

**Sea surface microlayer:
a field evaluation of
teflon plate, glass
plate and screen
sampling techniques.
Part 2. Dissolved and
suspended matter**

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Abstract

The similarities and differences in chemical composition of particular sub-layers of the sea microlayer are discussed on the basis of data obtained from the open sea region of the Gdańsk Basin. Three methods of microlayer sampling were tested simultaneously, resulting in sub-samples of different mean thickness (10, 90, 250 μm). Samples were analysed to determine dissolved NO_3^- , NH_4^+ , PO_4^{3-} , P_{tot} and DOC, as well as suspended components (chl *a*, phae *a*, ATP, POC, particle size distribution and algae). It was found that the stratified microlayer forms a medium extremely diversified chemically and biologically. Variable ranges of concentration as well as varying frequencies of the depletion or enrichment coefficient in each measurement period indicate disparate conditions in the microlayers, which are related to the intensity of primary production and the destruction of organic matter. Comparative studies provided no evidence for the preferential application of any of the sampling techniques to obtain the most accurate picture of dissolved organic and inorganic substances in the sea surface microlayer. In the case of particulate organic matter, satisfactory results were obtained with the classic screen technique. The article highlights the merit of simultaneous studies by various sampling techniques in answering questions related to the sea surface microstructure and the momentum of photochemical and biological processes at its different levels.

1. Introduction

The surface microlayer has been a subject of investigation for over 40 years now, and the results of this research have revealed the unique physical, chemical and biological features of the interface between air and seawater. In this environment various chemical and microbiological components of seawater undergo accumulation. In comparison to subsurface water, the surface microlayer is rich in dissolved organic matter (DOM) and suspended organic matter (POM) in both littoral and open-sea areas (Daumas *et al.* 1976, Sieburth *et al.* 1976, Wandschneider 1979, Carlucci *et al.* 1985, Williams *et al.* 1986, Marty *et al.* 1988, Mudryk *et al.* 1991, Hardy & Cleary 1992, Falkowska 1996, Liss & Duce 1997). Authors have stressed the fact that besides high coefficients of enrichment, considerable depletion in organic matter content in the surface microlayer also occurs, regardless of the sampling techniques (Dietz *et al.* 1976, Carlson 1982, 1983, de Souza Lima 1985).

It is well known that the natural organic matter present in the surface microlayer is composed of the material adsorbed in the interface layer and forms a surface film of molecular thickness. Surface slicks appearing on the sea surface support this finding well. Rarely visible on the open sea, such slicks are quite often encountered in coastal zones (Garabetian *et al.* 1993).

Hardy *et al.* (1988) indicated that the surface microlayer is rich in organic matter even when slicks are absent; on the other hand, in the presence of slicks, the coefficients of enrichment of *e.g.* DOC do not increase significantly. These observations suggest that the enrichment of the surface film in natural organic material cannot be regarded as a simple linear function. Besides the dissolved components, neuston organisms were also found to accumulate in the surface microlayer (Williams *et al.* 1986, Hardy *et al.* 1988, Hardy & Apts 1989). In many papers, authors stress the fact that the surface of the sea is a highly productive and metabolically active interphase layer, and underline the special role of bacteria in breaking down allochthonous and autochthonous organic matter. Some authors have put forward their views about the inhibition of neuston organisms and the considerable fluctuations of biological activity in the microlayer brought about by solar radiation (Daumas *et al.* 1976, Carlson 1982, 1983, Hardy & Gucinski 1989, Behrenfeld *et al.* 1993).

Comparative studies in which different sampling techniques have been applied within the surface microlayer yield information that is at times similar, but which can also be ambiguous with respect to the microlayer structure (Daumas *et al.* 1976, Carlson 1982, Estep *et al.* 1985, Hardy *et al.* 1988). Without taking into account laboratory analyses, since these cannot be transposed into natural conditions, the main conclusion can be

formulated as follows: samples from the thinner microlayer usually display much greater variability in comparison to those from subsurface water.

Obviously, the application of diverse sampling techniques makes the comparison of results difficult and as yet there is no straightforward answer to the question posed by Carlson (1982): How does the sampling technique affect microlayer results? The overall aim of the present project was to find out whether microlayers of different thicknesses, isolated from the sea surface, are identical as regards chemical composition, or whether they differ significantly in this respect, thus providing evidence for the stratification of dissolved and suspended substances within the tiny distance of a few hundred micrometres.

2. Material and methods

In this part of the project only the data collected in spring (May–June) 1994–1996 were analysed. Table 1 lists sampling dates, numbers of samples from individual layers and the scope of the chemical analyses.

Table 1. Number of samples from the sea surface microlayer and subsurface water layer collected for analysis in 1994–1996

Layer	29 April–4 May 1994	8–12 May 1995	16–20 June 1996
TPM		40	26
GPM	69	40	26
SM	86	41	26
SUB	110	41	26
Determinands			
	NO ₃ ⁻ , PO ₄ ³⁻ , particles	NO ₃ ⁻ , NH ₄ ⁺ , PO ₄ ³⁻ , P _{org} , DOC, chl <i>a</i> , algae	PO ₄ ³⁻ , NO ₃ ⁻ , NH ₄ ⁺ , DOC, POC, ATP, chl <i>a</i> , phae <i>a</i>

The location of the sampling area and the details of the sampling techniques were described in Part 1. Samples were collected from a platform protruding from the bows of the anchored research vessel. In addition to samples collected from the microlayer by teflon plate (TPM), glass plate (GPM) and screen (SM), water was taken from the subsurface layer with a polyethylene jar immersed to about 15 cm below the sea surface. Sampling by all techniques was repeated simultaneously every 1, 2 or 4 hours. Once samples had been hauled up on deck, they were protected against direct sunlight.

Nutrients, *i.e.* nitrate, nitrite, ammonia, phosphate and total phosphorus, were determined according to Grasshoff *et al.* (1983) directly on board ship.

The analytical precision in the medium concentration range of inorganic nitrogen, phosphate and total phosphorus analyses lay between 1–5%. The accuracy of the determination was $0.03 \mu\text{mol dm}^{-3}$ at low phosphate concentrations ($< 0.2 \mu\text{mol dm}^{-3}$) and $0.02 \mu\text{mol dm}^{-3}$ at low nitrate concentrations ($< 0.15 \mu\text{mol dm}^{-3}$). The detection limit of phosphate and nitrate analyses was $0.01 \mu\text{mol dm}^{-3}$, that of ammonia was $0.05 \mu\text{mol dm}^{-3}$. Organic phosphorus was calculated by subtracting the phosphate concentration from the total phosphorus content.

Dissolved organic carbon (DOC) was analysed in the land laboratory with a Shimadzu Analyser TOC 5000 (Kyoto, Japan) with 2% precision. Samples were prepared (filtering through roasted Whatman GF/F filters and conservation with HgCl_2) on board ship following the procedures of Sugimura & Suzuki (1988) and Benner & Strom (1993).

Suspended organic carbon (POC) was collected on roasted Whatman GF/F filters. To remove carbonates, the filters were kept in a dessicator over fuming sulphuric acid for 24 h. Filters with samples were weighed and then analysed in a Perkin-Elmer CHN-analyser.

The chlorophyll *a* concentration was determined after extraction in acetone solution according to the procedure recommended by BMB (Edler 1979). The water volumes necessary for complete analysis were 0.5 or 1.0 dm³ from the respective microlayers and 2 dm³ from the subsurface layer. Phaeophytin *a* was extracted from the same water samples with acetone and measured spectrophotometrically after acidification of the acetone extract (Parsons *et al.* 1985).

Samples for suspended particle size analysis were collected in late April and early May 1994 and stored deep-frozen for analysis in the land laboratory. The measurement was performed immediately after thawing in a Multisizer-II Coulter Counter. The methodology of measurement was described in detail in Falkowska & Latała (1995).

Adenosine triphosphate (ATP) was measured by the luciferone-lucrose method as originally described by Holm-Hansen & Booth (1966). Water samples (50 cm³) were filtered through Millipore filters of 0.22 μm pore size. The ATP having been extracted in TRIS buffer, the samples were deep-frozen (-20°C) for further analysis on land. The procedures in the land laboratory included the use of a Beckman LS 6000TA scintillation counter.

Samples for qualitative and quantitative determination of algae were conserved with Lugol solution and acetic acid, and analysed in the land laboratory under a reversed microscope.

Enrichment factors (EF) were calculated as the ratio of the concentration in the given microlayer to that in the bulk water. An enrichment factor of > 1.0 was termed enrichment, while a value of < 1.0 was designated as depletion.

3. Results and discussion

The studies of dissolved and suspended organic matter and nutrients at the sea surface were conducted on the open sea. No slicks were observed during the study periods.

The water layers collected at the sea surface were of different average thickness, *i.e.* $10\ \mu\text{m}$ thick (teflon plate technique – TPM), $90\ \mu\text{m}$ (glass plate technique – GPM) and $250\ \mu\text{m}$ (screen technique – SM). Their statistical estimators displayed a conspicuous diversity (Fig. 1): statistical analysis of the results indicated considerable differences in the concentrations of the chemical components between the three microlayers studied. The median values of nearly all the components examined were highest in the thinnest microlayer.

Despite differences in concentration ranges during the various periods of the study, nitrate and ammonia concentrations exhibited greater scatter in thicker microlayers and were often comparable, especially in the screen microlayer and subsurface water. Phosphate concentrations differed in all three measurement periods and in each one, the variations in statistical estimators in particular were irregular. In June 1996, despite the absence of slicks, extremely high DOC concentrations were measured in all the microlayers and in the subsurface water. While these values are not typical, the data between the upper and lower quartile fall within the range of concentrations from other marine areas (Dietz *et al.* 1976, Carlson 1982). Much lower DOC levels were found in May 1995 (Fig. 1).

The abundance of algae, regarded here as a component of suspended organic material, increased with growing distance from the boundary layer. However, on several occasions unparalleled abundance values were found in the uppermost microlayer ($3\ 000\ 000$ – $5\ 600\ 000\ \text{cells dm}^{-3}$), *Peridiniella catenata* being the dominant organism (88% of the total abundance). The contribution of the dominant taxon in the thicker microlayers was higher, making up 95–96% of the total number of algae cells (Fig. 2). Chlorophyll *a* concentrations differed in May 1995 and June 1996, this being indicative of the natural variability of biological processes in spring. Chlorophyll *a* and nutrient concentrations varied significantly during each sampling period. The estimators characterising the other parameters (ATP, POC, phaeophytin *a*, particles) are assumed also to have varied significantly, even though the concentrations of these substances were measured only

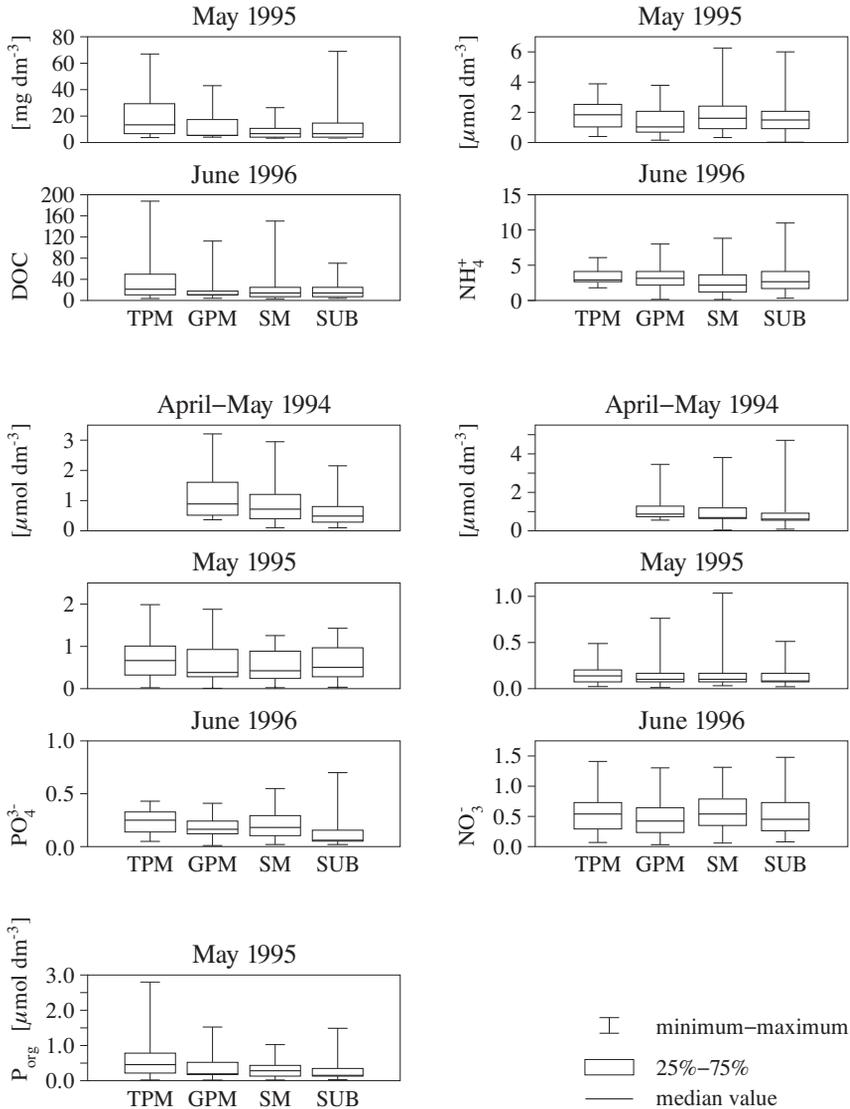


Fig. 1. Box and whisker plots of statistical estimators of dissolved substances in layers isolated from the sea surface

during one period. Such variations had been observed in earlier studies (Falkowska & Latała 1995). The differences in concentrations of dissolved and suspended substances in a particular sampling period seem to be a natural property resulting from the differences in biological activity, the succession of dominant species and the seasonally related rate of organic matter degradation.

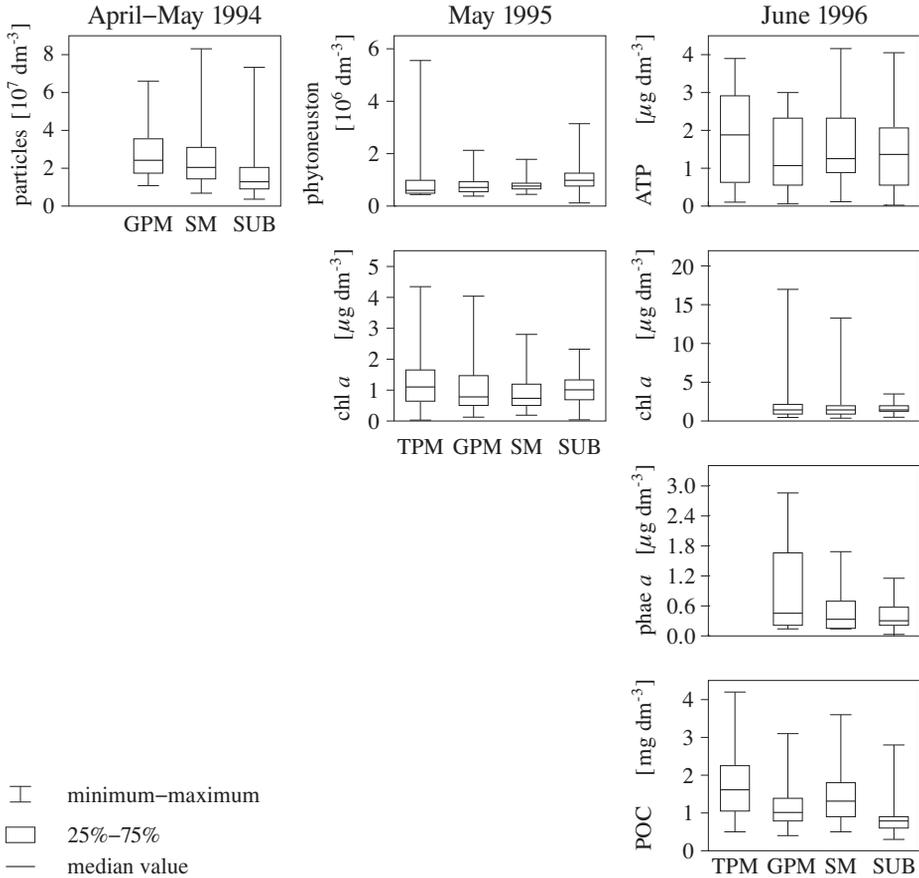


Fig. 2. Box and whisker plots of statistical estimators of suspended substances in layers isolated from the sea surface

In subsurface water from 15 cm depth, the enrichment factor (EF) differed according to parameter and microlayer (Figs. 3 and 4). The histograms of EF show both enrichment (values > 1) and depletion (values < 1). Of the dissolved substances, organic phosphorus and DOC reached high levels of enrichment in the thinnest TPM layer only in a few cases (Fig. 3a). The production of DOC by phytoneston in this microlayer can be 20–40 times higher than phytoplankton production in the bulk water (Carlucci *et al.* 1985). Marty *et al.* (1988) noticed that not only do active phytoplankton contribute significantly to the dissolved organic matter pool but the activity and decay of previous populations do so too. The EF of dissolved substances often decreases with increasing layer thickness (Figs. 3a,b). Carlson (1982) found similar DOC enrichment coefficients in GPM and SM microlayers and a comparable trend in variations in oceanic

waters, and de Souza Lima (1985) found enrichment coefficients of the same magnitude with regard to nutrients in a 60–100 μm thick microlayer in the Bay of Marseilles.

Enrichment factors of suspended organic compounds like ATP, POC and phaeophytin decreased with the increasing thickness of the microlayer (Fig. 4). Only in the thinnest layer (TPM) did a small percentage of results indicate extremely high enrichment. The EFs of ATP, DOC and phaeophytin correspond well with the results from the Bay of Marseilles (de Souza

a

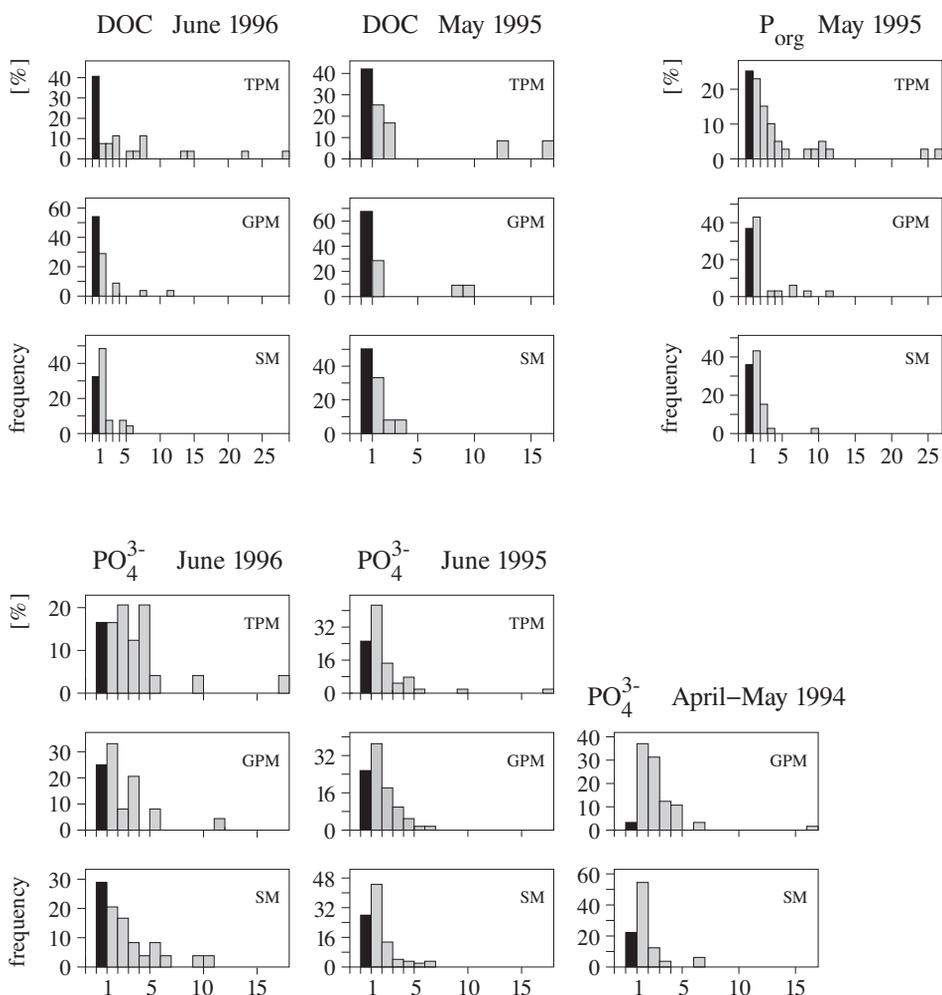


Fig. 3. Histograms of the enrichment factor (EF) of dissolved substances in layers isolated from the sea surface; DOC, PO_4^{3-} , P_{org} (a), NH_4^+ , NO_3^- (b)

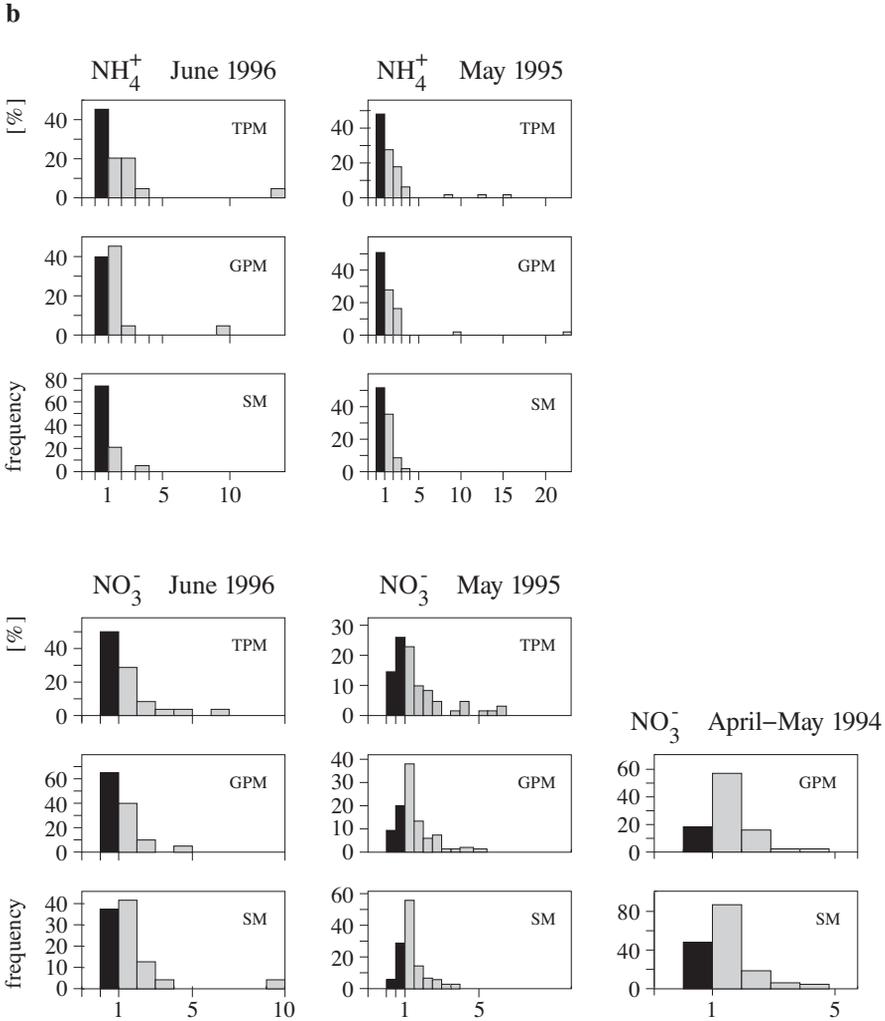


Fig. 3. (continued)

Lima & Romano 1983). Phytoneuston and chlorophyll *a* are frequently depleted, this tendency being particularly marked in the abundance of algae. Enrichment in the thinnest microlayer (TPM) was recorded in around 30% of cases, in the thicker layers only in 10%. On several occasions the maximum enrichment ($EF = 5$) was found in the screen microlayer. None of the analysed suspended components showed such a remarkable tendency to depletion as algae. The results of earlier projects indicate extremely high enrichment coefficients in the case of algae. In the North Sea, densities of dinoflagellates dominated by *Prorocentrum* sp. were more than 1000 times greater in the microlayer than in the bulk water (Brockmann *et al.*

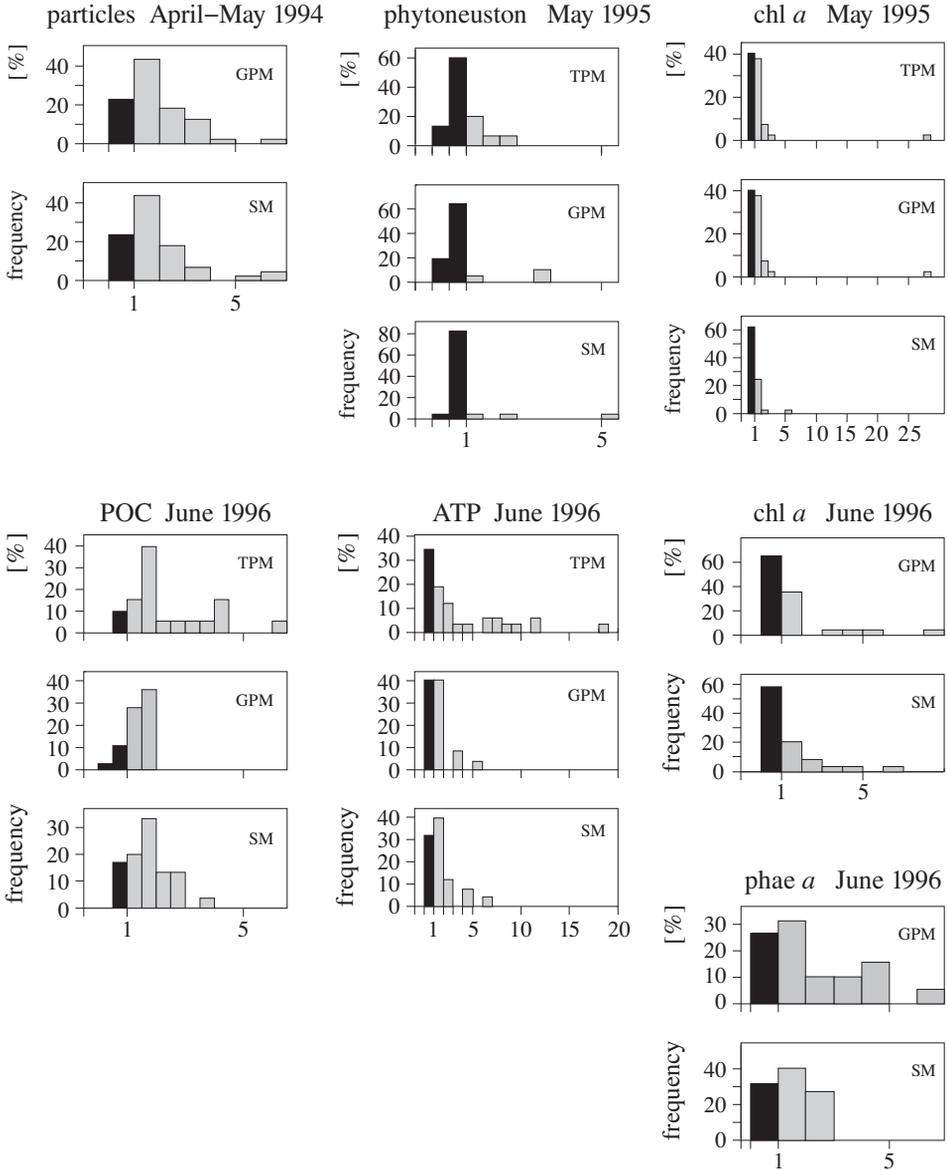


Fig. 4. Histograms of enrichment factors (EF) of suspended material in layers isolated from the sea surface

1976). Hardy & Apts (1989) recorded seasonally related variations in the magnitude of enrichment coefficients in Sequim Bay (Washington State). In spring (April, May) EFs fell to within the 3.7–9.0 range, but in July they increased to 1500. In the same basin in the summer of the following year, the enrichment coefficient of phytoneuston in the 50 μm microlayer

was 37 in the non-slick and 154 in the slick area (Hardy & Apts 1989). Concentrations of dissolved or suspended substances in the microlayer from the same basin can be very different in other months and years, despite the use of identical sampling methods. The results of the present project corroborate these earlier findings.

Friedman ANOVA & Kendall's concordance test was applied to verify the consistency of chemical composition between the isolated layers. The χ^2 test was chosen because of the non-parametric distributions of the substances analysed. The results of the verification are shown in Table 2.

Table 2. Statistical evaluation parameters of teflon plate, glass plate and screen samplers

Substance	Number of samples	χ^2 test	Probability
May 1994			
GPM/SM PO_4^{3-}	68	24.896	< 0.0001
GPM/SM NO_3^-	59	10.795	< 0.0010
GPM/SM particles	57	0.438	< 0.5078
May 1995			
TPM/GPM/SM PO_4^{3-}	40	22.850	< 0.0001
TPM/GPM/SM P_{org}	31	10.064	< 0.0060
TPM/GPM/SM NO_3^-	36	8.022	< 0.0180
TPM/GPM/SM NH_4^+	33	5.565	< 0.0618
TPM/GPM/SM DOC	32	6.500	< 0.0388
TPM/GPM/SM chl <i>a</i> ,	38	1.809	< 0.4040
TPM/GPM/SM algae	30	0.666	< 0.7165
June 1996			
TPM/GPM/SM PO_4^{3-}	25	10.473	< 0.0053
TPM/GPM/SM NH_4^+	22	12.315	< 0.0020
TPM/GPM/SM DOC	24	13.520	< 0.0011
TPM/GPM/SM POC	21	5.027	< 0.0809
TPM/GPM/SM ATP	24	1.272	< 0.5292
GPM/SM chl <i>a</i>	25	1.500	< 0.2206
GPM/SM phae <i>a</i>	23	1.000	< 0.3172

The phosphate, nitrate, ammonia and DOC data sets differ in each layer, hence the probability of the null hypothesis being true is minute. There was concordance between selected layers for chlorophyll *a*, phaeophytin *a*, suspended particles – particulate organic carbon, adenosine triphosphate – and the total number of algae, which was confirmed by a probability factor > 0.1. It can be concluded then that the distributions of suspended

substances are in accord regardless of the sampling method applied. However, the distributions of concentrations of dissolved components in the various layers do not accord, and this gives rise to the following questions:

1. Does the material of the sampler, which governs its selective adsorption or retention properties, affect the concentrations of dissolved substances but not of suspensions?
2. Are the thickness of the microlayer and the biological processes within it factors determining the concentrations of dissolved compounds?
3. Does the material contained in the bulk water in any way affect the concentrations of chemical substances in each of the microlayers examined?

If one assumes that the sampler material selectively adsorbs substances from the microlayer in accordance with its sorptive properties, the results obtained with the teflon or glass plates should yield extremely high enrichment coefficients, especially with respect to particulate organic substances. According to the diagram of the microlayer construction (Fig. 5), dissolved organic matter, surfactants and metals strongly bound to POM become concentrated in the interface layer, while the dissolved material ensuing from POM degradation is concentrated in the thicker water layer beneath (Sieburth *et al.* 1976, Daumas *et al.* 1976, Norkrans 1980, Hardy 1982, Hardy & Apts 1989). Since in both TPM and GPM layers EFs take positive and negative values, the sorptive properties of the sampler cannot supply an explanation, the more so that the depletion frequency of POM components was much greater than that of the dissolved substances. This observation lends support to some earlier findings of Carlson (1982), who observed that the majority of plate-collected ATP and chlorophyll *a* samples were depleted.

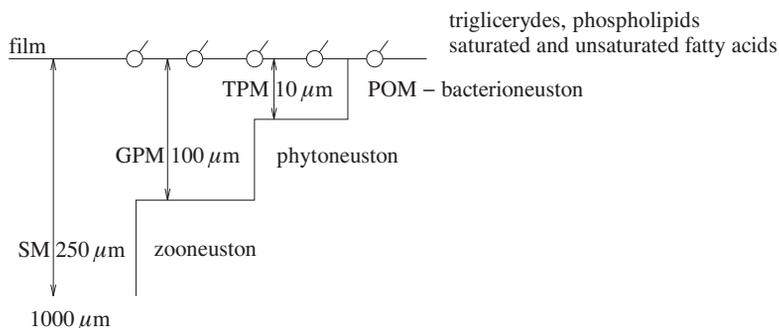


Fig. 5. Simplified diagram of the sea surface microlayer construction; the dominant ecological formation is indicated

The Garrett screen yielded results showing enrichment or depletion at the lowest level as compared to the thinner layer, probably because of a dilution effect. Algae were the exception. These were depleted in the thickest microlayer in 90% of cases and only in a few cases was a five-fold increment noted. Estep *et al.* (1985) pointed out that sampling with the screen technique yields highly erroneous results. In support of this view, these authors explain that macroscopic algal filaments often remain trapped on the screen during draining. Clearly, this affects the measurements of suspended matter concentrations and should be recorded as depletion. In the current project, the number of algae in the thicker screen layer was frequently greater than in the thinner layers, bearing out the view that the effect of bulk water has to be accounted for.

The sampling procedure (for details, see Part 1 of the paper) required the samplers to be hauled up, hence some phytoplankton cells could have been transferred from the subsurface to the microlayer. Still, the magnitude of errors ensuing from the transfer is difficult to evaluate. It is also perplexing why the other suspended material (particles, POC, chlorophyll *a* or phaeopigments) did not behave in the same way as algae. The author is of the opinion that only comparative studies of simultaneous 'downward' and 'upward' sampling using the screen technique can supply more information, on condition, of course, that the sea is calm (0°B), because only then is 'downward' sampling possible.

A number of authors (Hardy 1982, Sieburth 1983, Estep *et al.* 1985, Hardy & Apts 1989) have suggested that most of the organic substances from the sea microlayer are concentrated at the air-seawater interface or very close to it. Nutrients, the chemical compounds directly connected with the transformations of suspended and dissolved organic matter, should behave similarly. Owing to their polarity, however, high concentrations can be expected just below the surface film. This means that the sea surface microlayer is not homogenous and that the horizontally stratified layer of several hundred micrometres, in which laminar flows are dominant, is also chemically stratified. That this is indeed the case was confirmed by the χ^2 test (Table 2). The concentrations of dissolved organic and inorganic substances determined in isolated seawater layers of different thickness point clearly to the differences, not the similarities, between the microlayers. Stratification in the case of suspended organic substances was absent. To some extent, this fact can be explained by the active migration of neuston organisms caused by phototaxia or an abundance of food. Significance must also be attached to certain short-lived dynamic processes (Langmuir circulation cells, seiches) which enhance the accumulation of particulate material in the surface microlayer every few hours.

Stratification of chemical substances has also been discussed in the papers by Hardy (1982), de Souza Lima & Romano (1983), Estep *et al.* (1985), Hardy & Apts (1989) and Liss & Duce (1997). These authors draw attention to variable forms of stress to neuston organisms, such as nutrient depletion or photoinhibition. In consequence, a high radiation level at the air-sea interface leads to photodegradation of chlorophyll *a* and increased release of extracellular carbon by phytoplankton during photosynthesis, and the production of net particulate carbon declines. ATP is destroyed immediately after cell death. UV-B is thought to play a special role in the photodegradation of organic matter (Hardy & Gucinski 1989, Behrenfeld *et al.* 1993). The sea surface receives the maximum dose of this radiation, augmented by multiple reflections from wave action (Regan *et al.* 1992). Some neuston organisms, *e.g.* microheterotrophic neuston have developed protective mechanisms and adapted to high light energy and photic stress (Carlucci *et al.* 1985), but the efficiency of DNA radiation repair mechanisms in neustonic organisms has not been investigated (Hardy & Gucinski 1989).

The present author has not encountered any publications containing direct information on diel migrations of neustonic organisms, but the possibility that these organisms undertake such migrations cannot be excluded. Bovbjerg *et al.* (1976) observed the greatest rate of phytoplankton migration to the sea surface at sunrise, but the intensity of this migration declined rapidly in the next few hours. Similar observations, though from late evening, were described by Manzi *et al.* (1977) in estuarine water: there the maximum number of phytoplankton cells was recorded in the uppermost layer, 150–200 μm in thickness, while the water layer 1.5 m below the surface was very sparsely populated by dinoflagellates. Wandschneider (1979) recorded vertical differences in dinoflagellate sp. distribution along the shores of the island of Sylt. In the afternoon hours, *Prorocentrum micans* and *Ceratium furca* floated up to the sea surface and accumulated in a 60 μm -thick water layer, while other organisms concentrated in subsurface water (at 2–2.5 m) as a result of negative phototaxia. In studies of diel cycles of material in Lake Louise (Georgia), Freedman *et al.* (1982) recorded the highest concentrations of ATP and algae in the GPM layer at sunset, when radiation had dropped to zero. A great number of factors influence the diurnal migrations of organisms, which is why they are so complex in character, even though the mechanism related to illumination intensity is commonly recognised.

The difficulties encountered when results from different sampling techniques are compared has provoked research towards a method of sampling producing the most representative illustration of the microlayer's chemical composition and the concomitant biological processes. Estep *et al.* (1985)

attached great importance to the choice of sampling method appropriate to this phenomenon. While they considered the Garrett screen a satisfactory tool for analyses of dissolved substances (*e.g.* nutrients), they suggested the glass plate method for organic compounds and suspended particles. This approach is reliable when the study is focused on single compounds or a group of compounds of similar nature, but in more complex investigations the use of a single sampling technique is not advisable. Microlayer examination by three simultaneous methods of sampling has confirmed the influence of the sampling method on the results of an investigation. However, the effect of sampling methods relates rather to the thickness of a microlayer and the processes taking place there than to the material from which the sampler is made.

In every measurement period of this study, the differences in concentrations of the substances analysed and the variations in the magnitude and proportions of depletion and enrichment have shown that there is no point in searching for an ideal sampling method from among the existing *ex situ* methods which could produce comparable results from all three layers selected from the sea surface. It is the processes themselves, as well as their similarities and differences that have to be examined. In the author's experience, each new day's sampling results can reflect totally different conditions in the sea surface microlayer.

4. Conclusions

Simultaneous measurements of suspended and dissolved substances in the sea surface microlayer using three methods of sampling indicated the following:

- The distribution of dissolved substances depends on the thickness of the sampled microlayer. Dissolved organic compounds are contained in DOC, and organic phosphorus is concentrated in the uppermost layer (*ca* 10 μm). Concentrations of ammonia, nitrate and phosphate reach a maximum, albeit temporarily, in the thicker layers (90 and 250 μm). The distribution of chemical substances thus determined is a reflection of the internal stratification of the sea surface microlayer.
- Studies of concentrations of organic and inorganic substances show clearly that the layers differ considerably; therefore, individual layers should not be regarded as being representative of the entire microlayer.
- Depending on the time of the day, maximum concentrations of suspended organic substances appeared at various levels of the microlayer, but their distributions in isolated layers are similar.

- The fact that the concentrations of substances and their enrichment factors vary is probably due to the differing intensity of primary production or the rate of organic matter degradation at each level of the microlayer.
- No evidence was found for the preferential application of any of the methods of microlayer sampling with respect to dissolved substances, because each method yielded selective characteristics of the given layer.
- The samples collected by the classic screen technique gave a good characterisation of suspended organic matter.

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