



HAL
open science

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

Daffne Celeste Lopez-Sandoval, Emilio Marañón, Ana Fernández, Jose González, Josep M Gasol, Itziar Lekumberri, Manuel Varela, Alejandra Calvo-Díaz, Xosé Anxelu G. Morán, Xosé Antón Álvarez-Salgado, et al.

► To cite this version:

Daffne Celeste Lopez-Sandoval, Emilio Marañón, Ana Fernández, Jose González, Josep M Gasol, et al.. Particulate and dissolved primary production by contrasting phytoplankton assemblages during mesocosm experiments in the Ría de Vigo (NW Spain). *Journal of Plankton Research*, 2010, 32 (9), pp.1231. 10.1093/plankt/FBQ045 . hal-00587973

HAL Id: hal-00587973

<https://hal.science/hal-00587973>

Submitted on 22 Apr 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



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

Journal:	<i>Journal of Plankton Research</i>
Manuscript ID:	JPR-2010-019.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	16-Mar-2010
Complete List of Authors:	Lopez-Sandoval, Daffne; Universidad de Vigo, Ecología y Biología Animal Marañón, Emilio; Universidad de Vigo, Ecología y Biología Animal Fernández, Ana; Universidad de Vigo, Ecología y Biología Animal González, Jose; Universidad de Vigo, Ecología y Biología Animal Gasol, Josep; Institut de Ciències del Mar-CSIC, Biologia Marina i Oceanografia Lekumberrri, Itziar; Institut de Ciències del Mar-CSIC, Biologia Marina i Oceanografia Varela, Manuel; Instituto Español de Oceanografía, Centro Oceanográfico de A Coruña Calvo-Díaz, Alejandra; IEO Gijón-Xixón, Medio Marino Morán, Xosé Anxelu; IEO Gijón-Xixón, Medio Marino Álvarez-Salgado, Xosé Antón; Instituto de Investigaci3n Mariñas (CSIC), Oceanography Department Figueiras, Francisco; Instituto de Investigaci3n Mariñas (CSIC), Oceanography Department
Keywords:	Phytoplankton, dissolved organic carbon, Ria de Vigo



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

3
4 D. C. López-Sandoval¹, E. Marañón¹, A. Fernández¹, J. González¹, J. M. Gasol², I. Lekunberri², M.
5 Varela³, A. Calvo-Díaz⁴, X. A. G. Morán⁴, X. A. Álvarez-Salgado⁵, F. G. Figueiras⁵

6
7 Correspondence to: Daffne López (daffne@uvigo.es)

8
9 ¹ Departamento de Ecología y Biología Animal, Universidad de Vigo, 36210 Vigo, Spain

10 ² Institut de Ciències del Mar-CSIC, Pg. Marítim de la Barceloneta 37-39, 08003 Barcelona,
11 Catalunya, Spain

12 ³ Instituto Español de Oceanografía, Centro Oceanográfico de A Coruña, Aptdo. 130, 15080, A
13 Coruña, Spain

14 ⁴ Centro Oceanográfico de Xixón, Instituto Español de Oceanografía, Camín de L'Arbeyal, s/n,
15 33212, Xixón, Spain

16 ⁵ Instituto de Investigaciones Marinas-CSIC, Eduardo Cabello 6, 36208 Vigo, Spain

17
18
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.

1
2
3
4 39 Introduction

5 40
6
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
16
17 46 result, general patterns relating phytoplankton community composition, size structure, total
18
19 47 productivity and DOCp have been difficult to establish (Nagata, 2000).
20 48

21
22 49 Phytoplankton DOCp can originate from the passive diffusion of low molecular weight
23
24 50 compounds through the cell membrane but may also represent an adaptive process to cope with
25
26 51 high light and low nutrient conditions (Fogg, 1983; Wood and Van Valen, 1990). These
27
28 52 mechanisms are not mutually exclusive and can operate concurrently, but have different
29
30 53 implications. In the former case, DOCp will tend to be persistent whatever the growth conditions,
31
32 54 although a higher relative importance of DOCp could be expected when small cells dominate the
33
34 55 community, due to their higher surface/volume ratio (Bjørnsen, 1988; Kiørboe, 1993). In the latter
35
36 56 case, phytoplankton, by maintaining their full photosynthetic capacity, can prevent photochemical
37
38 57 damage and avoid any lag period in resuming carbon fixation when nutrients become available
39
40 58 (Fogg, 1983; Wood and Van Valen, 1990). This mechanism would result in increased relative
41
42 59 importance of DOCp during oligotrophic conditions.
43 60

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,
47
48 63 1991), 20% in (Marañón *et al.*, 2005)], variable PER data are recorded in the literature. Mean PER
49
50 64 values in coastal and open ocean waters typically range between 10 and 30% (Fogg, 1983; Karl *et*
51
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
57
58 68 phytoplankton community structure. Although some studies have found significant positive
59
60 69 relationships between PER and the relative importance of picophytoplankton (Malinsky-Rushansky
61
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

1
2
3
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.
7
8 75

9
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
16
17 81 under contrasting oceanographic conditions, has not yet been determined.
18
19
20 82

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
32
33 89 1-2 $\text{gC m}^{-2} \text{d}^{-1}$ (Tilstone *et al.*, 1999). A shift in phytoplankton size structure is observed during
34
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
40
41 93 column (Cermeño *et al.*, 2006). The marked seasonal and short-term variability of the Ría de Vigo
42
43 94 makes it an excellent scenario to test if the relative importance of DOCp varies among the different
44
45 95 phytoplankton communities that exist under the different hydrographic conditions.
46
47 96

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
51
52 100 phytoplankton communities in terms of physiological state, species composition and size structure.
53
54 101 Our main objectives were: i) to determine if the relative importance of DOCp changes during the
55
56 102 different growth phases of natural phytoplankton assemblages and ii) to assess if variations in the
57
58 103 taxonomical composition of the phytoplankton community, associated with different hydrographic
59
60 104 conditions during the year, result in changes in the relative contribution of DOCp to total primary
105
106 105 production.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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- μ m 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 μ m 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

138

1
2
3
4 139 Picophytoplankton abundance was determined in 1.8 mL-samples, fixed with
5
6 140 paraformaldehyde (1% final concentration) and glutaraldehyde (0.05% final concentration), using a
7
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
14
15 145 0.2- μm filtered formaldehyde (2% final concentration) and then filtered through 0.2- μm black
16
17 146 Millipore-Isopore filters placed on top of 0.45- μm Millipore backing filters. Epifluorescence
18
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
21
22 149 light were autotrophic, even though mixotrophic organisms are not correctly identified with this
23
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
28
29 153 and Strathmann (Strathmann, 1967) for small naked dinoflagellates belonging to the nanoplankton
30
31 154 size fraction.

32
33
34 For microphytoplankton determinations samples of 100 mL preserved in Lugol's iodine were
35
36 sedimented in composite sedimentation chambers and observed with an inverted microscope. The
37
38 organisms were counted and identified to the species level when possible. The small species were
39
40 enumerated from two transects scanned at $\times 400$ and $\times 250$, whereas the larger species were counted
41
42 by scanning the whole slide at $\times 100$. Phototrophic and heterotrophic species of dinoflagellates,
43
44 flagellates and ciliates were differentiated following Lessard and Swift (Lessard and Swift, 1986)
45
46 and also using epifluorescence microscopy. Cell biovolumes were estimated according to
47
48 Hillebrand *et al.* (Hillebrand *et al.*, 1999) and cell carbon calculated following Strathmann
49
50 (Strathmann, 1967) for diatoms and dinoflagellates, Verity *et al.* (Verity *et al.*, 1992) for flagellates
51
52 and Putt and Stoecker (Putt and Stoecker, 1989) for aloricate ciliates. All organisms containing
53
54 chloroplasts were assumed to be autotrophic. Dinoflagellates and ciliates $< 20 \mu\text{m}$ as well as single
55
56 diatoms $< 20 \mu\text{m}$ counted with this technique were assigned to the nanoplankton fraction.

57 155 Photosynthetic production of POC and DOC
58
59
60 156

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

1
2
3
4 159 incubators that were cooled with running seawater from the laboratory's continuous supply. The
5
6 160 incubator was located on the terrace of the Instituto de Investigaciones Marinas and the experiments
7
8 161 were thus conducted under natural irradiance conditions. For each sample, three light and two dark
9
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
12
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
16
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).
19

20 168
21
22 169 To remove the inorganic ^{14}C that was not incorporated into the cells, the filtrates were
23
24 170 acidified to a pH of ~ 2 with 100 μl of 50% HCl, and then maintained for ~ 12 h in 20-mL open
25
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
28
29 173 filters was removed by exposing them to concentrated HCl fumes for 12 h. The filters were then
30
31 174 placed in 5-mL scintillation vials to which 4 mL of scintillation cocktail were added. The
32
33 175 radioactivity in each sample was determined in a Packard Tri-Carb 3100TR scintillation counter
34
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
39
40 179 the concentration of dissolved inorganic carbon and a value of 1.05 for the isotopic discrimination
41
42 180 factor.

43 181 44 45 182 Bacterial production

46
47 183
48
49 184 Bacterial heterotrophic production (BP) was estimated using the ^3H -Leucine method (Kirchman
50
51 185 *et al.*, 1985) but in Eppendorf vials which were processed by centrifugation and trichloroacetic acid
52
53 186 (TCA) rinsing. Four replicates of 1.2 mL were taken for each mesocosm as well as two TCA-killed
54
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
57
58 189 with the addition of 120 μL of cold 50% TCA to the samples which, after mixing, were kept frozen
59
60 190 at -20°C until processing by the centrifugation method (Smith and Azam, 1992). The samples were
191
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

1
2
3
4 193 two replicate experiments. We gently filtered seawater from the mesocosms through 0.6 μm
5
6 194 polycarbonate filters (Millipore, DTTP) in order to remove predators. Then, we diluted the water
7
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
12
13 198 bacteria reached the stationary growth phase. The amount of biomass produced per unit Leucine
14
15 199 incorporated was computed with the cumulative method (Bjørnsen and Kuparinen, 1991), which
16
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
23
24 204 Cole (del Giorgio and Cole, 1998) is based on bacterial production (BP)

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

25
26
27 206
28
29 207
30
31 208
32
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

48
49 218
50
51 219 The initial conditions of each experiment reflect the seasonal variability in the hydrodynamic
52
53 220 conditions of the Ría de Vigo. In March 2005, the high nutrient concentrations (Table I) allowed the
54 221 development of a phytoplankton bloom. Chl-*a* concentration in this experiment reached more than
55
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

1
2
3
4 227 observed at the beginning of the September experiment (Table I) corresponded to the well-
5
6 228 documented upwelling events that occur in Ría de Vigo from April to October. The decay of a
7
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
24 238
25
26 239 The variability in both POCp and DOCp showed similar patterns to those observed in Chl-*a*
27
28 240 concentration (Fig. 2). POCp was lower in July ($<10 \text{ mgC m}^{-3} \text{ h}^{-1}$) and January ($<1 \text{ mgC m}^{-3} \text{ h}^{-1}$)
29
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
34
35 244 DOCp ($>30 \text{ mgC m}^{-3} \text{ h}^{-1}$) were measured in September during the upwelling season experiment.

36
37 245
38 246 A highly significant relationship was found between POC and DOC production rates ($r^2=0.71$,
39
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
43
44 249 contribution of DOCp to total primary production did not change across the range of POCp. No
45
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
48
49 252 March (13% [SD, 5]) (Table II). There were no significant differences in PER between experiments
50
51 253 (RMANOVA, $p=0.296$) (Table II). Similarly, we found no association between the changes in
52
53 254 taxonomic composition and the PER values (Table II).

54 255
55
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%,

1
2
3
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,
7
8 263 $p=0.099$).

9
10 264
11 265 In order to determine if dissolved primary production was favoured during the decaying phase
12
13 266 of the phytoplankton bloom, we compared the measurements conducted in the exponential growth
14
15 267 versus the senescent phases of the March and September experiments. The first three days of the
16
17 268 March experiment and the first two days of the September experiment were considered as belonging
18
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
21
22 271 (Table IV). The exponential phase was characterized by higher concentrations of dissolved
23
24 272 inorganic nitrogen and phosphate and by higher phytoplankton biomass, as inferred from the Chl-*a*
25
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)
32
33 277 in the exponential phase to a mean value of 23% (SD, 10) during the senescent phase (Table IV).

34 278
35
36 279 Dissolved organic carbon production and bacterial carbon demand

37
38 280
39
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
43
44 283 production. There was a lack of correlation between DOCp and BCD, irrespective of the model
45
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
48
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
52
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
55
56 290 lack of correlation between these variables suggests that bacterial metabolism and phytoplankton
57
58 291 exudation are largely uncoupled in this coastal ecosystem.

1
2
3
4 292
5
6 293
7
8 294
9
10 295
11 296
12
13 297
14
15 298
16
17 299
18 300
19 301
20 301
21
22 302
23
24 303
25
26 304
27 305
28
29 306
30
31 307
32
33 308
34 309
35
36 310
37
38 311
39
40 312
41 313
42
43 314
44
45 315
46
47 316
48
49 317
50 318
51
52 319
53
54 320
55
56 321
57 322
58
59 323
60
324
325

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

1
2
3
4 326 significant fraction of primary production in coastal, productive waters, irrespective of
5
6 327 phytoplankton productivity and species composition.
7

8 328
9
10 329 DOCp and size structure
11 330
12

13 331 There are physiological reasons to expect an effect of phytoplankton size structure on the
14
15 332 relative importance of DOCp. The increased surface to volume ratio of small cells should favour a
16
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
19
20 335 phytoplankton (Malinsky-Rushansky and Legrand, 1996). In contrast, Finkel (Finkel, 1998), using a
21
22 336 set of 8 diatoms species, ranging >5 orders of magnitude in cell volume, did not find any size
23
24 337 dependence on the volume or carbon specific exudation rates. In our study, we did not observe any
25
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
28
29 340 PER in oligotrophic environments such as the Atlantic subtropical gyres (Teira *et al.*, 2001), where
30
31 341 picophytoplankton are dominant both in terms of biomass and production (Marañón *et al.*, 2001),
32
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
35
36 344 phytoplankton production and growth.
37

38 345
39
40 346 DOCp and bloom development
41 347
42

43 348 It is now established that extracellular release of recent photosynthate is a normal function of
44
45 349 healthy cells, and that it is a process closely related to photosynthetic carbon assimilation (Mague *et*
46
47 350 *al.*, 1980; Bjørnsen, 1988; Nagata, 2000). However, it has also been observed that high percentages
48
49 351 of release are often associated with particular conditions experienced by the phytoplankton. These
50
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
53
54 354 phase after a phytoplankton bloom, when nutrients became scarce and limiting for growth (Norrman
55
56 355 *et al.*, 1995; Obernosterer and Herndl, 1995; Nagata, 2000). In our study, we did observe
57
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
60
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

1
2
3
4 360 to protect the cell's photosynthetic machinery, whereby organic carbon is excreted during periods of
5
6 361 energy excess and nutrient limitation. This mechanism would allow the cells to keep their
7
8 362 photosynthetic metabolism active for rapid growth whenever nutrients become available again
9
10 363 (Wood and Van Valen, 1990).

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

16
17 367 It has been estimated that nearly half of the daily photosynthetic production is released, through
18
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
21
22 370 bacterial carbon demand (BCD) strongly depends on the trophic structure of the microbial plankton
23
24 371 community and on the nature and magnitude of allochthonous sources of dissolved organic carbon
25
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
28
29 374 place (Teixeira and Figueiras, 2009), which is likely to lead to an important production of labile
30
31 375 DOC through egestion (Nagata, 2000). In addition, allochthonous inputs of dissolved organic matter
32
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,
35
36 378 therefore, should not support a significant portion of the estimated BCD. Consumption of
37
38 379 previously produced labile DOC seems more plausible, as demonstrated by Alvarez-Salgado *et al.*
39
40 380 (2001). Together, these processes may explain the lack of coupling between phytoplankton DOC
41 381 release and bacterial metabolism.
42

43 382 44 45 383 Conclusions 46 47 384

48
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
51
52 387 composition. However, the relative importance of dissolved primary production did tend to increase
53
54 388 during the decaying phase of phytoplankton blooms. Bacterial metabolism and phytoplankton
55
56 389 exudation were largely uncoupled, indicating that additional sources of DOC, both autochthonous
57
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,
60
392 productive ecosystem.
393

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

394

Acknowledgements

396

397

398

399

400

This research was funded by the Spanish Ministerio de Educación y Ciencia through research projects IMPRESION (grant VEM2003-20021 to F. G. F.) and PERSEO (grant CTM2008-03699/MAR to E. M.). D. C. López-Sandoval was supported by a postgraduate fellowship from the Mexican Council of Science and Technology (CONACyT).

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60**References**

- 401 **References**
- 402 Álvarez-Salgado, X.A., Gago, J., Míguez, B.M., *et al.* (2001). Net ecosystem production of
403 dissolved organic carbon in a coastal upwelling system: the Ría de Vigo, Iberian margin of
404 the North Atlantic. *Limnol. Oceanogr.*, **46**, 135-147.
- 405 Baines, S.B., Pace, M.L. (1991). The production of dissolved organic matter by phytoplankton and
406 its importance to bacteria patterns across marine and freshwater systems. *Limnol.*
407 *Oceanogr.*, **36**, 1078-1090.
- 408 Bjørnsen, P.K. (1988). Phytoplankton exudation of organic matter: Why do healthy cells do it?
409 *Limnol. Oceanogr.*, **33**, 151-154.
- 410 Bjørnsen, P.K., Kuparinen, J. (1991). Determination of bacterioplankton biomass, net production
411 and growth efficiency in the Southern Ocean. *Mar. Ecol. Prog. Ser.*, **71**, 185-194.
- 412 Borsheim, K.Y., Vadstein, O., Mykkestad, S.M., *et al.* (2005). Photosynthetic algal production,
413 accumulation and release of phytoplankton storage carbohydrates and bacterial production
414 in a gradient in daily nutrient supply. *J. Plankton Res.*, **27**, 743-755.
- 415 Calvo-Díaz, A., Morán, X.A.G. (2006). Seasonal dynamics of picoplankton in shelf waters of the
416 southern Bay of Biscay. *Aquat. Microb. Ecol.*, **42**, 159-174.
- 417 Cermeño, P., Marañón, E., Pérez, V., *et al.* (2006). Phytoplankton size structure and primary
418 production in a highly dynamic coastal ecosystem (Ría de Vigo, NW-Spain): seasonal and
419 short-time scale variability. *Estuar. Coast. Shelf. Sci.*, **67**, 251-266.
- 420 Cermeño, P., Marañón, E., Rodríguez, J., *et al.* (2005). Size dependence of coastal phytoplankton
421 photosynthesis under vertical mixing conditions. *J. Plankton Res.*, **27**, 473-483.
- 422 Cole, J.J., Likens, G.E., Strayer, D.L. (1982). Photosynthetically produced dissolved organic-carbon
423 an important carbon source for planktonic bacteria. *Limnol. Oceanogr.*, **27**, 1080-1090.

- 1
2
3
4 424 Crespo, G.B., Figueiras, G.F., Porras, P., *et al.* (2006). Downwelling and dominance of
5 autochthonous dinoflagellates in the NW Iberian margin: The example of the Ría de Vigo.
6 425 *Harmful Algae*, **5**, 770-781.
7
8 426
9
10
11 427 Chisholm, S.W. (1992). *Phytoplankton Size*. In: Falkowski, P.G., D., W.A. (Eds.), Primary
12 productivity and biogeochemical cycles in the sea. Plenum, New York, pp. 213-237.
13 428
14
15
16 429 del Giorgio, P.A., Cole, J.J. (1998). Bacterial growth efficiency in natural aquatic systems. *Annu.*
17 *Rev Ecol. Syst.*, **29**, 503-541.
18 430
19
20
21 431 Falkowski, P.G., Oliver, M.J. (2007). Mix and match: how climate selects phytoplankton. *Nat. Rev.*
22 *Microbiol.*, **5**, 813-819.
23 432
24
25
26 433 Finkel, Z.V. (1998). *Diatoms: size and metabolic processes*. , Dalhousie University, M.Sc. thesis.
27
28
29
30 434 Finkel, Z.V., Irwin, A.J., Schofield, O. (2004). Resource limitation alters the 3/4 size scaling of
31 metabolic rates in phytoplankton. *Mar. Ecol. Prog. Ser.*, **273**, 269-279.
32 435
33
34
35 436 Fogg, G.E. (1983). The ecological significance of extracellular products of phytoplankton
36 photosynthesis. *Bot. Mar.*, **XXVI**, 3-14.
37 437
38
39
40 438 Gago, J., Álvarez-Salgado, X.A., Nieto-Cid, M., *et al.* (2005). Continental inputs of C, N, P and Si
41 species to the Ría de Vigo (NW Spain). *Estuar. Coast. Shelf. Sci.*, **65**, 74-82.
42 439
43
44
45 440 Grasshoff, K., Ehrhardt, M., Kremling, K. (1983). *Methods of Seawater Analysis*. Verlag Chemie,
46 Weinheim, Germany.
47 441
48
49
50 442 Hillebrand, H., Durselen, C.D., Kirschtel, D., *et al.* (1999). Biovolume calculation for pelagic and
51 benthic microalgae. *J Phycol*, **35**, 403-424.
52 443
53
54
55 444 Karl, D.M., Hebel, D.V., Bjorkman, K., *et al.* (1998). The role of dissolved organic matter release
56 in the productivity of the oligotrophic North Pacific Ocean. *Limnol. Oceanogr.*, **43**, 1270-
57 445 1286.
58 446
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 447 Kiørboe, T. (1993). Turbulence, phytoplankton cell size, and the structure of the pelagic food webs.
448 *Adv. Mar. Biol.*, **29**, 1-72.
- 449 Kirchman, D., K'nees, E., Hodson, R. (1985). Leucine incorporation and its potential as a measure
450 of protein synthesis by bacteria in natural aquatic systems. *Appl Environ Microbiol*, **49**,
451 599-607.
- 452 Lancelot, C. (1983). Factors affectig phytoplankton extracellular release in the Southern Bight of
453 the North Sea. *Mar. Ecol. Prog. Ser.*, **12**, 115-121.
- 454 Lessard, E.J., Swift, E. (1986). Dinoflagellates from the North Atlantic classified as phototrophic or
455 heterotrophic by epifluorescence microscopy. *J. Plankton Res.*, **8**, 1209-1215.
- 456 López-Urrutia, A., Morán, X.A.G. (2007). Resource limitation of bacterial production distorts the
457 temperature dependence of oceanic carbon cycling. *Ecology*, **88**, 817-822.
- 458 Mague, T.H., Friberg, E., Hughes, D.J., *et al.* (1980). Extracellular release of carbon by marine
459 phytoplankton; a physiological approach. *Limnol. Oceanogr.*, **25**, 262-279.
- 460 Malinsky-Rushansky, N.Z., Legrand, C. (1996). Excretion of dissolved organic carbon by
461 phytoplankton of different sizes and subsequent bacterial uptake. *Mar. Ecol. Prog. Ser.*,
462 **132**, 249-255.
- 463 Malone, C.T. (1980). *Size fractionated primary productivity of marine phytoplankton*. In:
464 Falkowski, P. (Ed.), *Primary productivity in the sea*. Plenum Publishing Co., pp. 301-319.
- 465 Marañón, E., Cermeño, P., Fernández, E., *et al.* (2004). Significance and mechanisms of
466 photosynthetic production of dissolved organic carbon in a coastal eutrophic ecosystem.
467 *Limnol. Oceanogr.*, **49**, 1652-1666.
- 468 Marañón, E., Cermeño, P., Pérez, V. (2005). Continuity in the photosynthetic production of
469 dissolved organic carbon from eutrophic to oligotrophic waters. *Mar. Ecol. Prog. Ser.*,
470 **299**, 7-17.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 471 Marañón, E., Holligan, P.M., Barciela, R., *et al.* (2001). Patterns of Phytoplankton size structure
472 and productivity in contrasting open ocean environments. *Mar. Ecol. Prog. Ser.*, **261**, 43-56.
- 473 Morán, X.A.G., Estrada, M., Gasol, J.M., *et al.* (2002a). Dissolved primary production and the
474 strength of phytoplankton bacterioplankton coupling in contrasting marine regions.
475 *Microb. Ecol.*, **44**, 217-223.
- 476 Morán, X.A.G., Gasol, J.M., Pedrós-Alió, C., *et al.* (2002b). Partitioning of phytoplanktonic
477 organic carbon production and bacterial production along a coastal-offshore gradient in the
478 NE Atlantic during different hydrographic regimes. *Aquat. Microb. Ecol.*, **29**, 239-252.
- 479 Myklesstad, S. (1977). Production of carbohydrates by marine planktonic diatoms. II. Influence of
480 the ratio in the growth medium on the assimilation ratio, growth rate, and production of
481 cellular and extracellular carbohydrates by *Chaetoceros affinis* var. *willei* (Gran) Hustedt
482 and *Skeletonema costatum* (Grev.) Cleve. *J. Exp. Mar. Biol. Ecol.*, **29**, 161-179.
- 483 Nagata, T. (2000). *Production mechanisms of dissolved organic matter* In: Kirchman, D.L. (Ed.),
484 *Microbial Ecology of the oceans*. Wiley-Liss, pp. 121-152.
- 485 Nogueira, E., Pérez, F.F., Ríos, A.F. (1997). Seasonal patterns and long-term trends in an estuarine
486 upwelling ecosystem (Ria de Vigo, NW Spain). *Estuar. Coast. Shelf. Sci.*, **44**, 285-300.
- 487 Norrman, B., Zweifel, U.L., Hopkinson, C.S., *et al.* (1995). Production and utilization of dissolved
488 organic carbon during an experimental diatom bloom. *Limnol. Oceanogr.*, **40**, 898-907.
- 489 Obernosterer, I., Herndl, G.J. (1995). Phytoplankton extracellular release and bacterial-growth
490 dependence on the inorganic N-P ratio. *Mar. Ecol. Prog. Ser.*, **116**, 247-257.
- 491 Putt, M., Stoecker, D.K. (1989). An experimentally determined carbon-volume ratio for marine
492 oligotrichous ciliates from estuarine and coastal waters. *Limnol. Oceanogr.*, **34**, 1097-
493 1103.
- 494 Smith, C.D., Azam, F. (1992). A simple, economical method for measuring bacterial protein
495 synthesis rates in seawater using ³H-leucine. *Marine Microbial Food Webs*, **6**, 107-114.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
520
521

- 496 Strathmann, R. (1967). Estimating organic carbon content of phytoplankton from cell volume of
497 plasma volume. *Limnol. Oceanogr.*, **12**, 411-418.
- 498 Teira, E., Abalde, J., Álvarez-Ossorio, M.T., *et al.* (2003). Plankton carbon budget in a coastal
499 wind-driven upwelling station off A Coruña (NW Iberian Peninsula). *Mar. Ecol. Prog.
500 Ser.*, **265**, 31-43.
- 501 Teira, E., Pazó, M.J., Serret, P., *et al.* (2001a). Dissolved organic carbon production by microbial
502 populations in the Atlantic Ocean. *Limnol. Oceanogr.*, **46**, 1370-1377.
- 503 Teira, E., Serret, P., Fernández, E. (2001b). Phytoplankton size-structure, particulate and dissolved
504 organic carbon production and oxygen fluxes through microbial communities in the NW
505 Iberian coastal transition zone. *Mar. Ecol. Prog. Ser.*, **219**, 65-83.
- 506 Teixeira, I.G., Figueiras, F.G. (2009). Feeding behaviour and non-linear responses in dilution
507 experiments in a coastal upwelling system. *Aquat. Microb. Ecol.*, **55**, 53-63.
- 508 Tilstone, G.H., Figueiras, F.G., Fermin, E.G., *et al.* (1999). Significance of nanophytoplankton
509 photosynthesis and primary production in a coastal upwelling system (Ria de Vigo, NW
510 Spain). *Mar. Ecol. Prog. Ser.*, 13-27.
- 511 Tilstone, G.H., Figueiras, G.F., Fraga, F. (1994). Upwelling-downwelling sequences in the
512 generation of red tides in a coastal upwelling system. *Mar. Ecol. Prog. Ser.*, **112**, 241-253.
- 513 Verity, P.G., Robertson, C.Y., Tronzo, C.R., *et al.* (1992). Relationships between cell volume and
514 the carbon and nitrogen content of marine photosynthetic nanoplankton. *Limnol.
515 Oceanogr.*, **37**, 1434-1446.
- 516 Wood, A.M., Van Valen, L.M. (1990). Paradox lost? On the release of energy rich compounds by
517 phytoplankton. *Mar. Microb. Food Webs*, **4**, 103-116.
- 518 Worden, A.Z., Nolan, J.K., Palenik, B. (2004). Assessing the dynamics and ecology of marine
519 picophytoplankton: The importance of the eukaryotic component. *Limnol. Oceanogr.*, **49**,
520 168-179.

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]

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]

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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]

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

563 **Legends to figures**

564

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

567

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

570

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

573

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.

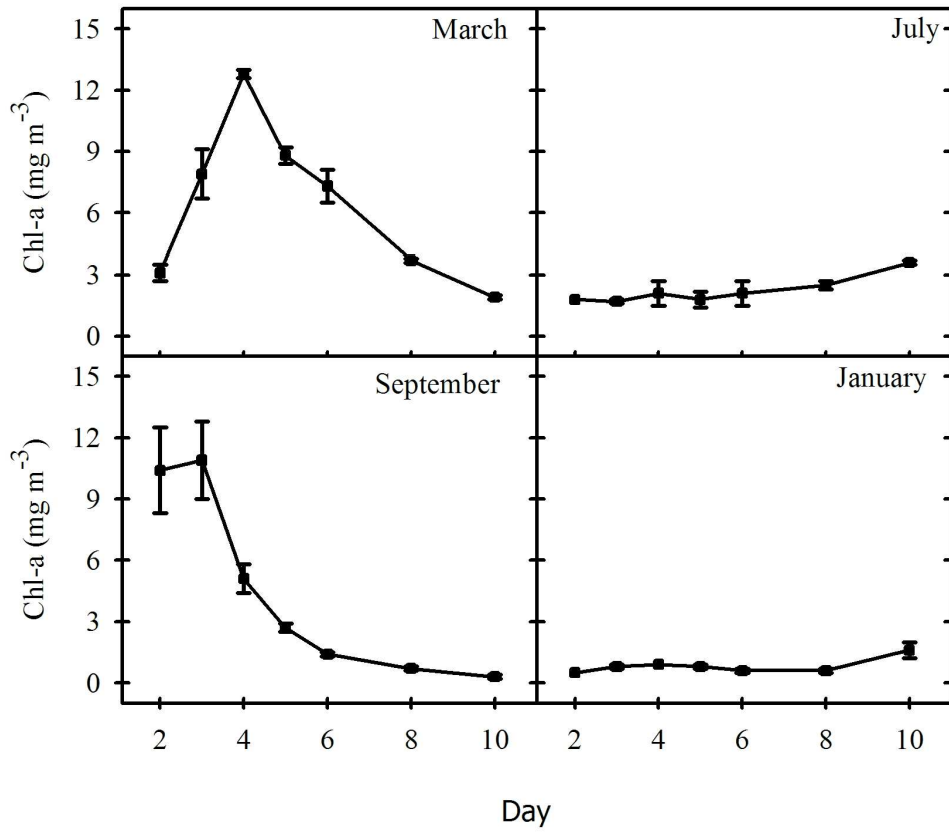
577

578

579

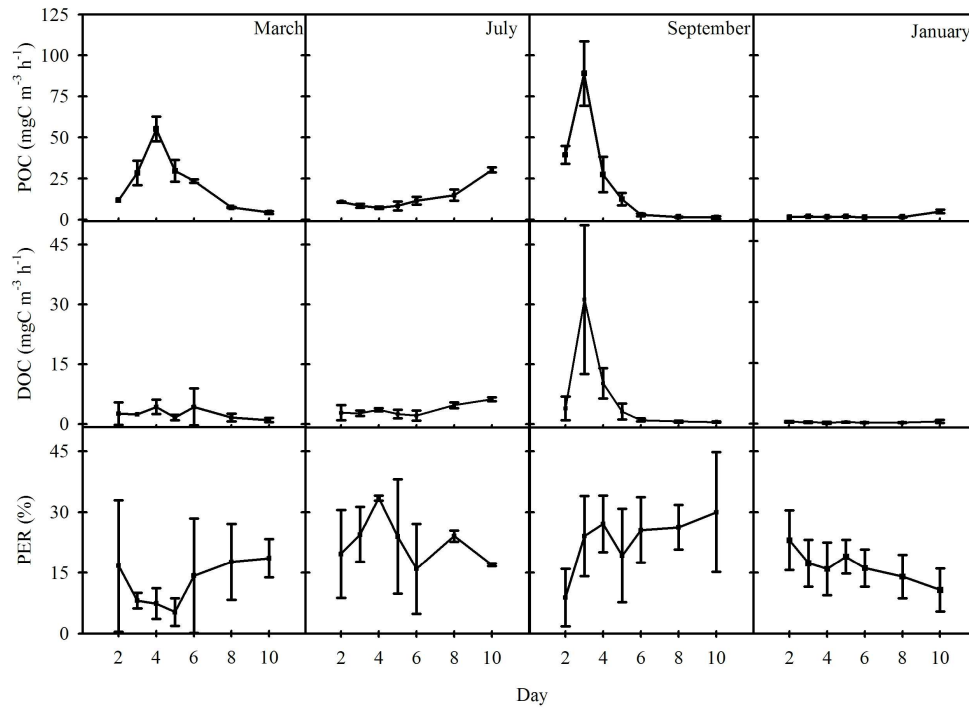
580

580

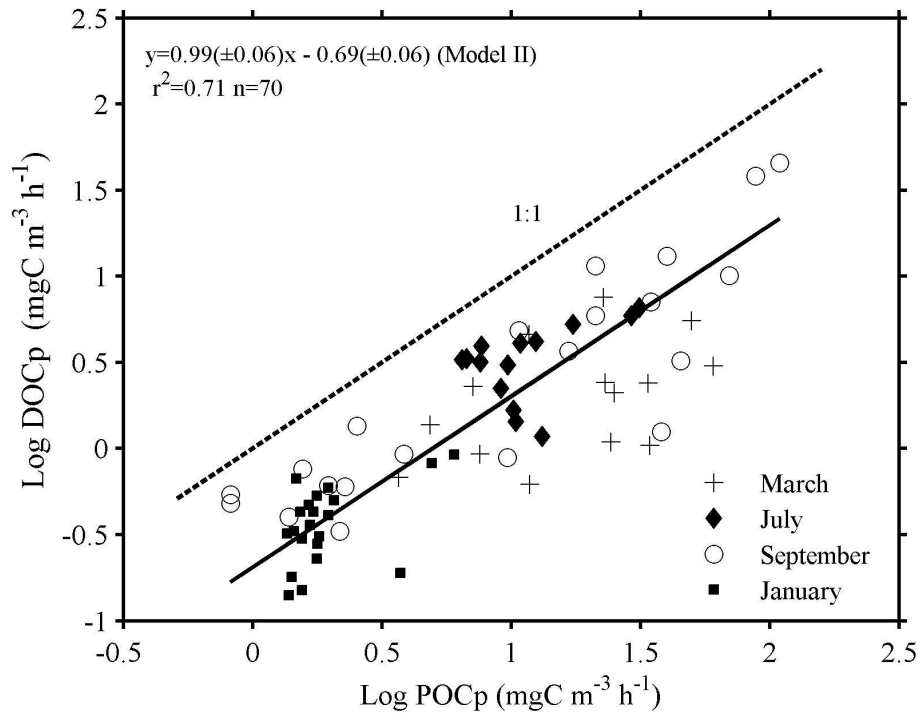


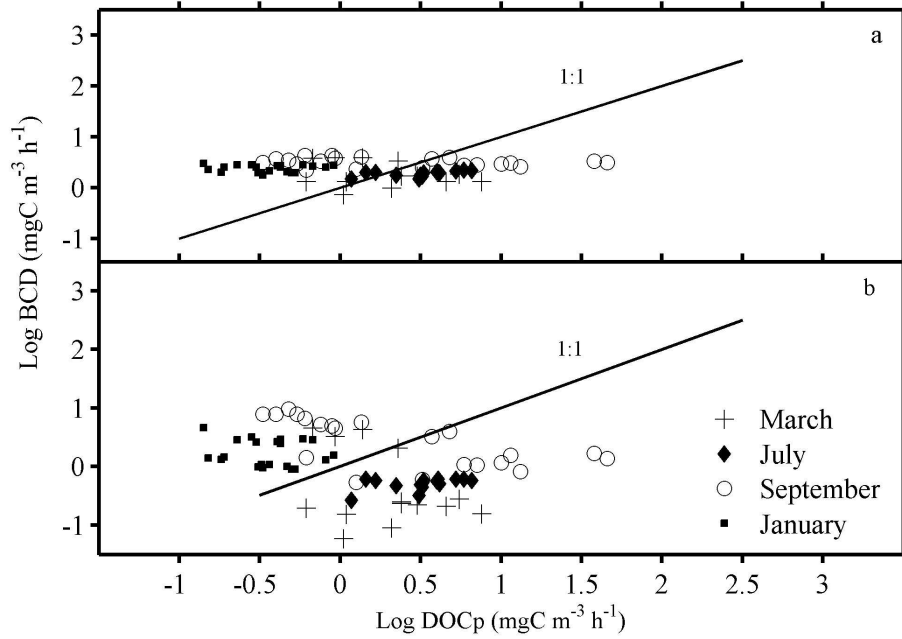
144x127mm (300 x 300 DPI)

iew



217x157mm (300 x 300 DPI)





181x125mm (300 x 300 DPI)