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The contribution of microorganisms to particulate carbon and nitrogen in surface waters of the Sargasso Sea near Bermuda

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Abstract—Seawater samples were collected from the euphotic zone of the Sargasso Sea near Bermuda in August of 1989 and March–April of 1990. Microbial population abundances, chlorophyll concentration, particulate carbon and particulate nitrogen were measured. Calculations were performed to establish the relative and absolute importance of the various microbial assemblages. The choice of conversion factors (g C and N $cell^{-1}$, or g C and N μm^{-3}) for the microbial populations dramatically affected the estimation of "living" and "detrital" particulate material in the samples, and the relative importance of the various microbial groups. Averaged over all samples on either of the two cruises, microbial biomass constituted a greater proportion of the total particulate carbon and nitrogen during March–April (55% and 63%, respectively), than during August (≈24% and 30%, respectively) using "constrained" conversion factors that were derived. Accordingly, detrital material constituted the bulk of the particulate material during August, but was similar to the amount of microbial biomass during March–April. The bacterial assemblage constituted the largest single pool of microbial carbon (35%) and nitrogen (45%) in the water, and a significant fraction of the total particulate carbon (≈10–20%) and nitrogen (≈15–30%). Phototrophic nanoplankton (microalgae 2–20 μm in size) were second in overall biomass, and often dominated the microbial biomass in the deep chlorophyll maxima that were present during both cruises. The results temper recent assertions concerning the overwhelming importance of bacterial biomass in the oligotrophic Sargasso Sea but still support a major role for these microorganisms in the open ocean as repositories for carbon and nutrients.

INTRODUCTION

Microorganisms constitute the majority of living biomass in most planktonic ecosystems. The microbial plankton (defined here as microorganisms ≤200 μm in size) is composed of a wide array of prokaryotic and eukaryotic species that exhibit phototrophic, osmotrophic, or phagotrophic nutrition. Most ecological studies of planktonic microorganisms divide

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these assemblages rather crudely into categories based on their size and (to some degree) their mode of nutrition. One popular convention (Sieburth *et al.*, 1978) divides these microorganisms into the picoplankton (0.2–2.0 μm), nanoplankton (2–20 μm) and microplankton (20–200 μm). Based on these simple criteria, the microorganisms contained within these categories roughly correspond to the chroococcoid cyanobacteria, prochlorophytes and bacteria in the picoplankton, the flagellated protozoa and smaller microalgae in the nanoplankton, and the ciliated protozoa, larger dinoflagellates, diatoms and larvae of metazoa in the microplankton. These size classes are often further separated into phototrophic (i.e. chlorophyll containing) and heterotrophic species, the autofluorescence of photosynthetic pigments being used as a diagnostic tool. Although the existence of symbiont-bearing and mixotrophic species presents some difficulties in characterizing free-living microorganisms in this manner (Estep *et al.*, 1986; Stoecker *et al.*, 1989; Taylor, 1990), these simplifying categories are still commonly employed.

Numerous studies have been published in which the abundances and biomasses of one or a few of these populations have been examined. For example, independent studies have been conducted on the importance of planktonic bacteria (Fuhrman *et al.*, 1980; Ducklow and Hill, 1985; Billen *et al.*, 1990; Ducklow and Carlson, 1992), chroococcoid cyanobacteria (Glover, 1985; Waterbury *et al.*, 1987), prochlorophytes (Chisholm *et al.*, 1988), phototrophic and heterotrophic nanoplanktonic protists (Davis and Sieburth, 1982; Caron, 1983; Sherr *et al.*, 1984), and microplanktonic protists (Lessard and Swift, 1986; Stoecker *et al.*, 1989; Pierce and Turner, 1992). In very few studies, however, has the entire microbial plankton been enumerated. For this reason the absolute and relative contributions of the various assemblages to total biomass in the plankton (or to total particulate material) are still poorly characterized.

One reason for our limited understanding of the absolute and relative importance of the various microbial assemblages to the total biomass in the open ocean is the difficulty associated with accurately determining the abundances of these various groups. Microbial population estimates are laborious and require a variety of preservative and microscopical techniques. Advances in these methods often have resulted in subsequent reappraisals of the importance of various planktonic microbial assemblages as well as the discovery of "new" microbial taxa in the plankton (Jannasch and Jones, 1959; Hobbie *et al.*, 1977; Johnson and Sieburth, 1979; Waterbury *et al.*, 1979; Chisholm *et al.*, 1988; Stoecker *et al.*, 1989).

Uncertainty also arises from the many (and varied) conversion factors used to calculate the biomass of these assemblages from estimates of their abundance. The choice of conversion factors, to a large degree, determines the relative importance of a microbial group to the total living biomass in the sample. A wide range of published conversion factors exist for each microbial assemblage. For example, Billen *et al.* (1990) reviewed conversion factors for bacterial biomass in the North Sea and found a range of approximately 2–50 fg C cell^{-1} (when converted from biovolume). Most studies, however, consider a more conservative range of 10–20 fg C cell^{-1} to span the range of bacterial carbon values in nature (Lee and Fuhrman, 1987; Fuhrman and Lee, 1989). Similarly, a six-fold range for the carbon content of chroococcoid cyanobacterial cells also exists (60–350 fg C cell^{-1}; Cuhel and Waterbury, 1984; Kana and Glibert, 1987; Waterbury *et al.*, 1987; Caron *et al.*, 1991). This wide range is due in part to the highly variable cell size in natural assemblages of chroococcoid cyanobacteria.

Published conversion factors for nanoplanktonic and microplanktonic protists (photo-

trophs and heterotrophs) range from at least 80 to 360 fg C μm^{-3} (Strathmann, 1967; Borsheim and Bratbak, 1987; Choi and Stoecker, 1989; Putt and Stoecker, 1989; Verity *et al.*, 1992). Part of this range is due to the fact that the volume of a phytoplankton cell (or frustule) that is actually occupied by cell plasma is variable among species (Strathmann, 1967). Shrinkage due to preservation is also an important factor affecting the estimated carbon and nitrogen content per unit volume of protistan cells (Choi and Stoecker, 1989; Verity *et al.*, 1992).

These wide ranges in conversion factors are justified to some degree. Both the average cell size and the average carbon (or nutrient) content $cell^{-1}$ for a microbial assemblage may vary with geographical location, depth or time of day. This variability may be due to differences in the species composition of assemblages from different locales, or to the physiological states of the species comprising the microbial communities. The variable nature of these conversion factors complicates the correct determination of microbial biomass in seawater and warrants direct, in-depth studies of the relative and absolute contributions of these assemblages to the particulate material of planktonic ecosystems.

Our knowledge concerning the relative importance of microbial assemblages is particularly poor for oceanic environments. The multi-investigator program ZOOSWAT was conducted in the Sargasso Sea near Bermuda in 1989 and 1990. The overall goal of the project was to measure simultaneously the biomass of all phototrophic and heterotrophic planktonic organisms in the euphotic zone of this oligotrophic environment, analyse the flows of carbon between these living compartments, and determine the flux of particulate material out of the surface waters. Numerous measurements of microbial population density were conducted, as well as concurrent measurements of particulate carbon, particulate nitrogen and chlorophyll. This manuscript analyses the absolute and relative importance of the microbial planktonic assemblages (microorganisms $\leq 200\,\mu m$ in size) to the total and living particulate material in this environment, and the effect of uncertainties in the microbial conversion factors on the estimated carbon and nitrogen content of the various microbial populations. A separate paper (Roman *et al.*, 1995) describes and compares the biomass of the meso- and macroplankton to the microbial plankton (pico-, nano-, microplankton).

MATERIALS AND METHODS

Cruise plan and sample collection

Two cruises were conducted (August 1989 and March–April 1990) near the location of the JGOFS time-series station south of Bermuda (approximately 32°N, 64°W) as part of the ZOOSWAT research program. The samples on which this paper is based were a subset of the data from this large program. For this portion of the study, our goal was to calculate and compare the biomass of the various microbial assemblages to each other and to the total particulate carbon and nitrogen in the water. The rationale in these analyses was that the microbial populations $\leq 200\,\mu m$ in size constituted most of the living biomass sampled in small (≤ 1 l) volumes in this oligotrophic environment. For this reason, particulate material filtered from 1 l samples reflected the carbon and nitrogen content of these microbial assemblages and the non-living particulate material in the water. Abundance and biomass estimates of organisms $>200\,\mu m$ were not included in these analyses because

it was believed that these large "rare" organisms were not precisely sampled in the 1 l volumes used for analyses of chlorophyll, particulate carbon and particulate nitrogen.

The sole criterion for including a sample in our analyses was that all of the population counts and chemical measurements were made on subsamples from the same sample. Because of scheduling conflicts and the volume requirements for some of the population counts, many samples were excluded from consideration. Nevertheless, one set of samples from one of the cruises for which there were no microplankton counts (but all other measurements) were included in the analyses because microplankton constituted an insignificant fraction of the total living biomass of other samples.

Water column structure (temperature, salinity, light transmission, fluorescence) was examined prior to selecting depths for each vertical profile to locate and sample pertinent physical and biological features (e.g. the deep chlorophyll maximum; DCM). Water samples from each depth were collected with two 30 l Niskin bottles (acid-rinsed and equipped with teflon-coated springs) in order to obtain enough water for all analyses. The contents of the two replicate bottles were combined into a single 50 l polyethylene carboy prior to sampling for chemical and biological parameters to ensure a homogeneous sample.

Chemical measurements

The particulate material retained on precombusted Gelman GF/F glass fiber filters was used as a measure of the chlorophyll *a* concentration, total particulate carbon (PC) and nitrogen (PN) in the water. Concentrations of these constituents were measured in duplicate 1 l subsamples from each depth. Duplicate subsamples from each depth for chlorophyll analysis were extracted and measured by the procedure of Parsons *et al.* (1984). Samples for particulate nitrogen and carbon were fumed with HCl (March–April cruise only), dried, and analysed by gas chromatography with a Controlled Equipment elemental analyser (model 240-XA).

Counting procedures

Samples for all microbial population counts were preserved with 1% glutaraldehyde and stored in the dark at 4°C until prepared and examined. Only microorganisms ≤ 200 μm were included in our analyses. Abundances of all microbial assemblages were determined directly by microscopy using standard procedures.

Bacteria (BACT) were counted by the DAPI technique (3–5 μg ml^{-1} final strain concentration; Porter and Feig, 1980). Chroococcoid cyanobacteria (CYAN) were visualized by the autofluorescence of the phycoerythrin and counted (Waterbury *et al.*, 1987). A total of 300–400 cells were counted for each sample in 10–15 microscope fields for the bacteria and 20–50 microscope fields for the cyanobacteria. Coefficients of variation for these measurements were 10–15% of the mean values for the bacteria and 5–15% for the cyanobacteria.

Assemblages of phototrophic and heterotrophic nanoplankton (PNAN, HNAN; 2–20 μm protists) were enumerated by epifluorescence microscopy within 24 h of preservation and after staining with DAPI (25 μg ml^{-1} final stain concentration) (Caron, 1983; Sherr *et al.*, 1993). Phototrophic (chloroplast-bearing) nanoplankton were distinguished from heterotrophs by the autofluorescence of chlorophyll *a*. At least 200 nanoplankton cells were

counted for each sample. Coefficients of variation for nanoplankton abundance estimates as determined from triplicate subsamples were 5–15% of the mean values. Although nanoplankton counts performed in this manner included cells up to 20 μm in size, it was found that extremely few cells in this size class were greater than 5 μm in diameter. For this reason, PNAN and HNAN abundances presented in this study were composed predominantly of cells $\leq$5 μm. Some prochlorophyte-like cells may have been included in the counts of phototrophic picoplankton or nanoplankton. However, these red-fluorescing cells are smaller than the nominal limit for nanoplankton, and epifluorescence microscopy apparently grossly underestimates the abundance of these cells (Chisholm *et al.*, 1988). Presumably, undetected prochlorophyte-like cells were included in the bacterial counts.

Phototrophic and heterotrophic microplankton (PMIC, HMIC; predominantly dinoflagellates and ciliates, 5–200 μm) were enumerated from two types of samples. Small and delicate microplankton were enumerated from unfiltered water samples (220 ml) stained with DAPI and proflavin (Haas, 1982: 1/1000 of recommended concentration) and filtered onto 2 μm blackened Nuclepore filters, which were then placed on slides and stored frozen. To enumerate the larger and more robust microplankton, a second sample (10–30 l) from each depth was concentrated by gentle reverse-flow filtration with 20 μm Nitex screening. Subsamples of the preserved concentrated samples were stained with DAPI and placed in small settling chambers. Both types of microplankton samples were counted on an inverted epifluorescence microscope equipped with a computer-aided digitizing system for tallying and sizing each individual microplankter. Whole slides were counted for both filtered and settled samples. The filtered samples were of fixed volume (220 ml) and the settled samples were adjusted to result in >100 protists counted per sample (except in a few deep euphotic zone samples where the numbers were lower. For triplicate subsamples from the same water sample, the coefficients of variation for the abundance estimates were $\pm$12% for the filtered samples and $\pm$15% for the settled samples.

Biovolumes of the nanoplankton and microplankton assemblages were calculated from microscopical measurements of cell diameters (nanoplankton), or from measurements of the linear dimensions of the cells and application of taxon-specific geometric formulae (microplankton). Biovolumes of all microplankton cells encountered in the microscopical examinations were estimated. Errors for these biovolume estimates were $\pm$18% of the mean values for the filtered samples and $\pm$33% for the settled samples. Biovolume estimates were not obtained from every depth for nanoplankton because of the laborious nature of these measurements. For nanoplankton, average biovolumes of 50–100 specimens per sample were obtained from two sampling depths (the shallowest sampling depth and the depth of the deep chlorophyll maximum), and were assumed to be representative of the biovolumes of specimens in the upper and lower euphotic zone, respectively. Coefficients of variation for replicate biovolume estimates of nanoplankton averaged 29%. Biovolumes of individual specimens within a size category (nanoplankton or microplankton) varied greatly, however, because of the breadth of these size classes. For example, coefficients of variation for individual averages of 50–100 nanoplanktonic protists ranged up to 240%.

Biomass conversion factors

The carbon contents of the microbial populations were estimated for each of the assemblages enumerated by converting population abundances to carbon biomass. The

Table 1. Summary of the "high", "low" and "constrained" conversion factors and C:N ratios used to calculate microbial biomass in this study. High and low conversion factors were obtained from the literature (see Introduction for citations)

Microbial assemblage	C:N ratio (by weight)	Conversion factors (*fg C cell^{-1} or †fg C μm^{-3})		
		"High"	"Low"	"Constrained"
BACT*	4.0	20	10	15
CYAN*	4.0	350	60	200
PNAN/HNAN†	7.0	220	80	183
PMIC/HMIC†				

biomass values of the bacterial and cyanobacterial assemblages were estimated directly from cell counts and published estimates of carbon and nitrogen cell^{-1} because of the large uncertainties associated with estimating the biovolume of picoplankton using epifluorescence microscopy. The carbon values of the eukaryotic assemblages (nano- and microplankton) were calculated from their abundances, average biovolume cell^{-1} and a carbon conversion factor derived from our data as described below. The nitrogen content of the microbial assemblages was calculated from their carbon contents using C:N values from the literature.

The choice of a conversion factor for converting microbial abundance or biovolume to biomass had a major effect on the estimated relative and absolute importance of each assemblage to particulate carbon and nitrogen in the water. Therefore, microbial biomass was estimated using a range of conversion factors and also using the best approximation of these conversion factors. The high and low conversion factors that we used span the ranges that have been commonly used for natural assemblages. The constrained set of conversion factors was determined by using ancillary measurements (chlorophyll, PC and PN concentrations, carbon:chlorophyll ratios, C:N ratios of the suspended particulate material) to constrain some of the conversion factors as described below. The overall ranges of the conversion factors, and the "constrained" conversion factors, are summarized in Table 1.

RESULTS AND DISCUSSION

Population abundance estimates

Population abundances of the six microbial assemblages enumerated in this study ranged from approximately 1–10^6 ml^{-1} (Tables 2 and 3, Fig. 1). Differences between the picoplankton and nanoplankton, and between the nanoplankton and microplankton, were approximately 1–3 orders of magnitude. Within a particular size class, phototrophic and heterotrophic assemblages were more proportionate except for the picoplankton size class, in which the densities of cyanobacteria and bacteria differed by approximately two orders of magnitude. On average, differences between the abundances of phototrophic and heterotrophic nanoplankton, and between the abundance of phototrophic and heterotrophic microplankton, were less than a factor of three.

Densities of heterotrophic nanoplankton showed the least amount of variability for all samples averaged over all depths and collection dates, while chroococcoid cyanobacteria

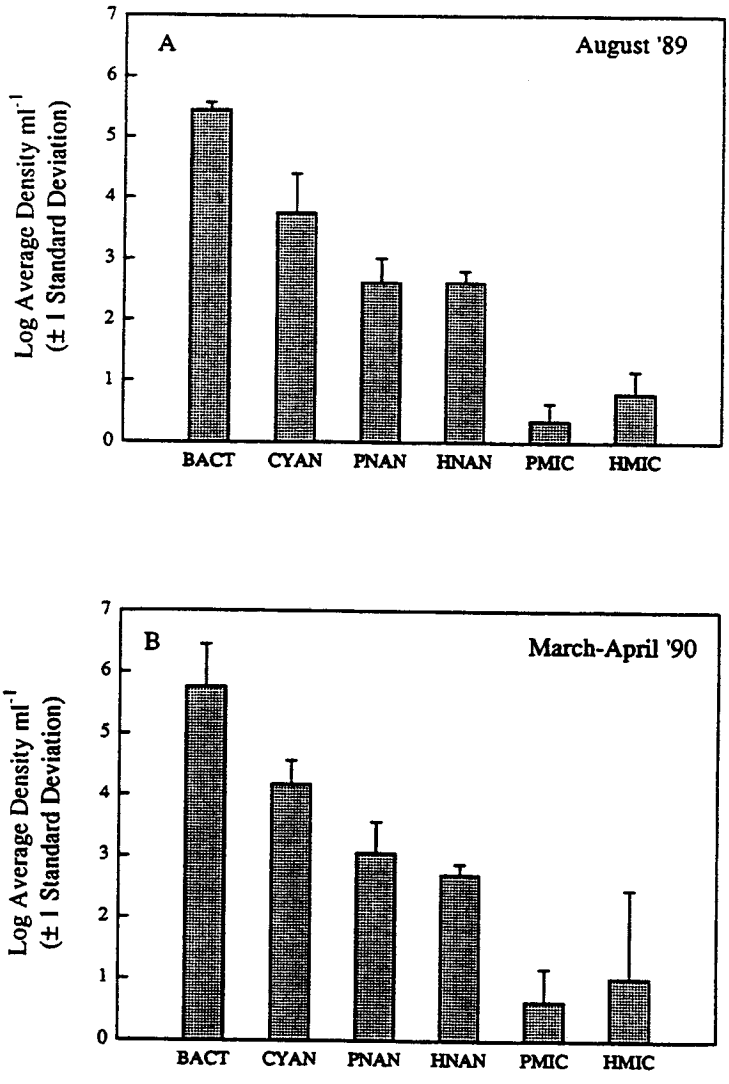

Fig. 1. Numerical averages (±1 standard deviation) of the microbial assemblages enumerated in samples collected during two cruises in the Sargasso Sea (see Tables 2 and 3 for individual sample dates and depths). Population identifications are: BACT = bacteria; CYAN = chroococcoid cyanobacteria; PNAN = phototrophic nanoplankton; HNAN = heterotrophic nanoplankton; PMIC = phototrophic microplankton; HMIC = heterotrophic microplankton.

were most variable during August and heterotrophic microplankton were the most variable during March–April (Fig. 1). Much of the variability associated with these latter assemblages was related to their depth-specific distributions (see below). The abundances of the various microbial groups observed in this study (Tables 2 and 3, Fig. 1) were similar to the abundances observed in other studies of these assemblages in oceanic environments (Azam *et al.*, 1983; Caron, 1983; Davis *et al.*, 1985; Waterbury *et al.*, 1987; Fuhrman *et al.*,

Table 2. Chlorophyll concentrations and microbial assemblage abundances in samples collected during August 1989. Population identifications are: BACT = bacteria, CYAN = chroococcoid cyanobacteria, PNAN = phototrophic nanoplankton, HNAN = heterotrophic nanoplankton, PMIC = phototrophic microplankton, HMIC = heterotrophic microplankton. Coefficients of variation for the individual counts were generally 10–15%. The overall average is the mean value of the individual measurements rather than the depth-integrated average

					Population density (ml^{-1})					
Date	Lat.	Long.	Depth (m)	Chl *a* $\mu g\ l^{-1}$	BACT $\times 10^5$	CYAN $\times 10^3$	PNAN $\times 10^2$	HNAN $\times 10^2$	PMIC	HMIC
5/8/89	31°51.90′	64°49.80′	2	0.05	3.0	19.7	5.16	6.19	—	—
			19	0.05	3.0	11.1	3.87	4.08	—	—
			37	0.07	3.6	8.04	3.12	5.03	—	—
			59	0.01	3.0	4.73	6.34	8.26	—	—
			102	0.23	2.3	0.34	26.1	4.60	—	—
			117	0.13	1.6	0.08	3.32	1.26	—	—
8/8/89	32°7.50′	64°20.10′	3	0.03	3.5	10.3	3.78	5.16	3.37	14.2
			15	0.05	3.9	7.33	3.73	5.02	3.40	10.1
			30	0.09	2.9	7.18	2.11	2.97	5.14	11.6
			52	0.20	3.9	9.92	7.30	7.73	4.77	11.3
			103	0.13	2.5	1.19	9.45	3.72	1.09	6.11
10/8/89	31°51.90′	64°19.30′	4	0.05	2.7	5.93	1.17	2.93	1.00	3.06
			15	0.05	3.5	2.74	0.85	4.45	1.38	8.19
			31	0.05	2.4	5.88	1.20	4.20	2.01	7.52
			52	0.08	2.2	9.34	1.88	4.57	3.49	17.7
			91	0.27	2.3	0.68	12.5	2.97	4.29	20.8
			101	0.22	1.9	0.48	1.68	3.04	2.65	14.7
11/8/89	31°53.58′	63°58.80′	3	0.04	2.8	4.42	1.11	4.96	1.02	7.61
13/8/89	32°7.58′	64°20.20′	3	0.045	2.9	6.72	0.64	5.18	1.14	6.25
			15	0.04	2.9	9.02	1.04	3.64	2.00	3.27
			31	0.04	3.0	4.62	1.02	2.64	1.63	3.73
			51	0.06	3.7	1.29	1.45	3.22	2.77	3.42
			120	0.18	1.5	0.44	1.79	2.34	1.11	1.71
15/8/89	32°6.80′	64°19.91′	4	0.05	2.9	8.94	2.72	5.35	0.66	1.10
			111	0.85	1.6	0.22	1.48	1.71	1.07	1.42
Overall average				0.12	2.78	5.63	4.19	4.21	2.31	8.09

Table 3. Chlorophyll concentrations and microbial assemblage abundances in samples collected during March–April 1990. Population identifications are the same as Table 1. Coefficients of variation for the individual counts were generally 10–15%. The overall average is the average of the individual measurements rather than the depth-integrated average

					Population density (ml^{-1})					
Date	Lat.	Long.	Depth (m)	Chl *a* $\mu g\ l^{-1}$	BACT $\times 10^5$	CYAN $\times 10^3$	PNAN $\times 10^2$	HNAN $\times 10^2$	PMIC	HMIC
27/3/90	31°43.98′	64°13.92′	25	0.09	2.60	17.8	8.40	5.20	1.80	4.68
			52	0.29	2.13	12.5	12.3	2.70	5.03	9.61
			59	0.35	5.20	8.23	27.2	4.66	4.59	4.68
29/3/90	31°43.02′	64°13.81′	2	0.13	6.20	23.6	10.0	4.80	2.25	6.06
			11	0.12	4.00	6.93	8.88	5.01	2.72	4.97
			27	0.12	3.40	18.3	8.64	6.48	1.58	7.08
			52	0.14	4.20	14.3	7.79	2.82	3.63	7.02
			62	0.45	10.9	15.7	22.0	4.59	4.64	9.74
31/3/90	31°43.89′	64°14.24′	2	0.27	9.10	24.6	8.28	4.14	3.95	9.76
			10	0.27	7.27	26.4	15.8	4.51	3.78	8.98
			25	0.27	5.00	29.2	13.3	5.58	3.38	5.91
			50	0.25	4.20	25.7	12.0	5.04	5.02	7.04
			89	0.27	11.93	9.05	16.6	5.94	2.49	3.21
			173	0.10	2.00	0.91	2.28	4.68	0.08	2.78
2/4/90	31°57.41′	64°18.54′	49	0.19	6.00	26.9	9.12	4.88	6.38	24.96
			74	0.27	5.20	7.54	6.00	3.72	7.05	20.34
			104	0.38	11.29	5.29	10.2	5.16	3.99	14.04
6/4/90	31°44.23′	64°14.16′	25	0.11	4.40	13.4	8.53	10.8	2.79	16.57
			52	0.32	8.67	11.2	11.5	8.88	7.73	18.97
			80	0.33	2.47	14.6	7.20	3.36	3.45	10.11
8/4/90	31°44.38′	64°14.21′	43	0.76	8.20	15.0	24.2	6.40	15.46	27.19
			64	0.04	2.53	3.17	1.02	3.12	2.17	7.78
Overall average				0.25	5.77	15.0	11.42	5.11	4.27	10.52

1989). Abundance ratios among the various assemblages also were similar to relationships observed in previous studies.

Microbial biomass based on a range of conversion factors

Published values for converting microbial abundances to carbon or nitrogen span a wide range. Therefore, the chosen conversion factor will affect conclusions on the relative importance of a microbial assemblage to the total particulate material in the water. as well as its relative importance to the biomass of other planktonic organisms. Microbial biomass for the two ZOOSWAT cruises, calculated using both high and low published conversion factors for each microbial group (see Materials and Methods) illustrate this effect. The summed microbial biomasses (BACT + CYAN + PNAN + HNAN + PMIC + HMIC) estimated for the two cruises using the set of high conversion factors were 2.6× (August) or 2.7× (March–April) greater than values calculated using the set of low factors (Fig. 2). That is, total microbial biomass values calculated for a given data set differed by as much as 2.7× as a consequence of which conversion factors were used to convert abundance to biomass. The slight difference (2.6 vs 2.7) between the two cruises were due to shifts in the composition of the microbial communities between seasons.

Constraining the conversion factors—the problem

Based solely on the microbial counts and PC/PN results averaged separately for each cruise, it was not possible to determine what combination of conversion factors might be most appropriate for our data. Using the high and low sets of conversion factors described above, averaged values were obtained of total microbial carbon and nitrogen that did not exceed the average total PC or PN measurements for either cruise (Fig. 2). When the individual samples were examined, however, microbial nitrogen determined using the high conversion factors exceeded the measured PN value in five out of the 22 individual samples during March–April, and in one of the samples for August. That is, the high conversion factors estimated more particulate nitrogen than was actually present in the sample.

The choice of conversion factors affected not only estimates of the absolute amount of living microbial biomass, but also estimates of the amount of non-living particulate material. Detrital carbon and nitrogen in the samples was estimated by summing the biomass estimates in the microbial assemblages and subtracting the values from the total PC and PN concentrations determined by CHN analysis. It was assumed in this calculation that microorganisms $\leq 200\,\mu m$ in size composed the vast majority of live particles caught on filters when relatively small volumes (≤ 1 l) of oceanic seawater are analysed. This approach would overestimate the detrital material somewhat because the contribution of crustacean zooplankton to PC and PN in the samples was not determined. These zooplankton were too rare to be accurately enumerated in the bottle samples. Using estimates of zooplankton abundance from pumped samples (Roman *et al.*, 1995), it was estimated that crustacean zooplankton contributed an average of $\leq 5\%$ of the particulate carbon and nitrogen.

Detrital carbon and nitrogen calculated in this manner were the dominant forms of PC and PN during August regardless of the conversion factors chosen. Detrital carbon during August was 88% or 69% of total PC and nitrogen was 83% or 58% of total PN using the low or high conversion factors, respectively (Fig. 2A,B). The situation was different for

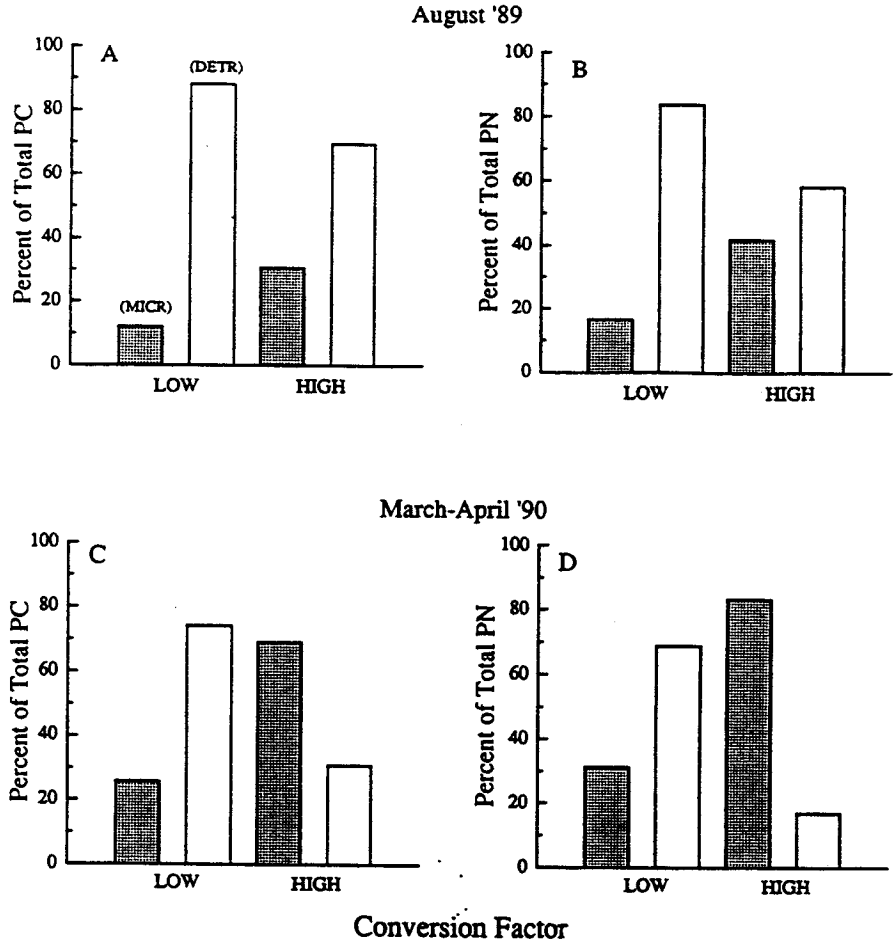

Fig. 2. Estimates of particulate carbon (A.C) and nitrogen (B.C) in the microbial assemblage ≤200 μm in samples collected during August 1989 (A.B) and March–April 1990 (C.D). Microbial biomasses, calculated using high and low published conversion factors, are shown by the shaded columns. Detrital material, shown by the open columns, was calculated as the difference between the total PC or PN and the amount of carbon or nitrogen contained in the microbial assemblage. The ranges of conversion factors were: 10 or 20 fg C bacterium^{-1} (C:N = 4.0 by weight), 60 or 350 fg C cyanobacterium^{-1} (C:N = 4.0 by weight), and 80 or 220 fg C μm^{-3} (C:N = 7.0 by weight) for nano- and microplankton.

March–April (Fig. 2C,D). Detrital carbon and nitrogen calculated for March–April exceeded the "live" carbon and nitrogen when the low conversion factors were applied, but resulted in the opposite conclusion ("live" exceeded "detrital") when high conversion factors were used. Detrital material averaged 31% of the PC and 17% of the PN during this cruise using the high conversion factors (not accounting for the contribution of crustacean zooplankton).

It could be argued that total PN was underestimated by the use of GF/F filters, which have been shown to pass a substantial fraction of the bacteria in some cases (Altabet, 1990). The measurements of bacterial abundance in the GF/F filtrate, however, did not

support a substantial effect of this artifact (see below). Alternatively, it is possible that the C:N ratios that were chosen (prokaryotes = 4 and eukaryotes = 7) were inappropriately low, and that these low ratios resulted in the discrepancies between the summed microbial biomass and the PN values. Because prokaryote biomass constituted nearly half of the living biomass averaged over the two cruises, much of the nitrogen was associated with this microbial group. It has been demonstrated that bacterial and cyanobacterial C:N ratios are typically low (≈4) and relatively stable (Nagata, 1986; Goldman *et al.*, 1987; Lee and Fuhrman, 1987). We therefore believe that this C:N value was appropriate. There is little published information for heterotrophic protists but it would appear that these microorganisms produce biomass in approximately the proportions of the Redfield Ratio (Caron *et al.*, 1990). Therefore a C:N ratio of 7 for the HNAN and HMIC assemblages seemed appropriate.

The PNAN and PMIC appear to be the only microbial groups whose C:N ratio might have substantially differed from our chosen value of 7. These assemblages constituted such a small percentage of the total microbial nitrogen, however, that a substantial increase in their C:N value had only a minor effect on the total microbial nitrogen values. For example, total microbial nitrogen calculated from the population abundances still exceeded the measured PN concentrations for four samples from the March–April cruise when a C:N ratio of 15 was used for the PNAN and PMIC assemblages. It was therefore concluded that the high conversion factors (i.e. cell abundances to carbon) for the microbial assemblages, and not the C:N ratios, were inappropriate for our study and we attempted to determine more realistic values by using the ancillary measurements made during the ZOOSWAT program to constrain these conversion factors.

Constraining the conversion factors—our solution

Carbon:chlorophyll ratios (C:Chl) for photosynthetically-fixed carbon were determined on the March–April cruise as a supporting measurement for the primary productivity studies (Malone *et al.*, 1992). These ratios were used to independently calculate the carbon content of the total phototrophic assemblage (CYAN + PNAN + PMIC).

$$\text{Total phototroph carbon} = (\text{C:Chl}) \times (\text{chlorophyll concentration}). \quad (1)$$

The samples from the vertical profiles (Tables 2 and 3) were grouped by depth range (<30 m, 30–60 m and >60 m) and the chlorophyll concentrations at each depth in the three groups were multiplied by C:Chl ratios of 60, 40 and 30, respectively. These C:Chl ratios were the approximate upper range of C:Chl ratios observed at these depths during the cruise. These ratios were chosen because they yielded the largest possible carbon values for the phototrophic assemblages.

Phototrophic microbial carbon values estimated in this manner were very similar to the carbon values estimated for these three phototrophic assemblages using the average of the high and low published conversion factors. Based on these results a conversion factor was chosen for the chroococcoid cyanobacteria that was approximately the average of the high and low published conversion factors (200 fg C cell^{-1}). This approximation seemed reasonable based on microscopical observations of the populations in the samples, and given that chroococcoid cyanobacteria (*Synchecococcus* spp.) in the Sargasso Sea display a wide range of cell sizes that are related to species composition of the assemblage, location, depth and time of day (Olson *et al.*, 1990a). The carbon content of the cyanobacterial assemblage in each sample was calculated in this manner.

$$\text{CYAN carbon} = (200 \text{ fg C cell}^{-1}) \times (\text{CYAN abundance}). \quad (2)$$

The values of cyanobacterial carbon (converted to μg C l^{-1}) in the samples were then subtracted from the total phototrophic carbon values (from equation 1). The carbon in the remaining phototrophs (PNAN and PMIC) was then divided by the measured biovolume of these assemblages to obtain a conversion factor of 183 fg C μm^{-3} for these assemblages.

$$\text{PNAN and PMIC conversion factor} = \frac{(\text{total phototroph carbon}) - (\text{CYAN carbon})}{(\text{total biovolume of PNAN and PMIC})} \quad (3)$$

This value is slightly greater than the average of the low and high published factors. The conversion factor calculated for phototrophic eukaryotes using equation (3) was then applied to the HNAN and HMIC assemblages to convert the biovolumes of these assemblages to carbon. The conversion factors determined above were designated as "constrained" values because the C:Chl ratios provided an independent means of estimating their magnitudes.

As a check on the validity of this approach, the "constrained" conversion factors determined from the March–April cruise data were used to calculate the microbial carbon present in the summed CYAN, PNAN and PMIC assemblages during the August cruise (conversion factor multiplied by the assemblage abundance or biovolume). These carbon values were used to calculate C:Chl ratios for each sample by dividing the calculated phototrophic carbon by the chlorophyll concentration in each sample. The samples were grouped by depth interval and an average C:Chl ratio was calculated for each depth interval. This procedure was essentially the reverse of the process used to calculate the conversion factors from the March–April data set. The resulting C:Chl ratios calculated for the August data set ranged from >100 at the surface to <30 at depths ≥100 m (overall average 84). These values are commensurate with values observed in oligotrophic oceanic environments (Laws *et al.*, 1984). We obtained two values, however, that appeared to be unreasonable. The sample from 59 m on 5 August had a C:Chl ratio of 669, and the sample at 111 m on 15 August had a C:Chl ratio of 1.6. The latter value may have been due to the predominance of prochlorophytes in the chlorophyll maximum. These methods of microscopical analysis undoubtedly underestimated the abundance of these cells (see Bacterial Biomass below). Underestimation of the phototrophic biovolume could have resulted in an unreasonably low C:Chl ratio. The high value on 5 August is unexplained, but the chlorophyll value for this sample was exceptionally low (4 times lower than any other chlorophyll value) and may have been in error (Table 2).

There was no independent means of examining the conversion factor for bacterial biomass. Based on the authors' experience, however, it was decided that the size of the bacteria from this open ocean environment did not warrant use of the high conversion factors in the literature. For a "constrained" conversion factor for this assemblage, therefore, 15 fg C $cell^{-1}$ was chosen. This values is at the lower end of the overall range of conversion factors employed for bacterial assemblages but it is intermediate between the commonly used values of 10 and 20 fg C $cell^{-1}$ (Van Duyl *et al.*, 1990).

Microbial and detrital biomass in surface waters of the Sargasso Sea

The "constrained" conversion factors described above were used to calculate the importance of the various microbial assemblages to total particulate carbon and nitrogen

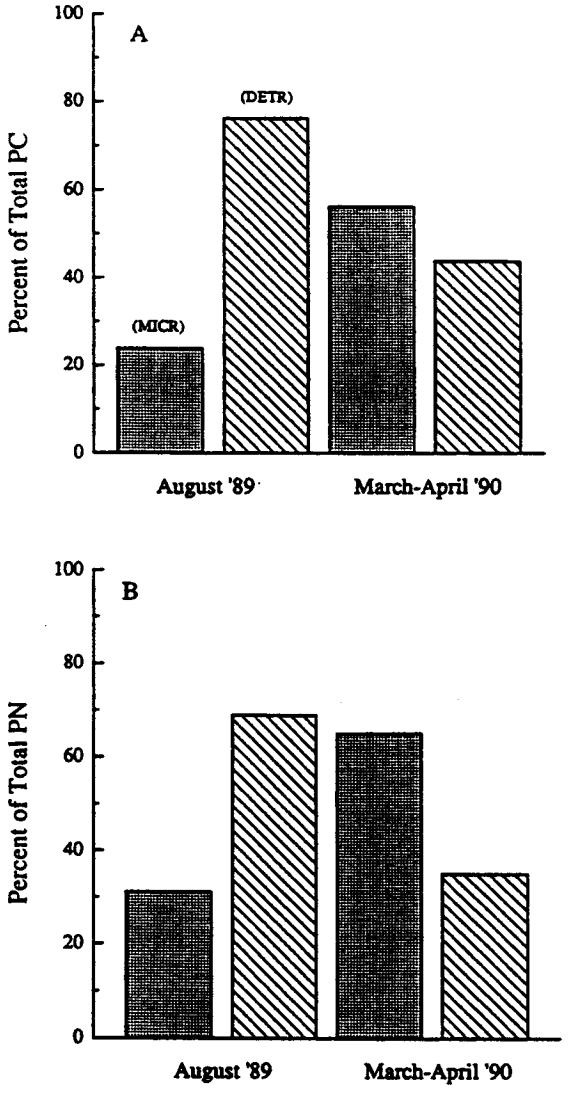

Fig. 3. Estimates of particulate carbon (A) and nitrogen (B) in the microbial assemblage ≤200 μm in samples collected during August 1989 and March–April 1990. Microbial biomass is shown by the shaded columns, detrital material by the hatched columns. The constrained conversion factors were used to calculate the microbial biomass (see text).

during the two cruises. Detrital carbon and nitrogen also were calculated by difference, as described above.

The partitioning of carbon and nitrogen among the "living" and "detrital" fractions of the particulate material differed markedly for the two cruises even though the averaged total PC and PN concentrations did not show great differences (Tables 4–7; Fig. 3). Measured PC and PN concentrations were approximately 25% and 10% greater during August than during March–April (Tables 4–7). The microbial biomass during March–April, however, was nearly twice that during August. For the two cruises, microbial

Table 4. Particulate carbon concentration (PC) and microbial carbon in samples collected during August 1989. The "constrained" conversion factors were used to calculate the microbial biomass (see text). Population identifications are the same as in Table 1. The overall average is the average of the individual measurements rather than the depth-integrated average

Date	Lat.	Long.	Depth (m)	PC $\mu gC\,l^{-1}$	Carbon biomass ($\mu gC\,l^{-1}$)					
					BACT	CYAN	PNAN	HNAN	PMIC	HMIC
5/8/89	31°51.90′	64°49.80′	2	58.34	4.50	3.94	4.67	5.28	—	—
			19	53.84	4.50	2.22	3.50	3.48	—	—
			37	62.65	5.40	1.61	2.82	4.29	—	—
			59	47.69	4.50	0.95	5.74	7.05	—	—
			102	96.21	3.45	0.07	23.62	3.92	—	—
			117	33.31	2.40	0.02	3.00	1.07	—	—
8/8/89	32°7.50′	64°20.10′	3	112.9	5.25	2.06	3.42	4.40	0.30	0.59
			15	51.14	5.85	1.47	3.38	4.28	0.38	1.74
			30	60.57	4.35	1.44	1.91	2.53	0.41	1.07
			52	61.13	5.85	1.98	6.61	6.59	0.18	1.05
			103	50.09	3.75	0.24	8.55	3.17	0.03	0.16
10/8/89	31°51.90′	64°19.30′	4	49.29	4.05	1.19	1.06	2.50	0.24	0.67
			15	58.8	5.25	0.55	0.77	3.80	0.17	0.41
			31	52.25	3.60	1.18	1.09	3.58	0.12	0.33
			52	53.91	3.30	1.87	1.70	3.90	0.28	0.85
			91	43.38	3.45	0.14	11.31	2.53	0.12	0.87
			101	54.00	2.85	0.10	1.52	2.59	0.07	0.89
11/8/89	31°53.58′	63°58.80′	3	28.61	4.12	0.88	1.00	4.23	0.36	0.84
13/8/89	32°7.58′	64°20.20′	3	59.94	4.35	1.34	0.58	4.42	0.39	0.34
			15	62.48	4.35	1.80	0.94	3.10	0.29	0.25
			31	54.38	4.50	0.92	0.92	2.25	0.39	0.35
			51	58.10	5.55	0.26	1.31	2.75	0.27	0.25
			120	41.03	2.25	0.09	1.62	2.00	0.05	0.28
15/8/89	32°6.80′	64°19.91′	4	45.78	4.35	1.79	2.46	4.56	0.21	0.22
			111	60.21	2.40	0.04	1.34	1.46	0.20	0.30
Overall average				56.40	4.17	1.13	3.79	3.59	0.23	0.60

Table 5. Particulate nitrogen concentration (PN) and microbial nitrogen in samples collected during August 1989. The "constrained" conversion factors were used to calculate the microbial biomass (see text). Population identifications are the same as in Table 1. The overall average is the average of the individual measurements rather than the depth-integrated average

Date	Lat.	Long.	Depth (m)	PN $\mu gN\ l^{-1}$	Nitrogen biomass ($\mu gN\ l^{-1}$)					
					BACT	CYAN	PNAN	HNAN	PMIC	HMIC
5/8/89	31°51.90′	64°49.80′	2	9.03	1.13	0.99	0.67	0.75	—	—
			19	7.70	1.13	0.56	0.50	0.50	—	—
			37	10.5	1.35	0.40	0.40	0.61	—	—
			59	6.82	1.13	0.24	0.82	1.01	—	—
			102	15.2	0.86	0.02	3.37	0.56	—	—
			117	5.24	0.60	<0.01	0.43	0.15	—	—
8/8/89	32°7.50′	64°20.10′	3	15.9	1.31	0.52	0.49	0.63	0.04	0.08
			15	7.20	1.46	0.37	0.48	0.61	0.05	0.25
			30	9.03	1.09	0.36	0.27	0.36	0.06	0.15
			52	9.10	1.46	0.50	0.94	0.94	0.03	0.15
			103	6.19	0.94	0.06	1.22	0.45	<0.01	0.02
10/8/89	31°51.90′	64°19.30′	4	6.32	1.01	0.30	0.15	0.36	0.03	0.10
			15	8.21	1.31	0.14	0.11	0.54	0.02	0.05
			31	8.08	0.90	0.30	0.16	0.51	0.02	0.05
			52	8.59	0.83	0.47	0.24	0.56	0.04	0.12
			91	7.70	0.86	0.04	1.62	0.36	0.02	0.12
			101	9.41	0.71	0.03	0.22	0.37	0.01	0.13
11/8/89	31°53.58′	63°58.80′	3	3.16	1.03	0.22	0.14	0.60	0.05	0.12
13/8/89	32°7.58′	64°20.20′	3	8.90	1.09	0.34	0.08	0.63	0.06	0.05
			15	10.2	1.09	0.45	0.13	0.44	0.04	0.04
			31	7.07	1.13	0.23	0.13	0.32	0.06	0.05
			51	6.69	1.39	0.06	0.19	0.39	0.04	0.04
			120	5.69	0.56	0.02	0.23	0.29	0.01	0.04
15/8/89	32°6.80′	64°19.91′	4	6.27	1.09	0.45	0.35	0.65	0.03	0.03
			111	9.59	0.60	0.01	0.19	0.21	0.03	0.04
Overall average				8.32	1.04	0.28	0.54	0.51	0.04	0.08

Table 6. Particulate carbon concentration (PC) and microbial carbon in samples collected during March–April 1990. The "constrained" conversion factors were used to calculate the microbial biomass (see text). Population identifications are the same as in Table 1. The overall average is the average of the individual measurements rather than the depth-integrated average

Date	Lat.	Long.	Depth (m)	PC $\mu gC\,l^{-1}$	Carbon biomass ($\mu gC\,l^{-1}$)					
					BACT	CYAN	PNAN	HNAN	PMIC	HMIC
27/3/90	31°43.98′	64°13.92′	25	30.39	3.90	3.56	5.60	3.90	0.35	0.91
			52	39.06	3.20	2.50	8.19	2.03	0.41	2.30
			59	60.89	7.80	1.65	18.12	3.50	0.27	1.95
29/3/90	31°43.02′	64°13.81′	2	37.11	9.30	4.72	6.66	3.60	0.42	0.97
			11	45.02	6.00	1.39	5.92	3.76	0.34	0.74
			27	43.82	5.10	3.66	5.76	4.86	0.28	0.65
			52	47.25	6.30	2.86	5.19	2.12	0.36	0.93
			62	54.94	16.35	3.14	14.7	3.44	0.62	0.90
31/3/90	31°43.89′	64°14.24′	2	45.06	13.65	4.92	5.52	3.11	0.41	0.84
			10	44.24	10.91	5.28	10.52	3.38	0.37	0.64
			25	47.07	7.50	5.84	8.86	4.19	0.95	0.58
			50	41.81	6.30	5.14	7.99	3.78	0.48	0.70
			89	42.52	17.90	1.81	11.06	4.46	0.67	0.55
			173	16.58	3.00	0.18	1.52	3.51	0.03	0.19
2/4/90	31°57.41′	64°18.54′	49	47.69	9.00	5.38	6.08	3.66	0.70	2.61
			74	54.77	7.80	1.51	4.00	2.79	0.48	1.92
			104	37.50	16.94	1.06	6.79	3.87	0.23	2.08
6/4/90	31°44.23′	64°14.16′	25	44.51	6.60	2.68	5.68	8.10	0.76	1.12
			52	67.05	13.01	2.24	7.66	6.66	0.54	0.98
			80	44.82	3.71	2.92	4.80	2.52	0.30	0.80
8/4/90	31°44.38′	64°14.21′	43	82.36	12.30	3.00	16.12	4.80	0.95	2.89
			64	14.5	3.80	0.63	0.68	2.34	0.09	1.30
Overall average				44.95	8.65	3.00	7.61	3.83	0.46	1.21

Table 7. Particulate nitrogen concentration (PN) and microbial nitrogen in samples collected during March–April 1990. The "constrained" conversion factors were used to calculate the microbial biomass (see text). Population identifications are the same as in Table 1. The overall average is the average of the individual measurements rather than the depth-integrated average

Date	Lat.	Long.	Depth (m)	PN $\mu gN\ l^{-1}$	Nitrogen biomass ($\mu gN\ l^{-1}$)					
					BACT	CYAN	PNAN	HNAN	PMIC	HMIC
27/3/90	31°43.98′	64°13.92′	25	5.65	0.98	0.89	0.80	0.56	0.05	0.13
			52	7.12	0.80	0.63	1.17	0.29	0.06	0.33
			59	12.59	1.95	0.41	2.59	0.50	0.04	0.28
29/3/90	31°43.02′	64°13.81′	2	5.29	2.33	1.18	0.95	0.51	0.06	0.14
			11	5.61	1.50	0.35	0.85	0.54	0.05	0.11
			27	6.09	1.28	0.92	0.82	0.69	0.04	0.09
			52	7.4	1.58	0.72	0.74	0.30	0.05	0.13
			62	10.42	4.09	0.79	2.10	0.49	0.09	0.13
31/3/90	31°43.89′	64°14.24′	2	7.76	3.41	1.23	0.79	0.44	0.06	0.12
			10	7.28	2.73	1.32	1.50	0.48	0.05	0.09
			25	7.46	1.88	1.46	1.27	0.60	0.14	0.08
			50	7.22	1.58	1.29	1.14	0.54	0.07	0.10
			89	7.40	4.48	0.45	1.58	0.64	0.10	0.08
			173	2.75	0.75	0.05	0.22	0.50	0.00	0.03
2/4/90	31°57.41′	64°18.54′	49	7.64	2.25	1.35	0.87	0.52	0.10	0.37
			74	7.70	1.95	0.38	0.57	0.40	0.07	0.27
			104	7.05	4.24	0.27	0.97	0.55	0.03	0.30
6/4/90	31°44.23′	64°14.16′	25	7.08	1.65	0.67	0.81	1.16	0.11	0.16
			52	10.85	3.25	0.56	1.09	0.95	0.08	0.14
			80	7.85	0.93	0.73	0.69	0.36	0.04	0.11
8/4/90	31°44.38′	64°14.21′	43	15.21	3.08	0.75	2.30	0.69	0.14	0.41
			64	2.71	0.95	0.16	0.10	0.33	0.01	0.19
Overall average				7.55	2.16	0.75	1.09	0.55	0.07	0.17

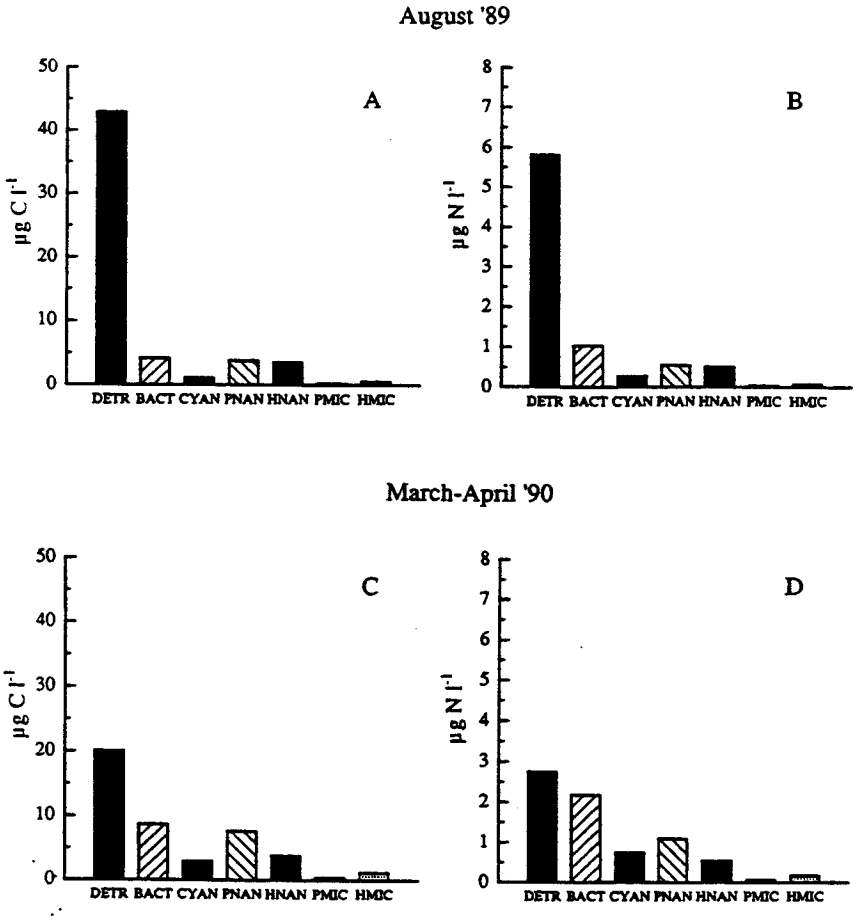

Fig. 4. Distribution of particulate carbon (A,C) and nitrogen (B,D) among the microbial assemblages and detrital particulate material (DETR) for cruises during August 1989 (A,B) and March–April 1990 (C,D). Population identifications are the same as in Fig. 1.

biomass constituted 24% (August) and 55% (March–April) of the total amount of particulate carbon and 30% (August) and 63% (March–April) of particulate nitrogen. The result was a dominance of detrital particulate material over microbial biomass during the summer cruise and a dominance of the microbial biomass over the detrital particulate material during the spring cruise (Fig. 3).

Absolute concentrations of detrital carbon and nitrogen, and the fraction of the total PC and PN constituted by detrital material, were roughly twice as great for August when compared to March–April (Fig. 4). The particulate carbon values for August may have been somewhat elevated because these samples were not fumed prior to CHN analysis. Not fuming the August samples may have contributed to the strong dominance of detrital carbon in these samples if $CaCO_3$ was a significant fraction of the total particulate carbon. Detrital nitrogen was also highly elevated in the August samples, however, and fuming would not have affected the number significantly. Moreover, detrital material was the

major component of the total PC and PN (relative to BACT, CYAN, PNAN, HNAN, PMIC or HMIC) for both cruises regardless of sample treatment (Fig. 4).

The average C:N ratios (by weight) for the particulate material caught on GF/F filters were 6.8 during August and 6.0 during March–April. The C:N ratios for the summed microbial biomasses were 5.4 and 5.2 for the summer and spring cruises, respectively. These relatively low values were a consequence of the contribution of prokaryotes with their low C:N to total microbial biomass. When the contribution of the microbial assemblages to total PC and PN were subtracted, the calculated C:N ratios for the remaining "detrital" particulate material were 7.4 and 7.3 for the summer and spring cruises, respectively.

The partitioning of particulate carbon and nitrogen between the prokaryotic and eukaryotic assemblages, and between the phototrophic and heterotrophic assemblages, was similar for both cruises when averaged over all depths and sampling dates (Fig. 5). The

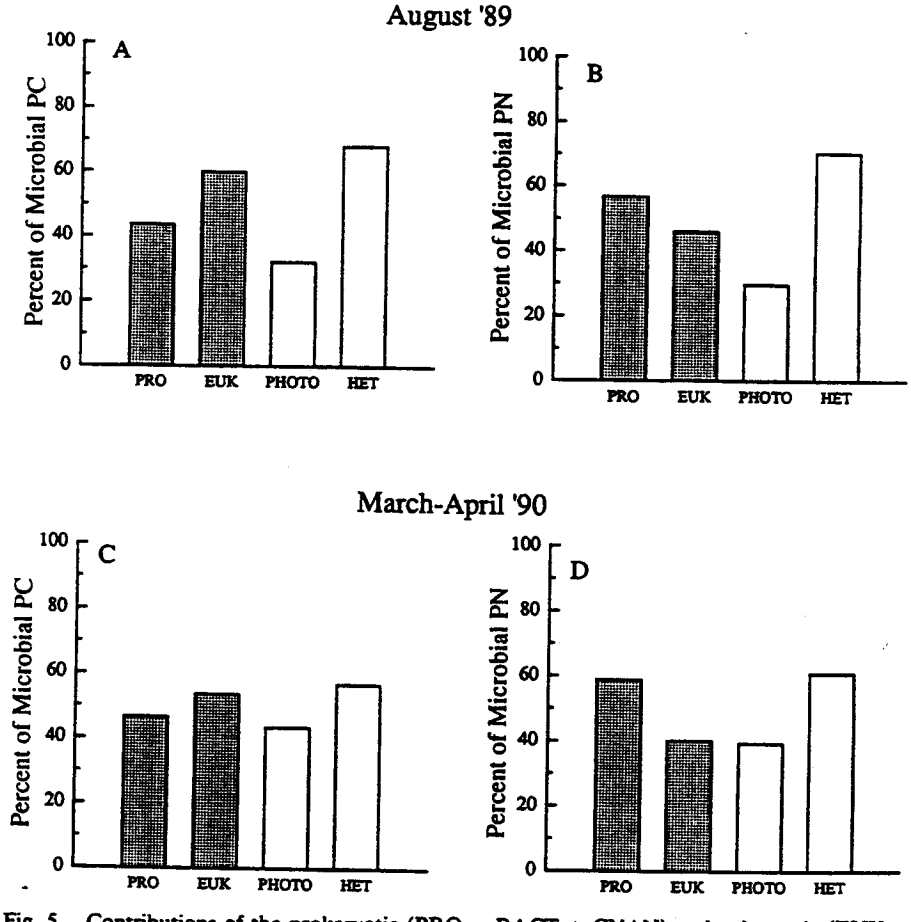

Fig. 5. Contributions of the prokaryotic (PRO = BACT + CYAN) and eukaryotic (EUK = PNAN + HNAN + PMIC + HMIC) assemblages (shaded columns), and the phototrophic (PHOTO = CYAN + PNAN + PMIC) and heterotrophic (HET = BACT + HNAN + HMIC) assemblages (open columns) to total microbial carbon and nitrogen. The comparisons are independent and therefore PRO + EUK = 100% and PHOTO + HET = 100%.

eukaryotic assemblage (PNAN, HNAN, PMIC, HMIC) contained slightly more carbon than the prokaryotic assemblage (BACT, CYAN), but the opposite was true for nitrogen (shaded columns in Fig. 5). This reversal was due to the lower C:N ratio of the prokaryote assemblage. The concentrations of particulate carbon and nitrogen in the heterotrophic microorganisms were greater than the corresponding values in the phototrophic microorganisms during both cruises (open columns in Fig. 5). The dominance of the heterotrophic biomass occurred during the spring cruise even though the average chlorophyll concentration in these samples was more than twice the average chlorophyll concentration in the summer samples. This dominance of the heterotrophic biomass was due primarily to the large contribution of bacterial biomass to heterotrophic biomass during both cruises.

Bacterial biomass

Bacterial biomass was the major "living" component of the particulate material during both cruises. Bacteria alone constituted roughly 10–20% of the total PC and 15–30% of the total PN during the two cruises (Fig. 4). The contribution of this assemblage to the microbial biomass, however, was considerably greater. Approximately 35% of the microbial carbon and 45% of the microbial nitrogen was bacterial biomass (Fig. 6).

The relative contribution of bacterial biomass to total microbial biomass was dependent on depth. The abundances of all microbial assemblages decreased below 150 m, but the decrease in bacterial abundance was not as great as decreases for the other assemblages. For this reason bacterial biomass greatly dominated the microbial biomass at depths between 150 and 250 m. The concentration of particulate material also decreased dramatically below 150 m, however, and the bacterial biomass constituted a fraction of the total particulate material that was similar to its value in surface waters. Bacteria constituted 21% of the measured PC and 28% of the measured PN in 12 samples collected from 250 m during March–April, while the overall averages for all of the shallower samples were 19% of the PC and 29% of the PN (Tables 6, 7 and Peele, unpubl. data).

Three factors may have resulted in possible errors in the estimations of the absolute and relative importances of bacterial biomass in this study. First, some fraction of the bacterial assemblage may not have been included in the particulate carbon and nitrogen analyses because they may have passed through the GF/F filters. Altabet (1990) noted the loss of 30% of the particulate material through GF/F filters relative to aluminum oxide filters. The number of bacteria in the GF/F filtrates were counted on several occasions during the March–April cruise. It was found that <10% of the bacteria ever passed through the filters. If bacteria passed through the GF/F filters and therefore were missed by the CHN analyses, the PC and PN values that were obtained were slight underestimations of the correct values. Bacterial biomass estimates would be unaffected by this problem, however, because biomass values were calculated from bacterial abundances which were determined by microscopical counts on 0.2 μm Nuclepore filters (which catch all the bacteria). The overall effect, therefore, would be a slight overestimation of the importance of bacterial biomass as a percentage of the total PC and PN.

Another factor that may have affected these estimates of the importance of bacterial biomass was the probable inclusion of prochlorophytes in the bacterial counts. The abundance of these phototrophs is underestimated in microscopical counts of DAPI-stained preparations, in which they appear as large picoplankton but cannot be easily identified as phototrophs because of their weak autofluorescence. Because they could not

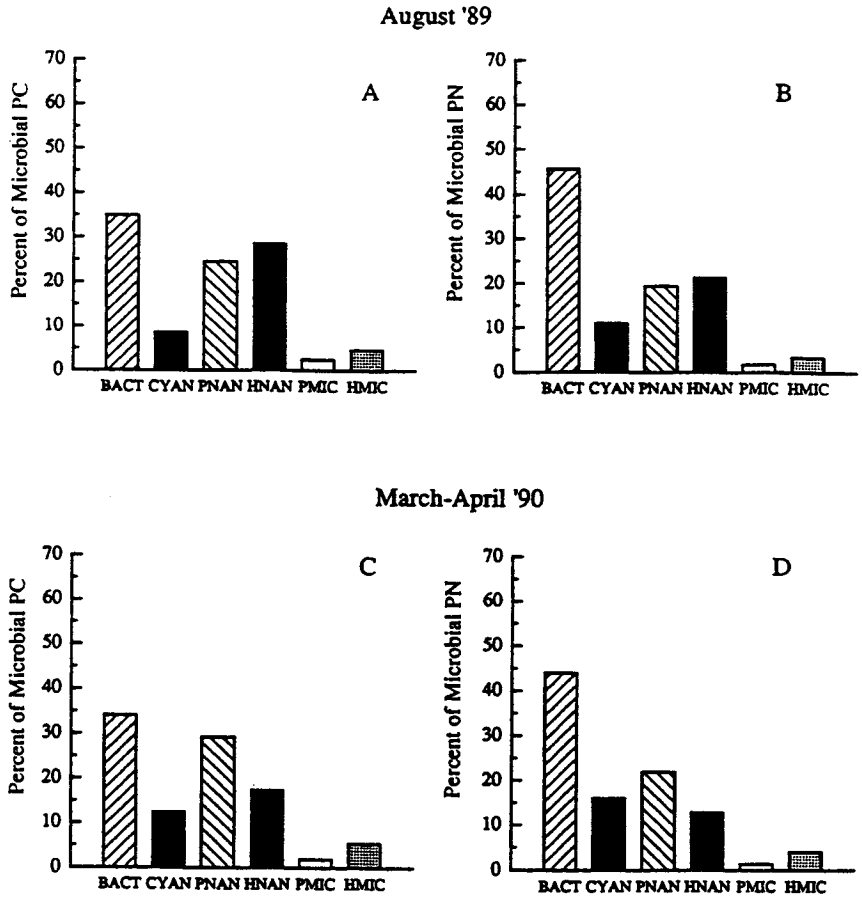

Fig. 6. Particulate carbon (A,C) and nitrogen (B,D) of the various microbial assemblages, expressed as a percentage of the total microbial biomass, for cruises during August 1989 (A,B) and March–April 1990 (C,D). Population identifications are the same as in Fig. 1.

be easily distinguished from bacteria, it must be assumed that prochlorophytes were included in the bacterial counts. Based on prochlorophyte abundances and vertical distributions near the study site during March and July of 1989 (Olson *et al.*, 1990b), it is probable that these cells contributed up to 20–30% of the bacterial counts in the samples from the deep euphotic zone during the August cruise. Contributions to bacterial abundances at other depths during August or during the March–April cruise, however, probably were <10%. Averaging this contribution over the entire data set and over both cruises, it is unlikely that prochlorophytes increased the overall average of the bacterial counts in this study by more than 10%.

A third factor that may have affected the relative importance of the bacterial assemblage was storage of the samples prior to staining for epifluorescence microscopy. One recent study has noted significant losses (≈40%) of bacterial numbers counted by epifluorescence microscopy when formalin-preserved samples are stored prior to staining (Turley and Hughes, 1992). This study differed from the procedures of Turley and Hughes in that the

samples were preserved with glutaraldehyde and stored at 5°C in the dark. Nevertheless, some undetermined percentage of the bacterial assemblage may not have been counted in the study. If this situation was the case, then the absolute bacterial biomass and the fraction of the PC and PN constituted by bacteria could have been underestimated. As a "worst case" calculation, the bacterial biomass values were multiplied by 1.67 (1.00 ÷ 0.6) to estimate the greatest possible contribution of bacteria to the samples. For March–April (during which bacterial biomass was the greatest percentage of the total PC and PN), bacteria may have constituted as much as 25% of the total PC (58% of the microbial carbon) and 50% of the total PN (75% of the microbial nitrogen).

It is interesting to note that the first two potential problems described above for estimating the biomass of bacteria in these samples would tend to nullify the effects of the third factor. Loss of PC and PN through GF/F filters, or the inclusion of prochlorophytes in the bacterial counts, would result in overestimation of the importance of bacteria in the samples while the loss of bacteria during storage of preserved samples would result in an underestimation of the importance of bacteria. When the uncertainty of the conversion factors is added to these problems, it is not surprising that past estimates of bacterial biomass in aquatic samples have varied greatly.

Phototroph biomass

PNAN biomass was the largest phototrophic microbial biomass observed during both cruises (Figs 4, 6). Overall, the carbon content of this assemblage was more than twice the chroococcoid cyanobacterial carbon and several times greater than the average PMIC carbon. PNAN accounted for up to 30% of the microbial carbon during the two cruises. PMIC averaged <5% of the microbial biomass during both cruises but did show occasional, episodic peaks in abundance (Malone *et al.*, 1992). Cyanobacteria on average constituted a relatively modest percentage of the microbial carbon and nitrogen (≤15%). The biomass values of chroococcoid cyanobacteria observed in this study overlap the values summarized for a number of marine and freshwater environments (Pick and Caron, 1987; Waterbury *et al.*, 1987; Stockner, 1988).

The perception of the distribution of biomass among the microbial phototrophic assemblages in this study may have been affected by underestimation of prochlorophytes. Although prochlorophytes probably constituted only a minor fraction of the bacterial counts (see above), they may have constituted a significant fraction of the biomass of the phototrophic microorganisms of similar size. Olson *et al.* (1990b) observed prochlorophyte abundances several times greater than *Synechococcus* abundances in the deep euphotic zone near the study site in 1989 (although *Synechococcus* often predominated in the shallow euphotic zone). Li *et al.* (1992) also estimated an overall prochlorophyte:cyanobacteria biomass ratio of approximately two in the northern Sargasso Sea. If this situation existed during the study, then phototrophic picoplankton biomass could have been significantly underestimated in the analyses.

Support for this possibility can be found in this report's chlorophyll data. Chlorophyll in the picoplankton size class accounted for 84% and 74% of the total chlorophyll during the August and March–April cruises, respectively, when integrated over the euphotic zone (Malone *et al.*, 1992). In contrast, CYAN (phototrophic picoplankton) in the present analysis accounted for 24% and 29% of the phototrophic biomass during August and March–April, respectively. The ratio between the chlorophyll values of Malone *et al.*

(1993) and our CYAN percentages are approximately the 2:1 ratio calculated by Li *et al.* (1992). The present analysis, however, was not an integration of biomass over the depth of the euphotic zone. The overall contribution of prochlorophytes in our individual samples is therefore difficult to estimate without some knowledge of the abundances of this component of the phototrophic assemblage.

Although our inability to enumerate prochlorophytes may have affected the proportions of phototrophic pico- and nanoplankton (and to a lesser extent the microplankton), it did not affect estimates of the total biomass of phototrophs in the samples. Total phototroph biomass was determined using the C:Chl ratio and the concentration of chlorophyll at each depth.

It has recently been reported that the carbon conversion factor (biovolume to carbon) for glutaraldehyde-preserved, cultured phytoplankton increases as phytoplankton size decreases (Verity *et al.*, 1992). The constrained conversion factor of 183 fg C μm^{-3} that was used for converting the biovolume of phototrophic and heterotrophic eukaryotes to carbon is intermediate to the range of values predicted by the regression of Verity *et al.* (1992) for nano- and microplankton protists. This result might be expected given that biovolumes of a wide size range of protistan species were averaged in these calculations.

The conversion factor for phototrophs was derived directly from C:Chl ratios and chlorophyll measurements, so it is unlikely that total phototroph biomass was underestimated in this study. It is probable, however, that the authors' method may have underestimated the contribution of nanoplankton to total biomass because of the size-dependent nature of the regression. This possibility was investigated by comparing nanoplankton biomass calculated using average biovolume and the constrained conversion factor to nanoplankton biomass calculated using the Verity *et al.* (1992) regression and the biovolumes of the individual nanoplankton cells in the analyses. Biomass of nanoplanktonic cells calculated by this latter method on average were 22% greater than biomass calculated using the constrained conversion factor. If the estimates of total phototroph biomass from the C:Chl ratios and chlorophyll measurements were accurate, the larger biomass of nanoplankton would indicate that the biomass of other phototrophs (CYAN, PMIC) might have been overestimated.

Heterotrophic eukaryote biomass

Most of the carbon and nitrogen content of the heterotrophic eukaryotes was contained in the heterotrophic nanoplankton. Overall, heterotrophic protists less then approximately 5 μm in size constituted slightly less than 10% of the total PC and approximately 15–30% of the microbial carbon (Figs 4, 6). HMIC generally was a very small fraction of the total PC and PN and 5% or less of the total microbial biomass. The decreasing contribution of heterotrophic eukaryotes to total microbial biomass that was observed in this study was an expected result. Decreasing amounts of heterotrophic biomass in the larger size classes is consistent with current conceptualizations of size-dependent grazing relationships within the microbial food web.

Losses of HMIC cells, particularly ciliates, probably occurred in the samples due to fixation with glutaraldehyde (Ohman and Snyder, 1991). However, the contribution of these cells to the total microbial biomass was small (Fig. 6). Doubling the contribution of ciliate protozoa (i.e. assuming cell losses of 50% in glutaraldehyde samples) would change the contribution of this assemblage to total microbial biomass only marginally. It was not

possible to determine if losses of HNAN occurred as a consequence of preservation. Shrinkage of HMIC and HNAN also may have occurred. The constrained conversion factor should have accounted for this artifact if the degree of shrinkage of these cells was similar to shrinkage in PNAN and PMIC cells.

Vertical distribution of microbial biomass

There were relatively few detectable changes in the averaged vertical profiles of the biomass of the microbial assemblages for the two cruises (Fig. 7). Examination of depth-averaged profiles for carbon and nitrogen during August showed that PNAN increased sharply in the deepest samples (≈100 m). This assemblage was the dominant phototrophic

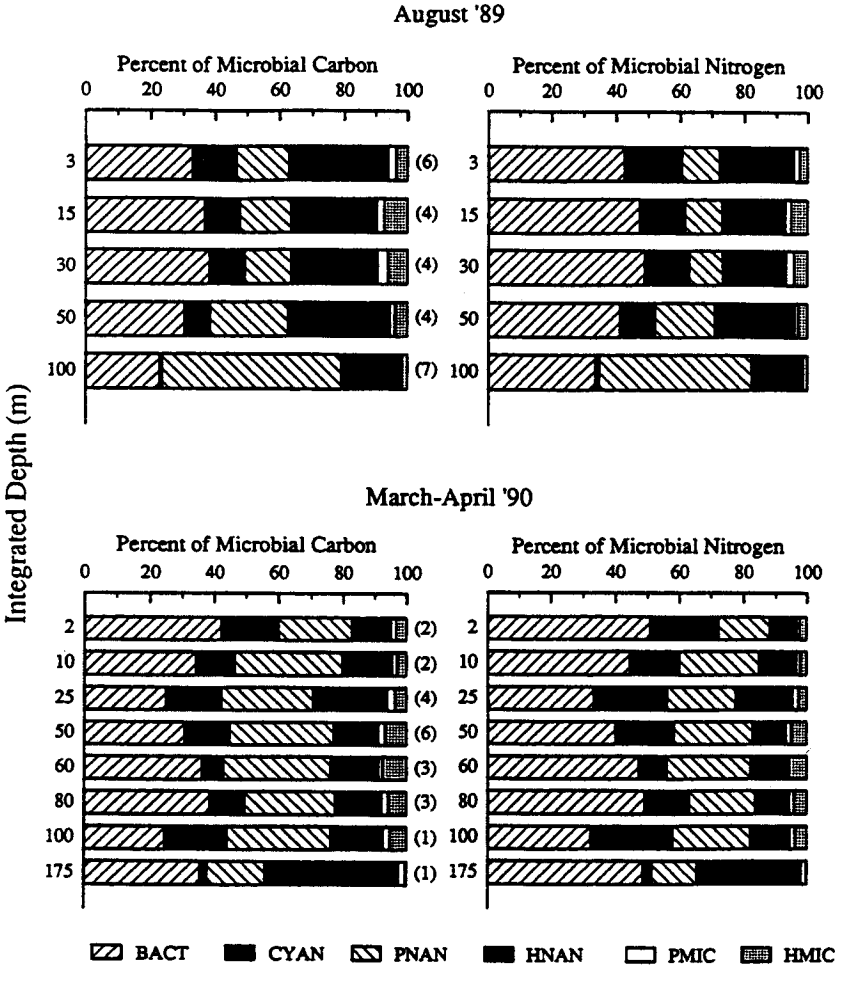

Fig. 7. Averaged depth distributions of the carbon and nitrogen biomass of the various microbial assemblages, expressed as a percentage of the total microbial biomass, during August 1989 and March–April 1990. Population identifications are the same as in Fig. 1. Samples were grouped into the depth interval closest to the depth given on the ordinate and averaged to obtain the biomass values displayed. Numbers in parentheses are the number of individual water samples on which the averages are based.

microbial group that was counted in the deep chlorophyll maximum. As mentioned above, however, these methods undoubtedly underestimated the importance of prochlorophytes which typically occur in the deep euphotic zone of this environment. The deep chlorophyll maximum was a conspicuous feature at approximately 100 m during August (Malone *et al.*, 1992). PNAN biomass was greater at 100 m than at the surface by a factor of three. This trend was similar for this microbial assemblage during the March–April cruise except that the depth of the deep chlorophyll maximum was shallower during the spring cruise, and therefore the peak in PNAN biomass was located at an intermediate depth.

Other depth-related features were not dramatic. The biomass of the CYAN was lower at the bottom of the euphotic zone during both cruises, while HMIC showed somewhat higher abundances at intermediate depths than at the surface and in the deepest samples.

The depth-related features described above resulted in perceptible shifts in the relative importance of some of the microbial groups with depth. In general, a greater importance of prokaryotic biomass in the surface waters during August gave way to a strong dominance of eukaryotic biomass in the deep euphotic zone. These biomasses were more equitable during March–April. The most notable shift in the biomass structure with depth was the increased relative importance of the PNAN in the deep euphotic zone during August. The PNAN alone constituted ≈55% of the microbial carbon at 100 m during the August cruise.

Evolving notions of planktonic microbial biomass

There has been a steady progression of reevaluations of the microbial assemblages comprising plankton communities over the last few decades. This progression has included a realization of the importance of nano-sized and pico-sized species among the phototrophic populations (Malone, 1971; Li *et al.*, 1983; Chisholm *et al.*, 1988), and an increased awareness of the heterotrophic components (bacteria, flagellated and ciliated protozoa) of the microbial plankton (Fenchel, 1986; Pierce and Turner, 1992). Modern conceptualizations of pelagic food webs now recognize and incorporate these populations as an integral and important component of planktonic communities, the so-called "microbial loop" (Pomeroy, 1974; Azam *et al.*, 1983; Sherr and Sherr, 1988). Determination of the correct apportionment of these various groups of microorganisms within the plankton is imperative if we are to accurately assess energy and elemental flow through these populations and through plankton communities in general. Accurate estimations of the biomasses of these various microbial assemblages, however, continue to be problematic.

Our knowledge concerning the relative importance of microbial assemblages is particularly poor for oceanic environments. Beers *et al.* (1969; 1971) provided some of the first detailed analyses of the absolute and relative importance of microzooplankton groups in oceanic plankton communities. However, these studies did not include counts of the picoplankton (bacteria and cyanobacteria), and nanoplankton may have been underestimated by the counting method employed in those studies. Davis *et al.* (1985) determined the abundances of phototrophic and heterotrophic components of the picoplankton and nanoplankton in the North Atlantic. This study provided more detail on the numerical relationship between these assemblages than was available up to that time. Unfortunately, biomass estimates were not derived from their abundance estimates (which spanned more than four orders of magnitude), and therefore is difficult to derive the relative importance of the various microbial assemblages from that study.

Recently, Fuhrman *et al.* (1989) reevaluated the importance of bacterial biomass in surface waters of the Sargasso Sea. Based on microscopical counts of the microbial assemblages and the conversion of these abundances to biomass, these investigators calculated that bacterial biomass constituted >70% of the living carbon and >80% of the living nitrogen in these waters. In addition, they concluded that, because bacterial biomass was much greater than algal biomass, the turnover of bacterial biomass must be much slower than the turnover of the algal biomass (i.e. the overall growth rate of the bacterial assemblage was less than the overall growth rate of the phytoplankton). This study indicated a major role for bacteria in the oligotrophic ocean as repositories for particulate carbon and nitrogen in surface waters. Cho and Azam (1990) reached similar conclusions with respect to bacterial biomass and growth rate. These results were generalized by Simon *et al.* (1992), who summarized data from freshwater and marine environments. Their summary demonstrated a sharp increase in the ratio between bacterial and phytoplankton biomass in ecosystems with low phytoplankton carbon concentrations relative to more eutrophic environments.

Other recent studies, however, have reached less dramatic conclusions concerning the role of bacteria than those suggested by Fuhrman *et al.* (1989) and Cho and Azam (1990). Li *et al.* (1992) concluded that phytoplankton and bacterial biomass may co-dominate the total microbial biomass in the northern Sargasso Sea. Kirchman *et al.* (1993) also noted about equal proportions of bacterial and phytoplankton biomass, and Garrison *et al.* (1993) observed a similar relationship at the ice edge in the Weddell Sea and Scotia Sea.

Central to the issue of establishing the relative and absolute amounts of biomass within the various assemblages composing the microbial plankton is the problem of converting microbial abundance to biomass. One goal of our study was to simultaneously measure the abundances of all major microbial assemblages, as well as obtain chemical measurements that could provide ancillary information with which to assess microbial biomass in the surface waters of an oceanic environment. Based on our measurements and calculations, we did not find strong support for the use of the highest published conversion factors for calculating microbial biomass from abundance. This paper's results indicated that conversion factors that were intermediate (in fact, almost average) between the extremes published in the literature provided the best approximation of microbial carbon and nitrogen. Yet, the biomass of the various microbial assemblages based on these "constrained" conversion factors still represented a major component of the total particulate carbon and nitrogen sampled from small volumes of Sargasso Sea surface waters.

A consensus is emerging that living microorganisms constitute a significant and sometimes dominant fraction of the total particulate material of oceanic plankton communities. The implications of this finding are significant. Fuhrman *et al.* reasoned that, because bacterial biomass appears to be considerably greater than phytoplankton biomass in the open ocean, on average, bacterial growth rates must be considerably slower than phytoplankton growth rates, because the small biomass of the latter assemblage must supply the substrate to support the large bacterial assemblage. The validity of this speculation must be confirmed, but it is consistent with the biomass relationships among microbial groups that are now emerging.

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