Stoichiometry of dissolved organic matter and the kinetics of its microbial degradation in a coastal upwelling system

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Abstract

The degradation of dissolved organic carbon (DOC), nitrogen (DON) and phosphorus (DOP) collected in the coastal upwelling system of the Ría de Vigo (NW Iberian Peninsula) was assessed following the time course of DOC, DON and DOP concentrations in laboratory incubations. Initial concentrations varied from 73 to 94 µM for DOC, 4.5 to 7.2 µM for DON and 0.18 to 0.26 µM for DOP. The bioavailable fraction (BDOM) represented 17 ± 6 % (average ± SE) of DOC, 38 ± 6 % of DON and 65 ± 9 % of DOP. BDOM was significantly correlated with temperature ($R^2 > 0.3$, p<0.05), and chlorophyll $a$ ($R^2 > 0.5$, p<0.05), indicating that the differences in DOM bioavailability were associated to the seasonal variations in plankton biomass and activity. The C: N: P stoichiometry of BDOM, 111 (± 38): 18 (± 6): 1 was not significantly different from the Redfield ratio (106: 16: 1), pointing to a phytoplankton origin of BDOM. Accordingly, exponential decay rates of BDOM suggest that this pool is very labile. Despite the reduced flushing times of the Ría de Vigo, from 3 to 8 days, as much as 68 ± 22 % of BDOC (average ± SD), 81 ± 8 % of BDON and 97 ± 7 % of BDOP are mineralized within the embayment. The remaining C–rich BDOM is exported to the adjacent oligotrophic waters, suggesting that the offshore transport of labile DOM must be accounted for when considering the carbon balance of ocean surface waters adjacent to productive coastal areas.

Keywords: DOM, bioavailability, degradation, stoichiometry, biochemical composition, coastal upwelling.
Introduction

Dissolved organic matter (DOM) is metabolically important in marine systems, supplying both energy (carbon) and nutrients (nitrogen, phosphorus) to prokaryotes (Azam 1998). The DOM consists of a small labile pool with short turnover times (from hours to days), a semi-labile pool with longer turnover times (from weeks to months) and a recalcitrant background (Carlson & Ducklow 1995). Recent studies have shown that both autochthonous and allochthonous DOM can be metabolized by bacteria (Moran & Hodson, 1990), with the bioavailability depending on molecular size (Amon & Benner, 1996) and chemical composition (Benner & Opsahl, 2001). In open oceans and coastal waters, not influenced by huge terrestrial inputs, bioavailable DOM has an autochthonous origin, which is considered as a mix of carbohydrates, lipids, proteins and phosphorus compounds (Álvarez-Salgado et al. 2006).

The old radiocarbon age of deep-water DOM (Williams & Druffel 1987, Druffel et al. 1992) indicates that it is resistant to biological utilization. The depth distribution of DOM has therefore been used to quantify the bioavailable DOM, by subtraction of deep-water DOM from surface water concentrations and the difference yielding the bioavailable pool (Carlson 2002). An alternative method used to quantify the bioavailable DOM is the application of microbial bioassay experiments, which provide an estimate of the labile fraction but also of the turnover rates. However, most studies using microbial incubations have only conducted single experiments with restricted spatial and time resolution.

DOM contains carbon, nitrogen and phosphorous in an average ratio of 300:22:1 (Benner 2002), and thus is depleted in nitrogen and phosphorous compared to the average phytoplankton biomass C:N:P ratio of 106:16:1 (Redfield et al. 1963). The turnover of DOM has been proposed to sequentially proceed with DOP being more bioavailable than DON, which in turn is more bioavailable than DOC.
(Hopkinson et al. 1997, 2002). This order has been used to explain why marine DOM is enriched in C over N and even more depleted in P compared with the Redfield ratio, suggesting that N and P depletion of DOM could be linked with age (Williams 1995). However, other studies have shown that freshly produced autochthonous DOM can have high C:N ratios due to large production of carbohydrates (Fajon et al. 1999), proposing that linking DOM age with N and P depletion is not valid under all conditions.

The study of the bioavailability and degradation rates of DOM is specially relevant in coastal upwelling systems because they are sites of large, episodic phytoplankton blooms, resulting from upwelling of nutrient rich bottom water, leading to enhanced DOM production resulting from upwelling of nutrient rich bottom waters (Chavez & Toggweiler 1995, Hansell & Carlson 1998a). In this study we (1) estimated the bioavailability of DOC, DON and DOP over an annual cycle, (2) assessed the biochemical composition of the DOM mineralized and (3) determined the DOM decay rates using laboratory incubations in the highly dynamic coastal upwelling area of the Ría de Vigo (NW Iberian Peninsula).

Material and methods

Study area. The Ría de Vigo has a length of 33 Km, a surface area of 176 Km², and a volume of 3.32 Km³. The hydrography of the ría is influenced by wind-driven upwelling and downwelling episodes; northerly winds, which predominate from April to October, produce upwelling of the cold and nutrient-rich Eastern North Atlantic Central Water (ENACW) resulting in a high productivity. On the contrary, from November to March, southerly winds dominate resulting in downwelling forcing warm and nutrient-poor shelf surface water into the ría (Álvarez-Salgado et al. 2003). Wind-driven upwelling enhances the positive residual circulation pattern of the ría, i.e. the inflow of cold and nutrient-rich bottom waters and the compensating outflow of warm and nutrient poor surface waters, resulting in a
proportional reduction of the renewal time of the ría (Álvarez-Salgado et al. 2001; 2008). A reversal of
the circulation occurs under wind driven downwelling conditions, which results in the entry of the warm
and nutrient poor shelf surface waters into the ría that is compensated by the evacuation of the water in
the ría through the bottom layer (Álvarez-Salgado et al. 2001; 2008). The river Oitabén–Verdugo, the
main tributary to the Ría de Vigo, flows into San Simon Bay, at the innermost reaches of the
embayment (Figure 1). With an average annual flow of 15 m$^3$s$^{-1}$ and a pristine composition (< 20 μM
inorganic nitrogen, < 0.3 μM phosphate, < 120 μM DOC, < 10 μM DON and < 0.4 μM DOP; Gago et
al. 2005), its impact on the circulation and biogeochemistry of the ría is minor and restricted to the inner
parts.

The study site was near the main channel in the middle sector of the ría (Figure 1). Previous
studies in the Ría de Vigo (Nogueira et al. 1997; Álvarez–Salgado et al. 2000; 2001) have shown that
this location is appropriate for evaluating the biogeochemical processes occurring in the pelagic Ría de
Vigo with the exception of the innermost reaches of the embayment, where the river Oitabén–Verdugo
enters the shallow sedimentary basin of San Simon Bay. Water for the laboratory incubation
experiments was collected in autumn (20 and 27 September, and 4 October 2007), winter (31 January, 7
and 14 February 2008), spring (17 and 24 April 2008), and summer (26 June, 3 and 7 July 2008) with a
25 l Niskin bottle at 5 m depth, and combined into a 50 l acid washed container. Salinity and
temperature profiles were recorded prior to water collection with an SBE 9/11 CTD probe. Chlorophyll
(Chl $a$) was measured by filtering between 100 and 200 ml of the sample water through GF/F filters,
which were frozen (-20°C) until analysis. Chl $a$ was determined with a Turner Designs 10000R
fluorometer after 90% acetone extraction (Yentsch & Menzel 1963). The precision is ±0.05 mg L$^{-1}$. 
Renewal time estimates. Following Wooster et al. (1976) daily offshore Ekman transport values (-Q_x, m^3 s^-1 km^-1), a rough estimation of the volume of water downwelled/upwelled per kilometre of coast, can be calculated as:

\[ -Q_x = \frac{\rho_{\text{air}} \cdot C \cdot |W| \cdot W_y}{\rho_{\text{sw}} \cdot f} \]  

(1)

where \( \rho_{\text{air}} \) is the density of air, 1.22 kg m^-3 at 15°C; C is an empirical drag coefficient (dimensionless), 1.3 \times 10^{-3}; f is the Coriolis parameter, 9.946 \times 10^{-5} s^-1 at 43° latitude; \( \rho_{\text{sw}} \) is the density of seawater, ~1025 kg m^-3; |W| is the wind speed; and \( W_y \) is the northwards component. Average daily geostrophic winds were estimated from atmospheric surface pressure charts, provided at 6 h intervals by the Spanish “Instituto Nacional de Meteorología”. Positive values indicate upwelling, with downwelling occurring when negative values are obtained.

The renewal time of the ría can be calculated from the offshore Ekman transport following Álvarez-Salgado et al (2008):

\[ t = \frac{n \cdot V}{\sum |Q_x| \cdot \frac{V}{L}} \]  

(2)

Where \( |Q_x| \) is the absolute value of the daily offshore Ekman transport. A 7 days running-mean centred on the sampling date was used (n = 7), V is the volume of the embayment from the inner reaches to the sampling site (0.53 \times 10^9 m^3) and L (2.50 \times 10^3 m) is the length of the open end of the embayment at the sampling site (see Fig. 1).

Experimental design. Filtration of the sample water started within 10 min of collection; one part was filtered through a pre-washed (> 10 l of MQ) dual-stage (0.8 µm and 0.2 µm) filter cartridge (Pall-Acropak supor Membrane); the second part was filtered through pre-combusted (450°C for 4 h) GF/C
filters to remove larger phytoplankton and grazers and establish a microbial culture. After filtration, the water was kept in the dark until arrival in the base laboratory (within 2 h). The water was transferred into a 20 l carboy and the microbial inoculum was added to the 0.2 µm filtrate corresponding to 10% of the total volume. The water was distributed into 16 glass bottles (500 ml) and incubated in the dark at a constant temperature of 15ºC, with four replicate bottles being analyzed for each sub-sampling at day 0, 4, 12 and 53 or 70 (summer experiments only). All glassware used in the experiments was acid washed and rinsed with Milli-Q water prior to use. At each time point samples from 4 replicate incubation bottles were filtered through 0.2 µm filters (Pall, Supor membrane Disc Filter) for subsequent analysis of the concentration of 1) dissolved inorganic nitrogen (DIN: NH₄, NO₂⁻ and NO₃⁻), 2) dissolved inorganic phosphorus (DIP: HPO₄²⁻), 3) dissolved organic carbon (DOC), 4) total dissolved nitrogen (TDN) and 5) total dissolved phosphorus (TDP). The DIN, DIP and TDP subsamples were collected into 50 ml acid washed polyethylene bottles and DOC and TDN samples into pre-combusted (450ºC, 12 h) glass ampoules and preserved with 50 µl 25 % H₂PO₄ to 10 ml sample.

**Sample analysis.** DOC and TDN samples were measured using a Shimadzu TOC analyzer (Pt–catalyst) connected with an Antek–TN measuring unit. Using the deep ocean reference (Sargasso Sea deep water, 2600 m) we obtained a concentration of 46.8 ± 2.8 µM (average ± SD) for DOC and 22.0 ± 2.0 µM for TDN. The nominal value for DOC provided by the reference laboratory is 44.0 ± 1.5 µM, while the TDN value provided was 21.8 ± 0.8 µM. Standards for DOC and TDN were made from potassium hydrogen phthalate and glycine, with the concentrations of DOC and TDN calculated using a daily calibration curve with 4 points and subtraction of a blank value. DON concentrations were calculated as the difference between TDN and DIN (DON = TDN – DIN) with the standard error (SE) calculated as the sum of the contributions: $SE^2_{DON} = SE^2_{TDN} + SE^2_{NH4} + SE^2_{NO2} + SE^2_{NO3}$. 


Inorganic nutrients (NH$_4^+$, NO$_2^-$, NO$_3^-$ and HPO$_4^{2-}$) were determined by standard segmented flow analysis (SFA). The precisions are ± 0.02 µM for nitrite, ± 0.1 µM for nitrate, ± 0.05 µM for ammonium, ± 0.02 µM for phosphate. TDP was measured by the ammonium molybdate method as inorganic phosphorus after a wet oxidation (120°C, 75 min) in acid persulphate (Hansen & Koroleff, 1999). The detection of TDP was performed in a SFA system using a daily calibration curve. The oxidation efficiency was tested daily with adenosine 5′–triphosphate (ATP) obtaining recoveries between 90 and 100 %. DOP was calculated as the difference between TDP and DIP (DOP = TDP – DIP) with the SE for DOP calculated as: SE$^2_{DOP} = SE^2_{TDP} + SE^2_{DIP}$. Unfortunately, the TDP samples from the 26-Jun-08 were under the detection limit of the method used, probably due to an unusual low recovery during that measuring day (ATP < 80%).

The decay of DOM during the course of the incubations was modelled by a first-order exponential decay function using the Marquardt-Levenberg algorithm and taking the refractory pool into account:

$$\text{DOM}(t) = \text{BDOM} \cdot e^{(-k_{\text{DOM}} \cdot t)} + \text{RDOM}$$

Where BDOM is the bioavailable pool (in µM), $k_{\text{DOM}}$ the degradation rate (in d$^{-1}$), t the time (in days) and RDOM the remaining pool after 53/70 days of incubation (in µM). In this study, BDOM was defined as: BDOM = DOM$_0$ - RDOM, where DOM$_0$ is the initial DOM concentration. Note that since BDOM and RDOM are calculated prior to adjusting the time evolution of DOM, the only parameter that is adjusted with eq. (3) is $k_{\text{DOM}}$. Days 0, 4, 12 and 53 or 70 (summer experiments only) were considered in the calculation of $k_{\text{DOM}}$.

**Conversion of C:N:P stoichiometry into biochemical composition.** Assuming that changes in the C:N:P composition of BDOM are due to variations in the proportions of the four major groups of biomolecules produced and released by phytoplankton (carbohydrates, lipids, proteins and phosphorus...
compounds) rather than variations in the molecular formula of each group (Alvarez-Salgado et al. 2006), it is possible to estimate their proportions as stated by Fraga et al. (1998). The typical elemental composition of proteins (Prt), carbohydrates (Cho), lipids (Lip) and phosphorus compounds (Pho) are shown in Table 1. The chemical formula of the phosphorus compounds results from the contribution of RNA, DNA, nucleotides, phospholipids, phosphoproteins, phosphosugars and cellular phosphates and polyphosphates in the average proportions provided by Miyata & Hattori (1986). From these chemical formulas, the contribution of the different biomolecules to the degraded BDOM can be calculated from changes in, DOC, DON and DOP with the following sets of equations:

\[
\begin{align*}
C_{138}H_{217}O_{45}N_{39}S + 144 O_2 + 103 OH^- &\rightarrow 138 HCO_3^- + 39 NO_3^- + SO_4^{2-} + 15 H_2O \\
C_6H_{10}O_5 + 6 O_2 + 6 OH^- &\rightarrow 6 HCO_3^- + 5 H_2O \\
C_{53}H_{89}O_6 + 72.25 O_2 + 53 OH^- &\rightarrow 53 HCO_3^- + 44.5 H_2O \\
C_{45}H_{76}O_{31}N_{12}P_{5} + 47.75 O_2 + 45 OH^- &\rightarrow 45 HCO_3^- + 12 NO_3^- + 5 HPO_4^{2-} + 13.5 H_2O
\end{align*}
\]

And the corresponding linear system of mass balance equations is:

\[
\begin{align*}
BDOC &= 138 \times Prt + 6 \times Cho + 53 \times Lip + 45 \times Pho \\
BDON &= 39 \times Prt + 12 \times Pho \\
BDOP &= 5 \times Pho
\end{align*}
\]

A system of 3 equations with 4 unknowns (Cho, Lip, Prt and Pho) has not a unique solution. Whereas the amounts of Prt and Pho are determined by eqs (9) and (10), the amounts of Cho and Lip are undetermined. Following Alvarez-Salgado et al. (2006), we calculated the range of possible
biochemical compositions compatible with a mineralization of at least 5% of Cho on one extreme and at least 5% of Lip on the other extreme.

**Statistical analysis.** Regression analyses were performed using the best-fit between the two variables X and Y obtained by regression model II as described in Sokal & Rohlf (1995). In the cases where the intercept was not significantly different from zero, it was set to zero and a new slope was calculated. Prior to the regressions, normality was checked, the confidence level was set at 95% with all statistical analyses conducted in Statistica 6.0.

**Results and discussion**

**Hydrography**

During the autumn surveys the Ekman transport indicated an initial strong upwelling event (-\(Q_x = 551 \text{ m}^3 \text{s}^{-1} \text{km}^{-1}\)), followed by upwelling relaxation (27-Sep-07) and moderate downwelling (04-Oct-07) resulting in a longer flushing time from 4 to 11 days. During this period, surface salinity was around 35.5 whereas temperature decreased from >16°C to <14°C. Ambient chlorophyll levels decreased from 3.2 to 2.8 mg m\(^{-3}\) and dissolved inorganic nitrogen (DIN) increased from 3 to 13 µM (Fig. 2). During the winter surveys, the conditions evolved from relaxation to strong downwelling and resulted in water flushing times varying between 3 and 18 days. Salinity was around 35 and surface temperatures were between 13.0 and 13.5°C. Chlorophyll levels were <1.5 mg m\(^{-3}\) and DIN concentrations maintained above 8 µM (Fig. 2). The spring surveys were dominated by moderate downwelling conditions, and flushing times varied between 6 and 9 days. Salinity was relatively low probably caused by high precipitation (153.7 mm during April were recorded in the terrace of the host laboratory). Salinity reached its lowest level (25) at the 24-Apr-2008 matching with the highest chlorophyll levels, > 8 mg m\(^{-3}\). Phosphate was relatively low, < 0.1 µM whereas DIN levels were > 5 µM (Fig. 2), possibly due to the high N/P molar ratio of the nutrient transported by terrestrial run-off (45; Gago et al. 2005). During
the summer surveys, initial strong upwelling was followed by moderate downwelling. The calculated average water flushing times varied between 3 and 9 days. Salinities were stable > 35 and temperatures were high > 17ºC. Low DIN levels, < 3 µM, and chlorophyll ranging from 1.1 to 4.5 mg m⁻³ were recorded (Fig. 2).

The hydrographic conditions at 5 m depth during this study were within the natural ranges of variability found in long–term studies conducted in the Ría de Vigo (Nogueira et al. 1997).

DOM bioavailability

Initial DOC concentrations varied between 73 and 94 µM, while DON and DOP ranged from 4.5 to 7.2 µM and from 0.18 to 0.26 µM respectively (Fig. 3), which is comparable with previous measurements performed in the Ría de Vigo (Doval et al. 1997, Álvarez-Salgado et al. 2006). BDOC varied between 7 to 29 µM representing 17 ± 6 % of DOC (average ± SE), BDON ranged between 1.3 and 3.6 µM corresponding to 38 ± 6 % of the DON and BDOP reached values between 0.11 and 0.19 µM representing 65 ± 9 % of DOP (Fig. 3). The percentages of bioavailable DOC were comparable with the 19 ± 12% reported for other coastal areas (Søndergaard & Middelboe 1995, Lønborg & Søndergaard 2009). The BDON values were also similar to previous estimates, ~30% (Bronk 2002, Lønborg & Søndergaard 2009). The same is applicable to BDOP: previously reported levels ranged from 60 to 80% (Hopkinson et al. 2002, Nausch & Nausch 2006, Lønborg et al. 2009). A positive linear relationship occurred between the BDOM and DOM pools (Table 2) with slopes not significantly different from 1. These slopes suggest that when BDOM concentrations increased by 1 µmol L⁻¹ total DOM increased 1 µmol L⁻¹, demonstrating that the seasonal variations in DOM were due to the BDOM pool, as previously found (Williams 1995). The origin intercepts of these regressions indicated the refractory concentrations of DOM: 65 ± 3 µM for DOC, 3.3 ± 0.2 µM for DON, and 0.09 ± 0.02 µM for
DOP (Table 2). These values are comparable with background levels measured in the bottom waters (40 m depth) of the Ría de Vigo (Doval et al. 1997, Álvarez-Salgado et al. 2006) and with the average refractory DOM end point concentrations in our incubations. Comparing our refractory levels with the concentrations found in the deep sea we find that they are around twice as high (35–45 µM, Hansell & Carlson 1998b), while the refractory DON and DOP of the Ría de Vigo are comparable with the levels found in the deep sea < 3 and 0.02 µM respectively (Hopkinson et al. 1997, Bronk, 2002).

BDOM significantly correlated with temperature, and chlorophyll a (Table 3), indicating that the differences in DOM bioavailability are related to the seasonal variations in plankton biomass and activity.

The contribution of DON and DOP to the mineralization of inorganic nutrients has previously been calculated as the slope between BDON/DIN and BDOP/DIP (Hopkinson et al. 1997) (Table 2). We found that 14 ± 9 % of DIN and 15 ± 8 % of DIP originated from the degradation of DON and DOP which is comparable with previous estimates for the Ría de Vigo (Álvarez-Salgado et al. 2006).

**DOM biochemical composition**

The range of possible biochemical composition of BDOM (Table 4) was calculated introducing the C: N: P molar ratios of BDOM into the system of linear equations 8 – 10 as described by Álvarez-Salgado et al. (2006).

The average biochemical composition of marine phytoplankton consists of 46% Prt, 41% Cho+Lip and 12% Pho (Table 1). Accordingly, the average composition of the BDOM degraded in the Ría de Vigo is comprised of 55 ± 13% Prt (average ± SD), 32 ± 15% Cho+Lip, and 13 ± 4% Pho (Table
It should be noted that the contribution of carbohydrates were inversely related with DIN and DIP and positively related with chlorophyll (Table 3), showing that when nutrients become exhausted phytoplankton respond by producing carbohydrates as observed before (Fajon et al. 1999). While protein compounds showed the contrary pattern (Table 3) with decreasing % contribution to the BDOM pool under N-deficient conditions.

DOM stoichiometry

The stoichiometry of DOM and BDOM was determined from the slopes of the DOM element–element plots (Fig. 4). These slopes are smaller than those of the elemental ratios, indicating that these C: N: P ratios only included the recently produced biomolecules containing nitrogen and phosphorus. The slope of the relations between DOC, DON and DOP suggested an average ratio of 133 (± 44): 14 (± 3): 1 for the DOM. The significant origin intercepts in these linear regressions (Fig 4) showed that a background level of DOC of 23 ± 10 and 54 ± 13 µM would persist when DON and DOP reached zero, respectively. Additionally, the origin intercept of the relationship between DON and DOP showed that 3 ± 1 µM of DON would remain when DOP is depleted (Fig 4), suggesting that degradation follows the sequence DOP > DON > DOC, which links DOM N and P depletion with age as found previously (Jackson & Williams 1985, Hopkinson et al. 1997,2002).

The C: N: P stoichiometry of BDOM, obtained from the slope of the significant (p < 0.05) linear relationships between BDOC, BDON and BDOP (Fig. 4), was 111 (± 38): 18 (± 6): 1 was not significantly different from the Redfield ratio (106: 16: 1, Redfield et al. 1963) and ratios found for recently produced organic matter (Garber 1984, Hopkinson et al. 1997). This stoichiometry is comparable to ratios previously reported for phytogenic DOM (Doval et al. 1997, Hansell & Waterhouse, 1997, Álvarez-Salgado et al. 2006).
DOM decay rates

Although the kinetics of DOM degradation can be modelled considering two (labile and refractory) or three (labile, semilabile and refractory) pools (e.g. Hopkinson et al. 1997, 2002), DOM should be considered as a continuum of pools with decreasing lability (Williams 2000). In our particular case, given the limited number of points of the degradation curves, the model used only considers a labile and a refractory DOM pool.

Average BDOM decay rates (Table 4) were 0.22 ± 0.08 d⁻¹ (average ± SE) for DOC (k_{DOC}), 0.29 ± 0.08 d⁻¹ for DON (k_{DON}) and 0.37 ± 0.11 d⁻¹ for DOP (k_{DOP}). The resulting half-live times of BDOM are as low as 1.4 ± 0.4 days for DOC, 1.1 ± 0.3 days for DON and 0.8 ± 0.1 days for DOP, which are characteristics of very labile DOM (Hopkinson et al. 2002). Maximum degradation rates were observed in autumn and summer and minimum in winter (Table 4) and they were positively correlated with BDOM (Table 2), demonstrating that higher BDOM concentrations would lead to faster mineralization rates as observed in other coastal systems (Hopkinson et al. 1997, Lønborg et al. 2009).

Positive correlation was observed between k_{DOC}, k_{DON} and k_{DOP}, and the corresponding linear regression slopes indicate that DOC and DON were degraded at a rate equivalent to 58 ± 21 % and 77 ± 13 % (slope ± SE) of DOP (Fig. 5). This is in agreement with the previous discussion of C:N:P stoichiometry with the degradation r of the DOM pools following the sequence DOP > DON > DOC (Garber 1984, Hopkinson et al. 1997, 2002).

The amount of BDOM degraded within the Ría de Vigo, calculated from the degradation rates and the average flushing time of every sampling date, were 68 ± 22 % of BDOC (average ± SE), 81 ± 8 % of BDON and 97 ± 7 % of BDOP. Comparing these values with the in-situ DIN and DIP levels it suggests that 35% and 36% of bioavailable dissolved N and P were bound in DON and DOP.
respectively. These calculations demonstrated that nitrogen and phosphorus budgets for this upwelling area based only on DIN and DIP measurements would underestimate the available nitrogen and phosphorus amounts considerably. The amounts degraded depended both on the lability of BDOM and the variable flushing times, suggesting temporal differences in the export of BDOM from this coastal upwelling system. The exported BDOM was more carbon rich than the DOM degraded within the ría having an average ratio of 282 (± 122): 28 (± 9): 1 compared to 111 (± 38): 18 (± 6): 1. The fate of the offshore exported C–rich BDOM, accumulation versus degradation, would depend on the demand of inorganic nitrogen and phosphate by the heterotrophic bacteria in the adjacent oligotrophic waters to process that BDOM. In this sense, Álvarez Salgado et al. (2007) have shown that less than 15% of the BDOM exported from the coastal upwelling region of W Iberian–NW Africa is consumed in the coastal transition zone.

Acknowledgement - This study was funded by a fellowship to C.L from the Early stage Training site ECOSUMMER (MEST-CT-2004-020501). We thank the captain, crew, and technicians of R/V *Mytilus* and the members of the Department of Oceanography of the Instituto de Investigaciones Marinas for the collaboration during the sampling program. Access to vessel time was provided by the RAFTING project (Impact of the mussel raft culture on the benthic-pelagic coupling in a Galician Ria, grant number: CTM2007-61983/MAR). The valuable suggestions and comments by four anonymous reviewers are greatly acknowledged.

References


1 Table 1. Chemical composition of the main organic products of the synthesis and early degradation of marine phytoplankton, according to Fraga et al. (1998). The percentages (in weight (w)) are corresponding to the average composition of marine phytoplankton.

<table>
<thead>
<tr>
<th>Chemical formula</th>
<th>contribution (w/w)</th>
</tr>
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<tbody>
<tr>
<td>Proteins</td>
<td>C_{138}H_{217}O_{45}N_{39}S</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>C_6H_{10}O_5</td>
</tr>
<tr>
<td>Lipids</td>
<td>C_{53}H_{89}O_{6}</td>
</tr>
<tr>
<td>Phosphorus compounds</td>
<td>C_{45}H_{76}O_{31}N_{12}P_{5}</td>
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<tr>
<td>Pigments</td>
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<tr>
<td>Average composition</td>
<td>C_{106}H_{17}O_{41}N_{16}P</td>
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Table 2: Significant regressions between BDOM and DOM, Inorganic nutrients (DIN, DIP), and degradation constants ($k_{DOC}$, $k_{DON}$ and $k_{DOP}$) obtained by fitting the exponential degradation of DOC, DON and DOP with time. Slope, incept, and standard error are values found by Model II regression. $R^2 = $ coefficient of determination, $p = $ significant levels and n.s. – not significant.

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>Slope (±SE)</th>
<th>Intercept (±SE)</th>
<th>$R^2$</th>
<th>p</th>
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</thead>
<tbody>
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<td>BDOC</td>
<td>DOC</td>
<td>1.1 ± 0.2</td>
<td>65 ± 3</td>
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<td>BDON</td>
<td>DON</td>
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<td>0.95</td>
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<td>BDOP</td>
<td>DOP</td>
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<td>0.09 ± 0.02</td>
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<td>&lt;0.001</td>
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<tr>
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<td>2.81 ± 0.21</td>
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<td>BDOP</td>
<td>DIP</td>
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<td>BDOC</td>
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<td>n.s</td>
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<td>&lt;0.0001</td>
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<tr>
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<td>0.13 ± 0.03</td>
<td>n.s</td>
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<tr>
<td>BDOP</td>
<td>$k_{DOP}$</td>
<td>2.8 ± 0.3</td>
<td>n.s</td>
<td>0.86</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>
Table 3: Matrix of the correlation coefficient ($R^2$) of the significant ($p< 0.05$) linear regressions between bioavailable DOC (BDOC), DON (BDON) DOP (BDOP) and proteins (Prt), Lipids+carbohydrates (Lip + Cho) with physical and chemical parameters from Ría de Vigo. n.s. – not significant.

<table>
<thead>
<tr>
<th>X/Y</th>
<th>BDOC</th>
<th>BDON</th>
<th>BDOP</th>
<th>Prt%</th>
<th>Lip+Cho%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
<td>-0.4</td>
<td>0.7*</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.8</td>
<td>0.5</td>
<td>0.3</td>
<td>-0.8*</td>
<td>0.7*</td>
</tr>
<tr>
<td>Chl $a$</td>
<td>0.5*</td>
<td>0.6*</td>
<td>0.5*</td>
<td>-0.7*</td>
<td>0.5*</td>
</tr>
<tr>
<td>DIN</td>
<td>-0.5</td>
<td>-0.3</td>
<td>-0.3</td>
<td>0.5*</td>
<td>-0.3*</td>
</tr>
<tr>
<td>DIP</td>
<td>-0.3</td>
<td>-0.3</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
</tbody>
</table>

*Data from the 24/04/08 have been omitted to reach significant levels.
Table 4. Degradation constants (± standard error) obtained by fitting the exponential decay model to the decrease in DOC (k_{DOC}), DON (k_{DON}) and DOP (k_{DOP}) over time. Average % (± SE) contribution of phosphorus compounds (Pho), Proteins (Prt) and Lipids + carbohydrates (Lip+Cho) to the degradable dissolved organic matter (DOM) during the sampling period in the Ría de Vigo. R^2 = coefficient of determination. n.d- not determined due to low recovery of DOP.

<table>
<thead>
<tr>
<th>Date</th>
<th>k_{DOC} (d^{-1})</th>
<th>R^2</th>
<th>k_{DON} (d^{-1})</th>
<th>R^2</th>
<th>k_{DOP} (d^{-1})</th>
<th>R^2</th>
<th>Pho %</th>
<th>Prt %</th>
<th>Lip+Cho %</th>
</tr>
</thead>
<tbody>
<tr>
<td>20/09/07</td>
<td>0.35 ± 0.04</td>
<td>0.99</td>
<td>0.41 ± 0.01</td>
<td>0.96</td>
<td>0.60 ± 0.10</td>
<td>0.95</td>
<td>12 ± 2</td>
<td>42± 6</td>
<td>46± 8</td>
</tr>
<tr>
<td>27/09/07</td>
<td>0.23 ± 0.05</td>
<td>0.99</td>
<td>0.27 ± 0.01</td>
<td>0.98</td>
<td>0.34 ± 0.04</td>
<td>0.95</td>
<td>10 ± 1</td>
<td>50± 6</td>
<td>40± 8</td>
</tr>
<tr>
<td>04/10/07</td>
<td>0.18 ± 0.03</td>
<td>0.98</td>
<td>0.28 ± 0.01</td>
<td>0.90</td>
<td>0.33 ± 0.03</td>
<td>0.91</td>
<td>13 ± 1</td>
<td>56± 5</td>
<td>31± 6</td>
</tr>
<tr>
<td>31/01/08</td>
<td>0.11 ± 0.01</td>
<td>0.97</td>
<td>0.20 ± 0.01</td>
<td>0.97</td>
<td>0.34 ± 0.02</td>
<td>0.92</td>
<td>16 ± 1</td>
<td>61± 3</td>
<td>23± 5</td>
</tr>
<tr>
<td>07/02/08</td>
<td>0.11 ± 0.02</td>
<td>1.00</td>
<td>0.20 ± 0.01</td>
<td>0.92</td>
<td>0.29 ± 0.03</td>
<td>0.81</td>
<td>23 ± 1</td>
<td>67± 1</td>
<td>10± 1</td>
</tr>
<tr>
<td>14/02/08</td>
<td>0.20 ± 0.01</td>
<td>0.95</td>
<td>0.22 ± 0.02</td>
<td>0.94</td>
<td>0.27 ± 0.04</td>
<td>0.85</td>
<td>7 ± 1</td>
<td>61± 6</td>
<td>32± 6</td>
</tr>
<tr>
<td>17/04/08</td>
<td>0.20 ± 0.02</td>
<td>0.99</td>
<td>0.28 ± 0.09</td>
<td>0.93</td>
<td>0.37 ± 0.04</td>
<td>0.86</td>
<td>15 ± 1</td>
<td>54± 5</td>
<td>31± 6</td>
</tr>
<tr>
<td>24/04/08</td>
<td>0.20 ± 0.02</td>
<td>0.99</td>
<td>0.26 ± 0.04</td>
<td>0.93</td>
<td>0.32 ± 0.05</td>
<td>0.90</td>
<td>12 ± 1</td>
<td>79± 1</td>
<td>9± 1</td>
</tr>
<tr>
<td>26/06/08</td>
<td>0.30 ± 0.08</td>
<td>0.94</td>
<td>0.37 ± 0.02</td>
<td>0.91</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>03/07/08</td>
<td>0.19 ± 0.05</td>
<td>0.93</td>
<td>0.35 ± 0.01</td>
<td>0.91</td>
<td>0.40 ± 0.04</td>
<td>0.92</td>
<td>11 ± 1</td>
<td>49± 6</td>
<td>40± 8</td>
</tr>
<tr>
<td>10/07/08</td>
<td>0.30 ± 0.05</td>
<td>0.98</td>
<td>0.39 ± 0.03</td>
<td>0.94</td>
<td>0.45 ± 0.20</td>
<td>0.86</td>
<td>12 ± 2</td>
<td>34± 6</td>
<td>54± 8</td>
</tr>
</tbody>
</table>
Figure legends.

Fig. 1. Map showing the sampling station (●) in the Ría de Vigo (NW Spain). V is the area used for calculating the volume of the embayment and L is the length of the open end of the embayment at the sampling site.

Fig. 2. Field conditions in the Ría de Vigo at the sampling site (5 m depth) during 2007 to 2008, with (a) salinity, temperature, and the seven-day running-mean of the offshore Ekman transport (-Qx), and (b) dissolved inorganic phosphorus (DIP) and inorganic nitrogen (DIN), and chlorophyll a concentration.

Fig. 3. Temporal variability in total, bioavailable and refractory DOM concentrations. Error bars represent standard errors.

Fig. 4. Plots of (a) DON versus DOC (●), BDON with BDOC (○), (b) DOP versus DOC (●), BDOP with BDOC (○) and (c) DOP versus DON (●), BDOP with BDON (○). Solid and dashed lines represent the corresponding regression and error bars are standard errors. $R^2 =$ coefficient of determination, $p =$ significant level.

Fig. 5. X–Y plots of the relationship between BDOM decay rates ($d^{-1}$) predicted from the exponential decay model with (a) $k_{DON}$ vs. $k_{DOC}$, (b) $k_{DOP}$ vs. $k_{DOC}$ and (c) $k_{DOP}$ vs. $k_{DON}$. The dashed line represents a 1:1 line where the decay rates would be equal and the solid lines represent the regression lines found. Error bars represent standard errors.
Lønborg et al, Figure 1.
Lønborg et al., Figure 2
a) DOC (µmol L⁻¹)

b) DON (µmol L⁻¹)

c) DOP (µmol L⁻¹)

Lønborg et al., Figure 3
\[ BDOC = 111 (\pm 38) \cdot BDOP, \quad DON, BDON (\mu M) \]

\[ DOC = 23 (\pm 10) + 10 (\pm 2) \cdot DON, \quad R^2 = 0.84, \quad p < 0.0001 \]

\[ DOC = 54 (\pm 13) + 133 (\pm 44) \cdot DOP, \quad R^2 = 0.52, \quad p < 0.003 \]

\[ DON = 3 (\pm 1) + 14 (\pm 3) \cdot DOP, \quad R^2 = 0.62, \quad p < 0.006 \]

\[ BDOC = 6 (\pm 1) \cdot BDON, \quad R^2 = 0.83, \quad p < 0.0001 \]

\[ BDON = 18 (\pm 6) \cdot BDOP, \quad R^2 = 0.72, \quad p < 0.002 \]

Lønborg et al., Figure 4
a) $k_{\text{DOC}} = 0.73 \pm 0.18 \cdot k_{\text{DON}}$
$R^2 = 0.75$, $p < 0.001$

b) $k_{\text{DOC}} = 0.58 \pm 0.21 \cdot k_{\text{DOP}}$
$R^2 = 0.64$, $p < 0.01$

c) $k_{\text{DON}} = 0.77 \pm 0.15 \cdot k_{\text{DOP}}$
$R^2 = 0.76$, $p < 0.0001$

1 Lønborg et al., Figure 5