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Light environment and synthesis of bacteriochlorophyll by populations of *Chromatium okenii* under natural environmental conditions

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Abstract

In the meromictic alpine Lake Cadagno a dense layer of phototrophic bacteria, mainly *Chromatium okenii* and *Amoebobacter purpureus*, develop annually at the chemocline at about 10 to 11 m depth. Radiometric spectral profiles of the incident sunlight demonstrate different attenuation coefficients in the mixolimnion and in the chemocline not only for the visible light effective at each depth (photosynthetically available radiation), but also for selected photosynthetically active wavelengths used by oxygenic and anoxygenic phototrophs. Phototrophic bacteria sampled from the upper part of the layer at the maximum of cell concentration were incubated in transparent bottles at the sampling depth and at a lower depth where the light intensity is only a few percent of the one at the sampling depth. Within 4 h the specific bacteriochlorophyll concentration (Bchl protein^{-1}) increased up to 50% depending on the difference in light intensity between the sampling and the incubation depth. The specific bacteriochlorophyll concentration in the upper part of the layer remained constant (53.0 mg Bchl g^{-1} protein, S.D. = 4.8) in spite of large changes in cell concentrations in the lake water over the season. These observations illustrate the phenomenon of light-regulated pigment synthesis under natural conditions.

Keywords: Bacteriochlorophyll; *Chromatium okenii*; Light intensity; Regulation; Spectral light distribution; In situ experiment

1. Introduction

In phototrophic bacteria the formation of the photosystem is regulated in response to environmental conditions, mainly the concentration of dissolved oxygen, the redox state and the light intensity [1–8]. Lowered oxygen partial pressure induces the synthesis of new chromatophore membranes and leads to a rapid expression of the pigment-proteins of the light-harvesting complexes and of the reaction centers. Increased light intensity suppresses the biosynthesis of photosynthetic units and their number and size per cell drop. In contrast, decreasing light intensities shift the cells into an energetically unfavorable state since the photosynthetic units are no longer light-saturated. Cells stop growing and the available

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light energy is used for the synthesis of new photosynthetic units. The pigment which senses the actual light intensity and the transmission of the signal to the effector molecules are largely unknown so far. It has been suggested that both the oxygen and light intensity influence the extent of the electrochemical gradient and the oxidation state of the cyclic electron transport system, a compound of which regulates the synthesis of the photosynthetic apparatus [5–7]. Besides light intensity and oxygen concentration the pigment content is governed by the nature of the electron donor, reducing substances increasing the specific bacteriochlorophyll content [1,2]. It has also been suggested that the Calvin cycle is involved in these regulatory events by acting as an electron sink for excess redox equivalents [8,9].

So far, regulation studies on the genetic and molecular level have been done with non-sulfur phototrophic bacteria, mainly *Rhodobacter capsulatus*, *Rb. sphaeroides* and lately also with *Rhodospirillum rubrum*. In these organisms the genes responsible for the expression of the photosynthetic apparatus are regulated by an oxygen-sensitive promoter (for reviews see [10–12]). A gene involved in the control of pigment protein gene expression by light intensity has been described recently [13]. Among the sulfur phototrophic bacteria oxygen inhibits bacteriochlorophyll synthesis in *Thiocapsa roseopersicina*, especially when illuminated [14,15], and in *Chromatium vinosum* the specific Bchl content drops with increasing light intensity [16]. In both groups organisms are found to contain not only the light-harvesting complex I (λ_{max} around 890 nm) but also a second light-harvesting system absorbing at wavelengths between 800–850 nm. The latter system is highly variable in concentration relative to the reaction center depending on environmental conditions. Since the genetic control mechanism of light and oxygen on pigment synthesis has been studied only in a small number of non-sulfur phototrophic bacteria, a generalization for in vivo conditions may not be allowed.

In this paper we investigate, in a natural environment, how cells react to lowered light intensities under anoxic conditions with respect to pigment synthesis. Lake Cadagno, a meromictic alpine lake in Switzerland which contains a plume of predominantly *Chromatium okenii* and *Amoebobacter purpureus* at the chemocline, is selected as a natural model system to follow the changes in bacteriochlorophyll concentration initiated by changes in light intensity. The ecology of phototrophic sulfur bacteria in general and the typical vertical structure of the chemistry and microbiology in various meromictic lakes has been recently summarized [17].

2. Materials and methods

2.1. Site of investigations and sampling

All measurements were carried out in Lake Cadagno, a small meromictic lake in the southern Swiss Alps [18]. Its anoxic monimolimnion is rich in hydrogen sulfide due to the high activity of sulfate-reducing bacteria present in the monimolimnic water column and in the sediments. A redoxcline at a depth of approximately 11 m separates the oxic mixolimnion from the anoxic monimolimnion. In this transition region a dense layer of phototrophic bacteria, mainly *Chromatium okenii* and *Amoebobacter purpureus*, is found during the summer season, as first observed by Düggeli 80 years ago [19].

Direct measurements and sampling were done from a floating platform placed above the deepest point of the lake. Water containing the bacterial suspension was taken from the upper part of the bacterial layer and filled into 50 ml tubes. Care was taken that neither light nor air had access to the bacterial suspension during the sampling operations. The tubes were supplemented with H_2S (final concentration 30 μM) to allow for non-limiting electron donor concentrations for photosynthesis during the subsequent incubation period [20]. The samples were then held in a specially built lowering frame and exposed in the lake during 4 h at two different depths in the bacterial layer. Control tubes were kept completely dark and incubated at the same depths.

2.2. Light measurements

The solar radiation at the site was measured continuously during the period of field work with a LI-190 SB Sensor (Li-Cor, Lincoln, NE), integrated over 10 min, recorded by means of a LI-550 B Printing Integrator and calculated as mol quanta m^{-2}

h^{-1}. The intensities of scalar irradiances of the photosynthetic active radiation (PAR) in the profile of the water column were measured at the following depths (m): 0.05, 0.5, 1.0 and then in 1 m steps down to 12 m using the 4π sensor LI-192 SB (Li-Cor, Lincoln, NE) giving the flux of quanta as μmol quanta m^{-2} s^{-1}. We calculated the vertical attenuation coefficients of PAR light (Table 1) separately for (1) the depth interval 0 to 7 m (= K_0(PAR,a)); and (2) the bacterial layer 11 to 12 m (= K_0(PAR,b)).

2.2.1. K_o(PAR,a)

There is no influence of phototrophic bacteria in this interval; normally 9 data points were used to calculate the linear regression. All relationships are significant almost at the 0.1% level. For details of the calculation procedure see [21].

2.2.2. K_o(PAR,b)

The interval represents the maximum density of the phototrophic bacteria. K_0(PAR,b) is the difference of the natural logarithms of the scalar irradiance at depth 11 m ($\ln(E_0(PAR,11))$) and 12 m ($\ln(E_0(PAR,12))$).

In situ spectroradiometric measurements were performed with an ASD general purpose spectroradiometer LabSpec VNIR-512 (Analytical Spectral Devices, Boulder, CO) fitted with a 25 m Fujikura fiberoptic cable GC.600/750 (C. Itoh, Zürich, Switzerland) with an entrance angle of 28°. Spectra were recorded from 365 to 1050 nm and stored on a computer (Toshiba 3200 SX) at intervals of 0.5 m from 0.05 m down to 20 m depth using a self-constructed lowering frame which kept the end of the fiber in a vertical upward directed position.

2.3. Measurement of the physical and chemical parameters

Temperature, pH, conductivity and oxygen concentration, as well as turbidity were measured by a submersible multisensor unit (Hydropolytester HPT, Züllig, Rheineck, Switzerland). Hydrogen sulfide was determined photometrically [22]. Pigment and protein concentrations were determined before and after incubation. The bacterial photosynthetic pigments were determined photometrically after filtration of 40 ml lake water through glassfiber filters (GF/F Whatman) and extraction with acetone/methanol (7:2) [23]. Spectra from 350 to 850 nm (Uvikon 810, Kontron, Zürich, Switzerland) of the extract were

Table 1
Vertical attenuation coefficient of photosynthetically active radiation K_0(PAR), calculated for the mixolimnion (mean depth interval a, 0–7 m) and the bacterial layer (depth interval b, 10–12 m) and the scalar irradiance at sampling depth z_1 (E_0(PAR),z_1) in percentage of the subsurface scalar irradiance (depth = 0.05 m)

Date (1987)	K_0(PAR,a) [a]		K_0(PAR,b) [b] (m^{-1})	Sampling depth, z_1, (m)	E_0(PAR,z_1) in % of E_0(PAR,0.05)
	(m^{-1})	(S.D.)			
August 20	0.304	(0.007)	4.68	10.9	0.5
August 21	0.303	(0.002)	4.09	11.0	0.6
September 1	0.327	(0.010)	2.81	10.8	0.7
September 2	0.310	(0.008)	1.79	10.8	1.1
September 10	0.308	(0.004)	4.01	11.0	0.6
September 11	0.303	(0.004)	3.95	11.0	0.6
September 15	0.307	(0.005)	4.83	10.8	0.9
September 16	0.317	(0.005)	4.50	11.0	0.4
September 24	0.264	(0.003)	2.17	10.8	1.0
September 25	0.269	(0.003)	2.11	11.0	0.6
Mean (S.D.)	0.301	(0.020)	3.49 (1.16)	10.9 (0.1)	0.7 (0.2)

S.D. = standard deviation (in brackets).
Light measurements were done between 11.30 and 12.30 h.
[a] All results of regression analysis significant at level $< 0.1\%$.
[b] Difference of the natural logarithm of downward scalar irradiance at depth 11 m and 12 m.
Sampling was done at the depth (z_1) of highest bacterial cell density which was usually in the upper part of the layer.

used to calculate the pigment concentrations (Bchl at 770 nm, using the extinction coefficient of Clayton [23] of 75 mM^{-1} cm^{-1}). In vivo spectra were made on the same instrument using the second sample position close to the photomultiplier to minimize the loss of scattered light.

Protein was determined according to Lowry [24] from the filter residue of 20 ml lake water after precipitation with trichloroacetic acid (TCA) and boiling with 0.25 N NaOH. Calibration was obtained with bovine serum albumin (BSA).

2.4. Statistical analysis

Paired *t*-test, Wilcoxon Signed Rank test and paired *t*-test from the StatView program package (Abacus Concepts Inc., Berkeley, CA, 1992) were used to compare the analytical data. To picture the effect of light intensity on the bacteriochlorophyll concentration the data were plotted as an x-y-graph. Where linear relationships were indicated the StatView program was used for the evaluation of linear correlations (*R*-squared, *t*-test and ANOVA statistics).

3. Results

The bacterial layer is easily detected by its high turbidity, it is situated at around 11 m in the upper region of the anoxic zone which is still reached by light but where all the H_2S is absent. Cell densities between 10^5 and 10^6 cells ml^{-1} are usually found equal to a biomass of 200 mg (dry weight) l^{-1}. The light penetrating the mixolimnion to the upper boundary of the bacterial zone is attenuated by the oxygenic algal plankton, suspended non-photosynthetic organic and inorganic particles and dissolved organic substances to a few percent of the surface radiation. It then decreases rapidly in the transition zone due to the strong absorption and scattering by the bacterial suspension. At the lower edge of the layer the light intensity is about 10^{-4} of the subsurface value ($= E_0$ (PAR, 0.05 m)). The attenuation coefficient K_0 (PAR) above the bacterial layer is around 0.3 m^{-1} with small deviations during the investigation period. It increases more than tenfold within the layer as demonstrated in Table 1 for the various sampling dates.

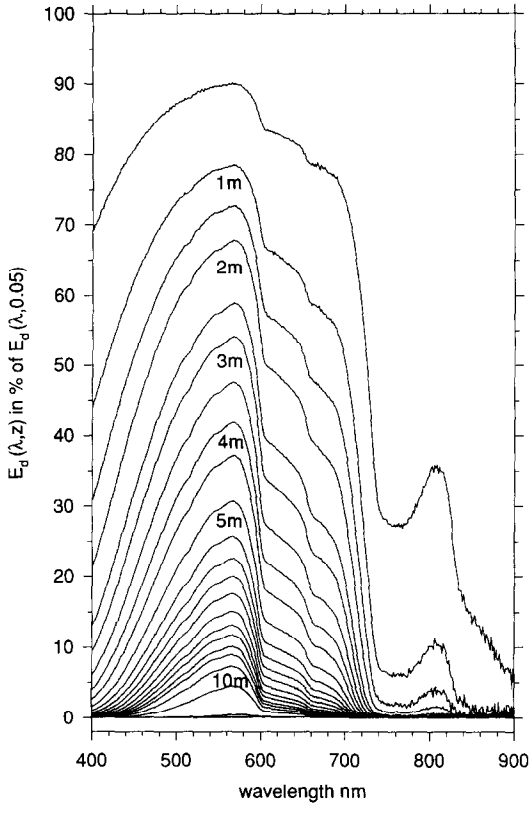

Fig. 1. Spectral distribution of the incident light taken in steps of 0.5 m to 10 m. Values are given in % of the radiation 0.05 m below the surface, data from 29 July 1994.

The light intensity reaching the sampling depth is between 0.4% and 1.1% of the subsurface radiation (0.05 m below the water surface). Light reaching this depth belongs mainly to the green wavelength region, the absorption range of the dominant carotenoid okenone and the β band of the bacteriochlorophyll a.

Fig. 1 gives a set of incident light spectra taken in 0.5 m intervals on a sunny summer day. When the attenuation profile is presented for selected wavelengths, the calculated K_d values for sets of groups of wavelengths differ and are characteristic for the organisms responsible for the light attenuation. Since cyanobacteria have been found to be rare in the mixolimnion 560 nm can be used as a marker for the bacterial carotenoids; 560 nm shows a small K_d value in the mixolimnion but a large one in the bacterial layer. 680 nm as a marker for plant chloro-

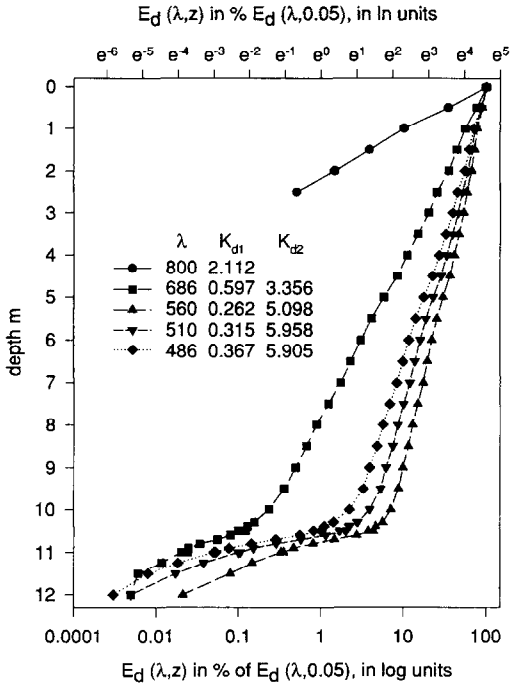

Fig. 2. Attenuation of light at selected wavelengths and calculated mean K_d values for the mixolimnion and the bacterial layer in Lake Cadagno. 686 nm is a marker for chlorophyll a, 560 nm for the bacterial carotenoid okenone, 800 nm indicates the strong absorption of the near infrared by water, data from Fig. 1. Significance for K_d-values given at a level $< 0.1\%$. $K_{d1} = K_d$ value calculated for the depth interval 0–10 m, K_{d2} = values for the interval 10–12 m.

phyll a clearly gives, in the oxic part, a larger K_d than the one of 560 nm (Fig. 2).

The rather high K_d-value of the 680 nm band in the bacterial layer where almost no chlorophyll a containing organisms are present is due to extensive light scattering by the bacterial suspension. The K_d value for wavelengths above 700 nm is determined by the high absorption coefficient of water for this wavelength range (0.65 m^{-1} at 700 nm up to 2.07 m^{-1} at 800 nm [25]), and almost no quanta above this limit reach the depth of 10 m (Fig. 1).

A solid state spectrum on filter disks of a few drops of the mixed population from the bacterial layer of the lake was taken with the Labspec instrument as described elsewhere (M. Wiggli et al., manuscript submitted). It shows the dominant absorption of the carotenoid okenone (500–600 nm) and of the long-wavelength band of bacteriochlorophyll (800–900 nm) (Fig. 3). A difference spectrum between the light recorded at 10 m depth minus the light recorded at 12 m depth shows the dominant carotenoid peak; however, due to the strong absorption of the upper layers in the red no information can be obtained above 600 nm (see Fig. 1).

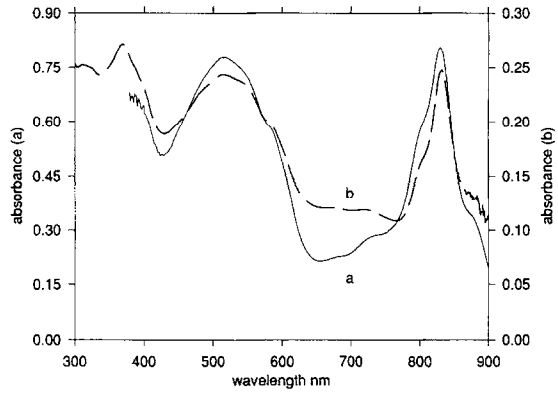

Fig. 3. Absorption spectrum of cells of the mixed bacterial population at the redox transition zone (a) on filter disks (Whatman GF/F) with the Labspec (left ordinate) and (b) measured as suspension (right ordinate, Uvikon 860). Sampling date: 26 September 1995.

Fig. 3 clearly shows that, at 12 m depth, light which could be absorbed by bacteriochlorophyll a in the spectral region of 800 to 900 nm, the main absorption bands of Bchl a, is completely attenuated

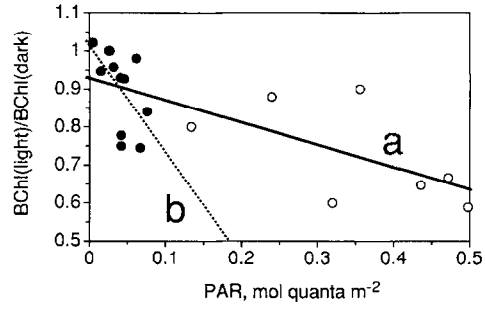

Fig. 4. Influence of light on the synthesis of bacteriochlorophyll a within a 4 h incubation period. (a) in the whole experimental range (0 to 0.5 mol quanta m^{-2}), whole data set, open circles; (b) at low irradiance (0 to 0.1 mol quanta m^{-2}), closed circles. Values below 1: Bchl synthesis suppressed by light. Bchl (light) = concentration of bacteriochlorophyll after incubation in the light (mg Bchl m^{-3}), Bchl (dark) = concentration of bacteriochlorophyll after incubation in the dark. For statistical analysis see text.

by water in the uppermost layers of the lake and will never reach the layer of the phototrophic bacteria.

We assume that the light intensity present at the sampling depth is not only characteristic for the moment of sampling but also for the short period before. The relationship between light intensity and the specific concentration of bacteriochlorophyll (mg Bchl g^{-1} protein) can give information on the daily adaptations to light. Although cell density and thus Bchl and protein concentrations at the sampling depth in the layer varied by a factor of up to five during several weeks in midsummer and fall, and the preceding light conditions differed between each sampling, the specific Bchl content was astonishingly constant at 53.0 (S.D. = 4.8) mg Bchl g^{-1} protein (Table 2).

During the in situ incubations of about 4 h at a temperature of around 6°C no increase in protein concentration indicating growth was observed independently of whether the samples were incubated at the depth of sampling, a few dm lower at a light intensity of about 1/10 of the one at the sampling site or in complete darkness. In contrast, a remarkable increase in the concentration of Bchl a of up to 50% occurred when cells from the upper part of the bacterial layer (= sampling depth, Table 1) were exposed either at the decreased light intensity in the lower part of the bacterial plume or in the dark.

The influence of light on Bchl synthesis is summarized in Fig. 4. To eliminate the variations in absolute values of the specific bacteriochlorophyll concentration, the ratio of the bacteriochlorophyll concentration after incubation in the light over the bacteriochlorophyll concentration after incubation in the dark is plotted against the amount of light (PAR) gathered during the 4 h of incubation. The values of this ratio vary between 1 (indicating no influence of light) and 0.61 for exposition to irradiances 0.3 to

Table 2
Changes in specific bacteriochlorophyll concentration (mg Bchl g^{-1} protein) before and after in situ incubations at different light intensities and in the dark

Date (1987)	Sampling depth z_1 (m)	Depth of exposition z (m)	Exposition time (h)	E_o(PAR,z) during 4 h exposition (mol quanta m^{-2})	Protein concentration (g m^{-3})	Specific concentration of Bchl before exposition (mg Bchl g^{-1} protein)	Specific concentration of Bchl after exposition in the light (mg Bchl g^{-1} protein)	Specific concentration of Bchl after exposition in the dark (mg Bchl g^{-1} protein)
August 20	10.8	10.8	4	0.320	2.07	58	57	96
		11.4	4	0.042			72	97
August 21	11.0	11.0	4.08	0.436	1.89	55	54	83
		11.6	4.08	0.042			69	89
September 1	10.8	10.8	4	0.134	1.57	56	56	70
		11.4	4	0.026			68	68
September 2	10.8	10.8	3	0.041	1.68	49	47	51
		11.3	3	0.005			55	54
September 10	11.0	11.0	3.96	0.498	2.70	42	43	73
		11.5	3.96	0.067			51	68
September 11	10.8	10.8	3.86	0.472	2.27	52	52	65
		11.7	3.86	0.015			79	83
September 15	10.8	10.8	3.92	0.356	1.60	56	53	58
		11.6	3.92	0.027			65	65
September 16	10.9	10.9	4	0.240	1.65	54	50	57
		11.2	4	0.062			60	61
September 24	10.8	10.8	3.83	0.076	0.79	53	53	63
		11.5	3.83	0.032			61	63
September 25	11.0	11.0	4	0.046	0.67	55	55	41
		11.3	4	0.027			51	51

E_o(PAR,z) = total scalar irradiances of PAR during the exposition period at depth z.

0.5 mol quanta m^{-2} during 4 h. Line (a) shows the regression curve for all data points. The ratio Bchl (Light)/Bchl (Dark) decreases with increasing light intensity during incubation (slope −0.598, 95% confidence interval −0.313 and −0.882, $P < 0.0004$, $n = 18$). This demonstrates that the larger the light intensity while sampling and during incubation, the larger is the increase in the Bchl content in the corresponding dark sample. The distribution of the data points in Fig. 4 suggest that incubation at low irradiance (0 to 0.1 mol quanta m^{-2}) might result in a stronger dependence on the light intensity during incubation (curve (b)). There a small increase of light intensity during incubation had a large effect upon the synthesis of Bchl thus reducing the ratio rapidly to values below 1 (curve b, slope −2.94, 95% confidence interval −0.32 and −5.56, $P = 0.03$, $n = 11$). This hypothesis is discussed below.

4. Discussion

While the specific bacteriochlorophyll content (Bchl protein^{-1}) seems to be rather independent of the light intensity before sampling, as well as of cell concentration and the season, the increase in Bchl either by lowering the light intensity to a few percent or in the dark highly correlates with the light before the experiment. The data set given in Table 2, obtained during a summer season under a variety of weather conditions, lends itself to statistical treatment. Under in situ conditions the specific pigment content shows low variability and no significant correlation to the light intensity at the sampling time. In contrast, the Bchl concentration (per volume of water in the lake) and thus the cell density in the lake is strongly dependent on the light as indicated by a more compact and less diffuse bacterial layer on sunny days (unpublished).

In laboratory experiments with cells of *R. rubrum* the bacteriochlorophyll to protein ratio has been shown to increase from 3.3 to 31.5 during pigment synthesis [26]. It varied between 20 and 60 mg Bchl g^{-1} protein in chromatophores of a variety of species [27]. For *Rb. capsulatus*, Bchl per cell protein was in the range of 4.8 to 24.2 mg Bchl g^{-1} protein depending on growth conditions [28]. At low irradiance a value of 30 mg Bchl g^{-1} cell protein has been reported and within 8 h the Bchl concentration increased from 4 mg g^{-1} to 18.2 mg g^{-1} cell protein [29]. For isolated chromatophores from various bacteria a ratio of 46 to 307 mg Bchl g^{-1} protein has been determined [30]. Similar values are given for *Chromatium vinosum* and *Thiocapsa roseopersicina* [14–16]. These data support the observation that in the lake the population at the upper edge of the bacterial layer has a rather high Bchl per protein ratio, typical for low light conditions. Furthermore it suggests that the biomass in the layer is mainly formed by the pigmented large *Chromatium okenii* cells while non-phototrophic cells, although more numerous, constitute only a minor portion of the biomass. Cell counts revealed that about 1/10 of the organisms are large *Chromatium okenii*; the rest of the biomass is made up of much smaller phototrophic (*Amoebobacter purpureus*) and various non-phototrophic organisms (unpublished data).

The potential to synthesize new bacteriochlorophyll when transferred to low light conditions is dependent on the light regime before incubation. The increase in the concentration of Bchl a at low light intensities is related to the difference in irradiance between the site of sampling and the site of incubation. It also relates to the light intensity previously experienced by the cells at the sampling site. Furthermore, the dark controls show similar or higher Bchl a concentrations as compared to the low light ones confirming the important roles of both the absence of oxygen and the decreased light intensity as signals for the synthesis of the components of the photosynthetic apparatus. The more light has been measured before incubation, the more bacteriochlorophyll is observed (linear regression significant at a level of 0.25%). A drop of the ratio of bacteriochlorophyll determined before and after incubation (Fig. 4) indicates induced synthesis of bacteriochlorophyll at low light and in the dark, respectively. The negative slope when the ratio Bchl (Light)/Bchl (Dark) is plotted against the irradiance at the incubation site means, that the capacity to form new bacteriochlorophyll is higher for cells which stayed at higher light intensities before the experiment. A much steeper slope is observed when only irradiances (0 to 0.1 mol quanta m^{-2}), obtained mostly with cells incubated at the lower edge of the bacterial layer, are considered. These very low light inten-

sities seem to be sufficient to negatively affect the massive Bchl synthesis observed in incubations in complete darkness. The lower the light during incubation, meaning the closer to complete darkness, the smaller is the difference to the amount of bacteriochlorophyll synthesized in cells kept in the dark resulting in ratios close to 1. Since data are lacking for irradiances above 0.06 mol quanta m^{-2} it may not be allowed to extrapolate to higher light intensities.

Adaptation to phototrophic conditions by cell differentiation takes 2 to 12 h under laboratory conditions [31] which seems therefore hardly suitable for short term adaptations to a changing environment. However, the results presented here clearly show that under in situ conditions, this adaptation occurs in an unexpectedly short time period. Results obtained from fluorescence kinetics [32] give additional evidence that the physiological adaptation to the environmental conditions within the layer of the phototrophic bacteria is rapid and faster than the displacement of the cells in the layer by diffusion, convection, sedimentation or active swimming. This may be different for slower processes such as pigment synthesis. The rather constant pigment to protein ratio over the season which is statistically independent (at 5% level) on the light intensity present at the sampling time suggests that also the cells of the upper part of the layer are 'averaged dark cells'. They are able, however, to increase the pigment to protein ratio further when they are transferred for several h to the even lower light intensities found in the lower part of the plume or into the dark for a few hours. It must be noted that these observed regulation processes are induced by small changes at very low light intensities. Obviously the effects of seasonal variations are not large enough from mid – August to the end of September to induce an increase in the relative Bchl concentration in the layer.

In summary, the statistical treatment of the data demonstrate that: (1) no significant Bchl synthesis occurs when the light intensity remains the same as before in the sampling depth; (2) significant Bchl synthesis occurs when the light intensity is drastically reduced; and (3) no significant differences in Bchl synthesis are observed in dark incubations at different levels of the bacterial plume.

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