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Effect of nutrient enrichment and temperature on intracellular partitioning of $^{14}CO_2$ in a summer phytoplankton community in the northern Baltic

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ABSTRACT: During a 20 d period in summer 1988, two 30 m^3 mesocosms were used to study the effect of nutrient enrichment (NH_4^+ plus PO_4^{3-}) on the short-term (6 h daylight) and diel (day-night) patterns of incorporation of ^{14}C into different cell constituents of the phytoplankton [proteins, polysaccharides, low molar mass compounds (LMC) and lipids] in the northern Baltic. After 6 h incubation, the clearest effect of the nutrient enrichment was an increase in the proportion of the ^{14}C-LMC pool. The nutrient additions led to a decrease in the proportion of ^{14}C-proteins in the whole algal assemblage, while the opposite was true with autotrophic picoplankton. The proportions of ^{14}C-polysaccharides and ^{14}C-lipids seemed to be unaffected by the nutrient enrichment, but a sudden decrease of 5 °C in water temperature temporarily increased the proportion of ^{14}C-polysaccharides by 10 to 20 percentage points. Our results demonstrate that determination of intracellular biochemical ^{14}C-fractions provides ecologically important information about the different metabolic pathways of algal cell constituent synthesis in a fluctuating environment, hence supplementing primary productivity measurements based on total incorporation of ^{14}C.

INTRODUCTION

The classical ^{14}C method of Steemann Nielsen (1952) is still a generally accepted standard in the measurement of phytoplankton primary productivity. A limitation on the primary productivity measurements based on total incorporation of ^{14}C is, however, that they do not take the biochemistry of algal cells into account. For example, the rate of photosynthesis remains virtually unchanged over large variations in the biochemical state of algal cells (Morris et al. 1974), and hence the actual physiological response of algae to changes in external conditions may not be detected by the ^{14}C method until after several days.

The end products of photosynthesis can be divided according to their function into regulating (mainly proteins), structural and storage (carbohydrates and lipids), and intermediate products (LMC: low molar mass compounds) (Morris 1981). The composition of the macromolecules synthesized indicates the dominant biochemical pathways and reflects the physiological state of algal cells under varying growth conditions. Accordingly, the protein:carbohydrate ratio has been used as an indicator of nutritional state in algae (Healey & Hendzel 1980).

Proteins are synthesized continuously in phytoplankton cells during the diel cycle, while polysaccharides are produced during the day to be used as storage products for night growth (Cuhel & Lean 1987a). This difference in synthetic pattern led Morris (1981), and Lancelot & Mathot (1985) to suggest that the turnover of the protein pool, rather than the turnover of the whole algal carbon pool, should be used as an index of phytoplankton growth.

Moreover, Scott (1980) showed that the transfer efficiencies of the major algal cell constituents between herbivores and their phytoplankton food differ considerably, ranging from 5 to 10 % (carbohydrates and lipids) to >50 % (proteins). Seasonal fluctuations in the chemical composition of the phytoplankton cells are to be expected (Morris & Skea 1978) and hence information on the proportions of the main macromolecules in algal cells is of vital importance for understanding the varying nutritional value of algae.

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In order to obtain more detailed information about the biochemistry of ^{14}C primary production, the effect of nutrient enrichment on the pattern of diel incorporation of $^{14}CO_2$ into the major end-products of photosynthesis was studied in a natural Baltic phytoplankton community.

MATERIAL AND METHODS

The study site was located at Tvärminne Storfjärd, in the outer Tvärminne archipelago zone off the SW coast of Finland. The sampling/incubation station was ca 15 m deep, with salinity of 5 to 6 ‰ and pH of 8. The area is characterized by occasional inflows of fresh water from the inner archipelago or of cold Baltic deep water during upwellings. The annual succession of phytoplankton closely resembles that of the adjacent open sea zone in the northern Baltic. A detailed description of the hydrography and phytoplankton ecology of the area is presented by Niemi (1975).

On 22 July 1988, on the evening preceding the 20 d experiment, two 30 m^3 mesocosms made of transparent plastic (diameter 2.15 m, depth 9 m, closed at the bottom) were filled by lifting their collar from 10 m depth to a pontoon on which they were mounted. The mesocosms were alternately subjected to 5 d treatment periods, during which they received daily nutrient additions of 16 mg NH_4^+-N m^{-3} and 4 mg PO_4^{3-}-P m^{-3}; moreover, 90 sticklebacks were added as top predators to both mesocosms for the last 2 manipulation periods (Table 1). In earlier experiments a period of 5 d has proved to be long enough to reveal the response of the algal community to nutrient enrichment. The water temperature, the attenuation of light in the water column (Licor LI-1000 irradiance meter, USA) and the cumulative daily solar irradiation (Kipp & Zonen solarimeter, The Netherlands) were followed continuously during the experiment.

^{14}C measurements. The mesocosms were sampled twice in every treatment period (Days 1, 5, 8, 10, 13, 15, 18 and 20), the sampling taking place between 07:00 and 08:00 h with a Ruttner sampler, just before the addition of nutrients. The sampling/incubation depth was 2 m. Pooled samples (4 to 6 l) were filtered through a 20 µm plankton net before incubation in order to remove large herbivorous grazers. $NaH^{14}CO_3$ at 370 kBq (specific activity of the undiluted stock 2.14 GBq $mmol^{-1}$, Amersham, UK) was added to 100 ml samples (glass bottles). Triplicate light bottles and 2 zero-time blanks (filtration immediately after addition of ^{14}C) were used in molecular fractionation. The *in situ* incubations started at about 10:00 h and lasted for 6 and 20 h, the latter period comprising a day and night cycle. On Days 4 and 7, molecular fractionation was done with picoplankton samples (< 3 µm prefiltration by gravity, Nuclepore polycarbonate filters) in the same way as that of the whole samples, except that a 200 ml incubation/filtration volume was used. Moreover, on Day 1 a time course ^{14}C incubation (1, 3, 6, 10, 20, 34 h) was performed with untreated samples in the laboratory (*in situ* temperature, irradiation ca 12 W m^{-2}; Dr Bruno Lange luxmeter, Germany) with a dark period between the 10th and 20th hours of incubation.

Table 1 Experimental design of the 20 d mesocosm experiment. Top predators (90 sticklebacks) were added to both mesocosms for the last 2 periods. 'NP+': daily nutrient enrichment with 16 mg NH_4^+-N m^{-3} and 4 mg PO_4^{3-}-P m^{-3}; 'NP-': no nutrient additions; 'F+': fish added; 'F-': no fish added

Mesocosm	Experiment days			
	1-5	6-10	11-15	16-20
1	NP-, F-	NP+, F-	NP-, F+	NP+, F+
2	NP+, F-	NP-, F-	NP+, F+	NP-, F+

After incubation the samples were filtered directly on Whatman GF/C glass-fibre filters (< 3 µm prefiltered samples on Whatman GF/F filters) and the filters were rinsed with about 30 ml prefiltered (Whatman GF/F) seawater (pressure differential < 50 mm Hg). The filters were stored frozen (-20 °C) in glass scintillation vials.

Extraction of the ^{14}C-labelled intracellular compounds was done by the techniques of Morris et al. (1974) and Rivkin (1985), with a few modifications. Briefly, the filters were thawed at room temperature and 3 ml methanol-chloroform solution (2:1 v:v) was added to the scintillation vials. According to microscopy, the freezing-thawing stage breaks algal cell walls effectively (H. Kuosa pers. comm.), and to promote the breakage and make the intracellular material fully extractable, the filters were exposed to ultrasonic vibration (intensity 50 W; Bransonic, USA) for 10 min in the methanol-chloroform solution. The samples were cooled in an ice-bath, and 1 ml distilled water was added. The lipids dissolved in the chloroform phase and the LMC pool (compounds of ca 100 to 600 g mol^{-1}) in the methanol/water phase. The polysaccharides were then extracted first for 30 min with 3 ml hot (85 to 90 °C) 5 % trichloroacetic acid (TCA) and then with 2 ml cold 5 % TCA. The proteins remained as a precipitate on the filters.

Net primary productivity (particulate plus dissolved organic ^{14}C) was measured by allowing an acidified 4 ml subsample (pH < 2) to stand in an uncapped glass scintillation vial for 24 h (no bubbling) before radioactivity measurements (Niemi et al. 1983).

Lumagel scintillation cocktail (LUMAC, Belgium) was added to the glass scintillation vials containing the filters or liquid fractions. The radioactivity of the samples was measured with a 1215 Rackbeta liquid scintillation counter (LKB Wallac, Finland), using the external standard ratio method.

Chlorophyll *a* and biomass measurements. For chlorophyll *a* (chl *a*) measurements, 50 ml samples were filtered on Whatman GF/F filters; on Days 4 and 7, 3.0 µm polycarbonate filters (Nuclepore) were also used. Chl *a* was extracted with 94 % ethanol for 24 h in darkness and measured fluorometrically (Sequoia-Turner 450; calibrated with pure chl *a*; Sigma). No correction was made for phaeopigments.

Algal species were determined every 3rd day (starting on Day 1; pooled samples from 0 to 6 m depth) on Lugol-preserved samples by the method presented by Utermöhl (1958), using phase contrast microscopy (Leitz Diavert, Germany). The nomenclature follows Edler et al. (1984).

RESULTS

During the experiment, the irradiance fluctuated and there were 2 periods of relatively stable water temperature separated by a period during which it decreased steeply (Fig. 1). An upwelling caused an inflow of cold deep water from the sea zone, resulting in a decrease of 7 °C in the water temperature in the mesocosms between Days 7 and 9 [the temperature in the mesocosms followed closely (within 1 °C) the temperature of the open water].

During the first nutrient enrichment periods in Mesocosm 2 (from Day 1 to Day 5) and in Mesocosm 1 (from Day 6 to Day 10), the plankton communities were unable to use up the added nutrients, which resulted in elevated concentrations of NH_4^+ and PO_4^{3-} (from an initial ca 7 to 40 mg N m^{-3}, and from 2 to 15 mg P m^{-3}). After Day 12, NH_4^+-N had diminished below the detection level in both mesocosms, however, and from then on the nutrient enrichment showed only in increased PO_4^{3-}-P concentrations (though a major part of the added PO_4^{3-}-P was taken up by the plankton community), while NH_4^+-N remained below the detection level.

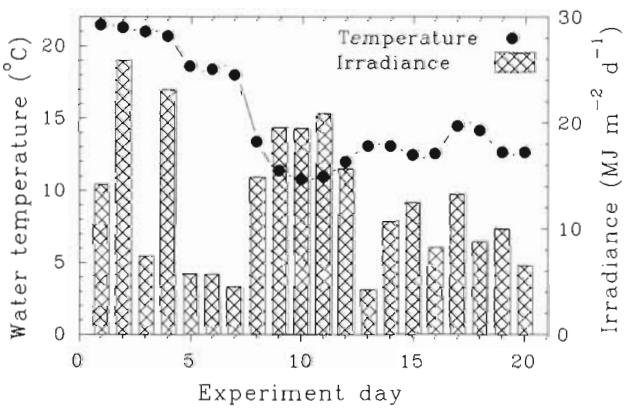

Fig. 1 Total daily irradiation and water temperature during the mesocosm experiment, 23 July to 11 August 1988, off the SW coast of Finland

Biomass development and succession of phytoplankton

The chl *a* concentration was at a low level (ca 2 mg m^{-3}) in the original plankton community (Fig. 2). Nutrient enrichment led to distinct fluctuations in phytoplankton biomass in the mesocosms. During the first 2 nutrient enrichment periods from Day 1 to 10, the chl *a* values clearly increased as a consequence of nutrient additions, but the peak chl *a* values of 16 to 17 mg m^{-3} were not recorded until the last 2 manipulation periods (Fig. 2). When the added NH_4^+-N was exhausted in the mesocosms, the chl *a* values decreased to about the initial level during the subsequent 5 d without nutrient additions.

Sedimentation of phytoplankton biomass was probably small during most of the study, since total phosphorus values (mineral P plus dissolved and particulate organic P) from 2 m depth showed high recovery of the PO_4^{3-} additions; a clear decline in total P values was recorded only during the last 5 d period in Mesocosm 2, which indicated increased sedimentation (data not shown). The sticklebacks (added on Day 10) were unable to remove the metazooplankton grazers

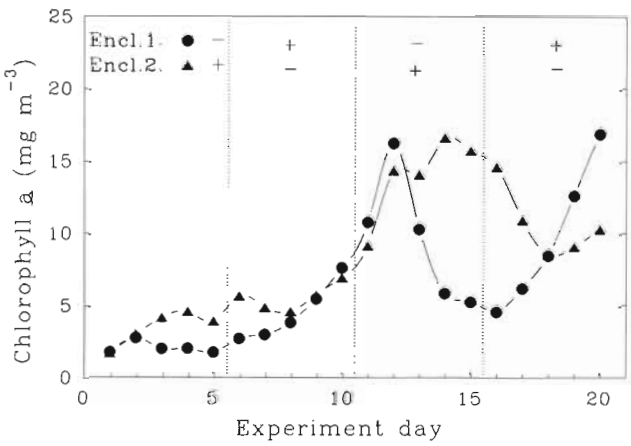

Fig. 2. Concentration of chl *a* in Mesocosms 1 and 2. Mesocosm 1 received daily nutrient additions ('+') during the 2nd and 4th quarter of the experiment and Mesocosm 2 during the 1st and 3rd quarter. ('–'): no nutrient additions. Other details of experiment are in Table 1

(copepods and rotifers; data not shown), and hence the steep decrease in chl *a* values after Day 12 in Mesocosm 1 may at least in part have reflected control by grazers.

The pattern of the development of algal primary productivity was similar to the course of chl *a*, with slight modifications in the shape of the peaks. Nutrient enrichment evoked an increase in the net primary productivity values from the initial ca 0.08 up to 2.28 g C m^{-2} d^{-1}. The net primary productivity and chl *a* values in the < 20 µm prefractionated samples varied respectively between 88 and 99 %, and between 83 and 94 % of the corresponding values in the whole samples.

The original phytoplankton community consisted predominantly of the filamentous blue-green alga *Aphanizomenon flos-aquae* (L.) Ralfs, *Cryptomonas* spp. flagellates and picoplanktonic blue-greens, together corresponding to about 77 % of the total biomass. During the first 5 d period, the abundance of most taxa decreased in both mesocosms. Small centric diatoms and the flagellate *Pseudopedinella elastica* Skuja increased strongly, however, while *A. flos-aquae* and picoalgae fluctuated less and remained an important component of the algal assemblage throughout the study in both mesocosms. Later on, the nutrient additions mainly enhanced the growth of small centric diatoms and *P. elastica*, and at the end of the second nutrient enrichment period (Day 9) these algae corresponded to > 50 % of the total algal biomass in both mesocosms. After Day 10, the response of the phytoplankton community to nutrient enrichment was mainly due to the small centric diatoms.

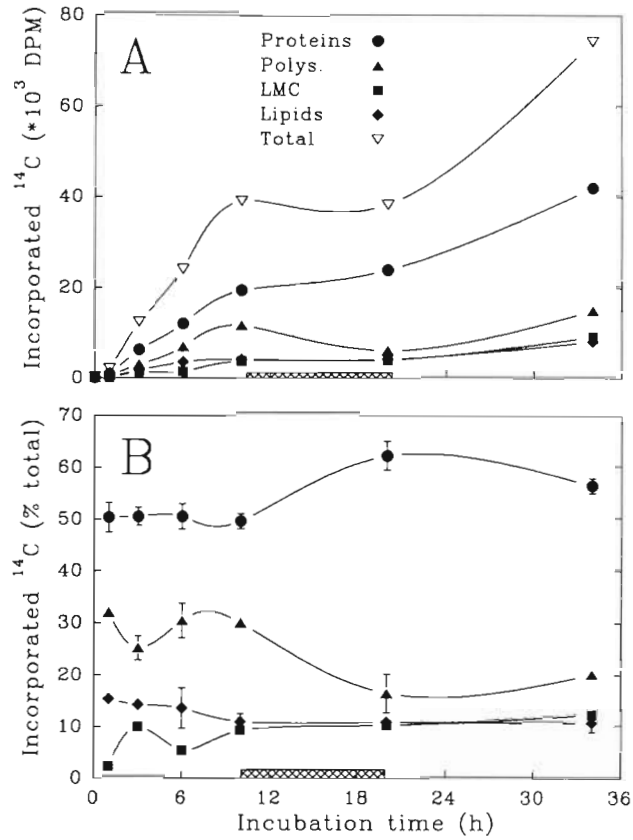

Fig. 3. Time course (mean ± SE) of incorporation of ^{14}C into the major cellular constituents of the initial phytoplankton community in *in vitro* conditions (A) in dpm and (B) as percentage of total ^{14}C. Hatched area above *x*-axis denotes dark period

Diel patterns in the distribution of fixed ^{14}C

In the 34 h time course incubation on Day 1, the proportion of ^{14}C incorporated into the protein fraction increased during the dark and decreased during the light period, whereas the opposite was true with the polysaccharide fraction (Fig. 3B). The proportions of ^{14}C-lipids and the ^{14}C-LMC pool remained stable. The total uptake of ^{14}C (sum of the molecular ^{14}C fractions) was linear in the first light period, and it continued at about the same rate in the second light period, reflecting weak grazing pressure due to the < 20 µm prefractionation of the samples (Fig. 3A).

The inverse diel relation between the proportions of ^{14}C-proteins and ^{14}C-polysaccharides was observed in all experiments (Fig. 4). The proportion of ^{14}C-proteins was on average 7 percentage points higher after the 20 h incubations than after 6 h ($p < 0.001$, Friedman's 2-way nonparametric ANOVA), while the corresponding values for ^{14}C-polysaccharides were on average 5 percentage points lower ($p < 0.01$, Friedman's 2-way nonparametric ANOVA). The ^{14}C-LMC pool increased during periods of nutrient enrichment and it constituted a somewhat larger fraction of the total particulate ^{14}C after the 6 h incubations than after 20 h. In the 6 h incubations ^{14}C-protein synthesis was inversely related to ^{14}C-LMC synthesis (Fig. 4). The proportion of ^{14}C-lipids remained about the same in the 6 h and 20 h incubations.

Effect of nutrient enrichment and environmental factors

The proportion of ^{14}C-proteins decreased on average from about 60 to 35 % of the total fixed ^{14}C during the first 8 d, regardless of the treatment of the mesocosms (20 h incubations; Fig. 4). After Day 10, the proportion of ^{14}C incorporated into proteins was lower in the mesocosm where nutrients were being added (Fig. 4).

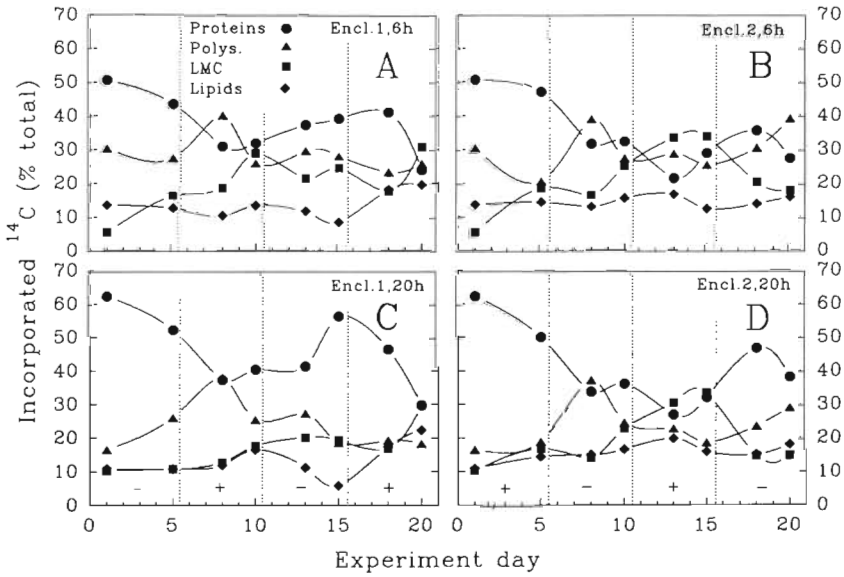

Fig. 4. Relative incorporation of ^{14}C into the major constituents of phytoplankton cells in 6 h (daylight) and 20 h (day-night) *in situ* incubations. Other explanations as in Fig. 2

The proportions of ^{14}C-polysaccharides showed parallel fluctuations between 20 and 40 % in the mesocosms, indicating that they were not controlled by the nutrient additions (Fig. 4). On Day 8, the highest proportion (almost 40 %) of the incorporated ^{14}C was found in the polysaccharide fraction in both mesocosms (Fig. 4), and this coincided with an abrupt decrease of 5 °C in the water temperature between Days 7 and 8 (Fig. 1). The proportion of the ^{14}C-LMC pool increased as a consequence of the nutrient additions, showing peak values of about 30 % at the end of the nutrient enrichment periods (Day 5 excluded; Fig. 4). The proportion of the lipid fraction remained fairly steadily at about 10 % throughout the experiment (Fig. 4).

The ratios of particulate organic carbon (POC) to particulate organic nitrogen (PON) ranged from 6.3 to 11.1, with a mean of 7.9. The POC:PON ratios had similar patterns in the 2 experimental units and they seemed to be independent of the development of the phytoplankton biomass and of the variations in the proportions of ^{14}C-protein.

Incorporation of ^{14}C by picoplankton

On Days 4 and 7, the chl *a* concentrations in the picoalgal (< 3 µm) size fraction were respectively 24 and 12 % of the total chl *a* in Mesocosm 1. As in the larger phytoplankton, the proportion of ^{14}C-proteins seemed to increase at night in the pico-size fraction, while the proportions of the other labelled compounds decreased (Fig. 5). In contrast to the pattern observed in the whole algal community, however, the nutrient enrichment led to an increase in the proportion of ^{14}C-proteins in picoalgae (Fig. 5). The summed proportion of labelled proteins and polysaccharides in the picoplankton was between 75 and 85 % and about 10 % was in both lipids and the LMC pool. These values were similar to those recorded with the whole algal community on Day 1 (Fig. 3B).

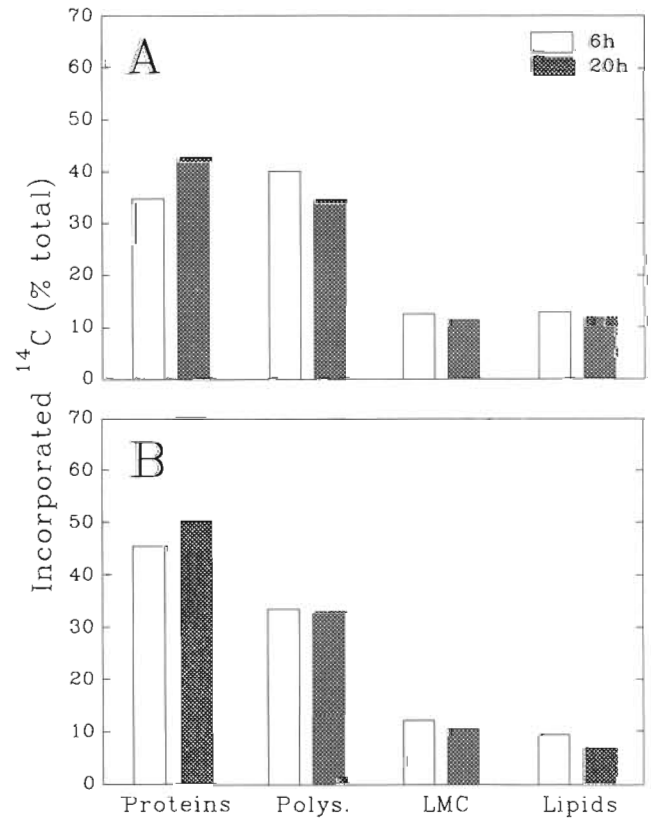

Fig. 5. Incorporation of ^{14}C into the major cellular constituents in < 3 µm picoplankton in Mesocosm 1 on (A) Day 4, without nutrient enrichment, and (B) Day 7, with nutrient enrichment. See Table 1 for details of experiment

DISCUSSION

Response of phytoplankton to nutrient enrichment

At the start of the experiment the primary productivity and chl *a* values were at the low levels (Fig. 2) typically prevailing in the northern Baltic in summer, when the nutrient concentrations are low (e.g. Niemi 1975, Lignell 1990). As expected, daily nutrient additions during the first 2 enrichment periods (Days 1 to 5 in Mesocosm 2 and Days 6 to 10 in Mesocosm 1) led to an increase in algal productivity and biomass. However, the original algal standing stock was too small and its response too slow for immediate exploitation of all the added nutrients, which resulted in accumulation of free mineral nutrients in both experimental units. A simultaneous shift was observed in the composition of the phytoplankton community in the mesocosms (probably at least partly due to the confinement of the plankton community), which may in part explain the lag in the responses. The algal growth was evidently further delayed by the steep decrease of about 7 °C in the water temperature between Days 7 and 9 (Fig. 1).

After Day 9, the algal communities showed parallel increases in biomass in the mesocosms (Fig. 2), consuming the mineral nutrients that had accumulated during the preceding enrichment periods. On Day 12, the NH_4^+-N concentrations had fallen below the detection level in both mesocosms and after that they remained negligible, the nutrient enrichment showing only in elevated PO_4^{3-}-P concentrations.

Nitrate concentrations were not measured during the present study. The possibility that chemoautotrophic bacteria had transformed our NH_4^+ additions to NO_3^- can be excluded, however, since experiments covering the phytoplankton growth season in our study area have invariably shown that depletion of NH_4^+ additions in enclosed surface samples is not associated with a concurrent increase in the NO_3^- pool (T. Tamminen pers. comm.). Thus, our results strongly suggest that during the last 2 enrichment periods the size of the phytoplankton communities was N-limited in both units.

The high recoveries of PO_4^{3-} additions in total P measurements (with the exception of the last 5 d period in Mesocosm 2) suggested that sedimentation was small in the mesocosms. Thus, the slow increase in algal biomass and the simultaneous increase in the pools of mineral nutrients as a consequence of nutrient enrichment observed during the first 2 manipulation periods suggest that grazing control by zooplankters (cf. Thingstad & Sakshaug 1990) or viral lysis (Suttle et al. 1990) were important. This view is supported by the results from Mesocosm 1, where the chl *a* values decreased steeply, coming close to the initial level, after the exhaustion of NH_4^+-N on Day 12 (Fig. 2).

Intracellular partitioning of ^{14}C in a fluctuating environment

The proportion of ^{14}C-proteins increased at night (Figs. 3b & 4). This increase was a consequence of the continuous synthesis of proteins, which took place although carbon fixation had ceased, at the expense of the other products of photosynthesis, mainly polysaccharides (Fig. 3A). A similar pattern has frequently been observed in earlier studies (Morris & Skea 1978, Morris et al. 1981, Cuhel & Lean 1987a). The total particulate ^{14}C decreased by about 10 % during the 10 h dark period, evidently mainly due to algal respiration of the carbohydrate storage products (and in part due to leakage of dissolved organic ^{14}C), as most large algal grazers were removed by < 20 µm prefractionation.

Nutrient enrichment led to a strong increase in the proportion of ^{14}C channelled into the LMC pool in the 6 h light incubations (Fig. 4). During the subsequent dark period the proportion of the ^{14}C-LMC pool decreased, but it still remained high compared to the corresponding value in the mesocosm without nutrient enrichment. The increase in the ^{14}C-LMC pool in the unit with nutrient enrichment was probably due to the enhanced photosynthetic production of precursors of macromolecules during the day and to increased transport of carbon skeletons from storage compounds via the LMC pool to macromolecules at night (cf. Cuhel & Lean 1987b). The proportions of ^{14}C-proteins remained relatively low, even after 20 h incubation, compared to the periods with no nutrient enrichment (Fig. 4). Therefore, the products of the LMC pool were evidently not used solely for building proteins at night, but the algal cells also enhanced the synthesis of structural components and pigments necessary for promotion of growth (Fig. 4; cf. Cuhel & Lean 1987a).

During the last two 5 d manipulation periods, the proportion of ^{14}C channelled into proteins increased in the mesocosm with no nutrient additions (Fig. 4). Corresponding results have been recorded in natural marine environments, where the relative incorporation of ^{14}C into protein was highest in oligotrophic conditions and lowest in waters with higher nutrient concentrations and biomass (Morris & Skea 1978, Morris et al. 1981, Priscu & Priscu 1984).

These results are surprising, as they seem to contradict the generally accepted view of higher C:N ratios and lower protein contents in N-limited algal cells compared to N-sufficient ones (e.g Morris 1981). Possibly, the relatively low proportions of ^{14}C-proteins

during periods with nutrient enrichment reflected the formation of large intracellular pools of labelled amino acids, because with NH_4^+ as the main N source protein synthesis is the slowest step in algal N assimilation (Dortch 1982). This hypothesis seems to be supported by the strong inverse relationship between the proportions of ^{14}C-proteins and ^{14}C-LMC pools during the last two 5 d periods in the units with nutrient enrichment (Fig. 4).

The proportions of ^{14}C-polysaccharide pools showed parallel fluctuations in the mesocosms, irrespective of the alternating nutrient enrichment periods (Fig. 4). A ^{14}C-polysaccharide peak, accounting for 40 % of total ^{14}C, was observed on Day 8 in both mesocosms (Fig. 4). The peak was associated with a steep decrease of 5 °C in the water temperature between Days 7 and 8 (Fig. 1). These observations are parallel to those of Cuhel & Lean (1987a), who found a decrease in lake water temperatures to be associated with increased channelling of ^{14}C into carbohydrates. Moreover, the simultaneous strong increase in irradiance (Fig. 1) may have further facilitated the temporary excess channelling of ^{14}C into polysaccharide storage products, as protein synthesis seems to be more temperature-dependent than photosynthesis (Hawes 1990) and also saturates at lower light levels (Cuhel & Lean 1987a).

Partitioning of ^{14}C incorporated into picoalgae

The proportion of ^{14}C channelled into picoalgal proteins (Fig. 5) was within the range of 30 to 60 % reported by Glover & Smith (1988) and Howard & Joint (1989). The picoalgae showed a diel pattern similar to that of large algae, with an increase in the proportion of ^{14}C-proteins during the night (Fig. 5). Smith et al. (1985) also found that the proportion of ^{14}C-proteins increased nocturnally in the < 1 µm fraction. However, in contrast to large algae, the picoalgae exposed to nutrient enrichment showed an increase in the proportion of ^{14}C-proteins of 7 to 10 percentage points (Figs. 4 & 5). This difference may be related to the small size and short generation times of picoalgae, which seem to lead to relatively smaller storage pools in picoalgal cells compared to large algae (Raven 1986). Moreover, Cuhel & Waterbury (1984) suggested that the TCA-soluble 'polysaccharide' fraction, which also contains nucleic acids, consists mostly of RNA in picoalgae. Thus, the chemical composition of the cells and the patterns of ^{14}C incorporation into macromolecules may follow more closely changes in the external nutrient conditions in the case of picoalgae than with large algae (resulting in lower C:N ratios and higher protein content under nutrient sufficient conditions; cf. Morris 1981), and this may explain the differences in the synthetic patterns observed between large and pico-sized algae.

Interpretation of the intracellular ^{14}C labelling patterns

The proportion of ^{14}C-lipids remained fairly constant in the whole samples throughout the experiment (Fig. 4), reflecting the fundamental role of these compounds as components of algal structures (mainly membranes and chlorophyll complexes). On the other hand, Mague et al. (1980) showed that the LMC pool reaches an isotopic equilibrium faster than the bulk cell material (but see Jensen et al. 1985), and hence the proportion of the LMC pool may have been overestimated in our ^{14}C incorporation measurements, especially with the 6 h incubations. Accordingly, the higher proportion of ^{14}C-proteins observed in the mesocosms without nutrient enrichment did not necessarily reflect increased absolute synthesis of proteins in relation to bulk algal carbon, as algae may have responded to nutrient scarcity by increasing their protein turnover rates. The interpretation of the ^{14}C labelling patterns of macromolecules was further complicated by the dynamics of phytoplankton succession in the mesocosms, since phytoplankton species differ in their chemical composition (Parsons et al. 1961).

However, the chemical composition of phytoplankton seems to be influenced more by its physiological state than its species composition (Goldman 1980). As discussed above, the increase in the proportion of ^{14}C-proteins which was observed during the last two 5 d manipulation periods in the mesocosms without nutrient enrichment (Fig. 4) may have reflected physiological stress caused by temporary N-deficiency in the algae. If this is true, then the gradual decrease in the proportion of ^{14}C-proteins during the first 2 enrichment periods (Fig. 4) may have reflected the diminishing importance of those algae that declined due to the 'confinement effect' and the simultaneously increasing importance of those algae that thrived in the mesocosms (mainly small centric diatoms and flagellate *Pseudopedinella elastica*). During the 2nd manipulation period (Days 6 to 10), the nutrient concentrations were high in both mesocosms, and the proportions of ^{14}C-proteins ranged from 30 to 40 % (6 and 20 h incubations respectively), being similar in the 2 mesocosms. From then on, the proportions of ^{14}C-proteins attained similar values (30 to 40 %) at the end of the nutrient enrichment periods (Days 15 and 20 in Units 2 and 1, respectively), which indicates that these values actually reflected 'balanced growth' of the prevailing phytoplankton communities with regard to N and P.

The POC:PON ratios of 6.3 to 11.1 were higher than the Redfield ratio (5.7) in both mesocosms. This indicates a moderate N deficiency in the algal cells (cf. Goldman 1980), though interference of detritus with a high C:N ratio cannot be ruled out. If we use the mean PON:POC ratio of 0.13 (these ratios did not show any covariation with our biological variables), and assume that the percentage protein C in phytoplankton is 280 times this ratio (DiTullio & Laws 1983; hence assuming that the effect of detritus on the PON:POC ratios was small), the mean protein C content of algal cells was about 35 %. This value is within the range of ca 30 to 55 % reported for the protein C content of various exponentially growing marine algal species (Parsons et al. 1961). Moreover, the percentage protein C value of 35 % based on the mean PON:POC ratio was within the range of 30 to 40 % found for the proportions of ^{14}C-proteins during 'balanced growth' (see above) in the mesocosms with added nutrients (Fig. 4). Thus, our results seem to agree with those of DiTullio & Laws (1986), who found with 5 unicellular algae grown in cyclostats that after a 12:12 h light:dark cycle the proportion of ^{14}C-proteins in the total incorporated ^{14}C provided an accurate estimate of the percentage protein C in algal cells.

Protein C-specific growth rates of phytoplankton (based on the turnover rate of protein C in the 20 h ^{14}C incubations, assuming a carbon content of 11 % of wet weight and a percentage protein C of 35 % in algal cells) were ca 0.4 d^{-1} in the original algal assemblage, and these values varied between 0.2 and 0.5 d^{-1} during the experiment with no clear patterns in their temporal development (data not shown). Thus, while the net effect of the diel fluctuations in the channelling of newly incorporated inorganic carbon into polysaccharides and proteins (Figs. 3 & 4) may have been small, the changes in the external nutrient conditions and temperature were probably also reflected in the chemical composition of the algae. This was especially true with prolonged nutrient enrichment, as nutrient additions led to a considerable change in the ^{14}C labelling pattern of algal cell constituents which lasted for several days (Fig. 4). Our results indicate that during periods with abrupt changes in growth conditions, such as vernal blooms and upwellings, natural phytoplankton communities may show ecologically important fluctuations in their nutritional value as food for zooplankton (cf. Scott 1980). This conclusion was confirmed in 1988, when a considerable increase in the proportion of ^{14}C-lipids was recorded after the culmination of the spring bloom in our study area, which was followed by increased proportions of ^{14}C-proteins during the summer period with low ambient nutrient concentrations (Lindqvist & Lignell unpubl.).

Concluding remarks

The ^{14}C labelling patterns of phytoplankton showed in general terms how the algae were responding biochemically to their fluctuating environment. The concurrent succession of the phytoplankton community seemed to explain in part the observed labelling patterns. The interpretation of the data was not straightforward, however, as temperature, light, nutrients and the composition of algal species all changed simultaneously during the time course.

The particulate organic ^{14}C pool examined in our molecular extractions does not comprise all the products of photosynthesis, as a significant part (from 2 to 15 %) of the incorporated carbon was lost as dissolved organic ^{14}C via exudation (data not shown). While the excreted products provide an important source of carbon for bacteria in the summer in our study area (Lignell 1990), the biomass production of large (> 2 µm) algae (contributing ca 75 % of primary production; Lignell 1990) is probably the key process sustaining the higher trophic levels of the plankton food web, as the microbial loop seems to be inefficient in returning the organic carbon from bacteria and picoalgae to large zooplankters (cf. Sherr & Sherr 1988).

Our results showed that the general biochemical patterns of phytoplankton primary production vary in a fluctuating environment. Thus, determination of intracellular biochemical ^{14}C-fractions supplements primary productivity measurements based on total incorporation of ^{14}C by providing ecologically important information about the physiological state of natural phytoplankton communities and the nutritional value of newly produced algal biomass.

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LITERATURE CITED

Cuhel, R. L., Lean, D. R. S. (1987a). Influence of light intensity, light quality, temperature, and daylength on uptake and assimilation of carbon dioxide and sulfate by lake plankton. Can. J. Fish. Aquat. Sci. 44: 2118-2132

Cuhel, R. L., Lean, D. R. S. (1987b). Protein synthesis by lake plankton measured using *in situ* carbon dioxide and sulfate assimilation. Can. J. Fish. Aquat. Sci. 44: 2102-2117

Cuhel, R. L., Waterbury, J. B. (1984). Biochemical composition and short term nutrient incorporation patterns in a unicellular marine cyanobacterium, *Synechococcus* (WH7803). Limnol. Oceanogr. 29: 370-374

DiTullio, G. R., Laws, E. A. (1983). Estimates of phytoplankton N uptake based on 14CO2 incorporation into protein. Limnol. Oceanogr. 28: 177–185

DiTullio, G. R., Laws, E. A. (1986). Diel periodicity of nitrogen and carbon assimilation in five species of marine phytoplankton: accuracy of methodology for predicting N-assimilation rates and N/C composition ratios. Mar. Ecol. Prog. Ser. 32: 123–132

Dortch, Q. (1982). Effect of growth conditions on accumulation of internal nitrate, ammonium, amino acids, and protein in three marine diatoms. J. exp. mar. Biol. Ecol. 61: 243–261

Edler, L., Hällfors, G., Niemi, Å. (1984). A preliminary checklist of the phytoplankton of the Baltic Sea. Acta bot. fenn. 128: 1–26

Glover, H. E., Smith, A. E. (1988). Diel patterns of carbon incorporation into biochemical constituents of *Synechococcus* spp. and larger algae in the Northwest Atlantic Ocean. Mar. Biol. 97: 259–267

Goldman, J. C. (1980). Physiological processes, nutrient availability, and the concept of relative growth rate in marine plankton ecology. In: Falkowski, P. G. (ed.) Primary productivity in the sea. Plenum, New York, p. 179–194

Hawes, I. (1990). Photosynthate partitioning in Antarctic freshwater phytoplankton: effects of light and temperature. J. Plankton Res. 12: 513–518

Healey, F. P., Hendzel, L. L. (1980). Physiological indicators of nutrient deficiency in lake phytoplankton. Can. J. Fish. Aquat. Sci. 37: 442–453

Howard, K. M., Joint, I. R. (1989). Physiological ecology of picoplankton in the North Sea. Mar. Biol. 102: 275–281

Jensen, L. M., Jørgensen, N. O. G., Søndergaard, M. (1985). Specific activity. Significance in estimating release rates of extracellular dissolved organic carbon (EOC) by algae. Verh. int. Verein. Limnol. 22: 2893–2897

Lancelot, C., Mathot, S. (1985). Biochemical fractionation of primary production by phytoplankton in Belgian coastal waters during short- and long-term incubations with ^{14}C-bicarbonate I. Mixed diatom population. Mar. Biol. 86: 219–226

Lignell, R. (1990). Excretion of organic carbon by phytoplankton: its relation to algal biomass, primary productivity and bacterial secondary productivity in the Baltic Sea. Mar. Ecol. Prog. Ser. 68: 85–99

Mague, T H., Friberg, E., Hughes, D. J., Morris, I. (1980). Extracellular release of carbon by marine phytoplankton; a physiological approach. Limnol. Oceanogr. 25: 262–279

Morris, I. (1981). Photosynthetic products, physiological state, and phytoplankton growth. Can. Bull. Fish. Aquat. Sci. 210: 83–102

Morris, I., Glover, H. E., Yentsch, C. S. (1974). Products of photosynthesis by marine phytoplankton: the effects of environmental factors on the relative rates of protein synthesis. Mar. Biol. 27: 1–9

Morris, I., Skea, W. (1978). Products of photosynthesis in natural populations of marine phytoplankton from the Gulf of Maine. Mar. Biol. 47: 303–312

Morris, I., Smith, A. E., Glover, H. E. (1981). Products of photosynthesis in phytoplankton off the Orinoco River and in the Caribbean Sea. Limnol. Oceanogr. 26: 1034–1044

Niemi, Å. (1975). Ecology of phytoplankton in the Tvärminne area, SW coast of Finland. II. Primary production and environmental conditions in the archipelago and the sea zone. Acta bot. Fenn. 105: 1–73

Niemi, M., Kuparinen, J., Uusi-Rauva, A., Korhonen, K. (1983). Preparation of algal samples for liquid scintillation counting. Hydrobiol. 106: 149–156

Parsons, T. R., Stephens, K., Strickland, J. D. M. (1961). On the chemical composition of eleven species of marine phytoplankton. J. Fish. Res. Bd Can. 18: 1001–1016

Priscu, J. C., Priscu, L. R. (1984). Photosynthate partitioning by phytoplankton in a New Zealand coastal upwelling system. Mar. Biol. 81: 31–40

Raven, J. A. (1986). Physiological consequences of extremely small size for autotrophic organisms in the sea. In: Platt, T., Li, W. K. W. (eds.) Photosynthetic picoplankton. Can. Bull. Fish. Aquat. Sci. 214: 1–70

Rivkin, R. B. (1985). Carbon-14 labelling patterns of individual marine phytoplankton from natural populations. Mar. Biol. 89: 135–142

Scott, J. M. (1980). Effect of growth rate of the food alga on the growth/ingestion efficiency of a marine herbivore. J. mar. Biol. Ass. U.K. 60: 681–702

Sherr, E., Sherr, B. (1988). Role of microbes in pelagic food webs: a revised concept. Limnol. Oceanogr. 33: 1225–1227

Smith, J. C., Platt. T., Li, W. K. W., Horne, E. P. W., Harrison, W. G., Subba Rao, D. V., Irwin, B. D. (1985). Arctic marine photoautotrophic picoplankton. Mar. Ecol. Prog. Ser. 20: 207–220

Steemann Nielsen, E. (1952). The use of radio-active carbon (C^{14}) for measuring organic production in the sea. J. Cons. perm. int. Explor. Mer. 18: 117–140

Suttle, C. A., Chan, A. M., Cottrell, M. T. (1990). Infection of phytoplankton by viruses and reduction of primary productivity. Nature, Lond. 347: 467–469

Thingstad, T. F., Sakshaug, E. (1990). Control of phytoplankton growth in nutrient recycling ecosystems. Theory and terminology. Mar. Ecol. Prog. Ser. 63: 261–272

Utermöhl, H. (1958). Zur Vervollkommnung der quantitativen Phytoplankton Methodik. Mitt. int. Ver. Limnol. 9: 1–38

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