

Photoheterotrophy and light-dependent uptake of organic and organic nitrogenous compounds by *Planktothrix rubescens* under low irradiance

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SUMMARY

1. *Planktothrix rubescens* is the dominant photoautotrophic organism in Lake Zürich, a prealpine, deep, mesotrophic freshwater lake with an oxic hypolimnion. Over long periods of the year, *P. rubescens* accumulates at the metalimnion and growth occurs *in situ* at irradiance near the photosynthesis compensation point. Experiments were conducted to evaluate the contribution of photoheterotrophy, heterotrophy and light-dependent uptake of nitrogenous organic compounds to the carbon and nitrogen budget of this cyanobacterium under conditions of restricted availability of light quanta.

2. We used both purified natural populations of *P. rubescens* from the depth of 9 m and an axenic culture grown under low irradiance at $11 \mu\text{mol m}^{-2} \text{s}^{-1}$ on a light : dark cycle (10 : 14 h) to determine the uptake rates of various amino acids, urea, glucose, fructose, acetate and inorganic carbon. The components were added to artificial lake water in low amounts that simulated the naturally occurring potential concentrations.

3. The uptake rates of acetate and amino acids (glycine, serine, glutamate and aspartate) were strongly enhanced at low irradiance as compared with the dark. However, no difference was observed in the uptake of arginine, which was taken up at high rates under both treatments. The uptake rates of glucose, fructose and urea were very low under all conditions. Similar results were obtained for both axenic *P. rubescens* and for purified natural populations of *P. rubescens* that were separated from bacterioplankton and other phytoplankton.

4. Metalimnetic *P. rubescens* that was stratified at low irradiance for weeks exhibited much higher uptake rates than filaments that were entrained in the deepening surface mixed layer and experienced higher irradiance. The added organic compounds contributed up to 62% to the total carbon uptake of metalimnetic *P. rubescens*. On the basis of a molar C : N ratio of 4.9, the nitrogen uptake as organic compounds satisfied up to 84% of the nitrogen demand.

5. The experiments indicate that photoheterotrophy and light-dependent uptake of nitrogenous organic compounds may contribute significantly to the carbon and nitrogen budget of filaments at low irradiance typical for growth of *P. rubescens* in the metalimnion and at the bottom of the surface mixed layer.

Keywords: amino acid uptake, carbon and nitrogen budget, low irradiance, metalimnion, photoheterotrophy, *Planktothrix rubescens*

Introduction

Deep chlorophyll maxima are widely distributed in marine, freshwater and saline aquatic ecosystems and indicate the uneven distribution of photosynthetically

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active microorganisms within the water column. Areas where marine deep chlorophyll maxima have been reported include the Pacific Ocean (Anderson, 1969), English Channel (Sharples *et al.*, 2001) and Mediterranean Sea (Dolan & Marrasé, 1995). In limnetic waters, they have been observed in saline (Gaevsky, Zotina & Gorbaneva, 2002) and freshwater lakes with oxic (Tilzer, Paerl & Goldman, 1977; Konopka, 1982; Abbott *et al.*, 1984; Lindholm, 1992; Zhou *et al.*, 2001) and anoxic hypolimnia (Reynolds, Tundisi & Hino, 1983; Pick, Nalewajko & Lean, 1984; Gasol, Guerrero & Pedrós-Alió, 1992; Adrian, Wickham & Butler, 2001). With few exceptions, the deep chlorophyll maxima were located in the zone covered by the metalimnion.

Mass developments of *Planktothrix rubescens* take place every year in Lake Zürich, Switzerland, and most of the population grows or accumulates in the metalimnion for long periods of the year. The same organism and similar behaviour is found in many other mesotrophic prealpine lakes. The toxicity of *P. rubescens* (Blom, Robinson & Jüttner, 2001) and the extensive use of water from these lakes warrant better understanding of the biology of this cyanobacterium and its prerequisites for growth and stratification in lakes. Previous investigations conducted in Lake Zürich (Micheletti, Schanz & Walsby, 1998; Bright & Walsby, 2000; Walsby & Schanz, 2002) clearly demonstrated that *P. rubescens* accumulates at the deep chlorophyll maximum primarily by active growth in the metalimnion rather than by recruitment through sinking or floating of filaments, although such movements do contribute to the formation of the spring maximum and the beginning of the summer population in the metalimnion. Quantitative analysis shows that the calculated growth rate at the measured irradiance is often sufficient to account for the increase in the population (Walsby & Schanz, 2002). However, any loss caused by crustacean and protozoan grazers and attack by cyanophages would necessitate a higher growth rate to explain the observed balance of calculated and measured increase of *Planktothrix*.

The energetic conditions for active growth in the metalimnion are fundamentally different from those that dominate in higher layers of the euphotic zone. As a consequence of light attenuation, growth of *P. rubescens* takes place in the metalimnion near the photosynthesis compensation point. Fluctuating

weather conditions and internal waves give rise to alternating periods of photosynthesis and respiration (Walsby *et al.*, 2001). To deal with these conditions, an economic use of energy is of high priority for a phototrophic organism. As growth of *P. rubescens* on various substrates was unsuccessful in the dark (Feuillade & Feuillade, 1989), this cyanobacterium was considered not to be a heterotroph. However, this observation does not rule out that heterotrophy and/or photoheterotrophy may contribute to the cellular requirements for reduced carbon. In addition, uptake of nitrogenous organic compounds may contribute to the demand for reduced nitrogen. Such mechanisms would improve the energy budget in periods when light availability is around the photosynthesis compensation point.

Several strategies have been used to determine whether cyanobacteria are obligate photoautotrophs or photoheterotrophs. Photoheterotrophy of cyanobacteria was demonstrated by growth on glucose-containing media in the presence of 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU) (Rippka, 1972) and the ability to take up organic compounds in the presence of DCMU (Paerl, 1991). The addition of the specific PS II inhibitor DCMU inhibits the photosynthetic electron flow in PS II and stops the carbon dioxide incorporation mediated by the reductive pentose phosphate cycle. A further method is the comparison of growth rates at low irradiances on mineral media and media supplemented with organic substrates (Feuillade & Feuillade, 1989).

In the present study, we performed experiments to show to what extent *P. rubescens* can utilise photoheterotrophy, heterotrophy and light-dependent uptake of nitrogenous organic compounds to meet the C and N demand when irradiance is either around the photosynthesis compensation point or not available.

Methods

Study site

Lake Zürich, Switzerland, is a prealpine mesotrophic lake with a maximum depth of 136 m. The filamentous cyanobacterium, *P. rubescens*, is a major component of the phytoplankton throughout the year. During the second half of the year, when a particularly strong development occurs, it constitutes more than 80% of the biomass. *Planktothrix rubescens* is

frequently stratified and responsible for the deep chlorophyll layer that develops in the metalimnion (Walsby, Avery & Schanz, 1998). Experiments were performed mid-lake at a depth of 9 m; the chemical environmental factors of the lake water at this depth on 9 November 2001 were: $29 \pm 3.2 \mu\text{M NO}_3^-$, $0.29 \pm 0.003 \mu\text{M NO}_2^-$, $0.34 \pm 0.042 \mu\text{M NH}_4^+$, $87 \pm 1.6 \mu\text{M Cl}^-$, $131 \pm 4.0 \mu\text{M SO}_4^{2-}$ (means \pm SD, $n = 3$) and $2500 \mu\text{M HCO}_3^-/\text{CO}_2$.

Sampling of lake water

Lake water was obtained from Lake Zürich 300–400 m offshore near the Limnological Station. The samples were removed from a depth of 9 m through a black tube (Viton, 5 mm i.d., neoLab, Heidelberg, Germany) with a battery-powered peristaltic pump (easy load head, model 7529-00, Masterflex). The flow rate was 2.5 L min^{-1} . The lake water was stored in light-protected, black plastic bottles after passage through a net (240 μm mesh size) to remove the macrozooplankton. Before each sampling, the water of the tube was replaced with two tube volumes of lake water from the particular sampling depth.

Determination of environmental factors

Depth profiles of temperature and oxygen concentration were measured with electrodes (model Oxi 197, WTW, Weinheim, Germany). Photon irradiance (PAR, photosynthetic active radiation) was determined with a spherical quantum sensor (SPQA 1178; LI-193 SB; Li-Cor, Nebraska, NB, U.S.A.). Vertical light attenuation coefficients were calculated from these data for every *in situ* incubation experiment. Incident irradiance data were obtained from the Swiss Meteorological Station No 44 at Zürich-SMA (47°23'N; 8°34'E; 556 m a.s.l.) as W m^{-2} in 10-min intervals. The concentrations of nitrate, chloride and sulfate were determined by ion chromatography (Ionospher 5 A, $250 \times 4.6 \text{ mm}$, Chromsep, Varian; isocratic 4 mM potassium hydrogen phthalate pH 4.5, 2 mL min^{-1}), those of ammonium (Solórzano, 1969) and nitrite (Grasshoff, 1983) by photometry.

Determination of biotic factors

The biovolume concentration of *P. rubescens* was determined by image analysis (epifluorescence micro-

scope Leica DM RXE (Wetzlar, Germany), camera Kappa CF 15 MCC (Gleichen, Germany)). The lengths of filaments, concentrated on membranes (cellulose nitrate, AE 99, 8 μm pore size, Schleicher & Schuell, Dassel, Germany), were automatically calculated from the fluorescence signals as described by Walsby & Avery (1996). A value of $25 \mu\text{m}^2$ was used as a mean cross-sectional area of the filaments (Micheletti *et al.*, 1998). The growth of axenic cultures was measured as absorption change on a spectrophotometer (Spectro 2, Riedel-de Haën, Seelze, Germany) at 570 nm in round cells (16 mm diameter). The values were corrected for scattering by the 750 nm absorption.

Chlorophyll *a* concentrations were determined on a spectrofluorometer (F-2000, Hitachi, Tokyo, Japan). To obtain 90% acetone extracts, filaments were concentrated on glass fibre filters (GF 6, Schleicher & Schuell), ground in a homogeniser (5-mL Potter S, B.Braun Biotech International, Melsungen, Germany) and extracted as described by Schanz (1982). The excitation wavelength was 430 nm, the emission wavelength was 668 nm. Chlorophyll *a* from Fluka (Buchs, Switzerland) served as the standard.

The abundance of bacterioplankton was determined with glutaraldehyde-preserved samples (2% final concentration). The bacteria (10 mL suspension) were stained for 15 min with 300 μL of a DAPI solution (4', 6-diamidino-2-phenylindol-dihydrochloride, 10 $\mu\text{g}/\text{mL}$) and collected on a membrane (0.2 μm pore size, Anodisc 25, Whatman) by filtration. A drop of Citifluor (Citifluor Ltd., London, U.K.) was added and covered with a cover slide. Another drop of Citifluor was added to count the bacteria at $1000 \times$ magnification (PL Fluotar 100, Leica, Wetzlar, Germany) under an epifluorescence microscope.

Artificial lake water

Artificial lake water was used for the incorporation experiments. The concentrations of macroelements of artificial lake water were: NaHCO_3 (2000 μM), KNO_3 (30 μM), $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ (200 μM), $\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ (40 μM) and $\text{CaSO}_4 \times 2 \text{ H}_2\text{O}$ (160 μM). Citric acid, ferric ammonium citrate and other microelements were added in a 1000-fold dilution of the concentrations given for the cyanobacterial medium BG-11 (Rippka *et al.*, 1979). The final concentration of NH_4^+ was 23 nM. The pH was adjusted to 8.0 with H_3PO_4 (final concentration $\approx 9\text{--}18 \mu\text{M}$). Sterilisation was

performed by filtration through a hollow fibre filter (MediaKap-2, 0.2 µm pore size; Microgon, Laguna Hills, CA, U.S.A.). The hollow fibre filters were washed with 1.5 L of purified water ($>18 \text{ M}\Omega \text{ cm}^{-1}$ Nanopure, Dubuque, IA, USA) and 500 mL of artificial lake water before use.

Separation of natural *P. rubescens*

Metalimnetic *P. rubescens* was separated from pelagic bacteria and viruses as far as possible by filtration. Very few bacteria were observed attached to the filaments. *P. rubescens* was concentrated by passing lake water through a cellulose nitrate membrane (50 mm *d*, 8 µm pore size, AE 99, Schleicher & Schuell) under pressure that was reduced by 100 mbar using a hand vacuum pump. The filaments collected on the membrane were washed with 100–150 mL of artificial lake water. The filaments were removed from the membrane by a stream of 5 mL of artificial lake water from an Eppendorf pipette and the obtained suspension was diluted with 40 mL of artificial lake water. This procedure was repeated twice. Finally, the filaments were suspended in 500–800 mL of artificial lake water. The separated *P. rubescens* was relatively free of bacterioplankton, picoplankton and eukaryotic algae. Typical background counts of DAPI positive particles were $1.3 \times 10^3 \text{ mL}^{-1}$ for artificial lake water and $2.1 \times 10^3 \text{ mL}^{-1}$ for purified natural *P. rubescens*, a thousand-fold reduction compared with untreated lake water containing 4×10^6 bacteria mL^{-1} . The separation from other plankton species was easy to achieve because the metalimnetic population of *P. rubescens* was already nearly pure.

For acclimation, unlabelled organic compounds were added to the suspension to give the concentrations, as used in the experiments. This suspension was preincubated in the dark for 30–40 min in a thermostat at 15 °C. All manipulations were done under sterile conditions and dim light (less than $0.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$ emitted from the flame of a Bunsen burner).

In situ incubations

For *in situ* incubation experiments, separated filaments of *P. rubescens* were taken from a population at 9 m depth. The preincubated filaments were concentrated on an 8 µm-membrane, washed with 100–150 mL and

resuspended in 800–900 mL of artificial lake water. This suspension was distributed among ten incubation flasks (65-mL sterile tissue culture polystyrene flasks; Semadeni, Ostermündingen, Switzerland; 25 cm² surface exposed to the downward irradiation; 2.5 cm height). After 30–40 min, the flasks were supplemented with aqueous concentrates that contained all organic compounds investigated (Table 1). However, in each flask, one component was replaced by an equimolar concentration of the corresponding ¹⁴C-labelled compound. Fructose uptake was determined in a mixture in which unlabelled glucose was replaced by ¹⁴C-labelled fructose. The labelled compounds obtained from Amersham Pharmacia Biotech exhibited the specific activities: sodium [¹⁴C] hydrogen carbonate (2.04 GBq mmol⁻¹), L-[U-¹⁴C] serine (5.74 GBq mmol⁻¹), [U-¹⁴C] glycine (3.74 GBq mmol⁻¹), L-[U-¹⁴C] glutamic acid (9.40 GBq mmol⁻¹), L-[U-¹⁴C] aspartic acid (7.66 GBq mmol⁻¹), D-[U-¹⁴C] glucose (11.5 GBq mmol⁻¹), [U-¹⁴C]fructose (11.7 GBq mmol⁻¹), L-[U-¹⁴C] arginine monohydrochloride (12.9 GBq mmol⁻¹), [¹⁴C]urea (2.11 GBq mmol⁻¹) and [1(2)-¹⁴C] acetic acid, sodium salt (2.07 GBq mmol⁻¹). The flasks were mounted on a transparent Plexiglas platform (25 × 36 cm) and exposed for 1.5 h at a defined depth. Precautions were taken to exclude any daylight during sampling and processing of the samples. The addition of labelled and unlabelled compounds, and filtration after termination of the incubation experiments were done on a boat beneath a black plastic foil to prevent exposure to light. To exclude even brief access of light during lowering and retrieving, the platform with its incubation vessels was covered with a black bin (65 cm *d*, 40 cm height) that

Table 1 Final concentrations of carbon- and nitrogen-containing compounds in the artificial lake water used for the uptake experiments

Compound	Concentration (nmol L ⁻¹)
Sodium hydrogen carbonate	2×10^6
Ammonium	33
L-Serine	25
Glycine	25
L-Glutamate	25
L-Aspartate	25
L-Arginine	25
Acetate	250
Citrate	54
Urea	250
D-Glucose	25

could be moved independently. The platform was protected from light by this bin during transfer to the incubation depth and was again covered with the bin before both were returned to the surface.

After termination of the incubation, the filtration and sample preparation were performed on board under cover of black plastic foil to restrict further light exposure. The lake water in each flask was immediately filtered using a glass fibre filter (GF/C, 25 mm diameter, Whatman) under reduced pressure of 250 mbar below ambient pressure. The filter was washed with 15 mL of a sterile washing solution. The washing solution contained 100-fold higher concentrations of all organic compounds used in the experiments, 200 mM sodium hydrogen carbonate, 2.5 mM N-[tris(hydroxymethyl)methyl]-glycine buffer, and had a pH of 9.1. The filtration device (Millipore) allowed the simultaneous filtration through 12 filters. Thirty minutes later, in the lab, the wet filters were washed again with 55 mL of washing solution. The filters were transferred to 20-mL glass scintillation vials. To each vial, 750 μ L of Soluene-350 (Packard, Meriden, CT, U.S.A.) and 5 mL of a scintillation cocktail (Hionic-fluor, Packard) were added. The vials were shaken for several hours. Filters that were acidified were treated with 200 μ L of 0.1 M HCl and the vials were left exposed to the air for 10 h, before Soluene-350 was added. Radioactivity was measured on a Quanta Smart Liquid Scintillation Analyser (Packard) with a counting efficiency of 95%.

Dark incubations of natural populations of *P. rubescens* were performed in an environmentally controlled growth chamber at 15 °C. All other manipulations were done as described above.

Experiments with axenic cultures

The origin and preculture of an axenic strain of *P. rubescens* BC 9307 ax. from Lake Zürich were recently described (Blom *et al.*, 2001). Experimental cultures were grown in 300-mL Erlenmeyer flasks on a light : dark cycle (L : D, 10 : 14 h) over a period of several weeks. Fluorescent tubes (L 15W/41-827, Interna, Osram, München, Germany) provided light from below and above and were adjusted to a total level of 11 μ mol m⁻² s⁻¹ from both directions. At the transition between light and dark (and dark and light), a 1-h period of reduced photon irradiance (5.4 μ mol m⁻² s⁻¹) was supplied. A Macrolon screen

excluded UV light emitted from the fluorescent tubes. The growth temperature was 15 °C, and the growth medium was BG-11 (Rippka *et al.*, 1979). One day before the incorporation experiments, an axenic culture was homogenised with a magnetic stirring bar. The filaments were concentrated on a membrane (8 μ m pore size), washed with 100–150 mL of artificial lake water, and resuspended in 800–900 mL of artificial lake water. The mixtures of all organic compounds were added as described above for separated *P. rubescens* from the lake and incubated in a light : -dark cycle for 20 h at 15 °C. The light cultures were removed 5.5 h after the beginning of the light phase and the dark cultures 0.5 h after beginning of the dark phase. The incubation with ¹⁴C-labelled compounds was performed at 15 °C and 3.5 μ mol m⁻² s⁻¹ (measured in the centre of the flask with a spherical micro quantum sensor, US-SQS, Walz GmbH, Effeltrich, Germany) provided by fluorescent tubes (Interna, Osram) from below and above. The same flasks were used as for the *in situ* incubation experiments. As a control of every incubation experiment, all labelled carbon compounds were added to one sample. Experiments were only accepted if the sum of the individually incorporated radioactive compound differed by not >15% from this control experiment. In the majority of experiments, this difference was <5%.

Elemental analysis

For elemental analysis, *P. rubescens* was grown for several weeks on a light : dark cycle as stated above. About 600 mL of suspension were harvested by filtration through an 8- μ m membrane; while maintaining the sample moisture, the cells were washed with 500 mL deionised water and carefully concentrated with a spatula. The wet biomass (119 mg) was freeze dried (24 mg) and subsequently dried over P₂O₅ under vacuum for 5 days to yield a dry mass of 21 mg. The combustion analysis of C, H, N, S and O (directly) was performed commercially (Solvias AG, Basel, Switzerland) in triplicate.

Results

Environmental and biotic factors

P. rubescens, obtained from the depth of 9 m, was used for the incorporation experiments. Some environmen-

	Date in October 2001			
	9	10	15	20
Start of exposure (local time)	16:20	14:05	13:50	13:25
Period of exposure (min)	85	100	90	87
Temperature (°C)	14.8	14.9	15.2	14.7
PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.7–1.6	1.2–1.9	1.5–2.	0.5–1.3
Oxygen concentration ($\mu\text{mol L}^{-1}$)	263	269	284	219
Biovolume of <i>P. rubescens</i> in the lake water ($\text{cm}^3 \text{m}^{-3}$)	1.8	3.3	3.4	4.6
Biovolume of <i>P. rubescens</i> in the incubation flasks ($\text{cm}^3 \text{m}^{-3}$)	4.6	3.5	10.3	6.0
Chlorophyll <i>a</i> concentration in the incubation flasks (nmol L^{-1})	33	22	41	20

Table 2 Environmental and biotic factors in the lake at 9 m depth on the dates of the experiments and the biotic factors in the incubation flasks. Photosynthetic active radiation (PAR) is the maximum and minimum amounts of downward irradiation observed at 9 m depth during the incubation period

tal and biotic factors for the *in situ* experiments are presented in Table 2. For comparison, the same experiments were conducted with an axenic strain of *P. rubescens* isolated from Lake Zürich that was grown at low irradiance on a light : dark cycle.

Uptake and storage of hydrogen carbonate

To differentiate between uptake and storage of $\text{CO}_2/\text{HCO}_3^-$ (inorganic carbon) in the dark and at low photon irradiance, radioactivity in filaments was measured without and after acidification of the filaments before counting. Differences between both treatments (Fig. 1) were statistically insignificant or very small (natural populations at low photon irradiance $P \geq 0.01$ and for the dark $P \geq 0.05$; axenic cultures at low photon irradiance $P \geq 0.01$). However, a statistically significant, small difference (even at $P \geq 0.01$) was observed for axenic cultures in the dark.

Vertical gradients

The incorporation of inorganic carbon and organic compounds was studied between 9 and 30 October. The weather conditions in that period allowed the comparison of two different growth histories of *P. rubescens*. During the incubation experiments on 9 and 10 October, the lake was stratified, as is normal for this time of the year. The onset of a bad weather period (15 October) induced a deepening of surface mixed layer, shown in changes in the vertical gradients of temperature and concentration of oxygen (Fig. 2). The two situations resulted in different light acclimation histories of the separated *P. rubescens*. The sampling depth of 9 m was in the first period just below the mixed layer but in the second period (15 October and later) within the mixed layer. The different light acclimations of the separated filaments of these two periods resulted in a shift of biovolume-

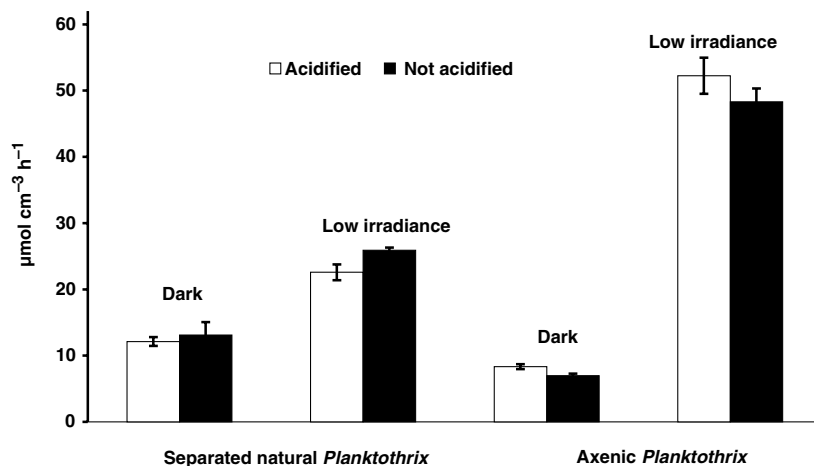


Fig. 1 Biovolume-specific incorporation rates of ^{14}C -labelled hydrogen carbonate in the dark and under low photon irradiance ($3.5 \mu\text{mol m}^{-2} \text{s}^{-1}$) in separated and axenic *P. rubescens* (means \pm 95% confidence interval, $n = 3$). Filaments were acidified and not acidified before counting to differentiate accumulated HCO_3^- from covalently bound carbon.

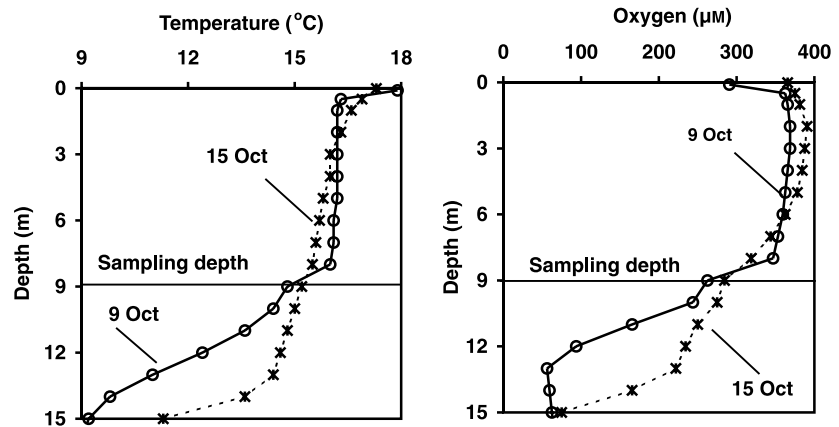


Fig. 2 Vertical profiles of temperature and concentration of oxygen ($\mu\text{mol L}^{-1}$) before (9 October) and after deepening of the surface mixed layer (15 October) of Lake Zürich.

specific chlorophyll *a* concentrations. *Planktothrix rubescens* grown under stratified conditions (on 9 and 10 October) had a higher biovolume-specific chlorophyll *a* concentration ($6.3\text{--}7.2 \text{ mmol L}^{-1}$) than the filaments that were taken from the water body that had experienced mixing ($3.5 \pm 0.3 \text{ mmol L}^{-1}$ (mean \pm SD, $n = 5$)). The axenic strain grown on a light : dark cycle exhibited a constant concentration of biovolume-specific chlorophyll *a* ($2.1 \pm 0.1 \text{ mmol L}^{-1}$ (mean \pm SD, $n = 6$)).

Uptake of inorganic carbon and organic trace compounds by separated natural Planktothrix

The different histories of the separated *Planktothrix* filaments from the lake had a strong effect on the uptake rates of the organic compounds (Fig. 3). Cells acclimated to low irradiance under stratified conditions exhibited higher uptake rates than those that experienced higher irradiance by mixing conditions. This difference was found for all organic compounds but did not hold for inorganic carbon. Different organic compounds were taken up to different extents. The highest rates were found for acetate, serine, glycine, and arginine; glutamate and aspartate showed intermediate rates; glucose and urea (data not shown) showed very low rates of uptake and the uptake of fructose was below the detection limit (data not shown). For the light-acclimated cells (15 October and onwards) the biovolume-specific uptake rates were reduced.

Under dark conditions the uptake rates of arginine were similar to that at low irradiance, while the uptake rates of serine and glycine were slightly smaller. Large differences in uptake rates between

dark and low irradiance conditions were found for glutamate, aspartate, acetate and glucose, which all were taken up in very low rates in the dark. No clear trend was observed for the incorporation rates of inorganic carbon.

Uptake of inorganic carbon and organic trace compounds by axenic Planktothrix

For comparison the uptake of inorganic carbon and organic compounds was studied with axenic cultures of *P. rubescens*. Under the applied growth conditions, the doubling time of *P. rubescens* was 7.0 d. Only marginal differences were observed for the uptake rates of inorganic carbon in dark and low irradiance incubations (Fig. 4). However, the amino acids were taken up much more rapidly at low irradiance than in the dark. Arginine was an exception because it was taken up at high rates under both regimes. The incorporation of glucose and urea was insignificant. These compounds were not taken up in significant amounts either in the dark or at low irradiance. The calculated incorporation of organic carbon as percent of total carbon incorporation on the different dates was between 8–62% (Fig. 5).

Elemental composition of Planktothrix

The washed biomass of *P. rubescens* that was cultivated over several weeks on a light : dark cycle at low photon irradiance was used for elemental analysis. Oxygen was determined directly rather than from the difference. The elemental composition of *P. rubescens* grown under nutrient saturating conditions is given in Table 3. The molar ratios of C : N, C : O and C : S

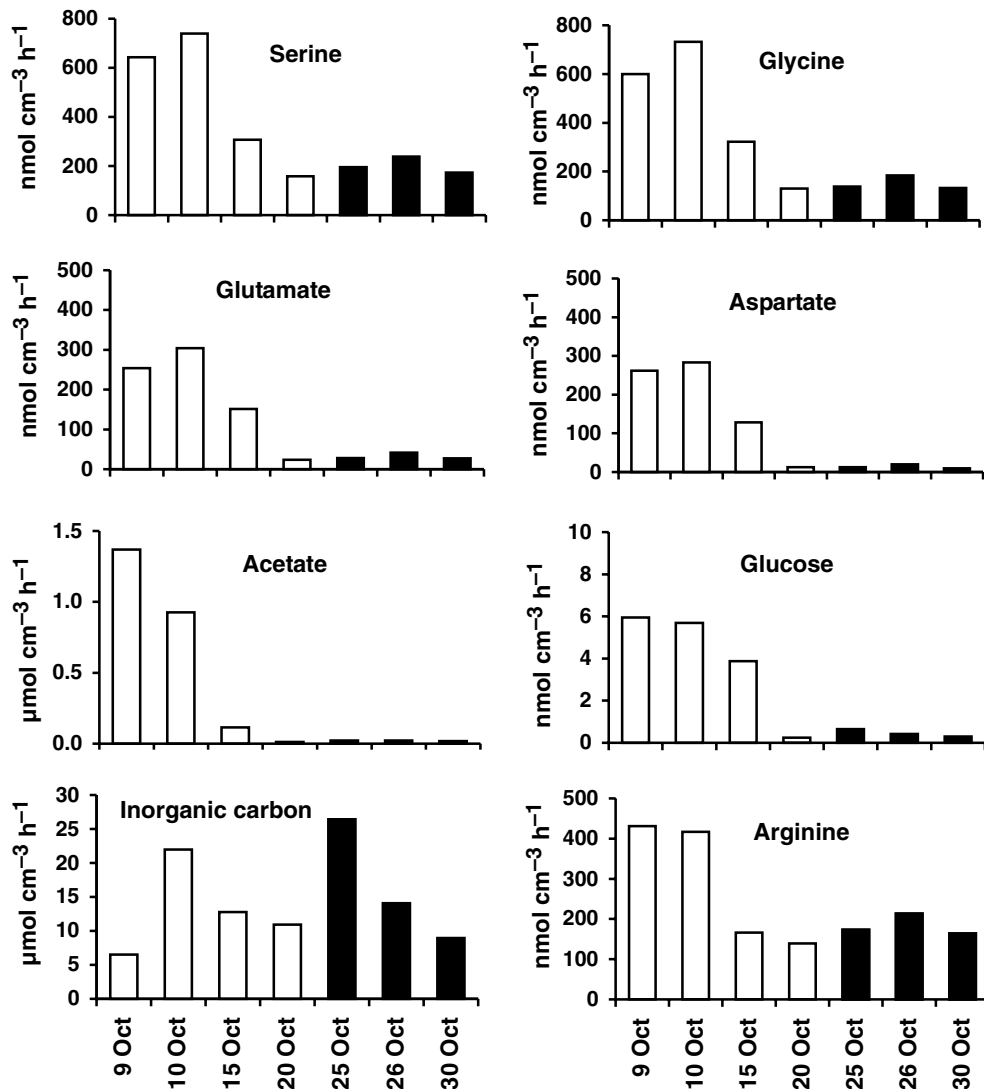


Fig. 3 Biovolume-specific incorporation rates of ^{14}C -labelled inorganic carbon (hydrogen carbonate) and organic compounds by separated natural *P. rubescens* of Lake Zürich. The cells of the incubation experiments on 9 and 10 October were from a natural population that was acclimated to stratified conditions, the cells of the later experiments from lake water after deepening of the surface mixed layer. Open bars – incubation under low irradiance, dark bars – incubations in the dark.

were 4.89, 2.04 and 199, respectively. Other elements accounted for 8% of the dry mass. The carbon content per dry mass was 45.2% (w/w); the dry mass per cm^3 was 0.27 g.

Discussion

Simulation of in situ conditions

Measurements of the uptake and metabolism of inorganic and organic carbon in the light and dark

gave estimates of photoautotrophic, photoheterotrophic and heterotrophic growth although, in the strict sense, only growth experiments provide definitive proof. For certain periods during the summer Bright & Walsby (2000) calculated that stratified populations of *P. rubescens* in Lake Zürich are within or below the compensation depth. This observation warrants analysis of the ability of *P. rubescens* for photoheterotrophy, heterotrophy and uptake of nitrogenous organic compounds, which may improve the energy budget of *P. rubescens* in the metalimnion

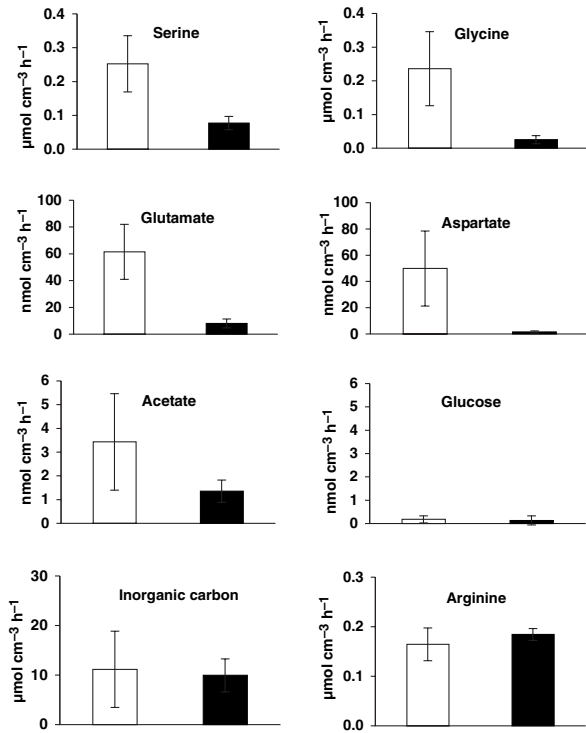


Fig. 4 Biovolume-specific uptake rates of ¹⁴C-labelled inorganic carbon (hydrogen carbonate) and organic compounds by axenic *P. rubescens* (means ± 95% confidence interval, *n* = 3). Open bars – incubation under low photon irradiance (3.5 μmol m⁻² s⁻¹), dark bars – incubations in the dark.

under microphotoc conditions. Experiments in this study simulated natural conditions in Lake Zürich as closely as possible.

To perform experiments under defined conditions, we used artificial lake water that resembled the water of Lake Zürich in its composition and concentration of inorganic ions. The most prominent features of this lake water were the high concentrations of nitrate and inorganic carbon. This contrasted with the situation of the organic compounds of which amino acids, urea, glucose, fructose and acetate were studied. The

concentrations applied were deduced from measurements performed in the pelagic zone of freshwater lakes.

Investigations of the concentrations of amino acids in the lake water of Lake Zürich have shown that concentrations varied, but never exceeded 10–25 nM of individual amino acids and 100 nM of the total dissolved free amino acids (unpubl. data). The concentrations were determined in lake water that was separated via hollow fibre filtration. This separation technology proved extremely gentle and maintained the integrity even of delicate cells (Jüttner, Meon & Köster, 1997). The measured concentrations of free amino acids in Lake Zürich were consistent with the lower concentrations previously determined by paper chromatography (Schürmann, 1964). Furthermore, the amino acid concentrations at the lower edge of the concentration range reported for Lake Mendota (Gardner & Lee, 1975), Frederiksborg Slotssø (Jørgensen, 1987) and Lake Constance (Simon & Rosenstock, 1992) were of the same magnitude as in Lake Zürich. In all these studies, the lake water was obtained by dead end filtration which can affect the integrity of cells and hence makes the lower concentration values more reliable than the higher concentrations also reported. Determinations of free monosaccharides in lake water separated by hollow fibre filtration typically revealed the presence of concentrations of about 20 nM of aldohexoses and ketohexoses with no detectable correlation with the chlorophyll *a* concentration and the time of day (Meon & Jüttner, 1999). These low concentrations corresponded to the concentrations of monosaccharides observed in the mesotrophic Lake Furesø (Jørgensen & Jensen, 1994) and the lower concentrations reported for Lake Constance (Bunte & Simon, 1999). Concentrations in the low nanomolar range have also been reported by Mopper *et al.* (1992) for a freshwater lake. Very few

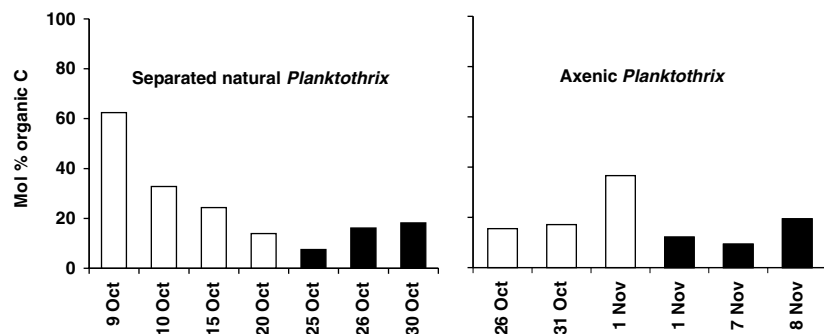


Fig. 5 Uptake of organic carbon in mol percentage of total carbon uptake. Open bars – incubation of natural populations under low irradiance at 9 m depth and of axenic *P. rubescens* at 3.5 μmol m⁻² s⁻¹, dark bars – incubations in the dark.

Table 3 Mol percentage and mass percentage of carbon, hydrogen, nitrogen, oxygen and sulphur of axenic *P. rubescens* dried over P₂O₅ (means \pm SD, $n = 3$). *P. rubescens* was acclimated to low irradiance for weeks

Element	Mol (%)	Mass (%)
Carbon	29.5 \pm 0.27	45.2 \pm 0.18
Hydrogen	49.8 \pm 0.27	6.4 \pm 0.070
Nitrogen	6.0 \pm 0.044	10.8 \pm 0.036
Oxygen	14.5 \pm 0.18	29.5 \pm 0.54
Sulphur	0.15 \pm 0.005	0.61 \pm 0.025
Others	–	8.3 \pm 0.63

data are available for concentrations of acetate in the pelagic zone of freshwater lakes. Hama & Handa (1981), who investigated short chain fatty acids in Lake Kizaki, found concentrations of formate, acetate and propionate to be around 1 mM in the upper layers of this lake. Obviously, the natural concentrations of organic acids are much higher than those reported for amino acids and monosaccharides. In accordance with this study, we added acetate in a 10-fold higher concentration than the sugars and amino acids.

Acclimation to low irradiance

During the study period, the changing environmental conditions had a strong effect on the uptake rates of the compounds being studied. At the start of the experimental period, the lake was thermally stratified, and *P. rubescens* within the metalimnion had been experiencing low irradiance for several weeks and was presumably acclimated to these conditions. The second part of the experimental period was characterised by strong winds that induced deeper mixing of the water body that now contained the metalimnetic *P. rubescens*. An unknown portion of the filaments of *P. rubescens* taken from the bottom of the surface mixed layer during that period had experienced fluctuating light intensities and received much more light than under stratified conditions. The different light histories are consistent with the biovolume-specific chlorophyll *a* concentrations, which were much lower in the low irradiance-acclimated filaments.

The experiments showed that *P. rubescens*, acclimated to low irradiance, effectively took up acetate and various amino acids but little if any glucose, fructose and urea. Irradiances that were so low that they did not support any surplus inorganic carbon uptake outside the dark conditions, enhanced

significantly the uptake rates of all organic compounds tested, with the exception of arginine. A comparison of the uptake rates of inorganic carbon with those of the assayed organic compounds on the basis of organic carbon clearly shows the importance of photoheterotrophy for life under metalimnetic conditions. Cells acclimated to low irradiance acquired as much as 62% of the total carbon at these low irradiances by the uptake of organic compounds. Light-acclimated cells, which probably had a higher intracellular pool of storage matter, took up much less organic carbon, but equivalent to at least 8% of the total carbon intake. The actual percentage of organic carbon taken up under *in situ* conditions can be assumed to be much larger because of the limited number of compounds studied and included in this calculation. Amino acid uptake experiments (Feuillade & Krupka, 1986) have shown that alanine and threonine are also taken up at high rates. However, these experiments were performed at an irradiance that was much higher than the ones used in this study. Light-stimulated uptake of free amino acids was also found for marine picoplankton in a microautoradiographic study on *Synechococcus* (Paerl, 1991). The molecular mechanism behind this light stimulation of uptake is not yet known.

Photoheterotrophy

The spectrum of organic compounds present in lake water is very wide and includes most proteinogenic amino acids (Gardner & Lee, 1975) and components of the arginine pathway, such as ornithine and citrulline (Jørgensen, 1987). Another group of compounds is represented by sugars of the pentose and hexose type (Jørgensen & Jensen, 1994), disaccharides and trisaccharides (Meon & Jüttner, 1999) and a variety of short chain fatty acids, like formate, acetate and propionate (Hama & Handa, 1981). Combining all these compounds, and the other classes not mentioned (combined amino acids, combined sugars, nucleotides, etc.), it becomes obvious that the supply of organic carbon may be of importance for maintenance and growth of *P. rubescens* at low irradiance. This assumption is consistent with growth experiments conducted under microphotoc conditions (Feuillade & Feuillade, 1989). Light-activated heterotrophic growth has been reported for *Synechocystis* PCC 6803 (Anderson & McIntosh, 1991) and recently a gene has been

isolated that is required for heterotrophic growth at low irradiance ($0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Kong, Xu & Hu, 2003). Experiments by Vonshak, Cheung & Chen (2000) with *Spirulina platensis* have shown that for growth, filaments of *S. platensis* required less light under photoheterotrophic conditions, than under photoautotrophic conditions and that the photosynthesis compensation point as measured by oxygen evolution shifted to a lower light value. However, in these experiments, glucose was added in concentrations much higher than expected under natural conditions. The amount of organic compounds taken up by *P. rubescens* from the lake water may induce the same effect on the photosynthesis compensation point. Uptake of dissolved organic nitrogen (Berman, 1997) and amino acids (Nilsson & Sundbäck, 1996) is well documented in planktonic cyanobacteria and photoheterotrophy has been described in an ice-covered lake (Wallen, 1990).

Photoheterotrophic growth of *P. rubescens* and possibly other cyanobacteria may be possible at low irradiance in the depths of lakes and may have great ecological importance. The uptake rates observed are sufficiently high to allow the fuelling of the maintenance energy and even slow growth of the filaments. However, when there is sufficient light to provide large amounts of energy, the reduction of CO_2 by the reductive pentose phosphate cycle will meet the organic carbon demand. At extremely low irradiances, it is conceivable that light is used as a regulator rather than energy source.

If amino acids are available in the lake water, photoheterotrophy may make a contribution to the productivity. The popular assumption that only CO_2 fixation rates determine the phytoplankton productivity is challenged when organic compounds are available as a substitute for the reduction of CO_2 . This is particularly true for populations growing at low irradiances.

Uptake of organic compounds in the dark

Experiments conducted by Feuillade & Feuillade (1989) demonstrated that *P. rubescens* could not grow in the dark when provided with a wide variety of organic nutrients, and heterotrophy was excluded for this organism. This is supported by the present experiments. The uptake rates in the dark were so low that it seems rather unlikely that organic

compounds can supply the cells of *P. rubescens* with sufficient energy to allow biosynthesis for cell growth.

Economical use of energy and nitrogen sources

The metalimnetic population of *P. rubescens* has developed strategies for extremely economical use of the limited light resource available in this habitat. Organic compounds (mean oxidation number around 0) are preferred as carbon sources because they are more similar in the oxidation state to the biomass of *P. rubescens* (mean oxidation number +0.08 calculated from the mol percentage) than CO_2 (oxidation number +4). This strategy also affects the uptake of nitrogenous compounds. Although Lake Zürich is rich in nitrate, assimilatory nitrate reduction need not necessarily be used for the nitrogen supply of the cells. The cellular demand for nitrogen can largely be satisfied by the uptake of organic nitrogenous compounds and ammonium. During stratification of *P. rubescens* the uptake of organic nitrogenous compounds satisfies as much as 84% of the nitrogen demand. In this calculation only those compounds are included that were investigated in the present study. Most of the nitrogen atoms of the amino acids taken up exhibit an oxidation number -3 and are energetically much more favourable than nitrate (oxidation number +5). The same holds for ammonium, which is present in lake water in the μmolar (0.5–1 μM) range (Köster & Jüttner, 1999). The molar C : N ratio of *P. rubescens* was 4.9 : 1 and identical to that described for marine bacterioplankton determined by single cell analysis (Fagerbakke, Heldal & Norland, 1996). When the fixation of CO_2 , uptake of amino acids and carbon compounds are taken together, in about one third of our experiments the C : N ratio of the compounds taken up were in balance with the cellular ratio. It can be assumed that the shortage of nitrogen as observed in the other cases can easily be compensated by the uptake of ammonium. Isotope dilution experiments with axenic *P. rubescens* under similar environmental conditions yielded uptake rates of 5–10 $\text{nmol NH}_4^+ \text{mm}^{-3} \text{h}^{-1}$ (O. Köster, Unpublished data) that would be sufficient to satisfy the cellular nitrogen demand.

It is well established that under low irradiance, aspartate and other derivatives of the incomplete citric acid cycle are labelled by $^{14}\text{CO}_2$ incorporation while the fixation via ribulose-*bis*-phosphate

carboxylase is extremely low (Krüger & Jüttner, 1980). A crucial reaction is β -carboxylation by PEP carboxylase that is necessary for complementation of the C₄-compound pool. The high uptake rates observed for acetate fit into the concept that this part of anabolic metabolism is very active under microphotic conditions while sugar synthesis is at a very low rate. This is supported by the finding that extremely low uptake rates of glucose and fructose are observed under this condition. The C₃-carbon source necessary to feed the anabolism of aspartate, glutamate and their derivatives may come from either extracellular substrates or intracellular carbon sources. Glycogen can, to a certain extent, serve as an internal source via the glycolytic pathway. The supply of glycogen is limited and must be accumulated in a previous light exposed period.

Amino acid uptake at low irradiance

The primary importance of arginine, which together with aspartate constitutes cyanophycin and consumes a particularly large amount of energy for biosynthesis, is indicated by the efficient uptake of this compound by high-affinity uptake transporters (Quintero *et al.*, 2001). To a certain extent, the efficient uptake also holds for aspartate, glutamate and other precursors of arginine. The high value of arginine for the N-metabolism is supported by the observation that the arginine transporter is also active in the dark.

A balanced nitrogen metabolism is of great importance for *P. rubescens* to stay stratified in the metalimnion because buoyancy control is directly affected by the C : N ratio (Klemer, Feuillade & Feuillade, 1982). It should be noted that the protein of gas vesicle membranes make up as much as 7.0% of the total cellular protein as determined for the related species *P. agardhii* (Walsby, 1994).

The energetically profitable uptake of amino acids, however, needs the existence of permeases that can collect amino acids from the extremely dilute solutions in lake water and transport them against a steep gradient across the cytoplasmic membrane. High affinity ATP-binding cassette transporter (ABC)-type transporters (Quintero *et al.*, 2001) and tripartite ATP-independent periplasmic transporter (TRAP)-type transporters (Forward *et al.*, 1997), that need energisation by ATP and a Na⁺ gradient, respectively, and can be saturated at substrate concentrations as low as about 100 nM, have been reported for several cyano-

bacteria and are likely also present in *P. rubescens*. The similar uptake rates found for serine and glycine, and glutamate and aspartate, can be easily explained by the action of a common neutral amino acid permease (Montesinos, Herrero & Flores, 1995) and an ABC-type transporter with a binding protein for glu/gln/asp/asn (Zheng & Haselkorn, 1996). Uptake of arginine, the proteinogenic amino acid with the highest nitrogen content per molecule, seems preferable, irrespective of a light or dark regime. A high-affinity permease for basic amino acids has been described for cyanobacteria and would fulfil the requirements for the effective uptake of arginine (Flores & Muro-Pastor, 1990). The high-affinity arginine permease in cyanobacteria is specific for the basic amino acids arginine, lysine and ornithine and eventually glutamine. Because the concentrations of these amino acids have an additive effect on the transport, the 25 nM concentrations of arginine applied in the uptake experiments actually represent the uptake of the total group of basic amino acids. This group shows notoriously lower concentrations in the lake water as compared with other amino acids. The low levels of these compounds are indicative of their extremely efficient uptake by microorganisms.

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