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C. A. Carlson

H. W. Ducklow
Virginia Institute of Marine Science

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Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea

Craig A. Carlson[1,*], Hugh W. Ducklow[2]

[1] Horn Point Environmental Laboratory, University of Maryland, PO Box 775, Cambridge, Maryland 21613, USA
[2] The College of William and Mary, Virginia Institute of Marine Sciences, PO Box 1346, Gloucester Point, Virginia 23062-1346, USA

ABSTRACT: Lability of the bulk dissolved organic carbon (DOC) pool and the amount available to bacterioplankton on short time scales (hours to days) were examined in oligotrophic Sargasso Sea water (near Bermuda). We examined bacterial growth and DOC utilization using seawater culture methodology in combination with measurements of bacterial abundance, cell volume, and DOC. Bulk DOC concentrations were determined by high temperature combustion (HTC) analysis, which proved to be a sensitive method for detecting small changes in natural concentration of DOC. Measurable bacterial growth and DOC utilization only occurred in unamended cultures when initial DOC concentrations were greater than observed in the mixed layer at the Bermuda Atlantic Time Series station. In unamended cultures exhibiting growth, approximately 6 to 7% of the bulk pool was available and considered a labile component. This material was utilized with an average bacterial growth efficiency (BGE) of 14 ± 6%. Nutrient enrichment experiments were also conducted with NH_4, PO_4, glucose, dissolved free amino acid (DFAA) and algal lysate additions. In all experiments bacterial growth rates, bacterial carbon production, and BGE increased with the addition of organic carbon supplements. There were no enhancements of bacterial production or DOC utilization above the control when inorganic nutrients were added, indicating that at the time these experiments were conducted bacterial growth was limited by available carbon.

KEY WORDS: Dissolved organic carbon · Bacterioplankton · Growth efficiency

INTRODUCTION

Dissolved organic carbon (DOC) represents the largest pool of reduced carbon in the ocean. Recent reports have demonstrated temporal and spatial variability of this bulk pool in the surface ocean (Copin-Montégut & Avril 1993, Carlson et al. 1994, Peltzer & Hayward in press), indicating that significant portions of this bulk pool are dynamic and consist of material of varying lability. While a large portion of the bulk DOC pool in the surface ocean consists of refractory material which turns over on the time scale of millennia, the remainder of the surface DOC pool consists of semi-labile material which turns over on the scale of months to years and labile material which turns over on the

scale of hours to days. Separating the refractory pool from the semi-labile and labile pool can be done by observing surface accumulation of DOC above background deep water (>1000 m) and assuming equal distribution of refractory material throughout the water column (see Carlson & Ducklow 1995). But separating the semi-labile and labile pools is more difficult because only about 30% of the bulk DOC pool can be identified chemically (Williams & Druffel 1988); thus, we must depend on biological assays to further partition these pools (Servais et al. 1989).

Bacterioplankton mediate the flux of dissolved organic matter (DOM); thus, factors which regulate bacterial growth can ultimately affect the magnitude of semi-labile and labile carbon accumulation. Several studies have suggested that bacterial growth may be limited by the availability of inorganic nutrients such as N and P (Toolan et al. 1991, Kroer 1993, Zweifel et al. 1993, Cotner et al. 1994), indicating that there is

*Present address: Bermuda Biological Station for Research Inc., Ferry Reach, St. Georges GE01, Bermuda
E-mail: ccarlson@bbsr.edu

a surplus of available labile C but that its uptake is limited by the supply of inorganic nutrients. Other studies suggest that oceanic bacterioplankton are indeed limited by the supply of labile carbon (Kirchman et al. 1990, Kirchman 1994). To better understand the dynamics of the bulk DOC pool of the oligotrophic ocean, we must first understand how much of the bulk pool is available to bacteria and at what efficiency this material is used. Second, we must determine if the availability of DOC and the efficiency of its use are affected by the presence of inorganic nutrients or if labile organic carbon is limiting bacterial growth in oligotrophic marine systems.

Pure culture and seawater culture methodology (Ammerman et al. 1984) have been used to address bacterial growth and C uptake. Previous experiments which investigated labile C uptake and bacterial growth efficiencies (BGE) used ^{14}C-labeled substrates in short-term incubations and yielded BGE ranging from 50 to 95% (Williams 1970, 1981). The shortcoming of this approach is that C uptake and BGE are based on the conversion of a few model compounds instead of a natural complex organic medium; thus, these estimates may be more representative of assimilation efficiency rather than growth efficiency (Bjørnsen & Kuparinen 1991). Indeed, lower BGEs have been determined for bacteria grown on naturally occurring substrates. Several methods are used in which bacterial production is monitored along with the disappearance of substrate. One approach using naturally occurring substrate estimates DOC depletion indirectly by estimating changes in respiration in a series of parallel incubations in gas-tight bottles (Bjørnsen 1986, Hopkinson et al. 1989, Bjørnsen & Kuparinen 1991, Coffin et al. 1993, Biddanda et al. 1994, Hansell et al. 1995). A second approach monitors DOC depletion directly by measuring changes in substrate concentrations (Kirchman et al. 1991, Kroer 1993, Zweifel et al. 1993) from a single large volume incubation chamber, which helps minimize bottle effects. However, little work has been conducted to address BGEs and DOC utilization in oligotrophic marine systems. In the case of the latter approach this is largely due to the difficulty in achieving high precision in the DOC analysis. Recent work with the high temperature combustion (HTC) method has resulted in improved accuracy and precision in the determination of DOC (Benner & Strom 1993, Peltzer & Brewer 1993, Sharp et al. 1993, 1994) and has allowed the detection of small changes in the bulk DOC pool (Carlson et al. 1994, Carlson & Ducklow 1995, Peltzer & Hayward in press).

In the present study, we used seawater cultures (Ammerman et al. 1984) to investigate bacterial growth and DOC utilization in oligotrophic seawater. We directly measured changes in DOC concentrations together with changes in several bacterial parameters including thymidine and leucine incorporation, cell abundance, and cell volume. The objectives were: (1) to test the sensitivity of the HTC method for detecting small changes in the bulk DOC pool; (2) to obtain estimates of available DOC in the bulk pool and the BGE with which it was utilized by natural assemblages of oligotrophic bacterioplankton grown in unamended seawater; and (3) to determine how inorganic and organic nutrient enrichments affected bacterial growth and DOC utilization.

METHODS

Seawater culture preparation. Oligotrophic seawater was collected from several stations between Bermuda and Hydrostation S (32° 10' N, 64° 30' W) in July 1992, October 1992, March 1993, July 1993 and January 1994. All stations were located 3 to 12 miles off Bermuda in water at least 1000 m deep. Water was collected from a depth of approximately 10 m using 12 l Niskin bottles equipped with epoxy coated springs on a CTD (conductivity, temperature, depth probe) rosette or by using GO-FLO bottles on Kevlar line (both bottle types were General Oceanics, Miami, Florida, USA). Upon recovery, seawater was transferred to polycarbonate carboys via non-contaminating Teflon tubing. All carboys, Teflon tubing, and filtration devices were washed in 5% HCl and rinsed with copious amounts of Millipore Q-Water (Milli-Q) prior to each experiment.

All seawater cultures, except the March 1993 experiment, which was a whole water treatment, were prepared by inoculating 0.22 μm filter sterilized seawater with either whole (unfiltered) water, or a 0.8 μm filtered inoculum at a dilution of 0 to 90% (see Table 1). Preparation of the 0.22 μm filtrate began within 2 h of collection. To minimize cell breakage and contamination, all filtrates were generated by gentle gravity filtration though Nuclepore Membra-Fil filters and a 142 mm plastic filtration manifold. The potential organic and inorganic contamination from the membrane filters was minimized by flushing the filters with approximately 1.5 to 2 l of Milli-Q water and approximately 2 l of sample water before filtrate collection. The 10 l polycarbonate collection carboys were rinsed several times with filtrate after the filter had been well flushed. After 20 to 25 l of 0.22 μm filtrate had been generated, the water was distributed to respective carboys (Table 1) and inoculated. This process generally took 6 to 8 h.

Cultures of 5 to 10 l were incubated in polycarbonate carboys in the dark at *in situ* temperatures and swirled frequently. All cultures, except those used in July and

Table 1. Experimental parameters for Sargasso Sea DOC utilization/seawater culture from 1992 to 1994. All cultures were incubated in the dark. October 1992 experiments included 2 culture preparation treatments: a 0.8 μm filtrate culture and a culture in which the 0.8 μm inoculum was diluted with particle-free water. –: not applicable

Expt date	Treatment	Final concentration (nominal)	Inoculum type	% inoculum diluted with particle-free water	Incubation temp. (°C)	Time until end of log phase (h)	Expt duration (h)
Jul 1992	Control	–	0.8 μm	90	23–25	41	180
	NH$_4$	0.25 μM N	0.8 μm	90	23–25	41	180
	DFAA	30 μM C	0.8 μm	90	23–25	31	180
	Glucose	50 μM C	0.8 μm	90	23–25	31	180
Oct 1992	0.8 filtrate	–	0.8 μm	0	23	–	87
	90% diluted	–	0.8 μm	90	23	–	87
Mar 1993	Whole water	–	Whole water	0	19.5	94	216
Jul 1993	Control	–	0.8 μm	80	26	–	180
	Glucose	20 μM C	0.8 μm	80	26	48	180
	DFAA	20 μM C	0.8 μm	80	26	57	180
	Algal lysate	20 μM C	0.8 μm	80	26	57	180
Jan 1994	Control	–	Whole water	70	20	85	111
	NH$_4$	1 μM N	Whole water	70	20	85	111
	PO$_4$	0.1 μM P	Whole water	70	20	85	111
	Glucose	10.5 μM C	Whole water	70	20	52	111

October 1992 experiments, were incubated at *in situ* temperatures. July and October 1992 cultures were incubated at room temperature, which was ±2°C of *in situ* temperatures. Samples were drawn periodically over 4 to 5 d through the carboy spigots.

Enrichment experiments. Final concentrations of organic and inorganic enrichments are reported in Table 1. Since our primary objective was to measure changes in bulk DOC rather than individual compounds, we added organic carbon to seawater media in concentrations of 10 to 50 μM C to ensure that changes in bulk DOC would be measurable. The final concentration of DOC after additions was less than twice the ambient concentration of DOC, however the final concentrations of individual compounds such as glucose and amino acids were much greater than their ambient concentrations. The dissolved free amino acid (DFAA) treatments contained a mixture of leucine, glycine, phenylalanine, arginine, proline, threonine, alanine, histidine, tyrosine, serine, isoleucine, valine, asparagine, lysine, and methionine in equal molar concentrations. The mixture of amino acids (AA) was similar to commercial AA standard mixtures (Pierce, Rockford, Illinois, USA) (Shiah & Ducklow 1994). The AA concentrations added to the culture are reported in carbon units as determined by HTC. The algal lysate, used in the July 1993 experiment, was prepared from a culture of *Isochrysis galbana* grown on F/2 medium and harvested at stationary growth phase. The cells were placed into several centrifuge tubes and spun at 1000 rpm (≈90 × g) for 20 min. The supernatant was decanted and the pellet was resuspended in low carbon isotonic solution. This rinse procedure was

conducted 3 times with each tube to minimize nutrient carry-over from media. Following the last rinse, the pellets were frozen at –20°C to freeze-fracture the cells. The pellets were combined into 1 centrifuge tube, thawed, and resuspended in isotonic solution. The majority of cellular debris was removed by centrifugation at 14 000 rpm (16 000 × g) for 10 min, and the supernatant was used as the primary algal lysate. The concentration of organic enrichment used in the July 1993 experiment was determined by HTC prior to the experiment, ensuring that cultures were enriched with the same C concentration of varying quality.

The C:N ratios of the DFAA and algal lysate supplement were determined in triplicate by soaking a combusted GF/F filter in the primary stock solutions. The elemental composition was determined by a Control Equipment Corporation 440 elemental analyzer (Lowell, Massachusetts, USA). The C:N ratios, based on atomic ratios, for DFAA and algal lysate supplement were 2.6 ± 0.74 and 19.47 ± 6.4, respectively.

Bacterial and flagellate abundance and bacterial biovolume. Acridine orange (final concentration 0.005%; Hobbie et al. 1977) was used to enumerate bacteria. Samples were preserved with particle-free 25% glutaraldehyde (final concentration of 1.0%) and stored at 4°C until slide preparation, which was within 24 h of sampling. Details of slide preparation, cell enumeration and biovolume estimates are presented in Carlson et al. (1995). At least 200 cells per slide were counted and analyzed for bacterial biovolume and at least 125 fields were counted to estimate flagellate abundance. Cell volumes were calculated using the algorithm of Baldwin & Bankston (1988) and converted to biomass

by using a carbon conversion factor (CCF) of 120 fg μm^{-3} (Watson et al. 1977, Ducklow & Carlson 1992). This CCF falls in the midrange 80 to 220 fg μm^{-3} estimated by Gundersen et al. (1994) for bacterioplankton in the Sargasso Sea near Bermuda.

Specific growth rates were calculated from 2 properties: one based on changes in cell abundance and the other on changes in cell biomass. Cell biomass equaled (cell abundance × cell volume) × 120 fg μm^{-3}. The natural log of cell abundance or cell biomass was plotted vs time. The slope of the logarithmic portion of this curve is equal to the specific growth rate. The lag portion of the curves was ignored for the specific growth rate calculation, and the end of logarithmic growth was determined as the maximum value before the curve became stationary or declined.

Bacterial carbon production was considered to be the increase in cell carbon from the initial time point to the last point in logarithmic growth. The bacterial carbon production rates were determined by linear regression analysis of the increase in bacterial carbon from time-zero (t_0) through the end of the logarithmic growth phase (data were not log transformed). Coefficients of determination (r^2) varied from 0.714 to 0.975 (n = 12), and all slopes were significant at the 0.05 % significance level.

^{3}H-thymidine and ^{3}H-leucine incorporation. ^{3}H-thymidine incorporation (Fuhrman et al. 1980, Fuhrman & Azam 1982) and ^{3}H-leucine incorporation (Kirchman et al. 1985) were used as sensitive indicators of changes in bacterial growth within the seawater cultures. The procedures followed those of Ducklow et al. (1993). Duplicate or triplicate incubations were amended with methyl [^{3}H-methyl] thymidine (Amersham, specific activity > 80 Ci $mmol^{-1}$, 10 nM final conc.) or ^{3}H-leucine (Amersham, specific activity > 140 Ci $mmol^{-1}$, 20 nM unlabelled and 1 nM labelled final concentration); further details are presented in Carlson et al. (1995).

Dissolved organic carbon. To avoid potential contamination from filtration, samples were drawn directly from the carboy's spigot into combusted 40 ml vials (Wheaton #224833). The vials were rinsed with sample several times, filled $\frac{3}{4}$ full, capped with Teflon-faced silicone septa and open-top caps, and sealed with Teflon tape. Samples were quick frozen and stored at −20°C until analyzed at Horn Point Environmental Laboratory (HPEL). Details of vial preparation are discussed in Carlson & Ducklow (1995).

All DOC samples were analyzed by the HTC method using a modified Dohrmann DC-190 (Rosemount Analytical Inc., Santa Clara, California, USA). Details of the machine's configuration and operating parameters are discussed in Carlson & Ducklow (1995) and Sharp et al. (1994). Briefly, 100 µl of sample was injected manually into a quartz combustion tube packed with

CuO wire, Pt gauze and 1 to 5 % Pt on alumina catalyst and heated to 720 to 780°C. The temperature was reduced to 720°C to extend the operating life of the combustion tube. The temperature change had no effect on the final value of DOC concentration (data not shown). The resulting CO_2 was detected with a LiCor 6252 CO_2 analyzer, and the signal was integrated with chromatographic software (Dynamax Macintegrator I version 1.3; Rainin Inst.).

Extensive conditioning of the combustion tube was essential to minimize the machine blank (Benner & Strom 1993). The combustion tube was conditioned by increasing the temperature to 800°C for at least 24 h and flushing the tube repeatedly with low carbon water (LCW) injections until a total blank was reduced to approximately 10 µM C. The highly pure LCW was generated from HPEL's Milli-Q water system and independently determined to contain approximately 3 µM C (J. H. Sharp pers. comm.). The analysis was standardized daily with a 4-point calibration curve of glucose solution in LCW. The high purity LCW was injected after the calibration curve and periodically throughout a daily run to monitor the instrument blank and performance.

In order to avoid the small error associated with the instrumental day-to-day variability, all samples generated from one experimental treatment were run on the same day. Analysis of utilization experiment samples required high precision and stable baseline performance from the HTC machine. This was attained by conditioning the machine for several days with deep seawater samples. Upon achieving stability, frozen samples were thawed at room temperature and 85 % H_3PO_4 was added to original sample vials (10 µl/10 ml). Immediately prior to injection, samples were sparged with CO_2-free oxygen for at least 10 min to dissolve all the precipitate that had formed during freezing and to remove inorganic carbon. A volume of 100 µl was injected through a septum-less port 3 to 5 times per sample. DOC concentrations were determined by the following equation:

$$\mu M\ C = \frac{\text{Average sample area} - \text{Average machine blank area}}{\text{Slope of standard curve}}$$

DOC consumption rates were determined by linear regression analysis of the decrease in DOC concentration over the logarithmic phase of growth. Coefficients of determination (r^2) for all fast-growing cultures varied from 0.853 to 0.994 (n = 11) and slopes were significant. A value of 0.594 was generated for the March 1993 culture in which the slope was not significant at the 0.05 level.

Bacterial growth efficiency. To take advantage of all measured points along the curves of bacterial growth

and DOC removal, the BGE was calculated by the following formula:

$$BGE = \frac{Bacterial\ production}{-(DOC\ consumption)} \times 100$$

where bacterial production is the slope of the linear regression of bacterial C versus time and DOC consumption is the slope of the linear regression of DOC removed versus time. DOC concentrations were corrected for bacterial biomass. The BGE was calculated for both the log phase of growth and the entire incubation.

Data analysis. Statistical analysis including analysis of covariance (ANCOVA) and linear regression (Model 1) was performed using Super ANOVA (Abacus Concepts, Inc., Berkeley, California, USA, 1989). Parameters are described as significantly different when the significance level of ≤ 0.05 was met. The standard errors associated with bacterial production rates and DOC consumption rates were determined from the linear regressions of the measured parameters versus time. Due to logistical constraints only 1 culture could be grown for each treatment within an experiment leading to problems associated with pseudoreplication (Hulbert 1984). However, the experimental design, which compared labile C amendments to control cultures, was repeated 3 separate times, thus replicating the experiment, and all experiments produced a similar trend of enhanced growth due to the addition of labile carbon as compared to a control (see 'Results').

RESULTS

Contamination due to seawater culture preparation

Preliminary experiments demonstrated that a significant amount of DOC can be leached from the Nuclepore Membra-Fil filters. However, this contamination can be minimized by flushing filter with copious amounts of Milli-Q water prior to sample collection (Fig. 1).

DOC concentrations in cultures for July 1992, October 1992, March 1993 and July 1993 did not increase during preparation compared to samples taken from the water column at the time of collection (Fig. 2). These results indicate that with proper conditioning of filters and incubation vessels, DOC contamination on the μM C scale can be eliminated. However, several studies (Fuhrman & Bell 1985, Kirchman et al. 1989, Keil & Kirchman 1991) have demonstrated that dissolved primary amine concentrations may increase from gravity filtration due to cell breakage and, thus, contamination of selected dissolved compounds due to filtration cannot be ruled out.

In order to place the initial culture DOC levels in the context of oceanic ranges, we present surface DOC concentrations from oceanic stations in the vicinity of the Bermuda Atlantic Time-series Station (31° 10' N, 64° 10' W; BATS) in Fig. 2. The average concentration for the BATS mixed layer was ~67 to 69 μM C in all seasons except winter 1993. These winter 1993 samples were collected during a mixing event in which deeper, low carbon water was entrained into the surface resulting in a dilution of the surface DOC water to a concentration of ~64 μM C (see Carlson & Ducklow 1994). The water collected for the July 1992, March 1993, and January 1994 culture experiments all had higher initial concentrations (t_0) compared to reference field samples collected near the BATS station (Fig. 2). Seawater used for these experiments was collected from stations located between Bermuda and Hydrostation S. The reasons for high initial DOC concentrations in the seawater collected for culture work are unknown, but Fig. 2 shows that higher concentrations were not due to post-collection contamination. This increase may have been a result of contamination from the collection bottles during initial collection; however, it is unlikely due to the fact that the bottles were flushed thoroughly by moving the bottles up and down in an open position through approximately 100 to 250 m of the water column prior to triggering the bottle closed. The elevated DOC concentrations may be a result of mesoscale features which affected spatial variability in DOC stocks and DOC source-sink relationships.
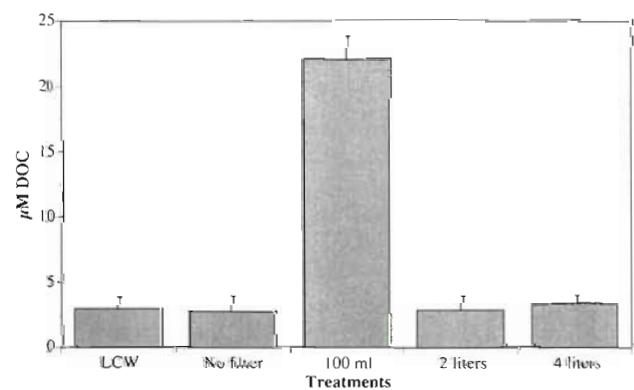

Fig. 1 Nuclepore Membra-Fil filters can contaminate the filtrate with DOC if not conditioned properly. Low carbon water (LCW) was used as a reference blank value. The no filter treatment represents the DOC concentration of water passing through the filter holder and tubing without a filter present. The 100 ml, 2 l and 4 l treatments refer to the amount of water flushed through the filter membrane before water was sampled for HTC analysis. Error bars represent 1 SD from the mean of 3 injections
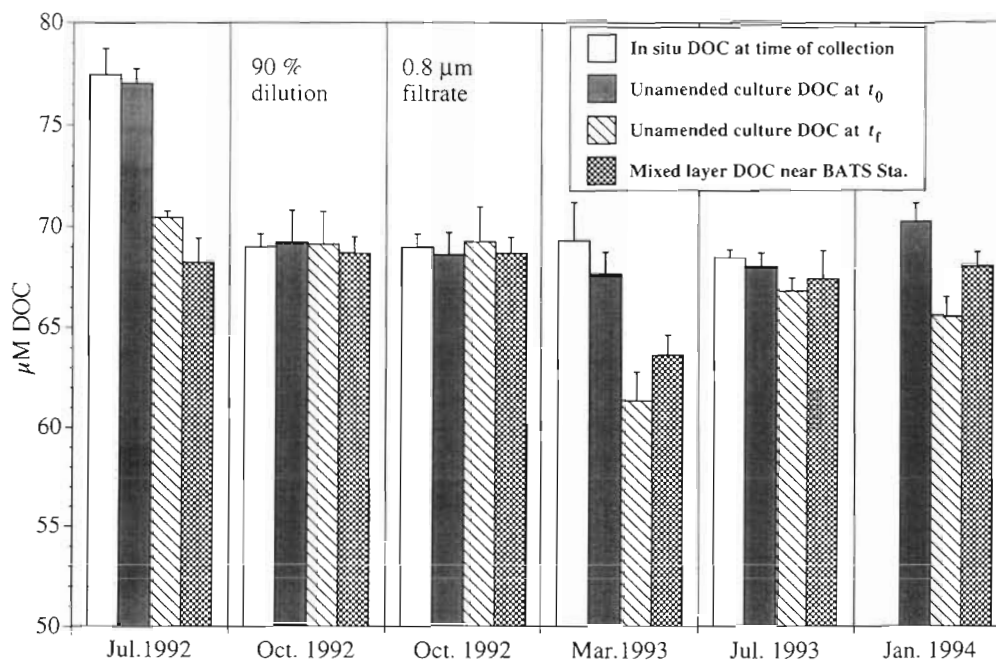
Fig. 2. DOC samples taken from the water column at the time of media collection are compared to the control DOC concentration after media preparation (t_0). DOC concentrations at t_0 and at the end of the culture (t_f) are also compared for each experiment. 90% dilution and 0.8 µm filtrate headings refer to the experimental set up for the October 1992 cultures (see Table 1). Concentration of DOC found in the mixed layer collected from the BATS site also shown. There was significant DOC removal from t_0 to t_f only in the July 1992, March 1993, and January 1994 experiments where the initial DOC culture concentrations were greater than the DOC concentration within the BATS mixed layer. Error bars represent 1 SD. Data presented are from cultures conducted in July 1992, October 1992, March 1993, July 1993, and January 1994

DOC utilization and bacterial growth in unamended seawater cultures

Cultures that demonstrated high growth also exhibited significant removal of bulk DOC (slope significant at the 0.05 level) (Fig. 3A). High bacterial growth and measurable DOC removal only occurred in cultures with initial DOC values higher than BATS mixed layer concentrations (Figs. 2 & 3). The growing seawater cultures in the July 1992, March 1993, and January 1994 experiments all demonstrated a classic sigmoidal growth curve that began with a lag phase of 10 to 20 h, was followed by a period of logarithmic growth, and terminated with a stationary growth phase (Fig. 3A). DOC utilization for these cultures followed a general pattern of rapid DOC removal during the lag phase and logarithmic growth, followed by a stabilizing period in which DOC utilization became insignificant during stationary growth phase. By the end of the experiments (111 to 216 h), DOC had been drawn down to concentrations similar to or slightly lower than those in the BATS mixed layer (Fig. 2). Although rates of bacterial production and DOC utilization varied between seasons, the fraction of DOC consumed through the end of the logarithmic growth period was 6 to 7% for all fast-growing cultures (Table 2). The

BGE for cultures able to grow on the slightly enhanced DOC levels in unamended seawater media averaged 14 ± 6% through log phase or 12 ± 6% over the entire incubation period. In cultures where DOC concentrations were similar to BATS mixed layer concentrations bacterial growth was low or not significant and DOC removal could not be resolved (Figs. 2 & 3B, Table 2).

Effects of nutrient additions

The effects of nutrient additions on bacterial growth and BGE were investigated by adding several inorganic and organic nutrients to a series of seawater cultures. A total of 3 complete nutrient enrichment experiments, each containing 3 nutrient treatments and an unamended control, were conducted in July 1992, July 1993, and January 1994 (Table 1). The time course responses of bacterial C, flagellate abundance, ^{3}H-thymidine incorporation, and ^{3}H-leucine incorporation to each nutrient supplement are presented in Fig 4. Growth patterns of bacterial C were similar to familiar batch culture patterns (described above) for all treatments except the July 1993 control, which did not grow well relative to other treatments. Bacter-
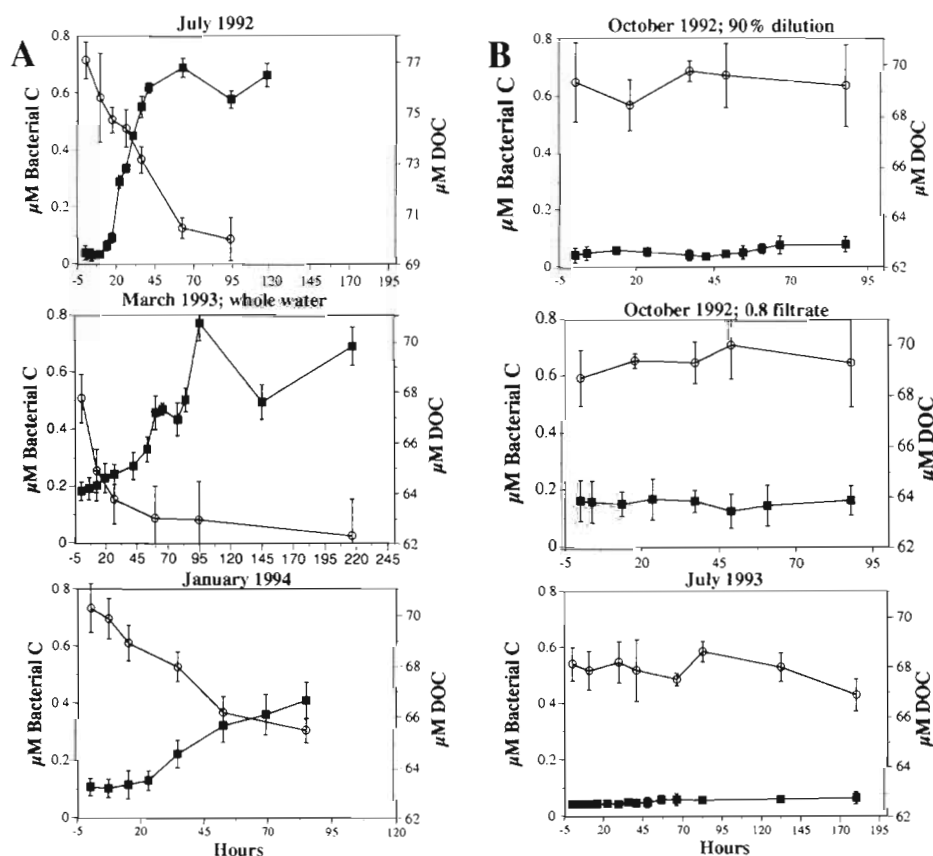
Fig. 3. Time course response of bacterial carbon (■) and DOC concentration (o) in unamended seawater cultures. (A) Cultures that demonstrated measurable growth utilized a significant portion of the bulk DOC pool. (B) Cultures that showed minimal growth did not significantly remove DOC

ial C decreased in several treatments after stationary growth. However, grazer concentrations remained consistently low and appeared to have little effect on the dynamics of bacterial C production for the first 100 h of culture growth (Fig. 4). Grazers increased after 100 h in only 4 of the 12 treatments.

Rates of ^{3}H-thymidine and ^{3}H-leucine incorporation varied greatly and were not coupled to changes in bac-

terial carbon (Fig. 4A, C, D). ^{3}H-leucine measurements could not be determined for DFAA treatments due to isotope dilution caused by the large addition of DFAA. The uncoupling between ^{3}H-thymidine incorporation, ^{3}H-leucine incorporation, and change in cellular biomass is evidence of unbalanced growth and has been discussed by several investigators (Chin-Leo & Kirchman 1990, Bjørnsen & Kuparinen 1991, Ducklow et al. 1992).

Table 2. Bacterial growth and DOC utilization for natural assemblages of bacterioplankton grown on oligotrophic seawater medium collected from the Sargasso Sea near Bermuda. ns: no significant change at the 5% significance level. –: no data

Expt	Bacterial production rate[a] μM C h^{-1} ($\pm$ SE)	DOC consumption rate[a] μM C h^{-1} ($\pm$ SE)	% DOC consumed		Growth efficiency % ($\pm$ SE)	
			Log phase	Entire incubation	Log phase	Entire incubation
Fast growth						
Jul 1992	0.016 (0.002)	0.101 (0.01)	6	9	19 (2)	9 (3)
Mar 1993	0.005 (0.001)	0.040 (0.02)	7	8	15 (3)	19 (7)
Jan 1994	0.003 (0.001)	0.059 (0.01)	7	7	7 (1)	7 (2)
Slow or no growth						
Oct 1992; 90%	0.0004 (0.0001)	−0.004 (0.008)	ns	ns	–	–
Oct 1992; 0.8 μm filtrate	−0.00004 (0.0002)	−0.007 (0.007)	ns	ns	–	–
Jul 1993	0.0002 (0.00002)	0.004 (0.003)	ns	ns	–	–

[a]Rates of bacterial production and DOC removal were estimated from the slope of linear regression of bacterial C vs time and DOC vs time, through the end of the logarithmic growth period
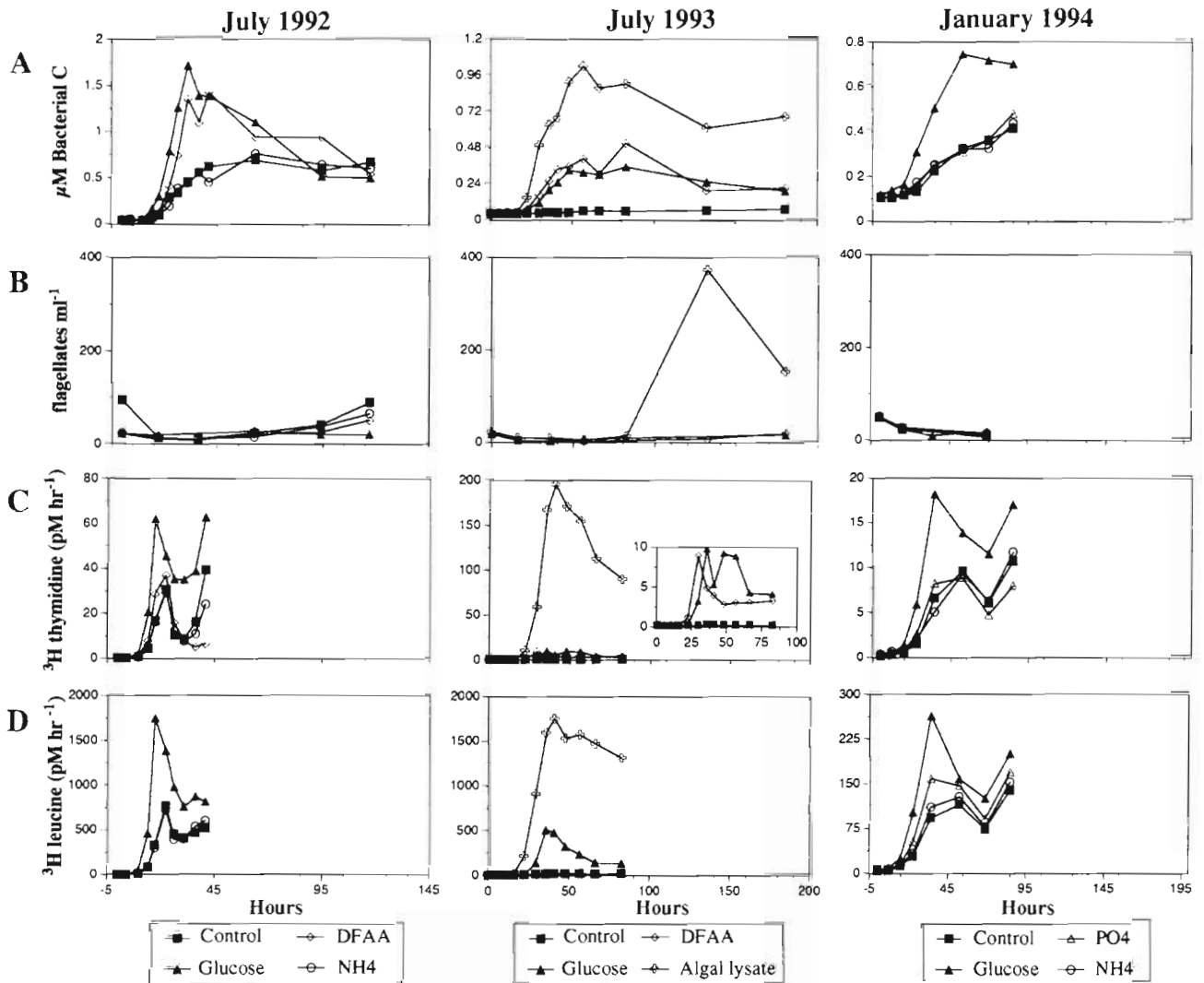
Fig. 4. Time course response of (A) bacterial C, (B) flagellates, (C) ³H-thymidine incorporation, and (D) ³H-leucine incorporation to nutrient enrichments in the July 1992, July 1993, and January 1994 experiments. The inset in the July 1993 ³H-thymidine panel is a rescaled plot of the glucose, DFAA, and control treatments

Difficulties in interpreting these dynamics led us to calculate bacterial C production from direct measurements of bacterial counts and cell volumes. However, we found the radioactive tracer techniques useful in illustrating general trends between treatments (Fig. 4C, D). Except for the DFAA treatment in the July 1992 experiment, the DOM enrichments resulted in a greater incorporation of ³H-thymidine and ³H-leucine compared to inorganic or control treatments.

Estimates of bacterial abundance, biovolume, and cell carbon obtained from the last point in logarithmic growth for each treatment (Table 1) are compared to t_0 background values (represented by the horizontal lines) in Fig. 5. Cell abundance was only slightly greater than the control for glucose and DFAA substrate enrichments and significantly higher with the algal lysate treatment. Cell volume increased significantly as cultures progressed from lag phase to logarithmic growth and either stabilized or decreased as stationary growth was attained. The magnitude of this increase varied from experiment to experiment.

Organic treatments yielded the larger cell volumes in all 3 experiments (Fig. 5) with little difference in the cell volume change between the control and inorganic N or P treatments. Changes in cell volume were accounted for by converting biovolume (cell abundance × cell volume) to biomass via a conservative volume-based conversion factor (120 fg C μm^{-3}). In all organic treatments, except the algal lysate treatment, bacterial C was greatly enhanced above the control by changes in cell volume relative to changes in cell abundance (Fig. 5).
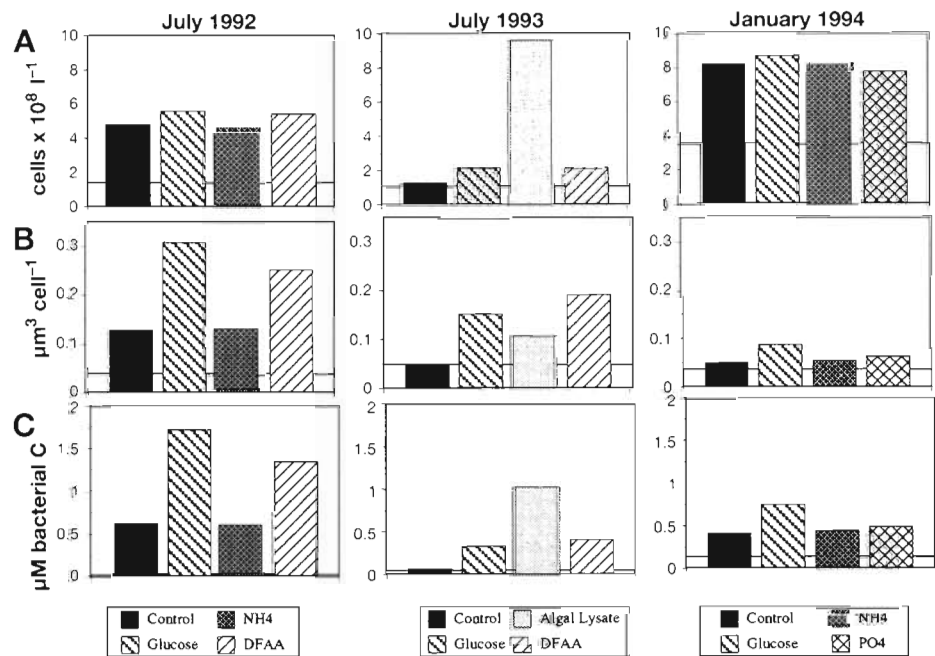
Fig. 5. July 1992, July 1993, and January 1994 nutrient enrichment experiments. (A) Bacterial cell numbers, (B) bacterial cell volume, and (C) biomass were estimated from the last point in logarithmic growth. Horizontal line represents the initial value t_0 of the control treatment

July 1992 nutrient enrichment experiment

In the July 1992 experiment, specific growth rates, bacterial C production, and DOC consumption rates were not significantly different in the control and NH$_4$ treatments (Table 3; ANCOVA; p > 0.05 significance level) but were substantially enhanced by DFAA and glucose supplements. The percentage enhancement over the control was calculated via the following formula:

% enhancement over control =

$$\frac{\text{Treatment value} - \text{Control value}}{\text{Control value}} \times 100\%$$

Table 3. Estimates of bacterial growth, DOC utilization, and growth efficiency for nutrient enrichment experiments. Treatments with the same letters within an experiment indicate no significant difference between the slopes at the 0.05 significance level according to analysis of covariance (ANCOVA)

Expt	Treatment	Growth property		Bacterial C production rate[c] $\mu M \, C \, h^{-1}$ (± SE)	DOC consumption rate[c] $\mu M \, C \, h^{-1}$ (± SE)	Growth efficiency % (SE)	
		Abundance	Biomass[b]			Log phase	Entire incubation
Jul 1992	Control	0.047 a	0.096 a	0.016 (0.002) a	0.101 (0.008) a	19 (2)	9 (3)
	NH$_4$	0.045 a	0.097 a	0.016 (0.002) a	0.108 (0.004) a	17 (2)	6 (3)
	DFAA	0.070 b	0.176 b	0.037 (0.010) b	0.216 (0.007) b	21 (6)	1 (1)
	Glucose	0.057 b	0.165 b	0.054 (0.010) b	0.236 (0.035) b	30 (6)	<1
Jul 1993	Control	0.002 a	0.003 a	1.6×10^{-4} (2.0×10^{-5}) a	0.004 (0.003) a*	–	4 (1)*
	Glucose	0.024 b	0.068 b	0.006 (0.001) b	0.148 (0.013) b	4 (1)	1 (1)
	DFAA	0.027 b	0.060 b	0.008 (0.001) b	0.126 (0.013) b	7 (1)	1 (1)
	Algal lysate	0.057 c	0.071 b	0.019 (0.002) c	0.092 (0.002) c	28 (3)	11 (6)
Jan 1994	Control	0.011 a	0.019 a	0.004 (0.001) a	0.059 (0.007) a	7 (1)	7 (2)
	NH$_4$	0.010 a	0.017 a	0.004 (0.001) a	0.077 (0.016) a	5 (1)	6 (2)
	PO$_4$	0.011 a	0.019 a	0.005 (0.001) a	0.066 (0.009) a	8 (0)	5 (2)
	Glucose	0.017 b	0.038 b	0.013 (0.001) b	0.154 (0.015) b	9 (1)	3 (2)

[a] Growth rates were calculated from the slopes of the regression of ln cell numbers or ln biomass vs time during the logarithmic growth phase. All units are h^{-1}

[b] Biomass = (abundance × mean cell volume) × (120 fg C μm^{-3})

[c] Rates of bacterial production and DOC removal were estimated from the slope of linear regression of bacterial C vs time and DOC vs time through the end of logarithmic growth

* Slope of the linear regression of DOC consumption was not significant at the 0.05 level; thus, BGE is not considered accurate

DFAA and glucose treatments stimulated bacterial C production by 131 and 237%, respectively, and DOC consumption rates by 114 and 134%, respectively, over the control (Table 3). Although cell abundances for the DFAA and glucose treatments were slightly higher than the control and NH_4 treatments, the large increase in bacterial C resulted from significant increase in cell volumes (Fig. 5). There were no significant differences between glucose and DFAA treatments with respect to specific growth rates, bacteria C production, or DOC consumption. BGE was higher for the organic enrichments, but only the glucose treatment was significantly greater than the control (Table 3).

DOC consumption was similar in both the NH_4 and control treatments, where DOC concentrations decreased to approximately 69 to 70 µM C by the time the stationary growth phase was reached and did not vary significantly for the remainder of the incubation period (Fig. 6). As in the unamended cultures described above, DOC concentrations at the end of the incubation were similar to the BATS mixed layer concentrations for the NH_4 and control treatments. In contrast, DOC consumption in both the DFAA and glucose treatments continued even after bacterial cell production ceased, but DOC consumption had not declined to the ambient level (67 to 69 µM C) by the end of the experiment.

July 1993 nutrient enrichment experiment

The objective of the July 1993 experiment was to determine if the response to equal DOC additions (actual concentrations of added DOC ranged from 22 to 26 µM C) of varying quality would affect the growth response and DOC consumption of a natural assemblage of oligotrophic bacterioplankton. Growth rates in all organically enriched cultures increased rapidly with significant increase in specific growth rates, bacterial C production, and DOC consumption compared to the control (Table 3, Fig. 7). The growth response to algal lysate was significantly greater than glucose and DFAA treatments by approximately 216%, yet the DOC consumption rate was 37 and 27% lower, respectively (Table 3). As a result, BGE determined for the algal lysate treatment was highest. Changes in cell volume contributed less to bacterial C increase in the algal lysate treatment than in the glucose and DFAA treatments (Fig. 5).

As in the July 1992 experiment, DOC removal continued in the DFAA and glucose treatments even after bacterial C growth ceased (Fig. 7). However, DOC consumption stabilized at approximately 82 µM C in the stationary phase for the algal lysate treatment. These results indicate that only 7.5 µM C of the added 21.5 µM C was readily available in ca 200 h of incubation. The remaining 14 µM C must have represented
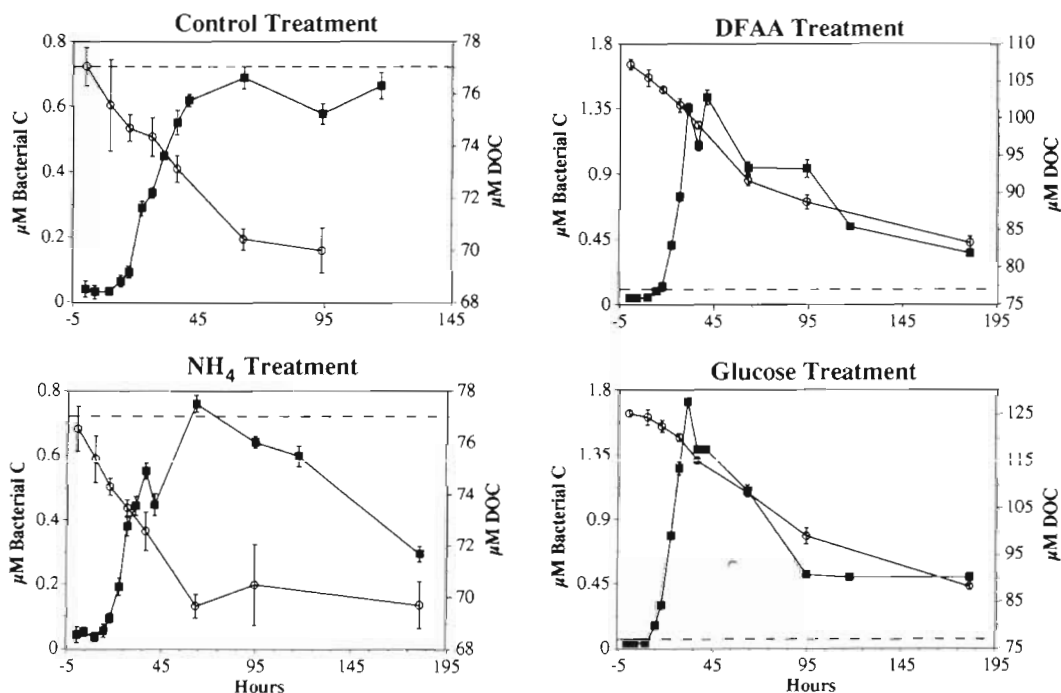

Fig. 6. July 1992 experiment. DOC concentration (o) and bacterial carbon (■) for various seawater cultures treatments including a control, NH_4, glucose, and DFAA treatments. Dashed line represents the ambient DOC concentration at time of seawater collection. Error bars represent 1 SD from the mean
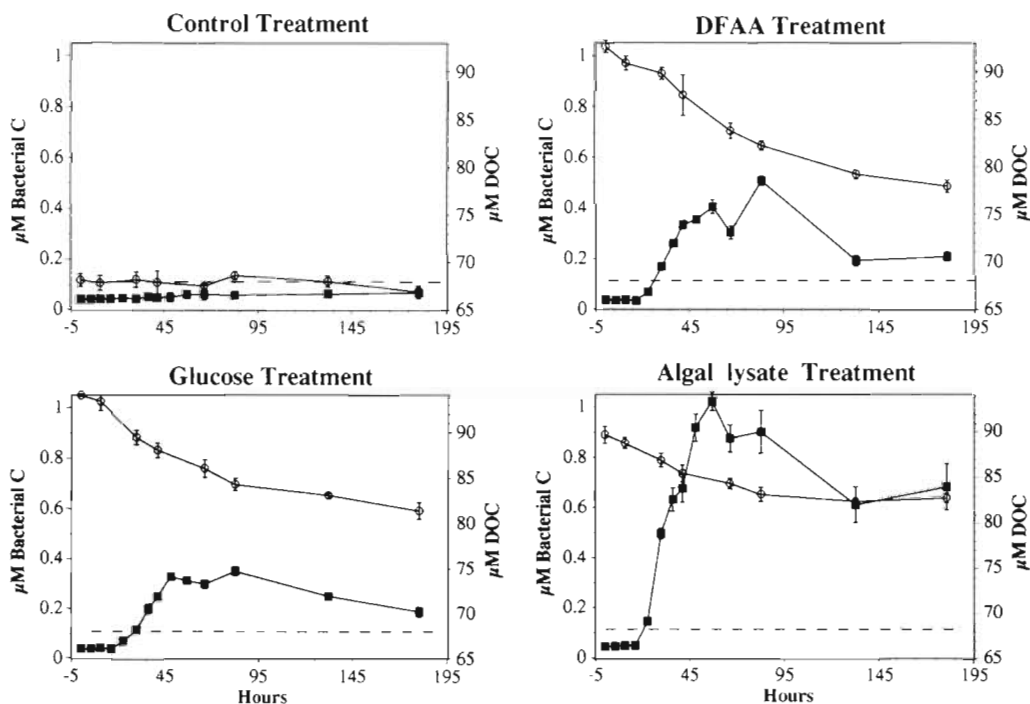
Fig. 7. July 1993 experiment. DOC concentration (o) and bacterial carbon (■) for various seawater cultures treatments including a control, glucose, DFAA, and algal lysate treatments. Dashed line represents the ambient DOC concentration at time of seawater collection. Error bars represent 1 SD from the mean

more refractory components that could not be oxidized on the time scale of this experiment. The algal lysate was a complex mixture of organic material; thus, a homogeneous lability of this material was not expected. Despite the lower percentage of available DOM in the algal lysate, the available portion was utilized more efficiently than glucose and DFAA enrichments.

January 1994 nutrient enrichment experiment

In the January 1994 experiment, the response of specific growth rates, bacterial C production, and DOC utilization to the inorganic enrichment (NH_4 and PO_4) was not significantly different from the control (ANCOVA; significance level of 0.05). However, the glucose supplement enhanced bacterial C production and DOC utilization by 225 and 161%, respectively, over the control (Table 3, Fig. 8). The changes in cell volume were similar in the inorganic and control treatments (Fig. 5). The overall changes in cell volume were relatively small compared to previous experiments, yet the contribution of this change significantly enhanced bacterial C production in the glucose treatment (Fig. 5).

DOC utilization was similar in the control and inorganic treatments, decreasing linearly for the first 53 h and reaching an asymptote of 64 to 66 µM C by the stationary growth phase (Fig. 8). Note that in this experiment, DOC declined to below the initial ambient level in all treatments. The high DOC value detected

for the last DOC time-point of the PO_4 treatment was attributed to contamination and was disregarded. As in previous experiments DOC continued to decline in the glucose treatment throughout the entire incubation period (Fig. 8). BGE determined through logarithmic growth was highest for the glucose treatment. However, the glucose BGE was only significantly different from the NH_4 treatment and not for the others. When BGE was determined for the entire incubation period, the glucose treatment had the lowest efficiency due to continued DOC removal after growth had ceased (Table 3).

DISCUSSION

DOC utilization in unamended seawater cultures

In the oceanic environment the production of 'fresh' DOC originates from in situ biological processes such as direct exudation from phytoplankton (Lancelot 1979, Goldman et al. 1992), zooplankton excretion and egestion (Lampert 1978, Nagata & Kirchman 1992), particle hydrolysis (Smith et al. 1992), and cell lysis from viral infection (Proctor & Fuhrman 1990, Fuhrman 1992). The application of size fractionation and dilution techniques combined with incubations in the dark minimizes these potential sources of DOC production in culture experiments (except for viral impact). Thus, our experimental design forces an uncoupling both of DOC production from consumption
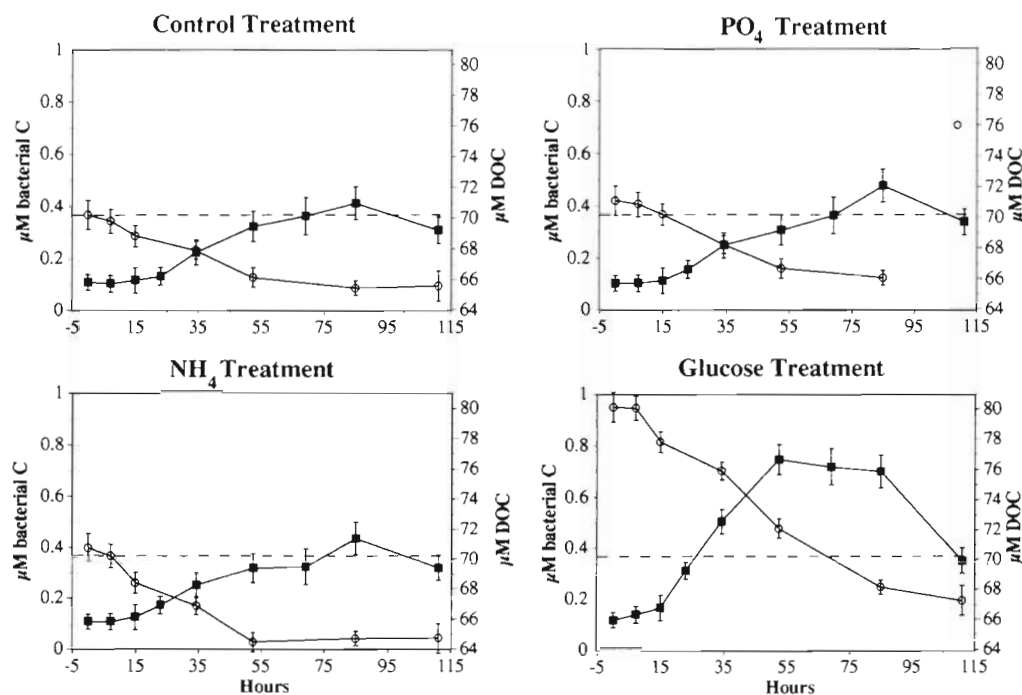
Control Treatment

PO₄ Treatment

NH₄ Treatment

Glucose Treatment

Fig. 8. January 1994 experiment. DOC concentration (o) and bacterial carbon (■) for various seawater cultures treatments including a control, NH_4, PO_4, and glucose treatments. Dashed line represents the ambient DOC concentration at time of seawater collection. Error bars represent 1 SD from the mean

and bacterial production from bacterivory. As a result, only the material present in the DOM pool upon collection is available for bacterial utilization. The quality of the biologically reactive component and the rate at which it accumulates depends on the *in situ* coupling of DOC production and consumption processes (Carlson & Ducklow 1995). If these processes are tightly coupled, then labile components of the DOC would quickly disappear soon after production processes were halted. Thus, due to the elimination of 'fresh DOC' production, the media collected from a tightly coupled system may not be entirely representative of the material *in situ* populations were utilizing. If DOC production was greater than consumption, 'fresh' DOC may accumulate, resulting in a longer residence time of available C within the bulk pool.

DOC stocks in the surface 250 m of the Sargasso Sea demonstrated significant seasonal variability (Carlson et al. 1994); however, the surface mixed layer concentrations did not change seasonally, except for the low concentrations in winter, indicating there was tight coupling between DOC production and consumption processes throughout most of the year. One might expect that if DOC production processes were halted the labile DOC would be removed rapidly leaving behind a more refractory medium for bacterial utilization. Interestingly, we observed growth of natural bacterial assemblages at the expense of ambient DOC only when initial DOC concentrations were greater than concentrations found in the BATS mixed layer. In contrast, cultures that exhibited little or no bacterial

growth contained initial DOC concentrations comparable to the BATS mixed layer DOC concentrations (Figs. 2 & 3). We hypothesize that the 67 to 69 μM C present in the BATS surface mixed layer and in nongrowing cultures represents DOC background concentrations consisting of both semi-labile and refractory components with little or no 'fresh' labile material present. When DOC production is halted, the quality of the remaining substrate could not support rapid bacterial growth. Bacterial cultures only grew and utilized DOC during transient episodes of accumulation of labile 'fresh' DOC. Although semi-labile material can be remineralized (Barber 1968, Carlson et al. 1994), turnover times were too long to be measured in our culture experiments, i.e. months.

Actively growing cultures removed 6 to 7% of the initial DOC by the end of logarithmic growth. This consumed material represented the labile portion of the bulk pool that was available after DOC production was eliminated (Servais et al. 1989). The removal rates of 0.04 to 0.1 μM C h^{-1} are similar to rates of carbohydrate removal (0.13 μM C h^{-1}) observed by Burney (1986) in the same region. Our rates are higher than those observed by Hansell et al. (1995), who used CO_2 accumulation as an index of DOC utilization. However, we suspect that due to the high sensitivity of their analytical procedure the low DOC utilization rates they observed were comparable to those we could not resolve in cultures starting with concentrations similar to the BATS mixed layer values (Figs. 2 & 3B). By the stationary growth phase, DOC concentrations were

drawn down to concentrations similar to BATS mixed layer concentrations and did not change significantly throughout the remainder of the experiments (110 to 219 h). We hypothesize that the quality of the DOC at the asymptote portion of the DOC utilization curve was representative of the semi-labile and refractory organic compounds.

The experimental approach used in this study yielded an average BGE of 14 ± 6% for fast-growing cultures (Table 2), a value considerably lower than the 34 to 95% observed in studies which traced the disappearance of model compounds through bacterial production (Williams 1970, Iturriaga & Zsolnay 1981, Goldman et al. 1987, Kirchman 1990). The growth efficiencies we observed in growing cultures varied from 7 to 19%, indicating that the quality of the utilizable DOM or the physiological status of the bacterial assemblage, or both, varied from experiment to experiment. Several studies in which naturally occurring DOM was used to support bacterial growth have demonstrated large temporal and spatial variability of BGE (Table 4). The average BGE of 14 ± 6% determined from this study was generally lower than estimates from coastal or inshore waters (Table 4), indicating that accumulated DOM in oligotrophic water was of lower quality than that in eutrophic environments. These values were similar to those of Hansell et al. (1995), who used respiration measurements to assess DOC utilization in the Sargasso Sea. Our data support the findings of Biddanda et al. (1994), who suggested BGE decreased

with increasing distance from shore due to decreasing DOM quality. Our data also lend support to the hypothesis of Ducklow & Carlson (1992) which stated that oligotrophic bacterioplankton must process a greater percentage of primary production to sustain bacterial to primary production ratios similar to those found in eutrophic environments. The high variability and relatively low BGE emphasize that the use of a constant bacterial conversion efficiency of 50%, often cited in carbon budgets, is inappropriate.

While our estimates of BGE fall within the range reported in the literature, such data must be used cautiously. In order to calculate BGE, accurate estimates of biomass production and DOC consumption are required. Bacterial production estimates in this study were determined by direct observations of changes in bacterial abundance and cell volume. Particulate organic carbon (POC) measurements were not made, due to the large sample volume needed to detect a signal. Thus, to quantify carbon production a CCF was applied to the biovolume measurements. A conservative CCF of 120 fg C μm^{-3} was chosen because Gundersen et al. (1994) found this to be a mid-range CCF for Sargasso Sea bacterioplankton (80 to 220 fg C μm^{-3}); however, a wide range of CCFs exist in the literature (see Ducklow & Carlson 1992). The BGE is directly related to the choice of CCF; for example, if the CCF was 60 or 240 fg C μm^{-3}, then the resulting BGE would be 7 or 28%, respectively. A higher CCF would result in a higher BGE; however,

Table 4. Bacterial growth efficiency values (%) selected from the literature, determined for natural assemblages of marine bacterioplankton grown on unamended seawater medium. ^{3}H-leu: ^{3}H-leucine; ^{3}H-TdR: ^{3}H-thymidine; DIC: dissolved inorganic carbon. ΔCO_2 was measured by high precision coulometric titration. ΔDOC was measured using the HTC method. ΔO_2 was measured by high precision titration. Bacterial POC was estimated from cell abundance and cell volumes

Location	Method	Mean	Range	Source
Gulf of Mexico; shelf	ΔO_2 and ^{3}H-leu	45	38–55	Biddanda et al. (1994)
Gulf of Mexico; slope	ΔO_2 and ^{3}H-leu	23	19–26	Biddanda et al. (1994)
Roskilde Fjord, Denmark	ΔDOC and ΔPOC	20	11–27	Bjørnsen (1986)
Southern Ocean	ΔDIC and ^{3}H-TdR	39	38–40	Bjørnsen & Kuparinen (1991)
Mississippi River plume	ΔO_2 and ^{3}H-TdR	19	10–22	Chin-Leo & Benner (1992)
Mississippi River plume	ΔO_2 and ^{3}H-leu	29	9–42	Chin-Leo & Benner (1992)
Northwestern Florida estuaries	ΔO_2 and ΔBacterial POC	43	28–58	Coffin et al. (1993)
Range Salt Marsh, Florida, USA	ΔO_2 and ΔBacterial POC	41	11–60	Coffin et al. (1993)
Santa Rosa Sound, Florida, USA	ΔO_2 and ΔBacterial POC	16	0.4–35	Coffin et al. (1993)
Georgia, USA; inshore	ΔO_2 and ^{3}H-TdR	11	11	Griffith et al. (1990)
Georgia, USA; nearshore	ΔO_2 and ^{3}H-TdR	6	6	Griffith et al. (1990)
Georgia, USA; shelf	ΔO_2 and ^{3}H-TdR	2	2	Griffith et al. (1990)
Sargasso Sea	ΔCO_2 and ^{3}H-TdR	6.6	3.8–8.9	Hansell et al. (1995)
Roskilde Fjord, Denmark	ΔO_2 and ^{3}H-TdR	42	42	Jensen et al. (1990)
North Atlantic (47° N, 18° W)	ΔDOC and ΔBacterial POC	5	2–8	Kirchman et al. (1991)
Inshore waters, Florida, USA	ΔDOC and ΔPOC	47	32–61	Kroer (1993)
Santa Rosa Sound, Florida, USA	ΔDOC and ΔPOC	30	26–33	Kroer (1993)
Gulf of Mexico	ΔDOC and ΔPOC	61	61	Kroer (1993)
Baltic Sea	ΔDOC and ΔBacterial POC	27	11–54	Zweifel et al. (1993)
Northwestern Sargasso Sea	ΔDOC and ΔBacterial POC	14	7–19	This study

even using a generous CCF of 240 fg C μm^{-3} would still produce low BGE relative to the often cited BGE of 50%.

DOC utilization was determined by direct measurements of DOC change over time. In cultures in which bacterioplankton did not grow DOC removal could not be resolved, indicating that the DOC changes observed in growing cultures were not due to a abiotic factor such as absorption to particles or to the bottle's wall. However, direct measurement of DOC change could lead to an underestimation of DOC utilization and BGE if DOM was excreted by bacterioplankton (Iturriaga & Zsolnay 1981) or if viral lysis caused production of DOC (Fuhrman 1992). Thus BGE must be considered a net BGE. In contrast, if viral lysis played a significant role in the loss of bacterial biomass after DOC consumption, then BGE would be underestimated. As discussed in Zweifel et al. (1993), decreases in substrate DOC concentrations may be a misleading measure of growth efficiency unless respiration, DOC decrease, POC, and bacterial numbers and volume are all determined. Thus the BGE determined in this study must be considered first approximations which require further study.

Is organic carbon a limiting nutrient?

In aquatic environments the assimilation of DOM is dominated by heterotrophic bacterioplankton (Azam & Hodson 1977); however, bacterioplankton are also capable of utilizing inorganic nitrogen (Wheeler & Kirchman 1986, Kirchman et al. 1989) and phosphorus (Suttle et al. 1990, Ammerman 1991). Due to the surplus of organic C in surface water of the Sargasso Sea (see Carlson et al. 1994) compared to undetectable concentrations of inorganic nitrogen and phosphate, one might suggest that carbon should not limit bacterial growth. However, it must be stressed that the availability of organic carbon to bacterioplankton is not determined by the absolute concentration of DOC but rather by the quality of the pool (Schaechter et al. 1958, Pomeroy & Wiebe 1988, Coffin et al. 1993, Biddanda et al. 1994). For example, deep-water DOC concentration values represent approximately 65 to 70% of the DOC present in the mixed layer in the Northwestern Sargasso Sea (Carlson et al. 1994), yet this material is approximately 4100 yr old (Bauer et al. 1992) and comprised of biologically refractory material (Barber 1968). Based on vertical and temporal distributions of bulk DOC in oceanic environments, Carlson & Ducklow (1995) separated the bulk DOC pool into 3 broadly characterized pools, defined as the refractory pool, which turns over on time scales of ocean mixing, the semi-labile pool, which turns over on scales of

months to years, and the labile pool, which turns over on scales of hours to days.

Ambient concentrations of individual DFAA in the waters surrounding Bermuda range from <1 to 30 nM (Liebezeit et al. 1980) and may not be sufficient to sustain bacterial N demand (Hagström et al. 1984). However, bacterial uptake of NH_4^+ has been shown to supplement this demand (Wheeler & Kirchman 1986, Kirchman et al. 1989). Except during brief periods of deep winter mixing, the concentrations of inorganic nitrogen and phosphorus in the waters surrounding Bermuda were generally below detection limits (Knap et al. 1994, Michaels et al. 1994). Indeed, Brezinski (1988) reported that concentrations of NH_4^+ <3.5 nM were not uncommon in the oligotrophic Sargasso Sea. Under such conditions, bacterial growth could be considered to be limited by both nitrogen (Kroer 1993) and phosphorus (Toolan et al. 1991, Cotner et al. 1994, Peele et al. 1994). Yet, when bacterial cultures were enhanced with inorganic NH_4^+ and $HPO_4^=$, there were no significant differences in bacterial growth rates, biomass production, or DOC consumption (Table 3) compared to control treatments. These results indicate that, despite low ambient concentrations, these nutrients were not the primary limiting nutrients to bacteria at the times these culture experiments were conducted.

The results of this study clearly demonstrate in the 3 separate experiments that, despite unmeasurable concentrations of NH_4^+ and $HPO_4^=$ in surface waters, the addition of labile DOM stimulated bacterial growth rates (Table 3) and biomass yield (Figs. 4 & 5) during our sampling periods. Organic enrichment yielded significantly higher BGE than the control in 4 of the 6 organic treatments. These results suggest organic carbon limitation and are consistent with the findings of Kirchman et al. (1990), who demonstrated carbon limitation of NH_4 uptake and Kirchman (1990), who showed that bacterial production was limited by the supply of amino acids in the subarctic Pacific.

Kirchman (1990, 1994) reported that in the subarctic Pacific and the North Atlantic (surface temperature ~12°C), DFAA enrichment stimulated bacterial production more than glucose addition and stated that 'it would be an oversimplification to conclude that heterotrophic bacteria were C-limited'. In contrast to these findings, Goldman & Dennet (1991) reported no difference in bacterial growth rate or BGE when glucose plus NH_4 was compared to DFAA additions in cultures grown on artificial medium. Hobbie & Crawford (1969) found that a lower percentage of total C uptake was respired with glucose additions than most AA in freshwater bacterial samples. In experiments conducted in the Kiel Fjord, Berman et al. (1994) demonstrated that glucose enrichment stimulated bacterio-

plankton more than several other organic compounds including alanine. In the present study, there were only slight differences between DFAA and glucose addition with respect to bacterial growth rate, bacterial C production, DOC utilization and BGE. Due to the low C:N ratio of DFAA and an energetic advantage of avoiding AA synthesis, one might expect this treatment to yield higher growth rates and BGEs than nitrogen-poor enrichments (Goldman et al. 1987). Surprisingly, we did not observe this.

One possible explanation is that the added concentrations of DFAA (~7.5 μM) were too high compared to the natural nM concentrations and the oligotrophic bacteria could not utilize the rich substrates sufficiently (i.e. 'oligotrophs' sensu Poindexter 1981). However, studies have demonstrated that most oligotrophic bacteria enriched with concentrations of AA and algal extract significantly higher than in this study grew effectively under high-nutrient conditions (Martin & MacLeod 1984, Carlucci et al. 1986). In the present study, bacterial growth increased significantly with all organic enrichments compared to the control, indicating that the natural assemblages were able to adjust to elevated nutrient concentrations. A second possibility is that the nutritional value gained from pre-formed AA may have been offset by the energetic costs of transporting AA across the cell membrane (Payne & Wiebe 1978, Goldman & Dennet 1991). A third potential explanation as to why bacterioplankton responded similarly to DFAA and glucose treatments may be that after the initial carbon limitation was alleviated, bacterial production became limited by a secondary nutrient. This hypothesis was supported by the results of the July 1993 experiment in which the phytoplankton lysate (C:N ≈ 19) produced a higher BGE and yield of bacterial C than glucose and DFAA enrichments. These results indicate that the complex molecular composition of the algal lysate provided a limiting nutrient not supplemented by glucose or DFAA additions, perhaps in the form of dissolved organic phosphorus (DOP), or an organic or inorganic micronutrient.

The algal lysate treatment produced the largest cell yield compared to any other treatment in this study, indicating that cell division was not limited (Fig. 5). DFAA and glucose treatments produced significantly more bacterial C than inorganic and control treatments; however, this elevated carbon production was attributed to a significant increase in cell volume rather than greater cell division. We hypothesize that when cell division became limited, the cells responded to the surplus labile carbon by synthesizing cell products and storing carbon, thus increasing biomass and cell volume. This hypothesis is supported by the findings of Baxter & Sieburth (1984), who described bacterial

strains which could adjust their metabolic strategies to high glucose concentrations by forming cellular storage products. In seawater culture experiments with glucose enrichments, Zweifel et al. (1993) observed that in N- and P-limited cultures cell division stopped but POC increased. They suggested this POC increase may have resulted from the synthesis of capsular material.

Our study demonstrated that even after cell biomass production became limited and reached the stationary phase, DOC removal continued in the glucose and DFAA treatments. Baxter & Sieburth (1984) suggested that the production of storage products or extracellular polymeric carbohydrates may result in high glucose uptake rates with low growth efficiencies. We suggest that in an attempt to sequester limiting nutrients, bacterioplankton participated in 'luxurious uptake' of labile carbon which was eventually respired. Our findings indicate that labile carbon in the form of glucose and DFAA was initially utilized as a carbon source, resulting in an elevated growth rate, and that upon exhaustion of a growth-limiting nutrient, the surplus of labile carbon was utilized as an energy source. While labile carbon may be the initial limiting nutrient, it is the availability of all limiting nutrients which dictates the growth efficiency of natural assemblages.

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