Vol. 183: 49–57, 1999 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published July 6

Effects of hydrostatic pressure on microbial activity through a 2000 m deep water column in the NW Mediterranean Sea

Olivier Tholosan, Jean Garcin, Armand Bianchi*

Microbiologie Marine, CNRS - INSU, EP 2032, Université de la Méditerranée, Faculté des Sciences de Luminy, case 907, F-13288 Marseille, cedex 9, France

ABSTRACT: Twenty-seven samples of undecompressed seawater were collected between 800 and 2000 m depth in the NW Mediterranean. Most of the samples (24) were collected at the same sampling station. Potential ^{14}C-glucose uptake rates and potential bacterial production rates were measured concomitantly under natural pressure conditions and under atmospheric pressure conditions. Compression of 5 surficial water samples suggested that metabolic activity of surface-borne bacteria would decrease when carried down by particle sedimentation or by a cascading seawater mass. Conversely, during the stratified water period, intermediate and deep-sea bacteria appeared adapted to the natural pressure conditions; for 90% of the samples metabolic rates were higher in samples kept in their natural pressure conditions than in their decompressed counterparts. Even if variable with time and depth, a global estimation of this adaptation to the natural pressure conditions resulted in a 3.5-fold increase relative to the measures done on decompressed samples. The pressure effect appeared at 800 m depth, a relatively shallow depth comparative to the average depth of the global ocean. In deep waters, potential glucose uptake rate and potential bacterial production rate varied greatly as a function of time and depth, ranging from 2 to 80 pmol C metabolised l^{-1} h^{-1}, and from 4 to over 400 pmol bacterial C produced l^{-1} h^{-1}, respectively.

KEY WORDS: Deep-sea bacteria · Bacterial activity · Pressure-retaining sampler · Pressure effect

INTRODUCTION

In the oceanic system, the productive area is limited to the superficial photic zone and most of the organic material produced there is recycled in the upper layers of the ocean. Nevertheless, a part of this organic production is exported out of the superficial layers and its remineralization depends on the microbial activities in the whole water column and at the water-sediment boundary layer (Deming 1985, Hoppe et al. 1993, Poremba 1994, Turley & Mackie 1994, Turley et al. 1995, Tholosan & Bianchi 1998). As depth increases in the water column, bacteria are submitted to increasing hydrostatic pressure conditions, decreasing temperature and decreasing nutrient concentrations. These conditions are known to slow down the growth rate of bacteria (Morita 1986, Turley 1993). Basically, out of the superficial productive layers, the metabolic velocities of microbial populations are found to be low (Deming 1985, Deming & Colwell 1985, Cowen 1989, De Angelis et al. 1991, Bianchi & Garcin 1993, 1994, Bianchi et al. 1998).

With reference to ETS (electron transport system) measurements done by Lefèvre et al. (1996) in the north-western Mediterranean, oxygen consumption by microplankton in intermediate and deep waters appears to be in the range of a few hundred pmol O_2 respired l^{-1} h^{-1}. Similarly, ^{14}C-glucose remineralization rates in intermediate and deep waters in the NE Atlantic are in the range of a few pmol CO_2 l^{-1} h^{-1}, or lower (Bianchi et al. 1998). However, depth integration of microbial activities measured through the water column shows that the potential remineralization flow

*Addressee for correspondence.
E-mail: a-bianchi@luminy.univ-mrs.fr

due to microbial populations located deeper than 200 m is far from negligible: in the north-western Mediterranean, 75% of the metabolic CO_2 is produced between 200 and 1000 m depth (Lefèvre et al. 1996), and in an oligotrophic area of the NE Atlantic 46% of the carbon dioxide potentially produced by glucose oxidation is due to intermediate and deep microbial populations (Bianchi et al. 1998). Therefore, to appreciate the actual role of the microbial compartment in the functioning of the global oceans, an accurate determination of the microbial activities is needed not only in the surficial productive layer, but throughout the whole water column, including the deep water masses.

In previous studies it has been shown that changes in hydrostatic pressure conditions during retrieval affected the glucose uptake rate of bacterial populations collected at 1100 m depth in the north-western Mediterranean (Bianchi & Garcin 1993, 1994). These studies, like all others already realized by using specific gears to collect undecompressed deep-sea water samples (Jannasch et al. 1976, Tabor et al. 1981), referred to a small number of samples. To our knowledge, the largest data set already reported in the literature in this domain (Jannasch & Wirsen 1982) concerns 16 deep-sea samples collected at 8 different sampling areas covering a large geographical zone in the north-western Atlantic. Thus, microbial activity measurements in deep-sea conditions suffer poor statistical significance. The main objectives of this study were to improve the representativeness of our data set by repetitively sampling from different depths at the same sampling station during different periods of the year, in order to estimate the extent to which microbial processed fluxes through the intermediate and deep-water masses vary throughout the year, and to ultimately determine the depth at which pressure effects on microbial activities begin.

MATERIAL AND METHODS

Sampling area and sampling period. All the samples were collected in the north-western Mediterranean Sea. Five surface water samples were collected at 20 m depth using a Niskin sampler. Two samples were collected in the coastal area near the city of Nice, 2 samples were collected 28 miles (45.05 km) south-east of Nice (43° 21' N, 07° 52' E), and 1 sample 30 miles (48.27 km) south-east of the city of Marseille (42° 57' N, 05° 26' E).

Twenty-four samples of intermediate and deep waters were collected between 800 and 2000 m deep at the DYFAMED sampling station (43° 21' N, 07° 52' E), 28 miles (45.05 km) south-east of Nice. Sampling was performed using a high-pressure sampler (see below) during 5 cruises (October 1994, February 1995, April 1995, November-December 1995, May 1996). Additionally, 3 undecompressed samples were collected between 900 and 1150 m depth in November 1994 in the Gulf of Marseille (42° 56' N, 05° 12' E).

Measurements of ^{14}C-glucose uptake rates in surface water samples. The effects of a downwelling process on superficial microbial populations were simulated by submitting superficial water samples to increased hydrostatic pressure conditions. Samples were transferred to 5 l sterile polycarbonate flasks and immediately labeled with a solution of D-(U-^{14}C)-glucose, specific activity 10.6 GBq mmol^{-1} (Amersham Corp.), to obtain a final concentration of 30.0 nM. After mixing, subsamples were distributed in sterile stainless steel syringes (150 ml) to be pressurized to 11 MPa (~1 MPa min^{-1}), or incubated under atmospheric pressure, at *in situ* temperature (±1°C). Incubation was conducted in strictly identical vessels and under the same culture conditions, except for pressure. Subsamples were taken at time zero (control), 30, 60 and 90 min. At the end of each incubation period, subsamples were fixed by the addition of formalin saturated with sodium tetraborate (1% final concentration, v/v). Bacterial cells were collected by vacuum filtration onto 0.2 µm polycarbonate filters (Nuclepore) prewashed with an unlabeled glucose solution. Filters were washed twice with 5 ml of 0.2 µm filtered seawater. The filtrate was acidified (1 ml HCl, 6 N), and released ^{14}C CO_2 was flushed using N_2 blowing (100 ml min^{-1}) for 30 min. CO_2 was trapped in 2 serial scintillation vials each containing 9 ml of a cocktail of ethanolamine/methanol/PCS (Amersham Corp.) scintillation liquid (ratio 1/1/7, v/v). Filters and vials containing the ^{14}C CO_2 were counted using a Packard 1600 TR liquid scintillation counter. The counting efficiency and quenching correction were determined by use of internal standards. Data were corrected against those obtained from control samples fixed immediately after label addition to determine ^{14}C-glucose uptake rates (sum of incorporated and respired label).

Sampling without decompression. To avoid the physiological stress due to decompression during retrieval of deep-sea water samples, we developed a 5 l high-pressure sampler (HPS, Metro Mesures, 92140 Clamart, France) able to maintain the natural pressure conditions during retrieval and incubation (Fig. 1). This equipment allows for high-pressure growth experiments to be performed in a time course fashion using a single undivided bacteriological culture, as preconceived by Taylor & Jannasch (1976). Furthermore, thanks to its large working volume, this gear allows the estimation of pressure effects on microbial activity using the same sample to measure metabolic rates under natural or atmospheric pressure conditions.

The HPS is a 20 mm thick 316 stainless steel cylinder with a 5 mm thick PEEK® coat. Its diameter is 190 mm (150 mm i.d.), and total length 750 mm. The screwed top and bottom-end are covered with a sheet of PEEK®. The PEEK® floating piston (total length = 180 mm) is fitted with 3 O-rings. The top end is fitted with a closing valve (Whitey SS-83KF4, Whitey Co., Highland Heights, OH, USA) connected to the filling circuit (1/8" stainless steel tubes). This circuit includes an externally adjustable relief valve (Nupro R3A, Nupro Co., Willoughby, OH, USA) previously calibrated at the pressure of the sampling depth, a check valve (SS-CHS2-1 Compact Series, Nupro) and an aero-hydraulic pressure accumulator (gas volume: 500 ml, maximal pressure delivery: 25 MPa, Metro Mesures). Its role is to avoid partial decompression of the sample due to a small change in volume of the stainless steel cylinder during upcast. A FGP 201-12 pressure sensor (FGP, Les Clayes-sous-Bois, France) is fitted on the filling circuit. All the tube fittings are from Swagelok Quick-Connect Co., (Hudson, OH, USA).

The bottom-end screwed cap of the 5 l HPS is connected via a 1/8 stainless steel tube and a Union Tee connection 1/8" Swagelok to a 6000 ml exhaust tank (Fig. 1). This circuit includes a closing valve (Whitey SS-83KF4, Whitey Co.). The exhaust tank is a 316 stainless steel tube (674 mm total length, 140 mm o.d., 110 mm i.d.) fitted with a bleed valve. Inlet into the exhaust tank is through a nozzle (0.010" i.d., stainless steel capillary, Touzart et Matignon, Les Ulis, Courtabeuf, France).

Sterilization: The piston of the HPS is pushed up, the lower chamber of the syringe is filled up with 0.2 µm filtered distilled water, and all the connecting tubes are accurately filled up with filtered seawater to avoid air bubbles. The HPS, with the attached tubes and valves, is autoclaved for 1 h at 110°C. Processing in this manner permits the sterilization of all the parts that will be directly in contact with the sample. To avoid contamination of the filling orifice by surface water bacteria the entry of the inlet valve, previously filled with filtered sterilized seawater, is capped with an alcohol sterilized Parafilm sheet.

Labeling: Just before immersion, the Swagelok connector connecting the filling circuit to the sampler's head is disconnected (Fig. 1). A volume of seawater equal to the tracer's volume to be injected is aseptically withdrawn (using a sterile syringe) from the dead volume of the sampler head. The tracer is injected in this volume and then the Swagelok connector is re-connected. Processing in this way provides a satisfactory distribution of the label into the whole volume of the sample.

Immersion and filling: The HPS is immersed at a rate of 1 m s^{-1}. When the selected sampling depth is reached, natural hydrostatic pressure causes the previously calibrated entry valve (Nupro R3A) to open, pushing down the piston and seawater to enter into the upper chamber of the HPS. Distilled water is flushed out of the lower chamber of the cylinder, through a nozzle, to the exhaust tank. The diameter of this nozzle determines the rate of flushing and keeps the sample undecompressed during the filling period (Jannasch et al. 1973).

Recovery onboard the research vessel: All the valves (Fig. 1) are immediately closed and the bottom end of the HPS is connected, by way of a 1/8" stainless steel tube fitted with a check valve, to a 5.5 l high-pressure reservoir (HPR, Metro Mesures). The HPR is filled with distilled water and pressurized to sample conditions by way of a nitrogen cylinder. The pressure sen-

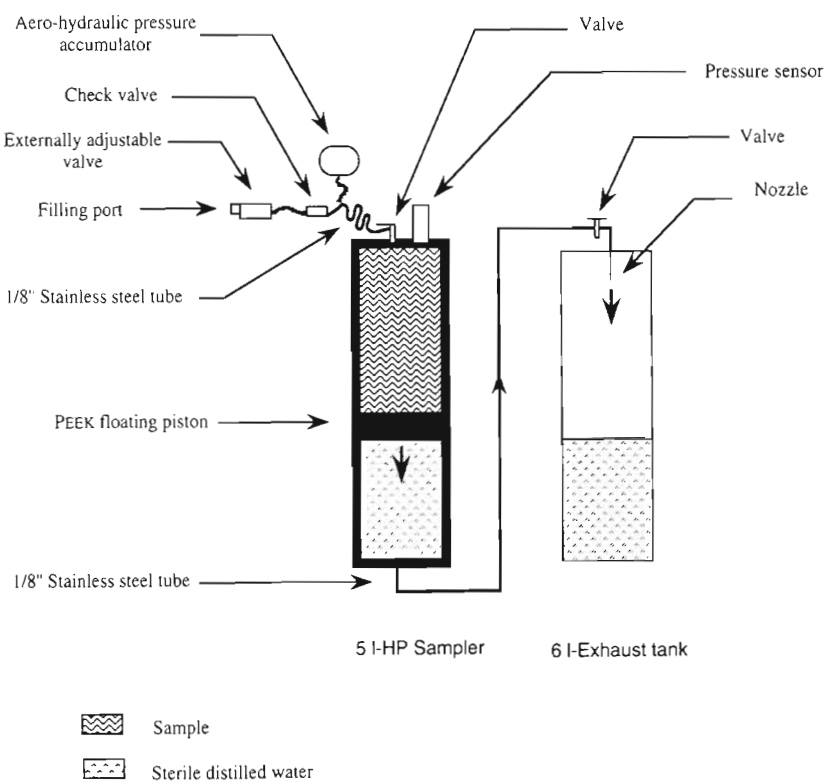

Fig. 1. Diagrammatic representation of the INSU-PACAC 5 l pressure-retaining sampler in configuration of sampling

sor is connected to a pressure meter allowing the control of the absence of leaks on the hydraulic circuit. Then, the HPS is immersed in a water bath kept at the sampling temperature ± 1°C. To subsample without decompression of the culture chamber, the filling circuit (externally adjustable valve, check valve and pressure accumulator) is disconnected and replaced by a PEEK® lamination pin valve (Metro Mesures). This valve provides a low and accurately regulated outflow rate minimizing the shear forces in the culture.

To withdraw a part of the sample without decompression of the bulk culture, the valve between the HPS and HPR is opened, then a slight increase in pressure (i.e. 0.6 MPa) in the HPR slowly pushes up the floating piston and allows the withdrawal of a part of the sample through the lamination pin valve at a rate of ~2 ml s^{-1}. For each sample a 2 l decompressed subsample was immediately withdrawn in this way to be incubated in the dark at the sampling temperature under atmospheric pressure conditions.

Precautions need to be taken when working with high-pressure equipment in order to prevent any leaks on the hydraulic circuit. Before each immersion it is necessary to carefully check all junctions. It is also recommended to frequently change the O-rings to avoid pressure loss. During sampling the aero-hydraulic pressure accumulator (gas volume: 500 ml, maximal pressure delivery: 35 MPa, Metro Mesures) avoids partial decompression of the sample due to a small change in volume of the stainless steel cylinder during its retrieval aboard the research vessel. During incubation and subsampling, pressure variations are controlled by the HPR connected to the bottom end of the HPS. Finally, it is obvious that pressure variations must be permanently checked by the pressure meter throughout the experiment. Processing in such a way allows the minimization of pressure variations in the culture chamber at ±10% of the initial pressure conditions.

Measurements of ^{14}C-glucose and ^{14}C-amino acid potential uptake rates. Samples were labeled using D-(U-^{14}C)-glucose (specific activity 10.6 GBq $mmol^{-1}$, Amersham Corp.), or L-(U-^{14}C)-amino acids mixture (specific activity 9.25 GBq, Amersham Corp.), for a final concentration of 10 nM. Previous studies showed that this concentration was above the saturation concentration of the glucose uptake systems for intermediate and deep Mediterranean waters.

Incubation was conducted at 13 ± 1°C (ambient temperature of intermediate and deep Mediterranean waters) for up to 12 h. During time course experiments 250 ml subsamples were withdrawn from both undecompressed and decompressed cultures after 1, 3, 6 and 12 h. Subsampling from the HPS was processed without decompression of the bulk culture. In order to stop the microbial activity as soon as the subsamples were decompressed, a volume of buffered formaldehyde (calculated for a final concentration of 1%) was previously added to the subsampling vials. Subsamples were then treated as described above for surface water samples.

Bacterial secondary production. This parameter was measured during time course experiments following the same protocol as described above. The labeled tracer was ^{3}H-thymidine, specific activity 3.07 TBq $mmol\ l^{-1}$ (Amersham Corp.), for a final concentration of 5 nMol. To calculate the bacterial production we used the empirical conversion factors of 2×10^{18} cells mol^{-1} (Ducklow & Carlson 1992) and 10 fg C $cell^{-1}$ (Bell 1993).

Bacterial counts. Subsamples for bacterial counts were collected immediately after retrieval of the HPS, following the protocol already described for the withdrawl of the decompressed 2 l subsample. They were immediately fixed in 2% formalin and stored for a few days at 4°C before staining with diamidinophenylindole (Porter & Feig 1980). Cells were enumerated by epifluorescence microscopy (Olympus, BH2).

All the samples were processed on board the research vessel immediately after retrieval.

RESULTS

Effects of increasing pressure on surface water bacteria

Table 1 shows that the potential bacterial glucose incorporation rate drastically decreases when surface water samples are submitted to increased pressure conditions. The activity measured in samples pressurized up to 11 MPa was only 32% of the rate obtained

Table 1. ^{14}C-glucose incorporation rate (pmol C l^{-1} h^{-1}, mean ± SD) of superficial water samples incubated under atmospheric pressure conditions and under increased hydrostatic pressure conditions (11 MPa). Incubation temperature: 13 ± 1°C (natural temperature)

Sampling area (20 m depth)	Atmospheric pressure (A)	Pressurized 11 MPa (HP)	Pressure effect (HP/A)
Nice coastal area	3350 ± 317 (n = 4)	1966 ± 37	0.59
Nice coastal area	2421 ± 74 (n = 4)	1329 ± 94	0.55
28 miles off Nice	199 ± 37 (n = 4)	43 ± 9	0.22
28 miles off Nice[a]	174 ± 24 (n = 6)	30 ± 4	0.17
30 miles off Marseille[a]	251 ± 35 (n = 10)	22 ± 1	0.09

[a]Data from Bianchi & Garcin (1994)

Table 2. Short-term variations of ^{14}C-glucose uptake rates (pmol C l^{-1} h^{-1}) measured on decompressed (DEC) and undecompressed (HP) seawater samples collected at a few days interval time at 1000, 1500 and 2000 m depth at the same sampling station (43° 21' N, 07° 52' E) in the Ligurian Sea. Values are mean ± SE (n = 3). Bacterial densities (×10^4 cells ml^{-1}) refer to subsamples collected immediately after retrieval of the high-pressure sampler

Depth (m)	Sampling date	Bacterial density (×10^4 cells ml^{-1})	Glucose uptake (pmol C l^{-1} h^{-1}) HP	DEC
1000	Oct 10	15.6	63.2 ± 2.7	53.3 ± 8.7
	Oct 13	10.9	15.9 ± 4.5	16.0 ± 2.0
	Variation	−30%	−75%	−70%
1500	Oct 09	23.2	8.4 ± 0.5	4.8 ± 0.6
	Oct 12	20.5	33.6 ± 2.1	18.9 ± 0.3
	Variation	−12%	+300%	+294%
2000	Oct 08	11.0	6.6 ± 0.6	3.4 ± 0.5
	Oct 11	12.5	37.9 ± 4.5	37.3 ± 4.3
	Variation	+14%	+474%	+1000%

Table 3. Effect of decompression on ^{14}C-glucose (or occasionally ^{14}C-amino acids) uptake rate: ratio of undecompressed to decompressed bacterial uptake rates. Values for November 1995 are ^{14}C-amino acid uptake rate. All the samples were collected in the Ligurian Sea (43° 21' N, 07° 52' E), except November 1994 samples, which were collected in the Gulf of Marseille (42° 56' N, 05° 12' E)

Depth (m)	Oct 1994	Feb 1995	Apr 1995	Nov 1995	Nov 1994	May 1996
800			5.50	0.94	11.20	
1000	0.99	1.48	1.53			
	1.25					
1150			1.97	2.15	5.20	
				0.99	8.00	
1500	1.75	1.25	2.08	2.07		
	1.80					
2000	1.94	1.51	28.12	1.29		2.50
	1.00					

from their counterparts kept under atmospheric pressure conditions (average value for 5 samples), suggesting a high fraction of surficial bacterial population are unadapted to increased pressure conditions.

Short-term variations of metabolic rates

Table 2 shows the variations of potential glucose uptake rates in intermediate and deep water samples during short time periods. Bacterial density varied up to 30% within a 3 d interval, whereas the uptake rate varied more drastically, with a possible increase of 1 order of magnitude within the same sampling interval.

Table 4. Effect of decompression on bacterial production rate: ratio of undecompressed (HP) to decompressed (DEC) bacterial biomass production rates defined by measuring ^{3}H-thymidine incorporation. Data refer to the same samples as those used for glucose uptake measurements (Table 3), except for 3 additional samples (*)

Depth (m)	Feb 1995	Apr 1995	Nov 1995	May 1996
800	2.76*		1.17	
1000	12.86	2.12		1.22
	1.49*			
1150			1.57	
			1.55*	
			0.89	
1500	2.26	2.30	1.56	
2000		1.74	1.61	17.08

Seasonal variations of microbial activities

Glucose (or occasionally amino acid mixture) uptake rates and bacterial production rates measured during different periods of the year are presented in Figs. 2 & 3, respectively. In most cases, uptake rates and bacterial production rates measured in undecompressed samples were higher than in their decompressed counterparts. The pressure effect (Pe = HP/DEC, where HP and DEC are the metabolic rates measured in the undecompressed and decompressed samples, respectively) describes the adaptation of bacterial consortia to their natural pressure conditions (Tables 3 & 4). Only 1 sample, collected at 1150 m depth during the November 1995 cruise, showed a negative effect of natural pressure conditions on microbial activity measurements (Pe = 0.89 and 0.99 for bacterial production and amino acid uptake rates, respectively), while 3 other samples (collected during October 1994 and November 1995) appeared unaffected by pressure conditions (Pe ≈ 1). An overall estimation of Pe shows that the 2 measured metabolic rates were nearly 3.5-fold higher in the undecompressed samples than in their decompressed counterparts: Pe = 3.72 ± 1.17, n = 24, and Pe = 3.48 ± 1.22, n = 15, for carbon uptake and bacterial production rates, respectively.

DISCUSSION

Behavior of surface water bacteria carried down in the water column

When surface water samples are submitted to increased hydrostatic pressure conditions, glucose incorporation rates drastically decrease (Table 1). Our experiment confirms previous observations (Oppenheimer & ZoBell 1952, Seki & Robinson 1969, Turley

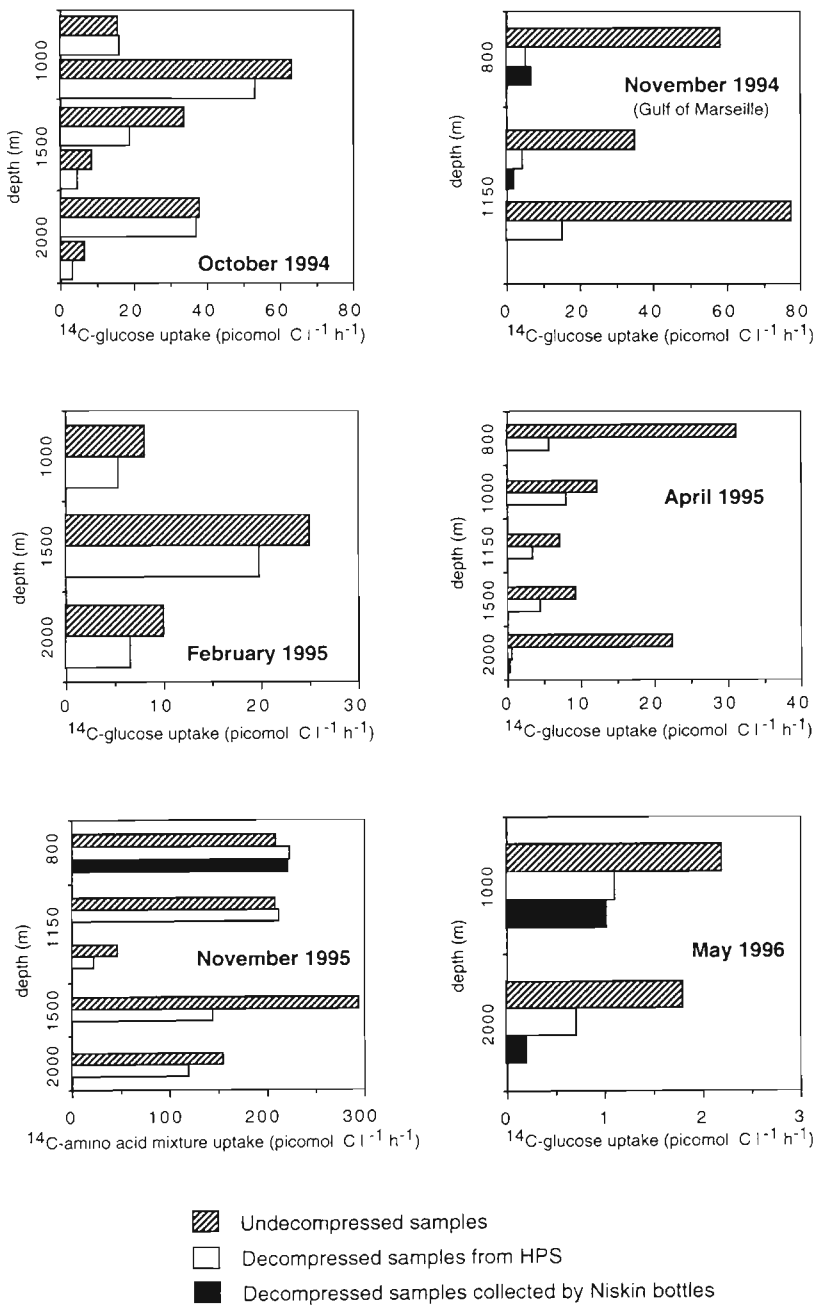

Fig. 2. Comparison of ^{14}C-glucose (or occasionally ^{14}C-amino acids) uptake rates in decompressed and undecompressed samples collected at different depths at diverse times of the year at the same sampling station in the Ligurian Sea, and additional data from the Gulf of Marseille

1993, Bianchi & Garcin 1994) showing that surface living bacteria are not adapted to high-pressure conditions.

These experimental data can be related to 2 processes naturally occurring in the ocean. The first one corresponds to downwelling or cascading processes carrying down superficial water masses, while the second refers to the settling of surface-produced particles. Detritic particles produced in the superficial layers of a marine system are rapidly colonized by highly diversified bacterial populations (Alldredge & Silver 1988, DeLong et al. 1993) possibly endowed with enhanced per cell activities (Kirchman & Mitchell 1982, Iriberri et al. 1987). When surficial water masses or surficial particles sink down, their environmental conditions, such as temperature and hydrostatic pressure, are progressively modified. If the metabolic rates of surface-borne bacteria drastically slow down with increasing pressure conditions, we could hypothesize that during the downwelling process or surface particle sedimentation, the primary colonizers, becoming progressively uncompetitive, are replaced by other bacterial species more efficiently adapted to increased pressure conditions (Poremba 1994, Turley et al. 1995). Consequently, the degradation of particulate organic materials composing the settling particles, and the remineralisation of dissolved organic compounds constituting the dissolved organic bulk of cascading watermasses, should depend on the rapidity and efficiency of colonization by pressure-adapted bacteria. Further studies need to be done to estimate the changes in bacterial diversity and to understand the physiological adaptation of natural microbial consortia to increasing pressure conditions when naturally carried down through the whole oceanic water column.

Adaptation of marine bacteria to their natural pressure conditions

Of the 27 samples of intermediate and deep waters we studied, 23 showed a decrease in their potential metabolic activities when kept out of their natural hydrostatic pressure conditions (Figs. 2 & 3). Globally, the slowing down of metabolic activities due to decompression is nearly a 3.5-fold decrease for the uptake rate as well as for the bacterial production rate. This decompression effect is in the same range as that already observed by Bianchi &

Garcin (1994) on the glucose incorporation by bacterial populations collected at 1100 m depth in the NW Mediterranean Sea, and by Cowen (1989) on the binding of Mn^{2+} by natural populations of bacteria in a deep-sea hydrothermal plume at 2000 m depth.

As determined by CTD casts (data not shown), all samples were collected during the water column stratification period. The observed decrease in bacterial activities due to decompression confirms previous observations (Deming 1985, Deming & Colwell 1985, Bianchi & Garcin 1993, 1994) that bacteria settling in intermediate and deep water masses are well adapted to the natural pressure conditions. This adaptation of marine bacteria to high-pressure conditions has been considered as a hallmark of their origin from deep-sea environments (Yayanos et al. 1982). These observations confirm the underestimation of microbial activity data measured, during the stratified water period, on deep water samples decompressed during retrieval.

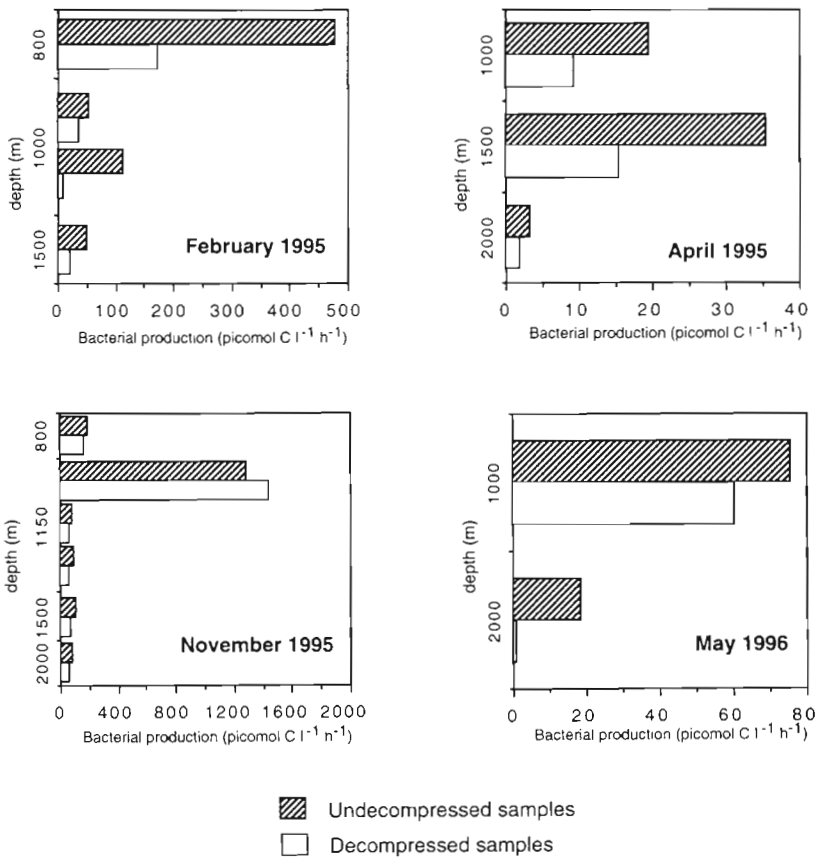

Fig. 3. Comparison of bacterial production (estimated by ^{3}H-thymidine incorporation) in decompressed and undecompressed samples collected at different depths at diverse times of the year at the same sampling station in the Ligurian Sea

Depth-integrated potential glucose uptake (determined by using the uptake rates which we measured in natural pressure conditions, Fig. 2) throughout the 800 to 2000 m depth layer during the April 1995 cruise amounted to 17.2 µmol C m^{-2} h^{-1}. This value is nearly equivalent to the flux (15.6 µmol C m^{-2} h^{-1}) defined by a similar integration of data obtained by Iriberri et al. (1996) in the 0 to 200 m depth layer at the same sampling station during the same cruise. In fact, theoretically (since the uptake rates were measured by adding labeled glucose at saturating concentration), nearly 25% of the glucose potentially used by bacteria in the whole 2000 m depth water column could be due to intermediate and deep water bacteria. This observation confirmed the importance of bacterial populations in intermediate and deep waters in the cycling of organic mater in the global oceans (Turley & Mackie 1994, Lefèvre et al. 1996, Bianchi et al. 1998).

Fluctuations of bacterial metabolic rates in intermediate and deep waters

In the superficial layers of the ocean, bacterial activities vary greatly throughout the year (Carney & Colwell 1976, Albright & McCrae 1987). These fluctuations appear associated to the seasonal cycling of nutrients in the productive layers (Fuhrman et al. 1980, Ducklow 1984, Ducklow et al. 1993, Hoppe et al. 1993). In intermediate and deep water masses, variability of environmental parameters should be considerably softened. Thus, Copin-Montégut & Avril (1993), who sampled at the same station as we did, reported that below 200 m depth dissolved organic carbon concentration remained rather constant (0.6 to 0.7 mg C l^{-1}) over a 2 yr period. Therefore, it could be expected that the metabolic activities of bacterial populations could be only slightly affected throughout the year. However, bacterial activities appear far from constant in deep water masses (Figs. 2 & 3). Since heterotrophic processes in the deep sea are fuelled by the organic nutrients exported from the sea surface, these variations could be linked to the particle flow through the water column. We cannot ascertain this discrepancy from the seasonal cycling because, as shown in Table 2, even at a very short time scale we observed the same

temporal variability: the measured glucose uptake rate may vary as much as 6-fold over a 3 d period. Such short-term variability in microbial activities of deep water masses has already been observed in the north-eastern Atlantic (Bianchi et al. 1998). Indeed, microbial activities processing the geochemical cycling of nutrients in intermediate and deep water masses appear as variable as in the surface productive layers. Such variability may be due to the patchy distribution of nutrients in oligotrophic conditions (Azam & Ammerman 1983); it may also be due to the characteristic time and space scales of coupling between nutrient sources and bacterial production on one hand, and between bacterial production and grazing on the other hand (Ducklow 1984). Evolution of microbial activities is governed by short- and long-term dynamic processes (Karl & Knauer 1984), not only in the surficial productive layers, but throughout the whole water column. Therefore, it is necessary to considerably enhance the sampling frequency, as suggested by Ducklow (1983), even in the deep water masses.

At what depth to bacterial populations adapt to deep-sea conditions?

Hydrostatic pressure, the main physical characteristic of deep environments, increases linearly with depth. Thus, it is difficult to locate the boundary between superficial and deep environments. Presently, most of the studies concerning the effect of decompression during retrieval on microbial activity measurements refer to samples collected deeper than 1000 m (Jannasch et al. 1976, Tabor et al. 1981, Jannasch & Wirsen 1982). As shown by the ratio of metabolic rates measured in undecompressed and decompressed conditions, adaptation to pressure conditions appears clearly in samples collected from 800 m depth (Tables 3 & 4). Since we did not sample from shallower depths, we have no indication of a possible pressure effect higher in the water column. Seki & Robinson (1969), using an *in situ* approach, described a pressure shock due to decompression on seawater samples collected at 400 m depth. Considering the average and extreme depths of the global oceans, 3800 and 11 000 m respectively, physiological adaptation of bacterial populations to natural pressure conditions appears at a relatively shallow depth. Nevertheless, at least in this study, the pressure effect on microbial activity does not correlate to increasing depth. This result confirms the high variability of the pressure effect on bacterial activity regulation in deep water masses, probably due to the temporal changes of the specific diversity of deep-sea bacterial populations.

Conclusion

In conclusion, this study confirms the importance of hydrostatic pressure on the regulation of metabolic activities of marine microbial populations. Metabolic activity of superficial bacteria drastically decreases with increasing pressure conditions. Therefore, when carried down by particle sedimentation or by a cascading seawater mass, superficial bacteria intervene very poorly in the geochemical cycles. Deep-sea bacteria, on the other hand, appear well adapted to their natural pressure conditions as confirmed by our results. During the stratified water column period this adaptation appears at nearly 800 m depth, a relatively shallow depth considering the average depth of the global oceans. This observation suggests the use of undecompressed samples for microbial activity measurements in intermediate and deep waters, in order to avoid an underestimation of the bacterial processes responsible for the nutrient cycling in the ocean. Sampling repetitively in the same area shows that bacterial densities and bacterial activities are far from constant throughout the year, even at 2000 m depth. Due to the patchy distribution of nutrients and bacteria through the water column, there is an interest for long-term and high-frequency sampling in the intermediate and deep water masses in order to determine the actual role of microbial processes in the flux of matter in the ocean.

Acknowledgements. This research has been undertaken in the framework of the Programme Flux Océaniques DYFAMED and the Mediterranean Targeted Project (MTP)–European Microbiology of Particulate Systems (EMPS) project. We acknowledge the support from the European Commission's Marine Science and Technology (MAST) Programme under contract MAS2-CT94-0090. The HPS was partially funded by the Institut National des Sciences de l'Univers and the Conseil Régional de la Région Provence Alpes Côte d'Azur Corse. The authors are grateful to J. C. Peyrus (CEA, DERS-SERE, Cadarache) and Metzo Mesure (Clamart) for assistance in high-pressure processing, and to Thalia Polychronaky for improving the English in the manuscript. Special thanks to the captain and crew of the RV 'Tethys II' for cruise assistance.

LITERATURE CITED

Albright LJ, McCrae SK (1987) Annual bacterioplankton biomasses and productivities in a temperate West Coast Canadian Fjord. Appl Environ Microbiol 55:1277–1285

Alldredge AL, Silver MW (1988) Characteristics, dynamics, and significance of marine snow. Prog Oceanogr 20: 41–82

Azam F, Ammerman JW (1983) Cycling of organic matter by bacterioplankton in pelagic marine ecosystems: microenvironmental considerations. In: Fasham MJR (ed) Flows of energy and materials in marine ecosystems. Theory and practice. Plenum Press, New York, p 345–360

Bell RT (1993) Estimating production of heterotrophic bacteri-

oplankton via incorporation of triated thymidine. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. CRC Press, Boca Raton, p 495–503

Bianchi A, Garcin J (1993) In stratified waters the metabolic rate of deep-sea bacteria decreases with decompression. Deep-Sea Res 40:1703–1710

Bianchi A, Garcin J (1994) Bacterial response to hydrostatic pressure in seawater samples collected in mixed-water and stratified-water conditions. Mar Ecol Prog Ser 111: 137–141

Bianchi A, Van Wambeke F, Garcin J (1998) Bacterial utilization of glucose in the water column from eutrophic to oligotrophic pelagic areas in the eastern North Atlantic Ocean. J Mar Syst 14:45–55

Carney JF, Colwell RR (1976) Heterotrophic utilization of glucose and glutamate in an estuary: effect of season and nutrient load. Appl Environ Microbiol 31:227–233

Copin-Montégut G, Avril B (1993) Vertical distribution and temporal variation on dissolved organic carbon in the North-Western Mediterranean sea. Deep-Sea Res 40: 1963–1972

Cowen JP (1989) Positive pressure effect on manganese binding by bacteria in deep-sea hydrothermal plume. Appl Environ Microbiol 55:764–766

De Angelis M, Baross JA, Lilley MD (1991) Enhanced microbial oxidation in water from a deep-sea hydrothermal vent field at stimulated in situ hydrostatic pressures. Limnol Oceanogr 36:565–570

DeLong EF, Franks DG, Alldredge AL (1993) Phylogenic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol Oceanogr 38:924–934

Deming JW (1985) Bacterial growth in deep-sea sediment trap and boxcore samples. Mar Ecol Prog Ser 25: 305–312

Deming JW, Colwell RR (1985) Observations of barophilic microbial activity in samples of sediment and intercepted particulates from the Demerara abyssal plain. Appl Environ Microbiol 50:1002–1006

Ducklow HW (1983) Production and fate of bacteria in the oceans. BioScience 33:494–501

Ducklow HW (1984) Geographical ecology of marine bacteria: bacteriological and physical variability at the mesoscale. In: Klug MJ, Reddy CA (eds) Current perspective in microbial ecology. American Society for Microbiology, Washington, DC, p 22–32

Ducklow HW, Carlson CA (1992) Oceanic bacterial production. In: Marshall KC (ed) Advances in microbial ecology, Vol 7. Plenum Press, New York, p 113–181

Ducklow HW, Kirchman DL, Quinby HL, Carlson CA, Dam HG (1993) Stocks and dynamics of bacterioplankton carbon during the spring bloom in the eastern North Atlantic Ocean. Deep-Sea Res 40:245–263

Fuhrman JA, Ammerman JW, Azam F (1980) Bacterioplankton in the coastal euphotic zone: distribution, activity and possible relationship with phytoplankton. Mar Biol 60: 201–207

Hoppe HG, Ducklow H, Karrasch B (1993) Evidence for dependency of bacterial growth on enzymatic hydrolysis of particulate organic matter in the mesopelagic ocean. Mar Ecol Prog Ser 93:277–283

Iriberri J, Unanue M, Barcina I, Egea L (1987) Seasonal variation in population density and heterotrophic activity of attached and free-living bacteria in coastal waters. Appl Environ Microbiol 55:2308–2314

Iriberri J, Unanue M, Ayo B (1996) Heterotrophic processes associated with the degradation of particulate material in Mediterranean Sea Waters. In: Bianchi M (ed) European microbiology of particulate systems. Final Report MAST II Programme, EC, Vol 2, contr no. 4. EC, Marseille, p 1–41

Jannasch HW, Wirsen CO (1982) Microbial activities in undecompressed microbial populations from the deep seawater samples. Appl Environ Microbiol 43:1116–1124

Jannasch HW, Wirsen CO, Winget CL (1973) A bacteriological pressure-retaining deep-sea sampler and culture vessel. Deep-Sea Res 20:661–664

Jannasch HW, Wirsen CO, Taylor CD (1976) Undecompressed microbial populations from the deep sea. Appl Environ Microbiol 32:360–367

Karl DM, Knauer GA (1984) Vertical distribution, transport, and exchange of carbon in the northeast Pacific Ocean: evidence for multiple zones of biological activity. Deep-Sea Res 31:221–243

Kirchman D, Mitchell R (1982) Contribution of particle-bound bacteria to total microheterotrophic activity in five ponds and two marshes. Appl Environ Microbiol 43:200–209

Lefèvre D, Denis M, Lambert CE, Miquel JC (1996) Is DOC the main source of organic matter remineralization in the ocean water column? J Mar Syst 7:281–291

Morita RY (1986) Pressure as an extreme environment. In: Herbert RA, Codd GA (eds) Microbes in extreme environments. Academic Press, London, p 171–185

Oppenheimer CH, ZoBell CE (1952) The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. J Mar Res 11:10–18

Poremba K (1994) Impact of pressure on bacterial activity in water columns situated at the European continental margin. Neth J Sea Res 33:29–35

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948

Seki H, Robinson DG (1969) Effect of decompression on activity of microorganism in sea water. Int Rev Ges Hydrobiol 54:201–205

Tabor PS, Deming JW, Ohwada K, Davis H, Waxman M, Colwell RR (1981) A pressure-retaining deep ocean sampler and transfer system for measurement of microbial activity in the deep sea. Microb Ecol 7:51–65

Taylor CD, Jannasch HJ (1976) Subsampling technique for measuring growth of bacterial cultures under high hydrostatic pressure. Appl Environ Microbiol 32:355–359

Tholosan O, Bianchi A (1998) Bacterial distribution and activity at the water-sediment boundary layer on NW Mediterranean continental margin. Mar Ecol Prog Ser 168:273–283

Turley CM (1993) The effect of pressure on leucine and thymidine incorporation by free-living bacteria and by bacteria attached to sinking oceanic particles. Deep-Sea Res 40:2193–2206

Turley CM, Mackie PJ (1994) Biogeochemical significance of attached and free-living bacteria and the flux of particles in the NE Atlantic Ocean. Mar Ecol Prog Ser 115:191–203

Turley CM, Lochte K, Lampitt RS (1995) Transformations of biogenic particles during sedimentation in the northeastern Atlantic. Phil Trans R Soc Lond B 348:179–189

Yayanos AA, Dietz AS, Van Boxtel RR (1982) Dependence of reproduction rate on pressure as a hallmark of deep-sea bacteria. Appl Environ Microbiol 44:1356–1361

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: September 7, 1998; Accepted: March 2, 1999
Proofs received from author(s): June 18, 1999