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Aerobic anoxygenic phototrophic bacteria in the Mid-Atlantic Bight and the North Pacific Gyre

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Abstract

The abundance of aerobic anoxygenic phototrophic (AAP) bacteria, cyanobacteria and heterotrophs was examined in the Mid-Atlantic Bight and the central North Pacific gyre using infrared fluorescence microscopy coupled with image analysis and flow cytometry. AAP bacteria comprised 5% to 16% of total prokaryotes in the Atlantic but only 5% or less in the Pacific. In the Atlantic, AAP bacterial abundance was as much as 2-fold higher than *Prochlorococcus* and 10-folder higher than *Synechococcus*. In contrast, *Prochlorococcus* outnumbered AAP bacteria 5- to 50-fold in the Pacific. In both oceans, subsurface abundance maxima occurred within the photic zone, and AAP bacteria were least abundant below the 1% light depth. Concentrations of bacteriochlorophyll *a* (BChl *a*) were low (~1%) compared to chlorophyll *a*. Although the BChl *a* content of AAP bacteria per cell was typically 20- to 250-fold lower than the divinyl-chlorophyll *a* content of *Prochlorococcus*, in shelf break water the pigment content of AAP bacteria approached that of *Prochlorococcus*. The abundance of AAP bacteria rivaled some groups of strictly heterotrophic bacteria and was often higher than the abundance of known AAP genera (*Erythrobacter* and *Roseobacter* spp.). The distribution of AAP bacteria in the water column, which was similar in the Atlantic and the Pacific, was consistent with phototrophy.

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INTRODUCTION

Prokaryotic microbes play a central role in carbon cycling and food web dynamics in the ocean. Much has been learned about the autotrophic prokaryotes and their contribution to primary production (15, 21) and the central role of heterotrophic prokaryotes in the consumption of dissolved organic materials (DOM) (34) and degradation of sinking particles in the ocean (25). Less is known about photoheterotrophic bacteria, such as the aerobic anoxygenic phototrophic (AAP) bacteria and proteorhodopsin-containing bacteria (1) that probably have phototrophic as well as heterotrophic metabolisms. These bacteria may have unique impacts on carbon cycling.

Kolber et al. (20) obtained the first evidence that AAP bacteria may be abundant in the ocean. Direct counts of infrared fluorescing bacteria suggested that AAP bacteria could comprise as much as 10% of the total microbial community (20). However, Schwalbach and Furhman (27) pointed out that this estimate may be too high because of problems in distinguishing cyanobacteria from AAP bacteria. Their direct count and quantitative PCR data indicate that AAP bacteria were usually a small fraction of total prokaryotic abundance in surface waters of several marine environments, but surface waters of the Chesapeake Bay and Long Island Sound did have relatively high AAP bacterial numbers (10% - 18%). In addition to some uncertainty about surface waters, it is not clear how AAP bacteria vary with depth in the oceans, except for one location in the Pacific Ocean (20). Furthermore, no study has compared AAP bacteria with the abundance of *Prochlorococcus, Synechococcus* and heterotrophic bacterial groups.

The abundance of heterotrophic bacterial groups has been useful in assessing their contribution to bacterial production (9). Similarly, abundance data will provide insight into the biogeochemical importance of AAP bacteria. Other characteristics of AAP bacteria, such as the concentration of bacteriochlorophyll *a* (BChl *a*) per cell, may provide insight into the importance of phototrophy to their metabolism. In this study we used pigment analysis to assess the phototrophic potential of AAP bacteria and infrared fluorescence microscopy, flow cytometry and fluorescence in situ hybridization to compare the abundance of AAP bacteria to cyanobacteria and heterotrophic bacteria in the Mid-Atlantic Bight and the central North Pacific Gyre. Abundance and cellular pigment content suggested that the contribution of AAP bacteria to bacterioplankton metabolism is comparable to recognized groups of heterotrophic bacteria and that the potential importance of photoheterotrophy varies in AAP bacteria.

MATERIALS AND METHODS

Environmental sampling. Seawater was collected in the Mid-Atlantic Bight in August 2003 and in the central North Pacific in February 2004. Samples for fluorescence in situ hybridization (FISH) and AAP bacterial abundance were preserved with 2% paraformaldehyde for 18 h at 4°C. The FISH samples were then filtered onto 0.2-μm white polycarbonate filters, rinsed with deionized water and stored at –20°C. The AAP samples were filtered onto 0.2 μm black polycarbonate filters and were not rinsed. The FISH sample filters were stored at –20 °C and the AAP bacteria and

Prochlorococcus filters were stored at –20°C for a few days until back in the lab where they were then stored at –80°C for up to two months prior to analysis.

Oceanographic parameters. Bacterial production was measured using the ^{3}H-leucine method (18). Samples were incubated with 20 nM leucine for 1 h at in situ temperature in the dark. Incubations were terminated by the addition of 5% trichloroacetic acid (TCA). Macromolecules were precipitated by TCA extraction, collected by centrifugation (28), rinsed with 80% EtOH, and radioassayed. Bacterial production was calculated assuming a ratio of 1.5 kg C per mol of leucine incorporated.

Samples for Chl *a* and BChl *a* analysis were collected by filtering 10 L of seawater onto GF/F glass fiber filters, which were then stored at –80 °C until analysis. Pigments were extracted in 95% acetone using a 1 minute sonication step followed by 4 h of incubation at -20°C (7). Pigments were analyzed by reverse-phase HPLC using an Agilent Technologies 1100 series system fitted with a Zorbax Eclipse XDB-C8 HPLC column. The mobile phase consisted of a binary gradient that went from a 70:30 mixture of methanol (95%) and tetrabutylammonium acetate buffer (28 mM) to 100% methanol (31). Pigment absorbance was monitored at 665 nm and 770 nm to quantify chlorophyll *a* and bacteriochlorophyll *a*, respectively. Pigments were quantified using chlorophyll *a* and bacteriochlorophyll *a* standards (Sigma-Aldrich).

Seawater samples for nutrient analyses were frozen on dry ice and stored at –20 °C until analysis. Concentrations of NO_3+NO_2, PO_4 and NH_4^+ were determined by automated, segmented flow colorimetric analysis, using a Flo-Solution IV analyzer (O/I Analytical, College Station, TX).

Fluorescence in situ hybridization. The abundance of selected bacteria was determined by fluorescence in situ hybridization (FISH) using probe Alf968 for alpha-proteobacteria (14), CF319a for *Cytophaga*-like bacteria (22), Ros537 for *Roseobacter* spp. (10) and Ery731 (TAA CTG TCC AGT GAG TCG) for *Erythrobacter* spp (this study). A slice of the filter was placed on a 30 µl drop of hybridization solution containing 75 ng of Cy3-labeled oligonucleotide probe, and incubated for 18 h at 46 °C. The hybridization solution contained 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 0.01% sodium dodecyl sulfate, and the concentration of formamide determined to achieve specificity for the targeted bacteria (14, 22). The Ery731 probe was used with a formamide concentration of 35%. The specificity of the *Erythrobacter* FISH probe, Ery731 was assessed using *Erythrobacter longus* (ATCC33941) and various negative controls, including *Roseobacter litoralis* (ATCC49566) and *Vibrio harveii* (ATCC700106), marine alpha-proteobacteria strains O21, E37 and *Silicobacter pomeroyi* (16), and *Cytophaga*-like bacteria strain IRI113 and IRI26 isolated from Delaware coastal waters. FISH samples were analyzed using image analysis as described previously (9).

AAP bacterial abundance. Samples for AAP bacterial abundance were stained with a solution containing 1 µg/mL 4',6-diamidino-2-phenylindole (DAPI) in 2X PBS (1 X PBS contains 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, and 0.24 g KH_2PO_4 in 800 ml water (pH 7.4)) for 5 min. After removing excess stain, the sample was then mounted on a glass slide with a cover slip using an antifade mountant comprised of Citifluor (Ted Pela) and Vectashield (Vector labs) mixed in a ratio of 4 to1 by volume. Stained samples were counted immediately or stored at –80°C and counted within 24 h.

AAP bacteria were counted using an Olympus Provis AX70 microscope and image analysis (ImagePro Plus, Media Cybernetics) to identify cells having DAPI and IR fluorescence, but not chlorophyll *a* (Chl *a*) or phycoerythrin (PE) fluorescence. A series of four images was acquired for each field of view using the following optical filter sets: DAPI (Ex. 360 ± 40, Em. 460 ± 50), IR (Ex. 390 ± 100, Em. 750 long pass), Chl *a* (Ex. 480 ± 30, Em. 660 ± 50) and PE (Ex. 545 ± 30, Em. 610 ± 75) (Chroma). Images were captured using a CCD camera (Intensified Retiga Extended Blue, Q Imaging) with the following exposure times: DAPI, 40 ms; IR, 200 ms; Chl 1500 ms; and PE, 50 ms. Focus was adjusted by approximately 0.8 μm between the DAPI and IR images using a computer controlled z-axis controller (Prior Instruments) to correct for chromatic aberration. Cells were identified by detecting edges with Laplacian and Gaussian filters applied in series (23). The filtered images were segmented into binary format and then overlaid to identify cells with DAPI and IR fluorescence but not Chl or PE fluorescence.

The method for counting AAP bacteria was tested using *Erythrobacter longus* (ATCC33941), which has BChl *a*. We also tested microbes with other photosynthetic pigments, including the cyanobacteria *Prochlorococcus marinus* (CCMP1375) and *Synechococcus* strain WH7803 (CCMP1334), and the picoeukaryotic alga *Aureococcus anophagefferens* (CCMP1706). A seawater sample collected from 2000 m in the Arctic Ocean was also examined.

Flow cytometry. Seawater samples for counting *Prochlorococcus* and *Synechococcus* by flow cytometry were preserved with 2% paraformaldehyde, frozen in liquid nitrogen and stored at –20 °C until analysis. Analysis was performed with a Beckton-Dickinson FACSCalibur using 488 nm laser excitation and 0.2-μm-filtered

seawater sheath fluid. *Synechococcus* and *Prochlorococcus* were identified in scatter plots of red (> 640 nm) versus orange (560 – 640 nm) fluorescence (4). Counts were calibrated using fluorescent beads (Molecular Probes, F-8823), which were counted by fluorescence microscopy and added to the sample.

RESULTS

Automated microscopic counting of AAP bacteria and *Prochlorococcus*. AAP bacteria were readily distinguished from microbes with photosynthetic pigments other than BChl *a*. The percentage of cells that were scored AAP-positive in an *Erythrobacter longus* culture was 84% ± 10% (Table 1). AAP bacterial abundance was not significantly different from zero in a *Prochlorococcus* culture, although some cells (0.3% ± 0.3%) were AAP-positive. No cells were AAP-positive in control cultures of *Synechococcus* and *Aureococcus anophagefferens*. The percentage of cells scoring AAP-positive in a sample from 2000 m in the Arctic Ocean was 0.3% ± 0.3%.

There was a good correspondence between microscopic counts and abundances of *Synechococcus* and *Prochlorococcus* determined by flow cytometry in the central North Pacific. Abundances of *Synechococcus* determined by microscopy were significantly correlated with flow cytometry ($r = 0.52$, $p = 0.003$), although microscopic enumeration tended to overestimate the number of *Synechococcus* when the abundance determined by flow cytometry was less than 10^3 cells/ml (Fig. 1). Microscopic counts of *Prochlorococcus* were significantly correlated with abundances determined by flow cytometry ($r = 0.75$, $p < 0.001$).

Environmental setting. In the Mid-Atlantic Bight, several biogeochemical parameters were indicated that the shelf break was mesotrophic whereas the coastal and Gulf Stream water was more oligotrophic. Concentrations of Chl *a* in surface water were 2-to 6-fold higher at the shelf break than in the Gulf Stream and coastal water due to higher concentrations of phosphate and nitrate + nitrite (Table 2). However, integrated primary production was almost 10-fold higher in the Gulf Stream than at the shelf break in part due to the deeper photic zone in the Gulf Stream (Table 2). In contrast, integrated bacterial production was 2-fold higher at the shelf break than in the Gulf Stream (Table 2).

Concentrations of inorganic nutrients and Chl *a* were low in the Pacific, indicative of oligotrophic conditions. The concentrations of nitrate + nitrite in the Pacific were comparable to concentrations in the Mid-Atlantic Bight, but phosphate in the Pacific was about 2-fold higher than in the Mid-Atlantic Bight (Table 2). Chl *a* in the Pacific was about 4-fold higher than in the Atlantic and the photic zone was approximately twice as deep.

Standing stocks of AAP bacteria. The abundance of AAP bacteria varied across regimes and with depth. In surface waters of the Mid-Atlantic Bight, AAP bacteria were most abundant in coastal waters (5.0×10^4 cell ml^{-1}) and the Gulf Stream (1.5×10^5 cells ml^{-1}) and less abundant at the shelf break (6.9×10^3 cells ml^{-1}) (Fig. 2A, 2B and 2C). AAP abundance varied more with depth than across regimes. AAP bacterial abundance was higher in the photic zone than at the surface and lowest below the photic zone. For example, at the shelf break the abundance of AAP bacteria at 5 m was 7-fold higher than at the surface (Fig. 2D). In the Gulf Stream and coastal water, AAP bacterial abundance

below the photic zone at 100 m was 3.0 X10^3 cells ml^{-1} compared to 1.0 X10^5 cells ml^{-1} at 20 m (Fig. 2B and 2C).

AAP bacteria were less abundant in the central North Pacific (Fig. 3) than in the Mid-Atlantic Bight. The number of AAP bacteria in the surface waters sampled near Oahu was 10 to 100-fold lower than in the Gulf Stream and coastal waters of the Atlantic (Fig. 2). However, sub-surface maxima in the Pacific were similar to the Atlantic. In the Pacific, AAP bacterial abundances were highest at depths ranging from 20 m to 100 m, and these subsurface maxima were 2 to 100-fold higher than the surface values (Fig. 3). Another similarity between the Atlantic and the Pacific was the low abundance at the 1% light depth and the even lower numbers below the photic zone (Fig. 3).

AAP bacteria comprised a substantially larger fraction of the total prokaryotic community in the mid-Atlantic Bight than in the central North Pacific Ocean. The maximum abundance of AAP bacteria in the Pacific was only 5% of the total prokaryotic community (Fig. 4), whereas AAP bacteria comprised from 5% to 15% of total prokaryotes in the mid-Atlantic Bight (Fig. 4). AAP bacteria in the surface waters of the Atlantic made up 5% - 10% of the total community compared to 3% in the Pacific. At depths below 100 m AAP bacteria made up 2% or less of the total community in the Atlantic and Pacific.

AAP bacteria versus cyanobacteria. In the Gulf Stream and Atlantic coastal waters, AAP bacteria were as much as 2-fold more abundant than *Prochlorococcus* and 10-fold more abundant than *Synechococcus* at depths shallower than 25 m (Fig. 2A, 2B and 2C). In contrast, at the bottom of the photic zone *Synechococcus* outnumbered AAP

bacteria by 2-fold (Fig. 2B and 2C). At the shelf break *Synechococcus* was the most abundant phototroph and outnumbered AAP bacteria 2- to 10-fold (Fig. 2D).

In contrast, in the central North Pacific *Prochlorococcus* outnumbered AAP bacteria and *Synechococcus* at all depths. *Prochlorococcus* was 5- to 50-fold more numerous than AAP bacteria at depths from the surface to 100 m (Fig. 3). However, similar to the Atlantic, AAP bacteria were more abundant than *Synechococcus* in the central North Pacific Gyre. In surface waters, the abundance of AAP bacteria was equal to or as much as 5-fold higher than that of *Synechococcus*. At depths ranging from 20 m to 200 m AAP bacteria outnumbered *Synechococcus* by 10- to 20-fold.

Contribution of AAP bacteria to prokaryotic community structure. We compared the abundance of AAP bacteria to the major groups of bacteria known to be active in DOM consumption. Alpha-*proteobacteria* and *Cytophaga*-like bacteria were major components of the bacterial communities in the Mid-Atlantic Bight as determined by FISH. At the shelf break alpha-*proteobacteria* and *Cytophaga*-like bacteria (comprising on average 27% and 23% of the prokaryotic community) outnumbered AAP bacteria by 10-fold (Table 3). In the Gulf Stream alpha-*proteobacteria* and *Cytophaga*-like bacteria on average made up just 6% to 12% of the total prokaryotes, similar to the average abundance of AAP (about 10%). However, the FISH estimates are probably conservative estimates due to limitations of FISH, since in the Gulf Stream only 40% - 60% of the total prokaryotes were detected by a general FISH probe (Eub338) for all bacteria.

We also compared AAP abundance to more narrowly defined bacterial groups such as SAR11, a type of alpha-*proteobacteria* (24). Overall, the abundance of AAP bacteria

was about one third of SAR11, which accounted for 20% to 30% of the prokaryotic community (Table 3). The relationship between AAP bacteria and *Roseobacter*, which is another type of alpha-*proteobacteria* with some cultured representatives that carry out AAP metabolism (36), varied between regimes and depth in the water column. In coastal and shelf break water *Roseobacter* comprised 3% to 6% of the total prokaryotic community regardless of depth, whereas AAP bacterial abundance did vary with depth. In the photic zone of coastal waters AAP bacteria were 3-fold more abundant than *Roseobacter*, but in surface water and below the photic zone the abundance of AAP bacteria and *Roseobacter* were about equal (<1% to 5% of total prokaryotes) (Table 3). In contrast, at the shelf break throughout the water column *Roseobacter* were on average three times as abundant as AAP bacteria (4% to 18%) (Table 3).

AAP bacteria were always more abundant than *Erythrobacter* spp., which is another alpha-*proteobacteria* group potentially involved in aerobic anoxygenic photosynthesis (19). In the Gulf Stream waters where AAP bacteria were most abundant, *Erythrobacter* comprised 1% - 6% of total prokaryotes compared to 5% - 15% AAP bacteria (Table 3 and Fig. 4). In contrast, in coastal waters and at the shelf break the abundance of *Erythrobacter* usually was not distinguishable from the negative control.

Photosynthetic pigments of AAP bacteria and primary producers. Similar to the variation in AAP bacterial abundance, the concentration of BChl *a* was highest at depths ranging from 15 m to 30 m within the photic zone. BChl *a* was not detected (limit of 0.05 ng L^{-1}) below the photic zone in the Gulf Stream and Atlantic coastal waters (Table 4). In contrast, the horizontal distribution of BChl *a* was different from the pattern in AAP bacterial abundance. BChl *a* concentrations were highest at the mesotrophic shelf

break (up to 6 ng L^{-1}) and decreased offshore to their lowest concentrations (< 2 ng L^{-1}) in the Gulf Stream (Table 4). However, at all sites in the Mid-Atlantic Bight the concentration of BChl *a* was low compared to Chl *a* concentration (0.3 % - 2.6%) (Table 4).

Estimates of BChl *a* per cell in AAP bacteria varied substantially among depths and sampling sites as well. BChl *a* per cell at the shelf break site was 0.24 fg $cell^{-1}$ at the surface and decreased almost 5-fold to 0.054 fg $cell^{-1}$ at the bottom of the photic zone (Table 4). In Atlantic coastal water and in the Gulf Stream, pigment concentrations per cell were typically 10-fold lower than at the shelf break (Table 4).

Concentrations of divinyl-chlorophyll *a* (div-Chl *a*) were much higher than BChl *a*. Offshore the average concentration of div-Chl *a* was 34 ng L^{-1} versus 0.7 ng L^{-1} for BChl *a* (Table 4). However, at the shelf break the concentration of the two pigments was similar (about 5 ng L^{-1}). Cellular concentrations of div-Chl *a* were also typically higher than BChl *a*. In the Gulf Stream div-Chl *a* per *Prochlorococcus* cell was 20- to 250-fold higher than the BChl *a* content of AAP bacteria. In contrast, at the shelf break, the photosynthetic pigment content of *Prochlorococcus* was only 2 to 10-fold higher than in AAP bacteria (Table 4).

DISCUSSION

We examined AAP bacteria in mesotrophic and oligotrophic regimes in the Mid-Atlantic Bight and in the oligotrophic central North Pacific Ocean to assess their abundance and contribution to bacterial community structure. We hypothesized that

AAP bacteria would be an abundant component of oceanic bacterial communities because the selective pressures for efficient DOM utilization by bacteria in the ocean would be substantial when bacterial growth is limited by the availability of DOM (3). Photoheterotrophic bacteria that use DOM and light could be more efficient than strictly heterotrophic bacteria because they supplement their energy requirements with light. Evidence for direct effects of sunlight on bacterial growth (6) and on community structure (32) suggests that phototrophic metabolism may be prevalent in marine bacteria. Larger numbers of proteorhodopsin genes uncovered by whole genome sequencing of Sargasso Sea bacteria also suggests an important role for photoheterotrophy (33).

Our data indicate that AAP bacteria are widespread in the Mid-Atlantic Bight and the oligotrophic central North Pacific Gyre and make up from 1% to 10% of the total prokaryotic community. A previous study in the Northeast Pacific also suggested that AAP bacteria constitute approximately 10% the total prokaryotic community in the photic zone (20). Although the data from the Northeast Pacific may be overestimates because no steps were taken to exclude *Prochlorococcus*, our measurements of AAP abundance do not include cyanobacteria. The possibility of *Prochlorococcus* contamination of AAP counts must be taken seriously when cyanobacteria are abundant, because Chl *a* is visible in the infrared (27). However, two lines of evidence indicate that our measurements of AAP bacterial abundance do not include cyanobacteria. In the central North Pacific, even though *Prochlorococcus* abundance was high our estimates of AAP bacterial abundance were low, averaging only about 4% of the *Prochlorococcus* abundance. In addition, only 0.3% ± 0.3% of cells were scored AAP positive in a *Prochlorococcus* culture, indicating that removing Chl *a* fluorescing cells from the

infrared image was highly effective at excluding *Prochlorococcus* and *Synechococcus* from the AAP bacterial counts. The inclusion of *Prochlorococcus* in AAP counts was less problematic in the Mid-Atlantic Bight where *Prochlorococcus* was not as abundant and in some samples were outnumbered 25-fold by AAP bacteria.

The ecology of AAP bacteria and their role in microbial food webs is potentially complex because they probably are both phototrophic and heterotrophic. Cultivated AAP bacteria are capable of purely heterotrophic growth in the dark, but grow more rapidly when exposed to a light-dark cycle (38). Previous measurements of infrared fluorescence transients suggest that AAP bacteria in the ocean are photosynthetically competent (20). Our data on the depth distribution of AAP bacteria is consistent with higher growth rates in the light since the abundance of AAP bacteria was higher in the photic zone than below the sunlit layers of the water column. AAP bacteria were distributed in the water column like other phototrophs whether they were abundant, as in the Mid-Atlantic bight, or rare as in the central North Pacific Ocean. AAP bacterial abundance did not appear to vary in the water column like heterotrophic bacteria. However, it is still unclear whether light is directly involved supporting AAP phototrophy or indirectly through heterotrophic consumption of phytoplankton DOM by AAP bacteria.

Our data on BChl *a* and divinyl Chl *a* can be used to explore further the importance of phototrophy in these bacteria. BChl *a* is the main photosynthetic pigment in AAP bacteria and serves in both light harvesting and reaction centers. Although carotenoids are abundant in AAP bacteria, they play only a minor role in harvesting light energy in culture (37) and in the ocean (20). The concentrations of BChl *a* per cell in coastal water and in the Gulf Stream were typically 20-fold lower than the divinyl Chl *a* content of

Prochlorococcus. Such low pigment content suggests that phototrophy is probably a smaller part of the metabolism of AAP bacteria than in *Prochlorococcus*, which is generally recognized as relying purely on autotrophy, although *Prochlorococcus* may have some heterotrophic activity (6, 39). However, at the shelf break concentrations of BChl *a* per cell were 20-fold higher than offshore and about 2-fold higher than has been reported for cultured AAP bacteria (37) and approached the concentrations of photosynthetic pigments in *Prochlorococcus*, which ranges from 0.22 – 1.83 fg $cell^{-1}$ (12). These data suggest that reliance on phototrophy varies in the Gulf Stream, coastal and shelf break waters.

A number of BChl *a*-producing bacteria are physiologically distinct from AAP bacteria. However, they are probably not an important source of BChl *a* in the ocean. Some aerobic methylotrophic bacteria and *Rhizobia* sp. are capable of BChl *a* synthesis (11, 30, 35), as well as the beta-*proteobacterium Roseateles depolymerans* (29). However, *Rhizobia* sp. are not abundant in marine systems (13) and *Roseateles* sp. occurs in freshwater (29). In addition, infrared direct counts and quantitative *pufM* gene PCR gave similar estimates of AAP abundance in the San Pedro Channel (27).

Cultivated marine AAP bacteria are restricted to just two genera of alpha-*proteobacteria*, *Roseobacter* spp. and *Erythrobacter* spp. (26), and recently cultivated oligotrophic gamma-*proteobacteria* (5), but the actual diversity of AAP bacteria in the ocean appears to be much greater. Our FISH and microscopic IR data indicate that the bulk of the AAP bacteria are not members of the *Erythrobacter* group. Furthermore, AAP bacteria were often more abundant than *Erythrobacter* and *Roseobacter* combined, indicating that the diversity of AAP bacteria extends beyond these two groups. Analysis

of *pufM* sequences obtained from the Sargasso Sea also indicates that the diversity of AAP bacteria in the ocean is not limited to *Roseobacter* spp. and *Erythrobacter* spp. (33). In addition, analysis of *pufM* genes in BAC clones containing environmental DNA from the Pacific Ocean revealed novel AAP bacteria related to beta-*proteobacteria* and gamma-*proteobacteria* (2). Identifying AAP bacteria based on *pufM* gene sequences has provided an initial assessment of AAP bacterial diversity, but problems caused by lateral gene transfer of photosynthetic genes has made definitive identification difficult (17). The distance between 16S rRNA and *pufM* genes in AAP bacterial genomes is apparently too great to capture both genes on a single genomic fragment using BAC vectors. However, the assembly of larger genome fragments from whole genome shotgun sequencing of bacterial communities (33) and more effective methods for cultivating marine bacteria (8) may allow direct determination of the phylogenetic identity of AAP bacteria.

Our study revealed that AAP bacteria are an abundant component of bacterial communities in the Mid-Atlantic Bight. The abundance of AAP bacteria was higher further offshore in the Gulf Stream than at the shelf break, which was consistent with the hypothesis that phototrophy provides AAP bacteria with an advantage in oligotrophic environments. Contrary to this hypothesis however, pigment content was lowest furthest offshore, and AAP bacteria were not abundant in the central North Pacific. It is not clear what role phototrophy might play in the high abundance of AAP bacteria in estuarine environments (27). The abundance of AAP bacteria revealed by our study suggests a potentially important impact on DOM cycling that may vary under different environmental conditions influencing cellular pigment content and thus phototrophy in

AAP bacteria. Additional information from experiments utilizing genomics, cultivation and in situ analysis will be necessary for assessing the role of phototrophy versus heterotrophy in determining the success of AAP bacteria in the ocean.

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Figure Legends

Figure 1. Abundance of *Synechococcus* (A) and *Prochlorococcus* (B) determined by microscopy and flow cytometry in the central North Pacific Gyre. Error bars are SE.

Figure 2. Abundance of AAP bacteria (AAP), *Prochlorococcus* and *Synechococcus* at Gulf Stream (A, B), coastal (C) and shelf break (D) sampling sites in the Mid-Atlantic Bight in August 2003. Error bars are SE.

Figure 3. Abundance of AAP bacteria and *Prochlorococcus*, *Synechococcus* and total prokaryotes in the central North Pacific Gyre in February 2004. The error bars indicate the variation (SD) in abundance among sampling sites located N of Oahu, NW of Oahu, W of Oahu, and SW of Oahu.

Figure 4. Contribution of AAP bacteria to community structure in the Mid-Atlantic Bight in August 2003 (black symbols) and the central North Pacific Gyre in February 2004 (white symbols). The dashed lines were drawn by hand. Error bars are SE.

Table 1. Testing the infrared microscopic method for counting AAP bacteria.

Sample	Description	BChl *a*	AAP-positive (%)	N*
Erythrobacter longus	AAP bacterium	+	84 ± 10	162
Prochlorococcus marinus	Cyanobacterium	-	0.3 ± 0.3	220
Aureococcus anophagefferens	Picoeukaryote	-	0 ± 0	3062
Synechococcus sp.	Cyanobacterium	-	0 ± 0	900
Arctic Ocean 2000 m	Deep-sea bacterial community	N.D.**	0.3 ± 0.3	10531

* Number of cells examined
** Not determined

Table 2. Characteristics of waters sampled in the Mid-Atlantic Bight in August 2003 and in the central North Pacific in February 2004.

Description	Geographic location	Photic zone* (m)	PO_4** (μmol L^{-1})	NO_3+NO_2* (μmol L^{-1})	Bacterial productivity (mgC m^{-2} d^{-1})	Primary productivity (mgC m^{-2} d^{-1})	Chl *a*& (μg/L)
Mid-Atlantic Bight							
Gulf Stream	35° 55' N, 73° 58' W	49	0.08 – 0.39	0.18 – 0.73	73 (4)	2266	0.13 – 0.25
Gulf stream	36° 00' N, 72° 60' W	65	0.07 – 0.17	0.41 – 2.7	38 (2)	900	0.04 – 0.11
Coast	36° 49' N, 73° 36' W	65	0.07 – 0.81	0.27 – 14.1	66 (16)	279	0.04 – 0.88
Shelf break	38° 00' N, 74° 26' W	35	0.37 – 0.76	1.48 – 8.0	96 (4)	259	0.23 – 0.83
Central North Pacific							
N of Oahu	22° 45' N, 157° 60' W	117#	0.11 – 0.11	0.17 – 0.52	N.D.***	466#	0.35 – 1.56
NW of Oahu	22° 27' N, 158° 5' W	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
W of Oahu	22° 10' N, 158° 10' W	N.D.	0.15 – 0.30	0.23 – 2.62	N.D.	N.D.	0.46 – 1.8
SW of Oahu	21° 53' N, 158° 14' W	109$	0.12 – 0.12	0.20 – 0.15	N.D.	N.D.	0.53 – 2.48
SW of Oahu	21° 21' N, 158° 16' W	100$	0.15 – 0.23	0.38 – 1.75	N.D.	N.D.	0.37 – 1.3

* Depth to 1% of surface irradiance
** Range of concentrations between the surface and the bottom of the photic zone
*** N.D. not determined
**** Hawaii ocean time-series station Aloha (http://hahana.soest.hawaii.edu/hot/hot.html)
# Hawaii ocean time-series February 24-28, 2003 (http://hahana.soest.hawaii.edu/hot/hotdogs/interface.html)
$ Hawaii ocean time-series February 23-26, 2004
& Range of concentrations between the surface and the sub-surface maximum in Chl *a* concentration

Table 3. Abundance of select bacteria in the Mid-Atlantic Bight in August 2003.

		% of Total Prokaryotes*						
Regime	Depth (m)	Eub338-positive**	Alpha-*proteobacteria*	SAR 11	*Cytophaga*-like	*Roseobacter*	*Erythrobacter*	AAP bacteria
Gulf Stream	0	58 (4.2)	14 (1.5)	34 (3.8)	11 (2.6)	7.0 (2.3)	3.7 (1.3)	12 (1.3)
	7	63 (3.8)	7.5 (2.2)	32 (6.3)	14 (12)	7.2 (1.9)	3.7 (2.8)	N.D.
	13	60 (3.8)	14 (12.2)	28 (6.8)	16 (1.5)	4.9 (1.6)	3.8 (1.5)	N.D.
	20	62 (4.6)	9.8 (2.4)	38 (5.2)	9.6 (1.3)	4.2 (1.0)	2.6 (0.9)	18 (2.1)
	50	N.D.***	N.D.	26 (4.9)	N.D.	N.D.	N.D.	N.D.
	80	N.D.	N.D.	28 (9.9)	N.D.	N.D.	N.D.	11 (1.4)
Gulf Stream	0	36 (5.5)	21 (7.6)	26 (5.4)	3.5 (0.9)	3.1 (1.5)	5.6 (1.9)	8.0 (0.8)
	8	56 (4.9)	8.2 (2.3)	20 (4.8)	8 (23)	4.3 (0.7)	1.6 (0.6)	9.6 (0.8)
	15	50 (4.3)	7.7 (1.2)	24 (4.4)	7.7 (1.2)	8.1 (12)	1.2 (1.3)	12 (1.1)
	30	38 (9.9)	1.9 (0.9)	19 (3.9)	1.8 (0.9)	3.3 (12)	0.9 (0.8)	8.6 (0.5)
	50	35 (11.0)	6.8 (1.9)	22 (4.7)	6.8 (1.9)	1.5 (1.1)	1.3 (1.2)	16 (1.8)
	100	23 (2.7)	0.7 (0.7)	11 (7.2)	0.5 (0.8)	1.0 (0.6)	0.4 (1.2)	2.0 (0.4)
Coast	0	60 (9.1)	6.4 (3.0)	27 (5.5)	17 (4.2)	3.5 (1.1)	1.4 (1.3)	5.4 (0.7)
	9	73 (15.7)	30 (12)	28 (3.8)	24 (12)	2.8 (1.2)	0.6 (0.5)	10 (0.8)
	17	69 (12.6)	18 (4.5)	29 (6.9)	39 (4.8)	3.4 (1.6)	0.9 (0.8)	17 (1.3)
	27	63 (9.5)	26 (13)	22 (4.4)	48 (14)	5.2 (2.0)	1.5 (0.9)	17 (1.0)
	50	37 (8.8)	5.4 (2.5)	20 (4.4)	16 (6.5)	6.0 (2.1)	2.9 (0.8)	2.6 (0.4)
	100	41 (11.1)	6.8 (3.6)	14 (5.4)	46 (18)	5.0 (4.2)	1.0 (1.5)	0.8 (0.2)
Shelf break	0	74 (3.8)	40 (6.1)	31 (3.7)	25 (4.3)	5.6 (0.9)	2.9 (1.5)	0.8 (0.1)
	5	73 (3.4)	32 (6.0)	32 (4.2)	22 (4.7)	4.3 (1.2)	1.8 (0.9)	2.1 (0.2)
	9	75 (8.2)	30 (6.8)	28 (5.0)	24 (2.8)	7.7 (1.5)	1.7 (0.6)	2.5 (0.2)
	15	61 (6.5)	27 (6.1)	34 (6.2)	18 (6.4)	18 (6.4)	2.2 (1.1)	3.1 (0.4)
	35	59 (6.1)	20 (5.7)	20 (4.0)	20 (4.8)	4.2 (2.1)	1.3 (0.8)	1.9 (0.3)
	45	N.D.	16 (4.3)	27 (4.7)	31 (5.6)	3.9 (1.2)	1.4 (1.0)	3.0 (0.8)

* Average (SE), n =10

** Eub338 is the general bacterial probe. The average negative control FISH probe was 3%

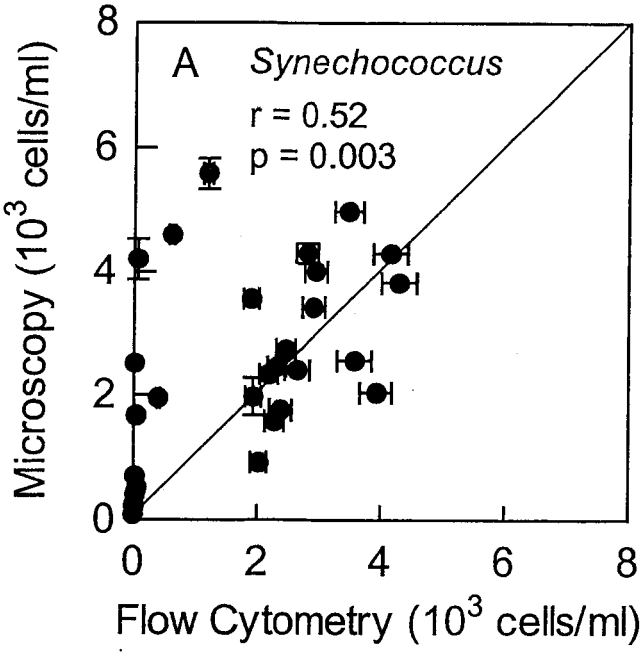
*** N.D. not determined

Table 4. Concentrations of photosynthetic pigments in the Mid-Atlantic Bight in August 2003.

Regime	Depth (m)	Chl *a* (µg/L)	Div-Chl *a* (ng/L)	Div-Chl *a* (fg/cell)	BChl *a* (ng/L)	BChl *a*/Chl *a* (%)	BChl *a* (fg/cell)
Gulf Stream	10	0.130	58.9	0.50	0.33	0.3	0.002
	20	0.148	78.2	0.67	1.56	1.1	0.007
	50	0.248	36.4	N.D.	0.76	0.3	N.D
	80	0.050	7.2	2.33	0.00	N.D.*	N.D.
Gulf Stream	0	0.042	18.2	0.35	0.42	1.0	0.008
	8	0.118	23.1	0.46	1.26	1.1	0.021
	15	0.063	22.1	0.43	0.40	0.6	0.005
	30	0.058	25.3	0.71	1.52	2.6	0.028
	50	0.106	85.7	2.45	0.69	0.7	0.006
	100	0.044	11.5	2.50	0.00	N.D.	N.D.
Coastal	0	0.044	32.4	53.3	0.60	1.3	0.016
	9	0.051	31.7	22.5	0.59	1.2	0.009
	17	0.058	34.1	14.8	0.45	0.8	0.005
	27	0.211	60.5	5.55	1.26	0.6	0.015
	50	0.885	17.5	1.29	2.15	2.4	0.084
	100	0.017	1.1	5.77	0.00	N.D.	N.D.
Shelf break	0	0.227	4.3	0.43	1.65	0.7	0.241
	5	0.371	5.9	0.45	2.23	0.6	0.105
	9	0.510	6.9	0.32	4.87	1.0	0.136
	15	0.825	19.6	1.19	5.84	0.7	0.121
	35	0.653	2.4	0.47	2.01	0.3	0.089
	45	0.457	3.3	1.62	1.88	0.4	0.054

* N.D. not determined

Figure 1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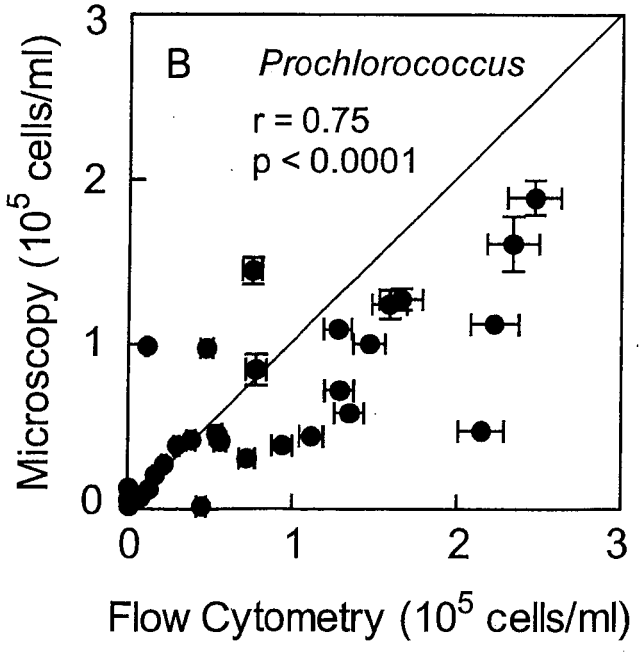
Flow Cytometry (10^3 cells/ml)
0
2
4
6
8


B
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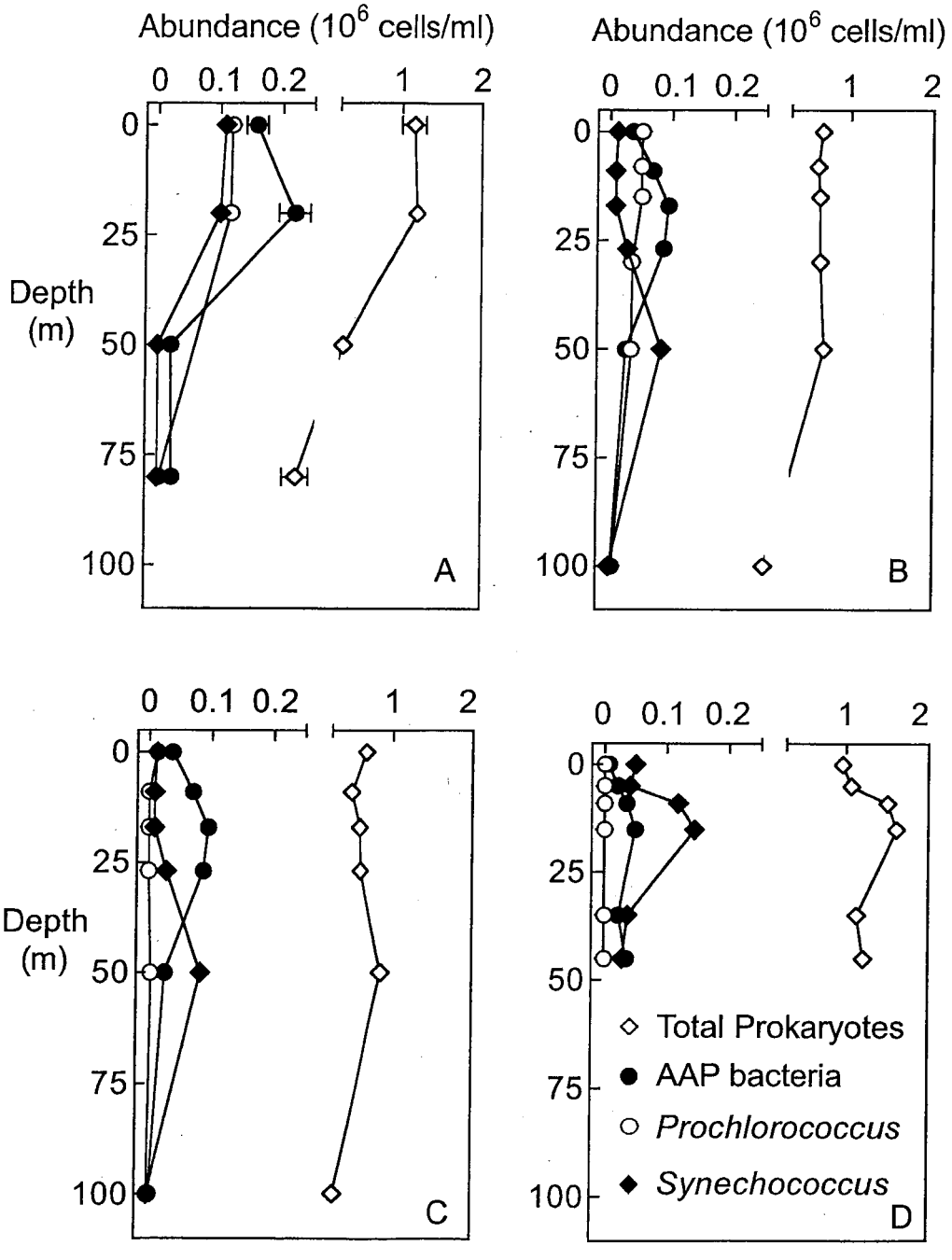
Flow Cytometry (10^5 cells/ml)
0
1
2
3

Figure 2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0 0.1 0.2 1 2
Depth (m)
0
25
50
75
100
A
B
C
D
Total Prokaryotes
AAP bacteria
Prochlorococcus
Synechococcus

Figure 3

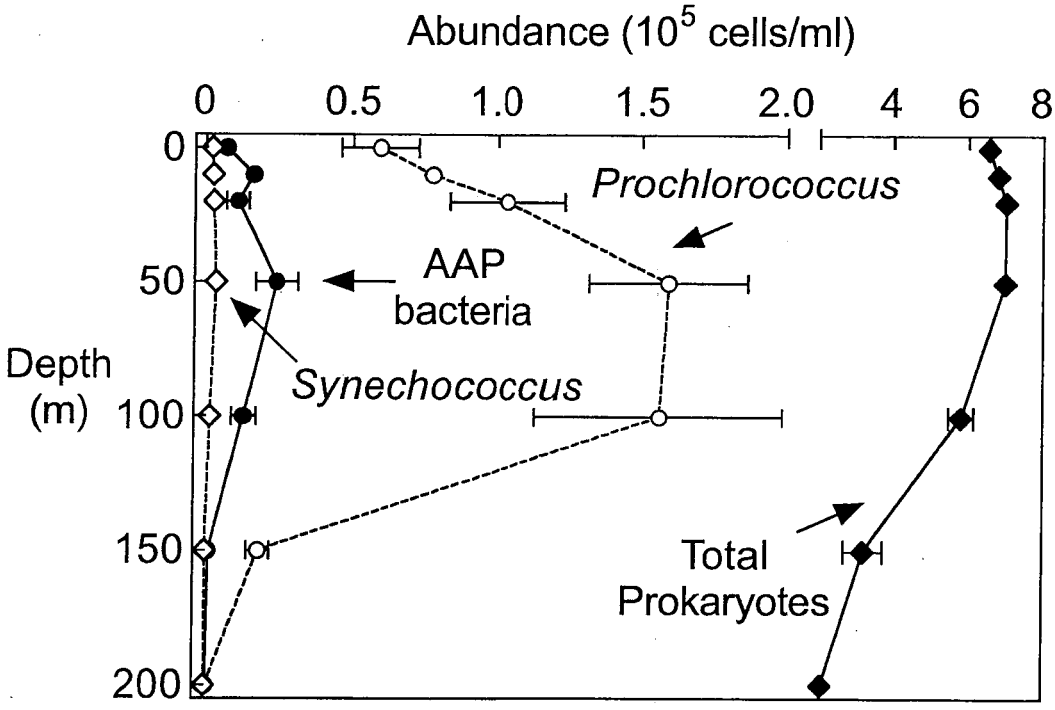
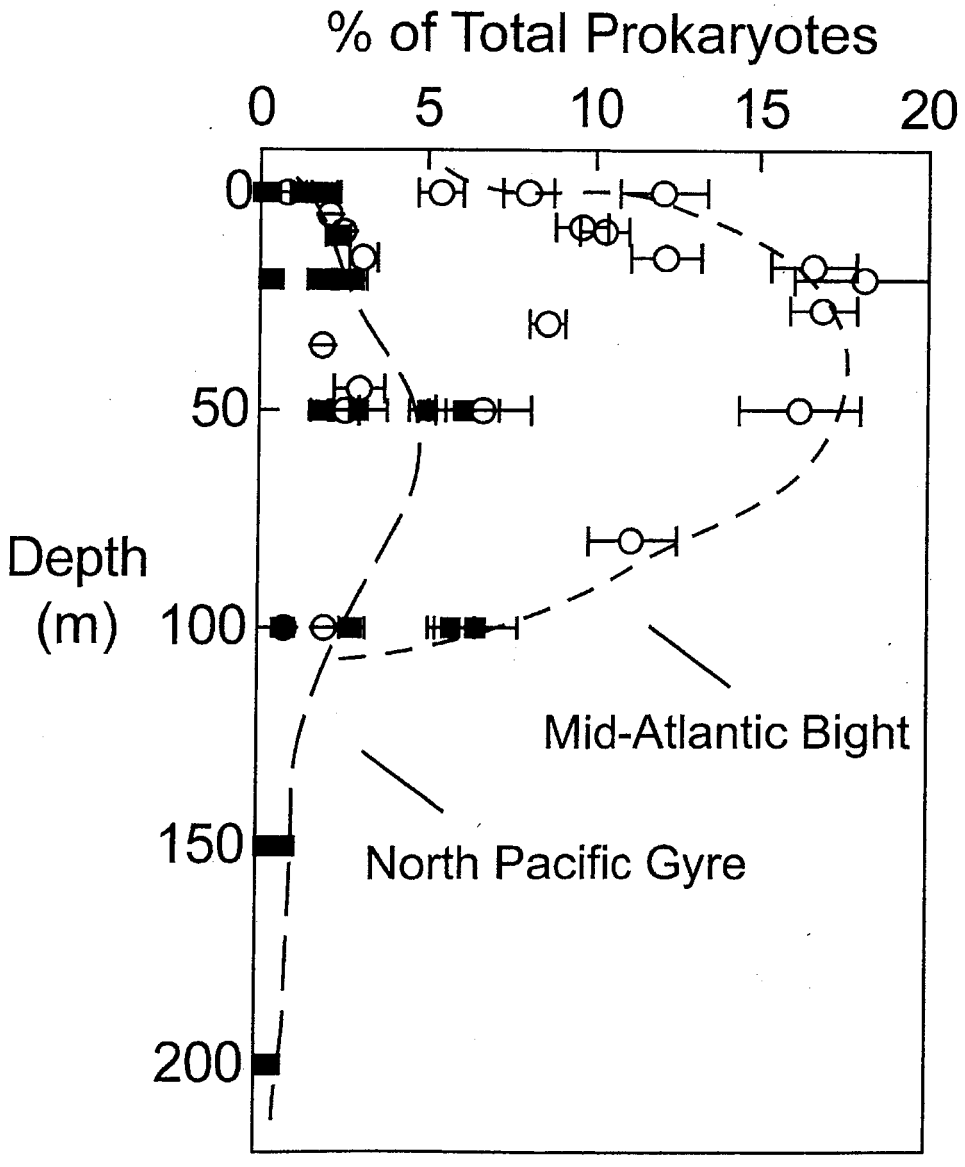

Abundance (10^5 cells/ml)
0
0.5
1.0
1.5
2.0
4
6
8
0
50
100
150
200
Depth (m)
Prochlorococcus
AAP bacteria
Synechococcus
Total Prokaryotes

Figure 4


% of Total Prokaryotes
0
5
10
15
20
0
50
100
150
200
Depth
(m)
Mid-Atlantic Bight
North Pacific Gyre

Popular Summary for the Cottrell, Mannino & Kirchman manuscript

Bacteria play a central role in carbon cycling and food web dynamics in the ocean. Photosynthetic bacteria, cyanobacteria, represent a major contribution to the ocean's primary productivity. At the base of the food web are the heterotrophic bacteria that utilize carbon released by phytoplankton and other organisms as food and decompose it to nutrients and carbon dioxide. Recent findings on the abundances of AAP bacteria suggest these bacteria have the potential to play an important role in the ocean's carbon cycle. AAP bacteria are considered heterotrophic bacteria but have the ability to derive energy from sunlight since they possess pigments similar to phytoplankton for sunlight absorption. Unlike photosynthetic organisms, AAP bacteria do not produce oxygen. In this study, we used pigment analysis to assess the phototrophic potential of AAP bacteria and microscopic techniques to compare the abundances of AAP bacteria to cyanobacteria and heterotrophic bacteria in the Mid-Atlantic Bight and the central North Pacific Ocean. AAP bacteria comprised 5 to 16% of total bacteria in the Mid-Atlantic but only 5% or less in the Pacific. Concentrations of bacteriochlorophyll a from AAP bacteria were low compared to chlorophyll a. The distribution of AAP bacteria in the water column, which was similar in the Atlantic and Pacific, was maximal in the sunlit portion of the water column, consistent with phototrophy. No previous study has compared the abundances of AAP bacteria with the abundance of cyanobacteria and other heterotrophic bacteria.

Significant Findings

No previous study has compared the abundances of AAP (aerobic anoxygenic phototrophic) bacteria with the abundance of cyanobacteria and other heterotrophic bacteria. AAP bacteria comprised 5% to 16% of total bacteria in the Atlantic but only 5% or less in the Pacific. In the Atlantic, AAP bacterial abundance was as much as 2-fold higher than the cyanobacteria *Prochlorococcus* and 10-folder higher than the cyanobacteria *Synechococcus*. In contrast, *Prochlorococcus* outnumbered AAP bacteria 5- to 50-fold in the Pacific. In both oceans, subsurface abundance maxima occurred within the photic zone, and AAP bacteria were least abundant below the 1% light depth. Concentrations of the AAP pigment bacteriochlorophyll *a* (BChl *a*) were low (~1%) compared to chlorophyll *a*. Although the BChl *a* content of AAP bacteria per cell was typically 20- to 250-fold lower than the divinyl-chlorophyll *a* content of *Prochlorococcus*, in shelf break water the pigment content of AAP bacteria approached that of *Prochlorococcus*. The abundance of AAP bacteria rivaled some groups of strictly heterotrophic bacteria and was often higher than the abundance of known AAP genera. The distribution of AAP bacteria in the water column, which was similar in the Atlantic and the Pacific, was consistent with phototrophy.