Seasonal dynamics of aerobic anoxygenic phototrophs in a Mediterranean coastal lagoon

D Lamy, P de Carvalho-Maalouf, M. T. Cottrell, Raphaël Lami, P. Catala, L. Oriol, J. Caparros, J. Ras, D. L. Kirchman, P. Lebaron

Vol. 62: 153–163, 2011
doi: 10.3354/ame01467

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online January 19

Seasonal dynamics of aerobic anoxygenic phototrophs in a Mediterranean coastal lagoon

D. Lamy[1,2,5,*], P. De Carvalho-Maalouf[1,2,6], M. T. Cottrell[3], R. Lami[1,2], P. Catala[1,2], L. Oriol[1,2], J. Caparros[1,2], J. Ras[4], D. L. Kirchman[3], 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]School of Marine Science and Policy, University of Delaware, Lewes, Delaware 19958, USA
[4]Laboratoire d'Océanographie de Villefranche-sur-mer, UMR 7093 CNRS et Université Pierre et Marie Curie, Villefranche-sur-mer Cedex, France
[5]*Present address:* University of Vienna, Department of Marine Biology, Althanstrasse 14, 1090 Vienna, Austria
[6]*Present address:* Centre d'Ingénierie des Protéines, Institut de Chimie, B6, Université de Liège, 4000 Liège, Belgium

ABSTRACT: Aerobic anoxygenic phototrophic (AAnP) bacteria are bacteriochlorophyll *a* (BChl *a*)-containing prokaryotes that can use both dissolved organic matter and light as energy sources. AAnP bacteria are widely distributed in aquatic environments where they are expected to play an important role in carbon cycling. However, little is known about their spatio-temporal distribution in marine ecosystems. In this study we examined the dynamics of AAnP bacteria in a coastal saline lagoon from November 2007 to September 2008. AAnP cells were enumerated by infrared (IR) microscopy, and BChl *a* concentrations were measured by both IR kinetic fluorometry and high-performance liquid chromatography (HPLC). The distribution of AAnP bacteria varied seasonally, but no clear spatial pattern emerged. The abundance of these bacteria ranged from 1.0 to 13.5×10^4 cells ml^{-1} from winter to summer, comprising 0.1 to 3% of total bacterial abundance. Size fractionation of the BChl *a* fluorescence signal showed that AAnP bacteria were mainly particle-attached in winter and free-living in spring and summer. BChl *a* concentrations (up to 108.7 ng l^{-1}), BChl *a* content per cell (up to 1.7 fg $cell^{-1}$) and the ratios of BChl *a* to chlorophyll *a* (chl *a*) (up to 15%) were high in spring and summer, suggesting that AAnP bacteria contributed significantly at this time to photosynthetically driven energy production in the lagoon. Temperature and light were the main factors driving seasonal variations in the abundance of AAnP bacteria, while total bacterial abundance was closely related to variations in the concentration of dissolved organic carbon. These results highlight for the first time the numerical importance and the dynamics of AAnP bacteria in a coastal lagoon.

KEY WORDS: Aerobic anoxygenic phototrophic bacteria · AAnP · Photoheterotrophy · Bacteriochlorophyll *a* · Seasonal dynamics · Coastal lagoon

INTRODUCTION

Aerobic anoxygenic phototrophic (AAnP) bacteria are photoheterotrophic prokaryotes able to combine light and dissolved organic matter (DOC) as energy sources (Yurkov & Beatty 1998, Beatty 2002, Suzuki & Béjà 2007). Their primary light-harvesting pigment is bacteriochlorophyll *a* (BChl *a*) (Kolber et al. 2001, Koblížek et al. 2005), but, unlike anaerobic anoxygenic phototrophic (AnAnP) bacteria, they depend on oxygen and organic carbon for energy. These bacteria are widely distributed in marine environments and their abundance varies greatly, accounting for 0 to 25% of the bacterial community (Schwalbach & Fuhrman 2005,

*Email: lamyd2@univie.ac.at

Lami et al. 2007, Salka et al. 2008, Cottrell & Kirchman 2009, Jiang et al. 2009). 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, Moran & Miller 2007) and suggest that AAnP bacteria play a significant role in aquatic food webs and biogeochemical cycles (Kolber et al. 2001, Koblížek et al. 2007).

Relatively little is known about the seasonality of AAnP bacteria in aquatic environments. Seasonal variations in the abundance of AAnP bacteria were observed in the East China Sea (Zhang & Jiao 2007), off the coast of Southern California (Schwalbach & Fuhrman 2005), in the Arctic Ocean (Cottrell & Kirchman 2009) and in the Delaware River estuary (Waidner & Kirchman 2007). However, few of these studies linked variation in the abundance of AAnP bacteria to variation in environmental variables. An initial hypothesis regarding the distribution of AAnP bacteria was that photoheterotrophy could be beneficial in nutrient-poor environments, such as oligotrophic zones (Kolber et al. 2000, Karl 2002), where these organisms would thrive. In contrast, various reports showed that these organisms are abundant in eutrophic as well as oligotrophic environments (Koblížek et al. 2006, Yutin et al. 2007, Zeng et al. 2009, Zubkov 2009). Other authors suggested that nutrient concentrations (Mašín et al. 2008), attachment to particles (Waidner & Kirchman 2007, Cottrell et al. 2010) or light intensity (Shiba et al. 1991, Koblížek et al. 2003) may affect the abundance of AAnP bacteria.

Coastal lagoons are appropriate environments in which to study the ecology and dynamics of AAnP bacteria. A study conducted in the Berre lagoon (Mediterranean Sea), and focused on sulphide-oxidizing AnAnP bacteria, showed a high abundance of AAnP bacteria (Ranchou-Peyruse et al. 2006). Lami et al. (2009) found relatively high concentrations of BChl *a* (22 ng l^{-1}) in the La Palme lagoon (Mediterranean Sea) in spring 2007. Coastal lagoons are interfacial areas between terrestrial and oceanic environments which retain, transport, and recycle large amounts of particulate and dissolved matter (Nixon 1995), and they are characterized by strong spatial and temporal gradients of environmental conditions. The La Palme lagoon, located on the French Mediterranean coast 60 km north of the French–Spanish border, is one of the coastal lagoons in this area that has been best preserved from anthropogenic impacts (Carlier et al. 2007). This lagoon is oxic, with oxygen concentrations between 2.1 and 16.2 mg l^{-1} from winter to summer (Wilke & Boutiere 2000), which prevents the dominance of anaerobic microorganisms such as AnAnP bacteria. Overall, the La Palme lagoon could serve as an appropriate ecosystem to follow the spatio-temporal dynamics of AAnP bacteria and to examine the environmental factors which could influence the dynamics.

The objective of this study was to determine the abundance and the dynamics of AAnP bacteria over the year and to evaluate the influence of environmental factors on the dynamics. To this end, the abundance and distribution of AAnP bacteria were monitored using infrared (IR) kinetic fluorometry, high-performance liquid chromatography (HPLC) and IR epifluorescence microscopy. Several environmental variables were also measured, including temperature, salinity, concentrations of chlorophyll *a* (chl *a*), nutrients, DOC and particulate organic carbon (POC). The rationale of the sampling was to examine the distribution of AAnP bacteria over (1) a short-term period encompassing 3 seasons and (2) a short spatial gradient of trophic conditions — from the internal part of the La Palme lagoon to the connection with the Mediterranean Sea — hypothesized to influence AAnP bacteria. This study found strong seasonal variations in AAnP bacteria over the 10 mo study period. The high total and per-cell concentrations of BChl *a*, and the high ratios of BChl *a*/chl *a* observed in spring and summer suggest that AAnP bacteria may greatly impact the carbon cycle and energy fluxes in Mediterranean coastal lagoons during this period of time.

MATERIALS AND METHODS

Sampling. Subsurface (0.3 m depth) sampling was conducted monthly from November 2007 to September 2008 at 5 stations distributed from the north end of the lagoon (Stn 1; Fig. 1) to the entrance channel (Stn 5). Samples were collected at the same time each day (10:00 to 12:00 h) to avoid within-day variations in pigment concentrations caused by diurnal variations and to allow between-month comparison. Samples were kept in the dark in an insulated container and processed at the laboratory within 2 to 4 h.

Environmental parameters. Temperature and salinity were measured using a WTW LF 196 thermosalinometer. The photosynthetically active radiation (PAR) was measured by the meteorological station SERVOB-2 of the Service d'Observation from the Observatoire de Banyuls-sur-mer using a datalogger LICOR LI-1400 equipped with a PAR sensor LI-190 (P. Conan unpubl. data). A Skalar Autoanalyzer (Skalar Analytical) was used according to Wood et al. (1967) and Bendschneider & Robinson (1952) to determine nitrate (NO_3^-) and nitrite (NO_2^-) concentrations, and used according to Murphy & Riley (1962) to determine phosphate (PO_4^{3-}) concentrations. Protocols were adapted to the Skalar Autoanalyser from Tréguer & Le

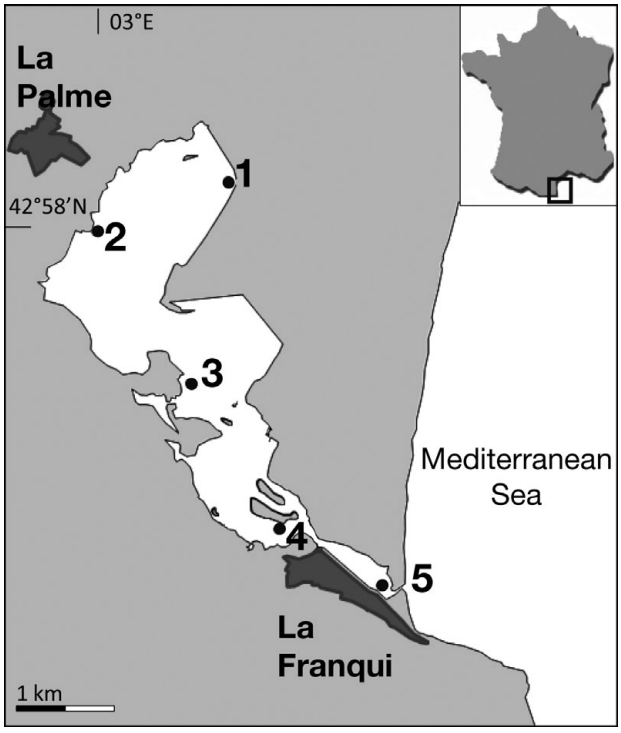

Fig. 1. Map of the 5 locations sampled in the La Palme lagoon, France, from November 2007 to September 2008

Corre (1975). DOC measurements were performed on a model TOC-V analyzer (Shimadzu) following Benner & Strom (1993). POC was quantified using a model CHN 2400 analyzer (Perkin Elmer) following Pregl (1924) and Sharp (1974).

Abundance of prokaryotes and bacterial production. Total heterotrophic prokaryotes (including AAnP bacteria) and autotrophic prokaryotes (*Synechococcus* and *Prochlorococcus*) were measured by flow cytometry using a FACSCalibur flow cytometer (Becton Dickinson) according to Marie et al. (2002). Subsamples, each 3 ml, were fixed with 2% formaldehyde (final conc.). For analysis of heterotrophic bacteria, samples were stained with the nucleic acid dye SYBR Green-I (Molecular Probes) at 0.025% (vol/vol) final concentration (Obernosterer et al. 2008). Stained prokaryotic cells were excited at 488 nm and enumerated according to their side scatter (SSC, related to cell size) and green fluorescence at 530 ± 15 nm. Based on a plot of green versus red fluorescence, photosynthetic prokaryotes were distinguished from non-photosynthetic prokaryotes. *Synechococcus*-like cells were discriminated by their strong orange fluorescence (585 ± 21 nm) and picoeukaryotic algae were discriminated by their scatter signals of the red fluorescence (>670 nm).

Bacterial production was estimated from the rates of incorporation of ^{3}H-leucine according to the microcentrifuge method (Smith & Azam 1992). Water samples were inoculated with 5 nM ^{3}H-leucine (specific activity 115 Ci mmol^{-1}; Amersham) and 25 nM of unlabelled leucine and were incubated for 2 h in the dark and at *in situ* temperature. Incubations were terminated by adding trichloracetic acid (TCA, 5% final conc.) and samples were stored at least 2 h at 4°C prior to centrifugation. The precipitates were rinsed once with 5% TCA and once with 70% ethanol. The precipitates were finally resuspended in 1 ml of FilterCount liquid scintillation cocktail (Perkin Elmer) and radioactivity was determined by an LS 6500 scintillation counter (Beckman Coulter). Bacterial carbon production (in gC ml^{-1} h^{-1}) was calculated according to Kirchman et al. (1993).

Abundance of AAnP bacteria. Samples were preserved with 2% paraformaldehyde for 1 to 4 h at 4°C in the dark and filtered onto black polycarbonate filters of pore size 0.2 µm. The filters were quickly frozen in liquid 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} as final conc.) in 1× phosphate-buffered saline for 10 min. Stained samples were counted immediately. AAnP bacteria were counted using an Olympus Provis AX 70 microscope and image analysis software (ImagePro Plus) 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) with the following exposure times: DAPI, 160 ms; IR, 400 ms; chl *a*, 1500 ms; PE, 50 ms. The biovolumes of total bacteria and AAnP bacteria were compared using microscopy data.

BChl *a* fluorescence. BChl *a* fluorescence was measured with an IR kinetic fluorometer using the method described in Koblížek et al. (2005). Briefly, the instrument was assembled using a standard PSI fluorometer control unit (FL200/PS) and custom-made optics (Koblížek et al. 2005). The absolute detection limit was 2 ng BChl *a* l^{-1}. To discriminate between the contributions of chl *a* and BChl *a* in the IR fluorescence signal (>850 nm), phytoplankton were selectively inhibited by adding 10^{-5} M (final conc.) of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Diuron, PESTANAL), which specifically inhibits Photosystem II of oxygenic phototrophs but does not affect the bacterial reaction centres; under these conditions only the variable part of the kinetic IR signal originating from bacterial reaction centres was determined (Koblížek et al. 2005). This approach avoids potential interference from other fluorescing compounds (not exhibiting variable fluorescence) or from electrical drifts within the detection system (occuring at different time scales). Size fractionation was conducted following the method of Lami

et al. (2009). Briefly, for each sample, BChl *a* fluorescence signals from the <0.8 µm and <3 µm fractions 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 & Kirchman 2007).

Concentration of pigments. Depending on trophic conditions, water samples of 1 to 2.5 l were filtered onto 2 GF/F Whatman filters. The filters were frozen immediately in liquid nitrogen and then stored at –80°C until HPLC analysis, as previously described (Ras et al. 2008, Cottrell et al. 2010).

Statistical analyses. Spearman correlations were performed to identify the significant links between all the measured parameters, including the distance of the stations from the mouth of the lagoon. Because the bacterial variables were not normally distributed (Shapiro–Wilk test, $p > 0.05$), dependent and independent variables were log-transformed in the regression analyses to meet the assumption of normality. Simple regression analysis was used to determine the influence of the PAR and the duration of daylight on the BChl *a*-related parameters. The correlation and regression analyses were performed using the XLSTAT 7.5.3 package.

A redundancy analysis (RDA) was used to determine which environmental variables were the most significant to explain variation in the abundance of autotrophic (*Synechococcus*), photoheterotrophic (AAnP) and heterotrophic (total) bacteria. We assumed a linear response of *Synechococcus*, AAnP and total bacteria to environmental variations. When a unimodal response was assumed, the percentage of explained variation is lower (canonical analysis not shown). Generally, linear models are more suitable for analysis of ecological data collected over a small gradient (ter Braak & Prentice 1988). The null hypothesis that the abundance of cyanobacteria and bacteria is independent of the environmental parameters was tested using constrained ordination with a Monte Carlo permutation test (499 permutations). Temperature, salinity, concentrations of chl *a*, DOC and nutrients, the duration of daylight, and the distance of the sampled stations from the mouth of the lagoon were used as explanatory variables. The RDA analysis was performed using the CANOCO version 4.5 software (ter Braak 1989).

RESULTS

Environmental setting

The surface water temperature ranged from 5.0°C in November 2007 to 25.8°C in August 2008. Surface water salinity varied greatly over the seasons, ranging from 15.4 to 78.3. Highest values were due to intense evaporation combined with little rainfall (500 mm yr^{-1}), intense sunshine in summer and strong wind during some periods over the year (>16 m s^{-1}) (Wilke & Boutiere 2000). The water depth varied between 0.5 and 1.5 m during the period studied due to evaporation, with minima in summer and maxima in winter.

Chl *a* concentrations were below 2 µg l^{-1}during the year, except at Stns 4 and 5 in September 2008 where they reached 2.84 and 6.34 µg l^{-1}, respectively (Fig. 2A). There was no clear spatial gradient over the sampling area (Spearman correlation with the distance from the mouth, $p > 0.05$). Within the lagoon, chl *a* concentrations decreased through winter (from 1.99 to 0.05 µg l^{-1}) and increased from spring to summer (from 0.11 to 6.34 µg l^{-1}), with a slight decrease in July (values comprised between 0.12 and 0.28 µg l^{-1} at the 5 stations). DOC concentrations ranged from 1.7 to 13.9 mg l^{-1} (Fig. 2B) and increased 3- to 4-fold from winter to summer in the whole lagoon. In contrast, POC concentrations decreased 3- to 4.5-fold during the same period, with a slight increase in March (Fig. 2C). Values varied between 30.7 and 76.4 µg l^{-1} in winter and between 12.9 and 36.4 µg l^{-1} during spring and summer. No clear spatial gradient of DOC and POC concentrations was observed along the length of the lagoon (Spearman correlation, $p > 0.05$).

The concentrations of inorganic nutrients (NO_3^- + NO_2^- and PO_4^{3-}) were highest in winter, reaching more than 7 µM in March and February (Table 1), and then dropped dramatically over the course of the study period to concentrations below 0.50 µM in September. Nutrient concentrations at Stn 1 were usually 3- to 100-fold higher than at any other station, and a significantly decreasing gradient of the $NO_3^- + NO_2^-$ concentration was observed from the internal part to the entrance channel of the lagoon (Spearman correlation, $R^2 = 0.12$, $p = 0.03$).

Total prokaryotes and the abundance of AAnP bacteria

Counts of total bacteria varied between 0.98 and 20.28×10^6 cells ml^{-1}. Total counts were lower in winter and higher in spring and summer (Fig. 3A), except at Stn 1 where the total bacteria were more abundant in May and decreased afterwards. Total bacterial production ranged from 0.73 to 14.93 mgC ml^{-1} h^{-1} (data not shown). Total bacterial production and abundance were significantly correlated (Spearman correlation, $R^2 = 0.17$, $p = 0.01$) and showed the same spatial and temporal patterns.

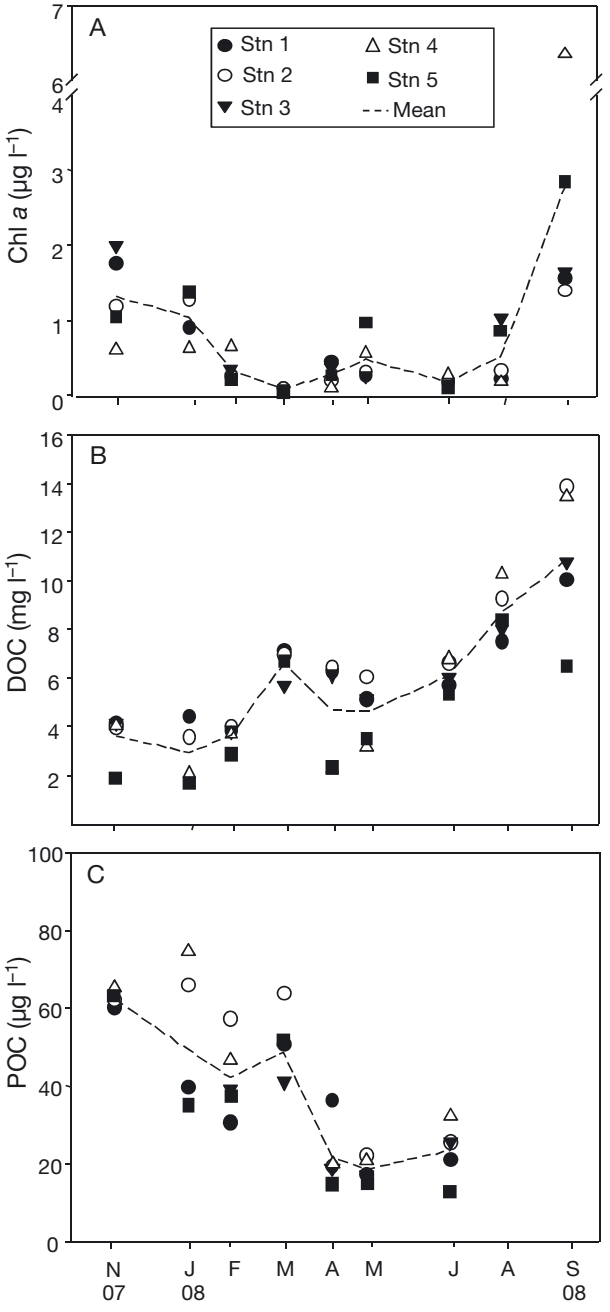

Fig. 2. Temporal evolution of (A) chl *a* , (B) dissolved organic carbon (DOC) and (C) particulate organic carbon (POC) concentrations at each sampling station (Stns 1 to 5) and the mean of the 5 stations at each sampling time from 29 November 2007 (N 07) to 24 September 2008 (S 08). Separation between consecutive months in julian days. Please note the discontinued *y*-axis in (A) due to high chl *a* concentrations at Stn 4 in September 2008

Counts of AAnP bacteria and total bacteria were significantly correlated (Spearman correlation, R^2 = 0.21, p = 0.006). Counts of AAnP bacteria ranged from 1.01 to 13.51 × 10^4 cells ml^{-1} during the study period (Fig. 3B). Although counts were sometimes high, AAnP bacteria comprised only 0.10 to 3% of the total bacterial counts, but they still outnumbered *Synechococcus* by 20- to 1000-fold. No *Prochlorococcus* was observed in the La Palme lagoon. The abundance of AAnP bacteria increased from winter to summer at Stns 2, 3 and 4. At Stns 1 and 5, the counts of AAnP bacteria were high (>4.0 × 10^4 cells ml^{-1}) in winter and peaked in spring, reaching values above 7 × 10^4 cells ml^{-1}. No clear spatial gradient of AAnP bacteria was observed (Spearman correlation with the distance from the mouth, p > 0.05). AAnP bacterial cell volumes ranged from 0.05 to 1.10 μm^3 while the biovolume of the total prokaryotic community ranged from 0.04 to 0.23 μm^3 (data not shown). AAnP bacteria were significantly larger (up to 8.0-fold and on average 2.3-fold) than total bacterial cells (paired *t*-test, p < 0.001, n = 28).

BChl *a* concentration

Concentrations were low in winter and varied between 3.2 and 28.5 ng l^{-1} from November 2007 to April 2008 (Fig. 4 A). They increased significantly in spring (Stn 1) and summer (Stns 2, 4 and 5), reaching values from 30 to more than 100 ng l^{-1}. At Stn 3, the BChl *a* concentrations varied little between 15.4 and 28.5 ng l^{-1} (Fig. 4A).

The BChl *a* content per cell (BChl *a* concentration divided by AAnP abundance) ranged from 0.05 to 1.70 fg $cell^{-1}$ (Fig. 4B). The BChl *a* content increased from winter to spring at Stns 2 to 5, reaching values above 1.40 fg $cell^{-1}$ in April and May. It then decreased from spring to summer at Stns 2, 3 and 5, while the highest value (1.70 fg $cell^{-1}$) was reached at Stn 4 in September 2008. At Stn 1, the BChl *a* content peaked in winter (1.14 fg $cell^{-1}$) and spring (0.87 fg $cell^{-1}$; Fig. 4B).

To compare the pigment concentration in AAnP bacteria and phytoplankton, the BChl *a*/chl *a* ratio was calculated for each sampling period and each station (Fig. 4C). The BChl *a*/chl *a* ratio increased from 0.67% in January to 15.23% in May 2008, showing a small decrease in April 2008 at all stations except Stn 2. The values then slightly decreased in summer and varied between 1.68 and 12.46%. On average, BChl *a* concentrations ranged from 0.49 ± 0.43% (mean ± SD) to 7.63 ± 4.66% of chl *a* concentrations.

BChl *a* fluorescence: size fractionation

Total BChl *a* fluorescence was significantly correlated with the abundance of AAnP bacteria (R^2 = 0.37,

Table 1. Environmental settings of the La Palme lagoon, France, from November 2007 to September 2008. The range of variation between the 5 sampled stations is given for temperature (Temp., °C), salinity, nitrate + nitrite concentration (NO_2^-+NO_3^-, µM) and phosphate concentration (PO_4^{3-}, µM). Lowest and highest values are in **bold** for these parameters. Daily mean (± SD) photosynthetically active radiation (PAR, W m^{-2}) and the duration of daylight (min) are given for each sampling date. Dates are mm/dd/yy. <DL: below the detection limit; nd: not determined

Sampling date	Temp. (°C)	Salinity (psu)	NO_2^-+NO_3^- (µM)	PO_4^{3-} (µM)	PAR (W m^{-2})	Duration of daylight (min)
11/29/07	**5.0**–5.5	46.0–51.0	0.12–5.49	**<DL**–0.23	nd	561
01/17/08	6.5–7.5	28.0–44.0	0.11–4.44	<DL–0.02	92.4 ± 66.2	566
02/14/08	8.6–10.3	**15.4**–41.3	0.16–4.06	0.53–**7.68**	nd	630
03/20/08	6.5–8.9	29.5–43.8	0.08–**7.91**	0.02–0.62	nd	730
04/21/08	11.0–12.5	36.6–45.0	0.09–4.23	0.06–0.14	144.2 ± 143.1	821
05/14/08	19.6–19.9	38.6–42.6	0.07–0.23	0.08–0.11	140.9 ± 112.9	877
07/08/08	17.3–20.5	40.3–50.5	0.06–2.45	0.08–0.13	197.6 ± 139.2	910
08/12/08	24.0–**25.8**	46.5–75.7	**0.02**–0.11	0.16–1.06	172.7 ± 112.6	842
09/24/08	16.0–17.8	54.4–**78.3**	0.22–0.30	0.25–0.45	nd	724

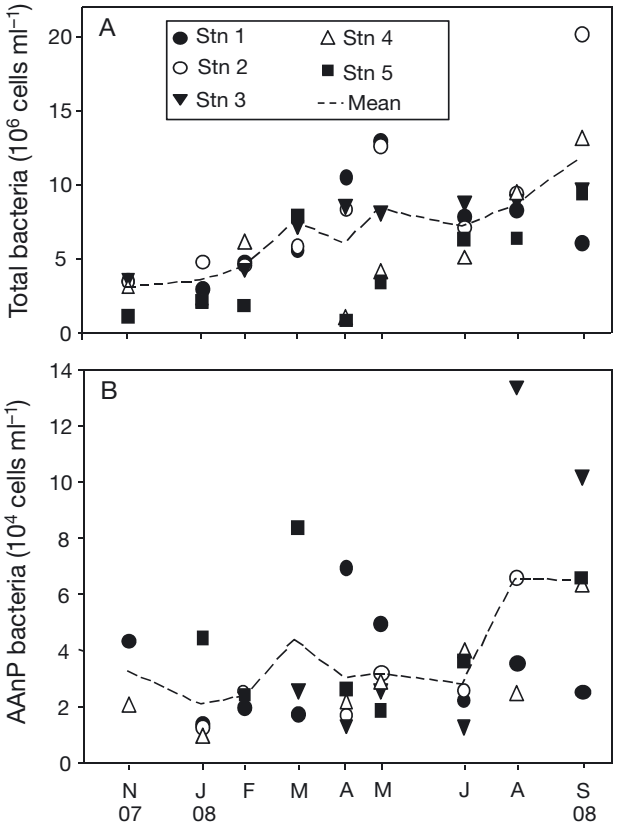

Fig. 3. Temporal evolution of (A) total bacteria and (B) AAnP bacteria at each sampling station (Stns 1 to 5) and the mean of the 5 stations at each sampling time from 29 November 2007 (N 07) to 24 September 2008 (S 08). Separation between consecutive months in julian days

$p < 0.0005$) and the concentration of BChl *a* ($R^2 = 0.23$, $p < 0.005$). Significant regressions were found between total BChl *a* fluorescence and the duration of daylight ($R^2 = 0.37$, $p < 0.0001$) and the mean PAR per day ($R^2 = 0.69$, $p < 0.0001$), meaning that a part of the BChl *a* fluorescence can be explained by light parameters.

The distribution of BChl *a* fluorescence in various size fractions was examined from February to September 2008 at all stations (Fig. 5). At each station, the >3 µm fraction (tracing particle-associated bacteria) comprised the largest fraction of the BChl *a* signal in winter, ranging from 28 to 92%. The <0.8 µm fraction (considered as free-living AAnP bacteria) was lowest in February 2008 (from 0 to 22%) and increased afterwards. In spring and summer, the <0.8 µm fraction comprised the main part of the BChl *a* signal, and varied between 25 and 99% (Fig. 5).

Redundancy analysis

The first 2 RDA axes explained 62.1% of the variability in the abundance of the bacterial and cyanobacterial groups and 99.3% of the relationship between abundance and the environmental variables (Fig. 6). The 2 first canonical axes were significant ($p = 0.002$). The AAnP bacteria appeared to be separated from the strict heterotrophic bacteria and the autotrophic *Synechococcus*. Fig. 6 shows a clear separation between the winter samples (circles, at the bottom) and the spring/summer samples (squares, at the top), indicating that seasonal variation was a significant explanatory variable. In contrast, the distance from the entrance of the lagoon was not a structuring factor. The chl *a* and DOC concentrations, and temperature and salinity, appeared to be the main explanatory factors in the variation in abundance of bacteria and cyanobacteria. This suggests that these environmental variables are those driving the greatest differences in the abundance of bacteria and cyanobacteria between the winter and spring/summer periods. It is noteworthy that

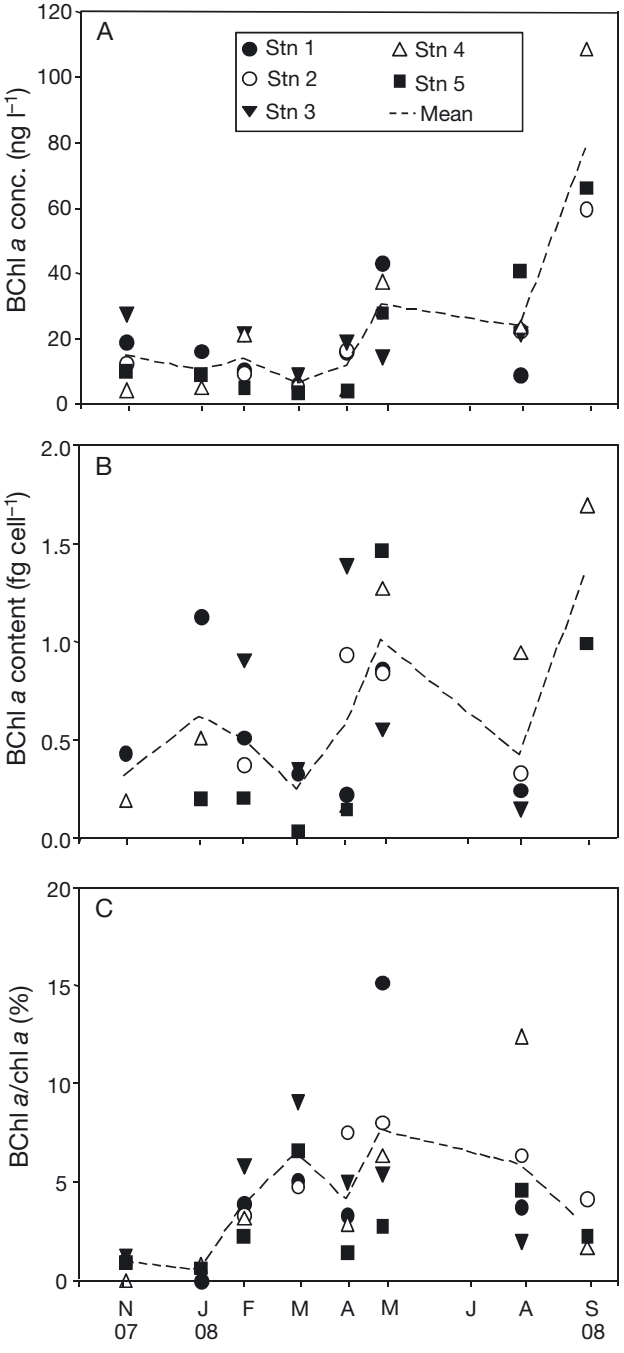

Fig. 4. Temporal evolution of (A) BChl *a* concentration (B) BChl *a* content per AAnP cell and (C) BChl *a*/chl *a* ratio at each sampling station (Stns 1 to 5) and the mean of the 5 stations at each sampling time from 29 November 2007 (N 07) to 24 September 2008 (S 08). Separations between consecutive months in julian days

the abundance of AAnP bacteria appeared to be closely linked to temperature and the duration of daylight, while there was a link between total bacteria and DOC concentrations (Fig. 6).

DISCUSSION

We investigated the distribution of AAnP bacteria by counting AAnP cells and determining both BChl *a* fluorescence and concentrations in a Mediterranean coastal lagoon. A few previous reports on an annual time scale highlighted a large range of variations in the abundance of AAnP bacteria (Schwalbach & Fuhrman 2005, Zhang & Jiao 2007); however the potential environmental factors controlling these temporal dynamics remain unclear. In our study, the simultaneous measurements of environmental parameters allowed us to examine which factors explain the temporal and spatial variation in the abundance of AAnP bacteria in a coastal lagoon. In addition, few studies have presented data on the dynamics of AAnP bacteria assessed using all 3 approaches, including IR fluorometry, epifluorescence microscopy and HPLC pigment analysis (Kolber et al. 2001, Lami et al. 2009). Furthermore, none of these studies included data on the spatial and temporal distribution of AAnP bacteria. The significant correlations between the abundance of AAnP bacteria, BChl *a* fluorescence values and concentration reported in this study demonstrate that the 3 methods yield highly complementary data on the distribution of AAnP bacteria.

Although AAnP bacteria made up only 0.1 to 3% of total bacterial abundance in the La Palme lagoon, their numbers were high, ranging from 1.0 to 13.5×10^4 cells ml^{-1}. These values were in the upper range of the abundance of AAnP bacteria observed in marine environments, comparable to the range reported by Cottrell et al. (2006) in the mid-Atlantic bight (0.69 to 15×10^4 cells ml^{-1}). The concentrations of BChl *a*, which ranged from 3.2 to 108.7 ng l^{-1}, were generally higher than the values reported in marine waters (e.g. Lami et al. 2007) and were comparable to those reported in freshwater systems in Central Europe (Mašín et al. 2008) and in the estuarine ecosystem of the Chesapeake Bay (Cottrell et al. 2010).

The microscopic counts of AAnP bacteria and the levels of BChl *a* reported in this study may include a few anaerobic anoxygenic phototrophic (AnAnP) cells which also contain BChl *a* (Imhoff 1995). These bacteria could originate from the anoxic sediments of the lagoon and may have been resuspended during periods of strong wind. Release of BChl *a* from sediments may be possible during winter when the BChl *a* signal was mainly in the >3 µm fraction. However, winter was the period when the BChl *a*-related parameters were the lowest. In addition, AnAnP bacteria are inhibited by oxygen and synthesize BChl *a* in anoxic environments; because this lagoon system is oxygen-rich from winter to summer (Wilke & Boutiere 2000), we effectively examined mostly AAnP bacteria in the present study.

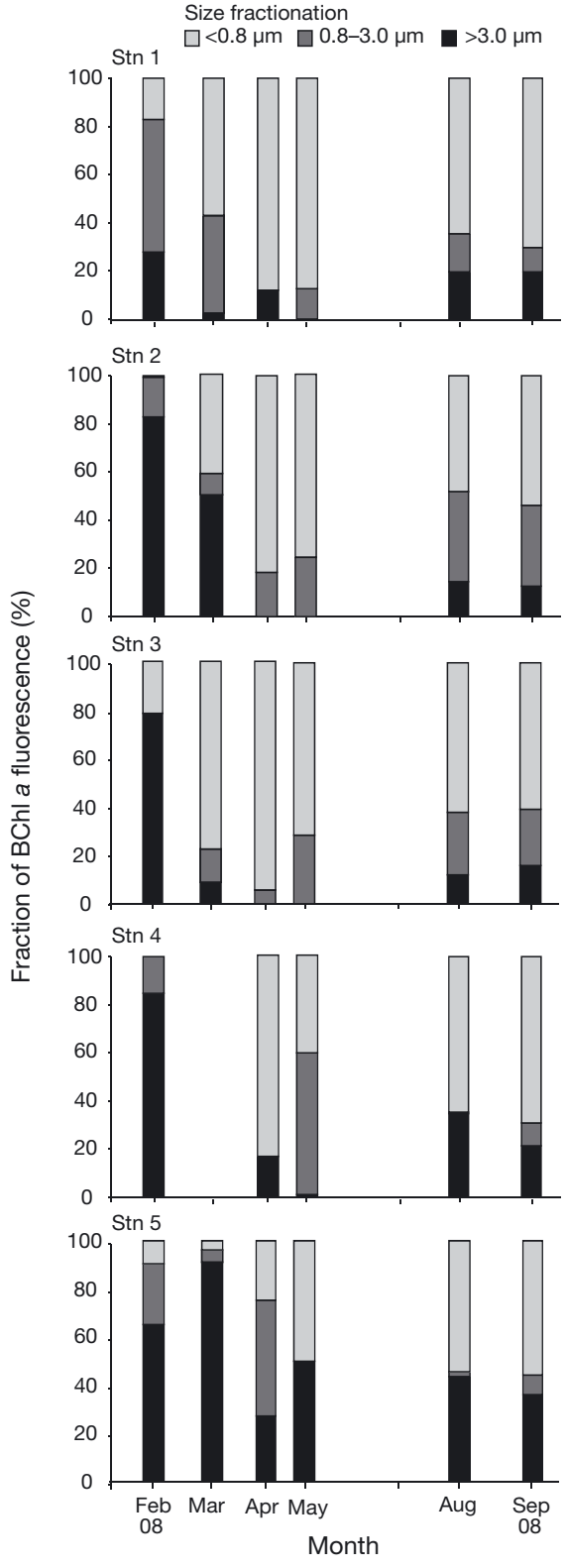

Fig. 5. Temporal evolution of size fractionation (<0.8 µm, 3.0–0.8 µm and >3 µm) of BChl *a* fluorescence at each sampling station (Stn 1 to Stn 5) from 14 February 2008 (Feb 08) to 24 September 2008 (Sep 08). Separation between consecutive months in julian days

The RDA analysis showed that temperature was the main factor explaining the abundance of AAnP bacteria, suggesting a strong seasonality in abundance, pigment concentration and pigment content per cell that was reflected by lower values in winter and higher values in summer. This is consistent with observations in the Gulf of Maine (Sieracki et al. 2006), in the Arctic Ocean (Cottrell & Kirchman 2009), in the southern Baltic Sea (Mašín et al. 2006) and in the Yangtze River estuary (Zhang & Jiao 2007). The production of the phototrophic apparatus of the AAnP bacteria can be affected by temperature (Yurkov & Beatty 1998) although cultured AAnP bacteria showed rather broad tolerance to variation in temperature (Rathgeber et al. 2004).

Among possible regulating factors, the availability of light could also explain our seasonal differences in the distribution of AAnP bacteria. The abundance of AAnP bacteria and the BChl *a* fluorescence were indeed closely linked to the duration of daylight and the mean daily PAR. The availability of light has been shown to enhance their growth (Shiba 1991) and to modulate the BChl *a* content strongly (Koblížek et al. 2003, Spring et al. 2009). Light attenuation in waters with high concentrations of particles, such as estuaries or lagoons, could lead to photoadaptation of AAnP bacteria, leading to particularly high pigment concentrations and content per cell (Cooney et al. 2006). However, the lowest BChl *a* concentrations and content per cell in the lagoon were observed when the POC concentrations were the highest and when the AAnP bacteria were mostly attached to particles. In contrast, the highest BChl *a* concentrations and content per cell were observed in spring and summer when the AAnP bacteria were mostly free-living. This suggests that AAnP bacteria do not show pigment regulation due to light attenuation by particles in the La Palme lagoon, as observed in Chesapeake Bay (Cottrell et al. 2010).

It has been suggested that attachment of AAnP bacteria to the particles in marine environments can be an important factor in explaining their distribution and ecology (Waidner & Kirchman 2007). As particles could be a source of labile organic substrates available for attached AAnP bacteria, we could speculate that the attachment of AAnP cells to particles in winter might lead to low concentrations of BChl *a* and content per cell, implying less phototrophy. It has been shown that light harvesting provides energy for AAnP bacteria in the absence of labile substrates (Koblížek et al. 2003). In contrast, our spring–summer results show that, even if dissolved organic substrates (DOC) were available, AAnP bacteria generated larger amounts of BChl *a* and exhibited higher pigment content per cell, indicating a higher reliance on phototrophy in this period of time. AAnP bacteria were found to be less versatile in utilising diverse

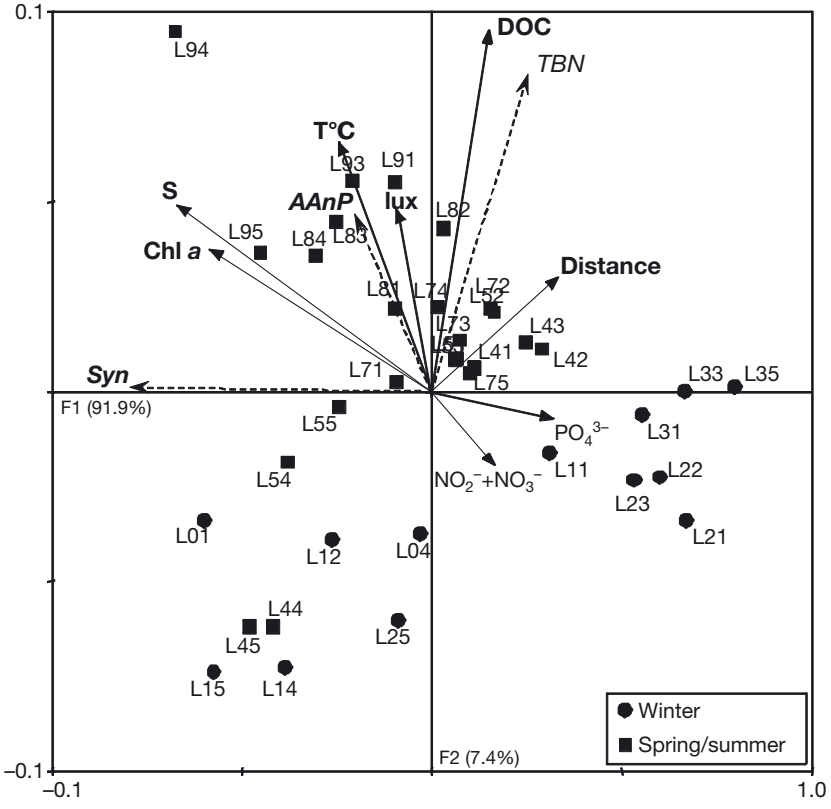

Fig. 6. Redundancy analysis using the abundances of AAnP bacteria (AAnP), total bacteria (TBN) and *Synechococcus* (*Syn*) as independent variables (dashed arrows) and salinity (S), temperature (T°C), chlorophyll *a* (chl *a*) concentrations, duration of daylight (lux), distance of the stations from the mouth of the lagoon (Distance), nitrite + nitrate concentrations (NO_2^- + NO_3^-) and phosphate concentrations (PO_4^{3-}) as explanatory variables (black arrows). The samples are identified as winter samples (black circles) or spring/summer samples (black squares). In the name of the samples, L stands for La Palme, the first number corresponds to the sampling month (0 for November 2007, 1 for January 2008 to 9 for September 2008) and the second number corresponds to the sampled station (Stns 1 to 5)

organic matter compared with most other bacterial groups (Jiao et al. 2007). We could thus hypothesise that the light-derived energy might help AAnP cells to use the available DOC. However, this capacity to rely on phototrophy in spring and summer did not allow them successfully to outcompete the strict heterotrophs. Their capacity to generate energy from light can double their organic carbon assimilatory efficiency (Yurkov & Csotonyi 2009) and/or reduce the metabolic requirements for organic carbon resources (Cho et al. 2007). Alternatively, the light-generated energy could serve for other metabolic processes, such as enhancing the acquisition of nitrogen (N) or phosphorus (P), as has previously been hypothesised (Mašín et al. 2008), particularly in an N- and P-depleted environment like the La Palme lagoon during spring and summer.

The ratio of BChl *a* to chl *a* in the lagoon ranged from 0.7 to 15.2%, similar to the ratios of 0.8 to 10% reported by Kolber et al. (2001) in the subtropical Pacific. Assuming that photosystems of anoxygenic phototrophic bacteria are as efficient as photosystems of oxygenic prokaryotes or eukaryotes (Kolber et al. 2001, Goericke 2002), these data suggest that AAnP bacteria might significantly contribute to photosynthetically driven energy production in the lagoon. The BChl *a* content per cell (>1.0 fg BChl *a* $cell^{-1}$) reached concentrations observed in the oxygenic phototroph *Prochlorococcus* (up to 2 fg $cell^{-1}$) (Gibb et al. 2001). Such a high content of pigment further supports our suggestion that BChl *a*-based phototrophy was probably a significant part of the metabolism of AAnP bacteria in spring and summer. Jiao et al. (2010) suggest that the BChl *a*-based phototrophy has an important role supplemental to chl *a*-based photosynthesis in the carbon cycle and would be critical for a region to be a sink or a source of atmospheric CO_2 (Jiao et al. 2010). Collectively, these results suggest that AAnP bacteria may widely impact the carbon cycling in coastal lagoon systems on a yearly time scale.

Our results showed seasonal changes in AAnP bacteria in a coastal lagoon and represent the first spatial and temporal survey of these bacteria in such an environment. The simultaneous measurements of BChl *a* fluorescence, concentration and abundance of AAnP cells, along with some environmental parameters, allowed us to examine potential controlling factors of the dynamics of AAnP bacteria over the year. The BChl *a* concentrations and the BChl *a*/chl *a* ratios were among the highest reported in marine and freshwater environments, suggesting that AAnP bacteria contribute to photosynthetically driven energy production in the La Palme lagoon. AAnP cells showed a particle-associated lifestyle in winter, which may have lead to the observed low phototrophy — given that particles could be a source of labile substrates. In contrast, the AAnP bacteria were mainly free-living in summer and showed a higher use of light. It was hypothesised that the light-generated energy during this time was used either to improve the use of available DOC in the lagoon or to enhance the acquisition of N or P in an N- and P-poor environment. A shift in community structure could be another factor that could explain the seasonal differences in the abundance of AAnP bacteria and BChl *a* content per cell.

Further studies should include phylogenetic investigations to identify the AAnP bacteria present in the lagoon over space and time scales and within each cell-size fraction.

Acknowledgements. We thank P. Conan for PAR measurements and A. Carlier for providing the map of the La Palme lagoon. This work was supported by the EC2CO program 'Dynamique et diversité des bactéries phototrophes anoxygéniques aérobies marines en milieu côtier' and partly by the Pierre Fabre Laboratories. D.L.K., M.T.C. and R.L. were supported by NSF grant MCB-0453993. We are grateful to Prof. M. T. Suzuki for useful criticism and comments which improved the manuscript.

LITERATURE CITED

Beatty JT (2002) On the natural selection and evolution of the aerobic phototrophic bacteria. Photosynth Res 73:109–114

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

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

Carlier A, Riera P, Amouroux JM, Bodiou JY and others (2007) A seasonal survey of the food web in the Lapalme Lagoon (northwestern Mediterranean) assessed by carbon and nitrogen stable isotope analysis. Estuar Coast Shelf Sci 73:299–313

Cho JC, Stapels MD, Morris RM, Vergin KL and others (2007) Polyphyletic photosynthetic reaction centre genes in oligotrophic marine Gammaproteobacteria. Environ Microbiol 9:1456–1463

Cooney MJ, Johnston WA, Pohl S, Bidigare RR (2006) Influence of photoperiod on pigmentation and metabolic efficiency of the marine aerobic anoxygenic photosynthetic bacterium *Erythrobacter longus* strain NJ3Y. Aquat Microb Ecol 43:303–309

Cottrell MT, Kirchman DL (2009) Photoheterotrophic microbes in the Arctic Ocean in summer and winter. Appl Environ Microbiol 75:4958–4966

Cottrell MT, Mannino A, Kirchman DL (2006) Aerobic anoxygenic phototrophic bacteria in the Mid-Atlantic Bight and the North Pacific Gyre. Appl Environ Microbiol 72: 557–564

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

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

Gibb SW, Cummings DG, Irigoien X, Barlow RG, Fauzi R, Mantoura C (2001) Phytoplankton pigment chemotaxonomy of the northeastern Atlantic. Deep-Sea Res II 48: 795–823

Goericke R (2002) Bacteriochlorophyll *a* in the ocean: Is anoxygenic bacterial photosynthesis important? Limnol Oceanogr 47:290–295

Imhoff JF (1995) Taxonomy and physiology of phototrophic purple bacteria and green sulfur bacteria. In: Blakenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer Academic, Dordrecht, p 1–15

Jiang HC, Dong HL, Yu BS, Lv G and others (2009) Abundance and diversity of aerobic anoxygenic phototrophic bacteria in saline lakes on the Tibetan plateau. FEMS Microbiol Ecol 67:268–278

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

Jiao N, Zhang F, Hong N (2010) Significant roles of bacteriochlorophyll *a* supplemental to chlorophyll *a* in the ocean. ISME J 4:595–597

Karl DM (2002) Microbiological oceanography: hidden in a sea of microbes. Nature 415:590–591

Kirchman D, Keil R, Simon M, Welschmeyers N (1993) Biomass and production of heterotrophic bacterioplankton in the oceanic subarctic Pacific. Deep-Sea Res I 40: 967–988

Koblížek M, Béjà O, Bidigare RR, Christensen S and others (2003) Isolation and characterization of *Erythrobacter* sp. strains from the upper ocean. Arch Microbiol 180:327–338

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

Koblížek M, Falkowski PG, Kolber ZS (2006) Diversity and distribution of photosynthetic bacteria in the Black Sea. Deep-Sea Res II 53:1934–1944

Koblížek M, Mašín M, Ras J, Poulton AJ, Prášil O (2007) Rapid growth rates of aerobic anoxygenic phototrophs in the ocean. Environ Microbiol 9:2401–2406

Kolber ZS, Van Dover CL, Niederman RA, Falkowski PG (2000) Bacterial photosynthesis in surface waters of the open ocean. Nature 407:177–179

Kolber ZS, Plumley FG, Lang AS, Beatty JT and others (2001) Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. Science 292:2492–2495

Lami R, Cottrell MT, Ras J, Ulloa O and others (2007) High abundances of aerobic anoxygenic photosynthetic bacteria in the South Pacific Ocean. Appl Environ Microbiol 73:4198–4205

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

Marie D, Simon N, Guillou L, Partensky F, Vaulot D (2002) Flow cytometric analysis of marine picoplankton. In: Diamond RA, De Maggio S (eds) Protocols in flow cytometry and cell sorting. Springer-Verlag, Berlin, p 421–454

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

Mašín M, Nedoma J, Pechar L, Koblížek M (2008) Distribution of aerobic anoxygenic phototrophs in temperate freshwater systems. Environ Microbiol 10:1988–1996

Moran MA, Miller WL (2007) Resourceful heterotrophs make the most of light in the coastal ocean. Nat Rev Microbiol 5:792–800

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

Nixon SW (1995) Coastal marine eutrophication: definition, social causes and future concerns. Ophelia 41:199–219

Obernosterer I, Catala P, Lami R, Caparros J and others (2008) Biochemical characteristics and bacterial community structure of the sea surface microlayer in the South Pacific Ocean. Biogeosciences 5:693–705

Pregl F (1924) Quantitative organic microanalysis. Translated from the 2nd German edn by E. Fyleman. J & A Churchill, London

Ranchou-Peyruse A, Herbert R, Caumette P, Guyoneaud R (2006) Comparison of cultivation-dependent and molecular methods for studying the diversity of anoxygenic purple phototrophs in sediments of an eutrophic brackish lagoon. Environ Microbiol 8:1590–1599

Ras J, Claustre H, Uitz J (2008) Spatial variability of phytoplankton pigment distributions in the Subtropical South Pacific Ocean: comparison between *in situ* and predicted data. Biogeosciences 5:353–369

Rathgeber C, Beatty JT, Yurkov VV (2004) Aerobic phototrophic bacteria: new evidence for the diversity, ecological importance and applied potential of this previously overlooked group. Photosynth Res 81:113–128

Salka I, Moulisová V, Koblížek M, Jost G, Jürgens K, Labrenz M (2008) 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

Schwalbach MS, Fuhrman JA (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

Sharp JH (1974) Improved analysis for particulate organic carbon and nitrogen from seawater. Limnol Oceanogr 19:984–989

Shiba T (1991) *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

Shiba T, Shioi Y, Takamiya K, Sutton DC, Wilkinson CR (1991) Distribution and physiology of aerobic bacteria containing bacteriochlorophyll *a* on the East and West Coasts of Australia. Appl Environ Microbiol 57:295–300

Sieracki ME, Gilg IC, Thier EC, Poulton NJ, Goericke R (2006) Distribution of planktonic aerobic anoxygenic photoheterotrophic bacteria in the northwest Atlantic. Limnol Oceanogr 51:38–46

Smith DC, Azam F (1992) A simple and economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Mar Microb Food Webs 6:107–114

Spring S, Lunsdorf H, Fuchs BM, Tindall BJ (2009) The photosynthetic apparatus and its regulation in the aerobic Gammaproteobacterium *Congregibacter litoralis* gen. nov., sp nov. PLoS ONE 4:e4866

Suzuki MT, Béjà O (2007) An elusive marine photosynthetic bacterium is finally unveiled. Proc Natl Acad Sci USA 104:2561–2562

ter Braak CJF (1989) CANOCO — an extension of DECORANA to analyze species-environment relationships. Hydrobiologia 184:169–170

ter Braak CJF, Prentice IC (1988) A theory of gradient analysis. Adv Ecol Res 18:271–313

Tréguer P, Le Corre P (1975) Manuel d'analyse des sels nutritifs dans l'eau de mer. Laboratoire d'Océanographie chimique. Université de Bretagne Occidentale, Brest

Waidner LA, Kirchman DL (2007) Aerobic anoxygenic phototrophic bacteria attached to particles in turbid waters of the Delaware and Chesapeake estuaries. Appl Environ Microbiol 73:3936–3944

Wilke M, Boutiere H (2000) Hydrobiological, physical and chemical characteristics and spatio-temporal dynamics of an oligotrophic Mediterranean lagoon: the Etang de La Palme (France). Vie Milieu 50:101–115

Wood ED, Amstrong FAJ, Richards FA (1967) Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J Mar Biol Assoc UK 47:23–31

Yurkov VV, Beatty JT (1998) Aerobic anoxygenic phototrophic bacteria. Microbiol Mol Biol Rev 62:695–724

Yurkov VV, Csotonyi JT (2009) New light on aerobic anoxygenic phototrophs. In: Hunter CN, Daldal F, Thurnauer MC, Beatty JT (eds) Advances in photosynthesis and respiration. Springer, Dordrecht, p 31–55

Yutin N, Suzuki MT, Teeling H, Weber M, Venter JC, Rusch DB, 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

Zeng YH, Shen W, Jiao NZ (2009) Genetic diversity of aerobic anoxygenic photosynthetic bacteria in open ocean surface waters and upper twilight zones. Mar Biol 156: 425–437

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

Zubkov MV (2009) Photoheterotrophy in marine prokaryotes. J Plankton Res 31:933–938

Editorial responsibility: Rutger de Wit, Montpellier, France

Submitted: May 5, 2010; Accepted: October 7, 2010
Proofs received from author(s): December 10, 2010