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Microbiomass structure and respiratory activity of microneuston and microplankton in the northwestern Mediterranean Sea influenced by Rhône river water

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ABSTRACT: Surface microlayers collected in the northwestern Mediterranean Sea showed significant enrichment of particulate organic carbon (POC), ATP, chlorophyll, muramic acid (a specific bacterial biomass indicator) and total and viable bacterial count. Mean enrichment factors for muramic acid (52.6) and total bacterial count (38.4) were clearly higher than that for chlorophyll (19.8). Although phyto- and bacterioneuston appeared to have lower ATP levels, particularly in brackish water areas, compared with plankton, the bacterioneuston exhibited higher viability than bacterioplankton found in the corresponding underlying waters. Biochemical Oxygen Demand (BOD) and Electron Transport Systems (ETS) measurements clearly showed enhanced respiratory acitvities (up to 765.1 µl O_2 l^{-1} h^{-1}) in the microlayers compared to underlying bulk waters. Data comparison between BOD, ETS, POC, salinity and microbiomass estimates suggests that the measured respiratory activities (BOD and ETS) were well associated with bacterial populations in both the microlayer and bulk waters.

INTRODUCTION

The surface microlayer has been well described as a site of intensive accumulation of dissolved and particulate organic matter involving phytoplankton and bacteria (reviewed by Hardy 1982). This environment has great importance for gaseous exchange at the sea-air interface (Sieburth 1983). Neustonic organisms (zoo-, phyto- and bacterioplankton) living in the surface microlayer may influence transfer of O_2 and CO_2 and thus play a role in respiratory CO_2 production. In oceanographic research, Biochemical Oxygen Demand (BOD) and Electron Transport Systems (ETS) measurements have been used for estimating the respiratory activity of marine microorganisms (Packard et al. 1971, Williams 1981). BOD-ETS comparisons also appear to give useful information about the structure of microplanktonic communities (Packard 1985). Very few studies have been made on the respiratory activity of neustonic microorganisms (de Souza-Lima 1985); therefore in this investigation we have focussed on the relationships between respiratory activity and accumulation dynamics of microneustonic communities.

Microneuston activity in surface films is a difficult subject to study because of the physical and chemical properties of this environment. Some authors have suggested that microneustonic organisms could be in a reduced metabolic state (Diet et al. 1976, de Souza-Lima 1981), Horrigan et al. 1981, Bell & Albright 1982, de Souza-Lima & Romano 1983, de Souza-Lima & Chretiennot-Dinet 1984), while others have reported significant bacterial activities (Harvey & Young 1980, Dahlbäck et al. 1982). Furthermore, the studies carried out in this biotope have often been limited to one functional planktonic group, i.e. phytoplankton or bacteria.

During the EUHROGLI cruise (June and July 1983), surface films were collected in the northwestern Mediterranean Sea, an area influenced by Rhône river waters. In these samples, we made a first attempt to estimate the structure of the microbial biomass by using specific biomass indicators such as ATP, chlorophyll, muramic acid and total and actively growing bacterial counts in order to define the proportions of plants and bacteria in the whole biomass and activity of

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the samples. Particular attention was given to the estimation of bacterial biomass by muramic acid concentrations, a specific cell wall compound of prokaryotic organisms, measured by high pressure liquid chromatography (HPLC) (Mimura & Delmas 1983, Mimura & Romano 1985). Biochemical Oxygen Demand (BOD) and Electron Transport System (ETS) were assayed in the same samples and compared to characterize neustonic and planktonic community structures. They were also compared with specific biomass estimates to establish relationships.

MATERIALS AND METHODS

Sampling. Samples were collected in the northwestern Mediterranean Sea (Golfe du Lion) at 11 stations (Fig. 1) during the EURHOGLI cruise (June and July 1983) aboard the RV 'Noroît'. Surface microlayers (about 100 µm thick) were collected by means of Harvey's rotating device (Harvey 1966) which was cleaned by the method of Brockmann et al. (1976) and pushed by an electric motor boat at low speed.

Corresponding bulk waters were sampled by a peristaltic pump fitted with a silicon tube immersed at desired depths (up to 0.5 m). For bacterial analysis, surface films were transferred directly from the blade fixed on the rotating drum into a sterile bottle, and bulk waters were collected by lowering a sterile bottle to the required depth, opening the bottle, and allowing it to fill.

The sampling was carried out preferentially in slicks, when present. All samples were collected between 08:00 and 18:00 h GMT, prefiltered for biochemical measurements on 150 µm mesh nylon net and immediately prepared aboard ship for analysis.

Enrichment factors were calculated as the ratio of microlayer concentrations to bulk water concentrations (Sieburth et al. 1976).

Analysis. Salinity values were obtained with a Beckman salinometer (mod. RS-7B). Particulate organic carbon (POC), collected on precombusted Whatman GF/C filters, was determined on a Perkin-Elmer elementary analyser (mod. 240). For adenylic nucleotide measurement, particulate matter was collected on 0.45 µm pore size acetate membrane (Sartorius SM 11106) and subjected to extration with 5 ml boiling Tris buffer (0.02*M*, pH = 7.75) for 5 min (Holm-Hansen & Booth 1966). Extracts were stored at −18 °C, and assayed by the bioluminescent method after an enzymatic transformation of ADP and AMP into ATP (Pradet 1967, Romano & Daumas 1981). Chlorophyll *a* was measured by the fluorometric method of Yentsch & Menzel (1963).

Muramic acid concentrations were determined by HPLC according to Mimura & Delmas (1983) as modified by Mimura & Romano (1985), after acid hydrolysis (2.5 h with 6*N* HCl at 105 °C) of the particulate matter collected on 0.2 µm pore size polycarbonate membrane (Nucleopore). ETS measurements as 'potential-respiration index' were conducted by filtration on Whatman GF/F glassfiber filters, stored in liquid nitrogen and analysed according to the procedure of Packard (1971), as modified by Kenner & Ahmed (1975a, b). Dissolved oxygen concentrations were determined by the Winkler method modified by Carrit & Carpenter (1966). For estimation of real oxygen consumtion in the samples, incubation for BOD (Biochemical Oxygen Demand) measurements were made in the dark at room temperature for 6 to 36 h depending on the particle load of the samples. Three aliquots (100 ml) were prepared for one sample: < 150 µm particle size fraction, < 20 µm

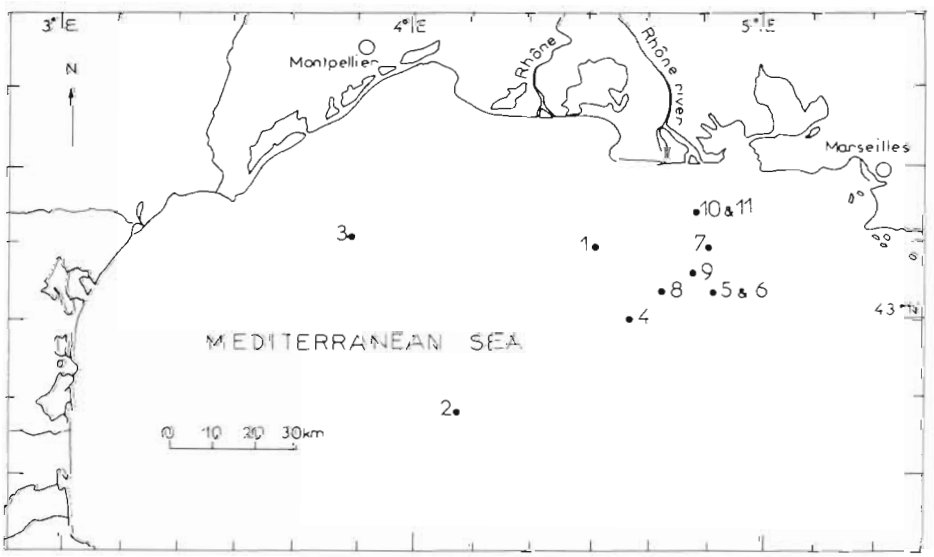

Fig. 1. Sampling locations in the northwestern Mediterranean Sea on the EURHOGLI cruise, June and July 1983

fraction and a control which was fixed with 1 ml of buffered formaldehyde solution (40 %) for estimating non-biological oxygen consumtion. Values obtained from the control samples were subtracted.

Total bacterial counts were made by DNA-binding fluorochrome DAPI or acridine orange and epifluorescence microscopy (Porter & Feig 1980). The percentage of actively growing heterotrophic Gram-negative bacteria was determined according to Kogure et al. (1979). Samples were incubated in the dark for 6 h with 0.025 % yeast extract and 0.002 % nalidixic acid. In the presence of nalidixic acid, a specific inhibitor of DNA polymerase for Gram-negative bacteria, living cells do not divide but increase their cell size. The elongated cells, in general strongly coloured reddish orange when acridine orange stain was used (Hobbie et al. 1977), can be easily counted by epifluorescence microscopy. By comparison with non-treated samples, it is possible to determine the percentage of these living cells.

Bacterial cell volume was approximatively estimated by epifluorescence microscopy according to Zimmerman (1977). Bacterial carbon (TBC-C) was estimated using TBC-C = total bacteria count (TBC) × average cell volume × 1.07 × 0.22 × 0.5, where 10.07 g cm^{-3} = specific density (Lamanna et al. 1973), 0.22 = dry wt/wet wt ratio and 0.5 = carbon/dry wt ratio (Luria 1960).

Microbiomass estimation. Total biomass, algal biomass and bacterial biomass were estimated by the following relations in order to compare them on a carbon basis.

Total living biomass:
ATP-C = 250 × ATP (Hamilton & Holm-Hansen 1967),

Algal biomass:
Chl-C = 60 × Chl *a* (Steele & Baird 1962),

Bacterial biomass estimated by muramic acid concentrations (MA):
MA-C = (MA/14) × 1000 (Mimura 1986).

Moriarty (1977) proposed C = (MA/12) × 1000 from a culture study on populations dominated by Gram-negative bacteria, but further studies carried out in our laboratory (Mimura 1986) suggest that the above formula is more appropriate for natural planktonic bacterial populations in the northwestern Mediterranean Sea.

Statistic. Because of high dispersion in values, the non-parametric Mann-Withney *U*-test was used for comparison of means between microlayer and related bulk water samples (Siegel 1956).

Stepwise multiple regression was used in order to explain the variation in BOD and ETS measurements by other variables. Salinity (S), particulate organic carbon (POC), total biomass (ATP-C), algal biomass (Chl-C) and bacterial biomass (MA-C) were taken into account as independent variables. The analysis was carried out at the 5 % significance level (Tomassone et al. 1983).

RESULTS

Salinity values (Table 1) from both the microlayer (I) and bulk water (B) samples were lower than those of classical Mediterranean coastal water (37.5 ‰) with the exception of Stns 1 and 2 (Table 1). Lower salinities were observed at the nearshore stations (Stns 4, 5, 6, 7, 10, 11), evidently influenced by the Rhône river water. No significant difference was found between microlayer (I) and related bulk waters (B), if all the samples are considered. However, considerable differences were recorded at Stns 7, 10, 11, the closest to the river mouth.

POC enrichment in surface microlayer

Mean values concerning particulate matter – POC, total living carbon (ATP-C), algal carbon (Chl-C) and bacterial carbon (MA-C) estimates and total bacteria count (TBC) – demonstrated significant enrichments in the microlayers (I) with regard to bulk waters (B) (Table 1). Important POC concentrations were obtained in microlayer samples collected in slicks which occurred under calm sea conditions: Stn 2 (21035 μg l^{-1}), Stn 4 (30175 μg l^{-1}), Stn 6 (28708 μg l^{-1}), Stn 7 (15654 μg l^{-1}) and Stn 9 (34739 μg l^{-1}) with enrichment factors (microlayer/bulk) of 149.2, 212.5, 66.5, 5.8 and 116.6 respectively. The low enrichment factor obtained at Stn 7 (5.8) is due to the fact that the related brackish bulk water was also highly loaded with POC (2690 μg l^{-1} at 0.5 m depth).

Bacterial biomass estimation

A good correlation has been previously reported (Mimura & Romano 1985) between MA concentrations and direct counts for microlayer and euphotic layer (0 to 50 m depth) samples collected during the same cruise. In the present work, bacterial carbon estimated by MA concentration (MA-C) was compared with that calculated from direct counts and average cell volume microscopically measured (TBC-C) at 5 stations (Table 2). At Stns 1, 2, 4 and 6, average cell volume ranged between 0.11 and 0.17 μm^3 in bulk waters and from 0.13 to 0.19 μm^3 in microlayers. In these samples, small bacteria with cell length < 0.5 μm represented about 50 % of the total counts (TBC), but only about 10 % of biovolume.

On the basis of biomass, the populations were dominated by large rods with an average cell volume > 0.16

Table 1. Comparison of salinity (S), particulate organic carbon (POC), total living carbon (ATP-C), algal carbon (Chl-C), bacterial carbon (MA-C), total bacteria count (TBC) and percentage of viable bacteria (% VB) in microlayer (I) and bulk water (B) samples

Stn	Depth (m)	S (‰)	POC	ATP-C[a] ($\times 10^3$ µg l^{-1})	Chl-C[a]	MA-C[a]	TBC ($\times 10^9$ l^{-1})	% VB (%)
1	I	37.625	2.546	0.235	0.014	0.114	1.7	2.0
	0.5	37.558	0.362	0.088	0.065	0.009	0.45	4.6
2	I	37.607	21.035	3.245	0.076	1.886	56.0	25.1
	0.5	37.551	0.141	0.030	0.011	0.007	0.46	3.0
3	I	36.744	6.374	2.478	0.025	–	–	–
	0.5	36.636	0.302	0.250	0.004	–	–	–
4	I	33.513	30.175	18.370	0.640	1.509	76.0	132.5
	0.05	33.458	0.280	0.049	0.014	0.048	4.0	3.9
	0.1	33.496	0.143	0.101	0.019	0.033	3.2	2.5
	0.2	33.492	0.214	0.093	0.023	0.038	2.4	4.9
	0.5	33.503	0.142	0.084	0.023	0.030	2.6	5.7
5	I	32.925	1.629	0.445	0.013	–	–	–
	0.5	32.821	0.400	0.102	0.013	–	–	–
6	I	32.951	28.708	3.350	0.549	1.049	49.0	–
	0.5	33.183	0.432	0.110	0.011	0.020	1.4	–
7	I	8.477	15.654	0.358	1.444	1.071	24.0	39.7
	0.2	14.105	1.252	1.183	0.557	1.144	–	–
	0.5	18.290	2.690	0.619	0.980	0.229	10.0	17.0
8	I	36.000	8.189	0.418	0.113	0.244	–	–
	0.5	35.723	0.467	0.037	0.043	0.021	–	–
9	I	37.362	34.739	2.349	0.557	0.683	–	–
	0.5	37.226	0.298	0.036	0.005	0.010	–	–
10	I	13.045	6.277	0.122	0.256	0.331	–	–
	0.5	21.384	0.664	0.149	0.159	0.051	–	–
11	I	10.495	0.578	0.067	0.179	0.076	–	–
	0.5	23.262	0.652	0.135	0.046	0.035	–	–
I	*n*	11	11	11	11	9	5	4
	Mean	30.625	14.173	2.849	0.384	0.773	41.0	50.0
	SD	10.140	11.960	5.306	0.440	0.647	31.0	49.0
B	*n*	15	15	15	15	13	8	7
	Mean	32.388	0.595	0.204	0.140	0.052	3.1	6.5
	SD	6.541	0.682	0.307	0.281	0.064	3.0	5.0
U-test	*U*	48	5	25	40	4	5	7
	p	ns[b]	<0.001	<0.01	<0.05	<0.001	<0.05	ns

[a] ATP-C = 250 × ATP; Chl-C = 60 × chlorophyll *a*; MA-C = 1000 × (MA/14); see also 'Materials and Methods'
[b] Not significant

µm³. This trend was particularly clear at Stn 7 where average cell volume was 0.27 µm³ in the bulk and 0.29 µm³ in the microlayer with a dominance of large rods in chains or in single cell form. Although average cell volume in the microlayers was found to be larger for each station, the overall statistical analysis (*U*-test) showed no significant difference between these 2 layers, probably due to the large data dispersion. Bacterial carbon estimated by muramic acid concentration (MA-C) was found to be very close to that calculated microscopically; MA-C/TBC-C ratios (Table 2) were < 2 with the exception of the microlayer sample from Stn 1.

MA content per cell (MA/TBC) (Table 2) was significantly higher in microlayers (I) (mean = 5.78×10^{-10} µg) than in bulk waters (B) (mean = 2.37×10^{-10} µg) ($U = 2$, $n_1 = n_2 = 5$; $p < 0.05$).

Biomass structure

Microplanktonic carbon to POC ratios (ATP-C/POC, Chl-C/POC and MA-C/POC) appear to be lower in the

Table 2. Comparison of bacterial carbon estimates in microlayer (I) and bulk water samples

Stn	Depth (m)	Average cell volume (μm^3)	TBC-C[a] ($\mu g\ l^{-1}$)	MA-C[b] / TBC-C	MA content per cell[c] ($\mu g\ cell^{-1}$)
1	I	0.13	27.1	4.2	9.4×10^{-10}
	0.5	0.11	6.0	1.4	2.7×10^{-10}
2	I	0.18	1238.3	1.5	4.7×10^{-10}
	0.5	0.11	5.6	1.3	2.5×10^{-10}
4	I	0.14	1263.8	1.2	2.8×10^{-10}
	0.5	0.11	35.8	0.8	1.6×10^{-10}
6	I	0.19	1128.8	0.9	3.0×10^{-10}
	0.5	0.17	28.3	0.7	2.0×10^{-10}
7	I	0.29	833.2	1.3	6.2×10^{-10}
	0.5	0.27	336.1	1.5	3.1×10^{-10}

[a] Bacterial carbon estimated microscopically by using TBC-C = total bacteria count (TBC) × average cell volume × 1.07 × 0.22 × 0.5 where: 1.07 = specific density (g cm^{-3}), 0.22 = dry weight/wet weight ratio, 0.5 = carbon/dry weight ratio (see also 'Materials and Methods')

[b] Bacteria carbon estimated from muramic acid concentration (MA) by using MA-C = 1000 × (MA/14). See also 'Materials and Methods'

[c] MA content per cell = MA/TBC

microlayer (Table 3): mean = 36.6 % (B) and 17.3 % (I) for ATP-C, mean = 13.3 % (B) and 4.8 % (I) for Chl-C and mean = 10.2 % (B) and 4.8 % (I) for MA-C, indicating a selective accumulation of detrital matter or photochemical destruction of cells by UV radiations. MA-C/ATP-C ratios (Table 3), which were significantly higher in microlayers (mean = 102.3 %) than in bulk waters (mean = 34.9 %), indicate an important bacterial contribution to total biomass in the microlayer.

Despite the small number of samples and the large range of variation (2 to 133 %), the percentage of actively growing bacteria (% VB) was higher in microlayers (Table 1) where intensive POC and TBC accumulations were recorded (Stns 2, 4 and 7). Values obtained in the bulk waters were relatively low and stable, ranging from 2.5 to 5.7 % with the exception of Stn 7 (10 %) where bacteria count was relatively high (10^7 cells ml^{-1}).

Energy charge ratios (EC; Table 3) exhibited high dispersion in the microlayer samples (range from 0.19 to 0.75) but were relatively stable in the bulk waters (0.54 ± 0.07). The lowest values were found at Stns 4, 6 and 7 (0.23, 0.19 and 0.27 respectively) where intensive POC accumulations occurred.

Respiratory activity measurements

BOD and ETS data are given in Table 4 together with the initial dissolved oxygen concentration. BOD measurements were carried out on < 20 and < 150 µm particle size fractions (< 150 µm fraction data not presented here). Comparison of the 2 fractions did not show significant differences either for all the samples ($n = 20$, $t = 1.134$) or for each layer ($n = 9$, $t = 1.274$ for microlayer and $n = 11$, $t = 2.201$ for bulk waters). As for the particulate matter enrichment described previously, BOD and ETS measurements were significantly higher in the microlayers. Values obtained at Stns 4, 6, and 7 are among the highest reported in the literature (Packard 1985, pers. comm.). The intensive respiratory activity was indicated also by significant deficiency of dissolved oxygen in the microlayers with regard to the bulk waters (Table 4), which may probably be partly explained by rapid oxygen consumption in the microlayer samples during the short period between sampling and sample treatment.

Comparison between BOD and ETS was carried out and relations obtained with linear adjustment are summarized in Table 5. In both layers (I and B), and also in whole samples, the 2 factors exhibit significant relations.

Relationships between respiratory activity, salinity, POC and microplanktonic biomass estimates

We attempted to explain the variation of BOD and ETS data from other studied factors. Correlation coefficients obtained after logarithmic transformation of data between salinity (S), POC, biomass estimates (ATP-C, Chl-C and MA-C), BOD and ETS are shown in Table 6.

Five points should be noted: (1) POC, ATP-C, Chl-C and MA-C are well correlated with each other in the bulk waters (B). In the microlayers (I), POC, ATP-C and

Table 3. Comparison of relative microplanktonic carbon ratios to particulate organic carbon (ATP-C/POC, Chl-C/POC and MA-C/POC in %) and to total living carbon (Chl-C/ATP-C and MA-C/ATP-C in %) and energy charge ratios (EC) in microlayer (I) and bulk water (B) samples

Stn	Depth (m)	ATP-C[a]/POC	Chl-C[a]/POC	MA-C[a]/POC	Chl-C[a]/ATP-C	MA-C[a]/ATP-C	EC
1	I	9.2	0.5	4.5	5.9	48.4	0.55
	0.5	24.4	17.9	2.4	73.3	9.7	0.51
2	I	15.0	0.4	9.0	2.4	60.0	0.75
	0.5	31.3	7.7	5.1	36.0	23.8	0.60
3	I	38.9	0.4	–	1.0	–	0.45
	0.5	82.8	1.4	–	1.7	–	0.56
4	I	60.9	2.1	5.0	3.5	8.2	0.23
	0.05	17.6	5.1	17.1	29.2	97.1	0.67
	0.1	70.5	13.1	23.0	18.4	32.6	0.53
	0.2	43.6	10.7	17.7	24.4	40.5	0.55
	0.5	58.9	16.5	21.2	28.0	35.9	0.52
5	I	27.2	0.8	–	3.0	–	0.56
	0.5	25.4	3.2	–	12.4	–	0.54
6	I	11.7	1.9	3.7	16.4	31.3	0.19
	0.5	25.4	2.5	4.6	9.8	18.2	0.50
7	I	2.3	4.2	6.8	403.7	299.2	0.27
	0.2	94.5	44.5	11.5	47.1	12.1	0.55
	0.5	23.0	36.4	8.5	158.4	36.9	0.45
8	I	5.1	1.4	3.0	27.0	58.3	0.50
	0.5	7.9	9.3	4.6	117.5	58.3	0.39
9	I	6.7	1.6	2.0	23.7	29.1	0.44
	0.5	12.0	1.6	3.4	13.4	28.0	0.48
10	I	1.9	4.1	5.3	210.4	371.6	0.60
	0.5	22.4	22.6	7.7	100.7	34.5	0.61
11	I	11.5	30.9	93.1	267.9	113.4	0.48
	0.5	20.7	7.1	5.5	34.2	26.4	0.59
I	*n*	11	11	9	11	9	11
	Mean	17.3	4.8	5.8	87.7	102.3	0.46
	SD	17.4	9.0	3.2	140.0	101.9	0.17
B	*n*	15	15	13	15	13	15
	Mean	36.6	13.3	10.2	47.0	34.9	0.54
	SD	26.1	13.3	6.9	45.5	22.6	0.07
U-test	*U*	37	29	ns[b]	ns	31	ns
	p	< 0.01	< 0.01			< 0.05	

[a] ATP-C = 250 × ATP, Chl-C = 60 × chlorophyll *a*; MA-C = 1000 × (MA/14); see also 'Materials and Methods' Ratios are expressed as %

[b] Not significant

MA-C still show strong correlations; they are however not significantly correlated with Chl-C. (2) Strongly negative correlations which appear in the bulk waters (B) between salinity (S) and biomass estimates (ATP-C, Chl-C and MA-C) are not found in the microlayers. (3) ETS activity is found to be well represented by POC, ATP-C, Chl-C and, in particular, MA-C in both layers (I and B). (4) BOD, on the contrary, is not correlated with Chl-C at significant levels. (5) BOD is more significantly correlated with POC, ATP-C and MA-C in the microlayers (I) than in the bulk (B).

The stepwise multiple regression analysis performed at the $p < 0.05$ level showed the following relations for BOD and ETS:

In the microlayers:

Log (BOD) = 0.913 Log (POC) − 1.579 (r = 0.916, p = 0.0017)

Log (ETS) = 1.340 Log (MA-C) − 1.693 (r = 0.884, p = 0.0017)

In the bulk waters:

Log (BOD) = 0.896 Log (MA-C) − 0.529 Log (POC) + 1.612 (r = 0.773, p = 0.017)

Log (ETS) = 1.376 Log (MA-C) − 1.007 (r = 0.967, p < 0.001)

Table 4. Comparison of dissolved oxygen concentration (O_2), Biochemical Oxygen Demand (BOD) and Electron Transport System (ETS) activity in microlayer (I) and bulk water (B) samples

Stn	Depth (m)		O_2 (ml l^{-1})	BOD ($\mu l\ O_2\ l^{-1}\ h^{-1}$)	ETS ($\mu l\ O_2\ l^{-1}\ h^{-1}$)
1	I		–	–	6.8
	0.5		–	–	1.6
2	I		4.7	313.8	245.5
	0.5		5.2	26.3	1.6
3	I		4.8	79.2	113.1
	0.5		5.1	–	2.5
4	I		3.5	583.3	510.9
	0.05		5.7	65.0	18.0
	0.1		5.9	86.7	14.4
	0.2		5.9	38.3	15.6
	0.5		5.8	66.7	18.6
5	I		4.8	48.8	52.9
	0.5		5.6	28.8	16.3
6	I		3.8	313.3	765.1
	0.5		5.3	65.8	–
7	I		4.8	253.8	420.4
	0.2		6.0	111.7	159.1
	0.5		8.0	112.5	94.7
8	I		4.4	120.0	18.5
	0.5		5.1	15.8	9.8
9	I		4.2	220.0	69.0
	0.5		4.9	12.9	1.4
10	I		5.3	26.7	21.1
	0.5		5.7	24.2	19.2
11	I		5.4	11.7	20.4
	0.5		5.4	17.5	10.0
	I	*n*	10	10	10
		Mean	4.6	197.1	204.0
		SD	0.6	168.9	243.0
	B	*n*	14	13	14
		Mean	5.7	51.7	27.0
		SD	0.7	34.2	43.0
	U-test	*U*	11	30	23
		p	< 0.001	< 0.05	< 0.01

Table 5. Comparison of relationships between Biochemical Oxygen Demand (BOD) and Electron Transport System (ETS) activity, obtained as linear regression BOD = a(ETS) + b in microlayer (I), bulk water (B) and combined (T) samples

Sample	*n*	*a*	*b*	*r*	*p*
(I)	10	0.523	82.213	0.748	< 0.05
(B)	12	0.612	31.211	0.780	< 0.01
(T)	22	0.577	48.564	0.814	< 0.001

Only 1 (MA or POC) or 2 (MA and POC) variables were taken into account because of strong dependences between the variables employed (Table 6).

Table 6. Table of correlation coefficients among log-transformed salinity (S), particulate organic carbon (POC), total living carbon (ATP-C), algal carbon (Chl-C), bacterial carbon (MA-C), Biochemical Oxygen Demand (BOD) and Electron Transport System (ETS), in microlayer (I) and bulk water (B) samples

Relation	I	B
	n = 9	*n* = 13
Log (S) vs Log (POC)	0.450	−0.812***
vs Log (ATP-C)	0.632	−0.916***
vs Log (Chl-C)	−0.402	−0.861***
vs Log (MA-C)	0.244	−0.842***
Log (POC) vs Log (ATP-C)	0.832**	0.758**
vs Log (Chl-C)	0.511	0.833***
vs Log (MA-C)	0.910***	0.701**
Log (ATP-C) vs Log (Chl-C)	0.327	0.859***
vs Log (MA-C)	0.812**	0.831***
Log (Chl-C) vs Log (MA-C)	0.548	0.779**
	n = 8	*n* = 12
Log (BOD) vs Log (S)	0.603	−0.411
vs Log (POC)	0.916**	0.213
vs LG (ATP-C)	0.894**	0.643*
vs Log (Chl-C)	0.360	0.453
vs Log (MA-C)	0.909**	0.653*
	n = 9	*n* = 12
Log (ETS) vs Log (S)	0.033	−0.807**
vs Log (POC)	0.735*	0.597*
vs Log (ATP-C)	0.736*	0.820**
vs Log (Chl-C)	0.701*	0.745**
vs Log (MA-C)	0.884**	0.967**
	n = 10	*n* = 12
Log (BOD) vs Log (ETS)	0.711**	0.434**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; otherwise not significant

DISCUSSION

Bacterial biomass estimation by muramic acid concentrations

MA content per cell (MA/TBC) is related to the respective proportions of Gram-negative and positive bacteria in natural populations, the latter containing in their cell walls 3 to 5-fold more MA (Miller & Casida 1970, Moriarty 1977, Mimura 1986). It is known that the majority of planktonic and neustonic bacteria in aquatic environments in offshore, coastal and estuarine waters are Gram-negative (ZoBell & Upham 1944, Sieburth 1971, Reinheimer 1974, Crow et al. 1975, Fehon & Oliver 1979). However, Sieburth (1983) described a possible presence of Gram-positive bacterial populations in sea surface films. In estuarine and fresh water environments, the relative proportion of Gram-negative and -positive bacteria needs to be

known for accurate biomass estimation by muramic acid because of possible inclusion of Gram-positive soil bacteria.

Mimura (1986) and Moriarty (1977), by monospecific culture studies, found that MA content per cell (MA/TBC) also varied with bacterial cell volume. Our previous work (Mimura & Romano 1985, Mimura 1986) showed that this factor decreased with sampling depth (0 to 50 m) in the Mediterranean Sea and paralleled the average cell volume of bacterial populations.

Thus, to study natural populations, MA data should be interpreted with those of microscopical observations such as the direct count and cell volume measurements. Taking this into account, MA analysis is shown to be useful for estimating the bacteria biomass in sea surface film where bacteria are often attached to detrital matter to form microcolonies, and are thus hard to count.

It is also possible that the higher MA content per cell (MA/TBC) in the microlayers is related to the presence of uncounted bacteria which are masked by particles.

When MA content per cell (MA/TBC) is compared with average cell volume, the value obtained in the microlayer sample from Stn 1 (9.39×10^{-10} µg) appears to be very high (Table 2). This suggests an enhanced proportion of Gram-positive population in this sample. Omitting the value from Stn 1, the 2 factors exhibited a significant correlation ($r = 0.720$, $n = 9$, $p < 0.001$).

Actively-growing bacteria fraction

The nalidixic acid incubation technique (Kogure et al. 1979) has been proposed for microscopically counting bacteria capable of synthesizing cellular materials under nutrient-sufficient conditions when cell division involving DNA synthesis is inhibited. Winogradsky (1949), Jannasch (1958) and Torella & Morita (1981) have described, for marine environments, 2 types of bacteria which can be distinguished by their growth rates under sufficient energy source, the first type exhibiting rapid growth and increase in cell size and the second being small and slow-growing without cell size changes. One may suppose that bacteria showing a positive response to the nalidixic acid test belong only to the first type of bacteria.

The percentage of actively growing bacteria (% VB; Table 1) obtained in the microlayer at Stn 4 (132.5 %) considerably exceeded the theoretical limit (100 %). This may be due to the presence of rapidly growing bacteria which divided at the beginning of the incubation period before the inhibiting effect of nalidixic acid could begin, or to the presence of viable bacteria which were not counted in non-treated sample but were detected, probably because of the cell size increase, in the incubated sample. Regardless of cause, the value indicates a high growth potential of this population. This may be related to a high ATP concentration (73 ng l^{-1}) obtained in the same sample.

Higher % VB appears to occur at stations where important POC enrichments were observed (Stns 2, 4 and 7), the lowest value of microlayer samples being obtained at Stn 1 (2 %) which was weakly enriched. However numerous missing values of % VB do not permit more conclusive discussion.

Accumulation dynamics in surface films

The mean enrichment factor (enrichment factor = microlayer/bulk water concentrations, by weight: Sieburth et al. 1976) varied according to the parameter determined: POC (55.5) > MA (52.6) > ATP (41.6) > TBC (38.4) > chl *a* (19.8), which suggests that POC and bacteria are preferentially accumulated in the microlayer or that accumulated phytoplankton organisms have a lower chlorophyll content (see also de Souza-Lima & Romano 1983).

A marked influence of the Rhône river on microlayer biomass structure can be found in the ratios of relative biomass (Chl-c; MA-C) to total living carbon (ATP-C) and in the values of energy charge (EC) ratios.

At Stns 7, 10 and 11, which were located in lower salinity waters (8.477, 13.045 and 10.495 ‰, respectively), Chl-C/ATP-C and MA-C/ATP-C ratios were very high (100 to 400 %) in the microlayers compared to values obtained in the bulk waters. These low ATP concentrations related to other compounds suggest that a part of both the phyto- and bacterioneuston are dead (see also Bell & Albright 1982, de Souza-Lima & Romano 1983). Interpretation of our EC ratios is delicate because both the highest (0.75) and the lowest (0.19) value were obtained in the microlayer samples. The highest value was found in a sample without river water influence (Stn 2), and the lowest at stations located in the brackish water areas (Stns 4, 6 and 7). According to culture studies performed by Chapman et al. (1971), Wiebe & Bancroft (1975), Chapman & Atkinson (1977) and Karl & Holm-Hansen (1978), low EC values (< 0.6) are found in a senescent or a starved state, but values as low as 0.1 to 0.3 obtained in our microlayer samples are probably due to the selective accumulation in this layer of dead or stressed populations containing detrital AMP (Romano & Daumas 1981, Romano 1982) which may have occurred in the river-ocean interface system. However, the coexistence in this detrital environment of a dead and an actively growing bacterial fraction is certain since the percentage of the latter was obviously high as frequently observed in particle-bound microcolonies (Harvey &

Young 1980, Mimura 1986). Values of chlorophyll concentrations show that accumulation of phytoplankton in the microlayer is relatively low in comparison with that of bacteria. Some authors, e.g. Hardy (1973) and Wanschneider (1979), however, reported active accumulations of microalgae involving phytoflagellates. Our recent study (de Souza-Lima unpubl.) indicates a specific accumulation of some flagellates belonging to the Chrysophycea which have phycoxanthine as a characteristic carotenoid pigment. According to Allen et al. (1960), *Ochromonas danica* could contain this pigment up to 75 % of its total pigment. In this case, the algal biomass calculated from chlorophyll concentrations should be revised.

Respiratory activity

Some methodological problems exist in respiratory studies using BOD and ETS measurements when applied to microlayer samples. The BOD measurement is performed assuming that the oxygen consumption rate is constant during incubation. Ogura (1975), Fuhrman & Azam (1980) and Williams (1981) reported no significant changes in metabolic activities during 48 h incubations with seawater samples. On the other hand, bacterial biomass increase during confinement is well known (ZoBell & Anderson 1936, Kirchman et al. 1982). Enhanced bacterial viability found in our microlayer samples suggests a possible bacterial development during confinement and, thus, an overestimation of BOD.

A problem of ETS measurements can be found in the utilization of glass fiber filters for concentrating samples. A significant part of ETS activity may have passed through the filters especially when working in oligotrophic offshore or deep-sea waters where most bacteria appear to be small and freefloating as was previously demonstrated by Gostan et al. (1985) on ATP measurements. However, a substantial proportion of bacteria are known to be attached in particle-rich microlayers (Harvey & Young 1980, Bell & Albright 1982) and brackish waters (Bell & Albright 1982, Goulder 1977). In microlayer samples, Mimura (1986) showed that more than 80 % of muramic acid was retained by 3 µm Nucleopore filters. We consider, therefore, that most of the bacterial biomass was retained on the filters in our microlayer and bulk brackish water samples. A possible loss of ETS activities can be suspected in bulk water samples with higher salinities (Stns 1, 2, 3 and 9) where values of ETS were very low when compared with those of BOD (Table 4). ETS activity can also be lost by an incomplete extraction of enzymatic systems from filtered cells but this is hard to calibrate in natural water samples.

The comparison between the real oxygen consumption (BOD) and the 'potential respiration' of a natural sample by ETS measurement has been found to be interesting for characterizing natural planktonic communities (Packard 1985). Slope values from our crude data regressions (Table 5), 0.523 for the microlayer (I) and 0.612 for the bulk (B), appear to be relatively high when compared with previously reported values (Packard 1985). Packard et al. (1974) obtained a slope value equivalent to 0.16 in upwelled waters of the northwest African coast, after correction of the original value (0.55) in order to compare data obtained by different methods (Christensen & Packard 1979), while Packard & Williams (1981) found in the Gulf of Maine a value of 0.34 which is 2 times higher than the precedent. According to Packard (1985), the second case is due to a mixed community of microzoo-, phyto- and bacterioplankton, and the former to a population dominated by *Chaetoceros socialis*. Taking this into account, our results suggest that the slope values increase with an increased proportion of bacteria populations in planktonic communities.

In our preceding papers, we attempted to estimate respective phytoplanktonic and bacterial contributions to total ETS measurements (Romano et al. 1987a, b), by using biomass indicators such as chlorophyll, adenylic nucleotides, bacteria cell count and muramic acid measurements. The proposed model for bacterial contribution (ETS_b) is: $ETS_b = ETS_m - (5.28 \times \text{chlorophyll})$ where ETS_m is measured ETS value. When the model is applied to our samples, estimated bacterial ETS values are 15.1 ± 27.1 µl O_2 l^{-1} h^{-1} (mean ± standard deviation) in the bulk waters and 173 ± 223.5 µl O_2 l^{-1} h^{-1} in the microlayers. These values are significantly different ($U = 41$, $n_1 = 11$, $n_2 = 14$, $p < 0.05$). Bacterial ETS per cell (ETS_b/TBC) is also higher in the microlayers than in the bulk waters (mean = 8.05 and 2.0 µl O_2 l^{-1} h^{-1} per cell respectively) at $p < 0.05$ level ($U = 7$, $n_1 = 5$, $n_2 = 7$).

Thus our results indicate that enhanced BOD and ETS values obtained in the microlayers are well associated to bacterial activity. Importance of microheterotrophic respiration in seawater has been reported by Williams (1981). Our results are in agreement with those obtained by Harvey & Young (1980) who, by using the INT technique ([2-(*p*-iodophenyl)-3(*p*-nitrophenyl)-5-phenyl tetrazolium chloride] dye) found that detectable respiring bacteria were 16 times more abundant in a salt marsh microlayer than in the corresponding underlying water.

Values for the microbial activity of the air-water interface found in the literature are difficult to compare. Difficulties may derive from: a possible seasonable fluctuation in heterotrophic activities (Bayley et al. 1983, Carlucci et al. 1985); different incubation methods used for estimating activities (incubation and

sampling time, temperature, added labelled substrates), studied sites (offshore, coastal or estuarine waters); and notably differences in enrichment between the studied microlayers (e.g. POC enrichment factor varied from 0 to 100 in our samples). In the present work, 6 out of 11 stations (Stns 2, 3, 4, 6, 7 and 9) were located in slicks which occurred in calm sea conditions, while other stations were investigated in choppy or calm sea without slicks. When enrichment factors of POC, BOD and ETS are compared (Table 7)

Table 7. Comparison of microlayer enrichment factor (microlayer/bulk) of particulate organic carbon (POC). Biochemical Oxygen Demand (BOD) and Electron Transport System (ETS) activity in slick (Stns 2, 3, 4, 6, 7 and 9) and non-slick (Stns 1, 5, 8, 10 and 11) samples

Sea surface state		POC	BOD	ETS
	n	6	5	5
Slick	Mean	95.3	9.0	56.0
	SD	88.7	5.8	57.3
	n	5	4	5
Non-slick	Mean	7.8	2.8	2.5
	SD	6.3	3.2	1.3
U-test	*U*	3	1	0
	p	< 0.05	< 0.05	< 0.05

between the 2 sampling station groups described above, mean values are clearly higher in slick stations as was previously demonstrated from microlayer samples by be Souza-Lima & Romano (1983). Thus, sea surface conditions during sampling are very important factors to consider in activity studies on the sea surface microlayer.

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