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# Major inter-annual variations in microbial dynamics in the Gulf of Trieste (northern Adriatic Sea) and their ecosystem implications

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ABSTRACT: Comprehensive, multi-year studies of microbial processes in an ecosystem context are important in understanding microbial regulation of ecosystem structure, function and variability. A 3 yr study on microbial community was carried out in a coastal area in the northern Adriatic Sea (Gulf of Trieste). It is a semi-enclosed shallow system, mostly controlled by pulsed external inputs, which determine a high variability of plankton communities. Samples were collected monthly at 2 stations from January 1999 to December 2001. Data illustrate remarkable inter- and intra-annual variability in parameters relevant to carbon biogeochemistry and ecosystem energy flow patterns. Integrated primary production (PP; 135 g C $m^{-2}$ $yr^{-1}$) in 1999 tripled (414 g C $m^{-2}$ $yr^{-1}$) in 2000, returning to a low level (150 g C $m^{-2}$ $yr^{-1}$) in 2001. Bacterial production (by $^3$H-leucine incorporation) accounted, on average, for 35.3% of net PP in 1999, 9.5% in 2000 and 29.4% in 2001. Flux into the microbial loop via bacterial C demand, assuming 30% growth efficiency, was the dominant C flow pathway in 1999 and 2001, accounting for almost 100% of the net PP but a lesser fraction (40%) of net PP in 2000. Total microbial respiration (<200 µm fraction, $R_{<200\mu m}$) exceeded PP for almost the whole period, indicating that the system was potentially net heterotrophic at the expense of external input of organic matter. Our results suggest that in 1999 and 2001, when PP was low, it mostly fuelled, and was respired within, the microbial loop. During 2000 there was a major shift in C flux partitioning. The coupling between PP and bacteria was loose, and larger fractions of PP were presumably channelled into other paths of C flow such as grazing food chain, sedimentation and 'storage' in the water column as dissolved and colloidal organic phases. Organic C in excess produced over the 2 mo preceding visible accumulation of gelatinous aggregates (mucilage) was sufficient in magnitude to account for the mucilage C pool. Our results are consistent with the hypothesis that mucilage is derived from accumulated slow-to-degrade dissolved organic matter.

KEY WORDS: Microbial dynamics · Mucilage · Northern Adriatic Sea



## INTRODUCTION

The northern Adriatic Sea episodically experiences, during summer, the formation of massive gels visible throughout the water column and covering significant fractions of the sea surface and bottom. Mucilage episodes have been reported for over a century (Fonda Umani et al. 1989), but their frequency has dramatically increased since 1988 to every 2 to 3 yr. Intriguingly, they have not been reported from elsewhere in other oceans. Mucilage events exact economic losses on coastal communities due to reduced tourism and

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fishery. The cause of the phenomenon remains unknown, but it is the subject of much investigation and debate (reviewed by Stachowitsch et al. 1990, Malej 1995, Vollenweider & Rinaldi 1995, Degobbis et al. 1999).

Mucilage is generally thought to consist of algal polysaccharides exuded during phosphorus (P)-limited growth of diatoms (e.g. Myklestad 1999 and references therein) or dinoflagellates (Pompei et al. 2003). Another proposal (Azam et al. 1999) is that bacterial processing of organic matter to produce slow-to-degrade dissolved and colloidal polysaccharide contributes significantly to the proximal source pool of mucilage. In either case, the sudden appearance of the huge mucilage carbon pool (Azam et al. 1999) suggests dissolved organic matter (DOM) as its proximal source; it is a sufficiently large and seasonally variable pool with a potential for transformation into particle or gel phases. Dissolved organic carbon (DOC) in the northern Adriatic ranged from 0.63 to 3.37 mg $l^{-1}$, with summer and winter average values of ca. 2.4 and ca. 1.2 mg $l^{-1}$, respectively (Pettine et al. 2001). This pool size translates into ca. 40 to 80 g C $m^{-2}$, commensurate in magnitude with the mucilage carbon pool (Malej et al. 2001). It could thus serve as a source of mucilage. Our study was intended to constrain this hypothesis on the possible relationship between DOM accumulation and mucilage formation.

DOM has been divided into 3 pools defined by biodegradability: labile (turnover time hours to days), semi-labile (turnover time of months) and refractory (turnover time of years to centuries) (see e.g. review of Kirchman et al. 1993). Long-lived DOM may be produced as a result of incomplete degradation of complex molecular species by microbial activity, particularly enzymatic actions that produce fragments from macromolecules not recognisable by bacterial enzymes (Azam et al. 1994, 1999). Ogawa et al. (2001) proposed a role for slow hydrolysis by non-specific ('promiscuous') enzymes acting on DOM components. Recently, DOC accumulation in the Mediterranean Sea has been proposed to be due to a malfunctioning of the microbial loop, wherein P-limited bacteria are unable to consume DOC as fast as it is produced (Thingstad et al. 1997). The Po River is also a source of substantial DOC (1.65 tons $km^{-2}$ $yr^{-1}$; Pettine et al. 1998). While the biodegradable fraction of Po-derived DOC has not been determined, this fraction in other riverine DOC has been estimated to be on the order of 10 to 20% (Søndergaard & Middelboe 1995, Becquevort et al. 2002). Whatever the mechanism of its production, the accumulation of slow-to-degrade DOC during the high-primary-productivity season is a common feature of many marine systems (e.g. Copin-Montegut & Avril 1993, Carlson et al. 1994, Williams 1995, Zweifel et al. 1995, Zweifel 1999), including the North Adriatic Sea (Pettine et al. 1999, 2001).

The shallow Gulf of Trieste (maximum depth: 23 m) is the northeasternmost edge of the Adriatic Sea. It has a surface area of about 600 $km^2$, with 10% average bottom depth <10 m (Malej & Malacic 1995) and a volume of 9.5 $km^3$ (Olivotti et al. 1986). Temperature shows a regular annual pattern from winter minima of 6°C to summer maxima >25°C (Cardin & Celio 1997). The highly variable main freshwater source (Isonzo River on the northwestern coast) controls salinity, which ranges in surface waters from 32.7 ± 0.5 to 37.6 ± 0.3 (Fonda Umani 1991, Celio et al. 2002). Late spring and autumn are characterised by the highest river discharges, while drought periods generally occur in winter and summer. The gulf is under the influence of the ascending Eastern Adriatic Current (EAC) that shows high interannual variability. The EAC reaches the gulf as a surface current after having received freshwater from the eastern coast and, consequently, is highly modified relative to its initial characteristics, i.e. Aegean Surface Waters. Sporadically, Modified Levantine Intermediate Water (MLIW) enters the gulf as bottom or intermediate waters, increasing the salt concentration of the local waters. The system appears to be controlled by 2 opposing forces: western freshwater inputs, all along the Italian western coast, and more oligotrophic water intrusions from the southeastern area. The concentration of inorganic nutrients, mainly due to riverine inputs, is highly variable (e.g. 0.5 to >30 µM surface $NO_3$) (Cantoni et al. 2003). Allochthonous inputs of P, which range between 0.05 and >3 µM at the surface, are mainly due to sewage input, since discharge from the karstic system is very low (Burba et al. 1994). The annual cycle of phytoplankton in the system is characterised by an intense late winter diatom bloom, a nutrient-depleted summer and a second short-lasting fall bloom (Mozetic et al. 1998, Harding et al. 1999).

In the last 17 yr the Gulf of Trieste, like the entire northern Adriatic Sea, has been affected by several mucilage events. After >50 yr of remission, mucilage reappeared in 1988 (Degobbis et al. 1995, 1999), and again in 1989, 1991, 1997, 2000, 2002 and 2004 (Precali et al. 2005).

The event in the summer of 2000 was one of the most widespread in the northern Adriatic (Precali et al. 2005). It was intense also in the Gulf of Trieste, where it began in mid-June and lasted until early July. However, the years 1999 and 2001 were mucilage free. Here, we consider some carbon cycle parameters in the Gulf of Trieste from January 1999 until December 2001, to seek insights into the significance of the temporal variability of the microbial cycling of organic matter for DOC dynamics and mucilage formation.

## MATERIALS AND METHODS

**Sampling and analyses**. Sampling was carried out monthly at 16 hydrological stations for CTD casts. At 9 of these stations, water samples were collected with Niskin bottles at surface, intermediate and bottom layers to assess chlorophyll *a* (chl *a*), DOC, particulate organic carbon (POC) and inorganic nutrient concentrations. In 2 of the latter stations, we analysed most of the biotic components and measured biogeochemical processes in the water column.

Pressure, temperature, conductivity and oxygen data were recorded with an Idronaut Ocean Seven (Model 316) multiparametric probe, calibrated every 6 mo. Underwater irradiance profiles were acquired by a PNF 300 Biospherical Instruments probe.

The residence time of freshwater in the region of interest (ROI) (Fig. 1), with an area of $10 \times 18$ km$^2$ and a total seawater volume of 2.7 km$^3$, was calculated as the ratio between the total volume of freshwater in this seawater volume and the freshwater loads of the Isonzo River. The freshwater content (FWC) was calculated in the first 5 m of the water column for each of the sampling stations, on the basis of the following formulas:

$$\mathrm{FW} = (S - S_{\mathrm{obs}})/S$$

$$\mathrm{FWC} = \int_0^{5\,\mathrm{m}} \mathrm{FW} \cdot \mathrm{d}z$$

where $S$ is the salinity of the water entering the gulf (37.2 to 38.5) and $S_{\mathrm{obs}}$ is the observed salinity (Hopkins et al. 1999). The kriging method applied to the FWC values obtained for each station permitted us to interpolate the FWC values on a grid of 70 cells covering the ROI area. In each cell (dimensions: $1955 \times 1393$ m$^2$), the FWC has been obtained as the average between the values of the grid points of the cell. The total volume of freshwater (TFV) has been obtained as:

$$\mathrm{TFV} = \sum_{\text{grid cell}} \mathrm{FWC} \cdot (\text{Cell Area})$$

Analyses of the dissolved nutrients were performed on seawater samples pre-filtered on glass-fibre filters (Whatman GF/F) using an Alliance Integral Segmented Flow Analyzer, according to Koroleff & Grasshof (1983).

For chl *a* measurements seawater samples of 1 l were filtered on board through Whatman GF/F glass-fibre filters (0.7 µm nominal pore size, 45 mm diameter) and immediately frozen (–20°C) until analysis, which was performed within 1 wk. Pigments were extracted overnight in the dark at 4°C with 90% acetone from the homogenate filter and determined spectrofluorometrically following the procedures described by Lorenzen & Jeffrey (1980). The measurements of chl *a*

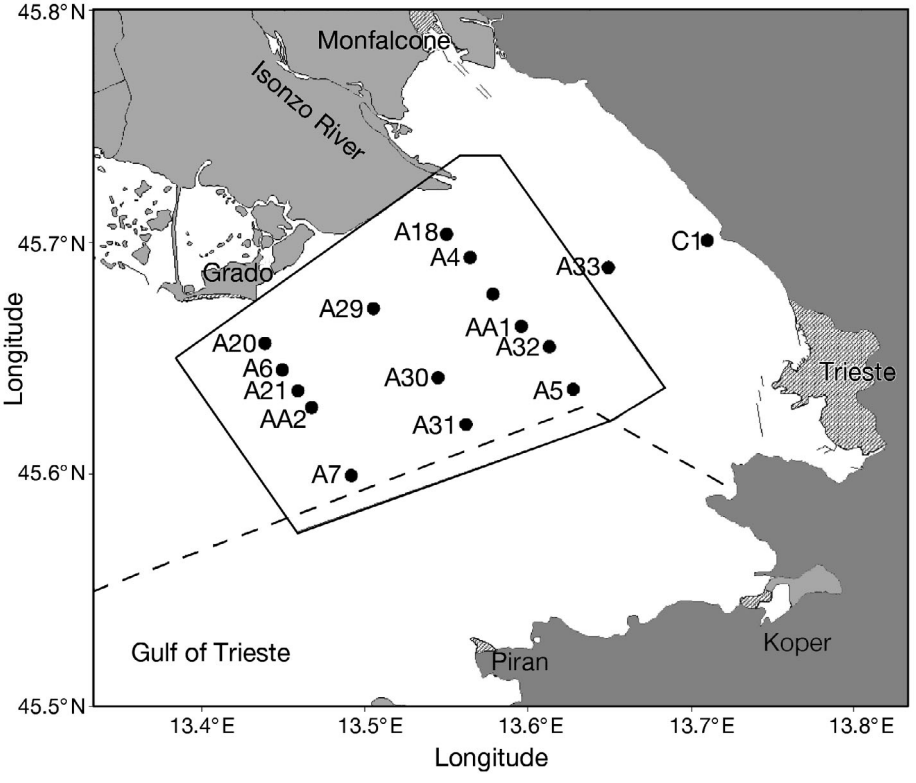


Fig. 1. Sampling stations in the Gulf of Trieste (northern Adriatic Sea). The region of interest is indicated by the polygon

were performed before and after acidification with 2 drops of HCl 1 N using a Perkin Elmer LS 50B (450 nm excitement and 665 nm emission wavelengths). Calibration was made with pure Sigma chl *a* standards and a linear instrumental response over the considered range.

Samples for DOC analyses were filtered immediately after collection through precombusted (4 h at 480°C) and acidified (HCl 1 to 2 N) Whatman GF/F glass-fibre filters (0.7 µm nominal pore size). Filtration was performed with a glass syringe and a filter holder, to minimise atmospheric contamination. Filtered samples were stored frozen (–20°C) in 20 ml glass vials (previously treated with chromic acid for 24 h, washed with Milli-Q water and precombusted for 4 h at 480°C). All vials were rinsed 3 times with the respective sample water before filling to the appropriate volume and immediately closed with Teflon-lined screw caps. Before analysis, the samples were acidified to pH < 2 using 100 µl HCl 6 N solution and purged for 8 min using high-purity oxygen bubbling (150 ml $min^{-1}$). The purging stage is required to remove the inorganic carbon initially present in the sample. It also removes the volatile organic carbon that might represent about 5% of the initial so-called DOC. The resulting DOC represents, therefore, the non-purgeable, 0.7 µm filtered, HTCO-analyzable organic carbon (Avril 2002). DOC analyses were made with an HTCO (high-temperature catalytic oxidation) method using a Shimadzu TOC 5000A with a quartz combustion column in the vertical position filled with 1.2% platinum on silica pillows with an approximate diameter of 3 mm (Cauwet 1994). Then, 100 µl of sample was injected. Carbon concentration was determined by automatic comparison with 4-point calibration curves. Standardisation was carried out every day using potassium hydrogen phthalate. The catalyst bed was preconditioned by injecting 100 µl of acidified and sparged Milli-Q water until the lowest stable integrated area was obtained (between 600 and 700 area units). Each value was determined from a minimum of 3 injections, with a variation coefficient of <2%.

For CHN analysis, seawater samples (1 l) were filtered onto precombusted glass-fibre filters (GF/F Whatman). POC was determined by high-temperature oxidation using a CHNS 2400 Perkin Elmer Elemental Analyzer after acidification with 1 N HCl to remove the inorganic carbon. Acetanilide was used to create the calibration curve.

Microbial community abundance, composition and activity were analysed at 2 stations (Stn C1: 200 m offshore of Miramare Marine Reserve, average depth 17 m, 4 depths [0.5, 5, 10 and 15 m] sampled; Stn AA1: centre of the gulf, depth 23 m, 3 depths [0.5, 15 and 21 m] sampled)

Samples (5 ml) for virus-like-particle (VLP) counts were immediately preserved with 0.02 µm filtered formalin (1% final concentration). Subsamples were filtered through a 0.02 µm Anodisc filter. The filters were then placed in 100 µl of the staining solution SYBR Green I (Molecular Probes) (1:2500 final concentration of original stock) and incubated for 15 min in the dark at room temperature. Following incubation, the backs of the filters were dried with a tissue paper and mounted onto glass slides with antifade solution (Noble & Fuhrman 1998). VLP abundances were detected by an Olympus BX60 epifluorescence microscope (at 1000×) using a blue light excitation filter set (BP 420/480 nm). We counted at least 10 fields $slide^{-1}$ and at least 200 cells $replicate^{-1}$.

Picoplankton samples (10 ml) were preserved in formaldehyde (2% final concentration), filtered onto black 0.2 µm polycarbonate filters (Nuclepore) laid over pre-wetted 0.45 µm cellulose backing filters (Millipore). Cells were stained in the dark with DAPI (5 µg $ml^{-1}$ final concentration) following a modification of the method of Porter & Feig (1980). Filters were stored at –20°C until processed. Enumeration was made as for VLP under a UV filter set (BP 330/385 nm).

Microphytoplankton samples (250 ml) were fixed with Ca $(HCO_3)_2$ buffered formaldehyde (1% final concentration). Samples were processed using sedimentation chambers (Utermöhl 1958) and observed with a Leitz-Diavert inverted microscope (at 320×). Cells were measured, and their linear dimensions were converted to cell volumes using standard geometric formulae. Cell volumes were converted to carbon content using standard conversion Strathmann formulae (Smayda 1978).

Phytoplankton productivity (PP) was measured by the $^{14}C$ technique (Steeman-Nielsen 1952). Water samples were poured into light and dark 70 ml polycarbonate bottles adding 222 KBq of $NaH^{14}CO_3$ per bottle. The samples were incubated at sea-surface temperature in a thermostatic bath exposed to natural light for 4 h around noon. To simulate the underwater irradiance at the sampling depth, bottles were screened with photographic filters. At the end of incubation, the samples were filtered on 0.2 µm polycarbonate filters (Nuclepore) and the filters were placed in scintillation vials and acidified with a few drops of 5 N HCl to remove residual ($^{14}C$) bicarbonate. Scintillation cocktail (5 or 10 ml) was added, and samples were radioassayed in a Canberra TriCarb 2900 TR liquid scintillation counter. Assimilation of carbon was calculated as described by Gargas (1975), assuming 5% isotope discrimination. Activity of the added $NaH^{14}CO_3$ and inorganic carbon concentration ($tCO_2$) were calculated on the basis of total alkalinity

measured in the same samples. To calculate PP per day, hourly data were integrated on the basis of the daylight surface PAR (photosynthetically active radiation) measured (µE $m^{-2}$ $d^{-1}$).

Microplankton community respiration ($R_{<200µm}$) was measured by following the changes in dissolved oxygen in sealed bottles during 24 h incubation. Water samples were incubated in acid-cleaned (10% HCl) and distilled-water-rinsed 60 to 250 ml BOD bottles in the dark at *in situ* temperature (±2°C). Oxygen concentrations were determined in triplicate bottles at the beginning and at the end of the incubation. Dissolved oxygen was measured with a Titrino Mettler titrator for automated Winkler titration based on potentiometric end point detection. Carbon respiration was estimated from rates of oxygen consumption assuming a respiratory quotient (RQ = $\Delta CO_2/\Delta O_2$) of 1 (Chin-Leo & Benner 1992).

Bacterial carbon production (BCP) was measured by $^3$H-leucine (Leu) incorporation (Kirchman et al. 1985 as modified for microcentrifugation by Smith & Azam 1992). Three replicates (1.7 ml aliquots) and 2 controls killed with 100% trichloracetic acid (TCA) were amended with 20 nM Leu and incubated at *in situ* (±2°C) temperature in the dark. Incubations were stopped with 100% TCA after 1 h. The extraction, with 5% TCA and 80% ethanol, was carried out by the microcentrifugation method (Smith & Azam 1992). Activity in the samples was determined by counts on a liquid scintillation counter (Packard Tri-Carb 2900 TR) after addition of 1 ml scintillation cocktail (Ultima Gold MV, Packard). Incorporation of Leu was converted into carbon produced via bacterial protein production according to Simon & Azam (1989), assuming a 2-fold intracellular isotope dilution for Leu.

Bacterial carbon demand (BCD) is the sum of BCP and bacterial respiration (BR). Because of methodological difficulties, BR is usually not measured directly and is instead computed from BCP and an assumed value of the bacterial growth efficiency (BGE). We used a medium BGE of 30% (e.g. Hoppe et al. 2002) for our computations.

Concentrations of inorganic nutrients and chl *a* were averaged over the whole water column at each station, then averaged among the stations and expressed as µM or µg $l^{-1}$. Concentrations of organic compounds were integrated over the whole water column at each station, then averaged among the stations and expressed as mg $m^{-2}$. PAR values at each depth were integrated over the whole water column, then averaged among the stations and expressed as µE $m^{-2}$ $s^{-1}$. PP, $R_{<200µm}$, BCP and BCD were also integrated per day (or month) and expressed as mg C $m^{-2}$ $d^{-1}$ (or g C $m^{-2}$ $mo^{-1}$).

## RESULTS

### Temperature, salinity and circulation

In 1999 temperature decrease began in January, to reach the annual minimum of 5.8°C in February, when inverse thermal stratification occurred. Modest freshwater inputs persisted throughout the spring and, together with seasonal warming, produced water column stratification. Beside in October, rainfall in the autumn was very scarce and led to a reduction in Isonzo River discharge. The low discharge caused a diminution in the percentage of diluted water in the gulf (only 25% of its total volume) and, together with intense north winds in the fall, induced intense advection from the south of the MLIW. Low freshwater discharge characterised the spring 2000 as well (data not shown). However, the retention time of surface diluted waters inside the gulf was particularly long (12 d in February) compared to retention times calculated for the same month in the other 2 yr (4 to 6 d) (Fig. 2), and the water column stability remained high throughout the spring. Seawater temperature began to rise in March 2000 and reached a maximum of 26°C in June, 2 mo earlier than usual. The period June to August was characterised again by a long retention time of diluted waters (8 to 16 d). From November 2000 to March 2001, the Isonzo River flow rates were very high. The temporal evolution of 2001 was characterised by 2 quite different behaviours. From January to June salinity was low due to continuous freshwater supply and temperatures were higher than in the previous years, remaining >10°C also in January and February. Conversely, from July to December 2001, we detected low temperatures and high salinities comparable to those in the MLIW, which entered the gulf in large amounts (data not shown).

### Irradiance

Integrated PAR showed a regular pattern, with summer maxima, but a relevant year-to-year variability (Fig. 3). In 2000, the annual mean PAR increased by 23% compared with the annual mean in 1999, and, in April and May 2000, maxima were >40% higher compared with the same months of the previous year.

### Nutrients

Nutrient concentration distributions matched those of diluted waters. Nitrate concentrations presented maxima in March and October 1999, November and

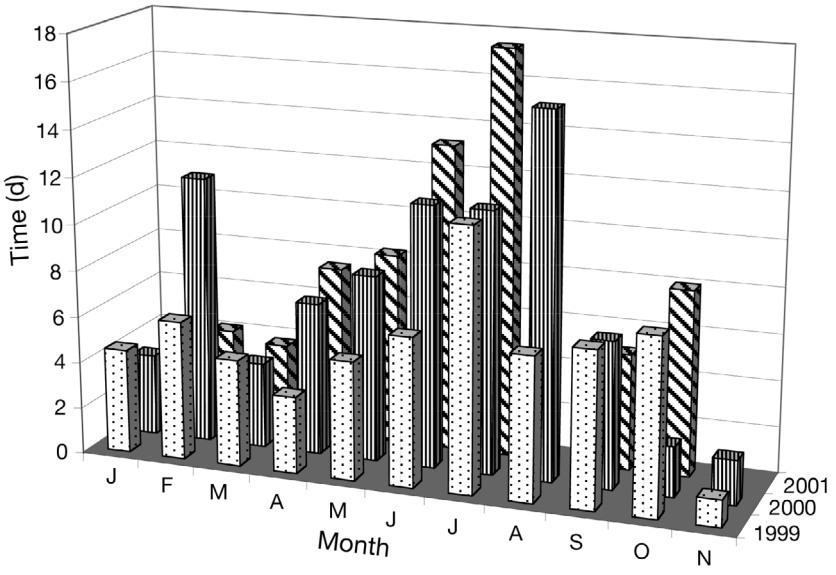


Fig. 2. Residence time of freshwater in the region of interest in the 3 years studied

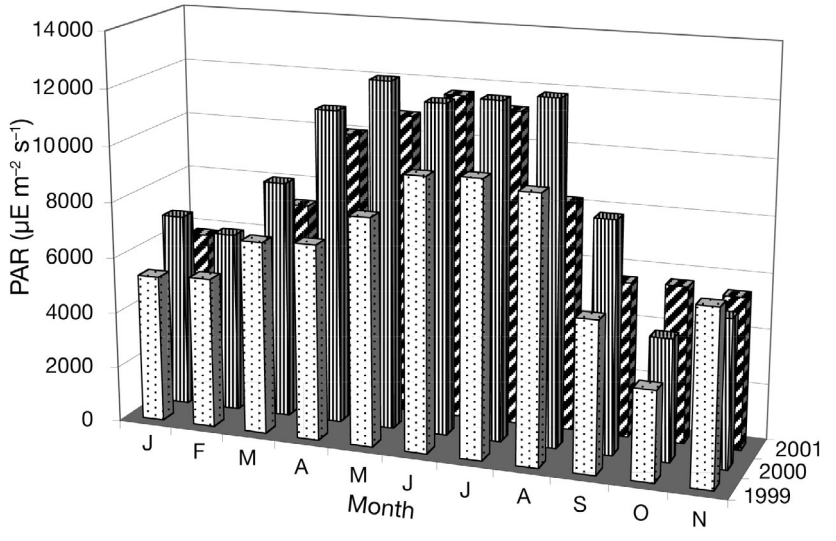


Fig. 3. Integrated photosynthetically available radiation (PAR) in the water column in the 3 years studied

December 2000 and January to April 2001. Phosphate concentrations ranged from 0.01 to 0.15 µM, with the highest values in October 2001 and the lowest values in 2000. Silicic acid maxima (>13 µM) occurred each year in October and November (data not shown).

### Dissolved and particulate organic carbon

DOC ranged from 15.5 to 30.06 g C $m^{-2}$. Over the 3 yr, DOC concentrations showed a clear annual periodicity, with winter minima and late summer maxima (Fig. 4). Accumulation was calculated as the difference between the averaged winter minimal value of 15.33 ± 3.25 g C $m^{-2}$ (Fig. 4 baseline) and the monthly integrated value. DOC accumulation varied from 0.06 g C $m^{-2}$ in February 2000 up to 15.33 g C $m^{-2}$ in September 1999, reaching 13.57 g C $m^{-2}$ in July 2000. POC fluctuations were larger in 1999 and 2001 than in 2000 (Fig. 4). The POC/chl *a* ratio averaged 310, ranging from 106 to 680, with minima during phytoplankton blooms.

### Chlorophyll *a*

The chl *a* temporal pattern showed regular seasonal dynamics, with late winter and autumn maxima (1.13 to 2.77 µg $l^{-1}$) and summer minima (<0.5 µg $l^{-1}$). The late winter peak in 2000 was less intense than in the other 2 years, whereas the autumn peak was much higher than in the other 2 years (data not shown).

### Phytoplankton community composition

The microphytoplankton community was dominated by diatoms and phototrophic and mixotrophic nanoflagellates throughout the study period. Phototrophic dinoflagelates were never abundant. In February 1999, a long-lasting diatom (e.g. *Lauderia annulata*) bloom wasrecorded. This large species accounted for a high fraction of phytoplankton C for 2 mo. In September a short outburst of the diatoms *Guinardia striata*, *G. flaccida* and *Pseudonitzschia seriata* was observed. In 2000, the February bloom was due to the diatoms *Skeletonema costatum* and *Thalassiosira* spp. (up to >2.5 × $10^6$ cells $l^{-1}$ at the surface). There was an intense bloom in October due to the diatom *Chaetoceros* spp., which reached 4.6 × $10^6$ cells $l^{-1}$. In March 2001, the bloom, though not intense, was due to the diatoms *Pseudonitzschia* cfr. *pungens*, *Asterionellopsis glacialis* and *Hemiaulus hauckii* (data not shown).

### Primary productivity

In 1999 the integrated PP was low, with a spring peak (1 g C $m^{-2}$ $d^{-1}$) and an autumn minimum (Fig. 5). In 2001 the average PP was generally <1 g C $m^{-2}$ $d^{-1}$, most values being <0.5 g C $m^{-2}$ $d^{-1}$. The year 2000 presents quite a different scenario. The PP rose beginning in May and high productivity persisted, with some oscillations, through October. The values ranged from 0.1 to 3.6 g C $m^{-2}$ $d^{-1}$. The low production during 1999 gave a mean annual value of 135 g C $m^{-2}$ $yr^{-1}$. In 2000 it tripled (414 g C $m^{-2}$ $yr^{-1}$), whereas in 2001 it was 150 g C $m^{-2}$ $yr^{-1}$. PP was significantly correlated with chl *a* concentration (1999: n = 104, r = 0.38, $p < 0.001$; 2000: n = 104, r = 0.5, $p < 0.001$; 2001: n = 65 r = 0.422, $p < 0.001$). The slope of PP/chl *a* was higher (0.82) in 2000 compared with 1999 (0.59) and 2001 (0.63).

### Respiration

$R_{<200\mu m}$ (Fig. 6) was highly variable in 1999 and 2000 (<2 to 19 g C $m^{-2}$ $d^{-1}$) (displaying a sharp peak in respiration in July, during the mucilage event). Respiration was lower in 2001, ranging between 0.09 and 2.27 g C $m^{-2}$ $d^{-1}$. The mean integrated PP/$R_{<200\mu m}$ ratio was >1 in October 2000 and in April, August and December 2001.

### Virus

Viral abundance displayed very high variability, by 2 orders of magnitude, during the study period. It was initially low at both stations (<$30 \times 10^{11}$ virus $m^{-2}$) (Fig. 7). It reached a first maximum (>45 ×

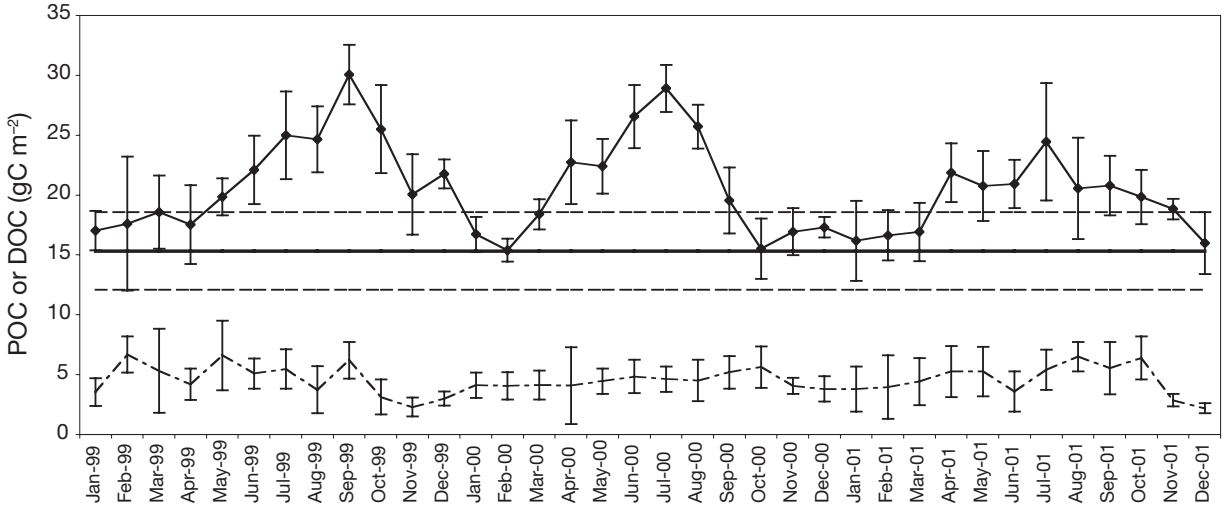


Fig. 4. Average particulate organic carbon concentration (POC, dot-dashed line) and dissolved organic carbon (DOC, solid line) accumulation at the 9 stations. The horizontal bold line corresponds to the DOC winter average (±SD, dashed lines)

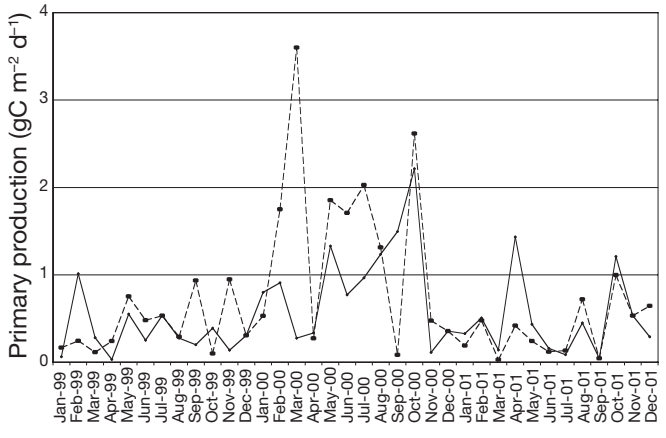


Fig. 5. Integrated primary production (PP) at Stn C1 (solid line) and at Stn AA1 (dashed line)

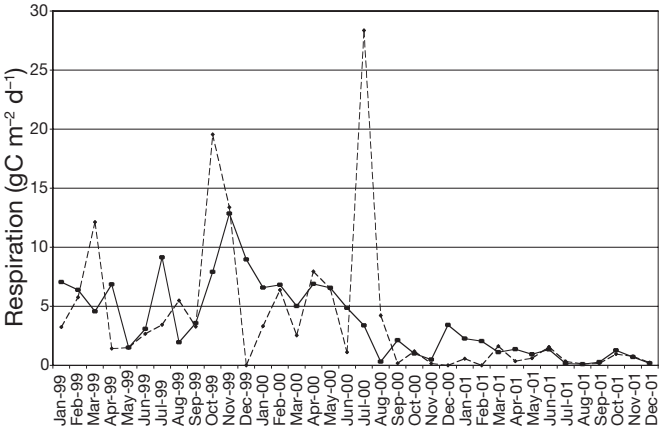


Fig. 6. Integrated respiration ($R_{<200\mu m}$) at Stn C1 (solid line) and at Stn AA1 (dashed line)

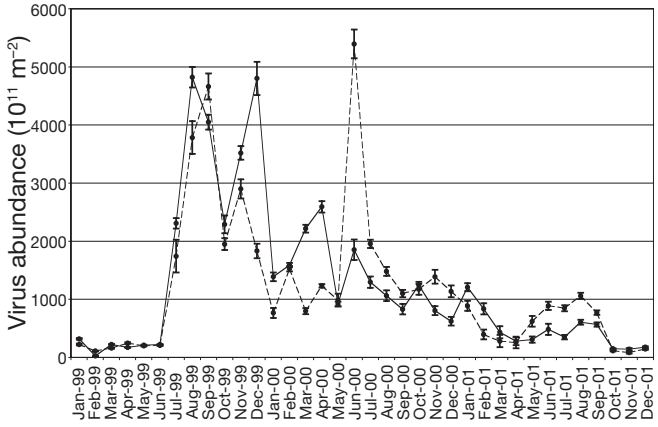


Fig. 7. Integrated virus-like-particle abundance (VLP) (±SD) at Stn C1 (solid line) and at Stn AA1 (dashed line)

$10^{13}$ virus m$^{-2}$) in August and September 1999, paralleling an increase in bacterial biomass (see the following subsection). Viral counts remained high (>$10 \times 10^{13}$ virus m$^{-2}$) until June 2000. Afterwards, except at Stn AA1 in July 2000, viruses continuously decreased to reach values as low as those of the first year.

### Bacteria

Bacterial abundance (Fig. 8) ranged between 30 and $358 \times 10^{11}$ cells m$^{-2}$. Before July 2000 bacterial counts remained <$100 \times 10^{11}$ cells m$^{-2}$, except 2 peaks in August and December 1999. A consistent pattern was that the numbers of bacteria were persistently higher during late spring to autumn than earlier in the year. This difference was more pronounced in the years 2000 and 2001.

### Bacterial carbon production

BCP showed regular maxima in August and September (Fig. 9). The rates were generally lower during winter and spring and increased 2- to 3-fold later in all 3 yr.

## DISCUSSION

### DOC dynamics

DOC levels of 15.5 to 30.06 g C m$^{-2}$ during our study were lower than those reported for a coastal northern Adriatic area (with comparable depth range as our study area) under the direct influence of the Po River (Pettine et al. 2001). In our study, DOC showed a regular and highly pronounced annual pattern: a progressive rise over summer, followed by a gradual decline, a pattern similar to that observed by Avril (2002) in the central Ligurian Sea. DOC accumulation was more pronounced during 1999 and 2000 than in 2001. Accumulation reached a maximum of 15.33 g C m$^{-2}$ (68.2 µM), but, on average, it was 24.1 µM, lower than that determined by Pettine et al. (2001) for the North Adriatic area off the Po River delta, by Doval et al. (2001) for the eastern North Atlantic–Azores Front region (168 µM) and by Cauwet et al. (2002) for the Black Sea (ca. 100 µM). In 1999, the DOC maximum was reached in September, while, in 2000, it occurred much earlier, in July. The decline of DOC was also more abrupt in 2000 than in 1999 (or 2001); DOC reached its absolute minimum value in October 2000. The DOC decline in 2000 occurred when the mass aggregation of OM reached its apex. In the Gulf of Trieste, the mucilage event, which was recorded all over the northern Adriatic Sea (Precali et al. 2005), began in the middle of June and ended by 10 July.

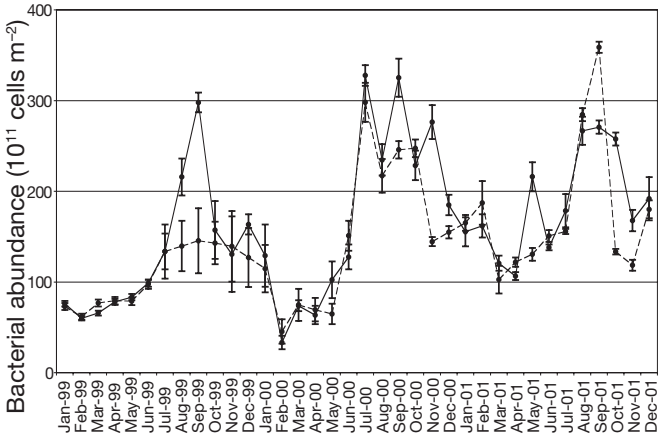


Fig. 8. Integrated heterotrophic bacterial abundance (±SD) at Stn C1 (solid line) and at Stn AA1 (dashed line)

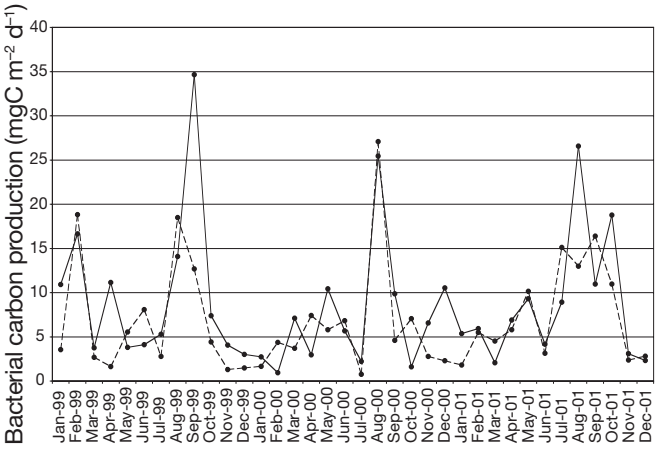


Fig. 9. Integrated bacterial carbon production (BCP) at Stn C1 (solid line) and at Stn AA1 (dashed line)

Whether there might have been a causal relationship between the temporally coincident abrupt DOC decline and the mass aggregation of OM to form mucilage is discussed later.

### DOC sources

In the Gulf of Trieste, a semi-enclosed basin, major allochthonous sources of DOC include river and sewage discharge. The main freshwater inputs in the gulf are derived from the Isonzo River, the outflow rates of which were high in October 1999 and even higher in October 2000. Therefore, the river probably made a significant contribution to the DOC increase in the autumn of 1999. However, the DOC increase resulting in the maximum in the summer of 2000, after a long drought, is likely to be essentially due to autochthonous inputs.

The ultimate source of autochthonous DOC — the phytoplankton — displayed quite low production during 1999; in 2000 it tripled, whereas in 2001 it was comparable to the 1999 level. (However, significant differences between stations were observed, and the offshore station [AA1], under a stronger riverine influence, was always characterised by higher productivity.)

Each year the late winter bloom was characterised by different diatom species. In the late winter of 1999, the dominant species was a large diatom (*Lauderia annulata*) that was not grazed (Fonda Umani & Beran 2003, Fonda Umani et al. 2005); this led to a long-lasting high-phytoplankton-carbon content in the water column. In 2000, the dominant species was *Skeletonema costatum*, which in terms of cellular abundance was very prevalent, but, due to its small size, contributed a phytoplankton carbon content lower than that in the previous year; furthermore, given its small dimensions, it is likely to be a good food source for small-sized predators, and indeed it was mostly grazed by microzooplankton (Fonda Umani et al. 2005). Consequently, the bloom was quickly removed from the water column, and it may also have been exported to the bottom through aggregation to rapidly sinking particles. A large part of the late winter bloom may have been exported to the bottom in the particulate phase in the shallow North Adriatic Sea. Non-predatory death of algae in pelagic marine systems is a large-scale process. Perhaps one-half of primary producers may be killed by lysis, possibly due to programmed cell death (Brussard et al. 1995, Berges & Falkowski 1998) or viral attack (Baldi et al. 1997). Algae also die or form dormant stages after sinking to the bottom in aggregates. As the aggregates sink rapidly through the shallow water column, the diatom detritus becomes rich in polysaccharides due to rapid recycling of their protein and organophosphorus content ('enzymatic fractionation'; Smith et al. 1992). The high POC/chl *a* (mean value of 310) ratios observed in the Gulf of Trieste may suggest that a large part of the particulate organic matter is usually in detrital form.

In the spring of 2000 polysaccharide production by phytoplankton (mostly composed by diatoms) could also have been enhanced due to the increase of P limitation; this is consistent with the findings of several authors (Guerrini et al. 1998, Myklelstad 1999, Alcoverro et al. 2000, Staats et al. 2000) that P-limited diatoms release copious amounts of polysaccharides.

Although it is highly variable, PP peaked in spring and autumn, and it was more evident in 2000 and 2001, whereas in all 3 yr BCP peaked in summer. Thus, each year PP was temporally loosely coupled with BCP, but in 2000 PP–BCP uncoupling was more evident on an annual scale. In 2000, PP may have increased due to higher PAR in the whole water column. Freshwater retention times were longer in the spring of 2000 than in the same period of the other years. This caused a longer exposure of nutrients to uptake by phytoplankton. These 2 concurrent factors may underlie the near-complete depletion of P. Phosphorus limitation could then have affected BCP (Obernosterer & Herndl 1995, Puddu et al. 2003). BCP accounted, on average, for 35.3% of PP in 1999, 9.5% in 2000 and 29.4% in 2001, with an annual average of 130.3 ± 124, 102.0 ± 98 and 120.6 ± 95 mg C $m^{-2}$ $yr^{-1}$ in 1999, 2000 and 2001, respectively.

In the early months of 2000, until July, both bacterial biomass and BCP were at the lowest levels recorded over the 3 years. Also, the cell-specific production was lower in the first 6 mo of 2000 (0.4 fg C $cell^{-1}$ $h^{-1}$) compared with the same period in 1999 (0.74 fg C $cell^{-1}$ $h^{-1}$), and this can be related to the strong P limitation (Puddu et al. 2003). In the meantime, viruses were present at high abundances, close to those reported by Corinaldesi et al. (2003) in the most eutrophic area of the North Adriatic. We can infer from these data that in the first part of 2000 bacterial biomass was under virus control, stronger than in the previous and following springs. Intense viral lysis may have maintained low bacterial biomass, as well as contributing to increased DOC production. The DOC processing by bacteria was likely incomplete, producing refractory DOC (DOC storage; Azam et al. 1999). Bacteria were also actively grazed by micro-consumers, as assessed by a parallel dilution experiment by Fonda Umani et al. (2005), who found a removal of 293% of the initial bacterial biomass per day.

For the calculation of BCD, a factor for BGE is necessary. Excluding extreme values, BGE is generally in the range from <10 to 50% (del Giorgio et al. 1997). Harris et al. (2001) applied a growth efficiency of 40%

to a large data set in the oligotrophic western Mediterranean Sea. Recently, Becquevort et al. (2002) reported a growth yield of 23% as estimated from bioassays performed in the Danube–Black Sea mixing zone. Using a medium BGE of 30% (e.g. Hoppe et al. 2002) and a conservative factor of $^3$H-leucine conversion to bacterial carbon production, the BCD varied, as an annual mean, between 147, 40 and 99% of PP in 1999, 2000 and 2001, respectively. These values translate into an apparent over-consumption of PP in 1999, a complete consumption in 2001 (carbon excess of 3 g C $m^{-2}$ $yr^{-1}$), but a carbon excess of 279 g C $m^{-2}$ $yr^{-1}$ in 2000. In July 2000, BCD reached its absolute minimum. The sum of carbon excess calculated for the 2 mo (May and June) before the mucilage event varied between 40 and 95 g C $m^{-2}$ (average 68 g C $m^{-2}$) at Stns C1 and AA1, respectively. It is relevant that at the offshore site (AA1), as well as in all offshore areas of the Gulf of Trieste, where the mucilage event persisted longer and aggregates were larger, our estimations yielded a greater difference between C production and consumption. Malej et al. (2001) calculated for the same mucilage event in an eastward area in the Gulf of Trieste that, at the maximum extension of mucilage coverage, the total integrated mucilage-associated particulate carbon was equal to 82 g C $m^{-2}$, which is numerically very close to our findings for the difference between C production and consumption. Given the high $R_{<200\mu m}$ rates, BGE possibly was <30%, but, even if we used a 5% efficiency value, at the offshore station, PP would exceed BCD in January, February, March, May and July 2000, giving a total C excess of 80 g C $m^{-2}$ over the first 7 mo. At the inshore station, PP would exceed BCD only in February and July, without any C excess.

DOC accumulation in July 2000 reached 13.57 g C $m^{-2}$, whereas the total DOC pool was 28.9 g C $m^{-2}$. This implies that the excess carbon produced in the 2 mo before the mucilage event was not completely transformed into DOC, but followed other routes (e.g. exported, settled, grazed, etc.). In this particular case, we can envision another mechanism to explain the ob-

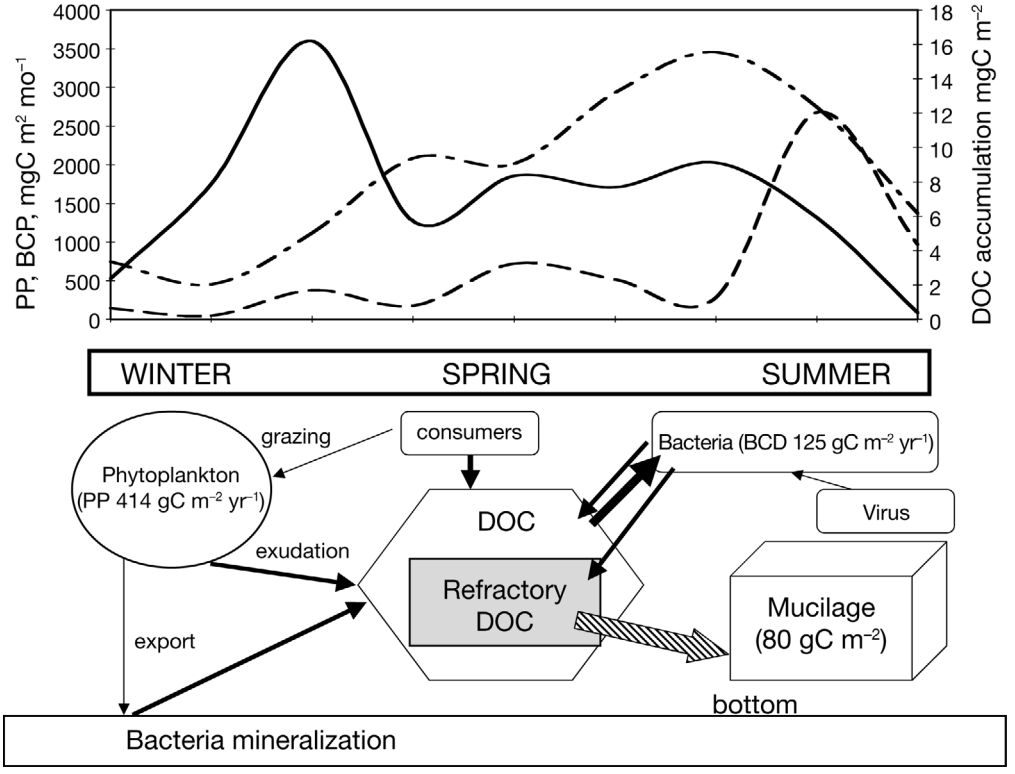


Fig. 10. Conceptual model of C fluxes in the pelagic ecosystem of the Gulf of Trieste in a year affected by mucilage. Upper panel: observed monthly integrated data of PP (solid line) and of BCP (dashed line, mg C $m^{-2}$ $mo^{-1}$) and DOC accumulation (dot-dashed line, mg C $m^{-2}$) at Stn AA1 in 2000. Lower panel: phytoplankton yearly production (414 g C $m^{-2}$ $yr^{-1}$) was exported to the bottom, exuded, or grazed by consumers. Bacterial activity at the bottom and in the water column modified organic carbon, increasing the refractory percentage. Bacteria were under virus control, which enhanced labile DOC production, increasing the storage of refractory DOC. BCD (125 g C $m^{-2}$ $yr^{-1}$) was <40% of PP, which implied an excess C production of 279 g C $m^{-2}$ $yr^{-1}$. At the maximum coverage, mucilage-associated C accounted for 80 g C $m^{-2}$ (Malej et al. 2001), which is less than one-third of the calculated excess C (arrows with medium shading: DOC production; arrows with heavy shading: DOC consumption; hatched arrow: mucilage production)

served difference: the progressive aggregation of DOC in the form of small to large flocks and stringers, which takes place before the large accumulation becomes visible (e.g. Degobbis et al. 1999, Precali et al. 2005). The first forms of aggregations are patchily distributed, although they tend to accumulate at the pycnocline (e.g. Degobbis et al. 1999). Such a patchy distribution does not allow representative sampling with the usual samplers (e.g. Niskin bottles mounted in a 'rosette'), which tend to disperse these initial aggregation states (Malej et al. 2001). They can, however, be sampled by divers using syringe samplers. Thus, a large part of organic carbon during the early aggregation stages of conversion to particulate organic matter probably escaped our sampling, and was not measured as POC.

In recent years, most research effort in the study of the mucilage phenomenon has been devoted to identifying the specific producers of polysaccharides (e.g. Herndl 1992), on the premise that such polysaccharides eventually stick together to generate the mucilage. Our findings support the hypothesis that mucilage formation may, in fact, be a manifestation of an uncoupling between organic carbon production and consumption to generate slow-to-degrade organic matter that later produced the mucilage.

Based on the above discussion, we propose the following conceptual model of C flux for the year 2000, which was affected by mucilage (Fig. 10). The PP peaked in late winter, while BCP lagged, reaching a peak in late summer. During this period, DOC accumulated, reaching a maximum in July, just preceding the apex of the mucilage event. We suggest that pathways followed by organic material led to the storage of DOC, which became increasing refractory over time and served as the source material for mucilage.

Mucilage aggregates have a maximum life span of 3 wk in the northern basin, after which they are mechanically destroyed by turbulence generated by wind stress, settle to the bottom, or are transported by the southward current into the deep layers of the eastern Mediterranean. If southward transport is a significant removal mechanism, then mucilage might be considered a mechanism to selectively decrease the level of refractory organic carbon produced and accumulating in the shallow North Adriatic, through transport and sinking into a deeper basin.

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