

Field study of the chemical characterization of the upper ocean surface using various samplers

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Abstract

The sea surface microlayer (SML) is the seawater layer (about 100 μm thick) located at the air-ocean interface, usually enriched in organic and inorganic matter compared to the underlying water (UW) sampled between 5 cm and 50 cm depth. This article is aimed at providing novel data for a better quantification of the SML enrichment and knowledge of associated processes. First, the mean 1 to 100 cm depth profile for dissolved total carbohydrates (DTCHO) established from a set of 10 stations in coastal northwestern Mediterranean waters (off Barcelona and Banyuls-sur-mer) indicates that (1) this layer is not necessarily homogeneous as generally admitted and (2) an exploratory survey is needed before undertaking an extensive sampling, to determine the most appropriate depth for sampling UW. Second, two samplers, glass plate (GP) and metal screen (MS), are compared for their ability to collect several classes of biomolecules at the first 50 μm and 440 μm layers below the interface, respectively. GP is shown to collect more efficiently (1) hydrophobic amino acids and phytoplankton-derived detrital matter, as shown by statistical results on a set of eight stations, and (2) fatty acids originating from bacteria and continental higher plants (inferred from a lower number of stations). Thus, it is well adapted for studying interactions between ocean, atmosphere, and continent. On the contrary, MS is better adapted for recovering phytoplankton organisms, as shown by data for chlorophyll *a*, pheophytin *a*, and unsaturated fatty acids. DTCHO are equally sampled by GP and MS.

The role of the boundary layer at the water atmosphere interface is recognized as playing a major influence on exchanges of matter and energy at various time and space scales (Liss and Duce 1997). Chemical and biological processes modify the sea surface, as observed by various types of remote sensors (Hartwig and Herr 1984; Hühnerfuss et al. 1983). Most

of these processes are conditioned by the structure of the upper ocean surface where natural materials and pollutants accumulate. The first attempts to collect the sea surface microlayer (SML) were performed by Garrett (1965) and Harvey (1966), who used a metal screen and a ceramic-covered metal rotating skimmer, respectively. Thereafter, various sampler tools have been proposed, and in situ and laboratory comparisons have been realized (for a recent review, see Zuev et al. 2001). As a result, one major question was addressed by Dumas et al. (1976) and Carlson (1982): How are particulate and dissolved material partitioned at the surface?

The SML is a structure usually enriched in dissolved and particulate organic matter compared to the layers below. Data in literature appear as rather "disparate" (Carlson 1982), however. This comes, from the variety of samplers used to lift up the most superficial seawater layers. Samplers differ mostly by the thickness of the layer they collect and by the material they are made of. Screens, either of metal (Garrett 1965; Marty et al. 1979), Nutex (polyethylene) (Duce et al. 1972), or polyester (Kuznetsova and Lee 2001), sample the thickest layer (250 to 500 μm , according to the authors). Plates of glass (Harvey and

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Burzell 1972), Teflon (Larsson et al. 1974; Hardy et al. 1988; Falkowska 1999b), and rollers (Harvey 1966; Daumas et al. 1976) sample a thinner layer (35 to 50 μm). Filtration membranes (Hardy and Apts 1984; Agogu e et al. 2004) collect the thinnest layer (5 to 50 μm). Samplers differ by specific bias caused by their unequal ability to collect dissolved molecules and particles, due to their chemical nature or size.

The chemical composition of dissolved organic matter (DOM) in the upper first 500 μm , as inferred from all the types of samplers used, appears vertically structured by at least a gradient of hydrophobicity (Daumas et al. 1976; Carlson 1982; Hardy 1982; Estep et al. 1985; Falkowska 1999a, 1999b). The most hydrophobic molecular species are concentrated at the very top of the air-water interface as an almost permanent multimolecular layer (100 Å) that is composed mainly of natural surface-active compounds (Ćosović et al. 1977), such as lipids (mainly fatty acids) and hydrocarbons, including occasionally oil spill components (Marty and Saliot 1976; Marty et al. 1979; Saliot et al. 1991; Gašparović et al. 1998; Cincinelli et al. 2001). Below this film, a layer of varying thickness comprises hydrosoluble proteins, amino acids (Williams et al. 1986; Carlucci et al. 1991, 1992; Kuznetsova and Lee 2001), and carbohydrates (Williams et al. 1986; Compiano et al. 1993). Proteins that are more hydrophobic than carbohydrates are more enriched in SML.

The inherent space and time variability of the processes controlling SML structure contribute significantly to the heterogeneity of the data, for example, the patchiness of the planktonic populations (Carlucci et al. 1992; Kuznetsova and Lee 2001; Agogu e et al. 2004), the upward increasing turbulence (Carlson 1982; Falkowska 1999a), and the light-dependence of biological and photochemical processes (Momzikoff et al. 1983; Zhou and Mopper 1997), including the hour of sampling.

An additional cause of this "disparate" character comes from the variety of depths used to sample the underlying water (UW). SML enrichment in a given parameter is quantified by the ratio of its concentration in SML to that in UW, so that depth position is, thus, crucial. UW has been sampled, however, at depths scattered between 5 and 50 cm (according to many authors, Estep et al. 1985; Williams et al. 1986; Falkowska 1999a; Carlucci et al. 1991) as if this layer was homogeneous, while SML enrichment implies gradients that are caused by upward fluxes of organic matter.

At the beginning of the AIRWIN program (see *Acknowledgments* for project title), we addressed this initial question: How do the sampling techniques affect the results and comparisons between biological and chemical data? To answer this question, biologists tested various samplers to optimize the characterization of the biological structure of the upper ocean surface (Agogu e et al. 2004). In conclusion, they recommend using MS for sampling microorganisms and to avoid membranes because of significant bias. The group of chemists working on natural substances defined the following objectives, keeping in mind previous intercomparison exercises: (1) better

assessment of the chemical structure of the first meter of the water layer in order to define an optimum depth for UW; (2) study of the microlayer structure, collected by several different samplers, in terms of thickness of water collected and efficacy in collecting dissolved and particulate chemical moieties; (3) analysis of organic compounds at different levels, from bulk parameters to the molecular level, and use of distribution patterns of amino acids and fatty acids to describe various characteristics of the organic matter, such as origins, degree of freshness, and degradation state; and (4) recommending a strategy for studying the top ocean surface, taking into account operational constraints.

To reach these objectives, we participated in two field campaigns, in pristine and polluted Mediterranean sites, in Banyuls and off the harbor of Barcelona, respectively. Several samplers were used, such as glass plate (GP), metal screen (MS), and nylon screen (NS).

Material and procedures

Sampling sites and sampling strategy—Samples were collected at two coastal stations in the NW Mediterranean Sea: the Bay of Banyuls-sur-Mer (France) and off the Olympic Harbour in Barcelona (Spain) (Fig. 1).

The Bay of Banyuls-sur-Mer is oligotrophic (Medernach et al. 2001; Gr emare et al. 2003). Most Banyuls samples were collected at the entrance of the bay, at the long-term observation station SOLA, which is monitored weekly for core biogeochemical and physical parameters. The Barcelona site is moderately eutrophic and heavily impacted by the urban sewage sludge outfall (Bayona et al. 1991). Samples were collected early morning between 0700 and 1100 h (in overall calm weather conditions with a wind speed < 5 m/s) during two field cruises in 2001 (end of winter, 13–18 March, and end of summer, 5–13 September). Samples were taken from an inflatable boat to avoid motor oil contamination.

Sampling the upper ocean layer—At each site, 20 L samples of SML were simultaneously collected by each sampler: MS, NS, and GP, respectively. The MS consists of a stainless steel screen first described by Garrett (1965). The MS used here (mesh size: 1.25 mm; wire diameter: 0.36 mm), stretched over a 60 \times 80 cm steel frame (Daumas et al. 1976; Marty et al. 1979), was lowered vertically through the surface of the water then oriented horizontally and lifted through the SML, collecting a 440- μm thick layer. To collect 20 L, about 200 to 250 successive dips were carried out during 2 h. The NS used (mesh size: 100 μm ; wire diameter: 0.32 mm; 57% open space) was stretched over a 72 \times 50 \times 3 cm nylon frame and was operated as for MS. The GP (500 \times 250 \times 4 mm), thoroughly cleaned with ethanol and Milli-Q water, was vertically introduced across the SML and withdrawn vertically (Harvey and Burzell 1972). The excess water was allowed to drain for about 20 s by holding the GP in a vertical position, and then the surface film and water adhering to the plate were removed from both sides of the GP with a Teflon wiper blade. About 1 L, corre-

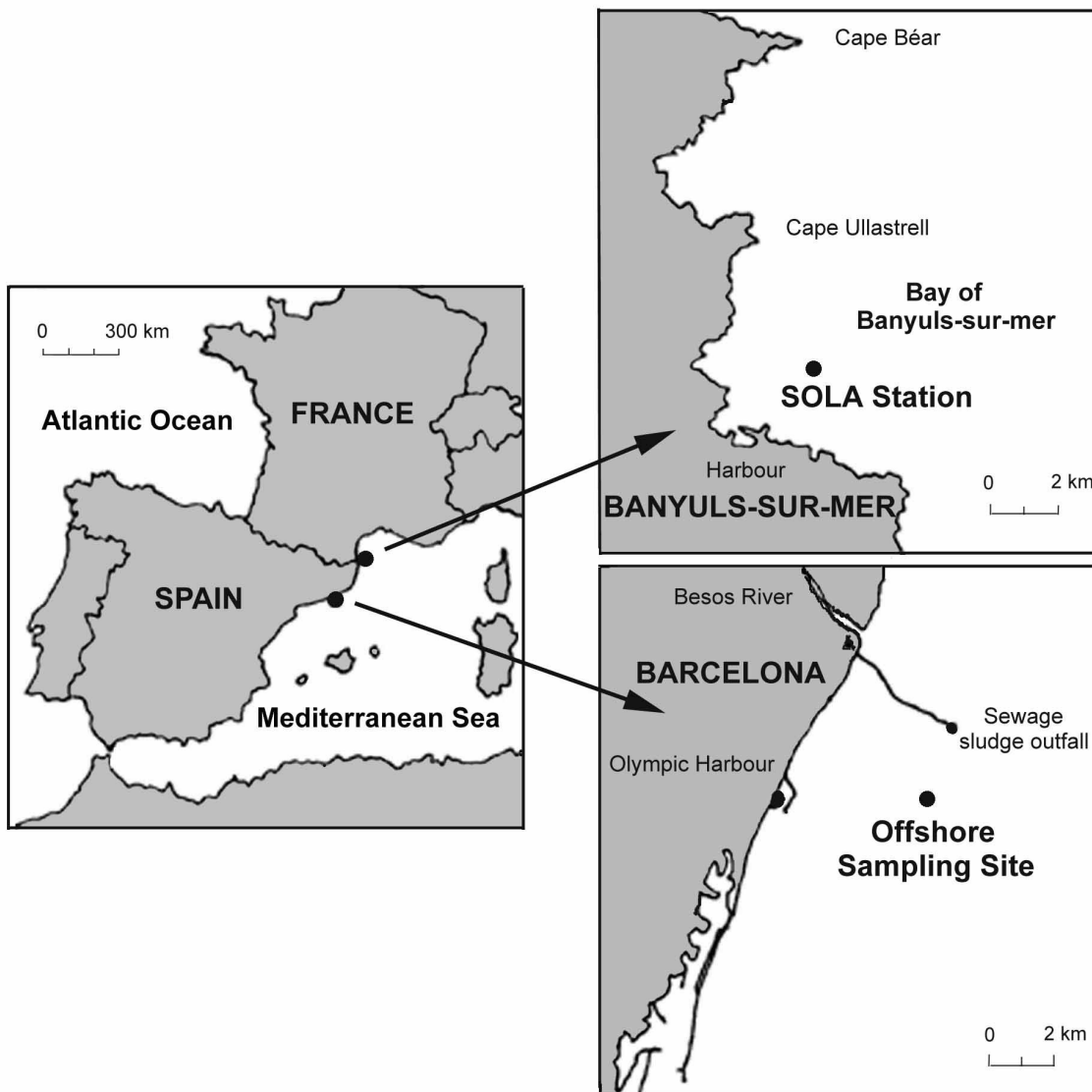


Fig. 1. Study area with the two sampling sites occupied during the AIRWIN program: off Banyuls-sur-mer (France) and off Barcelona (Spain)

sponding to the first 50 μm top surface, was collected within 1 h for each GP sampler. Samples from UW were collected by immersing a 5-L glass bottle and opening it at 0.5 m depth. All water samples were transported within 1 h of collection to the land-based laboratory, prefiltered on 200 μm void of mesh-bulking silk, and processed immediately.

The first meter below the surface was sampled by using a simple and robust device easy to handle at sea. It consists of 7 rigid Teflon tubes (5 mm internal diameter) perpendicular to a flat polypropylene floater with openings at 5, 10, 20, 30, 50, 70, 100 cm below the sea-surface, respectively, which allow for sampling at these depths. Seawater was collected with individual 50 mL syringes, immediately filtered on board onto two superposed Whatman GF/F glass fiber filters (precom-

busted at 400–450°C [10 h], and 2 p.1000 chlorhydric methanol washed), then transferred to the laboratory in an ice-box, and kept deep-frozen (-20°C) until analysis.

Analysis of bulk parameters: suspended particulate matter, particulate organic carbon, and dissolved organic carbon—Suspended particulate matter (SPM) was analyzed following the procedure of Aminot (1983) and particulate organic carbon (POC) and dissolved organic carbon (DOC) according to Cauwet (1975) and Sharp et al. (1993), respectively.

Analysis of nutrients—Samples for nitrate, nitrite, and phosphate were analyzed according to Woods et al. (1967) and to Tréguer and Le Corre (1975).

Analysis of chlorophyll a and pheophytin a—Seawater samples were filtered on GF/F glass fiber filters (47 mm diameter) and

precombusted 4 h at 450°C. Filters were immediately frozen in liquid nitrogen. Acetone extracts from filters were centrifuged. Supernatants were fluorimetrically assayed according to the method of Neveux and Lantoiné (1993) with a Perkin Elmer MPF66 model spectrofluorometer.

Analysis of dissolved amino acids and carbohydrates—Aliquots (400 μL) of seawater samples filtered on Whatman GF/F glass fiber filters (precombusted at 400–450°C, 10 h) were hydrolyzed in 6 N HCl (110°C, 20 h, under nitrogen) to liberate free amino acids from combined amino acids. Amino acids were analyzed at the molecular level by high-performance liquid chromatography fractionation of their *o*-phthalaldehyde (OPA) derivatives according to the procedure routinely used in the laboratory (Goutx et al. 2000). Briefly, a C18 column equipped with a guard-column (Alltech) was developed by 0.1 M Na acetate (10% methanol and 3 p.1000 tetrahydrofurane) as solvent A and a methanol gradient as solvent B. The individual concentrations of each one of the 19 amino acids identified (at a 10% precision level) were cumulated and represent the total dissolved amino acids (TDAA) amounts (expressed in μM). Amino acid percentages are expressed as mole percents.

Samples for carbohydrates were hydrolyzed in 0.1 N HCl (100°C, 20 h, under nitrogen) to liberate monomeric carbohydrates from polysaccharides. Carbohydrates were measured at the bulk level (7% precision) by the 2,4,6-tripyrindyl-s-triazine (TPITZ) colorimetric method (Myklestad et al. 1997). Concentrations are expressed as $\mu\text{M L}^{-1}$ of glucose-equivalent and referred to as total dissolved carbohydrates (TDCHO).

Analysis of dissolved and particulate lipids—10-L samples were used for lipid analyses. Particulate lipids were recovered by filtration on 142 mm diameter Whatman GF/F glass fiber filters (previously cleaned with appropriate organic solvents). Dissolved lipids were extracted by three sequential liquid-liquid extractions with dichloromethane, the latter at pH 2 to increase the recovery. The filters and the extracts were transferred frozen (-20°C) to the laboratory. Particulate lipids were extracted from filters following the Bligh and Dyer (1959) protocol. Particulate and dissolved fatty acids were transesterified with 14% boron trifluoride in methanol under argon (1 h at 65°C). The methyl esters were isolated on silica gel column, and then analyzed on a Varian 6000 gas chromatograph equipped with a polar capillary column BPX70 (30 m length, 0.22 mm inner diameter, 0.25 μm film thickness) and a flame ionization detector (the temperature-program was that routinely used in the laboratory [Brinis et al. 2004]). They were identified by gas chromatography/mass spectrometry (ionization energy 70 eV, source temperature 170°C) and quantified at a 10% precision level by using authentic standards and *n*-C23:1 acid as internal standard. The positions of the double bonds in the monounsaturated fatty acids were determined by gas chromatography/mass spectrometry after derivatization with dimethyldisulfide according to Scribe et al. (1988).

Statistical methods—The repeated measures analysis of variance (RMA) (Winer 1971) was chosen to compare GP and MS

in terms of their ability to collect the natural compounds studied. RMA is a parametric method particularly suitable when the amount of data is small, as here. In addition, it is more able than the nonparametric corresponding methods to answer the two questions that are to be addressed simultaneously, here: (1) do GP and MS give identical results? And (2) are the concentrations in SML and UW similar?

In this RMA model, we consider station ($n = 8$ in the case of DTAA and DTCHO, and varying according to the parameter) as a random factor and layer as a fixed factor ($n = 3$). For each station, the concentration of a given compound is measured once at each layer (GP, MS, and UW). We test for a null layer effect, splitting the 2 degrees of freedom *F* test into two one-degree of freedom planned comparisons, using contrasts and the local error term: H_{01} , there is no difference between the mean SML effect (GP + MS)/2 and the reference layer UW; H_{02} , there is no difference between the GP and MS samplers.

Systat software was used for all computations (Wilkinson 1989) on the log-transformed amino acids concentration (to remove the skewness of the score distributions). See Kirby (1993) for the reduced set of verification assumptions of the model.

A multivariate characterization of GP, MS, and UW groups of data were achieved by computing a multivariate canonical discriminant ordination of the amino acids data set (Cooley and Lohnes 1971). This was done by using some selected log-transformed variables centered by station, and the three layers as grouping categorical variable. The subset of amino acids used in the analysis was selected if at least one univariate RMA test indicated an associated probability less than or equal to 0.1. The method was used as a graphical tool only and corresponding multivariate testing was not performed, as the multinormality assumption was not met.

Assessment

DTCHO depth stratification—Biological, chemical, and physical arguments suggest that a small-scale chemical stratification may be found within the first meter below the surface (Hardy 1982). To our knowledge, no recent data documenting a possible vertical stratification of organic compounds in this layer are available. Position of the depth used for sampling UW is crucial, however, because even a 10-cm difference may significantly affect enrichment factors (EF) in the case of a pronounced vertical stratification within the first half-meter.

To check for this hypothesis we recorded 10 profiles between 5 and 100 cm depth (5 at Barcelona site and 5 at Banyuls, in March and September 2001) and measured DTCHO concentrations. Results are synthesized by calculating the average at each depth and using the respective standard errors (SE) to express the associated variability (Fig. 2). At Barcelona site, a gradual and pronounced decrease is found between 5 cm (1.82 μM) and 30 cm depth where the lowest concentrations are found (0.74 μM). Below 30 cm depth, concentrations are rather constant down to 100 cm depth (0.97 μM). Highest variability is located at 5 and 10 cm depth. Below this layer, SE are lower

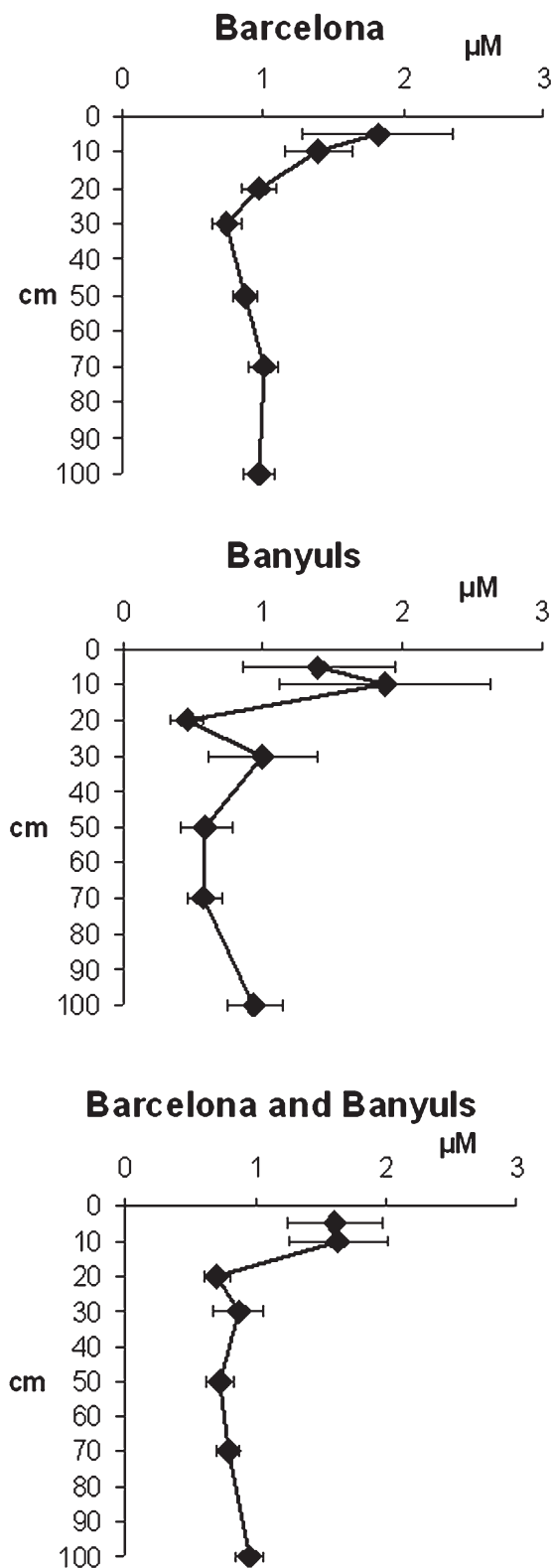


Fig. 2. Mean depth profiles for dissolved total carbohydrates (DTCHO) in the 0 to 100 cm layer below the air-ocean interface, established from 5 profiles at Barcelona, 5 at Banyuls, and from the pooled 10 profiles at both sites. Horizontal bars refer to corresponding SE.

and of the same order. At Banyuls, variations are less characteristic. The highest concentrations are located at the upper depths between 5 cm and 30 cm (1.40 and 1.01 μM , respectively), with an exception at 20 cm where concentrations drop to 0.46 μM . The highest SE coincide with the highest concentrations (5 cm and 30 cm depth). Lowest SE and concentrations are found between 50 cm and 70 cm depth (0.59 and 0.58 mM respectively). The profile inferred from the 10 cumulated stations shows that the highest means are located between 5 and 10 cm depth (1.61 and 1.63 μM , respectively). A rapid decline takes place between 10 and 20 cm (0.71 μM at 20 cm) and a rather constant concentration is found between 30 and 100 cm (0.87 and 0.96 μM , respectively). Below 20 cm depth, low means are found at 30 and 50 cm, and higher ones at 70 and 100 cm depth with SE of the same order. Present results thus show that a depth of 30 cm is appropriate to sample UW at Barcelona and of 50 cm at Banyuls, because lowest concentrations and variability coincide at these respective depths. When a common depth is required to be used as reference, 50 cm must then be preferred because both concentrations and variability are the lowest at both sites. One note also, these results justify a posteriori the choice of 50 cm we made at the beginning of the experiment.

Glass plate and metal screen comparisons—GP and MS have already been compared for their efficiency in recovering a large variety of particles and dissolved molecular species from SML. Such comparisons have still not been achieved when both amino acids and lipids were simultaneously studied at the molecular level. To provide a comparative background for our results, we used six additional variables (core parameters) measured simultaneously on the same seawater samples.

Data for GP and MS were compared by the RMA tests well adapted to the reduced number of stations available in our experiment. To get the number required by this method, we used a single set comprising all the stations explored at Barcelona and Banyuls sites during the two seasons studied.

Table 1 recapitulates for each variable the number of stations used, the periods of sampling, respective means, standard deviations, and value ranges for GP, MS, UW, and NS. Table 2 recapitulates the inferred EF with respective means, standard deviations, and value ranges. The results for the RMA tests are given in Table 3 for the six core parameters and in Table 4 for the amino acids.

Core variables—Concentrations of the core variables (Table 1) display a moderately large ecological gradient ranging from typical oligotrophic Mediterranean waters (Banyuls) to eutrophized neritic waters (Barcelona).

For nitrate + nitrite, significant contrasts are evidenced by RMA tests between GP and MS, and between SML (average score value for GP and MS) and UW (Table 3). SML is slightly enriched (EF = 1.4, $P = 0.0001$). Concentration is higher ($P = 0.02$) with GP than MS (EF = 1.6 and 1.2, respectively). Note the remarkably small scattering between GP and MS as indicated by the corresponding r square of a linear regression model ($R^2 = 0.940$).

Table 1. Chemical and biochemical variables measured with glass plate (GP), metal screen (MS), nylon screen (NS), and in underlying water (UW), with the number of stations used and the sampling seasons*

Variables†	n	Season	Mean (SD) [range]
GP			
NO ₃ +NO ₂ (μM)	12	March, September	1.35 (0.89) [0.31-3.04]
PO ₄ (μM)	6	September	0.22 (0.19) [0.11-0.61]
POC (mg L ⁻¹)	7	March, September	0.63 (0.50) [0.26-1.72]
DOC (mg L ⁻¹)	5	September	39.22 (73.26) [3.27-170.20]
SPM (mg L ⁻¹)	10	March, September	30.59 (14.21) [12.5-56-89]
Chl <i>a</i> (μg L ⁻¹)	6	September	0.25 (0.15) [0.16-0.54]
Pheo <i>a</i> (μg L ⁻¹)	6	September	0.09 (0.09) [0.02-0.27]
DTAA (μM)	8	March, September	5.52 (2.90) [1.87-9.21]
DTCHO (μM)	8	March, September	0.68 (0.38) [0.10-1.28]
DTFA (μg L ⁻¹)	3	March	32.00 (8.00) [24.00-40.00]
PTFA (μg L ⁻¹)	2	March	33.75 (3.64) [31.17-36.32]
Particulate PUFA (μg L ⁻¹)	3	March	4.03 (2.45) [1.90-6.70]
Particulate bacterial FA (μg L ⁻¹)	3	March	1.47 (0.87) [0.74-2.43]
Particulate terrigenous FA (μg L ⁻¹)	3	March	1.30 (0.86) [0.73-2.30]
Dissolved PUFA (μg L ⁻¹)	3	March	3.54 (0.48) [3.03-3.96]
Dissolved bacterial FA (μg L ⁻¹)	3	March	1.01 (0.30) [0.66-1.20]
Dissolved terrigenous FA (μg L ⁻¹)	3	March	0.62 (0.28) [0.41-0.94]
MS			
NO ₃ +NO ₂ (μM)	12	March, September	1.12 (0.77) [0.19-2.28]
PO ₄ (μM)	6	September	0.19 (0.20) [0.07-0.59]
POC (mg L ⁻¹)	7	March, September	0.31 (0.15) [0.21-0.63]
DOC (mg L ⁻¹)	5	September	5.76 (1.82) [3.84-8.28]
SPM (mg L ⁻¹)	10	March, September	20.89 (6.88) [10.04-23.75]
Chl <i>a</i> (μg L ⁻¹)	6	September	0.47 (0.17) [0.16-0.54]
Pheo <i>a</i> (μg L ⁻¹)	6	September	0.20 (0.16) [0.05-0.51]
DTAA (μM)	8	March, September	2.45 (2.62) [0.61-5.53]
DTCHO (μM)	8	March, September	1.00 (0.84) [0.33-2.86]
DTFA (μg L ⁻¹)	3	March	18.00 (6.00) [14.00-26.00]
PTFA (μg L ⁻¹)	2	March	49.13 (6.63) [44.44-53.81]
Particulate PUFA (μg L ⁻¹)	3	March	4.76 (2.64) [2.41-7.63]
Particulate bacterial FA (μg L ⁻¹)	3	March	0.88 (0.50) [0.71-1.43]
Particulate terrigenous FA (μg L ⁻¹)	3	March	0.70 (0.20) [0.48-0.89]
Dissolved PUFA (μg L ⁻¹)	3	March	3.77 (0.47) [3.29-4.23]
Dissolved bacterial FA (μg L ⁻¹)	3	March	0.42 (0.23) [0.22-0.67]
Dissolved terrigenous FA (μg L ⁻¹)	3	March	0.21 (0.11) [0.14-0.33]
UW			
NO ₃ +NO ₂ (μM)	12	March, September	0.90 (0.70) [0.15-2.04]
PO ₄ (μM)	6	September	0.17 (0.19) [0.08-0.58]
POC (mg L ⁻¹)	7	March, September	0.27 (0.15) [0.13-0.57]
DOC (mg L ⁻¹)	5	September	5.83 (2.33) [3.29-8.36]
SPM (mg L ⁻¹)	10	March, September	18.23 (8.56) [10.06-35.5]
Chl <i>a</i> (μg L ⁻¹)	6	September	0.26 (0.17) [0.16-0.61]
Pheo <i>a</i> (μg L ⁻¹)	6	September	0.08 (0.08) [0.03-0.23]
DTAA (μM)	8	March, September	4.31 (2.62) [0.51-8.04]
DTCHO (μM)	8	March, September	0.84 (0.60) [0.15-2.12]
DTFA (μg L ⁻¹)	3	March	8.00 (3.00) [5.00-13.00]
PTFA (μg L ⁻¹)	2	March	14.5 (9.74) [7.61-21.39]
Particulate PUFA (μg L ⁻¹)	3	March	0.79 (0.31) [0.70-1.13]
Particulate bacterial FA (μg L ⁻¹)	3	March	0.28 (0.28) [0.06-0.60]
Particulate terrigenous FA (μg L ⁻¹)	3	March	0.31 (0.43) [0.05-0.81]
Dissolved PUFA (μg L ⁻¹)	3	March	0.96 (0.73) [0.44-1.79]
Dissolved bacterial FA (μg L ⁻¹)	3	March	0.27 (0.08) [0.21-0.35]
Dissolved terrigenous FA (μg L ⁻¹)	3	March	0.17 (0.02) [0.16-0.20]
NS			
DTAA (μM)	8	March, September	5.75 (4.04) [2.08-10.94]
DTCHO (μM)	8	March, September	2.18 (1.20) [0.89-3.89]

*Mean concentrations are given with standard deviations (SD) and respective value ranges.

†POC, particulate organic carbon; DOC, dissolved organic carbon; SPM, suspended particulate matter; Chl *a*, chlorophyll *a*; Pheo *a*, pheophytin *a*; DTAA, dissolved total amino acids; DTCHO, dissolved total carbohydrates; DTFA, dissolved total fatty acids; PTFA, particulate total fatty acids; PUFA, polyunsaturated fatty acids; and FA, fatty acids, including bacterial and terrestrial sources.

Table 2. Enrichment factors (EF) of the SML versus UW measured by using glass plate (GP) and metal screen (MS)*

Variables†	n	Season	GP	MS
			Mean (SD) [range]	Mean (SD) [range]
NO ₃ + NO ₂	12	March, September	1.65 (0.55) [1.11-2.87]	1.22 (0.20) [0.79-1.55]
PO ₄	6	September	1.50 (0.34) [1.05-2.00]	1.12 (0.31) [0.83-1.56]
POC	7	March + September	2.82 (1.87) [0.89-5.55]	1.41 (0.78) [0.37-2.86]
DOC	5	September	5.12 (8.49) [0.76-20.29]	1.04 (0.19) [0.71-1.19]
SPM	10	March, September	1.89 (1.01) [0.86-3.68]	1.21 (0.72) [0.41-2.34]
Chl <i>a</i>	6	September	0.96 (0.09) [0.87-1.13]	1.98 (0.85) [1.10-3.03]
Pheo <i>a</i>	6	September	1.29 (0.67) [0.80-2.57]	2.82 (0.84) [1.60-3.79]
DTAA	8	March, September	1.99 (1.73) [0.38-5.37]	0.82 (0.60) [0.16-1.93]
DTCHO	8	March, September	1.02 (0.72) [0.30-2.58]	1.96 (2.09) [0.35-6.33]
DTFA	3	March	4.00 (1.50) [3.13-5.76]	2.59 (1.90) [1.24-4.75]
PTFA	2	March	2.89 (1.70) [1.70-4.10]	4.59 (3.50) [2.08-7.07]
Particulate PUFA	3	March	6.42 (5.61) [1.68-12.63]	7.01 (4.46) [2.14-10.90]
Particulate bacterial FA	3	March	15.69 (22.13) [2.04-41.23]	6.72 (5.66) [0.77-12.01]
Particulate terrigenous FA	3	March	18.0 (21.0) [1.10-41.94]	7.00 (5.00) [1.09-12.16]
Dissolved PUFA	3	March	5.22 (3.53) [2.03-9.02]	5.59 (3.79) [2.11-9.63]
Dissolved bacterial FA	3	March	3.81 (1.02) [3.19-5.00]	1.63 (0.99) [1.05-2.79]
Dissolved terrigenous FA	3	March	3.39 (1.08) [2.61-4.62]	1.25 (0.75) [0.70-2.11]

*For all the variables, number of stations used and sampling seasons are given with respective means, standard deviations (SD), and value ranges.

†Significance of abbreviations and units as in Table 1.

Phosphate concentration is significantly heterogeneous within SML ($P = 0.014$), i.e., higher with GP than with MS (EF = 1.5 and 1.1, respectively); however, the contrast between SML and UW is not significant. For POC, EF are greater with GP than with MS (EF = 2.8 and 1.4, respectively), but RMA tests are not significant. DOC is much more enriched with GP than MS (EF are 5.1 and 1.0, respectively) but not at a significant level. RMA tests were not achieved because of dispersed values and lower number of stations (5). SPM is significantly enriched in SML (EF = 1.5) as shown by its contrast with UW ($P = 0.018$). Higher concentration observed with GP than MS (EF 1.9 and 1.2, respectively) is not significant.

Chlorophyll *a* and pheophytin *a* display very similar vertical patterns: maximum concentrations are observed with MS

(EF = 2.0 and 2.8, respectively), a case never found with previous parameters. Accordingly, the contrast tests are significant.

Data for Barcelona and Banyuls were compared by computing for each parameter the respective mean at each site. Results are indicative of trends that were not tested because of the low number of stations at each site and the lowered associated power for testing (the more complex RMA model adding a between site factor was not computed). This comparison is of interest, however, because recurrent differences specific to each site are evidenced. Regarding core parameters, concentrations of most of the parameters (in SML sampled by GP and in UW) are higher in the eutrophized Barcelona waters than in the overall oligotrophic waters of Banyuls. With MS, in turn, concentrations are higher at Barcelona for all these parameters. EF

Table 3. Results for RMA tests on core variables*

Core variables†	n	Means			Contrast GP vs. MS		Contrast SML vs. UW	
		GP	MS	UW	GP/MS EF mean	Probability	SML/UW EF mean	Probability
NO ₃	12	1.43	1.25	1.06	1.36	0.019	1.44	1.20 10⁻⁴
PO ₄	6	0.23	0.19	0.18	1.40	0.014	1.31	0.06 NS
POC	7	0.63	0.31	0.27	2.31	0.16 NS	2.16	0.12 NS
SPM	10	31.05	18.7	18.5	1.84	0.10 NS	1.55	0.018
Chl <i>a</i>	6	0.25	0.47	0.27	0.57	0.014	1.47	0.039
Pheo <i>a</i>	6	0.09	0.20	0.08	0.46	0.023	2.06	0.022

*Mean concentrations for glass plate (GP), metal screen (MS), and underlying water (UW) with number of stations used and seasons. Probability of contrast between enrichment factors for GP/MS and between enrichment factors for SML/UW (note that enrichment factors is the mean of enrichment factors ratios). In bold characters, probability at least < 5%, NS = not significant.

†Significance of abbreviations and units as in Table 1.

Table 4. Results for RMA tests (as in Table 3) on 8 stations analyzed for dissolved total amino acids (DTAA) and dissolved total carbohydrates (DTCHO) (mean molar percentages of amino acids are included with standard deviations [SD])

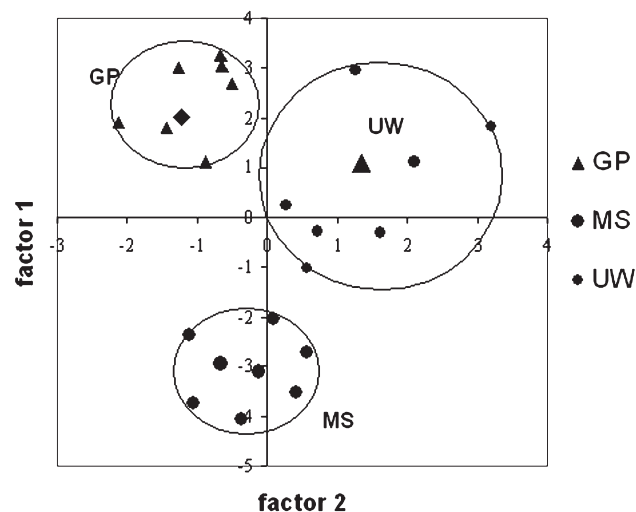
Amino acids	Mole percents		Means (μM)			Contrast GP vs. MS		Contrast SML vs. UW	
	Mean	SD	GP	MS	UW	GP/MS EF mean	Probability	SML/UW EF mean	Probability
glu	18.31	9.21	1.19	0.51	0.86	4.68	0.077 NS	1.80	0.94 NS
gly	13.63	6.14	0.72	0.26	0.62	3.15	0.04995	1.48	0.41 NS
ser	10.41	3.81	0.57	0.21	0.46	3.18	0.015	1.56	0.53 NS
asp	8.69	3.08	0.42	0.25	0.36	2.11	0.085 NS	1.55	0.71 NS
leu	7.36	5.23	0.44	0.18	0.23	4.15	0.061 NS	2.17	0.68 NS
ala	7.25	2.30	0.39	0.18	0.31	2.79	0.031	1.77	0.87 NS
lys	5.68	6.82	0.42	0.10	0.25	2.53	0.087 NS	2.99	0.74 NS
phe	5.64	3.75	0.21	0.18	0.19	3.08	0.46 NS	1.86	0.84 NS
thr	4.10	1.71	0.22	0.10	0.21	4.37	0.097 NS	1.22	0.31 NS
val	3.50	1.44	0.17	0.09	0.16	2.96	0.11 NS	1.43	0.68 NS
arg	3.49	3.22	0.24	0.15	0.12	5.16	0.14 NS	2.77	0.65 NS
ile	2.87	1.84	0.15	0.06	0.21	3.60	0.008	*	0.99 NS
tyr	1.98	1.79	0.08	0.05	0.09	4.01	0.44 NS	1.31	0.25 NS
gluN	1.95	2.51	0.06	0.04	0.04	1.19	0.35 NS	1.55	0.70 NS
his	1.53	2.65	0.04	0.01	0.10	1.98	0.91 NS	1.18	0.49 NS
bala	1.25	1.48	0.04	0.02	0.03	3.63	0.19 NS	0.88	0.44 NS
gaba	1.06	1.53	0.09	0.01	0.02	*	0.43 NS	1.49	0.77 NS
galN	0.87	1.53	0.02	0.02	0.02	0.87	0.72 NS	4.73	0.68 NS
met	0.47	0.80	0.04	0.01	0.03	3.50	0.06 NS	*	0.91 NS
DTAA	—	—	5.52	2.45	4.31	3.17	0.042	1.51	0.83 NS
DTCHO	—	—	0.68	1.01	0.96	0.68	0.24 NS	1.63	0.95 NS

*For isoleucine, gaba, and methionine, dubious values were deleted. Amino acids were abbreviated as follows: glutamate (glu), glycine (gly), serine (ser), aspartate (asp), leucine (leu), alanine (ala), lysine (lys), phenylalanine (phe), threonine (thr), valine (val), arginine (arg), isoleucine (ile), tyrosine (tyr), glucosamine (gluN), histidine (his), beta-alanine (bala), gamma aminobutyric acid (gaba), galactosamine (galN), and methionine (met).

for MS are consistent with this trend because they are always higher at Barcelona than at Banyuls, respectively: 1.2 versus 1.1 for nitrate + nitrite, 1.3 versus 0.9 for phosphate, 1.4 versus 0.8 for SPM, 2.2 versus 1.1 for POC, 2.4 versus 1.6 for chlorophyll *a*, and 3.0 versus 2.7 for pheophytin *a*. As regards DTAA and DTCHO, some differences are found relative to the core parameters. Their concentrations are always higher at Barcelona only when SML is sampled by GP. No difference is found for MS nor UW. Unlike core parameters, EF found are higher at Banyuls either with GP or MS. For DTAA, EF at Barcelona and Banyuls are 1.1 and 2.9 with GP, respectively, and 0.4 and 1.2 with MS, respectively. For DTCHO, one finds 0.7 and 1.2 with GP, respectively, and 0.5 and 3.4 with MS, respectively.

Dissolved total amino acids and dissolved total carbohydrates—DTAA and DTCHO make up the largest part of the DOM identified at the molecular level in the Ocean (Salot 1994). Their respective proportions are usually in favor of DTAA in the SML (Henrichs and Williams 1985; Williams et al. 1986) unlike the present case where the ratio DTAA/DTCHO varies between 2.5 and 8 according to the layer sampled (Table 1). Their respective enrichments in GP and MS are different. DTAA are ca. 2-fold enriched with GP (EF = 2.0) at less than 5% significant level of confidence, whereas they are not enriched with MS

(EF = 0.8). DTCHO are enriched with MS and not with GP (EF = 1.1 and 2.0, respectively) but not at a statistically significant level. The contrast between SML and UW is not statisti-

**Fig. 3.** Canonical discriminant scores for the 8 stations, amino-acid dataset. Points are the observations projected in the discriminant space between samplers. GP refers to glass plate, MS to metal screen, and UW to underlying water.

cally significant for DTAA or DTCHO, although MS is richer in DTCHO and depleted in DTAA.

Concentrations of DTAA and DTCHO collected by the NS are higher than by GP and MS (Table 2). DTCHO enrichment may be particularly high (EF = 3.3). Data are indicative because they refer to only 5 stations studied in March.

Amino acid molecular composition—The mean amino acid molecular composition computed on GP, MS, and UW cumulated data from 8 stations used (Table 3) is similar to that usually found in the SML within the pools of total and/or dissolved combined amino acids from various sites (Henrichs and Williams 1985; Coffin 1989; Carlucci et al. 1991, 1992). Six major amino acids (mole percent above 7%) account for 65.6% of the total amino acid pool. Three hydrophobic amino acids (glycine, alanine, and leucine) make up 28.2%, the hydroxylated serine 27.0%, and the two acidic (glutamate and aspartate) 27.0%. The RMA tests show that four amino acids are unequally distributed between GP and MS at a less than 1% level of significance. They comprise three hydrophobic (isoleucine, alanine, and glycine) and the hydroxylated serine. At an only approximate level of significance ($P < 10\%$), one finds six other amino acids among which the hydrophobic leucine and the hydroxylated threonine. Because hydroxylated amino acids are used as markers of detrital phytoplankton-derived matter (Hecky et al. 1973), our results indicate that GP collects matter with a more pronounced hydrophobic and detrital character than MS. Our data support those of Henrichs and Williams (1985) who sampled with MS. Moreover, they indicate that these characteristics both may be even more pronounced within the thinner SML layer sampled by GP.

The canonical discriminant analysis computed for the six more discriminant amino acids (among GP, MS, and UW) confirms that the three groups in GP, MS, and UW are distinct in the discriminant space, as each observation point is well classified into its own group (Fig. 3). Distances between the centroids of GP and MS, or between GP and UW, are greater than the distance between MS and UW.

Fatty acids—Data are considered at the total and molecular levels. At the total level, Table 1 and 2 show that the dissolved total fatty acids are better collected by GP than by MS (EF = 4.0 and 2.6, respectively). At the opposite, the particulate total fatty acids are better collected by MS than by GP (EF = 4.6 and 2.9, respectively) (results refer to means without associated statistical tests). At the molecular level, differences in composition reflect the various contributing sources, including marine living and dead organisms and continental inputs. Even saturated fatty acids with more than 22 carbon atoms are commonly used as markers of terrigenous material while branched fatty acids (*iso* and *anteiso*) with 13-17 carbon atoms are used as bacterial markers in sediments (Volkman et al. 1980) and in suspended particles (Salot et al. 1988). Table 1 and 2 show that GP collects more terrigenous and bacterial matter than MS both in the particulate and dissolved matter because of higher EF. MS, in turn, shows a trend to collect higher amounts of fresh plank-

tonic matter as indicated by the slightly enhanced amounts of polyunsaturated fatty acid originating from phytoplankton and zooplankton (Sargent and Falk-Petersen 1988; Viso and Marty 1993). In the particulate phase, EF for polyunsaturated fatty acid are 7.0 and 6.4 for MS and GP, respectively, and 5.6 and 5.2 in the dissolved one, respectively.

The trends we record here are determined from a small number of samples. They are novel, however, because such data are presently lacking in literature, especially for dissolved and particulate organic moieties simultaneously analyzed at a molecular level.

Discussion

DTCHO depth stratification—The large variety of depths used for sampling UW, ranging from 5 to 50 cm (Estep et al. 1985; Williams et al. 1986; Falkowska 1999a; Carlucci et al. 1991) indicates that organic matter distribution was assumed to be rather homogenous within this portion of the water column. To our knowledge, the only data describing a nonhomogenous distribution of chemicals in this layer are those of Danos et al. (1983), who recorded upward increasing gradients of nutrient within the first 5 to 10 cm. The unequal distribution of DTCHO we evidence in the upper first meter emphasizes the care to be taken when choosing the depth for sampling UW. DTCHO accumulation found between 5 and 10 cm depth suggests that this layer be excluded and 50 cm depth be used because it is where lowest values and lowest variability coincided at both sites. Literature and present data show that DOM depth distribution in the upper part of the water column may be significantly influenced by gradients of hydrophobicity. DTCHO are an appropriate probe for determining UW depth because they are less hydrophobic than amino acids and lipids and, consequently, their depth gradients below SML are expected to be less steep than those of the two other major components of the organic matter. The depth inferred from DTCHO profiles may thus be applicable, a priori, to all three classes. Note that the TPTZ method (Mykkestad et al. 1997) we used to measure carbohydrates is much less time-consuming than that of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) (Pakulski and Benner 1992). It also gives well-reproducible results provided that the analyses are carried out in strongly subdued light.

The results are interesting because they refer to means for various conditions, including two contrasted situations: the eutrophic conditions at the Barcelona site in March and the oligotrophic ones at Banyuls in September. However, a preliminary exploration of the DOM depth distribution is recommended, to take into account possible specific situations in the area to be studied, including sea conditions, various plankton blooms, and hour of sampling. SML is a dynamic structure. It receives inputs from atmosphere and from layers below it via rising bubbles and particles with positive buoyancy. It is also a layer of downward export of aggregated matter. The first upper meter is precisely the layer where all this matter is in transit and its depth distribution is, thereby, influenced by these fluxes.

DTCHO accumulation in the 5 to 10 cm layer provides novel data supporting the two above-mentioned functions of the SML system. DTCHO in the DOM fraction are the most prone to aggregate in turbulent conditions and to sediment out of the upper layers, in particular, when their concentrations become high (Passow et al. 1994). DTCHO are known also to increase the viscosity of seawater, in particular in the SML, as noted by Jenkinson and Biddanda (1999). One may then hypothesize that increased DTCHO concentration could entrap with efficiency both particulate and dissolved matter and, thereby, amplify organic matter enrichment in the uppermost layers.

Glass plate and metal screen comparisons—A comparison of GP and MS results must be done bearing in mind all the possible bias inherent to each sampler. Concentrations of organic matter collected by MS must be corrected by a factor of 9 because it collects a thicker layer than GP (440 μm vs. 50 μm), as shown by previous calibration experiments (Hatcher and Parker 1974; Van Vleet and Williams 1980; Estep et al. 1985) and by Henrichs and Williams (1985) for amino acids. Literature and present data show GP and MS enrichments differ by a factor in the order of 2 to 3 rather than 9, even for organics typically associated with the upper layers as lipids (Hunter and Liss 1981). This indicates, hence, the significant influence of dilution on the results. Further, GP and MS have unequal abilities to collect suspended particles according to their sizes: small-sized particles glide on the surfaces of the plates (Estep et al. 1985) whereas the largest ones may be entrapped within MS (Daumas et al. 1976). Moreover, molecules or particles may interact with the samplers, in particular, through hydrophobic interactions (more with GP than MS because of its larger area).

The difficulty of a precise quantification of all possible bias leads usually to the adoption of a more pragmatic approach. Such a method would compare the EF for the respective samplers without taking into account all the possible corrections and would use the sampler with the highest EF for the type of particle to be studied. This is precisely the objective of this study.

The significantly higher enrichment of nitrate and nitrite with GP than MS may be regarded as reflecting the process of their upward increasing concentration already recorded by Danos et al. (1983) between 10 and 5 cm depth. This enrichment may be regarded also as a result of a bias arising from their interactions with the molecules comprising the film absorbed on GP through mechanisms similar to ion exchanges (Kuznetsova and Lee 2001).

The trend for a higher enrichment of SPM with GP than MS indicates that GP is more appropriate to study continental inputs including aerodepositions. Our results for the fatty acids markers of continent-derived matter (with mean EF up to 18.0 for GP) are novel because they show that this type of input may be quantified by using these molecules within the SML system.

Our data for chlorophyll *a* and pheophytin *a* confirm previous observations of Henrichs and Williams (1985), who

found that phytoplankton is more efficiently sampled by MS than by GP, and the recent ones of Agogu e et al. (2004), who sampled at the same sites as we did. Our results for dissolved total fatty acids and polyunsaturated fatty acids are interesting because they show for the first time that fresh algal cells are better collected by MS than GP. This shows that these molecules may be used to achieve a diagnostic of the physiological state of the phytoplankton within the SML.

Regarding bacteria, our results are quite consistent with those of Agogu e et al. (2004), who found more bacteria collected by GP than MS. The higher enrichment of fatty acid bacterial markers found (EF up to 15.7 for GP) is most probably explained by an accumulation of dead bacteria cell walls.

Differences between the EF we recorded for the three main classes of the dissolved organic matter are consistent with what may be expected from their respective overall hydrophobicity. Indeed, fatty acids displayed highest EF particularly with GP. No significant difference was found between GP and MS for the less hydrophobic carbohydrates, whereas the situation was intermediate for the total amino acids with a selective accumulation of the most hydrophobic ones by GP. These differences may thus be regarded (1) as arguments for a fine-scale structuration within the SML structure itself, suspected to be oriented by an upward increasing vertical gradient of hydrophobicity, or (2) as reflecting the influence of bias varying according to the sampler and the type of molecule, or (3) a combination of both of them.

In conclusion, our data point to SML portion sampled by GP as being more enriched than MS in hydrophobic compounds, bacterial and algal debris, and continentally derived matter.

Comments and recommendations

Our data emphasize the need for a preliminary exploration of the depth distribution (within the first meter below the air-ocean interface) for one or several pertinent parameters prior to an extensive sampling being undertaken in a given area. This should be done to determine the depth the most appropriate for sampling UW used as reference for calculating the EF. This depth will be where both the values of the parameters and their spatial variability are the lowest, in order to maximize the EF and to reduce their variability.

The choice of the sampler most adapted to SML is determined by (1) the volumes of water required for analyzing the planed parameters and (2) the best efficiency for collecting both particulate and dissolved chemicals. Our data indicate MS as better adapted to multiparametric studies because of the 10-fold larger water volumes it allows to collect within the same time as GP (20 L per hour vs. 1 L). This holds mainly for parameters such as lipids, which require about a 10-L sample for each analysis. In addition, large water volumes collected in short time-intervals allow for integrating the spatial and temporal variability, thus making results more representative of the studied area. Moreover, MS allows for high frequency acquisition, which is required for under-

standing short-time processes biologically or chemically controlled. Our results indicate MS to better collect large-size particles, such as fresh phytoplankton, thus making it well-adapted for studying this biomass. GP is better adapted, in turn, to sample dissolved hydrophobic matter including various lipids, amino acids, and also nutrients (nitrate and nitrite) and suspended particulate matter. We thus recommend to use both samplers when processes at the upper layer of the water column are to be studied, especially, interactions with the continent including oil spills and aerodepositions.

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