Dissolved Organic Matter and Heterotrophic Microneuston in the Surface Microlayers of the North Atlantic

Abstract. *Dissolved organic carbon, carbohydrates, and adenosine triphosphate in the size fractions 0.2 to 3 micrometers and 3 to 1000 micrometers are significantly enriched in the upper 150-micrometer surface layer compared to subsurface water, mean enrichment factors being 1.6, 2.0, 2.5, and 3.1, respectively. When calculated as a 0.1-micrometer microlayer of wet surfactants, the mean concentration of organic matter was 2.9 grams per liter, of which carbohydrates accounted for 28 percent. The data for plant pigments and particulate adenosine triphosphate indicated that bacterioneuston was enriched at seven of nine stations while phagotrophic protists were enriched at five stations. Instances of enrichment and inhibition were verified by cultural data for bacteria and amoebas. The observations indicate that the surface microlayers are largely heterotrophic microcosms, which can be as rich as laboratory cultures, and that an appreciable part of the dissolved organic carbon is carbohydrate of phytoplankton origin, released and brought to the surface by migrating and excreting phagotrophic protists.*

The sea-air interface has attracted attention because of its importance in the detection and limitation of pollution (*1*). However, the sea surface is a major site of biological activity, and the organisms that have adapted to this specialized environment (*2*) have been termed neuston (*3*). Despite the importance of neuston in the diet of both sea birds and migrating nekton and the growing knowledge of the biology of the neuston (*4*), the chemical and microbiological nature of the surface film and the physical and biological mechanisms for its formation require further study. Light intensities at the sea surface are phototoxic to most phytoplankters; exceptions are *Trichodesmium*, *Sargassum*, and some pennate diatoms that are lifted onto the surface film in intertidal waters (*5*). Therefore the microneuston that nurtures the conspicuous blue invertebrate fauna on the open sea must be largely heterotrophic in nature and be based on bacterioneuston (*6*) growing on nutrients produced by the phytoplankton in the shaded euphotic zone below the sea surface.

It has been assumed that the surfactant nature of the surface layer is due to hydrocarbons, fatty acids and fatty alcohols, and similar dry surfactants (*1*, *7*), which produce a 0.01-μm microlayer above the sea-air interface (*8*). However, it has been shown that except for heavily traveled waterways polluted by hydrocarbons, the surface microlayer is dominated by a 0.1-μm-thick microlayer of wet surfactants below the sea-air interface that behaves like a polysaccharide-protein complex (*9*). The study reported here was designed to further characterize the nature and concentration of the dissolved organic carbon (DOC) and microorganisms in the surface microlayers in an attempt to show the relationship of the microbial plankton and neuston to the chemistry of the microlayer. Data are presented to show that there is a small but consistent and statistically significant enrichment of both DOC and microneuston in the surface film. If the dissolved materials are actually concentrated in a 0.1-μm microlayer (*8*, *9*) while the microorganisms are concentrated in a 1.0-μm microlayer, then the nutrient concentrations and microbial populations approach or equal those in laboratory cultures.

Stations were occupied at the locations shown in Table 1. A Nytex screen sampler (*1*) that had been scrubbed in alcohol was used to obtain the approximately 150-μm-thick surface layer. When the sea state allowed, an inflatable boat with low freeboard was used to sample away from the ship. Subsurface samples, taken with 5- and 30-liter Niskin bottles that had been scrubbed with 0.1*N* hydrochloric acid and rinsed in water in situ for 20 minutes before sampling (*10*), were filtered with a 1-mm Nytex screen and processed as rapidly as possible. The chemical and adenosine triphosphate (ATP) analyses were made in triplicate. The samples for DOC and carbohydrates were passed through Gelman glass-fiber filters (type A/E) without vacuum to remove suspended particles. Samples for total DOC (*11*) were prepared at sea and analyzed on shore. The determination of monosaccharides and total carbohydrates and the estimation of polysaccharides by difference were done aboard ship by using procedures based on precise and reliable chemical reactions (*12*). Organisms smaller than 1000 μm were divided into fractions < 3 μm and > 3 μm by Nuclepore filtration; the amount of ATP was determined at sea between stations (*13*, *14*), and plant pigments (*15*) were used to detect accumulations of phytoplankton. Large volumes of water (up to 200 liters) were passed through a continuous reverse flow concentrator (*16*) that reduced the volume containing the particles > 3 μm by a factor of 1000 in 2 hours or less. Fresh concentrates were examined at sea with phase contrast microscopes fitted with head braces (necessary on a rolling ship); portions fixed at sea by the addition of an equal volume of 6 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer (*p*H 7.20) with 0.25*M* sucrose were examined in greater detail on shore by light microscopy and scanning and transmission electron microscopy. Fresh concentrates were also used to prepare serial decimal dilutions for the extinction dilution culture of bacteria and amoebas. For bacteria, the peptone-yeast extract medium contained 1 g of each per liter of seawater, the carbohydrate medium contained 1 g each of glucose, mannitol, and glycolic acid per liter, and the chitin medium contained approximately 2 g of crab chitin suspended in 1 liter of seawater. Either a single rice grain or a 0.1-cm^3 1.5 percent agar plug of the peptone-yeast extract medium was used to sustain low bacterial populations for the amoeba cultures.

The data obtained for the screen and subsurface samples at nine stations from Block Island Sound along the outer continental shelf to the Grand Banks (stations 2, 5, 6, and 7) and across the North Atlantic to east of the Azores (stations 9, 11, 12, 13, and 14) are given in Table 1. The differences for all samples are significant at the 95 percent level except for two values. The concentration of materials in the surface microlayers was calculated by subtracting the subsurface (mean depth, 8 m) value from the screen value, and the remainder was multiplied by a factor based on a screen efficiency of 70 percent (*17*) and a dilution of the microlayer with the subsurface water (*1*). Assumed thicknesses of the components used to estimate the concentration of dissolved organic matter and microorganisms in the surface microlayer are shown in Table 1.

In contrast to the DOC concentrations of ≤ 1.3 mg of carbon per liter in the subsurface waters, the DOC in the microlayer has an estimated effective carbon concentration of 471 to 2250 mg/liter (mean value, 1427 mg/liter) or a dry weight of 2.9 g of organic matter per liter. This is the concentration of organic matter used to grow bacteria in seawater media. A mean value for total carbohydrates of 875 mg/liter indicates that carbohydrates account for some 28 percent of the DOC in the microlayer. The particulates < 3 μm are primarily bacteria, although some of the smaller apochlorotic microflagellates, which presumably func-

tion like bacteria, may also be present, depending on the sample. The large, chloroplast-containing phytoplankton were apparently not present in these preparations. An idea of the order of magnitude of the biomass of the bacterioneuston can be obtained by using the ATP : C conversion factor of 1 : 250 (*14*), which indicates concentrations of 0.08 to 5 mg of cellular carbon per liter.

The ATP concentration for the protists in the fraction > 3 μm, when present, was a third that of the bacterioneuston. Except at the shallow-water station 2, there was no appreciable concentration of chlorophyll a. These observations indicate that this > 3-μm fraction was usually due to phagotrophic protists, presumably grazing on the bacterioneuston. This was confirmed by microscopic observation of concentrates. At two stations the ATP concentration in the fraction < 3 μm was lower in the microlayer than in the subsurface sample; this may have been caused by grazing or by growth inhibition. The data on dilution extinction cultures for bacteria and amoebas at stations 2 and 12 are given in Table 2. The apparent inhibition at station 2 indicated in Table 1 is also con-

Table 1. Concentrations of dissolved organic carbon (DOC) and living particulates in the surface microlayers of the North Atlantic in July and August 1975. In the calculations a thickness commensurate with the dimensions of the material was assumed: a 0.1-μm layer for DOC gives a dilution factor of 1 : 1500; a 1.0-μm layer for the fraction < 3 μm, 1 : 150; and a 10-μm layer for the protists > 3 μm, 1 : 15. Microlayer concentration = (surface concentration − subsurface concentration) (70 percent screen efficiency × dilution with subsurface water). Enrichment is the screen enrichment factor, that is, (screen concentration)/(subsurface concentration). Differences between the means for the surface and subsurface determinations were statistically significant at the 95 percent level with two exceptions noted below. Letters in parentheses under station numbers indicate screen sampling method: Z means sampled from the Zodiac inflatable, and V means sampled from the bow of the research vessel.

Materials assayed	Station 2 (Z)	Station 5 (V)	Station 6 (V)	Station 7 (V)	Station 9 (V)	Station 11 (Z)	Station 12 (Z)	Station 13 (Z)	Station 14 (V)	Means
Location	41°48′N, 67°05′W	43°31′N, 59°29′W	43°19′N, 59°41′W	43°40′N 52°40′W	40°36′N 39°48′W	37°28′N, 28°43′W	37°28′N 28°44′W	36°59′N 21°22′W	36°47′N, 21°20′W	
Depth (m)	50	1794	160	296	4972	2000	1880	3803	4517	
Dissolved organic carbon (mg/liter)										
Microlayer	1714	471	2186	921	2207	1671	1029	600	1221	1336
Screen	1.88	1.55*	2.11	1.70	2.15	1.72	1.47	1.32	1.64	1.73
Subsurface	1.08	1.33	1.09	1.27	1.12	0.94	0.99	1.04	1.07	1.10
Enrichment	1.7	1.2	1.9	1.3	1.9	1.8	1.5	1.3	1.5	1.6
Carbohydrate carbon (mg/liter)										
Monosaccharides										
Microlayer	214	111	96	86	334	244	107	103	150	161
Screen	0.150	0.126	0.124	0.182	0.230	0.192	0.128	0.131	0.144	0.156
Subsurface	0.050	0.074	0.079	0.142	0.074	0.078	0.078	0.083	0.074	0.081
Enrichment	3.0	1.7	1.6	1.3	3.1	2.5	1.6	1.6	1.9	2.0
Polysaccharides										
Microlayer	221	NE†	193	257	491	154	139	103	411	246
Screen	0.220	0.154	0.190	0.205	0.273	0.168	0.137	0.141	0.301	0.199
Subsurface	0.117	0.171	0.100	0.085	0.044	0.096	0.072	0.093	0.109	0.099
Enrichment	1.9	0.9	1.9	2.4	6.2	1.8	1.9	1.5	2.8	2.3
Total carbohydrates										
Microlayer	435	75	289	343	825	398	246	206	561	375
Screen	0.370	0.280	0.314	0.387	0.503	0.360	0.265	0.272	0.445	0.355
Subsurface	0.167	0.245	0.179	0.227	0.118	0.174	0.150	0.176	0.183	0.180
Enrichment	2.2	1.1	1.7	1.7	4.3	2.1	1.8	1.5	2.4	2.0
Percentage of DOC										
Microlayer	25	16	13	37	37	24	24	34	46	28
Screen	20	18	15	23	23	21	18	21	27	21
Subsurface	15	18	16	18	10	18	15	17	17	16
Biomass (10^{-8} g ATP/liter)										
Fraction < 3 μm										
Microlayer	NE†	793	28	NE	1072	165	2145	362	1779	906
Screen	5.19	6.96	2.73	3.45	9.32	3.51	11.67	3.37	10.14	6.26
Subsurface	6.14	3.26	2.60	5.89	4.32	2.74	1.66	1.68	1.84	3.35
Enrichment	0.8	2.1	1.1	0.6	2.2	1.3	7.0	2.0	5.5	2.5
Fraction > 3 μm										
Microlayer	664	NE	NE	NE	273	58	204	NE	245	289
Screen	44.98	3.59	5.26*	3.81	21.18	4.58	11.10	4.71	12.64	12.43
Subsurface	14.00	13.45	5.87	7.44	8.43	1.88	1.59	5.72	1.23	6.62
Enrichment	3.2	0.3	0.9	0.5	2.5	2.4	7.0	0.8	10.3	3.1
Pigments (μg/liter)										
Chlorophyll a										
Microlayer	50	NE	NE	1.28	0.64	NE	0.21	0.08	1.50	8.95
Screen	3.78	1.09	0.00	0.19	0.14	0.05	0.07	0.08	0.14	0.62
Subsurface	1.46	2.05	0.10	0.13	0.11	0.07	0.06	0.08	0.07	0.45
Enrichment	2.6	0.5		1.5	1.3	0.7	1.2	1.0	2.0	1.4
Phaeopigments										
Microlayer	NE	NE	NE	0.02	NE	0.21	1.07	0.03	0.43	0.35
Screen	0.13	1.14	0.00	0.02	0.03	0.03	0.06	0.03	0.03	0.16
Subsurface	0.60	6.75	0.06	0.02	0.06	0.02	0.01	0.03	0.03	0.84
Enrichment	0.2	0.2		1.0	0.5	1.5	6.0	1.0	3.0	1.7

*Not significant. †NE = not enriched.

firmed by the cultural data. If inhibition rather than grazing occurred, it could have been due to substances (*18*) concentrated in the microlayer that were released from the appreciable *Nitzschia* bloom at this station indicated by the chlorophyll value. An instance of bacterial inhibition in the surface film has been reported (*19*). In contrast to the cultural data from station 2, the data from station 12 indicated a marked enrichment. When corrected for the thickness of the surface microlayer, bacterial populations on all three media were in line with the biomass estimates from ATP. Data on the amoebas associated with the microlayer correlated with those for the bacteria: extensive bacterial populations supported extensive amoeba populations.

The blue-pigmented pontellid copepods floating on the surface at several stations occurred in concentrations sufficient to be noticed during screen sampling. At station 12 half the specimens were infested with the suctorian *Ephelota gemmipara*, with as many as 80 individuals per copepod. A group of individuals from juvenile to an exogenously budding mature form are shown in Fig. 1A. The suctorians were present mainly in a band along the waterline of these surface-dwelling copepods. The only other copepod infested with stalked ciliates was *Acartia tonsa* from nearshore station 1, which was heavily infested with *Zoothamnium alternans* (*20*). The restriction of these epizoic stalked ciliates to nearshore waters and to the surface microlayer and fouled surfaces (*21*) of open ocean waters indicates that they probably occur only when there is an abundant source of microorganisms in the water. Thin sections of the suctorians (Fig. 1) indicate that bacteria are a substantial part of their diet and must occur in significant concentrations in the surface microlayer.

Carbohydrates accounted for 12 to 46 percent of the DOC (mean, 28 percent). They are produced by the phytoplankton as photosynthesate, reserve, extracellular, and cell wall materials (*22*), and may occur in part as side chains on protein backbones—that is, glycoproteins and mucopolysaccharides. The regular diurnal accumulation of monosaccharides, polysaccharides, and DOC in the subsurface waters at these stations was strongly associated with phagotrophic protist maxima occurring near phototrophic protist maxima (*23*). Such a continual replenishment of dissolved organic matter would be needed to sustain the microbial activity we observed. The enrichment of wet surfactants in the surface microlayers is probably achieved through a

Table 2. Cultivable populations of bacteria and amoebas in a theoretical 1-μm surface microlayer at station 2 (inhibitory) and station 12 (enriched).

Station	Sample	Bacteria per milliliter			Amoebas per milliliter	
		Peptone yeast medium	Monosaccharide medium	Chitin medium	Rice grain	Nutrient agar plug
2	Microlayer (0.1 μm)*	NE†	NE	NE	NE	NE
	Screen (150 μm)	5×10^2	5×10^1	5×10^1	<0.01	<0.01
	Subsurface (37 m)	1×10^3	5×10^1	1×10^2	0.50	2.30
12	Microlayer (0.1 μm)	$>2 \times 10^7$	$>2 \times 10^7$	1×10^7	1200	1350
	Screen (150 μm)	$>1 \times 10^5$	$>1 \times 10^5$	5×10^4	6.5	6.5
	Subsurface (30 m)	5×10^2	1×10^4	5×10^1	0.9	0.2

*Estimated from the screen minus subsurface concentrations × 214 (which allows for 70 percent screen efficiency and a 1 : 150 dilution with subsurface water). †NE = not enriched.

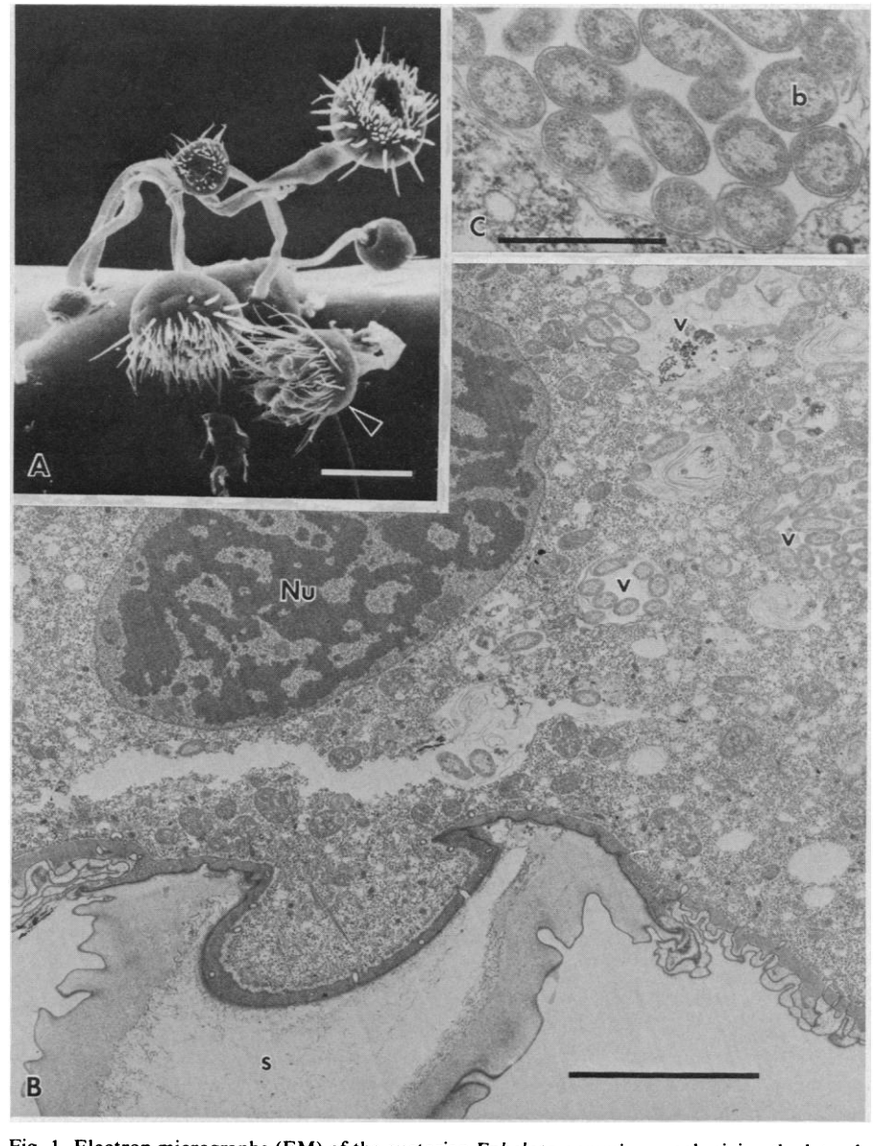

Fig. 1. Electron micrographs (EM) of the suctorian *Ephelota gemmipara* colonizing the lateral surface of the blue copepod *Pontella scutifer* floating in the surface microlayer of the North Atlantic. (A) Scanning EM of several *E. gemmipara* cells in various stages of development. Three of the cells show the prehensile (outer) and feeding (inner) tentacles used to capture and ingest food particles. One exogenously budding individual (arrow) has five ciliated telotrochs. Scale bar, 100 μm. (B) Transmission EM of a thin section of part of an *E. gemmipara* cell with nucleus (*Nu*) and stalk (*s*). Food vacuoles (*v*) show that these suctorians have been feeding mainly on bacteria. Scale bar, 5 μm. (C) Higher magnification of the ingested bacteria (*b*) in a vacuole. Scale bar, 1 μm.

combination of subsurface phototrophic production and phagotrophic release, Langmuir circulations (*24*), extraction and transport through bubble action during water turbulence (*25*), and the attraction of the hydrophobic groups to the sea-air interface.

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Bioenergetic Considerations in Cereal Breeding for Protein Improvement

Abstract. *The bioenergetic implications of changing cereal grain protein concentrations and amino acid compositions by plant breeding are examined. It is shown that increased inputs of carbon assimilates and nitrogen are necessary when increasing protein concentration in cereal grains while maintaining high yields. Also, energetic requirements for obtaining endosperm proteins with a high lysine content in genotypes of maize and barley are slightly higher than in normal lysine stocks. The implications for plant breeding are discussed.*

In plant breeding programs throughout the world considerable emphasis is being placed on improving the nutritional quality of cereals by increasing the protein concentration and altering the protein composition in grains to ameliorate the balance of lysine and other limiting amino acids. A major problem in such programs is combining high grain yield with increased or nutritionally better grain protein. Numerous observations indicate that the grain protein concentration is inversely correlated with yield in cereals (*1*). The underlying basis for this relationship has not been clearly enunciated. The fact that the gross energy (gram-calories per gram) in the dry matter of high-protein grains is higher than in low-protein grains (*2*), and that this energy differential has to be provided by the plant producing the grain, appears to have been neglected. To better understand the situation, an examination of the energetics of grain production is essential.

Recently, Sinclair and de Wit (*3*) considered seed biomass yield and the nitrogen requirement in 24 different crops with seed protein concentrations varying from 8 to 38 percent. Their study included cereals, pulses, and oilseeds. From their calculations, it can be inferred that in any species simultaneous increases in grain protein concentration and grain yield are incompatible from energetic considerations. To achieve both increments there is competition not only for the available carbon skeletons but also for the energy derived from photosynthates. The synthesis of more protein or more carbohydrate in the grain (or more grain) requires the availability of additional photosynthates to developing grains. Furthermore, an increment in nitrogen input is needed to produce additional protein. Alternatively, more efficient utilization of assimilates toward grain production would achieve the same result. In this report we examine, using the rationale of Sinclair and de Wit (*3*), the requirements for additional photosynthate and nitrogen likely to be associated with improvement in protein quantity or quality of cereals. The implications for breeding programs are discussed. Our purpose is to direct the attention of plant breeders and others to these considerations, which in the long term may align breeding expectations with the realities of the problems in breeding for altered chemical composition of grain. We wish to emphasize that these considerations would apply when the true genetic potential of the plant for energy conversion is expressed. This obtains only under favorable growing environments with the essential supplementary inputs.

Table 1. Energetic cost of increasing grain protein concentration in bread wheat.

Component	Amount (g/100g)	PV*	Equivalent glucose required† (g)
Standard cultivar			
Carbohydrate	82	0.83	98.80
Protein	14	0.40	35.00
Lipid	2	0.33	6.06
Minerals	2		
		Total	139.86
Cultivar with 1 percent more grain protein			
Carbohydrate	81	0.83	97.59
Protein	15	0.40	37.50
Lipid	2	0.33	6.06
Minerals	2		
		Total	141.15‡

*Production value (PV) is calculated as (weight of end product)/(weight of substrate required for carbon skeletons and energy production). †Equivalent glucose units are calculated as (amount of component)/PV, where the amount is expressed as grams per 100 g of grain biomass. ‡Thus, the differential glucose requirement is 141.15 − 139.86 = 1.29, or 0.92 percent greater for a cultivar with increased protein.

Our calculations are based largely on the analysis of Penning de Vries *et al.* (*4*) who, after extensive examination of biochemical pathways and the energy requirements of the component reactions, concluded that in plants, under aerobic conditions, 1 g of glucose can be used to produce 0.83 g of carbohydrates, or alternatively 0.40 g of proteins (assuming nitrate to be the nitrogen source) or 0.33 g of lipids. The derivation of these values and the assumptions made have been discussed (*4*). We realize that the biochemical pathways on which these calcula-