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Bacteriochlorophyll *a* in the ocean: Is anoxygenic bacterial photosynthesis important?

Abstract—Some groups of marine bacteria within the α -proteobacteria are capable of anoxygenic photosynthesis in oxic environments. Their primary photosynthetic pigment is bacteriochlorophyll *a* (BacChl *a*). The impact of these bacteria on flows of energy and carbon in the ocean has been difficult to ascertain in the past. Recently, however, Kolber et al. (2001) reported that such bacteria are abundant in the upper ocean and that these might contribute significantly to photosynthetically driven electron transport since measured and inferred ratios of BacChl *a* and chlorophyll *a* (Chl *a*) were about 0.8% in coastal environments and as high as 10% in the Eastern Tropical North Pacific. The authors suggested that the globally averaged BacChl *a*/Chl *a* ratio could be as high as 5 to 10%. To determine whether such high values are representative of other marine environments, concentrations of BacChl *a* were measured in samples collected in eutrophic nearshore and mesotrophic and oligotrophic offshore environments off Southern California. The average BacChl *a*/Chl *a* ratio was 1.1% in nearshore and 0.5% in mesotrophic shelf and oligotrophic offshore environments. Assuming that rates of photosynthesis scale with concentrations of photosynthetic pigments, these data suggest that the contribution of BacChl *a* driven anoxygenic bacterial photosynthesis to energy production in coastal eutrophic and offshore oligotrophic areas of the California current system is small, substantially smaller than the suggested global average of 5 to 10%.

Oxic and anoxic photic environments are colonized by dramatically different groups of photoautotrophs. In oxic environments we find cyanobacteria and eukaryotes whose primary photosynthetic pigment is chlorophyll *a* (Chl *a*, Fig. 1) or a close derivative thereof (Scheer 1991). Their mode of existence is oxygenic photosynthesis, using water as a photosynthetic electron donor. Photic anoxic environments are colonized by anoxygenic photosynthetic bacteria, which require anoxic conditions to synthesize their primary photosynthetic pigment bacteriochlorophylls *a*, *b*, *c*, *d*, *e*, and *g* (Imhoff 1992). Rather than water they use sulfide, other reduced sulfur compounds, hydrogen, or a number of small organic molecules as photosynthetic electron donors (Imhoff 1992). About 20 years ago marine bacteria containing bacteriochlorophyll *a* (BacChl *a*, Fig. 1) were isolated from oxic environments (Shiba et al. 1979). These bacteria, belonging to the α subclass of the *Proteobacteria*, are aerobic anoxygenic photosynthetic bacteria, i.e., they can synthesize BacChl *a* in the presence of oxygen and carry out photosynthesis under oxic conditions but do not produce oxygen in the process (Harashima et al. 1989; Yurkov and Beatty 1998). These bacteria are facultative phototrophs; they grow heterotrophically but can use light as an additional source of

energy. This implies that photosynthesis could be important for this group under conditions when the availability of dissolved organic carbon is limiting their growth.

Molecular studies of natural bacterial populations demonstrated that bacteria belonging to the α -proteobacteria are found in most marine environments, particularly the euphotic zone (Giovannoni and Rappe 2000). In spite of the abundance of these organisms in the marine environment, BacChl *a*, thought to be associated with some of these, was not detected by Mullins et al. (1995), who concluded that it is unlikely that photoheterotrophy is an important mode of metabolism for bacteria in the open ocean. Consistent with this conclusion is the observation that levels of photosynthesis in at least some members of this group are low (Harashima and Takamiya 1989). Recently, however, Kolber et al. (2000, 2001) reported that aerobic photoheterotrophic bacteria are ubiquitous in the coastal and open ocean. They reported that the average BacChl *a*/Chl *a* ratio was 0.8% off the coast of Oregon and Washington (U.S.A.) and, based on prior active fluorescence measurements (Kolber et al. 2000), estimated that the BacChl *a*/Chl *a* ratio is 5 to 10% in the Eastern Tropical North Pacific. Kolber et al. (2001) suggested that the globally averaged BacChl *a*/Chl *a* ratio could be as high as 5 to 10%.

To determine that BacChl *a* is indeed present in the oxic surface layer of the ocean and to validate the conclusion of Kolber et al. (2001), I developed sensitive methods for the identification and quantification of BacChl *a* in natural samples and studied the distribution and abundance of BacChl *a* in the ocean off Southern California (Fig. 2). The study domain is an extremely heterogeneous region that extends from the edge of the central gyre of the North Pacific to the shoreline. The trophic state of the system ranges from oligotrophic with low concentrations of Chl *a* to eutrophic upwelling with high concentrations of Chl *a* (Mantyla et al. 1995). In this environment ratios of BacChl *a* and Chl *a* were very low, less than 1% on the average, suggesting that BacChl *a* is not an important chromophore in this environment.

Samples of particulate matter were collected with Niskin bottles off the Scripps Pier (La Jolla, California) and during the January 2001 CalCOFI cruise (Fig. 2). Samples, 1 to 24 liters, were filtered on Whatman GF/F filters (25 or 47 mm) using a pressure differential of less than 15 mbar and were stored in liquid nitrogen. The filtrate of selected samples ($n = 24$) was collected, and 1 liter of the filtrate was filtered on 0.2- μ m Nuclepore filters to determine the fraction of Chl *a* and BacChl *a* passing Whatman GF/F filters.

All samples were extracted in acetone for 30 min, were

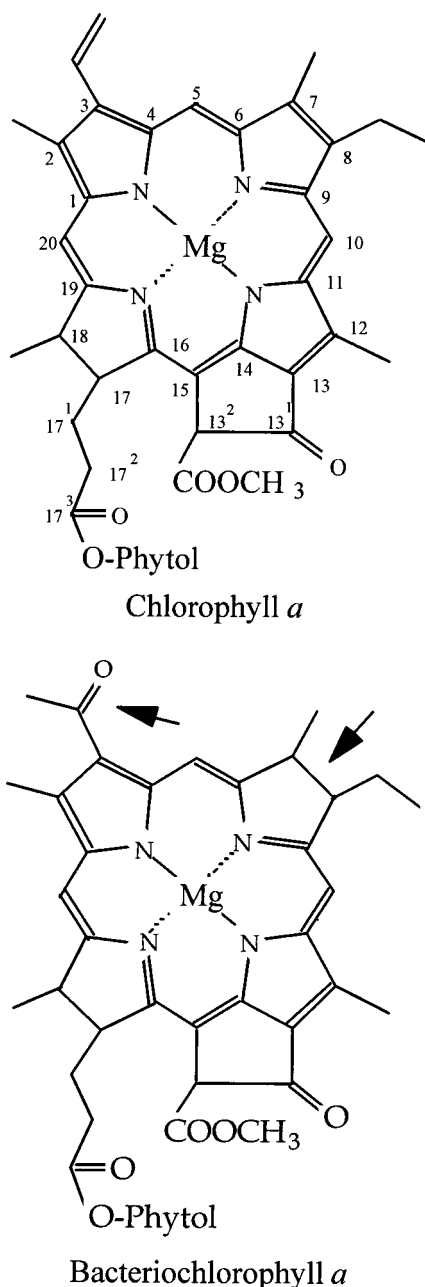


Fig. 1. The structures of chlorophyll *a* and bacteriochlorophyll *a*. The IUPAC carbon numbering system is used. The differences between the two compounds are the presence or absence of a double bond between carbon 7 and 8 (i.e., C7 and C8) and the functionalities at C3 (see arrows). The alcohol esterified to the propionic acid of BacChl *a* is usually phytol but can also be geranylgeraniol.

sonicated under a stream of nitrogen 3×1 min by inserting the sonicator tip into the extract containing the filter, and were subsequently extracted for an additional 30 min. The samples were filtered through a cotton-plugged pipette tip. Concentrations of BacChl *a* and Chl *a* in the extract were measured by reverse-phase high-pressure liquid chromatography (rp-HPLC) detecting pigments with fluorescence (Shimadzu RF 10 A_{xl}, Ex 360 and Em 780 nm), absorption (Shimadzu SPD AV, 770 nm) and photodiode-array (Waters

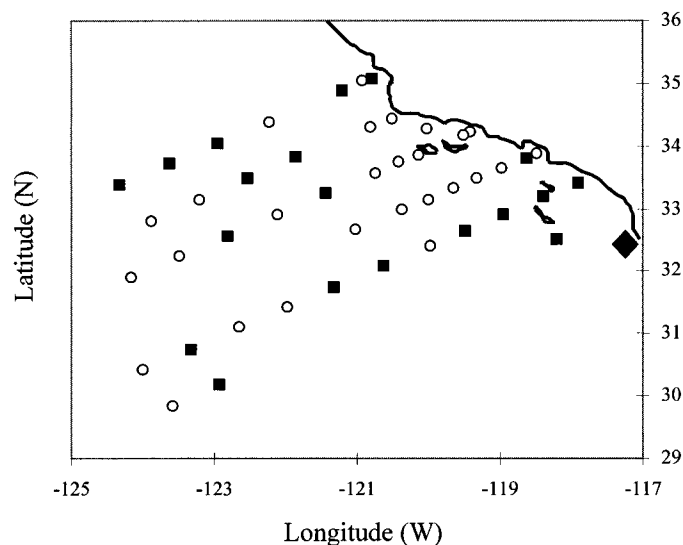


Fig. 2. The stations off Southern California where samples were taken for the analysis of BacChl *a*. Open circles denote samples that were analyzed by HPLC only. Filled squares denote samples that were analyzed by HPLC and LC/MS. The large filled diamond denotes the location of the nearshore sampling station (i.e., Scripps Pier).

991M, 350–800 nm) detectors. The 770 nm absorption maximum of BacChl *a* was used to detect and quantify the pigment. The C8 rp-HPLC system described by Goericke et al. (2000) was used for this study. Selected samples were also analyzed on a C18 rp-HPLC system [column, Supelco C18 Discovery 15 cm, $3 \mu\text{m}$, 4.6 mm; solvents, A—acetonitrile: water (60:40 v/v), B—acetone; gradient (time, % A), (0 min, 80%), (1 min, 50%), (10 min, 0%), (12 min, 0%), (14 min, 80%); flow, 1.5 ml min^{-1}]. This system was developed in order to eliminate ammonium acetate as a buffer, since it facilitates the formation of polymers in the presence of acetone, and to determine whether the presence of methanol induced the formation of BacChl *a* allomers during chromatography (c.f. Mauserall 1978). BacChl *a* injected as a pure compound was slightly unstable on the methanol-based C8 rp-HPLC system but not when added to a natural sample. Current limits of detection on the C8 rp-HPLC system (10:1 signal to noise) are ~ 50 pg BacChl *a* per injection using fluorescent detection (less desirable because it is less selective) and 200 pg BacChl *a* per injection using detection at 770 nm (desirable because of its high selectivity). Assuming a water sample of 24 liters, which can easily be processed, this translates to a detection limit of ~ 15 pg-BacChl *a* per liter seawater, which implies that in the oligotrophic ocean ($50 \text{ ng-Chl } a \text{ L}^{-1}$) BacChl *a*-Chl *a* ratios as low as 0.05% can be measured with a signal to noise ratio of $\sim 10:1$. The coefficient of variation determined from the analysis of replicate samples with low BacChl *a*-Chl *a* ratios (0.5%) was 5 and 1%, respectively ($n = 6$). BacChl *a* for synthesis and calibration was obtained from Sigma and quantified using its extinction coefficient in acetone (Mauserall 1978).

Selected samples were analyzed by liquid chromatography/electrospray mass spectrometry (LC/MS, see Goericke et al. 2000 for a description of the methods) using the rp-C18

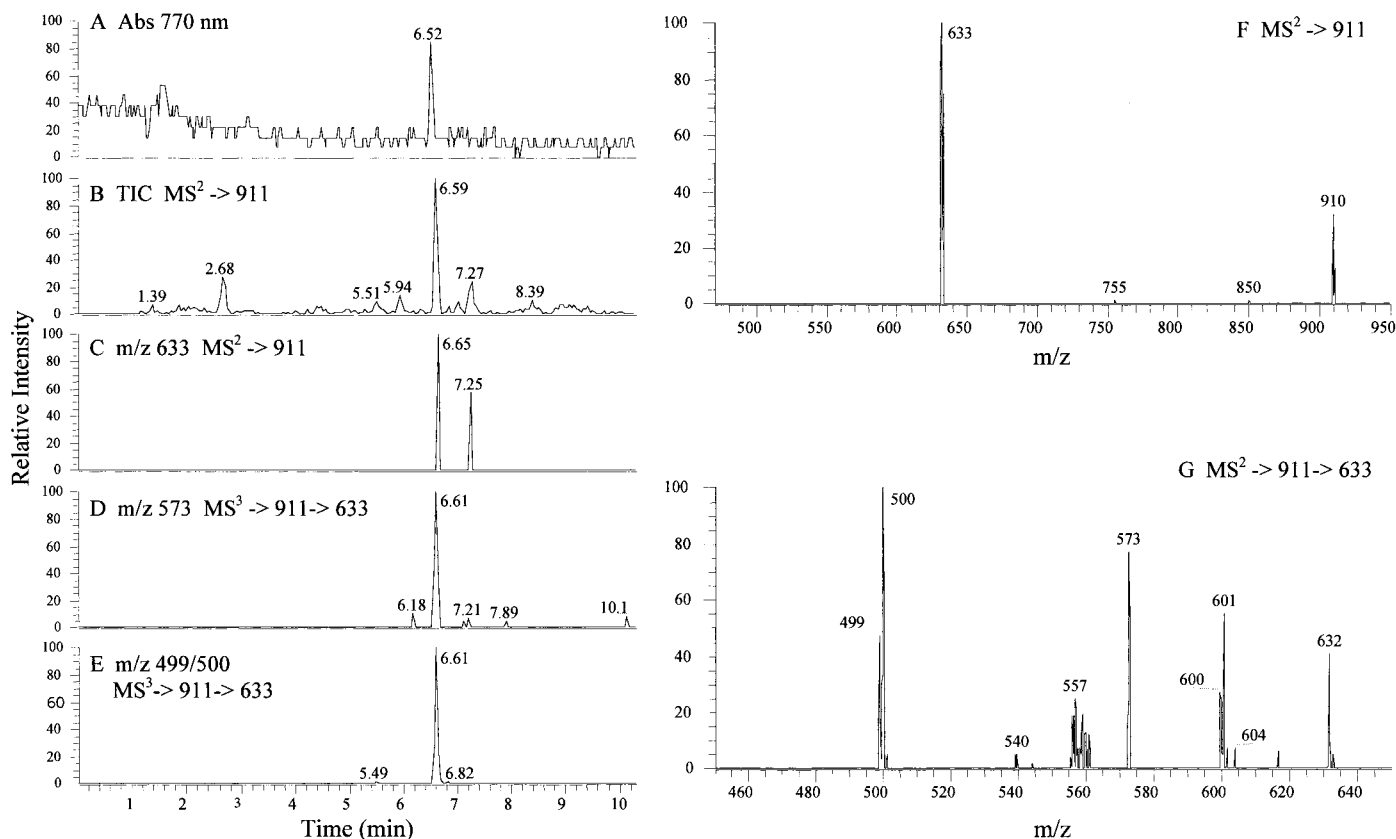


Fig. 3. LC/MS data for BacChl *a* in a natural sample (CalCOFI station 87.035, sampled January 2001). BacChl *a* was detected using absorption at 770 nm (A). The MS was set to only capture *m/z* 911 ions in the ion trap, perform first a fragmentation experiment on these ($MS^2 \rightarrow 911$ experiment), and subsequently perform a fragmentation experiment on any *m/z* 633 fragment ($MS^3 \rightarrow 911 \rightarrow 633$ experiment). (B) The total ion current (TIC) for the MS^2 experiment demonstrates that many different chemical species generate the *m/z* 911 ion. However, the MS^3 ions at (C) *m/z* 633, (D) 573, and (E) 499/500 are only generated by BacChl *a*, in this example and all other runs. The mass spectra for the *m/z* $MS^2 \rightarrow 911$ and *m/z* $MS^3 \rightarrow 911 \rightarrow 633$ experiments performed at $t = 6.6$ min (F and G, respectively) are identical to those of authentic BacChl *a*.

HPLC system coupled to a Finnigan LCQ ion-trap mass spectrometer. BacChl *a* was identified through the *m/z* 911 ion retention time and its characteristic MS^3 fragments produced through the MS^3 experiment *m/z* 911 (MH^+) \rightarrow 633 (MH^+ - phytol) \rightarrow 500 (-133), 573 (-60), 601 (-32). Note that \rightarrow designates an MS/MS experiment, i.e., the capture of a specific ion in the ion trap of the experiment and the subsequent fragmentation of the ion. The detection limit for this type of experiment is ~ 1 ng BacChl *a*.

Low levels of BacChl *a* and its derivatives are easily detected in natural samples, even in the presence of high concentrations of other chlorophylls, due to their characteristic and, as far as is known, unique absorption maximum at 770 nm. Chlorins, such as Chl *a*, have minimal absorption at 770 nm. For example, the mol-specific absorption of Chl *a* at 770 nm on the UV/Vis detector used for this study is $\sim 1,000$ times less compared to BacChl *a*. These properties allow the detection of pg quantities of BacChl *a* in the presence of μg quantities of chlorins. Fluorescence detection, even though it is more sensitive, is less selective since other chlorophylls still fluoresce in the infrared. In particular, allomers of chlorophyll *b*, which can coelute with BacChl *a*, could be mistaken for BacChl *a* or could contribute to its peak area.

Trace levels of BacChl *a* were detected in all samples collected off the Scripps Pier over a 6-month period in 2000 and in the California Current System (Fig. 2) during January 2001. The presence of BacChl *a* in 24 selected samples from the California Current System was further demonstrated by LC/MS (Fig. 3). BacChl *a* produced in all cases a molecular ion (MH^+) at *m/z* 911. The fragmentation pattern of this ion was in all cases identical to the fragmentation pattern of authentic BacChl *a* (data not shown). The *m/z* 278 loss from the molecular ion demonstrates that the alcohol esterified to the propionic acid of BacChl *a* is phytol rather than geranylgeraniol (expected primary MS^2 loss of -272 *m/z* for the latter). The presence of BacChl *a*'s with other alcohols cannot be ruled out, since other compounds absorbing at 770 nm and eluting close to BacChl *a* were consistently observed. However, these compounds, which were not further studied since their relative abundance was small, could also have been allomers of BacChl *a*, i.e., 13²-hydroxy or 13²-methoxy derivatives of BacChl *a*.

The dimensions of phototrophic bacteria with BacChl *a* in situ and their affinity for larger particles are unknown. To determine whether these bacteria can be sampled with the commonly used Whatman GF/F filters, seawater collected at

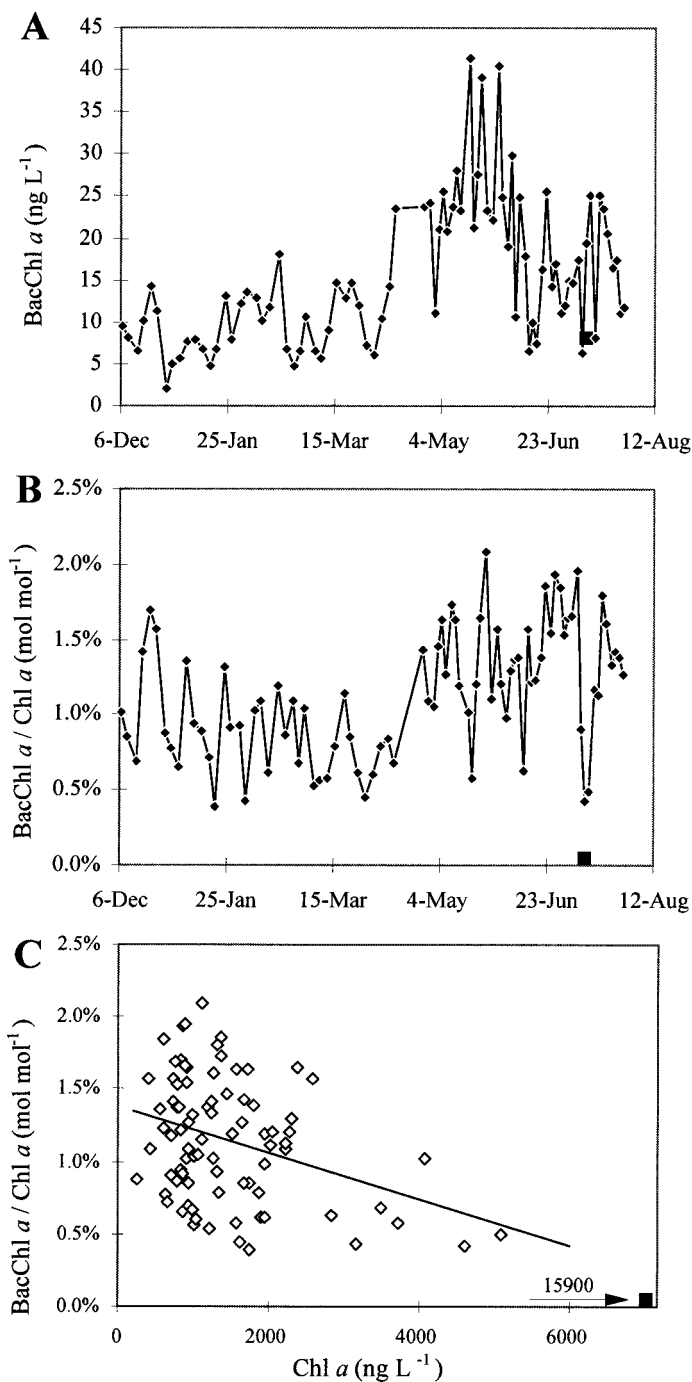


Fig. 4. BacChl *a* in a nearshore environment of the Southern California Bight (off Scripps Pier). (A) concentrations of BacChl *a* from December 2000 until July 2001. Sampling was every 3–4 d during the first half of the sampling period and every other day during the second half of the sampling period. (B) the molar ratio of BacChl *a* and Chl *a*. (C) the BacChl *a*–Chl *a* ratio plotted against concentrations of Chl *a*. The solid square represents the average of two samples collected during a dinoflagellate bloom.

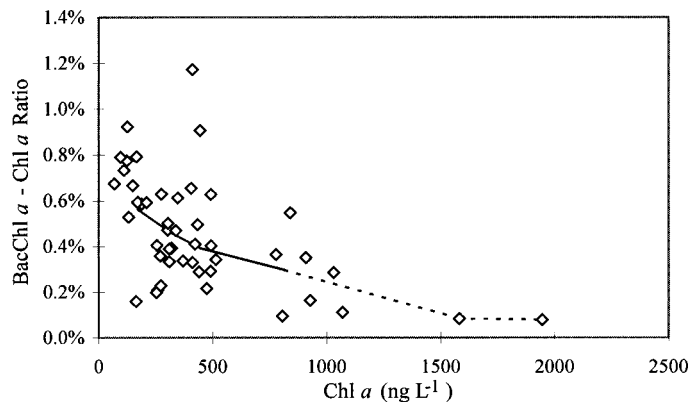


Fig. 5. The BacChl *a*–Chl *a* molar ratio for samples collected in the Southern California Bight and the southern California Current System (Fig. 2). The lines connect bin averages of the data; extrapolation beyond 800 ng-Chl *a* L⁻¹ is uncertain since few data points are available.

24 different stations off Southern California was filtered through GF/F filters and the filtrate was filtered through an 0.2- μ m Nuclepore filter. Both types of samples were assayed for BacChl *a* and Chl *a*. The retention efficiency of GF/F filters relative to 0.2- μ m Nuclepore filters was $98 \pm 2\%$ for Chl *a* and $95 \pm 3\%$ for BacChl *a*. However, the retention efficiency for BacChl *a* is an underestimate of the true efficiency since undetectable concentrations of BacChl *a* in filtrate samples (12 of 24) were set to values corresponding to twice the noise level of the analytical system. These data demonstrate that Whatman GF/F filters, which have at least a 95% retention efficiency for particles 0.7 μ m in diameter and larger (manufacturer's specifications), are adequate for the filtration of seawater for BacChl *a* analysis.

Concentrations of BacChl *a* ranged from 2 to 41 ng L⁻¹ in samples collected off the Scripps Pier from December 1999 until August 2000 (Fig. 4a). The molar ratio of BacChl *a* and Chl *a* was $1.1 \pm 0.4\%$ on the average, ranging from 0.4 to 2% (Fig. 4b; $n = 84$, not including the dinoflagellate bloom sample—see below). The ratio of BacChl *a* and Chl *a* decreased with increasing concentrations of Chl *a* (Fig. 4c). The concentration of BacChl *a* was 8 ng L⁻¹ in one sample collected during a dinoflagellate bloom, an average concentration; however the BacChl *a*–Chl *a* ratio was extremely low, 0.05% (solid squares in Fig. 4), due to the high concentrations of Chl *a*. In the California Current System concentrations of BacChl *a* were substantially lower, ranging from 0.26 to 4.8 ng L⁻¹. The BacChl *a*–Chl *a* ratio was $0.46 \pm 0.24\%$ on the average, ranging from 0.1 to 1.2% ($n = 47$). This ratio decreased with increasing concentrations of Chl *a* (Fig. 5).

The data presented here clearly establish that BacChl *a* can be present in eutrophic and mesotrophic nearshore and coastal environments and the oligotrophic open ocean. The diversity of environments covered by the sampling program—concentrations of Chl *a* in the study domain ranged from 0.07 to 15 μ g L⁻¹—suggests that this result may hold for the world's oceans in general. The measurements reported here are consistent with the direct measurements of Kolber et al. (2001), who reported that BacChl *a* concentra-

tions off the coast of Washington and Oregon were 0.8% of Chl *a* concentrations. The failure of Mullins et al. (1995) to detect BacChl *a* in samples collected in the Sargasso Sea is difficult to evaluate in the absence of more detailed descriptions of the methodology used. It is possible that Mullins et al. were not able to detect the low concentrations of BacChl *a* that one would expect in the Sargasso Sea based on the results presented here.

Likely sources of BacChl *a* in the euphotic zone of the open ocean are anaerobic anoxygenic photoautotrophic bacteria living in anaerobic microzones and aerobic anoxygenic phototrophic bacteria. Proctor (1997) showed that anaerobic photosynthetic bacteria (purple sulfur bacteria) were associated with copepods collected in the Caribbean Sea, but it was not determined whether these bacteria express BacChl *a* in the natural environment. It is conceivable that purple sulfur bacteria are also associated with marine particles where they might express BacChl *a* once anoxic microzones develop. However, these particles would have to be larger and it is unlikely that these or copepods would have been present consistently in our small volume water samples. Thus a more likely source of BacChl *a* in the open ocean are aerobic anoxygenic phototrophic bacteria belonging to the α -proteobacteria, e.g., those of the *Roseobacter* and *Erythrobacter* lineage. Indeed Kolber et al. (2001) have shown that such photoheterotrophic photosynthetic bacteria are ubiquitous in the surface layer of the open ocean, contributing about 10% to bacterial biomass off the coast of Washington and Oregon.

Concentrations of BacChl *a* off Southern California were low compared to concentrations of Chl *a*; the former were on the average 0.5% of the latter. Assuming that the photosystems of bacteria using anoxygenic photosynthesis are as efficient as the photosystems of oxygenic prokaryotes or eukaryotes (Kolber et al. 2001), these data suggest that BacChl *a* does not contribute significantly to total photosynthetic electron transport in the California Current System. The negative covariation of the BacChl *a*-Chl *a* ratio with Chl *a* in the California Current System (Fig. 5) suggests that BacChl *a* may well be more important in oligotrophic areas where heterotrophic bacteria are more likely to be limited by the availability of reduced carbon. The mixed layer of the Eastern Tropical North Pacific, where concentrations of Chl *a* are at times less than $0.05 \mu\text{g L}^{-1}$ (e.g. Goericke et al. 2000), may be such an area. However, the difference between BacChl *a*/Chl *a* ratios for the oligotrophic areas of the California Current System reported here (0.7% for samples with Chl *a* $< 0.2 \mu\text{g L}^{-1}$; $n = 11$) and the estimate of 5 to 10% of Kolber et al. (2001) for the Eastern Tropical North Pacific clearly shows that the values of Kolber et al. (2001) cannot be extrapolated to the global ocean without further study.

Off the Scripps Pier the BacChl *a*-Chl *a* ratio also declined with concentrations of Chl *a*, on the average the ratio was about two times higher than ratios observed in the California Current System. This difference may simply reflect a difference in bacterial communities between the nearshore (~ 500 m off the beach) and the offshore environments (5 to 500 km off the coast). The nearshore environment is also more dynamic; at times blooms of algae occur with concen-

trations of Chl *a* orders of magnitude higher than long-term averages. It is possible that the biomass of bacteria lags the biomass of phytoplankton during those blooms. The one sample taken during a dinoflagellate bloom may be such an example, as concentrations of BacChl *a* were only slightly below the average for the system even though concentrations of Chl *a* were almost one order of magnitude higher than its average concentration.

To summarize, the low BacChl *a*-Chl *a* ratios observed throughout the study domain suggest that the contribution of BacChl *a* to photosynthetically driven electron transport was small, about 0.5 to 1% of the contribution of Chl *a*. This conclusion is based on the assumption that rates of photosynthetic electron transport scale with concentrations of primary photosynthetic pigments, i.e., Chl *a* and BacChl *a*. This assumption is corroborated by measurements of Kolber et al. (2001), who concluded that the light use efficiency per unit chromophore is similar for aerobic anoxygenic photoheterotrophs and oxygenic photoautotrophs. Thus, the data presented here and those presented by Kolber et al. (2001) suggest that aerobic anoxygenic photoheterotrophs do not contribute significantly to photosynthetically driven energy fluxes in eutrophic and mesotrophic temperate and subtropical environments. The difference between results presented here and by Kolber et al. (2001) for oligotrophic environments clearly shows that it is not yet possible to determine the global significance of anoxygenic photoheterotrophs. Thus, more diverse environments have to be sampled before we can determine the contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean.

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UV-induced pigmentation in subarctic *Daphnia*

Abstract—The distribution of the planktonic crustacean *Daphnia* and the occurrence of ultraviolet (UV)-protective melanin pigmentation in its body wall were studied in relation to the UV transparency in subarctic ponds and lakes in Finnish Lapland. In shallow ponds, *Daphnia* only occurred in those with sufficient amounts of UV-screening dissolved organic matter. The highest pigment concentration was found in these *Daphnia* and in populations that occurred in lakes with low organic carbon content (<2 mg L⁻¹). Pigment synthesis was extremely low during the long winter and peaked immediately after the ice break-up at the time of the maximum underwater UV intensity. We propose that the predicted increase in UV irradiance from ozone depletion in the Northern Hemisphere spring, along with the earlier ice break-up associated with Arctic warming may favor the survival of those organisms with the ability to produce UV photoprotective pigments.

The most conspicuous feature of polar regions is the large seasonal variation in incoming solar radiation, from zero in winter months to 24 h of continuous sunlight in the summer. The poles therefore receive more solar radiation in summer than equatorial locations, yet the momentary radiation intensity never exceeds equatorial values. Ozone depletion in northern latitudes and the resultant changes in incident ultraviolet radiation have increased markedly during past decades, with some sectors of the Arctic experiencing upwards of 20% reductions in ozone and more than a 40% increase in ultraviolet (UV) radiation, the trend being most pronounced during spring months (WMO 1998). Although solar UV accounts for less than 5% of the total radiation reaching the surface of the earth, it contains the most energetic and biologically harmful wavelengths. These wavelengths are known to have broad effects on aquatic ecosystems, including the photoproduction of toxic compounds, mutagenesis, and physiological stress (Vincent and Neale 2000).

The penetration of UV radiation in lakes is known to be largely a function of the concentration of dissolved organic carbon (DOC; Morris et al. 1995). Because of poorly developed soils, sparse terrestrial vegetation, and low phytoplankton production, most of the waters in northern Fennoscandia situated above the tree line are poor in both allochthonous (watershed-derived) and autochthonous (originating within the lake) DOC, with values usually less than 5 mg L⁻¹. Northern Fennoscandian waters are also shallow, the mean depth being less than 5 m (Blom et al. 1998). This means that in many cases high levels of UV radiation can penetrate to the bottom of the water body. The open-water fauna of high-latitude lakes is therefore experiencing high enough UV intensities to cause increased mortality (Zellmer 1998).

To address the potential effect of increased UV radiation, we studied one of the most common and abundant groups of the subarctic open-water fauna, *Daphnia*, in relation to underwater UV conditions. First, we determined the occurrence of *Daphnia* in water bodies with different optical characteristics. Second, we measured the melanin pigment concentration of the body wall of *Daphnia*, which is suggested to depend on exposure to UV radiation (Hebert and Emery 1990; Hessen and Sørensen 1990). The growth of a crustacean takes place through the moulting of the shell and the formation of a new larger carapace. Melanin synthesis has to be repeated after each moult and has been considered to be energetically costly (Hebert and McWalter 1983; Hessen 1996). In laboratory experiments, the melanic morphs have been shown to have lower growth rates than the nonmelanic ones, thus being competitively inferior to the unpigmented forms (Hessen 1996). If pigment synthesis requires energy, the few resources available are not only allocated to growth and reproduction, but also to UV protection. In addition,