via the comparison of Pearson's correlation coefficients generated from a full factorial matrix of pairwise linear regressions of ARISA samples. The Sorenson's index (SI) was calculated as follows:

$$SI = 2W/(a_1 + a_2) \quad (1)$$

where W is the number of shared OTU and a_1 and a_2 are the total number of OTU in ARISA Fingerprints 1 and 2, respectively. Sorenson's index detects changes based upon presence/absence of identically sized OTU while the Pearson's coefficient determines the similarity of 2 ARISA fingerprints based upon the relative abundance of identically sized OTU. Sorenson indices and Pearson coefficients were multiplied by 100 and are expressed as percentages throughout. Therefore, in this study a Sorenson's index of 100% indicates that

all OTU are shared between 2 ARISA fingerprints while a value of 0% indicates that no OTU are shared between 2 fingerprints. Pearson correlation coefficients of 100% indicate that all OTU were shared and in identical proportion between 2 fingerprints while a value of 0% indicates that no identical peaks were shared between 2 fingerprints in similar proportion. The combination of both indices allows for the detection of changes due to the presence/absence of OTU as well as the detection of more subtle across treatment differences in the magnitude of shared OTU.

To complement similarity calculations based upon overall ARISA profile similarity, the difference in relative percent area and the magnitude of change in relative percent area was calculated for individual OTU within and between treatments as well. The difference between the relative percent area individual OTU comprised in the light versus the dark was calculated for all OTU as follows:

$$A_{nL} - A_{nD} \quad (2)$$

where A is the relative percent area of an OTU (n) comprised in the light treatment (L) or dark (D) treatment. For example, the difference between an ARISA OTU which comprised 10% of the relative percent area in light treatments but only 8% of the relative percent area in dark treatments was recorded as a difference of 2% (not 20%) between treatments. The magnitude of change in percent area was calculated for individual OTU which were relatively more abundant in light treatments as follows:

$$A_{nL}/A_{nD} \quad (3)$$

For example, an ARISA OTU which comprised 0.8% of the total ARISA area in light treatments and 0.1% in dark treatments was recorded as an 8-fold change. Eq. (3) was slightly modified if an OTU comprised a higher relative percentage of the integrated area in dark treatments to the following:

$$-(A_{nD}/A_{nL}) \quad (4)$$

For example, an ARISA OTU which comprised 0.1% of the total ARISA area in light treatments and 0.8% in dark treatments was recorded as a –8-fold change. The ratio was multiplied by negative one for consistency purposes; hence positive values denote OTU which comprised a larger relative percent area in light treatments, whereas negative values indicate an OTU which comprised a larger relative percent area in dark treatments.

Eqs. (2) to (4) were used to determine the reproducibility of replicates by quantifying how similar in relative percent area individual OTU were within replicate treatments. Eqs. (2) to (4) were then used to determine how similar in relative percent area individual OTU were between treatments. Differences between treatments were deemed significant when OTU differed in relative percent area between treatments by 2.5 standard deviations or more (which roughly correlates to a p-value of ≤0.01) of the mean reproducibility of replicates, similar to Tao et al. (1999). In this study, 1 standard deviation of the mean difference between replicates was calculated as ~0.8% in all experiments and the standard deviation of the mean magnitude of change between replicates was calculated as ~1-fold. Thus significance levels were calculated to equate to either a difference of ~2% in relative percent area between treatments or an approximate 2.5-fold magnitude of change between treatments.

Identification of ARISA peaks: Putative identifications of ARISA OTU were assigned using a clone library constructed from environmental DNA collected from the SPOTS station, the details of which are presented elsewhere (Brown et al. in press) and using GenBank. Typically, clones were obtained which corresponded to the most abundant OTU obtained via ARISA. However, many of the smaller OTU could not be identified and in a few instances a single OTU would correspond to more than 1 phylogenetic association and thus the identity could not be resolved. Briefly, clone libraries were constructed from PCR products which were amplified using the 16S 27-forward (*Escherichia coli* numbering), 5′-AGAGTTTGATCMTGGCTCAG-3′ (T_m: 59.4°C), (Lane et. al. 1985) and 23S 130-reverse (*E. coli* numbering), 5′-GGGTTBCCCCATTCRG-3′ (T_m: 59.7°C) (Borneman & Triplett 1997) primers. ITS sequences were determined as follows: environmental DNA was amplified in 100 µl PCR reactions that contained a final concentration of: 1X PCR AmpliTaq Gold® buffer (Applied Biosystems), 2.5 mM $MgCl_2$ (Applied Biosystems), 350 µM of each dNTP (Promega), 800 nM of each primer, 40 ng μl^{-1} BSA, 5 U AmpliTaq Gold®, and 0.1 ng μl^{-1} of template DNA. PCR reactions were held at 94°C for 10 min followed by 24 cycles of 94°C for 40 s, 55°C for 40 s and 72°C for 3 min with a final step at 72°C for 7 min. The amplified PCR products were purified using Zymo Clean and Concentrator-5 PCR purification columns and quantified by Pico Green fluorescence (Molecular Probes). Following quantification, PCR products were ligated into pGEM®-T Easy (Promega) plasmid vectors, which were subsequently used to transform JM109 High Efficiency Competent Cells (Promega). Plasmids containing inserts were isolated from 3 ml of cultures grown overnight using the QIAprep Spin Miniprep Kit (Qiagen). Sequencing reactions were conducted using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences) and contained 5 pmol of the M13 sequencing primer and the 16S Eubacteria specific 1392 primer. Sequences were run on an ABI 377XL automated sequencer according to the manufacturer's instructions.

Estimates of ARISA fragment lengths were determined directly from sequence data and include the 1392-forward and 130-reverse primer regions of the 16S and 23S small ribosomal subunits. Phylogenetic affiliations of clones were determined using the BLAST tool available on the GenBank website (http://www.ncbi.nlm.nih.gov).

RESULTS

General patterns of bacterial abundance were relatively similar between treatments within experiments; however cell counts were marginally higher in light treatments ($p = 0.05$) at the conclusion of Expt P1, but did not significantly differ at the conclusion of Expts P2 and P3 ($p = 0.07$ and 0.26, respectively) (Fig. 1A–C). The number of OTUs in ARISA profiles were also relatively similar between treatments in all 3 experiments (Fig. 1D–F). However, ARISA profiles collected from the oligotrophic CalCOFI station had, on average, fewer and different OTU than ARISA profiles collected at the mesotrophic SPOTS station. (Fig. 1D–F, Table 1). The Sorenson index (SI) and Pearson correlation coefficients (R^2) indicate that ARISA profiles were highly reproducible within and between treatments at the beginning of the experiments (mean ± SE; SI: 89 ± 5%, R^2: 88 ± 5%). In all experiments the similarity between treatments decreased after 5 or 10 d of light manipulation (mean ± SE; SI: 81 ± 5%, R^2: 74 ± 3%) (Table 1). Pearson correlation coefficients were consistently lower than SI for all experiments. ARISA profiles from final time points were highly dissimilar from initial ARISA profiles (SI: 78 ± 5%, R^2: 55 ± 2%) (Table 1).

Two separate statistical analyses indicate that the majority of individual OTU exhibited a minor response to light manipulation (Fig. 2). The relative percent area of the majority of individual OTU differed between treatments by less than 2%, with most OTU differing by less than 1% relative percent area between treatments (Fig. 2A–C). Similarly, the magnitude of change

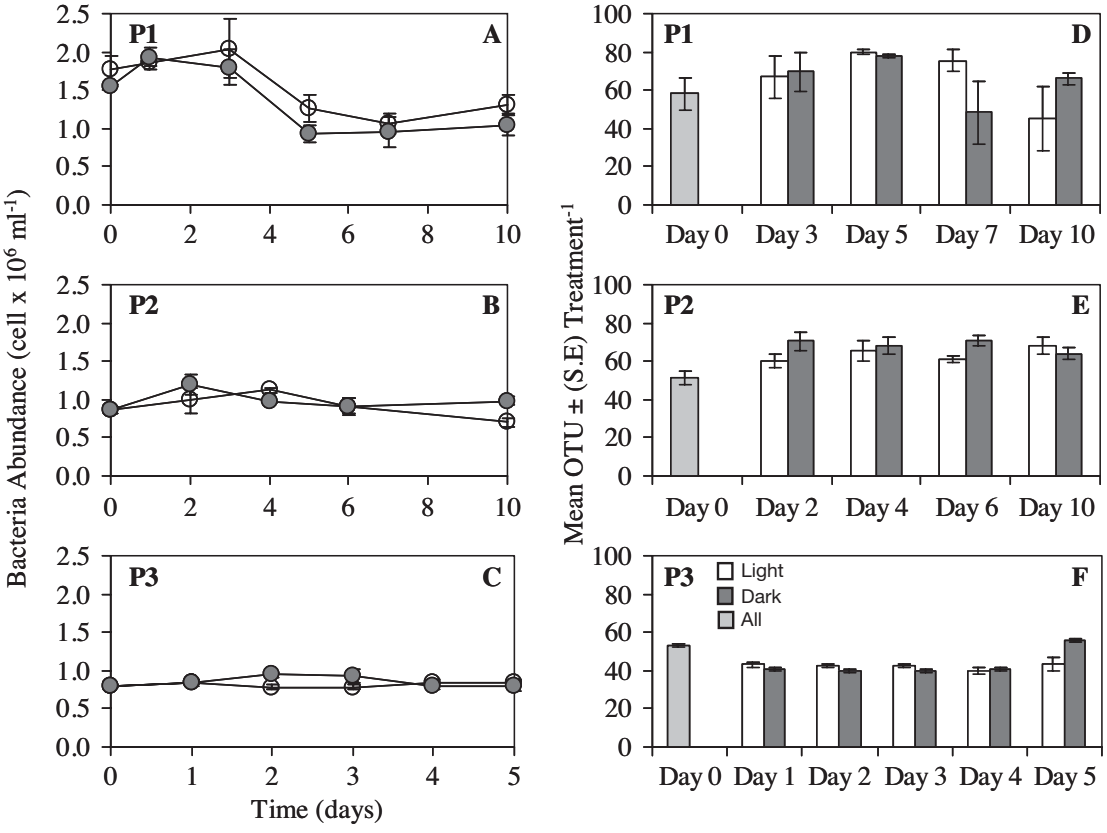

Fig. 1. (A–C) Cell counts from light manipulation experiments conducted in July 2002 (Expt P1), September 2002 (Expt P2), and April 2003 (Expt P3). Error bars indicate ±1 SD from the mean. Student's *t*-tests determined that cell densities were significantly greater in light treatments at the conclusion of Expt P1 ($p = 0.05$), but were not significantly different at the conclusion of Expts P2 ($p = 0.07$) or P3 ($p = 0.26$). (D–F) Number of operational taxonomic units (OTU) in ARISA profiles over time in phototroph experiments (P1, P2, P3). Error bars indicate standard error of the mean (±SE) of duplicate treatments. White bars indicate light treatments, dark gray bars indicate dark controls, and light gray bars indicate OTU abundance in both light and dark treatments at time zero

Table 1. Descriptive information and ARISA similarity statistics for all experiments (P1 to P3). Sorenson and Pearson similarity statistics are expressed as percentages (mean ± SE). A Sorenson's index or Pearson's coefficient of 100% indicates a perfect match between 2 ARISA profiles. Initial ($T_{initial}$) similarity calculations are based upon pairwise comparisons of ARISA profiles at the onset of the experiments while final (T_{final}) similarity calculations are based upon pairwise comparisons of ARISA profiles at the end of the mesocosm experiments. SPOTS: San Pedro Ocean Time Series station; CalCOFI: California Cooperative Oceanic Fisheries Investigations station; OTU: operational taxonomic units

	P1	P2	P3	Mean ± SE
Date	Jul 02	Sep 02	Apr 03	
Sample location	SPOTS	SPOTS	CalCOFI	
Initial chl *a* (µg l^{-1})	0.75	0.66	0.07	
Temperature (°C)	16	16	15	
Incubation duration (d)	10	10	5	
Avg. OTU # per ARISA profile	64 ± 4	63 ± 3	43 ± 2	
Sorenson (%)				
$T_{initial}$: Light vs. Dark	79 ± 4	94 ± 1	90 ± 2	88 ± 4
T_{final}: Light vs. Dark	72 ± 15	90 ± 1	82 ± 2	81 ± 5
$T_{initial}$ vs. T_{final}: all treatments	69 ± 8	78 ± 2	87 ± 4	78 ± 5
Pearson (%)				
$T_{initial}$: Light vs. Dark	80 ± 3	96 ± 1	92 ± 3	89 ± 5
T_{final}: Light vs. Dark	74 ± 8	68 ± 2	80 ± 9	74 ± 3
$T_{initial}$ vs. T_{final}: all treatments	54 ± 1	58 ± 2	53 ± 11	55 ± 2

in relative percent area between treatments also differed by less than 2.5-fold, with the majority of OTU varying by less than 2-fold between treatments (Fig. 2D–F). In Expts P1 and P2, 8 and 17 individual OTU, respectively, had either a 2% or 2.5-fold increase in light treatments or were absent from dark treatments, whereas in Expt P3, 5 individual OTU met the same criteria (Tables 2 & 3). This corresponds to approximately 13% of the OTU in Expt P1, 27% in Expt in P2 and 12% in Expt P3.

Clone libraries constructed at the SPOTS station allowed for putative identification of many of the most dominant OTU in ARISA profiles. Phylogenetic associations were assigned to OTU when possible; however, not all OTU within ARISA profiles were identified. Putative members of the cyanobacteria clade did consistently better in light compared to dark treatments and in all experiments the largest positive differences in relative percent area between treatments were associated with *Synechococcus* (OTU: 1052, 1055) and plastid-containing (OTU: 576) organisms (Tables 2 & 3). *Synechococcus* and *Prochlorococcus* virtually disappeared from dark controls in Expts P1 and P2 (OTU: 906, 820, 1052 and 1055; Table 2); however, in Expts P2 and P3 select members of the cyanobacteria clade were detectable via ARISA in dark treatments (OTU: 828, 1106 and 1130; Tables 2 & 3). The dominant *Prochlorococcus* OTU in Expt P3 (OTU 828, data not shown) decreased from 16 to 4% of the relative percent area in both light and dark treatments after 5 d of light manipulation.

In contrast to the cyanobacteria, members of major non-cyanobacterial groups (e.g. SAR11, *Roseobacter*, CFB, and SAR86) exhibited all possible responses to light manipulation (light preference, dark preference or no preference) (Tables 2 & 3). Putative members of the SAR11 clade (OTU: 636 and 710) exhibited a positive preference for light; however, a larger number of SAR11 (OTU: 664, 709, 712, 718) exhibited a preference for dark treatments (Tables 2 & 3). Similarly, putative members of the *Roseobacter* clade exhibited both a positive (OTU: 986) or negative (OTU: 947, 953, 995, 1154, 1192, 1217) preference for light at the conclusion of the mesocosms. OTU tentatively identified as members of the SAR86 clade mostly exhibited a preference for dark treatments (OTU: 459, 466, 527, 531, 535); however, one member of the SAR86 clade (OTU: 450) exhibited a preference for light treatments. Bacteria tentatively assigned to the CFB clade also had a non-uniform response to light removal. Positive preference for light was observed for CFB (OTU: 636, 641, 785) although other members of this group exhibited a preference for dark treatments (OTU: 623, 652, 728, 769, 786, 857, 966). Finally, marine bacteria tentatively assigned to the SAR116 (OTU: 657), SAR406 (OTU: 626) and *Marinobacter* (OTU: 591) clades exhibited some preference for light treatments as well. However, other members of the SAR116 (OTU: 655) and SAR406 (OTU: 623) clades exhibited a preference for dark treatments (Tables 2 & 3). Finally, 4 OTU (560, 626, 906, 1052), 9 OTU (510, 557, 591, 601, 604, 608, 785, 796, 820, 1055) and 1 OTU (840) were only present in light treatments in Expts P1, P2 and P3, respectively.

DISCUSSION

Four separate statistical analyses indicate that the majority of non-cyanobacteria exhibited only minor responses to light removal (Table 1, Fig. 2). The degree of similarity between treatments at the conclusion of the experiments ranged between 4 and 28% (Table 1) and, given that the oligotrophic CalCOFI mesocosms were incubated for 5 rather than 10 d, suggests that the response to light may have been nearly twice as strong in communities from more oligotrophic locations. The relatively minor changes in SI indicates that the majority of OTU were present in both treatments throughout

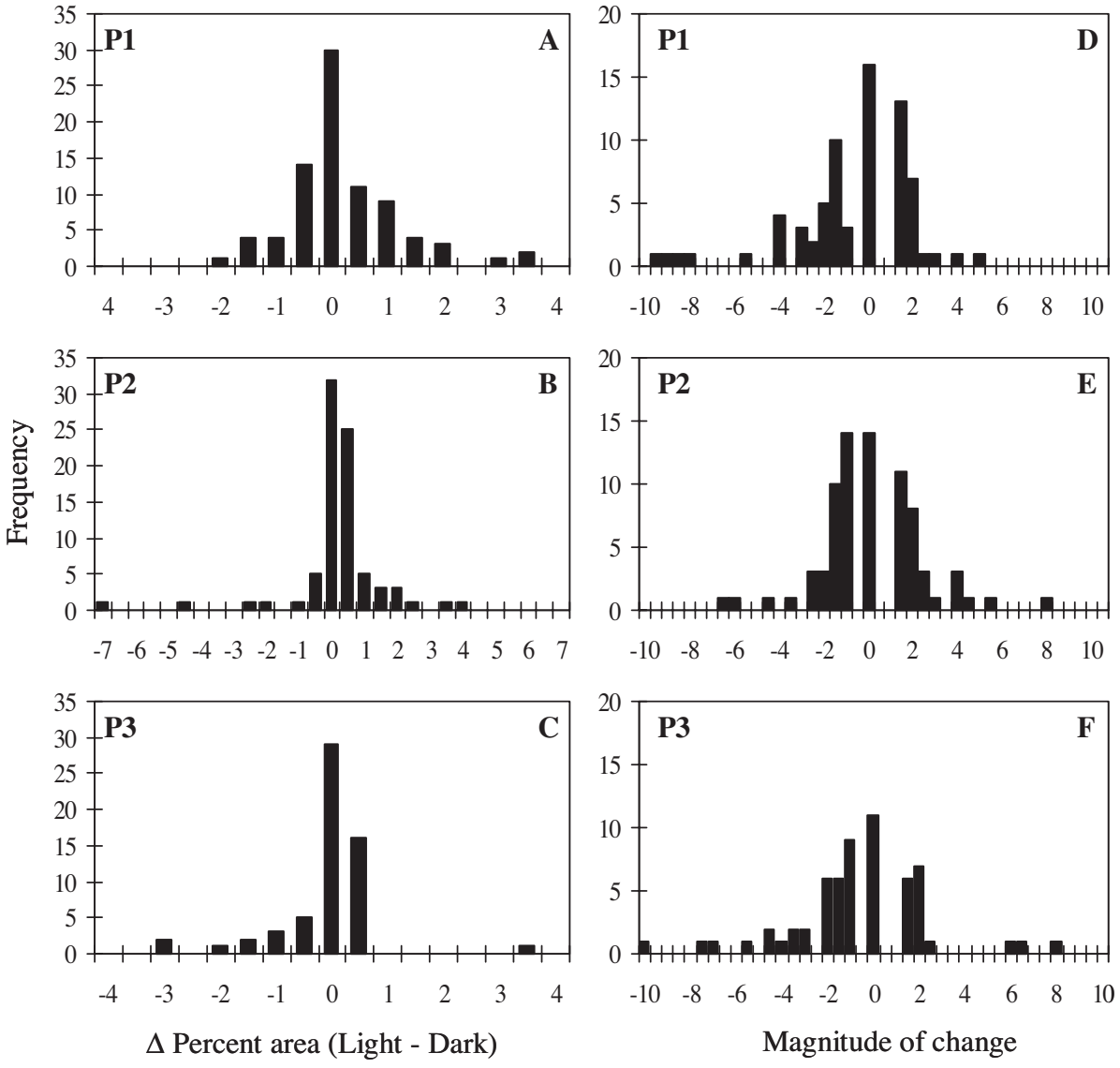

Fig. 2. Summary of extent of change of all OTU in all experiments, illustrating that most OTU changed very little following changes in ambient light levels. (A–C) Histograms representing the difference in mean percent area between light and dark treatments after 10 (Expts P1 and P2) or 5 d (Expt P3) of light manipulation. (D–F) Histograms representing the magnitude of change (multiplication factor) between light and dark treatments. OTU present in only 1 of the treatments are indicated with a zero. OTU which were more abundant in the light are represented by positive values whereas those more abundant in the dark are represented with a negative value. Note that the majority of changes between treatments were minor; hence values cluster closely to zero in all cases

the experiments. However, 14 OTU were only present in light treatments, but nearly half of these were identified as putative cyanobacteria or plastids (Table 2). Interestingly, the DNA of the dominant *Prochlorococcus* in Expt P3 was present in both treatments throughout the duration of the mesocosm. It has been reported recently that members of the *Prochlorococcus* genus can take up amino acids from the environment (Zubkov et al. 2003, 2004), suggesting that some degree of mixotrophy exists within this genus. The extended survival of the most abundant *Prochlorococcus* in the dark treatments in Expt P3 (OTU 828) may be partially due to a form of mixotrophy. However, in all experiments cyanobacteria and plastid-containing organisms exhibited the strongest preference for light and virtually disappeared from dark treatments, suggesting that most cyanobacteria are not capable of extended survival in total darkness in the presence of natural removal processes such as protistan grazing and viral lysis.

Table 2. Individual ARISA OTU from mesotrophic bacterial communities which were deemed most different between treatments at the conclusion of Expts P1 and P2. Δ Mag.: magnitude of change in relative percent area across treatments (e.g. 2-fold); Δ%: difference in relative percent area across treatments (e.g.%Light–%Dark). Positive values indicate light preference; negative values indicate dark preference. OTU present in one treatment but absent from the other lack a ΔMag. value. Phylogenetic groups of ARISA OTU were tentatively assigned when possible. Accession numbers of identifying clones are listed in parentheses. OTU values in **bold** were deemed significantly different ($p \approx 0.01$) between treatments at the conclusion of the experiment (see text for details). CFB: *Cytophaga-Flavobacteria-Bacteroides*

P1				P2			
OTU	Δ Mag.	Δ%	Putative group ID	OTU	Δ Mag.	Δ%	Putative group ID
560		0.5	Plastid (SPOTSDEC00_5m41)	**450**	7.5	0.2	SAR86 (AY033311)
569	3.0	1.5	Plastid (SPOTSDEC00_5m16)	**481**	2.5	0.8	
626		0.1	SAR406 (DQ009157)	510		0.2	
657	1.2	3.0	SAR116 (DQ009267)	**554**	3.6	1.9	
710	4.7	0.3	SAR11 (DQ009163)	**557**		3.6	Plastid (SPOTSDEC00_5m41)
906		0.3	*Prochlorococcus* (DQ009327)	**567**	2.6	0.2	Plastid (SPOTSDEC00_5m16)
986	3.9	2.7	*Roseobacter* (DQ009283)	591		1.3	*Marinobacter* (AF408811)
1052		3.3	*Synechococcus* (DQ009323)	601		0.1	
				604		0.3	
418	–3.3	–0.2	Actinobacteria (DQ009121)	**608**	3.7	0.4	
431		–0.4	Actinobacteria (DQ009124)	**636**	4.4	2.0	CFB (DQ009096) /
459		–0.1	SAR86 (AY033304)				SAR11 (AF151250)
479		–0.1		**641**	3.7	1.9	CFB (DQ009095)
483		–0.5		785		0.2	CFB (DQ009098) /
491		–0.3					γ-Proteobacteria (DQ009139)
515		–0.5		796		0.4	
521		–0.1		820		1.4	*Prochlorococcus* (AF397687)
527	–9.2	–0.5	SAR86 (AF268219)	**828**	1.8	2.2	*Prochlorococcus* (DQ009354)
531	–8.6	–0.6	SAR86 (DQ009145)	**1055**		3.4	*Synechococcus* (DQ009326)
550	–8.0	–1.7					
618	–5.6	–1.9	*Fibrobacter* (DQ009159)	**435**	–2.6	–2.2	Actinobacteria (DQ009124)
623	–9.9	–1.7	CFB (DQ009091) /	**466**	–6.1	–0.8	SAR86 (AY033326)
			SAR406 (DQ009157)	**573**	–6.9	–0.4	
630	–2.8	–0.6		**652**	–2.0	–2.8	CFB (DQ009097)
654	–3.5	–1.1	SAR116 (DQ009264)	**655**	–0.6	–7.2	SAR116 (DQ009264)
718	–2.6	–1.9	SAR11 (DQ009166)	**712**	–3.6	–4.5	SAR11 (DQ009160)
734	–4.0	–0.2	*Verrucomicrobia* (AY033323)	769		–0.1	CFB (SPOTSAPR01_5m120)
857	–4.0	–0.7	CFB (DQ009114)	847		–0.1	β-Proteobacteria (DQ009361)
887	–4.3	–1.0		**877**	–2.9	–0.4	
916	–4.2	–0.6		**887**	–2.9	–0.5	
947	–2.3	–2.1	*Roseobacter* (DQ009289)	**1217**	–4.8	–0.7	*Roseobacter* (DQ009307)
966		–1.4	CFB (DQ009115)				
995		–0.6	*Roseobacter* (DQ009279)				
1012		–0.3					

During comparison of ARISA profiles, excluding Sorenson calculations, we assumed that the integrated area under peaks in ARISA electropherograms was a measure of the relative quantity of DNA in those peaks, and that more initial template DNA results in more amplified PCR product and larger ARISA peaks. However, it is known that PCR may not proportionally reflect overall community structure for a variety of reasons (Suzuki & Giovannoni 1996, Polz & Cavanaugh 1998, Leuders & Friedrich 2003); thus fingerprints were interpreted and compared with caution. Yet despite the numerous caveats of PCR, ARISA fingerprints have also been shown to be highly reproducible (Fisher et al. 2000, Schwalbach et al. 2004); thus limited comparisons are likely possible among fingerprints based on more than a presence/absence criterion. In an effort to minimize the effects of PCR biases, only identically sized OTU within and between treatments in a given experiment were directly compared. Therefore, no comparisons between unequally sized OTU within or across experiments were made. Furthermore, ARISA profiles were more similar across treatments at the experiment's conclusion than final ARISA profiles were to initial ARISA profiles (Table 1), suggesting that similar containment effects were occurring in all mesocosms.

Van Mooy et al. (2004) recently reported a strong correlation between terminal restriction fragment length polymorphism (TRFLP) patterns of representative alpha- and gamma-*Proteobacteria* and %PAR along a transect in the eastern subarctic North Pacific. However, in this study members of broad non-

Table 3. Individual ARISA OTU from an oligotrophic bacterial community which were deemed most different between treatments at the conclusion of Expt P3. See Table 2 legend for further details

			P3
OTU	Δ Mag.	Δ%	Putative group ID
576	1.7	18.2	Plastid (SPOTSDEC00_5m12)
840		0.1	γ-Proteobacteria (DQ009128) / β-Proteobacteria (DQ009365)
853	1.8	3.3	α-Proteobacteria (DQ009126)
860	6.4	0.3	γ-Proteobacteria (DQ009315)
1106	6.0	0.3	*Prochlorococcus* (AF397704)
535	−4.6	−2.3	SAR86 (DQ009143)
607	−3.2	−0.6	CFB (DQ009082)
617	−4.0	−3.2	*Fibrobacter* (DQ009159)
664	−1.2	−3.1	SAR11 (DQ009202)
709	−7.6	−0.5	SAR11 (DQ009163)
718		−0.6	SAR11 (DQ009166)
728	−7.2	−0.3	CFB (DQ009085)
734		−0.3	*Verrucomicrobia* (AY033323)
786	−3.0	−0.2	CFB (DQ009098) / γ-Proteobacteria (DQ009139)
834	−5.9	−0.3	
871		−0.1	
889		−0.1	
946		−0.1	*Roseobacter* (DQ009289)
953	−4.8	−0.3	*Roseobacter* (DQ009291)
1024	−4.3	−0.1	α-Proteobacteria (AY033324)
1154		−0.6	*Roseobacter* (DQ009296)
1192		−0.2	*Roseobacter* (DQ009302)

cyanobacterial phylogenetic clades of marine bacteria did not respond uniformly to light manipulation (Tables 2 & 3). Rather, OTU putatively assigned to non-cyanobacterial clades exhibited mixed responses to light treatments, suggesting that only select subsets within broad phylogenetic groups may gain a direct benefit from sunlight. Mixed responses to light manipulation would be consistent with mounting evidence of high levels of microheterogeneity within broad marine groups (García-Martínez & Rodríguez-Valera 2000, Suzuki et al. 2001, Rocap et al. 2002, Acinas et al. 2004, Venter et al. 2004), and suggest that a variety of ecophysiologies may exist within a single phylogenetic clade of marine bacteria. ARISA fingerprinting is capable of delineating between much of the microheterogeneity within broad marine bacterial clades to a greater extent than other fingerprinting techniques (Fisher & Triplett 1999, Brown et al. in press, Brown & Fuhrman unpubl.), thus allowing for a more detailed analysis of community structure in this study than previous community analyses.

The sole member of the SAR86 cluster, which exhibited a stronger preference for light in Expt P2 (OTU 450), was tentatively assigned to the SAR86 subgroup I clade (Suzuki et al. 2001). Select members of SAR86 subgroup I have been shown to contain PR genes (Sabehi et al. 2004). It is currently unknown if all members of SAR86 subgroup I possess PR. If this is the case then the response of OTU 450 may suggest that organisms which possess PR may gain a direct ecological benefit from sunlight under certain circumstances. Similarly, PR-like genes have recently been reported in a putative member of the CFB (Venter et al. 2004), which may explain the increase of tentatively identified CFB (OTU: 636, 641, 785) in light treatments of Expt P2 (Table 2). Our results also indicate that select members of the SAR116 clade may potentially have a phototrophic component to their metabolism (Table 2). However, it is unknown to date if members of the CFB and SAR116 groups posses PR-like genes, highlighting the need for more directed sequencing efforts to determine the phylogenetic source of many of the rhodopsin-like genes reported by Venter et al. (2004). Finally, only a few (OTU: 636, 710) putative members of the widely distributed SAR11 group appeared to benefit from light to a measurable extent, with the majority exhibiting a stronger preference for dark treatments in all 3 experiments (Tables 2 & 3). Given that all non-cyanobacterial groups included in the mesocosm exhibited minor, mixed responses to changes in ambient light levels, newly discovered genes such as PR may confer alternative, unknown ecophysiological benefits other than phototrophy to marine bacteria.

Recent reports of widespread Bchl *a* genes among members of the *Roseobacter* group suggests that light may be important to the maintenance of these organisms (Allgaier et al. 2003, Oz et al. 2005). However, in these studies the majority of putative *Roseobacter* members exhibited a strong preference for dark treatments. This may be due to members of the *Roseobacter* group responding to increased phytoplankton decay products in dark treatments compared to light treatments since it has been shown that *Roseobacter* abundances are correlated with phytoplankton blooms in nature (Gonzalez et al. 2000, Zubkov et al. 2001). If phototrophic members of the *Roseobacter* group were present within these experiments, our results suggest that they do not require light under all circumstances or that they comprise a minor subset of this broad phylogenetic group. More work is needed to determine if and when members of this ubiquitous marine group engage in phototrophy.

It is difficult to know if the responses observed in these experiments are direct treatment effects resulting from light manipulation or indirect effects resulting from complex food web dynamics or containment. However, given the strong response of autotrophic cyanobacteria and plastid-containing organisms to light removal, it is unlikely that indirect effects would

allow obligate phototrophs, or even those depending significantly on light, to survive the normal turnover processes like grazing and viral lysis that occurred during the extended periods of darkness imposed in these experiments. The response of the cyanobacteria in this study is important because we assumed that obligate phototrophs such as cyanobacteria would exhibit a negative effect in the dark. Since the majority of ARISA OTU did not exhibit as strong an effect as cyanobacteria to light manipulation, we conclude that the majority of non-cyanobacteria in these mesocosms were not as dependent on light as cyanobacteria. However, our results do not allow us to deduce the underlying ecophysiology of non-cyanobacterial taxa; hence the genetic underpinnings of the observed responses in these mesocosms remain uncertain. Furthermore, how representative the results from these mesocosm studies are of natural bacterioplankton dynamics and other mesotrophic and oligotrophic waters is unknown. Experiments were analyzed on relatively short time scales (approximately 5 generations or less per experiment) and it may be likely that the removal of light over longer time scales would result in more substantial changes in community structure.

The ability to isolate putative phototroph genes from numerous locations around the globe suggests that augmenting a heterotrophic metabolism with light derived energy may be a common strategy among marine bacteria. Results from the present study suggest the opposite though, with only a small percentage of non-cyanobacteria in mesocosms exhibiting even a modest preference for light. More work is needed to determine the phylogenetic affiliations of marine bacteria containing phototrophy genes and to investigate the potential metabolic and ecological benefits that newly identified phototrophy genes, such as proteorhodopsin, may provide.

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