

Production and utilization of dissolved organic carbon during an experimental diatom bloom

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Abstract

Stable isotope measurements of carbon were used to study the transfer of carbon from phytoplankton to bacteria during an experimental diatom bloom. A large-volume tank was filled with 900 liters of 10- μm -filtered seawater from Woods Hole Harbor, and N, P, and Si nutrients as well as ^{13}C -enriched NaHCO_3 were added. This resulted in a high nutrient system with an isotope label of +85‰ in the dissolved inorganic carbon pool. Algal growth depleted nutrients to near-zero concentrations midway through the 2-week experiment with little net dissolved organic C (DOC) accumulation; algal chlorophyll concentrations peaked at 38 $\mu\text{g liter}^{-1}$ near the time of nutrient depletion. Decline of the algal peak was associated with diatom sedimentation to the bottom of the tank and rapid accumulation of DOC. In 3 d, DOC increased from ~130 to 250 $\mu\text{M C}$. The C:N ratio of this new DOC was >22 vs. 14 in background harbor water. ^{13}C labeling showed that bacteria preferentially assimilated the newly produced DOC in both the rapid growth and postbloom phases of the experiment, while DOC measurements indicated that very little if any of the background DOC from Woods Hole Harbor was metabolized by bacteria during the experiment. Overall, the experiment showed rapid DOC release following algal blooms, selective use of newly produced DOC by bacteria, and that DOC released during blooms is not rapidly or completely turned over following algal blooms.

Heterotrophic bacteria growth in aquatic ecosystems encounter a diverse suite of organic substrates of varying origin, chemical composition, and nutrient content. Some of these substrates are readily assimilated and incorporated into bacterial biomass, some are rapidly respired to CO_2 , and not incorporated into biomass (Ducklow et al. 1986), and others are extremely refractory and resistant to bacterial attack for thousands of years (Williams and Druffel 1987). Bulk dissolved organic C (DOC) measurements mostly reflect concentrations of refractory components. The ongoing slow metabolism of refractory substrates is unfortunately difficult to study without experi-

mental artifact in long-term bioassay experiments (Keys et al. 1935; Ogura 1972; Servais et al. 1987).

Primary producers supply intermittently new, potentially labile DOC to aquatic systems (Kirchman et al. 1991; Søndergaard et al. 1985). There is some evidence that dissolved organic matter (DOM) concentrations increase toward the end of blooms (Barlow 1980; Sellner et al. 1981) and that the nitrogenous content decreases upon nutrient starvation of the algae (McCarthy and Goldman 1979). However, the new carbon pool is usually small compared to more refractory carbon pools and is thus difficult to distinguish. Bacterial production and respiration yields are our best current indicators of the overall amounts and quality of these labile DOC pool sizes.

The present study used stable carbon isotope labeling to investigate bacterial utilization of newly produced organic C associated with a phytoplankton bloom relative to a background of more refractory "old" DOC. An algal bloom was initiated in a 1- m^3 mesocosm by adding inorganic N, P, and Si. We also added ^{13}C -enriched NaHCO_3 to the dissolved inorganic C (DIC) pool to follow algal incorporation of an environmentally safe tracer during normal photosynthetic carbon uptake. The coupling between bacteria and the new production of algal DOC was assayed by extracting nucleic acids from bacteria (Coffin et al. 1989, 1990) and testing the nucleic acids for the appearance of ^{13}C . DOC concentrations and isotopic compositions were also analyzed for turnover of the background, more refractory DOC present in water from Woods Hole Harbor. Previous decomposition experiments showed only a small loss of DOC (<30 $\mu\text{M C}$)

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during a 2-yr incubation in dark 20-liter glass carboys (Fry et al. in prep.). We were curious whether this refractory DOC would be metabolized more rapidly when bacteria were also supplied with labile DOC from the large algal bloom we induced.

Materials and methods

Mesocosm setup—The main mesocosm experiment was conducted in a 1-m³ fiberglass tank on a dock in Woods Hole from 20 May to 3 June 1992. The tank had been thoroughly cleaned before the experiment. All tubing used to transfer water during the experiment was preleached for at least 2 weeks prior to use. About 900 liters of Woods Hole Harbor water (salinity ~32‰) from 2-m depth was pumped into the tank through two coupled cartridge filters (Cole Parmer; 10- μ m mesh) by a peristaltic pump. To maintain ambient water temperatures (11–15°C), we placed the tank in a 30-cm-high outer tank through which harbor water was continuously pumped. The experimental tank was covered tightly with a transparent plastic sheet and a 50% light reduction shade cloth. This cover also reduced UV radiation to near-zero levels (Hullar pers. comm.).

Nutrients were added to the tank as KNO₃ (4.04 g), K₂HPO₄ (0.23 g), and Na₂SiO₃ (4.55 g). Nutrient concentrations were determined from filtered and acidified samples stored in the cold. A Technicon TRAACS autoanalyzer was used to analyze NO₃⁻ and PO₄³⁻ (Grasshof et al. 1983). DON (dissolved organic N) was determined by difference from total dissolved N assayed via persulfate digestion minus nitrate values. Ammonium was not analyzed, and to the extent ammonium was present, reported DON estimates are high.

Simultaneously with the fertilization, 99% ¹³C-pure NaH¹³CO₃ (Merck) was added to achieve a DIC δ^{13} C signal of about +85‰. The δ^{13} C values are given in reference to the PDB standard by the formula

$$\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1,000$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$.

Finally, a 10-liter inoculum of dense mixed diatom culture enriched from Woods Hole seawater was added to the tank at the beginning of the experiment.

Sampling—Samples were drawn from the tank with preleached plastic tubing which was present in the tank throughout the experiment. Several liters of water were drawn through the tubing before samples were collected. Mixing was provided once per day by bubbling the tank with air for ~15 min through the same tubing. Oxygen concentration and temperature were continuously recorded with an automated instrument (Orbisphere Laboratories model 2607).

Algal and bacterial numbers and biomass—Bacterial numbers were determined from formaldehyde-fixed samples stained with DAPI to a final concentration of 10 μ g liter⁻¹ (Porter and Feig 1980). Two to five milliliters of

sample were filtered onto black 0.2- μ m Nuclepore filters. A Zeiss standard microscope equipped with a 100 \times Neo-fluar objective was used for counting. At least 20 fields with >20 bacteria per field were counted for each sample. For enumeration of flagellates, 20 ml of sample was passed onto 0.6- μ m polycarbonate filters and the DAPI concentration increased to 100 μ g liter⁻¹. Flagellate enumeration by epifluorescence microscopy was verified by comparisons with flagellate counts from Lugol's-preserved samples. No significant differences were found (data not shown).

Algal groups were identified and enumerated from the preserved samples after concentration in a sedimentation tower. A Zeiss inverted microscope was used, and pigmented and nonpigmented cells were counted. The algal carbon content was calculated from biovolume determined with the microscope (Edler 1979).

Chlorophyll was determined from 1-liter water samples filtered on 47-mm Whatman GF/F filters. The chlorophyll was extracted from the filters with 90% acetone at 4°C in the dark, and chlorophyll concentration was determined after centrifugation by spectroscopy (Edler 1979). Particulate organic C (POC) was determined from 1-liter samples filtered onto 47-mm precombusted quartz filters (Whatman QM/A). Filters were dried at 60°C overnight and 11-mm-diameter circular subsamples punched from the filters and folded into aluminum boats and analyzed for carbon content as well as for δ^{13} C with an automated elemental analyzer-mass spectrometer system previously described (Fry et al. 1992). Bacterial nucleic acids spotted onto filters were also analyzed with this system, and blank corrections for mass and isotopic composition were made with precombusted filters. Overall precision of the δ^{13} C measurements was typically better than 0.2‰.

Primary production, community respiration, and net community production were calculated from the rate of oxygen change integrated over light and dark periods (Odum and Hoskins 1958). Diffusion was corrected for with a gas transfer coefficient (K) of 0.01 g O₂ m⁻² h⁻¹ atm⁻¹. This very low coefficient reflects the virtual absence of physical disturbance of the water surface due to the plastic sheet that sealed the tank.

Dissolved carbon and stable carbon isotope determinations—DIC was determined from 125-ml samples poisoned with mercuric chloride. At the time of analysis, samples were transferred to a vacuum line, acidified with phosphoric acid, and then sparged with nitrogen. The CO₂ released was collected by cryogenic separation and the concentration determined from gas pressure (Kroopnick 1974).

Samples for DOC analysis were filtered through Supor filters (Norrman 1993). DOC was determined by high-temperature catalytic oxidation (HTCO) (Sugimura and Suzuki 1988) with a Shimadzu TOC instrument and also by sealed tube combustion (Fry et al. 1993). The TOC-5000 was equipped with a "normal sensitivity" bead catalyst. Two or three 150- μ l injections were made for each sample by means of an autoinjector. DOC concentrations

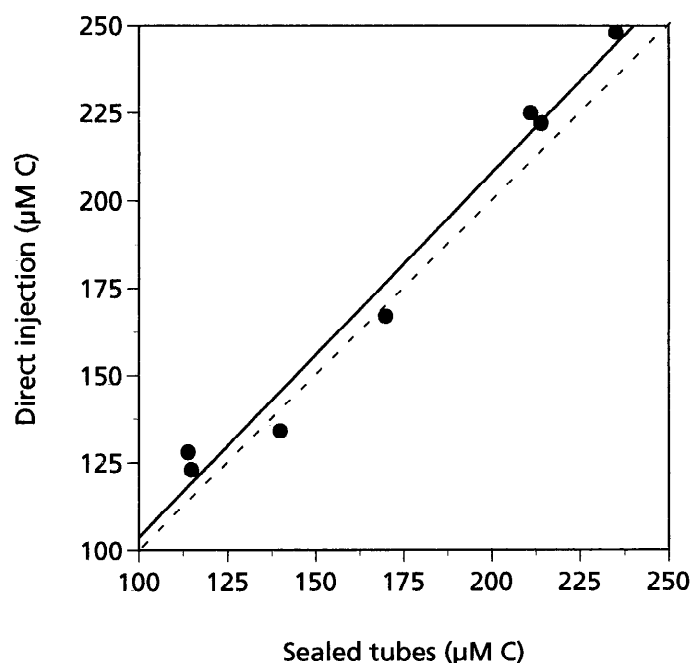


Fig. 1. Comparison between analysis of DOC concentrations by two high-temperature combustion techniques: sealed tubes and direct injection. Solid line shows the best fit by linear regression ($Y = -0.6 + 1.04X$; $r^2 = 0.98$); dotted line indicates a 1:1 relationship.

were calculated with the instrument software and a 4-point calibration curve with potassium biphtalate as standard. The upper limit for our system blank was 13 $\mu\text{M C}$, based on the measured C content of the Milli-Q water used to prepare standards. Triplicate injections showed good precision, with standard deviations of 0.1–1%. For duplicate samples, the combined error from all injections was <2%.

[^{14}C]DOC was also determined by combusting pan-dried salts overnight at 580°C in sealed, evacuated Pyrex tubes (Fry et al. 1993, in prep.). Tubes were cooled at room temperature, cracked under high vacuum, and a crude separation of CO_2 plus contaminating gases (HCl and some SO_2) was made by using dry ice and pentane-liquid nitrogen slushes that held back water vapor and most SO_2 . The volatile fraction containing CO_2 was frozen into a second tube containing CuO and MnO_2 , sealed, and fired 1 h at 450°C to remove the contaminating gases. These second sealed tubes were cracked again in a vacuum manifold, the water was removed, and CO_2 yields were measured with a calibrated capacitance manometer. Isotopic compositions were measured with a Finnegan 251 isotope ratio mass spectrometer. Various blank tests showed that <10 $\mu\text{M C}$ contamination typically occurs in sample handling, primarily during the long pan-drying step in the convection oven. Isotopic data have been corrected for blanks that averaged near -26.5‰ . Overall precision in the measurements is about $\pm 10 \mu\text{M DOC}$ (95% C.I.) and $0.3\text{‰ } \delta^{13}\text{C}$.

Comparison of the Shimadzu HTOC and sealed tube methods for DOC analysis showed that agreement be-

tween the two methods was very good, nearly 1:1 (Fig. 1).

Nucleic acid extraction for bacterial $\delta^{13}\text{C}$ analysis—Water samples were first prefiltered through a 20- μm -mesh net to remove large particles, then pressure filtered through a 1- μm Whatman Polycap cartridge filter to remove smaller plankton; bacteria were finally collected on a Gelman Microculture 0.2- μm capsule cartridge filter.

Prior to use, the filters were rinsed with 20 liters of Nanopure water and subsequently 50–75 liters of water were passed through the cartridges. After collecting the cells, the Gelman cartridge was air purged, and TEN buffer (2 mM Tris pH 8.0, 2 mM EDTA and 100 mM NaCl) containing 1% SDS was added. The cartridges were stored frozen until the time of nucleic acid extraction.

The cells were lysed in the cartridges by boiling for 5 min. The buffer-SDS solution was purged from the cartridges with air, and 20 ml of buffer was used to rinse the cartridge filters. The crude extracts were combined and precipitated with ethanol. After centrifugation, the pellet was dissolved in TEN buffer and extracted with a chloroform-phenol mixture (Sambrook et al. 1989) followed by precipitation of the buffer phase with ethanol. The resulting pellet was dissolved in 400 μl of TEN buffer and transferred to Eppendorf vials in which the subsequent steps were performed.

Nucleic acids were again extracted in chloroform-isomyl alcohol, and the purified nucleic acids were dialyzed against sterile Nanopure water overnight with at least two changes of water. The purified nucleic acids were precipitated with ethanol, dissolved in water, and spotted on precombusted QM/A filter dots for $\delta^{13}\text{C}$ analysis.

Natural water samples extracted from Woods Hole Harbor consistently showed nucleic acid $\delta^{13}\text{C}$ values of -21 to -22‰ . In control experiments performed with cells grown on dextrose with a $\delta^{13}\text{C}$ value of -9.8‰ , the extracted nucleic acids were 1‰ heavier than the dextrose (i.e. -8.8‰). In a separate experiment, $\delta^{13}\text{C}$ of nucleic acids extracted from filter capsules was compared with $\delta^{13}\text{C}$ of nucleic acids from cells concentrated with an Amicon ultrafiltration unit. Samples were collected at the Gulf Breeze Environmental Research Laboratory, Florida, from the dock or from a seawater tank that had been supplemented with glucose. For seawater samples, nucleic acids obtained from filter capsules were 0.7‰ heavier than nucleic acids from cells concentrated with the ultrafiltration unit. For glucose-grown cells, the difference was 0.2‰ between the methods and -1‰ vs. the $\delta^{13}\text{C}$ value of the glucose substrate.

Bacterial growth bioassay of labile DOC—Four times during the tank experiment, small-volume incubations were conducted to determine the ability of DOC to support bacterial growth. One-liter samples were filtered through acid-cleaned 0.2- μm Supor filters by means of a polysulfone filter apparatus (Nalgene). The filtrate was transferred to a 1-liter acid-cleaned polycarbonate bottle, and a 5% volume of 0.6- μm -filtered inoculate was added. Samples were periodically taken over 48 h for determi-

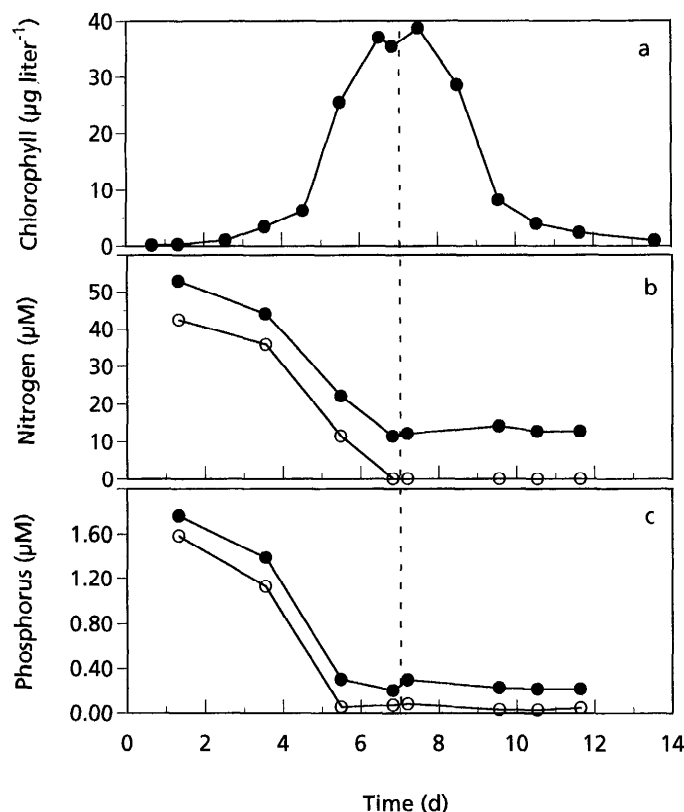


Fig. 2. Changes in chlorophyll and nutrients during the experiment. Nutrients were analyzed from filtered samples. [a.] Chlorophyll *a* in the particulate fraction. [b.] Total dissolved nitrogen (●) and nitrate (○). [c.] Total dissolved phosphorus (●) and phosphate (○). Dashed line indicates the peak of chlorophyll and biomass.

nation of bacterial growth, assuming 20 fg C cell⁻¹ (Lee and Fuhrman 1987).

Results

Algae, bacteria, and nutrients—Algae grew exponentially during the first week of the experiment, with peak chlorophyll *a* concentrations reaching 38 µg liter⁻¹ by day 8 (Fig. 2). Algal cells <10 µm were initially most abundant, but large diatoms rapidly became dominant. At the time of peak Chl *a*, large diatoms comprised 70–85% of total algal C (Table 1). Inorganic nutrient concentrations decreased during the accumulation of algal biomass, with phosphate and nitrate reaching near-zero levels on days 6 and 7, respectively (Fig. 2). DON estimates, which may be slightly inflated due to inclusion of any ammonium, increased slightly, from 8–10.5 µM early in the first week to 12.5–14.5 µM during the second week. Total dissolved phosphate concentrations after inorganic nutrient depletion were ~0.2 µM (Fig. 2).

Initial bacterial numbers were 2.5 × 10⁹ liter⁻¹ (Fig. 3) and doubled in 1 d to 4.8 × 10⁹ liter⁻¹ on day 2, probably due to introduction of labile DOC during filtration steps in the setup of the experiment. Bacterial numbers re-

Table 1. Cell counts (× 10⁵ cells liter⁻¹) of algae and diatoms. Algal cell counts were determined from Lugol's-preserved samples. Carbon content (µmol C liter⁻¹) was calculated from bio-volumes. Cells were separated into two groups: <10 µm and >10 µm. For the >10-µm group, diatoms were counted separately.

Day	Cells > 10 µm				Cells < 10 µm	
	Total cells		Diatoms		Total cells	
	No. cells	C	No. cells	C	No. cells	C
2	1.8	0.5	0.4	0.2	3.1	0.2
5	47	8.7	47	8.7	110	4.2
6	66	27.8	65	27.4	70	4.3
8	31	21.1	31	20.8	21	0.9
10	6.8	2.3	6.4	2.1	34	1.7

mained high for several days and then fell quickly in response to the mounting grazing pressure of an increasing population of heterotrophic flagellate grazers (Fig. 3). Flagellates and bacterial numbers were inversely correlated ($r^2 = 0.49$, $P < 0.01$), perhaps indicating grazing control of bacteria by flagellates. Bacterial numbers increased again between days 7 and 10, coincident with a large increase of DOC and moderate flagellate numbers (Fig. 3).

Carbon pools and estimating mesocosm production—DIC, POC, DOC, and O₂ flux measurements provided a biogeochemical view of the experiment. DIC concentrations decreased from 2,030 µM to <1,540 µM during the first 10 d of the experiment, then increased again (Fig. 4a) when respiration was greater than production. POC accumulated in the first week of the experiment, reaching its highest level on day 7 (Fig. 4b). DOC accumulation was low during the formation of the bloom before day 7 but increased considerably between days 7 and 10, when POC and chlorophyll levels dropped and diatoms sedimented out (Fig. 4c).

A conservative estimate of the atomic C:N ratio for this newly formed DOM was 22—substantially higher than the 14 estimated for background Woods Hole seawater (C. Hopkinson and B. Fry unpubl.). We derive this C:N estimate from the increase in DOC (120 µM C) and

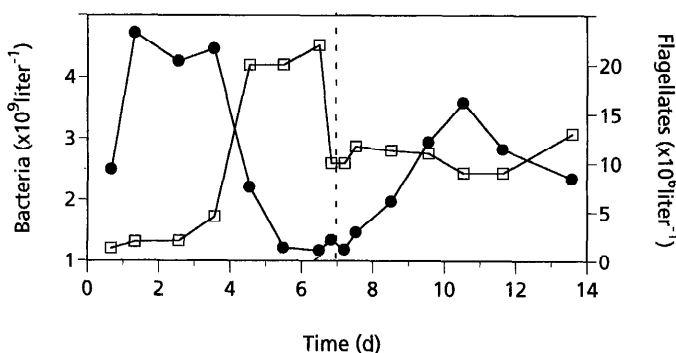


Fig. 3. Bacterial (●) and flagellate (□) numbers. Dashed line indicates the peak of chlorophyll and biomass.

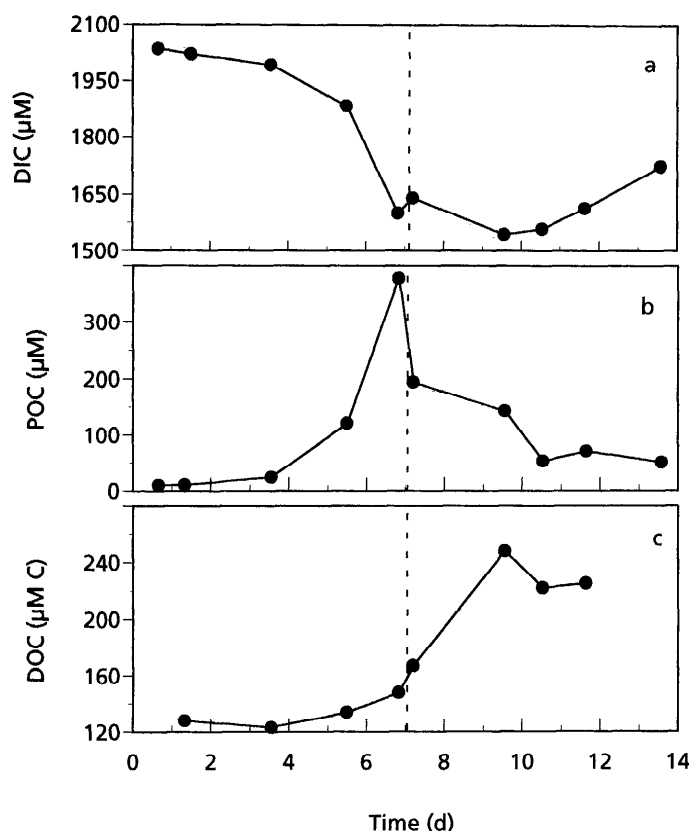


Fig. 4. Carbon fractions during the course of the experiment. [a.] Dissolved inorganic C determined by manometry after sparging CO_2 from mercuric chloride-preserved samples. [b.] Particulate organic C. [c.] Dissolved organic C determined by high-temperature catalytic oxidation. Dashed line indicates the peak of chlorophyll and biomass.

DON ($5.5 \mu\text{M}$ N or less). There are some uncertainties in the DON estimate because ammonium, which was not measured, was present. Specifically, if ammonium was present in the second week of the experiment, our DON estimate would be too high and our C:N estimate too low. Also, if we used the higher of our two estimates of baseline DON early in the experiment (10.5 and $8 \mu\text{M}$ N), we would estimate a DON increase of 3 rather than 5.5 and a C:N ratio of 40 rather than 22.

We used 2-d bacterial growth incubations to estimate labile DOC pools accumulating during the experiment. These experiments showed increasing stocks of labile DOC through the experiment, indicated by increasing yields of bacterial biomass. For days 1, 2, 5, and 9 of the experiment, the yields were 1.3 , 2.4 , 3.1 , and 3.6×10^9 bacteria liter^{-1} respectively. The growth yields alone are, however, underestimates of total labile DOC because some DOC is used for respiration, not growth. One set of respiration measurements made in conjunction with the last bioassay showed a DIC increase of $30 \mu\text{M}$ over 2 d; if an average bacterial C content of $20 \text{ fg C cell}^{-1}$ is assumed, the total labile C pool was $36 \mu\text{M}$ at a time when DOC had increased $\sim 120 \mu\text{M}$ over background levels (Fig. 4c). These

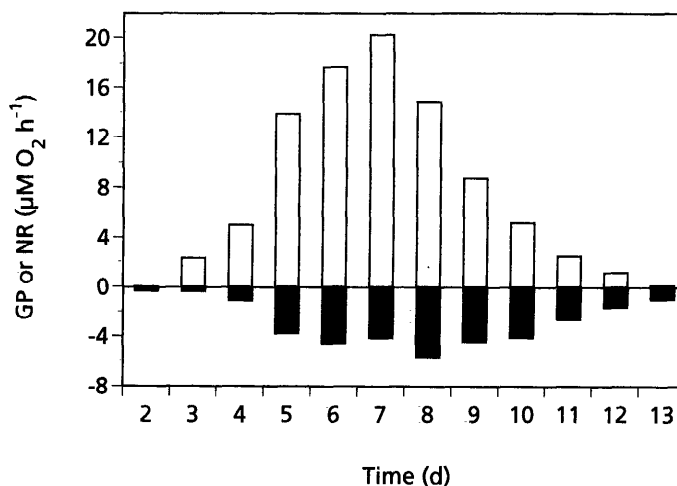


Fig. 5. Production and respiration in the system calculated from oxygen measurements. The daily averaged gross production (GP; white bars) and net respiration (NR; black bars) is shown.

data indicate that at this time, 30% of the accumulated DOC was labile toward degradation on a time scale of days.

Total production could be estimated from three different data sets: oxygen, organic C, and inorganic C. Gross primary production, community respiration, and net community production were calculated from continuous oxygen measurements. Gross production increased exponentially for the first 7 d (from 1.1 to $20.2 \mu\text{M O}_2 \text{ h}^{-1}$, Fig. 5) and then decreased through the rest of the experiment. Net community production during the net autotrophic portion of the experiment, days 1–10, was $558 \mu\text{M O}_2$. The low diffusion coefficient (0.01) used in these calculations was supported by concomitant ^{13}C DIC measurements which showed little exchange with the atmosphere. DIC calculations indicated that exchange was $<10\%$ during the first 8 d of the experiment. Specifically, because there was a small decrease in ^{13}C DIC toward atmospheric values vs. the expected increase in ^{13}C DIC due to algal removal of ^{13}C -depleted carbon (see Fig. 7), DIC invasion from the atmosphere could be calculated by isotope dilution. (Equations for residual DIC reactant used to estimate the expected photosynthetic ^{13}C increase in the DIC pool are similar to those used by Mariotti et al. 1981.)

New carbon production, estimated as the sum of the new POC and DOC, was $\sim 400 \mu\text{M}$ by day 7 (Fig. 6a). This C was withdrawn from the DIC pool, which declined by a roughly equivalent amount ($430 \mu\text{M}$) in these first 7 d (Fig. 4b). There was an imbalance in POC and DOC accumulation and DIC withdrawal after day 7 (Fig. 6b) which we attribute to the unquantified settling of diatoms to the bottom of the tank following the bloom. Total C withdrawn from the DIC pool during the net autotrophic portion of the experiment, days 1–10, was $490 \mu\text{M}$.

New production was also calculated independently from

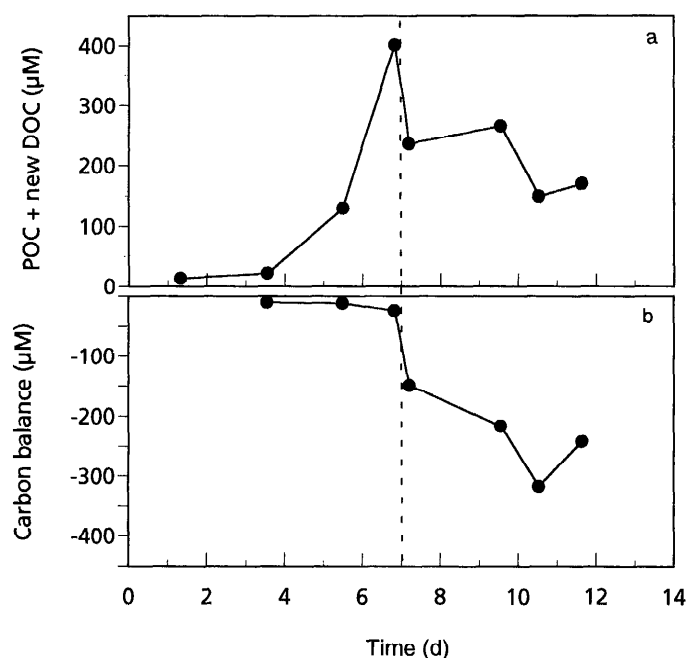


Fig. 6. Carbon balance during the experiment. [a.] Sum of new POC and DOC. [b.] Carbon balance = carbon produced – DIC consumed. Dashed line indicates the peak of chlorophyll and biomass.

initial nitrate concentrations (42 μM) and C:N ratios of accumulating POM pools (9.5 for the bulk of the POM formed in the experiment). This estimate is 400 μM over the first 7 d of the experiment, but should be increased somewhat to account for excreted DOC. An upper limit for new DOC production would be the 100 μM increase observed through the experiment; any fraction of this that is N-free, such as carbohydrate DOC, should be added to our N-based estimates. We add 120 μM C to account for DOC buildup between days 1 and 9.5, but subtract 52 μM C to account for POC remineralization estimated from accumulated DON+DIN (5.5 μM N accumulated × the 9.5 C:N ratio of POM). Our final estimate of new production calculated from nitrate, C:N, DOC, and DON was thus 400 + 120 – 52 = 468 μM over days 1–9.5.

Overall, there was fair agreement between the various estimates of new production, which ranged from 400 to 560 μM C or O₂. The calculated PQ (ΔO₂:ΔCO₂ ratio) for the system was 1.1–1.2, depending on which carbon estimate was used. These PQ values are within the range expected for marine systems in which the source of N supporting phytoplankton growth is NO₃⁻ (Parsons et al. 1984).

Propagation of stable carbon isotope signal—We used δ¹³C to sequentially follow carbon from the DIC pool to algae and then to bacteria. The addition of ¹³C-enriched NaHCO₃ gave an initial [¹³C]DIC signal of about +85‰ (Fig. 7). Algae began incorporating the ¹³C label, diluting out initial –22‰ carbon with newly synthesized ¹³C-rich carbon. The algal δ¹³C signal stabilized at +65‰, as ex-

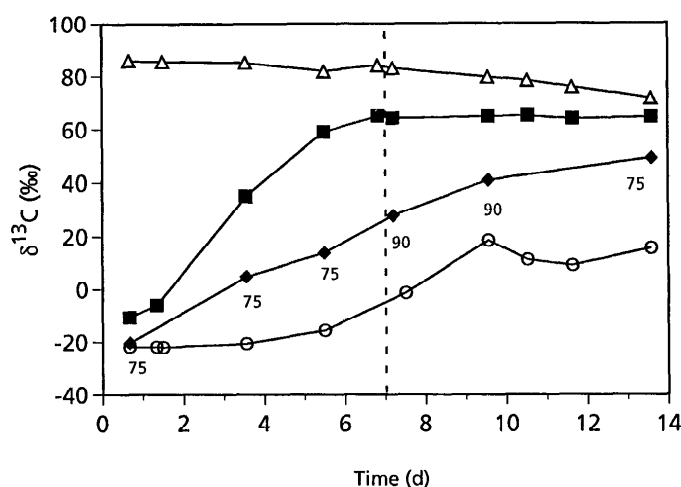


Fig. 7. Stable carbon isotope signal (δ¹³C) in different carbon pools. Time-courses are shown for DIC (Δ), DOC (○), POC (■), and extracted bacterial nucleic acids (◆) with volume filtered for the extraction of bacterial nucleic acids given beside the symbols. Dashed line indicates the peak of chlorophyll and biomass.

pected with a normal 20‰ fractionation between DIC and algal biomass (Fig. 7; see also Fry and Wainwright 1991).

The [¹³C]DOC started near –22‰—a typical value for DOC from Wood Hole Harbor (Fry et al. in prep.). Despite the rapid increase in the δ¹³C signal of the POC fraction, only minimal changes in [¹³C]DOC were found until day 7.5. The δ¹³C of the new DOC was assumed to equal that of the new POC, and this assumption was supported by the Y intercept of the calculated line in Fig. 8 (see below). Overall, the slow increase in [¹³C]DOC reflects substantial dilution of the new +65‰ [¹³C]DOC signal by a large pre-experiment background of –22‰ DOC from Woods Hole Harbor (Fig. 4c); [¹³C]DOC changes were larger between days 6 and 10 when DOC concentrations dramatically increased (cf. Fig. 4c and Fig. 7).

Nucleic acids extracted from bacteria showed a slow increase toward the +65‰ signal of newly produced algal photosynthetate during the experiment. At all times, the bacterial δ¹³C signal was higher than that of bulk [¹³C]DOC, indicating selective uptake of the new DOC (Fig. 7).

Potential degradation of the old DOC was quantified by means of a theoretical mixing curve between the new and old DOC (Fig. 8). This plot of [¹³C]DOC vs. the inverse of DOC concentration derives from mass balance equations for the mixing of two sources:

$$\delta_{\text{obs}} \times \text{mass}_{\text{obs}} = \delta_{\text{old}} \times \text{mass}_{\text{old}} + \delta_{\text{new}} \times \text{mass}_{\text{new}}, \quad (1)$$

and

$$\text{mass}_{\text{obs}} = \text{mass}_{\text{old}} + \text{mass}_{\text{new}}. \quad (2)$$

New and old refer to freshly produced vs. background harbor DOC, and obs refers to observed values. When

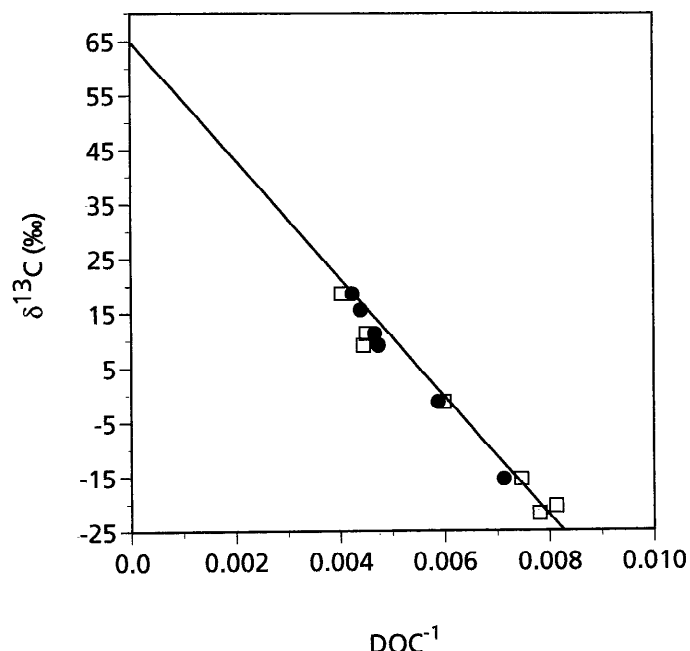


Fig. 8. $[\text{C}^{13}]\text{DOC}$ plotted as the reciprocal of DOC concentration. Data from determination of DOC with sealed tubes (●) and direct injection (□). Solid line indicates the theoretical mixing curve if no degradation of Woods Hole Harbor DOC occurred during the experiment.

one source is conservative and constant in isotopic composition and mass while the other increases in mass and has a constant isotopic composition, Eq. 1 and 2 can be rearranged to

$$\delta_{\text{obs}} = \delta_{\text{new}} + \text{constant}/\text{mass}_{\text{obs}}, \quad (3)$$

and plots of $\delta^{13}\text{C}$ vs. $1/\text{concn}$ should fall along a straight line. This linearity was in fact observed (Fig. 8), indicating little turnover of harbor DOC. Detailed day-by-day calculations showed that the degradation of this old DOC could not have exceeded 0.8% on a per day basis or 10% ($13 \mu\text{mol C}$) over the course of the experiment without markedly offsetting the observed $\delta^{13}\text{C}$ signal (Fig. 8).

Finally, we performed an additional tank experiment that also lasted 2 weeks but was less intensely monitored. Following nutrient addition, an algal bloom occurred, with a maximum chlorophyll concentration of $6 \mu\text{g Chl liter}^{-1}$. About $35 \mu\text{M C}$ as DOC had accumulated by the end of the bloom, and because H^{13}CO_3 was also added, the $[\text{C}^{13}]\text{DOC}$ signal increased from -22 to $+3.5\text{‰}$. Nucleic acids during the course of the bloom again showed selective uptake of new DOC, since nucleic acids had higher $\delta^{13}\text{C}$ values than bulk DOC ($+19$ vs. $+3.5\text{‰}$).

Discussion

This study demonstrated rapid production and accumulation of DOC following an experimental algal bloom. Bacteria selectively utilized the newly formed DOC throughout the development and collapse of the bloom.

Bacterial production and respiration were stimulated by the DOC, but not all newly produced DOC proved labile to bacterial use.

DOC production and accumulation—In the present experiment, a period of rapid DOC accumulation followed inorganic nutrient depletion and the collapse of the phytoplankton bloom (Fig. 2). Diatoms can continue to excrete polysaccharides for a considerable time after the halt of cellular protein synthesis (Jensen 1984). During the collapse of the bloom, microscopic examination revealed that diatom cells were in suboptimal condition; cells became bleached, and their contents shrank inside the frustules. Thus, the DOC increase is likely to be a combination of excretion and loss due to cell lysis. About 23% of total new production in our experiment accumulated as DOC. This percentage lies in the middle of many DOC excretion estimates for phytoplankton (Table 2) and of estimates of DOC release measured during a decade of short-term ^{14}C productivity studies along the U.S. east coast (O'Reilly et al. 1987). Interestingly, a previous study that used dock-water samples from Woods Hole found 4–16% excretion from natural healthy phytoplankton and 17–38% excretion at the end of a diatom bloom, when a large number of empty frustules were present (Hellebust 1965).

The magnitude of the DOC increase is comparable to other experimental studies. Calculations based on the experiments of Antia et al. (1963) suggest that $\sim 80 \mu\text{M}$ of DOC had accumulated in 2 d following nutrient depletion after a large bloom ($48 \mu\text{g Chl } a \text{ liter}^{-1}$) in a 125-m^3 mesocosm (Banse 1994). Substantial DOC can also accumulate in unconfined, natural offshore waters. Barlow (1982) followed DOC dynamics in the Benguela Current where carbohydrates accumulated following a diatom bloom of $19 \mu\text{g Chl } a \text{ liter}^{-1}$. Results of our study as well as the results of many laboratory and field studies support the idea that continued maintenance of photosynthetic machinery after nutrient exhaustion may be accompanied by excretion of DOM and especially carbohydrates that have a high C:N ratio (Hellebust 1965). An alternative hypothesis that DOC accumulation at the end of blooms occurs because of grazing or viral attack seems less likely because present evidence suggests that carbohydrates rather than mixed-cell lysates accumulate when nutrients are exhausted and blooms stop growing (Barlow 1982; Sellner et al. 1981). Overall, DOC excretion following the peak of a bloom could, if widespread, account for observations of Sambrotto et al. (1993) that DIC decreases often continue after nutrient exhaustion in many offshore systems. That DOC accumulation should be caused only by inorganic nutrient exhaustion limiting the bacterial degradation is not supported by data from subsequent degradation experiments (Fry in prep.). Despite nutrient additions, the new DOC was not fully degraded, even after more than a year of incubation.

These ideas pertain to DOC that accumulates, but DOC can also be respired and not accumulated. To gain a better view of overall DOC dynamics (including respired DOC), we used oxygen measurements to estimate microbial res-

Table 2. Examples of literature data on phytoplankton exudation.

Reference	Type of study	Measured exudation (%)	Comments
Baines and Pace 1991	Compilation of literature data from coastal areas	12 (5–18)	Measurable exudation doubled in absence of bacteria
Hellebust 1965	2–4-d incubation	6–12	Natural phytoplankton from Vineyard Sound
Hellebust 1965	6-h incubation	17–30	Vineyard Sound, end of diatom bloom
Larsson and Hagström 1982	In situ, 4-h incubation in Baltic water	10, 20, and 15	Compilation of seasonal results from spring, summer, and fall
Mague et al. 1980	In situ, 4-h incubation	5–10	Seasonal average
Mague et al. 1980	In situ, 4-h incubation	10–30	Depth profile
Møller-Jensen 1983	6-d culture with coastal water	3–10	30% in absence of bacterial activity, 3% in presence
Møller-Jensen 1983	In situ, 12-h incubation in coastal water	9–17	
Sharp 1977	Short (2–6 h) incubation	0–2	Coastal seawater, no exudation in log phase
Wood et al. 1992	Culture in dialysis bag system	10–25	
This study	10-d culture with coastal water	~23	

piration. The continuous oxygen measurements gave us total nighttime respiration (Fig. 5); we assumed that the portion of total respiration due to phytoplankton was 10% of gross primary production (Burris 1980) and the remainder was microbial use of DOC. We also used our bioassay growth experiments as a rough guide for DOC production that supported bacterial growth, assuming 20 fg C cell⁻¹. When these estimates are combined, respiration of DOC clearly dominates DOC dynamics (Fig. 9); DOC respiration increased not after bloom cessation, but during exponential growth of the bloom during days 4–7 (Fig. 9). During this period, the estimated average microbial respiration was 43% of total respiration, and it increased further to 75% for days 8–12.

Our estimates of total DOC production per liter during the experiment were 23 $\mu\text{mol C}$ in bacterial biomass, 100 $\mu\text{mol C}$ accumulated DOC, and 520 $\mu\text{mol C}$ respired DOC. A second way to estimate bacterial respiration is to use the 5 : 1 ratio of respired CO₂ to biomass observed in one of our bioassay experiments. If bacterial production was 23 $\mu\text{mol C}$ over the experiment, respiration would have been 115 $\mu\text{mol C}$, compared to the observed 100 $\mu\text{mol C}$ accumulated DOC.

These calculations show a wide range in estimates of microbially respired CO₂, depending on assumptions used, but indicate that respiration was likely an important sink for DOC throughout the experiment. We failed to observe any net DIN remineralization accompanying the calculated DOC respiration; this would be consistent with a nitrogen-poor structure for DOC, which of course is expected for carbohydrates. A hypothesis arising from these calculations is that primarily carbohydrates were extensively excreted and metabolized during and after this bloom and that accumulated DOC reflects a residue of this extensive metabolism.

Selective bacterial use of freshly produced DOM—The ¹³C-labeling experiments showed that bacteria selectively

consumed new DOC, having $\delta^{13}\text{C}$ values always higher than bulk DOC (Fig. 7). However, nucleic acid extracts used as biomarkers for bacterial $\delta^{13}\text{C}$ measurements never fully reached the +65‰ value of algal POC and in fact only gradually trended upward toward this value through the 2-week experiment (Fig. 7). How do we interpret this gradual isotope increase? A more immediate and full increase to +65‰ was expected for bacteria if they rely exclusively on freshly produced DOC.

The most likely explanation is that –22‰ carbon was present in the bacterial pool throughout the experiment, diluting the signal of bacteria growing exclusively on new +65‰ carbon. We can identify three possibilities for this –22‰ component. First, humic materials might have been coextracted in the nucleic acid procedure but gradually disappeared from solution due to precipitation, flocc-

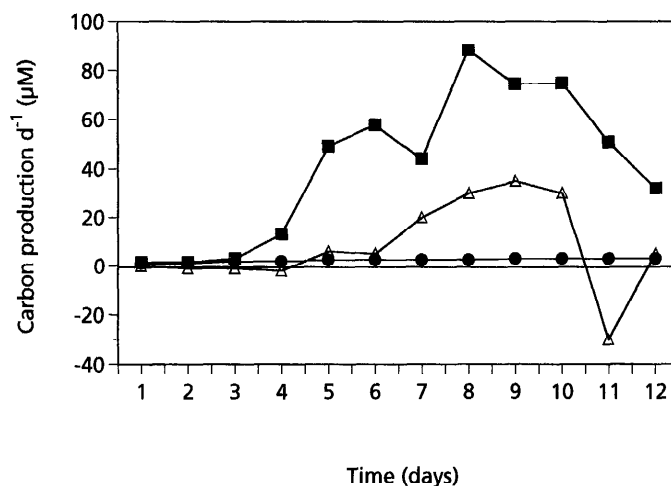


Fig. 9. Daily production estimates for three types of DOC: microbially respired (■), accumulating (△), and that supporting bacterial biomass growth (●).

culution, or sorption during the 2-week experiment. Second, a large population of bacterial resting cells might have been present at the beginning of the experiment and have been only gradually removed by grazing. Third, bacteria might in fact have used a mixture of old -22‰ and freshly produced $+65\text{‰}$ carbon, switching gradually from the former to the latter during the experiment. These alternatives are difficult to rigorously evaluate with existing data and may have all contributed to the observed pattern of gradual $\delta^{13}\text{C}$ increase. Our current data seem best viewed as showing selective uptake of freshly produced DOC rather than exclusive reliance on freshly produced DOC from phytoplankton. Further experiments that eliminate any bacterial resting cells by careful filtration ($0.2\text{-}\mu\text{m}$ instead of the $10\text{-}\mu\text{m}$ initial filtration used here) and that carefully monitor humic acid levels and blank contributions to nucleic acid extractions are required to more precisely quantify the reliance of bacteria on freshly produced DOC. Although only newly produced DOC could have supported the large increase in microbial respiration calculated in Fig. 9, bacteria may have used a few $\mu\text{mol C}$ of older Woods Hole Harbor DOC to support their growth because the harbor DOM was a relatively good source of N in this experiment.

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