Intracellular partitioning of $^{14}CO_2$ in phytoplankton during a growth season in the northern Baltic

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ABSTRACT During the phytoplankton succession in the northern Baltic in 1988, the distribution of $^{14}CO_2$ assimilated by algae into the main molecular groups [proteins, polysaccharides, lipids and low molar mass compounds (LMC)] after in situ light (6 h) and light to dark (20 h from ca 11:00 to 07:00 h) incubations at 2 m depth (just below maximum $^{14}CO_2$ fixation) was studied. By early May, the high winter levels of mineral nutrients were depleted from the water column, and in middle May the spring bloom predominated by large dinoflagellates (diatoms subdominant) peaked. The proportion of ^{14}C lipids was usually ca 15% of total $^{14}CO_2$ fixation, but it showed a distinct peak of 40% in middle May. The ^{14}C-lipid peak probably reflected nutrient stress of the algae, since nutrient (N+P) enrichment decreased this peak by 15 percentage points in 100 l enclosures. During the decline of the spring bloom, the proportion of ^{14}C proteins increased despite low ambient mineral N concentrations. In summer, the phytoplankton community (mainly small flagellates) consistently exhibited remarkable channelling of $^{14}CO_2$ into proteins (50 to 60%), which conformed to the low particulate organic C:N ratios of ca 7 (mol/mol). Summer upwellings, which introduced nutrients into the mixed layer, seemed to be accompanied by the highest proportions of ^{14}C proteins. The proportion of ^{14}C polysaccharides was usually ca 20%. After 6 h incubations, this proportion was significantly (on average 10 percentage points) higher than after 20 h, while the inverse was true with ^{14}C proteins, which reflected continuous nocturnal synthesis of proteins (enzymes) at the expense of polysaccharide storage products. In conclusion, the high proportions of algal ^{14}C proteins in summer suggest that phytoplankton is usually not physiologically N limited in our study area and provides N-sufficient food for herbivores, hence enabling high efficiency of algal C transfer to higher trophic levels.

KEY WORDS: Phytoplankton · Succession · ^{14}C-labelled macromolecules · Proteins · Lipids · Baltic Sea

INTRODUCTION

Primary productivity of phytoplankton usually forms the base of marine pelagic food webs. The composition of the freshly synthesised organic products reflects the activity of the photosynthetic apparatus and the regulated channelling of substances in the cells. These metabolic functions are very sensitive, but simultaneously show a conservative behaviour, responding to environmental changes according to a hierarchical pattern which depends on the amplitude and duration of the changes (Dortch & Postel 1989). As a result, the

physiological processes reflect integrated responses of algae to their growth conditions. Evidently, the classical ^{14}C method (Steemann Nielsen 1952), which measures total algal $^{14}CO_2$ fixation, provides only limited information about the physiological state and nutritional value of phytoplankton.

The extraction differentiation of incorporated ^{14}C into the main macromolecular pools [proteins, polysaccharides, lipids and low molar mass compounds (LMC); Morris et al. 1974] is an extension of the classical ^{14}C method and allows not only the quantity but also the quality of the synthesised organic substances to be evaluated. The extraction method has frequently been used in varying environments, as it measures the newly incorporated carbon, and hence only active phytoplankton is examined, without the hampering effect of detritus or other living particles. Its sensitivity

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in revealing qualitative changes in the algal standing stock should therefore be higher than analyses of the bulk particulate matter

Morris (1981) pointed out that extraction of ^{14}C photosynthates into major functional groups excludes the possibility of following the specific photosynthetic processes because the distinctions between those and other cellular reactions is not clear. Algal metabolism tends to compensate any deficiency, inducing metabolic pathways and cascade reactions which are hard to interpret by their end-products. Moreover, the results vary in response to the diel cycle, growth rate, algal size, species composition and long-term physical changes, making the interpretation of changes in natural phytoplankton communities complicated.

Nevertheless, the biochemical protein:carbohydrate ratio has been shown to sensitively indicate the physiological state of phytoplankton (Haug et al. 1973), while in some cases increased synthesis of lipids is a sign of nutrient deficiency (Taguchi et al. 1987, Reitan et al. 1994). Furthermore, partitioning of fixed ^{14}CO$_2$ in algal cells reveals several important aspects of the physiological and ecological status of phytoplankton. For example, since protein synthesis usually seems to be irreversible in algal cells, it has been suggested to be a more accurate index for phytoplankton growth than total carbon fixation (Morris 1981). The chemical composition of algal cells determines their nutritional value for herbivores as well, hence affecting the efficiency of algal C transfer in planktonic food webs (Scott 1980, Kiørboe 1989, Roman 1991, Sterner & Hessen 1994, Thompson et al. 1994).

Rapid biochemical changes in algal cells can be particularly expected during blooms and upwellings, when physical and chemical forcing is strong (Haug et al. 1973, Hitchcock 1978, Morris 1981). In support of this, we found rapidly changing irradiance, temperature and nutrient availability to be reflected in the accumulation of ^{14}C in algal macromolecules in a summer mesocosm study (Lignell & Lindqvist 1992). The development of biochemical ^{14}C-incorporation patterns in natural phytoplankton communities during spring blooms has been reported by some authors (e.g Hitchcock 1978, Parrish 1987, Smith & D'Souza 1993), and a few studies have described the seasonal development over longer periods (e.g Morris & Skea 1978, Taguchi & Laws 1987). However, information from European coastal waters is still sparse (e.g. Howard & Joint 1989, Lignell & Lindqvist 1992, Fernández et al. 1994) and seasonal studies seem to be lacking altogether

The present work gives an overview of the development of the ^{14}C-incorporation pattern in natural phytoplankton along with relevant physico-chemical and biological variables during the 1988 season of algal

growth in the northern Baltic. Upwellings take place frequently in our study area (Haapala 1994), drastically altering the growth conditions of the pelagic plankton community. Adaptation to the changing environment induces changes in the biochemistry of algal cells, and hence our investigation supplements the numerous total ^{14}CO$_2$-fixation studies carried out in the area.

METHODS

Sampling site and experiments. The sampling station (59° 47.6' N, 23° 19.5' E) was situated offshore of the eastern Hanko archipelago at the entrance to the Gulf of Finland, the northern Baltic. It is 46 m deep with a mixed layer depth of 10 to 15 m and no permanent halocline. The salinity and pH are ca 6‰ and 8, respectively. The area is not affected by major sewage outlets. Detailed information about the local hydrography is given in Niemi (1975) and Haapala (1994). The seasonal dynamics of nutrients and planktonic organisms and related state and process variables are well known in the Tvärminne area (see e.g. Niemi 1975 and references therein, Lignell et al. 1993 and references therein). Phytoplankton primary productivity usually follows the unimodal pattern commonly found in temperate waters, and the reported annual integral values vary between 74 and 111 g C m^{-2} (in situ ^{14}C incubations from twice a week to twice a month, acidified whole ^{14}C samples; see Lignell et al. 1993).

The study lasted from 19 April to 6 September 1988. Samples were collected weekly or every other week from the vernal phytoplankton bloom until the middle of June. Thereafter 4 experiments were performed: 2 in July, 1 in August and 1 in September. The samples were taken between 07:00 and 08:00 h from 2.5 m depth with a 9 l Sormunen sampler. Water temperature and the cumulative daily solar irradiation (Kipp & Zonen solarimeter, The Netherlands) were registered throughout the experiment.

On 17 May and 8 June, the time course of the diel ^{14}C-incorporation pattern was studied in vitro (triplicate natural phytoplankton samples, light intensity 60 µE m^{-2} s^{-1}, in situ temperature). The effect of light intensity on the ^{14}C partitioning was determined by covering half of the incubation bottles with neutral density screens, which allowed 20% of the irradiation (12 µE m^{-2} s^{-1}) to penetrate.

Finally, a 4 d enclosure experiment with daily nutrient additions was started on 10 May. Two transparent plastic bags (100 l each) were mounted in a floating rack and filled with water from the outer archipelago. Nutrients (0.5 µmol PO$_4$$^{3-}$-P l^{-1} and 4.3 µmol NH$_4$$^{+}$-N l^{-1}) were added daily to the NP enclosure while the other one served as an untreated control. The concen-

trations of mineral N and P, chlorophyll a (chl a) and the primary productivity were measured daily with samplings at 08:00 h.

Extraction of major cell constituents and primary productivity. During the vernal bloom the samples were incubated without any prefiltration due to predominance of large algae. From 26 May to the end of June, and from July to September, prefiltrations through 40 μm and 20 μm plankton nets, respectively, were performed to eliminate herbivorous zooplankton. After prefiltration the water was immediately dispensed to triplicate 100 ml glass bottles and 370 kBq NaH^{14}CO$_3$ (Amersham, UK) was added. The bottles were incubated *in situ* at 2 m depth (at or just below photosynthetic maximum on sunny days; e.g. Niemi 1975) in a bay near the laboratory from ca 11:00 h for 6 and 20 h (zero-time blank served as control). Water temperature deviated ±0.5°C from that at the sampling station.

Handling of samples and measurements were done according to Morris et al. (1974), with modifications as described by Lignell & Lindqvist (1992). Briefly, after incubation the samples were filtered onto Whatman GF/C glass-fibre filters. The filters were rinsed with 30 ml prefiltered (Whatman GF/F) sea water and stored frozen (−20°C) in glass scintillation vials until extractions were performed. The particulate organic ^{14}C on the filters was extracted into 4 fractions: lipids, LMC, polysaccharides and proteins. To complete the lipid extraction the samples were centrifuged in conical tubes (1000 × g, 5 min). The lower chloroform phase (lipid fraction) was carefully separated with a narrow pipette and filtered through a GF/F glass-fibre filter directly into a scintillation vial. (Due to potential health hazard the extraction was done in a fume cupboard.) The upper methanol/water phase was filtered through the same filter but gathered in another vial (LMC fraction) The GF/F filter was brought together with the original GF/C filter, and proteins were precipitated with 5% trichloroacetic acid. The combined filtrate was assumed to contain polysaccharides (Morris et al. 1974). With all ^{14}C samples, scintillation cocktail (10 ml Lumagel, LUMAC, The Netherlands) was added and the radioactivities were measured in a liquid scintillation counter (1219 Rackbeta, LKB Wallac, Finland) using the external standard channel ratio method.

Particulate primary productivity (Steemann Nielsen 1952) was measured by incubating 100 ml samples with 185 kBq NaH^{14}CO$_3$ for 24 h. The samples were filtered through GF/C glass-fibre filters, which were rinsed with 30 ml GF/F filtered sea water and placed in plastic scintillation vials, and 100 μl 1 M HCl was added to vaporise surplus inorganic ^{14}C. The vials were allowed to stand uncapped in a fume cupboard overnight, before 10 ml scintillation cocktail was added and the radioactivity was measured.

Nutrients, chl a and algal biomass. Dissolved ammonium-N, nitrate-N and phosphate-P were determined according to Koroleff (1976). In particulate organic carbon and nitrogen measurements, all filtration glassware and Whatman GF/F glass-fibre filters were acid-washed and precombusted (4 h at 500°C). The filters were dried and stored at room temperature until analysis with a Heraeus CHN-O-RAPID analyser. Chl a was filtered on GF/C filters (100 ml triplicate samples) and extracted in 94% ethanol for 24 h in darkness at room temperature (Jespersen & Christoffersen 1987), and then measured fluorometrically (Model 450, Sequoia-Turner Corporation, USA; calibrated with pure chl a; Sigma). Algal species were determined on Lugol-preserved samples according to Utermöhl (1958), using phase contrast microscopy (Leitz Diavert, Germany). The nomenclature follows Edler et al. (1984) with some modifications.

RESULTS

Nutrients, algal biomass and primary productivity

In early April, the ice broke up, and then the surface water gradually warmed up from 1°C at the end of April to over 20°C in the middle of July (Fig. 1). Several upwellings occurred during the study period, and due to a vigorous one on 23 July, the surface water temperature decreased by ca 10°C within 10 d (Fig. 1). Simultaneously the daily solar irradiance decreased to half of the values in July because of cloudy and unstable weather, changing the physical conditions for algal growth as well.
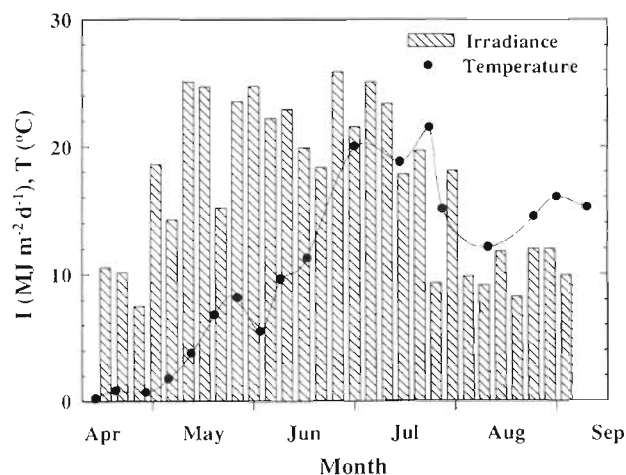

Fig. 1 Total daily solar irradiance on the surface (I) and the temperature (T) at the sampling depth (mean of 5 d) during the 1988 season of phytoplankton growth off the SW coast of Finland
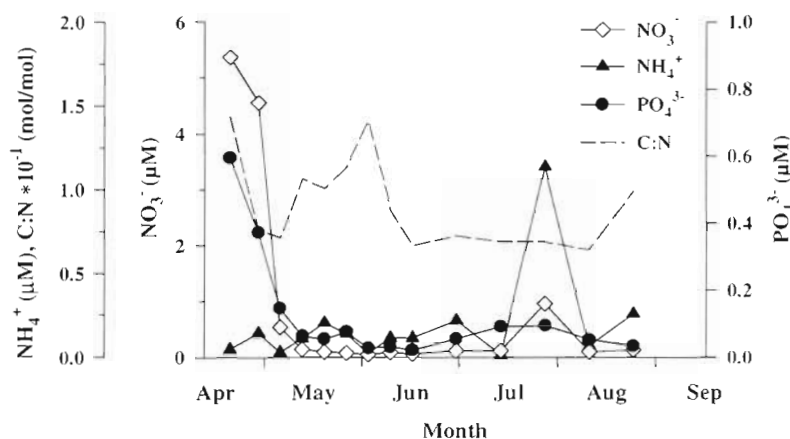
Fig. 2. Concentrations of major mineral nutrients (PO_4^{3-}-P, NH_4^+-N and NO_3^--N) and the particulate organic carbon to nitrogen ratio (C:N) in the surface layer off the SW coast of Finland in 1988. Note the different y-axis scales

ca 14 on 19 April and 2 June, while it was ca 7 at the beginning of May and again during most of the summer (Fig. 2; from March to early April the C:N ratio was 8 to 9; data not shown). The peak particulate C:N values were preceded by vigorous upwellings caused by strong NW winds in mid-April and at the end of May. The diatom *Skeletonema costatum* (G.) Cleve predominated at the end of May, being a typical late vernal bloom species in our study area. After the spring bloom (from 15 June) small flagellated Euglenophyceae predominated the phytoplankton biomass (60%), and they were followed by Cryptophyceae, Cyanophyceae and Dinophyceae in July and August.

At the beginning of May, mineral nitrogen and phosphorus were depleted from the water column (Fig 2), and the particulate primary productivity and chl a reached their respective maxima of 17.4 mg C m^{-3} h^{-1} and 24.1 mg m^{-3} a week or two thereafter (Fig. 3). At the end of May, chl a and primary productivity declined sharply due to sedimentation of the spring bloom and, after that, chl a exhibited typical low summer values of <5 mg m^{-3} The strong upwelling on 23 July resulted in a temporary increase in NO_3^- and NH_4^+ concentrations (both ca 1 µmol l^{-1}) in the surface layer.

During the spring bloom, the phytoplankton community consisted mainly of dinoflagellates (60 to 90% of the biomass) and diatoms (10 to 40%). The particulate organic C:N ratio (mol/mol) showed maximum values of
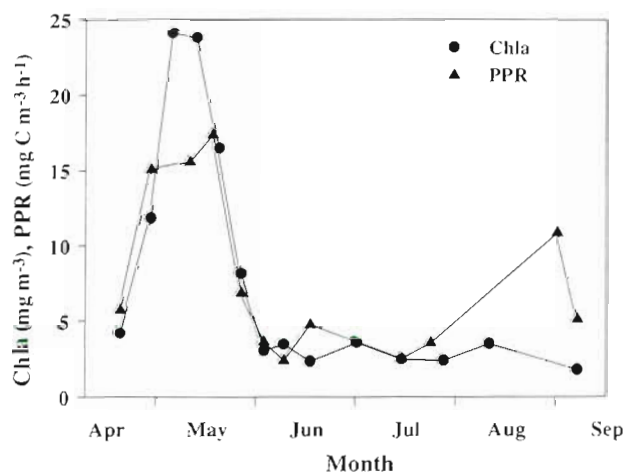
Partitioning of $^{14}CO_2$ in algal cells

The proportion of ^{14}C proteins was on average ca 10 percentage points higher after 20 h (day to night) than 6 h (daylight) incubations, whereas in summer the inverse was true with ^{14}C polysaccharides, suggesting that energy and carbon skeletons from ^{14}C polysaccharide storage products were then used for continuous ^{14}C protein synthesis during the night (Figs. 4 to 6). When the proportions of the 4 different biochemical ^{14}C fractions were calculated separately for each replicate sample, the coefficient of variation of 3 replicates (occasionally duplicates due to failed extraction or missing replicate) averaged from 4% (^{14}C proteins) to 9% (^{14}C LMC and ^{14}C lipids) (n = 11), which shows that our extractions were precise. For example, in the case of ^{14}C proteins with an average proportion of ca 50% of total particulate $^{14}CO_2$ fixation (Fig. 4B), the 95% confidence limits of these proportions were approximately $\pm(2 \times 0.04/\sqrt{3}) \times 50\% = \pm2$ to 3 percentage points, and hence the nocturnal increase of ca 10 percentage points was evidently also statistically significant.

The proportion of ^{14}C polysaccharides in 20 h incubations was highest (ca 30%) in early spring and early autumn, and ca 20% during the period in between. In contrast, the proportion of ^{14}C proteins was lowest (ca 30%) in middle May or about a week after depletion of mineral N from the mixed layer; after that, this proportion started to increase, being 50 to 60% most of the summer (Figs. 2 & 4). The peak ^{14}C-protein values in July were contemporary with enhanced nutrient availability caused by upwelling episodes.

Generally, the proportion of ^{14}C lipids was between 10 and 20%, but it increased to 40% in middle May,
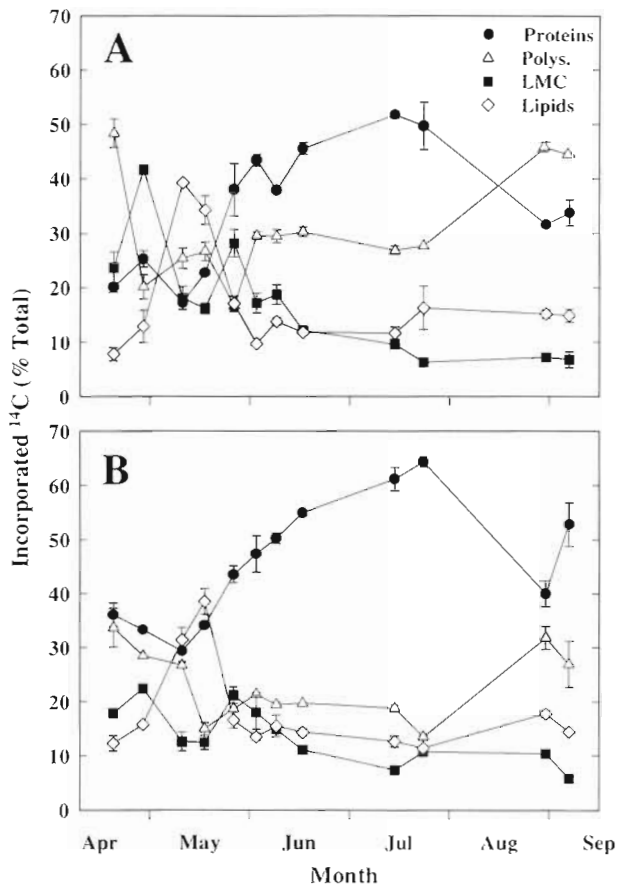

Fig. 3. Chlorophyll a (Chla) and particulate primary productivity (PPR, 6 h incubations) at 2 m depth off the SW coast of Finland in 1988

Fig. 4. Annual development of the $^{14}CO_2$-incorporation pattern into the main algal macromolecules. Percentages of summed ^{14}C incorporation after (A) 6 h (daylight) and (B) 20 h (day to night) *in situ* incubations at 2 m depth (just below maximum $^{14}CO_2$ fixation). Polys.: polysaccharides, LMC: low molar mass compounds. Error bars show ± SD
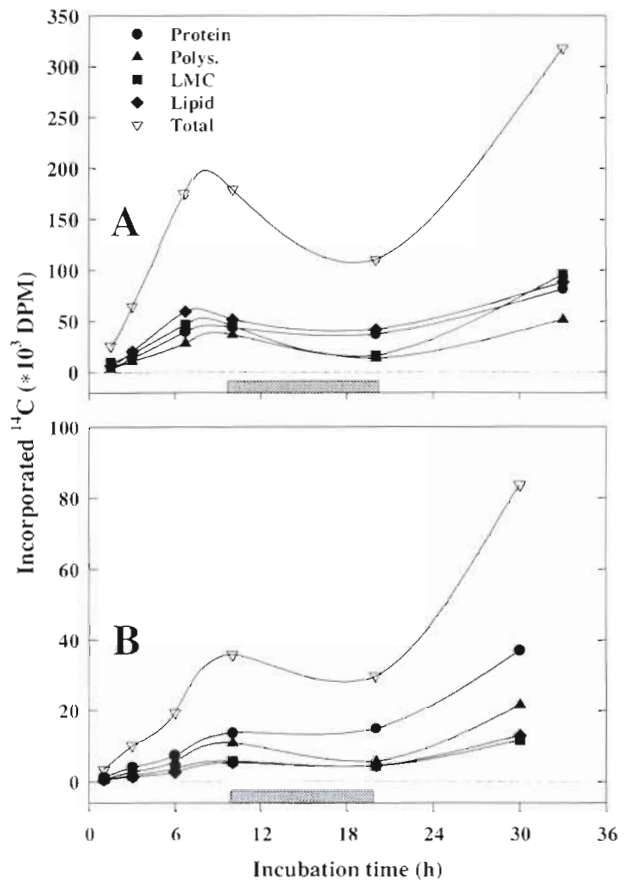
Fig. 5. Time course of ^{14}C labelling of the total biomass of natural phytoplankton communities and their main macromolecular groups *in vitro* on (A) 17 May (spring bloom) and (B) 8 June (summer minimum). Hatched area denotes dark period

during the period of peak chl *a* and primary productivity values (Figs. 3 & 4) and sedimentation of the most important early spring diatoms *Achnanthes taeniata* Grunow and *Chaetoceros holsaticus* Schütt (Heiskanen 1995). The proportion of ^{14}C LMC was ca 10 %, except for 2 peaks, the first one coinciding with the growing bloom (28 April) and the second one with the declining dinoflagellate spring bloom (late May), when the diatom *Skeletonema costatum* predominated.

Time course ^{14}C-incorporation patterns and effect of light reduction and nutrient enrichment

The temporal diel pattern in ^{14}C incorporation was studied *in vitro* with 2 natural phytoplankton communities in middle May (vernal phytoplankton peak) and early June (summer minimum period). Some loss of particulate fixed ^{14}C was discovered to have occurred

during the night, probably due to respiration of the newly synthesised carbohydrates and excretion of dissolved organic carbon (Fig. 5).

The diel and seasonal changes in the ^{14}C-incorporation patterns were also seen in the time course experiments (Fig. 6). The difference between the proportions of ^{14}C proteins and ^{14}C polysaccharides increased from May to June, because during the vernal primary productivity maximum in May, enhanced synthesis of lipids at the expense of polysaccharides and proteins took place (Fig. 6). Interestingly, polysaccharides, rather than lipids, seemed to be used for nocturnal respiration also in May, as judged from the lack of decrease in the proportion of ^{14}C lipids during the dark period (Figs. 4, 6 & 7; cf. the effect of reduced irradiation below).

During the first 20 h of incubation (light to dark period), 80% reduction of prevailing irradiation resulted in a slight increase in the proportion of ^{14}C
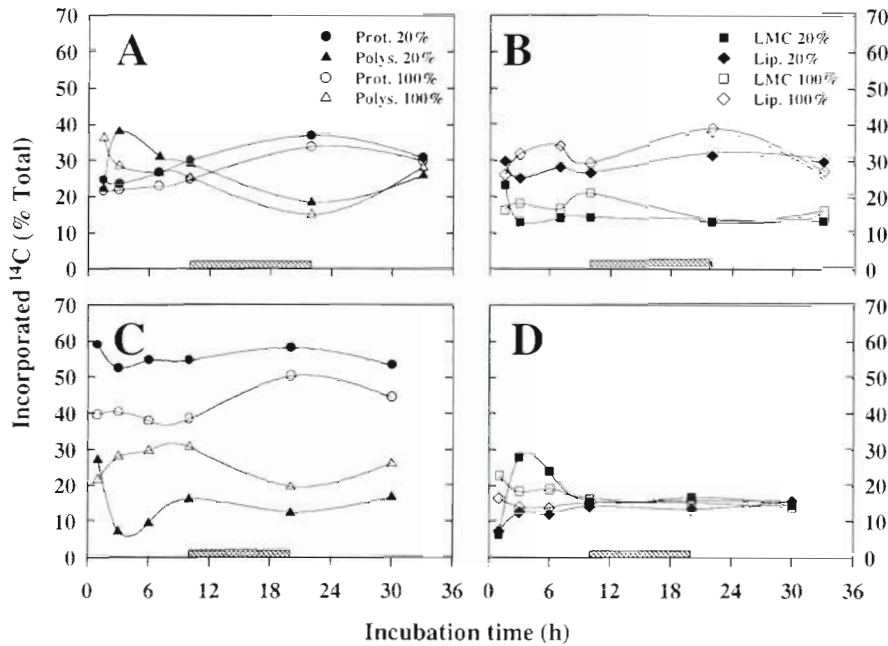
Fig. 6. Effect of light reduction (60 vs 12 $\mu E\ m^{-2}\ s^{-1}$ or 100% vs 20%) on the temporal development of ^{14}C partitioning in phytoplankton (% of summed ^{14}C incorporation) on (A, B) 17 May and (C, D) 8 June. Hatched area denotes dark period. Prot.: proteins, Lip.: lipids

proteins in the spring bloom samples, whereas in June the corresponding increase was more pronounced (8 percentage points; Fig. 6). This increase was accompanied by a decrease in the proportions of ^{14}C lipids and ^{14}C polysaccharides in May and June, respectively.

In the enclosure experiment started on 10 May, daily nutrient (N+P) enrichment resulted in respective increases of ca 100 and 200% in chl a and primary pro-

ductivity in 3 d. In samples from Day 3, nutrient enrichment had evoked a clear increase in the proportion of ^{14}C proteins (by 10 to 15 percentage points; Fig. 7), while the proportions of ^{14}C LMC and ^{14}C polysaccharides did not change remarkably. Incorporation of ^{14}C into lipids peaked during this period both in the natural community (Fig. 4) and in the Control enclosure (Day 3), whereas nutrient enrichment led to a decrease of >15 percentage points in the proportions of ^{14}C lipids in the NP enclosure (Fig. 7).
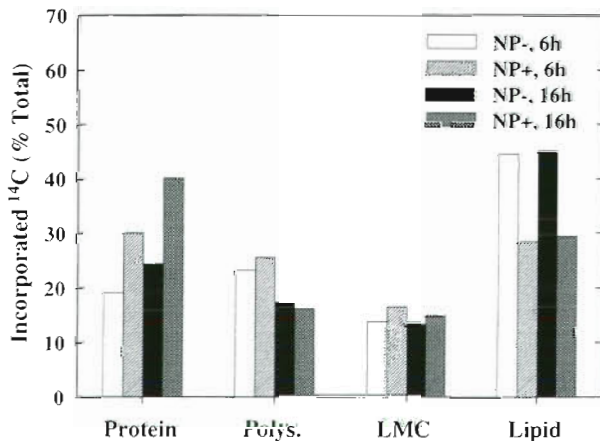

Fig. 7. Effect of nutrient additions (0.5 $\mu mol\ PO_4^{3-}$-$P\ l^{-1}\ d^{-1}$ and 4.3 $\mu mol\ NH_4^+$-$N\ l^{-1}\ d^{-1}$) on the patterns of ^{14}C incorporation into macromolecules in enclosed natural phytoplankton communities representing the spring bloom peak on 10 May 1988 off the SW coast of Finland. Results are from Day 3, and 6 h (daylight) and 16 h (last 6 h in darkness) incubations were used. (NP-) untreated control unit; (NP+) unit with nutrient enrichment

DISCUSSION

The diel incorporation pattern of ^{14}C into newly synthesised biomolecules (Figs. 4 to 6) followed a well-established one, showing intense production of carbohydrates in the daylight and a continuous protein synthesis, which at night goes on mainly at the expense of polysaccharides (e.g. Morris & Skea 1978, Morris et al. 1981, Lignell & Lindqvist 1992). Thus, the proportion of ^{14}C proteins increased significantly at night (in summer, on average by ca 10%), while the inverse was true with ^{14}C polysaccharides. The day to night incubations integrate the effect of the whole diel light cycle on the $^{14}CO_2$-incorporation pattern, and are therefore better related to the actual growth rate, physiological state and biochemical composition of the algae than short-term daylight incubations (Morris 1981, DiTullio & Laws 1986). Thus, when not otherwise stated, the following examinations are based on the results from 20 h incubations.

Intracellular partitioning of $^{14}CO_2$ during phytoplankton succession

^{14}C proteins

During the decline of the spring bloom at the end of May, the proportions of ^{14}C proteins started to increase to the high summer levels, though the mineral nutrients accumulated in winter were already exhausted from the water column (Figs. 2 to 4). A similar increase in the proportion of ^{14}C proteins was recorded during a declining vernal bloom by Morris & Skea (1978). High proportions of ^{14}C proteins generally relate to physiologically healthy algae with high relative growth rates (DiTullio & Laws 1986) and, accordingly, this pattern in ^{14}C incorporation probably reflected, at least in part (along with the simultaneous strong decrease in the proportion of ^{14}C lipids), the success of the diatom *Skeletonema costatum*, which became dominant at the end of this period (Heiskanen 1995). On 19 May, an upwelling took place (Haapala 1994) and may have further enhanced this development by providing an extra nutrient pulse (cf. Lignell & Lindqvist 1992).

The high particulate organic C:N ratios of ca 14 (by mole) recorded on 19 April and at the beginning of June (Fig. 2) indicate seriously (physiologically) N-deficient plankton communities (cf. Morris 1981), and hence seem to contradict the observed development of algal ^{14}C-incorporation patterns. Strong upwellings, which took place in mid-April and on 1 June (Haapala 1994, Heiskanen 1995), most likely explain the apparent discrepancy. These upwellings probably brought detritus with high C:N ratios from the nearby shallows back to the mixed layer. In support of this view, the ratios of algal C (determined by microscopy) to chl *a* decreased during both periods (the decrease was sharp from late May to early June; A.-S. Heiskanen pers. comm.), hence showing a trend opposite that suggested by the C:N ratios of the total particulate organic matter. Altogether, these examinations demonstrate the advantage of using the ^{14}C-incorporation patterns instead of the development biochemical composition of the bulk particulate matter in considerations of the physiological status of phytoplankton.

In summer, the proportions of ^{14}C proteins were high (50 to 60%) irrespective of the variations in the hydrographic conditions and in the species composition of the phytoplankton community. The values are similar to many of those recorded in marine coastal areas (Taguchi & Laws 1987, Howard & Joint 1989, Fernández et al. 1994), though somewhat lower values (30 to 40%) have also been reported, especially in more eutrophicated waters (Morris & Skea 1978, Morris et al. 1981). During the spring bloom of large dinoflagellates and diatoms, the proportions of ^{14}C proteins were

clearly lower than in summer (Fig. 4), which supports findings of an inverse relation between these proportions and algal cell size (Taguchi & Laws 1987, Howard & Joint 1989).

The high percentage of ^{14}C proteins in summer conformed to the elevated biomass-(chl *a*) specific $^{14}CO_2$-fixation rates (Fig. 3; cf. DiTullio & Laws 1986) and to the low particulate organic C:N ratios of ca 7 (Fig. 2), which were close to the Redfield ratio of 6.6 (Redfield et al. 1963) or the ratio exhibited by 'healthy' phytoplankton growing close to maximum rates (Goldman et al. 1979). DiTullio & Laws (1986) found with algal cultures that after 12 h light:12 h dark incubations the proportion of ^{14}C proteins provided a reliable estimate of the percentage protein C in algal cells (see also Lignell & Lindqvist 1992). Assuming that the percentage protein C in phytoplankton is about 280 times the particulate organic N:C ratio (by weight; DiTullio & Laws 1983), the corresponding summer N:C ratios of ca 0.17 give a protein C content of 47% of total algal C (assuming no serious interference by detritus and heterotrophic C). Thus, algal protein content seems indeed to have been high in summer, as was suggested by the high percentage ^{14}C-protein values.

The highest proportions of ^{14}C proteins were recorded in July (Fig. 4), when 2 upwellings (on 4 and 23 July) improved the availability of mineral nutrients in the upper water column (A.-S. Heiskanen pers. comm; this event remains partially uncovered in the sparse nutrient data presented in Fig. 2). Thus, these results seem to conform to those found in our mesocosm study in summer 1988, in which nutrient enrichment evoked an increase in the proportions of ^{14}C proteins after a time lag taken to use up the added nutrients (Lignell & Lindqvist 1992).

It seems surprising that the proportions of ^{14}C proteins showed highest values in summer, when the ambient mineral nutrient levels were lowest or close to the detection level. During periods of prolonged physiological N stress, algae are known to accumulate non-N containing compounds, mainly carbohydrates or lipids (e.g. Morris 1981). Moreover, in declining natural phytoplankton communities, which have shown biochemical (physiological) signs of nutrient deficiency, a decrease in the proportion of fixed $^{14}CO_2$ channelled into proteins has indeed been found (Hitchcock 1978, Smith & D'Souza 1993).

Enclosure (100 l each) experiments following factorial design (all combinations of mineral N and P additions, and < 100 µm prefractionation or removal of large zooplankton) suggested that the biomass of the phytoplankton community is regulated by the availability of mineral N during most of the summer in our study area (Kivi et al. 1993). The considerations above based on high proportions of ^{14}C proteins strongly suggest, how-

ever, not that the algal community is usually phys-
iologically N limited in summer in our study area,
but rather that the mineralisation processes in the
planktonic community supplemented by occasional
upwellings provide sufficient nutrients for the algal
community to show biochemically balanced growth.
Possibly, the high proportions of proteins in algal cells
reflect the large need of enzymes to cope with the poor
summer conditions.

^{14}C lipids

The proportion of ^{14}C lipids was mostly between 10
and 20% (Fig. 4), which fits the typical values reported
in the literature from 5 to 30% (mean 15 to 20%; see
Wainman & Lean 1992). In a mesocosm experiment
conducted in summer 1988 in our study area, the pro-
portion of ^{14}C lipids was virtually constant over sev-
eral-fold variations in phytoplankton biomass and
^{14}CO$_2$ fixation and changes in species composition
evoked by pulsed additions of nutrients and their sub-
sequent depletion (Lignell & Lindqvist 1992). The con-
sistency in these results seems to reflect the fundamen-
tal role of lipids as components of algal structures
(mainly membranes and chlorophyll complexes).

However, during the vernal phytoplankton produc-
tivity and biomass peak in middle May, the proportion
of ^{14}C lipids showed a distinct peak of 40% (Fig. 4).
Contrary to that of ^{14}C polysaccharides, the ^{14}C-lipid
pool did not show any nocturnal decrease during this
period (Figs. 4 to 7), suggesting that the ^{14}C-lipid pool
was not used as a 'primary' storage for energy or
carbon skeletons. Thus, the ^{14}C-lipid peak probably
reflected a true increase in the proportion of this pool
in algal cells, rather than increased turnover of ^{14}C
lipids. This view seems to be supported by the con-
comitant increase in the particulate C:N ratio (Fig. 2),
well above the Redfield ratio of 6.6. Moreover, Taguchi
et al. (1987) found the cellular lipid content in cultured
(SiO$_2$ limited) diatoms to be proportional to the in-
crease of the percentage of ^{14}C lipids.

Enhanced synthesis of polysaccharides or lipids at
the expense of N-containing compounds is to be
expected under severe ('physiological') N deficiency of
algal cells (see Morris 1981). Accordingly, increased
lipid accumulation has been observed during station-
ary growth phases or towards the end of natural
diatom blooms (Hitchcock 1978, Parrish 1987). In the
enclosure experiment conducted in middle May, the
proportions of ^{14}C lipids in algae decreased by ca 15
percentage points as a result of nutrient enrichment
(Fig. 7). The predominant and subdominant species
accounted for the biomass responses in the enclosures.
Thus, the ^{14}C-lipid peak (and the decrease in assimila-

tion number) during this period was most likely a con-
sequence of the depletion of nutrients from the mixed
layer in early May (Figs. 2 & 4).

The lipid content of diatom cells has been shown to in-
crease due both to P (Reitan et al. 1994) and to N (Shifrin
& Chisholm 1981) limitation. Silicate limitation has also
been found to result in accumulation of lipids in diatoms
(Taguchi et al. 1987), but the high SiO$_2$ concentrations
in spring–early summer (>4 µmol Si l^{-1}; A.-S. Heiskanen,
pers. comm.) evidently exclude this explanation.

The contribution of diatoms to total phytoplankton
biomass decreased from 30 to 10% during the period
of the vernal ^{14}C-lipid peak as the cold-water species
Achnanthes taeniata and Chaetoceros holsaticus sedi-
mented from the mixed layer (Heiskanen 1995). Little
seems to be known about the effects of nutrient stress
on the dinoflagellates predominant in the present
study [Peridiniella catenata (Levander) Balech and
Scrippsiella hangoei (Schiller) Larsen]. However, to
account for the ^{14}C-lipid peak of 40% of total ^{14}CO$_2$ fix-
ation, which coincided with the peak total algal bio-
mass and productivity values, nutrient stress must
have resulted in increased accumulation of lipids in the
predominant dinoflagellates.

Low molar mass ^{14}C compounds and ^{14}C polysaccharides

The proportion of ^{14}C LMC was generally ca 10%,
but it showed 2 peaks in spring, which were especially
pronounced (30 to 40%) after 6 h light incubations
(Fig. 4). These peaks preceded major shifts in the
metabolism of the phytoplankton community: the
development of the ^{14}C-lipid peak in mid-May and the
dominance of ^{14}C proteins in summer. Thus, the ^{14}C
LMC peaks seem to have reflected temporarily en-
hanced synthesis of precursors of macromolecules
required to commence the major shifts in algal metab-
olism. A similar temporary increase in percentage ^{14}C
LMC followed by changes in the proportions of ^{14}C
macromolecules was recorded shortly after pulsed
nutrient (N+P) additions in our mesocosm study car-
ried out in summer 1988 (Lignell & Lindqvist 1992).

The proportions of ^{14}C polysaccharides were 20 to
30% during most of the season of phytoplankton
growth (Fig. 4). These values are similar to those often
found in marine environments (Morris et al. 1981,
Smith & D'Souza 1993, Fernández et al. 1994).

Phytoplankton as food for zooplankton

If phytoplankton is indeed physiologically healthy
during most of the growth season, as suggested above

on the basis of the high proportions of ^{14}C proteins, an interesting question concerns its suitability (quality) as food for zooplankton. Zooplankton diets are diverse and composed of a variety of different kinds of foods to include substances essential for growth (Ahlgren et al. 1990, Kleppel 1993, Thompson et al. 1994). The proportions of the biochemical constituents in the diet are important as well (Scott 1980). For example, protein C seems to be incorporated with much higher efficiency into herbivore biomass than lipid and carbohydrate C due to the lower turnover rate of zooplankton proteins compared to the other macromolecular pools (Scott 1980, Roman 1991).

There seems to exist little consensus on which biochemical constituents of phytoplankton are important in determining their food value to herbivores (Thompson et al. 1994). Thompson et al. (1994) suggested that short-chain saturated fatty acids with a high energy yield determine the nutritional superiority of the algal food. In support of this, highest zooplankton ingestion rates, and gross and net growth efficiencies, during a spring bloom in Bedford Basin were found to coincide with a period when the proportions of lipids were highest in algal cells and the energy-rich storage component, triglyceride, had become the major particulate lipid class (Parrish 1987). This may well have been the case also during the 1988 vernal bloom in our study area, when the copepods more than tripled their biomass during the 2 wk period of the ^{14}C-lipid peak in middle May (Lignell et al. 1993).

During the summer minimum period of low ambient mineral nutrient concentrations, phytoplankton biomass and primary productivity are usually limited by N availability in our study area (Kivi et al. 1993). It has been suggested that N (as protein) limits copepod production at least in some coastal and nearshore ecosystems as well (Checkley 1980). Accordingly, when the copepod *Acartia tonsa* was fed with the diatom *Thalassiosira weissflogii* grown in N-limited continuous cultures at different dilution (growth) rates, the ingested N and the rate of egg production by *A. tonsa* increased by a factor of 6 over >2 orders of magnitude increase in algal growth rate and a concomitant decrease in C:N ratio, though ingested C remained constant (Kiørboe 1989).

Factors like morphological properties of algal species (size, shape, cell wall structure) and toxicity undoubtedly affect the quality of phytoplankton food for herbivores, but 2 aspects seem to be of special importance here. First, both protozoans and calanoid copepods (the most important herbivores in our study area) show selectivity in their feeding, which enables them to optimise their diets and for example, differentiate between detritus, and senescent and exponentially growing algae (see Verity 1991, Kleppel 1993).

Second, though zooplankton show seasonal changes in C and N content, the C:element ratios are considerably more homeostatic in zooplankton than in phytoplankton under varying nutritional conditions (see Båmstedt 1986, Sterner & Hessen 1994). While unicellular algae generally exhibit a large capacity for accumulating materials in excess of their immediate demands for growth and maintenance, zooplankton store little essential nutritional substrates (energy or lipid storage excluded), and excess ingested material is disposed of (see Goldman et al. 1987, Kleppel 1993, Sterner & Hessen 1994).

Calanoid copepods show a mean C:N ratio of ca 10 (SD 3) in temperate and boreal areas (Båmstedt 1986), while protozoans often exhibit C:N ratios clearly below 7 (see Stoecker & Capuzzo 1990). The high proportions of ^{14}C proteins recorded in summer 1988 in our study area suggest that the phytoplankton communities exhibited C:N ratios close to the Redfield ratio of ca 7 (cf. DiTullio & Laws 1986). Particulate organic C:N ratios recorded in summer were consistent with this view (Fig. 2; cf. DiTullio & Laws 1983). A carbon flow budget, which was based on local (mostly contemporary) measurements of the process and state variables governing the picoplankton-heterotrophic nanoflagellates-ciliates 'microbial loop' and the nanoplankton-metazooplankton 'grazing chain', has been constructed for summer 1988 in our study area. Metazooplankton (mostly calanoid copepods) and protozoans were both estimated to satisfy ca 60% of their C needs via direct feeding on phytoplankton, most of the rest being derived from heterotrophs at one trophic step 'below' the predator (A. Uitto pers. comm.). Thus, it seems unlikely that copepods are N limited in our study area, whereas with protists this may well be the case.

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