

# Microbial community structure in the sea surface microlayer at two contrasting coastal sites in the northwestern Mediterranean Sea

Fabien Joux<sup>1,\*</sup>, H el ene Agogu e<sup>1</sup>, Ingrid Obernosterer<sup>1</sup>, Christine Dupuy<sup>2</sup>,  
Thomas Reinthaler<sup>3</sup>, Gerhard J. Herndl<sup>3</sup>, Philippe Lebaron<sup>1</sup>

<sup>1</sup>Observatoire O c anologique de Banyuls, CNRS UMR 7621, Universit e Pierre et Marie Curie, BP 44,  
66651 Banyuls-sur-Mer Cedex, France

<sup>2</sup>Universit e de La Rochelle, P ole Sciences, Laboratoire de Biologie et d'Environnement Marins, FRE2727,  
Avenue Michel Cr epeau, 17042 La Rochelle, Cedex, France

<sup>3</sup>Department of Biological Oceanography, Royal Netherlands Institute for Sea Research (NIOZ), PO Box 59,  
1790 AB Den Burg, The Netherlands

**ABSTRACT:** In an attempt to compare the microbial community structure between the sea surface microlayer (SML) and subsurface waters (SSW), we determined the enrichment factors (EF: the ratio of abundance or activity in the SML to abundance or activity in SSW) of 13 biological parameters. Samples were taken at 2 contrasting coastal sites in the Mediterranean Sea, corresponding to a high (Barcelona, Spain) and low (Banyuls-sur-Mer, France) urbanized area. Principal component analysis showed that temporal variability was much higher at Barcelona than at Banyuls, and that the characteristics of the SML and SSW samples were more closely related at Barcelona. At both sites, the SML was weakly enriched in heterotrophic bacteria (on average 1.1-fold), *Synechococcus* spp. (on average 1.1-fold), and photosynthetic picoeukaryotes (on average 1.5-fold) relative to SSW. In contrast, the SML was considerably enriched in chlorophyll *a* (chl *a*) (on average 1.9-fold), phaeophytin *a* (on average 7.4-fold), autotrophic (on average 6.1-fold) and heterotrophic nanoflagellates (on average 5.1-fold) relative to SSW. Enrichments in bacterial production and culturable bacteria were highly variable. EF were significantly different between the 2 sites only for concentrations of chl *a*, *b*, and *c* and the abundance of autotrophic nanoflagellates, with higher EF at the Barcelona site. Except for autotrophic flagellates, the abundance or activity of the parameters determined in the SML was highly correlated with that determined in SSW, suggesting that enrichment of the SML results mainly from upward transport of microorganisms attached to buoyant particles or bubble scavenging. In contrast, the high contribution of autotrophic nanoflagellates to overall phytoneston biomass (mean = 26%) is likely due to their rapid colonization of the SML. The high abundances of auto- and heterotrophic nanoflagellates in the SML indicated that these organisms play a key role in the functioning of the microbial food webs at the air–sea interface.

**KEY WORDS:** Sea surface microlayer · Enrichment factor · Microbial structure · Virus-like particles · Bacteria · Microalgae · Nanoflagellates

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## INTRODUCTION

The neustonic realm is a vast habitat covering 70% of the earth's surface, and the surface microlayer (SML) of aquatic ecosystems was shown to have specific chemical and biological properties compared

to the subsurface waters (SSW) (see review by Liss & Duce 1997). Although the SML represents an extreme environment for living organisms, it is generally enriched in bacteria, microalgae, yeasts, molds, and protists, as well as a variety of organic and inorganic particulate and dissolved compounds (Hardy 1982,

\*Email: joux@obs-banyuls.fr

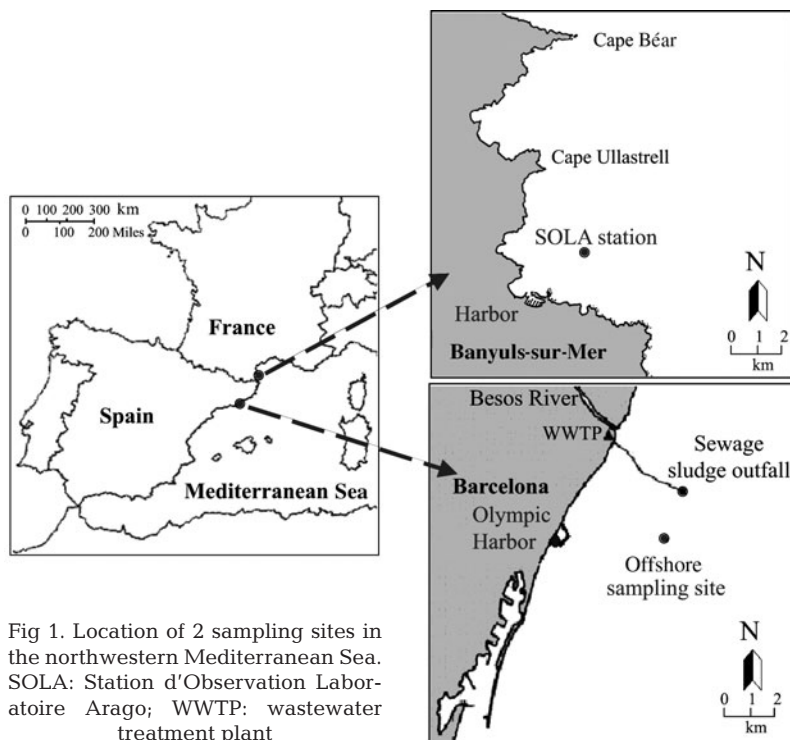


Fig 1. Location of 2 sampling sites in the northwestern Mediterranean Sea. SOLA: Station d'Observation Laboratoire Arago; WWTP: wastewater treatment plant

Williams et al. 1986). However, our knowledge of the biology of the SML is still in its infancy. Patterns in the distribution of neuston and the relationship between their abundance and environmental factors are poorly understood.

On the one hand, organisms present in the SML may have developed life strategies to survive in this habitat exposed to intense solar radiation, high temperature and/or salinity gradients, toxic organic substances, and heavy metals (see review by Maki 1993). On the other hand, the SML is enriched in organic material from natural and anthropogenic origins and this could fuel bacterial growth and the development of SML-bound microbial food webs (Williams et al. 1986, Kuznetsova & Lee 2001). However, there is no clear evidence to indicate that biogenic surfactants and other organic materials, which accumulate in the SML, stimulate biological productivity. The role of microorganisms in the SML is still unclear and controversial. Although higher abundances of microorganisms have been frequently reported in the SML compared to SSW (Marumo et al. 1971, Kuznetsova et al. 2004), lower microbial abundances in the SML have also been observed (Bell & Albright 1982). Variability in literature values of microbial abundances might be related to the use of different sampling devices to collect the SML and/or to variability in the formation of the SML (Carlson 1982, Hardy 1997, Agogue et al. 2004).

Microorganisms and their abiotic environment are linked and influence each other (e.g. concentration and quality of dissolved organic matter available for heterotrophic bacteria). Moreover, biotic interactions such as predation, viral infection, and antibiosis occur and we know little of these interactions in the SML. Light inhibition may affect viruses and also the physiological state of host organisms, and this inhibition may vary depending on the trophic status of ecosystems and on variations in light intensities. Several reports have shown that microalgae in the SML are sometimes photoinhibited (see review by Hardy 1997), and it is still unclear whether they form the basis of the food web in the SML (Obernosterer et al. 2005).

To our knowledge, no previous study has simultaneously investigated and compared most of the components of the microbial food web (i.e. viruses, bacteria, pico- to micro-algae, auto- and heterotrophic nanoflagellates) in the SML and SSW. In an effort to investigate the structure of the microbial food web operating in the SML, we sampled 2 coastal sites in the northwestern Mediterranean Sea characterized by different trophic conditions at different periods of the year. This data set was used to analyze temporal and spatial changes in the structure and productivity of microbial communities in both the SML and SSW.

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## MATERIALS AND METHODS

**Field sites.** Samples were collected at 2 coastal sites in the northwestern Mediterranean Sea: the Bay of Banyuls-sur-Mer (France) ( $42^{\circ} 29' N$ ,  $03^{\circ} 08' E$ ; Station d'Observation Laboratoire Arago, SOLA), and offshore from the Olympic Harbor in Barcelona (Spain) ( $41^{\circ} 23' N$ ,  $02^{\circ} 14' E$ ) (Fig. 1). The Bay of Banyuls-sur-Mer is oligotrophic (Médernach et al. 2001, Grémare et al. 2003). The Barcelona site is moderately eutrophic and impacted by an urban sewage sludge outfall (Bayona et al. 1991, Chalaux et al. 1994) (Fig. 1). Samples were collected during 4 cruises, each lasting 2 to 3 d (March 2001, September 2001, March 2002, and June-July 2002) (see Table 1).

**Sample collection.** Sampling of the SML and SSW was conducted early in the morning (05:00 to 09:00 h) from an inflatable boat. The SML was collected using 2 metal screens, which consisted of a stainless steel

screen (mesh size: 1.25 mm, wire diameter: 0.36 mm) stretched over a 60 × 80 cm steel frame (Garrett 1965, Daumas et al. 1976). The metal screen was lowered vertically through the water surface, then oriented horizontally and lifted up through the SML. Around 50 ml were collected for each screen dip. The volume of the SML required for analysis of all parameters (~20 l) was collected within 3.5 h. The thickness of the micro-layer sampled by this technique is about 250 to 440 µm (Daumas et al. 1976). Corresponding SSW samples were collected by submerging a polycarbonate bottle and opening it at 0.5 m depth. Upon return to the laboratory (generally within 30 min after completion of sampling), subsamples were taken for measuring chemical and microbiological parameters as described below. Enrichment factors (EF) were calculated as the ratio of the concentration or rate of the respective parameter in the SML to that in SSW. An EF > 1.0 indicated an enrichment in the SML relative to SSW; an EF < 1.0 indicated a depletion in the SML relative to SSW.

**Chemical analysis.** For the determination of suspended particulate matter (SPM), 1 l of seawater was filtered through glass fibre filters (GF/F, 0.7 µm nominal pore size). The SPM was determined gravimetrically once the filter was dried in an air-heated oven (55°C for 2 h) and brought to ambient temperature in a desiccator. Subsamples of the filtrate (100 ml) from GF/F filtration were collected in polyethylene bottles and frozen (-18°C) for later analysis. Nitrate (NO<sub>3</sub><sup>-</sup>) plus nitrite (NO<sub>2</sub><sup>-</sup>) were analyzed with a Skalar auto-analyzer according to Tréguer & Le Corre (1975). The method from Murphy & Riley (1962) was used for reactive phosphorus (PO<sub>4</sub><sup>3-</sup>) analysis. All chemical analyses were conducted in duplicate.

**Pigments.** Triplicate samples (200 ml) were passed through Whatman GF/F filters, which were immediately frozen in liquid nitrogen. Filters were extracted in acetone (90% final concentration). Extracts were kept at 4°C for 12 h and centrifuged before the absorbance of the supernatant was measured on a Perkin Elmer MPF66 spectrofluorometer to determine chlorophyll *a*, *b* and *c* (chl *a*, chl *b*, chl *c*) and phaeophytin *a* (Neveux & Lantoiné 1993). Pigment analyses were made in triplicate. Algal C biomass was estimated assuming that 1 µg chl *a* corresponds to 50 µg C biomass (Cho & Azam 1990).

**Flow cytometric enumeration of autotrophic and heterotrophic cells.** Duplicate or triplicate samples (3 ml) for flow cytometry analyses of both heterotrophic and autotrophic bacteria and photosynthetic eukaryotes were fixed with formaldehyde (2% final concentration) and stored in cryotubes. The samples were left at room temperature for 10 to 15 min, frozen in liquid nitrogen, and stored at -80°C until analysis. Samples were run through a flow cytometer (FAC-

Scan, Becton Dickinson) equipped with a 488 nm, 15 mW Argon laser. Phytoplankton cells were discriminated and counted according to Marie et al. (2000). The phycoerythrin of the cyanobacterium *Synechococcus* spp. fluoresces orange and was distinguished from red fluorescence of the chlorophyll. Photosynthetic picoeukaryotes were discriminated by scatter signals (a proxy of the cell size) and the more intense red fluorescence of their chlorophyll compared to that of *Synechococcus* spp. The carbon biomass of picophytoplankton was estimated assuming carbon contents of *Synechococcus* spp. and photosynthetic picoeukaryotes to be 250 and 2100 fg cell<sup>-1</sup>, respectively (Campbell et al. 1994).

Heterotrophic bacteria were stained with a nucleic acid dye (SYBR-Green I; final concentration 0.01% [vol:vol] of the commercial solution; Molecular Probes) and stored for at least 15 min at room temperature in the dark before counting. Stained bacteria were counted according to their right angle light scatter and green fluorescence measured at 515 to 545 nm (Marie et al. 1997, 2000). The number of bacteria with a high and low nucleic acid (HDNA and LDNA, respectively) content was determined according to Lebaron et al. (2002) and Servais et al. (2003). To convert bacterial abundance into carbon biomass, a mean bacterial C content of 20 fg cell<sup>-1</sup> was used (Lee & Furhman 1987).

**Culturable bacteria.** Samples for culturable bacteria were processed within 4 h after sampling. Serial dilutions (1:10 and 1:100) were prepared with filter-sterilized seawater, and 100 µl of each diluted and undiluted sample was plated on Marine Agar 2216 media (Difco) in triplicate. CFU (colony forming units) were counted after incubation at 25°C in the dark for 1 to 2 wk.

**Virus-like particles.** Samples (2 ml) were fixed with formaldehyde (2% final concentration) and filtered onto 0.02 µm filters (Anodisc, 25 mm diameter; Whatman). Virus-like particles (VLP) were stained on the filters using SYBR-Green I and enumerated under a Zeiss Axiophot microscope equipped for epifluorescence microscopy as previously described (Noble & Fuhrman 1998). At least 400 VLP were counted per filter.

**Autotrophic and heterotrophic nanoflagellates.** Samples for the enumeration of nanoflagellates were fixed, stained, and observed according to Dupuy et al. (1999). Triplicate 100 ml samples were preserved with paraformaldehyde (1% final concentration). Each sample was concentrated by filtration through 0.8 µm to a volume of 10 ml and stained with primulin (50 µg ml<sup>-1</sup> final concentration). Nanoflagellate abundance was determined by epifluorescence microscopy. Autotrophic nanoflagellates (AFLAG) were distinguished from non-pigmented heterotrophic nanoflagellates

(HFLAG) by autofluorescence signals. The mean cell volume of nanoflagellates was calculated by equating the shape to standard geometric configurations. The cell volume was converted into carbon units, using a theoretical carbon:volume ratio of  $0.14 \text{ pg C } \mu\text{m}^{-3}$  (Putt & Stoecker 1989).

**Bacterial production.** Bacterial production was estimated from the rates of protein synthesis using [ $^{14}\text{C}$ ]L-leucine ( $319 \text{ mCi mmol}^{-1}$ , Amersham) (Simon & Azam 1989). Duplicate samples (5 or 10 ml) and 1 formaldehyde-killed control were incubated with  $20 \text{ nmol l}^{-1}$  of [ $^{14}\text{C}$ ]L-leucine (final concentration) in the dark at *in situ* temperature for 0.5 to 1 h. Incubations were terminated by adding formaldehyde (2% final concentration). Subsequently, samples were filtered through  $0.45 \text{ } \mu\text{m}$  Millipore HAWP filters followed by 3 rinses with 5 ml of ice-cold 5% trichloroacetic acid. Filters were then dissolved in 1 ml ethyl acetate (Riedel de Haen) and after 10 min, 8 ml of scintillation cocktail (Insta-gel plus II, Canberra Packard) was added. The radioactivity of the filter was assessed after 18 h with a liquid scintillation counter (Canberra Packard Tricarb 2000).

**Data analysis.** Patterns among variables were assessed using principal component analysis (PCA) based on all parameters except for VLP,  $\text{PO}_4^{3-}$ , and SPM; these parameters were excluded due to the limited number of samples. Samples from March 2001 cruises were not included because pigment analysis of these samples was not completed. An initial PCA was carried out on all samples collected at the Banyuls and Barcelona sites. It aimed to compare levels of temporal variability between these 2 sites. Two other PCAs were conducted separately for samples collected at the Banyuls and Barcelona sites. They both aimed to distinguish the relationship between the characteristics of the SML and SSW for a given sampling date and/or cruise. This relationship was further assessed using a Mantel test, which compared similarity matrices of all SML and SSW samples.

The Wilcoxon rank sum test was used for each parameter to assess whether the SML was enriched relative to SSW. These tests were conducted on: (1) the whole data set, (2) the Banyuls data set, and (3) the Barcelona data set. Mann-Whitney *U*-tests were used for each parameter to assess possible differences in the EF between Banyuls and Barcelona. Correlations among parameters within the whole data set (i.e. Banyuls and Barcelona) were assessed using Spearman rank correlation coefficients and associated significance tests. Similar tests were also used for each parameter to assess the correlation between concentrations in the SML and SSW.

## RESULTS

### Characteristics of SSW at the 2 sites

For each cruise, temperature and salinity of SSW were similar at both sites (Table 1). However, differences in chemical parameters were evident between the 2 sites: concentrations of  $\text{NO}_3^- + \text{NO}_2^-$  (except in March 2001),  $\text{PO}_4^{3-}$ , and SPM in SSW were higher off Barcelona relative to the Banyuls site (Table 2). Bacterial abundance and activity, abundance of CFU, and chl *a* concentrations were generally higher in SSW off Barcelona (Table 2). However, bacterial abundance and chl *a* concentrations measured at the Barcelona site remained below  $2.5 \times 10^6 \text{ cells ml}^{-1}$  and  $3 \text{ } \mu\text{g l}^{-1}$ , respectively, indicating that eutrophication was rather modest here. The phaeophytin *a*:chl *a* ratios showed similar patterns between the 2 sites, with high (0.2), moderate (0.02 to 0.05), and low ( $<0.005$ ) values measured in September 2001, March 2002 and June-July 2002, respectively. Percentages of HDNA bacteria detected by flow cytometry were always higher off Barcelona relative to Banyuls, and showed a similar temporal trend at both sites, with higher values in March 2001 and 2002 (80 and 60% of total bacterial

Table 1. Location, sample codes, and physical parameters (temperature and salinity of surface water to 0.5 m depth) corresponding to sampling dates. ND: not determined; SML: sea surface microlayer; SSW: subsurface waters

| Location  | Date             | SML sample codes | SSW sample codes | Mean surface temp. ( $^{\circ}\text{C}$ ) | Mean surface salinity | Mean wind speed ( $\text{m s}^{-1}$ ) |
|-----------|------------------|------------------|------------------|---|-----------------------|---------------------------------------|
| Barcelona | 14–15 Mar 2001   |                  |                  | 13.2                                      | 37.9                  | 2–4                                   |
|           | 6–7 Sep 2001     | MB3–MB4          | UB3–UB4          | 24.2                                      | 37.9                  | 2–4                                   |
|           | 19–21 Mar 2002   | MB5–MB7          | UB5–UB7          | ND  | ND                    | ND                                    |
|           | 25–27 Jun 2002   | MB8–MB10         | UB8–UB10         | ND  | ND                    | <2                                    |
| Banyuls   | 16 & 18 Mar 2001 |                  |                  | 12.2                                      | 37.6                  | <5                                    |
|           | 12–13 Sep 2001   | Mb3              | Ub3              | 20.7                                      | 38.1                  | <4                                    |
|           | 25–27 Mar 2002   | Mb5–Mb7          | Ub5–Ub7          | 13.0                                      | 38.1                  | <3                                    |
|           | 1–3 Jul 2002     | Mb8–Mb10         | Ub8–Ub10         | 20.2                                      | 37.5                  | 2–5                                   |

abundance at Barcelona and Banyuls, respectively) than in September 2001 and June-July 2002 (58 and 47% of total bacterial abundance at Barcelona and Banyuls, respectively) (Table 2).

### Enrichment of the SML

When sampling sites were considered separately, 8 and 9 parameters out of a total of 16 were significantly enriched at the Barcelona and Banyuls site, respectively, relative to SSW (Table 3). Only 4 parameters (chl *a*, phaeophytin *a*, AFLAG, and HFLAG) were concurrently enriched at both sites. When data from the 2 sites were pooled, 10 parameters were significantly enriched in the SML relative to SSW. The EF of 4 parameters (chl *a*, chl *b*, chl *c*, and AFLAG) revealed significant differences between the 2 sites, with higher EF at Barcelona than at Banyuls (Table 3).

The EF for VLP were close to 1 for all samples except those taken at Barcelona in September 2001 (mean EF = 5.1) (Table 2). It was not possible to statistically test the enrichment of VLP per site due to low numbers of samples (i.e.  $n < 5$  per site). The EF of bacterial abundances were low at both sites (mean EF = 1.1). Significant differences between the SML and SSW were found at the Banyuls site and when all data were pooled (Table 3). The EF for culturable bacteria were much more variable (0.5 to 191), with a mean EF of 27.8 when all data were pooled (Tables 2 & 3). The EF for bacterial production were also highly variable, but no significant differences were detectable between the SML and SSW (Tables 2 & 3). Similarly, percentages of HDNA bacteria were not significantly different between the SML and SSW (Table 3). The concentration of chl *a* was always higher in the SML relative to SSW, and significantly higher EF were determined at Barcelona (EF = 2.1 to 3.0) relative to Banyuls (EF = 1.2 to 1.6) (Tables 2 & 3). Chl *b* and chl *c* were significantly enriched at the Barcelona site (average EF = 3.2 and 2.4, respectively), but generally not at the Banyuls site (Table 3). As for chl *a*, phaeophytin *a* was significantly enriched in the SML at both sites (EF = 1 to 27.1), and no difference was apparent in the EF between the 2 sites (Tables 2 & 3). At both sites, the abundance of *Synechococcus* spp. in the SML was close to that measured in SSW and did not differ significantly from 1 (Tables 2 & 3). Photosynthetic picoeukaryotes were only significantly enriched at the Banyuls site (EF = 1.1 to 1.8). The EF of AFLAG and HFLAG ranged from 1.1 to 18.5 and from 1.8 to 7.7, respectively (Table 2). For both types of nanoflagellates, EF were significantly higher than 1 and higher EF were observed at the Barcelona site for AFLAG (Table 3).

The cruise at the Barcelona site in June 2002 was characterized by large changes in enrichment of the SML over 3 consecutive days (Fig. 2). Low wind speeds ( $< 2 \text{ m s}^{-1}$ , Table 1) and cloudless skies were observed over the 3 sampling days. Under these conditions, EF for chl *a* concentrations increased from 1 to ~4, whereas EF for bacterial abundance did not change over the 3 days. Conversely, EF for CFU increased substantially (from 1 to 40). During the third day, CFU represented 100 and 4.2% of total bacterial abundance in

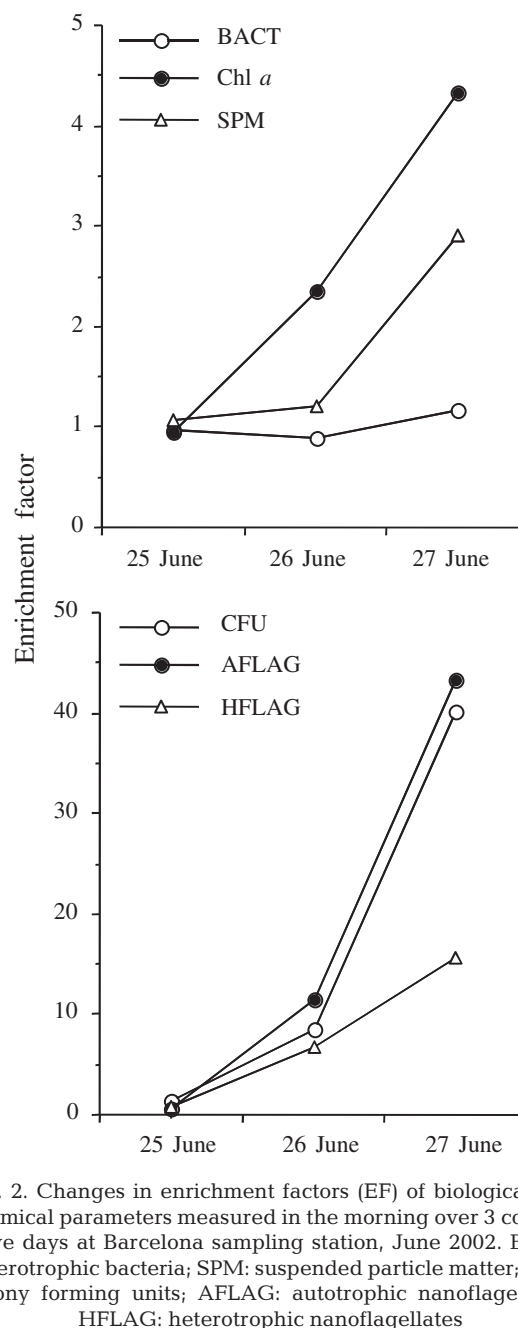


Fig. 2. Changes in enrichment factors (EF) of biological and chemical parameters measured in the morning over 3 consecutive days at Barcelona sampling station, June 2002. BACT: heterotrophic bacteria; SPM: suspended particle matter; CFU: colony forming units; AFLAG: autotrophic nanoflagellates; HFLAG: heterotrophic nanoflagellates

Table 2. Biological and chemical parameters measured in the sea surface microlayer (SML) and subsurface waters (SSW) at Barcelona and Banyuls sites, and respective enrichment factors (EF). Mean values and ranges over 2 to 3 consecutive sampling days are given. VLP: virus-like particles; %HDNA: percentage of high nucleic acid bacteria; CFU: colony forming unit; BP leucine: bacterial production measured by [<sup>14</sup>C]-leucine incorporation; AFLAG: autotrophic nanoflagellates; HFLAG: heterotrophic nanoflagellates; SPM: suspended particle matter; n: number of samples; ND: not determined

| Parameter   | Date         | Barcelona        |                  |      |   | Banyuls          |                  |      |   |
|---|--------------|------------------|------------------|------|---|------------------|------------------|------|---|
|   |              | SML              | SSW              | EF   | n | SML              | SSW              | EF   | n |
| VLP<br>(10 <sup>6</sup> ml <sup>-1</sup> )                          | Mar 2001     | ND               | ND               | ND   |   | ND               | ND               | ND   |   |
|   | Sep 2001     | 125 (46–204)     | 25 (24–26)       | 5.1  | 2 | 24.5             | 25.8             | 0.9  | 1 |
|   | Mar 2002     | 1.8 (1.0–2.5)    | 1.6 (1.1–2.1)    | 1.1  | 2 | 0.4 (0.3–0.4)    | 0.3 (0.3–0.4)    | 1.1  | 3 |
|   | Jun–Jul 2002 | ND               | ND               | ND   |   | ND               | ND               | ND   |   |
| Bacteria<br>(10 <sup>6</sup> ml <sup>-1</sup> )                     | Mar 2001     | 1.7 (1.9–1.5)    | 1.5 (1.2–1.8)    | 1.1  | 2 | 1.1 (1.0–1.1)    | 1.1 (1.1–1.1)    | 1.0  | 2 |
|   | Sep 2001     | 0.9 (0.9–0.9)    | 0.9 (1.0–0.9)    | 1.0  | 2 | 1.0 (0.9–1.1)    | 0.9 (0.9–1.0)    | 1.1  | 2 |
|   | Mar 2002     | 2.6 (1.7–4.0)    | 2.4 (1.3–3.9)    | 1.2  | 3 | 0.9 (0.8–1.2)    | 1.0 (0.8–1.1)    | 0.9  | 3 |
|   | Jun–Jul 2002 | 1.2 (1.0–1.5)    | 1.2 (1.0–1.6)    | 1.0  | 3 | 0.9 (0.8–1.0)    | 0.7 (0.7–0.8)    | 1.2  | 3 |
| %HDNA   | Mar 2001     | 80.7 (78.5–83.0) | 78.8 (74.6–83.0) | 1.0  | 2 | 61.1 (58.5–63.8) | 61.9 (61.1–62.7) | 1.0  | 2 |
|   | Sep 2001     | 58.1 (57.2–59.1) | 58.1 (56.5–59.8) | 1.0  | 2 | 47.8 (42.6–53.1) | 47.8 (47.0–48.7) | 1.0  | 2 |
|   | Mar 2002     | 85.6 (79.9–91.6) | 84.2 (77.3–89.6) | 1.0  | 3 | 62.2 (58.0–67.3) | 60.4 (57.5–63.3) | 1.0  | 3 |
|   | Jun–Jul 2002 | 54.1 (31.8–82.6) | 58.1 (54.6–62.9) | 0.9  | 3 | 61.6 (58.0–63.3) | 46.2 (31.9–54.7) | 1.4  | 3 |
| CFU<br>(10 <sup>3</sup> ml <sup>-1</sup> )                          | Mar 2001     | 3.3 (2.8–3.8)    | 0.8 (0.01–1.7)   | 191  | 2 | 7.0 (1.2–12.8)   | 0.5 (0.1–0.8)    | 12.3 | 2 |
|   | Sep 2001     | 35.7             | 71.0             | 0.5  | 1 | 7.9 (1.2–14.7)   | 7.1 (0.6–13.6)   | 1.5  | 2 |
|   | Mar 2002     | 119 (18.5–231)   | 124 (11.5–299)   | 1.4  | 3 | 22.3 (1.2–58.0)  | 1.6 (0.8–2.7)    | 9.8  | 3 |
|   | Jun–Jul 2002 | 611 (11–1745)    | 20.3 (8.5–43.5)  | 16.7 | 3 | 20.9 (9.1–27.0)  | 2.3 (1.2–4.5)    | 11.5 | 3 |
| BP leucine<br>(pmol leu l <sup>-1</sup> h <sup>-1</sup> )           | Mar 2001     | ND               | ND               | ND   |   | ND               | ND               | ND   |   |
|   | Sep 2001     | 481 (328–635)    | 199 (181–217)    | 2.4  | 2 | 2.4              | 35.9             | 0.1  | 1 |
|   | Mar 2002     | 1813 (776–3480)  | 1538 (966–9088)  | 0.5  | 3 | 299 (69–667)     | 198 (141–238)    | 1.9  | 3 |
|   | Jun–Jul 2002 | 2085 (56–6131)   | 1376 (727–2584)  | 0.8  | 3 | 246 (47–465)     | 876 (212–2149)   | 0.7  | 3 |
| Chl a<br>(µg l <sup>-1</sup> )                                      | Mar 2001     | ND               | ND               | ND   |   | ND               | ND               | ND   |   |
|   | Sep 2001     | 0.55 (0.51–0.60) | 0.19 (0.17–0.20) | 3.0  | 2 | 0.31 (0.18–0.45) | 0.19 (0.16–0.22) | 1.6  | 2 |
|   | Mar 2002     | 4.58 (3.16–5.30) | 2.20 (1.49–3.00) | 2.1  | 3 | 1.21 (0.97–1.51) | 1.01 (0.80–1.15) | 1.2  | 3 |
|   | Jun–Jul 2002 | 4.09 (1.50–8.42) | 1.69 (0.63–2.50) | 2.5  | 3 | 0.56 (0.54–0.61) | 0.47 (0.41–0.55) | 1.2  | 3 |
| Chl b<br>(µg l <sup>-1</sup> )                                      | Mar 2001     | ND               | ND               | ND   |   | ND               | ND               | ND   |   |
|   | Sep 2001     | 0.04 (0.04–0.05) | 0.02 (0.02–0.02) | 2.5  | 2 | 0.07 (0.02–0.13) | 0.02 (0.01–0.02) | 4.0  | 2 |
|   | Mar 2002     | 0.14 (0.10–0.16) | 0.08 (0.07–0.08) | 1.8  | 3 | 0.09 (0.08–0.10) | 0.12 (0.11–0.15) | 0.7  | 3 |
|   | Jun–Jul 2002 | 1.49 (0.28–3.81) | 0.26 (0.09–0.38) | 5.0  | 3 | 0.07 (0.06–0.07) | 0.06 (0.05–0.07) | 1.1  | 3 |
| Chl c<br>(µg l <sup>-1</sup> )                                      | Mar 2001     | ND               | ND               | ND   |   | ND               | ND               | ND   |   |
|   | Sep 2001     | 0.08 (0.07–0.09) | 0.03 (0.02–0.03) | 3.0  | 2 | 0.02 (0.02–0.03) | 0.02 (0.02–0.02) | 1.3  | 2 |
|   | Mar 2002     | 0.63 (0.43–0.74) | 0.25 (0.23–0.30) | 2.4  | 3 | 0.18 (0.14–0.23) | 0.16 (0.13–0.19) | 1.1  | 3 |
|   | Jun–Jul 2002 | 0.45 (0.22–0.75) | 0.26 (0.09–0.44) | 2.0  | 3 | 0.08 (0.08–0.09) | 0.08 (0.07–0.09) | 1.1  | 3 |
| Phaeophytin a<br>(µg l <sup>-1</sup> )                              | Mar 2001     | ND               | ND               | ND   |   | ND               | ND               | ND   |   |
|   | Sep 2001     | 0.12 (0.11–0.13) | 0.04 (0.04–0.04) | 3.3  | 2 | 0.11 (0.05–0.18) | 0.04 (0.03–0.05) | 2.7  | 2 |
|   | Mar 2002     | 0.42 (0.17–0.85) | 0.09 (0.03–0.16) | 5.1  | 3 | 0.09 (0.06–0.13) | 0.02 (0.02–0.04) | 27.1 | 3 |
|   | Jun–Jul 2002 | 0.00 (0.00–0.01) | 0.01 (0.00–0.02) | ~1   | 3 | 0.03 (0.02–0.04) | <0.01            | 18.2 | 3 |
| <i>Synechococcus</i> spp.<br>(10 <sup>3</sup> ml <sup>-1</sup> )    | Mar 2001     | 4.8 (4.7–5.0)    | 4.5 (4.1–4.8)    | 1.1  | 2 | 2.2 (2.2–2.2)    | 2.5 (2.2–2.7)    | 0.9  | 2 |
|   | Sep 2001     | 56.8 (53.1–60.1) | 42.8 (41.8–43.8) | 1.1  | 2 | 82.0             | 73.9             | 1.1  | 1 |
|   | Mar 2002     | 8.2 (2.0–13.5)   | 5.6 (3.4–8.2)    | 1.3  | 3 | 6.2 (3.6–7.8)    | 5.5 (3.4–7.8)    | 1.1  | 3 |
|   | Jun–Jul 2002 | 20.9 (9.1–34.7)  | 31.8 (16.6–41.8) | 0.6  | 3 | 33.3 (30.3–36.1) | 26.1 (15.8–34.1) | 1.4  | 3 |
| Picoeukaryotes<br>(10 <sup>3</sup> ml <sup>-1</sup> )               | Mar 2001     | 10.2 (9.8–10.7)  | 11.6 (10.0–13.1) | 0.9  | 2 | 3.1 (2.9–3.3)    | 2.8 (2.6–2.9)    | 1.1  | 2 |
|   | Sep 2001     | 1.0 (0.9–1.0)    | 0.8 (0.7–0.8)    | 1.1  | 2 | 1.3              | 1.1              | 1.1  | 1 |
|   | Mar 2002     | 1.8 (1.4–2.7)    | 1.1 (0.2–2.5)    | 3.5  | 3 | 1.2 (0.8–1.7)    | 0.9 (0.5–1.4)    | 1.4  | 3 |
|   | Jun–Jul 2002 | 4.1 (0.6–8.0)    | 9.5 (2.2–22.1)   | 0.5  | 3 | 1.1 (1.1–1.3)    | 0.7 (0.5–0.9)    | 1.8  | 3 |
| AFLAG<br>(10 <sup>5</sup> l <sup>-1</sup> )                         | Mar 2001     | ND               | ND               | ND   |   | ND               | ND               | ND   |   |
|   | Sep 2001     | 3.5 (1.9–5.1)    | 0.8 (0.6–1.0)    | 3.3  | 2 | 1.8 (1.2–2.4)    | 1.4 (0.8–1.9)    | 1.1  | 2 |
|   | Mar 2002     | 19.2 (15.9–24.3) | 3.1 (2.2–4.0)    | 6.1  | 3 | 6.7 (3.5–10.9)   | 5.1 (4.0–6.2)    | 1.2  | 3 |
|   | Jun–Jul 2002 | 118.6 (11.3–299) | 9.8 (3.4–18.5)   | 18.5 | 3 | 9.7 (4.2–12.5)   | 3.4 (3.2–3.7)    | 2.8  | 3 |
| HFLAG<br>(10 <sup>5</sup> l <sup>-1</sup> )                         | Mar 2001     | ND               | ND               | ND   |   | ND               | ND               | ND   |   |
|   | Sep 2001     | 2.3 (1.3–3.3)    | 0.6 (0.5–0.7)    | 3.0  | 2 | 2.0 (0.8–3.1)    | 0.3 (0.1–0.6)    | 4.0  | 2 |
|   | Mar 2002     | 5.9 (4.3–8.3)    | 0.9 (0.7–1.1)    | 7.4  | 3 | 0.6 (0.3–1.0)    | 0.2 (0.2–0.4)    | 2.7  | 3 |
|   | Jun–Jul 2002 | 7.8 (1.3–18.4)   | 1.2 (0.5–1.8)    | 7.7  | 3 | 0.7 (0.3–1.4)    | 0.4 (0.2–0.5)    | 1.8  | 3 |
| NO <sub>3</sub> <sup>-</sup> + NO <sub>2</sub> <sup>-</sup><br>(µM) | Mar 2001     | 1.09 (1.01–1.16) | 0.77 (0.75–0.79) | 1.4  | 2 | 1.76 (1.65–1.86) | 1.50 (1.39–1.62) | 1.2  | 2 |
|   | Sep 2001     | 0.32 (0.15–0.48) | 0.25 (0.19–0.31) | 1.2  | 2 | 0.24 (0.19–0.29) | 0.18 (0.15–0.21) | 1.3  | 2 |
|   | Mar 2002     | 2.16 (0.61–4.24) | 1.35 (0.31–2.94) | 1.8  | 3 | 1.03 (0.76–1.53) | 0.90 (0.45–1.38) | 2.0  | 2 |
|   | Jun–Jul 2002 | 0.37 (0.04–0.86) | 1.92 (0.73–3.12) | 0.1  | 3 | 0.13 (0.01–0.35) | 0.05 (0.00–0.16) | 2.7  | 3 |

Table 2 (continued)

| Parameter                             | Date         | Barcelona        |                  |     |   | Banyuls          |                  |     |   |
|---------------------------------------|--------------|------------------|------------------|-----|---|------------------|------------------|-----|---|
|                                       |              | SML              | SSW              | EF  | n | SML              | SSW              | EF  | n |
| PO <sub>4</sub> <sup>3-</sup><br>(µM) | Mar 2001     | ND               | ND               | ND  |   | ND               | ND               | ND  |   |
|                                       | Sep 2001     | 0.15 (0.14–0.16) | 0.10 (0.09–0.11) | 1.5 | 2 | 0.09 (0.07–0.10) | 0.09 (0.07–0.10) | 0.8 | 2 |
|                                       | Mar 2002     | 0.50 (0.33–0.64) | 0.20 (0.11–0.33) | 2.9 | 3 | 0.06 (0.05–0.08) | 0.01 (0.00–0.03) | 3.6 | 3 |
|                                       | Jun–Jul 2002 | ND               | ND               | ND  |   | ND               | ND               | ND  |   |
| SPM<br>(mg l <sup>-1</sup> )          | Mar 2001     | 23.7 (23.6–23.7) | 12.2 (10.2–14.2) | 2.0 | 2 | ND               | ND               | ND  |   |
|                                       | Sep 2001     | 22.5 (20.6–26.5) | 18.1 (13.0–23.1) | 1.4 | 2 | 11.8 (11.3–12.2) | 15.3 (11.2–19.4) | 0.8 | 2 |
|                                       | Mar 2002     | ND               | ND               | ND  |   | ND               | ND               | ND  |   |
|                                       | Jun–Jul 2002 | 20.6 (13.0–33.3) | 12.2 (11.5–12.8) | 1.7 | 3 | 12.5 (10.8–13.8) | 7.0 (6.0–9.3)    | 1.9 | 3 |

Table 3. Comparison of EF measured at Barcelona and Banyuls sites based on whole data set. (1): Wilcoxon test, (2): Mann-Whitney *U*-test. Asterisks denote significant levels where \**p* ≤ 0.05, \*\**p* ≤ 0.01, \*\*\**p* ≤ 0.001. n: number of samples; NS: not significant; NA: not applicable (n < 5)

| Parameter   | Barcelona |       |    | Banyuls |       |    | Barcelona + Banyuls |       |    | EF ratio Barcelona:<br>Banyuls (2) |
|---|-----------|-------|----|---------|-------|----|---------------------|-------|----|------------------------------------|
|   | EF        | p (1) | n  | EF      | p (1) | n  | EF                  | p (1) | n  |                                    |
| VLP   | 3.1       | NA    | 4  | 1.1     | NA    | 4  | 2.1                 | NS    | 8  | NS                                 |
| Bacteria  | 1.1       | NS    | 10 | 1.1     | *     | 10 | 1.1                 | **    | 20 | NS                                 |
| %HDNA   | 1.0       | NS    | 9  | 1.1     | NS    | 10 | 1.0                 | NS    | 20 | NS                                 |
| CFU   | 48.5      | NS    | 10 | 9.1     | **    | 10 | 27.8                | *     | 19 | NS                                 |
| BP leucine  | 1.1       | NS    | 8  | 1.1     | NS    | 7  | 1.1                 | NS    | 15 | NS                                 |
| Chl <i>a</i>  | 2.5       | *     | 8  | 1.3     | *     | 8  | 1.9                 | **    | 16 | *                                  |
| Chl <i>b</i>  | 3.2       | *     | 8  | 1.7     | NS    | 8  | 2.4                 | NS    | 16 | *                                  |
| Chl <i>c</i>  | 2.4       | *     | 8  | 1.1     | NS    | 8  | 1.8                 | *     | 16 | **                                 |
| Phaeophytin <i>a</i>  | 4.4       | *     | 8  | 9.2     | *     | 8  | 7.4                 | ***   | 16 | NS                                 |
| <i>Synechococcus</i> spp.                                   | 1.1       | NS    | 10 | 1.2     | *     | 9  | 1.1                 | NS    | 19 | NS                                 |
| Picoeukaryotes  | 1.6       | NS    | 10 | 1.4     | *     | 9  | 1.5                 | NS    | 19 | NS                                 |
| AFLAG   | 10.4      | *     | 8  | 3.7     | *     | 8  | 6.1                 | **    | 16 | *                                  |
| HFLAG   | 6.6       | *     | 8  | 3.7     | *     | 8  | 5.1                 | **    | 16 | NS                                 |
| NO <sub>3</sub> <sup>-</sup> + NO <sub>2</sub> <sup>-</sup> | 1.2       | NS    | 10 | 1.3     | **    | 9  | 1.3                 | **    | 19 | NS                                 |
| PO <sub>4</sub> <sup>3-</sup>                               | 2.3       | *     | 5  | 2.5     | NS    | 5  | 2.4                 | *     | 10 | NS                                 |
| SPM   | 1.7       | *     | 7  | 1.5     | NS    | 5  | 1.6                 | *     | 12 | NS                                 |

the SML and SSW, respectively. EF for AFLAG and HFLAG increased from 1 to 43 and from 1 to 16, respectively. EF for SPM also increased during this period from 1 to ~3.

### Microbial structure of SML and relationships with SSW

The projection of all Banyuls and Barcelona samples on the first 2 axes of PCA is presented in Fig. 3a. The first axis accounted for 41.7% of the total variance, while the second accounted for 18.6%. There was a clear outlier in the Barcelona samples, which corresponded to the 27 June 2002 SML sample for which high EF were observed (see above). Overall, and irrespective of this particular sample, Banyuls samples tended to group together much more than Barcelona samples (Fig. 3a). Temporal variability was thus much higher at Barcelona than at Banyuls. More specifically,

temporal changes in the Barcelona samples were mostly associated with the March 2002 cruise. Moreover, we observed significant daily changes in the characteristics of both SML and SSW samples during this cruise (Table 2). That is, samples collected on 20 March 2002 differed markedly in composition from those collected on 19 and 21 March 2002.

Separate projections of samples from Barcelona and Banyuls on the first 2 axes of PCA are presented in Fig. 3b & c, respectively. The first and second axis of the Barcelona PCA accounted for 37.5 and 22.1% of total variance, respectively. The first and second axis of the Banyuls PCA accounted for 40.1 and 18.2% of total variance, respectively. The Barcelona SML and SSW samples corresponding to the same sampling date clearly tended to group together. The only exception was again 27 June 2002 (MB10 and UB10), when SML and SSW samples differed markedly. There was also a connection between the characteristics of the SML and SSW samples in Banyuls. However, this relationship

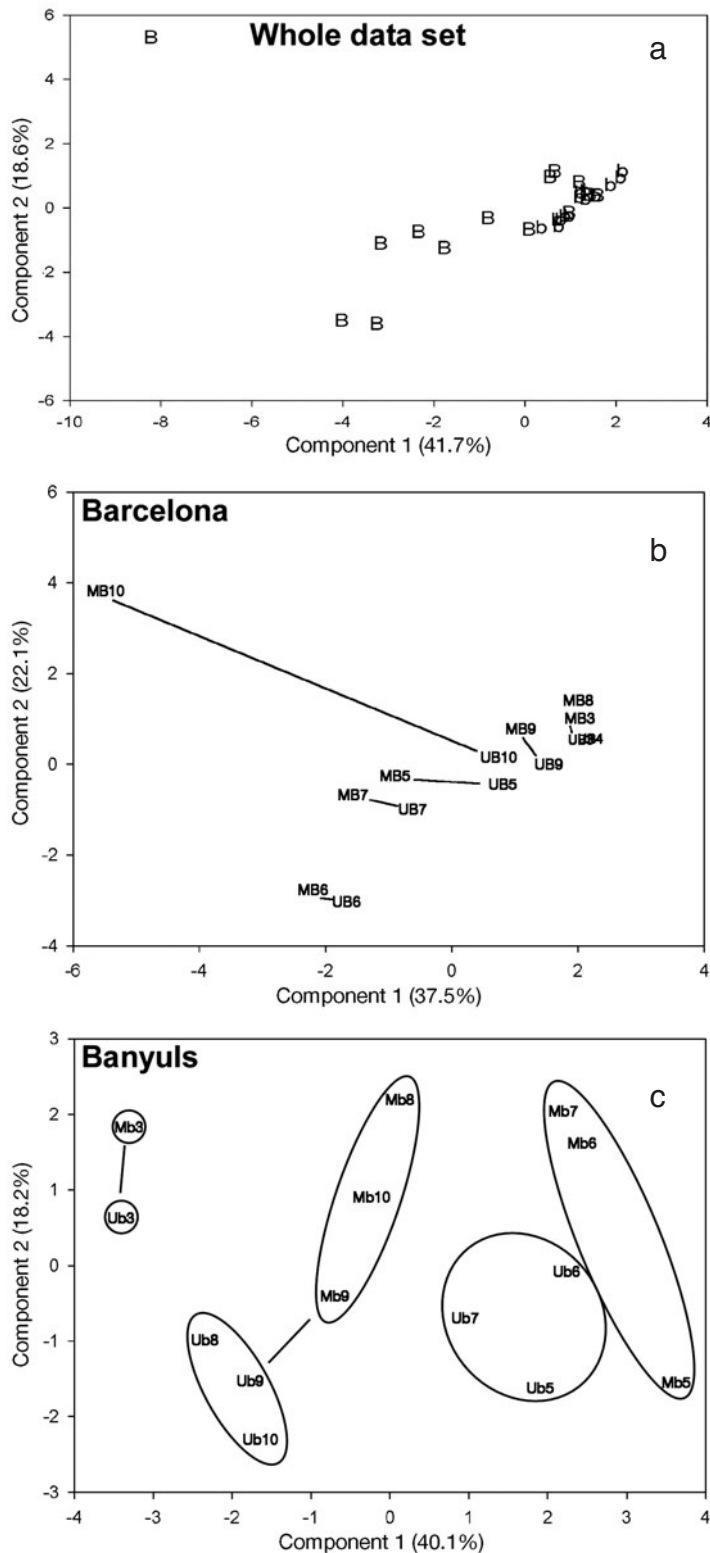


Fig. 3. Principal component analysis of (a) whole data set from Barcelona (B) and Banyuls (b); (b) Barcelona samples from the SML (MB prefix) and SSW (UB prefix); (c) Banyuls samples from the SML (Mb prefix) and SSW (Ub prefix). See Table 1 for sample codes and corresponding dates

was less apparent for individual daily samples and more obvious for each cruise. For each Banyuls cruise, SML and SSW samples tended to form distinct related groups. Moreover, and based on the whole data set, the Mantel test showed a significant correlation ( $p < 0.0001$ ) between similarity matrices of the SML and SSW samples. This further confirmed that the characteristics of the SML and SSW are highly significantly correlated. When relationships between the SML and SSW were examined on the basis of the whole data set for each parameter, we observed significant and positive correlations for all parameters except AFLAG and SPM (Table 4).

On the basis of whole data set, several relationships were found between parameters in both the SML and SSW (Table 5). Numbers of significant relationships observed for the SML and SSW were fairly similar ( $n = 40$  and  $36$ , respectively) (Table 5). A total of 15 correlations were specific to the SML and 11 to SSW. Chl *a* correlated with the greatest number of parameters in both the SML and SSW (10 and 8 parameters, respectively). Chl *a* was correlated with bacterial abundance and production in both layers, but negatively correlated with *Synechococcus* spp. abundance only in the SML. Bacterial abundance was correlated with bacterial production in SSW but not in the SML. CFU abundance was correlated with more parameters in the SML ( $n = 6$ ) than in SSW ( $n = 2$ ). VLP appeared to be negatively correlated with chl *a* and AFLAG, and positively correlated with *Synechococcus* spp., only in SSW. SPM was related to only 1 parameter in the SML (i.e. HFLAG) and none in SSW.

Table 4. Correlation coefficients between parameters in the SML and SSW (Spearman's rank correlation). Asterisks denote significant levels, where \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ . n: number of samples; NS: not significant

| Parameter                       | r     | p   | n  |
|---------------------------------|-------|-----|----|
| Virus                           | 0.923 | *   | 8  |
| Bacteria                        | 0.915 | *** | 20 |
| %HDNA                           | 0.840 | *** | 20 |
| CFU                             | 0.824 | *** | 19 |
| BP leucine                      | 0.604 | *   | 15 |
| Chl <i>a</i>                    | 0.918 | *** | 16 |
| Chl <i>b</i>                    | 0.743 | **  | 16 |
| Chl <i>c</i>                    | 0.909 | *** | 16 |
| Phaeophytin <i>a</i>            | 0.833 | **  | 16 |
| <i>Synechococcus</i> spp.       | 0.949 | *** | 19 |
| Picoeukaryotes                  | 0.711 | **  | 19 |
| AFLAG                           | 0.488 | NS  | 16 |
| HFLAG                           | 0.760 | **  | 16 |
| $\text{NO}_3^- + \text{NO}_2^-$ | 0.735 | **  | 19 |
| $\text{PO}_4^{3-}$              | 0.882 | **  | 10 |
| SPM                             | 0.364 | NS  | 12 |





## DISCUSSION

Enrichment of microorganisms in the SML could result from different processes: (1) enhanced growth in the SML due to elevated resource availability (Lion & Leckie 1981), (2) transport from subsurface waters to the SML by bubble scavenging of surface-active organic solutes and particulate matter (Carlucci & Bezdek 1972), or (3) lower loss processes in the SML due to inhibition of predators (viruses, heterotrophic protozoa, and metazoa). VLP, heterotrophic bacteria, autotrophic cells, and heterotrophic nanoflagellates are likely to be affected differently by these processes due to specific physical characteristics, growth requirements, and physiological adaptations. The enrichment of the SML in any of these members of the microbial community could therefore result in an SML-specific food web structure.

### Virus-like particles

In the present study, the enrichment of VLP in the SML was generally low (mean EF = 1.1), except for September 2001 off Barcelona (mean EF = 5.1). Using a polyester screen or rotating drum skimmer to sample the SML at coastal and open ocean sites in the north Atlantic Ocean, Kuznetsova et al. (2004) found an average EF for VLP of ~1.5. In our study, the virus:bacterium ratio (VBR) was similar between the SML and SSW, and ranged between 0.3 and 130, except for September 2001 when higher VBR were observed in the SML relative to SSW (130 and 27, respectively) off Barcelona. This confirms an earlier observation by Kuznetsova et al. (2004), who reported that VBR were similar between the SML and SSW (on average 2.6 and 2.4, respectively) and fairly constant on a spatial scale.

We did not observe any correlation between VLP and bacterial abundance in the SML and SSW. This is in agreement with Kuznetsova et al. (2004), but in contrast to the general view that these 2 parameters are strongly related in pelagic systems (Culley & Welschmeyer 2002 and references therein). Interactions between VLP and their hosts (heterotrophic bacteria, pico- to microautotrophic cells, and HFLAG) could be influenced by high levels of UV radiation in the SML and SSW. Based on light attenuation coefficients determined at the Banyuls site in summer (W. H. Jeffrey unpubl. data), we could estimate that intensities of UVB (at 305 nm) and UVA (at 340 nm) radiation were 24 and 14 % lower in SSW (at 50 cm depth) than in the SML. UV radiation can act in contrasting ways, either by decreasing viral abundance and infectivity (Suttle & Chen 1992, Noble & Fuhrman 1997, Wilhelm et al. 1998a,b), or by inducing the lytic cycle in lysogenic viruses (Weinbauer

2004). Furthermore, UV radiation could inactivate viruses or inhibit host activities, resulting in a disruption of the common relationship observed between viruses and host microorganisms.

### Heterotrophic bacterial abundance and activity

Bacterial abundance in the SML was not enriched at the Barcelona site; in contrast, low but significant enrichments were observed at the Banyuls site. A recent study that used epifluorescence microscopy to enumerate bacteria and a polyester screen to collect SML water reported comparably low EF for heterotrophic bacteria along a transect from coastal to open ocean waters (mean = 1.3) (Kuznetsova et al. 2004). As observed in our study, Bell & Albright (1982) reported significant correlations between bacterial abundance in the SML and SSW, which may be indirect evidence of the accumulation of bacteria in the SML due to passive transport from the underlying water column. This is in agreement with the absence of any correlation between bacterial abundance and production in the SML (Table 5), and with the generally high correlation of different parameters between the SML and SSW (Fig. 3).

Besides total bacterial abundance, we also determined the fraction of bacterial cells with a high nucleic acid content. These cells are reported to be more active (Lebaron et al. 2001) and more prone to grazing (Gasol et al. 1999). We did not observe differences in the HDNA fraction of the bacterial community between the SML and SSW samples at either site. Similarly, studies that used microautoradiography to detect metabolizing bacteria in the SML and SSW have reported lower or similar percentages of active cells in the SML compared to SSW (Bailey et al. 1983, Hermannson & Dahlback 1983, Williams et al. 1986). Using propidium iodide to enumerate bacteria with damaged cell membranes, Kuznetsova et al. (2004) found that the percentage of bacteria with damaged membranes was frequently lower in the SML than in SSW.

A number of reports suggest that bacterioneuston are not more active than bacterioplankton (Dietz et al. 1976, Bell & Albright 1982, Bailey et al. 1983, Carlucci et al. 1985). These results are in agreement with the bacterial production values reported herein, and support the assumption that most bacteria accumulate in the SML by physical processes such as flotation. However, lower bacterial production in the SML (as reported in our study) does not necessarily indicate a lower uptake of dissolved organic carbon: bacterial growth efficiencies (BGE) may differ in the SML compared to SSW. At present, the number of comparisons of BGE between the SML and SSW is too low to allow a conclusion to be drawn (Obenosterer et al. 2005).

### Culturable bacteria

CFU represent the fraction of viable bacterial cells which are able to grow on a synthetic culture medium. However, in most marine systems, they represent only a small fraction of total viable cells. Enrichment of culturable bacteria in the SML has been frequently reported (Dietz et al. 1976, Kjelleberg et al. 1979, Dahlbäck et al. 1981). The higher yield of culturable bacteria in the SML is generally explained by the fact that CFU yield increases with the nutrient concentration of the system (Zdanowski & Figueiras 1999). The EF for CFU obtained in the present study were the highest values reported for all parameters measured. The high abundance of CFU in the SML could result from electrostatic interactions between living bacteria and rising particles induced by bubble flotation. Because the pH of seawater is higher than the bacterial isoelectric point, bacteria in seawater have a net negative charge and passively attract cations (Grasland et al. 2003). This property may facilitate the adsorption of viable bacteria (including culturable cells) to rising particles as dead cells are not able to maintain their membrane potential.

Changes reported in the EF of CFU off Barcelona in June 2002 over 3 consecutive days demonstrate that CFU is a highly dynamic component of the total bacterial community. Surprisingly, CFU represented 100% of total bacterial counts on 27 June 2002. This exceptional situation was probably not due to an intensive proliferation of culturable bacteria at this time, but more likely to a passive accumulation of cells from underlying waters and from the release of wastewaters in this area (Fig. 1). Although we do not have taxonomic information on isolates from this date from Barcelona, the mean percentage of Gram-positive cells for isolates collected at this station during the year was much higher (27%) than that reported for underlying waters (6%) and isolates from the SML at Banyuls (6%) (Agogué et al. 2005). A high percentage of Gram-positive cells is generally found in wastewaters (Snaird et al. 1997). Consequently, we hypothesize that the stability of the water column during the sampling period, and the release of wastewater in proximity of the sampling area before or at the time of sampling, might have contributed to the accumulation of non-marine culturable bacteria in the SML.

### Autrophic cells

High chl *a* enrichments in the SML are occasionally reported in polluted areas (Daumas et al. 1976) and in slicks (Carlson 1982). However, chl *a* concentrations are more often reported to be only moderately higher

in the SML relative to SSW (Zaitsev 1971, De Souza Lima & Chrétiennot-Dinet 1984, Williams et al. 1986). Higher enrichments of chl *a* were observed at the most productive site (i.e. Barcelona) (Tables 2 & 3). As for bacteria, chl *a* concentrations in the SML were closely correlated to those of SSW, supporting the hypothesis of passive accumulation of phytoplankton at the surface rather than proliferation in the SML. Based on the whole data set, the ratio of bacterial C biomass to algal C biomass was significantly lower in the SML (0.6) than in SSW (0.9) ( $p = 0.0035$ ,  $n = 16$ , Wilcoxon rank sum test). Differences in this ratio between the 2 layers were more pronounced off Barcelona (0.3 vs. 0.8) than at Banyuls (0.8 vs. 0.9). The phaeophytin *a*:chl *a* ratio was highly variable depending on season (range: 0 to 0.40), but was significantly higher in the SML compared to SSW ( $p = 0.003$ ,  $n = 14$ , Wilcoxon rank sum test). High contributions of pheopigments in the SML could have resulted from: (1) photo-oxidation of chlorophyll under high surface light intensities, (2) a higher grazing rate and pigment digestion by zooneuston compared to zooplankton, or (3) an accumulation of less active or dead phytoplankton at the surface. Hardy & Apts (1984) rejected the latter because they found a unique phytoneuston species composition and similar percentage of dead cells in the phytoneuston compared to the phytoplankton.

The species composition of microalgae in the SML frequently differed from that in SSW (Williams et al. 1986, Hardy & Apts 1984, 1989). The tendency towards a dominance of small flagellates and small pennate diatoms in the SML has been previously reported (Hardy 1973, Hardy & Apts 1984, 1989). In our study, we did not observe significant differences in the chl *b*:chl *c* ratio between SML and SSW ( $p = 0.850$ ,  $n = 16$ , Wilcoxon rank sum test), which suggested that there was no preferential enrichment of chlorophytes or chromophytes in the SML. However, we observed that the contribution of AFLAG C biomass to overall algal C biomass was significantly higher in the SML than in SSW (25.7 vs. 12.2%) ( $p = 0.0113$ ,  $n = 16$ , Wilcoxon rank sum test). Differences between the 2 layers were more pronounced off Barcelona (26.5 vs. 9.1%) than at Banyuls (24.9 vs. 15.3%). AFLAG was also the only biological parameter for which no relationship in abundance between the SML and SSW was observed (Table 4). This fact supports the hypothesis that AFLAG do not only accumulate in the SML by advection but also proliferate in this environment. In support of this, we observed a high rate of enrichment in AFLAG at the Barcelona site during 3 consecutive days in June 2002 (Fig. 2). AFLAG cannot be viewed only as primary producers because mixotrophy is largely extended in autotrophic planktonic protists under conditions of inorganic nutrient limitation (Stoecker 1998). In coastal marine envi-

ronments, AFLAG may account for >50% of flagellate bacterivory in summer and somewhat less in winter (Epstein & Shiaris 1992, Hall et al. 1993, Havskum & Riemann 1996).

At present, little information is available on the abundance of picoautotrophs in the SML. High (~100%), moderate (~33%), and low (<8%) contributions of picophytoplankton to algal C biomass were observed in SSW at both sites in September 2001, June–July 2002 and March 2002, respectively. Our results obtained from flow cytometry demonstrated that *Synechococcus* spp. and picophotosynthetic eukaryotes were significantly but weakly enriched in the SML at Banyuls, but not at Barcelona. Consequently, the contribution of picoautotrophic cells to algal C biomass in the SML was in most cases fairly similar to that measured in SSW excepted off Barcelona in June 2002. During this cruise (characterized by a high enrichment of chl *a* in the SML), the contribution of picoautotrophic cells to total algal C biomass decreased in the SML (from 14 to 5%) and increased in SSW (from 15 to 58%) over the 3 d period. In contrast, the contribution of AFLAG to algal C biomass increased in the SML (from 17.2 to 72.5%) and decreased in SSW (from 32.3 to 11.8%) during the same period.

### Heterotrophic nanoflagellates

Our results showed that HFLAG are one of the most enriched microorganisms in the SML. The accumulation of HFLAG may have significant consequences for the structure of microbial food webs operating in the SML. Grazing activity of HFLAG was not measured in this study and, to our knowledge, has never been measured in the SML. However, the lower ratio between bacterial abundance and HFLAG (11 640 vs. 31 700) observed in the SML relative to SSW suggested that grazing rates of HFLAG on bacteria are higher in the SML. A positive correlation between the abundance of HFLAG and heterotrophic bacteria was observed in both the SML and SSW. This suggested that the low enrichment of bacteria observed in our study might have resulted from a high grazing activity of HFLAG. Microzooplankton were not considered in this study, due to the inadequacy of the metal screen to sample this type of organism (Agogu e et al. 2004), but should be considered in further studies to investigate their role in the regulation of nanoplankton at the air–water interface.

### CONCLUSION

We have shown that despite considerable differences in SSW characteristics between the Barcelona

and Banyuls sites, the EF of biological and chemical parameters were similar at both sites with the exceptions of chl *a*, *b*, *c*, and AFLAG. For those parameters, a greater EF was found at the more productive area (i.e. Barcelona site). Most of the biological parameters measured in the SML correlated well with corresponding SSW concentrations, which supported the idea that SSW are the major source of microorganisms for the SML. However, AFLAG did not fit into this general rule, which suggested that these organisms may also proliferate in the SML and do not only accumulate by physical transport.

Our results also clearly demonstrated that not all components of the microbial food web are equally enriched in the SML. Both heterotrophic and autotrophic organisms in the SML were less enriched in the pico cell size fraction compared to the nano cell size fraction. AFLAG and HFLAG might be better adapted to survive and/or proliferate in the SML than smaller cells, due to higher resistance to stresses like UV (Buma et al. 2001). This can lead to some modifications in the functioning of the microbial food web. The other characteristic of the SML is a lower ratio of bacterial C biomass to algal C biomass relative to SSW. However, due to distortions in the metabolism of both communities in the SML, this change in biomass ratio does not necessarily mean that the production:respiration ratio is higher in the SML. A recent study showed that heterotrophic processes effectively dominate over autotrophic production in the SML at oligotrophic sites in the Mediterranean Sea, whereas autotrophic processes balanced or exceeded heterotrophic processes in SSW (Obenosterer et al. 2005). Further investigations are required to measure interactions between biological components in the SML (e.g. grazing rates of bacteria and autotrophic cells, viral infection), to fully understand the functioning of microbial food webs in this environment. Furthermore, these studies should be performed within a broad range of trophic conditions including offshore waters (oligotrophic systems) to determine the potential role of the SML in oceans.

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