## METHODS

### Study area and sample collection

Samples were collected at approximately monthly intervals at the DYFAMED station located in the central part of the northwestern Mediterranean Sea (43°25' N, 7°52' E; Fig.1) from January 1999 to January 2000. Water was sampled between 12:00 and 14:00 GMT. Sampling was carried out at 5 depths for measurement of bacterial processes (5, depth of chlorophyll maximum (DCM), 50, 90 and 130 m) using 12-liter Niskin bottles mounted on a rosette coupled to a Seabird CTD system (SBE 9). Samples were also collected at 20 m depth when the DCM was close to 50 m.

### Determination of dissolved organic carbon concentrations

Samples for DOC analysis were collected in combusted (450°C for 4-5 h) glass ampoules, flame-sealed immediately after collection and stored frozen (-18°C) until analysis. The samples were not filtered as the POC content of the samples is low at this site (< 3.6 $\mu mol\ l^{-1}$ C, Copin-Montégut & Copin-Montégut 1983) and should not, therefore, contribute significantly to the DOC values. The DOC analysis was performed using high temperature combustion on a Shimadzu TOC-5000 total organic carbon analyser. A four-point calibration curve (range: 0 to 200 $\mu mol\ l^{-1}$ C) was constructed for each measurement day using potassium phthalate standards prepared fresh in UV-treated Milli-Q water. The instrument blank was assessed using two external standards (Certified Reference Materials, Hansell Laboratory, Bermuda Biological Station). It was between 10 and 12 $\mu mol\ l^{-1}$ C for all samples and was subtracted from the measurements. All DOC concentrations reported are the average of three injections from each sample.

### Bacterial biomass and production

Bacterial abundance was measured by a direct count method using epifluorescent microscopy and DAPI-stained samples (Porter & Feig, 1980). Samples (20 ml) were preserved with 0.2 µm pore-size filtered borax-buffered formalin (2% final concentration) and stored refrigerated in the dark. With the exception of two instances, when we had technical problems, slides were prepared within 24 hrs of sampling. For each slide, 5 ml of sample were stained with 0.2 µm filtered DAPI solution for 10 min and filtered onto a black 0.2 µm pore size Nuclepore membrane filter. Filters were mounted on glass slides with non-fluorescent immersion oil and stored frozen until enumeration. Samples were analyzed in duplicate and at least 10 randomly selected microscope fields on each filter were counted using a Zeiss microscope (magnification 1250x). Bacterial biomass was calculated from the number of bacteria and a carbon content per cell of 15 fg C $cell^{-1}$ (Caron et al, 1995).

Heterotrophic bacterial production was estimated from the rate of protein synthesis determined by the incorporation of $^{3}$H-leucine into trichloroacetic acid (TCA) insoluble macromolecular material (Kirchman et al. 1985). Three 20 ml replicates from each depth were spiked with 20 nM (2 nM $^{3}$H-leucine, specific activity 150 Ci $mmol^{-1}$ and 18 nM cold leucine mix) and were incubated in the dark at *in situ* temperatures (±1.5°C) for 2 h. Preliminary experiments confirmed that leucine incorporation is linear during this period. One of the replicates, to which formalin had been added (2 % final concentration) served as a control. The live incubations were terminated with formalin and all samples were filtered onto 0.2 µm, 25 mm diameter nitrocellulose filters. Samples were then extracted with 5 % trichloroacetic acid (TCA) for 10 min followed by five 3 ml rinses with 5 % TCA. The filters were placed in scintillation vials and 20 ml of Filter Count scintillation cocktail (Packard) was added. Radioactivity was counted with a Beckman LS 1800 counter and the counting efficiency was corrected for quenching. Concentration kinetic experiments showed that isotopic dilution was negligible when using 20 nM leucine in our study. Rates of bacterial production were then

calculated from leucine incorporation rates using the conversion factor of 1.5 kg C per mole leucine (Kirchman 1993). Daily BP were calculated as 24 times the hourly rates.

**Community and bacterial respiration**

Water samples were distributed into 60 ml BOD bottles (overflowing > 60 ml) as soon as the Niskin bottles arrived on deck. Three bottles were immediately fixed with Winkler reagents and a further 8 bottles were incubated in darkness at *in situ* temperature (±1.5°C). A second set of 11 BOD bottles (3 initials and 8 for incubations) were filled with filtered seawater (0.8µm, low vacuum pressure) from each depth, processed and incubated as described above. Two or 3 BOD bottles were fixed with Winkler reagents after approximately 30 and 48 h. At each timepoint, one bottle was processed, as described above, for subsequent determination of bacterial abundance.

Oxygen concentration was titrated with automated Winkler titration technique using a potentiometric end-point detection (Anderson et al. 1992) with an Orion redox electrode (9778-SC) and a custom built titrator. Reagents and standardizations were otherwise similar to those described by JGOFS (1996). The rate of respiration was determined by regressing $O_2$ against time for all three timepoints (0, 30 and 48h). We used a respiratory quotient of 1 and consider that respiration rates of non filtered water correspond to community respiration (CR) and respiration of filtered water (0.8 µm) correspond to bacterial respiration (BR).