

Significance and mechanisms of photosynthetic production of dissolved organic carbon in a coastal eutrophic ecosystem

*Emilio Marañón*¹, *Pedro Cermeño*, and *Emilio Fernández*

Departamento de Ecología y Biología Animal, Facultad de Ciencias del Mar, Universidad de Vigo, 36200 Vigo, Spain

Jaime Rodríguez and *Laura Zabala*

Departamento de Ecología y Geología, Universidad de Málaga, Campus de Teatinos, 29071 Málaga, Spain

Abstract

We have determined the seasonal (July 2001–July 2002) and vertical variability in the photosynthetic production of dissolved organic carbon (DOCp) and particulate organic carbon (POCp) in a coastal upwelling ecosystem (Ría de Vigo, Northwest Spain), together with the relationship between irradiance and DOCp and the time-course of DOCp over 24-h periods. Euphotic layer-integrated rates of DOCp and POCp ranged between 5 and 190 mg C m⁻² h⁻¹ and between 40 and 1,130 mg C m⁻² h⁻¹, respectively. Irradiance was the most important variable affecting the vertical variability of the percentage of extracellular release [PER, defined as DOCp/(DOCp + POCp)]. Whereas POCp decreased markedly below the surface, DOCp remained constant or even increased, thus causing a sharp increase in PER with depth. Biomass-specific rates of DOC production also increased with depth. These observations were confirmed by the results of photosynthesis–irradiance experiments, which consistently showed highest DOCp and PER values at subsaturating irradiances. Our results argue against the view that the release of DOC is an overflow mechanism occurring preferentially under conditions of high irradiance and low nutrient concentration. PER was uncorrelated with the size structure of phytoplankton biomass and productivity, and >80% of the variability in integrated DOCp was explained by POCp. These findings indicate that the relative importance of dissolved primary production was independent of the dominant type of planktonic trophic organization. Moreover, production of DOC stopped at night, which strongly indicates that trophic processes were not involved in the release of dissolved photosynthate. Our data support a purely physiological mechanism of passive DOC release by normally growing cells, which is enhanced under suboptimal irradiances but proceeds at a similar biomass-specific rate throughout the year. On an integrated basis, PER averaged 19 ± 1%, thus indicating that even in eutrophic waters, total primary productivity can be significantly underestimated if the dissolved products of photosynthesis are not taken into account.

The extracellular release of recently fixed photosynthate is a major process contributing to the production of dissolved organic carbon (DOC) in marine ecosystems. This source of DOC is particularly important for the trophic ecology of plankton, since the released compounds are susceptible to rapid uptake by heterotrophic bacteria (e.g., Cole et al. 1982; Norrman et al. 1995), giving way to a linkage between primary and bacterial production that is essential for the cycling of matter through the microbial loop and the microbial food web (Ducklow and Carlson 1992; Legendre and Rassoulzadegan 1996). Despite its widely acknowledged ecological and biogeochemical significance, DOC production is not routinely measured in most surveys of productivity in the sea, which typically include determinations of particulate organic carbon (POC) production only. As a result of the rel-

atively small number of good quality measurements, our knowledge of the dynamics of microbial DOC production in marine ecosystems is still limited. It is not clear which mechanisms are responsible for the extracellular release of recent photosynthate, what is the quantitative importance of DOC production relative to POC production, or how this importance changes with the productivity of the ecosystem and its trophic structure.

In their review of DOC production in marine and freshwater ecosystems, Baines and Pace (1991) concluded that the percentage of extracellular release (PER, defined as DOC production divided by the sum of DOC and POC production) was relatively constant at 13% and was not related to total productivity. These conclusions must be taken with caution, however, given that the original data set included very few measurements from low-productivity, open ocean waters, and given the methodological problems involved in the determination of DOC production. In particular, several studies included in Baines and Pace's (1991) review reported the use of glass-fiber filters, which are now known to adsorb significant amounts of DOC (Maske and García-Mendoza 1994; Karl et al. 1998; Morán et al. 1999) and thus lead to underestimates of the real rates of DOC production. In any case, most studies of DOC production covering wide geographical and productivity ranges have reported a strong negative relationship between PER and the total primary production of the ecosystem (e.g., Anderson and Zeutschel

¹ Corresponding author (em@uvigo.es).

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1970; Berman and Holm-Hansen 1974; Teira et al. 2001a; Morán et al. 2002a). The resulting widely accepted perception is that the omission of DOC measurements should not lead to significant underestimates of total primary productivity in eutrophic waters (e.g., Thomas 1971; Fogg 1983).

The relationship between PER and total productivity is intimately linked to the relative importance of the different mechanisms of photosynthetic DOC production. In marine microbial communities, the main mechanisms of labile DOC production are direct release from intact cells, cell lysis, and protozoan activity (*see* review by Nagata 2000). It has been suggested that trophic mechanisms such as microzooplankton grazing and egestion are a dominant DOC source in picophytoplankton-dominated, low-productivity systems (Nagata 2000), whereas purely physiological mechanisms, such as direct algal exudation, have been emphasized in the case of DOC release in productive waters dominated by larger phytoplankton (Mague et al. 1980; Lancelot 1983). Two main types of physiological mechanisms have been advocated to explain the exudation of recent photosynthate by phytoplankton: (i) an overflow mechanism whereby excess photosynthetic products are actively released to the external medium when the C fixation rate greatly exceeds the rate of macromolecular synthesis (Fogg 1983; Wood and Van Valen 1990) and (ii) passive diffusion of small metabolites through the cell membrane (Bjørnsen 1988). If the overflow mechanism dominates, then significant DOC production would preferentially occur in conditions of high irradiance and low nutrient concentration (Wood and Van Valen 1990). If passive diffusion is the main mechanism, DOC production will take place whenever a pool of small, recently photosynthesized metabolites is available.

The potential relationship between cell size and DOC production, formulated by Bjørnsen (1988) and Kiørboe (1993) on theoretical grounds, has recently been reinforced by the results of Teira et al. (2001a), who reported a strong inverse correlation between PER and the percentage of total primary production in the picophytoplankton size fraction. This relationship, however, may have also been caused by nutrient status, since conditions of picophytoplankton dominance also corresponded to highly stratified, open ocean environments in which nutrient supply was very low. It has been commonly reported that nutrient depletion tends to result in enhanced rates of DOC production (Lancelot 1983; Lignell 1990; Obernosterer and Herndl 1995; among others). Consequently, testing the hypothesis that size structure *per se* affects the relative rate of DOC production would require a set of observations in which size structure changes widely while nutrient concentrations remain sufficiently high that they are not severely limiting for primary production.

Our study system, the Ría de Vigo, is the southernmost of the Rías Baixas (Northwest Iberian Peninsula) coastal embayments characterized by a very active, wind-driven, two-dimensional circulation. During the upwelling-favorable season, which runs from April to October (Nogueira et al. 1997), the Ría de Vigo displays high rates ($>1\text{--}2\text{ g C m}^{-2}\text{ d}^{-1}$) of primary production (Tilstone et al. 1999; Moncoiffé et al. 2000), whereas reduced levels of phytoplankton biomass and production are encountered during the downwelling-favorable season, from November to March (Fraga

1976). Significant changes in the size structure of the phytoplankton assemblages also take place along this seasonal cycle: large phytoplankton ($>20\text{ }\mu\text{m}$) dominate during the productive season, whereas the importance of nano- and picophytoplankton increases markedly in winter. The dynamic nature of the Northwest Iberian upwelling region, and of the Ría de Vigo in particular, makes it an excellent study system for testing general hypotheses regarding the variability and mechanisms of microbial DOC production. In this regard, Álvarez-Salgado et al. (2001) have estimated, using a mass-balance approach, that net ecosystem production of DOC in this ecosystem is $\sim 20\%$ of net primary production during the upwelling season. Recently, Morán et al. (2002b) have reported on the relationship between particulate and dissolved primary production and bacterial production along a coastal-offshore gradient off Ría de Vigo.

Here we describe the seasonal and vertical changes in the production of particulate and dissolved primary production, with the intention of determining the significance of DOC production in highly productive waters and gaining further insight into the mechanisms involved in the extracellular release of recent photosynthate. In particular, we try to ascertain if physiological mechanisms alone can explain the observed rates of DOC production or if additional trophic mechanisms must also be invoked. With this purpose, we present the results of additional experiments specifically devoted to determining the kinetics of DOC production during 24-h light–dark cycles and the relationship between irradiance and PER. Finally, we test the hypothesis that the relative importance of DOC production is related to phytoplankton size structure, as determined both by size-fractionation experiments and by constructing continuous size-abundance spectra with a combination of flow cytometry and image analysis.

Materials and methods

Sampling—During the period extending from July 2001 to July 2002, we conducted 25 visits to a sampling station located in the central part of the Ría de Vigo ($42^{\circ}14.09'\text{N}$, $8^{\circ}47.18'\text{W}$), where the depth of the water column is 45 m at low tide. Typically, sampling was completed between 0700 h and 0900 h. Monthly visits were carried out in order to determine the seasonal variability, whereas a more intense sampling effort (two visits per week over 2–3 weeks) allowed us to determine the short-term temporal variability during four periods of contrasting hydrodynamic and biogeochemical conditions in July 2001, February 2002, April 2002, and July 2002.

Hydrography and irradiance—On each visit, we recorded vertical profiles (0–40 m) of temperature and conductivity with a SBE 25 CTD probe. The vertical distribution of photosynthetically active irradiance (PAR, 400–700 nm) was measured with a spherical quantum sensor connected to a LiCor datalogger. Seawater samples for chemical and biological measurements were collected using either single 5-liter Niskin bottles or a rosette equipped with ten 12-liter Niskin bottles. Samples for the analysis of dissolved inorganic nutrients (nitrate, nitrite, silicate, and ammonium) were

obtained from 0-, 5-, 10-, 15-, 20-, and 30-m depths. These samples were immediately frozen and stored at -20°C until they were analyzed in the laboratory following the methods described in Grasshoff et al. (1999).

Size-fractionated chlorophyll a and phytoplankton size-abundance spectra—Seawater samples for the determination of several variables related to plankton abundance, composition, and activity were collected between 0–20 m at 5-m intervals, thus covering the whole euphotic layer. Samples were stored in acid-washed, black, polypropylene carboys and transported to the laboratory within 2 h of sampling. Special care was taken to avoid any light shock to the plankton populations. For the determination of size-fractionated chlorophyll *a* (Chl *a*) concentration, 250-ml samples were filtered sequentially through 20-, 5-, 2-, and 0.2- μm polycarbonate filters, using low vacuum pressure (<13.5 kPa). Filters were stored frozen at -80°C until further analysis, which took place within 4 months of sampling. Pigment extraction was carried out by placing the filters in 90% acetone for 12 h. Chl *a* concentration was determined fluorometrically using a TD-700 fluorometer that had been calibrated with pure Chl *a* (Sigma Chemical).

Additional samples were taken from 0, 10, and 20 m for the determination of phytoplankton cell size and abundance by means of a combination of flow cytometry and microscopy image analysis, which enables the construction of size-abundance spectra. A detailed description of the protocols followed during flow cytometry and microscopy image analysis is available in Rodríguez et al. (1998). For flow cytometry, 5-ml samples were fixed with glutaraldehyde (1% v/v final concentration) and stored in liquid nitrogen until further analysis. Samples were analyzed with a FACScan (Becton Dickinson) flow cytometer, and the combined signals of light scattering and red fluorescence were used to determine the abundance and cell size of phytoplankton in the approximate size range of 0.5–13 μm (equivalent spherical diameter). For the image analysis of larger cells under the microscope, 100-ml samples were preserved with Lugol's solution. After sedimentation of a subsample (50 ml), cells and colonies were counted and measured at 100 \times and 200 \times on a Leitz Fluovert inverted microscope connected to a VIDS V (Analytical Measuring Systems) image analysis system. Cell volume (μm^3) was estimated as the revolution volume according to an ellipsoidal shape. The image analysis of the phytoplankton covered the size range from approximately 8 to 80 μm^3 of equivalent spherical volume. By combining the data obtained from flow cytometry and image analysis, it was possible to construct abundance–biovolume spectra, in which log cell abundance (cell ml^{-1} , y-axis) is plotted against log cell volume (μm^3 , x-axis). These spectra can be fitted to a linear regression model whose slope serves as an indicator of the relative dominance of small versus large cells in the community: smaller (i.e., less-negative) slopes are indicative of a more pronounced dominance of larger cells (Rodríguez et al. 1998).

Particulate and dissolved primary production—The production of DOC and POC was determined at 0, 10, and 20 m by running simulated in situ (SIS) incubations with the

radioisotope ^{14}C . We used an incubator equipped with a set of blue and neutral density filters that reproduced eight light levels from 100% to 1% of E_0 . Given that we knew the vertical distribution of irradiance prior to the experiments, it was possible to incubate each sample approximately under the irradiance level that was recorded at the sampling depth. A system of recirculating water connected to two refrigerators ensured that incubation temperature was maintained within 1.5°C of the original temperature at each sampling depth. The method used for the concurrent measurement of DOC and POC production is based on the protocol of Mague et al. (1980), as modified by Teira et al. (2001a), but with some important differences (*see* below). For each sampling depth, three light and two dark acid-washed Pyrex glass bottles (36 ml in volume) were filled with the sample and spiked with 15 μCi (555 KBq) of $\text{NaH}^{14}\text{CO}_3$. In addition, time zero blanks were run for each experiment by filling three bottles with 0.2 μm -filtered, sterilized seawater; spiking them with 15 μCi of ^{14}C ; and processing them immediately as normal samples. Light and dark bottles were incubated for 2 h.

At the end of the incubation period, two 5-ml aliquots from each incubation bottle were filtered through 0.2- μm polycarbonate (PC) filters (25 mm in diameter) using low vacuum pressure (<7 kPa). The choice to use PC filters instead of glass-fiber (GF/F) filters is based on the conclusive evidence presented by Maske and García-Mendoza (1994), which showed that GF/F filters adsorb up to 100 times more DOC than PC filters after a brief contact with a DO^{14}C extract. This means that the use of GF/F filters can lead to significant underestimates of DOC production and concurrent overestimates of POC production. After being acidified to a pH of ~ 2 with 100 μl of 50% HCl, filtrates were maintained for 12 h in open scintillation vials (20 ml in volume) placed on an orbital shaker. Preliminary experiments had demonstrated that this procedure was efficient at removing inorganic ^{14}C from the filtrates. After ^{14}C decontamination, 15 ml of a high sample capacity scintillation cocktail were added to each filtrate. The inorganic ^{14}C present in the filters was removed by exposing them to concentrated HCl fumes for 12 h. The filters were then placed in scintillation vials to which 4 ml of scintillation cocktail were added.

On 10 occasions during our study, we also measured the production of total organic carbon (TOC) in order to check the coherence between total primary production, obtained from unfiltered samples, and the sum of DOC and POC production. For TOC production measurements, additional 5-ml aliquots were taken from the incubation bottles and placed in scintillation vials, to which 100 μl of 50% HCl were added. These vials were then treated as previously described for the filtrates.

The radioactivity on each sample was determined on a 1409-012 Wallac scintillation counter that used an internal standard for quenching correction. Importantly, black-bottle DPMs were subtracted from the light-bottle DPMs (disintegrations per minute) in order to calculate the rates of DOC, POC, and TOC production. Typically, the light bottle to dark bottle DPM ratio was in the range of 3–4. The DPMs measured in the time zero samples were used to check that filtrate decontamination was efficient, but were not included in the productivity calculations. In fact, residual radioactivity

in the filtrates from the black incubation bottles was typically similar to, if not lower than, that measured in the filtrates from the time zero samples. In all our calculations, we used a constant value of $25,700 \text{ mg m}^{-3}$ for the concentration of dissolved inorganic carbon and a value of 1.05 for the isotopic discrimination factor. For all pooled determinations of the production rate of POC ($n = 75$), DOC ($n = 75$), and TOC ($n = 30$), the average coefficients of variation were 0.12, 0.22, and 0.10, respectively.

Size-fractionated primary production—The vertical distribution of primary production in four size classes was determined by running parallel SIS experiments with seawater samples collected from 0, 5, 10, 15, and 20 m. For each depth, three light and one dark acid-washed polystyrene bottles (100 ml in volume) were filled with the sample and spiked with $10 \mu\text{Ci}$ (370 KBq) of $\text{NaH}^{14}\text{CO}_3$. These size-fractionated productivity experiments and the POC and DOC production experiments described before had the same duration and were conducted simultaneously under identical conditions. At the end of the incubation period, samples were sequentially filtered through 20-, 5-, 2-, and 0.2- μm polycarbonate filters (47 mm in diameter) using low vacuum pressure ($<13.5 \text{ kPa}$). Filter processing, radioactivity measurements, and primary production calculations were performed as previously described for POC samples. This fractionation procedure allowed us to estimate primary production in four size classes: $>20 \mu\text{m}$, 5–20 μm , 2–5 μm , and 0.2–2 μm . Total production rate for the whole phytoplankton community was calculated as the sum of the primary production rate in each size class. The comparison between the results of these size-fractionation production experiments and the POC production estimates obtained with the DOC production technique allowed us to check the reliability of our experimental procedures (*see Results*).

Photosynthesis–irradiance experiments—In winter, spring, and summer of 2003, we took additional samples from the same station in the Ría de Vigo in order to conduct photosynthesis–irradiance (P-E) experiments and to determine the relationship between irradiance and the production of both POC and DOC. Fifteen acid-washed polystyrene bottles (100 ml in volume) were filled with seawater from 0 m and/or 15 m and spiked with $10 \mu\text{Ci}$ (370 KBq) of $\text{NaH}^{14}\text{CO}_3$. Samples were incubated for 2 h in a linear incubator equipped with a 100-W halogen lamp and were calibrated to provide a PAR range from 0 (black bottle) to $2,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The incubator was cooled with recirculating water that passed through a refrigerator, thus keeping the incubation temperature within 0.5°C of in situ temperature. Filtration, manipulation of filters and filtrates, scintillation counting, and calculations of primary production were all carried out as described before for the vertical profiles of POC and DOC production.

Time series experiments—In spring and summer 2003, we carried out two time series experiments aimed at monitoring the concurrent production of POC and DOC during 24 h under a 12 light (L):12 dark (D) photoperiod. Surface seawater collected at the sampling station was used to fill 12

dark and 24 light polystyrene bottles, which were inoculated with $10 \mu\text{Ci}$ (370 KBq) of $\text{NaH}^{14}\text{CO}_3$ and incubated in a temperature-controlled culture chamber under an irradiance of $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$. At each sampling time, one dark and two light bottles were processed for the determination of POC and DOC production.

Results

Hydrography and Chl a—The changes in the structure of the water column observed during our study reflected the seasonal alternation of mixing and stratification periods typically observed in temperate waters and the shorter-term upwelling and upwelling relaxation dynamics that are characteristic of the Rías Baixas (Fig. 1). During the first intense sampling period, carried out in July 2001, thermal stratification was persistent, and low ($<1 \mu\text{mol L}^{-1}$) nitrate concentrations were measured in surface and subsurface waters (Fig. 1A,B). This period corresponded to a phase of upwelling relaxation, when a well-developed subsurface Chl *a* maximum, with concentrations between 3 and 6 mg m^{-3} , was located at around 10 m in depth (Fig. 1C). An upwelling event was observed in early September, when relatively cold ($<15^\circ\text{C}$) and nitrate-rich ($>8 \mu\text{mol L}^{-1}$) waters reached the top of the water column, and maximum Chl *a* concentrations (6 mg m^{-3}) were measured at the surface. In October, we found high levels of phytoplankton biomass (Chl *a* $>7 \text{ mg m}^{-3}$ at the surface) and low nitrate concentrations (around $1 \mu\text{mol L}^{-1}$), which indicates that a postbloom situation was present at the time. Relatively strong vertical mixing was observed from November to February (Fig. 1A). During this period, typical Chl *a* concentrations were $0.5\text{--}1.5 \text{ mg m}^{-3}$, and high nitrate concentrations ($>4 \mu\text{mol L}^{-1}$) were measured throughout the water column (Fig. 1B,C). The development of an early spring bloom was observed in late February, when Chl *a* concentrations of up to 8 mg m^{-3} were measured in surface waters (Fig. 1C). Another phytoplankton bloom, associated with upwelling conditions, took place in April. In late May 2002, thermal stratification was again present, and low nitrate concentrations ($<1 \mu\text{mol L}^{-1}$) were measured in surface waters (Fig. 1A,B). A strong upwelling event was observed in July 2002, when the 13°C isotherm reached 10 m in depth and surface Chl *a* concentrations above 8 mg m^{-3} were measured (Fig. 1A,C).

Particulate and dissolved primary production—The temporal variability in the rates of production of both POC and DOC showed very similar patterns (Fig. 2A,B) and mirrored the changes in phytoplankton biomass, as inferred from Chl *a* concentration data. The vertical distribution of POC production rate was always characterized by the presence of a maximum at the surface. POC production in the upper water column ranged from values below $5 \text{ mg C m}^{-3} \text{ h}^{-1}$, observed during the winter period of low algal biomass, to values above $40\text{--}50 \text{ mg C m}^{-3} \text{ h}^{-1}$, which were measured during the various phytoplankton blooms that were sampled (Fig. 1A). During the upwelling relaxation phase observed in July 2001, POC production rates in the upper water column were in the range of $10\text{--}20 \text{ mg C m}^{-3} \text{ h}^{-1}$. Very high POC production rates ($>50 \text{ mg C m}^{-3} \text{ h}^{-1}$ at the surface) were mea-

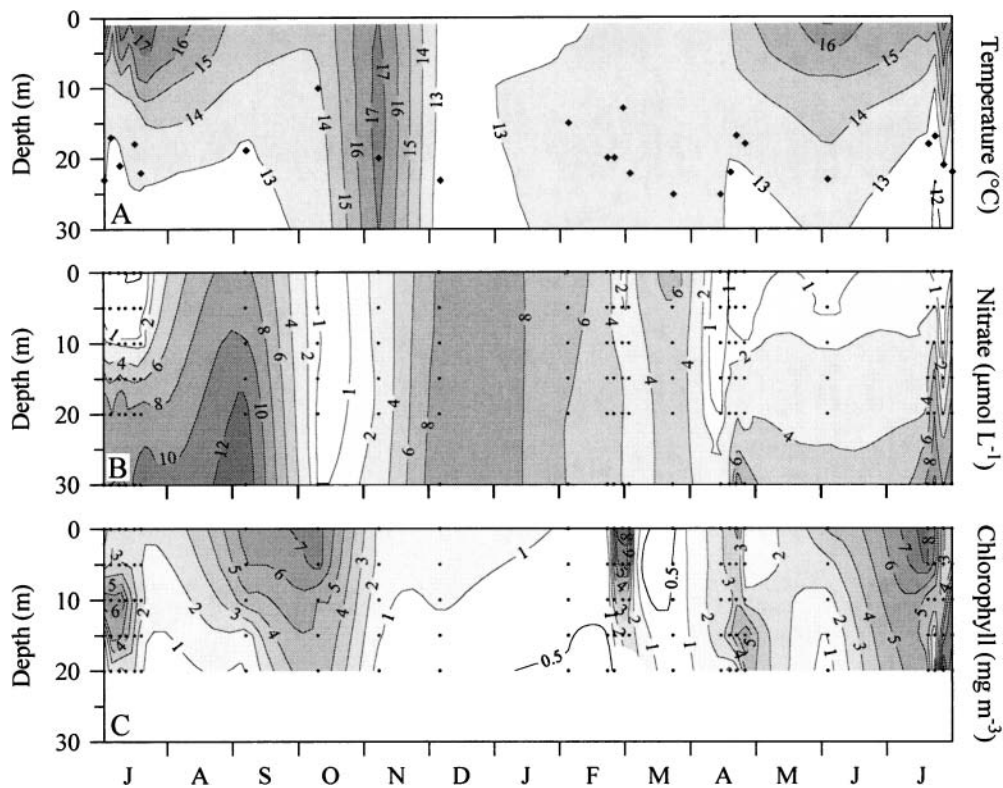


Fig. 1. Vertical and temporal distribution of (A) temperature, (B) nitrate concentration, and (C) chlorophyll *a* concentration from July 2001 to July 2002.

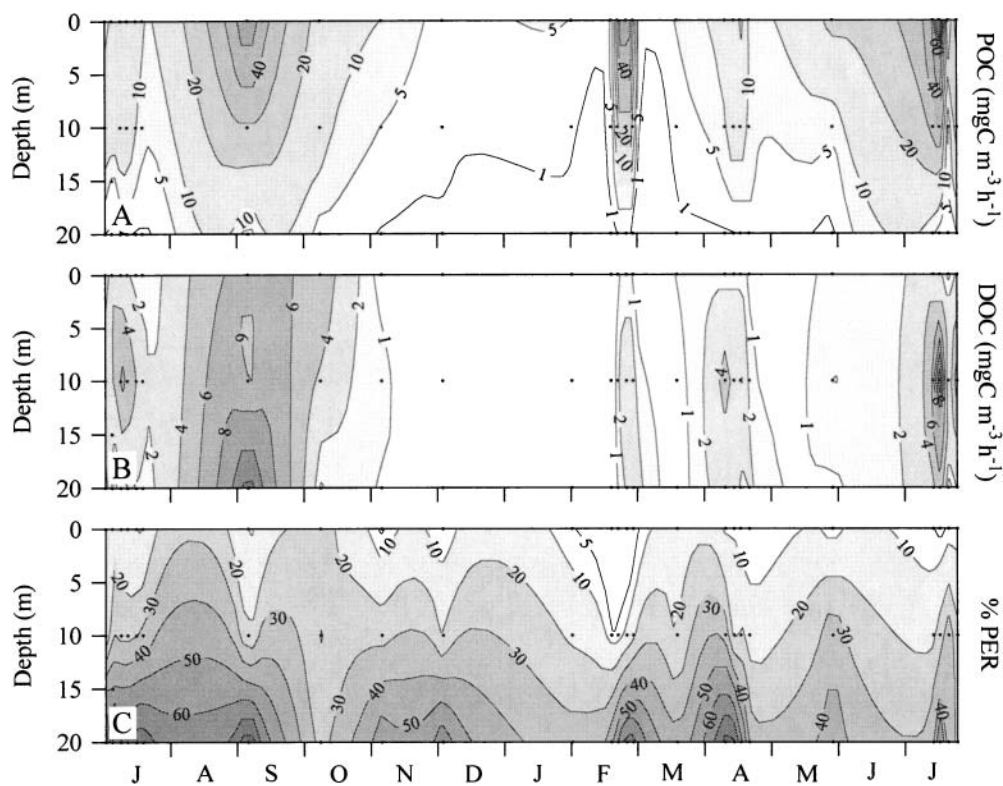


Fig. 2. Vertical and temporal distribution of (A) particulate organic carbon (POC) production, (B) dissolved organic carbon (DOC) production, and (C) the percentage of extracellular release (PER) from July 2001 to July 2002.

sured during the upwelling events of September 2001 and July 2002, which resulted in some of the highest integrated (0–20 m) rates of particulate primary production encountered during our study (634 and $1,007$ $\text{mg C m}^{-2} \text{h}^{-1}$, respectively).

Temporal changes in DOC production rates closely mirrored those found in Chl *a* concentration and POC production (Fig. 2B). Highest values (>6 $\text{mg C m}^{-3} \text{h}^{-1}$) were measured during the September 2001 and July 2002 upwelling events, whereas the lowest rates (<1 $\text{mg C m}^{-3} \text{h}^{-1}$) took place in winter. Relatively high rates of DOC production (>2 $\text{mg C m}^{-3} \text{h}^{-1}$) were also observed during the July 2001 upwelling relaxation period and during the spring blooms of March and April 2002. Using all vertically integrated data, we found a highly significant correlation between log Chl *a* concentration and log DOC production ($r^2 = 0.73$, $p < 0.01$, $n = 25$). The ratio between integrated DOC production and integrated Chl *a* concentration did not show any clear seasonal pattern and varied within a relatively narrow range (0.5 – 1.0 $\text{mg C mg Chl}^{-1} \text{h}^{-1}$) during most of the study. The vertical distribution of DOC production differed from that of POC production: typically, DOC production tended to remain constant or even increase with depth (Fig. 2B). This meant that, on a biomass basis, phytoplankton produced more DOC as depth increased. The average (± 1 standard error) ratio between DOC production and Chl *a* concentration throughout the study was 0.49 ± 0.08 , 1.05 ± 0.15 , and 1.14 ± 0.19 $\text{mg C mg Chl}^{-1} \text{h}^{-1}$ at 0, 10, and 20 m, respectively.

The percentage of extracellular release [PER, calculated as $\text{DOC}/(\text{POC} + \text{DOC})$] increased markedly with depth throughout the study period, changing from values around 10% at the surface to values above 40–50% at the bottom of the euphotic layer (Fig. 2C). No clear seasonal trends in PER were observed. Typically, integrated PER values fell within the range of 15–25%, with the exception of four visits conducted in January and February, when values between 5% and 10% were measured. On average, integrated PER took a value of $19 \pm 1\%$ during our study. To a first order, daily production rates can be estimated by multiplying the hourly rates by the duration of the photoperiod. Thus calculated, the average daily rates of euphotic layer-integrated POC and DOC production during our study would be 3.8 ± 0.8 and 0.8 ± 0.1 $\text{g C m}^{-2} \text{d}^{-1}$, respectively. These estimates should be taken with caution because of the uncertainty involved in extrapolating from short-term measurements to daily rates.

Despite the high degree of temporal variability in POC and DOC production rates, it was still possible to detect consistent average patterns in their vertical distribution through the water column (Fig. 3). POC production rates were significantly higher at 0 m and 10 m than they were at 20 m (one-way ANOVA and Games–Howell post-hoc test). In contrast, DOC production rate at 10 m was significantly higher than that measured at the surface, whereas no differences were found between surface and 20-m rates. As a result, PER increased markedly with depth, taking values of 9%, 21%, and 59% at surface, 10 m, and 20 m, respectively. The differences in PER between depths were all highly significant ($p < 0.001$, Games–Howell post-hoc test).

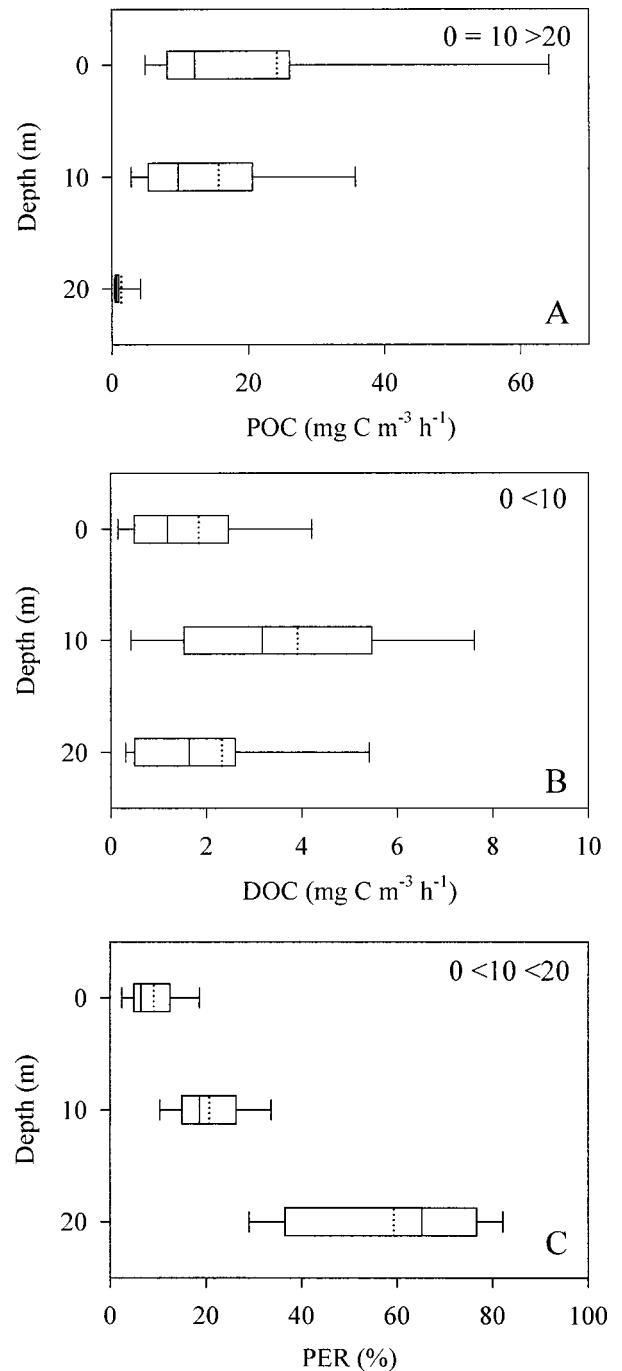


Fig. 3. Composite vertical profiles of (A) POC production, (B) DOC production, and (C) PER obtained from the 25 observations made during the period from July 2001 to July 2002. The boxes enclose the 25% and 75% percentiles of the data, bars encompass 90% of the data, the central solid line represents the median, and the central dotted line represents the mean. Significant differences between depths (one-way ANOVA and Games–Howell post-hoc test) are indicated in the upper right corner of each panel.

In order to test the validity of our measurements of POC production obtained with the DOC production technique (filtration through $0.2\text{-}\mu\text{m}$ polycarbonate filters of 25 mm in diameter and recovery of filtrates), we compared them with

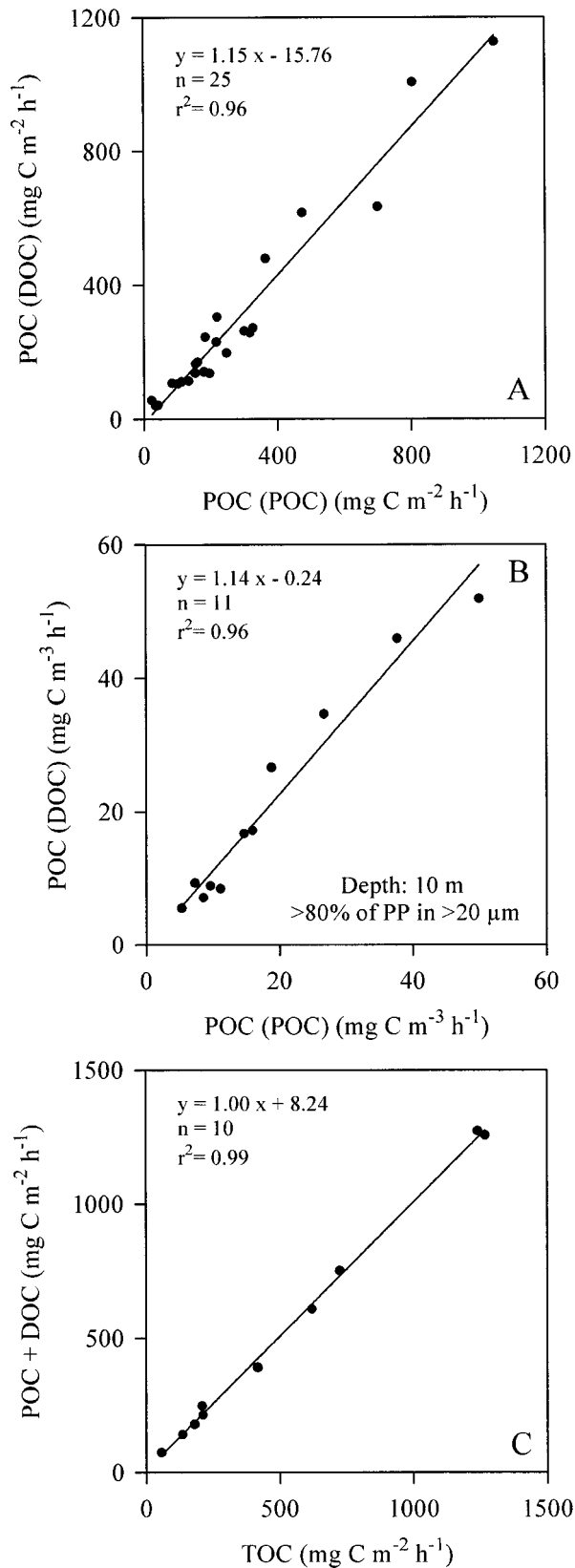


Fig. 4. (A) Relationship between vertically integrated POC production measured with the conventional ¹⁴C method (POC[POC]) and vertically integrated POC production measured with the method

the results obtained with the conventional method used to determine size-fractionated particulate primary production (sequential filtration through polycarbonate filters of different pore sizes and 47 mm in diameter). In particular, we wanted to check the possibility that part of the labeled DOC recovered in the filtrates might have been produced as a result of cell damage during filtration through the 25-mm, 0.2-μm polycarbonate filters. In contrast to this type of filtration, the use of larger filters (47 mm in diameter) and prefiltration through 20-, 5-, and 2-μm filters should minimize the risk of cell breakage and the ensuing loss of particulate, labeled carbon as 'artefactual' DOC. Figure 4A shows that a very good agreement existed between the estimates of vertically integrated, total POC production obtained with each method. There were no significant differences between the two types of estimates ($p = 0.43$, Wilcoxon signed-rank test).

Considering that the highest rates of DOC production were measured at 10 m (Fig. 3B), we conducted the same type of comparison with the samples from that depth. In this case, we selected the samples in which more than 80% of the total particulate primary production was in the >20-μm size fraction. Given that filtration through the 20-μm filters takes place by gravity, the chances of cell breakage in these samples was minimal. Therefore, we take the measurements of total POC production in these samples (measured as the sum of the primary production in each size class, see Materials and methods) as a valid reference, against which we can contrast the POC production results obtained with the DOC production technique. If our experimental procedure were overestimating DOC production, then we should expect anomalously low estimates of POC production. As shown on Fig. 4B, the estimates of 10-m-depth POC production obtained with the DOC production technique closely agreed with those obtained with the conventional POC production method. No significant differences were found between the two types of estimates ($p = 0.30$, Wilcoxon signed-rank test). In conclusion, there is no indication that our relatively high DOC production rates were the result of cell breakage during filtration.

Finally, we assessed the consistency between the estimates of POC and DOC production and the estimates of total organic carbon (TOC) production as obtained with unfiltered samples. One frequent problem in ¹⁴C-based, total primary production determinations involving filtration is that the sum of DOC and POC production does not coincide with TOC production measured in decontaminated, unfiltered samples (e.g., Lignell 1992). In our study, we obtained an excellent agreement between integrated TOC production and the sum of integrated DOC and POC production (Fig. 4C). There were no significant differences between the two types of

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to determine DOC production (POC[DOC]). (B) Same as in panel (A) but only for 10-m depth samples in which >80% of total POC production was due to phytoplankton in the >20-μm size fraction. (C) Relationship between vertically integrated total organic carbon (TOC) production, measured in unfiltered samples, and the sum of vertically integrated DOC and POC production. See text for details.

estimates ($p = 0.28$, Wilcoxon signed-rank test). This result provides further evidence that, in our experiments, filtration was not causing any interference in the determination of the real rates of DOC and POC production.

POC versus DOC production—A convenient way to explore the relationship between the production of POC and DOC is to plot the logarithms of the two variables against each other and to calculate the best fit to a linear model (Baines and Pace 1991). If the slope of the regression line is not significantly different from 1, it means that PER is constant across the observed range of primary production. If the slope is significantly larger or smaller than 1, it means that PER increases or decreases, respectively, with productivity. As we discuss later on (*see Discussion*), the interpretation of this analysis depends critically on the type of data used (i.e., volumetric vs. vertically integrated). Using all determinations of volumetric POC and DOC production rates, a poor correlation was found between the logarithms of the two variables ($r^2 = 0.20$, Fig. 5A), and the slope of the regression line (Model II) was significantly smaller than 1 (t -test, $p < 0.005$), implying that PER tends to increase with decreased POC production. However, when computed for each depth separately, the regression coefficients were higher (0.51, 0.72, and 0.26 for samples from 0, 10, and 20 m, respectively), and the slopes of the linear regression (Model II) were not significantly different from 1 (1.12, 1.13, and 1.22 for 0, 10, and 20 m, respectively). Using vertically integrated data in the euphotic layer, we found a strong correlation between POC and DOC production rates ($r^2 = 0.81$, Fig. 5B), and the slope of the regression line was not significantly different from 1 (t -test, $p > 0.2$). This means that when areal productivities were considered, PER was relatively constant across the whole range of measured POC production (from 42 to 1,127 $\text{mg C m}^{-2} \text{h}^{-1}$).

Time series experiments—In spring and summer 2003, we conducted additional visits to the sampling station in order to collect surface seawater samples and to perform ^{14}C -uptake time series experiments. The main objective of these experiments was to determine whether or not DOC production continued in the dark. This is a critical point if one wants to convert hourly rates of DOC production into daily rates. In addition, determining the diurnal variability in DOC production is most helpful in order to understand the mechanisms involved in the extracellular release of recently fixed carbon. Both Chl *a* concentration and size distribution differed markedly between the spring and summer experiments. In spring, surface Chl *a* concentration was 2.7 mg m^{-3} , and the $>5\text{-}\mu\text{m}$ size fraction accounted for 64% of total Chl *a*, whereas Chl *a* concentration in the summer experiment was 1.0 mg m^{-3} and the contribution of the $>5\text{-}\mu\text{m}$ size class to total Chl *a* was 82%. In both experiments, we found that the production of POC increased linearly with time during the light period and then stopped during the dark (Fig. 6). The production rate of DOC showed an acceleration after the first 4–6 h, resulting in a slightly exponential trend of increase during the light period, but it also stopped during the dark. Both experiments clearly demonstrated that, at least in the

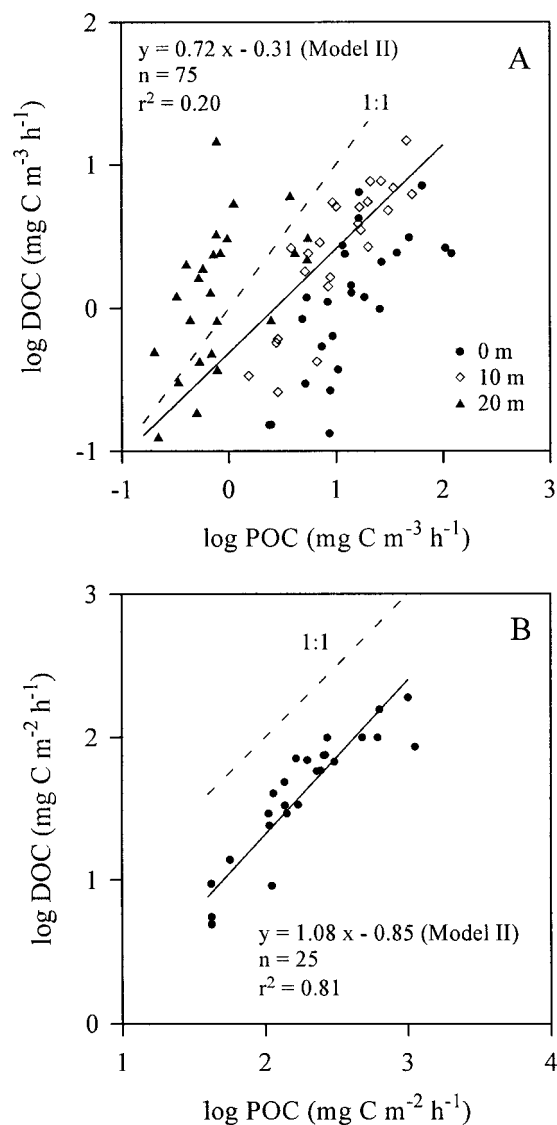


Fig. 5. (A) Relationship between the logarithms of the volumetric rates of POC and DOC production. (B) Same as panel A, but for vertically (0–20-m) integrated data.

ecosystem under study, the extracellular release of recent photosynthate is a strictly light-dependent process.

PER and irradiance—With the aim of determining the causes of the strong vertical variability in the relative importance of DOC release, we plotted all values of volumetric PER against incubation irradiance (Fig. 7). We found that an inverse relationship existed between the two variables: high values of PER ($>50\%$) were measured only at very low irradiances ($<100 \mu\text{mol m}^{-2} \text{s}^{-1}$), whereas typical values of PER at high irradiances were $<20\%$. Despite this strong relationship, it could well be possible that changes in other factors, such as temperature or species composition, were causing the observed vertical pattern in PER. In order to test this possibility, we conducted additional P-E experiments in 2003, using seawater samples collected from 0 or 15 m in the same sampling station during winter mixing (February),

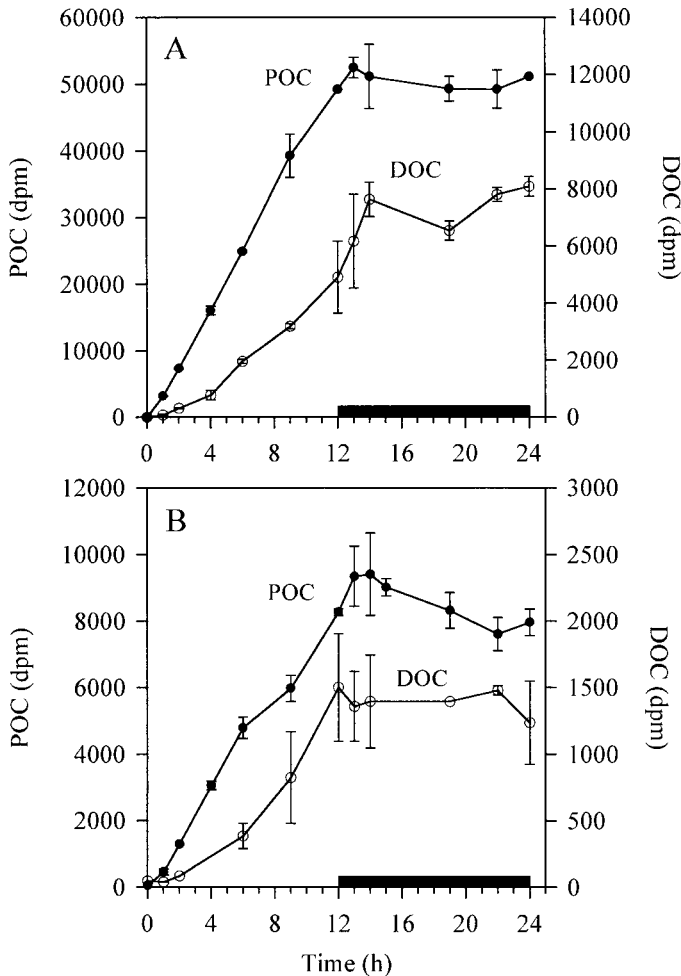


Fig. 6. Time series experiments of POC and DOC production by (A) spring and (B) summer phytoplankton assemblages collected from the surface. Vertical bars indicate ± 1 standard deviation ($n = 2$).

spring bloom (March), and summer upwelling (June) conditions. Phytoplankton biomass and size structure varied amply among these samples: Chl *a* concentration ranged from 2 to 11 mg m^{-3} and the percentage of Chl *a* in the $>5\text{-}\mu\text{m}$ size fraction varied from 50% to 90%. Despite these wide differences in phytoplankton biomass and size-structure, the results of the P-E experiments were fully consistent (Fig. 8). POC production increased with irradiance until saturation of photosynthesis was reached, which typically took place at an irradiance between 400 and 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 8A). In contrast, DOC production was relatively constant at intermediate and high irradiances but increased at irradiances below 200–300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 8B). In four of the five P-E experiments, DOC production at irradiance levels below I_k (light saturation index for POC production) was significantly higher than that measured at irradiance levels above I_k (t -test, $p < 0.05$). As a result of the contrasting behavior of POC and DOC production, PER showed a strong inverse relationship with irradiance (Fig. 9). In these experiments, the changes in POC and DOC production (and therefore PER) cannot be attributed to any other factor but irradiance.

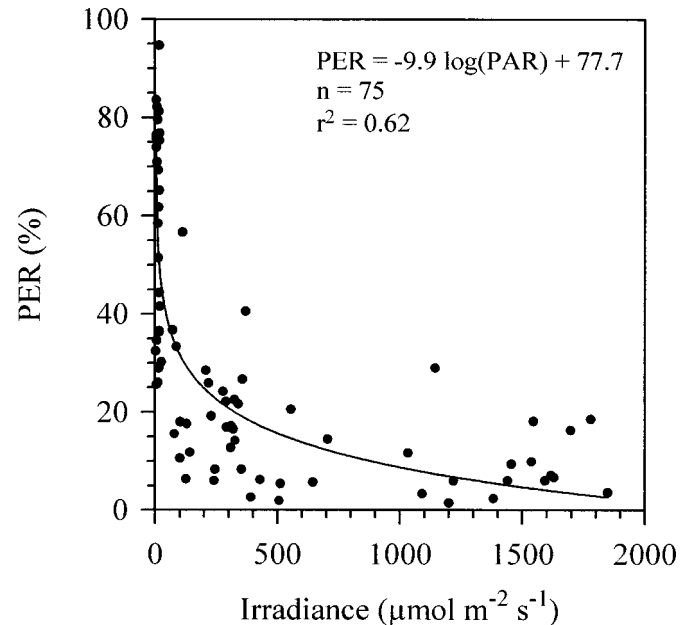


Fig. 7. Relationship between in situ irradiance and volumetric PER for all DOC production experiments conducted between July 2001 and July 2002.

PER and phytoplankton size structure—In an attempt to relate the dynamics of DOC production to the changes in the size structure of the phytoplankton assemblages, we plotted all volumetric PER values against the percentage of Chl *a* and POC production in the $>5\text{-}\mu\text{m}$ size fraction (Fig. 10A,B). There was no significant relationship between PER and the partitioning of Chl *a* and primary production in small and large phytoplankton using either the 5- μm threshold or any other size threshold (e.g., 2 or 20 μm). In addition, we did not find any relationship between Chl *a*-normalized DOC production and the percentage of Chl *a* or productivity in different size fractions (data not shown). Using vertically integrated data resulted in the same lack of correlation. Finally, we explored the relationship between PER and the slope of the abundance–size spectrum, which serves as an indicator of the relative importance of small and large cells and is based on a continuous approach to phytoplankton size structure. There was no significant relationship between PER and the slope of the abundance–size spectrum (Fig. 10C), again indicating that in the studied system, the relative importance of DOC production by the microbial populations is independent of phytoplankton size structure.

Discussion

Seasonal variability and quantitative importance of DOC production—The relative importance of dissolved primary production, expressed as a percentage of total, integrated PER, remained within a relatively constrained range (15–25%) during most of our study, and no marked seasonal patterns were detected. We shall now put these results in the context of the few existing reports on the seasonal variability of DOC production in temperate seas, keeping in mind that

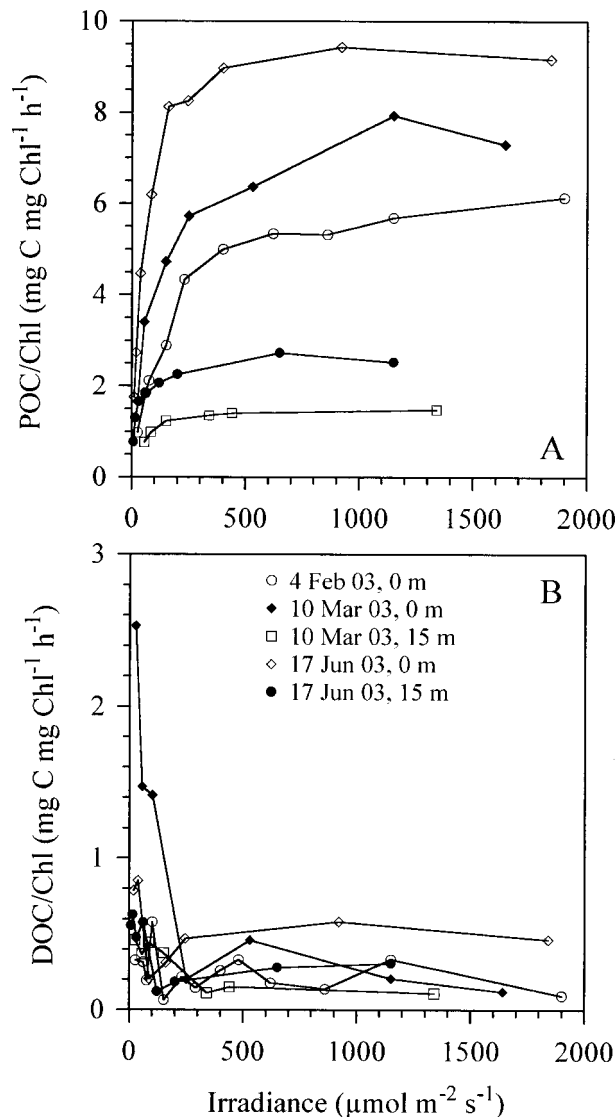


Fig. 8. Relationships between irradiance and (A) the ratio between POC production and Chl *a* concentration and (B) the ratio between DOC production and Chl *a* concentration, obtained from P-E experiments with winter, spring, and summer phytoplankton assemblages from 0 or 15 m in depth.

in some cases, methodological differences make it difficult to establish meaningful comparisons.

Two studies in the northern Baltic Sea have found an increase in the fraction of photosynthate that is recovered in filtrates during the summer period, coinciding with conditions of reduced primary production due to nutrient limitation (Larsson and Hagström 1979; Lignell 1990). In our study, however, we never encountered conditions of persistent stratification that could lead to severe nutrient limitation of primary production, which would explain the fact that PER never increased substantially over its mean value. The average PER values reported by Larsson and Hagström (1979) (8%) and Lignell (1990) (5%) were significantly lower than the ones we measured in our study. This discrepancy may have been caused, at least in part, by the fact that these

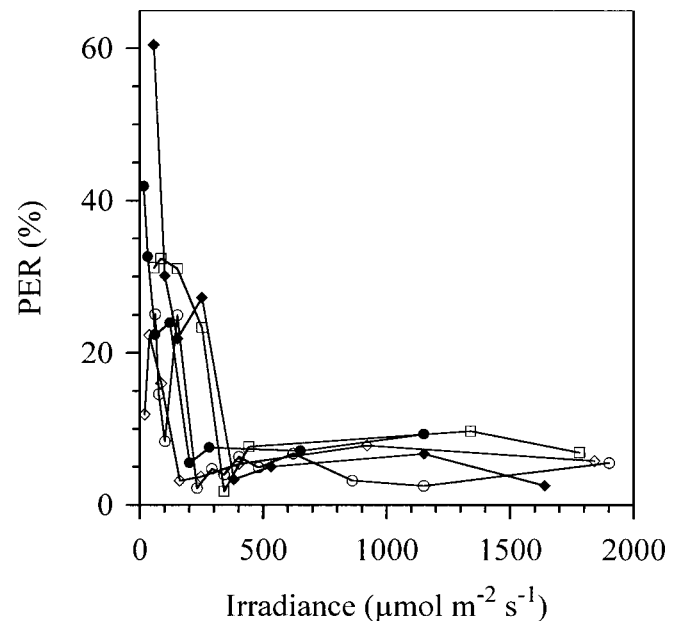


Fig. 9. Relationships between irradiance and PER, obtained from P-E experiments with winter, spring, and summer phytoplankton assemblages from 0 or 15 m in depth. Symbols as in Fig. 8.

authors incubated their samples for 4–7 h, whereas we incubated ours for only 2 h. This short incubation time has probably reduced the loss of labeled DOC due to bacterial uptake and respiration. On the other hand, it has been suggested that short incubation times might lead to anomalously high excretion rates, particularly when phytoplankton populations experience large environmental changes prior to incubation (Sharp 1977). However, there was no indication of this effect in our 24-h time series experiments, as the rate of DOC production, instead of decreasing, increased slightly during the light hours (Fig. 6).

After monitoring the extracellular release of recent photosynthate in coastal waters of the Gulf of Maine during 1 yr, Mague et al. (1980) concluded that a value of 5–10% could be taken as a valid average PER in their samples. This value is much lower than our measured average PER of 19%, but it has to be taken into account that in their study, only surface samples were collected. In fact, our average PER for surface samples (9%) falls within the range given by Mague et al. (1980). Another important difference between these studies is the type of filter used: 0.2- μm polycarbonate filters (this study) versus GF/F filters (Mague et al. 1980). The generally lower PER values reported in the latter study may have been caused by underestimating the real rates of release, because of adsorption of labeled DOC to the filters (Maske and García-Mendoza 1994; Karl et al. 1998; Morán et al. 1999).

The study with which we can most easily compare our results is that of Teira et al. (2003a), who determined the seasonal variability in DOC production in shelf waters off Northwest Spain using the same methodology described here (i.e., 0.2- μm polycarbonate filters and 2-h incubations). These authors concluded that, on average, DOC accounted for 37% of total primary production during the period of

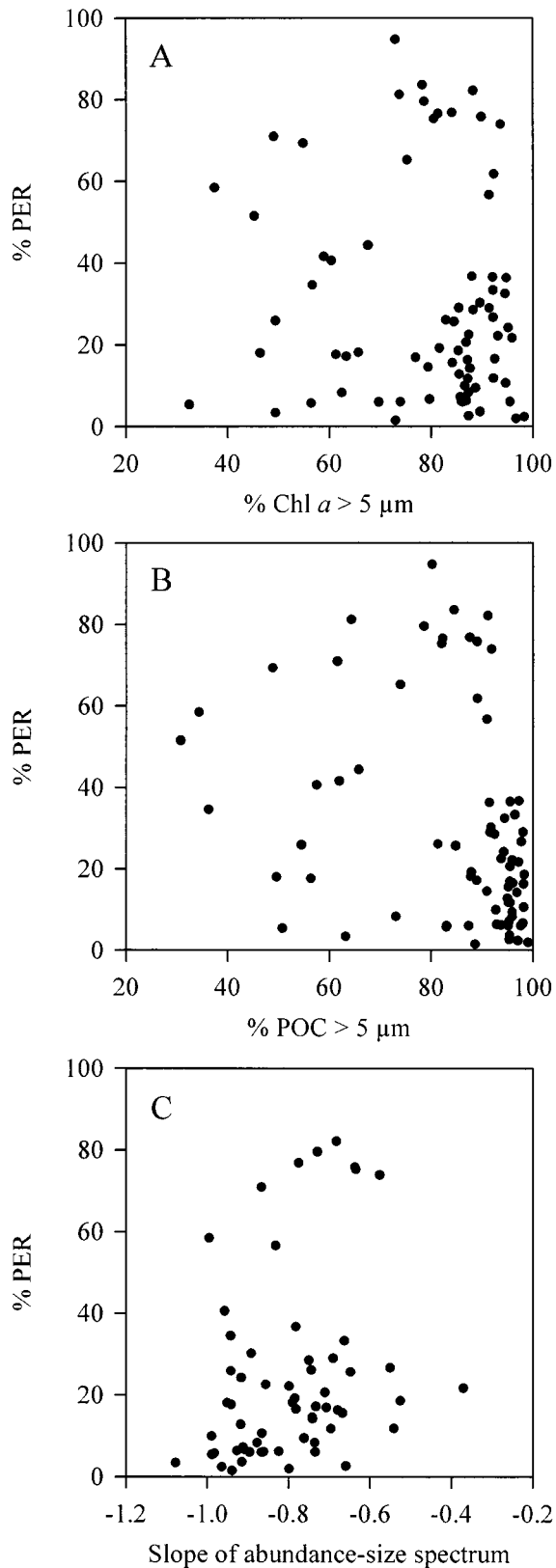


Fig. 10. (A) Relationship between the percentage of Chl *a* in the >5- μ m size fraction and PER (volumetric data, $n = 75$). (B) Same as panel A, but for the percentage of primary production in the >5- μ m size fraction. (C) Relationship between the slope of the abundance-biovolume spectrum and PER (volumetric data, $n = 62$).

study (from December 1998 to September 1999). This figure, however, is based on the untested assumption that DOC production, as determined with the ^{14}C technique, continues in the dark (Fig. 6). As we discuss below, this assumption is unsustainable by the available evidence. Considering only the release of DOC during the light period, the average PER obtained by Teira et al. (2003a) would then be 15% (i.e., close to our own estimate for the Ría de Vigo). As far as temporal variability is concerned, both studies agree in that no clear relationship was observed between PER and total integrated primary production, as evidenced by the fact that the slopes of the linear regression (Model II) between the logarithms of integrated POC and DOC production (1.08 in our study, 0.91 in the Teira et al. study) were not significantly different from 1.

In summary, both our results and those reported by Teira et al. (2003a) demonstrate that the release of recent photosynthate in dissolved form is a normal function of actively growing phytoplankton, proceeds at a similar relative rate throughout the year, and accounts for near 20% of the total annual primary production in coastal, highly productive waters of the Northwest Iberian peninsula. Interestingly, a recent study in the North Water Polynia (Arctic Ocean) reports an average PER value of >30% during the productive season (Mei et al. 2003). These findings contradict the view that the estimates of primary production in eutrophic waters are not seriously in error when DOC release is ignored (Thomas 1971; Fogg 1983). It can be concluded that the omission of DOC production measurements is likely to lead to significant underestimates of the real rates of total C fixation in highly productive ecosystems.

What explains the vertical PER gradient?—The increase in PER with depth is a pattern that has been observed frequently in estuarine (Thomas 1971), coastal transition zone (Teira et al. 2001b), and open ocean (Anderson and Zeutschel 1970) waters, although in all these studies there were also instances of PER remaining unchanged or even decreasing with depth. In the present survey, we consistently found that PER increased from the surface to the base of the euphotic layer, taking a mean annual value of 9%, 21%, and 59% at 0, 10, and 20 m, respectively. It is important to note that the relatively high PER values obtained at 10 and 20 m cannot be attributed to dark uptake processes (Fogg 1983), DO^{14}C contamination of the $\text{NaH}^{14}\text{CO}_3$ solution, or inefficient removal of inorganic ^{14}C from the filtrates (Sharp 1977), given that all these potential sources of error were countered by subtracting black-bottle DPMs from light-bottle DPMs (see Materials and methods). The increase in PER with depth was found also during conditions of strong vertical mixing in winter, when temperature, nutrient concentrations, and species composition were homogeneous throughout the euphotic layer. This indicated that these variables were not responsible for the vertical PER gradient. The results of the P-E experiments clearly confirmed the role of irradiance in controlling the vertical variability of PER. However, understanding the variability in PER ultimately requires understanding the variability in POC and DOC production separately.

The association between low irradiance and enhanced

PER, which has been found before (Zlotnik and Dubinsky 1989, Morán and Estrada 2001), reflects the fact that particulate primary production is drastically reduced at low light levels, whereas DOC release generally shows much smaller variability across irradiance gradients. Moreover, we observed an increase in the Chl *a*-specific rate of DOC production with depth, and our P-E experiments consistently showed an increase in the Chl *a*-specific rate of DOC production at irradiances below 200–300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 8B). These results indicate that DOC release was enhanced at suboptimal irradiances, which explains why absolute rates of DOC production peaked at 10 m (Fig. 3B). At this depth, phytoplankton biomass was generally similar to that present at the surface (average Chl *a* concentrations were 3.7 ± 0.6 and $4.0 \pm 0.6 \text{ mg m}^{-3}$ at 0 and 10 m, respectively), and typical irradiance levels (always $<200 \mu\text{mol m}^{-2} \text{s}^{-1}$, data not shown) were suboptimal for photosynthesis (see Fig. 8A). The combination of (1) a phytoplankton biomass that was still high and (2) an enhanced rate of biomass-specific DOC release gave way to the maximum in absolute rates of DOC production that we measured at 10 m.

The role of irradiance in controlling the partitioning of primary production among the dissolved and the particulate fraction bears an important consequence for the interpretation of DOC versus POC production relationships, such as those shown on Fig. 5. In these plots, a slope of <1 is interpreted to indicate that PER tends to increase with decreasing productivity of the ecosystem. However, if volumetric data corresponding to several depths in the euphotic layer are pooled together, then a slope significantly lower than 1 is likely to appear as a result of juxtaposing several data clouds (of slope near 1) that correspond to each sampling depth (effectively, each irradiance level). Each data cloud will have a similar range of variation for DOC production but progressively lower values of POC production as depth increases (Fig. 5A). We believe this irradiance effect may help explain the discrepancy observed by Baines and Pace (1991) between their within-studies and overall slopes (0.7 and 1.0, respectively) in the POC–DOC relationships. Similarly, the frequently reported observation that PER increases with decreasing particulate primary production, when based on volumetric data only (e.g., Mague et al. 1980; Morán et al. 2002a), should not be taken as evidence that the relative importance of DOC production increases with decreasing system productivity.

PER and size structure—We did not find any evidence to indicate that phytoplankton size structure per se had any influence on the relative rate of DOC production, despite the fact that there are both physiological and ecological reasons to expect a such an effect. From a physiological point of view, the increased surface-to-volume ratio of small cells should favor the passive diffusion across the membrane of low-molecular-weight metabolites (Bjorsen 1988; Kiørboe 1993), causing higher relative DOC release in smaller phytoplankton compared to larger ones. Malinsky–Rushansky and Legrand (1996), after measuring DOC release in three algal species and reviewing the available data from other studies with cultures, did show that PER was higher in picophytoplankton ($<2 \mu\text{m}$), but they did not observe clear

differences between nano- (2–20 μm) and microphytoplankton ($>20 \mu\text{m}$). Kiørboe's calculations also indicate that the size dependence of PER is much stronger in the picophytoplankton and small nanoplankton than in the large nanophytoplankton and microphytoplankton size range. In this regard, a laboratory study of eight diatom species with cell volumes ranging from ~ 10 to $\sim 10^5 \mu\text{m}^3$ failed to find any significant relationship between C-specific DOC production and cell size (Finke 1998). Having these results in mind, it is likely that we did not find a relationship between size structure and PER because we did not encounter any situations of strong picophytoplankton dominance of total biomass and productivity, such as those typically observed in the oligotrophic regions of the open ocean. In these conditions, however, nutrient limitation of primary production is usually present, which may confound the effects of cell size per se on the relative importance of DOC release. It thus seems that testing rigorously the hypothesis that cell size has a physiological effect on PER in natural phytoplankton populations will remain a difficult task.

Ecologically, it is well known that trophic coupling between zooplankton and phytoplankton is tighter when very small cells dominate the community of primary producers (Banse 1992; Kiørboe 1993). If microzooplankton sloppy feeding and egestion are important mechanisms of photosynthetic DOC production (Nagata 2000), then higher PER could be expected when pico- and nanophytoplankton are dominant. This is precisely what Teira et al. (2001a) found when pooling together several observations conducted across a wide geographical and productivity range in the Atlantic Ocean. These authors emphasized the role of trophic mechanisms in explaining the relatively high DOC release rates measured in oligotrophic picophytoplankton-dominated waters. As we shall discuss below, all of the available evidence indicates that, at least in our study system and most likely in other, less-productive ecosystems as well, trophic mechanisms are not involved in the release of recent photosynthate, as determined with the ^{14}C uptake technique. The lack of a relationship between PER and size structure in our data would then be fully consistent with this view.

Physiological and trophic mechanisms of DOC production—Our suite of simulated in situ, P-E, and time series experiments allows us to gain further insight into the mechanisms of DOC release by natural phytoplankton populations. The fact that POC production rate explains a high percentage of the variability in integrated DOC production rate has been taken by some authors as evidence that the release of recent photosynthate in dissolved form is an intrinsic part of the photosynthetic process and that physiological processes alone are involved (Mague et al. 1980; Fogg 1983; Baines and Pace 1991). However, it has to be born in mind that what we measure in ^{14}C experiments of DOC production is the appearance in dissolved form of labeled C that has recently been fixed intracellularly. This means that, regardless of the release mechanisms involved, some degree of relationship between POC and DOC production is to be expected. It is conceivable that zooplankton sloppy feeding and egestion are important mechanisms of DOC release and that, nevertheless, a strong relationship between POC and

DOC production is observed, particularly if grazing pressure is high and relatively permanent throughout the study.

More conclusive evidence can be obtained from the time series experiments of POC and DOC production. If microzooplankton activity or any other trophic processes were the main release mechanism, DOC production should not start immediately after the beginning of the labeling experiment, and, more importantly, it should continue at night. In both experiments, we detected significant DOC production only 30 min after ^{14}C addition, and the accumulation of labeled DOC stopped during the dark. It could be argued that DOC continued to be produced during the night but that it did not accumulate as a result of enhanced bacterial uptake. However, there is no reason to suppose that bacterial DOC uptake should suddenly increase at the end of the photoperiod. Besides, even if bacterial uptake did increase during the dark period, it is difficult to understand why its rate should equal the rate of DOC production so that the concentration of labeled DOC remains constant throughout the night. The simplest explanation for the observed time course of DOC production is that (i) photosynthate release is a purely physiological process and (ii) the liberated substances belong to a pool of small, recently synthesized metabolites, which have a relatively high turnover rate and whose intracellular abundance decreases promptly (1–2 h) once photosynthesis stops, thus causing extracellular release to cease. Our kinetics of DOC production, obtained in an eutrophic ecosystem, are similar to those obtained by Mague et al. (1980) in mesotrophic waters of the Gulf of Maine and by Karl et al. (1998) in oligotrophic waters of the North Pacific central gyre. The coherence between all these observations indicates that, regardless of the productivity and food web structure of the planktonic ecosystem, trophic effects play a minor role in the release of recent products of photosynthesis. This is not to negate the importance of trophic processes in DOC production, however, given that zooplankton grazing and viral lysis do cause the production of other types of dissolved organic substances (i.e., not recent photosynthate), particularly when longer time scales are considered (*see* Nagata 2000).

Finally, we can use our observations of the vertical distribution of PER and the results of the P-E experiments to try and elucidate which physiological mechanism (overflow or passive diffusion) was likely to be involved in phytoplankton exudation during our study. Baines and Pace (1991) rejected the passive diffusion mechanism on the basis of their observation that the correlation between POC and DOC production was much higher than the correlation between DOC production and phytoplankton biomass. This argument, used subsequently by many other authors, would be valid if the ^{14}C technique measured the release of all types of substances that exist inside the cell. In fact, what the ^{14}C technique measures is only the release of molecules containing recently fixed C. As explained above, in these conditions a relationship between DOC and POC production is bound to exist. Therefore, comparing the strength of the correlations between DOC production, POC production, and phytoplankton biomass does not give a clear-cut answer to the problem of which physiological mechanism is responsible for photosynthate exudation.

If DOC exudation were an overflow mechanism (Wood and Van Valen 1990), we should have found significant rates of DOC production only in surface waters, where irradiance is high and an uncoupling between energy and nutrient availability is more likely. In contrast, we consistently observed significant rates of DOC production at the base of the euphotic layer. Furthermore, our *in situ* observations and P-E experiments indicated that DOC production was actually enhanced under low, suboptimal irradiances. Increases in DOC production at low irradiances have been occasionally reported before, both for cultured (Zlotnik and Dubinsky 1989) and natural (Choi 1972) phytoplankton populations, although our data do not allow us to identify the underlying causes. This pattern would be consistent with our conclusion that the released substances belong to a pool of small, recently synthesized metabolites: it is known that the relative C incorporation into small-molecular-weight metabolites increases under low irradiances, as a result of a slowdown in macromolecular synthesis (Harding et al. 1985; Hawes 1990; Marañón and González 1998). In any event, the persistence of DOC production across a wide irradiance range, which has been reported by many other authors in a variety of marine environments (Thomas 1971; Mague et al. 1980; Teira et al. 2003b; among others) strongly indicates that as long as a pool of new products of photosynthesis is available, DOC release will occur as a background process of passive diffusion or 'leakage' (Raven 1986). This process, which seems to be enhanced under suboptimal irradiances, does not necessarily confer any particular adaptive benefit to phytoplankton, but rather reflects their inability to keep all recent photosynthate within the intracellular medium.

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