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Microbial decomposition of large organic particles in the northwestern Mediterranean Sea: an experimental approach

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ABSTRACT: Sediment trap particles, as well as particulate organic material including particles larger than 10 µm collected by *in situ* pumps (ISP) and fresh corpses of the gelatinous zooplankton species *Thalia democratica*, were collected in the northwestern Mediterranean Sea from April to July 1995, and incubated (after mixing with 0.2 µm filtered seawater) under laboratory conditions with their own bacterial assemblages for 6 to 24 d in batches under oxic conditions and in the dark. Particulate (POC > 0.7 µm), dissolved (DOC < 0.7 µm) and colloidal (0.02 < COC < 0.7 µm) organic carbon contents, as well as bacterial abundance and production, were quantified over time. In all experiments, total organic carbon (TOC = POC + DOC) decrease covaried with an increase in bacterial abundance and production, bacteria being the main mediators of particle decomposition. We found that COC accounted for 19 to 31% of DOC immediately after particle dilution in 0.2 µm filtered seawater, and always for less than 9% at the end of the experiments. As organic colloids comprised less than 7% of DOC in the 0.2 µm filtered seawater used to dilute the particles, this result suggests that COC was mainly produced from particle decomposition. Assuming that bacterial populations were the sole decomposer of organic matter in the batches, the results gave bacterial growth efficiencies (BGE) in the range of 3 to 21%, indicating that decomposition of these particles significantly produce CO_2 through bacterial respiration. The results showed that bacteria degraded 16 to 87% of the initial amount of POC within the first 48 h, whereas only 6 to 22% of POC was degraded in the second stages. Our data and modeling work based on such short incubation times suggest that salp bodies are composed of 1 labile and 1 refractory organic fraction, whereas both ISP- and trap particles are composed of 2 labile and 1 refractory organic fraction. A 1G-model (for salp) and a 2G-model for other particles was able to satisfactorily reproduce the data sets.

KEY WORDS: Mediterranean Sea · Bacteria · Particulate organic carbon

INTRODUCTION

The vectors of sinking particulate organic carbon (POC) are remains of plants and/or animals, faecal pellets, aggregates and exopolymer particles (Fowler & Knauer 1986, Martin et al. 1987, Turley & Lochte 1990, Asper et al. 1992, Passow & Wassman 1994, Silver et al. 1998). During settling of this particulate material, rates of particle destruction through break-up, remineralization and dissolution are critical variables that determine the amount and the lability of the organic matter ultimately reaching the seafloor. Moreover, the chemical and biological transformations which occur during the descent of these particles in the water column may affect trophic interactions of aggregate consumers and associated organisms and, thus, modify the chemistry of the surrounding water (Goldman 1984, Shaffer 1996). The magnitude of the sinking flux and the depth at which it is remineralized, mainly by heterotrophic

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bacteria, are both important factors in the oceanic carbon cycle. It is essential to understand not only the processes but also the kinetics of particle disaggregation, as well as bacterial growth efficiencies (BGE). This latter parameter being an important tool for carbon budget on a global scale (Sempéré et al. in press).

It is presently well known that intense hydrolytic activity on aggregate POC by attached bacteria is an important pathway for production of dissolved organic carbon (DOC) which is mainly taken up by free-living bacteria (Cho & Azam 1988, Herndl 1988, Karner & Herndl 1992, Smith et al. 1992, 1995, Hoppe et al. 1993). However, the turnover rates of large particles and the extent to which DOC and other degradation products such as sub-micron particles or colloids are produced from these particles are far from being understood. Some attempts to assess POC fates including bacterial degradation, zooplankton grazing and physico-chemical disaggregations and turnover rates were made by measuring particulate flux along the water column by sediment traps (Lee & Cronin 1982, Gardner et al. 1983, Walsh et al. 1988, Khripounoff & Crassous 1994) and by measuring adsorption rates of radionucleides on particles (Moran & Buesseler 1992, Van der Loeff et al. 1997).

The major biochemical processes and their degradation kinetics have also been examined in laboratory conditions. Such investigations showed that degradation of particulate material follows first-order kinetics and suggested that organic matter does not degrade as a single pool (Berner 1980, Westrich & Berner 1984, Henrich & Doyle 1986, Harvey et al. 1995, Nguyen & Harvey 1997). In addition, POC degradation as well as microbial parameters followed over time indicated that a part of organic matter is quickly and efficiently utilized by bacteria within the first days of the incubation period, whereas another part is slowly and less efficiently consumed in a later stage (Turley & Lochte 1990). However, all these studies were performed with zooplankton, phytoplankton debris or axenic cultures of phytoplankton (Westrich & Berner 1984, Henrich & Doyle 1986, Turley & Lochte 1990, Christaki & Van Wambeke 1995, Harvey et al. 1995, Van Wambeke 1995, Nguyen & Harvey 1997). To our knowledge, there is no report specifically dealing with the lability and the turnover rates of large particles (>10 µm) collected by *in situ* pumps and drifting traps which are commonly used by marine scientists.

In this paper, we present a series of experiments performed with large organic particles collected in the water column in the northwestern Mediterranean Sea. The objectives of our study are (1) to measure the amount of POC degraded by bacteria and subsequently the amount of DOC and colloidal organic carbon (COC) released by this process, (2) to determine BGE on the pool of labile total organic carbon (TOC) and (3) to provide turnover rates for the labile fraction of TOC.

MATERIALS AND METHODS

Study area. Seawater and particle collection was carried out from April to July 1995 in the northwestern Mediterranean Sea in the framework of EMPS (April), Dyfamed (May), and Euromarge (July) projects (Fig. 1). Three stations were sampled, 1 located in the Gulf of Lions (Stn M3, 43° 02′ N, 05° 12′ E, 900 m depth) and 2 others located in the Ligurian Sea: Stn 1, 43° 50′ N, 7° 8′ E, 1300 m depth and Stn 2, 43° 25′ N, 7° 52′ E, 2300 m depth. Stns M3 and 1 were coastal stations under the influence of the northwestern Mediterranean Current (Christaki et al. 1996, Yoro et al. 1997) whereas Stn 2 was the permanent 'Dyfamed station', which can be considered as typical of the whole northwestern Mediterranean Sea (Copin-Montégut & Avril 1993, Marty et al. 1994). Estimates of primary production in the northwestern Mediterranean basin averaged 78 g C m^{-2} yr^{-1} (Minas 1970), whereas the sole new production was 18 g C m^{-2} yr^{-1} (Minas et al. 1988). Transfer of organic material to deep layers takes place through diffusive processes and winter convection of DOC (18.4 g C m^{-2} yr^{-1} Copin-Montégut & Avril 1993) and from exportation of large sinking particles (5 g C m^{-2} yr^{-1}, Miquel et al. 1994).

Sampling. Particles were collected at 50 (Stn M3) and 200 m (Stn 1) depth by filtering 824 to 1000 l of seawater with *in situ* pumps (Challenger Oceanics) equipped with 10 µm pore-size filters (Nuclepore). Immediately after sampling, swimmers were discarded from the bulk of particle sets, then filters were maintained wet by adding a few ml of 0.2 µm filtered seawater and stored in the dark until incubation experiments in the land laboratory began (this time lapse never exceeded 6 h). Particles were removed by injecting with a syringe ca 200 ml of 0.2 µm filtered seawater on the surface of the filters. The mixture was then transferred to a 2 l glass bottle and stored in the dark, at *in situ* temperature. All glass bottles and vials used during this study were precombusted for 6 h at 450°C prior to utilization.

Large sinking particles were collected by a drifting Technicap-PPS5 sediment trap with a mouth of 1 m^2 deployed without preservative at 200 m depth at Stn 2 for 44 h. After removal of the trap, swimmers were discarded, and a 2 l glass bottle was filled with a part (100 ml) of the 6 cups held by the rotary collector. The cup supernatant was also decanted and discarded to remove free-living bacteria and heterotrophic flagellates as much as possible.

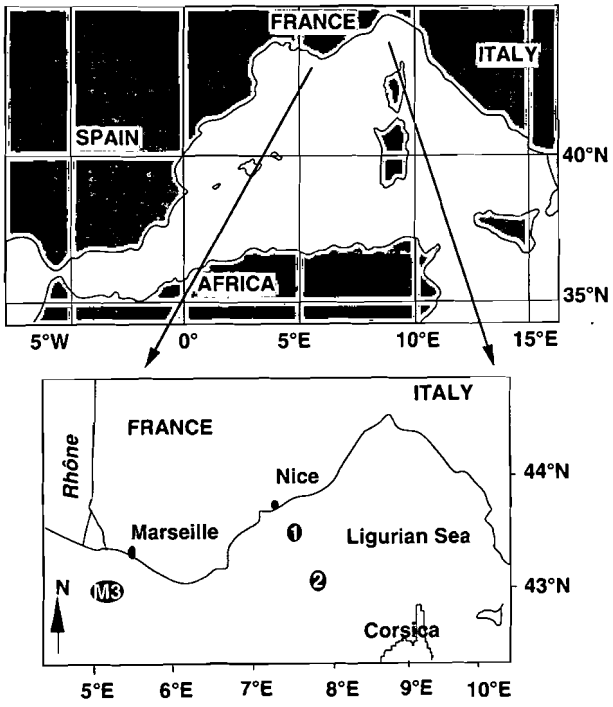

Fig. 1. Locations of the 3 stations studied (Stn M3: 43° 10′, 05° 12′ E, 900 m depth; Stn 1: 43° 50′ N, 7° 8′ E, 1300 m depth; Stn 2: 43° 25′ N, 7° 52′ E, 2300 m depth) during the Euromarge, EMPS and Dyfamed-Dynaproc cruises from April to July 1995 in the coastal northwestern Mediterranean Sea

The gelatinous zooplankton species *Thalia democratica* was collected using a zooplankton net at 10 m depth in the Bay of Villefranche-sur-mer. On board, these organisms were gently removed from the net, transferred into an aquarium and transported back to the laboratory (Villefranche-sur-mer Institute). Just before the laboratory experiment, they were rinsed several times with 0.2 µm filtered seawater and transferred to glass bottles. This experiment was conducted to study the bacterial response to inputs of fresh organic matter.

Incubation experiments. According to their abundance, the particles were diluted with 1 to 4 l of 0.2 µm filtered seawater collected at the same depth. Prior to filtration, the 0.2 µm pore-size filters were flushed with large amounts of Milli-Q water (2 l) and 0.5 to 1 l of seawater to minimize the potential organic contamination (Yoro et al. 1999). The solution including particles and 0.2 µm filtered seawater was distributed in 500 ml duplicate glass bottles with a headspace ratio of ca 150:500 ml to ensure oxic conditions throughout the incubation period. This was checked after the experiments by calculations taken from the TOC consumption during the whole incubations. The incubation bottles were closed with Teflon-lined screw caps and incubated in the dark at *in situ* temperature (15 to 18°C) for several days depending on bacterial growth, or decrease, and also on the quantity of material available for the incubation experiments. The incubation bottles were gently and regularly swirled upside-down to avoid particle deposition and to ensure oxic conditions. Each sub-sampling (each bottle corresponded to an incubation time) was performed by collecting aliquots for POC, DOC and COC as well as for bacterial production and/or abundance, after which the bottle was discarded. The standard deviation for the particle distribution, determined by measuring POC in various subsamples was about 8% at the start of the incubation. Experimental errors based on duplicate analyses for each experiment averaged 9% for TOC, 10% for bacterial abundance and 12% for bacterial production. Control experiments (3 sub-samples for ISP-50 m particle and 1 sub-sample for other stations) were performed by adding $HgCl_2$ at the start of the experiment (10 mg l^{-1} final concentration) to batches containing particles and processed as the live experiments.

POC. For each bottle, 50 ml was filtered through precombusted GF/F filters. The material collected on the GF/F filter (effective retention: 0.7 µm) was processed according to the method described by Tan & Strain (1979), and assayed on a CS analyzer (CS 125-Leco) to measure POC. In preparation for POC analysis, the GF/F filters were covered with a few ml of 2N H_3PO_4 and evaporated to dryness at 50°C for 12 h to remove inorganic carbon. The filters were then combusted, and the CO_2 generated was measured by a non-dispersive infra-red cell, with a standard deviation of 2%. POC values reported in this paper were calculated by subtracting bacterial biomass-C (determined from bacterial abundance and assuming that most of the bacteria were retained on the GF/F filters during the filtration procedure) to the POC measured by Leco.

DOC and COC. The filtrate passing through the GF/F filters was transferred into duplicate glass vials, poisoned with 50 µl of $HgCl_2$ (final concentration: 10 mg l^{-1}) and closed with Teflon-lined screw caps and stored in the dark at room temperature. A few ml of the remaining sample in the bottle was filtered upon 0.02 µm Anopore Whatman filters, previously cleaned with 1 to 2 l of Milli-Q water to minimize the potential organic contamination of the filters. The filtrate obtained was considered as true DOC (RDOC < 0.02 µm) and analyzed for DOC. The carbon blank of the Anopore filter (4 to 8 µM C, Yoro et al. 1999) was also measured and retrieved from the value of RDOC. COC was considered as the fraction between 0.02 (Anopore filter) and 0.7 µm (GF/F filter), and thus, obtained by applying the following formula: COC = DOC – RDOC. TOC was calculated as the sum of POC and DOC.

Samples for DOC analysis were measured by high temperature combustion on a Shimadzu TOC 5000 Analyzer. Details of this procedure have been previously described (Yoro et al. 1997, 1999). A 4-point calibration curve was performed daily with standards (83 to 332 µM C) prepared by diluting a stock solution of potassium hydrogen phthalate in Milli-Q water. The instrument blank ranged from 6 to 9 µM C.

Enumeration of bacteria. For each batch, a few ml of sample was preserved with 0.2 µm filtered borax-buffered formaldehyde (2% final concentration) for bacterial enumeration. Before slide preparation, samples were sonicated, diluted with 0.2 µm filtered seawater (1:10) and sonicated again to separate attached bacteria from particles and ensure good distribution on the filter. Slides were prepared within 24 h following particle sampling in the batches to avoid underestimation of cell numbers due to possible losses during storage (Turley & Hughes 1992). Bacteria were filtered onto 0.2 µm pore-size Nuclepore filters and stained by DAPI (final concentration: 2.5 µg ml^{-1}). Up to 30 fields were randomly counted with a Olympus BH2 epifluoresence microscope (Porter & Feig 1980), the standard error between replicates ranged from 3 to 20%. To compute the cell volume, we considered the rod-shaped cells to be cylinders with a hemispherical cap. Bacterial biovolume measurements ranged from 0.033 to 0.569 μm^3 (average: 0.18 ± 0.11 μm^3). This average biovolume is slightly lower than that observed by Turley & Lochte (1990): 0.2 to 0.3 μm^3 from biovolume measurements of bacteria incubated for 23 d with detritus of phyto- and zooplankton. Bacterial carbon biomass was estimated from abundance and biovolume using a power function calculated from Simon & Azam (1989):

$$y = 90V^{0.59} \quad (1)$$

where y is the amount of bacterial carbon per cell (fg C $cell^{-1}$) and V the mean cell volume. The average conversion factor estimated by this method was 32 fg C $bacterium^{-1}$.

Bacterial production. Bacterial production was determined from rates of DNA synthesis as measured from rates of tritiated thymidine incorporation (Fuhrman & Azam 1980). Two replicate samples (each 4 ml) and a formalin-killed control were incubated with 20.5 or 105 nM (methyl-^{3}H)-thymidine (specific activity: 44 to 65 Ci $mmol^{-1}$), according to the abundance of particles. Previous studies performed with bacteria attached to large particles indicated that the incorporation of (^{3}H)-thymidine was saturating at 20 nM (Simon et al. 1990, Becquevort et al. 1998). Samples were incubated at *in situ* temperature and in the dark for a predetermined period (1 to 2 h) that was in the linear phase of incorporation. Incubations were stopped with formalin (2% final concentration), samples were filtered through 0.2 µm Nuclepore filters and then extracted with ice-cold 5% trichloroacetic acid. Bacterial production was estimated from (^{3}H)-thymidine incorporation rates by applying the theoretical conversion factor of 2×10^{18} cells mol^{-1} (Ducklow & Carlson 1992), which is also comparable to the empirical factors previously used in particle degradation experiments (1.7 and 2.6×10^{18} cells mol^{-1}; Simon et al. 1990 and Becquevort et al. 1998, respectively). We applied a cell-to-carbon conversion value of 32 fg C $cell^{-1}$ as determined from bacterial biovolume measurements.

BGE. We determined BGE from TOC decreases and from the increases in bacterial biomass-C. Bacterial biomass was calculated either from the cell abundance or from thymidine-based bacterial production of bacteria over time, leading to the following formulae

$$BGE = (\Delta BB/\Delta TOC) \times 100 \quad (2)$$
$$BGE = (IBP/\Delta TOC) \times 100 \quad (3)$$

where IBP is integrated bacterial production cumulated over the successive interval periods and determined from rates of thymidine incorporation, ΔBB is the difference of bacterial biomass between the initial time and the end of the first period of biodegradation, and ΔTOC is the amount of TOC utilized during the same time interval. Whatever the method used, bacterial respiration is deduced from the BGE as follows:

$$\%\ respiration = 100 - BGE\ (\%) \quad (4)$$

Kinetics of particle decay. The kinetics of TOC and POC decomposition can be described by the first-order (exponential) decay of 2 reactive organic matter fractions. Hence, we considered the 2 classes (labile and less labile) of both TOC and POC concentration of any constituent at a point in time [$G(t)$] as the product of its original concentration (G_0) and the first-order decay constant (k) at that time as follows:

$$G(t) = G_0 \exp^{(-kt)} \quad (5)$$

We constructed a simple 2G-model in which each class (labile and less labile) is degraded with a first-order rate constant. In this model, we assume that an organic fraction is not degraded during experimental time (G_{NR}). Based on the formulation of Westrich & Berner (1984), we represent the TOC and POC data as a function of time by an equation of the form:

$$G_T(t) = G_{01}[\exp(-k_1 t)] + G_{02}[\exp(-k_2 t)] + G_{NR} \quad (6)$$

where G_T, G_{01} and G_{02} are the total concentrations, the labile-class concentration and the less labile-class concentration of organic carbon (POC or TOC), respectively (see Table 3). G_{NR} was defined as the concentration of the non-reactive class of POC or TOC fraction during the incubation times. The rate k_1 was defined as

Table 1. Net variations of the different pools of organic carbon (ΔTOC, ΔPOC, ΔDOC, ΔCOC) expressed as percentages of the initial amount of organic carbon. Δ% = 100 × (final concentration – initial concentration)/initial concentration. POC values were corrected for bacterial biomass assuming that most of the bacteria were retained on the GF/F filters used for filtration. ISP: particles larger than 10 µm collected by *in situ* pumps. Control: batches poisoned with $HgCl_2$. nd: not determined

Sample ID	Experiment duration (d)	TOC init. (µM)	POC init. (µM)	ΔTOC (%)	ΔPOC (%)	ΔCOC (%)	ΔDOC (%)
ISP-50 m	23	1113	906	−57	−68	nd	4
ISP-50 m (control)	23	860	750	−8	−30	nd	31
ISP-200 m	8.3	181	81	−17	−34	−81	−4
Trap particles	9.9	365	261	−31	−50	−50	18
Salps	5.7	794	683	−82	−93	−100	−12

the first-order decay constant of the most reactive TOC or POC and k_2 the first-order decay constant of the less reactive POC or TOC and t the time of decomposition.

RESULTS

The TOC concentrations (calculated as DOC+POC) in the batches were 2- to 10-fold the concentrations found in the water column. Attempts to balance the initial POC/TOC concentrations in the different incubation experiments were not successful because we did not have access to the POC analyzer when experiments were initiated immediately after sampling. Immediately after the particle dilution, DOC concentrations in the different types of batches ranged from 100 to 207 µM C, which are higher than those found in the surrounding water (70 to 100 µM C). Since the batches were made by mixing particles and 0.2 µm filtered seawater, this indicates that some DOC is released from particles immediately after their dilution in the batches. The DOC increases were also observed in the control experiments, suggesting that the mechanism leading to this initial production of DOC from the particles is likely to be also abiotic. During the incubation time, TOC decreased by 6 to 9% of the initial concentration in the batch controls (Table 1, Figs. 2 to 5) whereas bacterial growth was negligible, indicating that biotic mechanisms play the main role in the TOC decreases observed during non-poisoned incubations. TOC decrease in batch controls might also be due to adsorption of POC or DOC on the bottle wall or to experimental error.

Organic carbon consumption

Following enclosure of non-poisoned particle solutions, we observed an initial sharp decrease of POC concentration, i.e. 524 µM C (52% of POC initial concentration) in 0.8 d for ISP-50 m (Fig. 2), 13 µM C (16%) in 1 d for ISP-200 m (Fig. 3), 597 µM C (87%) in 1.9 d for salps (Fig. 4) and 81 µM C (31%) in 0.5 d for trap particles (Fig. 5), followed by a lower decline thereafter until the end of the incubation. By contrast, DOC concentrations showed little variation. Indeed, throughout the experiments, average DOC values in the experimental flasks were 219 µM C (±15%) for ISP-50 m, 90 µM C (±10%) for ISP-200 m, 107 µM C (±8%) for salp and 99 µM C (±8%) for trap particles. Basically, the highest decrease of the TOC (POC+DOC) content was observed for cadavers of salps (77% in 1.9 d; Fig. 4) whereas the lowest decrease was observed for ISP-200 m particles (14% in 1 d) (Table 1,

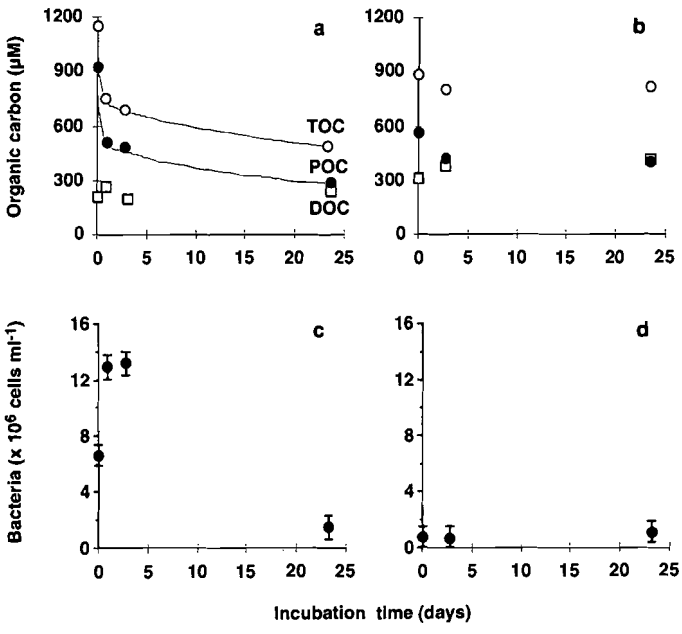

Fig. 2. Time-course responses of the different fractions of (a,b) organic carbon and (c,d) bacterial abundance from incubation experiments performed with ISP-50 m particles, i.e. material larger than 10 µm collected on July 7, 1995, by *in situ* pumps at 50 m depth at Stn M3 in the Gulf of Lions: (a,c) live incubation, (b,d) control (solution poisoned with $HgCl_2$). Lines represent the 2G-model concentrations for TOC and POC

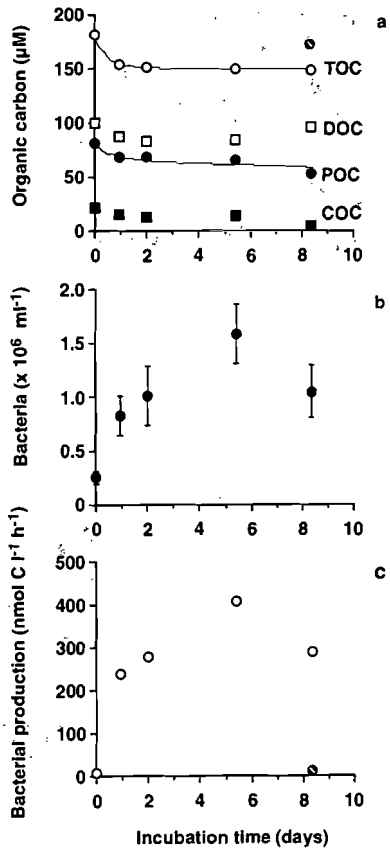

Fig. 3. Time-course responses of the different fractions of (a) organic carbon, (b) bacterial abundance and (c) production from incubation experiments performed with ISP-200 m particles, i.e. material larger than 10 µm collected on April 3, 1995 by *in situ* pumps at 205 m depth at Stn 1 in the Ligurian Sea. Lines in (a) represent the 2G-model concentrations of TOC and POC. Dashed circles: poisoned controls

Fig. 3). TOC decreases for ISP-50 m particles and 200 m trap particles were 34% (0.8 d) and 27% (0.5 d), respectively. In the second stages of decomposition (after ca 1 to 2 d) 18% of ISP-50 m particles were remineralized, whereas the TOC fraction remineralized in other batches was much lower (1 to 5%, Table 1).

Concentrations of COC only measured in salp, ISP-200 m particle and trap-particle experiments accounted for 19 to 31% of DOC at the beginning and for less than 9% of DOC at the end of the experiments. By contrast, they accounted for less than 7% of the DOC pool in surrounding water, indicating that they were rapidly released by particles at the beginning of the incubation period. Interestingly, 70 to 80% of the DOC produced by the dilution of the particle stage (t = 0) was in colloidal state, indicating that the abiotic and/or biotic processes that took place immediately after the introduction of particles into the experimental flasks produced essentially DOC in the colloidal size range 0.02 to 0.7 µm.

Bacterial abundance and production

Accompanying the initial decrease of TOC, we observed an increase of bacterial abundance and/or production (Figs. 2 to 5) in all experiments, indicating that activity of attached bacteria was essential to decrease TOC concentrations. It should be noticed that filtration of the seawater used to dilute the particles through 0.2 µm pore-size filters excludes the free-living bacteria and their grazers. Thus, in the initial conditions, particles were present with their natural microbial communities.

In such a way, initial bacterial densities for salp, ISP-200 m and ISP-50 m incubation bottles were ca 3.3, 2.5 and 1.1 times lower, respectively, than total counts found in seawater at the depth of collection (Bianchi et al. 1997). By contrast, an enhancement factor of 1.7 was obtained with trap particles. However, bacterial

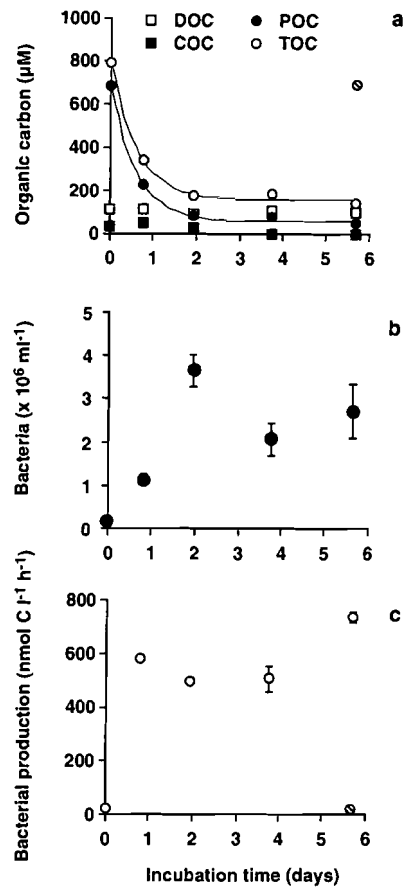

Fig. 4. Time-course responses of the different fractions of (a) organic carbon, (b) bacterial abundance and (c) production from incubation experiments performed with fresh bodies of salps collected at 10 m depth in April 4, 1995, in the Bay of Villefranche-sur-mer. Lines on (a) represent the 1G-model concentrations for TOC and POC. Dashed circles: poisoned controls

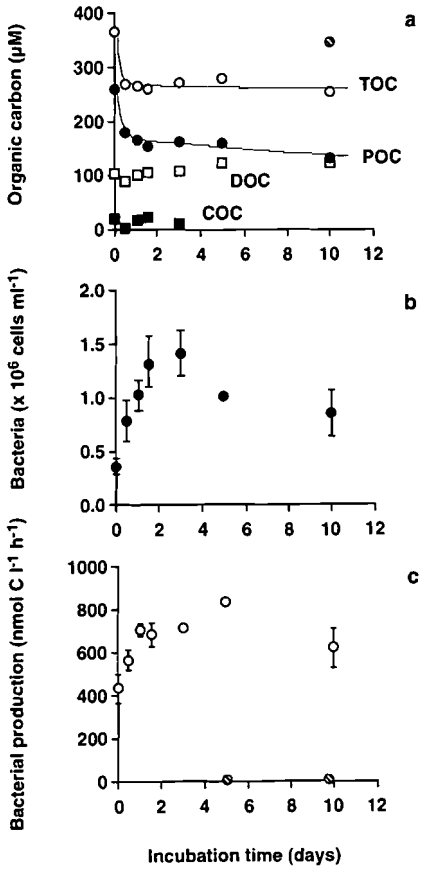

Fig. 5. Time-course responses of the different fractions of (a) organic carbon, (b) bacterial abundance and (c) production from incubation experiments performed with drifting trap particles deployed at 200 m depth at Stn 2 (DYFAMED station) and collected on June 1, 1995. Lines on (a) represent the 2G-model concentrations for TOC and POC. Dashed circles: poisoned controls

production rates in incubation bottles for ISP-200 m particles, salps and trap particles, were 17, 22 and 2088 times higher than that found in the water column. It should be kept in mind that bacterial production and biomass are related to the total population including free-living and attached bacteria. Bacterial abundance at the beginning of the experiments ranged from 0.25 to 6.60 × 10^6 cells ml^{-1}, the maximum being observed for ISP-50 m particles (6.60 × 10^6 cells ml^{-1}). The values observed at the peak of abundances ranged from 1.41 to 13.9 × 10^6 cells ml^{-1}. Bacterial densities in the batches increased by a factor of 5 to 10 during the first days of incubations. Bacterial production increased from 8.5 to 683 nm C l^{-1} h^{-1} during the same time interval (Figs. 3 to 5). The drastic decrease in bacterial abundance and production observed after 2 or 6 d of incubation could be due to the increase in bacterivores (heterotrophic flagellates) in the batches (Newell et al. 1981). In fact, random microscope transects checked on our slides did not show a significant number of heterotrophic flagellates at the beginning of the experiment but indicated that they were rather more abundant at the end of the experiment.

Bacterial growth efficiency

BGE (Table 2) determined from integrated bacterial production (3 to 31%) were higher than those calculated from bacterial biomass (1 to 6%), indicating that the use of bacterial abundance led to significant underestimation (3 to 10 times) of the BGE (Table 2). Even considering thymidine-based BGE, between 69 and 97% of the organic matter utilized by bacteria was respired. Surprisingly, BGE determined for salps (1 to 3%, Table 2) were among the lowest values observed, suggesting that only a small part of the labile TOC utilized by bacteria contributed to bacterial biomass.

Kinetics of particle decay

Our results showed that TOC was rapidly turned over by bacteria during the first days (<2 d) of the incubation period, and then slowly utilized during the following days (Figs. 2 to 5). This pattern suggests that at least 2 classes of TOC were being degraded with different mineralization rates: 1 rapidly degraded class (called labile) that causes the initial drop, and a more slowly degraded class of less labile TOC (hereafter called less labile). The so-called 'refractory fraction' (G_{NR}) is not degraded during the incubation time.

The measurements indicated that the time when rates changed suddenly occurred at 1.9 d for salps,

Table 2. Bacterial growth efficiencies (BGE %) calculated from TOC depletion and bacterial biomass increase (ΔBB) or TOC depletion and integrated bacterial production (IBP) during the first period (2 to 3 d according to the experiments). IBP was calculated from the rates of thymidine incorporation assuming a conversion factor of 2 × 10^{18} bacteria per mole of thymidine incorporated and 32 fg C bacterium^{-1}. ΔBB is the net increase of bacterial biomass determined from bacterial abundance assuming a conversion factor of 32 fg C bacterium^{-1} (see 'Materials and methods'). ISP: particles larger than 10 µm collected by using *in situ* pumps. nd: not determined

Sample ID	Period considered (d)	BGE % ΔBB	BGE % IBP
ISP-50 m	0–2.8	1.4	3.3
ISP-200 m	0–2.0	6.1	31
Trap particles	0–1.6	2.1	21
Salps	0–1.9	3.6	nd

0.5 d for trap particles, 1 d for ISP-200 m and 0.8 d for ISP-50 m. Then, we initially determined G_{01} as the concentrations of POC or TOC decomposed (or consumed) during these periods of time whereas the value of G_{NR} was initially calculated as the concentrations at the end of the experiments. In a first approach, the values of G_{02} were taken as the difference between total measured initial carbon and the sum of G_{01} and G_{NR}. By substituting these results in Eq. (6) as initial values and then by using least-square minimization method (Solver function of Excel), we obtained the adjusted values of k_1, k_2, G_{01}, G_{02} and G_{NR} for POC and TOC (Table 3). By contrast to the other decompositions, the best fit for salp decomposition (Fig. 4) was obtained for similar values of k_1 and k_2, indicating that the degradation (TOC and POC) can be approached by a 1G-model (k_{POC}= 1.65 d^{-1}, G_{NRPOC} = 61 µM C; k_{TOC} = 1.60 d^{-1}, G_{NRTOC} = 162 µM C). For the other experiments (Figs. 2, 4 & 6), the approximate values of k_{1TOC} ranged from 2.16 (ISP-200 m) to 6.14 d^{-1} (trap) whereas k_{2TOC} ranged from 0.007 (ISP-200 m) to 0.307 (trap) d^{-1}. These data are in general agreement with those found for POC (Table 3). It should be noticed that POC degradation rates (Table 3) are not equivalent to those found for TOC, because the POC decomposition produces DOC which is not completely consumed by bacteria.

Table 3. Coefficients of the 2G-model (1G-model for salp experiment) as determined by the model adjustment to the data (least-square minimization, Newton method). G_{01}: pool highly degradable (labile class), G_{02}: pool slowly degradable (less labile class), G_{NR}: refractory pool. These 3 terms are expressed in percentage of the initial amounts of POC and TOC concentrations specified in Table 2. Rates k_1 and k_2 are the exponential decay rates determined by the model adjustment according to the equation $G_T(t) = G_{01}[\exp(-k_1 t)] + G_{02}[\exp(-k_2 t)] + G_{NR}$. Errors estimated as percentages of the difference between observations and data calculated with the model are also given

Organic carbon	Coefficients	ISP-50 m	ISP-200 m	200 m trap	Salps
POC	G_{01} (%)	45	16	35	84
	G_{02} (%)	33	16	35	7
	G_{NR} (%)	22	68	30	9
	k_1 (d^{-1})	3.20	1.93	3.93	1.65
	k_2 (d^{-1})	0.058	0.13	0.045	
	CV (%)	2.8	3.4	2.2	6.4
TOC	G_{01} (%)	35	17	27	74
	G_{02} (%)	32	17	2	6
	G_{NR} (%)	34	66	71	20
	k_1 (d^{-1})	3.16	2.16	6.13	1.60
	k_2 (d^{-1})	0.058	0.0074	0.31	
	CV (%)	0.1	0.05	2.1	4.2

DISCUSSION

Mechanisms of particle decomposition

The small changes of DOC concentrations together with a high POC decrease observed over time for experiments indicate an intense biotic and/or abiotic DOC production followed by a rapid cycling of DOC in living batches during particle decomposition. Particle mineralization is likely due to an intense ectoenzymatic activity of attached bacteria which renders the particles soluble through macromolecular hydrolysis and produces dissolved organic compounds and small molecules which are subsequently taken up by attached and free-living bacteria (Karner & Herndl 1992, Smith et al. 1992). It is also interesting to note that DOC quantitatively increased in the poisoned batches, despite the lack of bacterial development. This strongly suggests that particles also produce abiotic DOC. These results are consistent with the recognition that a significant fraction of DOC is continuously released from POC (Biddanda & Pomeroy 1988, Williams 1990). Although they are not well quantified, the abiotic processes capable of aggregate destruction are numerous and could include physical breakdown (Karl et al. 1988, Wells & Goldberg 1993), cell lyses and diffusion of solutes from the particles (Jumars et al. 1989).

Incubation experiments performed with fresh corpses of salps provided evidence for a rapid response of bacteria to inputs of labile material. Hard parts accounted for a small part in the salp bodies. Hence, decomposition of the animals induced rapidly dissolved organic compounds readily utilizable by bacteria such as carbohydrates and proteins. Indeed, more than 80% of the initial amount of salp-C was mineralized within 6 d, which is significantly higher than the labile fraction found for trap (29%), ISP-50 m (39%) and ISP-200 m particles (16%). The results indicated that at 200 m, trap-TOC was more labile than ISP-TOC (see % of labile fraction, G_{01}, and k_1). Large aggregates are the main material deposited in the trap cups, whereas it is likely that particles collected with *in situ* pumps, even when equipped with 10 µm pore-size, comprised a wider spectrum of size classes since small particles can adsorb on the filter surface because of the clogging of pores during pumping. Since the pore-size filter (10 µm) is much higher than that of free-living bacteria (0.2 to 1 µm), attached bacteria are likely to be the main microbes present in the solution at the beginning of the experiment. Therefore, the material collected on the ISP filters probably includes smaller and older particles in addition to the >10 µm particles which lead to lower values of degradation rates. Moreover, trap particles comprise, in addition to other aggregates, faecal pellets which contain internal bac-

teria coming from the gut of animals (Lawrence et al. 1993) able to enhance bacterial activities (Turley & Mackie 1994). The labile fraction of ISP-50 m particles was higher than for ISP-200 m particles, indicating that the more labile part of the particulate material was probably already degraded during the sedimentation between 50 and 200 m.

Colloid production

High concentrations of sub-micron particles (0.4 to 1 µm) have been already observed in the euphotic layer in the Pacific Ocean (Koike et al. 1990, Yamasaki et al. 1998). In the Mediterranean Sea, we reported high concentrations of large colloids (>500 kD) in particle-rich environments such as the bottom nepheloid layer in the Gulf of Lions (Sempéré et al. 1994) or in the Krka estuary (Sempéré & Cauwet 1995), suggesting that high concentrations of particles promote abiotic/biotic production of large colloids. Ectoenzymatic activity of bacteria is one mechanism that might be responsible for sub-micron particle production during particle degradation (Smith et al. 1992). In the incubation bottles, release of metabolic waste in the colloidal size range (0.02 to 0.7 µm) by protozoans (Koike et al. 1990) cannot be precluded, although flagellates were found not to be abundant in the solutions. Small organic particles can also be generated during the physical or biological breakdown of large aggregates (McCave 1984, Karl et al. 1988, Wells & Goldberg 1993). Unfortunately, COC were not measured in control experiments and, therefore, the intitial abiotic and biotic origin of colloids cannot be explained here.

Efficiency of organic carbon utilization

The results indicated that carbon-based BGE was always lower when calculated from cell counting than from bacterial production experiments. This phenomenon, already observed for dissolved organic matter biodegradation (Kirchman et al. 1991, Amon & Benner 1994), is probably due to the grazing pressure of flagellates on bacteria and/or virus mortality which leads to an underestimation of the true bacterial biomass produced during the incubation experiments. Protozoans might have partly decomposed the particles studied. This would decrease the BGE values. Changes in the ratios (viable/total bacteria) which were already reported for marine samples (Van Wambeke & Bianchi 1990), may introduce bias in the calculation of BGE when calculated from abundance. At the beginning of the experiment (within ca 2 to 3 d), BGE ranged from 3 to 31 %, which is similar to published values (Bjornsen 1986, Tranvik 1988: 20 to 30 %, Turley & Lochte 1990: 5 to 80 %, Smith et al. 1995: 9 to 17 %). This study indicates that within the first 3 d, the organic carbon is utilized rapidly but with low efficiency, resulting in low biomass production and high microbial respiration, i.e. high CO_2 production.

Degradation rates of TOC

Multi-stage patterns of organic carbon decomposition have already been observed for POC (Westrich & Berner 1984, Turley & Lochte 1990, Harvey et al. 1995, Harvey & Macko 1997), for DOC (Kirchman et al. 1991, Amon & Benner 1994, Cherrier et al. 1996, Chen & Wangersky 1996) and for TOC in marine sediments (Rabouille et al. 1998). In our study, first-order TOC decay constants of G_{01} range from 2.16 to 6.13 d^{-1} whereas those of G_{02} range from 0.0074 to 0.31 d^{-1} (Table 3). As we approximated the salp decomposition by a 1G-model, there was only 1 rate (k_{TOC} = 1.60 d^{-1}), the G_{NR} being very small (20 % of the initial TOC).

The rates we found in this study were generally higher to those found for marine DOC in numerous studies. Previously reported degradation rates show values ranging from 0.038 to 0.50 d^{-1} for the first days of DOC degradation in seawater and ranging from 0.001 to 0.048 d^{-1} for the following days (Kirchman et al. 1991, Amon & Benner 1994, Cherrier et al. 1996, Chen & Wangersky 1996). However, it is difficult to compare the previous figures with our own data, since they were not calculated by a 2G-model. In the 2G-model organic carbon is treated as having multiple components with different decay constants acting together during the incubation time. Similarly, the degradation rates reported for phytoplankton-C during 3 mo incubations ranged from 0.035 to 0.041 d^{-1} (Harvey et al. 1995). However, these authors calculated the rates as the slopes of the logarithm of concentrations against time; therefore we cannot compare our data obtained from the 2G-model. However, if our rates were calculated in a similar manner to Harvey et al. (1995) they would range from 0.089 to 0.75 d^{-1} for the first days and from 0.002 to 0.06 d^{-1} thereafter, indicating a general agreement with the literature. Although Westrich & Berner (1984) used a 2G-model, their results were obtained for a much larger period of incubation time (ca 2 yr) and therefore it is difficult to compare their data with our own. Basically, comparison of labile fractions and degradation rates is difficult to establish without considering incubation times and methods of calculation (Sempéré et al. 1997).

Interestingly, we found that our k_2 (Table 3) were close to k_1 2G-model coefficients (0.038 and 0.045 d^{-1}) determined by Rabouille et al. (1998) for organic

carbon in marine sediments. Because organic material deposited on the sediment is likely to be partly degraded, the less labile fraction of surface-water column particles may correspond roughly to the labile fraction found in the sediment. We found that during the second stages only a small quantity (2 to 17%) of TOC was slowly mineralized (k ranged from 0.002 to 0.016 d^{-1}), which might be due to the exhaustion of the labile organic carbon. However, another explanation might be that our static system (bottles) became limiting in either the diversity of the microbial consortia or in an individual nutrient (Harvey et al. 1995). This would lead to an underestimation of the degradation rates of the less labile and refractory fractions. However, it should be noted that although the sampling area was different, the less labile part of ISP-50 m particles exhibits degradation characteristics (k_2 = 0.016; G_{02} = 17%) close to the labile fraction of the ISP-200 m particles (k_1 = 0.089 d^{-1}; G_{01} = 16%), giving confidence to our experimental protocol for short time (days) incubation experiments.

POC flux in the water column at the Dyfamed Stn 2

The Dyfamed Stn 2 is far enough from the Ligurian current to be considered representative of biogeochemical processes occurring vertically in the water column in the northwestern Mediterranean Sea (Marty et al. 1994). We are aware that only 1 result taken from experimental work is not enough to extend and explain annual POC flux at this station. However, in a first approach we compared our results (Dyfamed Stn 2) taken from experimental work and subsequent modeling to sediment trap results. At the Dyfamed Stn 2 at 200 m, and assuming a settling velocity of 100 m d^{-1}, it would take ca 8 d for sinking particles to reach 1000 m depth. According to the 2G-model and the parameters (k_1, k_2; G_{01}, G_{02}, G_{NR}) obtained for trap particles, 53% of the 200 m sinking POC would remain as POC after a microbial decomposition of 8 d. This number is higher than that taken from sediment trap studies, which indicate a POC flux at 1000 m comprising 35% of that measured at 200 m (Miquel et al. 1994). However, it is important to note that POC obtained from our experimental work and 2G-model, includes sinking POC but also suspended POC. As most of the suspended POC is not collected by sediment traps, the difference between the 2 estimates might be due to the release of suspended POC from sinking POC, which accumulates between 200 and 1000 m. Note that loss of POC by mesozooplankton, nekton grazers (Lampitt et al. 1993) and protozoans (Patterson et al. 1993) are not taken into account in the 2G-model and likely contribute to decrease the POC flux. Additionally, confinement effect in our biodegradation experiments could lead to nutrient limitation which is absent during the settling of particles in the water column. This may explain some discrepancies between experimental work and *in situ* observations based on sediment traps.

Although this model may probably provide acceptable estimates of microbial contribution to the particle decomposition such as CO_2, DOC production and bacterial carbon demand, an accurate model of the fates of large particles along the water column needs to be constrained by additional parameters such as suspended POC and DOC releases from sinking POC.

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