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The influence of photon flux density (PFD) on short term ^{14}C incorporation into proteins, carbohydrates and lipids in freshwater algae

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Abstract

The effect of photon flux density (PFD) on the partitioning of photosynthetically fixed $^{14}CO_2$-C into major intracellular end products was investigated for three species of freshwater planktonic algae (*Nitzschia palea*, *Monoraphidium minutum* and *Synechococcus elongatus* belonging to three different classes. This study was designed to investigate the phenomenon of polysaccharide synthesis associated with the saturation of protein synthesis and to test if this process is common to all three phytoplankton species. Protein synthesis was saturated at low PFD in all three species of algae studied. However, fixed carbon was differentially stored, namely in lipids in *Nitzschia palea* (Bacillariophyceae), in polysaccharides in *Monoraphidium minutum* (Chlorophyceae), and in low molecular weight metabolites (LMW) in *Synechococcus elongatus* (Cyanophyceae). The results of this transient state study indicate that the metabolic pathways of algae can easily be controlled by different irradiance. Furthermore, it appears that the difference in the patterns of synthesis is taxonomy dependent.

Introduction

Studies of marine phytoplankton have shown that ^{14}C incorporation into protein more closely reflects cell growth than does total ^{14}C uptake (Morris, 1981; Li & Harrison, 1982; Lancelot *et al.*, 1986). Under adverse conditions of low temperatures and/or nutrient limitation, only a small proportion of fixed carbon enters the protein pool and a large proportion accumulates as reserve products (Morris *et al.*, 1974; Morgan & Kalff, 1975). Similarly, a higher percent of fixed carbon enters the protein pool at low radiation fluxes. These results reflect a lower saturating PFD for protein synthesis than for total photosynthesis and support the view that polysaccharide is an energy reserve that is predominantly synthesized when excess fixed carbon is available within the cell (Morris *et al.*, 1974; Li & Harrison, 1982; Hawes, 1990; Lancelot *et al.*, 1986). The 'step' in polysaccharide synthesis associated with saturation of protein synthesis is a natural effect of this process (Hawes, 1990). In this paper experiments on ^{14}C-incorporation as a function of light intensity and photosynthate partitioning are used to test if polysaccharide synthesis after saturation of protein synthesis at low PFD is a common phenomenon in representatives of different phytoplankton classes.

Material and methods

In lake water it is not possible to separate algae quantitatively from the dead cells, detritus, and heterotrophs which make up a large portion of the biomass. For these reasons patterns of synthesis of particulate macromolecular fractions has a questionable relationship with algal content and production in natural waters. Therefore, I chose to work with pure cultures of algae belonging to three different classes.

Unialgal but not axenic cultures of *Nitzschia palea* (Bacillariophyceae), *Synechococcus elongatus* (Cyanophyceae), and *Monoraphidium minutum* (Chlorophyceae) were grown in 21 chemostats (con-

tinuous cultures) at 20 °C. Cultures were illuminated with cool white fluorescent tubes, providing an incident irradiance of *c.* 130 μmol m^{-2} s^{-1} in the middle of culture vessels (chemostat) The irradiance (light flux in the photosynthetically active 400–700 nm wave band) was measured using QSL-100 laboratory Quantum Scalar Irradiance Meter attached with 1.9 cm diameter solid Teflon sphere irradiance collector (Biospherical Instruments Inc., California, USA). All three cultures were grown at a 14-h light and 10-h dark cycle and were maintained at steady state, with a growth rate of $\mu = 0.38$–0.39 d^{-1}. Cultures were considered to be in a steady state when total cell counts and fluorescence varied less than 7% over a period of at least 8 days. Samples for cell counts (using an electronic particle counter, Cell Analyser System, CASY 1, Schärfe System, Germany), chlorophyll-*a* (Marker *et al.*, 1980) and fluorescence (using Turner 11̈1 - Fluorometer, Turner Associate, USA) were taken at 1 to 2 day intervals. Samples taken during the steady state growth phase yielded chlorophyll-*a* concentrations of *c.* 408 μg l^{-1} (*c.* 2.05×10^6 cells ml^{-1}) for *Synechococcus elongatus*, *c.* 746 μg l^{-1} (*c.* 1.38×10^6 cells ml^{-1}) for *Nitzschia palea* and *c.* 1092 μg l^{-1} (*c.* 13.07×10^6 cells ml^{-1}) for *Monoraphidium minutum*. Overall replicates agreed well with an accuracy of about ±10% within 95% confidence limits.

The incubation chamber (Phototron), has a capacity of 24 aluminium tubes, and maintains constant temperature (±0.5 °C). Glass vessels having an outer diameter of 4.0 cm and 8.5 cm in height (75 ml capacity) are inserted into the aluminium tubes. Light can be adjusted individually in each glass vessels by using perforated metal sieves of different mesh sizes, and neutral density filters (Schott, type NG4) mounted on the bottoms of the aluminium tubes. Irradiation was provided by a 12 V halogen lamp (Mazda-Pepite Type BA-150, 20W, 10 °) mounted under each glass vessel (Rai & Krambeck, 1992). To determine the photon flux density (photosynthetically active 400–700 nm wave band) experienced by the algae during incubation (in the Phototron), irradiance was measured in the incubation glass vessel containing algal culture using Laboratory Quantum Scalar Irradiance Meter, attached with 1.9 cm diameter solid Teflon sphere irradiance collector, Model QSL-100 (Biospherical Instruments Inc. USA).

Approximately 50 μ Ci of ^{14}C-labelled bicarbonate were added to 1 l of the algae culture (*c.* 145×10^3 DPM ml^{-1}), drawn into a sterile 1 l dispenser bottle from the chemostat *c.* 60 min before the onset of dark period and well mixed. Thirtyfive ml aliquots were then quickly dispensed into the sterile sample glass vessels that were in place in the incubation chamber of the Phototron. Two replicate aliquots were incubated at 10 levels of incident irradiance ranging from 13 to 1312 μ mol m^{-2} s^{-1}.

The samples were kept shaken throughout the duration of the experiment and incubated for 3 h at 20 °C. Preliminary experiments have shown that carbon flux change immediately upon a change in PFD. This change continues and the maximum incorporation is achieved after about 3 h exposure to light. However, when the cells are adapted to the new situation the carbon fluxes are back to 'normal' again, usually slightly different depending on the previous light history. Two unfiltered 0.5 ml aliquots were placed in scintillation vials to determine total radio activity. In addition two 5 ml aliquots were taken at time-zero, as controls, filtered through pre-combusted (450 °C for 4 h) glassfibre filters (Whatman GF/F), and rinsed three times with 10 ml of sterile distilled water. Filters covered with algae were immediately frozen (−20 °C) in micro test tubes with safety lid lock (Eppendorf) and stored at −20 °C until analysis. After a 3-h incubation, duplicate 5-ml subsamples from each of the two glass vessels at each light level, were filtered onto pre-combusted glassfibre filter, rinsed and frozen and stored until analysis as for controls.The material retained on the filters was separated by using the subcellular macromolecules fractionation procedure (Li *et al.*, 1980). Here the methanol-water-soluble fraction is referred to as low molecular weight-metabolites, (LMW), chloroform-soluble fraction is referred to as lipids, hot trichloroacetic acid (TCA) soluble fraction as polysaccharides and TCA-insoluble fraction as protein. The radioactivities of these fractions were counted by liquid scintillation using a Packard Tri-Cab-1900CA, LS System. An isotopic discrimination factor of 1.06 was used as suggested by Steemann Nielsen (1952).

The coefficient of variation (7 samples with four replicate each) was: for lipid replicates, 1.6–5.5; for LMW replicates, 0.65–5; for polysaccharide replicates, 1–13; and for protein replicates, 1.4–7.2.

The response of each macromolecular fraction in each species to light was summarized by fitting the relation between incorporation rates and light intensity to the Michaelis-Menten equation. The half saturation constant, K_{pfd} (μmol m^{-2} s^{-1}) provides a measure of the saturating light intensity whereas R_{max} (μg C

μg Chl-a^{-1} h^{-1}) is an estimate of the maximal rate of incorporation.

Photosynthetic rates from each of the four replicates at 10 light levels were pooled to construct each photosynthesis versus light intensity (P-I) curve. The photosynthetic parameters α, P_m^B, and I_k for C fixation into subcellular macromolecules fraction equivalents were calculated using a phytoplankton photosynthesis computer program (Fee, 1990). All parameters reported here are chlorophyll-*a* specific values.

Results and discussion

The three species differed in their ^{14}C incorporation in response to increasing light intensity. *Synechococcus elongatus* exhibited an increase in the relative proportion of LMW (*c.* 60%) at high PFD (>130 μmol m^{-2} s^{-1}-PFD higher than that in the cultures) and an increase in the percentage of protein at low PFD (Fig. 1A). Incorporation into lipids increased only slightly (<10%) with increasing PFD. Furthermore, the enhanced production of lipids at low light intensity in cyanobacterium might indicate (the first 'step' in) production of thylokoid membranes as a consequence of adaptation to a PFD lower than that in the cultures (Fig. 1A).

In contrast to the cyanobacteria, the diatom *Nitzschia palea* exhibited a sharp increase in lipid (>50%) under the same high light intensities (>130 μmol m^{-2} s^{-1}, – PFD higher than that in the cultures). The proportion of proteins and polysaccharides declined at high light intensities, whereas the fraction of LMW was independent of light intensity (Figs 1B and 2B).

Most previous data for the lipid fraction of total carbon fixation are from marine systems. Smith & Morris (1980) reported that lipid fraction in the Southern Ocean phytoplankton, dominated by diatoms, was 80% when light and temperature were low. In contrast Li & Platt (1982) reported that the lipid fraction in Arctic Ocean accounted for only 18% under similar conditions of light and temperature as Smith & Morris (1980). Palmisamo & Sullivan (1984) and Smith *et al.* (1989) found 8–30% lipids in sea-ice microalgae. Priscu *et al.* (1989) reported a lipid fraction of only 20% in the phytoplankton of two Antarctic lakes. In a temperate, eutrophic freshwater lake, Jensen (1985) reported 15–28% in the lipid fraction. Amblard & Bourdier (1990) found about 14–24% in the lipid fraction at different depths in Lake Pavin during spring bloom of the diatom *Melosira italica*. Wainman & Lean (1992) recently reported that the lipid fraction of total ^{14}C incorporation was 11–23% for phytoplankton of three lakes near Toronto, Canada. From the existing literature it appears that lipid accounted for 10–30% (excluding the results of Smith & Morris, 1980) of newly fixed carbon and this was related to either nutrients or physical factors like temperature and PFD. In marine systems (Smith & Morris, 1980) and for freshwater systems (Amblard & Bourdier, 1990; Wainman & Lean, 1992) suggested that carbon incorporation into lipid was related only to changes in temperature and PFD. These results are similar (but range is lower) to those observed in the present study. However, the patterns of photosynthesis illustrated for the three species of algae (Fig. 1) are rather different from those reported earlier (Smith & Morris (1980); Morris *et al.* (1974), Morris & Skea (1978); Li & Harrison (1982); Hawes (1990); Lancelot *et al.* (1986); Morgan & Kalff (1975); Cuhel & Lean (1987); Palmisano & Sullivan (1984); Priscu *et al.* (1989); Jensen (1985).

The data from this study suggest that relative more light was required to saturate the fixation of carbon into lipid especially in diatom (Figs 1B & 2B). In contrast to diatom (*Nitzschia palea*), in the cyanobacterium (*Synechococcus elongatus*) and green alga (*Monoraphidium minutum*) incorporation into lipids was not related to increase in PFD (Fig. 1 and 2). This finding differs from the results of Smith & Morris (1980) who while working on Southern Ocean plankton, dominated by diatoms reported that at higher light intensities, incorporation into lipids accounted for less than 20 percent of the fixed carbon, synthesis of polysaccharide and protein being more prominent.

In contrast to the diatom and cyanobacterium, the green algae *Monoraphidium minutum* accumulated more polysaccharide under high light intensities. Incorporation into lipids and proteins was independent of light intensity. The relative ^{14}C incorporation into LMW was maximal at lowest light intensity and low and stable at intermediate and high light intensities (Fig. 2C). The results illustrated in Fig. 2 show that protein saturation is found at low K_{pfd} values in all the three organisms. However, the proportion of saturated protein production is rather different in the three species (Fig. 1). Both phenomena could be the result of difference in the physiological status of the organisms.

In *Nitzschia palea* characteristic decreasing proportions of carbon fixation into proteins with increasing light intensity are offset by increasing proportion-

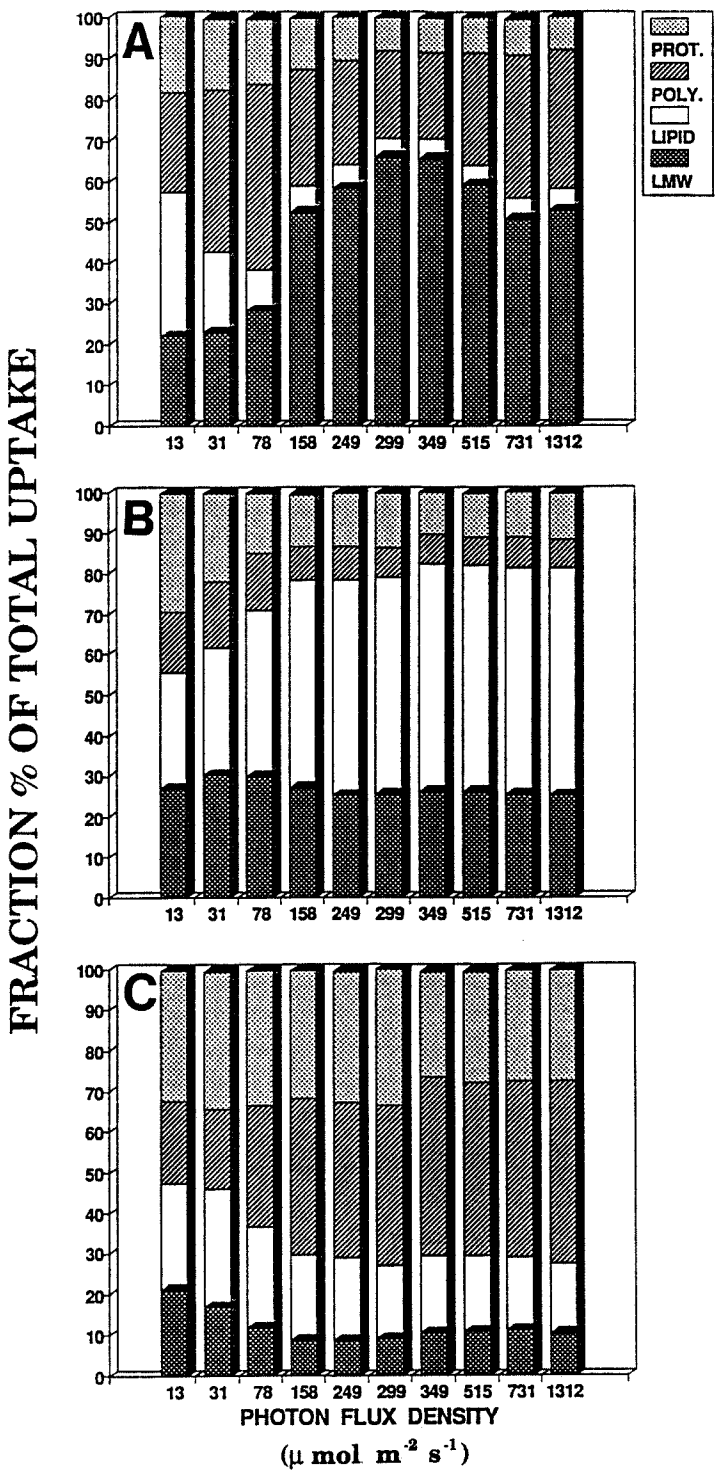

Fig. 1. The relationship between photon flux density and the proportion of fixed $^{14}CO_2$-C incorporation into protein (PROT.), polysaccharide (POLY.), LIPID and low molecular weight-metabolites (LMW) pools in: (A) *Synechococcous elongatus* (B) *Nitzschia palea* and (C) *Monoraphidium minutum*

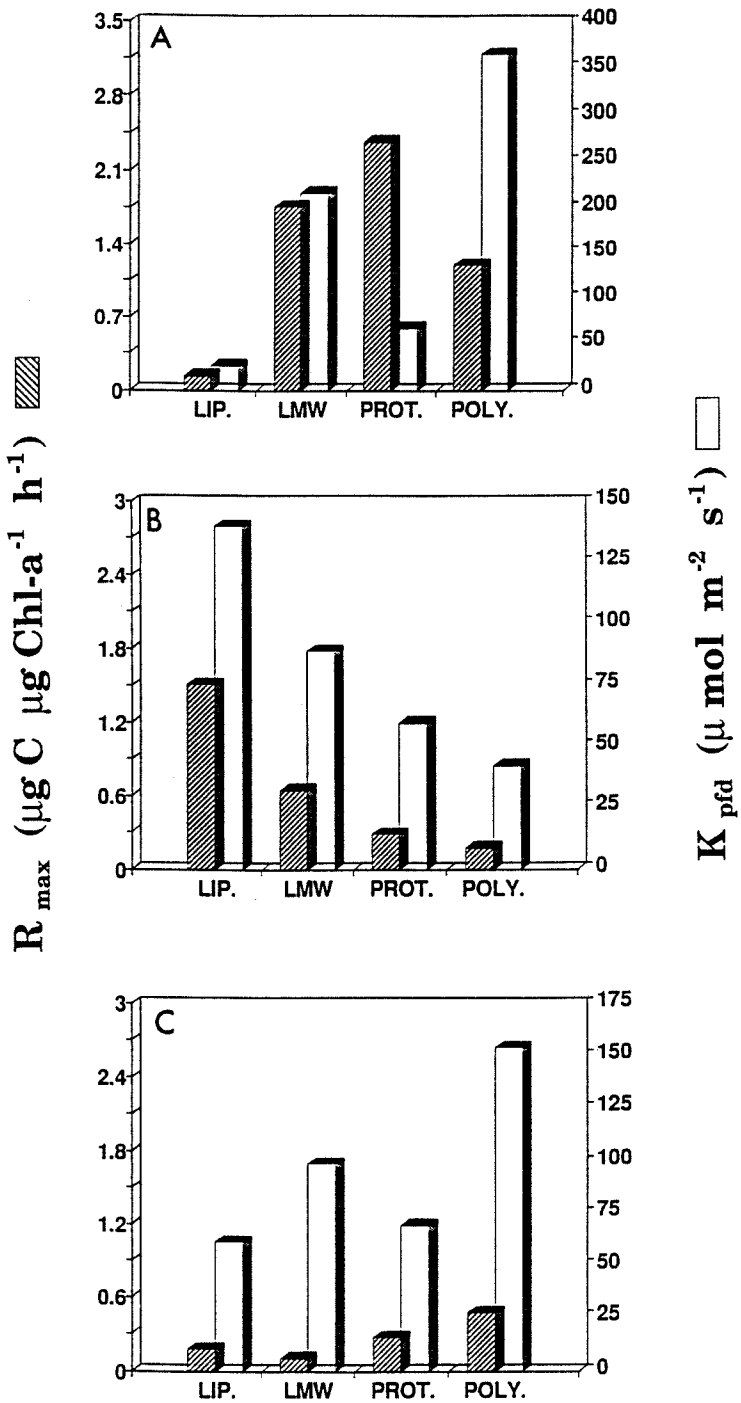

Fig. 2. Apparent half-rate constant K_{pfd} (μ mol m^{-2} s^{-1}) and R_{max} (μg C μg Chl-a^{-1} h^{-1}), calculated from the Michaelis-Menten equation, for major end-products of photosynthesis in: (A) *Synechococcous elongatus*. (B) *Nitzschia palea* and (C) *Monoraphidium minutum*

al lipid incorporation is clearly distinguishable from the light intensity-dependent assimilation pattern of carbon into lipids (Fig. 1B). Increased light intensity (>130 μ mol m^{-2} s^{-1}) caused increased relative labeling of lipids at the expense of incorporation into polysaccharide fraction. It is known that diatoms are characterized by larger lipid storage. For example Lee *et al.* (1971) observed that phospholipids and galactolipids accounted for as much as 57% of the total lipids in a diatom (*Skeletonema costatum*). Such materials are important structural components in the lamella of the chloroplast (Benson *et al.*, 1959). Furthermore, algal lipids are notably important to many species of herbivorous zooplankton that cannot synthesize large amounts of lipids or essential fatty acids yet rely on these lipids for reproduction and survival (Ahlgren *et al.*, 1990). Thus, lipids can be more than simple storage products.

Photosynthetic parameters (P_m^B, α, and I_k) for total cell C assimilation and C fixation into lipids, polysaccharides, and LMW were significantly different among algae studied. The three algae did not significantly differ in photosynthetic parameters for protein fraction (Table 1). The increase in the light saturation parameter $I_{k(\text{polysaccharides})}$ was greater than that in $I_{k(\text{total})}$, indicating that total C fixation was light saturated before C fixation into polysaccharides for *Synechococcus elongatus* and *Monoraphidium minutum*. In *Nitzschia palea*, $I_{k(\text{lipids})}$ was greater than $I_{k(\text{total})}$, so light-saturation decreased, showing that total C fixation was light saturated before C fixation into lipids. Thus, C fixation into lipids in *Nitzschia palea* required more light to be saturated and became less light efficient as compared with total C fixation. Similarly, for *Synechococcus elongatus* and *Monoraphidium minutum* C fixation into polysaccharides required more light to become saturated and became less light efficient compared with total C fixation (Table 1). These trends in C fixation are probably reflected in the physiological characteristics of the species of algae.

In *Monoraphidium minutum* increasing PFD resulted in a decrease in the ratio of protein:polysaccharide. Increasing PFD raised the maximum rate of polysaccharide synthesis in *Monoraphidium minutum* (Table 2). In *Nitzschia palea* increasing PFD for protein synthesis saturation resulted in high rates of lipids synthesis, which is associated with protein synthesis. At higher radiation fluxes ratios of protein:lipids and protein:LMW decreased (Table 2). In *Synechococcus elongatus* increasing PFD for protein synthesis saturation implies that high rates of polysaccharide and LMW synthesis, which are associated with protein synthesis saturation, and indicated by low ratios of protein:polysaccharide and protein:LMW, occurred at higher irradiation fluxes (Table 2).

Saturation of protein synthesis, hence growth, at low PFD will be of considerable significance to freshwater lake phytoplankton. For example, during bright sunny periods of the day (e.g., late morning and early afternoon, between 0 and 2 m depth) PFD will usually be higher than required to saturate protein synthesis. But during the period of low PFD (e.g., at dawn and dusk – and at deeper part of the euphotic zone), protein synthesis will be saturated at its maximum rate. If growth is directly related to protein synthesis then maximum growth rates are attained at low PFD for all three types of algae studied. It may then be concluded that growth rates are limited at high PFD, which is the case during late morning and afternoon periods, especially during sunny days in the upper photic zone in freshwater lakes. During these periods, high rates of photosynthesis observed in the lakes are poorly coupled to growth, thus resulting in accumulation of different products depending on the response of species. This study confirms that short-term exposure (3-h) could change the physiological response of algae and indicate that the metabolic pathways of algae can be easily controlled by different PFD (Fig. 1). Further, during the vertical fluctuations of the phytoplanktonic organisms the changes in the proportion of the major end-products of photosynthesis in the cells might be caused by fluctuating PFD at different depths. Hawes (1990) reported such changes in the proportion of macromolecules fraction in Antarctic freshwater phytoplankton, dominated by chlorophyceae and cryptophyceae. That protein synthesis was saturated at low PFD and at high PFD, the fixed carbon was increasingly stored as lipids and as polysaccharides.

The increasing percentage of fixed carbon which enters the protein pool at low irradiance intensity reflects the lower saturation level of PFD for protein synthesis than for total photosynthesis. This is a commonly observed phenomenon in phytoplankton photosynthesis (Morris *et al.*, 1974; Li & Harrison, 1982; Hawes, 1990). The increase in polysaccharide synthesis after protein saturation supports the view of polysaccharide as a carbon reserve, is predominantly synthesized when excess fixed carbon is available within the cell (Lancelot *et al.*, 1986; Hawes, 1990). Hawes (1990) reported this 'step' in polysaccharide synthesis associated with saturation of protein synthesis is a natural effect of this process.

Table 1. Photosynthetic parameters for C fixation in each subcellular macromolecules fraction

Phytoplankton	Macromolecules fraction	P_m^B	α^B	I_K	P_{Opt}	Sum. Sq.
Synechococcus elongatus	Total particulate	2.57	2.54	1.012	1051	0.462
	LMW	1.36	1.44	0.944	555.6	0.045
	Lipids	0.11	1.07	0.103	46.8	0.004
	Polysaccharides	0.91	0.62	1.468	373.8	0.141
	Proteins	0.2	0.67	0.299	82.9	0.005
Nitzschia palea	Total particulate	2.13	4.14	0.514	1588.7	0.035
	LMW	0.54	1.22	0.443	401.4	0.004
	Lipids	1.21	1.84	0.658	903.3	0.016
	Polysaccharides	0.15	0.68	0.221	115.5	0
	Proteins	0.25	0.66	0.379	184.5	0.003
Monoraphidium minutum	Total particulate	0.89	1.87	0.476	912	0.011
	LMW	0.08	0.17	0.471	91.7	0.001
	Lipids	0.15	0.47	0.319	166.9	0
	Polysaccharides	0.37	0.54	0.685	407.3	0.004
	Proteins	0.24	0.61	0.393	262.9	0.002

P_{Opt} = rate of phytoplankton photosynthesis at irradiance optimal (μg C l^{-1} h^{-1})
P_m^B = the rate of photosynthesis at optimal irradiance divided by B (μg C (Fraction) μg Chl-a^{-1} h^{-1})
B = chlorophyll concentration (μg l^{-1})
α^B = the slope of the photosynthesis vs irradiance curve, divided by B (μg C (Fraction) (μg Chl-a^{-1}) h^{-1} (μmol m^{-2} s^{-1})$^{-1}$)
$I_K = P_m^B/\alpha^B$ (μmol m^{-2} s^{-1})
Sum. Sq = sum of the squares of the deviations of the photosynthesis equation

Table 2. The effect of photon flux density (PFD) on the ratios of 14-C bicarbonate incorporation rates of protein: polysaccharide, protein: lipid and protein: LMW in a bluegreen alga (*Synechococcus elongatus*), a diatom (*Nitzschia palea*) and in a green alga (*Monoraphidium minutum*).

Photon Flux	Protein: Polysaccharide			Protein: Polysaccharide			Protein: LMW		
Density (PFD)	SYN.	NITZ.	MONO.	SYN.	NITZ.	MONO.	SYN.	NITZ.	MONO.
13	0.76	1.93	1.60	0.52	1.02	1.22	0.85	1.09	1.56
31	0.43	1.31	1.75	0.87	0.70	1.18	0.76	0.72	2.04
78	0.36	1.04	1.10	1.58	0.36	1.33	0.57	0.50	2.86
158	0.43	1.49	0.81	1.83	0.25	1.51	0.23	0.48	3.60
249	0.41	1.61	0.85	1.81	0.25	1.60	0.18	0.53	3.69
299	0.38	1.83	0.84	1.75	0.25	1.88	0.17	0.53	3.74
349	0.41	1.45	0.59	1.69	0.18	1.38	0.18	0.40	2.51
515	0.31	1.58	0.63	1.73	0.19	1.48	0.15	0.42	2.53
731	0.26	1.46	0.62	1.71	0.20	1.50	0.19	0.44	2.50
1312	0.24	1.62	0.60	1.47	0.21	1.60	0.15	0.46	2.69

SYN = *Synechococcus elongatus*
NIZ = *Nitzschia palea*
MON = *Monoraphidium minutum*

From this study it appears that this is not a common phenomenon in all phytoplankton photosynthesis. The patterns of ^{14}C-labeled incorporation into the different end-products of photosynthesis in various algal species consistently differed. The 'step' in polysaccharide synthesis in *Monoraphidium minutum* (Chlorophyceae) was associated with saturation of LMW synthesis at low PFD. But the major changes at light intensities greater than at growth conditions are in proportional fluxes to polysaccharides and LMW products in

this alga. In *Synechococcus elongatus* (Cyanophyceae) this 'step' was in LMW synthesis. In *Nitzschia palea* (Bacillariophyceae) the 'step' was in lipid synthesis. Such a variable 'step' in synthesis associated with protein saturation at low PFD has not been reported previously. It is assumed here that this difference in synthesis is taxonomy dependent. It may be concluded that low light intensity, low temperature and nutrient limitation (Morris *et al.*, 1974; Morris & Skea, 1978) are not the only environmental factors affecting the pattern of synthesis. Short-term (3-h in the present study) exposure to high light intensity could also affect the pattern of synthesis. Increasing PFD increased relative labelling of lipids at the expense of incorporation into polysaccharides and LMW in *Nitzschia palea*. Increasing PFD increased relative labelling of LMW at the expense of incorporation into lipids in *Synechoccous elongatus*. Increasing PFD increased relative labelling of polysaccharides at the expense of incorporation into lipids and LMW in *Monoraphidium minutum*. Thus high PFD alone could change the pattern of synthesis and fixed carbon stored differentially into photosynthetic end-products in the various species.

The significance of synthesis of lipids, LMW and polysaccharides to the food web interactions in the aquatic ecosystems is not sufficiently investigated. The high caloric value of lipids (in diatoms) might suggest that storage of high energy products, available during high PFD periods in freshwater lakes could be of significance in the dynamics of diatom and food web interactions. Cells may accumulate storage products during bright early morning and late afternoon, however when cells are adapted to new situation the carbon fluxes are back to normal again, usually only slightly different from the old 'ones'. The interaction between radiation flux and production and accumulation of reserves may be vital to the succession of phytoplankton in the lakes where the pressure of grazers is great. The varied chemical composition of algae at different depths in the water column and during different times of the day/night cycle may provide a useful range of foods from which zooplankton may select the optimal type required for their particular metabolic needs (Cuhel & Lean, 1987).

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References

Ahlgren, G., L. Lunstedt, M. Brett & C. Forseberg, 1990. Lipid composition and food quality of some freshwater phytoplankton for cladoceran and copepods. J. Plankton Res. 12: 809–818.

Amblard, C. & G. Bourdier, 1990. The spring bloom of the diatom *Melosira italica* subsp. *subarctica* in Lake Pavin: Biochemical, energetic and metabolic aspects during sedimentation. J. Plankton Res. 12: 645–660.

Benson, A. A., J. Wintermans & R. Wiser, 1959. Chloroplast lipids as carbohydrate reservoirs. Pl. Physiol. 34: 315–317.

Cuhel, R. L. & D. R. S. Lean, 1987. Influence of light intensity, light quality, temperature, and daylength on uptake and assimilation of carbon dioxide and sulphate by lake plankton. Can. J. Fish. aquat. Sci. 44: 2118–2132.

Fee, E. J., 1990. Computer programs for calculating in situ phytoplankton photosynthesis. Can. Tech. Rep. Fish. aquat. Sci. 1740: v + 27 p.

Hawes, I., 1990. The effect of light and temperature on photosynthate partitioning in Antarctic freshwater phytoplankton. J. Plankton. Res. 12: 513–518.

Jensen, L. M., 1985. ^{14}C-labeling patterns of phytoplankton: Specific activity for different product pools. J. Plankton Res. 7: 643–652.

Lancelot, C., S. Mathot & N. P. J. Owens, 1986. Modelling protein synthesis, a step to an accurate estimate of net primary production: *Pheocystis pouchetti* colonies in Belgian coastal waters. Mar. Ecol. Prog. Ser. 32: 193–202.

Lee, R., J. Nevenzel & G. Puffenhofer, 1971. Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar. Biol. 9: 99–108.

Li, W. K. W., H. E. Glover & I. Morris, 1980. Physiology of carbon assimilation by *Oscillatoria thiebautii* in the Caribbean Sea. Limnol. Oceanogr. 25: 447–456.

Li, W. K. W. & W. G. Harrison, 1982. Carbon flow into the end products of photosynthesis in short and long incubations of a natural phytoplankton population. Mar. Biol. 72: 175–182.

Li, W. K. W. & T. Platt, 1982. Distribution of carbon among photosynthetic end-products in phytoplankton of the eastern canadian Arctic. J. Phycol. 18: 466–471.

Marker, A. F. H., E. A. Nusch, H. Rai & B. Riemann, 1980. The measurement of photosynthetic pigments in freshwater and standardization of methods: Conclusions and recommendations. Arch. Hydrobiol. Beih./Ergebn. Limnol. 14: 91–106.

Morgan, K. & J. Kalff, 1975. The winter survival of an algal flagellate *Cryptomonas erosa* (Skuja). Verh. int. Ver. Limnol. 19: 2734–2740.

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

Morris, I., 1980. Paths of carbon assimilation in marine phytoplankton. In: Primary productivity in the sea. Ed. by P. G. Falkowski. New York: Plenum Press: 139–159.

Morris, I., 1981. Photosynthesis products, physiological state, and phytoplankton growth. In: Platt, T. (ed.), Physiological Bases of Phytoplankton Ecology. Can. Bull. Fish. aquat. Sci. 210: 83–102.

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

Palmisano, A. & C. W. Sullivan, 1994. Pathways of photosynthetic carbon assimilation in sea-ice microalgae from McMurdo Sound, Antarctica. Limnol. Oceanogr. 30: 674–678.

Priscu, J. C., L. R. Priscu, C. Haward-Williams & W. F. Vincent, 1988. Diel patterns photosynthate biosynthesis by phytoplankton in permanently ice-covered Antarctic lakes under continuous sunlight. J. Plankton Res. 10: 333–340.

Platt, T., C. L. Gallegos & W. G. Harrison, 1980. Photoinhibition and photosynthesis by phytoplankton in natural assemblages of marine phytoplankton. J. Mar. Res. 38: 687–701.

Rai, H. & H. J. Krambeck, 1992. Instrumentation for the measurement of physiological parameters of phytoplankton photosynthesis. Arch. Hydriobiol. 125: 295–309.

Smith, A. E. & I. Morris, 1980. Synthesis of lipid during photosynthesis by phytoplankton of the Southern Ocean. Ocean Science (Washington., D.C.). 207: 197–199.

Steemann Nielsen, E., 1952. The use of radioactive carbon ^{14}C for measuring production in the sea. J. Cons. Cons. int. Explor. Mer. 18: 117–140.

Wainman, B. C. & D. R. S. Lean, 1992. Carbon fixation into lipid in small freshwater lakes. Limnol. Oceanogr. 37: 956–965.