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

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ABSTRACT

Aerobic anoxygenic photosynthetic (AAP) bacteria are bacteriochlorophyll (Bchl *a*) containing microorganisms. Although these bacteria seem to be widespread in marine environments, little is known about their abundance, distribution and more generally their ecology, particularly in oligotrophic environments which represent 60% of the ocean. We investigated the importance of AAP bacteria across the South Pacific Ocean including the centre of the gyre, the most oligotrophic water body of the world ocean. AAP bacteria, *Prochlorococcus* and total heterotrophic bacterial abundance, as well as BChl *a* and Divinyl-Chlorophyll *a* concentrations were measured at several depths in the photic zone along a gradient of oligotrophic conditions. High abundances of AAP bacteria (up to 1.94×10^5 cells ml^{-1} and up to 24% of the overall bacterioplankonic community) as well as high Bchl *a* concentrations (up to 3.32×10^{-3} μg $liter^{-1}$) were found at all stations. Furthermore, the biovolumes of AAP bacteria were larger than those of other bacterioplanktonic cells. This study clearly demonstrates that AAP bacteria are ubiquitous in the marine environment and can be abundant in a wide diversity of trophic conditions including the most oligotrophic environments.

INTRODUCTION

Prokaryotic microbes play a key role in the carbon flow in aquatic ecosystems. Heterotrophic bacteria are central actors of the microbial loop and play an essential role in the transformation of dissolved organic matter (DOM) (2). In contrast, autotrophic bacteria like *Prochlorococcus* 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). It is unclear if AAP bacteria are capable of CO_2 fixation in the environment (17). The first marine AAP bacterium was isolated thirty years ago (28), but only recently these bacteria were 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 new 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 represent a significant fraction of the bacterioplanktonic community. Consequently, AAP bacteria could significantly contribute to the carbon cycle in the oceans (17).

It was suggested that AAP bacteria are adapted to oligotrophic waters, as light might supply 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 few studies combine both AAP cell enumeration together with BChl *a* concentrations. Moreover, little is known about the relative importance of AAP bacteria compared to autotrophic bacteria. Cottrell et al. (8) found that AAP bacteria were two-fold 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 across the South Pacific Ocean and to assess their relative importance in a gradient of oligotrophic conditions. Microscopic counts revealed high abundances of AAP bacteria across the South Pacific Ocean, yielding up to 24% of the overall bacterioplanktonic cell abundance.

MATERIALS AND METHODS

Study sites.

Samples were collected during the BIOSOPE (Biogeochemistry and Optics South Pacific Ocean Experiment) cruise that took place in October-December 2004. The sampling strategy was based on satellite imagery of ocean color with the aim of collecting samples across various trophic conditions. From the West to the East of the cruise transect, five stations, covering a distance of 6000 nautical miles and varying oligotrophic regimes were sampled (Fig.1): stations HNL1 (09°00S, 136°50W), STB2 (13°33S, 132°06N), GYR (26°00S, 114°00W), STB15 (30°041S, 95°25W) and STB17 (32°23S, 86°47W). Seawater samples were collected with General Oceanics 12 liters Niskin bottles mounted on a rosette equipped with a SeaBird SBE19+ CTD. 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). Integrated Chlorophyll *a* (Chl *a*) values (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, 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.

AAP bacterial abundance and cell volume determination

For the enumeration of AAP bacteria, seawater samples were fixed with paraformaldehyde 2% (final concentration). Samples were stored in the dark (18h, 4°C), before filtering onto 0.2µm 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, after a DAPI staining step (5 min) in a 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 water [pH 7.4]) the sample was mounted on a glass slide. We then added an antifading agent comprised of Citifluor (Ted Pela) and Vectashield (Vector Labs) mixed in a ratio 4:1 (vol/vol). AAP bacteria were counted on an Olympus Provis AX70 microscope. An image analysis software (ImagePro Plus, Media Cybernetic) was used to discriminate DAPI stained cells and AAP bacteria by infrared (IR) fluorescence, but not Chl *a* or Phycoerythrin (PE) fluorescence. For each field, 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 charged-coupled-device camera (Intensified Retiga Extended Blue; Q Imaging) with specific exposure times : DAPI, 40 ms; IR, 200 ms; Chl 1.500 ms; 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 or PE fluorescence. Cell volumes were measured by image analysis of DAPI-stained cells and measured from solids of revolution constructed by digital integration (29).

Pigment concentrations

Seawater samples were collected from the 12 liters Niskin bottles of the rosette sampler. Volumes between 5.6 and 1 liter (depending on the trophic conditions) were filtered onto 25 mm GF/F filters which were subsequently stored in liquid nitrogen then at –80°C

until analysis on land. The samples were extracted in 3 ml methanol for one hour minimum, with filter disruption by ultra-sonication. The clarified extracts were injected onto an *Agilent Technologies 1100* series High Performance Liquid Chromatography (HPLC) system equipped with a refrigerated auto sampler 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 actetate:methanol mixture (30:70) and 100% methanol. The chromatographic column, a Zorbax-C8 XDB (3 ×150 mm) was maintained at 60°C. Chl *a* and Divinyl-Chlorophyll *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 by both their retention times and absorption spectra. Quantification involved an internal standard correction (Vitamin E actetate) 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 abundance of *Prochlorococcus* and heterotrophic prokaryotes (*Bacteria* and *Archaea,* including AAP bacteria) were measured by flow cytometry (FACSCalibur, Becton Dickinson) following the proceedure outlined by Marie et al. (20) *Prochlorococcus* enumeration was done on board on 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 considered 12.4 fg of carbon per bacterial cell as in Fukuda et al. (11).

RESULTS

Abundance of AAP bacteria, *Prochlorococcus* and heterotrophic prokaryotes.

AAP bacteria were present at all stations, with the highest abundances (1.94×10^5 cells.ml^{-1} in surface waters of station STB2 (Fig 2,3). 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, 70m). 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, 5m) (Fig. 2a, Fig 3a). The relative contribution of AAP bacteria to the overall bacterioplanktonic abundance varied between 1% (STB2, 250 m) and 24% (STB2, 100 m) and *Prochlorococus* accounted 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) (respectively mean 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, with relative contributions of 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 non-photosynthetic prokaryotes (Table 2, Fig. 2a, Fig. 3a).

Bacteriochlorophyll *a* and Divinyl-Chlorophyll *a* (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 DvChl *a* were roughly fifty times higher and reached values of 0.135 μg.liter^{-1} (HNL1, 80 m) (Fig. 2b, Fig 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 – 0.135 μg.liter^{-1}), while low or undetectable concentrations were present at the surface and below the DCM (Fig. 2a., Fig. 3a). The BChl *a*/Chl *a* ratio decreased with depth, from 0.6 - 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 0.19-0.23 fg cell^{-1} in the upper layers of the euphotic zone to values greater than 2.00 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 1.296 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 all 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.

Prokaryotic cell abundances, 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 mmolC m^{-2}) and STB2 (18.9 mmolC m^{-2}) and lower at stations GYR (6.7 mmolC m^{-2}), STB15 (5.2 mmolC m^{-2}) and STB17 (4.3 mmolC 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 varying trophic conditions. Our sampling included the center of the gyre, the most oligotrophic water body of the world ocean. We found at all stations high abundances and important biomasses of AAP bacteria, along with high concentrations of Bchl *a.*

One problem in enumerating AAP bacteria by IR autofluorescence is that cyanobacteria are also potentially included in these estimates due to a emission 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* 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 *Prochorococcus* were highly abundant. These data indicate that our estimates of AAP bacterial abundance were not affected by *Prochlorococcus*.

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 constituted together with *Prochlorococcus* 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.

Concomittant with high abundances of AAP bacteria, we also determined high concentrations of Bchl *a* for oligotrophic waters, close to those reported in Kolber et al. (17). The BChl *a*/Chl *a* ratio (up to 2.3%) was lower than the 5-10% estimated by Kolber et al. (17) in the oligotrophic North Pacific Ocean but was close to ratios reported off the Californian coast (13), for the Baltic Sea (15), 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 concentrations conditions (17). The relationship between AAP bacteria and the nutrient status of water masses is still being debated in the literature, 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 the 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 an important 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 also report the presence of AAP bacteria in the photic zone, in agreement with their phototrophic abilities (8, 17). Clues of an *in situ* photosynthetic activity in the photic zone were previously described, as fluorescence data indicate that AAP bacteria account for 2-5% of the photosynthetic electron transport (16). Although cultured AAP bacterial isolates can survive under dark conditions, it was reported that light enhances their growth capacities (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.

All these data suggest that phototrophy is one possible adaptation making AAP bacteria extremely 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. *Roseobacter littoralis*, the first AAP bacteria 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) and was also described as a lithotroph that uses dissolved CO (for review, see (5)). 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). Among isolated *Erythrobacter* strains, previous studies reported that *Erythrobacter* sp. NAP1 and *Erythrtobacter* sp. OCh114 isolates are capable of CO_2 fixation (17, 28). 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 offshore California (3) revealed a high diversity of specific AAP bacterial sequences, demonstrating that AAP bacteria are distributed within several alpha- and gammaproteobacterial phylogenetic groups.

Our estimations of AAP bacterial biomass revealed that these organisms can represent an important stock of carbon in the euphotic zone, thus suggesting that AAP bacteria may play an important ecological role in the oceans. Our biovolume measurements reinforce this assertion. 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 suggest that these bacteria may be more active than other fractions of the bacterial community, if we assume that 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). Althrough further investigations are needed to better understand the ecological role of AAP bacteria, these observations reinforce their potential role in marine carbon cycling.

Conclusions

Our results clearly demonstrate that marine AAP bacteria can be abundant in oligotrophic and ultra-oligotrophic conditions. The importance of AAP bacteria in the world ocean might be explained by their high metabolic plasticity, combining autotrophy with a wide range of specific heterotrophic abilities. Previous studies revealed an important phylogenetic diversity among AAP bacterial populations, but little is still known on the diversity of photoheterotrophic bacteria in the water column and on the ecology of AAP bacterial populations. Further work is needed to isolate and to characterize AAP species and to determine their *in situ* activity as well as how environmental conditions affect their activity. Based on available data, AAP bacteria appear to be an essential link in the control of carbon fluxes, and should be considered when modeling biogeochemical processes in general and the carbon cycle in particular.

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Table 1. General oceanographic parameters: 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. n.d.- not detectable.

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

Table 2. Percentages of *Prochlorococcus* and AAP bacterial cell abundances of the overall bacterioplankton community.

Station	Depth (m)	*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, concentrations of Dv-Chl *a* per *Prochlorococcus* cell and BChl *a* per AAP bacterial cell.

Station	Depth (m)	Bchl *a* / Chl *a* (%)	DvChl *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	0.0		

FIGURE LEGENDS

Figure 1. Sampled stations along the transect of the BIOSOPE cruise in the South Pacific Ocean (October - December 2004).

Figure 2. Abundances of AAP bacteria, *Prochlorococcus,* total heterotrophic bacteria (a) and concentrations of BChl *a*, Dv-Chl *a* and TChl *a* (b) at stations HNL1 and STB2. For clarity, concentrations of BChl *a* are magnified by a factor of 30.

Figure 3. Abundances of AAP bacteria, *Prochlorococcus,* total heterotrophic bacteria (a) and concentrations of BChl *a* (×30), Dv-Chl *a* and TChl *a* (b) measured at stations GYR, STB15 and STB17.

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

Figure 5. Integrated (euphotic zone) Chlorophyll *a* (IChl *a*) (a), bacteriochlorophyll *a* (BChl *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} (Fukuda et al. 1998).

Figure 1

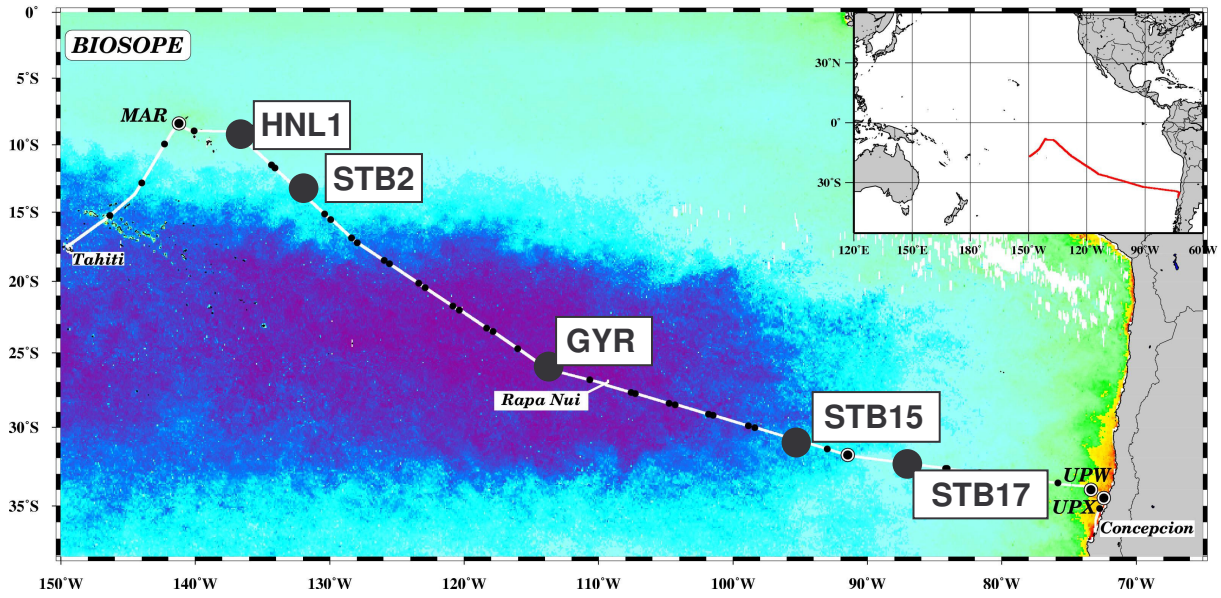

BIOSOPE
MAR
HNL1
STB2
Tahiti
GYR
Rapa Nui
STB15
STB17
UPW
UPX
Concepcion
0°
5°S
10°S
15°S
20°S
25°S
30°S
35°S
150°W
140°W
130°W
120°W
110°W
100°W
90°W
80°W
70°W
30°N
0°
30°S
120°E
150°E
180°
150°W
120°W
90°W
60°W

Figure 2

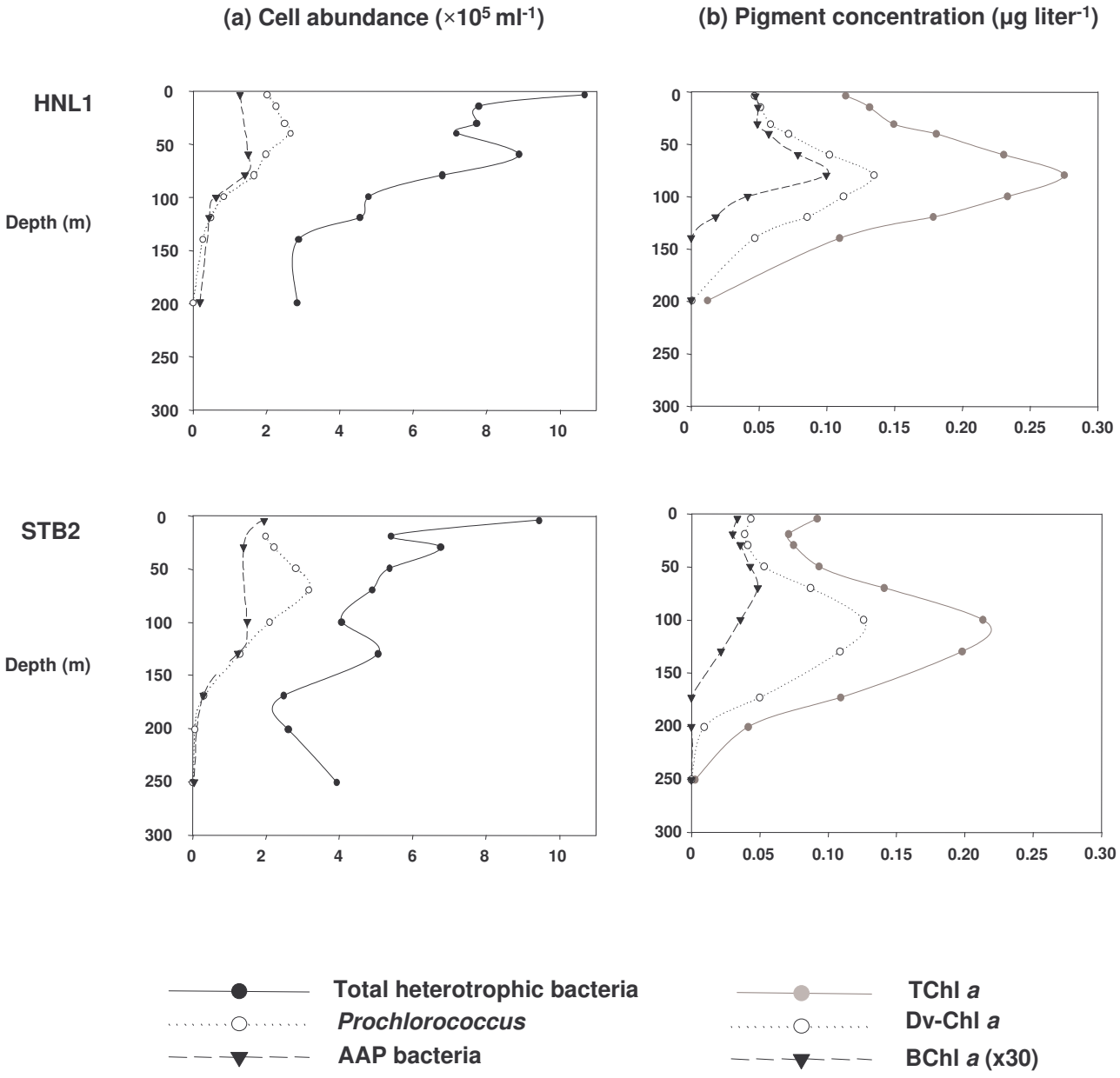

(a) Cell abundance (×10^5 ml^-1)
(b) Pigment concentration (µg liter^-1)
HNL1
STB2
Depth (m)
Depth (m)
0
50
100
150
200
250
300
0
2
4
6
8
10
0.05
0.10
0.15
0.20
0.25
0.30
Total heterotrophic bacteria
Prochlorococcus
AAP bacteria
TChl a
Dv-Chl a
BChl a (x30)

Figure 3

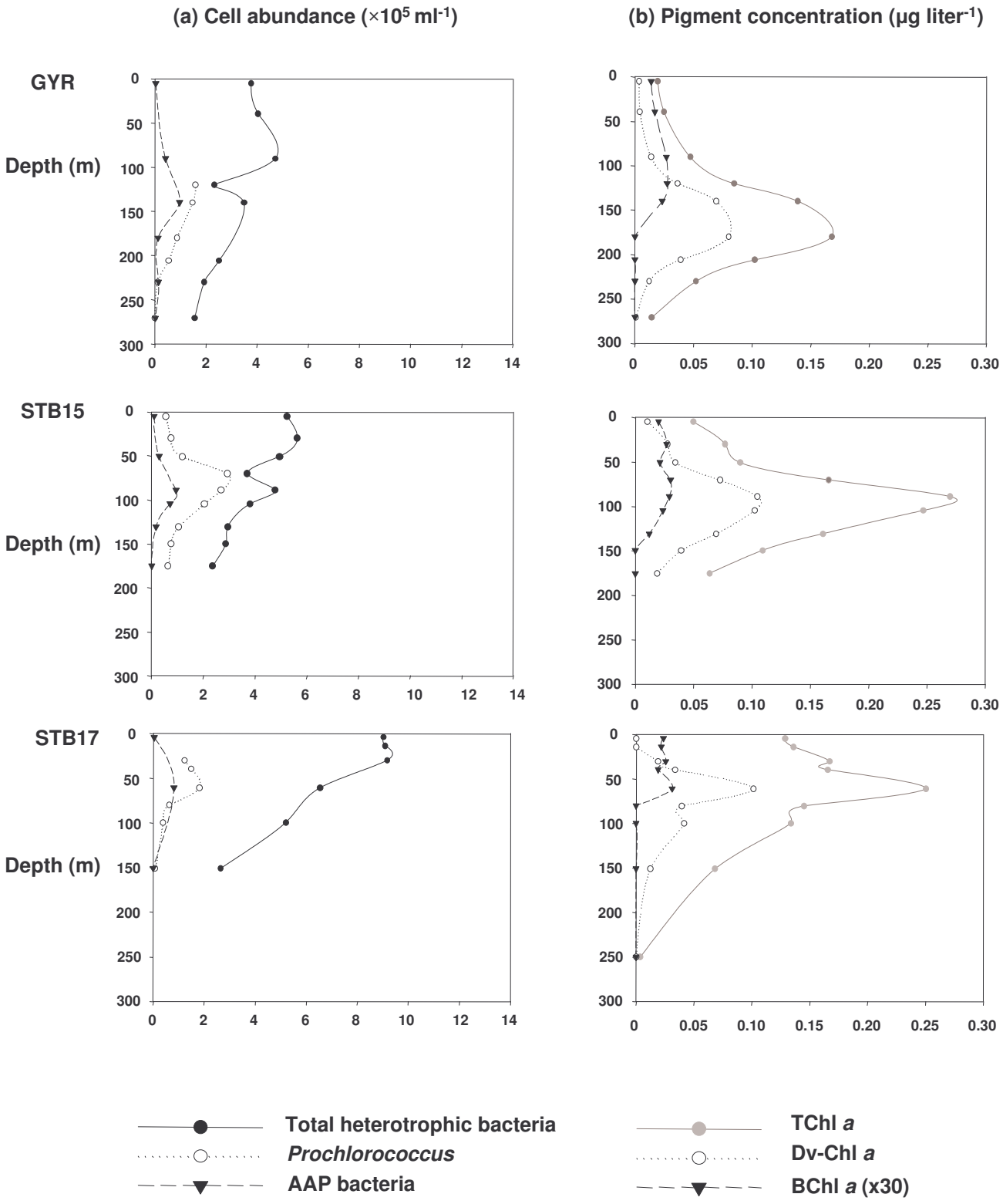
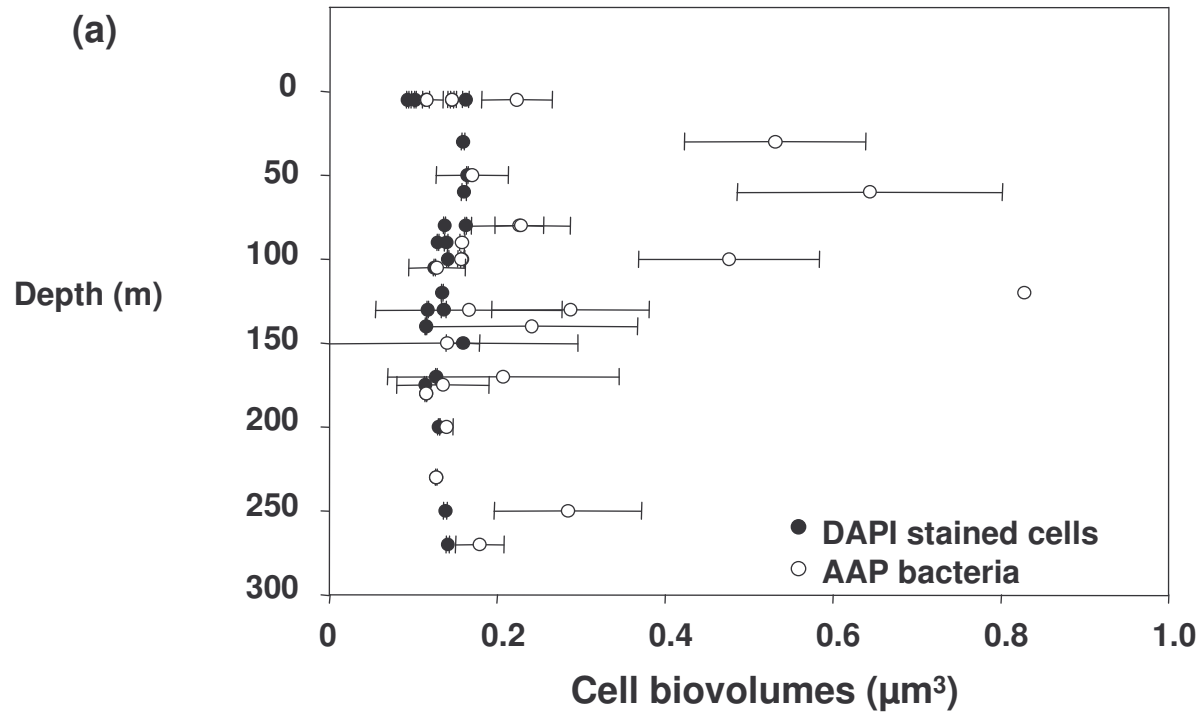

(a) Cell abundance (×10^5 ml^-1)
(b) Pigment concentration (µg liter^-1)
GYR
STB15
STB17
Depth (m)
0
50
100
150
200
250
300
0 2 4 6 8 10 12 14
0 0.05 0.10 0.15 0.20 0.25 0.30
Total heterotrophic bacteria
Prochlorococcus
AAP bacteria
TChl a
Dv-Chl a
BChl a (x30)

Figure 4


(a)
Depth (m)
0
50
100
150
200
250
300
0
0.2
0.4
0.6
0.8
1.0
DAPI stained cells
AAP bacteria
Cell biovolumes (µm³)

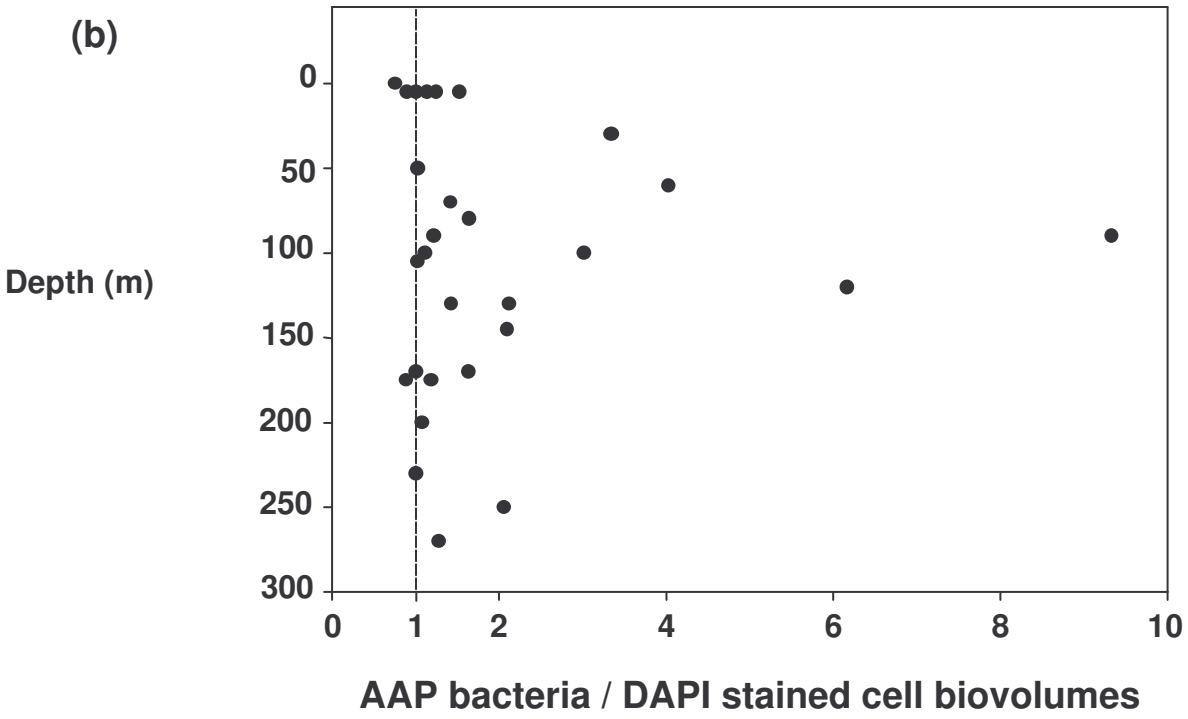
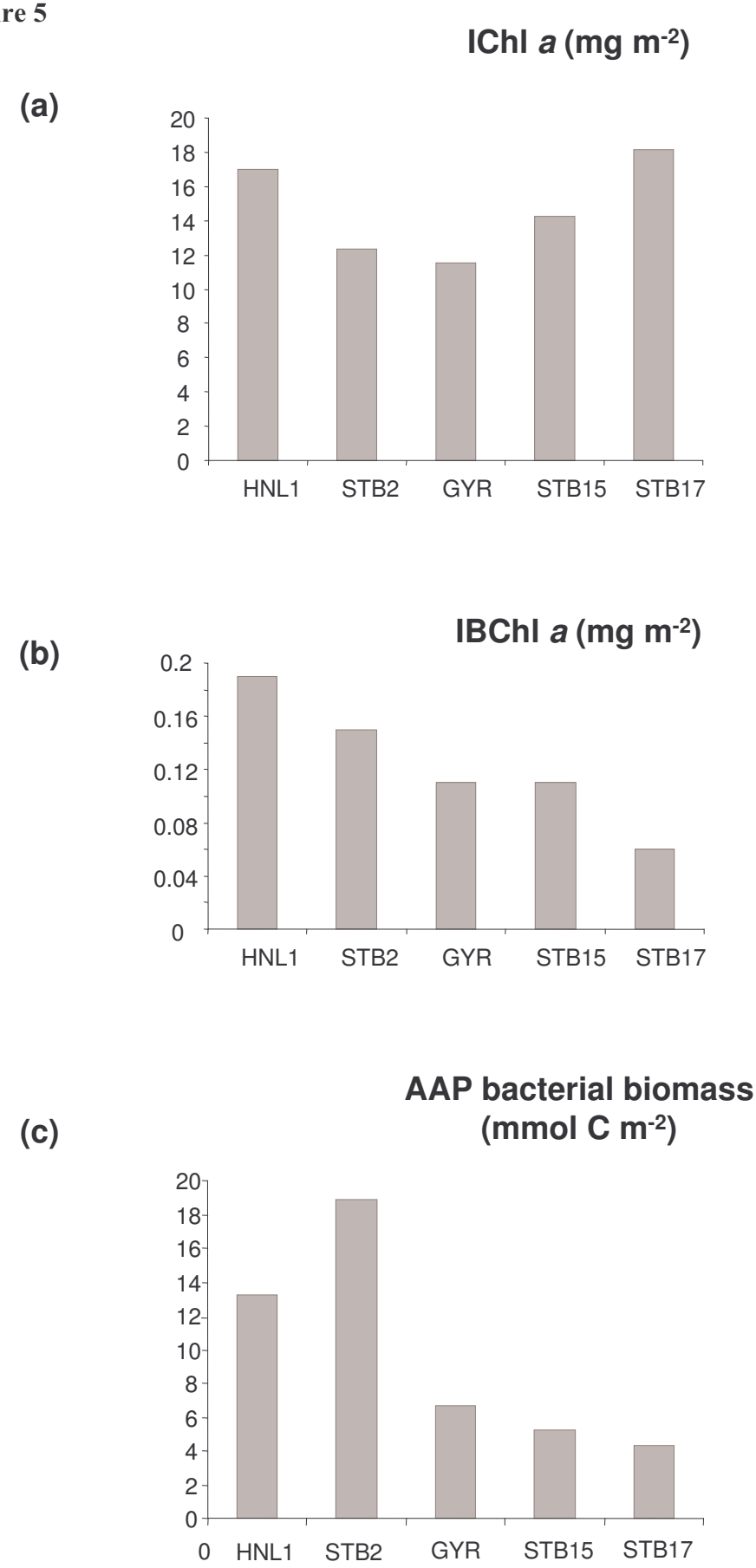

(b)
Depth (m)
0
50
100
150
200
250
300
0
1
2
4
6
8
10
AAP bacteria / DAPI stained cell biovolumes

Figure 5


(a)
IChl *a* (mg m-2)
20
18
16
14
12
10
8
6
4
2
0
HNL1
STB2
GYR
STB15
STB17
(b)
IBChl *a* (mg m-2)
0.2
0.16
0.12
0.08
0.04
0
HNL1
STB2
GYR
STB15
STB17
(c)
AAP bacterial biomass
(mmol C m-2)
20
18
16
14
12
10
8
6
4
2
0
0
HNL1
STB2
GYR
STB15
STB17