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Abundance, size, and activity of aerobic anoxygenic phototrophic bacteria in coastal waters of the West Antarctic Peninsula

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ABSTRACT: The objective of this study was to examine the abundance, size, and single-cell activity of aerobic anoxygenic phototrophic (AAP) bacteria in summer and fall over 2 yr in coastal waters of the West Antarctic Peninsula. Single-cell incorporation of ^{3}H-leucine was measured using a new microautoradiography approach coupled to infrared epifluorescence microscopy. The relative abundance of these photoheterotrophic bacteria was higher in January (1 to 8%) than in May (0.3 to 1%) but differed greatly between the 2 yr we sampled, as did several biogeochemical properties. The biovolume of AAP bacteria was nearly 3-fold larger than that of cells in the total bacterial community and ca. 2-fold larger than AAP bacterial biovolumes in low latitude coastal waters. A large fraction of AAP bacteria and of the total community incorporated ^{3}H-leucine in January (ca. 48% for both communities), which then decreased by 4-fold in May. The area of silver grains formed around active cells in microautoradiography preparations decreased nearly 5-fold from January to May. Silver grain area data indicated that AAP bacteria were about 40% more active than the total community in January but there was no difference in May, probably because of the nearly 20-fold decrease in light availability from January to May. These data suggest that AAP bacteria contribute more to biomass production in these polar waters than suggested by their abundance, but only in the austral summer.

KEY WORDS: Aerobic anoxygenic phototrophs · Photoheterotrophs · Bacterial growth · Leucine incorporation · Single-cell activity

INTRODUCTION

Polar waters are challenging environments for aerobic anoxygenic phototrophic (AAP) bacteria and other photoheterotrophs because light availability and other environmental properties potentially affecting phototrophy vary greatly. A quantitative PCR study found that AAP bacteria made up <0.1% of microbial communities in the Gerlache Strait and other Antarctic waters (Schwalbach & Fuhrman 2005), the lowest percentage observed to date for marine surface waters. In contrast, these bacteria are abundant in Arctic coastal waters (5 to 8%), although less so in deeper waters of the Beaufort Sea according to studies using epifluorescence microscopy (Cottrell & Kirchman 2009, Boeuf et al. 2013). AAP bacteria apparently are successful in the Arctic summer with 24 h of daylight even though the synthesis of bacteriochlorophyll (BChl) by AAP bacteria in cultures, except for a gammaproteobacterium (Spring et al. 2009), is repressed by light (Yurkov & Hughes 2013). Surprisingly, the relative abundance of AAP bacteria remained about the same in the Arctic winter in spite of near-complete darkness (Cottrell & Kirchman 2009). The lack of a difference in AAP bacterial abundance despite such extremes in light

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availability is consistent with the hypothesis that AAP bacteria gain relatively little energy from phototrophy (Kirchman & Hanson 2013).

The advantage afforded by phototrophy is more directly explored by data on the activity of AAP bacteria. Previous studies have used BChl turnover and other approaches to estimate growth rates of AAP bacteria in low-latitude marine systems (Koblížek et al. 2005, 2007, Liu et al. 2010, Ferrera et al. 2011, Hojerová et al. 2011). These studies suggest that AAP bacteria grow faster than the rest of the bacterial community. Recently, we developed a microautoradiographic method (AAP-MAR) to examine single-cell leucine incorporation by AAP bacteria in the Delaware estuary (Stegman et al. 2014). We found that AAP bacteria in the estuary were ca. 40% more active than the rest of the community — an enhancement lower than seen by most previous studies, but larger than predicted (Kirchman & Hanson 2013).

The objective of this study was to explore AAP bacteria in another polar environment, the coastal waters of the West Antarctic Peninsula (WAP). These waters have been extensively sampled by studies examining plankton dynamics (Ducklow et al. 2006), microbial metagenomics (Grzymski et al. 2012), and microbial activity at the single-cell level (Nikrad et al. 2014a,b). We measured the abundance of AAP bacteria using infra-red (IR) epifluorescence microscopy and single-cell incorporation of ^{3}H-leucine using the AAP-MAR approach (Stegman et al. 2014) in 2 mo over 2 yr in WAP waters. The cell sizes of AAP bacteria were also examined and compared with data from the Arctic Ocean and the Mid-Atlantic Bight (MAB) collected by previous studies (Cottrell & Kirchman 2009, Stegman et al. 2014). Previous studies found that AAP bacteria are bigger than other bacteria (Sieracki et al. 2006, Stegman et al. 2014) and that many bacteria in polar waters are bigger than bacteria in low latitude systems (Straza et al. 2009). We found that AAP bacteria were sometimes more active and bigger than other bacteria in WAP waters. These data add to accumulating evidence indicating that AAP bacteria have unique roles in the carbon cycle.

MATERIALS AND METHODS

Surface water was collected at 1 m depth in January and May 2011 and 2012 at a site 200 m (Station B; 64° 46.77 S, 64 ° 04.35 W) and another 3.2 km (Station E; 64° 48.90 S, 64° 02.43 W) offshore of Palmer Station on Anvers Island (64° 40 S, 64° 03 W). These 2 stations are being examined by a Long Term Ecological Research project (http://pal.lternet.edu/) (Ducklow et al. 2009) and were sampled by previous single-cell studies of organic compound uptake and bacterial growth (Nikrad et al. 2014a,b). Total prokaryote abundance was measured by microscopy after staining with 4′,6-diamidino-2-phenylindole (DAPI) and chlorophyll *a* (chl *a*) was measured by the standard fluorescence method. The abundance of AAP bacteria was determined by IR epifluorescence microscopy as described in Cottrell et al. (2006). Total leucine and thymidine incorporation rates were measured using the microcentrifuge method (Kirchman 2001). ^{3}H-leucine or ^{3}H-thymidine was added to triplicate incubations either in the dark or light for 4 h at the surface ocean temperature (20 nM, final concentration, 54.1 Ci mmol^{-1} and 80 Ci mmol^{-1}, respectively; Perkin Elmer). The light was provided by fluorescent lamps at an intensity of 100 µmol photons m^{-2} s^{-1}. Average surface light intensity in this environment was 1000 ± 400 µmol photons m^{-2} s^{-1} in January and 106 ± 75 µmol photons m^{-2} s^{-1} in May. The light data given in this paper are from www.esrl.noaa.gov/gmd/grad/antuv/Palmer.jsp. More experimental details are given elsewhere (Nikrad et al. 2014a).

Single-cell activity of AAP bacteria

We used the AAP-MAR method to examine single-cell use of leucine in January 2011, May 12 and 18, 2011, and May 9, 11 and 16, 2012. None of the single-cell activity measurements from May 2011 differed significantly from the May 2012 results (see 'Results'). Similar to the bulk assays, 20 nM ^{3}H-leucine was added to seawater and incubated in the dark or light for 4 h, after which paraformaldehyde was added (2% final concentration). Samples killed with paraformaldehyde before the ^{3}H-additions were also analyzed. The water was then filtered through polycarbonate filters (0.2 µm pore size) and stored at –80°C until AAP-MAR analysis as described elsewhere in detail (Stegman et al. 2014). In brief, sections of filters from the ^{3}H-leucine incubations were examined by IR epifluorescence microscopy to determine the location of microscope fields containing AAP bacteria. The sections were then taken off the microscope and subjected to the microautoradiography procedure. The exposure time during microautoradiography was 24 h, which was determined by time-course experiments to maximize detection of active cells while minimizing silver grain formation in killed controls (data not shown). The filter sections were

returned to the epifluorescence microscope, the previously analyzed fields were re-located automatically, and analyzed again for the presence of silver grains around DAPI-stained cells, indicating that the cell had taken up the ^{3}H-leucine. The image analysis program also measures the size of the silver grain area around active cells. Since few cells were labeled in the killed controls (<1%), it was not necessary to correct results from the live incubations.

The relative abundance and cell size of AAP bacteria reported here were estimated by standard IR epifluorescence microscopy (Cottrell et al. 2006). The current version of the AAP-MAR method does not give accurate biovolume estimates. We also analyzed biovolume data from coastal waters of the Arctic Ocean and MAB. The Arctic Ocean data were collected by a previous study but they were not published (Cottrell & Kirchman 2009). In addition to data from Stegman et al. (2014), the MAB data comes from monthly samples collected between February 2006 and August 2013 at 2 stations examined previously (Straza & Kirchman 2011). For all environments, the average number of cells examined to estimate biovolume was about 9000 per sample for the total community (range of 5011 to 23 468) and 500 for the AAP bacteria (range 30 to 2000). The number of AAP bacterial cells was low in samples from Palmer in May 2012 when these microbes were <1% of the total. The statistical analysis used the means and SD calculated from samples taken over time and among locations within the marine system; the SD calculated from the technical replicates was not used.

Statistical analyses

The results were explored with the nonparametric Kruskal-Wallis test or the parametric paired *t*-test. For the latter test, the percentage data were first subjected to the arcsin transformation while silver grain areas were log-transformed to satisfy the assumptions of the test. The number of samples reported here is the number of dates the 2 stations were sampled during the 4 mo of this study. All statistical analyses were done in R (www.r-project.org). The Kruskal-Wallis rank sum test was first used to see if a property differed significantly among the months we sampled. If significant differences were found, then the kruskalmc function in the pgirmess package (http://perso.orange.fr/giraudoux) was used for a multiple comparison test to explore which month was significantly different from the others.

Silver grain area around cells actively taking up ^{3}H-leucine is proportional to ^{3}H-leucine incorporation rates (Sintes & Herndl 2006). In addition to the silver grain area around active cells only, we obtained an estimate of silver grain area representative of either all bacteria or of all AAP bacteria. To do so, the measured silver grain area around active cells was multiplied by the proportion of active cells in either the entire bacterial community or the entire AAP bacterial community. The resulting value is a weighted average of the silver grain area around all cells in either community, reflecting both the active cells with silver grains and inactive cells with none.

RESULTS

We examined the abundance, cell size, and activity of AAP bacteria in coastal waters of the Antarctic Peninsula near Palmer Station in 2 seasons (January and May) over 2 yr. Although 2 stations were usually visited each sampling day, data from the two are not distinguished here because neither the oceanographic properties discussed here (Nikrad et al. 2014a) nor the AAP bacterial parameters (see below) differed significantly. The biophysical properties of the water column differed between January and May as expected, but temperature and general biological parameters also varied greatly between the 2 yr (Table 1). Temperature was nearly 3°C warmer in January 2011 than in May 2011, but the water in January 2012 was near 0°C, only 0.4°C warmer than in May 2012. Likewise, chl *a* was 4-fold higher in January 2011 than in May 2011, and was also more than 5-fold higher in January 2012 compared to January 2011. Bulk

Table 1. Summary of biogeochemical properties of coastal waters near Palmer Station, Antarctica. Means ± SD are shown. Temp.: water temperature; abundance refers to all prokaryotes; leu incorp.: leucine incorporation by the entire community; number of samples (N) includes trips to 2 stations visited 7 times in January 2011, 6 in May 2011, 7 in January 2012, and 4 in May 2012. Some of these data were reported previously in a different form (Nikrad et al. 2014a)

Date	Temp. (°C)	Chl *a* (µg l^{-1})	Abundance (10^5 cells ml^{-1})	Leu incorp. (pM h^{-1})	N
Jan 2011	1.6 ± 0.7	1.6 ± 0.9	7.9 ± 2.5	20 ± 9.9	14
May 2011	−1.2 ± 0.5	0.4 ± 0.03	5.1 ± 1.3	2.8 ± 1.8	9
Jan 2012	0.1 ± 0.5	10.4 ± 8.0	4.7 ± 1.3	39.0 ± 13	14
May 2012	−0.3 ± 0.1	0.4 ± 0.1	4.1 ± 3.6	11.0 ± 4.3	8

leucine incorporation roughly followed chl *a* concentrations. Total prokaryotic abundance significantly decreased by about 60% from January to May 2011 (Kruskal-Wallis test, $p < 0.005$), but then was similar during the other 3 mo we sampled (Table 1).

Abundance and biovolume of AAP bacteria

The relative abundance of AAP bacteria (% of total prokaryotic abundance) also varied greatly between the 2 mo for each year and between the 2 yr (Fig. 1). AAP bacteria made up about 8% of the community in January 2011, which then decreased to ca. 1% the following May. The abundance of these bacteria remained at ca. 1% in January 2012 before decreasing again the following May, this time to 0.3% on average. The differences between January and May 2011 and between January and May 2012 were statistically significant (Kruskal-Wallis test, $p < 0.001$).

The biovolume of an average AAP bacterium was generally bigger than that of a cell in the total bacterial community (Fig. 2), and overall the difference was about 2-fold (Table 2). However, there was much variation among the months we sampled. AAP bacteria were nearly 6-fold bigger than cells in the total bacterial community in January 2012, and cells in both communities were larger on average than bacteria examined during the other 3 mo (Table 2). At the other extreme, in January 2011 when relative AAP bacterial abundance was highest, the biovolume of AAP bacterial cells was the smallest, although the only statistically significant differences were seen when January 2011 was compared with May 2011 or with January 2012 (Kruskal-Wallis test, $p < 0.05$). In January 2011 AAP bacteria were actually significantly smaller than other bacteria by ca. 40% whereas AAP bacteria were substantially bigger than other bacteria in the other months (Fig. 2).

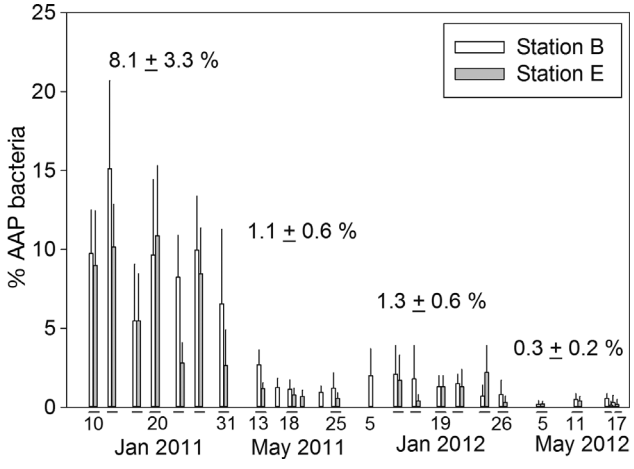

Fig. 1. Relative abundance of AAP bacteria in WAP coastal waters. Each bar represents one of 2 stations usually sampled together on the same day (indicated by the short lines under the horizontal axis). Numbers below the horizontal axis: some of the sample dates. Error bars: SD from the IR epifluorescence estimate. Data above bars: means ± SD for the entire month combining both stations

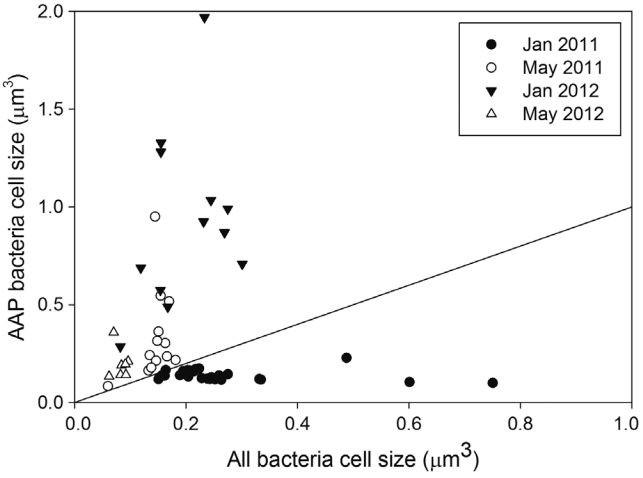

Fig. 2. Size of AAP bacteria versus size of all bacteria in WAP coastal waters. The line is the 1:1 line

We analyzed cell size data from 2 other marine systems: coastal waters of the western Arctic Ocean (Cottrell & Kirchman 2009) and MAB coastal waters (Stegman et al. 2014). The average relative abundance of AAP bacteria for these 2 systems was similar to the abundance we observed in WAP waters, except for the very low abundance levels seen in May 2012 (Table 2). As seen in the North Atlantic Ocean (Sieracki et al. 2006) and in WAP waters (see above), AAP bacteria were bigger on average than other bacterial cells in the Arctic and MAB (Table 2), although the 2-fold difference seen in the Arctic is not statistically significant (Kruskal-Wallis test, $p < 0.05$). In addition, AAP bacteria were bigger in WAP waters than AAP bacteria in the other systems, but only the comparison with MAB was statistically significant ($p < 0.05$). Finally, the cell size of all bacteria in WAP waters was about 2-fold bigger on average than the size in Arctic and MAB coastal waters (Kruskal-Wallis test, $p < 0.05$).

Leucine incorporation by AAP bacteria

Single-cell activity in January 2011 and May 2011 and 2012 was explored using a new microautoradiography approach to examine leucine incorporation

Table 2. AAP bacterial abundance and cell sizes in coastal waters of the West Antarctic Peninsula (WAP), the Arctic Ocean, and Mid-Atlantic Bight (MAB). %AAP: percentage of total microbial abundance contributed by AAP bacteria; All: total community; AAP/All: ratio of the cell size for AAP bacteria to that of the total community. The ratio was calculated for each sample and then the average and SD were calculated. The other SD values reflect the variation among N samples taken on different days and locations for each system. The Arctic data are from Cottrell & Kirchman (2009); the MAB data are from Stegman et al. (2014) and unpubl. sources

System	Date	% AAP	SD	Cell sizes (μm^3)				AAP/All	SD	N
				All	SD	AAP	SD			
Arctic	Jan & July	7.2	6.8	0.090	0.023	0.193	0.333	2.1	3.5	21
MAB	Entire year	4.0	4.0	0.090	0.028	0.146	0.232	1.5	1.3	76
WAP	Average	5.6	6.0	0.164	0.184	0.347	0.486	2.2	4.2	62
WAP	Jan 2011	8.1	3.3	0.188	0.099	0.098	0.019	0.6	0.2	26
WAP	May 2011	1.1	0.6	0.102	0.021	0.234	0.160	2.3	1.5	13
WAP	Jan 2012	1.3	0.6	0.228	0.335	0.854	0.590	5.8	4.2	15
WAP	May 2012	0.3	0.2	0.057	0.009	0.390	0.716	3.1	6.0	8

by AAP bacteria. Large fractions of both the total community and of AAP bacteria incorporated leucine, averaging nearly 50% in January for all microbes (Fig. 3). The percentage of active bacteria in light versus dark incubations did not differ significantly (Kruska-Wallis test, $p > 0.05$), nor did the percentages for the total community versus for AAP bacteria in January (see below). The fraction of bacteria taking up leucine declined substantially in May (to 12%), and again the active fractions were the same in the light and dark incubations (Fig. 3A). The silver grain area (SGA) around active cells (not weighted by inactive cells) was 5-fold larger in January than in May (Fig. 3B). The differences between the 2 mo for both percent active bacteria and SGA were statistically significant (Kruskal-Wallis test; $p < 0.0005$).

We plotted the percentage of active AAP bacteria versus for the total prokaryotic community to explore differences in leucine incorporation more closely (Fig. 4). This plot revealed that a higher fraction of AAP bacteria incorporated leucine than did the total community in May but not in January; in May all points except for one are above the 1:1 line (Fig. 4A), and the percent active AAP bacteria was significantly higher than the percent active total prokaryotes in May (paired *t*-test, $p < 0.02$). The average difference between AAP bacteria and the total bacterial community was nearly 2-fold.

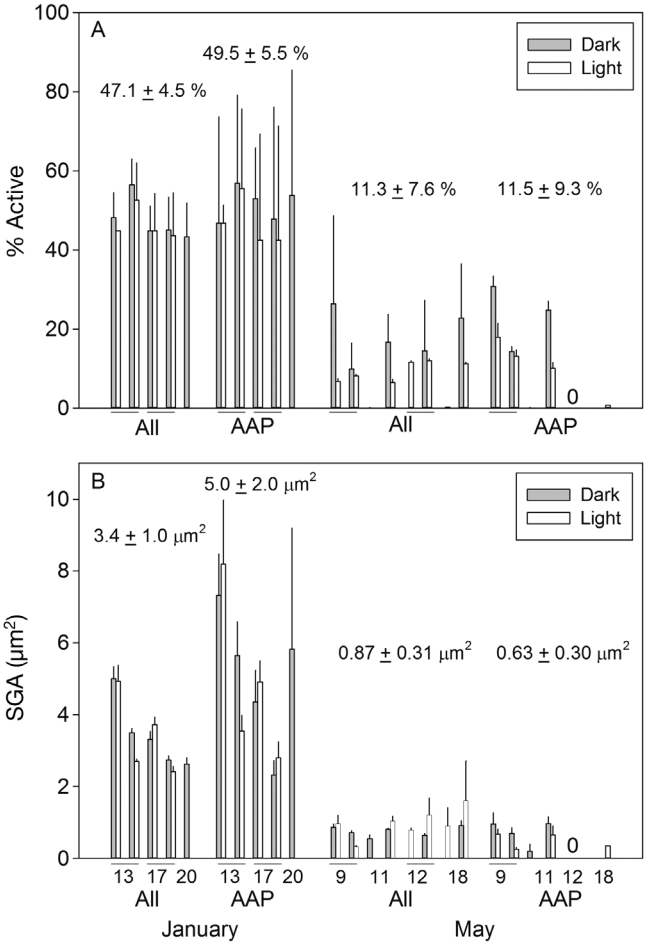

Fig. 3. Single-cell incorporation of ^{3}H-leucine by AAP bacteria and by all prokaryotes in light or dark incubations. (A) Percent of cells in the total community (All) or in the AAP bacterial community that assimilated ^{3}H-leucine, and (B) silver grain area (SGA) around active cells in the total community or the AAP bacterial community. Each pair of bars for light-dark incubations is a separate sample from either Stations B or E or both, taken on the indicated days (numbers under the horizontal axis in panel B) throughout the indicated months. Short lines under both horizontal axes indicate data taken on the same day. The error bars are SD in panel A and SE in panel B. Data above bars are means ± SD calculated with data from both light and dark incubations for the AAP bacteria or for the total community, combining both stations

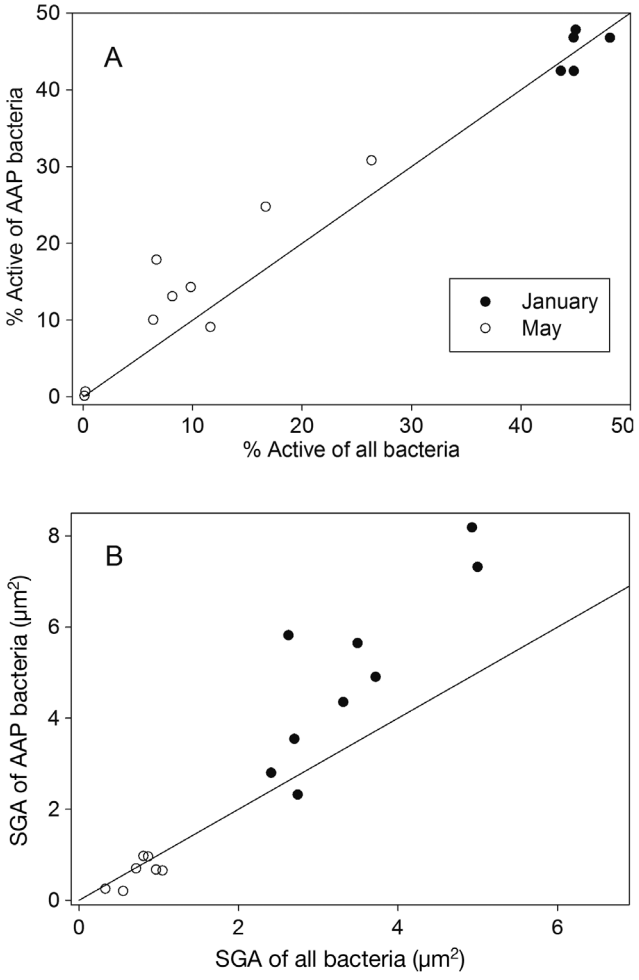

Fig. 4. Single-cell incorporation of ^{3}H-leucine by AAP bacteria versus by all prokaryotes in January and May. (A) Percent active cells, and (B) silver grain area (SGA) weighted by inactive cells. The line is the 1:1 line

We did a similar analysis for SGA, which scales with incorporation of ^{3}H-leucine by active bacteria (Sintes & Herndl 2006). The weighted SGA around all AAP bacteria was larger than that for the total community in January (Fig. 4B), and all points except for one were above the 1:1 line. The difference was 40% on average, which was statistically significant (paired *t*-test, $p < 0.01$). In contrast, there was no significant difference in SGA in May (paired *t*-test, $p > 0.05$). The overall difference for both months was ca. 30%, which was statistically significant (paired *t*-test, $p < 0.05$).

There was no difference in bulk leucine incorporation rates (n = 47) between light and dark incubations (data not shown). There was also no effect of light on bulk thymidine incorporation rates (n = 28).

DISCUSSION

Polar environments offer unique habitats to explore the ecology and ecophysiology of AAP bacterial communities, and they are important environments to study because climate change may lead to larger roles of heterotrophic and potentially photoheterotrophic bacteria in carbon fluxes (Kirchman et al. 2009, Sailley et al. 2013). One unique feature of polar environments is the large seasonal and interannual variation in light availability due to changes in ice cover, day length, and light intensity. It is usually assumed that light does not directly affect the microbes carrying out organic material mineralization, except for the negative effects of UV light on microbes and alteration of organic material by photochemical reactions (Moran & Miller 2007). However, light could influence mineralization if photoheterotrophic bacteria are abundant and sufficiently active. We found that AAP bacteria are generally larger and more active than other bacteria in Antarctic coastal waters, and are likely to contribute more to carbon fluxes than suggested by their abundance — but probably only in the austral summer.

The abundance of AAP bacteria in WAP coastal waters in January is similar to levels in another polar system, Arctic coastal waters. The abundance in both polar systems is similar to that seen in low latitude waters, such as the MAB coastal ocean, while all systems examined so far have more AAP bacteria than observed by a quantitative PCR study of Antarctic waters (Schwalbach & Fuhrman 2005). The difference in results is probably due to methodology. The primers used by PCR studies may not match all *pufM* genes found in AAP bacteria, leading to underestimates of AAP bacterial abundance (Schwalbach & Fuhrman 2005). Also, the 1.2 μm prefiltration step used by Schwalbach & Fuhrman (2005) may have removed more free-living AAP bacteria than other bacteria because AAP bacteria are usually larger (Sieracki et al. 2006, Stegman et al. 2014, this study). Unlike the Arctic where relative abundance did not vary between summer and winter (Cottrell & Kirchman 2009), we found in WAP waters that AAP bacterial abundance decreased substantially from the austral summer to fall in both years. The abundance of AAP bacteria may be kept high in the Arctic winter because AAP bacteria are carried into Arctic coastal waters by currents originating in lower latitude regions with higher light availability (Cottrell & Kirchman 2009). In contrast, WAP coastal waters are isolated from lower latitude systems by the Southern Ocean.

Single-cell activity of AAP bacteria and of the total community varied greatly between January and May in WAP waters. A large fraction (48%) of both AAP bacteria and the total community took up leucine in January, as has been observed before for the total community (Grossmann 1994, Straza et al. 2010, Nikrad et al. 2014a). The active fraction is even higher than observed in the much warmer, more eutrophic waters of the Delaware estuary and MAB coastal waters (Straza & Kirchman 2011, Stegman et al. 2014). The fraction of active bacteria then decreased to nearly 10% in May, probably due to the decrease in primary production and labile organic carbon inputs. One index of primary production, chlorophyll, decreased by >25-fold from January to May. Curiously, in May, relatively more AAP bacteria were active compared to the total community (the percent active data), but AAP bacteria appeared to take up about the same amount of leucine as did bacteria in the total community (according to the SGA data). These data imply that AAP bacteria were more active than some members of the total community but others were even more active than the AAP bacteria, resulting in similar SGA estimates for both communities. Two gammaproteobacterial clades have been shown to be active in WAP waters (Nikrad et al. 2014a), and other *Gammaproteobacteria* are highly active in coastal Mediterranean waters (Ferrera et al. 2011). The smaller overall advantage enjoyed by AAP bacteria in May is probably due to the 21-fold decrease in integrated irradiance in January versus May (14401 J m^{-2} versus 684 J m^{-2} in January and May 2012). The role of light in governing AAP bacteria was also evident in the Delaware estuary where AAP bacterial activity correlated with light availability in transects along the salinity gradient (Stegman et al. 2014). Light would also explain the variation of AAP bacterial abundance with depth (Cottrell et al. 2006, Sieracki et al. 2006, Salka et al. 2008) and the correlations with light intensity and day length over the course of a year in Mediterranean coastal waters (Ferrera et al. 2013).

In spite of light being the best explanation for the variation in relative AAP bacterial activity and abundance, direct experimental evidence for a light effect on natural communities is lacking. Light does not affect AAP bacterial activity in short incubations in WAP water (this study) nor in the Delaware estuary (Stegman et al. 2014). We hypothesize that the energetic advantage of photoheterotrophy over pure heterotrophy is too small to be measurable in a short incubation (Kirchman & Hanson 2013), yet this small advantage can accumulate. We can estimate the expected enhancement in a short incubation by assuming that the energetic advantage afforded by phototrophy accrues over a generation time and that this accrued energetic advantage is represented by the difference in the weighted silver grain area between AAP bacteria and other bacteria (ca. 30% on average). If the generation time in natural bacterial communities in WAP water is on the order of 1 d or longer (Ducklow 2000), the enhancement of AAP bacterial activity per hour due to phototrophy would be <2% (30%/24 h). Such a small difference would be difficult to detect.

Regardless of the role of phototrophy, variation in total activity probably contributes to the variation in cell size for both AAP bacteria and the total community we observed between January and May and between 2011 and 2012. The average cell size for AAP bacteria and for cells in the total community was largest in January 2012 when bulk leucine incorporation and chl *a* were highest. Our results are consistent with a previous study which reported that bacteria in polar waters are bigger than bacteria in low latitude systems (Straza et al. 2009). The difference in size was evident in our data and in the previous study of cells stained for DNA by DAPI (Straza et al. 2009). DAPI images of AAP bacteria are likely bigger than images of other bacteria in part because of genome size; cultivated representatives of AAP bacteria have large genomes, exemplified by strains in the *Roseobacter* clade (Brinkhoff et al. 2008). However, we think that some of the variation in biovolumes is due to bottom-up and top-down controls not directly tied to genome size. The size of the DAPI image depends on other cellular components, not just the nucleoid, because of nonspecific binding by DAPI (Zweifel & Hagström 1995). AAP bacteria are bigger in WAP than in MAB waters probably for the same reason evoked previously to explain latitudinal differences in cell size for the entire bacterial community. Straza et al. (2009) were able to account for some of the variation in average cell size for the entire community by combining data on the average size of cells in different major bacterial groups and their abundance in these waters. Abundance in turn varies because of the complex interplay between top-down and bottom-up factors governing microbes in these waters.

AAP bacterial abundance and cell size also varied greatly between 2011 and 2012, as did virtually all other biogeochemical properties examined by our study. Such year-to-year variation, well known for this polar system, is ultimately tied to the Southern Oscillation and climate change forcing (Ducklow et al. 2006). Variation in physicochemical properties

likely leads to variation in the bottom-up factors, such as the supply of labile organic material, that govern AAP bacterial communities.

Inter-annual variation in AAP bacterial abundance and cell size may also be due to changes in viral lysis and grazing. If bacterivores correlate with the larger grazers, which are known to vary from year to year in WAP water (Ducklow et al. 2006), then at least grazing if not viral lysis also likely differs between years. It is interesting to note that the relative abundance of AAP bacteria was highest (in January 2011) when the average biovolume was the smallest, although there is no overall relationship between relative abundance and biovolume in these data. The impact of top-down factors on AAP bacteria may differ from the impact on other bacteria since AAP bacterial cells are larger and thus are likely more susceptible to grazing and perhaps viral lysis. Ferrera et al. (2011) showed the importance of top-down factors in governing AAP bacterial growth and abundance in Mediterranean coastal waters.

Our results add to the growing evidence about the unique ecophysiology of AAP bacteria and their potential contribution to carbon cycling in aquatic systems. We speculate that the lack of terrestrial organic material inputs and low light availability in winter would select for differences in AAP bacterial communities compared with low latitude waters and the Arctic. We did find more complex patterns in AAP bacterial communities in WAP waters than observed by previous studies. While relative AAP bacterial abundance in WAP waters was similar to other systems in the austral summer, abundance decreased in the fall to some of the lowest levels observed to date. However, in fall a higher fraction of these AAP bacteria took up leucine than did the total prokaryotic community. We hypothesize that even the low availability of light in fall was sufficient to fuel phototrophic activity of AAP bacteria, but not enough to avoid decreases in abundance due to top-down control. We suspect similar controls operate in other systems. The few studies undertaken so far have not found large differences in the taxonomic composition of AAP bacteria in polar waters versus low latitude systems (Cottrell & Kirchman 2009, Koh et al. 2011). In both polar and low latitude waters, the contribution of AAP bacteria to the carbon cycle is probably greater than their abundance might suggest because of their larger cell size, faster growth, and higher activity. These data indicate that AAP bacteria and other photoheterotrophs should be included in models of the carbon cycle because they mediate a direct effect of light on organic carbon fluxes (Moran & Miller 2007).

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