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Picoplankton and nanoplankton and their trophic coupling in surface waters of the Sargasso Sea south of Bermuda

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Abstract

Studies were conducted during August and March–April in the Sargasso Sea south of Bermuda to examine rates of bacterial growth and picoplankton consumption by microbial consumers. Bacterial growth rates were estimated from [^{3}H]thymidine (TdR) incorporation rates, while grazing rates were determined using fluorescently labeled prey (FLP). In addition, net bacterial growth rates were calculated from changes in bacterial abundance during 24-h incubations. The latter measurements were compared to net growth rates obtained by subtracting picoplankton grazing mortality rates from bacterial TdR growth rate estimates (TdR minus FLP). Overall, bacterial growth rates determined by TdR uptake were similar to FLP grazing rates during the March–April cruise, indicating an approximate balance between production and removal processes. Bacterial growth rates during August, however, were approximately twice the rates of grazer removal. Net bacterial growth rates determined from TdR growth rates minus FLP grazing rates were similar to net growth rates estimated from changes in abundance for samples collected near the surface during both cruises. However, rates of change of bacterial abundances during incubations were generally greater than rates predicted from TdR minus FLP for samples collected in the deep euphotic zone during both cruises. These discrepancies might be explained by several factors, including the inclusion of prochlorophytes in the bacterial counts and/or the stimulation of bacterial growth during containment. The TdR conversion factor also was an important consideration when comparing net bacterial growth rates estimated from changes in bacterial abundance to net growth rates determined from TdR minus FLP. Small nanoplanktonic protists (<5 μm) were responsible for most of the picoplanktonic grazing activity. Doubling times of 0.9–18.3 d for the heterotrophic nanoplankton were estimated based on the removal rates of picoplankton. The complexity of the microbial food web of this oligotrophic ecosystem is such that relatively little carbon may be recovered from nonliving organic material and passed on to metazoa.

Numerous studies have firmly established the importance of picoplanktonic microorganisms (organisms $\leq$ 2 μm in size; primarily bacteria, cyanobacteria, prochlorophytes, and some protists) in energy flow and nutrient cycling in marine planktonic ecosystems. Phototrophic picoplankton contribute significantly to phytoplankton biomass and production, while nonphotosynthetic picoplankton are instrumental in carbon and nutrient transformation and remineralization. Consequently, research has focused on the rates of production of these minute primary and secondary producers in freshwater and marine habitats and on their fate in aquatic food webs.

Trophic relationships involving picoplankton in oceanic environments have unique significance in light of investigations that have concluded that picoplankton biomass and production are highly important in oligotrophic oceanic ecosystems (Fogg 1995). Bacterial biomass constitutes a major component of the total microbial carbon and nitrogen in these ecosystems (Fuhrman et al. 1989; Cho and Azam 1990; Li et al. 1992; Caron et al. 1995). Bacterial production is a substantial fraction of primary production in most pelagic communities, and this percentage increases with increasingly oligotrophic conditions (Cole et al. 1988; Ducklow and Carlson 1992; Simon et al. 1992; Carlson et al. 1996). Similarly, photosynthetic picoplankton are significant repositories of carbon and nitrogen and important primary producers in oligotrophic environments (Waterbury et al. 1987; Olson et al. 1990*a*,*b;* Li et al. 1992; Malone et al. 1992; Campbell et al. 1994).

The fate of picoplanktonic microorganisms in marine food webs has been linked repeatedly to the grazing activities of small protists (Sieburth 1984; Sanders et al. 1992; Sherr and Sherr 1994). This generalization is based on size-fractionation investigations, direct observations, and laboratory studies that have demonstrated that most grazing activity on picoplankton is associated with nanoplanktonic (2–20 μm) or microplanktonic (20–200 μm) consumers (Wright and Coffin 1984; Wikner and Hagström 1988; Sherr et al. 1989; Marrasé et al. 1992). These trophic interactions are consistent with the modern depiction of microbial plankton inter-

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actions as size-dependent trophic relationships (Sieburth et al. 1978; Azam et al. 1983; Moloney and Field 1991; Ducklow 1994). The role of viruses in picoplankton mortality also has received considerable attention in recent years, but the relative importance of viral lysis as a factor for reducing picoplankton abundance in oligotrophic waters is not clear (Fuhrman and Suttle 1993; Waterbury and Valois 1993).

Despite their significance, rates of consumption of picoplanktonic populations in many aquatic environments have not been characterized adequately. Most studies of bacterivory have been conducted in nearshore or estuarine environments, which are generally more accessible than the open ocean, and where relatively rapid rates of bacterial mortality facilitate measurement. Determinations of oceanic herbivory are more common than bacterivory; nevertheless, there are still few reports that specifically address removal of either phototrophic or heterotrophic picoplankton in oligotrophic environments (e.g., *see* Landry et al. 1995; Latasa et al. 1997; Reckermann and Veldhuis 1997).

This dearth of information on the fate of picoplankton biomass, particularly bacteria, in open-ocean food webs is in part a consequence of limitations related to extant methodologies for measuring this process. Methodologies for the estimation of microbial grazing activity on bacterial assemblages are controversial and problematic. These measurements may suffer from a variety of potential artifacts including containment, long incubation times, and the disruption of community structure/function (Caron et al. 1993). These possible complications are not limited to the measurement of bacterivory in the open ocean, but they may be exacerbated by low predator–prey abundances and slow rate processes, which often characterize oligotrophic communities.

In contrast, there are numerous reports of bacterial production from oceanic habitats (Ducklow and Hill 1985*a;* Cole et al. 1988; Ducklow and Carlson 1992; Simon et al. 1992; Ducklow et al. 1993; Carlson et al. 1996). The availability of relatively easy techniques for the measurement of bacterial production has resulted in their widespread application (Fuhrman and Azam 1982; Kirchman et al. 1985). Determination of production and growth rates from the results of these methods, however, requires several important assumptions and the application of conversion factors that remain difficult to corroborate. For these reasons, verification of measurements of bacterial production and growth rate by an independent approach would be highly beneficial.

The simultaneous determination of growth and grazing of a prey population in nature is desirable because these measurements provide complementary data on the movement of energy through the prey. If measurements of both parameters are accurate and precise, then direct comparisons of growth and mortality rates can provide information on the net change in abundance of the prey population. Conversely, if changes in prey abundance are measured directly during the experiments, then these changes can serve as a means of verifying comparisons of growth and grazing rates.

Experiments were conducted during two seasons in surface waters of the Sargasso Sea south of Bermuda as a part of the ZOOSWAT program (Roman et al. 1995). The goals of this multi-investigator program were to determine the distribution of carbon and nitrogen among the photosynthetic and heterotrophic components of this epipelagic community (≈0–200 m) and to estimate the rates of carbon flow among these biological compartments. Descriptions of the general population and biomass structure of the community have been reported previously (Caron et al. 1995; Roman et al. 1995). This manuscript is a synthesis of two components of this program designed to measure pico- and nanoplankton standing stocks (abundance and biomass), bacterial production, and picoplankton grazing by microbial assemblages in this environment. Independent measurements of bacterial growth and picoplankton removal by grazers were compared to investigate how closely these measurements agreed with each other and with net changes in bacterial abundance in incubated samples. In addition, rates of protozoan ingestion and growth were estimated from standing stocks of protozoa and observed rates of picoplankton consumption.

Materials and methods

ZOOSWAT cruise plan and sample collection—Cruises were conducted in August 1989 and March–April 1990 at a single location south of Bermuda (approximately 32°N, 64°W). Water samples were collected every 1–4 d for a 2-week period during each cruise to provide a time series of microbial abundances and activities (total of seven sampling days for each cruise). All samples for microbial abundance estimates and growth rate and grazing rate measurements were collected in acid-rinsed 30-liter Niskin bottles with Teflon-coated springs and O-rings. Two bottles were filled at each depth and their contents pooled into a single 50-liter acid-washed carboy to provide a single homogeneous sample for all biological measurements. Samples were removed and processed for particulate organic carbon as previously described (Caron et al. 1995). Profiles of water-column structure (temperature and salinity) were performed with a Seabird CTD conductivity-temperature-depth probe and in situ fluorescence with a Seatech in situ fluorometer, in conjunction with water sampling.

Population and biomass estimations—Samples for picoplankton (0.2–2.0 μm) and nanoplankton (2–20 μm) population counts were collected at five to six depths on each sampling day, preserved immediately with 10% glutaraldehyde (prepared with filtered natural seawater; final concentration of 1%), and prepared within 24 h for microscopy. Bacteria, chroococcoid cyanobacteria, phototrophic nanoplankton (chloroplast-bearing cells), and heterotrophic nanoplankton (flagellated protozoa) were enumerated by epifluorescence microscopy. Cyanobacteria were enumerated on unstained preparations using the autofluorescence of phycoerythrin to visualize the cells (Waterbury et al. 1987), while bacteria and nanoplankton were stained with fluorochromes (Porter and Feig 1980; Caron 1983; Bloem et al. 1986). Phototrophic nanoplankton were distinguished from heterotrophs using the autofluorescence of chlorophyll *a* (Chl *a*). The weak autofluorescence of prochlorophytes was not readily visible using epifluorescence microscopy, and these cells were presumably included in the counts of the bacteria. Their potential contribution to standing stock, bac-

terial growth rates, and protozoan ingestion is discussed below (*see Discussion*). Counting errors for microbial assemblages were typically ≤15% of population abundance.

Bacterial and cyanobacterial biomass was calculated directly from abundances of these assemblages using conversion factors of 15 fg C bacterium^{-1} and 200 fg C cyanobacterium^{-1}. Biomass of the nanoplankton assemblages was estimated from numerical abundances and the average volumes of the cells (determined from microscopical measurements and approximate geometric shapes). Nanoplankton biovolumes were determined for samples collected at 1 m and at the depth of the deep chlorophyll maximum (DCM). These biovolume estimates were applied to samples at and below the DCM and to all samples above this feature, respectively. A conversion factor of 183 fg C μm^{-3} was used to calculate nanoplankton biomass from biovolume. The basis for these conversion factors was an analysis of a larger data set including measurements of microplankton biomass, particulate carbon and nitrogen, and carbon : chlorophyll ratios measured during the ZOOSWAT program (Caron et al. 1995).

Bacterial production/growth and picoplankton grazing measurements—Experiments were performed on seawater samples collected near the surface (1–10 m) and approximately at the depth of the DCM (44–130 m, depending on the date and season) on each day that vertical profiles of microbial abundance and biomass were determined. Bacterial production was examined by measuring the incorporation of TdR (Fuhrman and Azam 1982). Subsamples from the 50-liter carboys were placed in 50-ml disposable centrifuge tubes, TdR was added at a final concentration of 20 nM, and samples were sealed and incubated in on-deck incubators covered with gray window screening to reduce light intensity to near-ambient conditions. Samples for bacterial productivity measurements were incubated for 1 h. Samples were subsequently extracted with cold trichloroacetic acid (TCA) (5% final concentration), filtered onto polycarbonate filters, rinsed with cold TCA, and assayed for TdR incorporation. TdR incorporation was converted to bacterial production based on a conversion factor of 4×10^{18} cells mol^{-1} determined empirically using the same technique in the northwestern Atlantic (Ducklow and Hill 1985*b*) and applied to this area in subsequent studies (Fuhrman et al. 1989; Børsheim 1990). Bacterial growth rates were estimated from the number of bacteria produced and the abundance of bacteria at the beginning of the incubation.

Measurements of picoplankton grazing by microbial consumers were performed in duplicate on subsamples of the seawater samples from which bacterial productivity was measured. Grazing rates were determined using the disappearance of FLP as a tracer of grazing activity (Sherr et al. 1987; Rublee and Gallegos 1989). Losses of FLP were monitored during 24-h incubations (endpoint determinations) in 1-liter aliquots held in 1.25-liter clear polycarbonate bottles and placed in the same incubators used for the bacterial production measurements. Control bottles (0.2-μm filtered seawater with FLP) were performed for each experiment to monitor nongrazing-related losses of the tracer (e.g., fading or sticking to bottle walls). Grazing rates on bacterial assemblages were estimated from the rates of loss of FLP assuming an exponential decrease during the incubation period.

Fluorescently labeled bacteria (*Halomonas halodurans*), chroococcoid cyanobacteria (*Synechococcus* strain WH8012; courtesy of J. B. Waterbury), and a picoplanktonic alga (a spherical 1–2-μm *Nannochloris*-like alga isolated from Great South Bay, New York) were tested as tracers to quantify the removal rates of picoplankton prey. In practice, we observed no consistent differences between the losses of FLP in the experiments, and the rates of loss of the labeled alga were used for calculating picoplankton grazing because changes in the densities of FLA in the control treatments (i.e., no grazing) were insignificant for the alga. The alga therefore provided a more sensitive rate measurement in this oligotrophic environment.

Net bacterial growth rates were determined for each grazing experiment performed in this study. Bacterial abundances were determined at the beginning and end of the 24-h incubation period by epifluorescence microscopy. We calculated net bacterial growth rate from these changes by assuming exponential growth during the incubation. Net bacterial growth rate calculated in this manner measures the outcome of growth and grazing processes in the bottles, but it is independent of the methods used to measure bacterial growth and picoplankton grazing.

The contribution of nanoplankton to total grazing pressure on picoplankton was examined by performing parallel FLP disappearance experiments with seawater prefiltered through 20-μm Nitex® screening. Filtrate was prepared by gentle gravity filtration. All experimental bottles were prepared and incubated in the same manner employed for the unfiltered seawater.

Picoplankton grazing also was investigated during one cruise by examining FLP disappearance in various size fractions to determine the size of the consumers of the picoplankton. Water for this experiment was collected at 61 m on 7 April. Filtrates of <1 and <2 μm were obtained by gentle (<25 mm Hg) filtration through polycarbonate filters. Filtrates of <5, <10, <20, and <200 μm were obtained by gravity filtration through Nitex® screening. Each of these filtrates and the unfractionated seawater were inoculated with FLP and incubated in an on-deck incubator. Abundances of FLP were determined at the beginning of the experiment and again after 24 h to determine the rates of disappearance of prey in the various filtrates and unfiltered samples.

Results

Water column structure, population abundances, and biomass—Stratification of the surface waters of the study area differed markedly for the two study periods (*see representative profiles, Fig. 1*). The August cruise was characterized by a strong seasonal thermocline. The severity of this feature was reduced somewhat by Hurricane Dean, which passed through the study site on 6 August, but surface water temperatures comparable to conditions prior to the storm were reestablished by 13 August. Fluorescence profiles during this cruise indicated a well-established DCM. The peak of this maximum ranged between ca. 90 and 125 m during the study

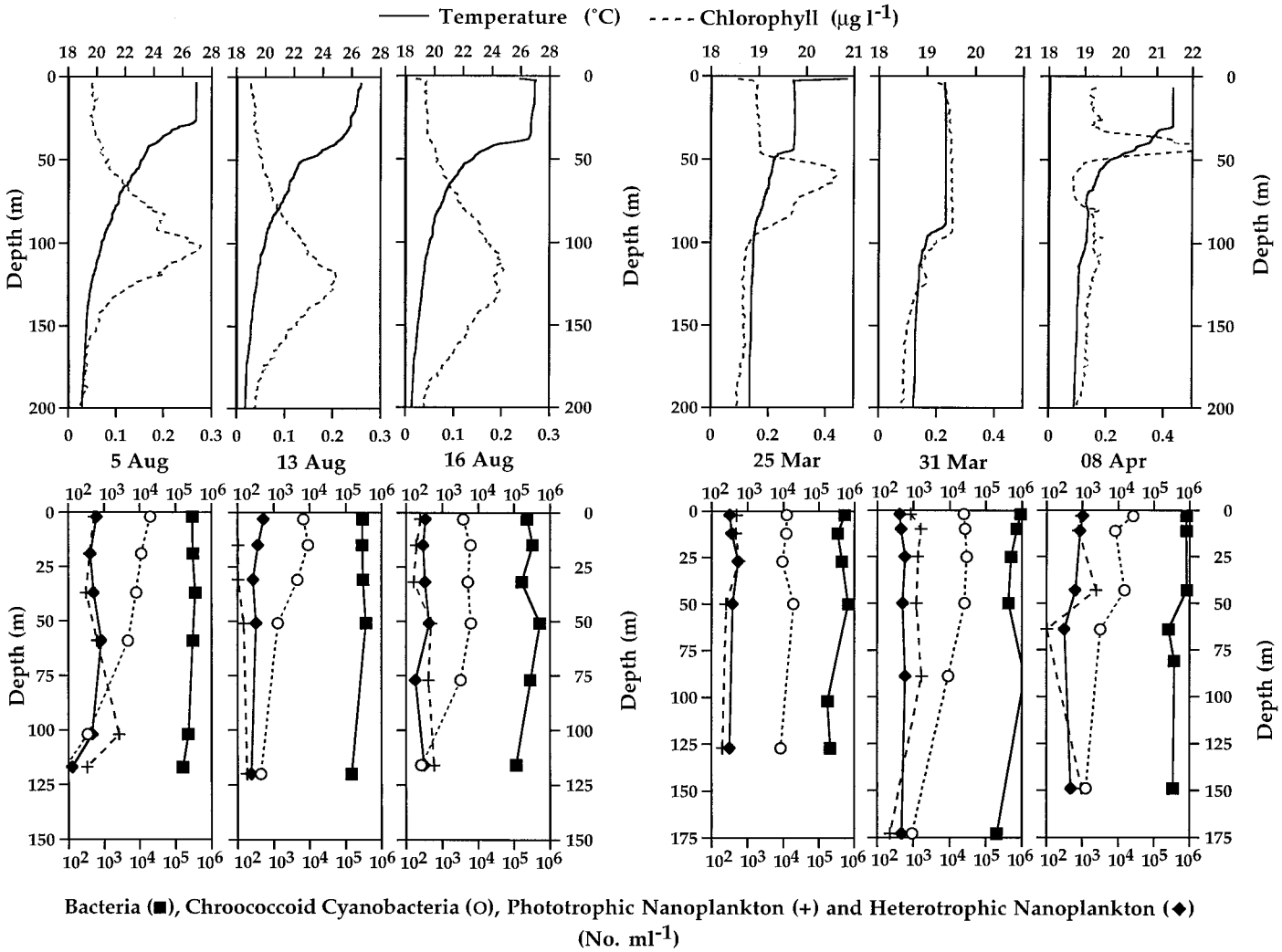

Fig. 1. Three representative vertical profiles of temperature, chlorophyll (from fluorescence), and population abundances during August 1989 and March–April 1990 in the Sargasso Sea south of Bermuda. (*See text for station location.*)

period and varied from a fairly discrete feature to a rather broad peak spanning 50 m or more (e.g., 16 August).

In contrast, water-column temperature structure during the March–April time series was quite variable and appeared to be the result of a succession of different hydrographic regimes passing through the study site (Fig. 1). The water column was weakly stratified to ≈50 m for the first 5 d of the study period (25–29 March) and had a well-defined DCM associated with the thermocline/nutricline. Temperature at the surface decreased slightly; the mixed layer deepened by 31 March, and a DCM was not apparent. A shallow, relatively warm (20.5–21.5°C) mixed layer followed and was associated with a DCM that became progressively shallower and more narrowly defined vertically (8 April). The later stages of this sequence may have indicated the passage of a mesoscale eddy or ring through the study area (Malone et al. 1992). Overall, these seasonal features of the hydrography of the study site are consistent with long-term time-series measurements at a nearby site (Michaels et al. 1994).

Pico- and nanoplankton assemblages indicative of an oligotrophic environment characterized the water column at the study site (Fig. 1). This situation was particularly true for the August cruise. Abundances of bacteria and chroococcoid cyanobacteria were typically $\leq 5 \times 10^5$ and $\leq 10^4$ ml^{-1}, respectively, while phototrophic and heterotrophic nanoplankton (PNAN and HNAN) generally ranged from approximately 10^2 to 5×10^2 ml^{-1}. Abundances of all assemblages were somewhat higher during the spring cruise. Bacterial and cyanobacterial abundances reached 10^6 and 5×10^4 ml^{-1}, respectively, while PNAN and HNAN occasionally occurred at abundances $\geq 10^3$ ml^{-1}. There were no clearly discernible patterns in the temporal changes among these populations during either cruise.

Order-of-magnitude differences in the abundances of these four assemblages were greatly reduced when cell number and biovolume were converted to particulate organic carbon (POC) (Fig. 2). Bacteria constituted the largest percentage of living microbial biomass in most samples. Bacterial POC was approximately 2–10 μg C liter^{-1} during the summer and 3–18 μg C liter^{-1} during the spring. PNAN carbon was high-

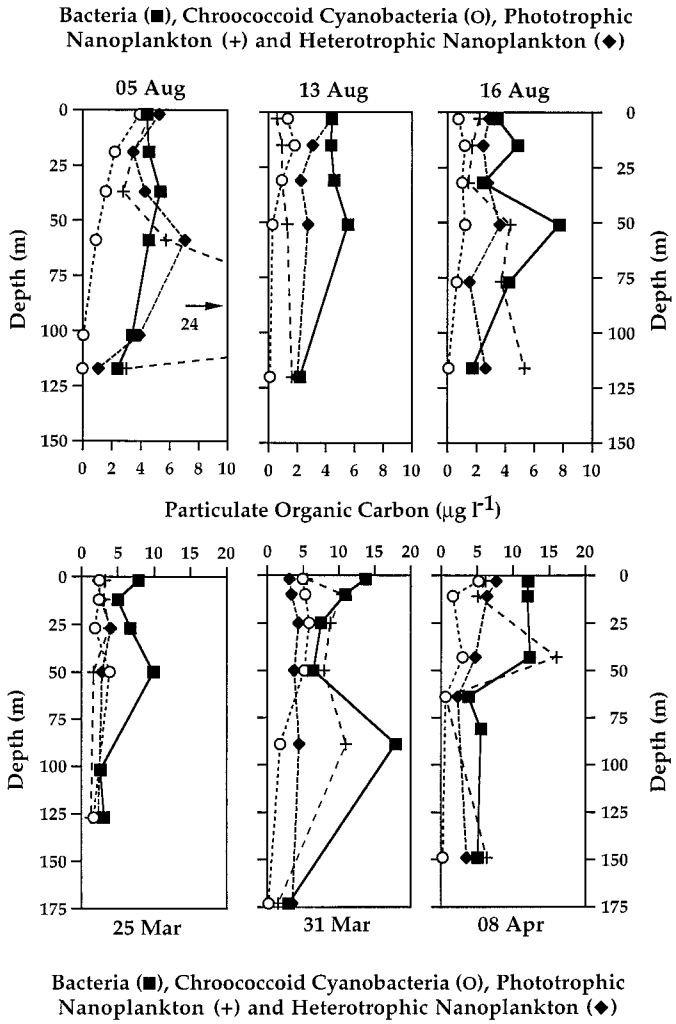

Fig. 2. Three representative vertical profiles of the contribution of phototrophic and heterotrophic picoplankton and nanoplankton to POC concentration during August 1989 and March–April 1990 in the Sargasso Sea south of Bermuda.

ly variable due to the large contribution of PNAN to the DCM in several of the vertical profiles (ranges ≈ 0.5–24 μg C liter^{-1} during August and 0.5–18 μg C liter^{-1} during March–April). HNAN carbon was more stable than PNAN during both cruises (ranges ≈ 1–7 μg C liter^{-1} and 2–14 μg C liter^{-1} for the summer and spring cruises, respectively) and exceeded the contribution of the other assemblages in only three samples. Chroococcoid cyanobacteria contributed an amount of carbon biomass similar to PNAN for most samples collected above 50 m, but the cyanobacterial contribution decreased in the samples from the deep euphotic zone. On average, the POC of the microbial assemblages was approximately twice as great during the spring cruise compared to the previous summer.

Depth integration (0–100 m) of the POC contributed by bacteria, chroococcoid cyanobacteria, PNAN, and HNAN revealed that biomass values of these assemblages differed by not more than one order of magnitude in this ecosystem during either season or on any sampling date (Fig. 3). Overall averages were approximately within a factor of two. Averaged over the seven sampling dates for both cruises, depth-integrated carbon biomasses were 450, 190, 480, and 410 mg C m^{-2} for the bacteria, cyanobacteria, PNAN, and HNAN, respectively. Temporal variability within a season was most dramatic for the nanoplankton. Depth-integrated PNAN and HNAN biomass, for example, varied approximately eightfold and twofold, respectively, during the August cruise. Bacterial and cyanobacterial carbon values were very stable throughout this same period. Pico- and nanoplankton assemblages varied temporally by factors of two to four during the spring cruise. Total depth-integrated pico- and nanoplankton carbon constituted 15–32% of the total POC during August and 33–60% of POC during March–April (Fig. 3).

Bacterial growth and grazing—Bacterial growth rates during the August cruise of this study averaged 3.3 times the growth rates observed during the March–April cruise (cruise averages = 0.56 d^{-1} vs. 0.17 d^{-1}; Fig. 4). This difference in growth rate, however, was balanced by a threefold greater bacterial biomass in these same samples during the March–April cruise. Thus, average bacterial production was comparable for the two cruises. There was no clear relationship between sample depth and bacterial growth rate.

Grazing rates on picoplankton during this study ranged from undetectable to 0.61 d^{-1} (Fig. 4). The latter value corresponds to a decrease in FLP abundance of approximately 46%. Counting error determined the lower limit of detection of the method (≈5–10%). Average rates for the two cruises were higher for the August cruise but not to the extent that bacterial growth in August exceeded March–April growth rates (grazing rates of 0.31 d^{-1} for August vs. 0.22 d^{-1} for March–April). Applying these average picoplankton grazing rates to the bacterial community implies that grazing pressure approximately balanced bacterial growth in samples examined during the spring (growth = 0.17 d^{-1} and grazing = 0.22 d^{-1}) but that bacterial growth rate was nearly twice the grazing rate in samples collected during August (growth = 0.56 d^{-1} and grazing = 0.32 d^{-1}).

As an independent test of the validity of the methods used to measure bacterial growth and grazing in these samples, we calculated net bacterial growth rates based on changes in bacterial abundance during the FLP disappearance experiments. We compared these bacterial growth rates with growth rates obtained from the difference between bacterial growth rate measurements (based on TdR incorporation) and picoplankton grazing rates (Fig. 5). Most of the net bacterial growth rate values calculated by this latter approach (TdR growth rate minus grazing rate) varied between −0.2 and +0.2 d^{-1}. That is, based on measurements of bacterial growth and grazing, these processes were in relative balance for most of the samples examined in the study. When plotted against net bacterial growth measured directly by changes in cell number, however, relatively few samples showed equality between net rates of growth based on changes in bacterial abundance with net growth rates determined by the difference between the TdR growth rates and FLP grazing rates (dotted line indicates the line of 1 : 1 correspondence in Fig. 5). TdR growth rates for these samples were obtained using

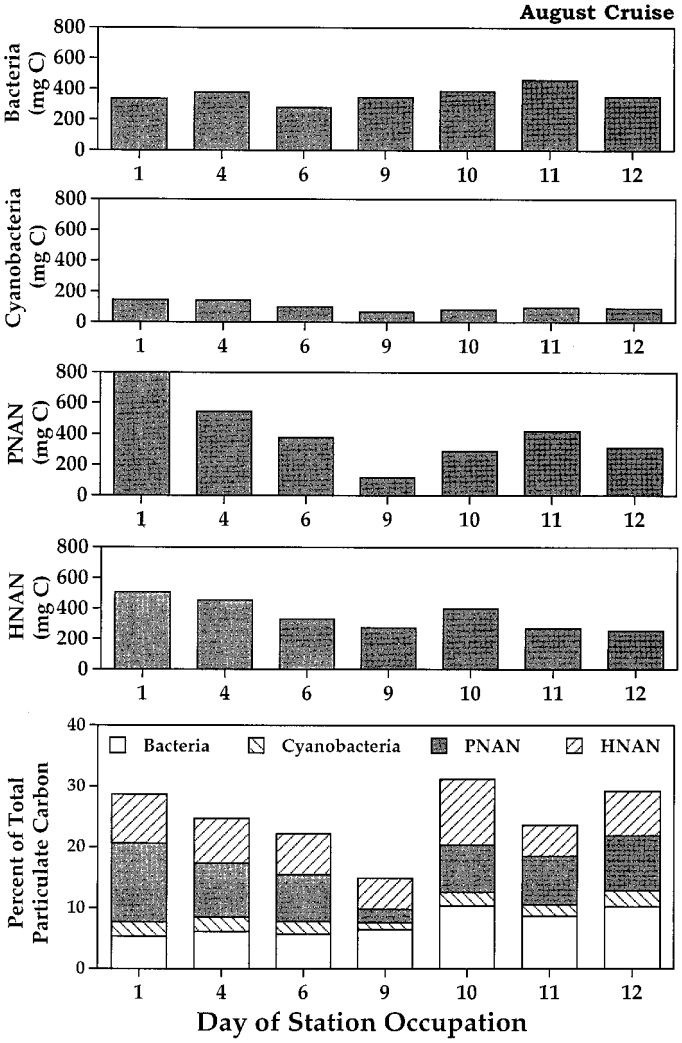

Fig. 3. Depth-integrated (0–100 m) carbon biomass (per square meter) of phototrophic and heterotrophic picoplankton and nanoplankton during August and March–April in the Sargasso Sea south of Bermuda. The contribution to POC (as a percentage of total POC) is given in the last panel for each cruise.

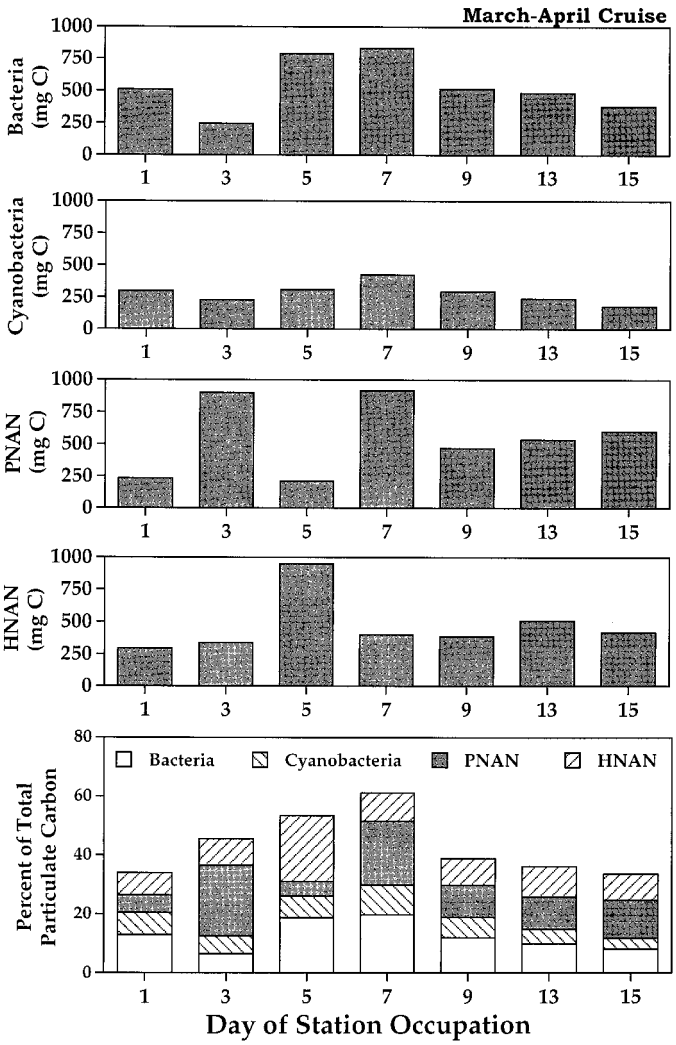

Fig 3. Continued.

4×10^{18} cells mol^{-1} as a conversion factor. Interestingly, the shallow samples from both cruises tended to cluster more or less equally on either side of the line of 1 : 1 correspondence (with one exception). Most of the deep samples displayed growth rates based on changes in abundance that exceeded growth rates based on TdR growth rate minus FLP grazing rate.

Size distribution of picoplankton consumers—Most of the grazing activity on picoplankton in this study could be attributed to microorganisms <20 μm in size (Table 1). Comparison of removal rates for individual sampling dates and depths revealed considerable variability between unfiltered seawater and seawater filtered through 20-μm screening, but the overall averages for the two treatments indicated that grazing rates were not greatly affected by the removal of plankton >20 μm in size. Grazing rates in 20-μm filtered seawater during August averaged 92 and 97% of the grazing rates in the unfiltered seawater for the shallow and deep samples, respectively. Respective averages for the March–April samples were 117 and 87%. The overall average (98%) indicates that prefiltration had little effect on picoplankton grazing. Grazing rates in the <20-μm filtrate that exceeded rates in corresponding unfiltered samples may have resulted from the release of nanoplankton from microplanktonic (>20 μm) predation.

A size-fractionation experiment was performed during the March–April cruise to more accurately define the size distribution of the major picoplankton consumers on that date. Seawater was passed through filters of different pore sizes, and the disappearance of FLP in each filtrate was determined during 24-h incubations (Fig. 6). The results of these experiments indicate that the primary consumers of picoplankton in these waters were exceedingly small. Removal rates of FLP were virtually unchanged for water samples passed through filters of 2-μm pore size or greater. Measurable grazing activity was eliminated, however, by filtration through a

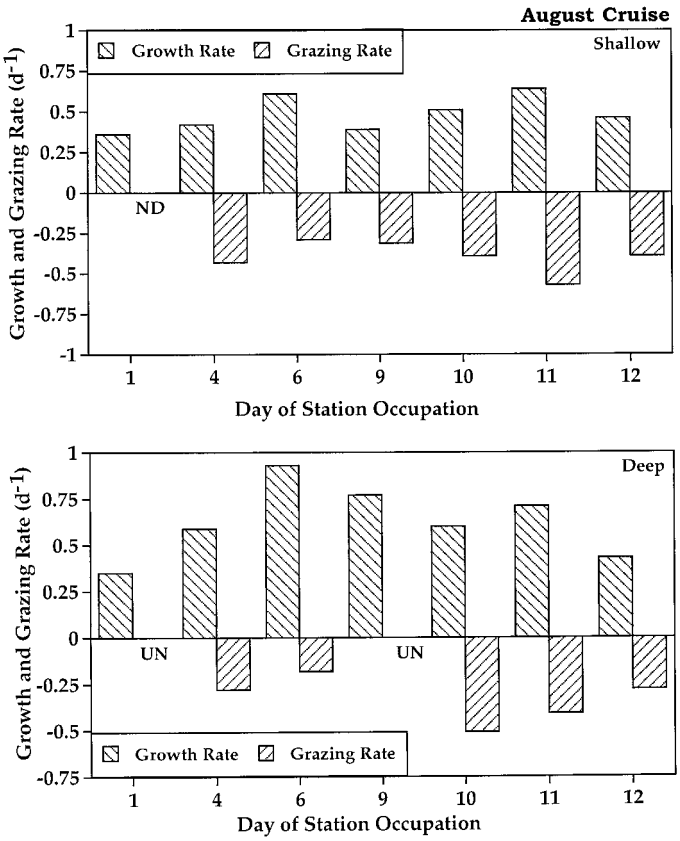

Fig. 4. Bacterial growth rates and picoplankton grazing rates on seven sampling days during August and March–April in the Sargasso Sea south of Bermuda. ND indicates that grazing rates were not detectable during the incubation period. UN indicates samples where grazing experiments were not performed.

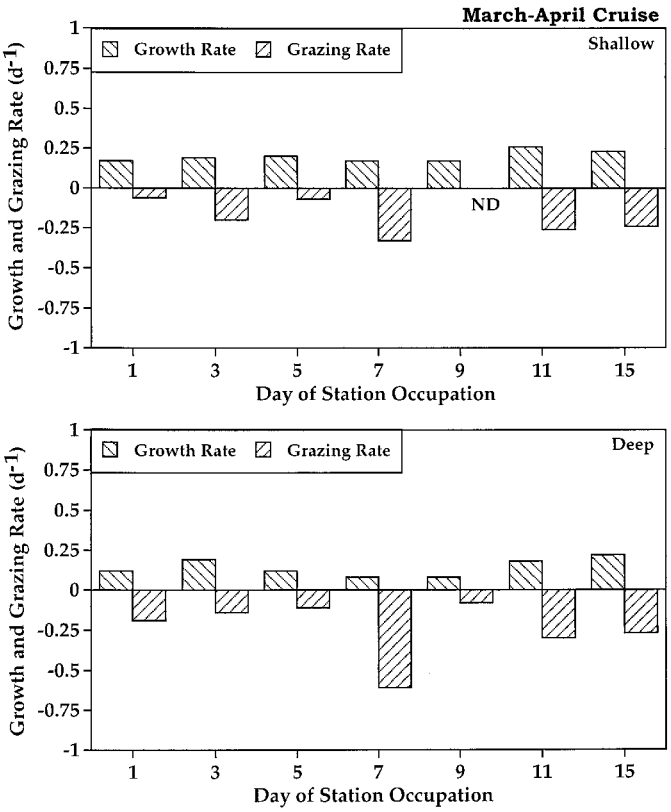

Fig. 4. Continued.

1-μm filter. FLP abundance in the <1-μm fraction did not decrease during the incubation period.

The minute size of the picoplankton consumers at the study site was confirmed by size measurements of HNAN (Fig. 7). Representative biovolume distributions of HNAN indicate that most cells were <5 μm in diameter (biovolume $\leq$ 40 μm^3). This situation was particularly true for the March–April cruise, where >50% of the HNAN were approximately 2–3 μm in diameter. Biovolume estimates were performed on preserved samples, and some shrinkage probably occurred because of preservation with glutaraldehyde (Choi and Stoecker 1989). Thus, cell volumes in Fig. 7 may underestimate the size of live nanoplankton. It is likely, however, that some of these cells passed through the 2-μm filters during the size-fractionation experiment (Fig. 6).

HNAN growth rates—Ingestion rates of HNAN in this study were estimated using the rate of disappearance of FLP as a measure of picoplanktonic grazing. Grazing rates and the abundances of bacteria (including undetected prochlo-

Table 1. Rates of disappearance of fluorescently labeled prey in seawater prefiltered through a 20-μm screen, expressed as a percentage of the rates of disappearance in unfiltered seawater (% unfilt.). ND indicates rates were not determined because grazing rates were not detectable. Grazing experiments were not performed on samples marked with a dash.

Date	Depth (m)	% unfilt.	Depth (m)	% unfilt.	Date	Depth (m)	% unfilt.	Depth (m)	% unfilt.
5 Aug	1	ND	100	—	25 Mar	10	131	51	40
8 Aug	1	62	100	93	27 Mar	10	199	60	64
10 Aug	1	87	100	117	29 Mar	10	192	63	26
13 Aug	1	108	120	—	31 Mar	10	82	90	33
14 Aug	1	59	130	25	2 Apr	10	ND	105	212
15 Aug	1	120	111	106	6 Apr	10	19	52	—
16 Aug	1	114	120	145	8 Apr	10	80	44	149
Average		92		97	Average		117		87

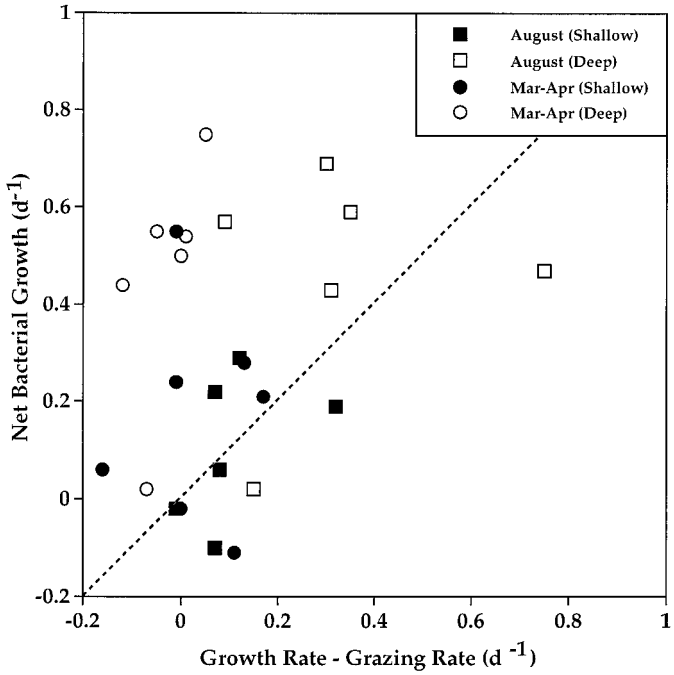

Fig. 5. Comparison of net bacterial growth (based on changes in bacterial abundances during the incubation period) with ''net'' growth rates derived from growth and grazing measurements. The dotted line is the line of 1:1 correspondence between the two growth rate estimates.

rophytes) and chroococcoid cyanobacteria were used to calculate ingestion of the HNAN assemblages. It was assumed that all picoplankton grazing was attributable to HNAN, but these rates do not take into account PNAN or HNAN consumption by HNAN. Ingestion rates were converted to carbon consumed using the conversion factors reported in ''Materials and methods.'' Doubling times for the HNAN assemblages (based on cellular carbon) were then calculated by assuming a 40% carbon gross growth efficiency for the HNAN (*see* summary in Caron et al. 1990).

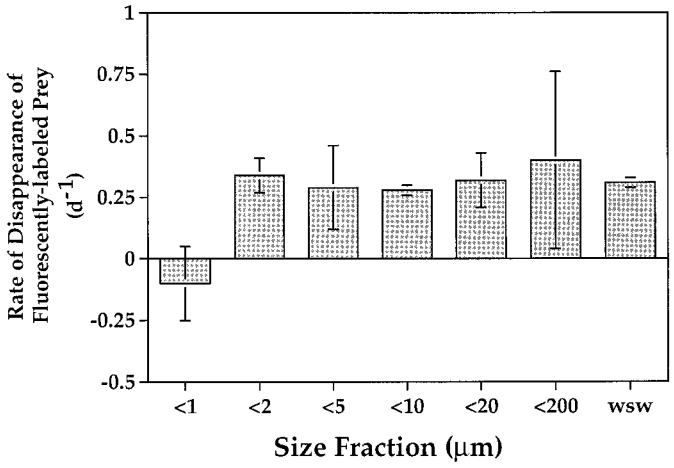

Fig. 6. Rates of disappearance of fluorescently labeled prey in filtrates and unfractionated seawater collected at 61 m on 7 April. Error bars indicate ±1 SD.

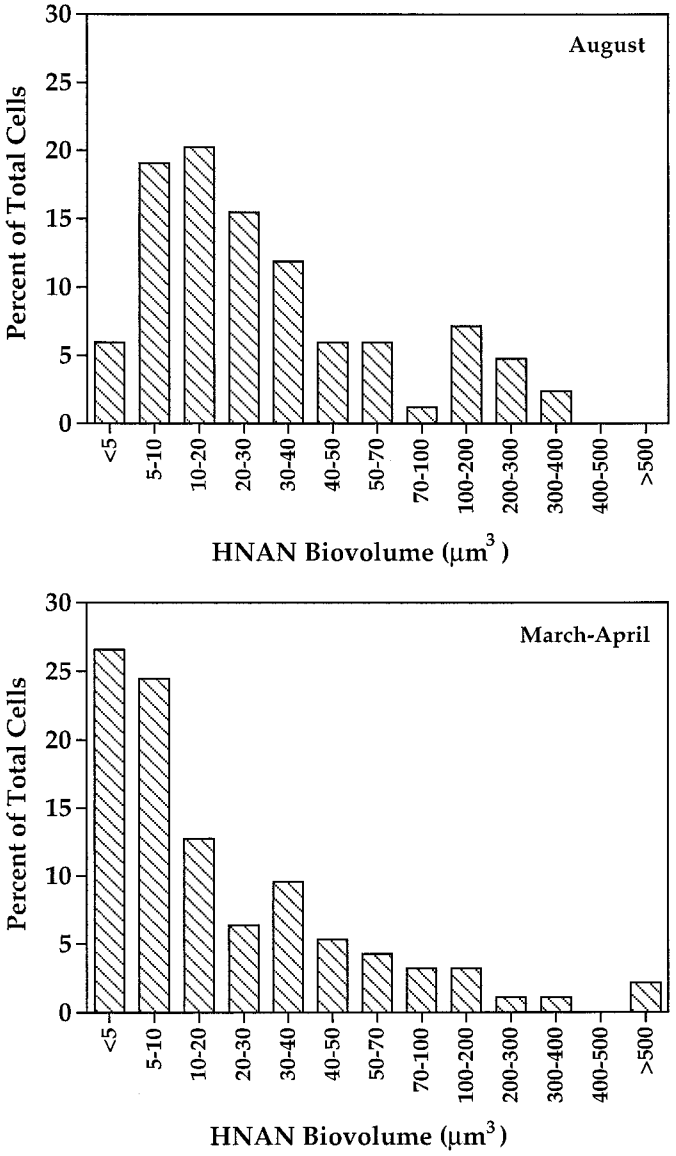

Fig. 7. Biovolumes of HNAN for representative samples from the August and March–April cruises.

Based on these calculations, doubling times for the HNAN assemblages ranged from 0.9 to 18.3 d for those samples in which grazing rates could be measured (Table 2). There were no clear trends in temporal distribution of doubling times, although the two fastest times were observed for the shallow and deep samples collected on 31 March (the date of an abrupt deepening of the mixed layer). In general, doubling times were shorter for the shallow samples relative to the deep samples during the August cruise, but they were consistently shorter for the deep samples relative to the shallow samples during the March–April cruise.

Discussion

Picoplankton/nanoplankton abundance and biomass—Abundances of pico- and nanoplankton assemblages during

Table 2. Estimated doubling times of the HNAN assemblage. Rates of disappearance of fluorescently labeled prey were used to estimate the rates of ingestion of picoplankton. Picoplankton ingestion rates were converted to rates of carbon incorporation by the HNAN based on conversion factors from bacterial biomass (15 fg C cell^{-1}), and cyanobacterial biomass (200 fg C cell^{-1}), assuming a gross growth efficiency for the HNAN of 40%. ND indicates rates were not determined because grazing rates were not detectable. Grazing experiments were not performed on samples marked with a dash.

Date	Depth (m)	Doubling time (d)	Depth (m)	Doubling time (d)	Date	Depth (m)	Doubling time (d)	Depth (m)	Doubling time (d)
5 Aug	1	ND	100	—	25 Mar	10	15.8	51	2.8
8 Aug	1	3.1	100	6.2	27 Mar	10	8.9	60	2.6
10 Aug	1	3.7	100	10.8	29 Mar	10	18.3	63	3.9
13 Aug	1	5.7	120	—	31 Mar	10	1.6	90	0.9
14 Aug	1	4.6	130	6.4	2 Apr	10	ND	105	6.6
15 Aug	1	2.8	111	3.2	6 Apr	10	9.8	52	3.6
16 Aug	1	2.8	120	4.0	8 Apr	10	4.9	44	2.9

the summer and spring cruises in this study (Figs. 1, 2) were indicative of the oligotrophic conditions that characterize this oceanic ecosystem (Michaels et al. 1994). Our results are consistent with observations at similar latitudes in the Sargasso Sea by other investigators (Davis et al. 1985; Fuhrman et al. 1989; Olson et al. 1990*a*,*b;* Buck et al. 1996; Carlson et al. 1996) and with patterns of phytoplankton and zooplankton abundance and biomass at this study site (Caron et al. 1995; Roman et al. 1995).

The North Atlantic Bloom Experiment (NABE) of the Joint Global Ocean Flux Study was conducted during 1989 and 1990 to the northeast of our study area (NABE site was 47–48°N, 18–20°W). Hydrographic conditions at that study site were quite different from conditions at our study site, and these differences were reflected in notably higher abundances and biomasses of nanoplankton during the NABE study. Ranges of microbial biomass (protozoa plus nanoplanktonic algae) in that study were 100–150 μg C liter^{-1} during 1989 and 150–550 μg C liter^{-1} during 1990 (Stoecker et al. 1994). Samples with the highest biomass were dominated by phototrophs. In contrast, phototrophs in our study generally did not exceed 20 μg C liter^{-1} during March–April (Fig. 2). Maximal values during August were approximately one-half the spring values.

The latitudinal differences in microbial biomass reported above are clearly evident in a recent survey along a north-south transect in the eastern North Atlantic (Buck et al. 1996). The latter study noted lower phytoplankton and protozoan biomass during summer in the subtropical region of the North Atlantic (25–45°N latitude) relative to biomass in tropical and temperate-subarctic regions. Average total phytoplankton and heterotroph biomasses from samples collected by Buck et al. in the subtropical region were 12.7 and 17.2 μg C liter^{-1}. Given that these latter values include microplanktonic algae and heterotrophs, their observations are concordant with ours in the present study during August (Fig. 2).

One caveat concerning the accuracy of our abundance and biomass data involves the presence of prochlorophytes in our samples. Flow cytometric analyses were not available during our study, and epifluorescence microscopy is not effective for obtaining accurate counts of these weakly fluorescing cells. Therefore, we did not count prochlorophytes. Their contribution to picoplankton biomass in this study, however, can be estimated from reported abundances of this phototrophic assemblage (Olson et al. 1990*a;* Goericke and Welschmeyer 1993). Based on prochlorophyte abundances and vertical distributions in those studies, the contribution of prochlorophytes may have been as much as 20–30% of the bacterial counts in the deep euphotic zone samples during August, but probably not more than 10% in the upper euphotic zone during August or in any of the samples during March–April.

Inclusion of prochlorophytes in the bacterial counts would have three consequences on our assessment of the distribution of biomass among the various microbial assemblages. First, the contribution of bacteria would be reduced because a portion of the cells counted as bacteria were actually prochlorophytes. Second, the contribution of picoplankton to phytoplankton biomass would be increased. Third, the total amount of microbial biomass would be increased slightly because prochlorophytes have higher carbon per cell content than the value we employed for bacteria in this study (Campbell et al. 1983; Olson et al. 1990*a*).

This caveat notwithstanding, depth-integrated phytoplankton biomass was strongly dominated by pico- and small nanoplankton species during the present study. Averaged over seven sampling dates for each of the two cruises, 74% (August) and 76% (March–April) of the chlorophyll passed through a 2-μm filter (Malone et al. 1992; Caron et al. 1995). Moreover, our determinations indicate that bacterial biomass constituted a major component of the standing stock of living microbial carbon in this plankton community during both study periods. If the contribution of prochlorophytes is included in our analysis, the overall contribution of picoplankton to total living carbon increases in this ecosystem. Taken together, these observations imply an important role for picoplankton consumers in the processing of primary and bacterial productivity in this oceanic ecosystem.

Comparison of bacterial growth and grazing—We compared our measurements of bacterial growth rate (from TdR uptake) and grazing rate (from FLP disappearance) to an independent measure of net bacterial growth rate (from

changes in bacterial abundance) to test the validity of these measurements. We hypothesized that net bacterial growth determined by the difference between TdR growth rates and FLP grazing rates should reconcile with net bacterial growth rates determined from changes in bacterial abundance during the 24-h incubations if all of the measurements were accurate and representative. Conversely, we would not expect close agreement if one or more of the methods either over- or underestimated rates of growth or grazing. Furthermore, systematic deviations from 1 : 1 correspondence might implicate specific aspects of the experimental protocols that could be addressed in future studies.

The agreement between net bacterial growth rate determined by changes in bacterial abundances in incubated samples and net bacterial growth rate determined from measurements of growth rate minus grazing rate was remarkable in this study, despite the potential artifacts inherent in the three methods (Fig. 5). Many of the samples had changes in bacterial abundance (net bacterial growth rates) that were approximated by TdR growth rates minus FLP grazing rates, as shown by their distribution close to the line of 1 : 1 correspondence in Fig. 5. In particular, most samples from the shallow euphotic zone during both cruises produced similar net bacterial growth rates by the two approaches. These results appear to indicate a relatively good concordance between the methods.

Several of the samples from the deep euphotic zone, however, had net bacterial growth rates determined from changes in abundance that exceeded net growth calculated from TdR growth minus FLP grazing (open symbols in Fig. 5). The presence of significant numbers of prochlorophytes in the deep samples may explain some of this discrepancy. Their presence would have resulted in overestimation of the initial abundances of bacteria, but they would not have affected measurements of bacterial production as measured by the rate of incorporation of TdR. Bacterial growth rates in our study were determined by converting TdR incorporation rates to the number of bacteria produced (using the factor 4×10^{18} cells mol^{-1}) and then comparing those values to the number of bacteria initially present in the samples. Overestimation of initial bacterial abundances (by including prochlorophytes) could have resulted in underestimation of bacterial growth rates by the TdR method up to a factor of two. One might expect a greater effect on TdR growth rate calculations for the deep samples in this study because prochlorophytes can constitute 20–30% in the deep euphotic zone of the Sargasso Sea (Olson et al. 1990*a*). Therefore, this explanation is consistent with our results.

Nevertheless, agreement (or disagreement) between the two approaches for estimating net bacterial growth rates in this study was necessarily dependent on the specific assumptions that we employed to calculate these rates (*see Materials and methods*). An examination of these assumptions provides some indication of the degree to which these measures of net bacterial growth might differ.

Net bacterial growth in 24-h incubations—Determination of net bacterial growth rate from changes in bacterial abundance are methodologically straightforward, but these estimates could have been affected by our use of an exponential model to describe growth during the 24-h incubations. Our approach would have resulted in an overestimation of net bacterial growth if growth was more closely approximated by a linear rather than an exponential model. Net bacterial growth rates recalculated using a linear model, however, differed from the exponential model by only a few percent on average (maximum difference of 6%). These variations are modest relative to the scatter of the data points in Fig. 5.

Changes in bacterial growth rates during the 24-h incubations, however, are a potentially important consideration for measurements of growth rates estimated by changes in cell abundances. Stimulation of bacterial growth rates could have occurred in these experiments due to a ''wall effect'' and/or enhanced release of organic material by confined plankton. An increase in bacterial growth rates during the incubations would not have been detected by the TdR method because it was performed only at the beginning of our experiments. Interestingly, many of the net bacterial growth rates determined by changes in bacterial abundance exceeded net growth rates determined from TdR growth minus FLP disappearance (values above the line in Fig. 5). This result may reflect, in part, stimulation of bacterial growth during the 24-h incubations.

We did not perform TdR uptake at the end of the 24-h incubations in the present study. However, these measurements were performed during experiments conducted in the Sargasso Sea during July and August of 1991 (Caron unpubl. data). Rates of TdR uptake after 24 h of containment ranged from −42 to +144% of the TdR uptake rate at the beginning of the incubation (overall average = +40% for four experiments). The effect of containment was highly variable among these latter experiments, although the average effect supported the view that long incubations may stimulate bacterial growth.

Measuring bacterial grazing in the open ocean—Community-level rates of bacterivory rarely have been measured in highly oligotrophic oceanic ecosystems (Hagström et al. 1988; Weisse and Scheffel-Möser 1991; Thingstad et al. 1996). We chose the FLP approach because we believed that, relative to the available methods, it would provide the most accurate assessment of removal of bacteria (and other picoplankton) in this oligotrophic environment. The FLP method allows the use of whole, unfractionated seawater and thus avoids resource limitation of the bacterial and picophytoplankton that may occur in size-fractionated or diluted water samples or samples to which metabolic inhibitors have been added. We reasoned that resource limitation in these perturbation experiments might be particularly important in oligotrophic environments. Also, evidence suggests that fluorescently labeled prey are digested similarly to natural prey items (Sherr et al. 1988; Dolan and Simek 1997) and thus should provide a reasonable index of protozoan grazing activity.

Nevertheless, several factors could have affected the accuracy of our grazing measurements. For example, feeding selectivity by protistan grazers for or against FLP based on size, chemical composition, or encounter rate has been reported. Feeding selectivity based on chemical composition or ''taste'' can result in rejection of FLP by some protozoa.

Heat-killing and labeling apparently reduce the acceptability of microbial prey to some protozoa (Landry et al. 1991). Likewise, our heat-killed cells are not motile, and nonmotile prey may have somewhat lower encounter rates with predators (and thus disappearance rates) than motile prey (Monger and Landry 1992). In contrast, selection for FLP based on size (the FLP were larger than most bacteria in this environment) could have resulted in higher rates of disappearance of our tracer than natural bacteria (Monger and Landry 1991, 1992; Sherr et al. 1992). The effects of these potential artifacts on overall grazing rates, therefore, are not easily predicted.

Long incubation periods (24 h) also could have affected protozoan feeding activities during our experiments (e.g., by stimulating bacterial growth; *see previous section*). However, bottle incubations and their potential artifacts are presently unavoidable in all measurements of bacterivory. Even methods that do not require incubation per se must be calibrated using methods that rely on incubation (e.g., González et al. 1993). Given the potential problems described above, it is clear why there are few reports of bacterivory in oligotrophic marine ecosystems and why a comparison of rates of bacterivory with estimates of bacterial growth rates and net population growth might be beneficial.

Underestimation of bacterial mortality by the FLP method would occur if factors other than microbial grazing (e.g., viral lysis) were a significant source of bacterial mortality. Nongrazer mortality would manifest itself in our data as changes in bacterial growth rates (based on changes in abundance) that were less than growth rates predicted from TdR growth rate minus FLP grazing rate. That is, data points in Fig. 5 would have occurred below the line of 1 : 1 correspondence if all other measurements were accurate. This situation was clearly not the case. Few samples had changes in bacterial abundance that resulted in growth rates less than TdR growth rates minus FLP grazing rates (i.e., below the dotted line on Fig. 5). Protozoan grazing appeared to dominate bacterial mortality in our experiments. It is possible that nongrazer mortality was significant and that the FLP method fortuitously overestimated grazing by an amount equivalent to the nongrazer mortality, but compensatory errors seem unlikely.

Several samples apparently had higher net bacterial growth rates based on abundance changes than net growth rates determined from TdR growth rate and FLP grazing rates (i.e., values above the line of 1 : 1 correspondence in Fig. 5). If we assume that the thymidine method accurately measured bacterial growth rate (*see next section*) and that bacterial growth rates did not change significantly during the incubations (*see previous section*), then these results imply that grazing rates may have been overestimated in some of the samples collected from the deep euphotic zone. This situation could have occurred because the FLP are relatively large picoplankton, and they may have had rates of consumption by grazers that were greater than consumption rates for bacteria. This explanation, however, probably would not account for the fact that samples from the shallow euphotic zone were distributed reasonably close to the line of 1 : 1 correspondence in Fig. 5, while most samples from the deep euphotic zone occurred above the line.

Another possible cause for overestimating rates of bacterivory would be the use an exponential model for FLP disappearance. We counted the number of labeled and unlabeled bacteria at the beginning and end of the incubations in our experiments, so we were able to examine other models of FLP removal (Salat and Marrasé 1994). Recalculation of our grazing rates using other models, however, had only modest effects (<10%) on our rates relative to other potential sources of error.

Measuring bacterial growth using TdR—The use of TdR to measure bacterial production is a common albeit controversial technique. Major concerns with the TdR method include the potential for nonspecific incorporation of label into bacterial cells, the inability of some bacteria to take up thymidine, dilution of added thymidine by endogenous sources, and the proper conversion factors for relating isotope uptake to bacterial carbon and cell production (Bell 1993). In addition to these issues, our estimates of bacterial growth rates using this method presumably were influenced by diel fluctuations in the rate of TdR uptake, the choice of an exponential growth model for calculating growth rates from the production data, and the inclusion of prochlorophytes in the bacterial abundance estimates.

As previously noted, the effect of the inclusion of prochlorophytes in the bacterial counts presumably was most important for samples from the deep euphotic zone, where prochlorophytes may have constituted 20–30% of those counts. Overestimation of the initial bacterial abundances in those samples could have resulted in twofold underestimations of net bacterial growth rates. The overall effect of this artifact would be closer agreement between the two methods used to estimate net bacterial growth rate (i.e., most open symbols in Fig. 5 would move closer to the line of 1 : 1 correspondence). In contrast, the use of a linear growth model to calculate growth rates rather than an exponential model would cause slightly more disparity between the two measures of net bacterial growth rates because TdR growth rates would decrease approximately 2–4%. We did not attempt to examine diel fluctuations in TdR uptake during this study. However, a significant effect has been noted in coastal ecosystems (Riemann et al. 1984) and might be expected in oceanic plankton communities.

By far the most significant methodological issue involving the calculation of bacterial growth rates from the uptake of TdR was our use of a thymidine conversion factor of 4×10^{18} cells mol^{-1}. That value was empirically determined in a North Atlantic warm core ring study using the same technique applied in this investigation (Ducklow and Hill 1985*b*). It is intermediate among published conversion factors (Ducklow and Carlson 1992), but these values vary considerably (<1–60×10^{18} cells mol^{-1}). Subsequent work in the Sargasso Sea near our study site obtained an average conversion factor of 1.6×10^{18}, less than half the value employed in our study (Carlson and Ducklow 1996; Carlson et al. 1996). Methodology in the latter studies differed slightly from ours, and it is difficult to determine the applicability of their conversion factor to our data.

The use of 1.6×10^{18} cells mol^{-1} as a conversion factor, if appropriate, would greatly reduce our estimates of bacte-

rial growth rates by TdR uptake. Our estimates of net bacterial growth rates determined from TdR growth minus FLP grazing would be reduced accordingly, greatly exacerbating differences between net bacterial growth rates calculated by changes in bacterial abundances (i.e., all data points would move up in Fig. 5). For example, the average net bacterial growth rate based on changes in bacterial abundance in 24-h incubations was 0.28 d^{-1} during the August cruise. The net bacterial growth rate determined from TdR growth minus FLP disappearance for the same samples was 0.22 d^{-1} using a conversion factor of 4×10^{18} cells mol^{-1}, but this value was -0.10 d^{-1} using 1.6×10^{18} cells mol^{-1}. This exercise demonstrates the sensitivity of production/consumption calculations to conversion factors inherent in these methods. This issue, rather than specific experimental details or the growth/grazing model employed, may be the greatest potential source of error in our comparison of net bacterial growth rates. Unfortunately, these conversion factors are difficult to ascertain with certainty under field conditions.

HNAN growth rates and the microbial food web in the Sargasso Sea—Determination of the grazing mortality of the bacterial assemblage, combined with net changes in bacterial abundance, provides an independent means of assessing bacterial growth rates in nature. If rates of bacterial growth and mortality are accurate, then they should accurately predict net changes in the abundance of this assemblage.

Bacterial grazing mortality measurements also provide a means of assessing the prey biomass consumed by bacterial predators. Growth rates of HNAN in this study were calculated from measured rates of the disappearance of FLP (as an estimate of the amount of picoplankton prey consumed), the standing stocks of picoplankton (bacteria and chroococcoid cyanobacteria), and the standing stock of HNAN. Clearly, these rates are crude estimates of the growth rates of this assemblage because there were several simplifying assumptions necessary for making these calculations. For example, we assumed that all picoplankton were consumed by HNAN and that picoplankton were the sole source of nutrition for the HNAN. While the former assumption seems reasonable given the results of our size-fractionation experiments (Table 1; Fig. 6), the latter assumption was not examined experimentally and probably was in error to some extent. The preponderance of very small nanoplankton in this study (Fig. 7) implies that some nanoplankton probably were consumed by larger nanoplankton. Also, we did not correct our estimates of HNAN growth rates for the contribution of prochlorophytes in these samples.

Given this situation, it is perhaps not surprising that growth rates of HNAN estimated in this study (Table 2) were well below maximal growth rates that have been observed for small protozoa at comparable temperatures (Caron et al. 1986). These observations imply that picoplankton consumers are strongly food-limited in the plankton. Our results are consistent with reports of HNAN growth rates estimated in other studies (Sherr et al. 1984; Kuuppo-Leinikki 1990; Solic and Krstulovic 1994) and with the notion that bacterivorous protozoa in many pelagic environments are food-limited (Sanders et al. 1992; Weisse 1997). However, more verification using new experimental approaches will be required to firmly establish these rates.

Our results indicate that the food web present at the depths and times sampled in this ecosystem was strongly dominated by minute producers and consumers. Much of the phytoplankton biomass occurred in the picoplankton size class, and heterotrophic bacteria also contributed significantly to living carbon in this size class (Caron et al. 1995). Very small (<3 μm) protists dominated the bacterivore assemblage on at least two dates (Fig. 7). This latter situation apparently is a common feature of other oligotrophic ecosystems as well (Wikner and Hagström 1988; Zohary and Robarts 1992). The structure of the Sargasso Sea community in this study was such that more than one trophic step probably was required before most of the energy in these small primary and secondary producers became available as food for metazoan zooplankton. This scenario is consistent with one in which organic carbon and nutrients are remineralized and recycled efficiently within a complex microbial food web (Goldman and Caron 1985). Therefore, we conclude based on our analysis of food web structure that little energy was recovered from the microbial loop and passed on as food to metazoan zooplankton of this Sargasso Sea community.

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