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Changes of algal biomass as carbon, cell number and volume, in bottles suspended in Lake Constance*

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Abstract. Changes of algal biomass, as carbon, cell numbers and volume were determined for phytoplankton of Lake Constance suspended *in situ* in 2 l glass bottles. Phytoplankton placed at the 6% surface penetrating light level (photosynthetically available radiation) were close to the compensation depth for growth estimated as total particulate carbon and total cell volumes. Cell counts of individual algal species however, showed appreciable growth of diatoms offset by the decline of flagellates. Bottles suspended at two shallower depths in a separate experiment showed some growth of all species and indicated a vertical niche separation of growth of *Rhodomonas minuta* Skuja and *R. lens* Dascher and Ruttner in accordance with their vertical distribution.

Introduction

The major effort in determining aquatic productivity is generally through measurements of photosynthesis, either by carbon isotope or oxygen methods. These estimations are usually by short-term (hours) incubations and rarely encompass the generation time of individual algal species, even less of phytoplankton populations. Although there is much debate over methodology and incubation periods, measurements of photosynthesis do indicate relative rates of carbon fixed and oxygen evolved. There are, however, considerable problems in converting these rates into rates of increase of cellular biomass due to losses by respiration, excretion, grazing, sinking and death (e.g., Talling, 1983; Harris, 1983). Also, in mixed assemblages of phytoplankton there may be great differences between species in rates of photosynthesis and growth.

Here we have endeavoured to bridge the gap between measurements of photosynthesis and biomass increase. Changes of algal biomass of phytoplankton from Lake Constance suspended *in situ* were determined at the same time as measurements of photosynthesis were being made by other workers and reported in this volume. Two experiments are described where change in algal biomass, or lack of it, measured as particulate carbon and cell numbers, was determined in samples suspended in the lake in glass bottles. The results are compared with the corresponding measurements of photosynthesis and rates of change for algal populations within the lake.

Methods

In the first experiment (20–23 April 1982), water was collected from the

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Uberlinger See bay of Lake Constance at the same position used in the First Workshop of the Group on Aquatic Primary Productivity (Sakamoto *et al.*, 1983). Two samples of water were collected from 7 m depth using a 9 l van Dorn sampler, filtered through a coarse mesh (200 μm) filter to remove zooplankton, well mixed and used to fill seven ~2 l Pyrex glass bottles. The bottles were individually placed in plastic net shopping bags and quickly suspended at 7 m depth to avoid damage to the cells by high surface irradiance. Water for the second experiment (27–29 April 1982) was obtained from 2, 4 and 7 m depths at the centre of the Uberlinger See bay to avoid the high concentrations of detritus near the shore, mixed and treated as before. In both experiments the bottles were suspended from an anchored buoy close to the Limnological Institute but beyond the turbid waters of the wave washed zone.

At the beginning of each experiment and thereafter at twice daily intervals, a bottle was removed from each depth. The contents of the bottles were well mixed and samples removed for cell counts and particulate carbon determinations. Samples for cell counts were immediately fixed in Lugol's iodine solution and counted according to Utermöhl's (1958) technique.

400 units (cells or colonies) of each species mentioned in this study were counted, thus giving a counting accuracy of roughly ± 10% (Lund *et al.*, 1958). Other species were counted at lower precision, but combined they contributed <2% to total biomass. Biomass was estimated as cell volume, for which the linear dimensions of 50 cells per species were taken. Geometrical calculation of the cell volume followed the recommendations by Rott (1981).

Specific growth rates (k) for individual algal species during the experiments were estimated by judging by eye periods of near exponential increase of cells and calculated as

$$k = \frac{dN}{N.dt} = \frac{1}{t_1 - t_0} \ln \frac{N_1}{N_0}$$

where N_0 is number of cells at time t_0 and N_1 is number of cells at time t_1. For species other than diatoms which do not leave readily recognizable remains of dead cells, the growth rates must be considered as net specific rates of growth within the enclosures.

For particulate carbon analysis, aliquots of known volume (~1 l) were filtered through 30 mm diameter glass fiber filters (Scheicher and Schüll No. 6) and lyophilized. The carbon content of the particulate matter on the filter was analysed after dry combustion and chromatographic detection of carbon dioxide using a F and M 185 (Hewlett-Packard) CHN analyser. The limit of detection, estimated as five times the standard deviation of blank filter determinations, was 39 μg carbon.

Various measurements of light during the first experiment are given by Jewson *et al.* (1983). Photosynthetically available radiation (PAR, 400–700 nm) was measured continuously on the roof of the Limnological Institute during both experiments using a spherical 4 π scalar quantum irradiance meter (Biospherical Instruments, San Diego, CA).

Water temperature was measured using a multiprobe (Hydropolytester, Züllig-Baerlocher AG, Rhineck, Switzerland).

Results

Experiment 1

Because of a shortage of bottles only one depth could be selected. The depth of 7 m was chosen to approximate the level of 10% surface penetrating light, measured as PAR-quantum flux. Light measurements at a position nearby at the beginning of the experiment (Jewson *et al.*, 1983) show that at 7 m depth there was ~6% surface-penetrating PAR determined by a spherical 4π scalar quantum sensor and a value of 8% was calculated indirectly from spectral readings with two flat-plate photometers. However, algal cells respond to the quantum flux at the depth of exposure. This can be ascertained from the continuous measurements of solar radiation (Figure 1) and the percentage light penetration. The temperature at 7 m was 5.7°C at the beginning of the experiment with negligible change throughout.

Changes in cell number of the dominant species of algae in the bottles are shown in Figure 2 with conversion to volume in Table I. Only the diatoms *Asterionella formosa* Hassal and *Stephanodiscus hantzschii* Grunow showed marked increases in numbers. The latter had a particularly rapid specific growth rate of 1.00 ln units of increase d^{-1} or 1.45 divisions d^{-1} compared to 0.30 ln units d^{-1} or 0.43 divisions d^{-1} for *Asterionella*. At the beginning of the experiment the two diatoms comprised 19% of the total biomass as cell volume increasing to 43% by the end.

The flagellates *Rhodomonas lens* and *R. minuta* decreased throughout the experiment. At the beginning they made up 48% of the total cell volume declining

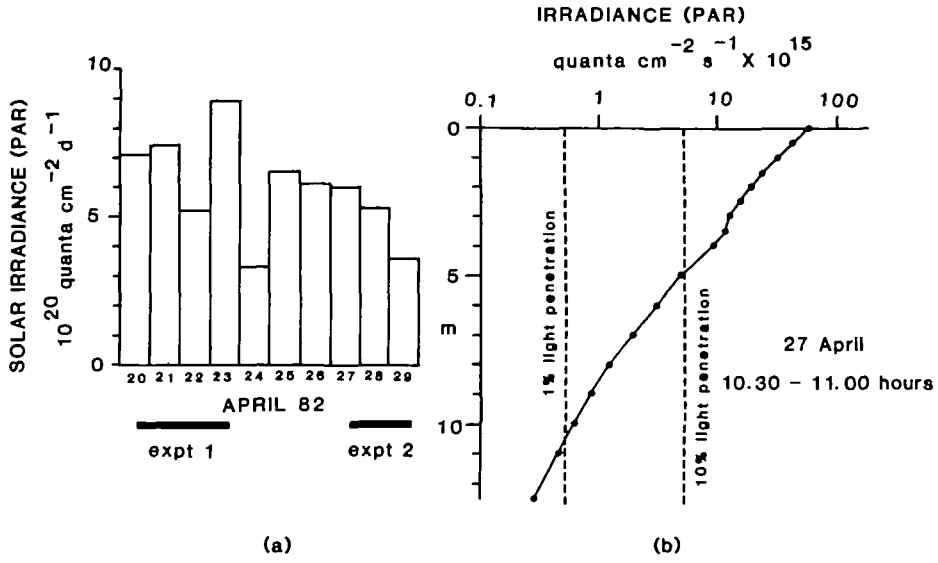

Fig. 1. (a) Solar radiation (PAR 400–700 nm) on the roof of the Limnological Institute, Konstanz during experiments 1 and 2. (b) The penetration of photosynthetically active radiation (PAR) in Lake Constance, 10.30–11.0 h 27 April 1982.

Table I. Calculated changes in cell volume ($mm^3 l^{-1} \times 10^3$) for algal species in 2 l bottles suspended in Lake Constance at 7 m depth, the average cell volumes for different species used in calculations are indicated.

Algal species	Single cell volume μm^3	Date and time						
		20.4.82	21.4.82		22.4.82		23.4.82	
		14.00	08.00	18.00	08.00	18.00	08.00	18.00
Rhodomonas lens	370	45.14	57.76	40.70	45.88	38.48	38.48	35.52
Rhodomonas minuta	70	14.28	12.39	8.89	7.63	4.34	4.34	3.43
Cryptomonas marssonii	600	4.80	3.00	1.50	2.76	2.10	2.10	1.80
Cryptomonas medium (c.f. *ovata*)	2400	8.40	4.80	4.80	5.76	4.80	4.80	4.80
Asterionella formosa	700	21.70	30.80	24.50	34.30	42.00	50.40	56.00
Stephanodiscus hantzschii	50	1.30	1.35	2.75	3.60	6.60	14.90	16.80
Synedra acus	2000	0.80	1.40	2.20	1.80	3.00	3.00	3.00
Diatoma elongatum	2500	5.00	5.00	5.00	5.00	4.75	5.00	5.00
Fragilaria capucina	400	0.40	0.40	0.40	0.44	0.40	0.40	0.40
Gymnodinium sp.	500	3.00	4.00	6.50	5.00	6.50	6.00	9.00
Chlamydomonas sp.	800	14.40	6.40	18.40	17.60	21.60	20.80	27.20
μ-algae	4	3.44	3.60	3.40	3.12	3.40	3.76	3.60
Total volume		122.66	127.90	119.04	132.89	136.40	153.98	166.55

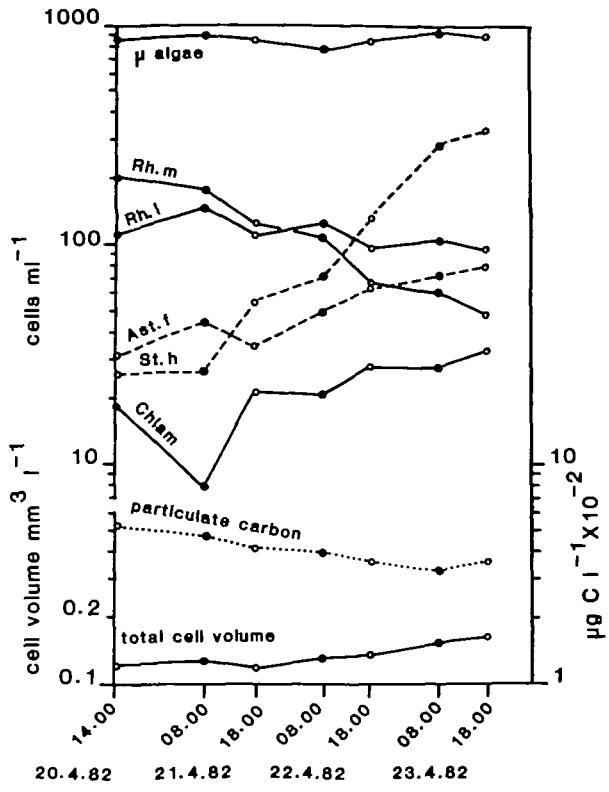

Fig. 2. Growth of dominant algal species (cells ml^{-1}) and change in total cell volume ($mm^3 l^{-1}$) and particulate carbon ($\mu g\ C\ l^{-1} \times 10^{-2}$) of phytoplankton from Lake Constance suspended in bottles at 7 m depth, 20–23 April 1982; open circles indicate afternoon values and closed circles morning values. *Rh. m* = *Rhodomonas minuta, Rh. l* = *Rhodomonas lens, Ast. f* = *Asterionella formosa, St. h* = *Stephanodiscus hantszchii, Chlam* = *Chlamydomonas* sp.

to 23%. There was a small, just significant, increase in *Chlamydmonas* sp. but no appreciable change in micro-algae.

Figure 2 also shows a small, 35% increase in the total cell volume over the duration of the experiment but a 29% decrease in the particulate carbon content.

Experiment 2

To give a greater coverage of the euphotic zone bottles were suspended at 2, 4 and 7 m depths. Corresponding to increased phytoplankton concentration in Lake Constance the depth of the euphotic zone (the depth of 1% surface-penetrating irradiance) had decreased from 13.7 m at the beginning of experiment 1 to 10.5 m (Figure 1) on 27 April 1982 at the beginning of experiment 2. Figure 1 indicates that phytoplankton exposed at 2 and 4 m depths would receive ~30 and 20%, respectively, of surface penetrating irradiance, while those at 7 m would be at the ~3% level close to the base of the euphotic zone. The water temperature was 7.2°C at the surface and 6.9°C at 7 m depth, but cloudy weather

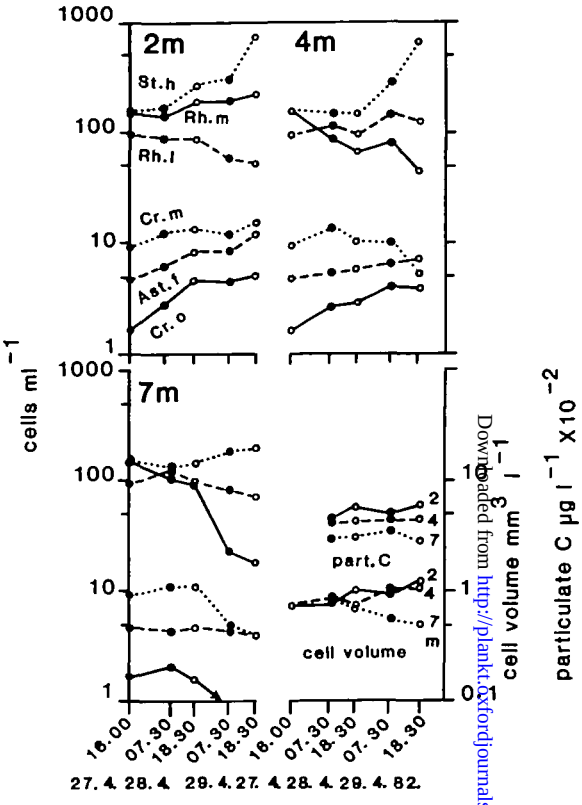

Fig. 3. Growth of dominant algal species (cells ml $^{-1}$) and change in total cell volume (mm^3 l^{-1}) and particulate carbon (μg C l^{-1} x 10^{-2}) of phytoplankton from Lake Constance suspended in bottles at 2, 4 and 7 m depth, 27–29 April 1982; open circles morning values. *St. h = Stephanodiscus hantzschii, Rh. m = Rhodomonas minuta, Rh. l = Rhodomonas lens, Cr. m = Cryptomonas marssonii, Cr. o = Cryptomona sovata, Ast f = Asterionella formosa.*

meant a large decrease in solar radiation compared to experiment 1 (c.f. Figure 1a and 1b).

Figure 3 shows the changes in cell numbers of the algal species, the total cell volume and the particulate carbon during the experiment. At 2 m depth there was a small, probably significant increase in total cell volume, at 4 m depth the increase in cell volume was not significant and at 7 m there was a significant decrease in cell volume. Changes of particulate carbon followed a similar pattern.

There was a wide variation in the ability of different species of algae to grow at the three depths. The calculated specific growth rates for diatoms and net specific growth rates for flagellates are given in Table II, although it should be realized that these are, at best, only guides at low growth rates where differences in cell numbers are small. With the exception of *R. lens*, all the major species grew at 2 m depth. By contrast this alga grew slowly at 4 m depth whereas both *R. minuta* and *Cryptomonas marssonii* Skuja which grew at 2 m decrease in numbers at 4 m. Only *S. hantzschii* grew at 7 m depth but at a much slower

Table II. Values of specific growth rate constant (k) during the periods and depths indicated for some species of algae suspended in bottles in Lake Constance.

Alga	k (ln units/ day^{-1})	Depth (m)	Time
Stephanodiscus hantzschii	1.04	7	21.4.82–23.4.82
Stephanodiscus hantzschii	1.10	2	28.4.82–29.4.82
Stephanodiscus hantzschii	1.50	4	28.4.82–29.4.82
Stephanodiscus hantzschii	0.16	7	28.4.82–29.4.82
Asterionella formosa	0.30	7	20.4.82–23.4.82
Asterionella formosa	0.45	2	27.4.82–29.4.82
Asterionella formosa	0.34	4	27.4.82–29.4.82
Rhodomonas minuta	0.18	2	27.4.82–29.4.82
Rhodmonas lens	0.13	4	27.4.82–29.4.82
Cryptomonas marssonii	0.25	2	27.4.82–29.4.82
Cryptomonas ovata	0.53	2	27.4.82–29.4.82
Cryptomonas ovata	0.41	4	27.4.82–29.4.82

growth rate compared with experiment 1.

Discussion

Enclosure of phytoplankton in small containers immediately places the algal cells in an artificial environment with movement in the water column curtailed. There have been numerous studies of *in situ* rates of photosynthesis and growth under such conditions and the possible consequences of containing phytoplankton in bottles for these purposes has been critically examined by Venrick *et al.* (1977). A major objection is the use of small bottles with associated high ratios of surface area to volume. In the present work bottles of ~ 2 l capacity were used to try to reduce this difficulty yet remain practical. The results of both experiments show that all species present could grow in the containers, albeit some slowly. At the time of the experiments the phytoplankton density in Lake Constance was low (~2.5–7.5 μg chlorophyll a l^{-1}) but the major nutrients C, N, P and Si were all abundant and would not have been limiting for algal growth.

In the first experiment the small increase in total cell volume indicated that 7 m depth was close to the compensation depth for phytoplankton production. This is in close agreement with the concurrent measurements of net photosynthesis using the oxygen method (Tschumi in Sakamoto *et al.*, 1983) which show no net production at 8 m depth. However, the apparent community compensation depth conceals appreciable growth of diatoms offset by the decline of the major flagellates.

Interesting interspecific differences in growth in relation to depth of exposure were found. The growth of *R. minuta* at 2 m depth and *R. lens* at 4 m depth agrees with observations of vertial niche separation of the two species in the lake during experiment 2 and previously described for an earlier year by Sommer

(1982). However, their growth rates in the bottles were low in relation to their net rates of population change in Lake Constance at similar times of the year during 1979/1980 and 1981 (C.O.5 ln units d^{-1}) recorded by Sommer (1981). Two weeks after experiment 2, from 11 – 18 May 1982, *Rhomodnas minuta* achieved a net growth rate of 0.47 ln units d^{-1}. This may in part be explained by the poor weather at the time of experiment 2 and the generally inferior growth of flagellates in bottles relative to diatoms (Venrick *et al.*, 1977 and references therein). *Cryptomonas marsonnii* showed a narrow depth preference similar to that of *R. minuta*. Besides growing more slowly than diatoms in the bottles, flagellates appeared to require higher levels of irradiance, perhaps to offset possibly higher rates of respiration or lower rates of photosynthesis.

Measurements of particulate carbon were less satisfactory for determining algal production than cell counts and volume estimates. This was more apparent in the first experiment where the lake water contained high levels of non-algal detritus. If one assumes that chlorophyll *a* is ~2% of cell carbon of phytoplankton (Talling and Heaney, unpublished results), then a chlorophyll *a* concentration of 2.5 $\mu g\ l^{-1}$ in Lake Constance at the beginning of experiment 1 would represent ~125 $\mu g\ l^{-1}$ algal carbon or only ~27% of the total particulate carbon. The total particulate fraction decreased during experiment 1, although total cell volume increased. Nevertheless, in the second experiment where the non-algal detrital matter was lower, appreciable increases in particulate carbon were found at 2 and 4 m depths reflecting changes in cell volumes. Further efforts in measuring particulate carbon in studies of algal production could prove a useful supplement to the more rapid determinations of photosynthesis and the tedious estimation of species changes and related cell volumes. This may be especially so using the micro-adaptation of the dichromate oxidation method for carbon equivalents (Mackereth *et al.*, 1978) which has a limit of detection of only 11 μg C (Butterwick *et al.*, 1982).

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