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Abstract

A High Frequency Flux (HFF) experiment was conducted during spring 1997 on the continental slope of the Gulf of Lions (Northwestern Mediterranean Sea) with the aim of examining the dynamical and biological processes controlling particle transfer in this margin environment. Within this general framework, a special attention was paid to short temporal and small spatial variations of phytoplankton and bacterial production through six hydrological and biological surveys performed during a 7-week period at nine sampling stations located on a 10 × 20-mile grid. Downward fluxes of particulate organic carbon at each station were measured by traps deployed at 240 m depth. The *f*-ratio and the ratio of integrated bacterial to primary production (IBP/IPP ratio), computed as indexes of biological export for each survey and station, did not provide a clear, unambiguous understanding of the importance of biological processes in the cycling of carbon in the upper water column. However, the data collected allowed to draw up carbon budgets for the different phases of the experiment. The comparison of primary production with measured and estimated organic carbon removal terms (sinking, cycling through the microbial food web, grazing by ciliates and metazoans) showed that a balance was never reached between fluxes of production and removal of organic carbon during the course of the experiment. The system shifted from an initial situation of 'missing' carbon (removal > production) to one of 'excess' carbon (removal < production). Factors such as horizontal advection of carbon into and out of the experimental area and accumulation of dissolved organic carbon (dissolved biological pump) are invoked to explain the observed imbalances. A sensitivity test of the budget to the variations of the different parameters involved showed that bacterial growth efficiency was the most important factor affecting the budget.

Keywords: Northwestern Mediterranean; Phytoplankton export; Bacterial production; Short term variability; Particulate detritus; Organic carbon flux; Carbon budget

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1. Introduction

The Gulf of Lions is a key area of the Western Mediterranean basin since it receives the Rhône river discharge, the most important riverine input to the Mediterranean Sea in terms of liquid (Martin and Saliot, 1992) and solid (Margat, 1992) discharges. The major physical factors affecting this region are the northwestern winds, which strongly influence coastal waters and the fate of the Rhône plume, and the Northern Mediterranean Current (NMC) and its associated front, which flows southwesterly along the Gulf of Lions slope (Conan and Millot, 1995). Such physical forcing significantly influences biological activity. It is thus important to obtain information on the temporal and spatial variability of the biological processes to test and run models for this area (Tusseau-Vuillemin et al., 1998; Diaz et al., unpublished results) or to validate budgets (Durrieu de Madron et al., 2000; Sempéré et al., 2000a). However, work performed so far on the biological activity in the Gulf of Lions concerned either snapshot studies (Yoro et al., 1997) and surveys at a scale of a few days (Christaki et al., 1996; Van Wambeke et al., 1996), or surveys with monthly to bimonthly sampling (Conan, 1996); this left aside, so far, intermediate terms at the scale of a few days to 1–2 weeks.

To fill this gap, a multidisciplinary study—the High Frequency Flux (HFF) experiment—was conducted in the Gulf of Lions in spring 1997, within the framework of the European 'MTP II-MATER program (Mass Transfer and Ecosystem Response)' and the French 'Programme National d'Océanographie Côtière (PNOC)'. HFF has been designed to examine various processes controlling short-term particle transfer on continental margins, from the sources to the final deposit. In particular, one of the general objectives assigned to HFF was to check the importance of transient hydrodynamic features and changes in the biology of the upper water column on the supply of particles to the continental slope. The work presented here aims at discussing the importance of some of the biological processes that could have been involved in that supply.

Studies simultaneously focusing on phytoplankton and bacteria remain scarce. However, it is crucial to examine the coupling/uncoupling of primary and heterotrophic productions to determine the fate of organic carbon. Indeed, the fate of spring blooms is influenced by the nature of the links between phytoplankton and the microbial food web. Links are established via the dissolved organic matter (DOM), whose characteristics determine the time lag between the peak of phytoplankton and the peak of bacterial abundance. The time lag between the two peaks is determined by the biodegradability of DOM, the combined effects of grazing pressure, the physical constraints that allow or not accumulation of suspended detritus in the euphotic zone, and, also, by the availability of N and P stochiometrically to C. For example, it has been recently shown that in summer, during water mass stratification, there is an accumulation of dissolved organic carbon (DOC) (Copin-Montégut and Avril, 1993), which has been attributed to phosphorus limitation (Thingstad and Rassoulzadegan, 1995). The more primary production enters the microheterotrophic food chain, the more the newly synthesised organic carbon will be mineralised or released as DOC. The converse is also true, indicating that the less primary production enters the microbial food chain, the more it is expected to reach higher trophic levels or bottom sediments via detritus settling. This tendency has been suggested on a large spatial scale in the Mediterranean (Bianchi et al., 1996; Conan et al., 1999; Turley et al., 2000).

The ratio of bacterial production to primary production is often used as an index of the flux of carbon channelled from phytoplankton to the microbial food web (Bianchi et al., 1996; Yoro et al., 1997; Pedros-Alio et al., 1999; Turley et al., 2000). Here we examine the variation of this ratio and its relation to *f*-ratios and to downward carbon fluxes below the euphotic zone. We also draw up a tentative carbon budget within the area to examine the importance of allochthonous detritus for bacterial carbon resources and DOC accumulation.

2. Materials and methods

2.1. Study site, sampling strategy

The HFF experimental site was located on the continental slope of the Gulf of Lions in the Northwestern Mediterranean basin (Fig. 1). Water-column processes were investigated in 1997 through a series

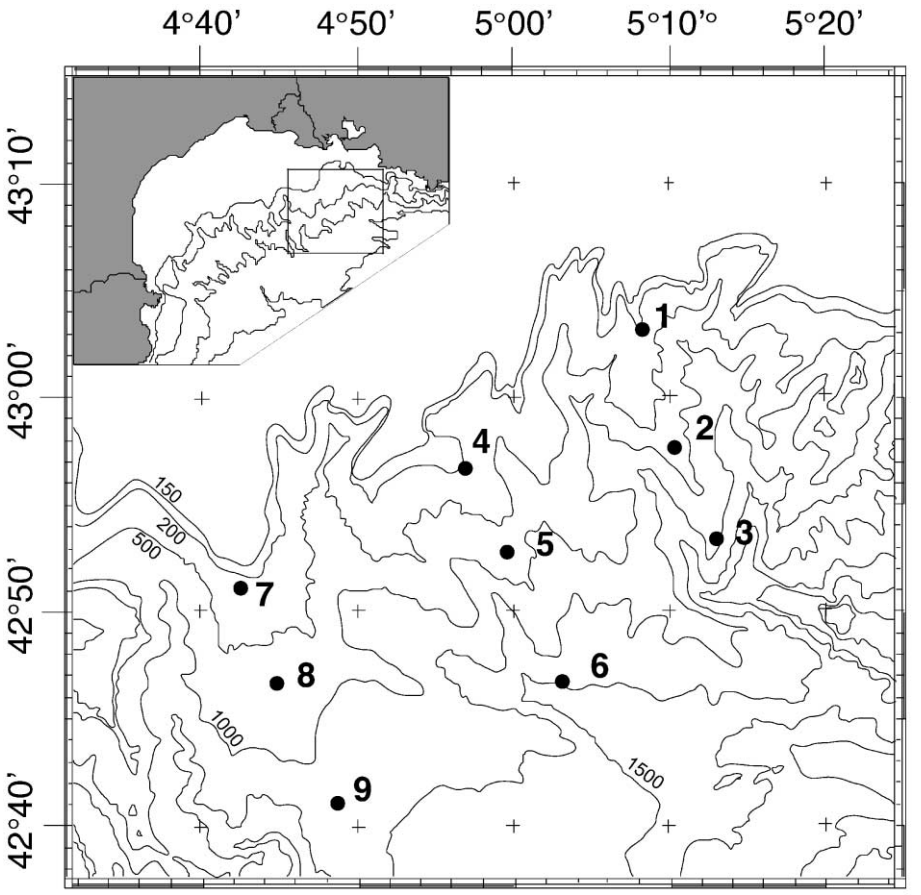


Fig. 1. Bathymetric chart of the HFF experimental area.

of six 1-day field cruises: HFF-1 (16 March), HFF-2 (23 March), HFF-3 (7 April), HFF-4 (14 April), HFF-5 (23 April) and HFF-6 (2 May). The site comprised, within an area of 10×20 miles, nine stations along three transects located at and beyond the shelf break in water depths of 300 to 1600 m. The station grid (Table 1) was systematically sampled over a <30-h period. Due to bad weather conditions, only the vertical profile of station 1 was sampled during HFF-1, as well as surface layers (5 m depth) at stations 4, 5 and 6. For the other cruises, all stations were covered.

Water samples were collected at standard depths (5, 10, 20, 40, 60, 80, 100, 165 m), using a CTD-rosette system with 8-l Niskin bottles. The base of the euphotic layer (taken as 1% of surface PAR) was located at around 60 m depth (Diaz et al., 2000). Temperature and salinity measurements were performed with a conductivity–temperature–depth–oxygen profiling system (Seabird, SBE 911+).

Table 1
Location and bottom depth of the nine sampling stations of the HFF experiment

Station	Latitude	Longitude	Depth (m)
1	43°02N	5°07E	395
2	42°57N	5°10E	800
3	42°51N	5°12E	1650
4	42°55N	4°57E	318
5	42°52N	4°59E	689
6	42°48N	5°02E	1350
7	42°50N	4°44E	329
8	42°46N	4°45E	730
9	42°41N	4°50E	1275

2.2. Nutrients, chlorophyll, primary production

Samples for dissolved inorganic nitrogen (DIN) were preserved with $HgCl_2$ immediately after sampling and were stored at 5 °C until analysis. Analysis of nitrate and nitrite was performed at the laboratory according to the working procedures described in Diaz et al. (2000). Samples for ambient ammonium were collected in glass flasks and the reagents were immediately added to the samples as detailed in Diaz et al. (2000); the ammonium concentrations were then measured manually at the laboratory 24 h later.

Samples for chlorophyll (250 ml) were collected at each depth and immediately filtered onto GF/F filters on board. Chlorophyll concentrations were determined by fluorimetry by using the methanol extraction procedure (Raimbault et al., 1988). Phyto-plankton

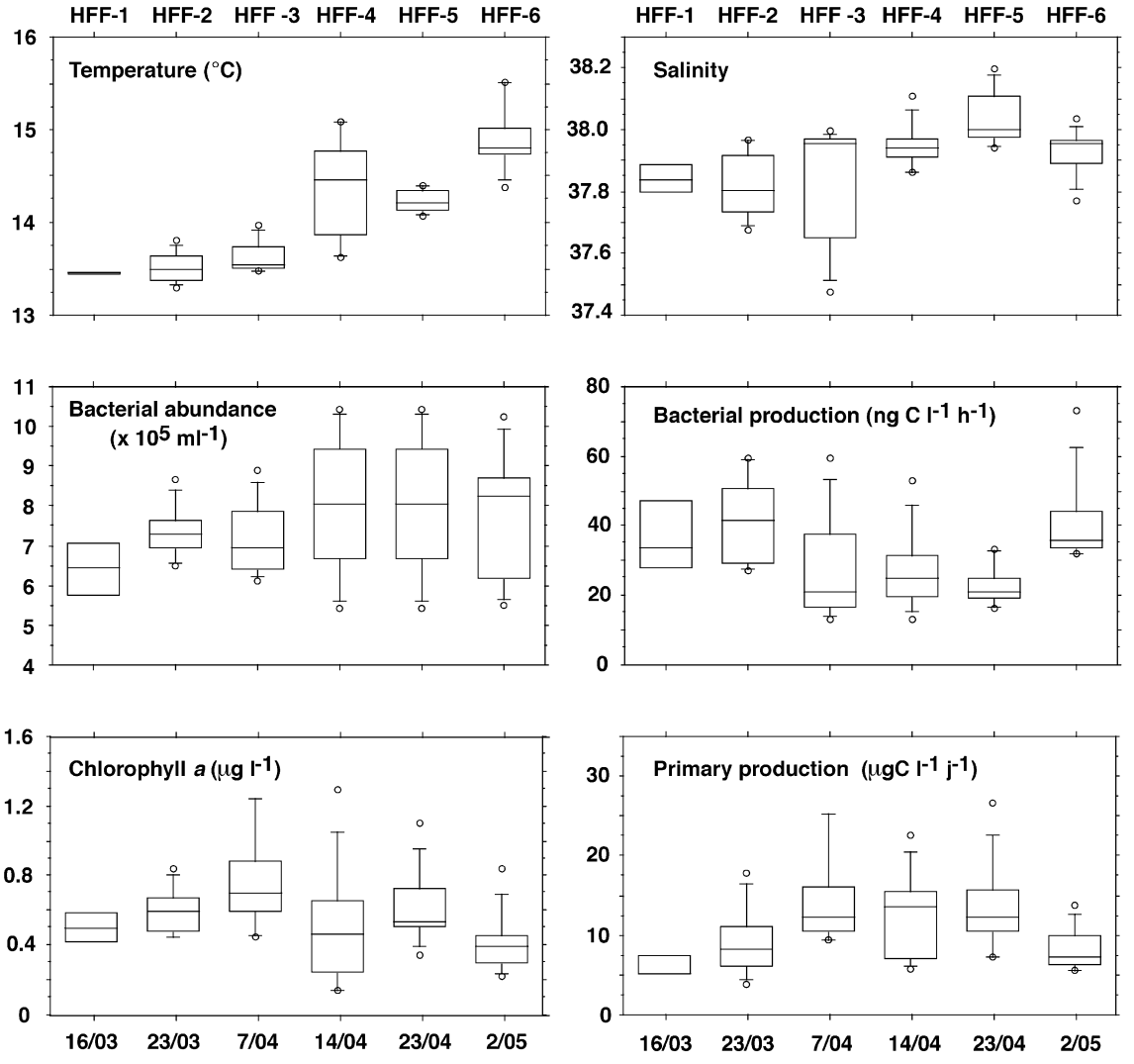


Fig. 2. Distributions of temperature, salinity, bacterial abundance, bacterial production, chlorophyll *a* and primary production at 5 m depth during the six HFF cruises. HFF-1: 16 March, HFF-2: 23 March, HFF-3: 7 April, 4: HFF-4: 14 April, HFF-5: 23 April, HFF-6: 2 May. The statistical distribution of box-plot is used: the horizontal line in each box represents the median. The other two horizontal lines contain 50% of the values between them. Vertical bars indicate the other 50% range of values excluding the outliers (points).

biomass (as carbon) was estimated using the carbon to chlorophyll ratio proposed by Delgado et al. (1992) for the NW Mediterranean basin (Catalano-Balearic province). The average conversion factors used were 32.5 for the upper mixed layer, 18.5 for the upper part of the chlorophyll maximum (when observed) and 12.7 below.

Nitrate (new production) and ammonium (regenerated production) uptake rates were measured with the N-tracer technique (Dugdale and Goering, 1967). N additions were set at 84 and 167 nM for nitrate and ammonium, respectively. The 5-, 20-, 40- and 60-m samples were then incubated under in situ simulated conditions for 24 h from the tracer inoculation. At the end of incubation, samples were poisoned with $HgCl_2$ (20 μg ml^{-1}) to stop biological activity. In the laboratory, samples were filtered onto GF/F Whatman® filters, which were used to determine the final ^{15}N-enrichment in the particulate organic nitrogen (PON) pool. The ^{15}N abundance in the PON pool was determined by using a mass-spectrometric method (see details in Diaz et al., 2000). In addition, the *f*-ratio was defined as the fraction of NO_3^- uptake rate to the total DIN ($NO_3^- + NH_4^+$) uptake rates (Eppley and Peterson, 1979).

Primary production (as carbon) was determined using the ^{14}C-tracer technique (Steeman-Nielsen, 1952). Acid-cleaned 0.25-l polycarbonate bottles (one dark, one light for each depth) were completely filled with the water sample from the 5, 20, 40 and 60 m depths. Each sample was spiked with 20 μCi of the $Na_2H\ ^{14}CO_3$ working solution and incubated under the same conditions as the ^{15}N samples. The light bottle measurement at each depth was corrected for dark uptake. The 24-h particulate primary production was calculated as described in Diaz et al. (2000).

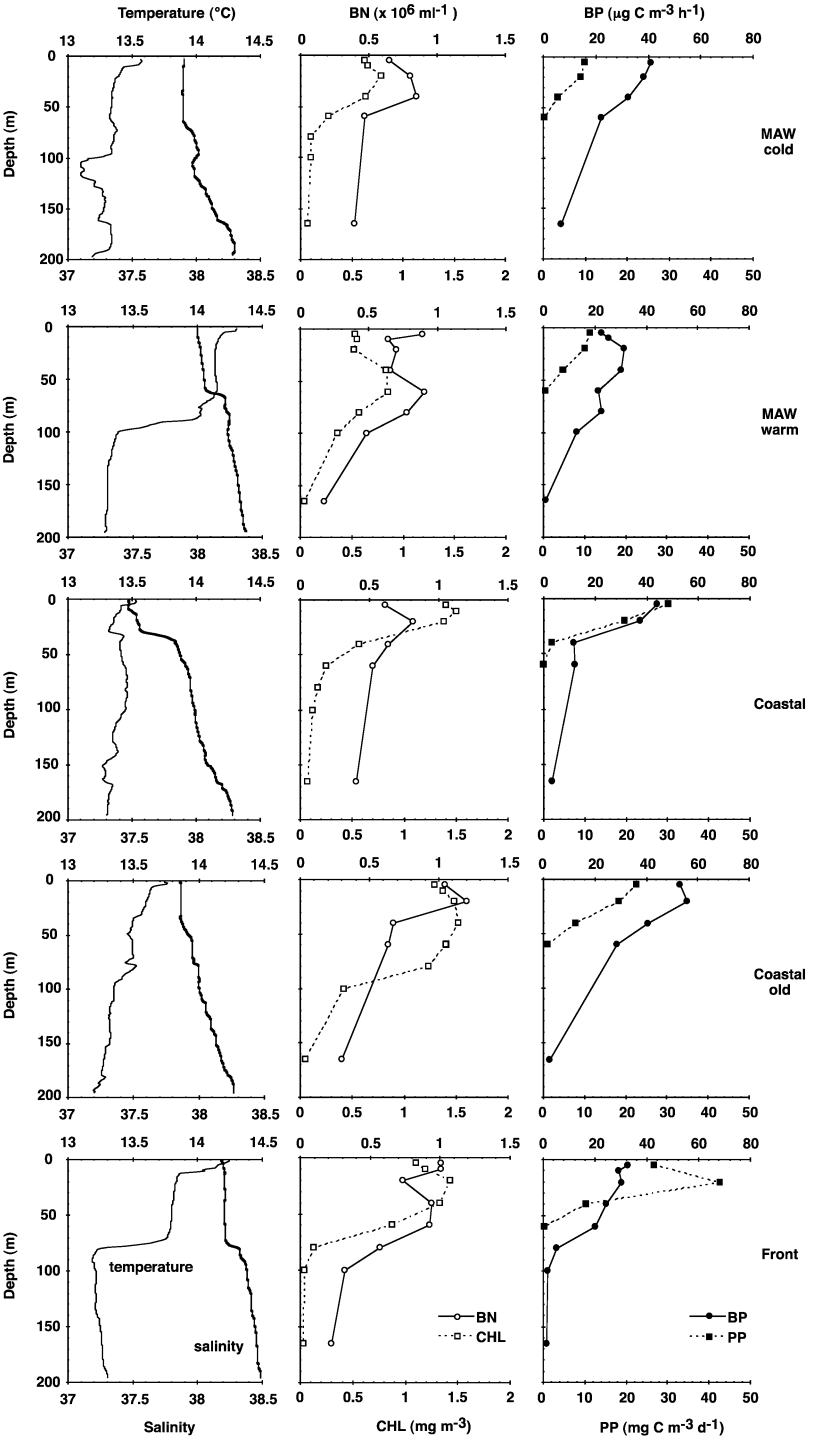
2.3. Bacterial parameters

Subsamples (20 ml) were fixed during the cruise with a 0.2-μm filtered formalin solution (2% final conc.) buffered with sodium tetraborate. Eight to twenty milliliters of the subsamples were filtered onto 0.2-μm dark polycarbonate filters after staining with DAPI (2500 μg l^{-1} final concentration) and stored at −20 °C until counting. Bacterial abundance was

Table 2
Mean ± SD of nitrite (NO_2), nitrate (NO_3), ammonium (NH_4) chlorophyll *a* (CHL), ^{14}C-primary production (PP), bacterial numbers (BN) and bacterial production (BP) in surface waters (5m depth)

Cruises	Stations	type	Surface salinity	NO_2 (nM)	NO_3 (nM)	NH_4 (nM)	CHL (μg l^{-1})	PP (mg C m^{-3} day^{-1})	BN ($\times 10^5$ ml^{-1})	BP (ng C l^{-1} h^{-1})
HFF-1	1, 4, 5, 6	MAW	nd	130 ± 30	690 ± 120	110 ± 30	0.50 ± 0.10	6.2 ± 2.1	6.4 ± 0.8	37 ± 14
HFF-2	1, 3, 4, 6, 8, 9	MAW	37.89 ± 0.08	100 ± 30	480 ± 90	50 ± 30	0.55 ± 0.08	8.5 ± 5.0	7.2 ± 0.5	37 ± 10
	2, 5, 7	CW	37.72 ± 0.05	110 ± 20	640 ± 190	60 ± 30	0.67 ± 0.2	10.4 ± 3.7	7.5 ± 1.0	53 ± 10
HFF-3	1, 2, 3, 6, 9	MAW	37.96 ± 0.01	70 ± 20	490 ± 150	70 ± 30	0.58 ± 0.12	10.4 ± 1.2	7.5 ± 0.9	17 ± 3
	4, 5, 7, 8	CW	37.60 ± 0.11	110 ± 30	670	180 ± 50	1.01 ± 0.28	19.2 ± 7.6	6.7 ± 0.9	43 ± 12
HFF-4	1, 2, 4, 5, 6	MAW	37.95 ± 0.03	bdl	60 ± 20	100 ± 60	0.25 ± 0.14	6.7 ± 0.7	7.2 ± 1.4	22 ± 7
	7, 8, 9	Old CW	37.89 ± 0.04	60 ± 50	150 ± 110	120 ± 50	0.86 ± 0.38	16.9 ± 4.9	9.9 ± 0.6	36 ± 17
	3	Open Sea	38.11	bdl	40	120	0.46	15.0	8.0	25
HFF-5	1, 2, 3, 4, 5, 7, 8	MAW	38.01 ± 0.05	bdl	110 ± 70	40 ± 40	0.53 ± 0.12	11.1 ± 2.9	8.0 ± 1.9	20 ± 2
	6, 9	FZ	38.17	bdl	382 ± 350	50 ± 10	0.92 ± 0.25	21.6 ± 7.1	8.0 ± 1.6	33 ± 1
HFF-6	2, 3, 4, 5, 6, 7, 8	MAW	37.95 ± 0.05	bdl	bdl	70 ± 30	0.35 ± 0.09	7.5 ± 1.9	7.8 ± 1.7	38 ± 6
	1	CW	37.77	30	50	170	0.84	13.7	6.2	73

MAW: Modified Atlantic Water-influenced stations. CW: coastal-influenced stations. FZ: frontal situations. Open Sea: open-sea influenced stations. Bdl: data below detection limit. nd: no data.


Temperature (°C)
BN (x 10^6 ml^-1)
BP (µg C m^-3 h^-1)
Depth (m)
MAW cold
MAW warm
Coastal
Coastal old
Front
temperature
salinity
BN
CHL
BP
PP
Salinity
CHL (mg m^-3)
PP (mg C m^-3 d^-1)

determined by epifluorescence microscopy coupled to an image analysis system (Van Wambeke, 1995). This method does not allow to discriminate prochlorophytes. However, these cells were counted by cytometry on the same samples (MATER data base; Angel Rodriguez, personal communication) and their abundances (500 to 2500 ml^{-1}) were never higher than 0.7% of epifluorescence counts. Bacterial biomass was calculated using the abundance relationship given by Lee and Fuhrman (1987), i.e. 20 fg C per cell.

Heterotrophic bacterial production was measured by ^{3}H-leucine incorporation into proteins (Kirchman, 1993). [4,5-^{3}H]-leucine (specific activity 126 Ci $mmol^{-1}$) and unlabeled leucine were added to 20-ml samples giving final concentrations of 1 and 19 nM, respectively. Samples (two replicates and one formalin-killed blank) were incubated in the dark at in situ surface temperature for 2 h. Optimal protocols were assessed by time and concentration kinetics (Van Wambeke et al., 2000). After incubation, the samples were fixed with formalin (1% final concentration), stored at 4 °C for not more than 36 h and filtered, on return to the laboratory, onto 0.2-μm Millipore GS filters (cellulose ester). Each filter was rinsed three times with 5% TCA, dissolved in 1-ml ethyl acetate and radioassayed in a Packard 1600 scintillation counter. Leucine uptake was converted into bacterial production by a factor of 1.5 kg C per mol of incorporated leucine, assuming a dilution factor of 1 since saturating conditions were respected (Kirchman, 1993; Christaki et al., 1999). Daily bacterial productions were calculated from hourly rates multiplied by 24. Bacterial turnover was calculated by dividing production by biomass, and the mean bacterial turnover through the euphotic zone was obtained by dividing integrated production by integrated biomass.

2.4. Sediment traps

The trap array used during the HFF experiment comprised nine mooring lines deployed at stations 1 to 9 and equipped with cylindroconical sediment traps at different depths (model PPS3 from Technicap, 0.125 m^2 collection area, 12 receiving cups; Heussner et al., 1990), coupled with Aanderaa current meters (for further details on the mooring design, see Flexas et al., 2002). Traps collected samples from 16 March to 2 May, with a sampling interval of 2 days for the first six samples and 6 days for the last six samples. Results presented here pertain only to the shallowest traps that were deployed at the nominal depth of 240 m.

The polyethylene receiving cups of the traps were filled before deployment with a buffered 5% (v/v) formaldehyde solution in 0.45-μm filtered sea water. The poisoning solution was used to minimise degradation of settled particles and to prevent mechanical disruption of "swimmers". Upon recovery, the cups were stored at 2–4 °C in the dark until processing. To facilitate subsampling, large "swimmers" were removed by wet sieving the sample through a 1-mm nylon mesh, and the smaller ones by handpicking under a dissecting microscope. The samples were then precisely divided into subsamples for subsequent analyses, using a high precision wet sample splitting system (Heussner et al., 1990). Total mass fluxes were determined on two to three replicates, depending on availability of material, by filtering the subsamples onto preweighed 0.45 μm Millipore membranes, rinsed with distilled water and dried overnight at 40 °C. The precision of mass estimates (coefficient of variation, CV%) was $<5\%$. Further subsamples were filtered onto Whatman GF/F filters, rinsed with distilled water, and dried at 40 °C. Total carbon was analysed by combustion in a CHN LECO 2000 carbon analyser, and organic carbon was analysed in the same way after acidification with 2 N HCl to remove carbonate. Due to the limited amount of material collected by the traps, no replicates could be obtained for total and organic carbon contents. However, the overall precision of carbon measurements from numerous samples collected in the same

Fig. 3. Vertical profiles of physical and biological parameters measured during HFF under typical situations. Horizontally, MAW cold: MAW-influenced station in March (station 1, HFF-2, 23 March); MAW warm: MAW-influenced station during April (station 3, HFF-5, 23 April); coastal: coastal-influenced station (station 7, HFF3, 7 April); coastal old: coastal-influenced station (station 7, HFF4, 14 April); front: frontal-influenced station (station 9, HFF-5, 23 April). Vertically, left profiles: temperature (light line, upper scale) and salinity (dark line, lower scale); middle profiles: bacterial numbers (BN, open rombs, upper scale) and chlorophyll (CHL, open squares, lower scale); right profiles: bacterial production (BP, dark rombs, upper scale) and primary production (PP, dark squares, lower scale).

region is generally $<2-3\%$ (Heussner, unpublished results).

3. Results

3.1. Hydrological conditions

The main hydrological features prevailing in the HFF area during the early spring period of 1997 have been reported in Diaz et al. (2000). The general trend was a regular warming of the Modified Atlantic Water (MAW) during the study period corresponding to the onset of thermal stratification. However, this trend was occasionally disrupted by the strong northwestern winds, before HFF-2 and HFF-5, as demonstrated by the variability of the surface temperature values among the station grid (Diaz et al., 2000; Fig. 2). The consequence of these northwestern winds was also to push the Coastal Water (CW, under the Rhône river influence) towards the HFF area as evidenced by surface salinity values <37.70 at stations 7 and 8 during HFF-3 (a few days after the northwestern wind gust). On the other hand, the surface salinity values >38.15 observed at station 9 during HFF-5 signed recently upwelled waters associated with the Frontal Zone (FZ) of the NMC. Considering these various hydrological conditions and the TS diagrams presented by Diaz et al. (2000), the sampling stations have been classified not only by survey, but also by the identified influences of the water masses, namely CW, MAW, FZ and open sea waters (Table 2). The biogeochemical variables at 5 m depth have been presented according to this partition.

Due to the predominance of MAW over the study period, the water column was generally nutrient-depleted (e.g. cold situation: station 1, HFF-2; warm situation: station 3, HFF-5) except at several stations under CW influence during HFF-2 and HFF-3 (e.g. station 7), respectively characterised by enrichments in nitrite and nitrate and in ammonium and nitrite (Table 2). During HFF-4, the CW influence was still noticeable, particularly at station 7, but corresponded to an older CW mass because nutrient enrichment was not clearly marked except in nitrate. The FZ conditions that were detected at the offshore stations (e.g. station 9) during HFF-5 clearly showed significant nitrate contents associated with high salinity values up to the surface.

3.2. Bacterial abundance and production, relations with primary producers

Bacterial abundance ranged from 3 to 12×10^5 bacteria ml^{-1} in the 0–100-m layer, with the highest values corresponding to stations under the CW influence (station 5, HFF-2), and under FZ conditions (station 9, HFF-5) (Table 2). Bacterial abundance generally decreased with depth, according to temperature and chlorophyll profiles (Fig. 3).

Bacterial production ranged from 1.4 to 72.9 ng C l^{-1} h^{-1} for the 0–100-m layer and from 0.02 to 9.8 ng C l^{-1} h^{-1} below this layer. During HFF-2, the highest values in surface waters were observed at stations 2 and 5 (58 and 59 ng C l^{-1} h^{-1}, respectively) under the CW influence (salinity <37.70). Profiles of bacterial production, showing a regular decrease with depth without any deep secondary peak, reflected a winter situation (see example of station 1, HFF-2, Fig. 3). Due to the strong northwestern wind gust just before HFF-3, stations 4, 5, 7 and 8 were largely under the CW influence. Bacterial production and chlorophyll biomass were high in the surface layer of stations 7 and 8 (see station 7, Fig. 3). During HFF-4, the onset of stratification was clearly evidenced by the appearance of a DCM around 40–60 m depth and the depletion of nitrate in surface layers (Table 2). During HFF-5, frontal conditions were depicted at station 9 with high primary production rates. Finally, during HFF-6, primary production decreased whereas bacterial production remained at levels around 35–40 ng C l^{-1} h^{-1} down to 60 m depth.

During all cruises, bacterial production and primary production at the CW-influenced stations were always higher than at stations under MAW influence. The higher bacterial production values mostly resulted from an increase in 'bacterial assemblage' turnover. Indeed, during HFF-2, mean bacterial turnover was 0.085 ± 0.012 h^{-1} in CW-influenced stations and 0.064 ± 0.018 h^{-1} in MAW-influenced stations. During HFF-3, the respective means were 0.079 ± 0.026 and 0.027 ± 0.006 h^{-1}. During HFF-4, the increase in BP resulted both from small increases in bacterial population turnover and bacterial biomass (10×10^5 bacteria ml^{-1}). On the contrary, the increase in primary production at the CW-influenced stations was generally combined with an increase in chlorophyll: on

average 1.0 ± 0.2 versus 0.58 ± 0.12 μg l^{-1} in MAW-influenced stations during HFF-3, 0.86 ± 0.38 versus 0.25 ± 0.14 μg l^{-1} during HFF-4 (Table 2). The phytoplankton efficiency was not statistically different for the different hydrological situations, except during the HFF-4 cruise when the highest values of efficiency were observed in the MAW-influenced stations and at station 3 under open-sea influence (mean for MAW-influenced stations: 2.7 ± 1.1 mg C mg chl^{-1} h^{-1}, with a maximum value of 3.7 mg C mg chl^{-1} h^{-1}).

3.3. Integrated standing stocks and fluxes

Spatial and temporal variability of primary production and chlorophyll data was high (CV of 47% and 34%, respectively) and was higher for bacterial production than for bacterial biomass (CV of 49% and 11%, respectively). The POC standing stock integrated over the euphotic zone (0–60 m) varied from 6.6 to 14.3 g C m^{-2}. Bacterial and autotrophic carbon ranged from 0.63 to 1.11 and from 0.42 to 2.82 g C m^{-2}, respectively. The ratio of bacteria to phytoplankton carbon varied between 0.38 and 1.35; around 30% of the ratios were >1 (bacterial carbon exceeding phytoplankton carbon), mostly during HFF-6, when phytoplankton carbon remained greater than bacterial carbon in only one station.

The percentage of bacterial carbon to POC remained relatively homogeneous ($11 \pm 2\%$, $n = 20$), with no significant increase with integrated primary production (IPP, $r^2 = 0.03$, $p > 0.05$). On the contrary, the ratio of autotrophic carbon biomass to POC increased significantly ($r^2 = 0.26$, $p < 0.05$) with IPP from 5% to 22% (Fig. 4). The living POC was assumed to be the sum of autotrophic and bacterial carbon. The ratio of living POC to total POC did not increase significantly with IPP ($r^2 = 0.12$, $p > 0.05$); however, the increase of this ratio was significant for IPP values lower than 450 mg C m^{-2} day^{-1} ($r^2 = 0.49$, $p < 0.05$). The detrital fraction of POC is calculated by the difference between total POC and living POC. Under this assumption, detrital POC ranged from 4.5 to 11.9 g C m^{-2} for the 0–60-m layer and from 11 to 20 g C m^{-2} when integrated over the 0–165-m layer. Detrital POC did not show a detectable increase at stations under coastal influence, neither in terms of absolute values nor in terms of percentage of total POC. The percentage of the detrital fraction represented at least two third of total POC, independently of the level reached by primary production.

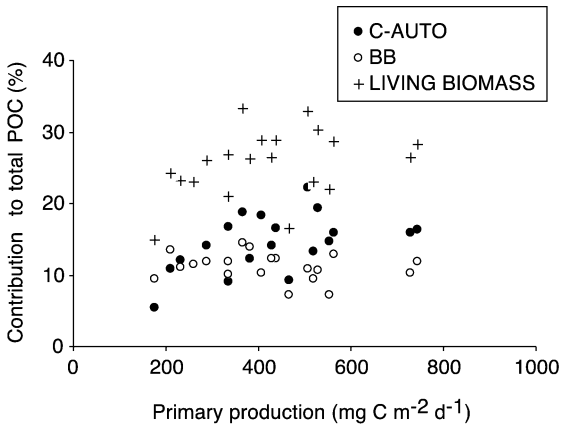


Fig. 4. Relation between bacterial biomass to POC ratio (BB/POC), autotrophic carbon biomass to POC ratio (C-AUTO/POC), living carbon to POC ratio (LIVING BIOMASS/POC) and primary production. All values integrated between 0 and 60 m.

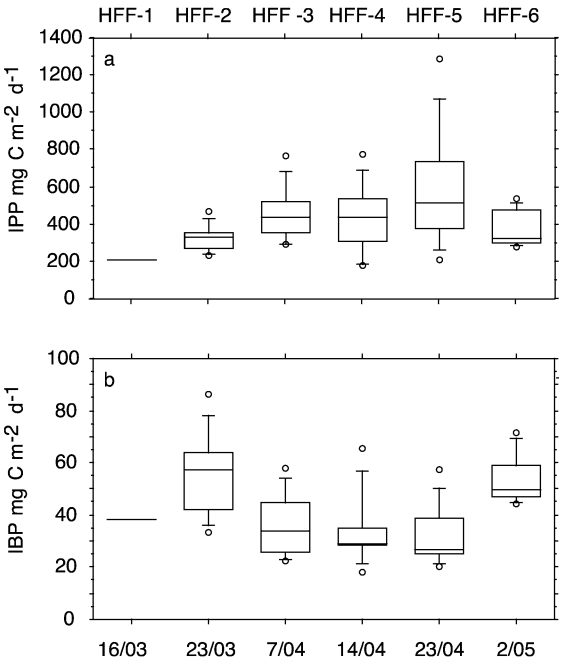


Fig. 5. Box plots of integrated primary production (upper panel) and integrated bacterial production (lower panel) during the HFF cruises. HFF-1: 16 March (station 1 only), HFF-2: 23 March, HFF-3: 7 April, 4: HFF-4: 14 April, HFF-5: 23 April, HFF-6: 2 May.

Integrated primary production (IPP) values ranged from 175 mg C m^{-2} day^{-1} to 1.28 g C m^{-2} day^{-1} from HFF-1 to HFF-5 while during HFF-6 IPP decreased down to values measured during HFF-4. The increase was concomitant with an increased variability among stations. Integrated bacterial production (IBP 0–60 m) ranged from 20 to 86 mg C m^{-2} day^{-1} (Fig. 5).

During HFF-2, a great variability of IBP/IPP ratios was observed, and the ratio ranged from 10% to 27% according to the stations, while IPP varied only by a factor of 2 (Fig. 6). Two weeks later (HFF-3), the variability of IBP/IBP was strongly reduced and the ratio ranged from 4.9% to 10.7%, whereas phytoplankton production was shifted towards slightly higher values (288–763 mg C m^{-2} day^{-1}). During HFF-4 and HFF-5, IPP showed a higher variability than during the previous cruises but IBP/IPP ratios remained low (5–16% and 3–12%, respectively) and varied independently from the IPP level. During HFF-6, the increasing IBP and decreasing IPP led to a greater IBP/IPP ratios than those specially observed during HFF-4 ($15 \pm 3\%$ versus $8 \pm 3\%$).

3.4. Downward fluxes of particulate carbon

The nine sediment traps deployed during the experiment at 240 m depth provided a total of 88 individual samples on which organic carbon could be determined. Organic carbon fluxes were generally low and ranged from 1.2 to 77 mg C m^{-2} day^{-1}, with 2/3 of the individual values below 20 mg C m^{-2} day^{-1} (Fig. 7). For the missing samples that were not analysed for carbon, due to the very low amounts of particles collected, organic carbon fluxes would have been even lower since total mass fluxes were very low, on the order of a few tens of mg m^{-2} day^{-1}. Stations 1, 2, 3 and 7 presented to some varying degrees increased values at the beginning of the experiment. An exceptionally high peak of 138 mg C m^{-2} day^{-1} was measured at station 7 on 24–25 March. The weighted mean concentration of

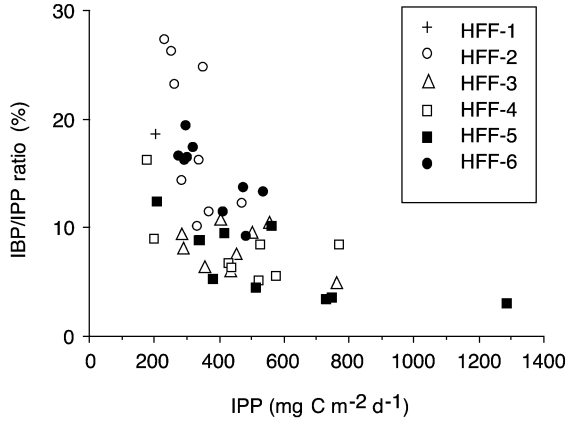


Fig. 6. Evolution of IBP/IPP ratio with primary production, all data included.

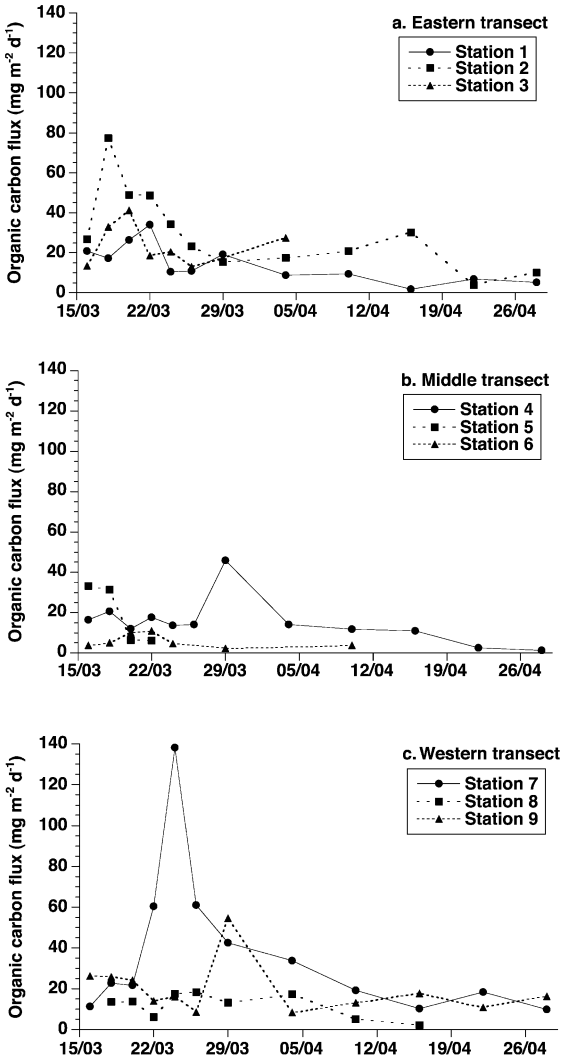


Fig. 7. Time series of organic carbon fluxes measured by the shallow HFF traps deployed at 240 m depth.

organic carbon in the settling particles was 3.7% and the overall time-weighted mean flux was 18 mg C m^{-2} day^{-1}. Variability of organic carbon fluxes was high (CV = 94%), and largely resulted from a limited number of short flux events lasting a few days.

Since the sampling frequency of the traps was much higher than that of primary production (6 × 2 days at the beginning of the experiment, then 6 × 6 days), the comparison of organic C fluxes with integrated primary production was based on time-integrated carbon fluxes in the traps between two consecutive HFF cruises and from means of primary production calculated at the beginning and at the end of the same period. Carbon fluxes varied independently of primary production (Fig. 8a). The ratio of organic C flux to integrated primary production, taken as a rough index of downward export since no corrections have been made to take into account settling delay between the production zone and the trap depth, was highly scattered and ranged from 0.3% to 18%. Despite the missing values that introduced some uncertainty towards the end of the experiment, downward carbon export showed an overall decrease from the beginning to the end of the experiment, with the only noticeable exception of station 2 that was characterised by a secondary peak in carbon flux (Fig. 8b).

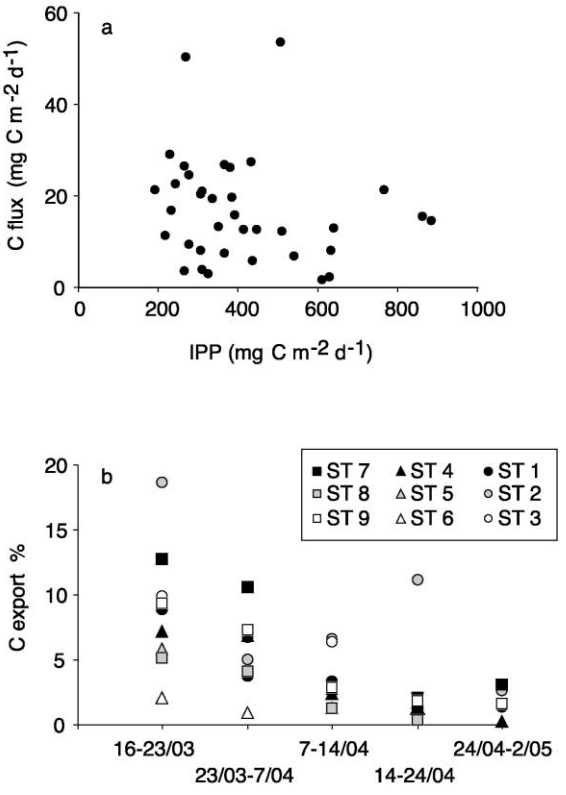


Fig. 8. Relations between trap-measured organic carbon and primary production. (a) Organic carbon flux at 240 m depth versus primary production (time-integrated values between cruises, see text). (b) Temporal evolution of carbon export expressed as the ratio of organic carbon flux at 240 m depth to primary production (%).

4. Discussion

4.1. Relations between bacteria and phytoplankton

At the scale of our study (a 7-week duration and a spatial grid of 10 × 20 miles), the large instability of the Northwestern Mediterranean Current and the propagation of its meandering along the slope described by Flexas et al. (2002) undoubtedly superimposed their effects on the temporal evolution of the bloom. For example, station 9, the southwesternmost station of the study area, was under the influence of MAW during HFF-2, -3 and -6, of old CW during HFF-4, and of FZ conditions during HFF-5. In addition, the northern winds allowed intrusion of CW which influenced both phytoplankton (by lateral input of nitrate) and bacteria (by lateral input of allochthonous sources of organic matter). In this context, integrated bacterial and phytoplankton production showed great variability (CV 49% and 47%, respectively) whereas phytoplankton biomass varied more than bacterial biomass (CV 34% and 11%, respectively). We therefore examined the evolution of biomass and production ratios in order to determine to which degree they were spatially and/or temporarily coupled.

Our observations followed the general rule of decreasing BB/CHL ratio with increasing chlorophyll concentration (Cho and Azam, 1990), with no particular outliers due to coastal or frontal situations. However, the use of such ratios is subject to debate, considering the bias related to bacterial abundance and chlorophyll conversion factors (Cho and Azam, 1990; Li et al., 1992; Buck et al., 1996; Van Wambeke et al., 2001). We therefore paid a particular attention

to the question and used the chlorophyll–carbon conversion factor proposed by Delgado et al. (1992) for the NW Mediterranean (Catalan Sea). This ratio is particularly well suited for our purpose since it was determined for conditions similar to ours: season, hydrological conditions (mixed layer 40–60 m depth, 1% incident light around 50 to 60 m depth) and on a similar phytoplankton composition, dominated by nanophytoplankton and with similar abundances of cyanobacteria (from 5 to 20×10^3 ml^{-1}, Angel-Rodriguez, personal communication). The decrease of the C/Chl*a* ratio with light matches those published for the Sargasso Sea, namely 40 for the mixed layer, 20 within the DCM and 15 below (Li et al., 1992; Carlson et al., 1996), though the 1% light was deeper there (110 m) and phytoplankton composition largely dominated by picophytoplankton. By comparison with published conversion factors (Cho and Azam, 1990; Li et al., 1992; Pedros-Alio et al., 1999), it appears that the factors used for HFF are in the range of those which favour bacterial biomass but not phytoplankton biomass. Despite such favourable conditions, bacterial biomass was most of the time lower than phytoplankton biomass and exceeded it only temporarily during HFF-6, which corresponded to a period that was recognised as a small post-bloom situation. Thus, our observations agree with the observations performed so far in the Mediterranean, where bacterial biomass seems to be always lower, or, at the best, equal to phytoplankton biomass (Fernàndez et al., 1994; Robarts et al., 1996; Pedros-Alio et al., 1999; Van Wambeke et al., 2001).

The ratio of integrated bacterial production to integrated primary production (IBP/IPP) is frequently used as an index to determine the amount of the carbon fixed by phytoplankton that is processed by heterotrophic bacteria (Ducklow et al., 1995; Carlson et al., 1996; Turley et al., 2000). The evolution of IPP depicted in Fig. 5 reflects a typical bloom evolution, with an increase from HFF-1 to HFF-5 followed by a decrease at the beginning of May (HFF-6). The classical scheme during such a temporal evolution first implies a low IBP/IPP ratio at the time phytoplankton is more productive (HFF-4, -5) and not limited by nutrients (Fig. 6). In that case, bacteria will essentially feed on phytoplankton exudates. At the time the bloom collapses (HFF-6) bacteria take advantage of new sources of phytoplankton-derived detritus and by-products from grazing, a situation that explains enhanced bacterial production, hence increased IBP/IPP ratios. However, to do so, bacteria must rearrange their degradation potentialities (Billen, 1990; Ducklow et al., 1995 and references therein). This phase corresponds to the delay that is generally observed between the peak in phytoplankton production and the peak in bacterial production. In our case this lag probably occurred during the period between HFF-5 and HFF-6.

Theoretically, uncoupling between phytoplankton and bacterial production, which translates into low IBP/IPP ratios, should correspond to a situation where exportation processes by grazing, lateral advection or sedimentation would be maximised. The index commonly used to estimate the exported fraction of primary production is the *f*-ratio. The highest mean *f*-ratio (0.38 ± 0.07) was observed during HFF-5, when IBP/IPP ratios showed the lowest values; this fact argues in favour of an uncoupled system. Theoretically, we could even expect to find a negative correlation between IBP/IPP ratio and *f*-ratio. With the spatio-temporal scale used here, however, such a relation was not obtained (log-transformed data, $r^2 = 0.06$, $p > 0.05$, Fig. 9). Several considerations could explain the lack of correlation between the two parameters.

A first explanation, of methodological nature, concerns the *f*-ratio itself. Indeed, the NH_4 assimilation rate could be an over-estimation of the phytoplankton uptake rate of NH_4, since 20% to 75% of the NH_4 uptake rate measured with the ^{15}N technique can

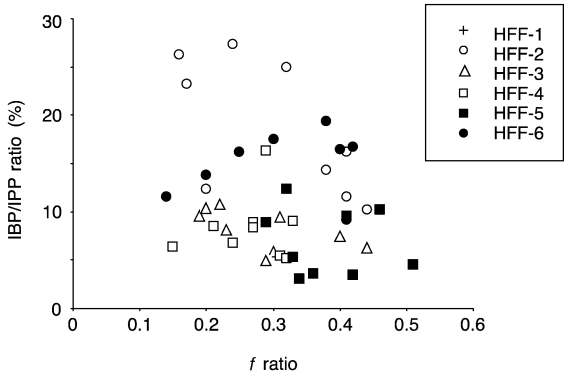


Fig. 9. Relation between the IBP/IPP ratio (%) and the *f*-ratio.

be due to bacterial uptake (Keil and Kirchman, 1991; Kirchman et al., 1994). Moreover, the NH_4 assimilation rate, although assumed to trace regenerated production, can also reflect new production when NH_4 is driven by lateral advection (Diaz et al., 2000). These facts render interpretation of the *f*-ratio within coastal zones difficult.

During HFF-2, in mid-March, IBP/IPP values were very high—like in a post bloom situation—but the low temperature, the mixed water column and the excess nutrients in surface waters characterised a winter situation. The second explanation could then rely on the implication of allochthonous sources, i.e. not depending directly on local, contemporaneous primary production. Such sources have been described to alternatively fuel bacterial growth, thus increasing the IBP/IPP ratio (Carlson et al., 1996; Yoro et al., 1997). The importance of lateral advection of POC has been recently shown in the area (Durrieu de Madron et al., 2000; Sempéré et al., 2000a). For example, in their tentative budget for the entire Gulf of Lions, Durrieu de Madron et al. (2000) estimated the annual input from the rivers (mainly the Rhône) and the atmosphere at around 25×10^4 T POC, a term that is not negligible when compared to the 260×10^4 T per year for primary production. One could have thus expected coastal waters to carry larger amounts of allochthonous sources of organic carbon than MAW, due to inputs from the Rhône river. But, surprisingly, IBP/IPP ratios in CW-influenced stations were not particularly high. Moreover, during HFF-2, these ratios were higher at stations that were not systematically under the influence of CW. In fact coastal waters, which carried POC and DOC but also nitrate, quite probably simultaneously enhanced bacterial production and primary production, resulting in the maintenance of IBP/IPP ratios comparable to that of MAW waters. The detailed examination of the relation between IBP/IPP ratios and IPP suggests that the dominant effect was the temporal evolution, which was detected because a large data set was sampled, thus dampening the effect of the spatial variability.

4.2. Carbon budget

It appears, from the preceding section, that neither IBP/IPP nor *f*-ratios were satisfying indexes to understand the fate of primary production during HFF and to determine the existence of allochthonous carbon sources. This failure largely results from the complex, highly dynamic system that was investigated (see Flexas et al., 2002). However, provided that a few assumptions on bacterial growth efficiency and grazing are made, our estimates of trap-measured carbon fluxes, primary production and bacterial production can be used to draw up a carbon budget for the experimental box, which can be of some help in solving the problem.

Bacterial growth efficiency, as determined by DOC biodegradation experiments, is generally low in oligotrophic to mesotrophic systems and ranges from 10% to 25% (Del Giorgio and Cole, 1998). Mediterranean values calculated from degradation experiments were in the range 1–20% for DOC and colloidal DOC (Sempéré et al., 1998; Lamy et al., 1999), and 3–31% for POC (Sempéré et al., 2000b). On the basis of these results, we considered a bacterial growth efficiency at 20% for the HFF area.

Concerning the grazing impact, a constant proportion of 20%, taken as an average of values cited in the literature, was considered to represent the proportion of primary production (mainly pico- and nano-sized cells here) grazed by ciliates in the area. This value of 20% is a good compromise for the 8–39% measured in the Ligurian Sea in May by Pérez et al. (2000) and the value of 14% given by Dolan et al. (1999) for Western Mediterranean stations. In our attempt of drawing up a carbon budget, direct predation of metazoans on phytoplankton was considered as negligible, since the levels of chlorophyll present were <1 μg Chl*a* l^{-1}. This has been previously demonstrated through computation of the potential sources of preys grazed by metazoans in the area (Van Wambeke et al., 1996).

Since the budget relies on biological data integrated down to 165 m, it was necessary to extrapolate the carbon flux data obtained at 240 m to estimate carbon fluxes at 165 m. To do so, we used the empirical relationship given by Martin et al. (1987), which describes the evolution of carbon flux with depth:

$$F_C(z) = \frac{F_C(100)}{\left(\frac{z}{100}\right)^{0.858}}, \quad (1)$$

where z is depth and $F_C(z)$ the carbon flux at that depth. This relationship leads to a correction factor of 1.38 for

fluxes extrapolated to 165 m depth. It is worthy to note that this average change with depth is very similar to the one that can be deduced from the mean organic carbon flux profile determined by Monaco et al. (1999) from their trap experiment in the nearby Grand-Rhône Canyon.

The comparison of primary production of carbon with its potential fate, i.e. sedimentation, ciliate grazing and cycling in the microbial food web through the bacterial utilisation of carbon (i.e. the bacterial carbon demand) shows that the budget, calculated on the basis of the above-mentioned assumptions and extrapolations, was not well-balanced during the HFF experiment (Table 3). We will first comment on these findings before discussing the effects of tuning up the budget by varying some of our assumed values for biological parameters.

The imbalance was more sensitive to time than space, since it was observed, to some varying degrees, at all stations. Two periods can be distinguished. The first one corresponds to a period of 'missing' carbon during which the sum of possible fates was higher than primary production. It was observed at all stations from HFF-1 to HFF-3 (16 March–7 April). On the contrary, the second period (14 April–2 May) from HFF-4 to HFF-6 showed for most stations (except 1 and 2) an 'excess' of carbon during which the removal of carbon was lower than primary production. The two periods were separated by a transition phase, from HFF-3 to HFF-4, for which mixed situations were found, either more or less balanced for stations 1 and 8 or positively/negatively unbalanced for the remaining stations.

The period of 'missing' carbon was observed at all stations and furthermore showed a trend towards values decreasing seaward (Table 3). The 'missing' carbon averaged 242 mg C m^{-2} day^{-1} during the HFF-1–HFF-2 interval and 117 mg C m^{-2} day^{-1} during the HFF-2–HFF-3 interval (Table 4). These values strongly suggest that during this period bacteria had to use additional sources of carbon that could have been only provided by lateral advection of DOC or POC. As shown by Sempéré et al. (2000a), there is a clear positive correlation between the Rhône river discharge and the corresponding DOC and POC inputs. At the time of our study, this discharge reached its maximum on 18 March (1400 m^3 s^{-1}), and decreased thereafter (600 m^3 s^{-1} on 6 April), indicat-

Table 3
Carbon budget for the different periods and stations considered during HFF

Period		PP	BP	C flux	C budget		PP	BP	C flux	C budget		PP	BP	C flux	C budget
16/03–23/03	st 7	228	102	40	−367	st 4	232	87	23	−271	st 1	276	96	34	−293
23/03–7/04		507	91	74	−123		382	92	36	−190		352	79	18	−133
7/04–14/04		767	81	29	180		512	64	17	72		278	41	13	3
14/04–24/04		642	69	18	150		632	43	11	277		268	48	5	−29
24/04–02/05		416	66	17	−14		610	73	2	119		437	95	8	−133
16/03–23/03	st 8	218	91	15	−298	st 5	336	85	27	−181	st 2	271	82	70	−265
23/03–7/04		393	94	22	−179		436	89				387	67	27	−53
7/04–14/04		541	86	9	−4		417	62				307	51	28	−36
14/04–24/04		628	68	3	162		405	38				192	52	29	−136
24/04–02/05		512	83				342	62				309	67	11	−97
16/03–23/03	st 9	243	82	31	−246	st 6	368	76	10	−98	st 3	268	67	37	−158
23/03–7/04		369	70	37	−89		327	79	4	−138		310	50	29	−31
7/04–14/04		446	48	17	102		312	61	5	−58		432	41	38	102
14/04–24/04		862	45	21	445		449	56				495	54		
24/04–02/05		884	65	20	364		418	86				354	79		

All data are expressed in mg C m^{-2} day^{-1} for the 0–165 m layer. For primary production (PP) and bacterial production (BP), values were computed as the mean of two consecutive cruises. Carbon fluxes (Cflux) at 165 m were calculated from time-integrated fluxes at 240 m depth, normalised to the period considered, and then extrapolated to 165 m using the Martin et al. (1987) relation. The carbon budget was calculated as the difference between possible sources and fates of primary producers as follows: $PP - 0.2 \times PP - Cflux - (BP \times 100/BGE)$, where BGE is the bacterial growth efficiency. See text for the various assumptions made to draw up this budget.

Table 4
Effect of varying bacterial growth efficiency (BGE) on the carbon budget

Period	PP	BP	C flux	C budget		
				BGE 10%	BGE 20%	BGE 30%
	mean					
16/03–23/03	271	85	32	−668	−242	−99
23/03–7/04	385	79	31	−506	−117	13
7/04–14/04	446	59	20	−251	45	143
14/04–24/04	508	53	15	−126	145	235
24/04–02/05	476	75	12	−336	48	169
	min					
16/03–23/03	218	67	10	−878	−367	−198
23/03–7/04	310	50	4	−650	−190	−37
7/04–14/04	278	41	5	−436	−58	41
14/04–24/04	192	38	3	−395	−136	−49
24/04–02/05	309	62	2	−608	−133	13
	max					
16/03–23/03	368	102	70	−476	−98	31
23/03–7/04	507	94	74	−281	−31	59
7/04–14/04	767	86	38	−102	180	315
14/04–24/04	862	69	29	219	445	519
24/04–02/05	884	95	20	37	364	471

Carbon fluxes are expressed in mg C m^{-2} day^{-1} (0–165 m layer) for each period separating two consecutive cruises. BP: bacterial production. PP: primary production. Cflux: trap-measured carbon flux. Mean, minimum and maximum values for the different parameters are calculated for the nine stations. BGE has been set at 10%, 20%, and 30% (see text).

ing that the importance of allochthonous inputs of DOC and POC within the HFF site was probably maximal in March, at the beginning of the experiment. Assuming that a mean of 18% of our estimated detrital POC was semi-labile (Sempéré et al., 2000a) and was used at a mean rate of 0.063 day^{-1} (Keil and Kirchman, 1999), hydrolysis of detrital POC could have provided from 120 to 200 mg C m^{-2} day^{-1}. Thus, hydrolysis of semi-labile detrital POC, in addition to the use of DOC driven by the Rhône, could have been sufficient to satisfy the missing part of the bacterial demand. Of course these calculations, essentially based on published data, are subject to some uncertainties; but, at least, they provide the order of magnitude of organic carbon issued from the hydrolysis of detritus that could have been used by bacteria. The remaining refractory detrital POC could have been further involved in the high episodic POC fluxes measured by the traps, e.g. the event of 18–19 March at station 2 (77 mg C m^{-2} day^{-1}) or at station 7 on 24–25 March (138 mg C m^{-2} day^{-1}, Fig. 7). Indeed, such high fluxes are not explained by classical 1D models describing, for open ocean situations, the fate of primary production with depth (e.g. Bishop, 1989). This inadequacy is illustrated in Fig. 10 that presents the ratio of measured to modelled carbon fluxes as a function of IPP. The figure shows that our measured fluxes can be up to 2.2 times higher than predicted by the model. Lateral import of continental shelf POC at depth, either directly linked to increased river discharge or resulting from resuspension processes of freshly deposited material, must be invoked to explain the increased fluxes observed in the sediment traps near the shelf break (Heussner et al., 1999).

Regarding the second period, from HFF-4 to HFF-6, budget calculations showed a large shift towards a situation of 'excess' carbon that affected more the central and western transect than the eastern one (Table 3). It is worthy to note that this second period corresponds to an important change in the along-slope circulation over the HFF site (Flexas et al., 2002). Indeed, the flow pattern derived from SST images shows the progressive installation of the Northern Mediterranean Current over the entire experimental box during the first half of April. Excess carbon

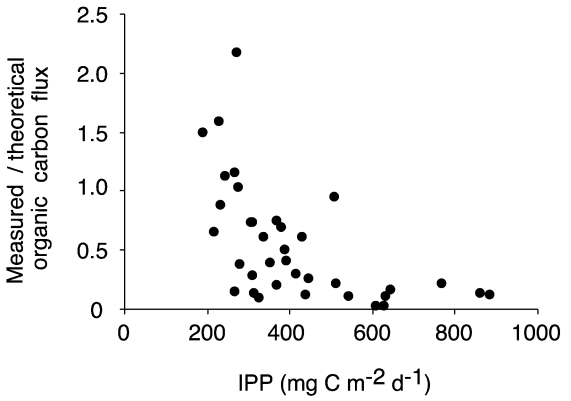


Fig. 10. Evolution of the measured to theoretical organic carbon flux ratio at 240 m depth as a function of primary production. The theoretical C flux at 240 m depth is computed from primary production according to the equation of Bishop (1989): C_z flux = $0.409 \times PP^{1.41}/z^{0.628}$, where PP and C_z flux are expressed in g C m^{-2} $year^{-1}$ and z is the depth in m.

occasionally reached values of 150 to 250 mg C m^{-2} day^{-1} at stations 4, 7 and 8, and up to 445 mg C m^{-2} day^{-1} at station 9. Meanwhile, stations 1 and 2 where still in the situation of missing carbon. Apart from the potential effects linked to the modifications of the circulation pattern, which still need to be unravelled, several mechanisms can be hypothesised to explain the observed discrepancy between production and fate of carbon during this period. Station 9 for example was located in a frontal zone during HFF-5. It has been shown that in such upwelling systems part of the primary production is exported laterally along isopycnal gradients (Peinert and Miquel, 1994), thus contributing to the observed local imbalance. In addition, direct predation of phytoplankton by zooplankton could also occur, since the chlorophyll values were >1 μg l^{-1}, a level above which blooms are mostly due to an increased participation of microphytoplankton that is more efficiently grazed by metazoans (Thibault et al., 1994; Van Wambeke et al., 1996). Finally, the transformation of POC in DOC through excess release due to bacterial hydrolysis of detritus and dissolution processes (Smith et al., 1992; Van Wambeke et al., 2001) could also explain the situation of excess carbon. In our experimental area, DOC can accumulate at a rate of 50 to 200 mg C m^{-2} day^{-1} over short periods (up to 20 days, Diaz, 2000). Such values correspond roughly to the excess fluxes observed during HFF. The DOC accumulated can be eliminated later on, either by the downward export through winter convection (Copin-Montégut and Avril, 1993), or through alongslope export processes (Durrieu de Madron et al., 2000). All in all, this second period seems to have been characterised by an increased DOC production and/or POC export through advection or grazing at the expenses of particle sedimentation.

The preceding discussion partly relies on the validity of the assumptions made and estimates used to complete the budget calculations. We therefore tested the degree to which the general trend observed was sensitive to changes in methodological assumptions and conversion factors. The basic principle of the test was to observe the changes in the final budget after having either maximised or minimised input and output terms. We considered first a potential underestimation of primary production due to the release of dissolved organic carbon during incubation, as we estimated only particulate primary production (Baines and Pace, 1991). We think that this bias was in fact reduced, as it was demonstrated that there is a rapid utilisation of photosynthetically released dissolved organic compounds during the incubation by heterotrophic bacteria (Fernàndez et al., 1994). However, we took into account a maximum correction factor of 15% to compensate for a possible underestimation of primary production values. Second, bacterial production exhibits daily variations that could range from 15% to 35% of the daily mean (Gasol et al., 1998; Van Wambeke et al., 2001) and thus introduce a bias in the computation of daily BP. More than 80% of our data were sampled between 1000 and 2200 h. After having assigned our results to five sampling periods (6–10, 10–14, 14–18 and 18–22 h), we could not find any outlying group of data (one factor ANOVA, $p>0.05$) that could have otherwise suggested a diel pattern among the different cruises and stations. However, we further tested if the budget was sensitive to a 20% change in BP. We also tested the effect of leaving out ciliate grazing. Finally, we used 10% and 30% as alternative bacterial growth efficiencies to the 20% used in our most probable case developed above. From all these combinations, which are not presented here, it clearly appears that the carbon budget was mainly sensitive to changes in BGE and that the various other parameters did no much affect the general trend described above. The effect of changing BGE is reported in Table 4. Applying a value of 30% decreases the 'missing' carbon observed between HFF-1 and HFF-2 by 2/3 on the average and balances the budget between HFF-2 and HFF-3. But, on the contrary, this increased value of BGE increases by up to 100% the 'excess' of carbon observed during the second period. Using a 10% value for BGE essentially results in budget values shifted almost entirely towards situations of 'missing' carbon.

All in all, it appears that the various combinations that we tried led to more or less pronounced imbalances between the amount of carbon introduced by PP into the system and the amount of carbon removed by the various processes considered. Now, coming back to one of the working hypotheses of the overall HFF experiment mentioned in introduction, this finding strongly suggests that, at least at the scale of the experiment, the supply of particulate carbon to the slope was not directly driven by the biological pro-

cesses acting in the upper water column of the slope of the Gulf of Lions.

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