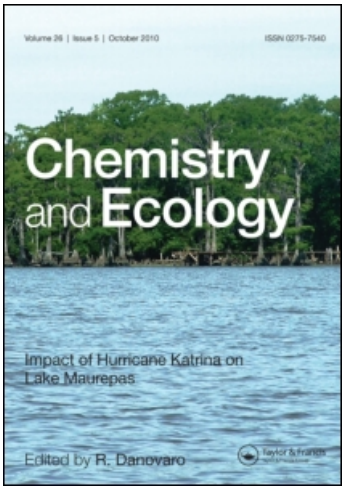


**Chemistry and Ecology**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

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Online publication date: 14 September 2010

**To cite this Article** Pettine, Maurizio , Capri, Silvio , Farrace, Maria Giuseppina , Manganelli, Maura , Patrolecco, Luisa , Puddu, Alberto and Zoppini, Annamaria(2002) 'Variability of dissolved organic matter in northern adriatic coastal waters', Chemistry and Ecology, 18: 1, 13 — 25
**To link to this Article: DOI:** 10.1080/02757540212690
**URL:** http://dx.doi.org/10.1080/02757540212690

*Chemistry and Ecology*, 2002, Vol. 18(1-2), pp. 13–25

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# VARIABILITY OF DISSOLVED ORGANIC MATTER IN NORTHERN ADRIATIC COASTAL WATERS

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*(Received 1 November 2001)*



The results obtained in the four seasonal cruises planned in the PRISMA II project are reported. These concern dissolved and colloidal organic carbon, free amino acids and total dissolved carbohydrates and heterotrophic activity. Main factors controlling organic matter degradation, resulting from laboratory tests not planned in the above project, are also discussed. Dissolved organic matter shows seasonal accumulation, which may be markedly different from year to year, and large contributions by colloidal and saccharide components. Heterotrophic activities play an important role in the carbon cycle, although laboratory runs highlight limitations caused by aging of organic matter and phosphorus deficiency.

*Keywords*: Organic matter; phosphorus deficiency; Adriatic Sea

## 1. INTRODUCTION

Dissolved organic carbon (DOC) is by far the major carbon pool in oceans, outweighing any other marine source of organic carbon by at least of a factor of 10 (Kepkay, 1994). While in oceanic waters dissolved organic matter results mainly from biological processes (the most relevant being cellular lysis, sloppy feeding and algal excretion), in coastal waters there is an additional source of organic matter which is given by terrestrial inputs. The northern Adriatic Sea is, in this context, a peculiar system since it receives large quantities of organic matter, estimated to be in the order of $50 \times 10^4$ tonnes per year (Pettine *et al*., 1998). The fate of carbon in this system has a large influence on the trophic food chain and is responsible for the two most serious environmental problems (anoxic crises and massive mucilage occurrences) affecting this basin. Despite the research effort devoted to this system, information on the distribution and variability of dissolved organic matter and its important biochemical components is limited and our knowledge of processes controlling the fate of organic matter is very scarce (Najdek, 1993).

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ISSN 0275-7540 print; ISSN 1029-0370 online 
DOI: 10.1080/0275754021000001925

This paper presents data on dissolved organic carbon concentrations and its molecular weight distribution, on two important DOC biochemical components (total dissolved carbohydrates TDCHO and amino acids DFAA) and on heterotrophic activity in northern Adriatic coastal waters. Measurements were performed to two frontal regions in the framework of PRISMA II – Biogeochemical Cycles project. Field results were paralleled by laboratory degradation runs on natural DOC samples aimed at highlighting possible limitations of bacterial activity BCP. Results obtained extend to our knowledge of chemical and molecular size composition of organic matter, providing information on temporal and spatial variability. Relationships among these variables are also discussed in order to provide synthetic descriptive tools for the acquired data sets.

## 2. MATERIAL AND METHODS

Water samples were collected with Niskin bottles on a rosette system equipped with a Sea-Bird CTD in two frontal regions in the northern Adriatic basin during four cruises on the R/V "Urania" (June, 1996, February and June, 1997 and February, 1998). In every area and in each cruise, sampling was conducted in a grid of stations along 2–4 transects across the front at 4–6 depths. A bench salinometer "Autosal Guildline" was used to measure the bottle salinities. The sampling generally took 6 days to each grid to be completed. Samples were chosen in order to be representative of different water masses in the frontal region and to highlight differences between surface and bottom layers. The former corresponded to 5–6 m surface water column and the latter to deep waters with similar salinity characteristics. The total number of measurements was 401 (DOC), 30 (COC), 186 (TDCHO), 156 (DFAA), and 232 (BCP).



For the analysis of dissolved organic carbon (DOC), water samples were filtered aboard, immediately after collection, through precombusted (4 h at 480°C) Whatman GF/F glass fibre filters (0.7 μm nominal pore size) and transferred directly in duplicate into 25 ml high density polyethylene (HDPE) bottles, previously treated with nitric acid 1.2 M at 50°C for 1 h. The HDPE bottles were quick-frozen in an aluminum block at −20°C. In the laboratory, filtered samples were thawed, acidified to pH 2 with ultrapure hydrochloric acid and purged with nitrogen for about 10 min to remove inorganic carbon. DOC was assayed by high temperature catalytic oxidation (HTCO) using a Shimadzu TOC-5000 A analyzer. Carbon concentrations were determined against potassium hydrogen phthalate standards after correction for total blank. This value, which is the analytical system blank plus a Milli Q water blank, was approximately 10–15 μM carbon under our experimental conditions and was mostly due to the experimental system. Samples were measured in triplicate and the relative standard deviation was within 2%. A limited number of samples were processed with a tangential flow ultrafiltration system after filtration through a 0.4 μm polycarbonate membrane, as described in Pettine *et al.* (1998), in order to isolate two different molecular weight colloidal organic carbon classes (COC > 10 and > 1 kDa) from truly dissolved organic arbon (DOC < 1 kDa). Dissolved free amino acids (DFAA) were analysed modifying the original method of Mopper and Lindroth (1982) based on high performance liquid chromatography (HPLC) and fluorimetric detection following

derivatization with o-phthaldialdehyde (OPA) and 2-mercaptoethanol. The gradient system used was methanol/tetrahydrofuran (90:10 v/v) – acetate buffer according to Cowie and Hedges (1992). A standard was prepared daily using aged sea water as a matrix. Standards, samples and blanks (aged sea water) were run in triplicate. The analytical precision under our experimental conditions was 5% for most individual amino acids and 10–15% for lysine and ornithine which give unstable OPA derivatives. Amino acids are given as individual amino acids (DFAA, nM) or their sum and also expressed as the sum of the corresponding molar carbon units (DFAA-C). Total dissolved carbohydrates (TDCHO), including mono-, oligo- and polysaccharides, were analysed by the MBTH (methyl-2-benzothiazolinone hydrazone) method after a preliminary hydrolysis with 0.09 N hydrochloric acid (20 h at 100°C) (Burney and Sieburth, 1977). Recently, stronger hydrolysis procedures using sulphuric acid have been proposed (Pakulski and Benner, 1992; Mopper *et al.*, 1992) and compared with the mild 0.09 M hydrochloric acid attack. Depending on the composition of oligo- and polysaccharides, results from these different hydrolysis methods have been found to vary markedly from one system to another (Borch and Kirchman, 1997; Pakulski and Benner, 1994). Only non-structural carbohydrates (e.g. starchlike polysaccharides and other storage polymers) and low-molecular-weight oligosaccharides should be hydrolysed following the 0.09 N hydrochloric acid treatment (Pakulski and Benner, 1994). Samples were analysed in triplicate against glucose standard solutions and results expressed in terms of carbon units by assuming 6 mol of carbon per mole of hexose. The relative standard deviation between replicate samples was usually below 5%.



Bacterial production was measured on natural samples by the incorporation of $^3$H-thymidine in all the cruises and $^3$H-leucine only in 1997 and 1998 cruises. Triplicate 1.7 ml samples and one killed control (TCA 5%) were amended with 20 nM radiotracer and incubated for 1 h, from 10 to 11 am local time, at *in situ* temperature. The extraction, with 5% ice-cold TCA for thymidine and room temperature, 5% TCA for leucine, and subsequent washing with 5% TCA and 80% ethanol, was carried out by the microcentrifugation method, according to Smith and Azam (1992). To estimate the bacterial carbon production from rates of thymidine incorporated ($BCPT_{TdR}$), the conversion factor of $2 \times 10^{18}$ cells mol$^{-1}$ (Bell, 1993) and a bacteria-to-carbon conversion factor of 20 fg carbon cell$^{-1}$ were used (Lee and Furhman, 1987). Rates of leucine incorporation were transformed in carbon ($BCP_{Leu}$) using the conversion factor of 3.1 kg carbon produced per mole of leucine incorporated (Kirchman, 1993). Measurements with the two methods gave similar results with an average $BCP_{TdR}/BCP_{Leu}$ ratio of $1.2 \pm 0.6$. In the discussion of our results we will refer only to $BCP_{TdR}$, for which a complete set of data for all the cruises is available.

The effect of nutrients on bacterial growth was investigated using samples filtered through a 0.4 μm polycarbonate membranes (Nucleopore). Batch cultures of predator-free natural bacterial assemblages ($< 1$ μm) were carried out in 100 ml BOD-type in replicate bottles in the dark and at *in situ* temperature. All glassware was acid treated (1 N HCl) and washed with ultrapure water (Millipore Milli-Q) and finally precombusted at 500°C. To evaluate the effect of inorganic nutrients on bacterial growth, parallel treatments were spiked with nitrogen (5 μM $NO_3^-$ + 5 μM $NH_4^+$) and phosphate ($PO_4^{3-}$, 1 μM) together. A further treatment with inorganic nutrient and concentration of glucose close to natural DOC levels was carried out. In the samples collected in

February, 1998, inorganic nutrients were also added separately, in the molar ratio C:N:P = 80:10:1, to investigate the different effects of phosphorus and nitrogen on the DOC uptake. In order to measure the time course of changes in the cultures, sample aliquots were withdrawn at 12–24 h intervals and monitored for 96 h. Bacterial numbers were counted with acridine orange staining (Hobbie *et al.*, 1977) and bacterial production was measured by $^3$H-leucine incorporation. Specific growth rates were calculated on changes in cell abundance in the exponential growth phase, as $\mu(t^{-1}) = (\ln((B_t - B_0) \cdot B_0^{-1})) \cdot t^{-1}$, where $B_0$ is the initial cell abundance and $B_t$ the abundance at the time $t$ (in days).

The significance of differences in the median values among different data groups was analyzed by using the Mann-Whitney Rank Sum Test.

## 3. RESULTS

### 3.1. Dissolved Organic Carbon and Molecular Weight Distribution

The overall set of DOC values ranged from 53 to 281 μM, while in winter cruises it was 53–124 μM (Tab. I). Concentrations of DOC in the upper and bottom layers were reasonably constant in February surveys in the two areas. Mean values in the north were 95 ± 8; 89 ± 7 μM in the upper layer and 74 ± 3; 75 ± 5 μM in the bottom layer in 1997 and 1998. Differences between layers were small, but significant ($p < 0.001$); in the south, the data set of DOC in February, 1997 was limited to only 4 surface values and the resulting average value is not representative of the area; it may not be compared then with the other averages derived from much more numerous data sets. In Feb-



TABLE I Summary of concentrations of dissolved organic carbon (DOC), total dissolved carbohydrate carbon (TDCHO), free amino acid carbon (DFAA-C) and bacterial carbon production rates ($BCP_{TdR}$) in the two sampled areas in different cruises. Values refer to variation ranges (VR) and total number ($n$) of measurements, and to mean ±95% confidence interval for upper (U) and bottom (B) layers

| | | *DOC (μM)* | | *TDCHO (μM)* | | *DFAA-C(μM)* | | *$BCP_{TdR}$ (μg C $l^{-1}$ $h^{-1}$)* | |
|---|---|---|---|---|---|---|---|---|---|
| *Survey* | | *North* | *South* | *North* | *South* | *North* | *South* | *North* | *South* |
| June 96 | VR | 74–281 | 88–219 | 8.0–72.4 | 10.8–57.4 | 0.64–2.44 | 0.37–1.70 | 0.03–1.26 | 0.02–0.92 |
| | n | 66 | 37 | 17 | 17 | 23 | 22 | 30 | 31 |
| | U | 191 ± 16 | 153 ± 15 | 43.7 ± 17.3 | 33.4 ± 12 | 1.56 ± 0.66 | 0.81 ± 0.34 | 0.60 ± 0.28 | 0.38 ± 0.11 |
| | B | 137 ± 13 | 123 ± 13 | 29.1 ± 23 | 19.9 ± 11.9 | 1.46 ± 0.48 | 0.95 ± 0.36 | 0.10 ± 0.02 | 0.10 ± 0.11 |
| Febr. 97 | VR | 53–123 | 84–153 | 6.0–30.7 | 6.4–39.2 | 0.44–1.81 | 0.4–1.81 | 0.03–0.67 | 0.05–1.61 |
| | n | 76 | 4 | 19 | 20 | 20 | 20 | 30 | 24 |
| | U | 95 ± 8 | 111 | 18.4 ± 7.7 | 16.1 ± 12 | 0.90 ± 0.22 | 0.96 ± 0.47 | 0.23 ± 0.08 | 0.62 ± 0.38 |
| | B | 74 ± 3 | | 11.2 ± 3 | 8.7 ± 1.6 | 0.81 ± 0.22 | 0.78 ± 0.16 | 0.21 ± 0.10 | 0.16 ± 0.09 |
| June 97 | VR | 66–162 | 71–142 | 10.0–50.0 | 10.8–24.2 | 0.14–0.57 | 0.14–0.60 | 0.09–7.09 | 0.08–0.62 |
| | n | 81 | 23 | 36 | 23 | 12 | 10 | 30 | 30 |
| | U | 123 ± 8 | 100 ± 27 | 27.4 ± 8.4 | 18.2 ± 1.7 | 0.39 ± 0.13 | 0.32 ± 0.16 | 2.45 ± 1.8 | 0.36 ± 0.39 |
| | B | 81 ± 9 | 84 ± 11 | 16.4 ± 2.8 | 16 ± 4.2 | 0.36 ± 0.13 | 0.30 ± 0.19 | 0.15 ± 0.05 | 0.11 ± 0.04 |
| Febr. 98 | VR | 65–124 | 58–115 | 7.1–39.4 | 6.7–19.4 | 0.16–0.39 | 0.23–0.82 | 0.02–1.19 | 0.01–0.45 |
| | n | 63 | 51 | 23 | 22 | 12 | 12 | 30 | 27 |
| | U | 89 ± 7 | 83 ± 7 | 18.5 ± 10.0 | 11.9 ± 3.3 | 0.25 ± 0.08 | 0.31 ± 0.06 | 0.37 ± 0.34 | 0.18 ± 0.10 |
| | B | 75 ± 5 | 73 ± 4 | 11.0 ± 6.4 | 10.5 ± 3.6 | 0.23 ± 0.06 | 0.44 ± 0.20 | 0.03 ± 0.01 | 0.01 ± 0.004 |

ruary, 1998 values in the south were $83 \pm 7$ and $73 \pm 4\,\mu M$ in the upper and bottom layers. The bottom average in February surveys is substantially the same in both years and areas, $74 \pm 2\,\mu M$ considering all data together, and can be considered as a threshold value for this coastal ecosystem. Contrary to the February results, concentrations of DOC in the June surveys varied in the two investigated years. They were $191 \pm 16$ and $137 \pm 13\,\mu M$ in the upper and bottom layers in 1996 compared to $123 \pm 8$ and $81 \pm 9\,\mu M$ in 1997, in the northern region. In the south, surface and bottom averages were $153 \pm 15$ and $123 \pm 13\,\mu M$ in 1996 compared to $100 \pm 27$ and $84 \pm 11\,\mu M$ in 1997. Mean value differences between layers were significant in the northern region in both the June cruises ($p < 0.001$) while in the south only in June, 1996 ($p < 0.05$); differences between corresponding layers in consecutive years were significant in the June cruises both in the north ($p < 0.001$) and in the south ($p < 0.01$). Differences between north and south frontal areas were limited to surface waters in June, 1996 ($p < 0.001$).

During our investigations, 30 samples from the two areas at various depths have been processed with the ultrafiltration system (12 in June, 1996 and 6 in each of other cruises). Since we did not find any significant difference between north and south regions and as a function of depth, results are discussed together. Concentrations of the overall COC (>1 kDa) ranged from 49 to 98 μM and from 44 to 80 μM in the June and February surveys. Average values calculated from all the data were $67 \pm 14\,\mu M$ and $39 \pm 13\,\mu M$ for the overall colloidal ($0.4\,\mu m > DOC > 1\,kDa$) and truly dissolved organic components ($DOC < 1\,kDa$). Overall COC concentrations made up 52 to 74% of total DOC with an average of $63 \pm 7\%$ in the June surveys and 54 to 83% with an average of $66 \pm 9\%$ in the February surveys, while the truly dissolved fractions (< 1 kDa) accounted for $38 \pm 7$ and $36 \pm 7\%$ of DOC in the two seasonal periods. Then, a major portion of the whole DOC pool is in the colloidal fraction in our system. Moreover, the high molecular weight colloidal fraction ($HMWC > 10\,kDa$) gave average contributions to DOC of $21 \pm 5$ and $18 \pm 6\%$ in June and February surveys respectively, while the low molecular weight colloidal fraction ($1\,kDa < LMWC < 10\,kDa$) accounted for $42 \pm 7$ and $48 \pm 6\%$ of DOC in the two seasons, with a major portion of COC existing as LMWC fraction. The sets of data (μM) of COC > 1 kDa and COC > 10 kDa showed very tight relationships as a function of DOC (Fig. 1), according to

$$\mathrm{COC}(>1\,\mathrm{kDa}) = 0.53(\pm 0.07)\mathrm{DOC} + 12.17(\pm 7.1)$$
$$n = 27;\ r = 0.84;\ \text{s.d.} = 7.5;\ p < 0.001$$

$$\mathrm{COC}(>10\,\mathrm{kDa}) = 0.33(\pm 0.04)\mathrm{DOC} - 12.50(\pm 5.1)$$
$$n = 27;\ r = 0.85;\ \text{s.d.} = 5.3;\ p < 0.001$$

### 3.2. Biochemical Compounds

Total dissolved carbohydrates are the major biochemical class of organic carbon compounds in waters. The overall set of values in carbon units ranged from 6.0 to 72.4 μM, while in February cruises it was restricted to 6–39.4 μM; average values corresponding to the previous ranges were $18.3 \pm 12.0$ and $12.9 \pm 7.4\,\mu M$. The distribution

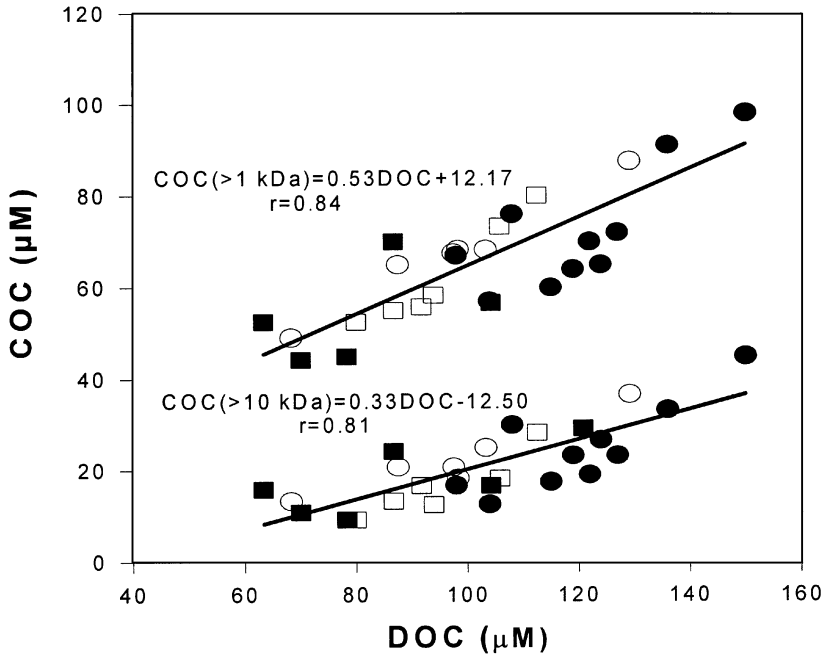


FIGURE 1 Relationship between concentrations of colloidal (COC) and dissolved organic carbon (DOC). ● June, 1996; □ February, 1997; ○ June, 1997; ■ February, 1998.



of TDCHO values showed a strict similarity with that of DOC. The June and February data groups were significantly different ($p < 0.001$) by considering the entire data sets. TDCHO average concentrations in the upper and bottom layers were reasonably constant in the two February surveys in the north, with surface values (18.4 and 18.5 μM in 1997 and 1998) higher than the corresponding bottom ones (11.2 and 11.0 μM, Tab. I). Differences between layers were significant ($p < 0.01$) and suggest the presence of a marked vertical gradient in the zone which is under the direct influence of the Po River. In the south, differences between surface and bottom layers were not significant. TDCHO averages in consecutive June surveys showed significant variations in the two investigated years in both the north and south regions, consistent with similar variations for DOC. Surface averages changed from $43.7 \pm 17.3$ to $27.4 \pm 8.4$ and $33.4 \pm 12$ to $18.2 \pm 1.7$ μM in the north and south regions. The two frontal areas showed significant differences in the distribution of TDCHO values in June, 1996 and a scarcely or not significant difference in the other cruises. The first survey was also characterized by significant frontal gradients in carbohydrate concentrations with values in the inshore side ($46 \pm 22$ and $37 \pm 4$ μM in the north and south) significantly different from those in the corresponding offshore side ($19 \pm 7$ and $25 \pm 22$ μM); in the other surveys differences between sides in the front were small and only scarcely significant.

The percent contributions of TDCHO to DOC were $20.3 \pm 10.7$; $15.1 \pm 4.9$; $18.4 \pm 4.3$ and $14.2 \pm 5.0$ in June, 1996, February, 1997, June, 1997 and February, 1998, with average values of $19.1 \pm 7.3\%$ in the June and $14.5 \pm 4.9\%$ in the February cruises; corresponding variation ranges were 5.3–49.9 and 7.7–30.9%. TDCHO concentrations from different areas and surveys give a good relationship as a function of DOC (Fig. 2)

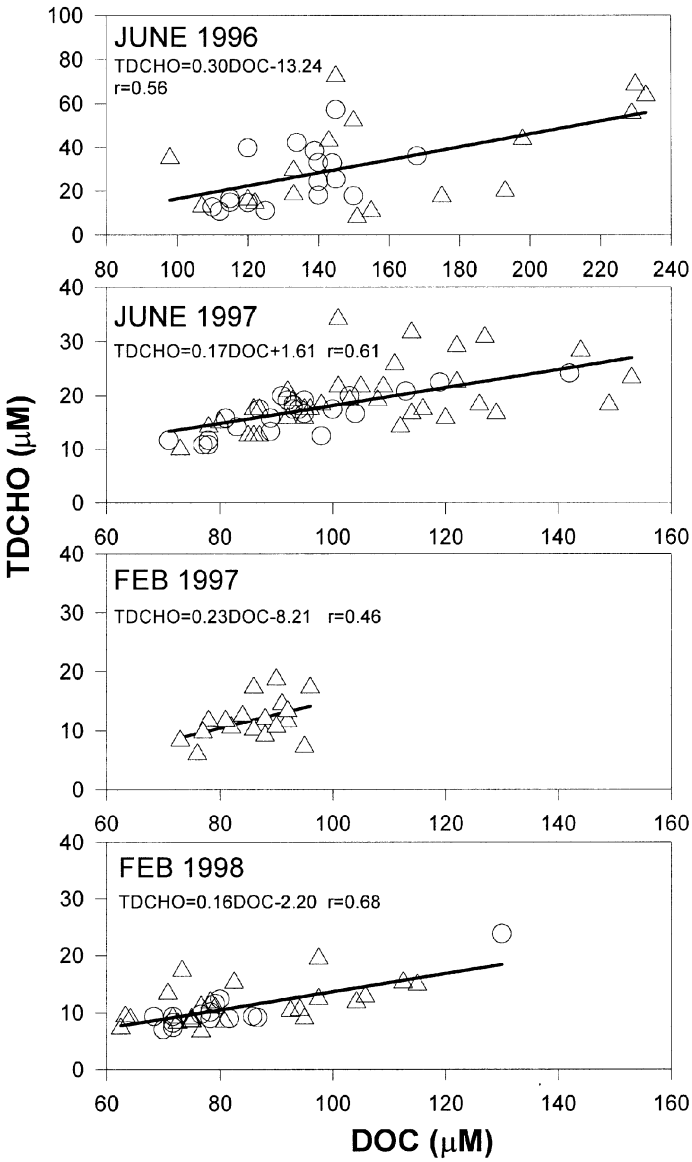


FIGURE 2 Relationships between the concentrations of total dissolved carbohydrates (TDCHO) and DOC measured in both the frontal areas. Δ northern area; ◯ southern area.

although a few data in June, 1996 showed a high scattering. The entire data sets were described by

$$\mathrm{TDCHO} = 0.28(\pm 0.02)\mathrm{DOC} - 10.46(\pm 2.22)$$
$$n = 155;\ r = 0.74;\ \text{s.d.} = 8.07;\ p < 0.001$$

The slope resulting from all the data indicates that TDCHO is responsible for 28% of the DOC variations in our system over the study period. This slope is in the high

range of values reported for TDCHO *vs.* DOC slopes which have been found to vary from 0.09 to 0.29 (Burney and Sieburth, 1977; Senior and Chevolot, 1991; Pakulski and Benner, 1994).

Concentrations of DFAA-C (amino acids in carbon units) from both frontal regions varied in the ranges 0.14 to 2.44 and 0.16 to 1.56 μM C in the June and February surveys (Tab. I). The corresponding average values were $0.92 \pm 0.61$ and $0.57 \pm 0.35$ μM C. These significant differences ($p < 0.01$) between the two seasonal periods are strongly dependent on variations in the northern area. The summer/winter ratio of 1.6 which is given by the above average values results from ratios of 1.8 and 1.6 in the north and south regions. Contrary to DOC and TDCHO data, DFFA-C average concentrations did not show any significant variations between layers. Differences between the two frontal areas were significant in June, 1996, with the northern average ($1.50 \pm 0.56$ μM) higher than the southern one ($0.89 \pm 0.35$ μM) less directly influenced by the Po River. In the other three cruises differences between areas were not significant. It is worth noting that the June, 1996 was characterized by higher concentrations of DFAA compared to the other cruises, both in the northern and southern areas. Differences between coastal and offshore frontal sides were only significant in the north in February, 1997 ($p < 0.001$), while they were not in all the other occasions.

DFAA-C data gave average contributions to DOC of $0.80 \pm 0.32$; $0.93 \pm 0.22$; $0.34 \pm 0.14$ and $0.37 \pm 0.15$% in June, 1996, February, 1997, June, 1997 and February, 1998, respectively, with marked differences in the two consecutive summer and winter periods. However, data collected in the two June and February surveys give rise to similar averages ($0.65 \pm 0.33$ and $0.64 \pm 0.34$ μM) and similar variation ranges (0.13 to 1.6 and 0.16 to 1.4 μM). The overall set of DFFA-C data was described as a function of DOC by

$$\mathrm{DFAA-C} = 0.009(\pm 0.001)\ \mathrm{DOC} - 0.30(\pm 0.12)$$
$$n = 113;\ r = 0.67;\ \text{s.d.} = 0.41;\ p < 0.01$$



### 3.3. Heterotrophic Activity

Values of $BCP_{TdR}$ from all the cruises and the two frontal regions varied in the range 0.01–7.09 μg C $l^{-1}$ $h^{-1}$. The variability in winter periods was much more limited with values in the range 0.01–1.61 μg C $l^{-1}$ $h^{-1}$. In the north, surface average values were similar in the two February cruises while significantly ($p < 0.05$) different in the two June cruises; on the contrary, bottom values were similar in the June cruises and significantly ($p < 0.001$) different in the February cruises. Surface and bottom averages in this region were significantly different ($p < 0.05$) except in February, 1997. In the south, both the surface and the bottom averages were similar in the June surveys while values in winter cruises were significantly different ($p < 0.01$), reflecting a higher productivity in 1997 compared to 1998. Surface and bottom averages, in the south, were also different in all cruises. Contrary to DOC and biochemistry, BCP was significantly higher in June, 1997 compared to June, 1996. The comparison between the two frontal regions generally highlights higher values in the north compared to the south with the exception of February, 1997.

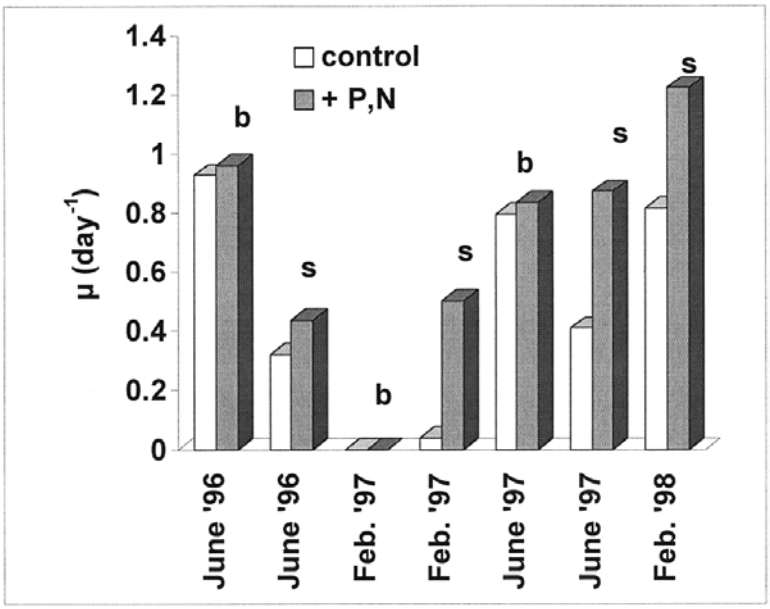


FIGURE 3 Bacterial growth rates in the exponential growth phase of sea water culture (b = bottom samples; s = surface samples).



### 3.4. Bioassays

The combined phosphorus and nitrogen additions to the predator-free batch cultures (Fig. 3) increased growth rates with respect to the control in surface samples, but not in bottom samples. The combined addition of glucose and inorganic nutrients produced in any experiment an extra increment in the growth rates proportional to the glucose carbon added ($r^2 = 0.7$ $n = 6$). In February, 1998 experiments were performed with separate additions of inorganic nutrients. Results clearly showed an increase in 3H-leucine incorporation rates with respect to the control (352 pM $h^{-1}$) in the case of the addition of phosphate (710 pM $h^{-1}$), while the only addition of nitrogen (308 pM $h^{-1}$) did not produce any significant effect.

## 4. DISCUSSION

DOC concentrations in northern Adriatic waters are characterised by seasonal changes with DOC accumulation in the summer period. Bottom and surface values recorded in our winter cruises are comparable to concentrations typical of surface oceanic waters (70–80 μM; Guo *et al.*, 1995) and to surface maxima recorded over a 12 months period by Copin-Montegut and Avril (1993) in Mediterranean waters (92 μM surface; 50 μM deep waters). Thus, degradation processes and water mass exchanges between the northern and middle Adriatic are able to keep DOC concentrations at a low level, despite the significant external and organic matter loads which affect this basin (Pettine *et al.*, 1998; Puddu *et al.*, 1998a). DOC values were strongly correlated with salinity

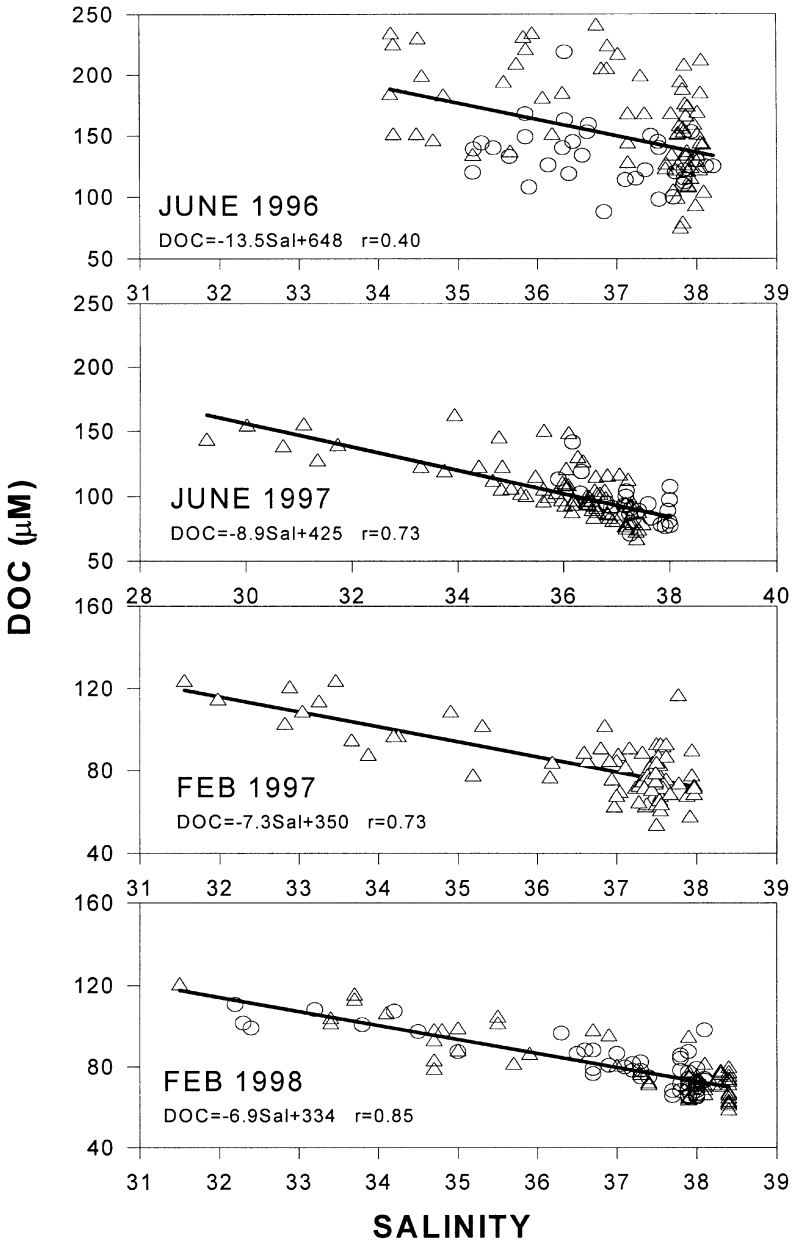


FIGURE 4 Behaviour of DOC values from both the frontal areas as a function of salinity. Δ northern area; ◯ southern area.

(Fig. 4), except than in the first survey where the scattering was high; however, the intercepts of fitting lines give unrealistically high values for the Po River end member which is the major terrestrial source. These higher values of the intercept reflect an increase in the slopes of the fitting lines which are driven by a stimulation of *in situ*

biological production processes in inshore eutrophied waters. Summer concentrations of DOC are significantly higher than winter values, although it may be noted that in the two investigated years, they were significantly different. Average surface values in June, 1996 and 1997 are a factor of 2.6–2.1 and 1.7–1.3 higher than the threshold value typical of bottom waters in February for the northern—southern areas. That DOC concentrations show seasonal changes also results from previous findings (Fonda Umani *et al.*, 1997; Faganeli and Herndl, 1991; Vojvodić and Cosović, 1996).

Two major characteristics of dissolved organic matter in the northern Adriatic system, which have been discussed in previous papers of our group (Pettine *et al.*, 1999; Pagnotta *et al.*, 1999; Puddu *et al.*, 2001), need to be remarked: the presence of a high colloidal fraction with a shift in the molecular weight distribution toward the large size fraction and a high contribution of carbohydrates to dissolved organic matter.

$BCP_{TdR}$ measurements showed a high variability with thymidine incorporation rates in the range 0.2–177 pM $h^{-1}$ and were tightly correlated with salinity ($p < 0.001$). This wide variability, which has a similarity only in the estuarine environment of the Chesapeake Bay (0.5–500 pM TdR $h^{-1}$, Ducklow & Shiah, 1993), reflects relatively quick adaptations of microorganisms to environmental changes driven by biological processes and external inputs. Two major factors controlling the fate of organic matter result from laboratory biodegradation experiments: the quality of the organic substrate and phosphorus limitation. The influence of the former variable was clear from bottom samples collected in February which did not give any increase in bacterial abundance and any growth even in the presence of added inorganic nutrients, while they have positively responded to the additions of both organic and inorganic nutrients. The influence of the latter variable was argued from the clear positive influence exerted by the addition of phosphorus on the degradation of freshly produced organic matter in surface samples which were insensitive to the additions of nitrogen salts. The strong correlations of both DOC and BCP values as a function of salinity along with estimates of a bacterial carbon demand higher than primary production in low productivity periods strengthen the role of allochthonous inputs in this basin. Riverine inputs are also responsible for the observed high colloidal fraction and the shift toward the large molecular size (Benner and Hedges, 1993; Whitehouse *et al.*, 1989).

Setting up of strong vertical stratification and eddy circulation which characterize the northern Adriatic in the summer period increase fresh water residence time and favor the accumulation of refractory organic matter directly discharged by rivers. However, the accumulation of organic matter in the refractory pool may be partially counteracted by photoxidation processes which tend to transform refractory into labile compounds. In spring-summer periods the *in situ* production of DOC is also strongly enhanced and often phosphorus becomes exhausted after algal blooms. Under these conditions, the fraction of biogenic carbon which is not immediately degraded may undergo, with aging, abiotic processes which are strongly driven by interactions with terrestrial-derived phosphorus DOM. The severe phosphorus limitation which also occurs during this period along with aging processes lower degradation rates of organic substrates, favouring an enrichment in organic matter. High polymer and particle concentrations which are typical of this system enhance the probability of spontaneous assembling of organic macromolecules to form microgels and their following annealing to produce larger aggregates (Chin *et al.*, 1998).

## 5. CONCLUSIONS

Seasonal increase in DOC concentrations along with its high colloidal fraction, high molecular weight size and high carbohydrate contributions result from data obtained during the PRISMA II project. An inefficient DOC uptake by bacterioplankton, due to the scarce bioavailability of DOC and/or to nutrient limitation, expecially phosphorus, possibly contribute to the above increase. The consequent accumulation of aged DOC is a necessary condition which concur in the formation of mucilaginous aggregates. However, the dynamic and fate of these aggregates will also be profoundly influenced by biological processes which take place within them and by wind-induced mixing of the water column which prevent their disaggregation and favour their high residence in the water column and their growth (Riebesell, 1992).

More results on the composition and variabilities of DOC, COC, TDCHO and DFAA are needed along with further data on combined amino acids, monosaccharides and structural carbohydrate components, as well as on the composition of colloidal matter, its terrigenous and marine components and bio-lability characteristics in order to characterize better the role of DOM in northern Adriatic waters.



### *Acknowledgements*

Thanks are due to Mr. F. Bacciu of this Institute for his valuable help in the collection of samples and analysis of DOC. This work was supported by the Italian Ministry of University and Scientific and Technological Research (MURST) and the National Research Council (CNR) in the framework of the PRISMA II – Biogeochemical Cycles Research Project.

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