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High Abundances of Aerobic Anoxygenic Photosynthetic Bacteria in the South Pacific Ocean[▽]

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Little is known about the abundance, distribution, and ecology of aerobic anoxygenic phototrophic (AAP) bacteria, particularly in oligotrophic environments, which represent 60% of the ocean. We investigated the abundance of AAP bacteria across the South Pacific Ocean, including the center of the gyre, the most oligotrophic water body of the world ocean. AAP bacteria, *Prochlorococcus*, and total prokaryotic abundances, as well as bacteriochlorophyll *a* (BChl *a*) and divinyl-chlorophyll *a* concentrations, were measured at several depths in the photic zone along a gradient of oligotrophic conditions. The abundances of AAP bacteria and *Prochlorococcus* were high, together accounting for up to 58% of the total prokaryotic community. The abundance of AAP bacteria alone was up to 1.94×10^5 cells ml^{-1} and as high as 24% of the overall community. These measurements were consistent with the high BChl *a* concentrations (up to 3.32×10^{-3} μg $liter^{-1}$) found at all stations. However, the BChl *a* content per AAP bacterial cell was low, suggesting that AAP bacteria are mostly heterotrophic organisms. Interestingly, the biovolume and therefore biomass of AAP bacteria was on average twofold higher than that of other prokaryotic cells. This study demonstrates that AAP bacteria can be abundant in various oligotrophic conditions, including the most oligotrophic regime of the world ocean, and can account for a large part of the bacterioplanktonic carbon stock.

Prokaryotic microbes play a key role in carbon cycling in aquatic ecosystems. Heterotrophic bacteria are central actors of the microbial loop and play an essential role in the transformation of dissolved organic matter (2). In contrast, autotrophic bacteria like *Prochlorococcus* spp. are significant primary producers in a number of oceanic ecoystems (23). Aerobic anoxygenic phototrophic (AAP) bacteria are able to combine both phototrophic and heterotrophic functions (35). Recent studies indicate that this group of bacteria is widely distributed in the oceanic environment. However, the ecological importance of AAP bacteria remains poorly understood.

These bacteriochlorophyll (BChl *a*)-containing prokaryotes require oxygen and can use reduced organic compounds as electron donors (17, 35). The first marine AAP bacterium was isolated 30 years ago (27), but only recently have these bacteria been found to be phylogenetically diverse (3). These photoheterotrophs were not expected to be abundant in the open ocean, but novel approaches, such as epifluorescence microscopy, quantitative PCR, and sensitive measurements of BChl *a* fluorescence and concentrations, revealed the presence of AAP bacteria in a wide diversity of marine ecosystems (8, 13, 17, 25, 30). AAP bacteria were also detected by metagenomic studies in various aquatic environments (3, 22, 32, 33). These data show that AAP bacteria are widely distributed and may make up a large fraction of the bacterioplanktonic community. Consequently, AAP bacteria could significantly contribute to the carbon cycle in the oceans (17).

It has been suggested that AAP bacteria are adapted to oligotrophic waters, as light might provide supplementary energy in low-nutrient conditions (17). This hypothesis has not been supported by recent data indicating high abundances of AAP bacteria in mesotrophic coastal and estuarine environments (8, 25). More data on the abundance and distribution of AAP bacteria are needed to better understand links between the trophic status of water masses and photoheterotrophy. AAP bacteria remain clearly undersampled in several areas, especially in oligotrophic environments, which represent 60% of the ocean (19).

BChl *a* data might provide interesting clues about the photophysiology of AAP bacteria (8), but only a few studies combine both AAP cell enumeration and BChl *a* concentrations. Moreover, little is known about the relative abundance of AAP bacteria compared to autotrophic bacteria. Cottrell et al. (8) found that AAP bacteria were twofold more abundant than *Prochlorococcus* in the Gulf Stream, while the abundance of AAP bacteria was considerably lower than that of *Prochlorococcus* in the central North Pacific and in the Sargasso Sea (8, 30).

The objective of the present study was to determine the vertical and spatial distribution of AAP bacteria in the South Pacific Ocean and to assess their relative importance in a gradient of oligotrophic conditions. Microscopic counts revealed high abundances of AAP bacteria representing up to

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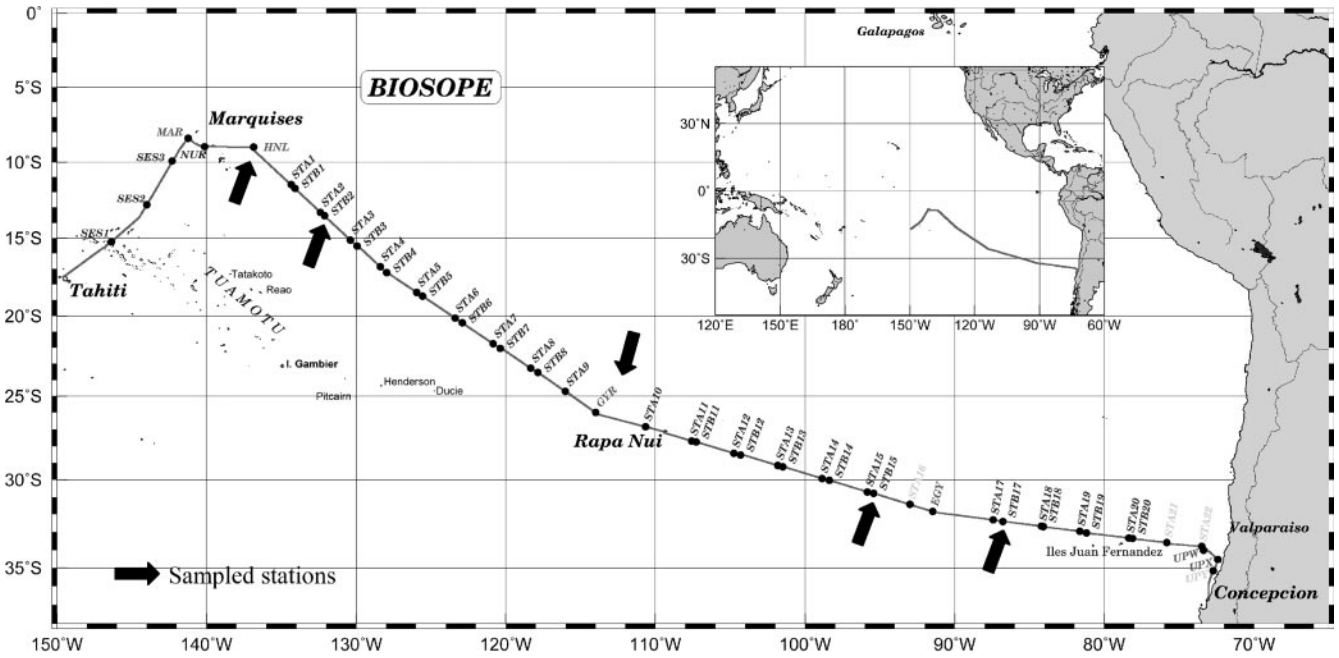

FIG. 1. Locations sampled during the Biogeochemistry and Optics South Pacific Ocean Experiment (BIOSOPE) cruise in the South Pacific Ocean (October to December 2004).

24% of the overall bacterioplanktonic cell abundance in the South Pacific Ocean.

MATERIALS AND METHODS

Study sites. Samples were collected during the Biogeochemistry and Optics South Pacific Ocean Experiment cruise that took place from October to December of 2004. The sampling strategy was guided by satellite imagery of ocean color with the aim of collecting samples across various trophic conditions. Five stations distributed over a distance of 6,000 nautical miles and various oligotrophic regimes were sampled (Fig. 1): stations HNL1 (09°00S, 136°50W), STB2 (13°33S, 132°06N), GYR (26°00S, 114°00W), STB15 (30°41S, 95°25W), and STB17 (32°23S, 86°47W). Seawater samples were collected with General Oceanics 12-liter Niskin bottles mounted on a rosette equipped with a SeaBird SBE19+ conductivity-temperature-depth instrument. Sea surface temperature (5 m) varied between 27.8°C at station HNL1 to 17.3°C at station STB17. The depth of the euphotic zone varied between 160 m at station GYR and 90 m at station HNL1 (Table 1). The chlorophyll *a* (Chl *a*) concentration integrated over the euphotic zone ranged between 11 mg m^{-2} (station GYR) and 18 mg m^{-2} (station HNL1). Concentrations of inorganic nitrogen in the mixed layer varied between undetectable levels at stations STB2, GYR, and STB15 and 3.35 ± 0.43 μM at station STB15, while phosphate concentrations ranged between 0.17 ± 0.015 μM at station GYR and 0.34 ± 0.040 μM at station HNL1 and STB17 (Table 1). Samples for all parameters described here were taken around noon.

TABLE 1. General oceanographic parameters[a]

Station	Parameter				
	SST (°C)	Ze (m)	Zm (m)	Nitrate (μM)	Phosphate (μM)
HNL1	27.8	90	74	1.59	0.340
STB2	27.4	124	21	ND	0.187
GYR	22.1	160	18	ND	0.130
STB15	18.7	108	30	ND	0.135
STB17	17.3	96	20	3.05	0.320

[a] SST, sea surface temperature; Ze, depth of the euphotic zone; Zm, depth of the wind-mixed layer; nitrate and phosphate concentrations are mean values of the wind-mixed layer; ND, not detectable.

AAP bacterial abundance and cell volume determination. For the enumeration of AAP bacteria, seawater samples were fixed with 2% paraformaldehyde (final concentration). Samples were stored in the dark for 18 h at 4°C and then filtered onto 0.2-μm-pore-size black polycarbonate filters. Filters were stored at −80°C before microscopic counts. AAP bacterial abundances were determined following the protocol previously described by Cottrell et al. (8). Briefly, the sample was stained for 5 min with DAPI phosphate-buffered saline (10 g NaCl, 0.25 g KCl, 1.8 g Na_2HPO_4, and 0.3 g KH_2PO_4 in 1 liter of water [pH 7.4]) and then mounted on a glass slide using an antifading agent comprised of Citifluor (Ted Pela) and Vectashield (Vector Labs) mixed in a ratio of 4:1 (vol/vol). AAP bacteria were counted on an Olympus Provis AX70 microscope. Image analysis software (ImagePro Plus; Media Cybernetic) was used to identify total DAPI-stained cells and AAP bacteria, which have infrared (IR) fluorescence but not Chl *a* or phycoerythrin (PE) fluorescence. For each field of view, a series of four images were taken: DAPI (excitation, 360 ± 40; emission, 460 ± 50); IR (excitation, 390 ± 100; emission, 750 long pass); Chl *a* (excitation, 480 ± 30; emission, 660 ± 50), and PE (excitation, 545 ± 30; emission, 610 ± 75) (Chroma). Each image was captured with a charge-coupled-device camera (Intensified Retiga Extended Blue; Q Imaging) with the following exposure times: DAPI, 40 ms; IR, 200 ms; Chl *a*, 1,500 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 (21). The filtered images were segmented into binary format and then overlaid to identify cells with DAPI and IR fluorescence but not Chl *a* or PE fluorescence. Cell volumes were measured from solids of revolution constructed by digital integration (29) by analyzing the DAPI image of the cells, including the AAP bacteria.

One problem in enumerating AAP bacteria by IR autofluorescence is that cyanobacteria are also potentially included in these estimates due to fluorescence of Chl *a* in IR (25, 36). In the present study, problems counting AAP bacteria in the presence of Chl *a*-containing cyanobacteria such as *Prochlorococcus* spp. were avoided by removing cells having Chl *a* fluorescence from IR images (8). Using this approach, only a low background of cells in a *Prochlorococcus* culture were identified as AAP bacteria (0.3% ± 0.3%). In previous work this approach detected low abundances of AAP bacteria in the North Pacific Gyre where *Prochlorococcus* spp. were highly abundant. These data indicate that our estimates of AAP bacterial abundance were not affected by *Prochlorococcus*.

Pigment concentrations. Between 1 and 5.6 liters of seawater (depending on the trophic conditions) was filtered onto 25-mm glass fiber filters (GF/F; Whatman) which were frozen immediately in liquid nitrogen and then stored at 80°C

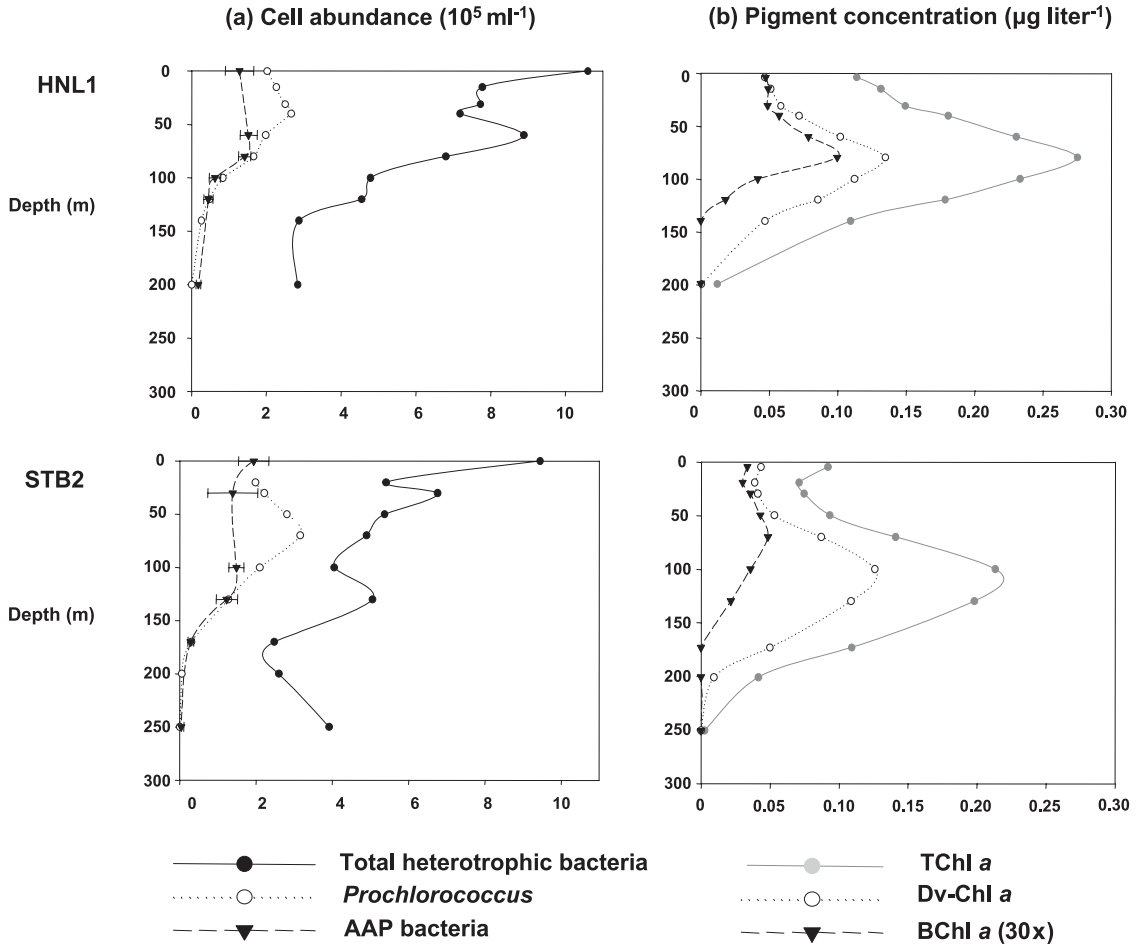

FIG. 2. Abundances of AAP bacteria, *Prochlorococcus* spp., and total heterotrophic bacteria (a) and concentrations of BChl *a*, Dv-Chl *a*, and total Chl *a* (TChl *a*) (b) at stations HNL1 and STB2. For clarity, concentrations of BChl *a* are multiplied by a factor of 30. Horizontal bars represent standard deviations of AAP counts. The coefficient of variation for the other variables was less than 5%.

until analysis back in the lab. The samples were extracted in 3 ml of methanol for a minimum of one hour, which included an ultrasonication step. The clarified extracts were injected onto an Agilent Technologies 1100 series high-performance liquid chromatography system equipped with a refrigerated autosampler and a column thermostat, according to a modified version of the method described by Van Heukelem and Thomas (31). Separation was achieved within 28 min during a gradient elution between a tetrabutylammonium acetate-methanol mixture (30:70) and 100% methanol. The chromatographic column, a Zorbax-C8 XDB (3 mm by 150 mm), was maintained at 60°C. Chl *a* and divinyl-Chl *a* (Dv-Chl *a*) were detected at 667 nm and BChl *a* at 770 nm using a diode array detector. The detection limit was 0.0001 μg liter^{-1}; the injection precision was 0.4%. The different pigments were identified both by their retention times and by absorption spectra. Quantification involved an internal standard correction (vitamin E acetate) and a calibration with external standards provided by DHI Water and Environment (Denmark) for Chl *a* and Dv-Chl *a* and by Sigma for BChl *a*.

***Prochlorococcus* and bacterioplankton abundances.** The abundances of *Prochlorococcus* spp. and heterotrophic prokaryotes (*Bacteria* and *Archaea*, including AAP bacteria) were measured by flow cytometry (FACSCalibur; Becton Dickinson) following the procedure outlined by Marie et al. (20). *Prochlorococcus* enumeration was done on board using fresh samples, while samples for the enumeration of heterotrophic prokaryotic cells were fixed with paraformaldehyde (1% final concentration), quick-frozen in liquid nitrogen, and stored at −80°C until analysis back in the lab. For heterotrophic prokaryotic counts, samples were stained with SYBR green I (Molecular Probes). Picoplanktonic populations were differentiated based on their scattering and fluorescence signals. When surface *Prochlorococcus* populations were not well defined because of their weak fluorescence, their abundance was determined by fitting a Gaussian curve to the data. To estimate bacterial carbon biomass, we assumed 12.4 fg of carbon per bacterial cell, as determined by Fukuda et al. (11).

RESULTS

Abundances of AAP bacteria, *Prochlorococcus* spp., and heterotrophic prokaryotes. AAP bacteria were present at all stations, within the first 300 m of depth, with the highest abundances (1.94×10^5 cells ml^{-1} in surface waters of station STB2 [Fig. 2 and 3]). All along the transect, AAP bacterial abundances were lower than those of *Prochlorococcus*, which ranged between less than 100 cells ml^{-1} and 3.16×10^5 cells ml^{-1} (station STB2, 70 m). Heterotrophic bacterial abundance ranged from 1.57×10^5 cells ml^{-1} at station GYR (270 m) and 1.07×10^6 cells ml^{-1} (station HNL1, 5 m) (Fig. 2a and 3a). The relative contribution of AAP bacteria to the overall bacterioplanktonic abundance varied between 1% (STB2, 250 m) and 24% (STB2, 100 m), and *Prochlorococcus* accounted for up to 44% (STB15, 70 m) of prokaryotic abundance (Table 2). At stations HNL1 and STB2, AAP bacteria were abundant from the surface to the deep chlorophyll maximum (DCM) (respec-

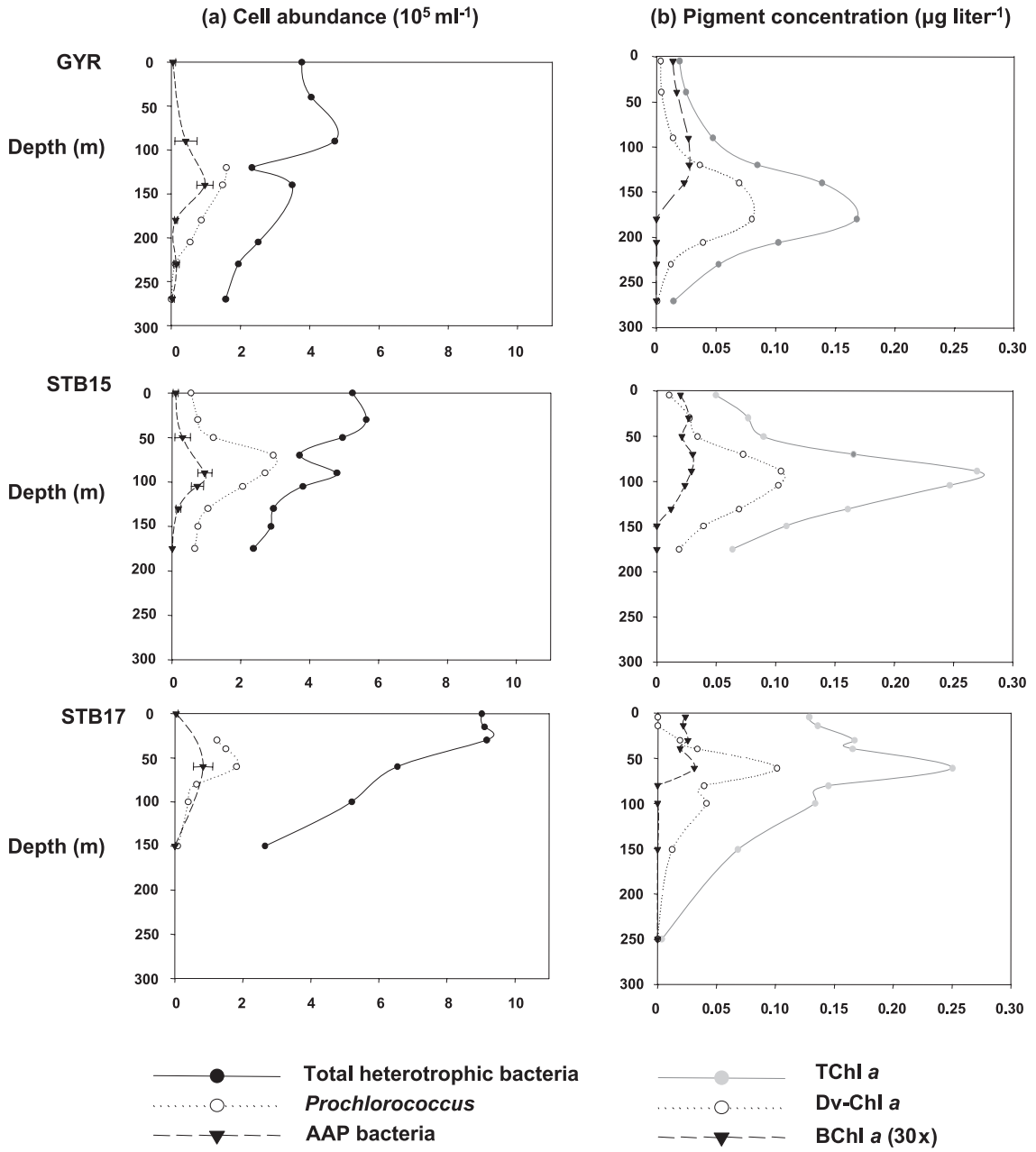

FIG. 3. Abundances of AAP bacteria, *Prochlorococcus* spp., and total heterotrophic bacteria (a) and concentrations of BChl *a* (30×), Dv-Chl *a*, and total Chl *a* (TChl *a*) (b) measured at stations GYR, STB15, and STB17. Horizontal bars represent standard deviations of AAP counts. The coefficient of variation for the other variables was less than 5%.

tively, means of 13.6% ± 3.3% and 20.1% ± 4.4% of bacterioplanktonic abundance) and rapidly decreased with depth below the DCM. At stations GYR, STB15, and STB17, AAP bacterial abundance was lowest in surface waters and highest just above the DCM, contributing 20%, 13%, and 10% of the total prokaryotic abundance, respectively. The vertical distribution of AAP bacteria was similar to that of *Prochlorococcus* but clearly different from that of nonphotosynthetic prokaryotes (Table 2; Fig. 2a and 3a).

BChl *a* and Dv-Chl *a*. Concentrations of BChl *a* ranged between undetectable levels and 3.32×10^{-3} μg liter^{-1} (HNL1, 80 m) in the euphotic zone across the transect. In contrast, concentrations of Dv-Chl *a* were roughly fifty times higher and reached values of 0.135 μg liter^{-1} (HNL1, 80 m) (Fig. 2b and 3b). The concentration of BChl *a* per AAP bacterial cell was up to 0.184 fg while *Prochlorococcus* yielded cell-specific Dv-Chl *a* concentrations of up to 2.91 fg (Table 3). BChl *a* concentrations were high from the surface to the DCM and then decreased with depth to low concentrations at 200 m. At Station GYR, BChl *a* peaked at 120 m, just above the DCM. These patterns were quite different from those of Dv-Chl *a*, as this pigment was abundant in the DCM (0.101 to 0.135 μg liter^{-1}), while low or undetectable concentrations were present at the surface and below the DCM (Fig. 2a and

TABLE 2. *Prochlorococcus* and AAP bacterial cell abundances expressed as percentages of the total bacterioplankton community

Station	Depth (m)	Abundance (%)		
		Prochlorococcus	AAP	SD
HNL1	5	15.9	10.2	3
	15	22.5		
	30	24.5		
	40	27.0		
	60	18.2	14.0	2.1
	80	19.6	16.8	1.9
	100	14.9	11.1	2.7
	120	9.6	8.9	2.5
	140	8.4		
	200	0.1	6.5	2.3
STB2	5		20.6	4.2
	20	26.9		
	30	24.7	15.5	7.3
	50	34.4		
	70	39.2		
	100	34.2	24.2	3.2
	130	20.2	19.5	4.4
	170	10.9	10.5	2.7
	200	2.2		
	250		1.1	1.7
GYR	5		1.4	2.1
	90		9.0	6.8
	120	40.6		
	140	29.8	19.6	4.7
	180		15.3	5.2
	200	17.9		
	230	5.7	7.5	3.8
	270	0.2	1.7	3.9
STB15	5	9.6	1.9	1.3
	30	11.8		
	50	19.5	5.0	3.7
	70	44.2		
	90	36.1	12.8	2.7
	105	35.1	12.6	3
	130	26.1	4.7	1.8
	150	20.8		
	175	21.9	0.3	0.2
STB17	5		0.5	0.5
	30	11.9		
	60	21.7	10.0	3.4
	100	7.0		
	150	3.0	0.1	0.3

TABLE 3. BChl *a*/Chl *a* ratios and concentrations of Dv-Chl *a* per *Prochlorococcus* cell and BChl *a* per AAP bacterial cell

Station	Depth (m)	BChl *a*/ Chl *a* (%)	Dv-Chl *a*/cell (fg cell^{-1})	Bchl *a*/cell (fg cell^{-1})
HNL1	5	1.36	0.23	0.012
	15	1.22	0.23	
	30	1.07	0.23	
	40	1.03	0.27	
	60	1.12	0.51	0.017
	80	1.18	0.81	0.023
	100	0.58	1.34	0.022
	120	0.33	1.76	0.013
	140	0.00	1.79	
	200	0.00	2.60	
STB2	5	1.20		0.006
	20	1.39		
	30	1.57	0.19	0.009
	50	1.51	0.19	
	70	1.13	0.28	
	100	0.55	0.60	0.008
	130	0.36	0.85	0.006
	170	0.00	1.64	
	200	0.00	1.58	
	250	0.00	0.00	
GYR	5	2.32		0.086
	40	2.28		
	90	1.86		0.021
	120	1.07	0.23	
	140	0.55	0.47	0.008
	180	0.00	0.92	
	200	0.00	0.72	
	230	0.00	1.04	
	270	0.00	2.91	
STB15	5	1.31	0.19	0.06
	30	1.14	0.37	
	50	0.77	0.28	0.023
	70	0.59	0.25	
	90	0.35	0.39	0.010
	105	0.31	0.50	0.011
	130	0.24	0.66	0.021
	150	0.00	0.52	
	175	0.00	0.28	
STB17	5	0.60		0.184
	14	0.52		
	30	0.50	0.16	
	40	0.38	0.23	0.012
	60	0.41	0.56	
	80	0.00	0.62	
	100	0.00	1.06	
	150	0.00	1.47	
	250	0.00		

3a). The BChl *a*/Chl *a* ratio decreased with depth, from 0.6 to 2.3% to 0% (Table 3). BChl *a* cell concentrations decreased with depth at stations GYR, STB15, and STB17 but peaked at 80 m and 30 m at stations HNL1 and STB2. In contrast, Dv-Chl *a* per cell increased with depth at all stations, from 0.19 to 0.23 fg cell^{-1} in the upper layers of the euphotic zone to values greater than 2 fg cell^{-1} above 200 m (HNL1 and GYR) (Table 3).

Biovolumes of AAP bacteria and heterotrophic prokaryotes. AAP bacteria were on average twice as large as other prokaryotic cells (Fig. 4), but this difference varied greatly. AAP bacterial cell volumes ranged from 0.115 to 0.828 μm^3, while that of other cells varied between 0.093 and 0.164 μm^3 (Fig. 4). Considering all data, the difference in biovolumes between AAP cells and total DAPI-stained cells was statistically significant (paired *t* test; $P < 0.01$, $n = 27$). AAP bacteria were significantly larger than other cells for 16 out of 25 samples, including 12 samples collected at stations HNL1, STB2, and GYR. Overall, AAP bacteria were 1.9-fold larger than other prokaryotes.

Prokaryotic cell abundance, biomass, and pigment distribution along the horizontal gradient. There was no clear relationship between AAP bacterial biomass or BChl *a* concentration and Chl *a* (Fig. 5). AAP bacterial biomass integrated over the euphotic zone was highest at stations HNL1 (13.2 mmol C

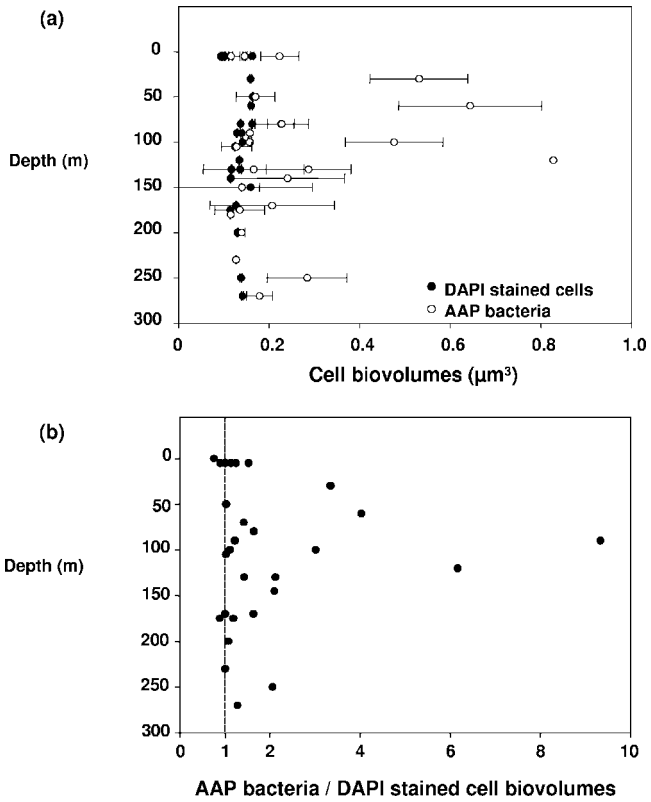

FIG. 4. (a) Cell biovolumes of AAP bacteria and DAPI-stained cells (all stations are pooled). Each point represents the mean value (± standard deviation) of biovolume determinations on each counted cell. (b) Ratio of the biovolume of AAP cells to DAPI-stained cells (all stations are pooled).

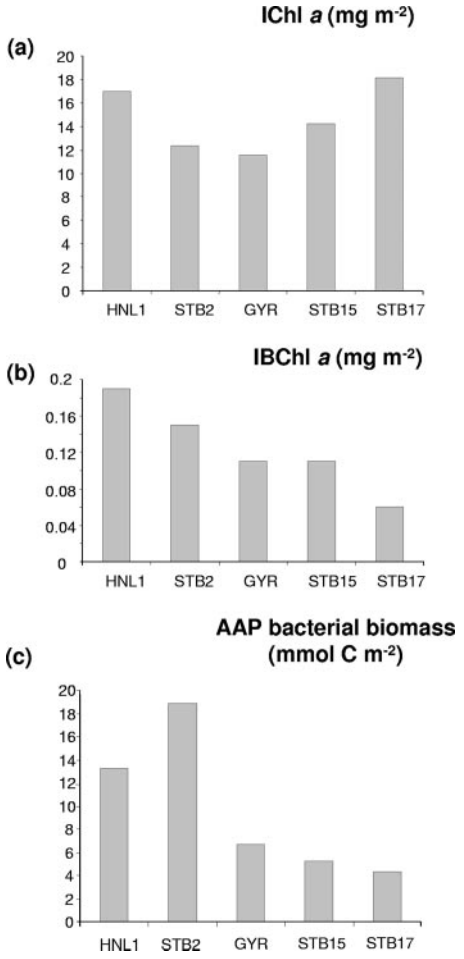

FIG. 5. Integrated (euphotic zone) Chl *a* (IChl *a*) (a), integrated BChl *a* (IBChl *a*) (b), and AAP bacterial biomass (c) along the sampling transect. AAP bacterial cells were converted to C-units using a conversion factor of 12.4 fg cell^{-1} (11).

m^{-2}) and STB2 (18.9 mmol C m^{-2}) and lower at stations GYR (6.7 mmol C m^{-2}), STB15 (5.2 mmol C m^{-2}), and STB17 (4.3 mmol C m^{-2}), while integrated Chl *a* concentrations were highest at the most eastern and the most western stations. There was also no relationship between Chl *a* and BChl *a*. Like AAP bacterial stocks, the highest integrated concentrations of BChl *a* were found only at the most eastern stations (HNL1 and STB2) and decreased from the east to the west of the transect, from 0.19 mg m^{-2} at station HNL1 to 0.058 mg m^{-2} at station STB17 (Fig. 5).

DISCUSSION

Biogeochemical processes in the South Pacific Ocean remain poorly documented, even though the importance of this oceanic area in global biogeochemical cycles is well recognized (7, 10). In this study, we determined the abundance and distribution of AAP bacteria in the photic zone of the South Pacific Ocean along a transect with various trophic conditions. Our sampling included the center of the gyre, the most oligotrophic water body of the world ocean. At all stations we found high abundance and biomass of AAP bacteria, along with high concentrations of BChl *a*.

We detected high abundances (up to 1.94×10^5 cells ml^{-1}) of AAP bacteria at each station along the transect and at several depths in the photic zone of the South Pacific Ocean. The high-standing stocks of AAP bacteria were found above the DCM at stations HNL1 and STB2 and closer to the DCM at stations GYR, STB15, and STB17. AAP bacteria were also a large fraction of the overall South Pacific prokaryotic community (up to 24%) and together with *Prochlorococcus* spp. constituted up to 58% of the overall prokaryotic community in these oceanic regimes. These abundances of AAP bacteria are the highest ever reported for oligotrophic waters. High relative abundances (11%) in the oligotrophic North Pacific Ocean were also reported by Kolber et al. (17). However, that study did not correct for possible inclusion of cyanobacteria in the AAP bacterial estimate. Cottrell et al. (8) did this correction and still found that AAP bacteria made up nearly 18% of the prokaryotic community in Gulf Stream waters and more than 10% in the shelf break waters of the Mid-Atlantic Bight. In contrast, Schwalbach and Fuhrman (25) report that AAP bacterial abundance was low in oligotrophic waters of Southern California.

Concomitant with high abundances of AAP bacteria, we also determined high concentrations of BChl *a* for oligotrophic waters, close to those reported by Kolber et al. (17). The BChl

a/Chl *a* ratio (up to 2.3%) was lower than the 5 to 10% estimated by Kolber et al. (17) in the oligotrophic North Pacific Ocean but was close to ratios reported off the California coast (13), for the Baltic Sea (15), and the Mid-Atlantic Bight and the Gulf Stream (8).

It was previously suggested that photoheterotrophy may be an adaptation to oligotrophic environments, as light might supply energy under low-nutrient concentration conditions (17). The relationship between AAP bacteria and the nutrient status of water masses is still unclear, since several studies report high abundances of these bacteria in estuarine environments (25) and in coastal waters (8). To our knowledge, this is the first study reporting such high AAP bacterial abundances in an oligotrophic environment. Our results clearly indicate that AAP bacteria are not adapted to a narrow range of trophic conditions and that these bacteria may represent a large fraction of the prokaryotic community, whatever the trophic status of the water masses.

AAP bacteria were detected in the upper layers of the photic zone, between the DCM and the sea surface. Several authors have also reported the presence of AAP bacteria in the photic zone, in agreement with their phototrophic abilities (8, 17). Indications of in situ photosynthetic activity in the photic zone were previously described, as fluorescence data indicated that AAP bacteria account for 2 to 5% of the photosynthetic electron transport (16). Although cultured AAP bacterial isolates can survive under dark conditions, light enhances their growth (28, 34). All these observations suggest that AAP bacteria are able to use their phototrophic potential. However, the measured low amounts of BChl *a* per cell—consistent with previous published observations (8, 29)—reinforce the hypothesis that AAP bacterial phototrophy is probably low in the ocean and that these organisms essentially use energy from dissolved organic matter, in contrast to *Prochlorococcus* cells, which are strictly phototrophs.

These data suggest that phototrophy is one possible adaptation making AAP bacteria competitive and therefore widespread in oceanic waters. However, this wide distribution of AAP bacteria is probably due to the fact that phototrophy in most AAP bacteria is combined with a wide range of other metabolic capacities. *Erythrobacter longus*, the first AAP bacterial strain isolated in the marine environment (27), was previously found in natural bacterial communities using organic material produced by phytoplankton in the Adriatic Sea (9). This high metabolic plasticity has been reported among other cultured AAP bacterial strains. In the *Roseobacter* clade, several AAP bacteria can combine phototrophy with other specific metabolisms (5). Several studies demonstrate that this photoheterotrophic strategy is not limited to a few AAP bacterial species. Although cultured AAP bacteria are restricted to some *Erythrobacter*, *Roseobacter*, and gammaproteobacterial strains (6, 24), recent metagenomic approaches conducted in the Sargasso Sea (32), in the Delaware estuary (33), and in offshore California (3) revealed a high diversity of AAP bacterial sequences, demonstrating that AAP bacteria are distributed within several alpha- and gammaproteobacterial phylogenetic groups.

Our estimates of AAP bacterial biomass revealed that these organisms are a large stock of carbon in the euphotic zone, thus suggesting that AAP bacteria may play an important ecological role in the oceans. In further support of this hypothesis, our biovolume data indicated that these bacteria were larger than other prokaryotic cells. Sieracki et al. (30) found a similar trend across a transect in the North Atlantic Ocean. The larger size of AAP bacteria also suggests that these bacteria may be more active than other fractions of the bacterial community, if there is a positive relationship between cell size and activity, as previously shown (4, 12, 18). Furthermore, AAP bacteria might be under intense grazing pressure, since larger cells are preferentially grazed on by bacterial predators (1, 14, 26). Although further investigations are needed to better understand the ecological role of AAP bacteria, these observations reinforce their potential role in marine carbon cycling.

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