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Spatio-temporal distribution and growth dynamics of phototrophic sulfur bacteria populations in the sulfide-rich Lake Arcas

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ABSTRACT

Lake Arcas exhibits a thermal stratification from April to October. A sulfide-rich anoxic hypolimnion is then formed between the deeper part of the thermocline and the lake bottom, and high population densities of phototrophic microorganisms are found at the oxic-anoxic interface. *Chromatium weissei*, a large rod, 8 × 4 μm in size, was the dominant phototrophic bacterium, reaching densities of up to 1.84×10^6 cells ml^{-1}. Other phototrophic sulfur bacteria, such as *Amoebobacter* cf. *purpureus*, *Thiocapsa* sp., and *Pelodictyon clathratiforme* were also present in the anoxic hypolimnion, but their cell size and population densities were much lower. Net growth rates (0.125 to –0.123 d^{-1}) and frequency of dividing cells, indicated that *C. weissei* grew most rapidly in the upper part of the phototrophic bacterial layer. The highest growth rates were found during the first half of the stratification period, with a marked decrease in population density as mixing approached. Our results suggest that purple sulfur bacteria in Lake Arcas are light limited, even though they possess okenone, which can efficiently harvest light at the wavelengths penetrating to the chemocline. High rates of carbon photoassimilation by phototrophic bacteria were measured (up to 200 mg C m^{-3} h^{-1}), but because of the narrow depth range in which anoxygenic photosynthesis occur, bacterial contribution to overall primary production during summer was estimated to be only 12–13%.

Introduction

Many freshwater ecosystems form spatially ordered structures, where growth of microorganisms is influenced by gradients of biologically active substances and physical parameters such as temperature, density, light, pressure and ionic strength. In karstic sulfated freshwater systems, sulfate reduction is the main mechanism of anaerobic respiration (Miracle et al., 1992; Van Gemerden and Mas, 1995). This

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process releases sulfide in high amounts, which accumulates during summer if the hypolimnion is anoxic. At the oxic-anoxic boundary sharp gradients of environmental factors are found in a narrow depth range. At these interfaces, dense populations of phototrophic sulfur bacteria can be found if sufficient light is available to sustain a positive net production (Van Gemerden and Mas, 1995). These bacteria can use sulfide as electron donor for anoxygenic photosynthesis. Light and sulfide are the main factors determining both the vertical distribution and photosynthetic activity of phototrophic bacteria (Parkin and Brock, 1980; Van Gemerden and Mas, 1995; Vila et al., 1996, 1998). Because of the low light availability at the oxic-anoxic boundary where phototrophic bacteria are most abundant, they possess light-harvesting complexes that can collect the dim light penetrating to this depth (Van Gemerden and Mas, 1995; Fischer et al., 1996; Schanz et al., 1998).

In this work we have studied the spatio-temporal distribution and growth dynamics of phototrophic bacterial populations in Lake Arcas in relation to vertical gradients of physical and chemical parameters. Bacterial populations in Lake Arcas are among the densest and most rapidly growing deep populations of the purple sulfur bacteria *Chromatium* that have been described so far (Van Gemerden and Mas, 1995). We determined population densities and growth rates of phototrophic bacteria, the frequency of dividing cells (FDC) as an additional measure of growth (Newell and Christian, 1981), carbon photoassimilation and light-harvesting pigment concentrations and composition.

Material and methods

Study site

Lake Arcas is a small karstic lake (45 m diameter, 14.2 m maximum depth) located in Cuenca (Central Spain). The central part of the lake contains a depression of 4 m in diameter and 2 m deep. The karst has been formed on gypsum bedrock, resulting in high sulfate concentrations (c a. 15 mM) of the lake water. Biogenically produced sulfide accumulates in the hypolimnion during the summer stratification period. Meteorological data were obtained from the weather station of Cuenca, located less than 5 km from the lake.

Sampling

A survey on the limnology of Lake Arcas, including the determination of physical and chemical variables (temperature, light profiles, dissolved oxygen, sulfide, water mineralisation, inorganic nutrients, etc.) and the study of plankton dynamics (especially the development of phototrophic microbial populations) was performed from September 1989 to January 1992. Sampling was conducted monthly during mixis and every two weeks during stratification. An improved version of a fine-layer sampler described by Jørgensen et al. (1979) was used to collect water samples at depth intervals as close as 10 cm within the chemocline. Wires crossing the lake were used to secure the boat to ensure minimal movement during sampling. Samp-

ling was always completed within the two hours around noon in order to avoid the influence of diel changes in the distribution of phototrophic microorganisms.

Physical and chemical variables

Some physical and chemical variables were determined *in situ* by using the appropriate sensors. Dissolved oxygen was determined with a portable WTW Oxi91 field oxymeter. Conductivity was measured with a WTW LF-191 conductivity meter, and its temperature probe was used to obtain temperature profiles. Eh and pH were also determined *in situ* with the respective electrodes placed in a flow-through chamber receiving water pumped directly from the sampling depth (Miracle et al., 1992). Inorganic nitrogen and phosphorus compounds were determined according to Golterman et al. (1978). Soluble sulfide was determined by the colorimetric method described by Cline (1969), the reagent was added immediately after sample collection and subsequent spectrophotometric measurements were performed in the laboratory.

Identification and counting of phototrophic bacteria

Phototrophic sulfur bacteria were identified according to Bergey's Manual of Systematic Bacteriology (Staley et al., 1989) following morphological and biochemical criteria. *Amoebobacter* cf. *purpureus* and *Thiocapsa* sp. were additionally assigned to their respective taxa following Eichler and Pfennig (1988) and Guyoneaud et al. (1998). These bacteria were easily differentiated under the light microscope because of the larger diameter of *A*. cf. *purpureus* and the gas vesicles shown by many of the *A*. cf. *purpureus* cells. Although the species *Pelodictyon clathratiforme* was recently proposed to be transferred to the genus *Chlorobium* because of similarities in 16S rDNA sequences (Imhoff, 1999), the classical species assignment is maintained here. Phototrophic sulfur bacteria were counted at 1250× with a Zeiss III phase contrast microscope on dried cellulose acetate filters (0.2 μm pore diameter) after treatment with erythrosine (Jones, 1979). Total bacterial counts (not including phototrophic bacteria) were made by epifluorescence microscopy on 0.2 μm black membrane filters after filtering 2 ml of sample and staining with DAPI (4′,6-diamidino-2-phenylindole) at a final concentration of 0.0013 μg ml^{-1}. Three hundred individual cells were measured in order to estimate biovolumes. The frequency of dividing cells (FDC) of *Chromatium weissei* was calculated by determining the cell phase in a minimum of 500 cells per sample. A cell was counted as dividing if a clear invagination of the cell wall could be seen, but not a clear separatory space between the daughter cells (Newell and Christian, 1981).

Light, photosynthetic pigments and photosynthesis

Photosynthetically active radiation (PAR, 400–700 nm) was measured in the vertical profile with a 4π scalar irradiance sensor (Li-Cor LI-193SA). A Li-Cor 1800UW underwater spectroradiometer was used to determine the spectral composition of

the light at different depths during the stratification period (Vila et al., 1996). Pigments were extracted with acetone: DMSO (1:1 v:v) (Shoaf and Lium 1976) after filtration of samples through Whatman GF/F glass fibre filters. The absorbance of the extract was determined spectrophotometrically and the value at 772 nm was used to calculate Bchl *a* concentration. HPLC pigment analyses were performed occasionally during the stratification period on 100% acetone extracts as described by Borrego and Garcia-Gil (1994).

In situ carbon photoassimilation was measured by the ^{14}C method as described in Camacho and Vicente (1998). Replicate water samples from the chemocline were incubated by using a floating hanger designed to place the samples at the incubation depth. Cylinders, one meter in length and made of meta acrylate, with adapters for up to eight bottles located at 10 cm depth intervals, were suspended from the hanger at exactly measured depths. Bottles were filled completely to avoid air bubbles. Replicate bottles were either left untreated or were treated with DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea; 10 μM final concentration) before incubation *in situ*. DCMU was used to inhibit PSII, so that oxygenic and anoxygenic photoassimilation could be distinguished. Additionally, two dark bottles and a dark bottle treated with formalin were assayed for inorganic ^{14}C assimilation at each depth. DCMU-treated bottles corrected for dark assimilation allowed for the estimation of anoxygenic photosynthesis by sulfur bacteria.

Calculation of population parameters

Bacterial population densities per m^2 of lake area were calculated by summing the number of bacterial cells in each depth layer of the water column. Data were also used to calculate the total population size of phototrophic sulfur bacteria by considering the lake hypsography. The entire lake population was used to calculate net growth rates, assuming exponential growth (Pedrós-Alió et al., 1987).

Results

Lake Arcas exhibited a thermal stratification from April to October. A sulfide-rich anoxic hypolimnion was found between the deeper part of the thermocline (8.5–9 m) and the lake bottom. Sulfide concentrations exceeded 2 mM near the bottom during certain periods. However, sulfide concentrations at the chemocline, where phototrophic bacteria reached their maximal population densities, ranged from undetectable to 0.4 mM. Inorganic nitrogen was fairly abundant. Ammonium concentrations during the stratification period ranged 10–100 μM in the epilimnion and 200–1000 μM in the hypolimnion, and 50–150 μM during the mixing period. Nitrate was found at concentrations of 15–30 μM during mixis, and 10–20 μM in the epilimnetic waters during the stratification period. Nitrite concentrations were under the detection limit of 0.3 μM. Phosphorus availability was also low in the oxic waters, with soluble reactive phosphorus (SRP) concentrations often less than 0.03 μM in the epilimnion. In contrast, SRP concentrations in the anoxic hypolimnion were usually between 0.1 and 1 μM. Inorganic N:P ratios were always higher

than 100, and usually ranged from 500–3000. In spite of the low SRP availability, maximal total phosphorus (TP) concentrations were found at the chemocline. Phototrophic microorganisms were the main phosphorus reservoir at these depths, since heterotrophic bacterial biovolume was 10-times lower than that of phototrophic microorganisms (data not shown). Figure 1 shows a typical profile of physical and chemical variables during the summer stratification period. Strong gradients of these variables occur at the oxic-anoxic boundary and the term "chemocline" is used in this article when referring to this zone of the vertical profile. In addition to the summer stratification period, anoxia with sulfide concentrations near 0.1 mM also occurred occasionally in the central depression in February and March 1990 when high air temperatures coincided with still and rainless periods. However, oxic conditions were rapidly re-established when weather conditions changed.

Chromatium weissei, a large (ca. 8 µm long, 4 µm wide) purple sulfur bacterium was the dominant phototrophic bacterium. Cell densities of these bacteria showed a steep gradient at the chemocline (Figs. 2 A and 3). Highest population densities of *C. weissei* were found during stratification (Fig. 4), with maxima at the chemocline (up to 1.84×10^6 cells ml^{-1}), although these bacteria were present throughout the anoxic hypolimnion. This presence in deep waters may have been a result of sedimentation, even though dividing cells were also observed in the hypolimnion. This explanation remains tentative because sedimentation rates were not determined. Phototrophic bacteria were also observed during short periods in winter, when anoxic conditions occurred in the central depression. During these periods, the population densities of *C. weissei* did not reach high levels (about 10^4 cell ml^{-1} and less

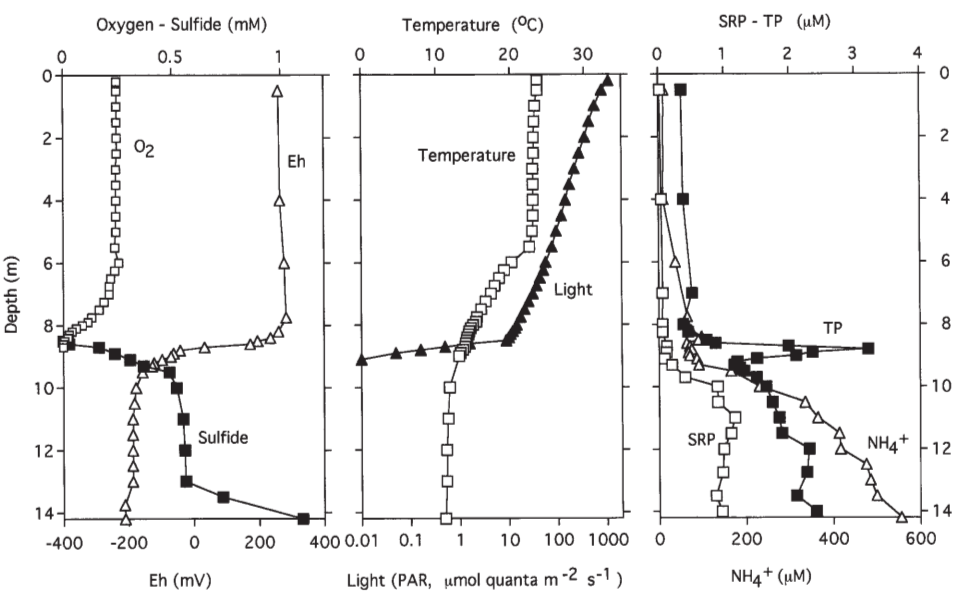
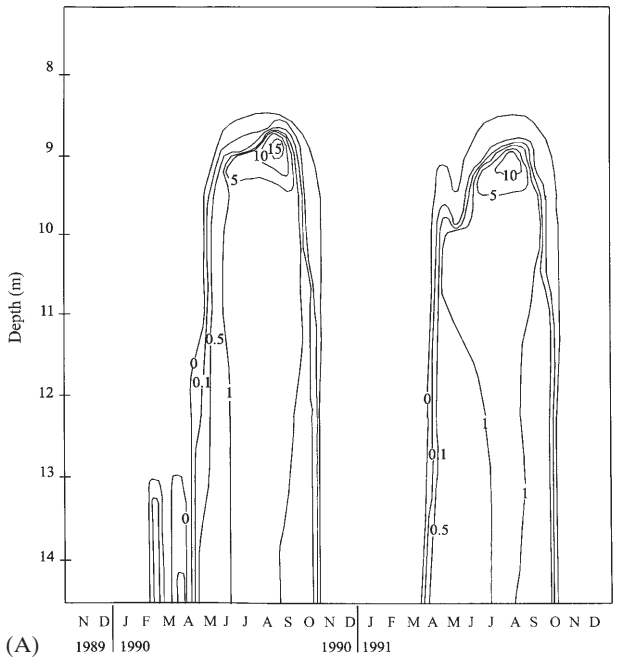


Figure 1. Vertical profiles of oxygen and sulfide, redox potential (Eh), temperature, photosynthetic active radiation, soluble reactive phosphorus, total phosphorus and ammonium in Lake Arcas, in August 1990



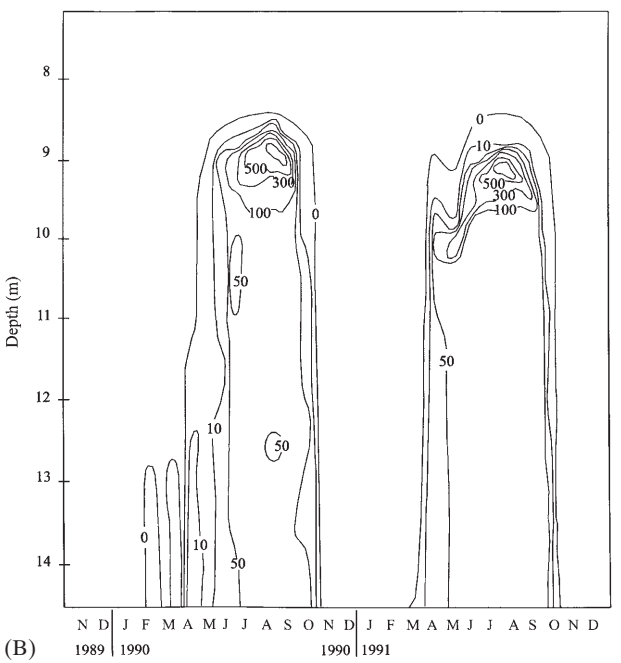


Figure 2. Isopleths of the (A) distribution of *Chromatium weissei* (cells $ml^{-1} \times 10^5$) and (B) bacteriochlorophyll *a* concentrations ($\mu g\ l^{-1}$) in Lake Arcas from October 1989 to December 1991

Table 1. Relative biovolume of the four dominant phototrophic sulfur bacteria found in Lake Arcas. Mean values for 1990 and 1991

	% of total biovolume of	
	Chemocline	Chemocline and hypolimnion
Chromatium weissei	96.48	98.01
Amoebobacter purpureus	1.42	1.61
Thiocapsa sp.	2.09	0.35
Pelodictyon clathratiforme	0.005	0.03

than 5 μg l^{-1} of bacteriochlorophyll *a*). Smaller phototrophic sulfur bacteria such as *Amoebobacter* cf. *purpureus*, *Thiocapsa* sp., and *Pelodictyon clathratiforme* were also present in the anoxic hypolimnion of the lake. However their numbers and biovolumes were much lower than those of *C. weissei* (Table 1), especially those of P. *clathratiforme*, which appeared only occasionally. *Thiocapsa* sp. was mainly located at the oxic-anoxic boundary, whereas *A.* cf. *purpureus* and *P. clathratiforme* occurred throughout the hypolimnion with no clear maximum at any depth (Fig. 3).

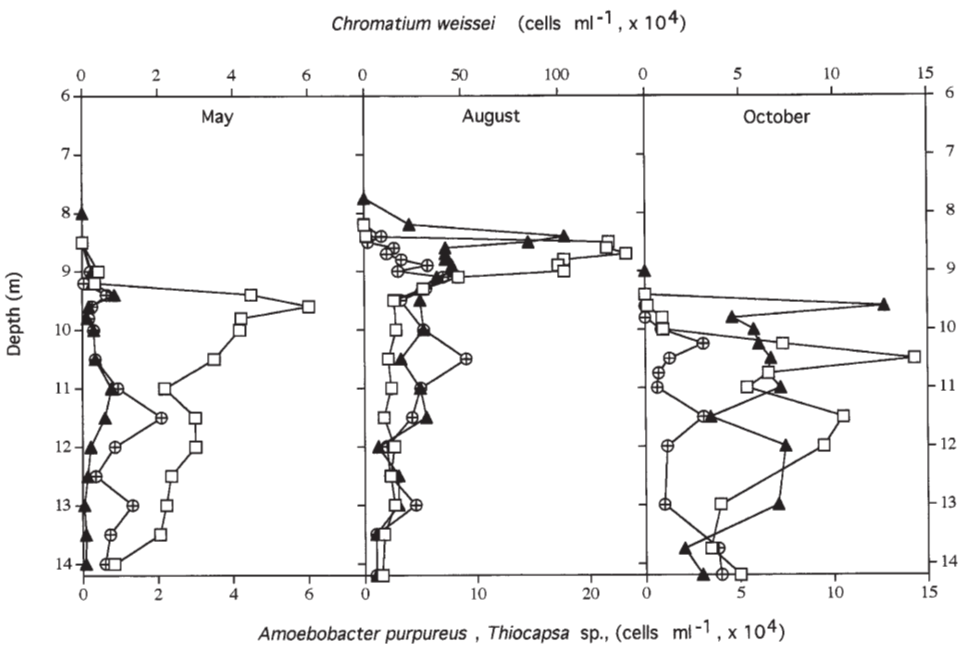


Figure 3. Vertical distribution of the dominant phototrophic bacterial species in Lake Arcas at the beginning, middle, and end of the 1990 stratification period. *Chromatium weissei* (□), *Amoebobacter* cf. *purpureus* (⊕) and *Thiocapsa* sp. (▲). Note the differences in scale for the different months

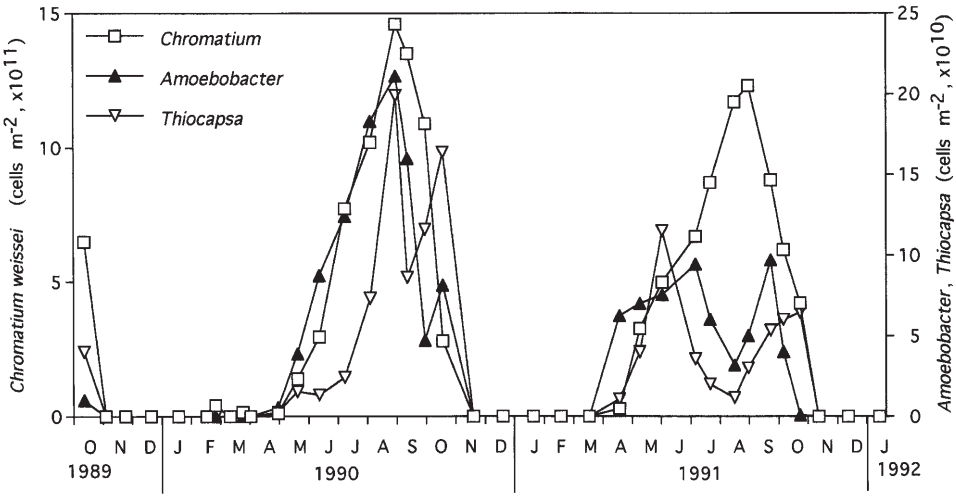


Figure 4. Numbers of *Chromatium weissei*, *Amoebobacter* cf. *purpureus*, and *Thiocapsa* sp. per lake surface area between October 1989 and January 1992

Net growth rates of *C. weissei* ranged from 0.125 to –0.123 d^{-1}. Highest rates were observed in spring soon after anoxic conditions had established (Fig. 5). Growth rates remained positive until the end of August, but became negative as mixing approached in autumn. *C. weissei* disappeared after mixis. *A.* cf. *purpureus* showed a similar pattern as *C. weissei* in 1990, but not in 1991, whereas changes in growth rates of *Thiocapsa* sp. and *P. clathratiforme* were somewhat erratic (data not shown). High frequencies of dividing cells (FDC) of up to 60% occurred in winter

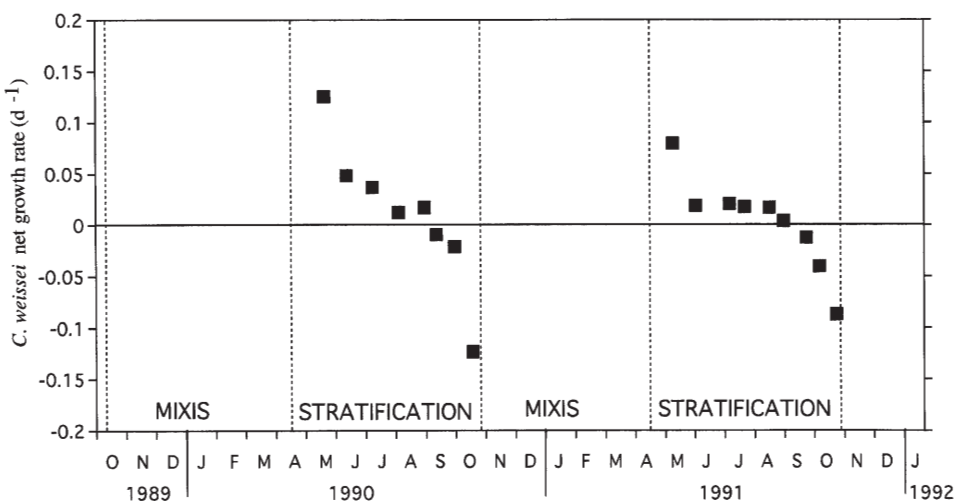


Figure 5. Net growth rates of *Chromatium weissei* in Lake Arcas, calculated for the entire lake population, between October 1989 and January 1992

1990 during the short periods of anoxia in the central depression of the lake, as well as during stratification in spring and early summer (Fig. 6). This result is in accordance with the rapid development of the *C. weissei* population during these periods. Subsequently, FDC decreased with the lowest values of 15–20% being recorded in the last weeks of stratification. FDC were always lower in the hypolimnion than at the chemocline, especially at the end of summer. As expected, temporal pattern of *C. weissei* matched that of net growth rates.

Purple sulfur bacteria from Lake Arcas possess bacteriochlorophyll *a* (Bchl a). This pigment was absent from the water column during the mixing period, but reached high concentrations during the stratification period in the upper part of the anoxic layers, where the population of *C. weissei* was densest (Fig. 2B). Bchl *a* distribution paralleled that of *C. weissei* cell density. Maximum Bchl *a* concentrations of about 700 μg l^{-1} were reached at the chemocline, with much lower values (50–100 μg l^{-1}) in the dark anoxic waters. All purple sulfur bacteria isolated from Lake Arcas contained okenone (Okn), which was the only purple bacterial carotenoid detected by HPLC. Okn/Bchl *a* molar ratios were slightly higher at the upper part of the chemocline (mean values of 0.77) and decreased with depth to values around 0.5. The green sulfur bacterium *P. clathratiforme* contained chlorobactene as its main carotenoid.

During summer stagnation light availability at the chemocline depth (8–9 m) was higher than during the rest of the year at the same depth, corresponding to lower epilimnetic phytoplankton densities in summer. However, a maximum of 0.5–1% of surface irradiance reached this depth (Fig. 1). Spectroradiometric determinations demonstrated that more than 70% of the photons available to the phototrophic bacteria at the chemocline were from the yellow-green range of the light spectrum.

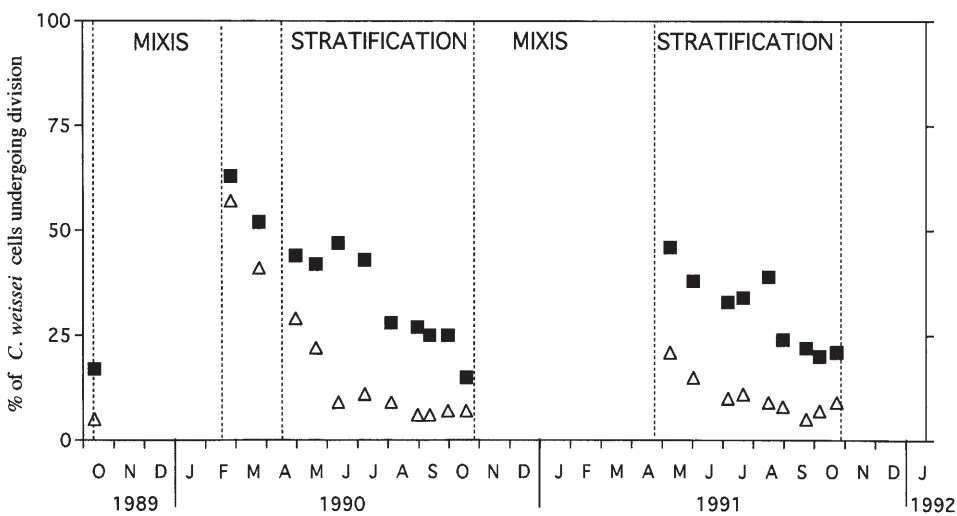


Figure 6. Frequency of dividing cells (%) in *Chromatium weissei* in the chemocline (■) and hypolimnetic waters (Δ) of Lake Arcas, between October 1989 and January 1992. Data included in the mixing period of 1990 correspond to the short periods of anoxia in the central depression of the lake

Table 2. Main features of the ^{14}C photoassimilation experiment carried out in September 20, 1991. Mean irradiance values during the incubation and standard deviations (±) are shown

Sample depth (m)	Oxygen (mM)	Sulfide (mM)	Chl *a* (mg m^{-3})	Bchl *a* (mg m^{-3})	Mean irradiance at depth (µmol photon m^{-2} s^{-1})	^{14}C Oxygenic photoasimilation (mg C m^{-3} h^{-1})	^{14}C Anoxygenic photoasimilation (mg C m^{-3} h^{-1})
0.5	0.22	0	2.6	0	796 ± 22	4.7	0
3	0.22	0	3.3	0	232.7 ± 6.7	10.4	0
7	0.22	0	3.6	0	43.6 ± 1.2	6.6	0
9.1	0.06	0	7.8	6.0	10.26 ± 0.27	9.2	0
9.2	0	0.08	119.1	62.9	6.85 ± 0.14	113.7	18.8
9.3	0	0.10	107.0	381.3	3.93 ± 0.10	6.8	190.8
9.4	0	0.14	59.6	206.0	1.33 ± 0.07	0.3	14.7
9.5	0	0.22	35.4	130.7	0.62 ± 0.04	0	12.5
9.8	0	0.83	19.3	68.8	0.19 ± 0.006	0	0

Table 2 shows the results of one of several *in situ* $^{14}CO_2$-photoassimilation experiments. The maximal rates of anoxygenic carbon fixation at the chemocline exceeded rates of oxygenic assimilation, accounting for 12–13% of the integrated photosynthetic carbon photoassimilation in the lake during the summer time. This relatively small percentage is due to the extremely narrow depth range in which photosynthesis by the phototrophic bacteria occurred. Oxygenic photoassimilation was observed at the chemocline, mainly due to *Oscillatoria* cf. *ornata* (Camacho et al., 1996, 2000) and *Cryptomonas* spp, accounting for more than 6% of the integrated summer carbon photoassimilation in the entire lake. When water samples from the chemocline were incubated 30 cm above, anoxygenic carbon photoassimilation increased by nearly 400%, indicating that light rather than sulfide availability controlled anoxygenic photosynthesis. Nevertheless, high assimilation numbers of up to 0.5 mg C (mg Bchl *a*)$^{-1}$ h^{-1} were calculated in late August, when phototrophic bacterial populations reached their maximal development.

Discussion

The population density of *Chromatium* found in Lake Arcas is one of the highest observed for this genus (see compilation in Van Gemerden and Mas 1995). Other *Chromatium* populations reaching even higher population densities were found in shallower waters with higher light availability. Several factors can explain the dominance of *Chromatium weissei* over other purple sulfur bacteria in Lake Arcas. According to Van Gemerden (1974), large *Chromatium* species dominate at the chemocline of stratified lakes due to their ability to oxidise sulfide into elemental sulfur that is subsequently stored intracellularly. This sulfur can later act as electron donor when photooxidation depletes sulfide over the course of the day (Veldkamp et al., 1984). In contrast, small purple bacteria oxidise sulfide directly to sulfate, and therefore lack this temporary reservoir of sulfur as electron donor. The conditions found in the upper part of the chemocline of Lake Arcas, with relatively low sulfide concentrations (< 0.1 mM) decreasing during the day to undetectable levels (Camacho et al., 2000) are thus advantageous to the larger *Chromatium* species. Flagellar motility may be another important advantage for *C. weissei*, allowing a faster response to directional stimuli, such as sulfide or light availability, than buoyancy regulation by either gas vesicles or changes in carbohydrate content. Phototrophic sulfur bacteria other than *C. weissei* exhibited much lower population densities and represented a very small part of the phototrophic bacterial biomass in Lake Arcas (Table 1). Since neither *Amoebobacter purpureus* nor *Pelodictyon clathratiforme* possess flagella, their response to diel changes in sulfide availability in the upper part of the chemocline is limited. This may represent a competitive disadvantage in relation to the dominant *C. weissei*. The presence of a dense plate of oxygenic phototrophs (Camacho et al., 2000) and purple bacteria at the chemocline of Lake Arcas is probably the reason why the development of dense green sulfur bacteria populations in deeper layers is prevented, because the multilayered phototrophic community at the chemocline absorbs most of the photosynthetically active radiation.

Maximal population densities of *Thiocapsa* sp. were found at the upper part of the chemocline. Since this organism lacks mechanisms to regulate its position in the

water column (Imhoff, 1992), photosynthetically supported growth of *Thiocapsa* sp. is limited to a few hours of the day when sulfide is available. Oxic conditions prevail several hours in the afternoon (Camacho et al., 2000), resulting in the lack of the electron donor for the anoxygenic photosynthesis of *Thiocapsa*. However, light availability is much higher at the top of the chemocline than in deeper waters. Then, *Thiocapsa* may fix carbon avoiding light limitation during the first hours of the day, when sulfide has not yet been depleted and light availability is sufficient. The presence in microaerobic waters during a part of the day should not be a problem for these bacteria, since some species of this genus have been described to possess metabolic capacities that allow them to survive in aerobic environments. These possibilities include chemoorganotrophy (Kondratieva et al., 1976), chemolithotrophy (De Wit and Van Gemerden, 1990; Overmann and Pfennig, 1992) or even phototrophy (Schaub and Van Gemerden, 1994). Although a prolonged presence in oxic conditions results in an inhibition of Bchl *a* synthesis (De Wit and Van Gemerden, 1990; Imhoff, 1992), the *Thiocapsa* population of Lake Arcas overcomes this problem, since the diel shifts of the oxic-anoxic interface prevent a long exposure to oxic waters. The ability of anaerobic microorganisms to withstand in the presence of oxygen during a limited time should be an advantageous feature in systems like the chemocline of stratified lakes (e.g. Lake Arcas), where diel changes in oxygen and sulfide concentrations occur. In contrast to these metabolic capabilities of *Thiocapsa*, chemolithotrophic growth has never been found in large species of *Chromatium* (Overmann and Pfennig, 1992) and oxygen is lethal for the later (Van Gemerden and Mas, 1995), highlighting the different ecological strategies followed by *C. weissei* and *Thiocapsa* sp.

The growth rate of the *C. weissei* population is higher in the beginning of the stratification period, whereas during the last weeks of the stagnation period the decrease in population density is quite rapid. Net growth rates of 0.0365 to –0.0719 d^{-1} were previously calculated for natural populations of *Chromatium minus* in Lake Cisó (Mas et al., 1990), and mean values of 0.05 d^{-1} have been reported from Lago di Cadagno for *Chromatium okenii* (Schanz et al., 1998). Both studies thus found lower growth rates than those observed for *C. weissei* in Lake Arcas (0.125 to –0.123 d^{-1}). These differences may be explained by the temporal stability of anoxic conditions throughout the year. Lake Cisó permanently shows anoxic conditions either in the hypolimnion during thermal stratification or in the entire lake during holomixis, whereas Lago di Cadagno is a meromictic lake with a permanently anoxic monimolimnion. The permanence of anoxic conditions in these lakes allows for the maintenance of phototrophic bacteria during the whole year and part of the population may overcome adverse conditions with low growth and loss rates. In contrast, in Lake Arcas these populations disappear after the autumnal mixis, when oxic conditions prevail through the water column. The withdrawal of phototrophic bacteria from the water column is a consequence of an aggregation mechanism (Van Gemerden and Mas, 1995). Bacterial aggregates, commonly observed in Lake Arcas immediately after mixing, reach rapidly the sediment and serve as an inoculum for the development of future populations. Sulfide can play a role in disaggregation in order to develop a new population when anoxic conditions are re-established (Overmann 1997). This may explain the observed winter development of *C. weissei* close to the lake bottom during short periods of

physical stability and anoxic conditions (Fig. 2). When these populations are in the early stages of development sulfide is abundant and light availability is high because self-shading does not yet occur. Consequently, growth rates can be high. Although the growth rates determined in the present study are relatively high for natural systems, they are much lower than those measured under optimal laboratory conditions, which ranged from 1.2 to 3.3 d^{-1} (Van Gemerden, 1984). The growth of *C. weissei* was evidently limited by factors, such as light availability for the population at the chemocline of Lake Arcas.

The frequency of dividing cells (FDC) in *C. weissei* was much higher than is usually found in heterotrophic bacteria. The use of equations proposed for the calculation of growth rates from FDC in heterotrophic bacteria (Hagström et al., 1979; Newell and Christian, 1981) would yield unreasonably high values. Therefore, these equations could not be used here; calibrations against growth rates determined in pure culture are thus required. The high FDC values could be due to the differences, among phototrophic and heterotrophic bacteria, in the duration of cell division, as well as to the large size of *Chromatium*, which allowed us to distinguish early phases of division. Nevertheless, FDC were always much higher at the chemocline than in hypolimnetic waters, indicating that growth of the *C. weissei* population mainly occurred in the bacterial plate where light availability was sufficient to allow a positive net photosynthesis (Van Gemerden et al.,1985). Although a portion of the *Chromatium* cells in the deeper part of the hypolimnion were found to be dividing, this does not necessarily imply active growth, because cell divisions may have begun in the upper layers and continued or stopped as the cells settled.

The contribution of phototrophic bacteria to overall primary production in Lake Arcas is relatively small (Camacho and Vicente, 1998). However, lakes where the contribution of sulfur bacteria to total production is higher usually present shallower populations (Van Gemerden and Mas, 1995) receiving a mean of 5–10% of surface irradiance, in contrast with the 10 times lower light availability in Lake Arcas. Light rather than sulfide availability limits photosynthesis of *C. weissei* in Lake Arcas, as demonstrated by the much higher photosynthetic rates of samples from the chemocline incubated above the bacterial plate. This limitation is commonly found for the dominant species of phototrophic sulfur bacteria (Parkin and Brock, 1980; Guerrero et al., 1985). Nevertheless, metalimnetic populations of Lake Arcas are relatively well adapted to the light climate they experience at the chemocline. This is demonstrated by P vs I curves, which determined an I_k value of 12 $\mu E\ m^{-2}\ s^{-1}$ (Camacho and Vicente, 1998), not much higher than light availability at the upper part of the chemocline. Most photons available at the depth where phototrophic bacteria develop are from the yellow-green range of the spectrum due to the selective absorption by the overlying water and phytoplankton (Fischer et al., 1996; Camacho et al., 2000). Like most purple bacteria in stratified lakes (Van Gemerden and Mas, 1995; Overmann, 1997), all the Chromatiaceae isolated from Lake Arcas contained okenone as the major carotenoid, which enables these bacteria to efficiently harvest a major fraction of the photons available at the chemocline (Imhoff, 1992).

Carbon fixed by phototrophic bacteria may be important for the maintenance of the food web in the microaerobic and anaerobic waters of the lake. Although phagotrophy of phototrophic bacteria by oxyclinal rotifer species and anaerobic

ciliates is not very important in Lake Arcas (Miracle and Armengol-Diaz, 1995; Guhl et al., 1996), the role of purple bacteria as a source of organic carbon for heterotrophic microorganisms is not limited to particulate material. Dissolved organic carbon (DOC) reach maximal values at the chemocline (Finlay et al., 1991). Most of this DOC may be excreted by phototrophic bacteria (Overmann, 1997) whereas organic carbon derived from phototrophs after cell death may be an additional carbon source, allowing for the better development of heterotrophic bacteria which are an important food source for anaerobic ciliates (Guhl et al., 1996). Moreover, the demand of organic matter for sulfate reduction can be satisfied in part by the carbon assimilated at the chemocline. This supposes a link in the processes of photosynthetic oxidation of sulfide and its production via sulfate reduction (Cohen, 1986; Overmann et al., 1991). Oxygenically fixed or allochthonous organic matter can also be used for sulfide production (Overmann, 1997), which implies that sulfide-driven anoxygenic photosynthesis could be considered as a way of recycling primary production.

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