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Carbon and nitrogen dynamics during growth and degradation of phytoplankton under natural surface irradiance

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Abstract. Under conditions of natural irradiance, the development and decline of a flagellate-dominated phytoplankton population was followed in a coastal North Atlantic pond over a 3 d period in summer 1986. Irradiance negatively affected phytoplankton biomass estimated as chlorophyll *a*, which decreased during the day at photosynthetically available radiation (PAR) levels above 600 to 1000 μmol m^{-2} s^{-1}; chlorophyll *a* increased at PAR values below this threshold. In addition, an inverse relationship was found between changes in chlorophyll *a* and changes in dissolved inorganic nitrogen, indicating synthesis of nitrogenous biomass mainly at night and degradation mainly during the day, with intense exchanges of material between the particulate and dissolved nitrogen fractions. The natural abundance of ^{13}C in particulate matter increased initially, and then remained constant, and was controlled mainly by the ratio β-carboxylases activity : ribulose biphosphate carboxylase activity. The hypothesis that the latter enzyme is broken down under high irradiance and is partly responsible for increases in external dissolved nitrogen was rejected.

Introduction

Previous investigations of phytoplankton dynamics in surface waters have revealed very intense fluxes of material between the dissolved and particulate phases in the water column of clay ponds during phytoplankton blooms. More specifically, it was shown that the degradation of microalgal populations was accompanied by increases in dissolved inorganic nitrogen (Collos et al. 1988a). As decreases in chlorophyll *a* and particulate nitrogen previously observed during the day (Collos et al. 1989) were not accompanied by similar changes in particulate carbon, we decided to confirm this by using both enzymatic (carboxylase activities) and isotopic (^{13}C:^{12}C) measurements of the particulate matter.

As ribulose biphosphate carboxylase is a major storage protein in plants, associated with chloroplasts (Huffaker and Peterson 1974, Ellis 1979), and is more easily mobilized than the rest of the protein pool during environmental changes (Ekman et al. 1989), it was hypothesized to act as a nitrogen reservoir and to be responsible for the observed decrease in particulate nitrogen during the day. In addition, carboxylase activities give an instantaneous estimate of carbon assimilation, while the isotopic carbon measurements yield an integrated view of all photosynthetic processes associated with inorganic carbon assimilation as well as gas-exchange processes occurring simultaneously (Descolas-Gros and Fontugne 1985, 1988). We report here on these phenomena, employing a sampling frequency which allows a good resolution of the processes involved in the carbon and nitrogen cycle of such environments.

Materials and methods

Samples were taken in July 1986 from a 2500 m^2 clay pond (No. 5 of the CREMA experimental site on the Atlantic coast of France near La Rochelle) which had been filled with fresh seawater (for a detailed description of the study site, see Collos et al. 1988b). The average depth was 1 m. As the pond had been recently dug in clay ground, there was practically no bottom sediment. We sampled directly from the pond (10 cm below the surface) during three successive 24 h periods. The sampling interval varied from 2 to 12 h. Previous results had indicated that, at the biomass levels and wind conditions prevailing during this study, the chemical and biological variables of the pond were horizontally and vertically homogenous (Collos et al. 1988b).

Surface irradiance was measured and recorded every 20 min with an Aanderaa pyranometer (Model 2770). The total energy values (in W m^{-2}) were converted to the visible part of the spectrum (400 to 700 nm) by multiplying by 0.42 (Jitts et al. 1976) and converted to total quanta by multiplying by 2.5×10^{18} (Morel and Smith 1974). The final values are expressed in μmol m^{-2} s^{-1}. Water temperature was between 18.8 and 25.0 °C.

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Samples for chlorophyll *a* analyses were filtered on Whatman GF/C filters. A preliminary study found no difference with samples filtered on GF/F filters. Within 30 min of collection, chlorophyll *a* was extracted in 90% acetone (Holm-Hansen et al. 1965), applying the recommendations of Holm-Hansen and Riemann (1978) concerning the final HCl concentration in the acidified sample, and measured on a fluorometer fitted with an F4T5B lamp (Baker et al. 1983) and an R136 photomultiplier. Excitation and emission filters were Corning models CS5-60, and CS2-64, respectively.

During the daytime, nutrients were analyzed immediately on a continuous-flow analyzer (Skalar Analytical, Breda, Netherlands) for nitrate, nitrite, ammonium, phosphate and silicate. Night samples were frozen and analyzed the following day.

Samples for cell counts were preserved in 4% (final concentration) formalin and enumeration was carried out in 5 ml chambers according to the Utermöhl (1958) technique, using a Diaphot inverted microscope (Nikon) with phase-contrast equipment. The whole bottom chamber was examined at 100× magnification for the presence of larger cells (mainly diatoms). Depending on their abundance, one or more diameter-transects were counted for smaller cells and flagellates at 400× magnification. Identification to species level was attempted whenever possible. Bacterial numbers were estimated from 18 ml samples of seawater fixed with phosphate-buffered formaldehyde (2% v/v), using the epifluorescence direct-counting technique (AODC) of Hobbie et al. (1977).

Carboxylating enzyme assays were performed according to Descolas-Gros and Fontugne (1985, 1988). Phytoplankton cells were collected by filtering 250 to 750 ml of seawater through GF/C glass-fiber filters which were placed immediately in cryotubes (Nunc R) and stored frozen at −196 °C in liquid nitrogen. Activities were determined by measuring the incorporation of radioactive bicarbonate into stable products, and expressed as nmol CO_2 fixed per liter seawater per hour (nmol CO_2 l^{-1} h^{-1}). Ribulose biphosphate carboxylase, phosphoenol pyruvate carboxylase, and phosphoenol pyruvate carboxykinase activities were measured in the same extract.

For determination of stable carbon-isotope ratios, phytoplankton was collected by filtering 350 ml of seawater through precleaned GF/C glass-fiber filters (Chesselet et al. 1981). The filters were decarbonated with 0.1 *N* HCl, dried at 60 °C, and stored in the dark at 4 °C before combustion in oxygen (Fontugne and Duplessy 1978, 1981). The CO_2 gas obtained was analyzed using a VG Micromass 602D mass spectrometer. Results are expressed relative to the PDB standard in the usual notation:

$$\delta^{13}C\ (‰) = \left[\frac{^{13}C:^{12}C \text{ sample}}{^{13}C:^{12}C \text{ standard}} - 1\right] \times 1000.$$

The reproducibility of the carbon isotope ratio is 0.1‰.

Table 1. Phytoplankton populations during study period. Values are cell counts (units of cells/liter). –: not detected

Species	24 July (11.00 hrs)	26 July (06.00 hrs)
Prorocentrum minimum	2×10^6	2×10^6
Scripsiella trochoidea	34×10^3	42×10^3
"Gymnodinium"-like	6×10^6	5×10^6
Pyramimonas sp.	–	5×10^6
3 to 5 μm cells	150×10^6	130×10^6

Results

The phytoplankton population consisted mostly of flagellates (Table 1). Bacterial counts increased from 2.55×10^7 cells per ml on the first day, to 3.19 on the second day, and to 3.30 on the third day, an overall increase of about 30%.

Fig. 1 shows the changes in surface photosynthetically available radiation (PAR), nitrate, nitrite, ammonium and chlorophyll *a* (chl *a*) in particulate matter suspended in the ponds as a function of time. There was a strong increase in chl *a* during the first dark period and during the following morning (15.6 to 27.1 μg l^{-1} in 14 h) with a concomitant decrease in dissolved inorganic nitrogen (DIN, 11.1 to 3.0 μg-at N l^{-1}). All three DIN forms were used up simultaneously. For the sake of clarity, nitrite is shown for the first 12 h only; thereafter, it always remained between 0.1 and 0.4 μg -at N l^{-1}. A decrease in chl *a* followed during the late morning and the afternoon, and this was again followed by an increase in the subsequent dark period. These oscillations continued throughout the final 24 h, but with a marked decline in amplitude.

During the first 24 h, there was a highly significant negative correlation between chl *a* and DIN ($r=0.938$,

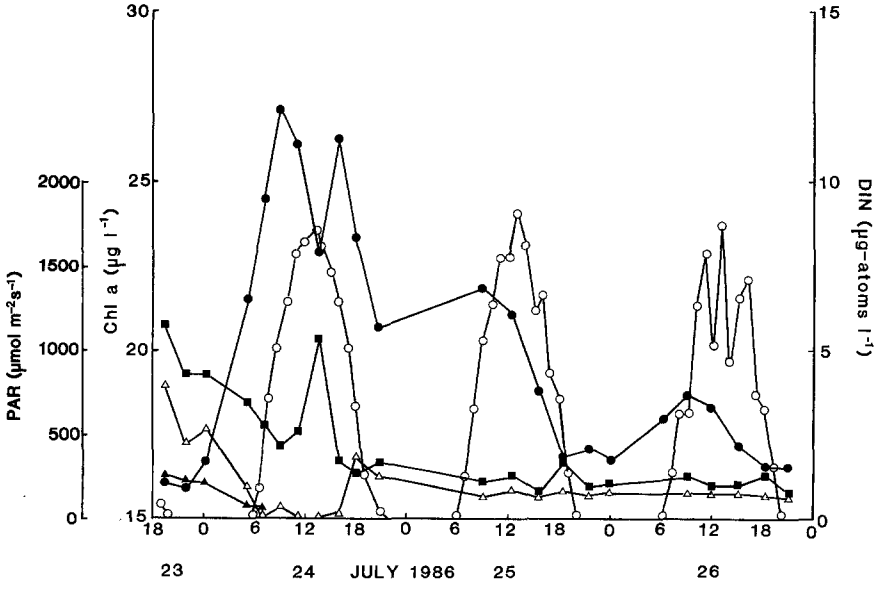

Fig. 1. Changes in photosynthetically available surface radiation (PAR, ○), nitrate (△), nitrite (▲), ammonium (■) and chlorophyll *a* (●) content of particulate matter suspended in pond seawater as a function of time. For clarity, nitrite is shown for first 12 h (18.00 to 06.00 hrs) only (see "Results" for further details). Abscissa shows time of day (hrs). DIN: dissolved inorganic nitrogen

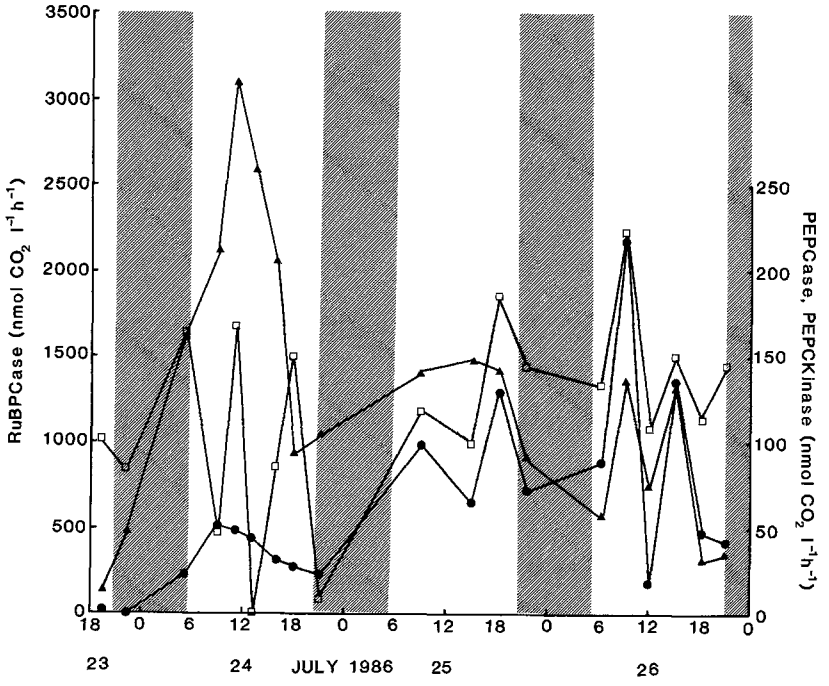

Fig. 2. Changes in ribulose biphosphate carboxylase (RuBPCase) activity (▲), phosphoenol pyruvate carboxylase (PEPCase) activity (●), and phosphoenol pyruvate carboxykinase (PEPCKinase) activity (□) as a function of time. Hatching indicates hours of darkness

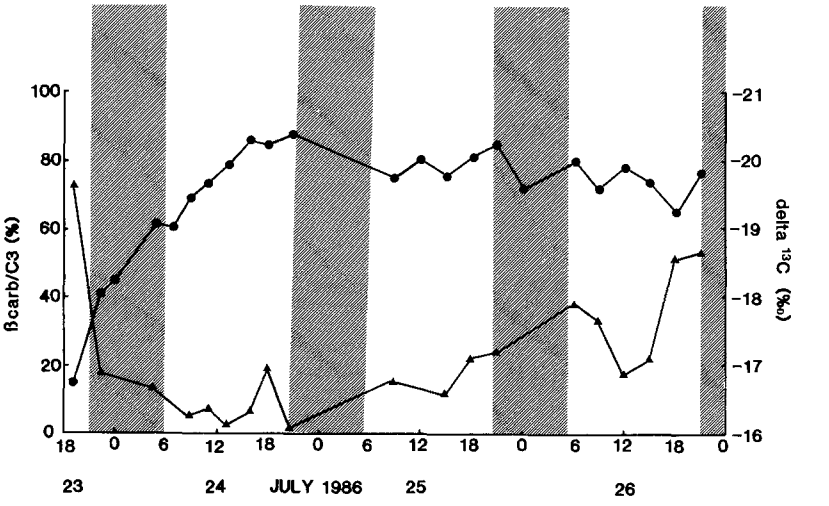

Fig. 3. Changes in ratio βcarboxylases:ribulose biphosphate carboxylase activities (βC:C3, ▲), and natural abundance of ^{13}C ($\delta^{13}C$, ●) in particulate matter as a function of time. Hatching indicates hours of darkness

$p<0.01$); the intensity of this relationship decreased after 48 h ($r=0.502$, $p<0.05$) and was not apparent when the data set was considered as a whole ($r=0.224$, NS).

Ribulose biphosphate carboxylase (RuBPCase) activity (Fig. 2) displayed patterns similar to those of the phytoplankton biomass estimated as chl *a*, with a lag of a few hours. These two variables were correlated in a highly significant way ($r=0.798$, $p<0.01$). Fig. 2 also shows the activities of the other carboxylases: phosphoenol pyruvate carboxylase (PEPCase) and phosphoenol pyruvate carboxykinase (PEPCKinase). In general, the activity of both βcarboxylases was lower than that of RuBPCase. The former displayed similar trends over the sampling period, but was not correlated with chl *a*.

Fig. 3 illustrates the changes in the ratio βcarboxylases: RuBPCase and the natural ^{13}C content of the particulate matter with time. The βC:C3 ratio decreased rapidly over the first 3 h (from 73 to 18%) due mostly to increased RuBPCase activity.

During the first 24 h, the $\delta^{13}C$ of the particulate matter became more negative in sign (Fig. 4), thereafter remaining fairly constant, or becoming slightly less negative as the bloom declined. The greatest changes occurred at the beginning of the experiment, during the period of active phytoplankton growth, and coincided with the largest changes in DIN (Fig. 4). $\delta^{13}C$ and DIN were inversely correlated in a highly significant way. $\delta^{13}C$ was also inversely correlated with the ratio βC:C3 ($r=0.563$, $p<0.05$).

RuBPCase activity per liter seawater (Fig. 5) or per unit chl *a* (data not shown) was positively correlated in a highly significantly way with surface irradiance. Al-

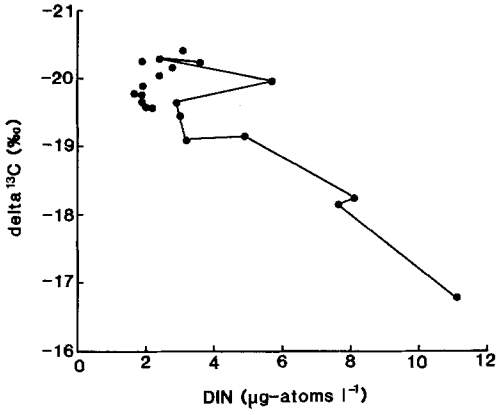

Fig. 4. Natural abundance of ^{13}C ($\delta^{13}C$) in particulate matter as a function of dissolved inorganic nitrogen (DIN). Linear correlation coefficient $r = -0.843$ ($p < 0.01$). Time zero is to right of graph. Line joins data points for first 24 h of incubation

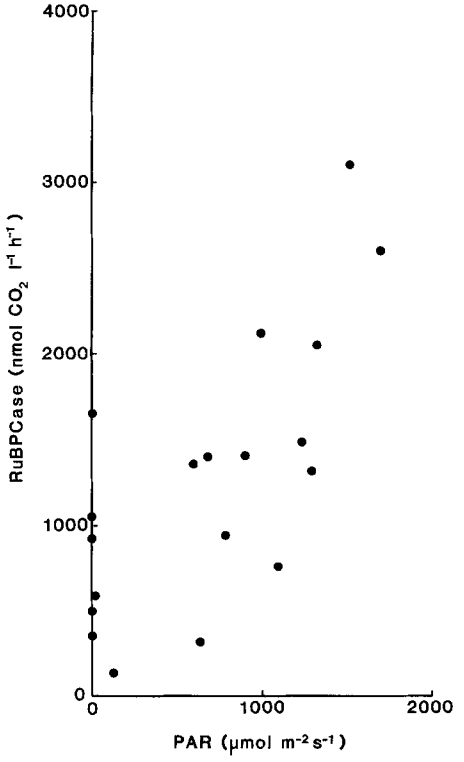

Fig. 5. Ribulose biphosphate carboxylase (RuBPCase) activity as a function of surface PAR over 3 d period. Linear correlation coefficient $r = 0.719$ ($p < 0.01$). PAR values measured and averaged for 20 min prior to sampling time

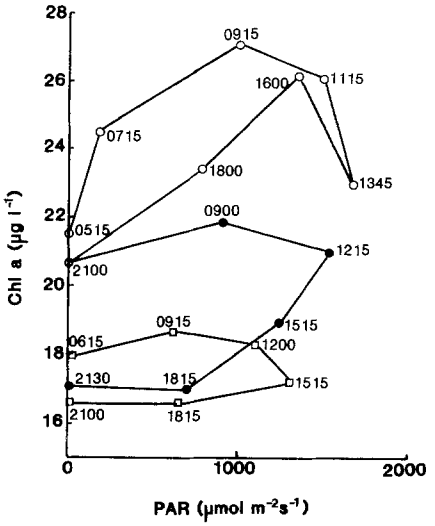

Fig. 6. Phytoplankton biomass (chlorophyll *a*) as a function of surface PAR over 3 d period. o: first day; •: second day; □: third day. Numbers by data points indicate local time (hrs). PAR values measured and averaged for 20 min prior to sampling time

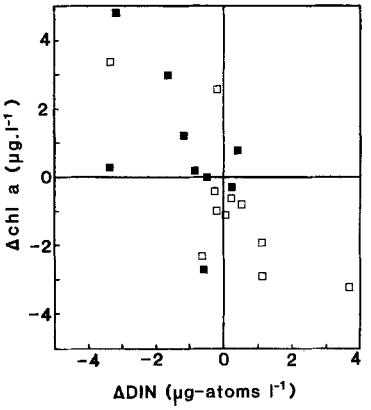

Fig. 7. Changes in phytoplankton biomass estimated as chlorophyll *a* as a function of changes in dissolved inorganic nitrogen (DIN) over three light (□) -dark (■) periods. Linear correlation coefficient $r = -0.70$ ($p < 0.01$)

though chl *a* was also positively correlated with PAR ($r = 0.512$, $p < 0.05$ for the whole data set), there was a negative effect of high irradiance on phytoplankton biomass estimated as chl *a* (Fig. 6). The threshold at which the decrease in chl *a* commenced ranged from 600 to 1000 µmol m^{-2} s^{-1} over the 3 d sampling period. In addition, changes in chl *a* and DIN over each sampling interval were inversely correlated (Fig. 7) in a highly significant way.

Discussion

The very clear effect of irradiance on phytoplankton biomass estimated as chl *a* (Fig. 6) was due not only to the well-known photoadaptation phenomena (Beardall and Morris 1976, Falkowski and Owens 1980, Perry et al. 1981), but also to degradation of phytoplankton nitrogen, reflected by the simultaneous increase in DIN (Figs. 1 and 7). Such an increase was postulated by van Rijn et al. (1984) following breakdown of nitrogen-containing pigments in unicellular algae, but the present paper documents it for the first time. Chlorophyll *a* metabolism has long been known to be associated with nitrogen cycling in algal cells (Strickland 1965). Pigments and protein or nitrogen content of unicellular algae have been shown to change in a parallel manner (Prézelin 1982, Döhler 1985).

The slope of the regression for the data shown in Fig. 7 indicates a ratio of about 0.86 µg at DIN for 1 µg chl *a*, which is consistent with the particulate nitrogen:chl *a* ratio of those populations (Maestrini and Robert 1981, Collos et al. 1989). Thus, the rates of change of chl *a* and DIN are internally consistent and also consistent with the hypothesis of particulate nitrogen (PN) decrease under high irradiance. We used the pooled DIN here, since it is known that transformations of one form of nitrogen into another can take place on the time scale of hours in such environments (Collos et al. 1988a). We believe that the ammonium peak at 13.45 hrs on 24 July was real, since it was followed by a peak in nitrate at 18.00 hrs on the same day, reflecting active nitrification. This confirms earlier results showing decreases in PN during day under high irradiance in these (Collos et al. 1989) and other (Dortch and Postel 1989) marine environments.

The simultaneous changes in nutrients were not observed in our previous study (Collos et al. 1989), probably because of the lower sampling frequency and the overall exponential biomass increase over the long term (several days). Assuming that PN is transformed to DIN with 100% efficiency, and taking the equivalent of 1 µg-at PN-N for 1 µg chl *a* to represent the chemical composition of phytoplankton (Strickland 1965, Maestrini and Robert 1981), then the maximum increase in DIN (3.7 µg-at N l^{-1} between 11.00 and 14.00 hrs on 24 July) would correspond to a relative decrease in PN of about 15% in 3 h.

The trends in $\delta^{13}C$ (Figs. 3 und 4) indicate a preferential assimilation of ^{12}C over ^{13}C during the development of the phytoplankton bloom (essentially the first 24 h). These changes were similar to those shown during batch growth of *Skeletonema costatum* (Descolas-Gros and Fontugne 1985). The carbon isotopic ratio paralleled the evolution of carboxylase activities (mainly RuBPCase) during the first 24 h, and reflected the relatively greater contribution of βcarboxylases towards the end of the incubation period. The correlation between the βC:C3 ratio and $\delta^{13}C$ illustrates the dominant impact of carbon pathways on the isotopic ratio of particulate carbon (Descolas-Gros and Fontugne 1990).

The changes in the βC:C3 ratio during the development of the phytoplankton bloom were similar to those observed by Descolas-Gros and Fontugne (1985) during the exponential growth phase of a culture and by Smith et al. (1983) and Descolas-Gros and Fontugne (1988) during a bloom of a natural population. As the phytoplankton bloom declined, the increase in this ratio reflected the greater contribution of βcarboxylases to carbon fixation. In our study (Fig. 3), the changes in βC:C3 could also have been due to species succession and, more particularly, to the development of flagellates such as *Pyramimonas* sp. (Table 1). In this taxonomic group, βcarboxylase activities relative to RuBPC activity appear to be greater than for green algae or diatoms (Descolas-Gros 1985).

From the data available, we cannot conclude that RuBPCase is being degraded under high irradiance and is partly responsible for the increase in DIN observed under such conditions. The assay we used does not allow discrimination between the total amount of the enzyme and its specific activity.

The present study confirms previous results (Collos et al. 1989) which have shown the nitrogen fraction of the phytoplankton biomass to be more labile than the carbon fraction. This rapid degradation is characteristic of phytoplankton in shallow environments (< 1 m depth) which cannot avoid high irradiance levels during daytime, and may also reflect conditions in incubation bottles held at fixed positions near the surface. Recent evidence on changes in chl *a* during measurements of photosynthesis as a function of irradiance (Cullen 1988) indicates that the processes described here are significant on short timescales. In particular, the increased nitrogenous nutrient supply which results from the degradation of algal biomass at high levels of irradiance seems to be important to the nitrogen dynamics of aquatic environments, such a nitrogen source becoming available for the synthesis of new phytoplankton biomass during subsequent periods of low irradiance.

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