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Particulate and dissolved primary production by contrasting phytoplankton assemblages during mesocosm experiments in the Ría de Vigo (NW Spain)

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1 **Particulate and dissolved primary production by contrasting phytoplankton**
2 **assemblages during mesocosm experiments in the Ría de Vigo (NW Spain)**

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19 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 chlorophyll *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.

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4 39 Introduction

5 40
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7 41 The photosynthetic production of dissolved organic carbon (DOCp) by phytoplankton can
8
9 42 represent a substantial fraction of total primary production (Baines and Pace, 1991; Nagata, 2000)
10
11 43 and plays an important role in food web interactions as a source of labile material to fuel bacterial
12
13 44 growth (Cole *et al.*, 1982; Fogg, 1983; Norrman *et al.*, 1995). In spite of its importance, dissolved
14
15 45 organic carbon production (DOCp) is not a routine measurement in most field studies and, as a
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17 46 result, general patterns relating phytoplankton community composition, size structure, total
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19 47 productivity and DOCp have been difficult to establish (Nagata, 2000).
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22 49 Phytoplankton DOCp can originate from the passive diffusion of low molecular weight
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24 50 compounds through the cell membrane but may also represent an adaptive process to cope with
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26 51 high light and low nutrient conditions (Fogg, 1983; Wood and Van Valen, 1990). These
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28 52 mechanisms are not mutually exclusive and can operate concurrently, but have different
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30 53 implications. In the former case, DOCp will tend to be persistent whatever the growth conditions,
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32 54 although a higher relative importance of DOCp could be expected when small cells dominate the
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34 55 community, due to their higher surface/volume ratio (Bjørnsen, 1988; Kiørboe, 1993). In the latter
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36 56 case, phytoplankton, by maintaining their full photosynthetic capacity, can prevent photochemical
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38 57 damage and avoid any lag period in resuming carbon fixation when nutrients become available
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40 58 (Fogg, 1983; Wood and Van Valen, 1990). This mechanism would result in increased relative
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42 59 importance of DOCp during oligotrophic conditions.
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44 61 While some analyses have suggested that the percentage of DOC extracellular release
45
46 62 [$PER=100*DOCp/(DOCp+POCp)$] is a relatively constant value [e. g., 13% in (Baines and Pace,
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48 63 1991), 20% in (Marañón *et al.*, 2005)], variable PER data are recorded in the literature. Mean PER
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50 64 values in coastal and open ocean waters typically range between 10 and 30% (Fogg, 1983; Karl *et*
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52 65 *al.*, 1998; Teira *et al.*, 2001b; Morán *et al.*, 2002a), with most of the higher values being measured
53
54 66 in oligotrophic environments (Obernosterer and Herndl, 1995; Teira *et al.*, 2001a). The differences
55
56 67 found in PER among contrasting systems suggest the existence of a relationship between DOCp and
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58 68 phytoplankton community structure. Although some studies have found significant positive
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60 69 relationships between PER and the relative importance of picophytoplankton (Malinsky-Rushansky
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62 70 and Legrand, 1996; Teira *et al.*, 2001a; Morán *et al.*, 2002b), others have found no relationship
63
64 71 between PER and phytoplankton cell size (Finkel, 1998; Marañón *et al.*, 2004). The variability of
65
66 72 DOCp in marine waters has rarely been addressed in conjunction with a detailed analysis of

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4 73 phytoplankton species composition (Lancelot, 1983). As a result, is it unclear if changes in the
5
6 74 dominant phytoplankton groups are also associated with differences in the importance of DOCp.
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10 76 Work with laboratory cultures has shown that phytoplankton respond to nutrient limitation
11 77 with increased synthesis of extracellular organic compounds such as carbohydrates (Mykkestad,
12 78 1977; Lancelot, 1983; Borsheim *et al.*, 2005). In this regard, a higher PER has been reported for
13 79 cells growing under phosphorus or nitrogen limitation (Obernosterer and Herndl, 1995). However,
14
15 80 the evolution of PER during the different growth stages of a given phytoplankton community, and
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17 81 under contrasting oceanographic conditions, has not yet been determined.
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21
22 83 The Ría de Vigo (NW Iberian Peninsula) is a productive ecosystem characterised by a seasonal
23
24 84 cycle of upwelling events between April and October, a downwelling period from October to March
25
26 85 (Nogueira *et al.*, 1997), and a transient period between the two phases. Blooms are dominated by
27 86 diatoms in spring and by dinoflagellates in autumn (Tilstone *et al.*, 1994; Crespo *et al.*, 2006).
28
29 87 During the upwelling season, the phytoplankton community is dominated by microphytoplankton
30
31 88 (>20 μm) (Cermeño *et al.*, 2006), and euphotic zone integrated primary production rates can reach
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33 89 1-2 $\text{gC m}^{-2} \text{d}^{-1}$ (Tilstone *et al.*, 1999). A shift in phytoplankton size structure is observed during
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35 90 downwelling events, when the contribution of pico (<2 μm) and nano (2-20 μm) phytoplankton to
36
37 91 total biomass increases. During this period, plankton community respiration accounts for more than
38
39 92 80% of the primary production, thus most of the organic matter is remineralized within the water
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41 93 column (Cermeño *et al.*, 2006). The marked seasonal and short-term variability of the Ría de Vigo
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43 94 makes it an excellent scenario to test if the relative importance of DOCp varies among the different
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45 95 phytoplankton communities that exist under the different hydrographic conditions.
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48 97 Our experimental approach was to collect distinct phytoplankton assemblages characteristic of
49 98 four contrasting oceanographic periods throughout the year and monitor their dynamics during 9-
50 99 day long mesocosm experiments. This approach ensured that we studied a wide range of
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52 100 phytoplankton communities in terms of physiological state, species composition and size structure.
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54 101 Our main objectives were: i) to determine if the relative importance of DOCp changes during the
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56 102 different growth phases of natural phytoplankton assemblages and ii) to assess if variations in the
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58 103 taxonomical composition of the phytoplankton community, associated with different hydrographic
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60 104 conditions during the year, result in changes in the relative contribution of DOCp to total primary
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106 105 production.

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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 m³ in volume (1.5 m in diameter and 2 m deep) were filled at a central station (42° 14.09'N, 8° 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 (-20°C) 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 -20°C. Chl-*a* concentration was determined fluorimetrically using a Turner-TD-700 fluorometer previously calibrated with pure Chl-*a*.

Phytoplankton community composition and biomass

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4 139 Picophytoplankton abundance was determined in 1.8 mL-samples, fixed with
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6 140 paraformaldehyde (1% final concentration) and glutaraldehyde (0.05% final concentration), using a
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8 141 FACSCalibur flow cytometer (Calvo-Díaz and Morán, 2006). Carbon biomass was estimated
9
10 142 assuming a spherical shape and using volume-to-carbon conversion factors: 230 fg C μm^{-3} for
11 143 *Synechococcus*, 240 fg C μm^{-3} for *Prochlorococcus* and 237 fg C μm^{-3} for picoeukaryotes (Worden
12
13 144 *et al.*, 2004). For the analysis of nanophytoplankton, subsamples of 10 mL were fixed with buffered
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15 145 0.2- μm filtered formaldehyde (2% final concentration) and then filtered through 0.2- μm black
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17 146 Millipore-Isopore filters placed on top of 0.45- μm Millipore backing filters. Epifluorescence
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19 147 microscopy was used to determine autotrophic organisms, which were enumerated under blue light
20 148 excitation. It was assumed that all organisms showing red autofluorescence when excited with blue
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22 149 light were autotrophic, even though mixotrophic organisms are not correctly identified with this
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24 150 technique. Dimensions were taken for several individuals and cell volumes were calculated
25
26 151 assuming a spherical shape or after approximation to the nearest geometrical shape (Hillebrand *et*
27 152 *al.*, 1999). Cell carbon was estimated following Verity *et al.* (Verity *et al.*, 1992) for nanoflagellates
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29 153 and Strathmann (Strathmann, 1967) for small naked dinoflagellates belonging to the nanoplankton
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31 154 size fraction.

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

57 155 Photosynthetic production of POC and DOC
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157 The production of particulate organic carbon (POCp) and dissolved organic carbon (DOCp)
158 was determined by carrying out *in situ* (SIS) incubations with the radioisotope ^{14}C . We used

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4 159 incubators that were cooled with running seawater from the laboratory's continuous supply. The
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6 160 incubator was located on the terrace of the Instituto de Investigaciones Marinas and the experiments
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8 161 were thus conducted under natural irradiance conditions. For each sample, three light and two dark
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10 162 acid-washed Pyrex glass bottles (50 mL) were filled and spiked with 10 μCi of $\text{NaH}^{14}\text{CO}_3$. At the
11 163 end of the incubation, which lasted 2-3 hours, two 5-mL aliquots from each incubation bottle were
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13 164 filtered through 0.2- μm pore size polycarbonate filters of 25-mm in diameter using low vacuum
14
15 165 pressure (< 50 mmHg) to avoid cell breakage and the loss of particulate, labelled material into the
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17 166 filtrate. Previous experiments conducted with the same method strongly indicate that the filtration
18 167 procedure used does not cause cell breakage (Marañón *et al.* 2004).
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20 168
21
22 169 To remove the inorganic ^{14}C that was not incorporated into the cells, the filtrates were
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24 170 acidified to a pH of ~ 2 with 100 μl of 50% HCl, and then maintained for ~ 12 h in 20-mL open
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26 171 scintillation vials placed on an orbital shaker. After inorganic ^{14}C removal, 10 mL of high sample
27 172 capacity scintillation cocktail were added to each 5 mL filtrate. The inorganic ^{14}C present in the
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29 173 filters was removed by exposing them to concentrated HCl fumes for 12 h. The filters were then
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31 174 placed in 5-mL scintillation vials to which 4 mL of scintillation cocktail were added. The
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33 175 radioactivity in each sample was determined in a Packard Tri-Carb 3100TR scintillation counter
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35 176 which used the external standard method for quenching correction. The dark bottle value of
36 177 disintegrations per minute (DPM) was subtracted from the light bottle DPMs in order to calculate
37
38 178 the rates of DOC and POC production. In all calculations, we used a value of 25,700 mgC m^{-3} for
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40 179 the concentration of dissolved inorganic carbon and a value of 1.05 for the isotopic discrimination
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42 180 factor.

43 181 44 45 182 Bacterial production

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49 184 Bacterial heterotrophic production (BP) was estimated using the ^3H -Leucine method (Kirchman
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51 185 *et al.*, 1985) but in Eppendorf vials which were processed by centrifugation and trichloroacetic acid
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53 186 (TCA) rinsing. Four replicates of 1.2 mL were taken for each mesocosm as well as two TCA-killed
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55 187 controls. The Leucine tracer was added at a 40 nM final concentration in incubations lasting
56 188 approximately 2 h at *in situ* temperatures and in dark conditions. The incorporation was stopped
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58 189 with the addition of 120 μL of cold 50% TCA to the samples which, after mixing, were kept frozen
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60 190 at -20°C until processing by the centrifugation method (Smith and Azam, 1992). The samples were
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192 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

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4 193 two replicate experiments. We gently filtered seawater from the mesocosms through 0.6 μm
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6 194 polycarbonate filters (Millipore, DTTP) in order to remove predators. Then, we diluted the water
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8 195 (1:9) with 0.2 μm filtered (Millipore, GTTP) seawater and incubated the mixture in 2-L acid-clean
9
10 196 polycarbonate bottles in the dark in a room adjusted to the *in situ* temperature. Subsamples were
11 197 taken for Leucine incorporation and bacterial abundance measurements at every 12-24 hours until
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13 198 bacteria reached the stationary growth phase. The amount of biomass produced per unit Leucine
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15 199 incorporated was computed with the cumulative method (Bjørnsen and Kuparinen, 1991), which
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17 200 maximizes the use of the available data. The obtained empirical factors were: 1.2 kgC mol Leu⁻¹
18 201 (March 2005), 0.18 kgC mol Leu⁻¹ (July 2005), 0.28 kgC mol Leu⁻¹ (September 2005) and 0.95 kgC
19 202 mol Leu⁻¹ (January 2006). Bacterial carbon demand (BCD) was calculated by adding the measured
20 202 BP rates and estimates of bacterial respiration (BR). In order to compute BR, bacterial growth
21
22 203 efficiency (BGE) was estimated with two different models. The model proposed by del Giorgio and
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24 204 Cole (del Giorgio and Cole, 1998) is based on bacterial production (BP)

$$\text{BGE} = (0.037 + 0.65\text{BP}) / (1.8 + \text{BP})$$

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33 209 whereas the model of López-Urrutia and Morán (López-Urrutia and Morán, 2007) is based on
34 210 chlorophyll *a* concentration (Chl-*a*):

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

35 36 211 37 38 212 39 40 213 41 214 42 43 215 **Results**

44
45 216
46
47 217 Nutrients, chlorophyll *a* and phytoplankton biomass

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51 219 The initial conditions of each experiment reflect the seasonal variability in the hydrodynamic
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53 220 conditions of the Ría de Vigo. In March 2005, the high nutrient concentrations (Table I) allowed the
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55 221 development of a phytoplankton bloom. Chl-*a* concentration in this experiment reached more than
56 222 12 mg m⁻³ (Fig. 1), and the phytoplankton community was dominated by diatoms (82%) (Table II).
57
58 223 In July, the warm temperatures (21° C) and low nutrient concentrations indicated that a marked
59
60 224 thermal stratification of the water column was present at the time of sampling. Lower Chl-*a*
225 concentrations (2 mg m⁻³) were measured (Table I), but the relative contribution of diatoms to the
226 total biomass was still large (77%) (Table II). The low temperature and high nutrient concentrations

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4 227 observed at the beginning of the September experiment (Table I) corresponded to the well-
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6 228 documented upwelling events that occur in Ría de Vigo from April to October. The decay of a
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8 229 bloom was observed during this experiment: Chl-*a* concentrations decreased from $>10 \text{ mg m}^{-3}$ on
9
10 230 the first two days of the experiment to $<1 \text{ mg m}^{-3}$ (Fig. 1), and the biomass was dominated by
11 231 diatoms (62%) and autotrophic dinoflagellates (25%) (Table II). A markedly different
12
13 232 phytoplankton community structure was observed during the January experiment, when the biomass
14
15 233 was dominated by flagellates and picophytoplankton (Table II). The high nutrient concentration and
16
17 234 low Chl-*a* concentration (Table I) reflected the low light conditions and the strong vertical mixing
18 235 that are characteristic of the winter season in the Ría de Vigo.

20 236 21 22 237 Dissolved and particulate organic carbon production 23

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25
26 239 The variability in both POCp and DOCp showed similar patterns to those observed in Chl-*a*
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28 240 concentration (Fig. 2). POCp was lower in July ($<10 \text{ mgC m}^{-3} \text{ h}^{-1}$) and January ($<1 \text{ mgC m}^{-3} \text{ h}^{-1}$)
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30 241 than during the March and September experiments, when values above $50 \text{ mgC m}^{-3} \text{ h}^{-1}$ were
31 242 recorded during the peak of the phytoplankton bloom. The variability in DOCp differed from that of
32
33 243 POCp in the March experiment, when no clear maximum was observed (Fig. 2). High rates of
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35 244 DOCp ($>30 \text{ mgC m}^{-3} \text{ h}^{-1}$) were measured in September during the upwelling season experiment.

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37 245
38 246 A highly significant relationship was found between POC and DOC production rates ($r^2=0.71$,
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40 247 $p<0.001$, $n=70$, Fig. 3). The slope of the regression line (Model II) between the logarithms of DOCp
41
42 248 and POCp was not significantly different from 1 (Clarke test, $p=0.915$), indicating that the relative
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44 249 contribution of DOCp to total primary production did not change across the range of POCp. No
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46 250 clear pattern of temporal variability in the percentage of extracellular release (PER) was found
47 251 during the experiments (Fig. 2). The mean PER was 19% (SD, 9), with the lowest value found in
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49 252 March (13% [SD, 5]) (Table II). There were no significant differences in PER between experiments
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51 253 (RMANOVA, $p=0.296$) (Table II). Similarly, we found no association between the changes in
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53 254 taxonomic composition and the PER values (Table II).

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56 256 We grouped all our observations into three groups according to the measured Chl-*a*
57
58 257 concentration ($<1 \text{ mg m}^{-3}$, $1-4 \text{ mg m}^{-3}$ and $>4 \text{ mg m}^{-3}$) in order to assess if PER changed with
59
60 258 phytoplankton standing stocks (Table III). POCp and DOCp increased progressively in groups with
259 higher Chl-*a* concentration, and the size structure also changed significantly: in low Chl-*a* samples
260 the pico- and nano-phytoplankton size classes showed the largest relative contribution (33 and 40%,

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4 261 respectively), whereas in high Chl-*a* samples the microphytoplankton was clearly dominant (87%).
5
6 262 In contrast, PER did not show any significant differences between groups of samples (ANOVA,
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8 263 $p=0.099$).

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10 264
11 265 In order to determine if dissolved primary production was favoured during the decaying phase
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13 266 of the phytoplankton bloom, we compared the measurements conducted in the exponential growth
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15 267 versus the senescent phases of the March and September experiments. The first three days of the
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17 268 March experiment and the first two days of the September experiment were considered as belonging
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19 269 to the exponential growth phase. The last two days from both experiments were considered for the
20 270 senescent phase. Clear differences between the exponential and the senescent phases were observed
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22 271 (Table IV). The exponential phase was characterized by higher concentrations of dissolved
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24 272 inorganic nitrogen and phosphate and by higher phytoplankton biomass, as inferred from the Chl-*a*
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26 273 concentrations. Changes in the phytoplankton community also occurred, with diatoms clearly
27 274 dominating during the exponential phase but sharing dominance with pigmented dinoflagellates
28
29 275 during the senescent phase. Rates of POCp and DOCp also decreased during the senescent phase,
30
31 276 while PER showed a significant increase (ANOVA, $p=0.022$) from a mean value of 14% (SD, 10)
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33 277 in the exponential phase to a mean value of 23% (SD, 10) during the senescent phase (Table IV).

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36 279 Dissolved organic carbon production and bacterial carbon demand
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40 281 In order to assess whether photosynthetic DOCp was the main source of organic matter for
41
42 282 bacteria, bacterial carbon demand (BCD) was calculated from measurements of bacterial
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44 283 production. There was a lack of correlation between DOCp and BCD, irrespective of the model
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46 284 used to estimate the bacterial growth efficiency. However, the dispersion of the BCD data points
47 285 changed between these two models. Changes in BCD between experiments were more evident
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49 286 when the model of López-Urrutia and Morán was used, as this model is based on Chl-*a*
50
51 287 concentration. During the January experiment, BCD clearly exceeded DOCp, indicating that
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53 288 phytoplankton exudation was not sufficient to sustain bacterial metabolism. The opposite occurred
54 289 during the July experiment, when in most cases DOCp was larger than BCD. However, the overall
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56 290 lack of correlation between these variables suggests that bacterial metabolism and phytoplankton
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58 291 exudation are largely uncoupled in this coastal ecosystem.
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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

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4 326 significant fraction of primary production in coastal, productive waters, irrespective of
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6 327 phytoplankton productivity and species composition.
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10 329 DOCp and size structure
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13 331 There are physiological reasons to expect an effect of phytoplankton size structure on the
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15 332 relative importance of DOCp. The increased surface to volume ratio of small cells should favour a
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17 333 higher diffusion of small molecular weight compounds through the membrane (Bjørnsen, 1988;
18 334 Kiørboe, 1993). In fact, increased PER values have been reported for cultures of small sized
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20 335 phytoplankton (Malinsky-Rushansky and Legrand, 1996). In contrast, Finkel (Finkel, 1998), using a
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22 336 set of 8 diatoms species, ranging >5 orders of magnitude in cell volume, did not find any size
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24 337 dependence on the volume or carbon specific exudation rates. In our study, we did not observe any
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26 338 relationship between PER and size structure, not even in the January experiment, when the relative
27 339 importance of picophytoplankton was much larger. Our results suggest that the observed increase in
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29 340 PER in oligotrophic environments such as the Atlantic subtropical gyres (Teira *et al.*, 2001), where
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31 341 picophytoplankton are dominant both in terms of biomass and production (Marañón *et al.*, 2001),
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33 342 may not necessarily reflect a direct effect of phytoplankton cell size on exudation, but result from
34 343 the very low nutrient concentrations prevailing in these regions, which are strongly limiting for
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36 344 phytoplankton production and growth.
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40 346 DOCp and bloom development
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43 348 It is now established that extracellular release of recent photosynthate is a normal function of
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45 349 healthy cells, and that it is a process closely related to photosynthetic carbon assimilation (Mague *et*
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47 350 *al.*, 1980; Bjørnsen, 1988; Nagata, 2000). However, it has also been observed that high percentages
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49 351 of release are often associated with particular conditions experienced by the phytoplankton. These
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51 352 include very high or very low irradiances and abrupt changes in nutrient concentrations (Fogg,
52 353 1983; Nagata, 2000). Several studies have shown increases in PER associated with the stationary
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54 354 phase after a phytoplankton bloom, when nutrients became scarce and limiting for growth (Norrman
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56 355 *et al.*, 1995; Obernosterer and Herndl, 1995; Nagata, 2000). In our study, we did observe
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58 356 differences between the exponential and the senescent phases of the two phytoplankton blooms. The
59 357 highest PER was found during the senescent period, when the concentration of dissolved inorganic
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358 nitrogen was low and presumably limiting for phytoplankton growth. This observation supports the
359 view that the release of dissolved photosynthate under nutrient limitation may serve as a mechanism

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4 360 to protect the cell's photosynthetic machinery, whereby organic carbon is excreted during periods of
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6 361 energy excess and nutrient limitation. This mechanism would allow the cells to keep their
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8 362 photosynthetic metabolism active for rapid growth whenever nutrients become available again
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10 363 (Wood and Van Valen, 1990).

11 364 12 13 365 Coupling between phytoplankton DOC release and bacterial production 14 15 366

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17 367 It has been estimated that nearly half of the daily photosynthetic production is released, through
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19 368 different mechanisms, as dissolved organic carbon that may be available for heterotrophic bacterial
20 369 consumption (Nagata, 2000). The importance of the photosynthetic production of DOC to fulfill the
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22 370 bacterial carbon demand (BCD) strongly depends on the trophic structure of the microbial plankton
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24 371 community and on the nature and magnitude of allochthonous sources of dissolved organic carbon
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26 372 (Morán *et al.*, 2002a; Borsheim *et al.*, 2005). Our experiments were conducted in an ecosystem that
27 373 sustains high standing stocks of phytoplankton and where intense microzooplankton grazing takes
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29 374 place (Teixeira and Figueiras, 2009), which is likely to lead to an important production of labile
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31 375 DOC through egestion (Nagata, 2000). In addition, allochthonous inputs of dissolved organic matter
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33 376 of continental origin have also been shown to be significant in this system (Álvarez-Salgado *et al.*,
34 377 2001; Gago *et al.*, 2005). However, the DOC of continental origin is mostly refractory and,
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36 378 therefore, should not support a significant portion of the estimated BCD. Consumption of
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38 379 previously produced labile DOC seems more plausible, as demonstrated by Alvarez-Salgado *et al.*
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40 380 (2001). Together, these processes may explain the lack of coupling between phytoplankton DOC
41 381 release and bacterial metabolism.
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43 382 44 45 383 Conclusions 46 47 384

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49 385 The release of recently fixed photosynthetic carbon appeared to be a relatively constant process
50 386 in the Ría de Vigo, irrespective of hydrographic period, phytoplankton size structure and taxonomic
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52 387 composition. However, the relative importance of dissolved primary production did tend to increase
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54 388 during the decaying phase of phytoplankton blooms. Bacterial metabolism and phytoplankton
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56 389 exudation were largely uncoupled, indicating that additional sources of DOC, both autochthonous
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58 390 and allochthonous, are likely to be used by bacteria. On average, DOC_p contributed 19% of total
59 391 primary production, which illustrates the importance of dissolved primary production in a coastal,
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392 productive ecosystem.
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For Peer Review

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

	Mar-05	Jul-05	Sep-05	Jan-06
	n=2	n=2	n=3	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 ($\text{NO}_3+\text{NO}_2+\text{NH}_4$)	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- <i>a</i>	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 μ m)		82 [4]	77 [9]	62 [25]	5 [6]
Autotrophic dinoflagellates (>20 μ m)		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]

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Table III. Mean values of the contribution of each phytoplankton size class to total Chl-*a* concentration, the rates of POCp and DOCp ($\text{mgC m}^{-3} \text{h}^{-1}$), 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- <i>a</i> 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]

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Table IV. Mean nutrient concentration ($\mu\text{mol kg}^{-1}$), particulate and dissolved organic carbon production (POCp and DOCp) ($\text{mgC m}^{-3} \text{h}^{-1}$), 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 ($\text{NO}_3+\text{NO}_2+\text{NH}_4$)	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- <i>a</i>	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]

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563 **Legends to figures**

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

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

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

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574 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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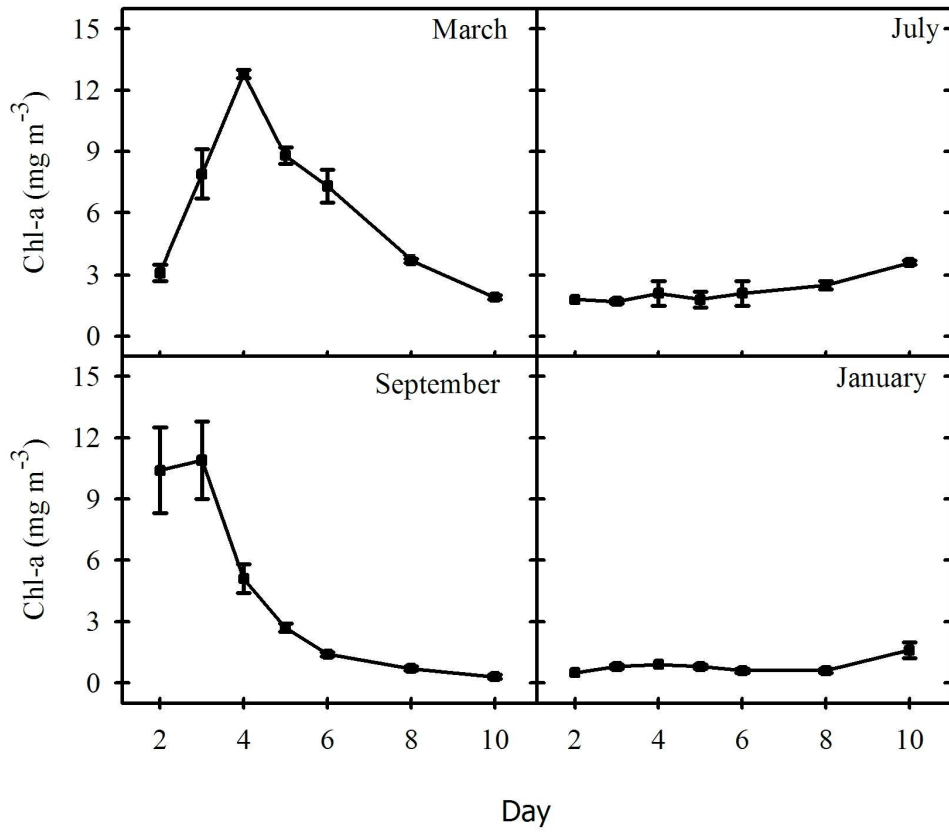
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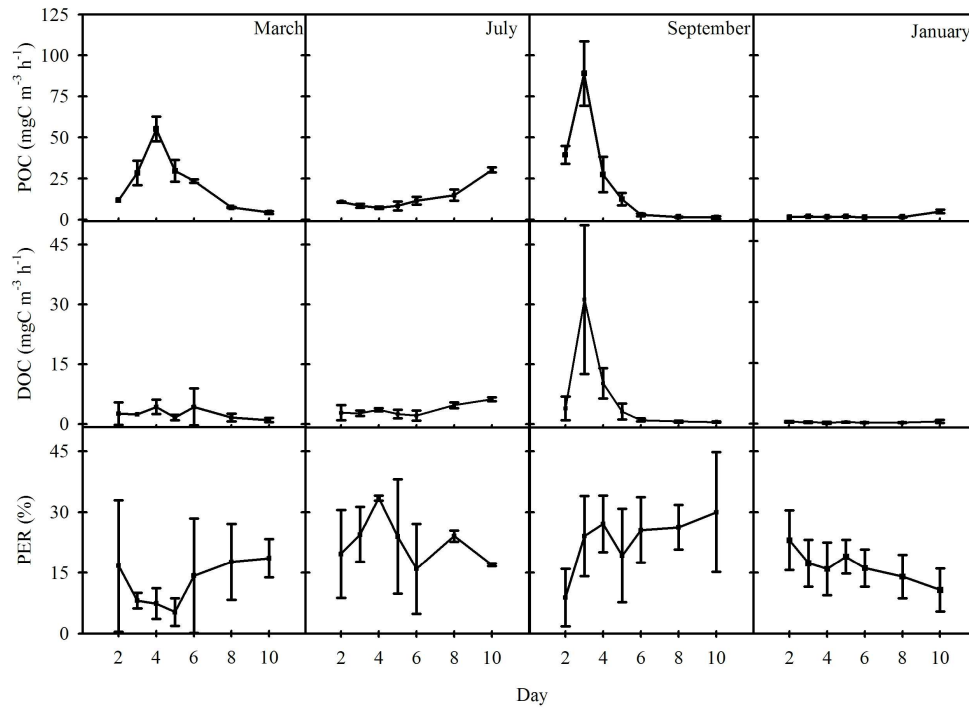
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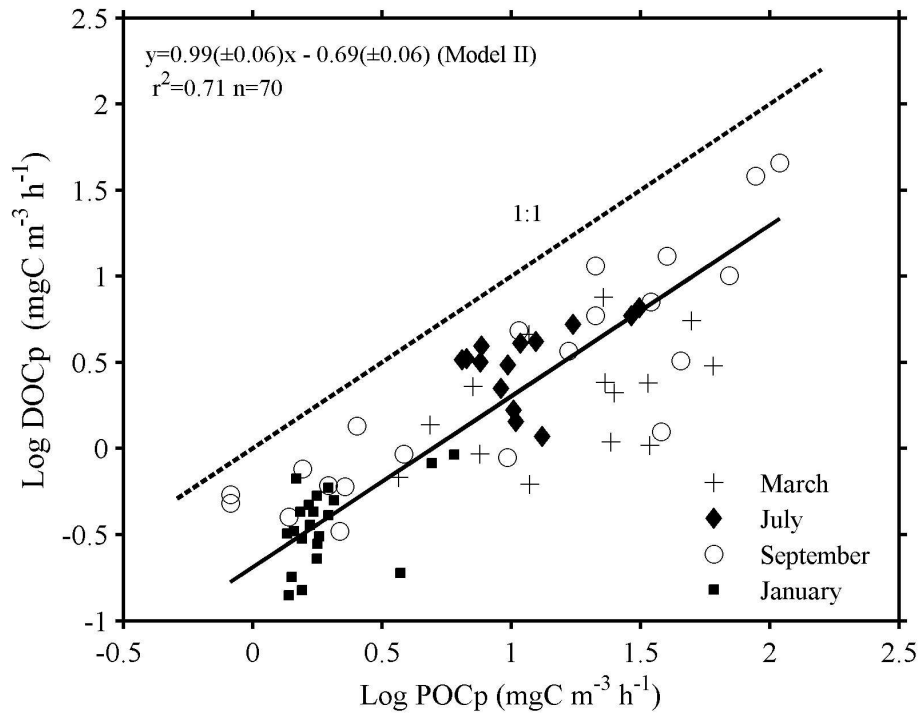


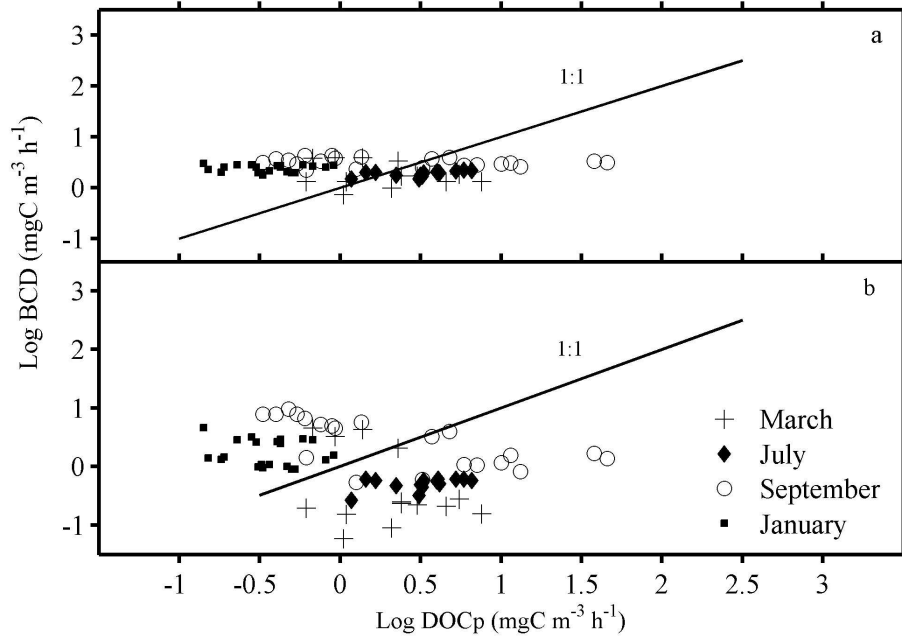
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217x157mm (300 x 300 DPI)





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