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Ecology of aerobic anoxygenic phototrophic bacteria along an oligotrophic gradient in the Mediterranean Sea

D. Lamy[1,2,*], C. Jeanthon[3,4], J. Ras[5], F. Van Wambeke[6], O. Dahan[3,4], M. T. Cottrell[7], D. L. Kirchman[7], and P. Lebaron[1,2]

[1]UPMC Univ Paris 06, Observatoire Océanologique de Banyuls, 66650 Banyuls-sur-mer, France
[2]CNRS, UMR 7621, Laboratoire d'Océanographie Microbienne, LOMIC, Observatoire Océanologique de Banyuls, 66650 Banyuls-sur-mer, France
[3]UPMC Univ Paris 06, UMR 7144, Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, 29680 Roscoff, France
[4]CNRS, UMR 7144, Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, 29680 Roscoff, France
[5]Laboratoire d'Océanographie de Villefranche-sur-mer, UMR 7093 CNRS et Université Pierre et Marie Curie, Villefranche-sur-mer Cedex, France

[6] CNRS, Université de la Méditerranée, Laboratoire de Microbiologie, Géochimie and Ecologie Marines, UMR 6117, Campus de Luminy, case 901, 13 288 Marseille cedex 9, France
[7] School of Marine Science and Policy, University of Delaware, Lewes, Delaware 19958, USA
* now at: University of Vienna, Department of Marine Biology, Althanstrasse 14, 1090 Vienna, Austria

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Correspondence to: D. Lamy (lamyd2@univie.ac.at)

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Abstract

Aerobic anoxygenic phototrophic (AAP) bacteria are photoheterotrophic prokaryotes able to use both light and organic substrates for energy production. They are widely distributed in coastal and oceanic environments and may contribute significantly to the carbon cycle in the upper ocean. To better understand questions regarding links between the ecology of these photoheterotrophic bacteria and the trophic status of water masses, we examined their horizontal and vertical distribution and the effects of nutrient additions on their growth along an oligotrophic gradient in the Mediterranean Sea. Concentrations of bacteriochlorophyll-*a* (BChl-*a*) and AAP bacterial abundance decreased from the western to the eastern basins of the Mediterranean Sea and were linked with concentrations of chlorophyll-*a*, nutrient and dissolved organic carbon. Inorganic nutrient and glucose additions to surface seawater samples along the oligotrophic gradient revealed that AAP bacteria were nitrogen- and carbon-limited in the ultra-oligotrophic eastern basin. The intensity of the AAP bacterial growth response generally differed from that of the total bacterial growth response. BChl-*a* quota of AAP bacterial communities was significantly higher in the eastern basin than in the western basin, suggesting that reliance on phototrophy varied along the oligotrophic gradient and that nutrient and/or carbon limitation favors BChl-*a* synthesis.

1 Introduction

Aerobic anoxygenic phototrophic (AAP) bacteria are photoheterotrophic prokaryotes able to combine light and dissolved organic matter as energy sources (Beatty, 2002; Suzuki and Béjà, 2007; Yurkov and Beatty, 1998). Their primary light harvesting pigment is bacteriochlorophyll-*a* (BChl-*a*) (Koblížek et al., 2005; Kolber et al., 2001) and they depend on oxygen and organic carbon for energy. These bacteria are widely distributed in marine environments and their abundances vary greatly, accounting for up to 25% of the bacterial community (Cottrell and Kirchman, 2009; Lami et al., 2007;

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Salka et al., 2008; Schwalbach and Fuhrman, 2005). The metabolic flexibility, the high abundance in various environments and the widespread occurrence of these organisms in both marine and freshwater environments challenge our view of carbon and energy budgets (Eiler, 2006; Lamy et al., 2011; Mašín et al., 2008; Moran and Miller, 2007) and suggest that AAP bacteria play a significant role in aquatic food webs and biogeochemical cycles (Koblížek et al., 2007; Kolber et al., 2001).

An initial hypothesis regarding AAP bacteria distribution was that photoheterotrophy could be beneficial in nutrient-poor environments, such as oligotrophic zones (Karl, 2002; Kolber et al., 2000). In contrast, various reports showed that these organisms are abundant in eutrophic as well as in oligotrophic environments (Jiao et al., 2007; Koblížek et al., 2006; Yutin et al., 2007). Other authors suggested that nutrient concentrations (Mašín et al. 2008), attachment to particles (Cottrell et al., 2010; Waidner and Kirchman, 2007) or light intensity (Koblížek et al., 2003; Shiba, 1991) may influence AAP bacterial abundance.

Increasing attention has been paid to AAP bacteria in marine environments exhibiting various trophic regimes. However, oligotrophic waters, which represent 60% of the oceans, have not been extensively studied. The Mediterranean Sea has a wide range of oligotrophic conditions including extreme oligotrophy in summer when the water column is stratified (Berman et al., 1985). The Mediterranean Sea is known to have low nutrient concentrations, especially phosphate (Moutin and Raimbault, 2002), making it one of the largest nutrient-depleted areas in the world (Ignatiades, 2005). Overall, the Mediterranean Sea could serve as a particularly relevant system to follow the spatial distribution of AAP bacteria and to examine the environmental factors that are hypothesized to influence their distribution. Few studies highlighted the large diversity of AAP bacteria in the Mediterranean Sea (Lehours et al., 2010; Oz et al., 2005; Yutin and Béjà, 2005; Yutin et al., 2008), but their quantitative variation with longitude and depth has never been explored.

The objectives of this study were to determine the vertical and longitudinal distribution of AAP bacteria in the Mediterranean Sea and to identify the factors limiting their

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growth across a wide longitudinal gradient of oligotrophy. We examined these variables along a 3000 km transect from the west to the east during the BOUM cruise carried out in June/July 2008. The abundance and distribution of AAP bacteria were monitored using infra-red (IR) kinetic fluorometry, high performance liquid chromatography (HPLC) and IR epifluorescence microscopy. The effects of inorganic nitrogen, phosphate and glucose additions on AAP and total bacterial growth were studied in surface seawater samples incubated under natural solar irradiance.

This study showed that AAP bacteria exhibited an obvious longitudinal gradient, decreasing in abundance from the oligotrophic western to the the ultra-oligotrophic eastern basins of the Mediterranean Sea. The higher concentrations of BChl-*a* per-cell in the eastern part of the Mediterranean Sea suggest that photosynthetic pigment synthesis by AAP bacteria may be favored in nutrient-depleted conditions.

2 Material and methods

2.1 Study area and sampling

Sampling was carried out during the "Biogeochemistry from the Oligotrophic to the Ultra-oligotrophic Mediterranean Sea" (BOUM) cruise on the R/V *Atalante* during June/July 2008. The cruise was planned as a 3000 km transect of stations encompassing a large longitudinal gradient in the Mediterranean Sea (Fig. 1, see also Moutin et al., 2011). Hydrological, biological and chemical parameters were studied at 27 short-term stations and three long-term stations designed by letters (A, B, and C), located at the centre of anticyclonic gyres, where lateral advection was expected to be minimum (Fig. 1). The east-to-west transect crossed the 3 main areas of the Mediterranean Sea, the western (stations 18 to 27), the Ionian (station 2 to 17) and the eastern or Levantine basins (stations 3 to C).

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2.2 Environmental parameters

Profiles of temperature, salinity and photosynthetically active radiation (PAR) were measured with a SBE 911 PLUS Conductivity-Temperature-Depth (CTD) system.

Nutrients concentrations were determined with a Skalar Autoanalyzer (Skalar Analytical B.V., The Netherlands) according to Wood et al. (1967) and Bendschneider and Robinson (1952) for nitrate (NO_3) and nitrite (NO_2) concentrations and according to Murphy and Riley (1962) for phosphate (PO_4) concentrations. Protocols were adapted to the Skalar Autoanalyser from Tréguer and Le Corre (1975). Dissolved inorganic phosphate was also quantified following the Rimmelin and Moutin procedure (2005), derived from the initial MAGIC method proposed by Karl and Tien (1992). Ammonium (NH_4) concentrations were determined onboard by fluorometry according to Holmes et al. (1999) on a fluorometer Jasco FP-2020.

Concentration of dissolved organic carbon (DOC) was measured on a model TOC-V analyzer (Shimadzu, Japan) following the procedure outlined by Benner and Strom (1993). Dissolved organic nitrogen (DON) and phosphate (DOP) were immediately analyzed by persulfate wet-oxidation according to Pujo-Pay et al. (1997). Particulate organic carbon (POC) was quantified using a model CHN 2400 analyzer (Perkin Elmer 2400, Waltham, MA) following Pregl (1924) and Sharp (1974). Particulate organic nitrogen (PON) and phosphate (POP) were analyzed simultaneously on board according to the wet oxidation procedure of Pujo-Pay and Raimbault (1994). The methods conducted for the determination of the nutrients and dissolved and particulate matter concentrations are described in Pujo-Pay et al. (2010).

2.3 AAP bacterial abundance

Water samples for the determination of AAP bacterial abundance were taken at 5–6 depths from 8 stations equally distributed in the 3 different basins. Samples were preserved with 2% paraformaldehyde for 1–4 h at 4 °C in the dark and filtered onto 0.2-µm-pore-size black polycarbonate filters. The filters were quickly frozen in liquid

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nitrogen and stored at −80 °C until processing. Samples were processed as described previously (Cottrell et al., 2006). Briefly, each filter was stained with 4′,6-diamidino-2-phenylindole (DAPI, 1 µg mL^{-1} final concentration) in 1X phosphate-buffered saline for 10 min. Stained samples were counted immediately. AAP cells were counted using an Olympus Provis AX 70 microscope and image analysis software (ImagePro Plus, Media Cybernetics) to identify cells having DAPI and IR fluorescence but not Chl-*a* or phycoerythrin (PE) fluorescence. Twenty images per sample were captured using a charge-coupled-device camera (Intensified Retiga Extended Blue; QImaging, Surrey, BC) with the following exposure times: DAPI, 160 ms; IR, 400 ms; Chl-*a*, 1500 ms; PE, 50 ms. The biovolumes of total bacteria and AAP bacteria were compared using cell sizes obtained by image analysis (Cottrell et al., 2006).

2.4 Fluorometry

BChl-*a* fluorescence was measured at the same stations and depths as those sampled for AAP bacterial abundance. BChl-*a* fluorescence was determined using an IR kinetic fluorometer using the method previously described (Koblížek et al., 2005). Briefly, the instrument consisted of a standard PSI fluorometer control unit (FL200/PS, Photon Systems Instruments, Brno, Czechia) and custom-made optics (Koblížek et al., 2005). The absolute detection limit was 2 ng BChl-*a* L^{-1}. To discriminate between the Chl-*a* and Bchl-*a* contribution in the IR fluorescence signal (>850 nm), phytoplankton fluorescence was selectively inhibited by adding 10^{-5} M (final concentration) of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Diuron, PESTANAL, Fluka), which specifically inhibits Photosystem II of oxygenic phototrophs but does not affect the bacterial reaction centers. Then, only the variable part of the kinetic IR signal originating from bacterial reaction centers was determined (Koblížek et al., 2005). This approach avoids potential interferences from other fluorescing compounds not exhibiting variable fluorescence or from electrical drifts within the detection system that occur on different time scales. Size fractionation, following Lami et al. (2009), was conducted at the same stations and at the depths of maximum BChl-*a* fluorescence. Briefly, for each sample,

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<0.8 µm and <3 µm BChl-*a* fluorescence signals were recorded along with the original whole water sample and the percentage of BChl-*a* signal in individual fractions was calculated. Gravity filtration was used to minimize potential particle dislodging by filtration (Waidner and Kirchman, 2007).

2.5 Bacterioplankton abundance and bacterial production

The abundances of total heterotrophic prokaryotes (including AAP bacteria) and autotrophic prokaryotes (*Synechococcus* and *Prochlorococcus*) were measured by flow cytometry using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) according to Marie et al. (2002). The bacterial production was determined by [^{3}H] leucine incorporation applying the centrifugation method (Smith and Azam, 1992) and is detailed in Van Wambeke et al. (2010). Production by Archaea would be included in this measurement, but their abundance is low in Mediterranean surface waters (De Corte et al., 2009), particularly during the stratification period (Winter et al., 2009).

2.6 Pigment concentrations

Samples for the determination of the pigment concentrations were collected at the 30 stations at 7–12 depths. Seawater sample (1 to 2.5 L) was filtered onto a stack of two GF/F Whatman filters which were frozen immediately in liquid nitrogen and then stored at −80 °C until analysis as previously described (Ras et al., 2008). Briefly, the samples were extracted in 3 mL of methanol for at least one hour and the clarified extracts were injected into a 1100 series high-performance liquid chromatography system (Agilent Technologies) according to a modified version of the method described by Van Heukelem and Thomas (2001). Separation was achieved during a gradient elution between a tetrabutylammonium acetate-methanol mixture (30:70) and 100% methanol. Chl-*a* and BChl-*a* pigments were detected at 667 and 770 nm, respectively, using a diode array detector. The detection limit was 0.1 ng L^{-1} and the injection precision was 0.4%. The different pigments were identified both by their retention times and by absorption spectra.

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2.7 Effects of nutrient and glucose amendments on bacterial production and growth of AAP and total bacteria

To identify the factors limiting heterotrophic bacterial production and cell growth of AAP and total bacteria, on board enrichments were conducted. Selected surface seawater samples (3–5 m depth) were collected and dispensed into acid-washed transparent polycarbonate flasks (250 ml, Nalgene). Nutrients were added in order to obtain a final concentration of 1 µM NH_4Cl+1 µM $NaNO_3$ (N), 0.25 µM Na_2HPO_4 (P), and 10 µM C-glucose (C). For these experiments, a series of five flasks (control, +P, +N, +C, +NPC) were incubated for 48 h under simulated in situ conditions, in on-deck incubators with running surface seawater and neutral density screens to mimic in situ 55% light conditions. After incubation, samples for determining the abundance and growth of AAP bacteria and total bacteria and for measuring bacterial production were processed as described above.

2.8 Statistical analyses

A redundancy analysis (RDA) was used to determine which environmental variables were the most significant to explain variation in the abundance of autotrophic (*Synechococcus and Prochlorococcus*), photoheterotrophic (AAP) and heterotrophic (total) bacteria. We assumed a linear response of *Synechococcus*, AAP and total bacteria to environmental variations since the gradient length, expressed as standard deviation units of species turnover (SD) along the first ordination axis and determined through a detrended canonical correspondence analysis along the first ordination axis, was <1 SD. The gradient length is a measure of how unimodal or linear the species responses are along an ordination axis and a gradient length under 1 SD would indicate a clear linear response of the species along this gradient (Ramette, 2007; ter Braak and Smilauer, 2002). The null hypothesis that the cyanobacterial and bacterial abundances were independent of the environmental parameters was tested using constrained ordination with a Monte Carlo permutation test (499 permutations).

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Temperature, salinity, depth, concentrations of Chl-*a*, nutrients and organic matter concentrations (DOC, DOP, DON and POC) and PAR were used as explanatory variables. The RDA analysis was performed using the CANOCO version 4.5 software (ter Braak, 1989).

Spearman correlations were performed to identify the significant links within the BChl-*a*-related parameters. Multiple regression analysis was performed to determine which proportion of the variation in AAP bacterial abundance could be explained by the variation in environmental parameters, used as explanatory variables. A multiple stepwise regression with forward selection was performed to identify major environmental or extrinsic factors controlling the variation of AAP cell abundances. Since the variables were not normally distributed (Shapiro-Wilk test, $p>0.05$), dependent and independent variables were log-transformed to meet the assumption of normality. The explanatory variables were the same as those used in the RDA analysis. An ANOVA analysis followed by a LSD pairwise-comparison test was performed to compare BChl-*a* quotas along the transect. The correlation, regression and ANOVA analyses were performed using the XLSTAT 7.5.3 package.

3 Results

3.1 Environmental setting

The physical and hydrologic characteristics of the water masses in the Mediterranean Sea have been previously described and full details for the BOUM cruise can be found in Moutin et al. (2011). During the summer BOUM cruise, surface seawater temperatures ranged from 24 °C in the western basin (station A) to 27.5 °C in the central-eastern part (station B). Depth distributions of Chl-*a* concentrations included a well defined deep chlorophyll maximum (DCM), more pronounced in the western basin, and deepening from west (~40 to 80 m) to east (from 80 to >100 m) (Fig. 2; see also Crombet et al., 2010). Surface Mediterranean waters were depleted in nutrients and the thickness of this depleted layer increased towards the east from about 10 m in the

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Gulf of Lion to more than 100 m in the Levantine basin (for details see Pujo-Pay et al., 2010).

3.2 Abundance and size fractionation of AAP bacteria along the BOUM transect

Concentrations of BChl-*a* ranged between undetectable levels and 3.1 ng L^{-1}, averaging 0.4 ± 0.5 ng L^{-1} between 0 and 150 m (Fig. 2). The concentrations decreased from west to east, with maximum values (from 1.1 to 3.1 ng L^{-1}) measured in the Rhône River plume toward the western basin (until station 19) at depths comprised between 5 and 60 m. The maximum values of BChl-*a* concentrations deepened toward the east, following the deepening of the DCM. The BChl-*a* concentrations were low (<0.5 ng L^{-1}) below the DCM. In the Ionian and eastern basins, highest BChl-*a* values (0.7–1.0 ng L^{-1}) occurred at depths between 25 and 100 m, and concentrations were low (<0.5 ng L^{-1}) in subsurface waters.

At eight stations equally distributed along the transect, AAP bacteria were investigated by measuring BChl-*a* concentrations, BChl-*a* fluorescence signal and AAP bacterial abundance (Fig. 3a). The three AAP bacterial parameters clearly showed similar longitudinal and vertical patterns, demonstrating that AAP bacteria were mostly found in the euphotic zone along the whole transect. The BChl-*a* concentrations were significantly and positively correlated with the AAP bacterial abundance (Spearman correlation, r=0.61, p<0.001) and the BChl-*a* fluorescence signal (Spearman correlation, r=0.73, p<0.0001). AAP bacterial abundance and BChl-*a* fluorescence signal were also positively correlated (Spearman correlation, r=0.81, p<0.0001).

AAP bacterial abundance ranged from 3.0×10^2 to 3.5×10^4 cells mL^{-1}, accounting for 0.1 to 4% of total bacterial abundance. Although AAP bacteria were present at all stations within the first 150 m depth, their abundance varied along the longitudinal transect and with depth (Fig. 3a). AAP bacteria reached up to 3.5×10^4 cells mL^{-1} in the western part (station 21 at 50 m depth) whereas they did not exceed 9.0×10^2 cells mL^{-1} in the eastern part (station 9 at 50 m). Abundances in the Ionian Basin (stations 1 and 13) were intermediate up to 1.9×10^4 cells mL^{-1}.

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The depth of AAP bacterial abundance maxima increased from west to east, as previously noticed for the maximum values of BChl-*a* concentrations and the DCM. At stations 27 and 25 in the western basin, the DCM corresponded to the depth of highest AAP bacterial abundance, reaching 1.6 and 2.8×10^4 AAP cells mL^{-1} at 30 m and 50 m depth, respectively. At station 21, the highest numbers of AAP bacteria were found just above the DCM at 50 m, while at stations 13 and 9 AAP bacteria peaked at 60–70 m above the DCM (at 80 m and 50 m depth, respectively). The highest numbers of AAP bacteria were measured below the DCM at stations 1, 5 and 17 at 85 m, 110 m and 90 m, respectively.

BChl-*a* fluorescence in selected size fractions was examined at the same eight stations (Fig. 3b). The contribution of each size fraction was measured only at depths with the highest BChl-*a* fluorescence. The <0.8 µm fraction containing free-living AAP bacteria accounted for generally most (64 to 93%) of the total BChl-*a* fluorescence. The only exception was station 1 where the particle-associated BChl-*a* fluorescence in the 0.8–3 µm and >3 µm fractions accounted for 87% and 77% of the total signal at 70 m and 85 m, respectively.

3.3 AAP, cyanobacteria and total heterotrophic bacteria

The abundance of AAP bacteria was compared to that of the two main genera of photoautotrophic bacteria, including the cyanobacteria *Prochlorococcus* and *Synechococcus* and of total heterotrophic bacteria, including AAP bacteria (Fig. 4a).

At the three stations selected in the western basin (station 21), the Ionian basin (station 13) and the Levantine basin (station 9), total heterotrophic bacteria outnumbered cyanobacteria and AAP bacteria, with abundances ranging between 2.3 and 6.90×10^5 cell mL^{-1} (Fig. 4a). Total bacteria were more abundant in the western than in the eastern part of the Mediterranean Sea, and in the subsurface than in deep waters. AAP cells were about 1.2-fold larger on average (± 0.6) and up to 4-fold larger than cells in the total bacterial community (data not shown).

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In surface waters (5 m) *Synechococcus* outnumbered AAP bacteria by 2- to 4-fold. In contrast, from 25 to 150 m, AAP bacterial abundance was equal to or 9-fold higher than that of *Synechococcus*. *Prochlorococcus* cells were not detected by flow cytometry in surface waters at the three stations, However, *Prochlorococcus* was 2- to 22-fold more abundant than AAP bacteria at deeper depths at stations 13 and 9. In contrast, AAP bacteria abundances were in the same order of magnitude than that of *Prochlorococcus* at station 21, with values up to 4.0×10^4 cell mL^{-1}.

3.4 Photosynthetic pigment concentrations and cell quotas

Photosynthetic pigments were compared at the same three stations (Fig. 4b). Total Chl-*a* and Div-Chl-*a* were up to 300-fold and 50-fold higher than BChl-*a*, respectively. Div-Chl-*a* peaked at the DCM (51 to 82 ng L^{-1}), while BChl-*a* concentration was generally at its highest level just above the DCM and then decreased with depth. BChl-*a*/Chl-*a* ratio decreased with depth, from 1 to 1.5% to undetectable values (Fig. 4c). The Div-Chl-*a* content per *Prochlorococcus* cell increased with depth at all stations from undetectable values to 2.50 fg $cell^{-1}$. The BChl-*a* quota varied substantially along the transect (Fig. 4c). It decreased with depth and peaked at the bottom depth of the euphotic zone. This peak is more pronounced at stations 13 and 9 at 90 and 100 m, respectively, than at station 21 at 70 m. The highest BChl-*a* quota reaching up to 0.44 fg $cell^{-1}$ was observed at station 9 where a second peak occurred at 150 m. The BChl-*a* quota was significantly higher in the eastern basin (stations 1, 5 and 9) than in the central (stations 13 and 17, ANOVA, p=0.007, LSD pair-wised test, p=0.005) and the western (stations 21, 25 and 27, LSD pair-wised test, p=0.009) basins.

3.5 Relationship between AAP bacterial abundance and environmental factors

The first two RDA axes explained 78% of the variability in the abundances of bacteria and cyanobacteria and 99% of the relationship between these abundances and the environmental variables (Fig. 5). The two first canonical axes were significant (p=0.002).

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AAP bacteria appeared to be closely linked to the strict heterotrophic bacteria and the autotrophic *Synechococcus*. There was a clear separation between the samples collected below the euphotic zone (black down-triangles, on the top-side) and those obtained in the euphotic zone (open up-triangles, on the bottom-side, Fig. 5), indicating that depth and light availability were significant explanatory variables. Concentrations of Chl-*a*, nutrient and organic pools and the temperature and salinity appeared to be main factors accounting for the variation in AAP and total bacterial abundances with depth and longitude.

A multiple regression analysis indicated that the concentrations of Chl-*a*, NO_3+NO_2 and DOC were the main parameters controlling the AAP bacterial abundance. The combination of these three parameters explained 54% (p<0.0001) of the variability in the AAP bacterial abundance.

3.6 Effects of nutrient and glucose amendments on bacterial production and growth of AAP and total bacteria

In surface waters, nutrient (NO_3+NO_2, NH_4 and PO_4) concentrations were initially close or below the detection limit (Table 1). PO_4 turnover time is the most broadly-applicable measurement of PO_4 availability because it has the potential to identify variations in P availability even when PO_4 concentrations become analytically undetectable (Moutin et al., 2002, 2008). Turnover times of phosphate in surface waters were very short at the four stations. This rapid turnover, along with very low concentrations, indicated that phytoplankton was experiencing conditions where phosphate was scarce. Concentrations of Chl-*a*, POP and POC, abundance of heterotrophic prokaryotes and bacterial production were significantly lower in stations 9 and C than in stations 17 and A, reflecting the west-east gradient in oligotrophy.

Bacterial production relative to the control significantly increased in all P, N and NPC enrichments, the latter always showing the highest stimulation (Table 2). A strong positive effect of glucose on bacterial production was only observed at station C. As previously noticed by Van Wambeke et al. (2008), when they increased, bacterial

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numbers always increased less than the corresponding bacterial production. Significant increases in bacterial abundance were observed in N amended bottles at station C and in NPC enrichments at both analyzed stations.

The intensity of the AAP bacterial growth response generally differed from that of the total bacterial growth response at the stations they could be compared (stations C and 9). The main differences were the stronger increase after N and C additions for AAP bacteria compared with the total community. In contrast to the strong positive effect on bacterial production at all stations, the P addition only weakly stimulated net AAP and total bacterial growth at stations C and 9.

4 Discussion

Our study is the first to investigate the biogeographical patterns of AAP bacteria in the Mediterranean Sea by using IR fluorometry, epifluorescence microscopy and HPLC pigment analysis. Few studies have used all these methods together (Kolber et al., 2001; Lami et al., 2009). In this study, the data obtained by the three methods showed significant correlations between AAP bacterial abundance, BChl-*a* fluorescence values and concentrations revealing new insights in the distribution of AAP bacteria.

The fine spatial scale resolution obtained with measurement of BChl-*a* concentrations, hitherto never shown in other studies, provides a comprehensive picture of biogeographical trends of AAP bacteria along different trophic regimes. The values (up to 3.1 ng L^{-1}) are consistent with other studies investigating oligotrophic environments (Cottrell et al., 2006; Kolber et al., 2001; Schwalbach and Fuhrman, 2005; Sieracki et al., 2006). Both AAP bacterial abundance and BChl-*a* concentrations significantly decreased with increasing longitude and oligotrophy from west to east, though both varied less with location than with depth. AAP bacteria were more abundant in the upper layers of the photic zone than below the sunlit layers of the water column and strongly followed the depth distribution of cyanobacteria. Other studies also reported that AAP bacteria are major bacterial components in the photic zone, in agreement with their phototrophic abilities (Cottrell et al., 2006; Kolber et al., 2001).

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Our statistical analyses indicated a strong positive correlation between AAP bacteria and various indices of organic matter supply, suggesting that AAP bacteria were more abundant when organic carbon was available. Furthermore, their abundance and BChl-*a* concentrations were highest in the western basin which is a bit richer in nutrients (Pujo-Pay et al., 2010) and in phytoplankton-derived organic material (López-Sandoval et al., 2010) than the eastern basin. The distribution of AAP bacteria may be determined by the availability of specific organic compounds provided by phytoplankton (Jiao et al., 2007). Consistent with their growth stimulation by glucose additions in most stations, this supports the view that AAP bacteria represent a physiological group of heterotrophs able to use light-derived energy to rapidly grow when organic carbon sources are available. This is in agreement with their high abundances in estuarine waters (Schwalbach and Fuhrman, 2005; Waidner and Kirchman, 2007) and their common association with phytoplankton blooms (Gonzalez et al., 2000; Suzuki et al., 2001).

It has been shown that AAP bacteria are mainly free-living in surface offshore Mediterranean waters and that a large fraction (50%) can be attached to particles at the DCM (Lami et al., 2009). By examining the distribution of particle-attached and free-living BChl-*a* fluorescence on a larger dataset, we found that the majority of AAP bacteria living at the DCM of Mediterranean waters were free-living cells. This supports the view that this free-living lifestyle may reflect cellular and physiological adaptations to oligotrophic waters (Cottrell et al., 2010), with a low and intermittent supply of dissolved substrates.

Our data on BChl-*a* and Div-Chl-*a* can be used to further explore the importance of phototrophy in AAP bacteria in this part of the Mediterranean Sea. The concentrations of BChl-*a* per AAP cell in the eastern basin of the Mediterranean Sea was 4- to 16-fold lower than the Div-Chl-*a* content per *Prochlorococcus* cell. This may suggest that phototrophy was probably a lower part of the AAP cell metabolism when compared to *Prochlorococcus*, which mainly relies on phototrophy. However, the BChl-*a* quotas reached notably high values along the transect. These values are up to 2.5-fold higher

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than the values observed in the oligotrophic South Pacific Ocean (Lami et al., 2007), 3-fold higher than those observed in the Sargasso Sea (Sieracki et al., 2006) and until 10-fold higher than those observed in the Mid-Atlantic Bight (Cottrell et al., 2006). Moreover, the BChl-*a* content per cell was significantly higher in the ultra-oligotrophic eastern basin than in the two other basins. Assuming that BChl-*a* quotas indicate the extent of phototrophic potential (Cottrell et al., 2006), the data suggest that reliance on phototrophy varied along the oligotrophic gradient and that nutrient and/or carbon limitation favors BChl-*a* synthesis in natural communities as it has been reported for AAP cultures (Koblížek et al., 2003; Yurkov and Csotonyi, 2009).

In the Mediterranean Sea, which is one of the world's most oligotrophic bodies of water, inorganic nutrients and particularly phosphate (P) have been shown to limit both phytoplankton and bacteria in certain areas during stratification (Pinhassi et al., 2006; Thingstad et al., 1998; Van Wambeke et al., 2002). It has been hypothesized that the capacity of AAP bacteria to generate energy from light could reduce the metabolic requirements for recycling the organic carbon resources, such as the nutrient requirements (Cho et al., 2007). Mašín et al. (2008) suggested that the light-generated energy could serve for enhancing the P acquisition in P-depleted environments. On the basis of our nutrient-addition experiments, we cannot speculate for a P limitation of AAP bacteria in the Mediterranean Sea since growth of AAP and total heterotrophic bacteria were not stimulated by P addition, although higher heterotrophic bacterial activity was observed. The hypothesis that light-derived energy could serve for supporting the nutrient acquisition in a nutrient-depleted environment is not consistent with our observation that net growth of AAP bacteria was enhanced by nitrogen and glucose additions in the eastern basin whereas net growth of total bacteria was not stimulated by these additions. The problem may be in trying to deduce the impact of the additions by measuring net growth, which is a function of the bacterial response and mortality factors.

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5 Conclusions

Our study gives a comprehensive picture of the longitudinal and vertical distribution of AAP bacteria in the Mediterranean Sea. AAP bacteria were more abundant in the euphotic zone than in deeper waters and in the western than in the eastern basin of the Mediterranean Sea. As a significant part of the total bacterial abundance, AAP bacteria abundances decreased with the increasing oligotrophic gradient from the western to the eastern basin of the Mediterranean Sea. The simultaneous measurements of BChl-*a* fluorescence, pigment concentration and AAP cell abundance examined along with some environmental parameters support the notion of the prominence of the heterotrophic metabolism in AAP bacteria. However, the high BChl-*a* quotas observed in the samples from the ultra-oligotrophic eastern basin suggest that their phototrophic lifestyle may be especially advantageous under nutrient- and carbon-depleted environmental conditions. Assessing the impact of photoheterotrophy in aquatic systems will be needed to better understand the role of this functional group into elemental cycling.

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References

Beatty, J. T.: On the natural selection and evolution of the aerobic phototrophic bacteria, Photosynth. Res., 73, 109–114, 2002.

Bendschneider, K. and Robinson, R. J.: A new spectrophotometric determination of nitrite in seawater, J. Mar. Res., 11, 87–96, 1952.

Benner, R. and Strom, M.: A critical-evaluation of the analytical blank associated with DOC measurements by high-temperature catalytic-oxidation, Mar. Chem., 41, 153–160, 1993.

Berman, T., Walline, P. D., Schneller, A., Rothenberg, J., and Towsend, D. W.: Secchi disk depth record: a claim for the Eastern Mediterranean, Limnol. Oceanogr., 30, 447–448, 1985.

Cho, J. C., Stapels, M. D., Morris, R. M., Vergin, K. L., Schwalbach, M. S., Givan, S. A., Barofsky, D. F., and Giovannoni, S. J.: Polyphyletic photosynthetic reaction centre genes in oligotrophic marine gammaproteobacteria, Environ. Microbiol., 9, 1456–1463, 2007.

Cottrell, M. T. and Kirchman, D. L.: Photoheterotrophic microbes in the Arctic Ocean in summer and winter, Appl. Environ. Microbiol., 75, 4958–4966, 2009.

Cottrell, M. T., Mannino, A., and Kirchman, D. L.: Aerobic anoxygenic phototrophic bacteria in the Mid-Atlantic Bight and the North Pacific Gyre, Appl. Environ. Microbiol., 72, 557–564, 2006.

BGD
8, 323–354, 2011

Cottrell, M. T., Ras, J., and Kirchman, D. L.: Bacteriochlorophyll and community structure of aerobic anoxygenic phototrophic bacteria in a particle-rich estuary, ISME J., 4, 945–954, 2010.

Crombet, Y., Leblanc, K., Quéguiner, B., Moutin, T., Rimmelin, P., Ras, J., Claustre, H., Leblond, N., Oriol, L., and Pujo-Pay, M.: Deep silicon maxima in the stratified oligotrophic Mediterranean Sea, Biogeosciences Discuss., 7, 6789–6846, doi:10.5194/bgd-7-6789-2010, 2010.

De Corte, D., Yokokawa, T., Varela, M. M., Agogue, H., and Herndl, G. J.: Spatial distribution of bacteria and Archaea and amoA gene copy numbers throughout the water column of the Eastern Mediterranean Sea, ISME J., 3, 147–158, 2009.

Eiler, A.: Evidence for the ubiquity of mixotrophic bacteria in the upper ocean: Implications and consequences, Appl. Environ. Microbiol., 72, 7431–7437, 2006.

Gonzalez, J. M., Simo, R., Massana, R., Covert, J. S., Casamayor, E. O., Pedros-Alio, C., and Moran, M. A.: Bacterial community structure associated with a dimethylsulfoniopropionate producing North Atlantic algal bloom, Appl. Environ. Microbiol., 66, 4237–4246, 2000.

Holmes, M. R., Aminot, A., Kérouel, R., Hooker, B. A., and Peterson, B. J.: A simple and precise method for measuring ammonium in marine and freshwater ecosystems, Can. J. Fish. Aquat. Sci., 56, 1801–1808, 1999.

Ignatiades, L.: Scaling the trophic status of the Aegean Sea, Eastern Mediterranean, J. Sea Res., 54, 51–57, 2005.

Jiao, N., Zhang, Y., Zeng, Y. H., Hong, N., Liu, R., Chen, F., and Wang, P.: Distinct distribution pattern of abundance and diversity of aerobic anoxygenic phototrophic bacteria in the global ocean, Environ. Microbiol., 9, 3091–3099, 2007.

Karl, D. M.: Microbiological oceanography – hidden in a sea of microbes, Nature, 415, 590–591, 2002.

Karl, D. M. and Tien, G.: MAGIC: a sensitive and precise method for measuring dissolved phosphorus in aquatic environment, Limnol. Oceanogr., 37, 105–116, 1992.

Koblížek, M., Béjà, O., Bidigare, R. R., Christensen, S., Benitez-Nelson, B., Vetriani, C., Kolber, M. K., Falkowski, P. G., and Kolber, Z. S.: Isolation and characterization of *Erythrobacter* sp. strains from the upper ocean, Arch. Microbiol., 180, 327–338, 2003.

Koblížek, M., Stoñ-Egiert, J., Sagan, S., and Kolber, Z. S.: Diel changes in bacteriochlorophyll-*a* concentration suggest rapid bacterioplankton cycling in the Baltic Sea, FEMS Microbiol. Ecol., 51, 353–361, 2005.

BGD
8, 323–354, 2011

Koblížek, M., Falkowski, P. G., and Kolber, Z. S.: Diversity and distribution of photosynthetic bacteria in the Black Sea, Deep-Sea Res. Pt. II, 53, 1934–1944, 2006.
Koblížek, M., Mašín, M., Ras, J., Poulton, A. J., and Prášil, O.: Rapid growth rates of aerobic anoxygenic phototrophs in the ocean, Environ. Microbiol., 9, 2401–2406, 2007.
Kolber, Z. S., Van Dover, C. L., Niederman, R. A., and Falkowski, P. G.: Bacterial photosynthesis in surface waters of the open ocean, Nature, 407, 177–179, 2000.
Kolber, Z. S., Plumley, F. G., Lang, A. S., Beatty, J. T., Blankenship, R. E., VanDover, C. L., Vetriani, C., Koblížek, M., Rathgeber, C., and Falkowski, P. G.: Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean, Science, 292, 2492–2495, 2001.
Lami, R., Cottrell, M. T., Ras, J., Ulloa, O., Obernosterer, I., Claustre, H., Kirchman, D. L., and Lebaron, P.: High abundances of aerobic anoxygenic photosynthetic bacteria in the South Pacific Ocean, Appl. Environ. Microbiol., 73, 4198–4205, 2007.
Lami, R., Čuperová, Z., Ras, J., Lebaron, P., and Koblížek, M.: Distribution of free-living and particle-attached aerobic anoxygenic phototrophic bacteria in marine environments, Aquat. Microb. Ecol., 55, 31–38, 2009.
Lamy, D., De Carvalho-Maalouf, P., Cottrell, M. T., Lami, R., Catala, P., Oriol, L., Caparros, J., Ras, J., Kirchman, D. L., and Lebaron, P.: Seasonal dynamics of aerobic phototrophs in a Mediterranean coastal lagoon, Aquat. Microb. Ecol., doi:10.3354/ame01467, in press, 2011.
Lehours, A.-C., Cottrell, M. T., Dahan, O., Kirchman, D. L., and Jeanthon, C.: Summer distribution and diversity of aerobic anoxygenic phototrophic bacteria in the Mediterranean Sea in relation to environmental variables, FEMS Microbiol. Ecol., 2010.
López-Sandoval, D. C., Fernández, A., and Marañón, E.: Dissolved and particulate primary production along a longitudinal gradient in the Mediterranean Sea, Biogeosciences Discuss., 7, 8591–8617, doi:10.5194/bgd-7-8591-2010, 2010.
Marie, D., Simon, N., Guillou, L., Partensky, F., and Vaulot, D.: Flow cytometric analysis of marine picoplankton, in: Protocols in flow cytoemtry and cell sorting, edited by: Diamond, R. A. and De Maggio, S., Springer-Verlag, Berlin, Germany, 421–454, 2002.
Mašín, M., Nedoma, J., Pechar, L., and Koblížek, M.: Distribution of aerobic anoxygenic phototrophs in temperate freshwater systems, Environ. Microbiol., 10, 1988–1996, 2008.
Moran, M. A. and Miller, W. L.: Resourceful heterotrophs make the most of light in the coastal ocean, Nat. Rev. Microbiol., 5, 792–800, 2007.
Moutin, T. and Raimbault, P.: Primary production, carbon export and nutrients availability in

BGD
8, 323–354, 2011

Western and Eastern Mediterranean Sea in early summer 1996 (MINOS cruise), J. Mar. Syst., 33–34, 273–288, 2002.

Moutin, T., Thingstad, T. F., Van Wambeke, F., Marie, D., Slawyk, G., Raimbault, P., and Claustre, H.: Does competition for nanomolar phosphate supply explain the predominance of the cyanobacterium *Synechococcus*?, Limnol. Oceanogr., 47, 1562–1567, 2002.

Moutin, T., Karl, D. M., Duhamel, S., Rimmelin, P., Raimbault, P., Van Mooy, B. A. S., and Claustre, H.: Phosphate availability and the ultimate control of new nitrogen input by nitrogen fixation in the tropical Pacific Ocean, Biogeosciences Discuss., 5, 95–109, doi:10.5194/bg-5-95-2008, 2008.

Moutin, T., Van Wambeke, F., and Prieur, L.: Introduction to the Biogeochemistry from the Oligotrophic to the Ultraoligotrophic Mediterranean (BOUM) experiment, Biogeosci. Disc., in preparation, 2011.

Murphy, J. and Riley, J. P.: A modified single solution method for the determination of phosphate in natural waters, Anal. Chim. Acta, 27, 31–36, 1962.

Oz, A., Sabehi, G., Koblížek, M., Massana, R., and Béjà, O.: Roseobacter-like bacteria in Red and Mediterranean Sea aerobic anoxygenic photosynthetic populations, Appl. Environ. Microbiol., 71, 344–353, 2005.

Pinhassi, J., Gomez-Consarnau, L., Alonso-Saez, L., Sala, M. M., Vidal, M., Pedros-Alio, C., and Gasol, J. M.: Seasonal changes in bacterioplankton nutrient limitation and their effects on bacterial community composition in the NW Mediterranean Sea, Aquat. Microb. Ecol., 44, 241–252, 2006.

Pregl, F.: Quantitative organic microanalysis. Translation from Fyleman E., edited by: Churchill, J. and A., London, 69 pp., 1924.

Pujo-Pay, M. and Raimbault, P.: Improvement of the wet-oxydation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters, Mar. Ecol. Prog. Ser., 105, 203–207, 1994.

Pujo-Pay, M., Conan, P., and Raimbault, P.: Excretion of dissolved organic nitrogen by phytoplankton assessed by wet oxidation and N-15 tracer procedures, Mar. Ecol. Prog. Ser., 153, 99–111, 1997.

Pujo-Pay, M., Conan, P., Oriol, L., Cornet-Barthaux, V., Falco, C., Ghiglione, J.-F., Goyet, C., Moutin, T., and Prieur, L.: Integrated survey of elemental stoichiometry (C, N, P) from the Western to Eastern Mediterranean Sea, Biogeosciences Discuss., 7, 7315–7358, doi:10.5194/bgd-7-7315-2010, 2010.

BGD
8, 323–354, 2011

Ramette, A.: Multivariate analyses in microbial ecology, FEMS Microbiol. Ecol., 62, 142–160, 2007.
Ras, J., Claustre, H., and Uitz, J.: Spatial variability of phytoplankton pigment distributions in the Subtropical South Pacific Ocean: comparison between in situ and predicted data, Biogeosciences, 5, 353–369, doi:10.5194/bg-5-353-2008, 2008.
Rimmelin, P. and Moutin, T.: Re-examination of the MGIC method to determine low orthophosphate concentration in seawater, Anal. Chim. Acta, 548, 174–182, 2005.
Salka, I., Moulisová, V., Koblížek, M., Jost, G., Jürgens, K., and Labrenz, M.: Abundance, depth distribution, and composition of aerobic bacteriochlorophyll-*a* – producing bacteria in four basins of the Central Baltic Sea, Appl. Environ. Microbiol., 74, 4398–4404, 2008.
Schwalbach, M. S. and Fuhrman, J. A.: Wide-ranging abundances of aerobic anoxygenic phototrophic bacteria in the world ocean revealed by epifluorescence microscopy and quantitative PCR, Limnol. Oceanogr., 50, 620–628, 2005.
Sharp, J. H.: Improved analysis for particulate organic carbon and nitrogen from seawater, Limnol. Oceanogr., 19, 984–989, 1974.
Sherr, B. F., Sherr, E. B., and McDaniel, J.: Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblages, Appl. Environ. Microbiol., 58, 2381–2385, 1992.
Shiba, T.: *Roseobacter litoralis* Gen-Nov, Sp-Nov, and *Roseobacter denitrificans* sp-nov, aerobic pink-pigmented bacteria which contain bacteriochlorophyll-*a*, Syst. Appl. Microbiol., 14, 140–145, 1991.
Sieracki, M. E., Gilg, I. C., Thier, E. C., Poulton, N. J., and Goericke, R.: Distribution of planktonic aerobic anoxygenic photoheterotrophic bacteria in the Northwest Atlantic, Limnol. Oceanogr., 51, 38–46, 2006.
Smith, D. C. and Azam, F.: A simple and economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine, Mar. Microb. Food Webs, 6, 107–114, 1992.
Suzuki, M. T., Preston, C. M., Chavez, F. P., and DeLong, E. F.: Quantitative mapping of bacterioplankton populations in seawater: field tests across an upwelling plume in Monterey Bay, Aquat. Microb. Ecol., 24, 117–127, 2001.
Suzuki, M. T. and Béjà, O.: An elusive marine photosynthetic bacterium is finally unveiled, P. Natl. Acad. Sci. USA, 104, 2561–2562, 2007.
ter Braak, C. J. F.: CANOCO – an extension of DECORANA to analyze species-environment relationships, Hydrobiologia, 184, 169–170, 1989.

BGD
8, 323–354, 2011

ter Braak, C. J. F. and Smilauer, P.: CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5), Microcomputer Power, Ithaca, NY, 500 pp., 2002.
Thingstad, T. F., Zweifel, U. L., and Rassoulzadegan, F.: P limitation of heterotrophic bacteria and phytoplankton in the Northwest Mediterranean, Limnol. Oceanogr., 43, 88–94, 1998.
Tréguer, P. and Le Corre, P.: Manuel d'analyse des sels nutritifs dans l'eau de mer, in: Utilisation de l'Autoanalyzer II Technicon. 2nd edition, edited by: Laboratoire d'Océanographie chimique, U. d. B. O., Brest, France, 110 pp., 1975.
Van Heukelem, L. and Thomas, C. S.: Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments, J. Chromatogr., 910, 31–49, 2001.
Van Wambeke, F., Christaki, U., Giannokourou, A., Moutin, T., and Souvemerzoglou, K.: Longitudinal and vertical trends of bacterial limitation by phosphorus and carbon in the Mediterranean Sea, Microb. Ecol., 43, 119–133, doi:10.1007/s00248-001-0038-4, 2002.
Van Wambeke, F., Nedoma, J., Duhamel, S., and Lebaron, P.: Alkaline phosphatase activity of marine bacteria studied with ELF 97 substrate: success and limits in the P-limited Mediterranean Sea, Aquat. Microb. Ecol., 52, 245–251, 2008.
Van Wambeke, F., Catala, P., and Lebaron, P.: Relationships between cytometric characteristics of high and low nucleic-acid bacterioplankton cells, bacterial production and environmental parameters along a longitudinal gradient across the Mediterranean Sea, Biogeosciences Discuss., 7, 8245–8279, doi:10.5194/bgd-7-8245-2010, 2010.
Waidner, L. A. and Kirchman, D. L.: Aerobic anoxygenic phototrophic bacteria attached to particles in turbid waters of the Delaware and Chesapeake estuaries, Appl. Environ. Microbiol., 73, 3936–3944, 2007.
Winter, C., Kerros, M. E., and Weinbauer, M. G.: Seasonal changes of bacterial and archaeal communities in the dark ocean: evidence from the Mediterranean Sea. Limnol. Oceanogr., 54, 160–170, 2009.
Wood, E. D., Amstrong, F. A. J., and Richards, F. A.: Determination of nitrate in sea water by cadmium-copper reduction to nitrite, J. Mar. Biol. Ass. UK, 47, 23–31, 1967.
Yurkov, V. V. and Beatty, J. T.: Aerobic anoxygenic phototrophic bacteria, Microbiol. Mol. Biol. Rev., 62, 695–724, 1998.
Yurkov, V. V. and Csotonyi, J. T.: New light on aerobic anoxygenic phototrophs, Adv. Photosynth. Respir., 28, 31–55, 2009.

BGD
8, 323–354, 2011

Yutin, N. and Béjà, O.: Putative novel photosynthetic reaction centre organizations in marine aerobic anoxygenic photosynthetic bacteria: insights from metagenomics and environmental genomics, Environ. Microbiol., 7, 2027–2033, 2005.

Yutin, N., Suzuki, M. T., Teeling, H., Weber, M., Venter, J. C., Rusch, D. B., and Béjà, O.: Assessing diversity and biogeography of aerobic anoxygenic phototrophic bacteria in surface waters of the Atlantic and Pacific Oceans using the Global Ocean Sampling expedition metagenomes, Environ. Microbiol., 9, 1464–1475, 2007.

Yutin, N., Béjà, O., and Suzuki, M. T.: The use of denaturing gradient gel electrophoresis with fully degenerate pufM primers to monitor aerobic anoxygenic phototrophic assemblages, Limnol. Oceanogr. Methods, 6, 427–440, 2008.

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Table 1. Initial chemical conditions and biological parameters prevailing in surface (5 m depth) seawater samples before nutrient amendments.

Parameter[a]	Station C	Station 9	Station 17	Station A
NO_3+NO_2 (nM)	ld[b]	ld	20	40
NH_4 (nM)	4	ld	3	4
PO_4 method 1/method 2[c] (nM)	60/14	ld/20	10/5	10/6
DOC (µM)	70	71	59	66
DON (µM)	4.9	4.4	5.1	4.9
DOP (µM)	0.03	0.02	0.04	0.07
POC (µM)	2.7	3.2	5.0	4.5
PON (µM)	0.37	0.23	0.41	0.37
POP (µM)	0.016	0.013	0.026	0.026
PO_4 turnover time (h)	11.1	3.4	3.0	1.6
Chl-*a* (µg L^{-1})	0.03	0.03	0.06	0.06
BChl-*a* (ng L^{-1})	<2	3	6	<2
Bacterial abundance ($\times 10^5$ cells mL^{-1})	2.51	2.69	5.51	4.43
AAP bacterial abundance ($\times 10^4$ cells mL^{-1})	nd	0.12	0.63	nd
Bacterial production (ng C L^{-1} h^{-1})	12	12	33	26

[a] See in the text for the meaning of the abbreviations;
[b] ld, below the detection limit (see Material and methods);
[c] In method 1 (ld=10 nM), phosphate was analysed on board using the automated colorimetric technique (Tréguer and Le Corre, 1975; Wood et al., 1967). In method 2 (ld=2 nM), phosphate was analysed on board using the MAGIC method (Rimmelin and Moutin, 2005).

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Table 2. Bacterial production, growth of total and AAP bacteria after nutrient (P for phosphate, N for nitrogen, C for glucose and NPC for N+P+C) amendments of surface (5 m depth) seawater samples at 4 stations (St.) of the BOUM transect. Results are expressed relative to the unamended controls.

	Bacterial production				Bacterial growth				AAP bacterial growth			
	P	N	C	NPC	P	N	C	NPC	P	N	C	NPC
St. C	2.4	10.6	15.0	75.5	0.9	2.0	1.1	5.9	1.0	2.4	1.9	3.8
St. 9	3.5	2.0	1.2	18.7	1.3	1.1	1.1	2.1	1.1	4.6	2.4	4.6
St. 17	1.8	1.9	1.3	6.9	nd[a]	nd	nd	nd	1.3	1.6	1.2	3.1
St. A	1.7	2.2	1.1	32.8	nd	nd	nd	nd	1.4	1.8	1.7	3.2

[a] nd: not determined.

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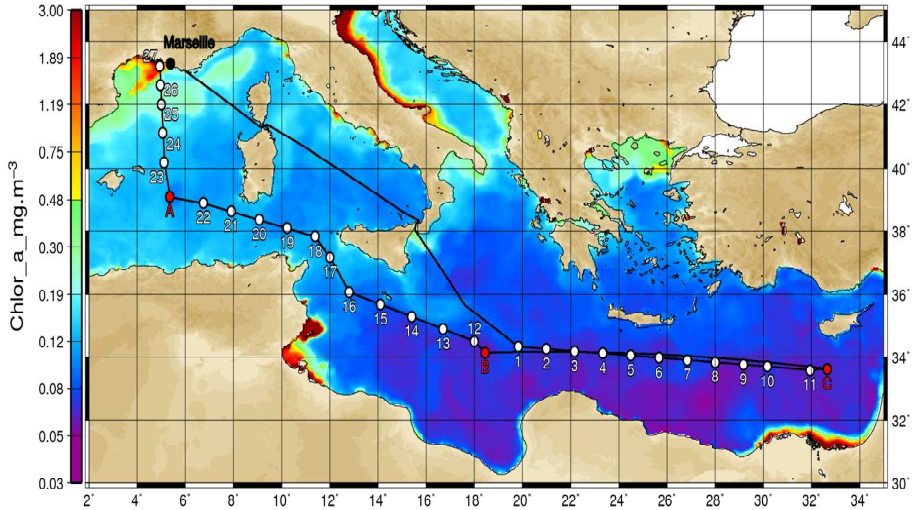

Fig. 1. Transect of the BOUM cruise superimposed on a SeaWiFS 10 surface Chl-*a* 11 composite image (June 2008) and location of the 30 sampled stations.

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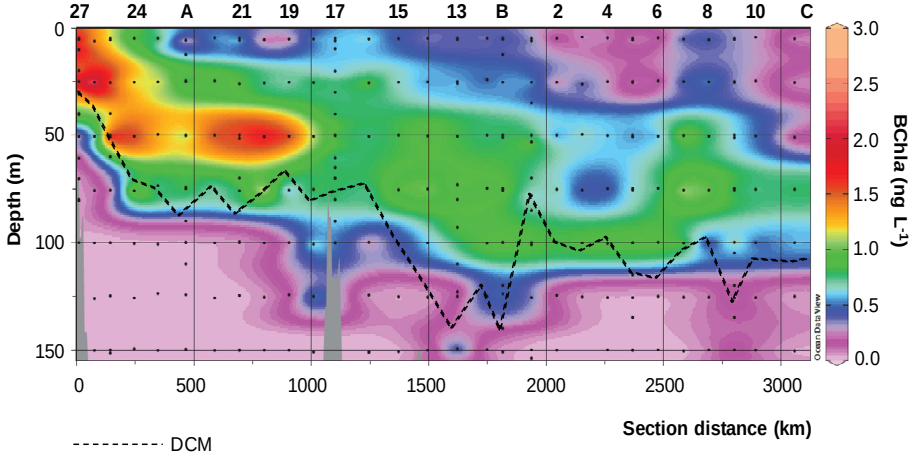

Fig. 2. Longitudinal cross section of BChl-*a* concentration along the BOUM transect, from the most north-western station (left) to the most eastern station (right) and for the 0–150 m layer. Depth of the deep chlorophyll maximum is also indicated (dashed line).

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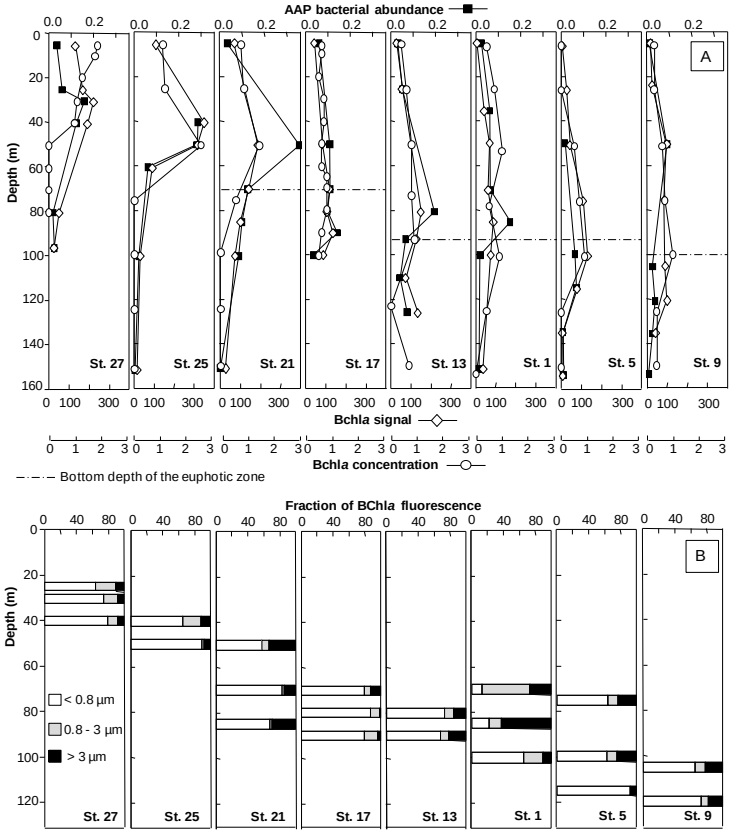

Fig. 3. Vertical profiles at 8 stations along the BOUM transect of **(A)** AAP bacterial abundance (10^5 cell mL^{-1}), BChl-*a* concentration (ng L^{-1}) and BChl-*a* fluorescence signal (arbitrary units) and **(B)** size fractionation (<0.8 µm, 0.8–3 µm and >3 µm) of BChl-*a* fluorescence (in %). The bottom of the euphotic zone is the depth to which 1% of PAR reached. No PAR data were available at stations 25 and 27.

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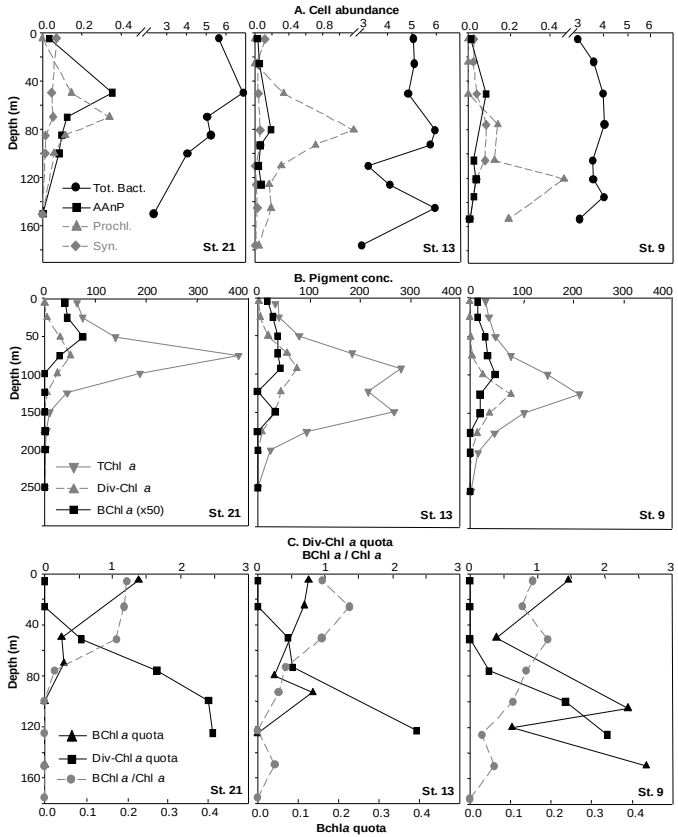

Fig. 4. Vertical profiles at stations 21, 13 and 9 of **(A)** total bacteria, AAP bacteria, *Prochlorococcus* and *Synechococcus* abundances (10^5 cell mL^{-1}), **(B)** Chl-*a*, Div-Chl-*a* and BChl-*a* concentrations (ng L^{-1}) and **(C)** Div-Chl-*a* and BChl-*a* content per cell (fg $cell^{-1}$) and BChl-*a*/Chl-*a* ratios (%).

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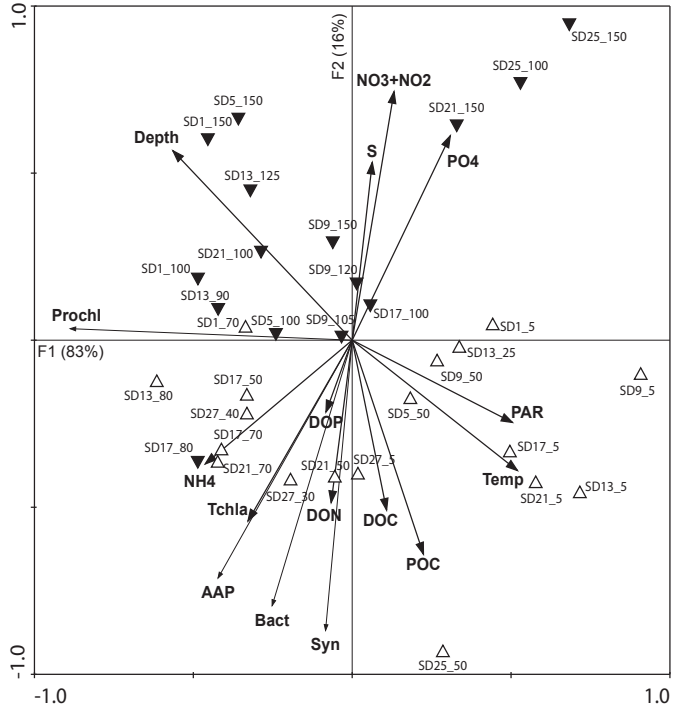

Fig. 5. Redundancy analysis using the abundances of AAP bacteria (AAP), total bacteria (Bact), *Prochlorococcus* (Prochl) and *Synechococcus* (Syn) as independent variables (thin arrows) and temperature (Temp), salinity (S), depth, photosynthetically active radiation (PAR), concentrations of Chl-*a* (Tchl-*a*), nitrite+nitrate (NO_2+NO_3), phosphate (PO_4), ammonium (NH_4), dissolved organic carbon (DOC), nitrogen (DON) and phosphorous (DOP) and particulate organic carbon (POC) as explanatory variables (thick arrows). The samples are identified as "below the euphotic zone" (black down-triangles) or "euphotic zone" (open up-triangles) samples. In the name of the samples, the first number corresponds to the number of the sampling station and the second number to the sampling depth.

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