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Photosynthate allocation in a temperate sea over an annual cycle: the relationship between protein synthesis and phytoplankton physiological state

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Abstract

The seasonal and vertical variations in the patterns of photosynthate allocation into biomolecules by natural phytoplankton assemblages were determined, together with their species composition, in a coastal station of the central Cantabrian Sea (southern Bay of Biscay). Chlorophyll-*a* concentration ranged from values below 20 mg m⁻² in winter to values above 80 mg m⁻² during spring and during an upwelling event in summer. Low primary production rates (< 300 mgC m⁻² d⁻¹) were measured during winter and during summer stratification periods. The rate of C fixation during summer upwelling conditions exceeded 3500 mgC m⁻² d⁻¹. In terms of photosynthate partitioning, proteins were the dominant fraction, as they typically accounted for >30% of total photo-assimilated C, with polysaccharides and low molecular weight metabolites showing incorporation percentages around 10–30%. Relative C incorporation into lipids was generally < 15%. Recurrent patterns of vertical variability in photosynthate partitioning were observed: the relative synthesis of proteins increased toward the bottom of the euphotic zone, whereas the relative C incorporation into polysaccharides and lipids tended to be higher near the surface. When primary production decreased, the synthesis of proteins was maintained more than that of other molecules. Throughout the year, the relative synthesis of proteins was inversely correlated with phytoplankton biomass, production and growth rate. The conservation of protein synthesis under growth-limiting conditions and the enhancement of lipid and polysaccharide synthesis when irradiance is high seem to constitute general patterns of photosynthate partitioning in marine phytoplankton. In our study, these patterns represented metabolic strategies of phytoplankton in response to changing environmental factors, rather than the effect of variations in the species composition of the community.

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1. Introduction

The study of the patterns of photosynthetic carbon (C) incorporation into biochemical pools (e.g. pro-

teins, polysaccharides and lipids) allows a deeper understanding of phytoplankton physiological state than can be obtained from the simple determination of total C fixation rates (Morris, 1981). Different molecular fractions are associated with different cell functions: lipids and polysaccharides serve mainly as intracellular reservoirs of carbon and energy, whereas proteins are more directly linked with basic functions

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of biosynthesis and cell division (Geider et al., 1996). Thus, photosynthate C partitioning in different fractions informs us about the various metabolic strategies that phytoplankton adopt when facing variability in environmental factors such as irradiance or nutrient concentration.

The patterns of C allocation into each biochemical pool also have trophic implications because they ultimately determine the biochemical composition of phytoplankton, which strongly affects herbivore assimilation efficiencies (e.g. Scott, 1980). In particular, proteins tend to be conserved along food chains, due to the high energetic cost involved in their synthesis (Lean et al., 1989). From a biogeochemical point of view, it has been noted that differences in photosynthetic C partitioning by phytoplankton can make the C to nitrogen (N) uptake ratio depart significantly from the Redfield ratio (Marañón and Fernández, 1995). This process, dependent on the physiological state of the phytoplankton, could help explain the observed inconsistencies in the stoichiometry of C and N cycling in upper ocean waters (Sambrotto et al., 1993).

A problem that has received particular attention in the study of phytoplankton C metabolism is the linkage between protein synthesis, primary production and growth rate. Di Tullio and Laws (1983) proposed the use of the percentage of C incorporation into the protein fraction as a proxy for phytoplankton relative growth rate. The rationale behind this approach is that in N-limited continuous cultures, a positive relationship exists between the ratio of realised to maximum growth rate and the phytoplankton N/C ratio (Goldman et al., 1979), which in turn is closely related to the ratio between C incorporation into proteins and total C fixation. Despite the fact that many measurements of photosynthetic C incorporation into biochemical fractions have been conducted during the last two decades, the evidence remains conflicting as to how protein synthesis is related to production and growth in natural phytoplankton assemblages.

While some authors stress the association between relatively high percentages of C incorporation into proteins and situations of enhanced phytoplankton production and growth (Hama et al., 1988; Madariaga and Fernández, 1990), other reports have shown that under conditions of low irradiance or low nutrient availability the synthesis of proteins tends to be

conserved more than that of lipids or polysaccharides (Barlow, 1984; Marañón et al., 1995; Marañón and González, 1997). These discrepancies might be caused by the fact that each study typically focuses on one particular group of species and/or one particular oceanographic setting over short periods of time. Species-specific differences in C metabolism may confound the interpretation of natural variability (Madariaga, 1992; Fernández et al., 1994), unless a sufficiently high number of observations over longer time scales are used. Studies of photosynthate partitioning over seasonal (Furgal et al., 1998) and annual (Charpin et al., 1998) time scales in lakes have emphasised the importance of different environmental factors, in particular nutrient availability and temperature, in the control of C allocation into biomolecules. However, no study exists where the changes in photosynthate allocation by marine phytoplankton are described over an entire annual cycle.

Here we report on the results of an annual survey of phytoplankton abundance, composition and C metabolism in a coastal, temperate ecosystem located in the central Cantabrian Sea (southern Bay of Biscay). The region has been extensively studied in the past, mainly from the point of view of the relationship between hydrodynamic forcing and phytoplankton composition and production (e.g. Fernández and Bode, 1991, 1994). During summer, this area is affected by a highly dynamic, wind-driven coastal upwelling (Botas et al., 1990; Marañón et al., 1995), which gives way to a high degree of temporal variability in phytoplankton abundance and production. Thus, the system seems to be an ideal one in which to study the patterns of photosynthetic C allocation into biomolecules under a wide range of hydrographic and ecological conditions. By determining the seasonal and vertical variability in the allocation of photosynthate into biomolecules, we aim to identify the existence of recurrent patterns of phytoplankton C metabolism that are observed throughout the annual cycle. In particular, we will try to confirm if there is a consistent relationship between protein relative synthesis and phytoplankton production and growth. By also analysing the taxonomic composition of the phytoplankton assemblages, we will try to ascertain to which extent the observed patterns of C metabolism are mainly driven by responses of physiological acclimation or by changes in species composition.

2. Methods

A coastal station located at a distance of 3.2 km from the shore in the central Cantabrian Sea (southern Bay of Biscay, 43°36'N 6°08'W, depth = 60 m, Fig. 1) was visited monthly between December 1992 and November 1993. On each cruise, vertical profiles of temperature and salinity were obtained with a SeaBird CTD probe. The vertical distribution of photosynthetically active radiation (PAR) was measured with a Li-Cor spherical quantum sensor connected to a LI-1000 datalogger. Using Niskin bottles, water samples were collected from 0, 10, 20, 30, 40 and 50 m depth for the determination of the vertical distribution of nutrient concentration, Chlorophyll-a (Chl-a) concentration, phytoplankton species composition and the rate of photosynthetic carbon incorporation into different biochemical fractions.

For the determination of the concentration of nitrate, ammonium, phosphate and silicate, duplicate 8-ml samples were stored at $-20\text{ }^{\circ}\text{C}$ until they were analysed colorimetrically following the methods described in Grasshoff et al. (1999). Prior to analysis, nitrate was reduced to nitrite in a copper-cadmium

reduction column. Nitrite was then analysed following a method that is based on the reaction of nitrite with sulphanilamide leading to the formation of a diazonium compound, which couples with another aromatic amine to give an azo dye. The determination of ammonium was based on the reaction of phenol and hypochlorite in the presence of NH_3 , which leads to the formation of indophenol. The determination of phosphate was done using a method based on the reaction of phosphate ions with an acidified molybdate reagent to yield a phosphomolybdate acid. The method for silicate analysis was based on the formation of silicomolybdic acid when the acidified sample is treated with a molybdate solution. The detection limit of these analyses was $0.05\text{ }\mu\text{M}$ for nitrate and ammonium, $0.01\text{ }\mu\text{M}$ for phosphate and $0.1\text{ }\mu\text{M}$ for silicate.

In order to determine Ch-a concentration, samples of 100 ml were filtered through Whatman GF/F filters, which were stored at $-20\text{ }^{\circ}\text{C}$ until further analysis. Pigment extraction was accomplished by grinding the filters and then keeping them in 90% acetone at $4\text{ }^{\circ}\text{C}$ for 10 min. Fluorescence in the extracts was measured with a Turner fluorometer, which had been calibrated using pure chlorophyll-a.

In order to identify and count nano- and micro-phytoplankton cells, samples of 50 or 100 ml (depending on Chl-a concentration) were preserved in acid Lugol's iodine solution, allowed to settle in sedimentation chambers and then observed with an inverted microscope. Cell counts of each species were converted into carbon biomass by using the conversion factors obtained by Holligan et al. (1984) in waters of the English Channel. These authors obtained their estimates of cellular C content for each species by determining cell volumes according to Kovalá and Larrance (1966) and applying the cell volume/C relationship of Eppley et al. (1970). We found that the correlation between total estimated phytoplankton C and Chl-a concentration was highly significant ($r=0.71$, $p<0.01$, $n=27$) when all samples were considered. However, it is well known that the C to Chl-a ratio changes greatly with depth (Taylor et al., 1997), which is likely to confound the comparison between Chl-a concentration and estimated phytoplankton C when samples from different depths are considered. Using data from surface samples only, we found that the correlation between estimated

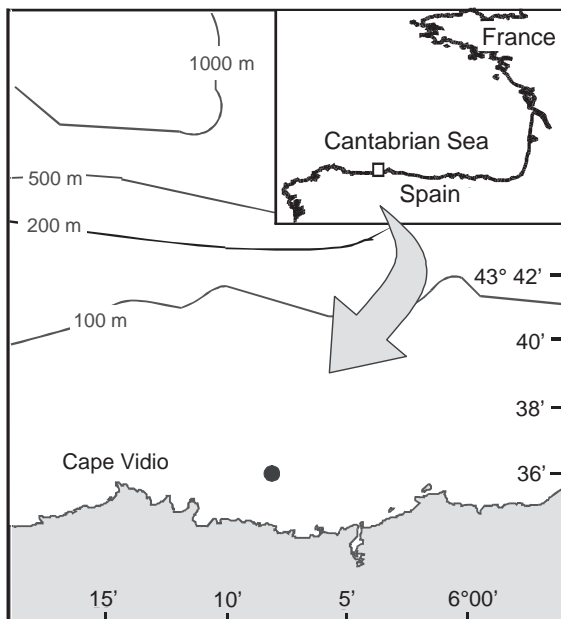


Fig. 1. Location of the sampling station in the central Cantabrian Sea (southern Bay of Biscay).

phytoplankton C and Chl-a concentration improved markedly ($r=0.83$, $p<0.001$, $n=9$).

Measurements of primary production were obtained in the laboratory by conducting simulated 'in situ', light-dark incubations with ^{14}C for 24 h. These 24-h, light-dark incubations, as opposed to short (6–8 h) incubations under continuous light, allow the determination of the integrated patterns of photosynthate partitioning taking into account also the C reallocation processes that occur during the night (e.g. Smith et al., 1990). In addition, the use of 24 h incubations circumvents the problems of computing daily C fixation rates from hourly rates measured during short incubations (Mingelbier et al., 1994). Water samples were transported in 5-L carboys to the laboratory, and stored in the dark at a temperature within $1.5\text{ }^{\circ}\text{C}$ of the 'in situ' temperature. Typically, 12–14 h elapsed between collection of the samples at sea in the late afternoon and inoculation of the samples in the laboratory the next morning. For each sampling depth, duplicate water samples were transferred to 70-ml, acid-washed polycarbonate bottles, spiked with 370 KBq ($10\text{ }\mu\text{Ci}$) of $\text{NaH}^{14}\text{CO}_3$ and incubated for 24 h in temperature-controlled chambers. Throughout the study, incubation temperature was kept within $\pm 1.5\text{ }^{\circ}\text{C}$ of 'in situ' temperature. Bottles were illuminated with cold white light from 'Sylvania' fluorescent tubes, and a 12L:12D photoperiod was used. Seasonal changes in the photoperiod, which were not simulated in our experimental setup, did not exceed $\pm 16\%$ of the 12L:12D photoperiod. 'In situ' irradiance levels were simulated using neutral density screens and taking into account the vertical profile of PAR measured at the station. At the end of the incubations, samples were filtered through Whatman GF/F filters under low vacuum pressure ($<100\text{ mmHg}$). The filters were stored at $-20\text{ }^{\circ}\text{C}$ until further analysis.

The determination of the amount of ^{14}C incorporated into the different molecular fractions was carried out as described in detail in Marañón et al. (1995). The procedure of biochemical fractionation separates the cellular material into four fractions: methanol/water-soluble compounds (low molecular weight metabolites, LMWM), chloroform-soluble compounds (lipids), hot trichloroacetic acid (TCA)-soluble compounds (polysaccharides and nucleic acids) and hot trichloroacetic acid (TCA)-insoluble compounds (proteins). Total primary production was estimated as the

sum of the C incorporation in the 4 molecular fractions (proteins, polysaccharides, lipids and low molecular weight metabolites). In preliminary experiments, the sum of the ^{14}C activity in the 4 fractions was found to account for 95–104% of the total ^{14}C activity measured in parallel, non-fractionated samples. We also evaluated the methodological variability of our fractionation procedure by calculating, for each station and depth, the coefficient of variation (CV) of the percentage of C incorporated into each biochemical fraction. For the whole study, we obtained a pooled CV of 0.077 ± 0.009 (mean \pm standard error) for proteins, 0.090 ± 0.014 for polysaccharides, 0.142 ± 0.021 for lipids and 0.094 ± 0.011 for LMWM. Vertically integrated rates of C incorporation were obtained using trapezoidal integration. Given that on some dates we did not have C incorporation data for the 50 m sample, we considered the 0–40 m depth range in the vertical integration in order to obtain data that are comparable throughout the study period. Integrated percentages of C incorporation into each biochemical fraction were calculated by dividing the integrated C incorporation rate into each fraction by the integrated, total C fixation rate.

All statistical analyses were carried out using the SPSS statistical package. Model II was used in all linear regression analyses. In the statistical treatment of the slopes of the regression lines, we applied the method of Clarke (1980). We used principal component analysis (PCA) in order to reduce the variability in the phytoplankton species composition into a smaller number of variables. We conducted the PCA on the correlation matrix obtained from the list of cell abundances (cells per litre) of each phytoplankton taxa. Cell abundances (x) were transformed logarithmically [$y=\log(x+1)$] and the PCA was performed without axis rotation. Rare species that were present in less than 10% of the samples were excluded from the analysis, leaving a total of 32 phytoplankton taxa that were used in the PCA.

3. Results

3.1. Temperature and nutrient distribution

The variations in the hydrographic conditions along the sampling period reflected the seasonal pat-

terns typically found in shelf waters of the Cantabrian Sea. The water column showed strong vertical mixing during December–March, with temperatures typically in the range 12–13 °C (Fig. 2A). From April onwards, the warming of the surface waters gave rise to an increasing vertical stratification which was particularly intense during summer, when gradients of up to 5 °C were observed in the upper 50 m. Surface water temperature during the summer months was in the range 16–18 °C. From September onwards, temperature started to decrease throughout the water column and the thermal stratification weakened

progressively during the rest of the study. The effects of an upwelling pulse were noticeable during the August survey, when relatively cold (<14 °C) and nitrate-rich waters were detected at the base of the euphotic layer. A similar upwelling event may also have taken place during May, as indicated by the relatively high nitrate concentration measured at 40–50 m depth.

The concentration of nitrate, phosphate and silicate showed broadly similar spatial and temporal distributions that reflected the variability in the structure of the water column and the consumption

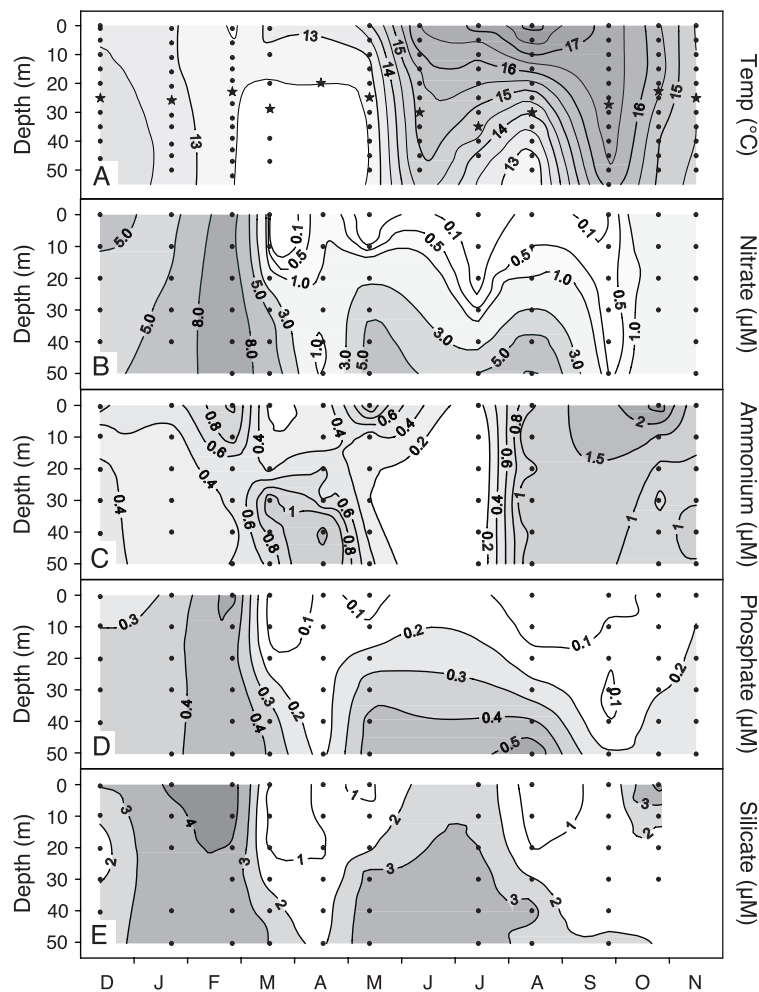


Fig. 2. Temporal and vertical distribution of (A) temperature (°C), and the concentration (µM) of (B) nitrate, (C) ammonium, (D) phosphate and (E) silicate. Stars in (A) indicate the depth where PAR was 1% of E_0 .

by phytoplankton (Fig. 2B, D, E). The patterns in the distribution of ammonium were somewhat different, indicating the importance of local regeneration processes at particular times (Fig. 2C). During winter, nutrient concentrations were high and relatively constant throughout the water column, owing to the strong vertical mixing. The highest nutrient concentrations were measured in February, when nitrate values above $9 \mu\text{M}$ were found in the whole water column (Fig. 2B). Phosphate and silicate concentrations during winter were above $0.3 \mu\text{M}$ and $2 \mu\text{M}$, respectively (Figs. 2D and E), whereas typical ammonium concentrations were in the range 0.4 – $0.8 \mu\text{M}$. The onset of stratification and the subsequent enhancement of phytoplankton growth in spring gave way to a progressive decrease in nitrate, phosphate and silicate concentrations, particularly in surface waters. Relatively high ($>1 \mu\text{M}$) ammonium concentrations were measured in subsurface waters during spring and in surface waters during autumn (Fig. 2C). In the upper 20 m, nitrate concentrations below $1 \mu\text{M}$ were typically measured during summer. The vertical distributions of nitrate and phosphate during August, showing relatively high concentrations in subsurface waters, indicated the effects of an upwelling event which brought cold, nutrient-rich waters to the euphotic zone. Nitrate and phosphate concentrations started to increase again in October and November, when vertical mixing became progressively more intense.

Overall, the concentrations of dissolved inorganic nitrogen ($\text{DIN} = \text{nitrate} + \text{nitrite} + \text{ammonium}$) and phosphate (P) were strongly correlated, according to the linear regression $\text{DIN} = 18.49 * \text{P} - 0.74$ ($r^2 = 0.87$, $n = 52$, $p < 0.001$). The N/P molar ratio generally took values between 10–20, except during March–April and September, when values below 5 were recorded throughout the water column.

3.2. Chlorophyll-*a* concentration and primary production

Chl-*a* concentration ranged from values below 0.2 mg m^{-3} typically observed in winter to values over 2 mg m^{-3} registered at depths of 10–20 m in March, May and August (Fig. 3A). Integrated Chl-*a* concentration over the upper 50 m (Fig. 4A) were in the range 10 – 30 mg m^{-2} during the less productive periods and reached values above 80 mg m^{-2} in May and August.

The temporal distribution of primary production reflected the variations in Chl *a* concentration, showing a moderate increase in March (around $50 \text{ mgC m}^{-3} \text{ d}^{-1}$ at the surface) and very marked peaks in May and August, when values above $100 \text{ mgC m}^{-3} \text{ d}^{-1}$ were measured (Fig. 3B). During most of the study, the highest rates of primary production took place in surface waters, with the exception of August, when C fixation rate peaked at 20 m, coinciding with a marked chlorophyll maximum. Typical production

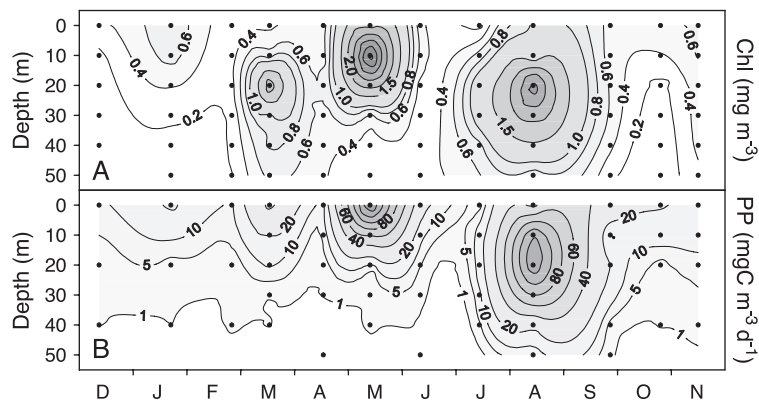


Fig. 3. Temporal and vertical distribution of (A) chlorophyll-*a* concentration (Chl, mg m^{-3}), and (B) rate of primary production (PP, $\text{mgC m}^{-3} \text{ d}^{-1}$). High Chl-*a* contour levels in (A) are 2.0, 2.5, 3.0, 3.5 and 4.0 mg m^{-3} . High PP contour levels in (B) are 80, 100, 120 and $140 \text{ mgC m}^{-3} \text{ d}^{-1}$.

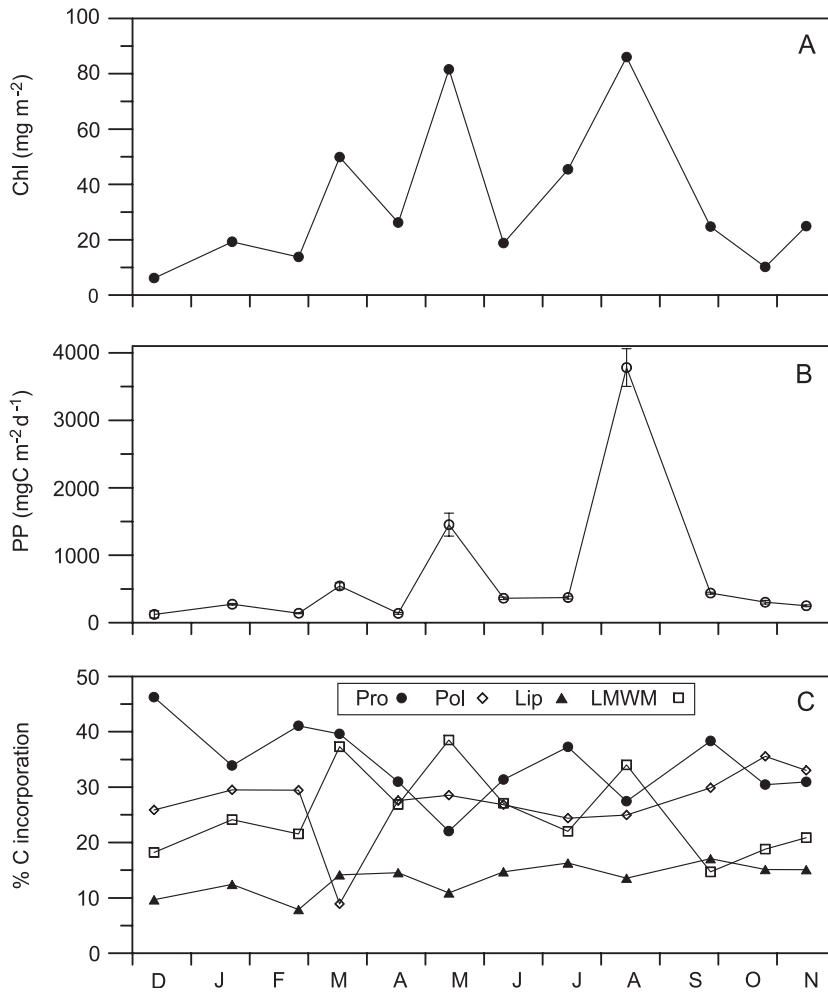


Fig. 4. Temporal distribution of integrated (A) chlorophyll-a concentration (Chl, mg m⁻²), (B) primary production rate (PP, mgC m⁻² d⁻¹) and (C) percentage of carbon incorporation into proteins (Pro ●), polysaccharides (Pol ◇), lipids (Lip ▲) and low molecular weight metabolites (LMWM □). Vertical bars represent ± 1 standard error.

rates in surface waters during winter and late autumn were in the range 10–30 mgC m⁻³ d⁻¹. Enhanced primary production conditions found during March, May and August resulted in integrated C fixation rates of 500, 1500 and >3500 mgC m⁻² d⁻¹, respectively. During the rest of the year, integrated primary production was typically in the range 100–400 mgC m⁻² d⁻¹.

3.3. Phytoplankton species composition

The relative contribution of diatoms, dinoflagellates and microflagellates to total phytoplankton C

biomass showed marked temporal and vertical variability (Fig. 5). Diatoms tended to dominate the phytoplankton assemblages during the episodes of increased Chl-a concentration and primary production in spring, with relative biomass contributions above 40% (Fig. 5A). Dinoflagellates showed their highest relative abundances (>30%) from April to September (Fig. 5B). Both groups displayed opposite patterns of vertical variability: diatoms tended to be more abundant in subsurface waters, whereas dinoflagellates were typically more abundant at the surface. Small flagellates represented a background upon which the

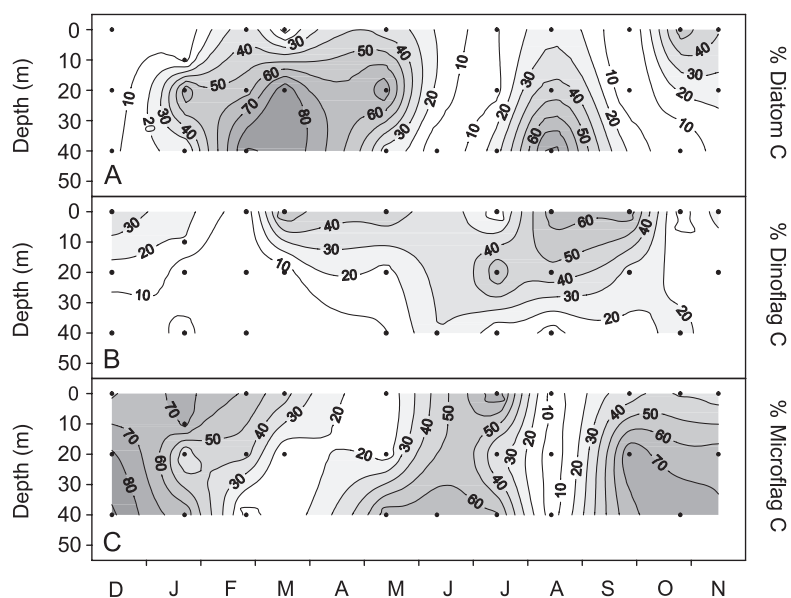


Fig. 5. Temporal and vertical distribution of the percentage of phytoplankton carbon contributed by (A) diatoms, (B) dinoflagellates and (C) microflagellates.

changes in diatoms and dinoflagellate abundance were superimposed. Thus, the relative contribution of microflagellates to total phytoplankton biomass was relatively low (<20%) when total phytoplankton abundance was high (e.g., March–May and August), but increased noticeably during periods of reduced phytoplankton biomass, such as autumn and winter (Fig. 5C).

In terms of particular phytoplankton species, we also observed significant compositional differences between each episode of enhanced Chl-a concentration and primary production. The dominant diatoms at the subsurface Chl-a maximum found in March were *Pseudonitzschia* spp., *Leptocylindrus danicus* and *Thalassiosira rotula*. During the May bloom, the diatoms *Pseudonitzschia* spp. and *L. danicus* were still important, but high numbers of *Rhizosolenia alata* and *Chaetoceros* spp. were also found. The distinct phytoplankton bloom found during August was composed by a diverse assemblage of diatoms and dinoflagellates. The most abundant diatoms at this time were *Chaetoceros* spp., *L. danicus*, *Pseudonitzschia* spp., *Rhizosolenia stollerfothi* and *R. alata*. Among the dinoflagellates, *Prorocentrum micans*, *Ceratium furca* and *Gyrodinium* spp. were the most numerous species. In September, very few diatoms

were present in the water column, whereas the dinoflagellates *Ceratium furca*, *C. fusus*, *Gyrodinium* spp., *Gymnodinium* spp. and *Oxytosum* spp. were highly abundant. During the low biomass and primary production period of December–February, the most abundant diatoms were *Thalassionema nitzschioides* and *Thalassiothrix frauenfeldii*, together with *Pseudonitzschia* spp. and *L. danicus*. The two latter species were also the most abundant diatoms in October and November.

3.4. Photosynthate partitioning into biomolecules

In terms of the relative allocation of photosynthate into biomolecules, proteins were the dominant fraction, as they typically accounted for >30% of the total C fixation (Figs. 4C and 6A). Percentages of C incorporation into polysaccharides and small metabolites were in the range 10–30% (Fig. 6B and D), whereas C flow into the lipid fraction was usually less than 15% of total photosynthesis (Fig. 6C). The percentage of C incorporated into proteins tended to increase with depth, frequently reaching values >40% at 40–50 m. C incorporation into lipids showed the opposite behaviour, as it tended to decrease with increasing depth (Fig. 6C, Table

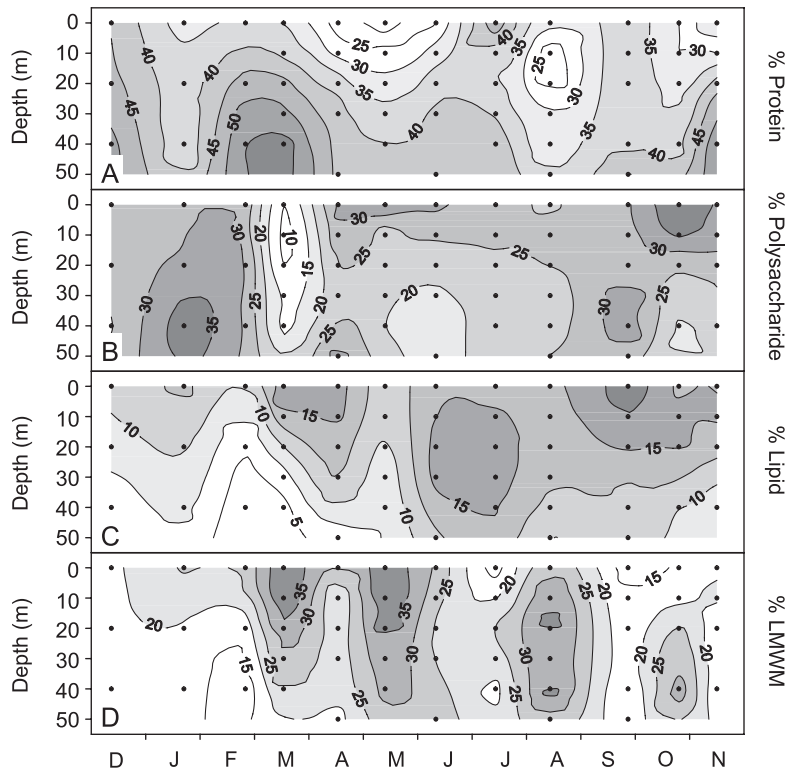


Fig. 6. Temporal and vertical distribution of the percentage of photo-assimilated carbon incorporated into (A) proteins, (B) polysaccharides, (C) lipids and (D) low metabolic weight metabolites (LMWM).

1). Relative C incorporation into polysaccharides decreased with depth during the summer stratification period (Fig. 6B). During the two main episodes of high phytoplankton biomass and production (May and August), we observed a reduction in the percentage of C incorporated into the protein fraction (Fig. 6A) and a relative increase in the fraction of C flowing into the pool of small metabolites (Fig. 6D).

The main patterns of temporal variability in photosynthetic C partitioning are more easily recognised in Fig. 4C, which shows the percentage of the total integrated production accounted for by each biochemical fraction. Protein relative synthesis was high (>30–40%) during periods of low phytoplankton biomass and production. The lowest percentages of C incorporation into proteins (<30%) were measured during spring and summer, particularly in May and August, when the highest primary production rates were observed (Fig. 4B and C).

The temporal variations of the percentage of C incorporated into LMWM showed the opposite pattern: higher values tended to occur during spring and summer, and the lowest values were measured during low primary production conditions in winter and autumn.

We found a significant, inverse relationship between the percentage of C incorporated into proteins and the Chl-a concentration, both with volumetric ($r = -0.36$, $p < 0.05$, Table 1) and integrated data ($r = -0.61$, $p < 0.05$, Table 1). We also assessed the relationship between the patterns of C allocation among biomolecules and an index of the photosynthetic efficiency of phytoplankton, namely the rate of C fixation per unit chlorophyll, or assimilation number (Table 1). There was a significant, inverse relationship between the percentage of C incorporation into proteins and the assimilation number ($r = -0.49$, $p < 0.01$). The relative C assimilation into lipids was positively correlated with the assimilation

Table 1

Correlation coefficient (r) between the percentage of C incorporation into different biomolecules and depth, chlorophyll-*a* concentration (Chl-*a*), assimilation number (AN) and growth rate

Variables	r	p	n
% protein \times depth	0.50	**	53
% polysaccharide \times depth	-0.22	ns	53
% lipid \times depth	-0.52	**	53
% protein \times Chl- <i>a</i> (volumetric data)	-0.36	*	53
% protein \times Chl- <i>a</i> (integrated data)	-0.61	*	12
% protein \times assimilation number	-0.49	**	53
% polysaccharide \times assim. number	0.07	ns	53
% lipid \times assimilation number	0.37	*	53
% protein \times growth rate	-0.49	**	53
% polysaccharide \times growth rate	0.07	ns	53
% lipid \times growth rate	0.37	*	53

The statistical significance (p) of the correlation and the number of samples (n) in each analysis are also shown. *: $p < 0.05$; **: $p < 0.01$; ns: not significant. For each group of multiple correlation tests, a Bonferroni correction was applied to calculate the significance levels.

number (Table 1). Furthermore, we estimated phytoplankton growth rates by dividing the daily C fixation rates by the phytoplankton C biomass, which was estimated from Chl-*a* concentration using a C to Chl-*a* ratio of 50 (Taylor et al., 1997). We found a significant inverse relationship between phytoplankton growth rate and the percentage of C incorporation into proteins ($r = -0.49$, $p < 0.001$, Table 1).

Finally, we represented, on a log-log basis, the rate of C incorporation into proteins against the sum of the rates of C incorporation into the other biochemical pools, using volumetric (Fig. 7A) and vertically integrated values (Fig. 7B). A few data points in Fig. 7A and B fall very close to or even above the 1:1 line, indicating that on some occasions when primary production was low the rate of C incorporation into proteins was very close to or even higher than that of C incorporation into all the other pools together. Both with volumetric and vertically integrated data, the slope of the Model II regression line was significantly lower than 1, indicating that as the C incorporation into polysaccharides, lipids and LMWM decreased, the C incorporation into proteins decreased at a slower pace. This means that the relative C incorporation into proteins, expressed as a percentage of the total C fixation, increased with decreasing primary production. In absolute terms, of course,

the rate of C incorporation into proteins decreased when phytoplankton biomass and production were low.

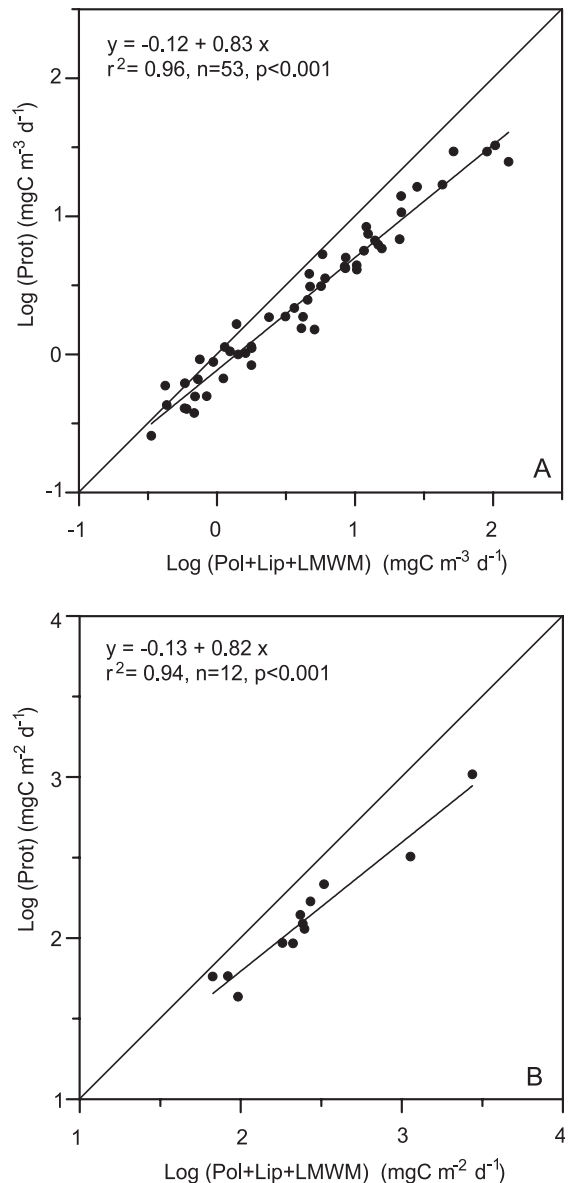


Fig. 7. Log-log relationship between carbon incorporation into proteins and the sum of the carbon incorporation into polysaccharides, lipids and low molecular weight metabolites, for (A) volumetric and (B) integrated data. The slope of the regression line (Model II) was significantly lower than 1 both for the volumetric ($p < 0.001$, $n = 53$) and the integrated data ($p < 0.05$, $n = 12$).

3.5. Relationship between photosynthate partitioning and species composition

We tested the hypothesis that the temporal changes in the integrated patterns of C allocation were correlated with the relative contribution of diatoms, dinoflagellates and microflagellates to total integrated phytoplankton biomass. We conducted a multiple correlation test between the percentage of algal biomass in each main group (diatoms, dinoflagellates and microflagellates) and the percentage of C incorporated into each biochemical fraction. We did not find any significant correlation for any combination of phytoplankton group and biochemical fraction.

In order to summarise the patterns of variability in phytoplankton species composition, and relate them with the changes in C metabolism, we conducted a principal component analysis (PCA) on the abundances of the 32 taxa that were present in >10% of the samples. Our intention was not to explore the causes of variability in species composition, but to assess if that variability, as represented in a small number of variables (the principal components), showed any relationship with the changes in C partitioning patterns among biomolecules. The first (PC1), second (PC2) and third (PC3) principal components accounted, respectively, for 27%, 13% and 8% of the total variance. As is often the case when PCA is applied to data of phytoplankton species composition, PC1 was mainly related to total cell abundance. The temporal and vertical distributions of PC1 closely resembled those of Chl-a concentration and primary production (data not shown). PC1 was significantly correlated with Chl-a concentration ($r=0.73$, $n=27$, $p<0.01$) and primary production ($r=0.77$, $n=27$, $p<0.01$).

The second and third principal components were not related to Chl-a concentration or primary production, but to phytoplankton species composition. In particular, PC2 clearly separated diatoms from dinoflagellates (Table 2). Most diatoms had correlation coefficients with PC2 between 0.4 and 0.8, whereas for dinoflagellates all species had correlation coefficients between 0.2 and -0.4. Consequently, PC2 showed very highly significant correlations with the relative biomass contribution of diatoms and dinoflagellates (Table 2). PC3 separated different groups of diatoms and dinoflagellates. The diatoms *Chaetoceros*

Table 2

Correlation coefficient (r) between several variables (percentage of C incorporation into each type of biomolecule and percentage of C biomass in different phytoplankton groups) and the second (PC2) and third (PC3) principal components obtained from a principal components analysis of the phytoplankton cell counts (see text for details)

Variable	PC2			PC3		
	r	p	n	r	p	n
% protein	0.03	ns	25	0.00	ns	25
% polysaccharide	0.06	ns	25	-0.06	ns	25
% lipid	-0.29	ns	25	0.23	ns	25
% LMWM	0.05	ns	25	-0.05	ns	25
% diatoms	0.70	**	27	-0.09	ns	27
% dinoflagellates	-0.52	**	27	-0.15	ns	27
% microflagellates	-0.37	ns	27	0.23	ns	27

The statistical significance of the correlation and the number of samples (n) in each analysis are also shown. *, $p<0.05$; **, $p<0.01$; ns, not significant.

decipiens, *Thalassiosira* spp. and *Pseudonitzschia* spp. were positively correlated with PC3, whereas *Dytilum brightwelli*, *Thalassiothrix frauenfeldii* and *Thalassionema nitzschioides* were negatively correlated with PC3. Most dinoflagellates, except *Oxytosum* spp. and *Pyrocystis lunula*, showed negative correlations with PC3. These results strongly suggested that PC2 and PC3 were representing the variability in the species composition of the phytoplankton assemblages, so it seemed appropriate to test whether or not these 2 principal components were also correlated with the patterns of photosynthate partitioning. Neither PC2 nor PC3 showed any significant correlation with the relative C incorporation into each biochemical pool (Table 2), which suggests that the changes in phytoplankton C metabolism were largely independent of the species composition.

4. Discussion

The patterns of temporal variability in phytoplankton abundance and production that we observed during the present survey were typical of temperate, coastal waters, and have been previously described for the same region (Fernández and Bode, 1991, 1994). Our study benefited from a high degree of temporal variability in phytoplankton biomass, species composition and primary production rates, which reflected the changes in incident irradiance and hydrographical

regime encountered during the sampling period. This variability enabled us to test whether the observed patterns of photosynthetic C allocation are recurrent and thus generally applicable to phytoplankton, or whether they depend on the particular hydrographical setting or type of phytoplankton community. To the best of our knowledge, the present study is the first one to describe a full seasonal cycle of photosynthetic C incorporation into biomolecules by marine phytoplankton, taking also into account the species composition of the microalgal assemblages. In what follows we shall consider the existence of general patterns of C assimilation in phytoplankton, the relationship between protein synthesis and phytoplankton physiological state, and the relationship between photosynthetic C allocation and the species composition of the community.

Irradiance was a major factor affecting C allocation among biomolecules. The percentage of C incorporated into the protein fraction increased with depth, whereas the opposite was true for C incorporation into lipids. During the summer stratification period, relative C incorporation into polysaccharides also tended to decrease with depth. These vertical patterns, which have been described before both for marine (Morris, 1981; Fernández et al., 1994) and freshwater (Hama et al., 1990) phytoplankton, are consistent with the lower saturation irradiance of protein synthesis as compared with the synthesis of polysaccharides or lipids (Hawes, 1990; Turpin, 1991; Marañón and González, 1997). As a result, carbon to nitrogen uptake ratios are expected to be lower at depth (Kudela et al., 1997). It has to be taken into account, however, that other environmental factors, such as nutrient concentration or temperature, could be responsible for the observed vertical variability in phytoplankton C metabolism. The enhanced relative synthesis of protein at larger depths could conceivably be caused by lower temperatures or an increased availability of dissolved inorganic nitrogen at the bottom of the euphotic layer. Our results strongly suggest this is not the case, though, given that the pattern of enhanced protein synthesis at depth was also observed during winter, when high and almost constant nitrate concentrations were measured throughout the water column, and vertical differences in temperature were very small.

The relative C incorporation into the protein fraction increased as phytoplankton abundance, as esti-

mated by Chl-a concentration, decreased. The linear relationship between Chl-a concentration at each depth and the percentage of C channelled into proteins was highly significant ($r = -0.36$, $p < 0.01$). In principle, this relationship could just be a reflection of the previously discussed linkage between irradiance (or depth) and protein synthesis, given that Chl-a concentration typically decreases markedly at the bottom of the euphotic layer.

However, the inverse relationship between Chl-a concentration and relative protein synthesis was also significant ($r = -0.61$, $p < 0.05$) when irradiance effects were removed by using vertically integrated data. This indicates that the synthesis of proteins is maintained, relative to that of other macromolecules, whenever phytoplankton biomass is low. Furthermore, we found that the relative C incorporation into proteins was also negatively correlated with phytoplankton photosynthetic efficiency and estimated growth rate. Similar results had been obtained for summer phytoplankton assemblages from the same location both during microcosms experiments (Marañón et al., 1995) and along a transect that crossed an upwelling front (Marañón and Fernández, 1995). The present results show that the association of low phytoplankton biomass, production and growth with higher percentages of C incorporation into protein prevails also during the rest of the year.

When primary production decreased, the synthesis of proteins was reduced less than the synthesis of other molecules, thus causing higher percentages of relative protein synthesis. This result, which was first reported by Morris et al. (1974) for laboratory cultures, has also been obtained in natural assemblages of marine (Priscu and Priscu, 1984; Barlow, 1984; Marañón et al., 1995) and lacustrine (Charpin et al., 1998) phytoplankton. Conservation of protein synthesis under growth-limiting conditions ensures that basic cellular functions (e.g. enzymatic activities) are maintained. Conversely, when resources are plentiful, more C is channelled into the synthesis of storage compounds such as polysaccharides and lipids.

It could be argued that nutrient limitation inside the experimental bottles during our incubations (which lasted for 24 h) could have caused the observed inverse correlation between primary production and relative protein synthesis, given that nutrient depletion is more likely when production is high. In order to test

this possibility, we compared the slope of the log-log relationship between integrated C incorporation into protein and integrated C incorporation into [lipids + polysaccharides + LMWM] in 2 groups of observations: months when nutrients were abundant (from December to March plus October and November) and months when nutrient concentrations were relatively low (from April to September). We found that the slope was in both cases lower than 1 and, furthermore, that it was lower (0.81) for the ‘high’ nutrient months than for the ‘low’ nutrient months (0.85). These results strongly suggest that nutrient depletion in the incubation bottles was not the cause of the observed relationship between primary production and relative protein synthesis.

Our results have implications for the use of C incorporation into protein as an estimate of phytoplankton relative growth rates (Di Tullio and Laws, 1983; Di Tullio, 1993). These authors have found that the relative C incorporation into proteins is positively correlated with growth rate of N-limited phytoplankton. If relative protein synthesis were positively correlated with growth rate in our study, we would have to conclude, unrealistically, that phytoplankton grow faster under low irradiance conditions (at the bottom of the euphotic layer and during periods of intense vertical mixing) and also under low nutrient conditions (e.g. summer stratification periods). The alternative explanation is that, in natural conditions at sea, high relative C incorporation into proteins by phytoplankton is indicative of the presence of adverse growth conditions that cause low algal biomass, low primary production rates, low photosynthetic efficiencies and, consequently, low growth rates. It is unlikely that the differences between our observations and the results of Di Tullio and Laws (1983) are due to methodological differences in the procedure of photosynthate fractionation, because we have all used the same solvents in the sequential extraction of each group of compounds. The discrepancy between our observations and the laboratory results of Di Tullio and Laws (1983) might stem from the fact that the latter have been obtained with N-limited, continuous cultures experiencing balanced growth. Observations at sea, however, reflect dynamic and transient algal responses to ever changing conditions that preclude phytoplankton from attaining balanced growth. In any event, our results lead us to recommend against the use of the percent-

age of C incorporation into proteins as an indicator of phytoplankton growth rate in coastal waters.

While the inverse relationship between total production and relative protein synthesis has been reported before, our study provides some novel insights into this metabolic pattern. First, the inverse relationship is observed both with volumetric and vertically integrated measurements, which suggests that the maintenance of protein synthesis under adverse growth conditions is independent of the nature of limiting factor (i.e. light at the bottom of the euphotic layer or nutrient availability during stratification periods). Second, we have shown that this pattern is robust and persistent over the entire annual cycle, despite the occurrence of major changes in the species composition of the phytoplankton assemblages.

In the study of the patterns of photosynthate partitioning in phytoplankton, the importance of species-specific responses has often been stressed (Rivkin and Voytek, 1987; Madariaga, 1992; Fernández et al., 1994). However, Morris (1981), when reviewing the results obtained with laboratory cultures of phytoplankton, noted that species differences are less important than environmental factors in controlling the fate of recently photo-assimilated carbon. In our study of C allocation patterns over an entire annual cycle, we have found that photosynthate partitioning could not be related to the species composition of the phytoplankton assemblages. Recurrent features such as the increased relative synthesis of proteins at depth, the maintenance of protein synthesis under adverse growth conditions, and the enhancement of C incorporation into storage compounds when irradiance is high, were not caused by the presence of particular assemblages of species, but reflected what probably are general metabolic strategies of marine phytoplankton. We conclude that the importance of environmental factors that control phytoplankton production and growth overrides the importance of phytoplankton species composition in explaining the general patterns of photosynthate partitioning among biomolecules.

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