

## Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea

Craig A. Carlson<sup>1</sup>

Bermuda Biological Station for Research, Ferry Reach, St. George's GEO1, Bermuda

Stephen J. Giovannoni

Department of Microbiology, Oregon State University, Corvallis, Oregon 97331

Dennis A. Hansell

Bermuda Biological Station for Research, Ferry Reach, St. George's GEO1, Bermuda; University of Miami, Rosenstiel School of Marine and Atmospheric Science, Miami, Florida 33149

Stuart J. Goldberg<sup>2</sup> and Rachel Parsons

Bermuda Biological Station for Research, Ferry Reach, St. George's GEO1, Bermuda

Kevin Vergin

Department of Microbiology, Oregon State University, Corvallis, Oregon 97331

### Abstract

At the Bermuda Atlantic Time-Series Study (BATS) site, the field observations of dissolved organic carbon (DOC) dynamics indicate that seasonally produced "semilabile" DOC is resistant to rapid microbial degradation in the surface waters but available for microbial remineralization once it is delivered into the mesopelagic zone after convective overturn. In this study, we employed an experimental simulation of convective overturn events to determine whether the remineralization of semilabile DOC would occur in a controlled laboratory setting. Seawater culture experiments were conducted in which surface ( $\leq 10$  m) and mesopelagic (250 m) 0.2- $\mu\text{m}$  filtrates were inoculated with unfiltered water from  $\leq 10$  and 250 m in an assortment of combinations to simulate various mixtures of nutrients, DOC quantity and quality, and microbial assemblages. Results indicate that (1) microbial inocula from the upper euphotic zone were incapable of remineralizing the seasonally accumulated semilabile DOC ( $\mu\text{mol C L}^{-1}$  resolution) on the timescales of the incubations; (2) the utilization of semilabile DOC was greatest when the inoculum source was from 250 m and the filtrate source was from the upper 10 m; and (3) the decrease in bacterioplankton diversity, estimated with the Shannon–Wiener diversity index, was greater in treatments in which inoculum from 250 m was mixed with filtrate from 10 m than in treatments in which the surface inoculum was mixed with the surface filtrate. Our findings are that a portion of the surface semilabile DOC can be metabolized by microorganisms in a laboratory setting and that mesopelagic nutrients alone are insufficient to stimulate DOC drawdown  $> 1.3 \mu\text{mol L}^{-1}$ . Transformations of microbial community structure were associated with the drawdown of surface DOC in simulated mixing events and suggest that microbial community structure is a factor in surface-layer DOC dynamics.

The production of oceanic dissolved organic carbon (DOC), regardless of mechanism (*see* Carlson 2002), is ul-

<sup>1</sup> To whom correspondence should be addressed. Present address: Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, California 93106-9610 (carlson@lifesci.ucsb.edu).

<sup>2</sup> Present address: Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, California 93106-9610.

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timely constrained by the magnitude of primary production. DOC uptake by heterotrophic prokaryotes is the dominant DOC removal mechanism in the sea (Azam 1998). However, it is estimated that 17% of global new production escapes rapid microbial degradation and accumulates in the surface waters as DOC (Hansell and Carlson 1998). DOC that resists rapid microbial remineralization, with lifetimes of weeks to years, is referred to as semilabile DOC (Kirchman et al. 1993; Carlson 2002). If the DOC turnover time is greater than the intervals between major vertical mixing events, then the DOC can be exported into the ocean interior (Copin-Montégut and Avril 1993; Carlson et al. 1994; Hansell 2002). The majority of exported DOC is remineralized

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within the mesopelagic zone (100–1,000 m) (Hansell 2002). Doval and Hansell (2000) found that the remineralization of exported DOC explained up to 47% of the apparent oxygen utilization (AOU) in the upper 500 m of the South Pacific and Indian Oceans. Similarly, DOC oxidation accounted for 15–41% of the annual AOU signal between 100 and 400 m at the Bermuda Atlantic Time-Series Study (BATS) site in the northwestern Sargasso Sea (Hansell and Carlson 2001). Factors that control the uncoupling of dissolved organic matter (DOM) production and consumption processes can ultimately affect the magnitude of DOC accumulation in and export from the surface ocean.

DOC dynamics at BATS demonstrates a regular annual pattern. Coincident with convective overturn and subsequent nutrient entrainment is the development of spring phytoplankton blooms (Michaels and Knap 1996; Steinberg et al. 2001) and a net accumulation of DOC within the surface 250 m (Carlson et al. 1994; Hansell and Carlson 2001). The magnitude of DOC production in the surface 250 m is influenced by the strength of the annual convective overturn event, with annual net DOC production ranging from  $>1$  to  $<0.4$  mol C m<sup>-2</sup> (Hansell and Carlson 2001). After water-column stratification in the spring, DOC stocks accumulate rapidly within the euphotic zone and reach a maximum by the end of May. The DOM that accumulates within the surface 40–60 m resists rapid microbial attack by surface assemblages and persists at relatively constant and elevated concentrations from the end of May until late autumn, when it is diluted by convective overturn (Carlson et al. 2002).

During convective overturn, a portion of the seasonally accumulated DOC is mixed out of the euphotic zone and into the upper mesopelagic zone (defined here as 140–250 m) (Carlson et al. 1994; Hansell and Carlson 2001). After a restratification of the water column, the entire exported DOC pool is removed on the timescale of weeks to months. Microbial remineralization is hypothesized to be responsible for a significant portion of the DOC removal within the upper mesopelagic zone at BATS (Hansell and Carlson 2001). If true, then the DOC that accumulates in the surface is resistant to rapid microbial degradation in the upper euphotic zone but available for microbial remineralization, on the timescale of weeks, in the upper mesopelagic.

Specific constraints on bacterial production could prevent the rapid utilization of surface DOC and lead to its accumulation. Depending on the marine system, factors that may control bacterial production include temperature (Shiah and Ducklow 1994), grazing (Thingstad et al. 1997), and competition for limiting nutrients (Rivkin and Anderson 1997; Thingstad et al. 1997). In the Sargasso Sea, several studies have demonstrated that the addition of inorganic nutrients (specifically phosphorous) can lead to increased bacterial production (Cotner et al. 1997; Rivkin and Anderson 1997; Caron et al. 2000) or bacterial respiration (Obernosterer et al. 2003) in seawater cultures. However, in other Sargasso Sea seawater culture experiments, the addition of nitrogen and phosphorous was insufficient to stimulate bacterial production or surface DOC utilization during the time frame in which the experiments were conducted (Carlson and Ducklow 1996; Carlson et al. 2002). Together, these studies indicate that the effect of nutrient limitation on bacterial pro-

duction and the magnitude of DOC utilization in the Sargasso Sea varies with respect to space and time.

The accumulation of DOC in surface waters has also been attributed to the net production of biologically recalcitrant compounds that are resistant to rapid degradation (McCarthy et al. 1998; Aluwihare and Repeta 1999; Ogawa et al. 2001), leaving the surface heterotrophic microbial community energy limited (Kirchman 1990; Cherrier et al. 1996; Carlson et al. 2002). In addition to these factors, microbial community structure may play a role in DOC dynamics.

Small-subunit ribosomal ribonucleic acid (rRNA) genes, employed as phylogenetic markers, have shown significant diversity among the prokaryote assemblages found in the upper water column of the ocean (Giovannoni and Rappé 2000; Venter et al. 2004). Additional studies have demonstrated that major prokaryote groups are highly stratified within the upper 300 m of the ocean (Giovannoni et al. 1996; Karner et al. 2001; Morris et al. 2002). A marked shift in microbial community structure occurs at the transition between the euphotic and aphotic zones (Giovannoni and Rappé 2000). However, it is unclear whether the community structure shift between the two depth horizons is a result of bottom-up controls, top-down controls, or a combination of the two.

In the present study, we employed an experimental simulation of convective overturn events to develop an understanding of how microbial community structure, in combination with naturally occurring nutrient fields, affects the remineralization patterns of semilabile DOC. Our experimental design was to mix inocula (unfiltered water) and media (a 0.2- $\mu$ m filtrate) from surface and mesopelagic depth horizons in various combinations to evaluate the effect of mixing on (1) bacterioplankton production, (2) remineralization of semilabile DOC, and (3) to determine if shifts in bacterioplankton community structure were associated with mixing treatments and utilization of semilabile DOC.

## Methods and materials

*Study site and water collection*—We present data from four seawater culture experiments conducted in the northwestern Sargasso Sea from 1997 to 2001. Water was collected from Hydrostation S (32°10'N, 64°30'W) or the BATS site (31°40'N, 64°10'W), located 26 and 82 km southeast of the islands of Bermuda, respectively. We have not observed any systematic differences in microbial biomass or production, or DOC concentrations, between Hydrostation S and BATS (Carlson et al. 1996; Carlson and Hansell unpubl. data); thus, we felt it was appropriate to use either sampling site as representative of northwestern Sargasso Sea open-ocean conditions. Water from the surface 10 m and from 250 m was collected from the RV *Weatherbird II*, via Niskin bottles, on a conductivity, temperature, and depth rosette. Prior to use, all carboys were washed with 5% HCl and rinsed with copious amounts of Millipore Q (Milli-Q) water.

*Mixing experiments*—The experimental design included mixtures of surface and mesopelagic media (a 0.2- $\mu$ m filtrate) with unfiltered surface and mesopelagic inocula in various combinations (Table 1). All experiments were per-

Table 1. Experimental and water-column conditions for mixing experiments conducted in the northwestern Sargasso Sea from 1997 to 2001. Duration represents the length of the incubation to the final DOC time point. MLD is mixed-layer depth. Experiment ID indicates the location (HS is Hydrostation S, and B is BATS) and cruise designation.

Experiment ID	Date	Treatment	Duration (days)	Depth of inoculum source (m)	Depth of 0.2- $\mu$ m media source (m)	MLD* (m)	Incubation temperature ( $^{\circ}$ C) $\dagger$
HS 852	Aug 97	Surface inoculum–in-surface filtrate	31	5	5	13	28
		Deep inoculum–in-surface filtrate	31	250	5	13	19
HS 875	Aug 98	Surface inoculum–in-surface filtrate	69	10	10	21	28.8
		Deep inoculum–in-surface filtrate	69	250	10	21	21.2
HS 893	Jul 99	Surface inoculum–in-surface filtrate	37	10	10	28	27.5
		Deep inoculum–in-surface filtrate	37	250	10	28	20
		Surface inoculum–in-mix filtrate	37	10	10+250	28	27.5
		Deep inoculum–in-deep filtrate	37	250	250	28	20
B155	Aug 01	Surface inoculum–in-surface filtrate	7	10	10	12	27.8
		Deep inoculum–in-deep filtrate	7	250	250	12	20.1

\* Mixed-layer depth (MLD) during the experimental period was determined as the depth where  $\sigma_t$  is equal to sea-surface  $\sigma_t$  plus an increment in  $\sigma_t$  equivalent to a 0.2 $^{\circ}$ C temperature decrease (Sprintall and Tomczak 1992).

$\dagger$  All cultures were incubated in the dark at the in situ temperatures of the inoculum source.

formed in the summer when the physical separation of distinct nutrient fields and community structures through thermal stratification was ensured (i.e., mixed-layer depths were <30 m) (Table 1). Natural assemblages of bacterioplankton collected from the surface 10 m and the upper mesopelagic (250 m) were incubated in grazer-diluted cultures and allowed to grow on various mixtures of the naturally occurring substrates (Ammerman et al. 1984). Seawater was gravity filtered through an in-line 142-mm Costar Membra-Fil filter (0.2  $\mu$ m) housed in an all-plastic filter holder attached directly to a Niskin bottle. Membra-Fil filters leach a measurable amount of DOC, so they were flushed with at least 2 liters of Milli-Q water and an additional 0.5 liter of sample water prior to the collection of experimental filtrate (Carlson and Ducklow 1996). For each treatment, 7 liters of a 0.2- $\mu$ m filtrate was inoculated with 3 liters of unfiltered seawater collected from the same cast. We avoided the pre-filtration of bacterial inoculum because pre-filtration significantly reduces Sargasso Sea bacterioplankton activity (Carlson et al. 2002). Throughout, the treatments will be described as inoculum source–in-filtrate source (e.g., deep inoculum–in-surface filtrate).

In the present study, we created treatments in which a deep inoculum was mixed with a surface filtrate. Because the deep inoculum contained elevated inorganic nutrient concentrations as well as differing prokaryotic populations relative to the surface waters, we designed an additional treatment (surface inoculum–in-mix filtrate) (Table 1) to evaluate the importance of elevated nutrient concentrations in the remineralization of DOC. In the surface inoculum–in-mix filtrate treatment, the ratio of inorganic nutrients, DOC concentration, and quality was kept the same as in the deep inoculum–in-surface filtrate treatment (i.e., both treatments contained 7 liters of surface water and 3 liters of deep water); only the source and numbers of the inoculated cells differed. This was achieved in the surface inoculum–in-mix filtrate treatment by combining 4 liters of a surface 0.2- $\mu$ m filtrate with 3 liters of a deep 0.2- $\mu$ m filtrate and inoculating the mixed filtrate with 3 liters of unfiltered surface water

(Table 1). To ensure the maximal removal of bacteria from the filtrate, water was twice filtered through 0.2- $\mu$ m filters for experiment HS 893.

All cultures were placed in an environmental chamber and incubated in the dark at in situ temperatures of the inoculum (Table 1). Samples for bacterial abundance, DOC concentrations, and environmental deoxyribonucleic acid (DNA) were drawn at regular intervals for 5–10 d. An additional DOC sample was collected 1–2 months after the culture initiation to assess the effect of long-term incubations on DOC removal.

All statistical analyses were performed with the Statview 4.5 statistical program (Abacus Concepts), and the standard error was propagated through calculations according to Bevington (1969).

*Prokaryote abundance*—All experimental cell abundance samples were fixed with particle-free 25% glutaraldehyde (final concentration = 1.0%) and stored at 4 $^{\circ}$ C until preparation within 48 h of collection. Cells were filtered onto Ir-galan black-stained 0.2- $\mu$ m polycarbonate filters; samples were stained with either acridine orange (Hobbie et al. 1977) or 4'-6'-diamidino-2-phenylidole (DAPI) (Porter and Feig 1980) and enumerated with an Olympus AX-70 epifluorescence microscope (1,000 $\times$ ). Experiments comparing the acridine orange with the DAPI preparation did not show systematic variation in total prokaryote counts (unpubl. data). A carbon conversion factor (CCF) of  $1 \times 10^{-14}$  g C cell $^{-1}$  (Fuhrman 1981) was used to convert DAPI cell counts into carbon units. This often-cited CCF is within the range of 6– $12 \times 10^{-14}$  g C cell $^{-1}$  that is reported for open-ocean bacterioplankton (Fukuda et al. 1998; Gundersen et al. 2002). The slope of the regression of cell abundance from the initial time point until the end of the log-phase growth was used to assess whether cell production was significant ( $p < 0.05$ ). The change in cell carbon (biomass yield) was calculated as the difference between the average lag-phase concentration measured over the first 24 h and the maximum cell carbon concentration measured during the first week of incubation.

**DOC analysis**—DOC samples were analyzed in shore-based laboratories at the Bermuda Biological Station for Research or the University of California at Santa Barbara with a custom-built high-temperature combustion analyzer (Hansell and Carlson 2001) or a Shimadzu TOC-V. The operating conditions of the Shimadzu TOC-V were slightly modified from the manufacturer's model system. Condensation coils and an internal water trap were removed to reduce the system's dead space. The combustion tube contained 0.5-cm platinum (Pt) pillows placed on top of Pt alumina beads to improve peak shape and to reduce alteration in the combustion matrix throughout the run.

Potential DOC contamination from further filtration of culture medium was minimized by drawing samples directly from the incubation carboy's spigot into precombusted glass vials. Unfiltered DOC samples included bacterial carbon, which accounted for  $<1 \mu\text{mol C L}^{-1}$  (Carlson and Ducklow 1996; Carlson et al. 2002). To avoid the small error associated with day-to-day instrument variability, all samples from a given experiment were analyzed in a single day's run. All samples were systematically checked against low carbon water and deep Sargasso Sea reference waters (2,600 m) every fourth to sixth analysis (Hansell and Carlson 1998).

**Community structure analysis**—Initial DNA samples were collected within 12 h of inoculation and at subsequent time points for experiments HS 875 and HS 893. Aliquots of 250–500 ml were filtered onto 0.2- $\mu\text{m}$  Supor filters (Gelman Scientific) under gentle vacuum ( $\approx 100$  mmHg). Filters were placed in sealed plastic bags containing 2 ml of sucrose lysis buffer (20 mmol  $\text{L}^{-1}$  ethylenediamine-tetraacetic acid; 400 mmol  $\text{L}^{-1}$  NaCl; 0.75 mol  $\text{L}^{-1}$  sucrose; and 50 mmol  $\text{L}^{-1}$  Tris-HCl, pH 9.0) and stored at  $-70^\circ\text{C}$ . Total cellular nucleic acids were isolated from the samples by lysis with proteinase K (100  $\mu\text{g ml}^{-1}$ ) and sodium dodecyl sulfate (final 1%), followed by a phenol:chloroform:isoamyl alcohol (25:24:1) extraction (Giovannoni et al. 1990).

Ten nanograms of purified environmental DNA was used as a template for the length heterogeneity–polymerase chain reaction (LH-PCR) (Suzuki et al. 1998). The forward bacterial primer 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') was 5' labeled with phosphoramidite dye 6-FAM (6-carboxyfluorescein, Applied Biosystems). The reverse primer used was 338RPL (5'-GCW GCC WCC CGT AGG WGT-3'). Fluorescently labeled amplicons were separated electrophoretically on an ABI-377 automated DNA sequencer (Applied Biosystems). ABI Genescan software 3.01 was used to infer relative 16S rDNA gene abundances in microbial communities from DNA fragment lengths, as described by Suzuki et al. (1998). The PCR cycles were limited to 20 to minimize reannealing bias and ensure the highest-quality amplified product (Suzuki and Giovannoni 1996).

The peaks in an LH-PCR electropherogram correspond to rRNA genes that have been amplified and electrophoretically separated, with molecule length indicated on the horizontal axis and relative gene abundance on the vertical axis (measured as relative fluorescence). In this study, we used the raw electropherogram area data to calculate the percent area contribution of each peak to the sum of all peak areas between 300 and 380 base pairs (bp). The relative contribution

of each peak was used to demonstrate whether the overall community structure varied in space (depth), time, and as a function of mixing treatments.

We used the proportion of total peak area represented by each amplicon length to calculate the Shannon–Wiener index of diversity ( $H'$ ) (Krebs 1985). Here, we use amplicon length as a proxy for bacterial diversity. The calculated  $H'$  value represents an index of diversity and assesses both the richness and evenness of the bacterioplankton community over time and between treatments.  $H'$  was calculated according to the following formula:

$$H' = \sum_{i=1}^S (p_i)(\ln p_i)$$

where  $p_i$  is the relative abundance of amplicon  $i$  in proportion to the total electropherogram amplicon area,  $\ln$  is the natural log, and  $S$  is the total amplicon area.

## Results

**Prokaryote production and DOC utilization**—Care was taken to reduce potential DOC contamination by gently gravity filtering, thoroughly flushing filters, and minimizing the number of water transfers by directly filtering in-line from the Niskin into the filtrate collection carboy. Slight DOC contamination from experimental preparation and water handling could result in enhanced bacterial production and DOC removal rates if the heterotrophic prokaryotic assemblage were initially carbon limited. Direct measurements of DOC concentrations indicated that for all experimental treatments, except for B155 deep inoculum–in-deep filtrate, initial DOC concentrations were not different from in situ concentrations (at  $\mu\text{mol C L}^{-1}$  resolution) (Table 2). The observed prokaryote biomass production and subsequent DOC removal in these experiments were not, therefore, a result of the inadvertent contamination of labile DOC ( $\mu\text{mol L}^{-1}$  level) from experimental preparation.

**Prokaryotic production and DOC utilization**—Prokaryotic biomass production during the course of the incubation was significant in all but the B155 experiment. Biomass yield was calculated for treatments with significant cell production (Table 2). Biomass yield was greatest for all of the deep inoculum–in-surface filtrate treatments, averaging 0.21  $\mu\text{mol C L}^{-1}$  during the first 3–6 d of the incubation compared to  $\leq 0.07 \mu\text{mol C L}^{-1}$  for all other treatments (Figs. 1, 2; Table 2). DOC removal was significant (3–5  $\mu\text{mol C L}^{-1}$ ) during the course of a week to months in all of the deep inoculum–in-surface filtrate treatments (Table 2; Figs. 1, 2). The majority of the DOC removal was observed within the first 7–10 d of the incubation and was used at a growth efficiency of 5–13% for the deep inoculum–in-surface filtrate treatments (Table 2). The first-order decay constants ( $k$ ) of semilabile DOC in the deep inoculum–in-surface treatments ranged from 0.003 to 0.008  $\text{d}^{-1}$  (Table 2).

The potential effect of nutrient enrichment on biomass yield and DOC utilization due to mixing surface inoculum with nutrient-enriched deep waters was tested in the HS 893 experiment. Compared to the surface inoculum–in-surface

Table 2. Heterotrophic prokaryotic biomass yield, DOC concentrations at times of collection and various time points during the incubations, prokaryotic growth efficiency (GE), and first-order decay constant ( $k$ ) for DOC measured in the mixing experiments. Treatments are described in Table 1. The initials ns and nd indicate not significant and not determined, respectively. Values in parentheses are standard error. Blank spaces indicate that calculations could not be made because one of the necessary variables was insignificant.

Experiment ID	Treatment	Biomass yield* ( $\mu\text{mol C L}^{-1}$ )	DOC ( $\mu\text{mol C L}^{-1}$ )				GE‡	$k$ § ( $\text{day}^{-1}$ )
			In situ	Initial	$\approx 1$ week†	$\geq 1$ month		
HS 852	Surface inoculum–in-surface filtrate	0.06 (0.02)	68.1 (0.5)	68.5 (0.7)	68.6 (0.6)	67.8 (0.7)	0.13	0.003
	Deep inoculum–in-surface filtrate	0.24 (0.01)	63.7 (0.7)	63.3 (0.3)	61.4 (0.3)	59.8 (0.4)		
HS 875	Surface inoculum–in-surface filtrate	0.07 (0.02)	69.6 (0.6)	68.6 (0.6)	69.2 (0.5)	68.9 (0.5)	0.07	0.006
	Deep inoculum–in-surface filtrate	0.21 (0.01)	64.8 (0.9)	65.7 (0.4)	62.6 (0.5)	62.6 (0.5)		
HS 893	Surface inoculum–in-surface filtrate	0.04 (0.01)	68.2 (0.7)	68.7 (0.5)	68.5 (0.5)	67.8 (0.8)	0.05	0.008
	Deep inoculum–in-surface filtrate	0.17 (0.01)	64.2 (0.9)	64.7 (0.8)	61.0 (0.7)	59.6 (0.7)		
	Surface inoculum–in-mix filtrate	0.07 (0.01)	64.2 (0.9)	64.7 (0.7)	64.8 (0.7)	63.4 (0.6)		
	Deep inoculum–in-deep filtrate	0.03 (0.01)	54.4 (0.4)	53.8 (0.6)	52.8 (0.5)	53.6 (0.3)		
B155	Surface inoculum–in-surface filtrate	ns	67.6 (0.6)	68.2 (0.6)	68.5 (0.6)	nd		
	Deep inoculum–in-deep filtrate	ns	51.2 (0.3)	53.1 (0.5)	53.6 (0.4)	nd		

\* A carbon conversion factor of  $1 \times 10^{-14}$  g C cell $^{-1}$  (Fuhrman 1981) was used to convert DAPI cell counts to carbon units.

† The elapsed time for the  $\approx 1$ -week time point was 7 d for all experiments, except for HS 852, which was 10 d.

‡ GE =  $\Delta \text{cell carbon} / \Delta \text{DOC}$ . GE was calculated from changes in prokaryotic cell C and DOC during the first 7–10 d of the incubation.

§ The first-order decay constant ( $k$ ) was calculated for the  $\approx 1$ -week decay period by the following formula:  $k = (2.303/t) \times \log(A_0/A_t)$ , where  $t$  is the decay period,  $A_0$  is the initial DOC concentration, and  $A_t$  is the final DOC concentration.

|| Represents predicted in situ DOC concentrations of a simulated mixed water column as determined by diluting surface DOC concentrations by 30% with lower DOC concentrations from 250 m.

filtrate treatment, the biomass yield was enhanced (albeit small) in the surface inoculum–in-mix filtrate treatment, and  $1.3 \mu\text{mol L}^{-1}$  DOC was removed during the course of 37 d (Fig. 2; Table 2). However, compared to the deep inoculum–in-surface filtrate treatment, the biomass yield and DOC removal in the surface inoculum–in-mix filtrate treatment were three- and fourfold less, respectively (Table 2). Changes in bulk DOC concentrations could not be resolved for any other treatments (Table 2).

**Community structure**—The molecular technique LH-PCR was used to display the relative variability in the bacterioplankton community structure among various mixing treatments in experiments HS 875 (Fig. 3) and HS 893 (Fig. 4). The Shannon–Wiener diversity index ( $H'$ ) was used to assess the richness and evenness of amplicon length diversity, a proxy for bacterial species diversity, over time and between treatments (Table 3).

A 5-d time series of community structure variability demonstrated significant differences between the surface inoculum–in-surface filtrate treatment (white bars) and the deep inoculum–in-surface filtrate treatment (black bars) in experiment HS 875 (Fig. 3). The deep inoculum–in-surface filtrate treatment demonstrated a dramatic shift in bacterial community structure within 2 d of incubation. The diversity of the bacterial assemblage was greatly reduced after only 2 d and persisted at lower  $H'$  values compared to the surface inoculum–in-surface filtrate treatment through day 5 (Fig. 3; Table 3). Despite some variability in the percentage of contribution of individual peaks to total integrated peak area in the surface inoculum–in-surface treatment over time, the diversity index remained higher than in the deep inoculum–in-surface treatment (Table 3).

In experiment HS 875, the deep inoculum–in-surface treatment, the two major peaks found at amplicon lengths

342 and 350 bp composed  $>60\%$  of the total peak area from day 2 onward. Bacterial species represented by the amplicon 342 and 354 bp represented 1% and an undetectable fraction of total peak area, respectively, upon initiation of the surface inoculum–in-surface filtrate treatment and were undetectable at all subsequent time points of the surface inoculum–in-surface filtrate treatment.

The bacterial diversity in the surface inoculum–in-surface filtrate treatment of the HS 893 experiment increased slightly over 7 d (Table 3). However, as observed in the HS 875 experiment, the deep inoculum–in-surface filtrate treatment of HS 893 demonstrated a dramatic shift in community structure with a decrease in the Shannon–Wiener diversity index over time (Table 3). Bacterial species represented by amplicon 342 and 343 bp composed  $>60\%$  of the total peak area by day 7 in the deep inoculum–in-surface filtrate treatment of experiment HS 893 (Fig. 4b). The dominant 342- and 343-bp peaks and a minor peak at 353 bp found in the deep inoculum–in-surface filtrate treatment (Fig. 4b) were not detected in the surface inoculum–in-surface treatment (Fig. 4a), demonstrating a significant difference in the bacterial community that developed between the two treatments (Fig. 4a,b). The species represented by amplicon lengths 342 and 343 bp in experiment HS 893 were also present in the initial deep inoculum–in-deep filtrate sample (Fig. 4d). These data indicate that seed populations of these ribotypes were present in the mesopelagic water at the time the water was collected for this experiment but undetectable in the surface sample (Fig. 4a).

## Discussion

The experimental design used in our seawater culture studies uncoupled DOC production and consumption pro-

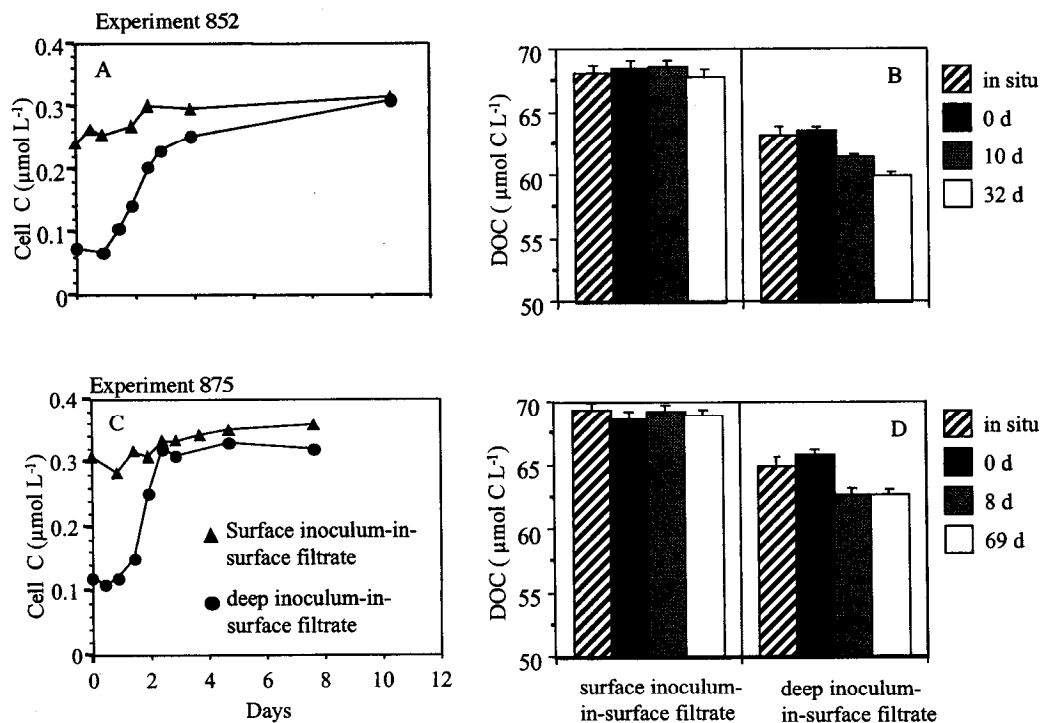


Fig. 1. Changes in (A, C) prokaryote cell biomass and (B, D) DOC concentrations during seawater culture experiments HS 852 and HS 875, respectively. See Table 1 for details about treatments. Panels B and D show DOC concentrations from in situ samples and samples taken at various time points throughout each of the experiments, including a sample measured after an extended incubation of  $\geq 1$  month. Error bars represent standard error.

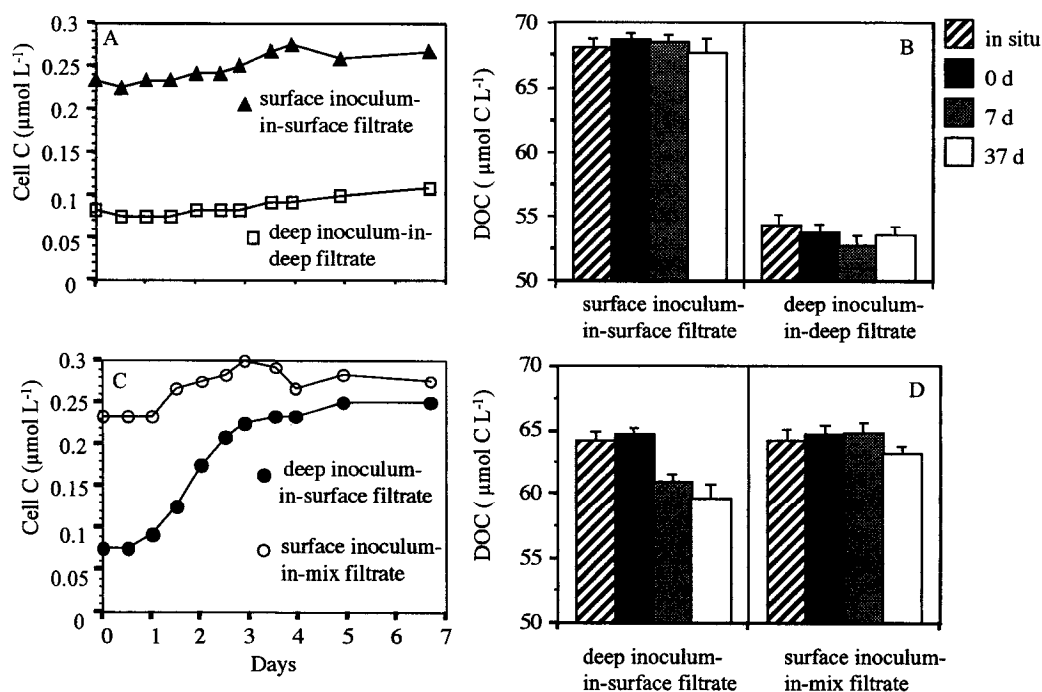


Fig. 2. Changes in (A, C) prokaryote cell biomass and (B, D) DOC concentrations during seawater culture HS 893. See Table 1 for details about treatments. DOC concentrations were also measured after an extended incubation (day 37). Error bars represent standard error.

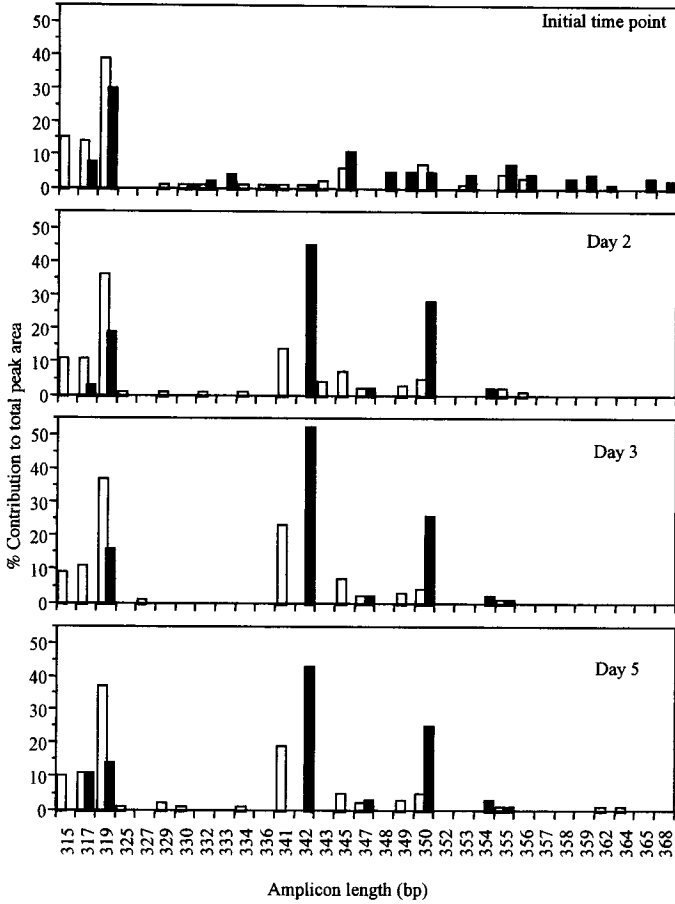


Fig. 3. Five-day time series of the percentage of area contribution of specific amplicon lengths to total electropherogram peak area between 300 and 380 bp for surface inoculum-in-surface filtrate treatment (white bar) and deep inoculum-in-surface filtrate (black bar) of experiment HS 875.

cesses, shut down new DOC production, and forced the natural heterotrophic prokaryotic assemblage to use the previously accumulated DOM. Our results are consistent with earlier Sargasso Sea experiments that reported little or no measurable change in DOC or respiration measurements when surface prokaryotes were forced to grow on unamended surface semilabile DOC from the northwestern Sargasso Sea (Carlson and Ducklow 1996; Carlson et al. 2002; Obnersterer et al. 2003). Our results are also consistent with

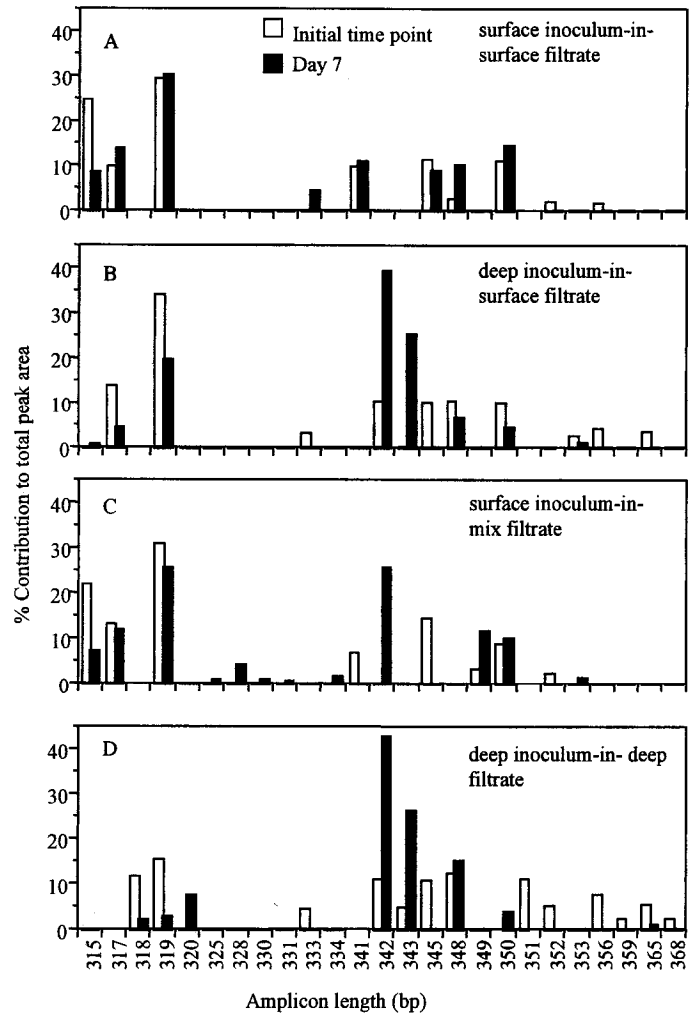


Fig. 4. Percentage of area contribution of specific amplicon lengths to total electropherogram peak area between 300 and 380 bp for samples collected for the initial sample (0.5 d) (white bars) and day 7 (black bars) from the treatments of experiment HS 893. Each of the four panels represents an individual treatment.

the field observation that shows that semilabile DOC in the upper euphotic zone at BATS is resistant to rapid microbial utilization and is maintained at relatively constant concentrations during stratified periods during the summer and au-

Table 3. A quantitative assessment of bacterial diversity for the HS 875 and HS 893 experiments calculated from amplicon length data using the Shannon–Wiener diversity index ( $H'$ ). See text for Shannon–Wiener index formula.

Experiment	Time point (days)	Shannon–Wiener diversity index ( $H'$ values)			
		Surface inoculum-in-surface filtrate	Deep inoculum-in-surface filtrate	Surface inoculum-in-mix filtrate	Deep inoculum-in-deep filtrate
HS875	0	1.96	2.51		
	2	2.09	1.29		
	3	1.76	1.19		
	5	1.99	1.48		
	7	1.93	1.54		
HS 893	0.5 (initial)	1.85	1.99	1.82	2.41
	7	1.93	1.54	1.96	1.52

tumn of each year (Hansell and Carlson 2001; Carlson et al. 2002).

Semilabile DOC removal was observed in treatments in which mesopelagic inoculum was added to surface filtrate and is consistent with the systematic removal of semilabile DOC within the upper mesopelagic after thermal stratification measured at BATS (Carlson et al. 1994; Hansell and Carlson 2001). The prokaryotes in the deep inoculum-in-surface filtrate treatments used the DOC at an efficiency of 5–13% (Table 2), an estimate comparable to oceanic surface assemblages (Carlson and Ducklow 1996; del Giorgio and Cole 1998).

The first-order decay constants ( $k$ ) of DOC in the upper mesopelagic at BATS ranged from 0.0002 to 0.003 d<sup>-1</sup> (Hansell and Carlson 2001) and were less than or equal to those observed in the deep inoculum-in-surface filtrate treatments. However, despite these comparable  $k$  values, DOC was drawn down to an average of only  $60.7 \pm 1.7 \mu\text{mol L}^{-1}$  in the deep inoculum-in-surface filtrate treatments (Table 2), compared to an average background concentration of  $54 \pm 2 \mu\text{mol C L}^{-1}$  measured for the mesopelagic zone (250 m) at BATS. Thus, in our experiments, DOC removal by prokaryotic production became limited by unknown factors. These factors may have included inorganic nutrient limitation and viral or grazing mortality, or perhaps the resulting community structure in our experiments differed from that present in situ. Alternatively, other mechanisms may be responsible for a portion of DOC removal in situ, such as advection or sorption of DOC to sinking particles (Druffel et al. 1998).

Nevertheless, the experimental results demonstrate that mixing mesopelagic inoculum with surface filtrate of the northwestern Sargasso Sea enhanced prokaryotic biomass yield and net removal of semilabile DOC. Potential factors that contribute to these responses include (1) the addition of a limiting inorganic nutrient present in the 250-m inoculum that stimulates microbial production; (2) the introduction of surface semilabile DOM that was more bioreactive than the DOM normally present in the mesopelagic zone; (3) the stimulation of a specialized mesopelagic prokaryotic community; and (4) some combination of these factors.

**Nutrient limitation:** Several experimental studies conducted in the Sargasso Sea have reported enhanced bacterial production (Cotner et al. 1997; Rivkin and Anderson 1997; Caron et al. 2000) or respiration rates (Obernosterer et al. 2003) in response to inorganic nutrient additions (specifically phosphorus). These studies indicated that DOC bioavailability was not limiting at the time their experiments were conducted. However, other studies did not detect enhanced bacterial production or changes in DOC concentrations ( $\mu\text{mol L}^{-1}$  level) after cultures were amended with inorganic N and P, indicating that bioavailable DOC was limiting at the time of their experiments (Carlson and Ducklow 1996; Carlson et al. 2002). DOC concentrations were not measured in the former studies, making it difficult to assess how in situ and initial DOC concentrations compared to those of the latter study. Recent Sargasso Sea seawater culture work (Oliver, Carlson, and Ducklow data not shown) demonstrated that N plus P amendments stimulated bacterial production in a cul-

ture initiated coincident with the passage of a mesoscale cyclonic eddy. However, the same treatment failed to stimulate bacterial production in a culture conducted 1 week prior to the passage of the mesoscale feature. The sum of all these results indicates that the bioavailability of DOC and nutrient limitation vary in space and time. Indeed, short-lived aperiodic bursts of net autotrophy have been shown to give rise to O<sub>2</sub> supersaturation in oceanic systems (Karl et al. 2003). These net autotrophy events could affect DOC source-sink relationships, resulting in the short-term accumulation of bioavailable DOC.

In the present study, mixing nutrient-rich mesopelagic filtrate with a mixture of filtered and unfiltered surface water (surface inoculum-in-mix filtrate) did produce an increase in biomass yield and DOC removal compared to the surface inoculum-in-surface filtrate treatment, perhaps signifying some level of nutrient limitation. However, mixing mesopelagic nutrients with a surface inoculum (surface inoculum-in-mix filtrate treatment) was not sufficient to promote the magnitude of DOC drawdown observed in the deep inoculum-in-surface filtrate treatments (Table 2), indicating that another factor was involved. The lack of semilabile DOC removal by surface-water prokaryotic assemblages observed in the present study and previous studies (Carlson and Ducklow 1996; Carlson et al. 2002) cannot be attributed solely to nutrient limitation.

**DOM quality:** Changes in DOC concentrations could not be resolved in the deep inoculum-in-deep filtrate treatment despite the presence of elevated inorganic nutrients concentrations (Table 2). In comparison, the enhanced biomass yield and DOC utilization in the deep inoculum-in-surface filtrate treatment (Fig. 4; Table 2) indicated that carbon limitation was alleviated when surface DOM was introduced to the deeper prokaryotic assemblage. Using a ratio of aldose to total organic carbon as an index of bioreactivity, Skoog and Benner (1997) demonstrated greater organic matter bioreactivity at the surface than at deeper depths. Similar vertical trends in DOC bioreactivity have been observed in the upper 500 m at the BATS site (Goldberg and Carlson unpubl. data). Thus, although the DOC in the surface waters was not available for surface microbial degradation, it was of higher quality relative to the DOC found in the mesopelagic and was available to the deeper microbial assemblage after introduction through mixing.

Significant O<sub>2</sub> respiration has been reported for a 10-d incubation of BATS water from 300 m (Obernosterer et al. 2003). DOC was not measured in that study, so it is unknown whether the difference between this and the present study was due to physiologic differences or if there was a pulse in bioreactive DOC during their sampling period.

**Community structure:** LH-PCR is a high-resolution electrophoretic method used to monitor prokaryotic community structure shifts. Natural-length polymorphisms in the 5' region of these molecules are reflected in the lengths of DNA fragments of the PCR amplicon (Suzuki et al. 1998). LH-PCR is highly reproducible and is insensitive to growth rate (Suzuki et al. 1998); however, peak areas do not quantitatively reflect microbial abundances because of variations in



the copy number of rRNA genes in cells and biases in the PCR amplification, and there is some overlap in the lengths of genes originating from different species (Suzuki et al. 1998). A quantitative approach such as fluorescence in situ hybridization (FISH) is an obvious next step to assess the dynamics of specific contributors of the prokaryotic community; however, this technique requires prior identification of the target organisms and will not detect unsuspected changes in community structure. Nonetheless, LH-PCR data effectively show changes in the microbial communities, provide useful information about the phylogenetic composition, and are being used to design specific FISH probes for future experiments.

Results of the deep inoculum-in-surface filtrate treatments demonstrated enhanced bacterial production, significant DOC removal (Figs. 1, 2), and dramatic shifts in bacterial community structure within a few days of incubation (Figs. 3, 4b). Suzuki et al. (1998) identified several species of beta, delta, and gamma proteobacteria as well as flavobacteria that produced LH-PCR amplicons lengths of 342, 343, and 353 bp. We did not identify the species that grew in the deep inoculum-in-surface filtrate treatments; however, the data were sufficient to prove that they were undetectable at later time points of the surface inoculum-in-surface treatments.

It may seem suspicious that such a large shift (undetectable to >40%) for a single ribotype (342 bp) occurred over a period of 2 d (Fig. 3). Because LH-PCR is a measure of the relative contribution of amplicons, this increase could be significantly greater than the actual increase in the abundance of the replacement species if the replacement species has a higher rRNA gene copy number than the average rRNA gene copy number for the species originally present. However, previous studies have also shown similar rapid shifts in bacterial community structure and reduction in bacterial diversity coincident with enhanced bacterial cell production in grazer-reduced dilution cultures (Lebaron et al. 1999; Fuchs et al. 2000; Massana et al. 2001).

Using group-specific FISH probes, Fuchs et al. (2000) quantitatively monitored changes in bacterial community structure. In their seawater culture experiments, in which a 0.8- $\mu\text{m}$  filtrate was diluted 90% with a 0.2- $\mu\text{m}$  filtrate, they observed an increase in gamma proteobacteria densities from 8% of total cells in undiluted coastal seawater to >60% in their 90% diluted cultures in just 2 d. The specific growth rate of such an increase would have to have been approximately 2.7  $\text{d}^{-1}$ . We have relatively persuasive evidence based on FISH measurements that specific growth rates of some *Alteromonas* species can be as high as 2–4  $\text{d}^{-1}$  in seawater cultures, provided that substrates are available (data not shown). We are not suggesting that cells in nature grow at these rates commonly, but rather that rapid transitions in microbial community structure are entirely plausible and occur commonly in seawater cultivation experiments such as these. In fact, it has been suggested that microbial communities include r and k growth strategists, the former being species with high rRNA gene copy numbers, and that the number of rRNA genes correlates with the rate at which phylogenetically diverse bacteria respond to resource availability (Klappenbach et al. 2000). This would suggest that phenotypic effects associated with rRNA gene copy number

are indicative of ecological strategies that influence the structure of natural microbial communities (Klappenbach et al. 2000).

Reports of community structure shift in response to the addition of potentially limiting nutrients are conflicting. Hutchins et al. (2001) reported minimal shifts in the bacterial community after Fe was added to cultures, despite significant changes in bacterial production in high-chlorophyll low-nutrient regions. Carlson et al. (2002) observed little difference in resulting community structure upon amending Sargasso Sea culture with N and P compared to an unamended culture. However, in other experiments, shifts in community structure have been observed when deep nutrients were mixed with surface inoculum (Fig. 4c) or when inorganic nutrients were added to surface-water seawater cultures (unpubl. data). Note also, however, that qualitative shifts were observed in the previous experiments and that those displayed in Fig. 4c,d were associated with little biomass yield. Because LH-PCR displays relative changes in community structure, these results imply that the rRNA gene content of the inoculated biomass was altered by a process in which rRNA genes of some species were displaced with other populations having a different rRNA gene profile, initially minor, with minimal changes in total biomass.

The qualitative shifts observed in the present study account for changes in the bacterial domain only, because we did not use archaeal primers. *Archaea* organisms are potentially important contributors to microbial processes in the oceanic water column (Karner et al. 2001). Little is known about the metabolism of *Archaea* organisms in the mesopelagic zone, but it is possible that *Archaea* organisms actively oxidize recalcitrant organic matter found at depth. It is also possible that some of the biomass yield observed in the deep inoculum-in-surface filtrate treatments was due to archaeal production.

Vertical stratification of major prokaryotic groups between the oceanic euphotic and aphotic zones has been observed (Giovannoni et al. 1996; Karner et al. 2001; Morris et al. 2002). The mechanisms responsible for spatial variabilities of group or domain-specific profiles are not well understood. Conceptual models provide compelling reasons to believe that the growth of specific microbial populations in planktonic ecosystems is linked to the composition and amount of DOM as well as to the availability of inorganic nutrients. We suggest that the quality of DOC introduced into the mesopelagic zone as well as the nutrient regime and the microbial community structure all create a condition in which surface-derived semilabile DOC can be remineralized.

We hypothesize that the heterotrophic microbial assemblages present in the euphotic zone at BATS are adapted to low-nutrient concentrations and obtain their carbon and energy requirements from constantly produced, labile DOM, ultimately driven by daily primary production. Tight coupling of DOM production and consumption maintains relatively constant DOC stocks within the upper euphotic zone (Hansell and Carlson 2001; Carlson et al. 2002). If prokaryotes adapted to use recalcitrant DOM are in competition for inorganic nutrients with those that are adapted to use labile DOM, they may grow at slower rates and may be further

suppressed by grazing pressure, thereby limiting semilabile DOM remineralization within the euphotic zone.

Controls on community structure and DOC utilization likely vary in space and time. The most salient observation from the present study is that, when deep water was mixed with surface filtrate, a drawdown of DOC and a concomitant shift in bacterial community structure were observed. These data do not prove that DOC drawdown is determined by community composition alone. Indeed, to be complete, such a scenario would need to explain why some deep-water microorganisms are not prominent in surface waters under summer-stratified conditions. Therefore, any explanation for the observations accumulated to date must assume that multiple factors working together are involved in DOC accumulation in the upper surface layer and in DOC removal at depth. It appears that these factors do not come together in the surface waters during summer stratification at BATS.

### References

- ALUWIHARE, L. I., AND D. J. REPETA. 1999. A comparison of the chemical characteristics of oceanic DOM and extracellular DOM produced by marine algae. *Mar. Ecol. Prog. Ser.* **186**: 105–117.
- AMMERMAN, J. W., J. A. FUHRMAN, A. HAGSTRÖM, AND F. AZAM. 1984. Bacterioplankton growth in seawater: I. Growth kinetics and cellular characteristics in seawater cultures. *Mar. Ecol. Prog. Ser.* **18**: 31–39.
- AZAM, F. 1998. Microbial control of oceanic carbon flux: The plot thickens. *Science* **280**: 694–696.
- BEVINGTON, P. R. 1969. Data reduction and error analysis for the physical sciences. McGraw.
- CARLSON, C. A. 2002. Production and removal processes, p. 91–151. *In* D. A. Hansell and C. A. Carlson [eds.], *Biogeochemistry of marine dissolved organic matter*. Academic Press.
- , AND H. W. DUCKLOW. 1996. Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. *Aquat. Microb. Ecol.* **10**: 69–85.
- , ———, AND A. F. MICHAELS. 1994. Annual flux of dissolved organic carbon from the euphotic zone in the North-western Sargasso Sea. *Nature* **371**: 405–408.
- , ———, AND T. D. SLEETER. 1996. Stocks and dynamics of bacterioplankton in the Northwestern Sargasso Sea. *Deep-Sea Res. II* **43**: 491–515.
- , AND OTHERS. 2002. The effect of nutrient amendments on bacterioplankton growth, DOC utilization, and community structure in the northwestern Sargasso Sea. *Aquat. Microb. Ecol.* **30**: 19–36.
- CARON, D. A., E. L. LIN, R. W. SANDERS, M. R. DENNETT, AND U. G. BERNIGER. 2000. Response of bacterioplankton and phytoplankton to organic carbon and inorganic nutrient additions in contrasting oceanic ecosystems. *Aquat. Microb. Ecol.* **22**: 175–184.
- CHERRIER, J., J. E. BAUER, AND E. R. M. DRUFFEL. 1996. Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern North Pacific surface waters. *Mar. Ecol. Prog. Ser.* **139**: 267–279.
- COPIN-MONTÉGUT, G., AND B. AVRIL. 1993. Vertical distribution and temporal variation of dissolved organic carbon in the North-Western Mediterranean Sea. *Deep-Sea Res.* **40**: 1963–1972.
- COTNER, J. B., J. W. AMMERMAN, E. R. PEELE, AND E. BENTZEN. 1997. Phosphorus-limited bacterioplankton growth in the Sargasso Sea. *Aquat. Microb. Ecol.* **13**: 141–149.
- DEL GIORGIO, P., AND J. J. COLE. 1998. Bacterial growth efficiency in natural aquatic systems. *Annu. Rev. Ecol. Syst.* **29**: 503–541.
- DOVAL, M. D., AND D. A. HANSELL. 2000. Organic carbon and apparent oxygen utilization in the western South Pacific and central Indian Ocean. *Mar. Chem.* **68**: 249–264.
- DRUFFEL, E. R. M., S. GRIFFIN, J. E. BAUER, D. M. WOLGAST, AND X.-C. WANG. 1998. Distribution of particulate organic carbon and radiocarbon in the water column from the upper slope to the abyssal NE Pacific Ocean. *Deep-Sea Res. II* **45**: 667–687.
- FUCHS, B. M., M. V. ZUBKOV, K. SAHM, P. H. BURKILL, AND R. AMANN. 2000. Changes in community composition during dilution cultures of marine bacterioplankton as assessed by flow cytometric and molecular biological techniques. *Environ. Microbiol.* **2**: 191–201.
- FUHRMAN, J. A. 1981. Influence of method on the apparent size distribution of bacterioplankton cells: Epifluorescence microscopy compared to scanning electron microscopy. *Mar. Ecol. Prog. Ser.* **5**: 103–106.
- FUKUDA, R., H. OGAWA, T. NAGATA, AND I. KOIKE. 1998. Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. *Appl. Environ. Microbiol.* **64**: 3352–3358.
- GIOVANNONI, S. J., E. F. DELONG, T. M. SCHMIDT, AND N. R. PACE. 1990. Tangential flow filtration and preliminary phylogenetic analysis of marine picoplankton. *Appl. Environ. Microbiol.* **56**: 2572–2575.
- , AND M. RAPPÉ. 2000. Evolution, diversity and molecular ecology of marine prokaryotes, p. 47–84. *In* D. L. Kirchman [ed.], *Microbial ecology of the oceans*. Wiley.
- , ———, K. VERGIN, AND N. ADAIR. 1996. 16S rRNA genes reveal stratified open ocean bacterioplankton populations related to the green non-sulfur bacteria phylum. *Proc. Natl. Acad. Sci. USA* **93**: 7979–7984.
- GUNDERSEN, K., M. HELDAL, D. A. PURDIE, AND A. H. KNAP. 2002. Elemental C, N, and P cell content of individual bacteria collected at the Bermuda Atlantic Time-Series Study (BATS) site. *Limnol. Oceanogr.* **47**: 1525–1530.
- HANSELL, D. A. 2002. DOC in the global ocean carbon cycle, p. 685–716. *In* D. A. Hansell and C. A. Carlson [eds.], *Biogeochemistry of marine dissolved organic matter*. Academic Press.
- , AND C. A. CARLSON. 1998. Deep ocean gradients in dissolved organic carbon concentrations. *Nature* **395**: 263–266.
- , AND ———. 2001. Biogeochemistry of total organic carbon and nitrogen in the Sargasso Sea: Control by convective overturn. *Deep-Sea Res. II* **48**: 1649–1667.
- HOBBIE, J. E., R. J. DALEY, AND S. JASPER. 1977. Use of Nuclepore filters for counting bacteria by epifluorescence microscopy. *Appl. Environ. Microbiol.* **33**: 1225–1228.
- HUTCHINS, D. A., B. J. CAMPBELL, M. T. COTTRELL, S. TAKEDA, AND S. C. CARY. 2001. Response of marine bacterial community composition to iron additions in three iron-limited regimes. *Limnol. Oceanogr.* **46**: 1535–1545.
- KARL, D. M., E. A. LAWS, P. MORRIS, P. J. L. WILLIAMS, AND S. EMERSON. 2003. Metabolic balance of the open sea. *Nature* **426**: 32.
- KARNER, M. B., E. F. DELONG, AND D. M. KARL. 2001. *Archaeal* dominance in the mesopelagic zone. *Nature* **409**: 507–510.
- KIRCHMAN, D. L. 1990. Limitation of bacterial growth by dissolved organic matter in the subarctic Pacific. *Mar. Ecol. Prog. Ser.* **62**: 47–54.
- , C. LANCELLOT, M. FASHAM, L. LEGENDRE, G. RADACH, AND M. SCOTT. 1993. Dissolved organic matter in biogeochemical models of the ocean, p. 209–225. *In* G. T. Evans and M. J. R.

- Fasham [eds.], Towards a model of ocean biogeochemical processes. Springer.
- KLAPPENBACH, J. A., J. M. DUNBAR, AND T. M. SCHMIDT. 2000. rRNA operon copy number reflects ecological strategies of bacteria. *Appl. Environ. Microbiol.* **66**: 1328–1333.
- KREBS, C. J. 1985. *Ecology: The experimental analysis of distribution and abundance*, 3rd ed. Harper.
- MASSANA, R., C. PEDRÓS-ALIÓ, E. O. CASAMAYOR, AND J. M. GASOL. 2001. Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. *Limnol. Oceanogr.* **46**: 1118–1188.
- MCCARTHY, M. D., J. I. HEDGES, AND R. BENNER. 1998. Major bacterial contribution to marine dissolved organic nitrogen. *Science* **281**: 231–234.
- MICHAELS, A. F., AND A. H. KNAP. 1996. Overview of the U.S. JGOFS Bermuda Atlantic Time-Series Study and Hydrostation S program. *Deep-Sea Res. II* **43**: 157–198.
- MORRIS, R. M., S. A. CONNON, M. RAPPÉ, K. L. VERGIN, W. A. SIEBOLD, C. A. CARLSON, AND S. J. GIOVANNONI. 2002. High cellular abundance of the SAR11 bacterioplankton clade in seawater. *Nature* **420**: 806–809.
- OBERNOSTERER, I., N. KAWASAKI, AND R. BENNER. 2003. P-limitation of respiration in the Sargasso Sea and uncoupling of bacteria from P-regeneration in size-fractionation experiments. *Aquat. Microb. Ecol.* **32**: 229–237.
- OGAWA, H., Y. AMAGAI, I. KOIKE, K. KAISER, AND R. BENNER. 2001. Production of refractory dissolved organic matter by bacteria. *Science* **292**: 917–920.
- PORTER, K. G., AND Y. S. FEIG. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* **25**: 943–948.
- RIVKIN, R. B., AND M. R. ANDERSON. 1997. Inorganic nutrient limitation of oceanic bacterioplankton. *Limnol. Oceanogr.* **42**: 730–740.
- SHIAH, F. K., AND H. W. DUCKLOW. 1994. Temperature regulation of heterotrophic bacterioplankton abundance, production, and specific growth rate in Chesapeake Bay. *Limnol. Oceanogr.* **39**: 1243–1258.
- SKOOG, A., AND R. BENNER. 1997. Aldoses in various size fractions of marine organic matter: Implications for carbon cycling. *Limnol. Oceanogr.* **42**: 1803–1813.
- SPRINTALL, J., AND M. TOMCZAK. 1992. Evidence of the barrier layer in the surface layer of the tropics. *J. Geophys. Res.* **97**: 7305–7316.
- STEINBERG, D. K., C. A. CARLSON, N. R. BATES, R. J. JOHNSON, A. F. MICHAELS, AND A. H. KNAP. 2001. Overview of the U.S. JGOFS Bermuda Atlantic Time-Series Study (BATS): A decade-scale look at ocean biology and biogeochemistry. *Deep-Sea Res. II* **48**: 1405–1447.
- SUZUKI, M., AND S. J. GIOVANNONI. 1996. Bias caused by template annealing in the amplification of 16S rRNA genes by the PCR. *Appl. Environ. Microbiol.* **62**: 625–630.
- , M. S. RAPPÉ, AND S. J. GIOVANNONI. 1998. Kinetic bias in estimate of coastal picoplankton community structure obtained by measurements of small-subunit rRNA gene PCR amplicon length heterogeneity. *Appl. Environ. Microbiol.* **63**: 983–989.
- THINGSTAD, T. F., A. HAGSTROM, AND F. RASSOULZADEGAN. 1997. Accumulation of degradable DOC in surface waters: Is it caused by a malfunctioning microbial loop? *Limnol. Oceanogr.* **42**: 398–404.
- VENTER, J. C., AND OTHERS. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. *Science* **304**: 66–72.

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