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Phytoplankton, dissolved organic carbon, Ria de Vigo

Abstract

We studied the importance of dissolved primary production in a coastal, productive ecosystem in relation to phytoplankton biomass, community structure and productivity. The photosynthetic production of dissolved organic carbon (DOCp) and particulate organic carbon (POCp) was determined in mesocosm experiments during four contrasting oceanographic periods in the Ría de Vigo (NW Iberian Peninsula). We also determined the size-fractionated chorophyll *a* concentration and primary production, phytoplankton taxonomic composition and bacterial production. Phytoplankton biomass was dominated by the >20 µm size fraction (mostly diatoms), except in winter, when the 2-20 and <2 µm size fractions (flagellates and picophytoplankton) increased in importance. The percentage of extracellular release (PER) had an average value of 19% and was independent of oceanographic period, phytoplankton biomass and production, taxonomic composition and size structure. During phytoplankton blooms, PER increased significantly from 14% in the exponential growth phase to 23% in the senescent phase. Bacterial carbon demand and DOCp were uncoupled, suggesting that other processes in addition to photosynthate exudation contribute the majority of labile carbon to fuel bacterial metabolism. Dissolved primary production remains an important process in coastal phytoplankton assemblages throughout the year, irrespective of size-structure and community composition, but attaining higher significance during the decaying phase of blooms.

Introduction

The photosynthetic production of dissolved organic carbon (DOCp) by phytoplankton can represent a substantial fraction of total primary production (Baines and Pace, 1991; Nagata, 2000) and plays an important role in food web interactions as a source of labile material to fuel bacterial growth (Cole *et al.*, 1982; Fogg, 1983; Norrman *et al.*, 1995). In spite of its importance, dissolved organic carbon production (DOCp) is not a routine measurement in most field studies and, as a result, general patterns relating phytoplankton community composition, size structure, total productivity and DOCp have been difficult to establish (Nagata, 2000).

Phytoplankton DOCp can originate from the passive diffusion of low molecular weight compounds through the cell membrane but may also represent an adaptive process to cope with high light and low nutrient conditions (Fogg, 1983; Wood and Van Valen, 1990). These mechanisms are not mutually exclusive and can operate concurrently, but have different implications. In the former case, DOCp will tend to be persistent whatever the growth conditions, although a higher relative importance of DOCp could be expected when small cells dominate the community, due to their higher surface/volume ratio (Bjørnsen, 1988; Kiørboe, 1993). In the latter case, phytoplankton, by maintaining their full photosynthetic capacity, can prevent photochemical damage and avoid any lag period in resuming carbon fixation when nutrients become available (Fogg, 1983; Wood and Van Valen, 1990). This mechanism would result in increased relative importance of DOCp during oligotrophic conditions.

While some analyses have suggested that the percentage of DOC extracellular release [PER=100*DOCp/(DOCp+POCp)] is a relatively constant value [e. g., 13% in (Baines and Pace, 1991), 20% in (Marañón *et al.*, 2005)], variable PER data are recorded in the literature. Mean PER values in coastal and open ocean waters typically range between 10 and 30% (Fogg, 1983; Karl *et al.*, 1998; Teira *et al.*, 2001b; Morán *et al.*, 2002a), with most of the higher values being measured in oligotrophic environments (Obernosterer and Herndl, 1995; Teira *et al.*, 2001a). The differences found in PER among contrasting systems suggest the existence of a relationship between DOCp and phytoplankton community structure. Although some studies have found significant positive relationships between PER and the relative importance of picophytoplankton (Malinsky-Rushansky and Legrand, 1996; Teira *et al.*, 2001a; Morán *et al.*, 2002b), others have found no relationship between PER and phytoplankton cell size (Finkel, 1998; Marañón *et al.*, 2004). The variability of DOCp in marine waters has rarely been addressed in conjunction with a detailed analysis of

phytoplankton species composition (Lancelot, 1983). As a result, is it unclear if changes in the dominant phytoplankton groups are also associated with differences in the importance of DOCp.

Work with laboratory cultures has shown that phytoplankton respond to nutrient limitation with increased synthesis of extracellular organic compounds such as carbohydrates (Myklestad, 1977; Lancelot, 1983; Borsheim *et al.*, 2005). In this regard, a higher PER has been reported for cells growing under phosphorus or nitrogen limitation (Obernosterer and Herndl, 1995). However, the evolution of PER during the different growth stages of a given phytoplankton community, and under contrasting oceanographic conditions, has not yet been determined.

The Ría de Vigo (NW Iberian Peninsula) is a productive ecosystem characterised by a seasonal cycle of upwelling events between April and October, a downwelling period from October to March (Nogueira *et al.,* 1997), and a transient period between the two phases. Blooms are dominated by diatoms in spring and by dinoflagellates in autumn (Tilstone *et al.*, 1994; Crespo *et al.*, 2006). During the upwelling season, the phytoplankton community is dominated by microphytoplankton (>20 µm) (Cermeño *et al*., 2006), and euphotic zone integrated primary production rates can reach 1-2 gC m^{-2} d^{-1} (Tilstone *et al*., 1999). A shift in phytoplankton size structure is observed during downwelling events, when the contribution of pico (<2 µm) and nano (2-20 µm) phytoplankton to total biomass increases. During this period, plankton community respiration accounts for more than 80% of the primary production, thus most of the organic matter is remineralized within the water column (Cermeño *et al.,* 2006). The marked seasonal and short-term variability of the Ría de Vigo makes it an excellent scenario to test if the relative importance of DOCp varies among the different phytoplankton communities that exist under the different hydrographic conditions.

Our experimental approach was to collect distinct phytoplankton assemblages characteristic of four contrasting oceanographic periods throughout the year and monitor their dynamics during 9-day long mesocosm experiments. This approach ensured that we studied a wide range of phytoplankton communities in terms of physiological state, species composition and size structure. Our main objectives were: i) to determine if the relative importance of DOCp changes during the different growth phases of natural phytoplankton assemblages and ii) to assess if variations in the taxonomical composition of the phytoplankton community, associated with different hydrographic conditions during the year, result in changes in the relative contribution of DOCp to total primary production.

Method

Sampling and experimental setup

Mesocosm experiments were conducted in the Ría de Vigo during March 2005, July 2005, September 2005 and January 2006, thus covering four relevant hydrographic periods of this ecosystem: spring bloom, summer stratification, autumn upwelling and winter mixing. In each experiment, polyethylene bags of 3.5 m^3 in volume (1.5 m in diameter and 2 m deep) were filled at a central station (42º 14.09´N, 8º 47.18´W). The bags were gently filled from their bottom with seawater passing through a 200-µm mesh, in order to exclude mesozooplankton. Once they were filled, a diver closed the bags with a stopper at the bottom. Afterwards, they were transported to a sheltered bay where they were attached to a pontoon. The bags were open from the top and therefore the enclosed seawater was subjected to natural irradiance conditions.

Two mesocosms (true replicates) were used in the March and July experiments, whereas in the September and January experiments three bags were filled. Each experiment lasted nine days and samples were taken every day during the first five days, and thereafter every two days. Daily sampling was conducted at 08:00 hours using 1.5-m long methacrylate tubes, which were filled in a vertical position in order to sample the upper half of the water mass enclosed in each mesocosm. The water was gently dispensed into 10-L polycarbonate carboys, which were then carried to the laboratory, where small volume samples were collected for each particular analysis.

Inorganic nutrients and size-fractionated chlorophyll *a*

Water samples for nutrients were collected into 50-mL polyethylene bottles and kept frozen (-20ºC) until determination using standard segmented-flow analysis with colorimetric procedures (Grasshoff *et al*., 1983). For the determination of size-fractionated chlorophyll *a* (Chl-*a*), 250-mL samples were filtered sequentially through polycarbonate filters of 20, 2, and 0.2 µm pore size, using low vacuum pressure (<100 mmHg). Pigment extraction was carried out by placing the filters in 90% acetone for 24h at -20ºC. Chl-*a* concentration was determined fluorimetrically using a Turner-TD-700 fluorometer previously calibrated with pure Chl-*a*.

Phytoplankton community composition and biomass

Picophytoplankton abundance was determined in 1.8 mL-samples, fixed with paraformaldehyde (1% final concentration) and glutaraldehyde (0.05% final concentration), using a FACSCalibur flow cytometer (Calvo-Díaz and Morán, 2006). Carbon biomass was estimated assuming a spherical shape and using volume-to-carbon conversion factors: 230 fg C μm^{-3} for *Synechococcus*, 240 fg C μm^{-3} for *Prochlorococcus* and 237 fg C μm^{-3} for picoeukaryotes (Worden *et al*., 2004). For the analysis of nanophytoplankton, subsamples of 10 mL were fixed with buffered 0.2-µm filtered formaldehyde (2% final concentration) and then filtered through 0.2-µm black Millipore-Isopore filters placed on top of 0.45-µm Millipore backing filters. Epifluorescence microscopy was used to determine autotrophic organisms, which were enumerated under blue light excitation. It was assumed that all organisms showing red autofluorescence when excited with blue light were autotrophic, even though mixotrophic organisms are not correctly identified with this technique. Dimensions were taken for several individuals and cell volumes were calculated assuming a spherical shape or after approximation to the nearest geometrical shape (Hillebrand *et al*., 1999). Cell carbon was estimated following Verity *et al*. (Verity *et al.*, 1992) for nanoflagellates and Strathmann (Strathmann, 1967) for small naked dinoflagellates belonging to the nanoplankton size fraction.

For microphytoplankton determinations samples of 100 mL preserved in Lugol's iodine were sedimented in composite sedimentation chambers and observed with an inverted microscope. The organisms were counted and identified to the species level when possible. The small species were enumerated from two transects scanned at ×400 and ×250, whereas the larger species were counted by scanning the whole slide at ×100. Phototrophic and heterotrophic species of dinoflagellates, flagellates and ciliates were differentiated following Lessard and Swift (Lessard and Swift, 1986) and also using epifluorescence microscopy. Cell biovolumes were estimated according to Hillebrand *et. al.* (Hillebrand *et al*., 1999) and cell carbon calculated following Strathmann (Strathmann, 1967) for diatoms and dinoflagellates, Verity *et. al.* (Verity *et al.,* 1992) for flagellates and Putt and Stoecker (Putt and Stoecker, 1989) for aloricate ciliates. All organisms containing chloroplasts were assumed to be autotrophic. Dinoflagellates and ciliates <20 µm as well as single diatoms <20 µm counted with this technique were assigned to the nanoplankton fraction.

Photosynthetic production of POC and DOC

The production of particulate organic carbon (POCp) and dissolved organic carbon (DOCp) was determined by carrying out *in situ* (SIS) incubations with the radioisotope ^{14}C. We used

incubators that were cooled with running seawater from the laboratory's continuous supply. The incubator was located on the terrace of the Instituto de Investigaciones Marinas and the experiments were thus conducted under natural irradiance conditions. For each sample, three light and two dark acid-washed Pyrex glass bottles (50 mL) were filled and spiked with 10 µCi of $NaH^{14}CO_3$. At the end of the incubation, which lasted 2-3 hours, two 5-mL aliquots from each incubation bottle were filtered through 0.2-µm pore size polycarbonate filters of 25-mm in diameter using low vacuum pressure (< 50 mmHg) to avoid cell breakage and the loss of particulate, labelled material into the filtrate. Previous experiments conducted with the same method strongly indicate that the filtration procedure used does not cause cell breakage (Marañón *et al*. 2004).

To remove the inorganic ^{14}C that was not incorporated into the cells, the filtrates were acidified to a pH of ~2 with 100µl of 50% HCl, and then maintained for ~12 h in 20-mL open scintillation vials placed on an orbital shaker. After inorganic ^{14}C removal, 10 mL of high sample capacity scintillation cocktail were added to each 5 mL 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 5-mL scintillation vials to which 4 mL of scintillation cocktail were added. The radioactivity in each sample was determined in a Packard Tri-Carb 3100TR scintillation counter which used the external standard method for quenching correction. The dark bottle value of disintegrations per minute (DPM) was subtracted from the light bottle DPMs in order to calculate the rates of DOC and POC production. In all calculations, we used a value of 25,700 mgC m^{-3} for the concentration of dissolved inorganic carbon and a value of 1.05 for the isotopic discrimination factor.

Bacterial production

Bacterial heterotrophic production (BP) was estimated using the ^{3}H-Leucine method (Kirchman *et al.,* 1985) but in Eppendorf vials which were processed by centrifugation and trichloroacetic acid (TCA) rinsing. Four replicates of 1.2 mL were taken for each mesocosm as well as two TCA-killed controls. The Leucine tracer was added at a 40 nM final concentration in incubations lasting approximately 2 h at *in situ* temperatures and in dark conditions. The incorporation was stopped with the addition of 120 µL of cold 50% TCA to the samples which, after mixing, were kept frozen at -20ºC until processing by the centrifugation method (Smith and Azam, 1992). The samples were counted on a Beckman scintillation counter, 24 h after addition of 1 mL of scintillation cocktail. To convert Leucine uptake rates to BP we determined empirical conversion factors in each season in

two replicate experiments. We gently filtered seawater from the mesocosms through 0.6 µm polycarbonate filters (Millipore, DTTP) in order to remove predators. Then, we diluted the water (1:9) with 0.2 µm filtered (Millipore, GTTP) seawater and incubated the mixture in 2-L acid-clean polycarbonate bottles in the dark in a room adjusted to the *in situ* temperature. Subsamples were taken for Leucine incorporation and bacterial abundance measurements at every 12-24 hours until bacteria reached the stationary growth phase. The amount of biomass produced per unit Leucine incorporated was computed with the cumulative method (Bjørnsen and Kuparinen, 1991), which maximizes the use of the available data. The obtained empirical factors were: 1.2 kgC mol Leu^{-1} (March 2005), 0.18 kgC mol Leu^{-1} (July 2005), 0.28 kgC mol Leu^{-1} (September 2005) and 0.95 kgC mol Leu^{-1} (January 2006). Bacterial carbon demand (BCD) was calculated by adding the measured BP rates and estimates of bacterial respiration (BR). In order to compute BR, bacterial growth efficiency (BGE) was estimated with two different models. The model proposed by del Giorgio and Cole (del Giorgio and Cole, 1998) is based on bacterial production (BP)

$$BGE= (0.037+ 0.65BP)/(1.8+BP)$$

whereas the model of López-Urrutia and Morán (López-Urrutia and Morán, 2007) is based on chlorophyll *a* concentration (Chl-*a*):

$$BGE= 1-[1/(0.727 \times [Chl\text{-}a/(Chl\text{-}a + 4.08)] + 1.02)]$$

Results

Nutrients, chlorophyll *a* and phytoplankton biomass

The initial conditions of each experiment reflect the seasonal variability in the hydrodynamic conditions of the Ría de Vigo. In March 2005, the high nutrient concentrations (Table I) allowed the development of a phytoplankton bloom. Chl-*a* concentration in this experiment reached more than 12 mg m^{-3} (Fig. 1), and the phytoplankton community was dominated by diatoms (82%) (Table II). In July, the warm temperatures (21° C) and low nutrient concentrations indicated that a marked thermal stratification of the water column was present at the time of sampling. Lower Chl-*a* concentrations (2 mg m^{-3}) were measured (Table I), but the relative contribution of diatoms to the total biomass was still large (77%) (Table II). The low temperature and high nutrient concentrations

observed at the beginning of the September experiment (Table I) corresponded to the well-documented upwelling events that occur in Ría de Vigo from April to October. The decay of a bloom was observed during this experiment: Chl-*a* concentrations decreased from >10 mg m^{-3} on the first two days of the experiment to <1 mg m^{-3} (Fig. 1), and the biomass was dominated by diatoms (62%) and autotrophic dinoflagellates (25%) (Table II). A markedly different phytoplankton community structure was observed during the January experiment, when the biomass was dominated by flagellates and picophytoplankton (Table II). The high nutrient concentration and low Chl-*a* concentration (Table I) reflected the low light conditions and the strong vertical mixing that are characteristic of the winter season in the Ría de Vigo.

Dissolved and particulate organic carbon production

The variability in both POCp and DOCp showed similar patterns to those observed in Chl-*a* concentration (Fig. 2). POCp was lower in July (<10 mgC m^{-3} h^{-1}) and January (<1 mgC m^{-3} h^{-1}) than during the March and September experiments, when values above 50 mgC m^{-3} h^{-1} were recorded during the peak of the phytoplankton bloom. The variability in DOCp differed from that of POCp in the March experiment, when no clear maximum was observed (Fig. 2). High rates of DOCp (>30 mgC m^{-3} h^{-1}) were measured in September during the upwelling season experiment.

A highly significant relationship was found between POC and DOC production rates (r^2=0.71, p<0.001, n=70, Fig. 3). The slope of the regression line (Model II) between the logarithms of DOCp and POCp was not significantly different from 1 (Clarke test, p=0.915), indicating that the relative contribution of DOCp to total primary production did not change across the range of POCp. No clear pattern of temporal variability in the percentage of extracellular release (PER) was found during the experiments (Fig. 2). The mean PER was 19% (SD, 9), with the lowest value found in March (13% [SD, 5]) (Table II). There were no significant differences in PER between experiments (RMANOVA, p=0.296) (Table II). Similarly, we found no association between the changes in taxonomic composition and the PER values (Table II).

We grouped all our observations into three groups according to the measured Chl-*a* concentration (<1 mg m^{-3}, 1-4 mg m^{-3} and >4 mg m^{-3}) in order to assess if PER changed with phytoplankton standing stocks (Table III). POCp and DOCp increased progressively in groups with higher Chl-*a* concentration, and the size structure also changed significantly: in low Chl-*a* samples the pico- and nano-phytoplankton size classes showed the largest relative contribution (33 and 40%,

respectively), whereas in high Chl-*a* samples the microphytoplankton was clearly dominant (87%). In contrast, PER did not show any significant differences between groups of samples (ANOVA, p=0.099).

In order to determine if dissolved primary production was favoured during the decaying phase of the phytoplankton bloom, we compared the measurements conducted in the exponential growth versus the senescent phases of the March and September experiments. The first three days of the March experiment and the first two days of the September experiment were considered as belonging to the exponential growth phase. The last two days from both experiments were considered for the senescent phase. Clear differences between the exponential and the senescent phases were observed (Table IV). The exponential phase was characterized by higher concentrations of dissolved inorganic nitrogen and phosphate and by higher phytoplankton biomass, as inferred from the Chl-*a* concentrations. Changes in the phytoplankton community also occurred, with diatoms clearly dominating during the exponential phase but sharing dominance with pigmented dinoflagellates during the senescent phase. Rates of POCp and DOCp also decreased during the senescent phase, while PER showed a significant increase (ANOVA, p=0.022) from a mean value of 14% (SD, 10) in the exponential phase to a mean value of 23% (SD, 10) during the senescent phase (Table IV).

Dissolved organic carbon production and bacterial carbon demand

In order to assess whether photosynthetic DOCp was the main source of organic matter for bacteria, bacterial carbon demand (BCD) was calculated from measurements of bacterial production. There was a lack of correlation between DOCp and BCD, irrespective of the model used to estimate the bacterial growth efficiency. However, the dispersion of the BCD data points changed between these two models. Changes in BCD between experiments were more evident when the model of López-Urrutia and Morán was used, as this model is based on Chl-*a* concentration. During the January experiment, BCD clearly exceeded DOCp, indicating that phytoplankton exudation was not sufficient to sustain bacterial metabolism. The opposite occurred during the July experiment, when in most cases DOCp was larger than BCD. However, the overall lack of correlation between these variables suggests that bacterial metabolism and phytoplankton exudation are largely uncoupled in this coastal ecosystem.

Discussion

Seasonal variability in phytoplankton community structure and DOCp

The different environmental conditions present in the Ría de Vigo prior to each experiment resulted in differences in phytoplankton biomass, community structure and productivity. During spring (March experiment), the confinement of nutrient-rich seawater allowed the development of a phytoplankton bloom, dominated mostly by large cells (diatoms), which are characteristic of high turbulence and increased nutrient conditions (Malone, 1980; Chisholm, 1992; Falkowski and Oliver, 2007). The summer (July) and autumn (September) experiments showed the shift from low nutrients and chlorophyll *a* concentration typical of a stratification event in the Ría (Nogueira *et al*., 1997) to higher nutrients and biomass, which was dominated by microphytoplankton and nanophytoplankton, characteristic of a coastal upwelling event (Cermeño *et al.,* 2006). In January, when high nutrient concentrations were available, the low phytoplankton standing stocks and primary production, together with the increased importance of pico- and nano-phytoplankton, could be attributed to the low incident irradiance and the enhanced vertical mixing of the water column. Low irradiance conditions limit more strongly the metabolic activity of large phytoplankton, which suffer a stronger package effect than the pico- and nano-phytoplankton (Finkel *et al.*, 2004; Cermeño *et al.*, 2005). As a result, pico- and nano-phytoplankton may contribute up to 70% of total phytoplankton biomass and particulate primary production during winter (Cermeño *et al.,* 2006).

In spite of this wide variability in hydrographic conditions and the ensuing changes in the composition of phytoplankton assemblages, a relatively constant PER value of, on average, 19% (SD, 9) was found. When we pooled all our data, we found that the slope of the regression line between log POCp and log DOCp was not significantly different from 1, indicating a constant PER across the productivity range considered. In addition, we did not find significant differences in mean PER among seasons. Our results agree with the mean PER value reported before (19%, SD 1) for the Ría de Vigo, in a study which included 25 vertical profiles of particulate and dissolved primary production obtained throughout a year (Marañón *et al*., 2004), and also with the value of 15% reported for a coastal station located further North in the NW Iberian Peninsula (Teira *et al.*, 2003). Another study conducted mainly in shelf waters off the Ría de Vigo but including also some measurements from the Ría, reported lower mean PER values (9% in spring and 6% in late summer) (Morán *et al.*, 2002b). Overall, these results confirm that the release of DOC is a

significant fraction of primary production in coastal, productive waters, irrespective of phytoplankton productivity and species composition.

DOCp and size structure

There are physiological reasons to expect an effect of phytoplankton size structure on the relative importance of DOCp. The increased surface to volume ratio of small cells should favour a higher diffusion of small molecular weight compounds through the membrane (Bjørnsen, 1988; Kiørboe, 1993). In fact, increased PER values have been reported for cultures of small sized phytoplankton (Malinsky-Rushansky and Legrand, 1996). In contrast, Finkel (Finkel, 1998), using a set of 8 diatoms species, ranging >5 orders of magnitude in cell volume, did not find any size dependence on the volume or carbon specific exudation rates. In our study, we did not observe any relationship between PER and size structure, not even in the January experiment, when the relative importance of picophytoplankton was much larger. Our results suggest that the observed increase in PER in oligotrophic environments such as the Atlantic subtropical gyres (Teira *et al.*, 2001), where picophytoplankton are dominant both in terms of biomass and production (Marañón *et al.*, 2001), may not necessarily reflect a direct effect of phytoplankton cell size on exudation, but result from the very low nutrient concentrations prevailing in these regions, which are strongly limiting for phytoplankton production and growth.

DOCp and bloom development

It is now established that extracellular release of recent photosynthate is a normal function of healthy cells, and that it is a process closely related to photosynthetic carbon assimilation (Mague *et al.*, 1980; Bjørnsen, 1988; Nagata, 2000). However, it has also been observed that high percentages of release are often associated with particular conditions experienced by the phytoplankton. These include very high or very low irradiances and abrupt changes in nutrient concentrations (Fogg, 1983; Nagata, 2000). Several studies have shown increases in PER associated with the stationary phase after a phytoplankton bloom, when nutrients became scarce and limiting for growth (Norrman *et al.*, 1995; Obernosterer and Herndl, 1995; Nagata, 2000). In our study, we did observe differences between the exponential and the senescent phases of the two phytoplankton blooms. The highest PER was found during the senescent period, when the concentration of dissolved inorganic nitrogen was low and presumably limiting for phytoplankton growth. This observation supports the view that the release of dissolved photosynthate under nutrient limitation may serve as a mechanism

to protect the cell's photosynthetic machinery, whereby organic carbon is excreted during periods of energy excess and nutrient limitation. This mechanism would allow the cells to keep their photosynthetic metabolism active for rapid growth whenever nutrients become available again (Wood and Van Valen, 1990).

Coupling between phytoplankton DOC release and bacterial production

It has been estimated that nearly half of the daily photosynthetic production is released, through different mechanisms, as dissolved organic carbon that may be available for heterotrophic bacterial consumption (Nagata, 2000). The importance of the photosynthetic production of DOC to fulfill the bacterial carbon demand (BCD) strongly depends on the trophic structure of the microbial plankton community and on the nature and magnitude of allochthonous sources of dissolved organic carbon (Morán *et al.*, 2002a; Borsheim *et al.*, 2005). Our experiments were conducted in an ecosystem that sustains high standing stocks of phytoplankton and where intense microzooplankton grazing takes place (Teixeira and Figueiras, 2009), which is likely to lead to an important production of labile DOC through egestion (Nagata, 2000). In addition, allochthonous inputs of dissolved organic matter of continental origin have also been shown to be significant in this system (Álvarez-Salgado *et al.*, 2001; Gago *et al.*, 2005). However, the DOC of continental origin is mostly refractory and, therefore, should not support a significant portion of the estimated BCD. Consumption of previously produced labile DOC seems more plausible, as demostrated by Alvarez-Salgado *et al.* (2001). Together, these processes may explain the lack of coupling between phytoplankton DOC release and bacterial metabolism.

Conclusions

The release of recently fixed photosynthetic carbon appeared to be a relatively constant process in the Ría de Vigo, irrespective of hydrographic period, phytoplankton size structure and taxonomic composition. However, the relative importance of dissolved primary production did tend to increase during the decaying phase of phytoplankton blooms. Bacterial metabolism and phytoplankton exudation were largely uncoupled, indicating that additional sources of DOC, both autochthonous and allochthonous, are likely to be used by bacteria. On average, DOCp contributed 19% of total primary production, which illustrates the importance of dissolved primary production in a coastal, productive ecosystem.

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Table I. Mean initial values for temperature (ºC), salinity, nutrient concentration (µmol kg^{-1}) and chlorophyll *a* concentration (mg m^{-3}) on each experiment. Standard deviation is indicated in brackets.

	Mar-05 n=2	Jul-05 n=2	Sep-05 n=3	Jan-06 n=3
Temperature	11 [0]	21 [0.1]	15 [0]	12 [0]
Salinity	35.5 [0.02]	35 [0.03]	35.7 [0.01]	35.6 [0.01]
DIN (NO_3+NO_2+NH_4)	4.4 [0.08]	0.6 [0.11]	5.7 [0.73]	7.7 [0.44]
PO_4	0.5 [0.01]	0.1 [0]	0.4 [0.1]	0.5 [0.04]
SiO_4	3.2 [0]	0.5 [0.1]	0.4 [0.1]	3.7 [0.15]
Chl-*a*	3 [0.5]	2 [0]	10 [2]	0.5 [0.03]

Table II. Mean biomass contribution of the different phytoplankton groups and percentage of extracellular release ([PER=100*DOCp/(DOCp+POCp)]) for each experiment. Standard deviation is indicated in brackets (n=7 for all experiments).

%	Mar-05	Jul-05	Sep-05	Jan-06
Diatoms (>20 µm)	82 [4]	77 [9]	62 [25]	5 [6]
Autotrophic dinoflagellates (>20 µm)	10 [3]	16 [6]	25 [21]	48 [12]
Autotrophic nanoflagellates	6 [2]	4 [2]	10 [7]	29 [7]
Picophytoplankton	2 [2]	3 [2]	3 [1]	18 [8]
PER	13 [5]	23 [6]	23 [7]	17 [4]

Table III. Mean values of the contribution of each phytoplankton size class to total Chl-*a* concentration, the rates of POCp and DOCp (mgC m^{-3} h^{-1}), and the percentage of extracellular release (% PER) for three groups of samples having different Chl-*a* concentrations (mg m^{-3}). Standard deviation is indicated in brackets.

Chl-*a* concentration range (mg m^{-3})	<1 (n=24)	1-4 (n=29)	>4 (n=17)
>20 µm (%)	29 [19]	59 [14]	84 [9]
2-20 µm (%)	39 [8]	29 [11]	12 [7]
0.2-2 µm (%)	32 [15]	12 [6]	4 [3]
POCp	2 [0.3]	10 [7]	44 [25]
DOCp	0.4 [0.2]	3 [2]	9 [13]
PER (%)	20 [8]	20 [9]	15 [11]

Table IV. Mean nutrient concentration (µmol kg^{-1}), particulate and dissolved organic carbon production (POCp and DOCp) (mgC m^{-3} h^{-1}), the percentage of extracellular release (PER), chlorophyll *a* concentration (mg m^{-3}), and the biomass contribution (%) of different phytoplankton groups during the exponential (n=5) and senescent phases (n=4) of the March and September experiments. Standard deviation is indicated in brackets.

	Exponential	Senescent
DIN (NO_3+NO_2+NH_4)	2.9 [0.8]	0.8 [0.3]
SiO_4	1.1 [1.2]	1.0 [0.3]
PO_4	0.4 [0.1]	0.2 [0]
Chl-*a*	12 [6]	3 [4]
POCp	45 [12]	4 [5]
DOCp	9 [5]	1 [0.4]
PER	14 [10]	23 [10]
Diatoms (>20 µm)	81 [16]	54 [31]
Autotrophic dinoflagellates (>20 µm)	9 [4]	34 [25]
Autotrophic nanoflagellates	6 [3]	10 [8]
Picophytoplankton	4 [1]	1 [1]

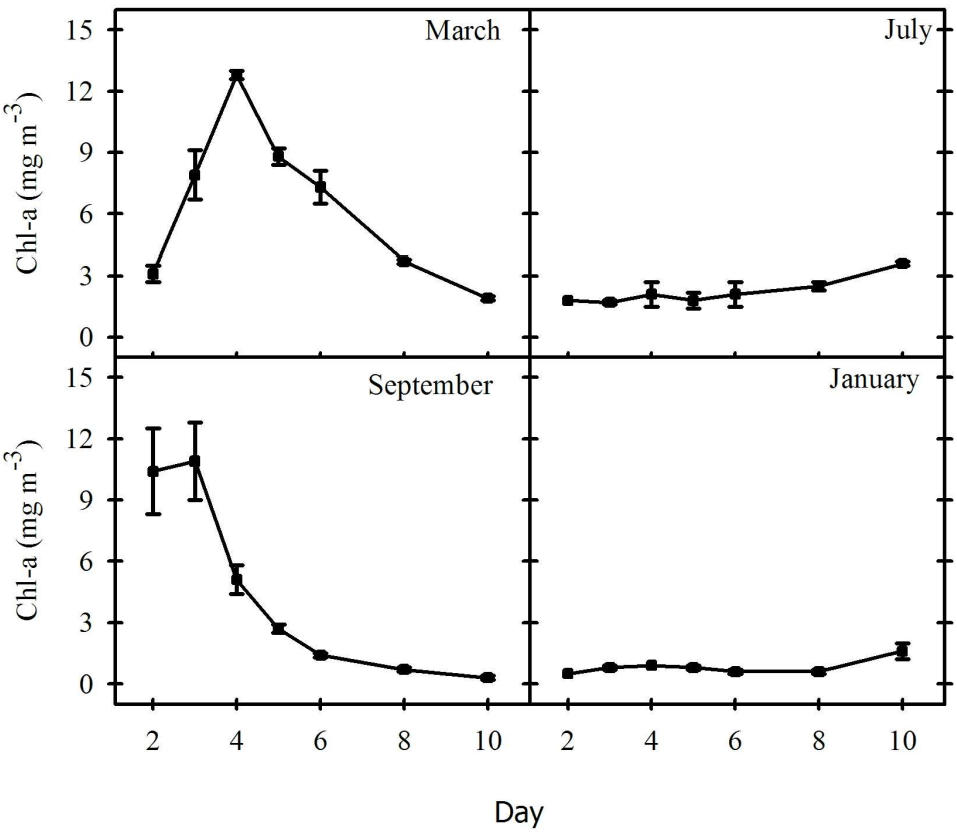
Legends to figures

Figure 1. Chlorophyll *a* concentration during the mesocosm experiments conducted in March, July and September 2005 and January 2006.

Figure 2. Particulate and dissolved primary production rate and percentage of extracellular release (PER) during each mesocosm experiment.

Figure 3. Relationship between particulate (POCp) and dissolved organic carbon production (DOCp) with all pooled measurements.

Figure 4. Relationship between DOCp and bacterial carbon demand (BCD). To compute BCD, bacterial growth efficiency was estimated with the models of a) del Giorgio and Cole (1998) and b) López-Urrutia and Morán (2007). See Methods for details.



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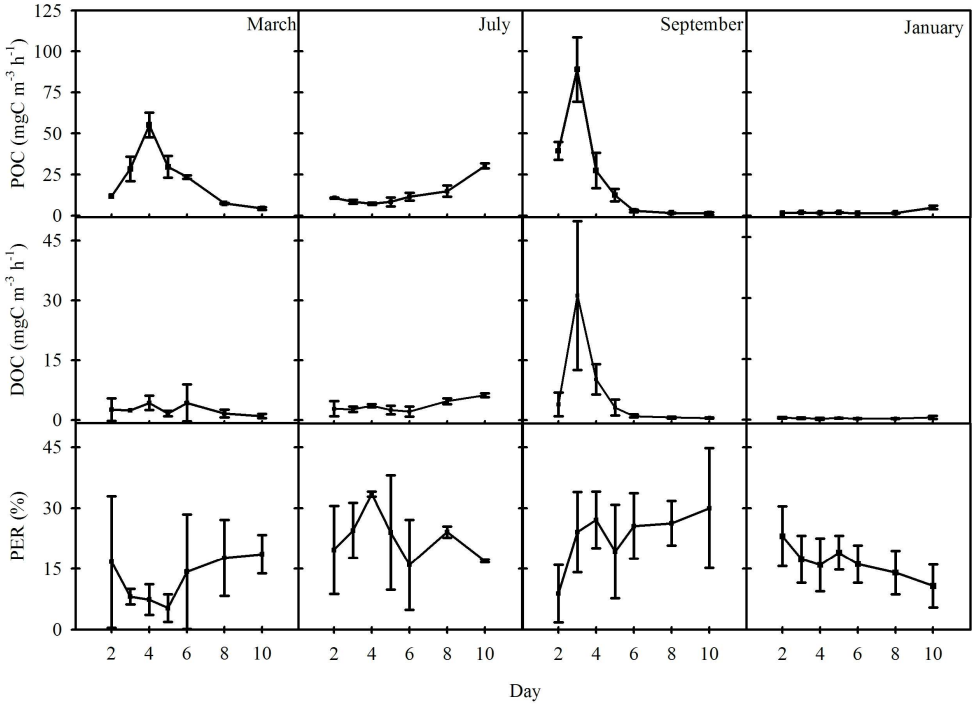

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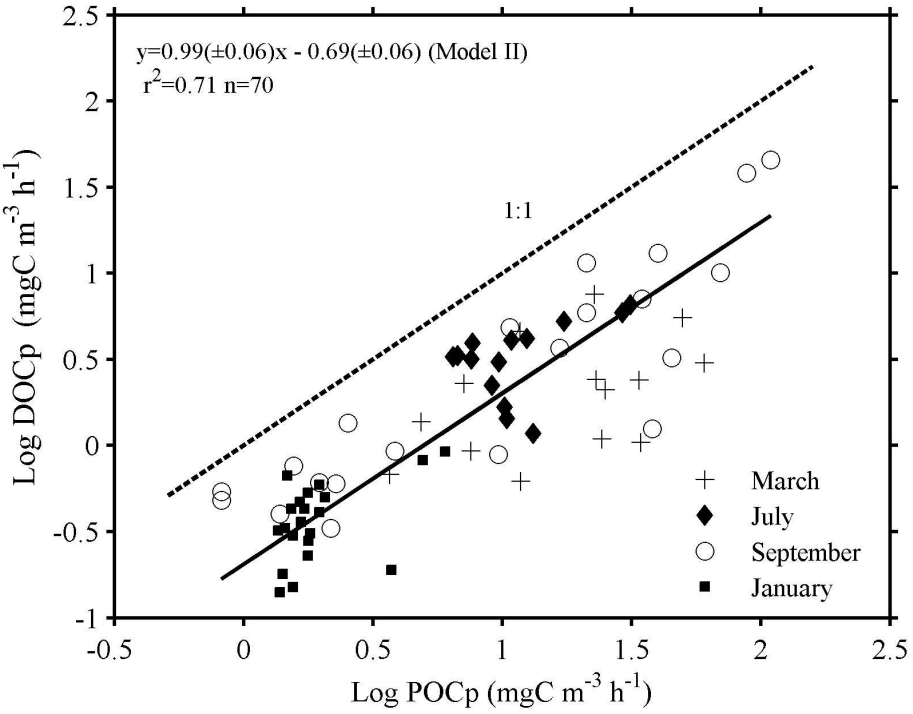

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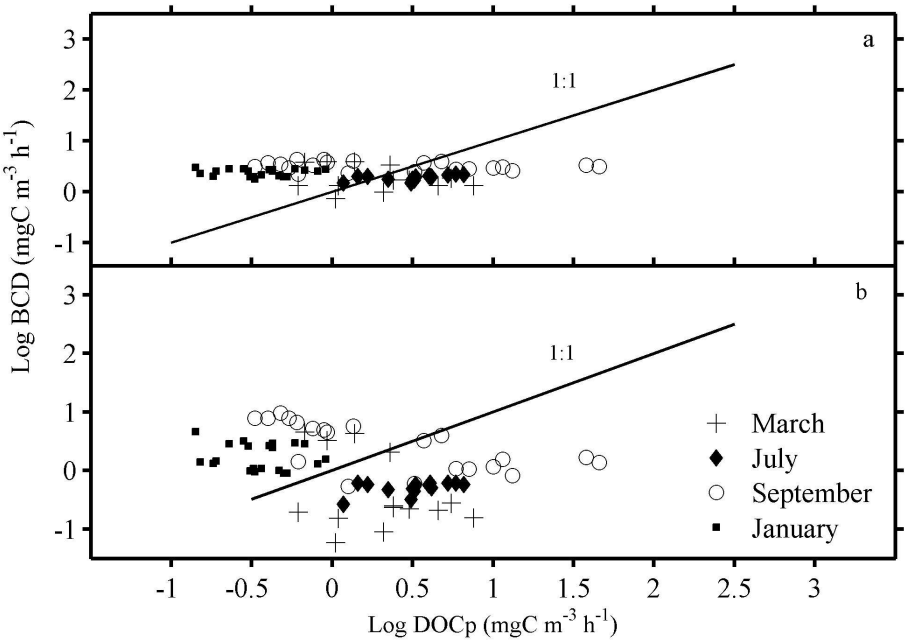

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