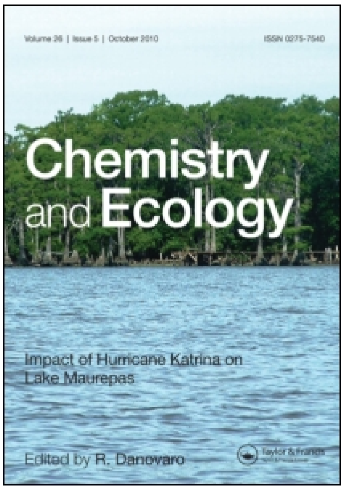

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BENTHIC RESPONSE TO MUCILAGINOUS AGGREGATES IN THE NORTHERN ADRIATIC SEA: BIOCHEMICAL INDICATORS OF EUTROPHICATION

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In summer 1997, gelatinous aggregates appeared in the Adriatic Sea, covering large areas of the northern basin. This study deals with the comparison between the biochemical composition of the sedimentary organic matter in summer, 1996 (when no aggregates appeared), and in summer, 1997 (during the appearance of aggregates). The biochemical composition of organic matter in surface sediments (determined in terms of proteins, carbohydrates, lipids, phytopigments and nucleic acids) has been investigated in two areas along the coast of the NW-Adriatic Sea in order to characterize benthic processes during aggregate deposition on the sea floor. During mucilage accumulation, a significant increase of biochemical compounds was observed, and chlorophyll-a and carbohydrate concentrations doubled their concentrations. In contrast, protein concentrations decreased, so that overall biopolymeric carbon content (expressed as the sum of lipid, protein and carbohydrate carbon equivalents) did not display significant differences between sampling periods (1579.3 in June, 1996 1678.8 $\mu gC\,g^{-1}$ and June, 1997). The protein to carbohydrate ratio decreased from 4.9 in June, 1996 to 1.8 in June, 1997. Mucilage production in June, 1997, modified significantly the biochemical composition of the sedimentary OM, thus affecting the potential availability of OM to benthic consumers. We hypothesise that the production of highly refractory composition of the sedimentary OM during mucilage accumulation might have an important biogeochemical implications.

Keywords: Sediment organic matter; Mucilage; Adriatic Sea

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INTRODUCTION

The northern Adriatic Sea is a semi-enclosed system, characterized by a peculiar hydrological features (Orlic, 1987). The reduced summer hydrodynamism often drives then this system towards a eutrophic condition (Solazzi and Boni, 1989). Large inputs of land derived nutrients, with non-balanced nitrogen to phosphorus ratios, lead to an "over-flow reaction" in phytoplankton primary production (Fogg, 1990). In addition the peculiar physical characteristics (*i.e.*, highly stratified waters, low-turbulence and developed pycnocline) contribute to exacerbate these processes, favouring the formation of large masses of mucilage. This phenomenon has been described as mucus aggregations, in the form of stringers (elongate, comet-shaped aggregates), macroflocs (subspherical, irregular, whitish aggregates), or dense clouds (large, elongate to subspherical aggregates), that are transported horizontally and vertically in the water column. These aggregates tend to settle on the bottom and, as previously observed for organic inputs derived from frontal systems, such large amounts of organic matter, are expected to induce a significant benthic response (Josefson and Conley, 1997). During the summers of 1988–1989–1991 and 1997 floating gelatinous aggregates appeared in large areas of the northern Adriatic basin, causing anoxia and mechanical threats to the benthic system (Welker and Nichetto, 1996). Despite large efforts have been devoted to the study of mucilage's effects on the marine environment, little information is available of sediment benthic response (Fadda *et al.*, 1994). In particular, no information is available on the effects of mucilage events on the quality and quantity of the sedimentary organic matter, but this issue is of crucial importance for a predictive understanding of the biogeochemical cycling in stressed ecosystems.

In the present study the biochemical composition (*i.e.*, proteins, carbohydrates, lipids, chlorophyll-a and nucleic acids) of organic matter in surface sediments was investigated in June, 1997 (during the appearance of aggregates) and compared with analyses carried out in June, 1996 (when no aggregates appeared), in order to identify biochemical descriptors of the altered benthic processes during aggregate deposition on the sediments.

MATERIAL AND METHODS

Sampling Strategy and Sediment Sampling

Sediment sampling was carried out in the northern Adriatic Sea on board of the R/V "Urania", in June, 1996 and in June, 1997 (Fig. 1). Sampling schedule included two study areas along the north-western Adriatic sea coasts (the first close to the Po delta and the second in the northern Marche Region coasts). Each sampling area included a grid of 6 stations. In each grid, 6 stations were sampled: 2 coastal stations, 2 central and 2 open sea stations. Coastal and offshore stations were both about 10–12 miles far from the intermediate stations. Undisturbed sediment cores were collected using a multiple corer (Mod. Midi, $n=4$ with 5.7 and 9.5 cm inner diameter). For the analysis of the biochemical composition of the sedimentary organic matter, 2–3 corers were collected at each station, and sectioned into a sediment layer of 0–1 cm, and frozen at −20°C.

Phytopigments and Biochemical Composition of Sedimentary Organic Matter

Chlorophyll-a analysis was carried out according to Lorenzen and Jeffrey (1980). Lipids were extracted according to the Bligh and Dyer

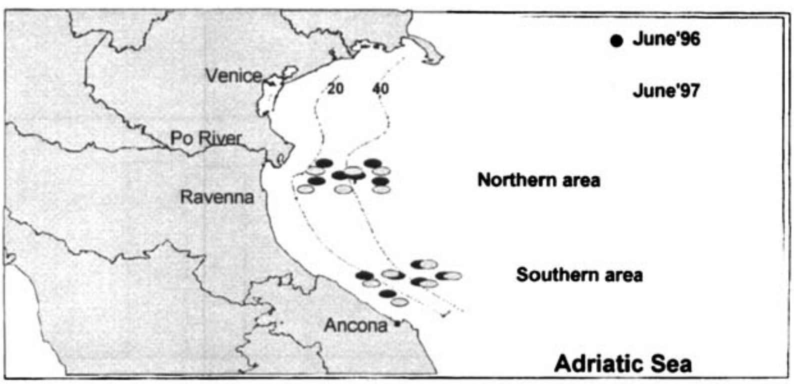

FIGURE 1 The sampling stations in the Adriatic Sea in June, 1996 and 1997.

method (1959) and measured following Marsh and Weinstein (1966). The absorbance was evaluated at 375 nm. Tripalmitine solutions were used as standard. Carbohydrates were analysed according to Dubois *et al.* (1956). The absorbance was measured at 485 nm. D (+) glucose solutions were used as a standard. Protein analysis was conducted according to the Hartree method (1972). The absorbance was evaluated at 650 nm. Bovine albumin solutions were used as a standard. Sedimentary organic matter (BPC) was expressed as the sum of carbohydrates, proteins and lipids converted to carbon content using: 0.40, 0.49 and 0.75 $\mu g\,C\,\mu g^{-1}$ conversion factor, respectively. Sedimentary nucleic acids (DNA and RNA) were determined according to Dell' Anno *et al.* (1998). The absorbance of total nucleic acids was measured at 260 nm.

RESULTS

As no significant quantity differences were observed comparing the two areas, data reported here have considered values from June, 1996 and 1997, of the two areas pooled together. Chlorophyll-a in the top sediment layer (0 – 1 cm) exhibited a significant marked increase from June, 1996 ($3.2 \pm 0.7\,\mu g\ g^{-1}$) to June, 1997 ($5.6 \pm 0.9\,\mu g\ g^{-1}$; *t*-test, $P < 0.05$). By contrast, no significant differences (*t*-test, $P > 0.05$) have been observed comparing phaeopigment concentrations in the two sampling periods (7.1 ± 1.2 June, 1996 and $7.3 \pm 1.0\,\mu g\ g^{-1}$ and in June, 1997 in Fig. 2).

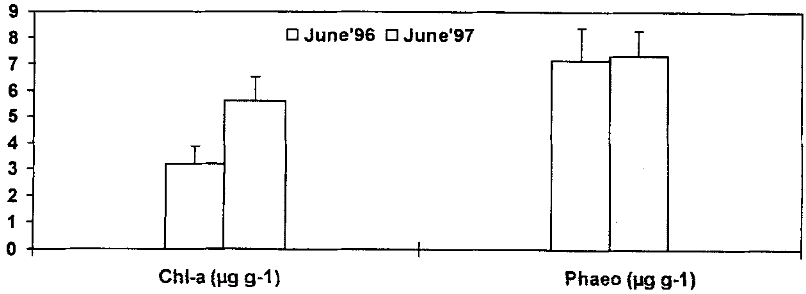

FIGURE 2 Chlorophyll-a (Chl-a) and Phaeopigments (Phaeo) concentrations in the two sampling periods.

Biopolymeric carbon concentrations (BPC), used to estimate the organic carbon fraction potentially available to sediments-ingesting organisms, did not show significant differences between 1996 and 1997 (average values, 1579.3 in June, 1996 1678.8 μg C g^{-1} in June, 1997). OM composition was dominated by proteins, followed by carbohydrates in both sampling periods (together accounted for 73 and 63% of the BPC, in June, 1996 and June, 1997), but the concentrations of the three biochemical classes of organic compounds showed different values in the two years. Highest protein concentrations were observed in June, 1996 (on average 2016.0 ± 351.1 μg g^{-1}, 1447.1 ± 154.8 μg g^{-1} in June, 97). By contrast carbohydrate concentrations doubled from 418.5 ± 68.4 μg g^{-1} (June, 1996) to 910.1 ± 135.5 μg g^{-1} (June, 1997). Also lipid concentrations increased from 565.3 ± 90.7 μg g^{-1} (in June, 1996) to 807.5 ± 117.5 μg g^{-1} (in June, 1997; Fig. 3). As

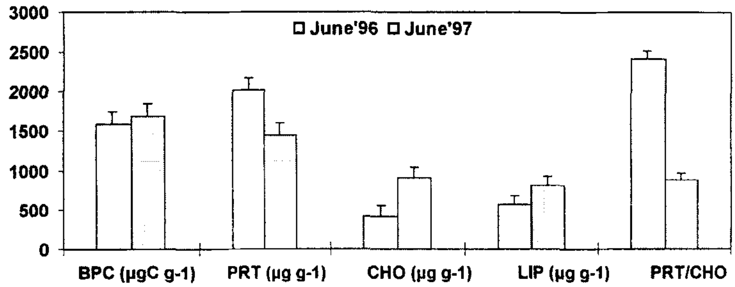

FIGURE 3 Biochemical composition of sedimentary organic matter in the two sampling periods. Biopolymeric carbon (BPC), total proteins (PRT), total carbohydrates (CHO), total lipids (LIP), protein to carbohydrate ratio multiply by 500 (PRT/CHO).

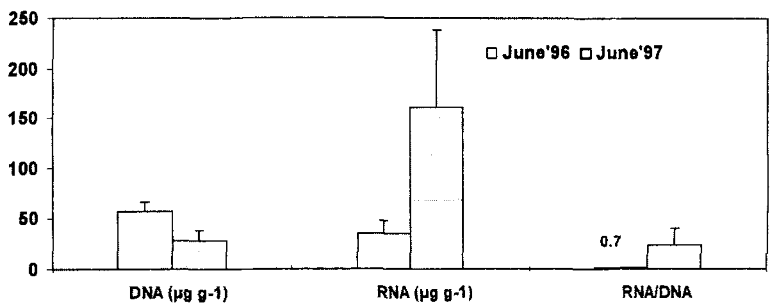

FIGURE 4 DNA, RNA concentrations and RNA/DNA ratio in the two periods of sampling.

a result, the ratio of protein to carbohydrate decreased from June, 1996 (4.9) to June, 1997 (1.8).

Nucleic acid (DNA and RNA) content of the sediments and the RNA : DNA ratio are reported in Figure 4. Highest DNA concentration were observed in June, 1996 (57.1 ± 10.3 μg g^{-1}) in correspondence to the lowest RNA concentration (35.0 ± 12.7 μg g^{-1}). In June, 1997, DNA concentrations decreased for about one half (28.6 ± 9.54 μg g^{-1}) and displayed a marked peak in RNA concentration (160.7 ± 77.7 μg g^{-1}). As a result the RNA : DNA ratio showed the highest value in June, 1997 (23.9).

DISCUSSION

Data reported in the present study clearly suggest that the appearance of mucilage aggregates along the Adriatic coasts during summer in 1997 determined evident changes in biochemical features of the benthic system. Comparing June, 1996 to June, 1997, we observed rather constant biopolymeric carbon concentrations, that therefore appeared of limited importance for characterizing changes induced by mucilage production, but evident differences in terms of chlorophyll-a content and biochemical composition of the sedimentary organic matter. High chlorophyll-a contents associated to mucilage have been previously reported by comparative studies with free marine waters (Prezelin and Alldredge, 1988). In this study, sedimentary chlorophyll-a concentrations exhibited a marked increase during mucilage deposition on to the sediments, reaching values comparable or higher than those reported by Plante *et al.* (1986) in the highly productive Gulf of Fos, which receives the inputs from the Rhone river (4.9 μg g^{-1} in September). Chlorophyll-a concentrations were significantly higher (about 3-times) than those reported (Fabiano *et al.*, 1995) in the subtidal sediments of the Ligurian Sea (1.3 μg g^{-1}) and about double that those reported from rich seagrass beds of the NW Mediterranean (Manini *et al.*, 1996).

The most evident changes in the biochemical composition of the sedimentary OM are related to the decrease in the protein to carbohydrate ratio and to the significant increase of the lipid content during mucilage appearance. Polysaccharides are thought to play an important role in formation of larger organic aggregates. Large-scale gelatinous mucous aggregations observed in the Adriatic Sea mainly consist of polymeric carbohydrates, produced by algae especially in response to environmental/nutritional stress (Posedel and Faganelli, 1991) and produced toward the end of blooms when particle aggregation is pronounced (Myklestad *et al.*, 1989). The northern Adriatic coastal waters represent a highly productive system; previous studies demonstrated that protein to carbohydrate ratios >1 are typical of highly productive environments (Fabiano *et al.*, 1997). This was confirmed in this study as the coastal sediments of the northern Adriatic were always dominated by sediment protein concentrations. Despite the presence of high carbohydrate concentrations, such protein dominance was observed throughout the investigation period. Previous studies suggested that the relationship between carbohydrates and protein content can be used to evaluate the quality of the material potentially available to consumers (Fabiano *et al.*, 1993). Comparing the two sampling periods, the much lower mean PRT/CHO ratio observed in June, 1997 compared to June, 1996 suggests a strong nutritional depletion (*i.e.*, a more refractory composition) of the sedimentary OM during mucilage production and decomposition (Fabiano *et al.*, 1995).

The detailed analysis of the temporal changes in nucleic acid concentrations may provide additional information on the characteristics of the sediment organic matter. In general terms, DNA represents an index of biomass while RNA content might change in relation to the processes of synthesis (Danovaro *et al.*, 1995). Therefore, RNA : DNA ratios has been recently proposed as a metabolic activity index of the microbial communities associated to sedimentary organic matter (Danovaro *et al.*, 1993; Fabiano *et al.*, 1997) and seems to be a sensitive parameter with respect to gradients of environmental stress (Danovaro *et al.*, 1995). In June, 1997, very high values of the RNA : DNA ratios were observed. Such changes appear to be primarily due to increased RNA concentrations, so that higher RNA : DNA ratios would indicate enhanced metabolic activity in the system during mucilage production.

In conclusion, in both investigated periods, the quantity of sedimentary organic material was quite constant over time, but evident differences in terms of OM quality were observed. In June, 1997, sedimentary organic matter was largely composed by carbohydrates. Due to the different turnover of the protein and carbohydrate pools in marine sediments (Dell'Anno *et al.*, 2000), such mucilage-induced changes, might have important biogeochemical implications, possibly altering processes of OM diagenesis in the northern Adriatic.

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