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Distribution and Growth of Aerobic Anoxygenic Phototrophs in the Mediterranean Sea

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Summary

The distribution of aerobic anoxygenic phototrophs (AAPs) was surveyed in various regions of the Mediterranean Sea in spring and summer. These phototrophic bacteria were present within the euphotic layer at all sampled stations. The AAP abundances increased with increasing trophic status ranging from 2.5×10^3 cells per ml in oligotrophic Eastern Mediterranean up to 90×10^3 cells per ml in the Bay of Villefranche. Aerobic anoxygenic phototrophs made up on average 1–4% of total prokaryotes in low nutrient areas, whereas in coastal and more productive stations these organisms represented 3–11% of total prokaryotes. Diel bacteriochlorophyll *a* decay measurements showed that AAP community in the Western Mediterranean grew rapidly, at rates from 1.13 to 1.42 day^{-1}. The lower AAP abundances registered in the most oligotrophic waters suggest that they are relatively poor competitors under nutrient limiting conditions. Instead, AAPs appear to be metabolically active organisms, which thrive better in more eutrophic environments providing the necessary substrates to maintain high growth rates.

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Introduction

Photoheterotrophic bacteria represent an important part of microbial communities inhabiting the euphotic zone of world oceans (Béjà *et al*., 2000; Kolber *et al*., 2000; Campbell *et al*., 2008; Gasol *et al*., 2008). These organisms require a supply of organic substrates for growth, but they are able to derive a significant portion of their energy requirements from light (Koblížek, 2011). One group of these organisms, the aerobic anoxygenic phototrophs (AAPs) contain photosynthetic reaction centres composed of bacteriochlorophyll *a* (BChl *a*) (Yurkov and Csotonyi, 2009; Koblížek *et al*., 2010). AAPs have been previously found in open ocean waters (Kolber *et al*., 2001; Cottrell *et al*., 2006; Sieracki *et al*., 2006; Jiao *et al*., 2007) as well as in shelf and coastal environments (Koblížek *et al*., 2005; Mašín *et al*., 2006; Zhang and Jiao, 2007; Cottrell *et al*., 2010). Phylogenetically, they consist of several groups distributed across the *Alpha*-, *Beta*- and *Gammaproteobacteria* (Yutin *et al*., 2007; Salka *et al*., 2008).

In his pioneering work, Kolber and colleagues (2000) suggested that AAP bacteria might be abundant in nutrient-poor areas of the ocean where their ability to utilize light energy could provide an ecological advantage over chemoheterotrophic organisms. The Mediterranean Sea is an example of such environment, combining both oligotrophic character and high solar input. A characteristic feature of this semi-enclosed marine basin is the pronounced longitudinal gradient with decreasing nutrient levels and microbial biomass from west to east. The inflow of Atlantic waters and its circulation into the basin is one of the main hydrological features bringing nutrients into the surface waters of the western basin. The intense solar heating and water evaporation result in a salinity gradient with increasing temperatures and salinity from west to east. The eastern Mediterranean Sea is one of the most oligotrophic regions in the world (Turley, 1999) with severe phosphorus deficiency (Krom *et al*., 1991; Thingstad *et al*., 1998).

The presence of AAPs in the Mediterranean Sea was first revealed by isolation of two strains of AAP bacteria from the French Mediterranean coast (Koblížek *et al*., 2003; 2010), and later confirmed by the presence of *puf*M genes encoding the M subunit of bacterial photosynthetic reaction centres (Oz *et al*., 2005; Lehours *et al*., 2010). A

Table 1. List of the sampling stations.

Station	Context	Latitude, longitude	Date	Surface temperature	Max depth
Villefranche Bay	Coastal waters	43°42′N, 7°18.5′E	14–15 September 2005	24.0°C	7 m
Station 6	Ionian Sea, EB	38°29.7′N, 18°2′E	14 June 2007	22.6°C	2328 m
Station 7	Open Sea, EB	35°8.2′N, 20°51′E	16 June 2007	22.9°C	2825 m
Station 8	Open Sea, EB	34°53.4′N, 22°31.8′E	17 June 2007	22.7°C	2964 m
Station 9 Ieri	Open Sea, EB	34°25.2′N, 26°3.2′E	24 June 2007	24.3°C	3000 m
Station C, BBMO	Shelf near coast, CS	41°39.1′N, 2°48.1′E	18–20 September 2007	23.2°C	33 m
Station CM	Off coast, CS	41°24.1′N, 2°48.1′E	21 September 2007	23.8°C	570 m
Station M	Continental margin, CS	41°9′N, 2°49′E	21 September 2007	23.0°C	1427 m
Station MD	Open Sea, CS	40°54.9′N, 2°50.7′E	22 September 2007	21.5°C	1962 m
Station D	Open Sea, CS	40°39.1′N, 2°51′E	23–25 September 2007	24.0°C	2181 m
Station 12	Open Sea, GL	41°30.7′N, 3°57′E	3 May 2009	15.3°C	2376 m
Station 27	Open Sea, GL	42°2.3′N, 4°2.7′E	8 May 2009	15.3°C	2107 m
Station 32	Open Sea, GL	42°2.8′N, 4°9.9′E	10 May 2009	15.6°C	2075 m
Station 41	Open Sea, GL	41°23.1′N, 4°30.4′E	12 May 2009	16.8°C	2557 m

Regions: EB, Eastern Basin (TransMed cruise); CS, Catalan Sea (Modivus cruise); GL, Gulf of Lion (Famoso2 cruise); BBMO, Blanes Bay Microbial Observatory.

subsequent study performed in Banyuls-sur-Mer (France) in spring 2007 first enumerated AAP bacteria in this environment (Lami *et al*., 2009).

For all its characteristics, the Mediterranean Sea was chosen as an excellent site to study the ecological role of AAP bacteria in a variety of environments ranging from the highly oligotrophic eastern basin to highly productive coastal areas. We took advantage of four sampling campaigns in the different parts of this Sea and used infrared (IR) epifluorescence microscopy and IR fluorometry to test the hypothesis that AAPs should be favoured in nutrient-poor environments. In addition, the activity of AAP bacteria was assessed from diel changes of BChl *a*, which allows to calculate their growth rates (Koblížek *et al*., 2005; 2007).

Results

AAP abundances

We first measured the abundance of AAPs in the Mediterranean Sea during a diurnal sampling in the Bay of Villefranche-sur-Mer, France in September 2005 (Table 1). The AAP abundance at the surface layer ranged from 23×10^3 to 88×10^3 cells ml^{-1} forming 5–11% of total prokaryotes (Table 2). Further research was conducted in the eastern part of the Mediterranean Sea during the Italian TransMed cruise Leg 3 in June 2007 (Fig. 1 and Table 1). The summer conditions resulted in pronounced stratification with deep chlorophyll maxima located between 85 and 105 m (240–630 ng Chl *a* l^{-1}). The surface chlorophyll concentration ranged 34–80 ng Chl *a* l^{-1}. At all stations the euphotic zone (upper 100 m) was depleted in phosphate (< 0.025 μM). The total inorganic nitrogen concentrations ranged from 0.08 to 0.5 μM. The AAP bacteria were observed within the euphotic zone in all sampled stations. The AAP distribution had two subsurface maxima. The first one was found at around 25 m depth, whereas the second one overlapped with the deep chlorophyll maximum located between 80 and 100 m (Fig. 2). At the majority of stations AAP abundances were relatively low, between 2.6 and 29×10^3 cells ml^{-1}, which represented 0.5–3.5% of total prokaryotes.

The western part of the Mediterranean was surveyed during the Spanish Modivus cruise in September 2007.

Table 2. Main characteristics of microbial community during diel measurement in the Bay of Villefranche (14–15 September 2005), Station 'C' – Blanes Bay (18–20 September 2007) and Station 'D' in the Catalan Sea (23–25 September 2007).

Parameter	Villefranche Bay	Station C	Station D
Chl *a* content (ng l^{-1})	444 ± 121	115 ± 20	85 ± 5
BChl *a* content (ng l^{-1})	8.0 ± 2.5	1.6 ± 0.4	1.1 ± 0.2
BChl *a*/Chl *a* ratio (%)	1.7 ± 0.8	1.4 ± 0.3	1.3 ± 0.2
Total DAPI (10^3 cells ml^{-1})	614 ± 97	528 ± 84	408 ± 132
AAPs (10^3 cells ml^{-1})	55.7 ± 19.1	19.7 ± 6.0	8.2 ± 2.5
Fraction of AAP cells	8.9 ± 2.1%	3.8 ± 1.2%	3.1 ± 1.1%
BChl *a* per cell (ag BChl *a*)	150 ± 39	81 ± 20	131 ± 24
Primary production (μg C l^{-1} day^{-1})	n.d.	39.0 ± 3.5	27.6 ± 2.0

Samples were collected at 0.5 m or 5 m depth respectively during 24 h with 2 to 4 h intervals. The provided values report averages± standard deviations. n.d., no data.

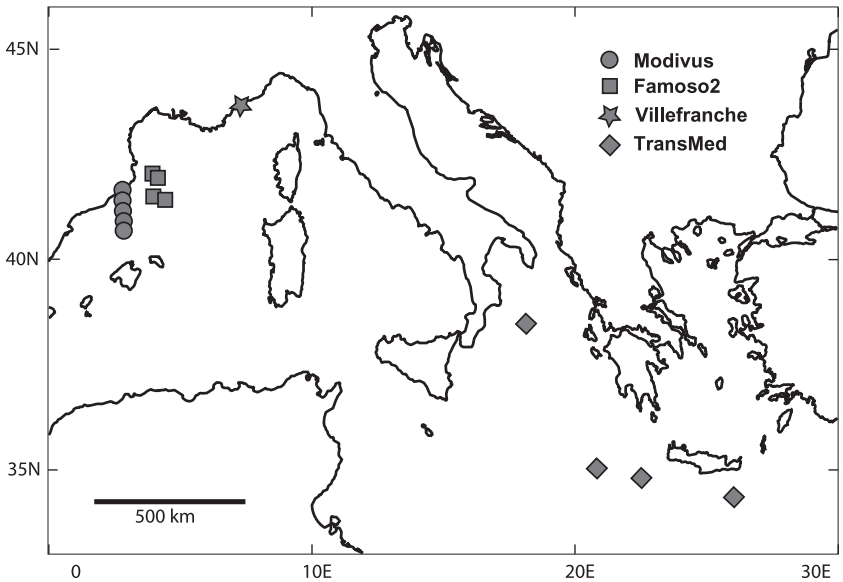

Fig. 1. Map of the Mediterranean Sea with sampling stations. The TransMed cruise – June 2007, Modivus cruise – September 2007, Famoso2 cruise – May 2009, Bay of Villefranche – September 2005.

Here, in addition to IR epifluorescence microscopy, the AAP distribution was analysed also by means of IR kinetic fluorometry. The transect from the coastal Blanes Bay (station 'C') to the offshore station 'D' sampled from 20 until 23 September 2007 revealed a relatively uniform pattern of AAP distribution. Aerobic anoxygenic phototrophs were located in the upper 100 m with a distinct subsurface maximum between 25 and 40 m. In contrast, the deep chlorophyll maxima (270–420 ng Chl *a* l^{-1}) were found significantly deeper, between 60 and 70 m (see Fig. 2). At all stations the euphotic zone (upper 80 m) was depleted in phosphate (< 0.05 μM) while the total inorganic nitrogen concentrations ranged from 0.5 to 4.2 μM. Based on IR epifluorescence microscopy there were $8–15 \times 10^3$ AAP cells ml^{-1} at the surface layer and $20–25 \times 10^3$ AAP cells ml^{-1} (4% of the total prokaryotes) in the subsurface maximum (Fig. 2). The surface BChl *a* concentration ranged 1–1.5 ng BChl *a* l^{-1} whereas the BChl *a* subsurface maxima (25–50 m) varied between 2–2.3 ng BChl *a* l^{-1}. In general the vertical distribution of AAPs was similar to the vertical distribution of *Synechococcus*, whereas picoeukaryotes and *Prochlorococcus* were found deeper in the water column forming the deep chlorophyll maximum. A simple size fractionation conducted at station 'D' (5–65 m) revealed that 55.3 ± 6.7% (mean ± SD, $n = 3$) of the BChl *a* signal was associated with the < 0.8 μm fraction, 26.2 ± 9.5% with the 0.8–2 μm fraction and 8.5 ± 7.0% of the signal was in > 2 μm fraction.

The last survey of the western Mediterranean waters was conducted during Famoso2 cruise in the Gulf of Lion in May 2009 in a diatom post-bloom situation. In contrast to the previous measurements the surface water temperature was significantly lower (15.3–16.8°C) and the water-column was less stratified. The chlorophyll maxima (380–690 ng Chl *a* l^{-1}) were typically located at the surface with exception of Station 12 where it was placed at 40 m. Interestingly, the AAP maxima were located below the surface at depths between 18 and 30 m (Fig. 2). Both AAP abundances ($7.6–51.9 \times 10^3$ cells ml^{-1}) and percentages (1.8–10%) were significantly higher than those observed in the Catalan Sea during the Modivus cruise (see Fig. 3). It is noteworthy that the recorded numbers in the Gulf of Lion corresponded well with the previously published values of $4.4–65 \times 10^3$ cells ml^{-1} from the same area and season reported by Lami *et al.*, (2009).

Diurnal measurements of bacteriochlorophyll a

In our previous work we have shown that the diel changes of BChl *a* signal can be conveniently used to measure AAP mortality rates (Koblížek *et al.*, 2005). Assuming day-to-day stability, the recorded mortality rates equal growth rates. The diurnal measurements were conducted during the Modivus cruise in September 2007. BChl *a* decays were followed at station 'C' (Blanes Bay) for two consecutive days and at station 'D' (deep station) for three consecutive days at 5 m depth (Table 2). In addition, control samples obtained from a pre-dawn sampling were placed in an on-deck incubator. The decay of the signal observed in the incubator samples was virtually the same as the decay observed *in situ* using CTD recovered water samples (see Fig. 4). This indicates that on-deck incubations faithfully reflect *in situ* conditions of AAP growth. The BChl *a* signal decay rates at station 'C' were 1.42 ± 0.26 day^{-1} during the first and 1.15 ± 0.23 day^{-1} during the second day of measurement. Together with the changes of BChl *a* concentration we observed also significant diurnal changes

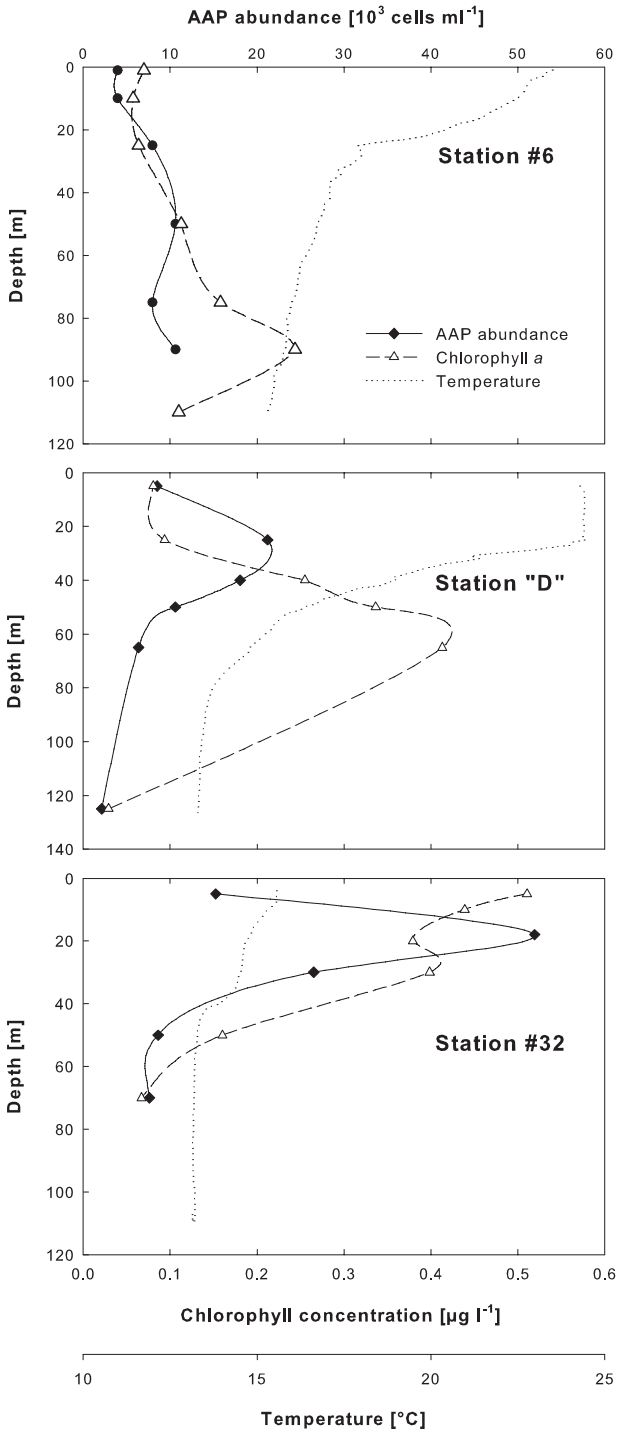

Fig. 2. Depth profiles of AAP abundance, chlorophyll *a* concentration and temperature at three stations in the Mediterranean Sea. Upper panel: Station #6, Ionian Sea, June 2007; central panel: Station 'D' (CTD #27), Catalan Sea, September 2007; lower panel: Station #32, Gulf of Lion, May 2009.

in AAP cell numbers, which varied between 15.2 and 24.3×10^3 cells ml^{-1}. The rates recorded at station 'D' were somewhat lower: 1.13 ± 0.14 day^{-1} during the first and 1.15 ± 0.23 day^{-1} during the second day of the measurement. The last day, the recorded decay rate was stopped because of an afternoon storm, which shaded the sun and caused partial mixing of the water column.

The growth rates obtained from BChl *a* decays can be used to calculate AAP production at the respective stations. Using the known AAP abundances at the respective stations (see Table 2) and assuming a conservative AAP cell quota of 30 fg *C* (Koblížek *et al.*, 2010), we can estimate that the AAP production at station 'C' ranged between 0.5 and 1.1 µg *C* l^{-1} day^{-1}, and at station 'D' between 0.2 to 0.4 µg *C* l^{-1} day^{-1}. The calculated productions represent 35–69% of bacterial production (1.6 µg *C* l^{-1} day^{-1}) at station 'C' and 27–56% of bacterial production (0.74 µg *C* l^{-1} day^{-1}) at station 'D'. Using the previously published decay rates of 2.35–3.01 day^{-1} (Koblížek *et al.*, 2007) and determined abundances we can calculate the AAP production also for the Bay of Villefranche. Here, in this more productive coastal environment, AAP production was an order of magnitude higher, ranging between 2.6 and 6.8 µg *C* l^{-1} day^{-1}.

Discussion

Over the past decade, AAPs have attracted an elevated interest of marine microbiologists. A lot of controversy was stirred by the first contradictory reports regarding AAP abundance. In the first study using IR epifluorescence microscopy, Kolber and colleagues (2001) reported that AAPs formed 11% of total bacteria in North East Pacific. This number was later questioned by Schwalbach and Fuhrman (2005), claiming that the fraction of AAPs was much lower (1.66 ± 0.55%). The AAP percentages found in this study (0.2–11.1%) are somewhere between those two extremes and resemble values reported from the North Atlantic (0.8–18%) by Sieracki and colleagues (2006) and Cottrell and colleagues (2006). The AAP abundances ranged from 0.2×10^3 to 90×10^3 cells ml^{-1}, which is similar to previously published values ($0.5–155 \times 10^3$) from the Atlantic and Pacific Oceans (Cottrell *et al.*, 2006; Sieracki *et al.*, 2006). In general, the AAP distribution in the Mediterranean Sea showed a clear positive trend connected with the trophic status (Fig. 3). The minimum numbers (both absolute and relative) were observed at the most oligotrophic stations in the Eastern Mediterranean. The higher proportion of AAPs was registered in the Catalan Sea and the Gulf of Lion. The maximum values were observed in the Bay of Villefranche (Fig. 3). The clear preference of AAP bacteria for higher trophic environments was confirmed also by statistical analyses, which documented a significant positive correlation between absolute AAP abundance and chlorophyll content (Pearson's $r = 0.510$, $P < 0.0001$, $n = 61$) as well as between AAP percentage and chlorophyll ($r = 0.570$,

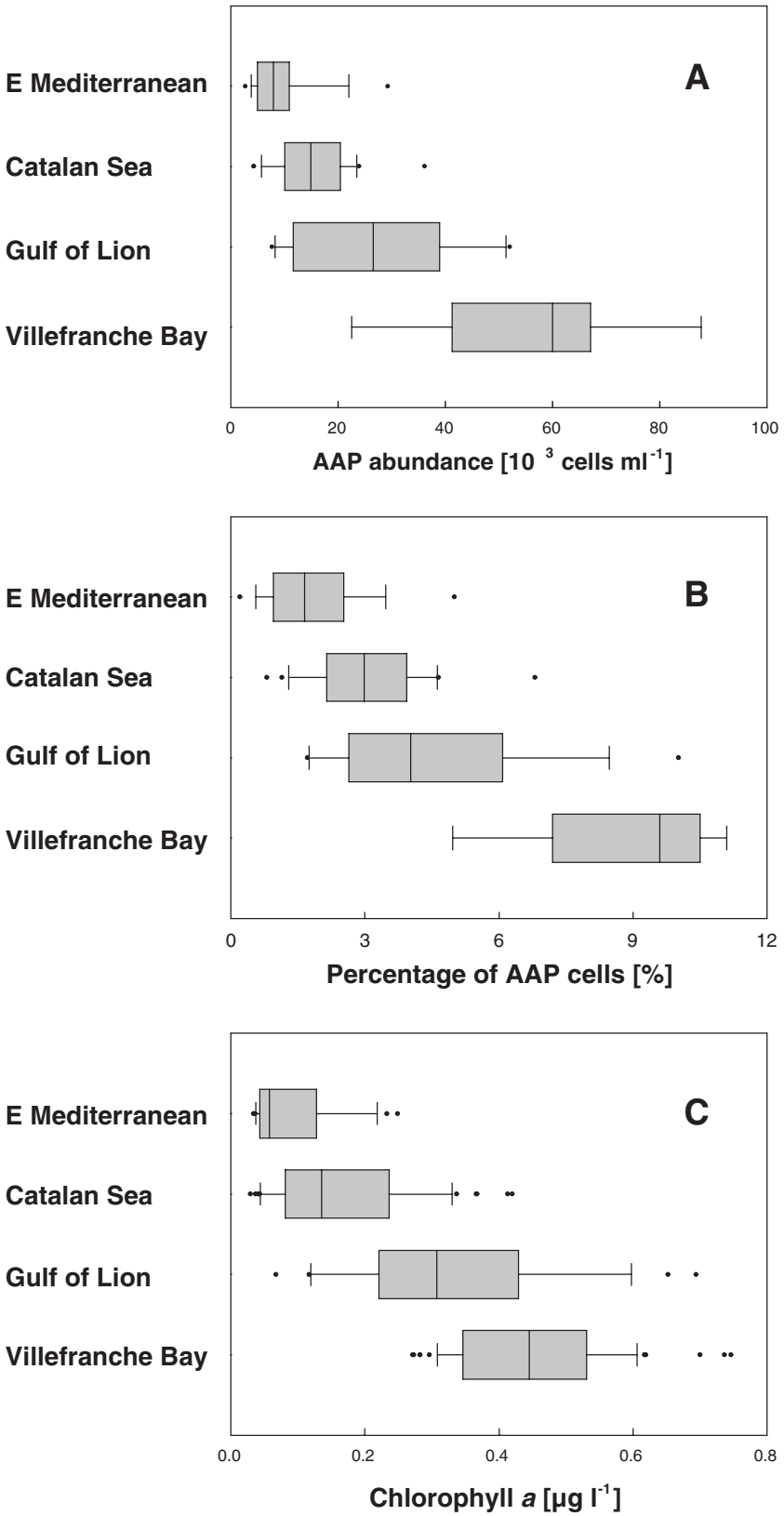

Fig. 3. AAP distribution in different regions of the Mediterranean Sea. (A) Absolute abundances in 10^3 cells ml^{-1}; (B) AAP abundance as a fraction of total prokaryotes; (C) chlorophyll concentrations. Small dots indicate outlier values. The individual boxes depict: E Mediterranean – the TransMed cruise ($n = 18$), Catalan Sea – Modivus cruise ($n = 26$), Gulf of Lion – Famoso2 cruise ($n = 15$), Bay of Villefranche sampling ($n = 9$).

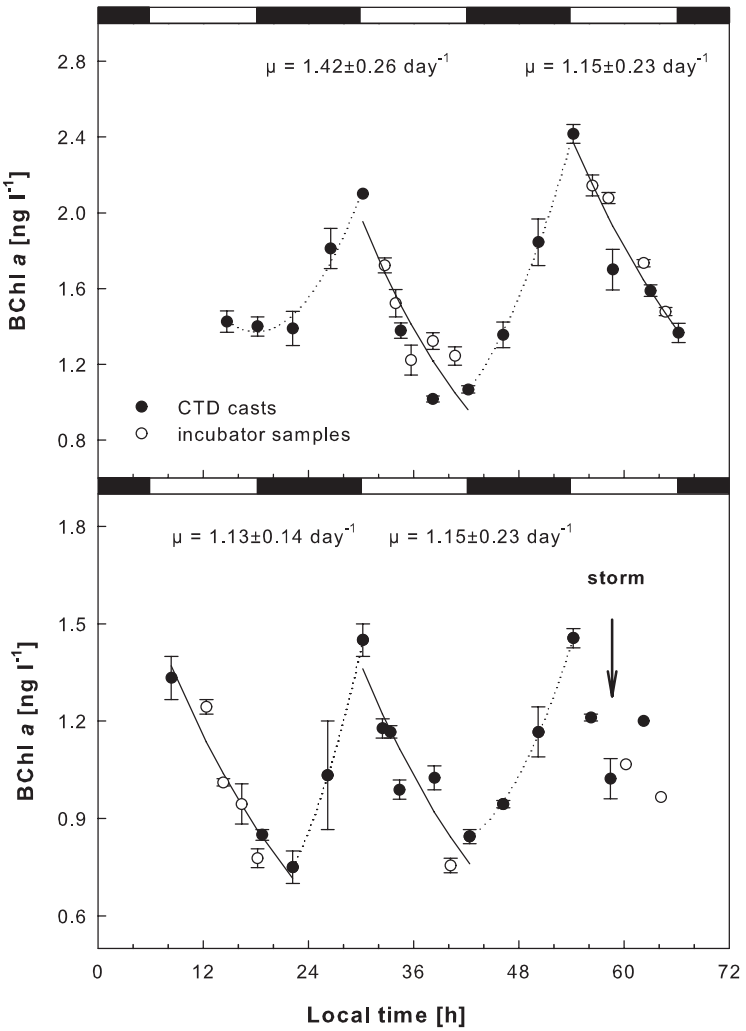

Fig. 4. Diel changes of BChl *a* fluorescence signal recorded at station 'C' (Blanes Bay, upper panel) on September 18–20, 2007) and station 'D' (lower panel) on September 23–25, 2007. Closed symbols represent measurements with CTD recovered samples, the open symbols were samples obtained from on-deck incubations. The dark periods are marked by black bars on the top of the figure. The provided μ-values represent calculated BChl *a* signal decay rates.

$P < 0.0001$, $n = 61$). A positive correlation was found between AAP abundance and total bacterial counts ($r = 0.377$, $P = 0.0011$, $n = 72$) and also between AAP percentage and ammonium concentration ($r = 0.378$, $P = 0.0211$, $n = 37$). All these results are consistent with previous reports which also found smaller AAP percentages in the more oligotrophic areas when compared with the more productive regions or shelf seas (Cottrell *et al*., 2006; Mašín *et al*., 2006; Sieracki *et al*., 2006; Jiao *et al*., 2007).

Clearly, the obtained distribution data are in conflict with the view that the ability to use light should be especially advantageous in nutrient-poor environments. This observation might be explained by the fact that AAP bacteria use light energy only to supplement their primarily heterotrophic metabolism. The use of light energy allows them to store more carbon (which would otherwise be respired) in form of biomass, which has been confirmed in laboratory cultures (Shioi, 1988; Kolber *et al*., 2001). Thus, AAPs likely have an ecological advantage over heterotrophs under conditions when bacterial growth is energy or carbon limited. However, the Mediterranean Sea is a mostly phosphorus-limited ecosystem (Krom *et al*., 1991; Thingstad *et al*., 1998). Low phosphate concentrations in the euphotic zone were observed in this study. Under such conditions the presence of a photosynthetic apparatus probably offers only little metabolic advantage.

The BChl *a* decay measurements showed that the AAP community in the Catalan Sea grew at rates of 1.13–1.42 day^{-1}. Those are only slightly faster than the rates of 0.91–1.08 day^{-1} previously determined in the Sargasso Sea. To compare the obtained data with previously published values from the North Atlantic (Koblížek *et al*., 2007), the BChl *a* turnover rates were plotted against chlorophyll concentrations (Fig. 5). The regression analysis showed that both variables were strongly linearly correlated with a slope $k = 5.27 \pm 0.39$

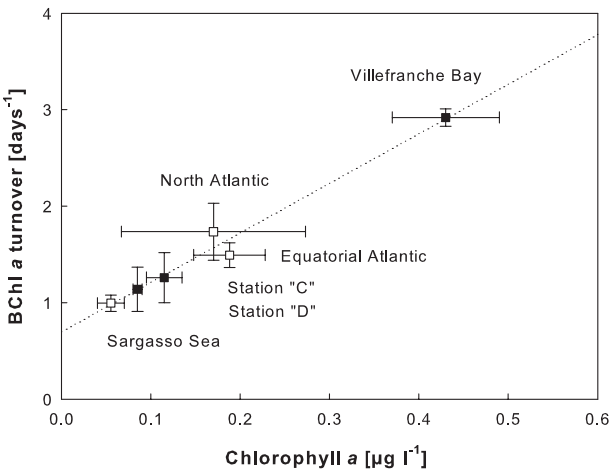

Fig. 5. Relationship between BChl *a* turnover and chlorophyll content. The Atlantic Ocean data and BChl *a* turnover in Villefranche Bay were taken from Koblížek and colleagues (2007). Each data point represents an average value from two individual diel measurements in case of Mediterranean data (closed symbols) or from three to five measurements for the Atlantic (empty symbols). The obtained values give a linear relationship $y = 5.27x + 0.66$ ($r^2 = 0.977$).

day^{-1} µg *Chl*$^{-1}$ l^{-1} ($r = 0.987$, $f = 184.3$, $P < 0.001$, $n = 7$) and *y*-axis intercept 0.66 ± 0.08 day^{-1}. The interpretation of the observed relationship is not straightforward. The correlation of BChl *a* turnover times with chlorophyll might reflect the dependence of AAP growth rates on phytoplankton primary production. However, an analysis performed previously directly with primary production data did not yield such a correlation (Koblížek *et al.*, 2007). The second possibility is that both chlorophyll and BChl *a* turnover are controlled by the same common factor such as nutrient availability. The low phosphate concentrations (< 0.05 µM) and high N:P ratios (18–112) suggest that at least during the Modivus cruise the system was mostly limited by phosphorus availability. In any case, the observed linear relationship indicate that AAP growth rates were controlled primarily by bottom-up factors. Top-down factors (grazing, viral attack) are probably of secondary importance. In addition, we have found a strong linear correlation between calculated growth rates from the Mediterranean and the Sargasso Sea and the percentage of AAP bacteria in the respective environments (Fig. 6). This result indicates that in environments supporting faster AAP growth, AAP bacteria form higher percentage of total prokaryotes. The observed correlations of chlorophyll concentration with AAP growth rates and growth rates with AAP percentages are consistent with the correlation of AAP percentage and chlorophyll concentration.

It has been previously shown that AAP bacteria are on average larger cells when compared with heterotrophic bacteria (Sieracki *et al.*, 2006; Koblížek *et al.*, 2010). Larger cell sizes of AAPs were also indicated by the size fractionation conducted during Modivus cruise. The larger cell size may make AAPs more vulnerable targets for protistan grazing, which would result in higher loss among AAP cells. The higher grazing pressure would explain the apparent paradox, that, in spite of their rapid growth, AAPs constitute only a relatively small part of bacterioplankton community.

The observed rapid growth rates and larger cell sizes also mean that an individual AAP cell would require much more substrates (per unit of time) for its growth when compared with an average bacterium. Such an organism has to be highly metabolically active, processing large quantity of organic matter and other nutrients (in spite of the fact that even among various AAP groups may exist significant heterogeneity in their activity). Thus, in spite of their smaller numbers AAP bacteria might be responsible for a significant amount of bacterial production in the euphotic zone. This is consistent with our calculation of AAP production in the Catalan Sea (see Results), where we estimated that AAP bacteria might have represented one third to one half of the bacterial production determined by Leucine incorporation.

In conclusion, all the presented data suggest that AAP bacteria are not particularly good competitors under strong nutrient limiting conditions. In contrast to the original Kolber's hypothesis, they thrive better in higher trophic environments that provide sufficient substrates to maintain high growth rates. Aerobic anoxygenic phototrophs are metabolically active organisms contributing largely to bacterial production in the euphotic zone of the Mediterranean Sea.

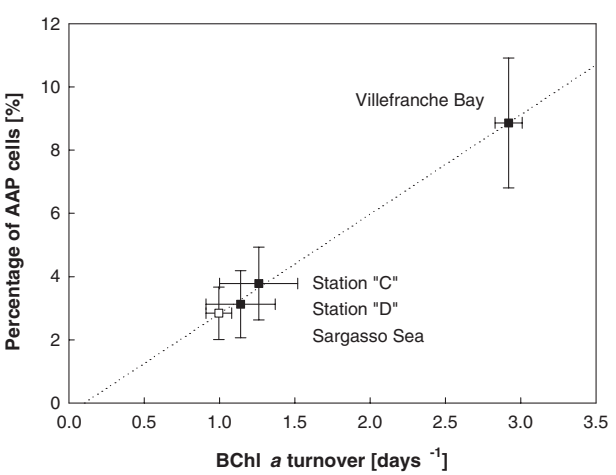

Fig. 6. Relationship between AAP percentage and BChl *a* turnover. The Sargasso Sea (empty symbols) and BChl *a* turnover in Villefranche Bay were taken from Koblížek and colleagues (2007). The obtained values give a linear relationship $y = 3.10x - 0.22$ ($r^2 = 0.998$, $P < 0.0001$, $n = 4$).

Experimental procedures

Sampling

Samples were collected (i) in September 2005 in the Bay of Villefranche, (ii) in June 2007 during the TransMed Leg 3 cruise (conducted in the framework of the Italian 'VECTOR' Program aboard R/V *Universitatis*), (iii) in September 2007 during the Spanish cruise Modivus aboard R/V *García del Cid*, and (iv) in May 2009 during cruise Famoso2 on board R/V *Sarmiento de Gamboa* (Table 1). During the cruises samples were collected from Niskin bottles, and occasionally (indicated) from the ship's underway system. In the Bay of Villefranche, samples were collected from a small boat manually from 0.5 m depth. Samples for IR epifluorescence microscopy were fixed with 2% formaldehyde and stored in the fridge (+4°C).

Fluorometry

BChl *a* concentration was estimated from its IR fluorescence. The signals were recorded by an IR kinetic fluorometer with modified flashing units, which were populated with six blue-green Luxeon Rebel diodes (505 nm) as described previously (Koblížek *et al*., 2007). To separate the chlorophyll and BChl *a* signal we used the herbicide DCMU (10^{-5} M) as described earlier (Koblížek *et al*., 2005). The instrument was calibrated using a diluted culture of *Roseobacter* strain COL2P (Koblížek *et al*., 2010), where the BChl *a* content was determined spectroscopically in acetone : methanol 7:2 (v/v) pigment extracts. The BChl *a* signal size fractionation was performed using 0.8 and 2 μm Nucleopore filters as described earlier (Lami *et al*., 2009).

At two stations in the Western Mediteranean the decay of BChl *a* signal during the day-light period was followed. Samples were regularly taken every 2–4 h and changes of BChl *a* signal were followed for two or more consecutive days. The signal decay observed during the day-light hours was analysed by curve fitting assuming simple exponential kinetics, as described earlier (Koblížek *et al*., 2005).

Microscopy

The AAP bacterial counts were performed using previously described methods (Mašín *et al*., 2006). Briefly, bacterial cells were fixed using 2% formaldehyde and collected onto 0.2 μm polycarbonate filters, dried and stained with 4′,6-diamidino-2-phenylindole (DAPI) using a 3:1 mixture of Citifluor AF1 and Vectashield containing 1 μg ml^{-1} DAPI. First, total DAPI-stained bacteria were recorded in the blue part of the spectrum (100–200 ms exposure). Then, an IR emission (> 850 nm) image was captured, showing both AAPs and phytoplankton (15 s to 35 s exposition). Finally, chlorophyll *a* autofluorescence was recorded to identify chlorophyll-containing organisms (0.5 to 1 s exposure). The acquired images were saved and semi-manually analysed with the aid of AnalySiS software (Soft Imaging Systems) to distinguish the number of heterotrophic bacteria, picocyanobacteria and AAPs for each sample. For each individual sample, 8–12 frames were recorded and analysed (~ 400 to 600 DAPI cells).

Other analyses

In the TransMed cruise, two litres of water were filtered onto Nuclepore filters of 0.2 μm pore size and immediately stored in liquid nitrogen. Extracted pigments were analysed using the Hewlett Packard series 1100 HPLC (Hewlett-Packard, Wilmington, NC, USA) equipped with a 3 μm C_8 BDS column (ThermoHypersil, Runcorn, UK). The mobile phase was composed of two solvents mixture: A, methanol, aqueous ammonium acetate (70:30) and B, methanol (Dimier *et al*., 2009). Pigments were detected at 440 nm and quantified using purchased standards from the V.K.I. (Water Quality Institute, Horsholm, Denmark). In the Modivus and Famoso2 cruises, 250 ml samples were filtered through Whatman GF/F filters. The filters were homogenized in 90% acetone and extracted for 24 h in the dark at 4°C. Fluorescence of the extracts was measured in a Turner Designs fluorometer.

Primary production was measured in acid-cleaned polycarbonate bottles filled with 160 ml water samples and spiked with approximately 3.7×10^5 Bq of ^{14}C-bicarbonate (VKI, Denmark). The samples were placed at the sun rise in an on-deck incubator simulating the light conditions at 5 m depth and cooled with running surface water. After dusk, the samples were collected onto Whatman GF/F filters (25 mm diameter) and fumed overnight with HCl 35%. Finally, incorporated radioactivity was determined in a Beckman LS6500 liquid scintillation counter using ReadySafe liquid scintillation cocktail. Dark bottle activities were subtracted, no isotope discrimination factor was used. The conversion to carbon units was done assuming the inorganic C concentration of 25 g C m^{-3}.

Bacterial production was measured using the leucine incorporation method. The three replicates and a trichloroacetic acid killed control were incubated in the dark with ^{3}H-leucine (40 nM final conc.) for 2 h at *in situ* temperature. Activity was converted into bacterial production using the theoretical conversion factor of 1.55 kg C mol^{-1} Leu, which corresponds to the average empirical conversion factors measured throughout a year in the Blanes Bay Microbial Observatory (Alonso-Sáez *et al*., 2010).

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References

Alonso-Sáez, L., Pinhassi, J., Pernthaler, J., and Gasol, J.M. (2010) Leucine-to-carbon empirical conversion factor experiments: does bacterial community structure have an influence? *Environ Microbiol* **12:** 2988–2997.

Béjà, O., Aravind, L., Koonin, E.V., Suzuki, M.T., Hadd, A., Nguyen, C., *et al*. (2000) Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science* **289:** 1902–1906.

Campbell, B., Waidner, L.A., Cottrell, M.T., and Kirchman, D.L. (2008) Abundant proteorhodopsin genes in the North Atlantic Ocean. *Environ Microbiol* **10:** 99–109.

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

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

Dimier, C., Brunet, C., Geider, R., and Raven, J. (2009) Growth and photoregulation dynamics of the picoeukaryote *Pelagomonas calceolata* in fluctuating light. *Limnol Oceanogr* **54:** 823–836.

Gasol, J.M., Pinhassi, J., Alonso-Sáez, L., Ducklow, H., Herndl, G.J., Koblížek, M., *et al*. (2008) Towards a better understanding of the microbial carbon flux in the sea. *Aquat Microbial Ecol* **53:** 21–38.

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

Koblížek, M. (2011) Role of photoheterotrophic bacteria in the marine carbon cycle. In *Microbial Carbon Pump in the Ocean*. Jiao, N., Azam, F., and Sanders, S. (eds). Washington, DC, USA: Science/AAAS, pp. 49–51.

Koblížek, M., Béjà, O., Bidigare, R.R., Christensen, S., Benetiz-Nelson, B., Vetriani, C., *et al*. (2003) Isolation and characterization of *Erythrobacter* sp. strains from the upper ocean. *Arch Microbiol* **180:** 327–338.

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

Koblížek, M., Mašín, M., Ras, J., Poulton, A.J., and Prášil, O. (2007) Rapid growth rates of aerobic anoxygenic prototrophs in the ocean. *Envir Microbiol* **9:** 2401–2406.

Koblížek, M., Mlčoušková, J., Kolber, Z., and Kopecký, J. (2010) On the photosynthetic properties of marine bacterium COL2P belonging to *Roseobacter* clade. *Arch Microbiol* **192:** 41–49.

Kolber, Z.S., Van Dover, C.L., Niederman, R.A., and Falkowski, P.G. (2000) Bacterial photosynthesis in surface waters of the open ocean. *Nature* **407:** 177–179.

Kolber, Z.S., Plumley, F.G., Lang, A.S., Beatty, J.T., Blankenship, R.E., Van Dover, C.L., *et al*. (2001) Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. *Science* **292:** 2492–2495.

Krom, M.D., Kress, N., Brenner, S., and Gordon, L.I. (1991) Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. *Limnol Oceanogr* **36:** 424–432.

Lami, R., Čuperová, Z., Ras, J., Lebaron, P., and Koblížek, M. (2009) Distribution of free-living versus particle-attached aerobic anoxygenic phototrophic bacteria in marine environments. *Aquat Microbial Ecol* **55:** 31–38.

Lehours, A.-C., Cottrell, M.T., Dahan, O., Kirchman, D.L., and Jeanthon, C. (2010) Summer distribution and diversity of aerobic anoxygenic phototrophic bacteria in the Mediterranean Sea in relation to environmental variables. *FEMS Microbiol Ecol* **74:** 397–409.

Mašín, M., Zdun, A., Stoń-Egiert, J., Nausch, M., Labrenz, M., Moulisová, V., and Koblížek, M. (2006) Seasonal changes and diversity of aerobic anoxygenic phototrophs in the Baltic Sea. *Aquat Microbial Ecol* **45:** 247–254.

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

Salka, I., Moulisova, V., Koblížek, M., Jost, G., Jürgens, K., and Labrenz, M. (2008) Abundance, depth distribution, and composition of aerobic bacteriochlorophyll *a*-producing bacteria in four deeps of the central Baltic Sea. *Appl Environ Microbiol* **74:** 4398–4404.

Schwalbach, M.S., and Fuhrman, J.A. (2005) Wide-ranging abundances of aerobic anoxygenic phototrophic bacteria in the world ocean revealed by epifluorescence microscopy and quantitative PCR. *Limnol Oceanogr* **50:** 620–628.

Shioi, Y. (1988) Growth characteristics and substrate specificity of aerobic photosynthetic bacterium, *Erythrobacter* sp. (Och 114). *Plant Cell Physiol* **27:** 567–572.

Sieracki, M.E., Gilg, I.C., Their, E.C., Poulton, N.J., and Goericke, R. (2006) Distribution of planktonic aerobic anoxygenic photoheterotrophic bacteria in the northwest Atlantic. *Limnol Oceanogr* **51:** 38–46.

Thingstad, T.F., Zweifel, U.L., and Rassoulzadegan, F. (1998) P limitation of heterotrophic bacteria and phytoplankton in the northwest Mediterranean. *Limnol Oceanogr* **43:** 88–94.

Turley, C.M. (1999) The changing Mediterranean sea – a sensitive ecosystem? *Prog Oceanogr* **44:** 387–400.

Yurkov, V.V., and Csotonyi, J.T. (2009) New light on aerobic anoxygenic phototrophs. In *The Purple Phototrophic Bacteria, Advances in Photosynthesis and Respiration*, Vol. 28. Hunter, C.N., Daldal, F., Thurnauer, M.C., and Beatty, J.T. (eds). Dordrecht, The Netherlands: Springer Verlag, pp. 31–55.

Yutin, N., Suzuki, M.T., Teeling, H., Weber, M., Venter, J.C., Rusch, D.B., and Béjà, O. (2007) 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.

Zhang, Y., and Jiao, N. (2007) Dynamics of aerobic anoxygenic phototrophic bacteria in the East China Sea. *FEMS Microbiol Ecol* **61:** 459–469.