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Enrichment of amino acids in the sea surface microlayer at coastal and open ocean sites in the North Atlantic Ocean

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Abstract

We investigated how regional differences in environmental parameters influenced enrichment of amino acids in the sea surface microlayer relative to underlying bulk seawater. Concentrations and compositions of dissolved free (DFAA), dissolved combined (DCAA), and particulate (PAA) amino acids were measured in the sea surface microlayer and corresponding subsurface waters along a transect from coastal Massachusetts to open ocean waters of the Sargasso Sea. We also measured total bacteria concentrations, the percent bacteria with damaged membranes, and concentrations of virus-like particles. Microlayer samples taken by two different techniques—screen sampler (thickness 200–400 μm) and rotating drum (thickness 30–60 μm)—were compared. On average, concentrations of bacteria and amino acids in both subsurface and microlayer water were higher in water masses with higher fluorescence, lower temperature and salinity, and historically higher chlorophyll. The microlayer was enriched with amino acids and bacteria and, in most instances, virus-like particles. Consistent microlayer features, such as non-uniform preferential enrichment with DFAA and PAA and a uniform difference in dissolved amino acid composition between microlayer and subsurface water, were unrelated to environmental parameters measured. Wind, humidity, and light also had no apparent effects on amino acid concentration, composition, or enrichment in the microlayer in this study.

Enrichment of the sea surface microlayer with dissolved and particulate organic matter affects physical and chemical properties of the air–sea interface (Liss and Duce 1997) and influences exchange processes between the ocean and atmosphere. It has been known for decades that the extent of microlayer enrichment at any given moment generally results from a combination of processes: inputs from bubble scavenging and biological production; losses from diffusion, aerosol production, and biological degradation; stabilization by surface tension; and exchanges between dissolved and particulate pools (Liss and Duce 1997). Yet how these processes control enrichment of the microlayer with individual components is not clear. We report here how the concentration and composition of one class of compounds, the amino acids, are distributed in the sea surface microlayer along a range of hydrological zones and how their variations are related to different environmental parameters. Amino acids represent one of the most easily recognizable fractions of dissolved organic matter and one of the most important classes for marine biota (Hansell and Carlson 2002). The marine microlayer is enriched with dissolved and particulate amino acids by up to a factor of 50 (Henrichs and Williams 1985; Carlucci et al. 1992; Kuznetsova and Lee 2002). The rates of major processes of amino acid transformation, such as uptake, respiration, extracellular hydrolysis, and adsorption to solids, differ between microlayer and subsurface waters (Henrichs and Williams 1985; Kuznetsova and Lee 2001).

This study was designed to determine how the variations in microlayer enrichment of amino acids along a coastal to open ocean transect vary with environmental parameters. Relative fluorescence, water temperature, and salinity were measured as indicators of the hydrological and biological status of sampling areas. Relevant meteorological parameters monitored included air temperature, wind speed, and intensity of short-wave radiation. Water and air temperatures influence the rates of many biological processes as well as the intensity of evaporation at the air–water interface. Wind is responsible for mixing the microlayer with bulk water and affects scavenging intensity by controlling the number of bubbles in the water column. Ultraviolet (UV) light affects dissolved organic matter (DOM) concentration and composition (De Mora et al. 2000). Shorter wave radiation absorbs quickly in seawater; thus, the intensity of UV radiation is higher in the microlayer than other areas of the water column. Because bacterial and viral cells are direct sources of amino acids, intact and membrane-compromised bacteria and viruses were enumerated in all samples. Bacteria also alter organic matter in general and amino acids in particular; bacteria with damaged membranes can leak amino acids and thus alter the dissolved amino acid pool. Viruses can affect bacteria concentrations and the dissolved organic pool in general (Bratbak and Heldal 1995). Tapper and Hicks (1998) found that viruses can be enriched in the microlayer up to 15-fold compared with subsurface water. The effect of these biotic and abiotic parameters on amino acid pools was evaluated statistically by principal component analysis (PCA).

In addition, two different microlayer sampling techniques

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were compared. Sampling technique can greatly influence the concentration and composition of material sampled (Liss and Duce 1997). As in our previous studies (Kuznetsova and Lee 2001, 2002), we sampled with a screen sampler that is traditionally used in many microlayer studies and that samples a microlayer thickness of 200–400 μm (Garrett 1965). For comparison, we used a rotating drum sampler that allows sampling of a finer (30–60 μm) surface microlayer (Liss and Duce 1997; Frew et al. 2002).

Methods

Sample collection—Microlayer and subsurface water samples were collected during a cruise aboard the RV *Oceanus* from 18 to 29 June 2001. Twenty-two locations were sampled on a transect in the North Atlantic Ocean from Woods Hole, Massachusetts, to the Sargasso Sea and back (Table 1; Fig. 1). Both drum and screen samples were taken, and prescreening and length of sampling time differed between the types of collection.

Sixteen ''screen'' microlayer samples were taken from an inflatable boat with an electric motor 100–300 m from the ship with a polyester screen (1-mm mesh) as described previously (Kuznetsova and Lee 2001). As mentioned earlier, the thickness of the microlayer sampled by this technique is about 200–400 μm (Garrett 1965). Corresponding subsurface water samples were collected by submerging a polypropylene bottle by gloved hand 15 cm ($\pm$2 cm) below the sea surface; the bottle was opened and closed in place to avoid surface microlayer interference. Sampling took about 15 min; samples were kept on ice in the dark during transport (10–15 min) to the ship-based laboratory. Subsurface samples taken during microlayer screen sampling will be called ''screen subsurface samples,'' even though no screen was used in their collection, to differentiate them from subsurface samples taken during drum sampling. Six ''drum'' microlayer samples were taken with a rotating drum skimmer (Liss and Duce 1997; Frew et al. 2002), which was transported to the sampling point (>300 m from the ship) and controlled from the inflatable boat. Subsurface samples were collected simultaneously from 10–15 cm below the surface with a pump. Subsurface samples taken during microlayer drum sampling will be called ''drum subsurface samples,'' even though the drum was not used in their collection. Drum microlayer and subsurface samples were passed through a 60-μm screen and collected in 40-liter stainless steel containers kept on ice. The microlayer sampling thickness was 30–60 μm; the length of sampling was 3–4 h.

On board ship, subsamples (10–15 ml) of microlayer and subsurface water (for measurements of ambient dissolved free amino acid [DFAA] and dissolved combined amino acid [DCAA] concentrations) were filtered through 0.2-μm sterile cellulose acetate syringe filters (Sigma, cat. #F-0139), placed in sterile polypropylene tubes, and frozen at -20°C. The appropriateness of syringe filters for these measurements was discussed by Kuznetsova and Lee (2002). Subsamples of microlayer and subsurface water for particulate amino acid (PAA) measurements were frozen unfiltered. Unfiltered subsamples (7.5 ml) for bacterial and viral counts were placed in cryogenic vials (Nalgene) and preserved with 25% glycerol at -20°C.

Hydrographic and meteorological data—Sea surface temperature, salinity, and fluorescence were monitored in seawater from the ship's uncontaminated seawater intake. An IMET meteorological sensor system (Hosom et al. 1995) was used to collect meteorological data once each minute. These data were processed by the Athena data logging system on the RV *Oceanus* (www.marine.whoi.edu/ships/athena.htm). Table 1 presents data that were averaged over the sampling period (15 min for screen sampling; 3–4 h for drum sampling). One of the objectives of the study was to sample areas of different biological production, which was expected to differ significantly between the coastal and open ocean waters we sampled. Typical chlorophyll values in coastal areas are usually 5 to 10 times higher than in the Sargasso Sea, as can be seen in a time-averaged map of historical (1978–1986) June chlorophyll values (Fig. 1). We measured fluorescence rather than chlorophyll as an indicator of algal biomass. During our cruise, fluorescence generally followed the chlorophyll contours shown in Fig. 1. Our sampling sites are shown on Fig. 1 relative to the historical chlorophyll data. During June 2001, sites 1–5 and 16–23 had cooler, less salty waters with higher fluorescence than sites 6–15 (Fig. 2). Temperature and salinity were clearly negatively correlated with the high-fluorescence water and could also be used to characterize these water masses.

Amino acid measurements—Concentrations of individual DFAA were measured by high-performance liquid chromatography with the following modification of Lindroth and Mopper (1979). Fluorescent *o*-phthaldialdehyde amino acid derivatives were separated on a 25-cm-long Alltima C18 (5-μm) column with a gradient of 0.05 mol L^{-1} sodium acetate/5% tetrahydrofuran solvent and methanol (from 20% to 65% MeOH in 35 min, from 65% to 100% methanol in 4 min, and a 2-min hold at 100%). The separated derivatives were quantified with the use of an FL-750 fluorometer (McPherson Instruments) with an excitation wavelength of 330 nm and emission wavelength of 418 nm. Amino Acid Standard H (Pierce Chem., #20088) was diluted to approximate sample concentrations for use as a standard. Analytical reproducibility for measurement of total DFAA in standards was $\pm$7.5%. Detection limit for the standard was 0.002 μmol L^{-1}. Relative standard deviations of three to four replicate DFAA measurements for selected microlayer and subsurface water ranged from $\pm$10% (>1 μmol L^{-1}) to $\pm$30% (<0.3 μmol L^{-1}). Duplicates of every sample were run, and we report the average value.

Individual amino acids in the DCAA pool were measured after modified vapor-phase hydrolysis (Tsugita et al. 1987; Keil and Kirchman 1991). First, 75 μl of 12 mol L^{-1} HCl were added to 75 μl of sample in a small sample vial. Ascorbic acid was added (0.114 μmol ascorbic acid ml^{-1} sample) before hydrolysis to prevent sample oxidation (Robertson et al. 1987; Kuznetsova and Lee 2002). Two sample vials were put into a larger reactor vial. A mixture of 10.5 mol L^{-1} HCl, 10% trifluoroacetic acid, and 0.1% phenol was

Table 1. Location, local time, and hydrographic conditions of microlayer and subsurface water sampling.

Sample*	Date	Sampling start time	Position		Sampler	Fluorometer (relative units)	Temperature (°C)	Salinity	SWR ($W\ m^{-2}$)†	Humidity (%)	Wind ($m\ s^{-1}$)	Comment
			Longitude	Latitude								
1	18 Jun 01	1405	70°57′W	41°18′N	Screen	169.6	18.6	31.44	880.6	24.15	5.00	Visible slicks
2	19 Jun 01	0550	70°28′W	40°51′N	Screen	190.85	16.17	32.09	60.1	18.5	5.15	
3	19 Jun 01	1015	70°30′W	40°51′N	Screen	153.15	16.5	32.27	807.7	17.95	4.80	
4	19 Jun 01	0630	70°23′W	40°51′N	Drum	168.7	17.04	32.28	533.0	17.9	5.80	
5	19 Jun 01	1750	70°23′W	40°51′N	Screen	169.35	17.05	32.28	507.9	19.7	7.63	
6	20 Jun 01	0550	70°02′W	40°18′N	Screen	114.4	18.8	34.21	88.6	20.65	6.18	
7	20 Jun 01	1300	69°42′W	39°48′N	Screen	95.65	20.86	34.75	969.2	21.8	6.52	
8	20 Jun 01	1915	69°46′W	39°49′N	Screen	109.85	21.08	34.97	64.1	21.95	5.48	
9	21 Jun 01	0540	69°22′W	39°06′N	Screen	86.65	23.81	35.91	81.0	23.15	5.69	
10	21 Jun 01	0600	69°20′W	39°12′N	Drum	84.2	23.94	35.96	434.9	23.5	3.90	
11	21 Jun 01	1430	69°20′W	39°12′N	Screen	82.2	24.2	35.94	872.2	23.9	5.12	
12	22 Jun 01	0545	68°47′W	38°02′N	Screen	83.4	25.3	35.31	784	23.45	4.57	
14	22 Jun 01	0600	68°47′W	38°02′N	Drum	83.97	25.30	35.31	372.5	23.8	7.00	Whitecaps
15	25 Jun 01	1300	72°09′W	38°31′N	Screen	108.15	22.75	35.25	395.5	22.3	6.05	After storm/rain
16	26 Jun 01	0530	73°22′W	38°3′N	Screen	184.85	22.13	32.19	10.8	21.5	4.07	Visible slicks
17	26 Jun 01	0600	73°21′W	38°21′N	Drum	173.9	22.52	32.17	173.9	21.9	2.41	
18	26 Jun 01	1415	73°29′W	38°28′N	Screen	156.55	23.52	31.88	919.4	23.3	1.48	Visible slicks
19	27 Jun 01	0545	73°28′W	38°27′N	Screen	184.25	22.31	32.07	16.95	23.1	6.77	
20	27 Jun 01	0600	73°28′W	38°25′N	Drum	181.3	22.31	32.07	181.3	23.2	6.80	
21	28 Jun 01	1200	70°40′W	41°15′N	Screen	149.55	19.44	31.29	926.8	21.8	5.22	Visible slicks
22	28 Jun 01	1230	70°40′W	41°14′N	Drum	161.2	20	31.30	764.0	22.6	3.00	Visible slicks
23	28 Jun 01	1630	70°39′W	41°12′N	Screen	190.35	20.7	31.31	429.9	23.1	1.95	Visible slicks

* Sample 13 was taken from 50 m with a Niskin bottle and not used in this study.
† SWR, short-wave radiation.

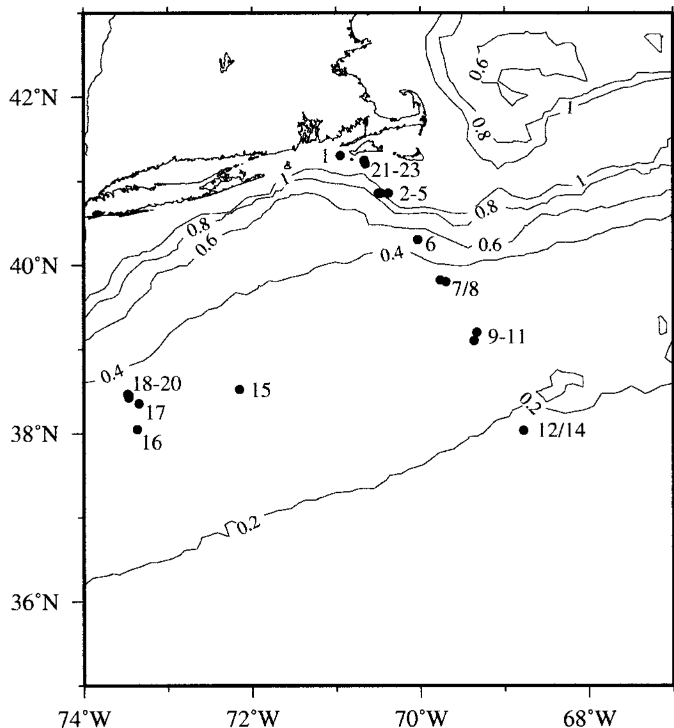

Fig. 1. Map of sampling locations during cruise OC367. Historical climatological Coastal Zone Color Scanner (CZCS) chlorophyll values (mg m^{-3}) (Feldman et al. 1989) for June 1978–1986 are shown, contoured at 0.2-mg m^{-3} intervals. Data were obtained from http://daac.gsfc.nasa.gov/data/dataset/CZCS/index.html.

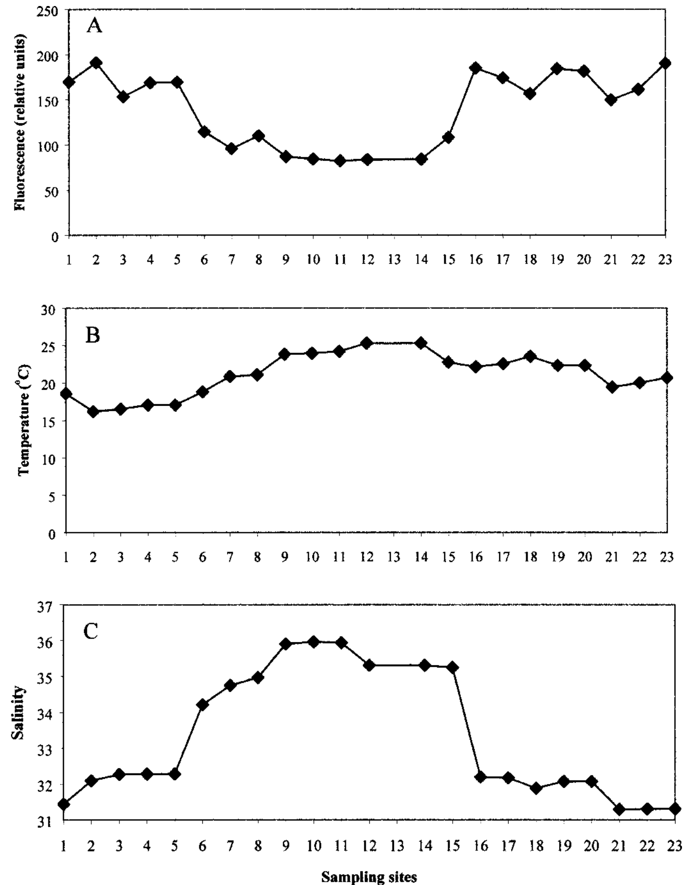

Fig. 2. (A) Relative fluorescence, (B) temperature, and (C) salinity at microlayer and subsurface water sampling sites.

added to the bottom of the reactor vial, which was then flushed with nitrogen, sealed, and heated to 160°C for 45 min. Resulting total dissolved amino acids were measured as described above. DCAA were calculated as the difference between total dissolved amino acids and DFAA. Standard deviations of DCAA concentrations ranged from ±18% to 55% for two to four replicates.

Total (particulate and dissolved) amino acid concentrations were measured in unfiltered samples after hydrolysis and analysis by the procedure described above, except that samples were filtered (0.2 μm) before injection onto the chromatography column. PAA were calculated as the difference between total and dissolved (DFAA + DCAA) amino acids. Standard deviations for PAA also ranged from ±18% to 55% for two to four replicates.

Bacterial and virus-like particle counts—Total bacterial abundance and abundance of bacteria with damaged membranes were evaluated with the use of the fluorescent dye DAPI (4′,6-diamidino-2-phenylindole) for total counts and propidium iodide for counts of damaged bacteria (Joux and Lebaron 2000; Howard-Jones et al. 2001). Cells were counted with a ×100 Nikon Eclipse E400 microscope with a MetaMorph Imaging System. Duplicate slides and a minimum of 100 cells per slide were counted for each sample; counting precision was ±5%.

Virus-like particles (VLP) were counted with a modified version of the methods of Noble and Fuhrman (1998). Prefiltered samples (0.2 μm) were filtered onto a 0.02-μm Anodisc filter and stained with a 100-μl drop of SYBR gold (2.5× concentration) for 15 min. A minimum of 200 VLP and 10 fields were counted for each slide. Counting precision was ±5%.

Statistical analysis—A number of different parameters were measured for microlayer and subsurface samples at each location: DFAA, DCAA, and PAA concentrations; mole percentage of each individual amino acid in these three pools; total bacteria concentration; percentage of bacteria with permeable membranes; and concentration of VLP. PCA, a multivariate regression analysis that reduces a large number of variables to a few principal components, was used to identify patterns in the data. PCA transforms the original data set into a scores matrix that contains information about any sample patterns in the data and a loadings matrix that shows how different variables influence the scores. The matrices are called principal components (PC) and are calculated in order of decreased explained variance in the data set. PCA is frequently used for analysis of amino acid data to allow the detection of subtle relationships not easily seen visually in such a complicated data set (e.g., Dauwe and Middelburg 1998; Sheridan et al. 2002; Ingalls et al. 2003). We used Sirius for Windows (version 1.1) for our PCA applications.

We present here two separate PCA analyses. In a first

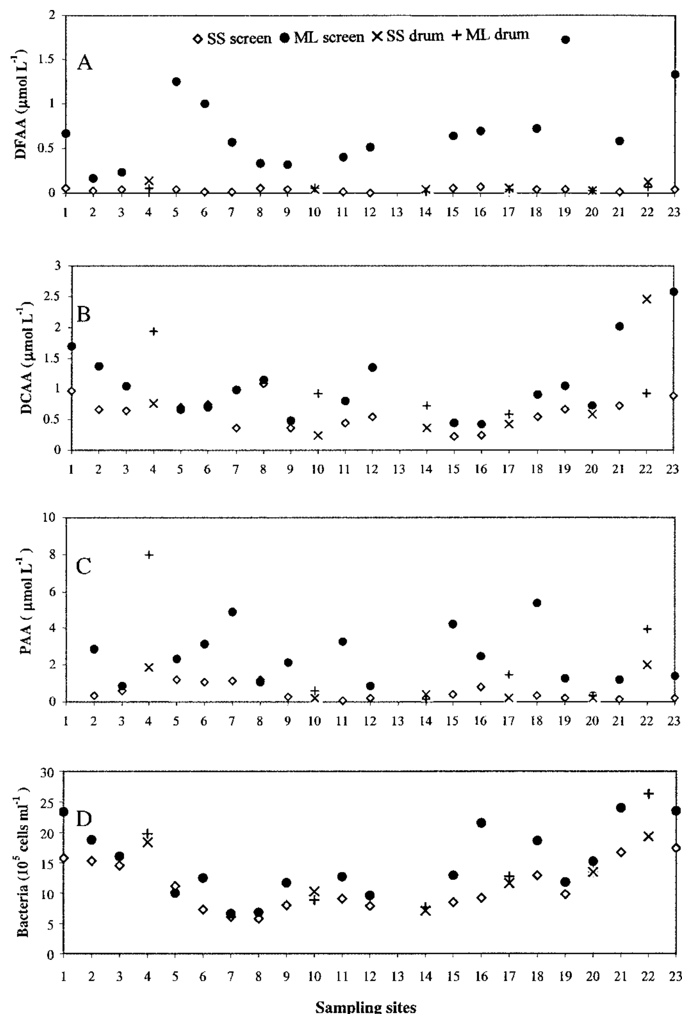

Fig. 3. (A) DFAA, (B) DCAA, (C) PAA, and (D) bacteria concentrations in microlayer (ML) and subsurface (SS) water collected by screen and drum samplers. Standard deviations are stated in the text.

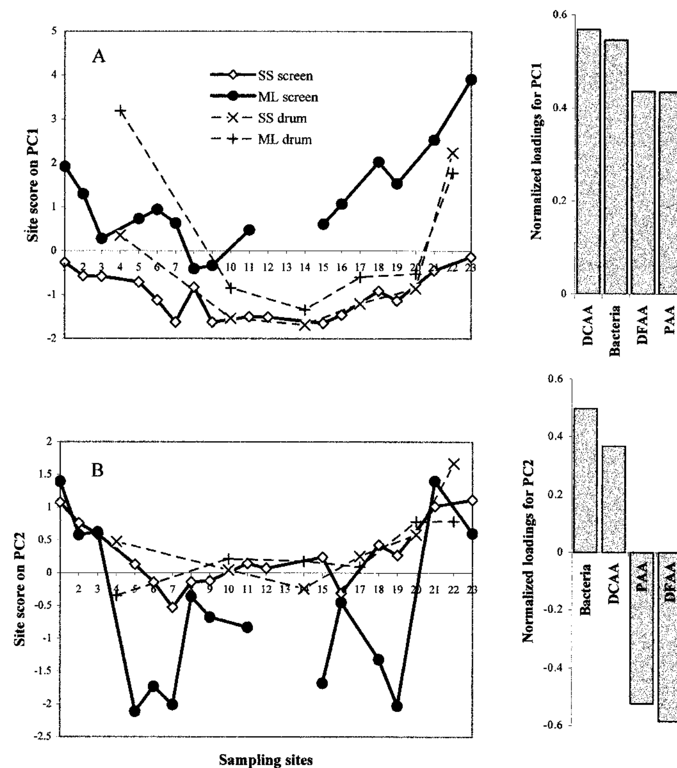

Fig. 4. Concentration PCA site scores for microlayer (ML) and subsurface (SS) water samples on (A) PC1 (explained variance 50.9%), and (B) PC2 (explained variance 21.6%). ML12 was dropped as an outlier.

(concentration) PCA, we included total concentrations of DFAA, DCAA, PAA, and bacteria. We did not include concentrations of bacteria with permeable membranes and VLP abundance to simplify data interpretation, because little is known about these parameters. Data were preprocessed by subtracting the mean and dividing by the standard deviation. In a second (composition) PCA, we analyzed amino acid compositions. In this analysis, PCA was applied separately to DFAA, DCAA, and PAA mole percent data. During preprocessing, the data set was block normalized so that amino acids with higher mole percent values that had higher loadings affected the PCA results the most.

Correlation coefficients were calculated between concentrations of various parameters measured and between PC site scores and environmental parameters.

Results

Relation of amino acid, bacteria, and VLP concentrations to environmental parameters and sampler type—DCAA and bacteria concentrations in both microlayer (ML) and subsurface (SS) samples were on average higher at the more coastal stations than in the more oligotrophic Sargasso Sea (Fig. 3). DFAA and PAA showed no clear relation to location. The relation between amino acid and bacteria concentrations and sampling location was explored with PCA, and each sample was assigned a unique site score on the basis of a data set that combined DFAA, DCAA, PAA, and bacteria concentrations. Principal component 1 (PC1) explained half (50.9%) of the variance in the microlayer and subsurface water concentration data and was defined by positive loadings for DFAA, DCAA, PAA, and bacteria concentrations (Fig. 4A). Although PCA does not suggest a cause for the variation it quantifies, PC1 showed a pattern with location similar to that of fluorescence and salinity, larger at stations 1–5 and 16–23 than at stations 7–15, and the inverse of temperature. This suggests that the major variability in the data is from environmental parameters. In addition, PC1 site scores were correlated with relative fluorescence (R_{SS} = 0.510, R_{ML} = 0.555, n = 22), temperature (R_{SS} = −0.535, R_{ML} = −0.467, n = 22), and salinity (R_{SS} = −0.645, R_{ML} = −0.641, n = 22) at the 0.95 significance level.

Differences between drum and screen samples were further explored by PCA. PC2 revealed inherent differences between microlayer samples obtained by the two types of samplers, explaining 21% of the variability of the data set. PC2 site scores showed that microlayer screen and drum samples were different along the transect, whereas PC2 site

scores for subsurface water samples were similar for both types of samples (Fig. 4B). Screen microlayer samples were clearly nonuniformly enriched in DFAA, DCAA, PAA, and bacteria. They often had much more negative site scores, reflecting higher DFAA and PAA, and lower bacteria and DCAA concentrations than microlayer samples taken by drum or either type of subsurface water sample.

Statistically significant correlations were found between amino acid, bacteria, and VLP concentrations and environmental parameters (Table 2). In screen samples, microlayer and subsurface DFAA and DCAA, but not PAA, concentrations were correlated with relative fluorescence (Table 2A). Microlayer DCAA, but not DFAA or PAA, concentrations were correlated with corresponding subsurface values. In drum samples, no correlation was found between microlayer and subsurface amino acid concentrations (Table 2B). Subsurface DFAA, and PAA, microlayer DCAA, and PAA were negatively correlated with temperature.

Bacteria and VLP abundances in the microlayer were significantly correlated with their corresponding subsurface values in both screen and drum samples (Table 2). Bacteria concentrations in screen samples were also correlated with relative fluorescence (Table 2A). The negative correlation between screen bacteria concentrations and wind suggested that wind mixing lowered bacteria concentrations in both microlayer and subsurface waters; the wind effect was stronger in the microlayer (Table 2A). In both microlayer and subsurface screen samples, the percentage of bacteria with permeable membranes decreased as total bacteria concentration increased (Table 2A). No such relationship was found for bacteria in drum samples, possibly because of the limited number of samples or lengthy sampling times (Table 2B). Bacteria were probably not a major component of the particulate proteinaceous matter (PAA) in microlayer and subsurface screen samples because there were no significant correlations between their concentrations and PAA contents. In drum samples, however, bacteria and PAA concentrations in microlayer and subsurface water were positively correlated.

VLP abundances in microlayer and subsurface samples were not correlated with amino acids, bacteria, or the environmental parameters measured. Dissolved amino acid concentrations did not depend on VLP abundance, even though, by definition (0.2-μm filtration), VLP are a component of the high–molecular weight fraction of dissolved organic carbon (Hansell and Carlson 2002). A rough estimate of virus contribution to the DCAA pool can be made if we assume that viruses are spheres of pure protein with an average diameter of 50 nm (Bratbak and Heldal 1995) and density approximately equal to that of seawater. Subsurface and microlayer VLP concentrations varied from 10 to 100 $\times$ 10^5 VLP ml^{-1}. Thus, the amount of DCAA constituted by viruses ranged from 0.6 to 6 nmol L^{-1}, which is insignificant compared with observed microlayer and subsurface DCAA concentrations (Table 3).

Microlayer enrichment of amino acids and bacteria—Greater than 90% of the surface microlayer samples collected by screen were enriched with both dissolved and particulate amino acids (Table 3; Fig. 3). In addition, both drum and screen microlayer PC1 site scores based on amino acid and bacteria concentrations were always larger than corresponding subsurface site scores throughout the region sampled (Fig. 4A), reflecting microlayer enrichment. In drum samples, however, DFAA concentrations were similar in microlayer and subsurface samples, and DCAA and PAA were only sometimes enriched in the microlayer (Table 3; Fig. 3). This difference in enrichment between drum and screen samples was related to differences in concentrations in the microlayer rather than subsurface waters. Screen and drum subsurface samples had very similar average amino acid concentrations, whereas microlayer samples showed larger differences in measured parameter concentrations. Drum and screen collection methods differed in the length of handling time, prescreening (60 μm for drum samples and none for screen samples), and thickness of the microlayer collected. These differences would affect subsurface and microlayer samples to different degrees because of higher enrichment with biota and particles and faster biodegradation in the microlayer (Liss and Duce 1997).

Microlayer enrichment of bacteria in screen samples was also greater than in drum samples (Table 3; Fig. 3). Although bacteria concentrations were correlated with fluorescence in both the microlayer and subsurface waters, microlayer enrichment of bacteria was independent of location of the sample. Bacteria were less enriched in the microlayer than particulate amino acids at most stations (Table 3). To determine the effect of bacteria on PAA enrichment in the microlayer, we estimated the contribution of bacteria to particulate amino acids by assuming that the protein content of bacterial cells is typically 12–30 fg $cell^{-1}$ (Fukuda et al. 1998; Zubkov et al. 1999). Thus, bacteria could supply 0.06–0.43 μmol L^{-1} PAA to subsurface waters and 0.07–0.60 μmol L^{-1} PAA to the microlayer. Observed concentrations of subsurface PAA varied from 0.08 to 2 μmol L^{-1} (average 0.6 μmol L^{-1}), whereas microlayer PAA varied from 0.12 to 8 μmol L^{-1} (average 2.5 μmol L^{-1}) (Table 3). Thus, although bacteria accounted for 10–70% of the PAA in subsurface samples, they contributed a lower percentage (3–25%) to microlayer PAA. The amino acid molar mass used in these calculations is 116 g mol^{-1}, which was determined with the use of average mole percent composition for microlayer and subsurface water PAA.

Determination of DFAA, DCAA, and PAA compositions—Concentrations of individual amino acids in the DCAA and PAA pools were not measured directly but were calculated by difference: DCAA from the difference between total dissolved amino acids and DFAA and PAA from the difference between total amino acids and total dissolved amino acids. For this reason, standard deviations of calculated concentrations were sometimes high at the lowest amino acid concentrations. Thus, in our statistical analysis of amino acid compositions, we excluded those that had standard deviations comparable to or higher than their actual concentration and with average concentrations less than three times the concentration of the hydrolysis blank. Generally, the remaining amino acids (aspartic acid, glutamic acid, serine, arginine,

Table 2. Correlation coefficients for statistically significant correlations between the amino acid and bacterial concentrations (Bac. SS or ML), concentration of bacteria with permeable membrane (Perm. Bac. SS or ML), viral concentrations (VLP SS or ML), and environmental parameters. —, Correlation is not significant at a confidence level >0.90.

	DFAA SS	DCAA SS	PAA SS	DFAA ML	DCAA ML	PAA ML	Bacteria SS	Bacteria ML	Perm. Bac. SS	Perm. Bac. ML	VLP SS	VLP ML
(A) Samples taken by screen*												
DFAA SS	1											
DCAA SS	—	1										
PAA SS	—	—	1									
DFAA ML	—	—	—	1								
DCAA ML	—	0.627	—	—	1							
PAA ML	—	−0.521	—	—	−0.436	1						
Bac. SS	—	—	−0.483	—	0.702	—	1					
Bac. ML	—	—	−0.485	—	0.540	—	0.847	1				
Perm. Bac. SS	—	—	—	—	—	—	−0.663	−0.661	1			
Perm. Bac. ML	—	—	0.713	—	—	—	−0.563	−0.561	—	1		
VLP SS	—	—	−0.573	—	—	—	—	—	—	—	1	
VLP ML	—	—	—	—	—	—	—	—	—	—	0.687	1
Relative fluorescence	0.426	—	—	0.441		—	0.698	0.659	—	—	—	—
Temperature	—	−0.456	—	—	—	—	−0.497		—	—	—	—
Salinity	—	—	—	—	−0.523	—	−0.813	−0.748	—	—	—	—
SWR	−0.473	—	—	—	—	—	—	—	—	—	—	—
Humidity	—	—	—	—	—	—	—	—	—	—	—	—
Wind	—	—	—	—	—	—	−0.457	−0.588	—	—	—	—
(B) Samples taken by drum†												
DFAA SS	1											
DCAA SS	—	1										
PAA SS	0.982	0.772	1									
DFAA ML	—	—	—	1								
DCAA ML	—	—	—	—	1							
PAA ML	0.903	—	0.871	—	0.907	1						
Bac. SS	0.833	0.75	0.868	—	—	0.803	1					
Bac. ML	0.824	0.892	0.869	—	—	0.684	0.960	1				
Perm. Bac. SS	—	—	—	—	—	—	—	—	1			
Perm. Bac. ML	—	—	—	—	—	—	—	—	0.889	1		
VLP SS	—	—	—	—	0.840	—	—	—	—	—	1	
VLP ML	—	—	—	—	—	—	—	—	—	—	0.841	1
Relative fluorescence	—	—	—	—	—	—	—	—	—	—	—	—
Temperature	−0.841	—	−0.83	—	−0.816	−0.948	−0.920	−0.805	—	—	—	—
Salinity	—	—	—	—	—	—	−0.780	−0.821	−0.886	—	—	—
SWR	0.808	0.779	0.847	—	—	—	—	—	—	—	—	—
Humidity	—	—	—	—	−0.887	−0.927	—	—	—	—	—	—
Wind	—	—	—	—	—	—	—	—	—	—	—	—

* $|R| > 0.497$, 0.95 confidence level; $|R| > 0.426$, 0.90 confidence level; $n = 16$.
† $|R| > 0.811$, 0.95 significance level; $|R| > 0.729$, 0.90 significance level; $n = 6$.

Table 3. Free (DFAA), combined (DCAA), and particulate (PAA) amino acid concentrations; bacterial numbers and percent bacteria with permeable membranes; and concentration of virus-like particles (VLP) in microlayer (ML) and subsurface (SS) water. Subsamples for PAA measurements in sample 1 were lost.

Sample no.	Sample location	Amino acid concentration (μmol L^{-1})			Bacteria		VLP
		DFAA	DCAA	PAA	Number ($\times 10^5$ cells ml^{-1})	% permeable membrane	($\times 10^5$ ml^{-1})
1	ML	0.660	1.70		23.4	5	23.8
	SS	0.051	0.97		15.8	1.8	8.8
2	ML	0.162	1.36	2.86	18.8	3.1	19.4
	SS	0.032	0.67	0.31	15.3	4.3	17.2
3	ML	0.235	1.05	0.89	16.1	4.2	28
	SS	0.040	0.65	0.58	14.6	4.7	39.3
4	ML	0.058	1.94	7.98	19.8	6.4	55.2
	SS	0.132	0.76	1.91	18.4	8.9	28.8
5	ML	1.254	0.67	2.36	10.0	4.5	16.3
	SS	0.035	0.71	1.21	11.2	4.1	10.1
6	ML	1.001	0.7	3.14	12.5	4.7	45.6
	SS	0.020	0.74	1.07	7.3	5.8	22.3
7	ML	0.565	0.98	4.93	6.6	11.5	30.3
	SS	0.010	0.36	1.17	6.1	7.1	12.4
8	ML	0.334	1.15	1.06	6.8	8.5	23.9
	SS	0.057	1.09	1.21	5.8	8.2	19.8
9	ML	0.322	0.49	2.17	11.7	5.0	25.3
	SS	0.044	0.36	0.26	8.0	2.4	24.4
10	ML	0.062	0.92	0.58	8.9	7.7	32.1
	SS	0.037	0.25	0.19	10.3	8.8	27.9
11	ML	0.397	0.81	3.32	12.7	3.3	31.4
	SS	0.012	0.45	0.08	9.1	4.3	40.9
12	ML	0.518	1.34	0.89	9.6	4.2	38.1
	SS	0.004	0.54	0.19	7.9	9.6	38.7
14	ML	0.017	0.72	0.12	7.7	17.1	34.6
	SS	0.047	0.36	0.37	7.1	10.7	23
15	ML	0.639	0.45	4.24	12.9	4.8	68.5
	SS	0.061	0.23	0.43	8.5	6.3	38.3
16	ML	0.695	0.43	2.48	21.5	7.0	37.8
	SS	0.074	0.24	0.82	9.2	7.9	34.3
17	ML	0.039	0.59	1.51	12.7	21.0	19.3
	SS	0.053	0.42	0.20	11.6	14.7	16.8
18	ML	0.728	0.9	5.34	18.6	2.5	28.3
	SS	0.043	0.55	0.36	12.9	3.1	16.7
19	ML	1.716	1.05	1.29	11.8	4.5	34.5
	SS	0.041	0.67	0.21	9.8	7.5	32
20	ML	0.033	0.72	0.31	15.2	15.3	27
	SS	0.026	0.59	0.21	13.5	11.3	19.7
21	ML	0.583	2.01	1.21	24.0	2.3	109.4
	SS	0.009	0.72	0.12	16.7	2.5	51
22	ML	0.063	0.93	3.96	26.3	26.6	17
	SS	0.130	2.45	2.02	19.3	13.4	10
23	ML	1.330	2.57	1.39	23.5	2.5	31
	SS	0.044	0.89	0.22	17.4	3.9	20.3

glycine, threonine, and alanine) contributed more than 85% of total DCAA or PAA concentrations. A bias in composition because of concentration is also unlikely because we observed little relationship between amino acid composition and concentration within the DFAA, DCAA, or PAA pools, except for DFAA in microlayer samples taken by screen and PAA samples in subsurface water (Table 4).

The drum sample at Sta. 4 was dropped from the data set because DFAA and DCAA compositions there were highly unusual for ambient, nonincubated seawater. Glutamic acid made up 59 to 85 mole percentage of total DFAA and DCAA in both microlayer and subsurface samples at this site. Bacteria and VLP concentrations were also much higher than at nearby stations. Later, we discuss possible microbial effects on drum samples. DFAA, DCAA, and PAA compositions of microlayer samples taken by screen and drum were statistically different (*t*-test, $p = 0.95$, heteroscedastic). Subsurface water samples taken by hand (during microlayer screen sampling) and by pump (during microlayer drum sampling) were similar in DFAA and DCAA composition

Table 4. Correlation coefficients for statistically significant correlations between the amino acid compositional site scores and environmental parameters. —, Correlation is not significant at a confidence level >0.90.

Axis (% explained variation)	ML or SS	DFAA	DCAA	PAA	Bacteria	VLP	Permeable bacteria	Fluorescence	Temperature	Salinity	SWR	Humidity	Wind
(A) Samples taken by screen*													
PC1 DFAA	ML	0.548	—	—	—	—	—	—	—	—	—	—	—
(65.8%)	SS	—	—	0.616	—	—	—	—	—	—	—	—	—
PC2 DFAA	ML	−0.558	—	—	−0.413	—		−0.641	—	−0.667	—	—	—
(20.1%)	SS	−0.600	—	—	—	—	—	—	—	—	—	—	—
PC1 DCAA	ML	−0.472	—	—	—	—	—	—	—	—	—	—	—
(36.7%)	SS	−0.441	—	—	—	—	—	—	—	—	—	—	—
PC2 DCAA	ML	—	—	—	—	—	—	—	—	—	—	—	—
(33%)	SS	—	—	—	—	—	—	—	—	—	—	—	—
PC1 PAA	ML	—	—	—	—	—	—	—	—	—	—	—	—
(59.7%)	SS	—	—	—	—	—	—	—	—	—	—	—	—
PC2 PAA	ML	—	—	—	−0.421	—	—	−0.580	—	0.643	—	—	0.457
(21.3%)	SS	—	—	0.684	—	−0.555	−0.646	—	−0.470	—	—	−0.544	—
(B) Samples taken by drum†													
PC1 DFAA	ML	—	—	—	—	—	—	—	—	—	—	−0.857	—
(65.8%)	SS	—	—	—	—	—	—	—	—	—	—	—	—
PC2 DFAA	ML	—	0.903	—	—	—	—	—	—	—	0.913	−0.832	—
(20.1%)	SS	—	—	—	—	—	—	—	—	—	—	—	—
PC1 DCAA	ML	—	—	—	—	—	—	0.905	—	−0.878	—	−0.854	—
(36.7%)	SS	—	—	—	—	—	—	—	—	—	—	—	—
PC2 DCAA	ML	—	—	—	—	—	—	—	—	—	—	—	—
(33%)	SS	—	—	—	—	—	—	—	—	—	—	—	—
PC1 PAA	ML	—	—	—	—	—	—	—	—	—	—	—	—
(59.7%)	SS	—	—	—	—	—	—	—	—	—	—	—	—
PC2 PAA	ML	—	—	—	—	—	—	—	—	—	—	—	—
(21.3%)	SS	—	—	—	—	−0.881	—	—	—	−0.858	—	—	—

* $|R| > 0.497$, 0.95 confidence level; $|R| > 0.426$, 0.90 confidence level; $n = 16$.
† $|R| > 0.878$, 0.95 confidence level; $|R| > 0.805$, 0.90 confidence level; $n = 5$.

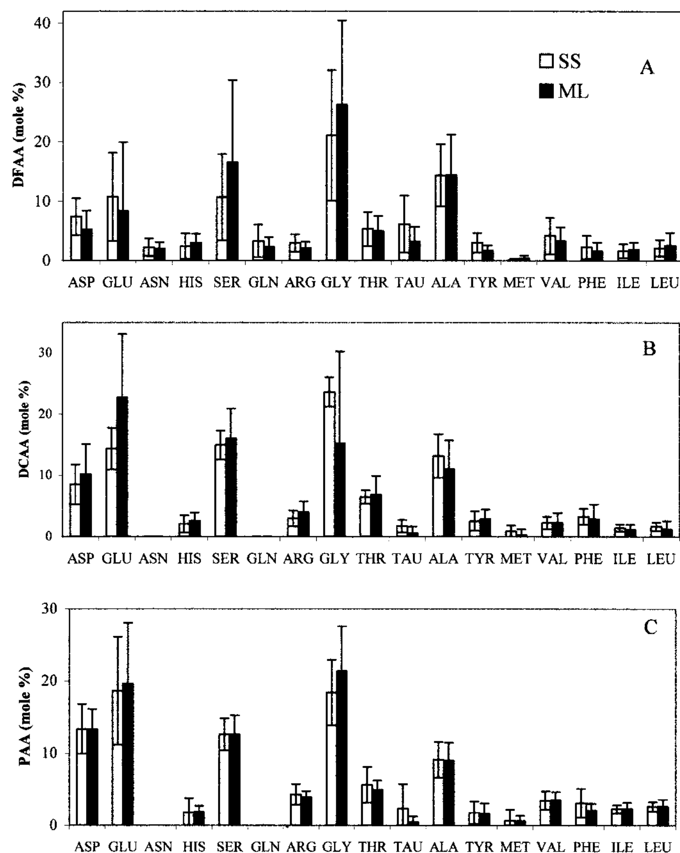

Fig. 5. Average mole percent composition of (A) DFAA, (B) DCAA, and (C) PAA for all sampling sites. Bars indicate sample variability and not error.

(t-test as above). Subsurface PAA compositions, however, were statistically different.

Variation in DFAA, DCAA, and PAA microlayer and subsurface water compositions: DFAA, DCAA, and PAA compositions in both subsurface water and microlayer samples varied significantly over the region sampled, but their average values (Fig. 5) were typical for bulk and microlayer seawater (Lee and Bada 1975; Henrichs and Williams 1985; Meon and Kirchman 2001). PCA of the composition data set allowed detailed examination of the complex compositional differences that existed among the sampling sites (Fig. 6). Even though amino acid compositions changed along the cruise track, a PC could always be found in which microlayer and subsurface scores for screen samples correlated with each other, compatible with the idea that subsurface waters are the major source for microlayer water. These PC were PC1 for DFAA composition site scores (Fig. 6A), PC2 for DCAA composition site scores (not shown), and PC1 for PAA composition site scores (Fig. 6C). In drum samples, site scores between the subsurface and microlayer were significantly correlated only for DCAA composition in PC1 (Fig. 6B). PC1 explained a larger proportion of the variability for DFAA and PAA than for DCAA (Table 4).

In addition to the correlation of PCA composition site scores with location, microlayer DFAA PC1 site scores were higher than corresponding subsurface water scores in 17 sites out of 21 (Fig. 6A), and microlayer DCAA PC1 scores were lower than corresponding subsurface scores in 19 sites out of 21 (Fig. 6B). PC loadings indicate that microlayer DFAA had relatively higher mole percentages of glycine and serine and lower mole percentages of alanine, glutamic and aspartic acids, valine, glutamine, threonine, asparagine, taurine, and histidine than corresponding subsurface DFAA. Microlayer DCAA tended to have relatively higher mole percentages of glutamic acid, serine, and arginine and lower mole percentages of alanine, glycine, aspartic acid, and threonine than corresponding subsurface DCAA. No significant difference was found between particulate subsurface and microlayer amino acid compositions (t-test, $p = 0.95$, $n = 21$). We attempted to determine what was responsible for the difference between microlayer and subsurface DFAA and DCAA site scores but found little correlation between that difference and a number of parameters in our data set (Table 5).

Relation of DFAA, DCAA, and PAA compositions to environmental parameters—PCA allowed us to extract possible relationships between amino acid composition and environmental parameters. The transition from the productive to oligotrophic area was reflected in screen microlayer DFAA PC2 composition site scores and PAA PC2 composition scores, which were negatively correlated with fluorescence and positively correlated with salinity (Table 4A). Normalized loadings shown in Fig. 6 show how amino acid compositions change as scores go from lower to higher values. Similarly in drum samples, microlayer DCAA compositional scores on PC1 correlated positively with relative fluorescence and negatively with salinity; subsurface water PAA compositional scores correlated negatively with salinity (Table 4B). Wind effects might be visible in the positive correlation between wind speed and screen microlayer PAA composition PC2 scores (Table 4A). There also can be a connection between VLP concentration in subsurface water and PAA composition here for both screen and drum samples because there was some negative correlation between PAA composition and VLP (Table 4A,B).

Discussion

Characterization of microlayer enrichment of amino acids, bacteria, and viruses—While supporting previous studies that show enrichment of the marine microlayer with organic matter and microorganisms (Liss and Duce 1997), this study newly establishes consistent features and interactions of amino acids, bacteria, and viruses in the sea surface film. The striking nonuniform preferential enrichment of the microlayer with dissolved free, compared with combined, amino acids has been observed previously in nearshore and some offshore areas (Henrichs and Williams 1985; Carlucci et al. 1992; Kuznetsova and Lee 2002). Here, we observed such enrichments at multiple locations (Table 3) that included highly oligotrophic areas that have not routinely been sampled for amino acid enrichment before. We showed that preferential DFAA, compared with DCAA, enrichment is a consistent microlayer feature. The consistent relationship be-

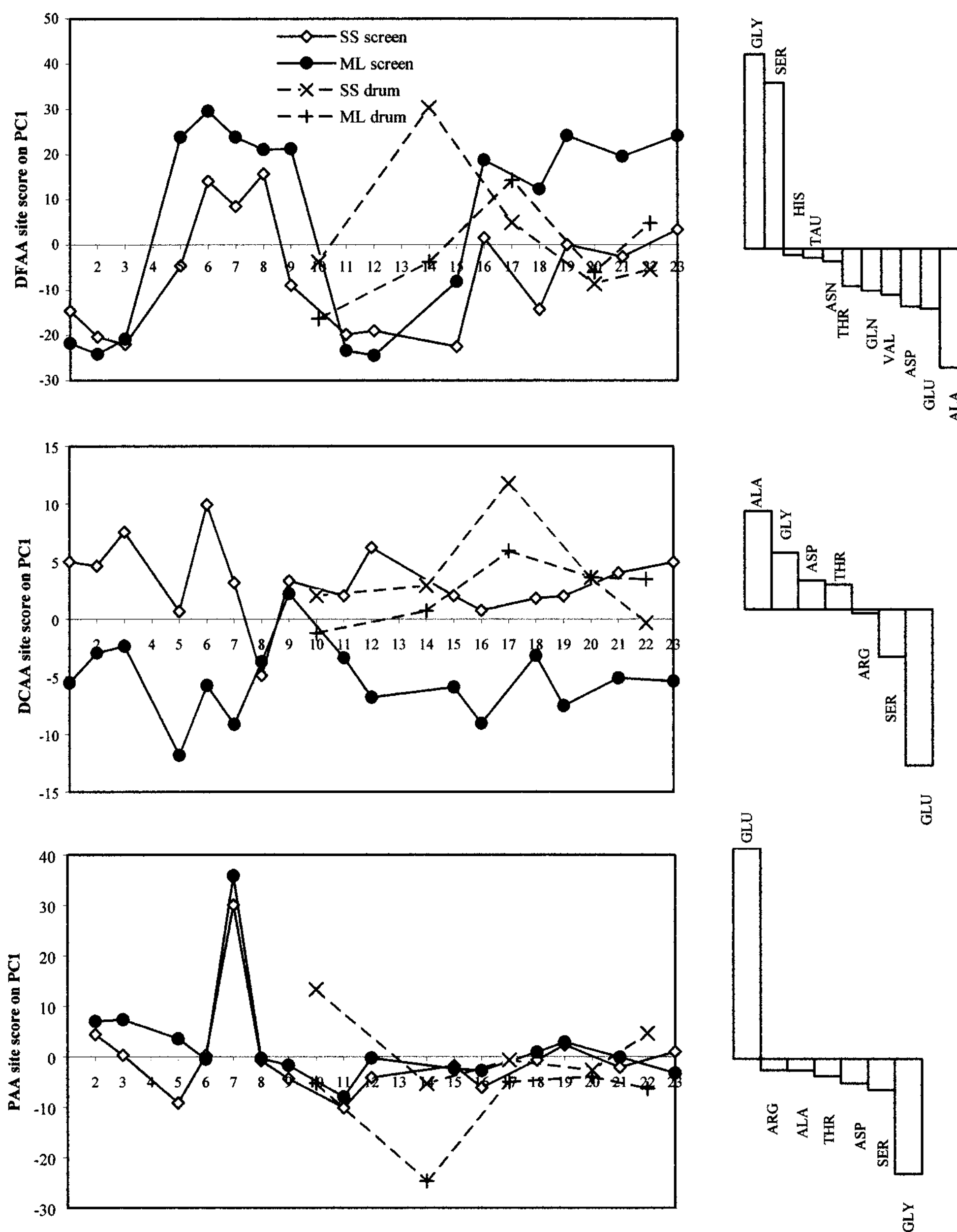

Fig. 6. Composition PC1 site scores in microlayer (ML) and subsurface (SS) samples taken by screen and drum and corresponding normalized loadings for (A) DFAA (65.8% explained variations) and (B) DCAA (36.7% explained variations) and, (C) PAA (59.7% explained variations).

tween microlayer and subsurface DFAA and DCAA compositions over the whole sampling range for both screen and drum samples suggests a subsurface source of material to the microlayer (Fig. 6).

Microlayer particulate matter also showed consistent patterns of enrichment. Bacteria were less enriched in the microlayer than PAA at most of our stations (Table 3); that is, bacteria/PAA in the microlayer was consistently lower than in subsurface water. Most of the particulate proteinaceous matter enriching the microlayer does not appear to be bacterial in origin because no correlation was observed between bacteria and PAA enrichment, and microlayer PAA concentrations were typically much higher than the amount of protein contributed by bacteria. Estimates based on typical bacterial protein contents suggest that bacteria accounted for 10–70% of the PAA in subsurface samples, but only 3–25% in microlayer PAA. Williams et al. (1986) during March 1979, October 1980, and July 1981 expeditions to the Gulf of California and off the west coast of Baja California found that bacterial carbon represented about 16% of the particu-

Table 5. Correlation coefficients for statistically significant correlations between the differences (dif) between microlayer (ML) and subsurface (SS) water site scores and environmental parameters. —, Correlation is not significant at a confidence level >0.90.

Axis (% explained variation)	DFAA dif	DCAA dif	PAA dif	BAC dif	Fluorescence	Temperature	Salinity	SWR	Humidity	Wind
(A) Samples taken by screen*										
PC1 DFAA (65.8%)	0.524	—	—	—	—	—	—	—	—	—
PC1 DCAA (36.7%)	−0.451	—	—	—	—	—	—	—	—	—
(B) Samples taken by drum†										
PC1 DFAA (65.8%)	—	—		—	0.871	−0.888	−0.850	—	−0.835	—
PC1 DCAA (36.7%)	—	−0.804	—	0.809	—	—	—	—	—	—

* $|R| > 0.497$, 0.95 confidence level; $|R| > 0.426$, 0.90 confidence level; $n = 16$.
† $|R| > 0.878$, 0.95 confidence level; $|R| > 0.805$, 0.90 confidence level; $n = 5$.

late organic carbon in the microlayer and about 19% in the subsurface waters. Microlayer PAA have not been well characterized but are likely, at least in open ocean waters, to originate from phytoplankton debris, aggregation of colloidal material, and adsorption of DFAA and DCAA onto particles.

A small fraction of bacteria in our microlayer and subsurface samples were permeable to propidium iodide dye, presumably resulting from damaged membranes. Such bacteria are of interest for this study because the bacterial intercellular pool is rich in protein, and leaching of organic matter from bacteria could increase the dissolved amino acid pool significantly. Damage to bacteria might occur within the microlayer because of viral lysis or other stress factors typical in the microlayer, including UV light, increased toxicity (Liss and Duce 1997), evaporation, and aerosol formation. Damaged bacteria are also preferentially scavenged from the water column (Wallace et al. 1972). We therefore expected to see a larger number of damaged bacteria in the microlayer compared with subsurface water samples. However, the percentages of such bacteria in the microlayer were often smaller than in subsurface waters, and both microlayer and subsurface samples had a percentage of damaged bacteria that was much smaller than normally observed deeper in the water column. Howard-Jones et al. (2001) reported that the fraction of permeable bacteria in deep water is 30–40% and not dependent on total bacteria concentrations. Bacteria with damaged membranes in our subsurface and microlayer samples ranged from 2% to 27%. Moreover, the concentration of damaged bacteria was negatively correlated with total bacteria concentration in both subsurface and microlayer samples. The relatively low number of damaged bacteria in surface water compared with deeper water might be a result of high bacterial division rates in the surface layers where bacteria can take advantage of higher concentrations of nutrients. Indeed, the highest bacteria concentration is reported in the uppermost sample in most studies of vertical distribution of bacterial abundance (Amon and Benner 1998; Culley and Welschmeyer 2002).

Viruses were enriched in the microlayer at most of our sites. At present, little can be said about causes of viral enrichment because their natural history is poorly known in the marine environment. There were no statistically significant correlations between microlayer and subsurface VLP abundance and other parameters measured. Unlike in deeper waters in other study areas (Culley and Welschmeyer 2002; Middelboe et al. 2002), VLP abundance in the surface microlayer and in 10–15-cm subsurface water at our sampling sites was not related to total bacteria concentration or fluorescence. Viral lysis is one of the causes of bacterial death (Proctor and Fuhrman 1990); however, concentrations of damaged bacteria were not dependent on VLP concentration. Viral dynamics in the microlayer are probably complex and suggest the necessity of further study.

Sources of microlayer enrichment—Our data provide additional evidence that scavenging from bulk seawater, rather than in situ production, is a major source of material enriching the microlayer. Concentrations of DCAA, bacteria, and VLP in screen microlayer samples were well correlated with corresponding subsurface water concentrations. Amino acid compositions for all fractions (DFAA, DCAA, and PAA) tended to cluster according to geographical location rather than by location in the microlayer or subsurface.

Although there was sufficient correspondence between microlayer and subsurface water parameters to conclude that most of the microlayer material originates in the bulk water, microlayer DFAA and PAA concentrations were not correlated with corresponding subsurface values. This absence of correlation could be because processes within the microlayer especially influence DFAA and PAA concentrations. DFAA might be altered in the microlayer by the same processes as in bulk seawater (microbial uptake; production by or leaching from phytoplankton, zooplankton, and bacteria; adsorption or desorption on particles), but at significantly different rates (Carlucci et al. 1992; Kuznetsova and Lee 2001). Competition between production and loss by these processes leads to a complex pattern of DFAA enrichment in the microlayer (Kuznetsova and Lee 2002). The lack of correlation between microlayer and subsurface PAA concentrations might well be because of formation in the microlayer of new

particles from dissolved material scavenged from bulk water by bubbles or adsorption of that dissolved material onto existing particles. These processes would vary depending on local physical conditions.

Relation of amino acids to environmental parameters—Consistent microlayer features such as nonuniform preferential enrichment with DFAA and PAA and a uniform difference in dissolved amino acid composition between microlayer and subsurface water did not depend on location or on any of the measured environmental parameters. We explored the dependence of other features on these parameters.

Microlayer and subsurface waters generally had higher DCAA and bacteria concentrations (Fig. 3) in areas of higher fluorescence, lower temperature and salinity (Fig. 2), and higher historical chlorophyll concentrations (Fig. 1). DFAA and PAA concentrations, however, appeared to be unrelated to these parameters. PCA of dissolved and particulate amino acid concentrations, on the other hand, showed that 51% of the variability of the data could be explained by a first PC that correlated positively with fluorescence and negatively with temperature and salinity. Thus, a major relation that we observe is between amino acid concentrations and these characteristics of the water mass. If we assume that areas where higher fluorescence was observed represent areas of higher biomass at the time we sampled, then we might conclude that a significant part of seawater DFAA or PAA has sources other than immediate in situ production. Compositions of amino acids in the microlayer, but not subsurface water, also might be somewhat related to hydrological status (Table 4).

Other relationships that might have been expected between amino acids, microorganisms, and environmental parameters were not observed. Amino acid concentrations or compositions were not generally correlated with bacterial abundance, except for DFAA and DCAA concentrations in subsurface drum samples. As will be discussed in ''Comparison between Screen and Drum Samplers'' below, however, those correlations might result from longer drum sampling times and not reflect ambient conditions. Organic matter leaching from damaged bacteria was not great enough to be reflected in any correlations of damaged bacteria concentration with amino acid concentration or composition. VLP made an insignificant contribution to the dissolved amino acid pool, even though they are a component of the ''dissolved'' pool (Hansell and Carlson 2002).

Wind can reduce microlayer enrichment as a result of turbulent mixing, which increases with wind speed. Enrichment of some components can, however, be enhanced by wind because wind promotes bubble formation and, consequently, scavenging of dissolved and particulate matter from bulk water to the microlayer (Blanchard 1975; Blanchard and Syzdek 1982). In this study, bacteria numbers in both subsurface and microlayer samples were reduced at higher wind speeds; the effect was stronger in the microlayer than in subsurface water (Table 2A). This suggests that mixing of the microlayer with underlying layers that contain lower numbers of bacteria is more important than bubble scavenging in controlling bacteria concentrations. Wind mixing did not appear to be an important control of amino acid concentrations because we observed no apparent correlation between wind speed and any amino acid concentration.

Light greatly affects many of the processes controlling bacteria, virus, and DOM concentrations (De Mora et al. 2000). We collected screen samples at different short-wave radiation (SWR) intensities: before sunrise, at noon, and in the afternoon. No effect of SWR on microlayer and subsurface amino acid concentration or composition or on bacteria or virus concentration was apparent (Table 2A). Apparently, variations in microlayer enrichment caused by processes such as bubble scavenging, diffusion, adsorption or desorption, and metabolism can effectively mask the influence of UV light.

Comparison between screen and drum samplers—Results of microlayer studies often depend on the type of samplers employed (Liss and Duce 1997). Our microlayer screen samples often differed from microlayer drum samples, even when collected at nearby locations. Direct comparison between samples is complicated by considerable natural variability in DOM microlayer enrichment (Frew et al. 2002). ''Patchiness'' in concentrations of particles, chlorophyll *a,* nutrients, and, specifically, DFAA and DCAA in the microlayer were demonstrated earlier (Falkowska and Latala 1995; Kuznetsova and Lee 2002). Even samples collected at the same location by the same sampler minutes apart can differ by as much as four- to fivefold in terms of microlayer amino acid concentration (Kuznetsova and Lee 2002).

Although we made an effort to collect screen and drum samples in the same location, the distance between sampling points could be as great as 5 km because of ship drift. Time intervals between collections were up to 1 h. Natural variability might explain much of the observed compositional differences between screen and drum microlayer samples. There are, however, also inherent differences between the two types of microlayer samplers that could lead to further variability. For example, sampling depth is a major difference between the two samplers, with the drum sampler collecting almost an order of magnitude thinner layer than the screen sampler (30–60 μm vs. 200–400 μm, respectively). The thickness of the portion of the microlayer where chemical and physical characteristics differ significantly from the bulk water is on the order of 50 μm. Thus, we would expect to see higher enrichment of microlayer samples collected by drum than by screen. However, the apparent enrichment of microlayer collected by screen was higher than for drum samples. In addition, PCA analysis revealed that microlayer samples collected by screen differed from subsurface water much more than drum microlayer samples did, even though corresponding subsurface waters were similar (Fig. 4).

In addition to different sampling thickness, the drum sampler automatically passed the samples through a 60-μm screen, whereas no prefiltering was done for screen samples. Exclusion of particles of >60 μm might account for our observation that four of six microlayer PAA concentrations were lower in drum samples than in screen samples taken at the same locations. Bacteria and VLP concentrations in drum samples were also lower in four of six cases.

Collection time was 3–4 h for drum samples versus 15–

20 min for screen samples. The long drum sampling time was required to collect large enough volumes for a separate study and is not a requirement of the sampler. These differences in collection time could greatly influence amino acid concentrations and compositions. In previous studies, we found that DFAA in microlayer samples usually decreased to background concentrations after a few hours when incubated in the dark at room temperature (Kuznetsova and Lee 2002). Even though the microlayer drum-sampling container was kept on ice, DFAA could be lost during the extended sampling time. This could explain the higher enrichment of DFAA observed in screen compared with drum samples. Bacterial abundance, however, could increase during longer sampling times if bacterial growth occured. Indeed, drum microlayer and subsurface samples taken at Stas. 4 and 22 had significantly higher bacteria concentrations than corresponding samples taken at nearby stations. In DFAA and DCAA fractions in drum samples 4 and 22, a high mole percentage of glutamic acid was observed, which often correlates with exponential bacterial growth (Yamada et al. 1972; Henrichs and Cuhel 1985; Kuznetsova and Lee 2002). However, most (four of six) drum samples had lower bacteria concentrations than in nearby screen samples. Perhaps bacterial predator communities had time to develop; bacteria with damaged membranes made up a significantly higher proportion of the total in both microlayer and subsurface drum samples (Table 3). To avoid problems caused by long sampling times, a new sample processing system has been coupled to the drum sampler, which allows extraction of microlayer DOM on short time scales (~10 min) for further characterization by liquid chromatography–mass spectrometry (Nelson et al. 2002). This system, however, was not configured for use in amino acid or bacteria sampling at the time of this study. Further development could in time allow better comparison of enrichment in thinner and thicker layers of the surface film.

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