

P-limitation of respiration in the Sargasso Sea and uncoupling of bacteria from P-regeneration in size-fractionation experiments

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ABSTRACT: Community respiration rates were measured in unfiltered seawater collected in the upper 75 m of the water column along a transect in the Sargasso Sea and at the Bermuda Atlantic Time-series Study (BATS) station (31° 50' 00 N; 64° 10' 00 W) during a cruise in June and July 2001. Community respiration rates in the upper 75 m of the water column averaged $1.1 \pm 0.4 \mu\text{M O}_2 \text{ d}^{-1}$ and exhibited significant spatial and temporal variability. Concurrent determination of the heterotrophic and autotrophic community revealed no relationship between community respiration and the abundance of any of the major metabolic groups. Addition of inorganic nutrients (NO_3 and PO_4) and organic carbon (glucose) indicated that community respiration was P-limited in the surface mixed layer. Size-fractionation experiments indicated that the abundance of heterotrophic bacteria in the $<0.6 \mu\text{m}$ fraction was ~80% of the abundance in unfiltered seawater, but respiration rates in the $<0.6 \mu\text{m}$ size fraction accounted for only 23% of community respiration. Addition of P to the $<0.6 \mu\text{m}$ size fraction increased respiration rates ~2-fold, indicating bacterial respiration was P-limited. It appears that the uncoupling of bacteria from nutrient regeneration in size fractionation experiments resulted in a reduction of bacterial metabolism in the P-limited surface waters of the Sargasso Sea.

KEY WORDS: Community respiration · Size-fractionation · P-limitation · Sargasso Sea · BATS

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INTRODUCTION

About half the marine primary production passes through the reservoir of dissolved organic carbon (DOC) and is processed by heterotrophic bacterioplankton (Cole et al. 1988, Ducklow 2000). A strong spatial and temporal coupling between these processes has been suggested by concurrent measurements of phytoplankton and bacterial production and biomass (Kirchman et al. 1991, White et al. 1991). However, there is increasing evidence suggesting that DOC may accumulate in the photic zone on both daily and seasonal timescales (Copin-Montégut & Avril 1993, Carlson et al. 1994, Zweifel et al. 1995). This indicates that the processing of DOC by heterotrophic bacterioplankton might be temporally lim-

ited by the availability of nitrogen or phosphorus (Williams 1995).

To determine the factors limiting the processing of DOC, most previous studies have investigated heterotrophic bacterial production (Zweifel et al. 1993, Kirchman & Rich 1997, Rivkin & Anderson 1997), while a limited number of studies have addressed the factors limiting heterotrophic bacterial respiration (Pomeroy et al. 1995, Smith & Kemp 2003). A large fraction of community respiration is attributable to the oxidation of DOC, making respiration measurements a relatively straightforward and quantitative estimate of the biological processing of organic carbon (Williams 1984, Hopkinson et al. 1989).

Limitation of heterotrophic bacterial production by inorganic nutrients has been reported for both fresh-

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water (Currie & Kalff 1984, Vadstein et al. 1988, Toolan et al. 1991) and marine systems. Several studies performed in the Atlantic Ocean (Cotner et al. 1997, Rivkin & Anderson 1997, Kuipers et al. 2000) and in marginal seas (Pomeroy et al. 1995, Thingstad & Rassoulzadegan 1995, Zweifel et al. 1995) indicate that P is often the primary limiting nutrient for marine heterotrophic bacterial production. In contrast, heterotrophic bacterial production in the Pacific Ocean has been characterized by several authors as organic carbon- or energy-limited (Kirchman 1990, Cherrier et al. 1996, Kirchman & Rich 1997, Donachie et al. 2001). These studies show that the factors limiting heterotrophic bacterial production vary spatially and temporally. They further indicate that the degree to which bacterial metabolism is limited by sources other than carbon often influences the coupling between primary production and the processing of DOC.

The bacterial contribution to community respiration is commonly assessed by size fractionation (Williams 1981, Griffith et al. 1990, Danieri et al. 1994, Sampou & Kemp 1994). However, separation of the microbial community by size fractionation bears a major disadvantage. Interactions among microbial communities of different size classes are inhibited by size fractionation, which in turn can affect their overall metabolic activity (Sherr et al. 1988). The interaction of members of different metabolic groups and size classes might be particularly important in oligotrophic environments that are characterized by low concentrations of substrates and rapid cycling of inorganic nutrients (Legendre & Rassoulzadegan 1995).

In the present study we report that community and bacterial respiration is P-limited during the summer in surface waters of the Sargasso Sea, and our results further indicate that bacterial respiration could be underestimated in size-fractionation experiments due to the separation of various functional groups of the microbial community.

MATERIALS AND METHODS

The present study was carried out during a cruise aboard RV 'Cape Hatteras' along a transect in the Sargasso Sea and at the Bermuda Atlantic Time-series Study (BATS) site between 20 June and 11 July 2001 (Table 1). Seawater samples were collected using 30 l Niskin bottles mounted on a General Oceanics rosette sampler equipped with a CTD (Seabird 911).

Respiration rate measurements. Community respiration rates were measured in dark incubations of unfiltered seawater in 300 and 60 ml BOD bottles. Seawater was collected from the upper 25 m at all stations

and from the surface to 75 m depth at BATS and Stns 4 and 5 (Table 1). The depth of the surface mixed layer varied from 6 to 26 m. The depth of the surface mixed layer was determined based on the temperature profile. Seawater was collected within the surface mixed layer in 10 out of 14 experiments. Except for Stns 1 and 3, water samples were collected between 07:00 and 10:00 h throughout the cruise, and on 2 consecutive days water samples were additionally collected at 16:30 and 20:00 h.

The BOD bottles were filled directly from Niskin bottles with unfiltered seawater using silicon tubing. Prior to sampling, the BOD bottles were rinsed with 1N HCL, Milli-Q water and seawater (3×) from the respective depth layer. Continuous water flow from the Niskin bottle was allowed throughout the sampling procedure. Respiration measurements were performed in triplicate or quadruplicate in the dark at *in situ* temperatures ($\pm 1^\circ\text{C}$). Except for 3 time-course experiments (see below) all BOD bottles were incubated for 24 h. The respiration rate was calculated as the difference between initial and final concentrations of dissolved oxygen. The concentration of dissolved oxygen was determined by Winkler titration of a 50 ml subsample with an automated potentiometric end-point detection system (Biddanda et al. 1994). No statistical difference in respiration rates between 60 and 300 ml BOD bottles was observed (*t*-test, $p > 0.05$).

To evaluate the kinetics of respiration during a 48 h incubation period, time-course experiments were performed at Stns 1 and 3 and at BATS (Table 1). Unfiltered seawater collected at 25 m depth at Stns 1 and 3 and at 22 m depth at BATS was incubated in BOD bottles as described above. The decrease in dissolved oxygen concentration was determined at Stns 1 and 3 after 24 and 48 h and at BATS after 12, 24, 36 and 48 h, each sample in triplicate BOD bottles. Respiration rates were determined as the slope of a linear regression of the time-course data.

Size fractionation. The contribution of bacterioplankton to community respiration was determined in water samples that were passed through 0.6 μm pore-size Nuclepore QR polycarbonate filter cartridges

Table 1. Location of sampling stations in the Sargasso Sea. BATS: Bermuda Atlantic Time-series Study

Station	Date (mm/dd)	Lat ($^\circ\text{N}$)	Long ($^\circ\text{W}$)
1	06/21	34° 26' 30	72° 34' 19
2	06/22	36° 10' 25	70° 43' 07
3	06/23	36° 46' 76	69° 09' 00
BATS	06/27–07/07	31° 50' 00	64° 10' 00
4	07/08	31° 50' 07	66° 14' 73
5	07/09	32° 16' 71	69° 00' 99

using a peristaltic pump. The $<0.6 \mu\text{m}$ filtrate was incubated in 60 ml BOD bottles for 24 h as described above.

Long-term experiments. In addition to 24 h incubations, respiration rates were also measured during long-term experiments performed at BATS. Seawater was collected from 50, 150 and 300 m and filtered through $0.6 \mu\text{m}$ pore-size Nuclepore QR polycarbonate filter cartridges and incubated in 60 ml BOD bottles at $20 \pm 1^\circ\text{C}$ for 5 to 10 d.

Nutrient enrichment experiments. The effect of organic carbon (glucose) and inorganic (NO_3 and PO_4) nutrient additions on respiration rates was determined during short- (24 h) and long-term (5 d) experiments at BATS and at Stn 5. Unfiltered seawater samples (24 h incubations) were transferred to BOD bottles as described above and subsequently spiked with nutrients resulting in final concentrations of $1 \mu\text{M}$ glucose and NO_3 and $0.1 \mu\text{M}$ PO_4 . Either single nutrients or a combination of all nutrients were added to seawater collected in the surface mixed layer, at 30, 40, 50 and 75 m. Additionally, at BATS, PO_4 -amended ($0.1 \mu\text{M}$ final conc.) $0.6 \mu\text{m}$ filtered seawater collected at 10 and 50 m was incubated for 1 and 5 d, respectively. All nutrient stocks were prepared in deionized Milli-Q water and kept frozen until used. Incubation of the nutrient-amended treatments followed the protocol described above.

Enumeration of the heterotrophic and autotrophic community. For enumeration of the plankton community in unfiltered seawater and the $<0.6 \mu\text{m}$ size fraction, duplicate 15 ml subsamples were fixed with 2% formaldehyde (final conc.) and stored at 4°C for ~ 3 wk. In the laboratory, 10 ml subsamples were stained with DAPI (Porter & Feig 1980) and filtered onto black Nuclepore filters ($0.2 \mu\text{m}$ pore size). Subsequent enumeration was performed with an epifluorescence microscope (Olympus BX) at $1250\times$ magnification. The abundance of heterotrophic bacteria and heterotrophic protists was determined using an excitation filter of 365 nm, while an excitation wavelength of 480 nm was used for the enumeration of autofluorescent cells (Sherr et al. 1993). For bacterial abundance at least 300 bacteria per sample were counted and 60 fields were enumerated for the abundance of heterotrophic protists, cyanobacteria and autotrophic nanoplankton.

RESULTS

Community respiration rates

Time-course experiments with unfiltered seawater revealed a fairly linear decrease in the concentration of dissolved oxygen over a 48 h incubation period at Stns 1 and 3 and at BATS (Fig. 1). The average respira-

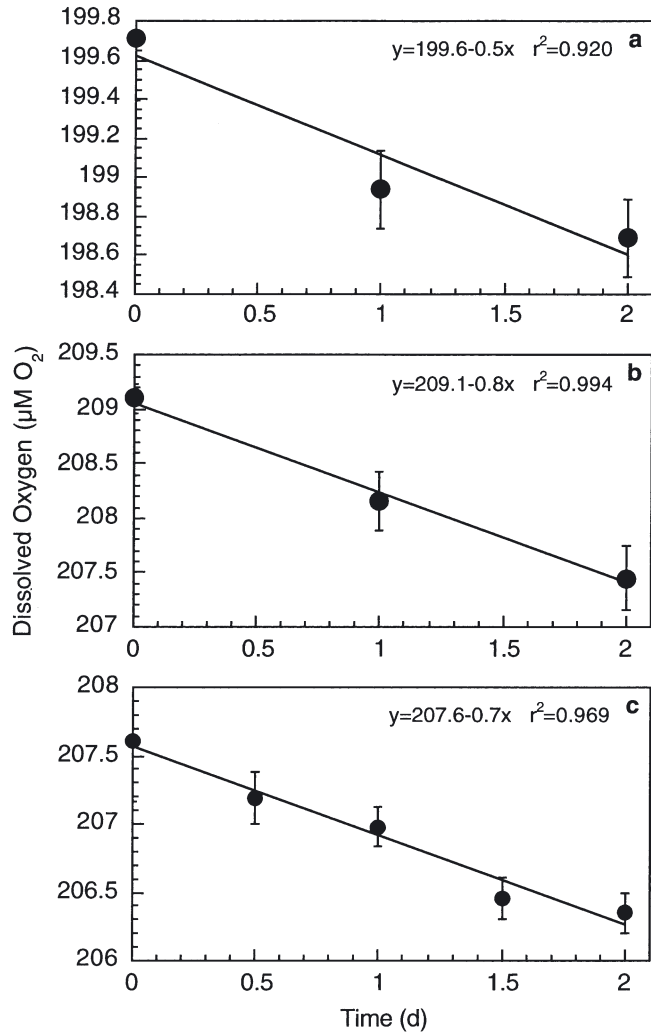


Fig. 1. Changes in dissolved oxygen concentration over time in unfiltered seawater collected at (a) 25 m at Stn 1, (b) 25 m at Stn 3, and (c) 22 m at the Bermuda Atlantic Time-series Study (BATS) station. Error bars represent the SD of 3 replicate bottles

tion rate derived from the 3 time-course experiments was $0.7 \pm 0.2 \mu\text{M O}_2 \text{ d}^{-1}$. Based on these results, it appears respiration rates were linear during the 24 h incubations used in this study.

Community respiration rates averaged $1.3 \pm 0.5 \mu\text{M O}_2 \text{ d}^{-1}$ ($n = 8$) in the surface mixed layer and $1.1 \pm 0.4 \mu\text{M O}_2 \text{ d}^{-1}$ ($n = 20$) in the upper 75 m of the photic zone of the Sargasso Sea (Table 2). The average respiration rate in the upper 75 m at BATS ($1.1 \pm 0.4 \mu\text{M O}_2 \text{ d}^{-1}$, $n = 10$) was similar to that determined for the other stations sampled in the Sargasso Sea ($1.0 \pm 0.4 \mu\text{M O}_2 \text{ d}^{-1}$, $n = 10$; Table 2). In the present study, respiration rates $\leq 0.2 \mu\text{M O}_2 \text{ d}^{-1}$ were not significantly different from zero (Table 2; t -test, $p > 0.05$). Respiration rates $\leq 0.2 \mu\text{M O}_2 \text{ d}^{-1}$ are not included in the average rates given above.

Abundance of the major groups of the heterotrophic and autotrophic community

Bacterial abundance in unfiltered seawater from the surface mixed layer of the Sargasso Sea was $2.3 \pm 0.5 \times 10^8$ cells l^{-1} ($n = 10$). A similar average bacterial

Table 2. Community respiration rates in the Sargasso Sea in June and July 2001. Respiration rates \pm pooled SD of initial and final dissolved oxygen concentration are given; $n = 3$ or $n = 4$. SML: surface mixed layer; BATS: Bermuda Atlantic Time-series Study

Study station	Date (mm/dd)	Time of sampling	Sampling depth (m)	Depth of SML (m)	Respiration rate ($\mu M O_2 d^{-1}$)
1	06/21	12:30 h	25	11	0.8 ± 0.3
2	06/22	09:00 h	25	26	0.5 ± 0.2
3	06/23	14:30 h	25	15	1.0 ± 0.2
BATS	06/27	07:00 h	2.5	10	1.5 ± 0.6
	06/28	07:00 h	5	10	1.4 ± 0.3
	06/28	16:30 h	2	6	1.2 ± 0.2
	06/29	07:00 h	10	10	1.9 ± 0.3
	06/29	20:00 h	10	7	0.8 ± 0.3
	06/30	10:00 h	10	10	0.1 ± 0.0^a
	06/30	10:00 h	50	10	0.6 ± 0.2
	07/02	10:00 h	22	9	0.7 ± 0.5
	07/03	10:00 h	50	10	0.7 ± 0.3
	07/03	10:00 h	75	10	1.3 ± 0.5
4	07/07	08:00 h	5	10	1.2 ± 0.5
	07/08	10:00 h	5	19	0.2 ± 0.2^a
	07/08	10:00 h	50	19	0.7 ± 0.3
5	07/08	10:00 h	75	19	1.1 ± 0.5
	07/09	08:00 h	5	21	0.8 ± 0.5
	07/09	08:00 h	15	21	1.7 ± 0.4
	07/09	08:00 h	30	21	1.2 ± 0.9
	07/09	08:00 h	40	21	1.0 ± 0.3
	07/09	08:00 h	50	21	1.2 ± 0.7
07/09	08:00 h	75	21	0.0 ± 0.2^a	

^aNot significantly different from zero

abundance was observed for the upper 75 m of the photic zone ($2.4 \pm 0.5 \times 10^8$ cells l^{-1} , $n = 23$). Cyanobacteria and autotrophic nanoplankton averaged $0.22 \pm 0.19 \times 10^7$ cells l^{-1} ($n = 10$) in the surface mixed layer and $0.32 \pm 0.22 \times 10^7$ cells l^{-1} ($n = 23$) in the upper 75 m of the photic zone. Heterotrophic protists averaged $0.70 \pm 0.41 \times 10^6$ cells l^{-1} ($n = 10$) in the surface mixed layer and $0.63 \pm 0.41 \times 10^6$ cells l^{-1} ($n = 23$) in the upper 75 m of the photic zone. No correlation between community respiration and abundance of any of the major metabolic groups of microorganisms was observed in the photic zone of the Sargasso Sea (Fig. 2).

Size fractionation

Respiration rates in the $<0.6 \mu m$ size fraction of water collected in the surface mixed layer at BATS exhibited minimal variability among 3 experiments performed on separate days (0.3 to $0.4 \mu M O_2 d^{-1}$; Table 3). Respiration in the $<0.6 \mu m$ size fraction accounted for $23 \pm 4\%$ of community respiration. In contrast, heterotrophic bacterial abundance in the $<0.6 \mu m$ size fraction accounted for $\sim 78\%$ of the abundance in unfiltered seawater samples (Table 3). Addition of P to the $<0.6 \mu m$ size fraction from the surface mixed layer (29 June) stimulated respiration by a factor of 1.8, resulting in a respiration rate of $0.7 \pm 0.2 \mu M O_2 d^{-1}$ (Table 3).

Long-term experiments

Respiration in $0.6 \mu m$ filtered seawater collected from 50 m at BATS was $1.3 \pm 0.3 \mu M O_2$ during the 10 d

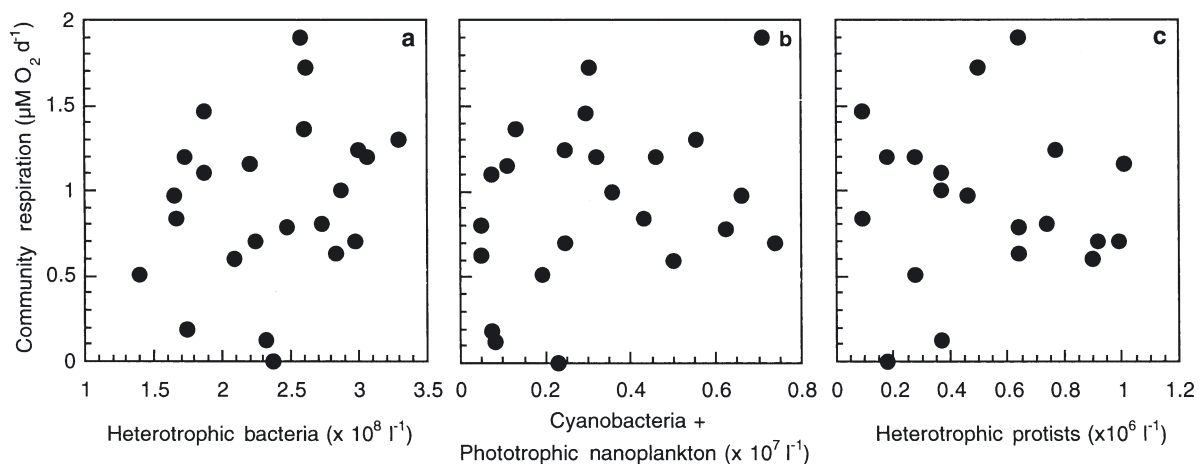


Fig. 2. Relationship between community respiration and the abundance of (a) heterotrophic bacteria, (b) cyanobacteria and phototrophic nanoplankton, and (c) heterotrophic protists

Table 3. Respiration rates and bacterial abundance in unfiltered seawater and in the $<0.6 \mu\text{m}$ size fraction collected in the surface mixed layer (SML) at the Bermuda Atlantic Time-series Study (BATS) station. On one occasion (29 June) P was added to the $<0.6 \mu\text{m}$ size fraction ($0.1 \mu\text{M PO}_4$ final conc.). Respiration rates \pm pooled SD of initial and final dissolved oxygen concentration are shown; $n = 3$

Date (mm/dd)	Respiration rate ($\mu\text{M O}_2 \text{ d}^{-1}$)		Bacterial abundance ($\times 10^8 \text{ cells l}^{-1}$)	
	Unfiltered seawater	$<0.6 \mu\text{m}$ size fraction	Unfiltered seawater	$<0.6 \mu\text{m}$ size fraction
06/27	1.5 ± 0.6	0.3 ± 0.2	2.2	1.9
06/28	1.4 ± 0.3	0.4 ± 0.2	2.6	1.7
06/29	1.9 ± 0.3	0.4 ± 0.2	2.6	2.1
06/29 (+P)		0.7 ± 0.2	2.6	2.1

Table 4. Respiration and changes in bacterial abundance during long-term experiments with $0.6 \mu\text{m}$ filtered seawater from different depths at the Bermuda Atlantic Time-series Study (BATS) station incubated at $20 \pm 1^\circ\text{C}$. On 1 occasion P was added to the $<0.6 \mu\text{m}$ size fraction ($0.1 \mu\text{M PO}_4$ final conc.). Respiration \pm pooled SD of initial and final dissolved oxygen concentration are shown; $n = 4$

Depth (m)	Respiration (in $\mu\text{M O}_2$)	Bacterial abundance ($\times 10^8 \text{ cells l}^{-1}$)		Duration (d)
		Initial	Final	
50	1.3 ± 0.3	2.1	2.1	10
150	2.1 ± 0.5	1.0	1.9	10
300	2.6 ± 0.4	0.7	2.5	10
50	1.4 ± 0.2	2.0	2.6	5
50 (+P)	3.5 ± 0.3	2.0	2.6	5

incubation (Table 4). An increase in bacterial respiration was observed with depth during the 10 d incubation. At 300 m, dissolved oxygen consumed during the 10 d incubation was 2-fold higher than at 50 m. Bacterial abundance increased 2- and 4-fold in the 10 d incubations from 150 and 300 m, respectively, while no net increase in bacterial abundance was observed in $0.6 \mu\text{m}$ filtered seawater from 50 m. In a separate experiment, the addition of P to the $<0.6 \mu\text{m}$ size fraction from 50 m increased respiration 2.5-fold during 5 d as compared to the unamended control (Table 4). Bacterial abundance increased by a factor of 1.3 in the unamended and P-amended $<0.6 \mu\text{m}$ size fraction over the 5 d incubation period.

DISCUSSION

Community respiration rates provide a direct measure of catabolic activity and are therefore excellent integrators of the cycling of organic carbon. Although the need for respiration measurements was emphasized previously (Williams 1981, Biddanda et al. 1994, Jahnke & Craven 1995), measurements of respiration in ocean waters are scarce. This is particularly true for the oligotrophic regions of the open ocean that cover about 30% of the Earth's surface. The current debate whether the consumption of organic carbon exceeds its production in the oligotrophic ocean, and subsequently whether the open ocean can be considered net heterotrophic (del

Nutrient enrichment experiments

Addition of P to unfiltered seawater from the surface mixed layer at BATS and Stn 5 resulted in a significant increase (up to 2.8-fold) in respiration rates (paired *t*-test, $p < 0.05$, $n = 5$), while no stimulatory effect was observed upon addition of N or C (Fig. 3). However, stimulation of respiration rates by P-addition was limited to the upper 30 m (Fig. 3). At 40, 50 and 75 m respiration rates were unaffected by the addition of P. Similarly, neither N nor C alone resulted in enhanced respiration rates at these depths. If unfiltered seawater was amended with a combination of P, N and C, respiration rates increased by a factor of 3 in the surface mixed layer, and by factors of 1.3 and 1.4 at 50 and 75 m, respectively (Fig. 3).

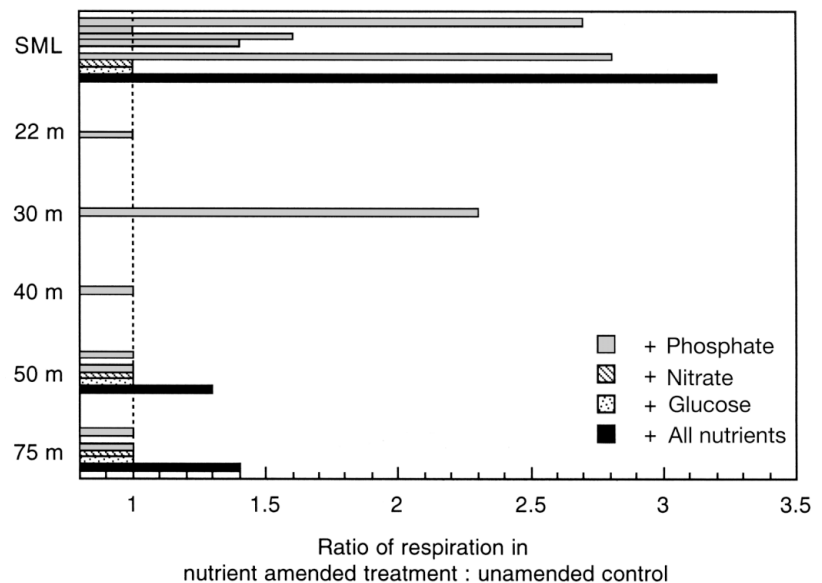


Fig. 3. Ratio of community respiration rates in nutrient-amended seawater to unamended controls at different depths at the Bermuda Atlantic Time-series Study (BATS) station and Stn 5. The surface mixed layer extended to 10 and 21 m at these stations, respectively. SML: surface mixed layer

Giorgio et al. 1997, Duarte & Agusti 1998, Williams 1998), further indicates the importance of understanding the mechanisms controlling these processes.

Variability in community respiration

An important observation of the present study is the considerable variability in community respiration rates in the photic zone of the Sargasso Sea. Spatial and temporal changes were also observed in the abundance of the major metabolic groups of the microbial community; however, none of these changes was significantly correlated with changes in community respiration rates. Even though the biomass of the major metabolic groups might provide a stronger relationship to respiration than abundance, we assume that the conversion of abundance to biomass would not result in a substantial change of the observed spatial and temporal variability within each of the major metabolic groups.

Measurements of biological activity in bottle incubations are often criticized for a variety of possible artifacts. A major concern is the uneven distribution of larger organisms or organic aggregates resulting in large variability among replicate incubation bottles. In the present study, the relative standard deviation of dissolved oxygen concentrations among replicates averaged ~0.2%, suggesting fairly uniform distributions of organisms and particles in the incubation bottles. In addition, incubations in 60 and 300 ml bottles yielded similar results, and a linear decrease in the concentration of dissolved oxygen was observed over a 48 h incubation period. Thus, it appears that spatial and temporal variability in community respiration rates was not an artifact of bottle incubations, but resulted from *in situ* shifts in the metabolic activity of the plankton community. Shifts in bacterial metabolism have been shown to be attributable to highly variable percentages of active cells (Gasol et al. 1995, Zweifel & Hagström 1995). Rapid stimulation of dormant or slow-growing cells was observed upon the addition of nutrients (Sherr et al. 1999), indicating a wide range in the metabolic activity of heterotrophic bacteria. In the present study, shifts in community respiration were very sensitive to changes in P-availability in the surface mixed layer, while co-limitation of substrates prevailed at deeper layers of the photic zone. Furthermore, the response of community respiration to P-addition in the surface mixed layer revealed substantial variability among days. The observed spatial and temporal variability in community respiration therefore appears to be related to changes in nutrient and substrate supply and availability. We therefore suggest that the availability of P, and possibly other substrates, is variable on short temporal and spa-

tial scales, resulting in variable community respiration rates.

The average community respiration rate for surface waters of the Sargasso Sea determined in the present study ($1.1 \pm 0.4 \mu\text{M O}_2 \text{ d}^{-1}$) is somewhat lower than the average rate for the upper 25 m of the Sargasso Sea ($1.7 \pm 0.3 \mu\text{M O}_2 \text{ d}^{-1}$) reported by Williams & Jenkinson (1982). Respiration rates reported for surface waters of the oligotrophic North Pacific Ocean averaged $0.84 \pm 0.5 \mu\text{M O}_2 \text{ d}^{-1}$ (Williams & Purdie 1991). The community respiration rates determined in the present study are within the range of values reported for surface waters of the oligotrophic ocean.

Size-fractionation and bacterial respiration

Estimating the bacterial contribution to overall metabolism has often been studied using size fractionation experiments (Azam & Hodson 1977, Williams 1981, Hopkinson et al. 1989). Respiration measurements with size-fractionated water samples have mainly been performed with coastal seawater, revealing that respiration is dominated by the heterotrophic microbial fraction at low to moderate chlorophyll concentrations (Williams 1981, Kuparinen 1984, Iriarte et al. 1991, Chin-Leo & Benner 1992). In a recent survey of lakes of varying trophic status, bacterial respiration (<1 μm size fraction) accounted for ~90% of plankton community respiration in the most oligotrophic systems (Biddanda et al. 2001). In the present study, bacterial respiration determined in size-fractionation (<0.6 μm) experiments was on average $0.4 \mu\text{M O}_2 \text{ d}^{-1}$ and contributed 23% to overall plankton community respiration at BATS. A similar respiration rate ($0.37 \mu\text{M O}_2 \text{ d}^{-1}$) was determined by Kepkay et al. (1990) in size-fractionated (2 μm filtered) water collected at 10 m in the Sargasso Sea. Hansell et al. (1995) measured carbon mineralization rates ($0.45 \mu\text{M C d}^{-1}$) in size-fractionated (0.8 μm pore size) water from 20 m at BATS.

The low bacterial contribution to plankton community respiration in the Sargasso Sea is surprising given that ~80% of heterotrophic bacteria pass a 0.6 μm pore-size filter. A similar observation was reported from the Gulf of Mexico, where respiration rates in the <1 μm size fraction were ~10-fold lower than rates in unfiltered seawater, although bacterial abundance was similar in both treatments (Biddanda et al. 1994). This pattern was only evident at the more oligotrophic site on the continental slope, whereas on the shelf bacterial respiration accounted for ~50% of community respiration based on size-fractionation experiments (Biddanda et al. 1994). In the oligotrophic Mediterranean Sea, large seasonal variations in the contribu-

tion of bacteria (<0.8 μm size fraction) to community respiration (16 to >100%) were observed in size fractionation experiments in the euphotic zone (Lemée et al. 2002). The relative contribution of the bacterial abundance in the <0.8 μm size fraction, however, did not vary substantially during the year, accounting for ~80% of the bacterial abundance in unfiltered seawater. These results clearly indicate that size fractionation can substantially impact bacterial metabolic activity. Similarly, rates of bacterial carbon production can be dramatically reduced in size-fractionation experiments (Carlsson & Caron 2001). These authors report a 70 to 90% decrease in bacterial production (measured as ^3H -leucine incorporation) in 0.8 μm filtered water as compared to unfiltered lake water, while size fractionation removed only ~20% of the bacterial abundance.

Previous studies indicate a tight coupling among the various members of the microbial loop (e.g. Sherr et al. 1988), and we considered the potential consequences of the separation of these microorganisms in size-fractionation experiments with oligotrophic waters. Heterotrophic protists are known to efficiently regenerate inorganic nutrients which are essential for maintaining biological activity in oligotrophic environments (Andersson et al. 1985, Caron 1994). However, size fractionation separates heterotrophic protists from bacterioplankton, thereby uncoupling processes that regenerate inorganic nutrients. We therefore suggest that the absence of heterotrophic protists in size-fractionated samples, particularly from the P-limited surface mixed layer, is responsible for the low bacterial respiration rates in 0.6 μm filtered seawater. Addition of P to <0.6 μm filtered water resulted in a ~2-fold increase in bacterial respiration rates in the present study.

Several other possibilities could, at least to some extent, contribute to the low bacterial respiration rates observed in the present study. Even though only ~20% of bacterial abundance was retained by 0.6 μm filtration, this fraction of the bacterioplankton could account for more than 20% of bacterial respiration. Likewise, heterotrophic protists, cyanobacteria and phototrophic nanoflagellates could be responsible for a greater fraction of community respiration than previously recognized. In terms of biomass, these members of the microbial community have been reported to account for up to 60% of the overall microbial biomass in surface waters of the Sargasso Sea (Caron 1994). If community respiration in the surface mixed layer is predominantly attributable to these metabolic groups, a relationship between their abundance and community respiration would be expected. However, no such relationship was observed in the present study.

The determination of bacterial growth efficiency combines measurements of bacterial production and respiration to better understand the flow of carbon

through heterotrophic bacterioplankton (del Giorgio & Cole 2000). The experimental approaches commonly used in growth efficiency experiments, namely size fractionation and dilution, are designed to minimize the contribution of other microorganisms to metabolism. The present and previous studies (Biddanda et al. 1994, Carlsson & Caron 2001, Lemée et al. 2002) raise the question of whether heterotrophic bacterial metabolism determined in size-fractionation and dilution experiments is representative of bacterial metabolism in unfiltered water. The complex structure of the microbial community and the interactions among the various members of the community are disrupted in these experiments. Our results indicate the potential for experimental artifacts when microbial groups are separated for measurements of activity in oligotrophic aquatic environments.

P-limitation of community and bacterial respiration

Nutrient enrichment experiments performed in the present and previous studies (Cotner et al. 1997, Rivkin & Anderson 1997, Caron et al. 2000) support the idea that specific biological processes in surface waters of the Sargasso Sea are P-limited during the stratified summer period. In addition to bioassay experiments that suggest P-limitation, P-limitation is also indicated by high dissolved N:P (60:1) ratios (Wu et al. 2000) and short P turnover times (5 h) in planktonic biomass in surface waters of the Sargasso Sea (Cotner et al. 1997). Soluble reactive phosphorus (SRP) concentrations are below the limit of detection (0.03 μM) throughout the upper 100 m water column during summer at BATS (data available at www.bbsr.edu/cintoo/bats/bats.html). P is a vital nutrient for all living organisms and is rapidly assimilated to form compounds that have critical structural (e.g. lipid membrane) and metabolic functions, such as protein synthesis, cell production and energy transport. Thus, P is an essential nutrient for energy production and biosynthesis, and P likely limits both growth and respiration. In the present study, P was the primary factor limiting community respiration in the surface mixed layer of the Sargasso Sea. Bacterial respiration was also P-limited during size fractionation experiments with Sargasso Sea surface water. These results suggest that phytoplankton production is also likely P-limited in the surface mixed layer. Carlson et al. (2002) observed that bacterial carbon production and DOC mineralization were co-limited by P, N and C in dilution experiments with water from the surface mixed layer of the Sargasso Sea. Differences between these studies could result from temporal and spatial variability or differences between size fractionation and dilution culture experiments.

Bacterial respiration was also P-limited in long-term experiments from 50 m at BATS. The overall respiration in 0.6 μm filtered seawater incubated for 5 to 10 d was similar to respiration measured in unfiltered seawater during 24 h incubations ($\sim 1 \mu\text{M O}_2 \text{ d}^{-1}$). P-addition to the long-term experiments from 50 m resulted in a 2.5-fold increase in bacterial respiration. Based on results obtained from time-course and nutrient addition experiments we estimate that bacterial respiration in the unamended treatments probably decreases dramatically after 3 d due to P-limitation. The present study therefore confirms previous suggestions (Cotner et al. 1997, Rivkin & Anderson 1997) that P-limitation could in part be responsible for the observed accumulation of DOC in the surface Sargasso Sea during summer (Carlson et al. 1994, Michaels 1994).

Bacterial respiration in the mesopelagic zone is likely limited by bioavailable C, as the concentrations of nutrients increase and concentrations of DOC decrease rapidly below ~ 125 m. The relatively high bacterial respiration rates at 150 and 300 m indicate the potentially important contribution of mesopelagic metabolism to overall water-column metabolism, as has been pointed out by Biddanda & Benner (1997).

Implications for the oligotrophic ocean

The oligotrophic ocean is often considered a stable and uniform environment. In contrast, these open ocean environments are characterized by a diverse and heterogenous plankton community (Giovannoni & Rappe 2000, Sherr & Sherr 2000). The present and previous studies further indicate that rates of metabolic activity of the plankton community exhibit substantial temporal and spatial variability in the euphotic zone of the oligotrophic ocean. This relatively fine-scale variability in biological processes likely reflects the spatial diversity of the open ocean (Azam 1998). Metabolic and spatial diversity appear to be important in open ocean environments and should be considered in future studies. Discovering the factors influencing fine-scale variability in biological processes will enhance the understanding of the dynamics of C cycling and the balance between the production and mineralization of C in the upper ocean.

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