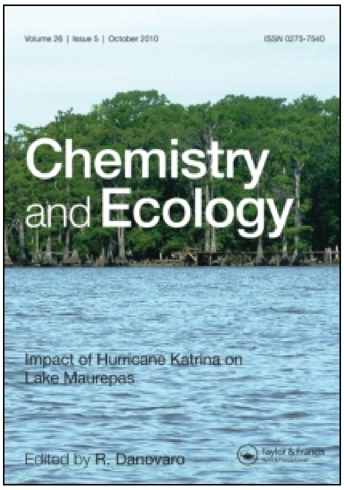

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MESOSCALE VARIABILITY OF ORGANIC MATTER COMPOSITION IN NW ADRIATIC SEDIMENTS

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(In final form 2 December 2002)

We investigated the spatial variability of sediment organic matter content and composition in three areas (A, B and C) of the Northwestern Adriatic Sea, subjected to a putative gradient of trophic state (*i.e.*, increasing distance from the Po river outflow) in order to determine the appropriate sample size and replication. The analysis of the mesoscale variability was carried out comparing variability on the scale of meters (*i.e.* among different deployments) with the variability observed on a scale of several kilometres (*i.e.* among different sampling areas). Sediment samples, collected on April 1999, October 1999, April and October 2000, were analysed for chloropigment content (chlorophyll-a and phaeopygments) and protein, carbohydrate and lipid concentrations. Chloropigment, protein, carbohydrate and lipid concentrations were high, indicating that this system shares trophic conditions typical of highly productive environments. All organic matter components displayed a distribution independent from the increasing distance from the Po river outflow and a clear spatial variability, characterised by significant differences among different areas, but not among deployments. Carbohydrates were the biochemical compound displaying the highest spatial variability among the three areas. Chloropigment, protein, carbohydrate and lipid concentrations displayed also significant temporal changes. When spatial and temporal variability were compared, chlorophyll-a, phaeopigment and protein concentrations displayed a higher temporal than spatial variability. Conversely, for carbohydrates and lipids spatial and temporal variability was of the same order of magnitude. Organic matter composition displayed limited changes among areas, but a strong temporal variability. The results from the Adriatic sea suggest that analyses from sediments collected from a single deployment are sufficient for assessing organic matter concentration and composition over areas of several hundreds of square meters. However, for estimating organic matter composition over larger spatial scales (*i.e.* miles) the identification of different sampling areas is needed.

Keywords: Mesoscale; Sediment; Chloropigments; Organic matter composition

INTRODUCTION

The biochemical composition of sediment organic matter is the result of the dynamic equilibrium between external inputs, autochthonous production and heterotrophic utilisation. Quality and quantity of organic matter in surface sediments have been considered of primary importance in determining the amount of organic material potentially available to consumers, thus affecting community structure and benthic metabolism (Graf *et al.*, 1983; Grant and Hargrave, 1987; Thompson and Nichols, 1988; Graf, 1992). Microphytobenthos is also a substantial food source for benthic invertebrates, from protozoan to meio- and macrofauna

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(Koop and Griffith, 1982; Montagna *et al.*, 1983; Bianchi and Levinton, 1984; Plante-Cuny and Plante, 1986). Therefore, the knowledge of organic matter and phytopigment distribution is crucial in benthic ecological studies (Mayer, 1989; Fabiano *et al.*, 1995). However, so far, most studies dealing with sedimentary organic matter have been performed on selected sites, repeatedly investigated for temporal-trend analysis (Danovaro *et al.*, 1994; Fabiano *et al.*, 1995; Danovaro, 1996; Danovaro *et al.*, 1999a). The spatial distribution of benthic key-parameters (*i.e.* organic matter composition, bacteria, microphytobenthos) also received little attention, and generally was limited to small scale investigations (*i.e.*, cm-metre; Findlay, 1981; Plante *et al.*, 1986; Decho and Fleeger, 1988; Blanchard, 1990; Pinckney and Sandulli, 1990; Sandulli and Pinckney, 1999; Danovaro *et al.*, 2001).

The distribution of organic matter on the sea floor largely depends upon primary productivity of the water column which, in turn, is controlled by a complex array of factors including water column structure and circulation (Levy *et al.*, 1999; Spall and Richards, 2000). Recent studies carried out in the Mediterranean reported a tight coupling between mesoscale circulation and primary production (Andersen and Prieur, 2000). However, a certain confusion arises when oceanographers and benthonologists carry on investigations on mesoscale, because these two disciplines use different spatial scales to investigate mesoscale processes (*i.e.*, 1 to 100 km or more for oceanographic studies and 1–100 m or more for benthic investigations).

It is well known that benthic marine assemblages are characterised by aggregate distributions, which can be related to local environmental, trophic, mineralogical, and hydrodynamic conditions (Bell *et al.*, 1978; Eckman, 1979; Fleeger *et al.*, 1984; Cerrano *et al.*, 1999). Also vertical fluxes of settling particles may change on spatial scale of meters (Crassous and Krihpounoff, 1994). Therefore it is likely that sedimentary organic matter concentration and composition also depend from mesoscale variability of the overlying water column.

In the present study we investigated the spatial variability of sediment organic matter content and composition in order to determine the appropriate sample size and replication. The analysis of the mesoscale variability was carried out comparing variability on the scale of meters with the variability observed on a scale of several kilometres. The study was conducted in a sector of the Northwestern Adriatic Sea, subjected to a putative gradient of trophic state (*i.e.*, increasing distance from the Po river outflow). Finally, sediment sampling was repeated on seasonal basis in order to compare the relative importance of the temporal *vs.* spatial variability.

MATERIALS AND METHODS

Study Area and Sampling

Sediment sampling was carried out on three different areas of the NW Adriatic Sea using the R/V Daphne (Fig. 1). The first area A (*ca.* 12 m depth) is close to the Po delta, the second area B (*ca.* 11 m depth) and the third area C (*ca.* 10 m depth) are located at increasing distance from the Po delta, at about 15 miles distance from each other. Each sampling area (*i.e.*, each square, Fig. 1) had a surface of *ca.* 400 m^2. At each sampling site, temperature and salinity of surface water column were synoptically measured by a multiparametric CTD-probe (Seabird).

Undisturbed sediment samples were collected using a box-corer on April 1999, October 1999, April and October 2000. These two periods were selected on the basis of the regime of the Po river outflow and typical seasonality of the sampling area. During each cruise, 4 to 7

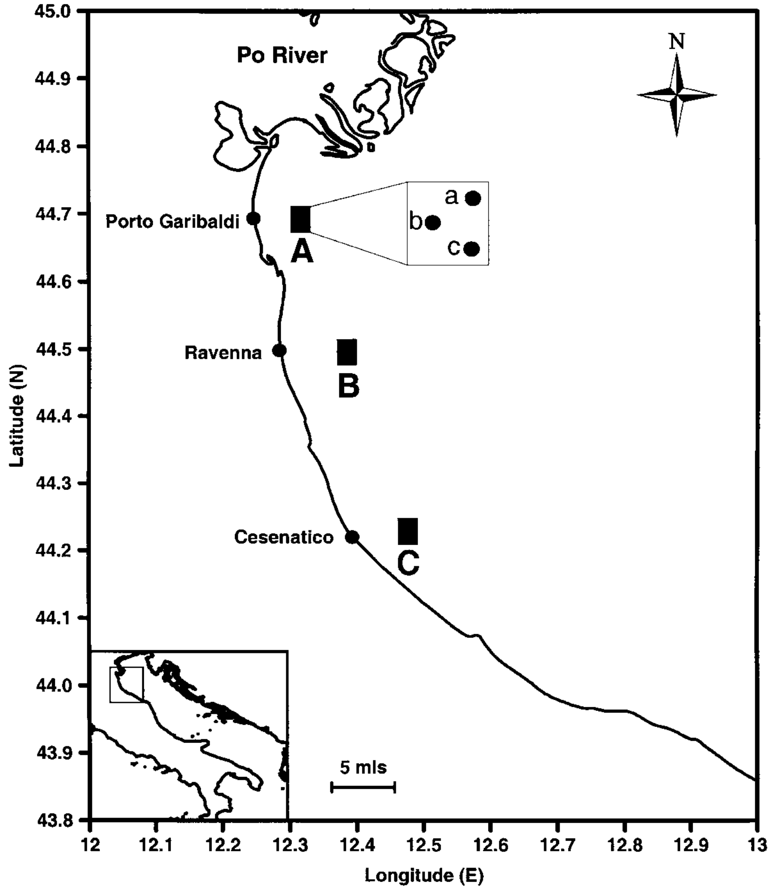

FIGURE 1 Sampling area and station location. Reported are the three sampling areas: area A (Porto Garibaldi), area B (Ravenna) and area C (Cesenatico). The position of three box corer deployments are indicated as an example.

deployments were carried out at each site. From three different deployments, 3 different cores were randomly collected. In this way the spatial scale of meters and tens of miles was compared in different sampling periods. Upon recovery cores were vertically divided into 3 layers (0–1, 1–3 and 3–6 cm) and deep frozen at −20 °C until analysis. All analyses were carried out only on the top 1 cm of the sediment cores.

Sedimentary Parameters

Total lipids were extracted by direct elution with chloroform–methanol according to Bligh and Dyer (1959) and Marsh and Weinstein (1966). Analyses were carried out on about 1 g of sediment sonicated for 30 minutes in deionised water. This treatment allows to increase the extraction efficiency of lipids of about 30% (Danovaro, 1996). Data are reported in tripalmitina equivalents.

Protein analysis were conducted following extraction with NaOH and were determined according to Hartree (1972), modified by Rice (1982) to compensate for phenol interference. Concentrations are presented as bovine serum albumin (BSA) equivalents. Carbohydrates were analysed according to Gerchacov and Hatcher (1972) and expressed as glucose equivalents. The method is based on the same principle as the widely used method of Dubois *et al.* (1956), but is specifically adapted for carbohydrate determination in sediments.

Analyses of chlorophyll-a and phaeopigments in the sediments were carried after extraction with 90% acetone (24 h in the dark at 4 °C, Lorenzen and Jeffrey, 1980). After centrifugation ($800 \times g$, 10 min), the supernatant was used to determine functional chlorophyll-a and phaeopigments were determined after acidification with 0.1 N HCl to estimate the amounts of phaeopigments (Plante-Cuny, 1974).

All analysis were carried out on three replicates. For each biochemical analysis, blanks were made using the same sediments previously treated in a muffle furnace (550 °C, 4 h). All data were normalised to sediment dry weight after desiccation (60 °C, 24 h).

Biopolymeric organic carbon (BPC; *sensu* Fabiano *et al.*, 1995) was defined as the sum of the carbon equivalents of total carbohydrates, proteins and lipids (utilizing conversion factors of 0.4, 0.49 and 0.75, respectively).

Data Analysis

A Spearman-Rank correlation analysis was performed to test for possible relationships among the investigated variables. Analyses of variance (ANOVA) were carried out to test for temporal and spatial differences among areas and among different deployments in the investigated variables.

RESULTS AND DISCUSSION

The Po river, accounting alone for about 30–50% of the total watershed input of the entire Adriatic basin, is recognised to be one of the most important components influencing trophic state, physical characteristics and biogeochemical processes of the Northern and Middle Adriatic Sea (Franco and Michelato, 1992). This was confirmed by salinity distribution, which displayed a clear increasing trend with increasing distance from the Po delta. Salinity values were characterised by significant differences among sampling periods ($F = 11.957, p < 0.01$; ranging from 31.96 in October 1999 to 37.26 in October 2000) related to the river outflow regime. Temperature displayed a typical seasonality (from 10.6 in April 1999 to 20.6 °C in October 1999).

All organic matter components displayed a spatial pattern apparently independent from the salinity gradient and the increasing distance from the Po river outflow. Chloropigment (chlorophyll-a and phaeopigments) concentrations were high and, indeed, comparable to values previously reported for highly productive environments (Danovaro, 1996; Boon *et al.*, 1999; Danovaro *et al.*, 1999a; Pusceddu *et al.*, 2000). Also proteins, carbohydrates and lipids displayed very high concentrations (see Pusceddu *et al.*, 1999 and data therein for comparison). This is not surprising since these variables reflect the general trophic state and the level of sediment organic enrichment, which is expected to be high in this coastal area influenced by the river outflow (Moodley *et al.*, 1998; Danovaro *et al.*, 2000).

All variables displayed a clear spatial variability, characterised by significant differences (Tab. I) among different areas (A, B, C), but not among different deployments (a, b, c). The spatial variability of chloroplastic pigments is illustrated in Figures 2 and 3 (for chlorophyll-a and phaeopigments, respectively). Highest chlorophyll-a concentrations

TABLE I Results of the ANOVA Analyses for the Benthic Variables.

Source of variation	*Variable*	*F*	*P-value*
Sampling period	Proteins	20.316	***
	Carbohydrates	3.328	*
	Lipids	25.788	***
	Chlorophyll-a	10.577	***
Inter-areas	Proteins	8.327	***
	Carbohydrates	5.385	**
	Lipids	3.286	*
	Chlorophyll-a	10.577	***
Inter-deployments	Proteins	0.120	ns
	Carbohydrates	0.204	ns
	Lipids	0.366	ns
	Chlorophyll-a	0.075	ns

Note: (*) $= P < 0.05$; (**) $= P < 0.01$; (***) $= P < 0.001$; ns = No Significant Difference.

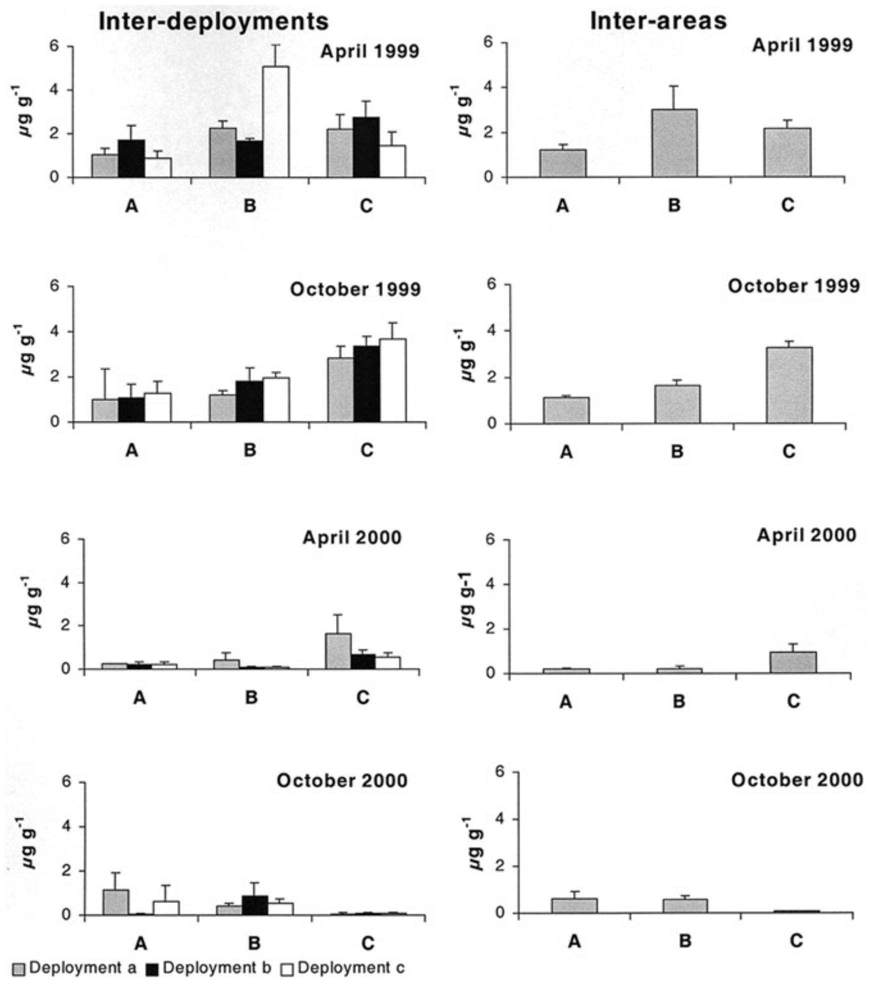

FIGURE 2 Chlorophyll-a concentrations (expressed as $\mu g\, g^{-1}$) as average of different deployments (±standard deviation; left side) and average values for each area (±standard error; right side) are reported in the sediments of area A (Porto Garibaldi), area B (Ravenna) and area C (Cesenatico) during the four sampling periods.

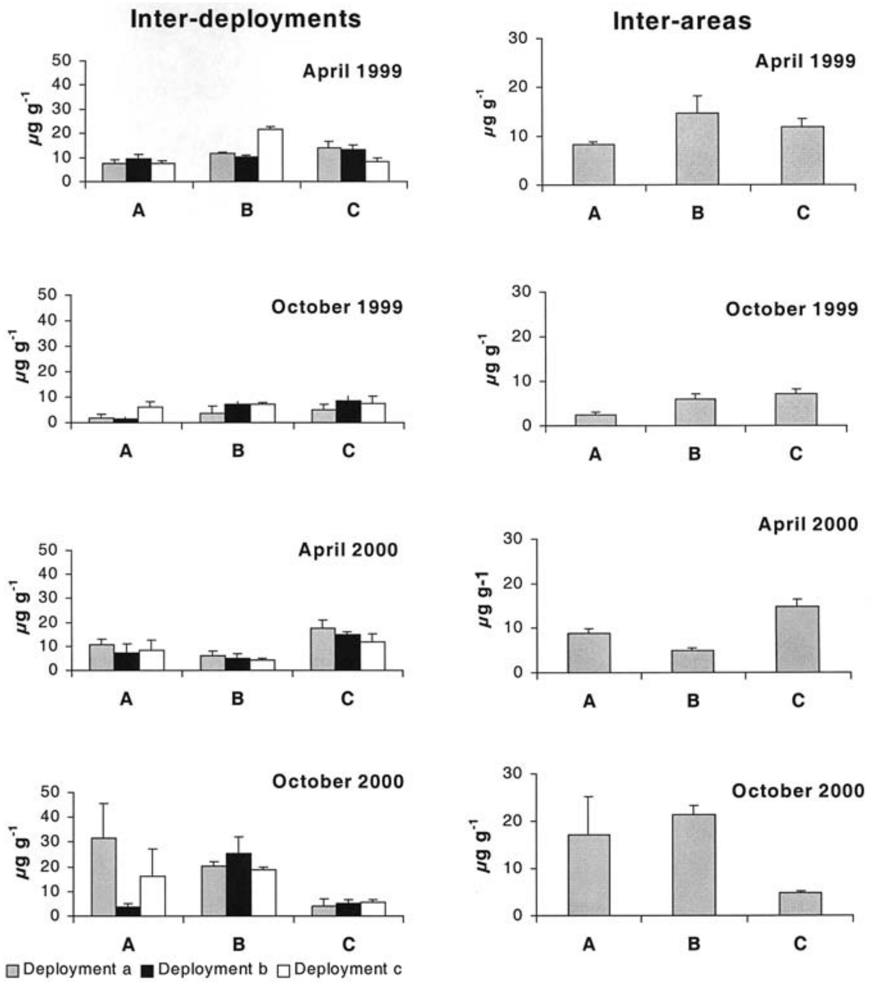

FIGURE 3 Phaeopigment concentrations (expressed as $\mu g\,g^{-1}$) as average of different deployments (±standard deviation; left side) and average values for each area (±standard error; right side) are reported in the sediments of area A (Porto Garibaldi), area B (Ravenna) and area C (Cesenatico) during the four sampling periods.

(as average of the four sampling periods) were observed in area C ($1.6 \pm 0.7\,\mu g\,g^{-1}$), whilst lowest values were observed in area A ($0.8 \pm 0.2\,\mu g\,g^{-1}$). Previous studies carried out in Adriatic sediments reported a similar distribution, characterised by increasing chlorophyll-a concentration with increasing distance from the Po river outflow (Tahey *et al.*, 1996; Danovaro *et al.*, 2000), and suggested that benthic autotrophic biomass was somehow inhibited under the freshwater mass. Moodley *et al.* (1998) found a similar gradient of chlorophyll-a concentrations (from 5.8 to $8.2\,\mu g$ Chl-a cm^{-3} wet sed. at stations close to area B and C, respectively). Also Totti (1998) reported high microphytobenthic densities (up to 9278 cells cm^{-3}), especially in coastal areas far from the Po river outflow. By contrast, phaeopigment concentrations did not display a clear spatial distribution, with highest average values at area B ($11.7 \pm 3.9\,\mu g\,g^{-1}$) and lowest at area A ($9.2 \pm 3.0\,\mu g\,g^{-1}$). Therefore, higher concentrations of photosynthetic pigments are generally observed in coastal areas characterised by relevant nutrient discharge. This is the case of Ravenna (one of the most important ports and industrialised areas of the Adriatic basin), and of Cesenatico (one of the most important tourist resorts of the NW Adriatic coast).

Previous studies reported that the biochemical composition of the sedimentary organic matter could be assumed as an estimate of the material potentially available to benthic consumers (Fichez, 1991; Fabiano *et al.*, 1995; Danovaro *et al.*, 1999a; Tselepides *et al.*, 2000). Biopolymeric organic carbon concentrations found in this study were relatively high at all stations ranging on average from 2035 ± 459 to $2870 \pm 632\ \mu g\ g^{-1}$ at area A and B, respectively (Fig. 4). Proteins were the dominant biochemical class of biopolymeric organic carbon (on average $57 \pm 2\%$) followed by lipids ($23 \pm 3\%$) and carbohydrates ($20 \pm 3\%$). The dominance of proteins among the different biochemical components of organic matter has been previously reported for the NW Adriatic sediments and appears a specific feature of eutrophic environments (Danovaro *et al.*, 2000).

All biochemical classes of organic compounds displayed higher concentrations in the sediments of area B (on average 3306 ± 968, 1729 ± 450 and $745 \pm 73\ \mu g\ g^{-1}$ for proteins,

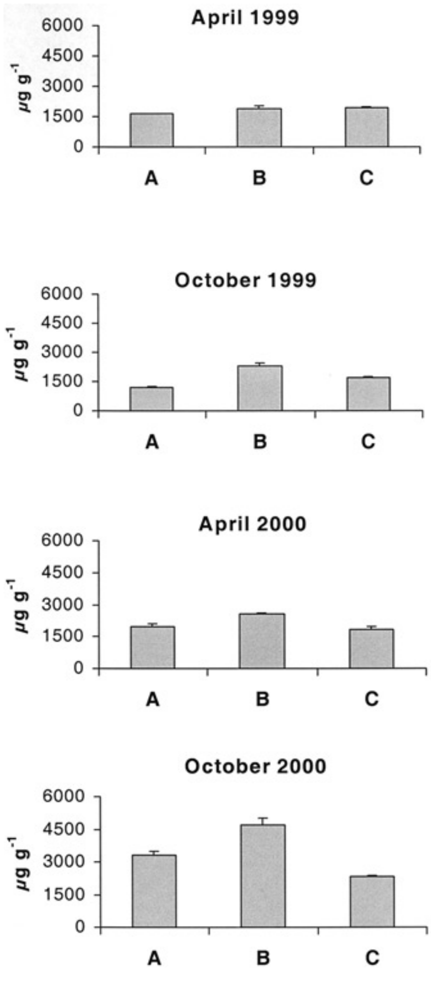

FIGURE 4 Biopolymeric organic carbon concentrations (expressed as $\mu g\ g^{-1}$) in the sediments of the three areas at the four sampling periods. Standard deviations are reported.

carbohydrates and lipids, respectively). Carbohydrates were the biochemical compounds displaying the highest spatial variability among areas (on average of the 4 sampling periods, high to low ratio = 2.1). Protein and carbohydrate concentrations (Figs. 5 and 6, respectively) co-varied both spatially and temporally whereas lipids displayed different spatial and temporal patterns (Fig. 7).

Therefore, it is likely that different classes of organic compounds are subjected to different accumulation and/or degradation rates. A significant relationship between protein and phaeopigment concentrations was found ($r = 0.596$; $p < 0.05$). Such relationship was also observed in other studies (Danovaro *et al.*, 1999b), which suggested that protein concentration in the sediment was a good descriptor of the amount of fresh organic detritus (*i.e.*, primary organic material reaching the sediment surface).

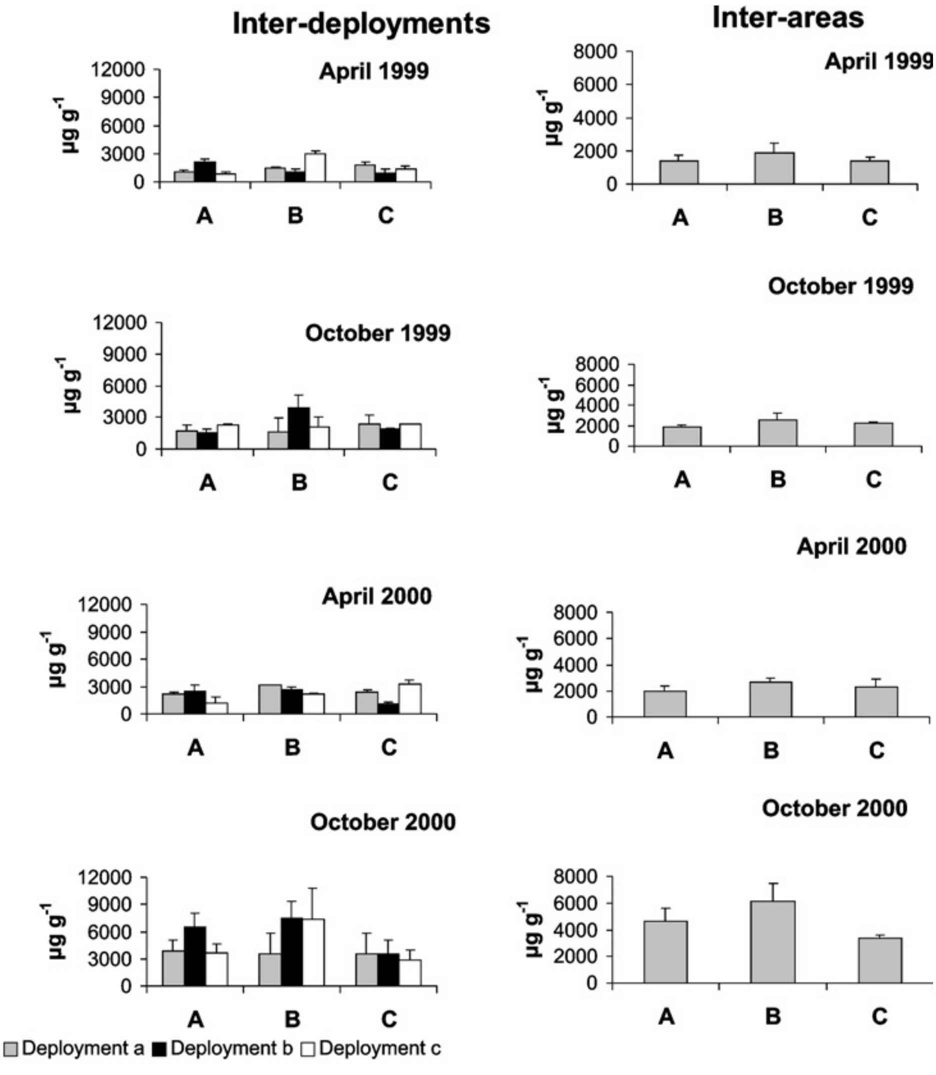

FIGURE 5 Protein concentrations (expressed as $\mu g\, g^{-1}$) as average of different deployments (±standard deviation; left side) and average values for each area (±standard error; right side) are reported in the sediments of area A (Porto Garibaldi), area B (Ravenna) and area C (Cesenatico) during the four sampling periods.

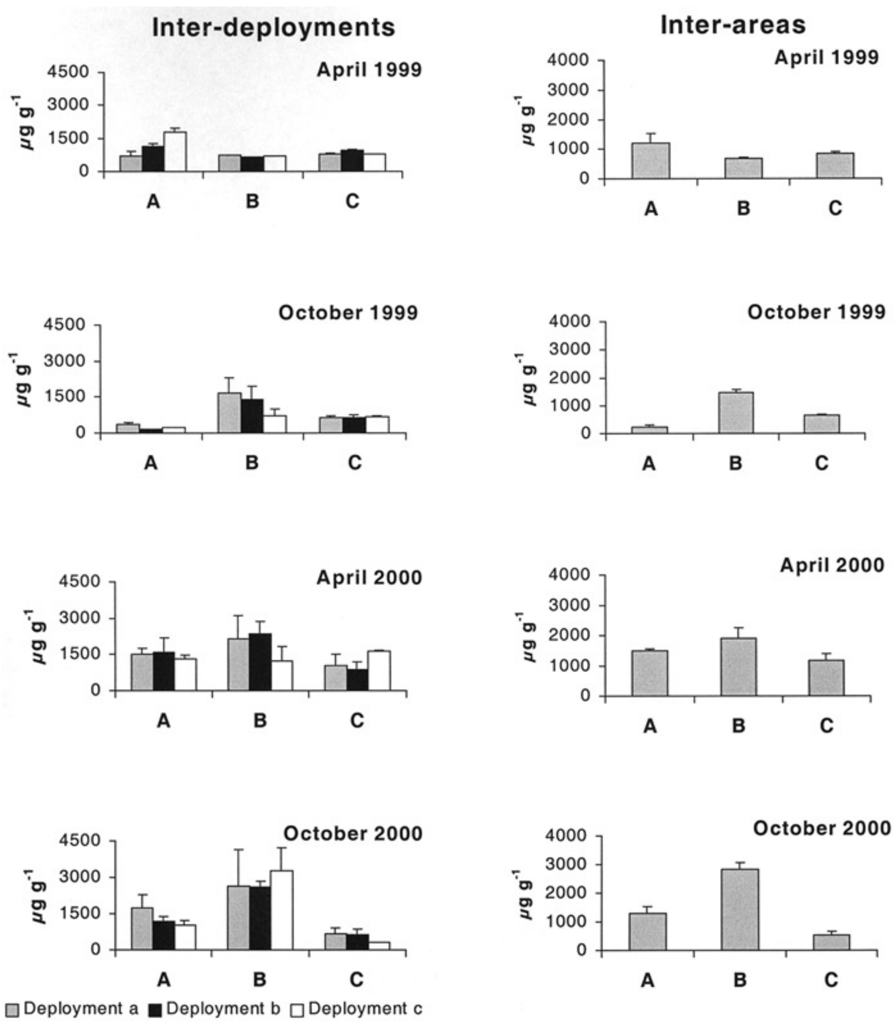

FIGURE 6 Carbohydrate concentrations (expressed as $\mu g\, g^{-1}$) as average of different deployments ($\pm$standard deviation; left side) and average values for each area ($\pm$standard error; right side) are reported in the sediments of area A (Porto Garibaldi), area B (Ravenna) and area C (Cesenatico) during the four sampling periods.

Protein, carbohydrate and lipid pools displayed significant spatial and temporal changes. In particular, significant differences were observed among sampling areas. This is in contrast with other studies dealing with total organic carbon and nitrogen distribution in NW Adriatic sediments, where no significant spatial and temporal changes were observed (Moodley *et al.*, 1998). However, it is well known that OC and ON are rather conservative measures of sedimentary organic matter (Fabiano *et al.* 1995).

Differences observed among sampling areas were not significant among different deployments, which displayed an average high to low ratio of 2.8, 3.0, 2.6 for proteins, lipids and carbohydrates, respectively.

All parameters displayed significant differences among sampling periods (Tab. I), with generally higher values in October 2000 and lower concentrations in October 1999. When spatial and temporal variability (as high to low ratio) were compared, chlorophyll-a, phaeopigment and protein concentrations displayed a higher temporal than spatial (*i.e.* among

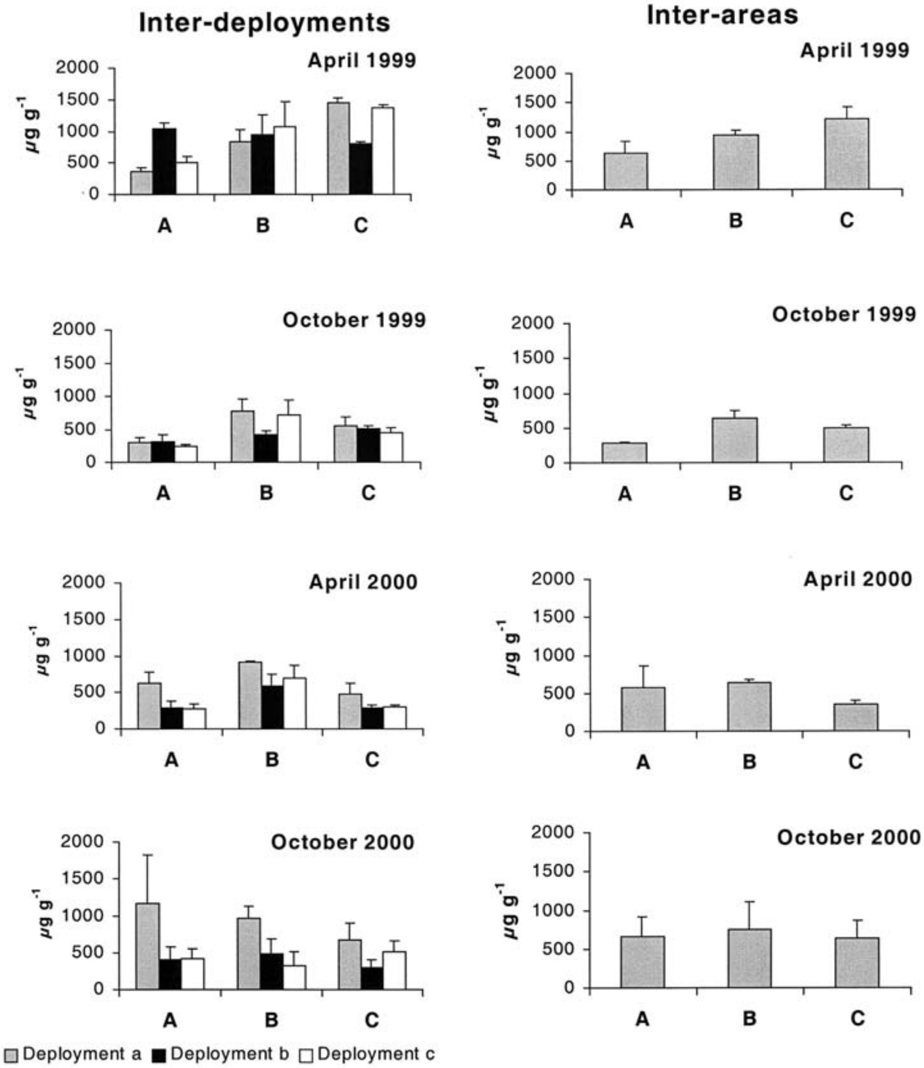

FIGURE 7 Lipid concentrations (expressed as $\mu g\,g^{-1}$) as average of different deployments (±standard deviation; left side) and average values for each area (±standard error; right side) are reported in the sediments of area A (Porto Garibaldi), area B (Ravenna) and area C (Cesenatico) during the four sampling periods.

areas) variability. For all these variables, indeed, variability among sampling periods was more than double than variability among areas. Conversely, carbohydrates and lipids displayed a similar spatial and temporal variability.

Organic matter composition (as relative contribution of the protein, carbohydrate and lipid carbon equivalents to the BPC pools) displayed limited spatial variability, but much stronger temporal changes (Fig. 8). Protein carbon accounted for about 65% of BPC pools in October 1999 and 2000, and for 42–53% in April 1999 and 2000, respectively. Such changes in organic matter composition might have important implications in the benthic trophodynamics, since consumers subjected to different diet regimes might display diverse adaptive mechanisms to optimise food exploitation (Dell'Anno *et al.*, 2000).

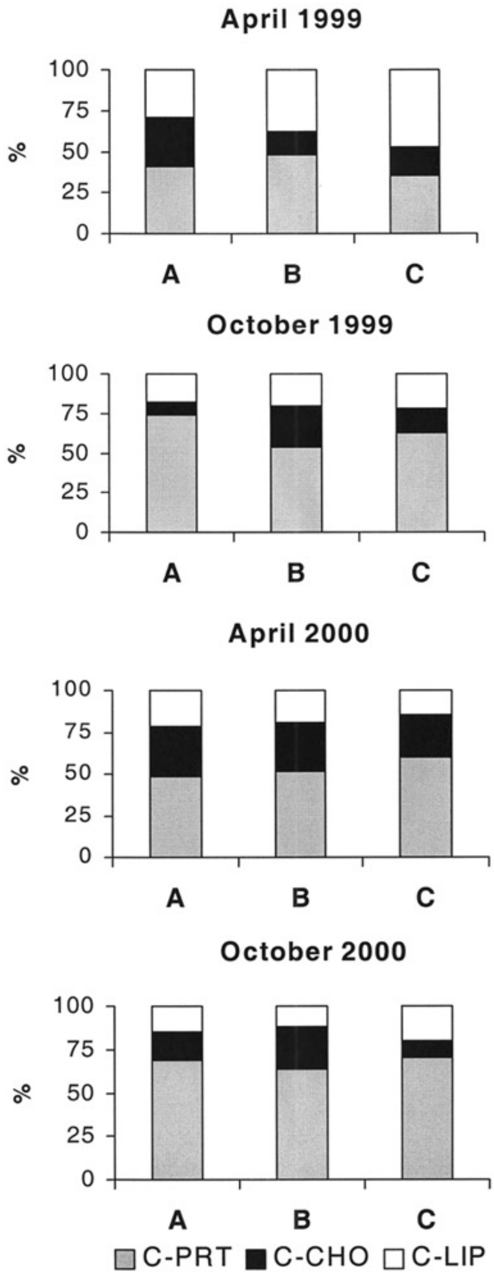

FIGURE 8 Relative contribution (expressed as percentage) of proteins (C-PRT), carbohydrates (C-CHO) and lipids (C-LIP) to the carbon pool in the sediments of the three areas in the four sampling periods.

CONCLUSIONS

This study from Adriatic sediments pointed out that replicate analyses from a single deployment are adequate for defining organic matter concentration and composition over a scale of several meters. However, for estimating organic matter composition over larger spatial scales (*i.e.* miles) the identification of different sampling areas is needed, thought it is sufficient one deployment per area. This study also indicated that temporal variability, in an area subjected to strong autochthonous inputs, might be higher than spatial variability, so that temporal changes must be taken into account as major factors controlling changes in quantity and quality of sedimentary organic matter.

The North Adriatic is a highly productive, highly exploited (fisheries), shallow (average depth of about 35 m) sea subjected to strong and frequent resuspension processes (strong northern winds), which make this a peculiar ecosystem. Therefore, further investigations are needed to assess mesoscale variability of organic matter composition in other marine systems.

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