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Dynamics of dissolved and particulate organic matter during the spring bloom in the Oyashio region of the western subarctic Pacific Ocean

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ABSTRACT: We evaluated the dynamics of dissolved organic matter (DOM) and particulate organic matter (POM) at various stages of the spring bloom in the Oyashio region of the western subarctic Pacific Ocean by means of samples obtained during 3 cruises in April, May, and June 2003. In April, the average concentration (±1 SD) of nitrate plus nitrite ions was 23.3 ± 0.39 µmol l^{-1} in surface waters, and this decreased to below the detection limit (<0.05 µmol l^{-1}) during the bloom period. About 80% of newly accumulated organic carbon was partitioned into POM. Whereas the C:N ratios of bulk DOM ranged from 13 to 20, the average C:N ratio of newly accumulated DOM was 7.3 ± 1.5. The lability of DOM was assessed by seawater culture-type incubation experiments on surface seawater samples from each observation site. Although the newly accumulated DOM was relatively rich in nitrogen, most of it escaped rapid (d to wk) bacterial consumption in the seawater culture experiments.

KEY WORDS: Dissolved organic matter · Particulate organic matter · Oyashio region · Spring bloom

INTRODUCTION

Primary production in the ocean surface plays a key role in the global carbon cycle and regulates the uptake of atmospheric CO_2 into the ocean. A great deal of effort has therefore gone into the precise estimation of primary production throughout the entire ocean (e.g. Longhurst et al. 1995, Polovina et al. 2008, Tilstone et al. 2009).

Photosynthesis by the planktonic community produces particulate organic carbon (POC) and dissolved organic carbon (DOC) at the surface; these 2 types of organic carbon materials have markedly different biogeochemical and ecological characteristics (Karl et al. 1998). Large particles can sink out of the surface water, and some of the POC fraction is transported by vertical migration of zooplankton (Steinberg et al. 2000, 2008a,b). DOC is mainly exported by advection and diffusion (Copin-Montégut & Avril 1993). POC is available as a source of carbon and energy for many heterotrophs, whereas most cannot utilize DOC, which is mainly utilized by bacteria (Azam et al. 1983). Therefore, the partitioning of organic carbon production between POC and DOC is a primary constraint on marine carbon cycles.

The primary production of marine phytoplankton involves a fixed C:N ratio, known as the Redfield ratio (Redfield et al. 1963). It has been suggested that a C:N ratio of 6.6 will permit the use of the nitrogen flux as a proxy for the carbon flux; by distinguishing between new production and regenerated production it should be possible to assess carbon export production (Dug-

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dale & Goering 1967, Eppley & Peterson 1979). However, this simple relationship has been challenged by recent observations that more dissolved inorganic carbon than would be expected from the Redfield ratio is taken up in the euphotic layer (Sambrotto et al. 1993, Copin-Montégut 2000, Kähler & Koeve 2001). One of the most probable consequences of this carbon overconsumption (Toggweiler 1993) is accumulation of C-rich dissolved organic matter (DOM) in the surface ocean (Banse 1994, Williams 1995, Kähler & Koeve 2001). The presence of this C-rich DOM implies that organic carbon export is decoupled from nitrogen export (Williams 1995, Hopkinson & Vallino 2005). These studies, therefore, force us to reconsider the C:N ratios of newly accumulated organic products.

The Oyashio region of the western subarctic Pacific is a well-studied marine environment (Saito et al. 1998, Kasai et al. 2001) that has predictable seasonal cycles of primary production (Kasai 2000). It is also known that primary production in this region is comparatively high among known oceanic values (Takahashi et al. 2002). Our objective in the present study was to examine the dynamics of DOM and particulate organic matter (POM) and their C:N ratios during the spring bloom. We also discuss the lability of DOM accumulated in surface waters.

MATERIALS AND METHODS

Sampling. The sampling program was carried out during survey cruises to the Oyashio region in 2003, along the monitoring line known as the A-line (Saito et al. 1998, Kasai et al. 2001). Along the A-line, Oyashio water coexists with the mixed water that originates partly from the subtropical Kuroshio current. Stations within the Oyashio waters were selected according to the criterion of the presence of a temperature of <5°C at a depth of 100 m (Kawai 1972). Water samples were collected from April 15 to 16, 2003, by the RV 'Tankai-maru' and from May 17 to 22 and June 9 to 10, 2003, by the RV 'Hokko-maru'. The sampling stations were A4 (42° 15′ N, 145° 08′ E), A5 (42° 00′ N, 145° 15′ E), and A7 (41° 30′ N, 145° 30′ E) in April; A45 (42° 08′ N, 145° 11′ E), A55 (41° 53′ N, 145° 19′ E), and A7 in May; and A4 and A5 in June (Fig. 1).

Seawater samples were collected with a plastic bucket (surface) or a CTD rosette system equipped with 5 l Niskin bottles (other depths). Samples for nutrient and DOM analyses were taken at 5 to 8 depths in the upper 500 m in the water column, whereas samples for POM and chlorophyll *a* (chl *a*) analyses were taken at 4 to 6 depths in the upper 200 m.

For determination of NH_4^+, NO_x (NO_3^- + NO_2^-), PO_4^{3-}, DOC, and dissolved organic nitrogen (DON), a subsample (100 ml) was GF/F filtered (47 mm; precombusted at 450°C, 3 h), and the filtrate was frozen for subsequent analyses in a 20 ml glass vial (precombusted at 550°C, 5 h). For the analyses of POC and particulate organic nitrogen (PON), a subsample (1000 to 2000 ml) was GF/F filtered (47 mm; precombusted at 450°C, 3 h), and the filter was frozen until its carbon and nitrogen contents were determined. For chl *a* analysis, a subsample (120 ml) was GF/F filtered (25 mm) and stored with N,N-dimethylformamide at –20°C. Vacuum-filtration onto a GF/F filter was performed at a pressure of <50 mm Hg to minimize cell disruption.

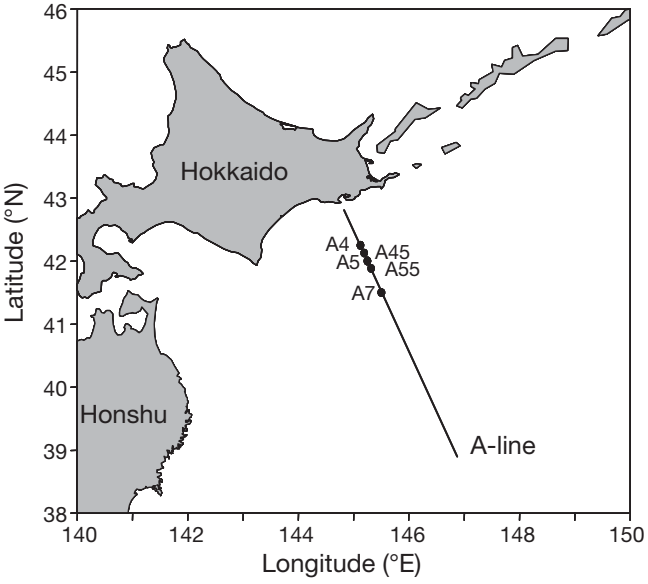

Fig. 1. Study site of the Oyashio region in the western subarctic Pacific. Solid line shows the monitoring line (A-line)

Seawater culture experiments. To evaluate the lability of DOM, seawater culture-type incubation experiments were performed by using surface seawater (0 m) from each observation site. The filtrate from a 47 mm GF/F filter (precombusted at 450°C, 3 h) was dispensed into 6 or 7 Teflon bottles that had been acid-washed and rinsed thoroughly with Milli-Q water before use. The samples were then incubated in darkness at 5°C. Although the incubation experiments were performed at 5°C, the surface seawater temperatures ranged from 1.6 to 7.5°C. However, the effect of the temperature on the estimation of the lability of DOC is likely to be minimal, because the incubation periods were long (15 to 38 d) and most of the labile DOC would have been consumed. Samplings were performed at intervals of 0.5 to 21 d (see Fig. 5). At each sampling stage (including Day 0), 1 bottle was used as a sample for DOC analyses and bacterial counts. For determination of NH_4^+, NO_x, PO_4^{3-}, DOC, and DON, subsamples were frozen for later analyses in

20 ml glass vials (precombusted at 550°C, 5 h) without filtration. For bacterial counts, subsamples (10 ml) were fixed with formalin (final concentration 1%) and stored at 4°C.

Chemical and bacterial analyses. Concentrations of NH_4^+, NO_x, and PO_4^{3-} were measured with a Bran + Luebbe Autoanalyzer III using a modified version of the method of Strickland & Parsons (1972). Concentrations of DOC and total dissolved nitrogen (TDN) were measured using a high-temperature catalytic oxidation method with a modified Shimadzu TOC-5000 unit, according to the method of Ogawa et al. (1999). Glucose and potassium nitrate were used as standards for DOC and TDN, respectively. Typical blank values for DOC and TDN analyses (i.e. values for Milli-Q water) were 3.2 to 5.5 µmol l^{-1} and 0.6 to 1.0 µmol l^{-1}, respectively. Replicate analyses (usually 4 instances) were performed continuously for each sample. The DOC and TDN were measured with average standard deviations of 0.7 and 0.3 µmol l^{-1}, respectively. DON concentrations were calculated by subtracting the concentrations of NH_4^+ and NO_x from those of TDN. The concentrations of POC and PON were measured by means of a CHN analyzer (MT-5, Yanaco). Before this analysis, the GF/F filter was acidified with HCl vapor to remove carbonate. Chl *a* concentrations were determined by the fluorometric method of Strickland & Parsons (1972), as modified by Suzuki & Ishimaru (1990), using a Turner Designs fluorometer. Bacteria were counted directly by means of epifluorescence microscopy after staining with DAPI (Porter & Feig 1980).

RESULTS

Vertical profiles of sigma-t, NO_x, chl *a*, and organic carbon

Sea surface temperature ranged from 1.7 to 2.7°C in April, from 3.6 to 5.6°C in May, and from 6.0 to 8.1°C in June. The sea surface salinity ranged from 33.0 to 33.1 in April, from 32.8 to 32.9 in May (no data for A45), and from 32.7 to 32.9 in June. In April, sigma-t values were distributed almost uniformly through the upper 100 m at all stations, suggesting that the waters were vertically and horizontally well-mixed at that time (Fig. 2, upper panels). In this region, good mixing of the waters in the upper 100 m have also been observed during March to April in other years (Saito et al. 1998, Kasai et al. 2001). The high concentrations of NO_x (23.3 ± 0.39 µmol l^{-1}) and the low concentrations of chl *a* (0.31 ± 0.035 µg l^{-1}) at the surface showed that April was a pre-bloom period. The DOC and POC concentrations at the surface were 54.1 ± 1.5 µmol l^{-1} and 6.0 ± 1.1 µmol l^{-1}, respectively (Figs. 2 & 3).

Later, the sigma-t values and NO_x concentrations gradually decreased in the shallow layers (Fig. 2, middle and lower panels). As the NO_x concentration decreased, the DOC and POC concentrations increased (Figs. 2 & 3). In June, the POC concentrations decreased to <20 µmol l^{-1} at station A4 (Fig. 3, lower panels). During the observation period, many concentration changes were observed at depths of <20 m (Figs. 2 & 3). An explanation for the changes in the above parameters following decreases in NO_x concentrations is given in the next section. The progress of blooming was arbitrarily divided into 3 phases corresponding to various NO_x concentrations, i.e. pre-bloom (NO_x 23.3 ± 0.39 µmol l^{-1}), bloom (NO_x 0.63 to 11.6 µmol l^{-1}), and post-bloom (NO_x <0.05 µmol l^{-1}).

Changes in chl *a*, DOM and POM

Relationships between NO_x and chl *a* concentrations in the shallow layer (depth <20 m) during the observation period are shown in Fig. 4a. The shallow layer was considered to be the euphotic layer during most of the observation period (H. Kasai unpubl. data). As the blooming condition progressed, the NO_x concentration decreased from 23.3 to 11.5 µmol l^{-1}, and the chl *a* concentration increased from 0.31 to 10.5 µg l^{-1} (Fig. 4a). Later, the chl *a* concentrations were relatively high (5.9 to 9.1 µg l^{-1}), and they ranged from 0.67 to 4.2 µg l^{-1} when the NO_x concentration was <0.63 µmol l^{-1}. When NO_x concentrations were <0.05 µmol l^{-1} at the surface, the NH_4^+ concentrations were also <0.05 µmol l^{-1}, but the PO_4^{3-} concentrations were >0.1 µmol l^{-1}, suggesting that N-limited conditions existed in the regions that we studied.

The relationships between shallow-layer NO_x concentrations and those of organic matter are also shown in Fig. 4b,c. The concentrations of both carbon and nitrogen in the DOM were higher than those in the POM during the pre-bloom period. As NO_x was consumed, the concentrations of both types of organic matter increased, and the magnitudes of the increases were much greater for the particles (Fig. 4b,c). POM concentrations frequently exceeded those of DOM in the nitrogen pool, but not in the carbon pool.

To calculate the concentrations of newly accumulated POM and DOM, the average pre-bloom POM and DOM concentrations were subtracted from the corresponding observed POM and DOM concentrations (Table 1, Fig. 4b,c). Under bloom conditions, the proportions of newly accumulated POC and PON were 78 ± 16% and 81 ± 16%, respectively, of the total concentrations (newly accumulated POM + DOM), and these values decreased to 65 ± 15% and 62 ± 17%, respectively, under post-bloom conditions (Table 1).

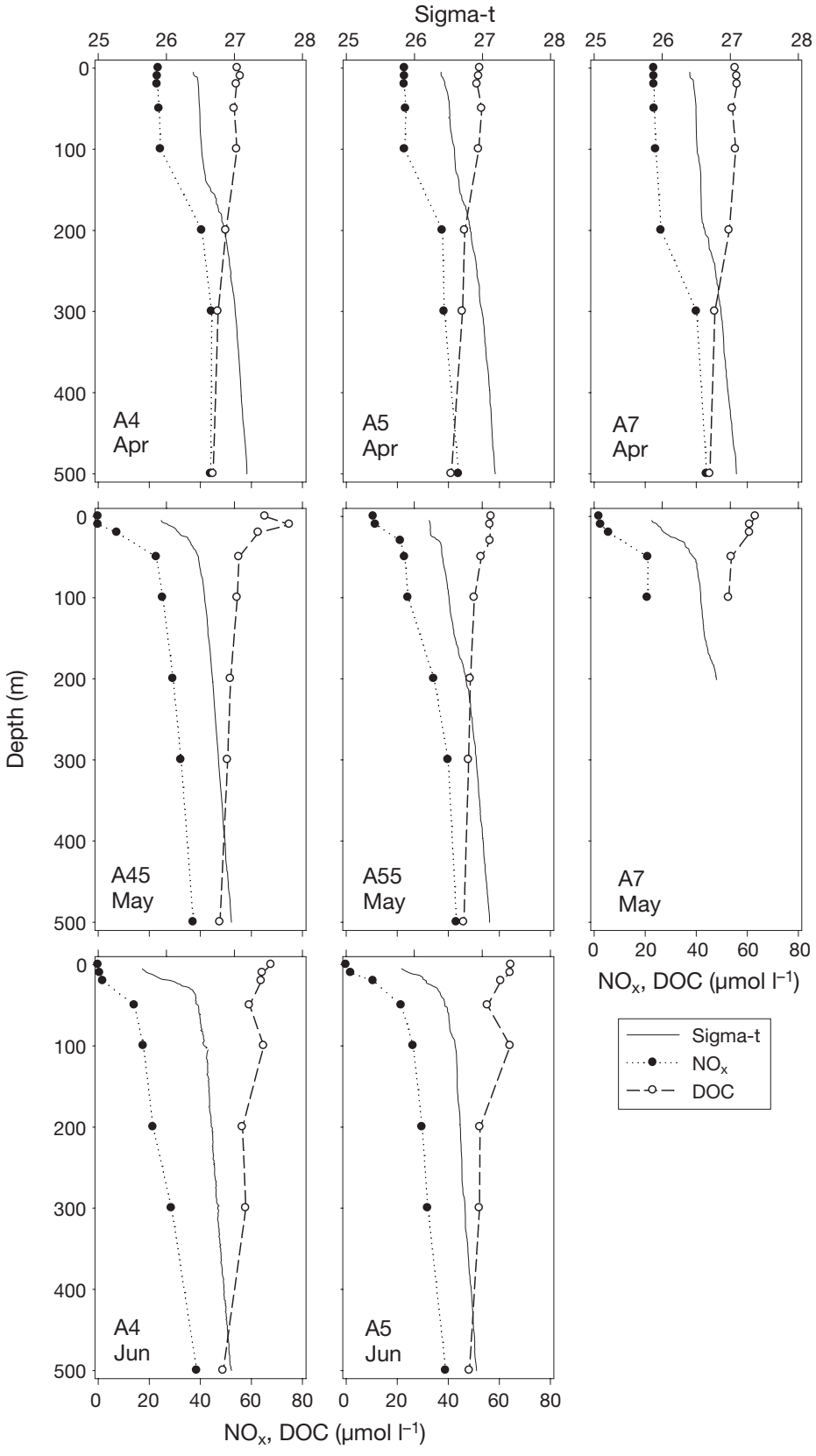

Fig. 2. Vertical distributions of sigma-t and concentrations of NO_x (NO_3^- + NO_2^-) and dissolved organic carbon (DOC) at 3 (April and May) or 2 (June) sampling stations. Upper, middle, and lower panels correspond to measurements taken in April, May, and June, respectively

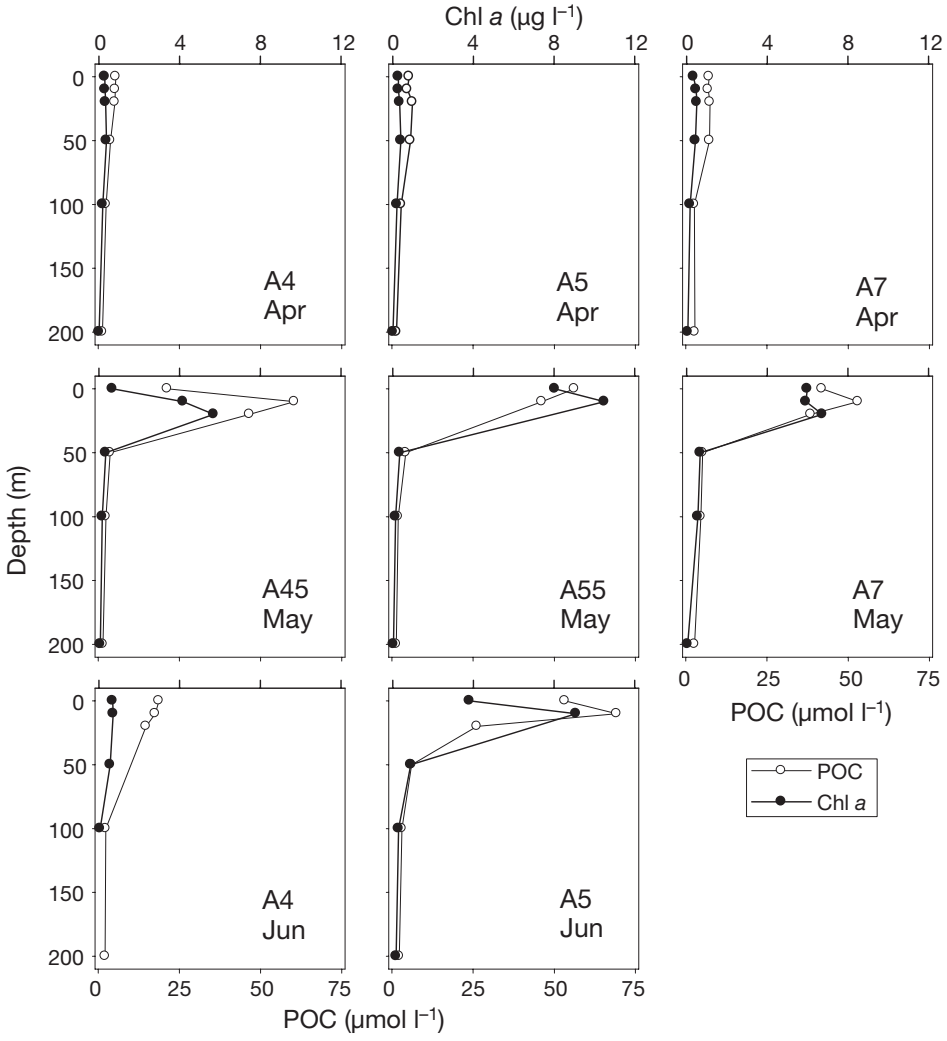

Fig. 3. Vertical distributions of concentrations of particulate organic carbon (POC) and chlorophyll *a* (chl *a*) at 3 (April and May) or 2 (June) sampling stations. Upper, middle, and lower panels correspond to measurements for April, May, and June, respectively

In the pre-bloom period, the average C:N ratio for POM was 6.0 ± 0.30. Under bloom and post-bloom conditions, the C:N ratios were 5.9 ± 0.51 and 8.7 ± 0.62, respectively, and the averages were significantly different (Kruskal-Wallis test, $p < 0.01$). The average C:N ratios of DOM were 15.9 ± 2.2, 13.9 ± 1.4, and 13.5 ± 0.83 in the pre-bloom, bloom, and post-bloom periods, respectively, and the decrease in the value is significant (Kruskal-Wallis test, $p < 0.05$); however, the ratios exceeded the Redfield ratio throughout the observation period.

Seawater culture experiment

Seawater culture experiments were performed using surface seawater samples (0 m) that were GF/F filtered at the start of the incubation. Filtration removed 69 to 85% of bacterial cells from the original seawater. The changes in the DOC concentrations and bacterial cell numbers that occurred during incubation experiments are shown in Fig. 5. The numbers of bacterial cells increased 2- to 7-fold, and exponential growth appeared to be complete before Day 20. Relatively small changes were observed for DOC concentrations during the incubation periods, and the maximum decrease was 5.6 µmol C l^{-1} for station A5 in June. In the all cases, the major decrease in DOC concentration occurred within the first few days of incubation (Fig. 5).

DISCUSSION

Organic carbon partitioning and C:N ratios of dissolved organic matter

The spring phytoplankton bloom resulted in an accumulation of organic matter in the shallow region. POC and DOC concentrations increased by up to 12-fold

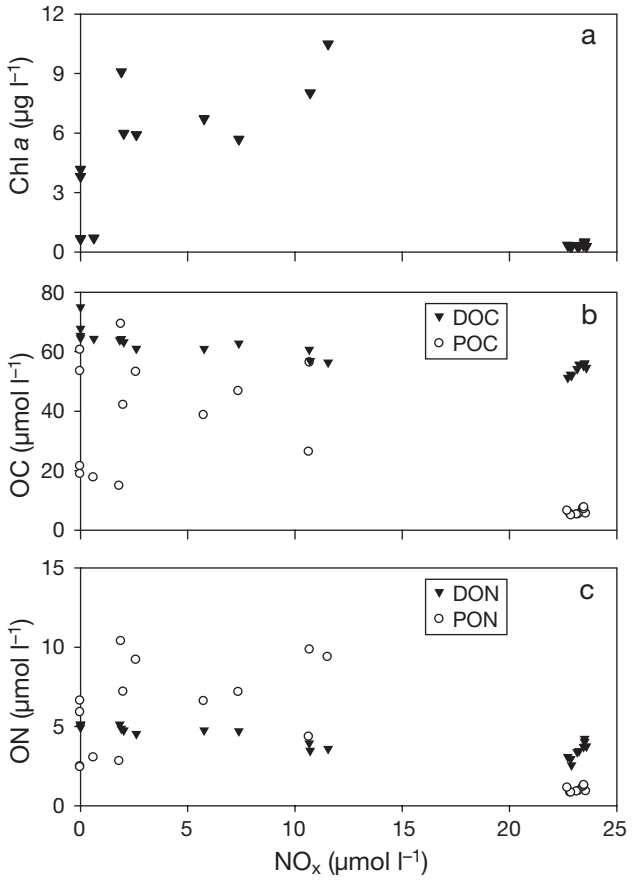

Fig. 4. Relationships between surface layer NO_x (NO_3^- + NO_2^-) and (a) chl *a*: chlorophyll *a*, (b) OC: organic carbon, and (c) ON:organic nitrogen. DOC: dissolved organic carbon; POC: particulate organic carbon; PON: particulate organic nitrogen; DON: dissolved organic nitrogen

and 1.4-fold, respectively (Fig. 4b,c). In this region, 47 to 95% (average 78%) of newly accumulated organic carbon was partitioned into particulate forms in the bloom (Table 1).

In agreement with the results of our study, Carlson et al. (1998) have shown that 89% of newly accumulated organic carbon during a bloom in the Ross Sea was present as POC (Table 2). Körtzinger et al. (2001) have also shown the importance of POC (66 to 100%) as newly accumulated organic carbon in the northeast Atlantic Ocean. Conversely, some studies have reported that accumulation of DOC was of the same magnitude as that of POC or that it exceeded that of POC during the bloom period. In the English Channel, 83% of newly accumulated carbon in the surface water was present as DOC (Williams 1995). In the Sargasso Sea, the average of newly accumulated DOC was 86% in 1992, 1993, and 1995, whereas from 1996 to 2000, it was 47% (Carlson et al. 1998, Lomas & Bates 2004).

These studies clearly show the existence of large spatial and temporal variations in the proportion of newly accumulated POC to total newly accumulated organic carbon. However, the variations cannot be explained in terms of the region (oceanic or coastal) or the dominant phytoplankton (Table 2). For example, in the Sargasso Sea, although Haptophytes dominated throughout the period that was studied, the percentage of newly accumulated POC increased considerably after 1995 (Lomas & Bates 2004; Table 2).

It has been suggested that healthy cells of phytoplankton release some 0 to 10% of their primary production as DOC (Sharp 1977, Smith et al. 1977, Mague et al. 1980), and therefore, phytoplankton in both the English Channel and the Sargasso Sea could have been stressed by nutrient limitation (Lancelot 1983) and/or extreme irradiance (Zlotnik and Dubinsky 1989). Also, the release of DOC mediated by heterotrophs and viruses (Nagata 2000) could be dominant.

Generally, C:N ratios for bulk DOM are considerably higher than the Redfield ratio, and in surface ocean, a typical C:N ratio of 14 has been reported (Benner

Table 1. Average (±SD) concentrations (µmol l^{-1}) of particulate organic carbon (POC), dissolved organic carbon (DOC), particulate organic nitrogen (PON), and dissolved organic nitrogen (DON). Phases were arbitrarily divided by correspondence to NO_x concentrations in the same seawater, i.e. pre-bloom (NO_x: 23.3 ± 0.39 µmol l^{-1}), bloom (NO_x: 0.63 to 11.6 µmol l^{-1}), and post-bloom (NO_x: <0.05 µmol l^{-1}). Values in parentheses represent concentrations of newly accumulated POC, DOC, PON, and DON (estimated by subtracting the average pre-bloom POC, DOC, PON and DON concentrations from the corresponding observed POC, DOC, PON and DON concentrations) and the proportions of newly accumulated POC and PON are expressed as percentages of total newly accumulated organic carbon and nitrogen, respectively

Phase	POC (µmol l^{-1})	DOC (µmol l^{-1})	POC (%)	PON (µmol l^{-1})	DON (µmol l^{-1})	PON (%)	n
Pre-bloom	6.0 ± 1.0	54.2 ± 1.9		1.0 ± 0.19	3.5 ± 0.53		6
Bloom	41.0 ± 17.3	61.5 ± 2.9		7.0 ± 2.8	4.5 ± 0.60		10
(Newly accumulated)	(35.0 ± 17.3)	(7.3 ± 2.9)	(78 ± 16)	(6.0 ± 2.8)	(1.0 ± 0.60)	(81 ± 16)	(10)
Post-bloom	38.5 ± 21.5	68.2 ± 4.7		4.3 ± 2.2	5.1 ± 0.08		4
(Newly accumulated)	(32.5 ± 21.5)	(14.0 ± 4.7)	(65 ± 15)	(3.3 ± 2.2)	(1.6 ± 0.08)	(62 ± 17)	(4)

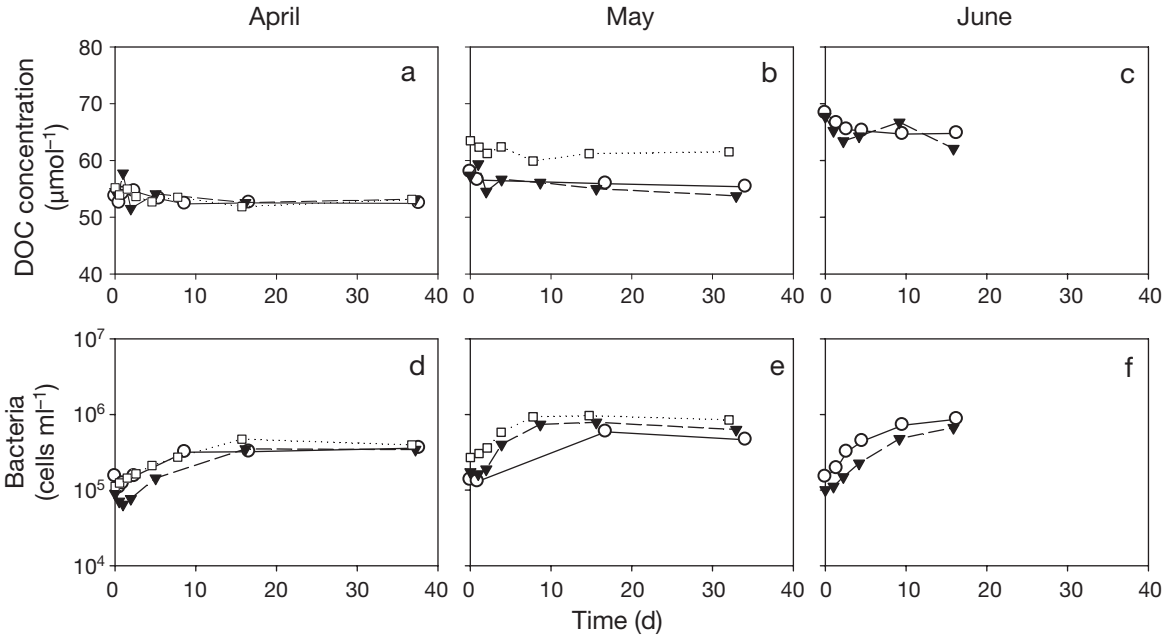

Fig. 5. (a–c) Dissolved organic carbon (DOC) concentrations and (d–f) bacterial cell numbers during the seawater culture experiments. Seawater was sampled at Stns A4 (○), A5 (▲), and A7 (□) in April; A45 (○), A55 (▲) and A7 (□) in May; and A4 (○) and A5 (▲) in June

2002). However, a wide range of ratios is known to exist for newly accumulated DOM (Table 2). Hopkinson & Vallino (2005) showed relatively high ratios of 9.8 for the Georges Bank and 10 to 14 for the middle Atlantic Bight. Very high C:N ratios of accumulated DOM (up to 25) have been reported for the English Channel and the north Atlantic Ocean (Williams 1995, Körtzinger et al. 2001).

In contrast to the above, the average C:N ratio of newly accumulated DOM was 6.2 in the southern Ross Sea (Carlson et al. 2000), 8.4 in the East China Sea (Hung et al. 2003), and 8.7 the Hawaiian Ocean (Hop-

Table 2. Proportions of newly accumulated particulate organic carbon (POC) to total newly accumulated organic carbon, expressed as percentages, and the C:N ratios of the newly accumulated dissolved organic matter (DOM). Numbers in parentheses are ranges or SD

Site	POC (%)	C:N ratio	Dominant phytoplankton	Description	Source
Ross Sea	89	6.2	*Phaeocystis*	Oceanic region	Carlson et al. (1998) Carlson et al. (2000)
Northeast Atlantic Ocean	84 (66–100)	24 (23–25)	Diatom flagellates (ultraplankton)	Oceanic region	Körtzinger et al. (2001)
English Channel	17	20		Coastal region	Williams (1995)
Sargasso Sea	14 (9.5–22)		Haptophytes	Oceanic region in 1992, 1993, and 1995	Carlson et al. (1998) Lomas & Bates (2004)
	53 (38–100)		Haptophytes	Oceanic region from 1996 to 2000	Lomas & Bates (2004)
Georges Bank		9.8(1.4)		Coastal region	Hopkinson & Vallino (2005)
Middle Atlantic Bight		10(0.9)–14(0.6)		Coastal region	Hopkinson & Vallino (2005)
East China Sea		8.4		Coastal region	Hung et al. (2003)
Hawaiian Ocean		8.7(0.6)		Oceanic region	Hopkinson & Vallino (2005)
Southern Ocean		2.7(1.7)–52(49)		Oceanic region	Ogawa et al. (1999)
Subarctic western North Pacific	74[a] (47–95)	7.3 ± 1.5[b]	Diatoms[c]	Oceanic region	Present study

[a]Average during the study period, [b]95% confidence interval, [c]Saito et al. (2002)

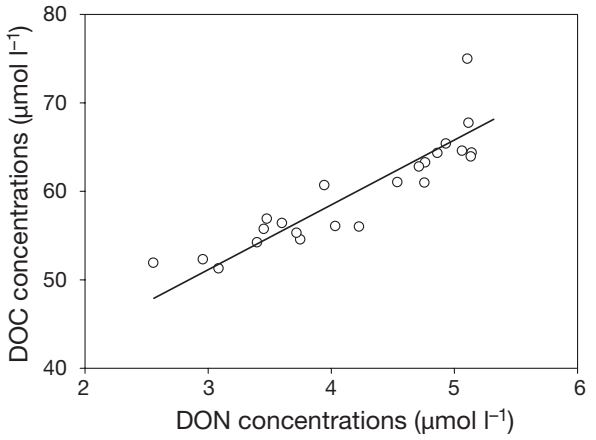

Fig. 6. Relationship between dissolved organic nitrogen (DON) and carbon (DOC) concentrations. Solid line is the regression (Model II) line. Resulting slope (± 95% confidence interval) is 7.3 ± 1.5 (n = 23, R^2 = 0.80, p < 0.01)

kinson & Vallino 2005). In the present study, the property–property plot for DON and DOC in the shallow layer (<20 m depth) suggests that the average C:N ratio of newly accumulated DOM was 7.3 ± 1.5 (the slope of Model II regression; n = 23, p < 0.01; Fig. 6 and Table 2).

Although, C:N ratios for newly accumulated DOM showed a considerable degree of variation (Table 2), the ratios were all lower than the bulk C:N ratio of 14 that is typically found near the surface of oceans (Table 2, Benner 2002), except for the northeast Atlantic Ocean and the English Channel. The bulk C:N ratios were lower in surface waters than in deep waters, implying that nitrogenous compounds undergo selective decomposition during the downward transport of DOM (Benner 2002, Ogawa & Tanoue 2003). It is likely that selective decomposition also partially explains the lower C:N ratios of newly accumulated DOM in comparison with the C:N ratio for typical bulk DOM in surface waters.

Hopkinson & Vallino (2005) suggested that, for a given mass of new nitrogen entering the surface layer, newly accumulated DOM enriched with carbon can efficiently export more carbon than would be expected from the Redfield stoichiometry. However, the C:N ratios of newly accumulated DOM showed a wide range of values (Table 2); the efficiency of export may therefore change accordingly.

Bacterial use of newly accumulated DOC

DOC can, conceptually, be partitioned into 3 pools: labile, semi-labile, and refractory (Kirchman et al. 1993). These have turnover times of hours to days, months to years, and centuries to millennia, respectively. This concept provides us with information on the lability of DOC on the basis of the vertical profile of DOC concentrations (Carlson 2002). In the pre-bloom period, the average DOC concentration of the upper 100 m was 54 ± 1.6 μmol l^{-1}, and the vertical profiles suggest that at least about 10 μmol l^{-1} of labile to semi-labile DOC persisted in the surface waters (Fig. 2). The value of 10 μmol l^{-1} is the excess DOC concentration compared with the value at a depth of 500 m (44 ± 2.3 μmol l^{-1}).

DOC concentrations in the surface layers increased to 75 μmol l^{-1} during the bloom periods (Fig. 2). Other studies have also shown that DOC accumulation occurs in the upper water column during and/or after the productive season (Copin-Montégut & Avril 1993, Carlson et al. 1994, Williams 1995, Sohrin & Sempéré 2005, Mathis et al. 2007). These results suggest that decoupling occurs between DOC production and the consumption of DOC by bacteria.

Whereas recent studies suggest that the levels of biomolecules such as polyunsaturated fatty acids and amino acids are predictors of the bacterial availability of DOM (McCallister et al. 2006, Davis & Benner 2007), seawater culture-type experiments are a common and direct method for assaying the utilization of DOM by bacteria. Our seawater culture experiments showed that a small proportion of the bulk DOC pool consisted

Table 3. Range of initial and consumed dissolved organic carbon (DOC) concentrations, initial dissolved inorganic nitrogen (DIN) and PO_4^{3-} concentrations, DOC consumption rate, percentages of consumed DOC fraction, increases in bacterial cells, increased bacterial carbon, and bacterial growth efficiencies (BGE) in the seawater culture experiments. All incubation experiments were performed in darkness at 5°C

Month	Initial DOC (μmol l^{-1})	Consumed DOC (μmol l^{-1})	Initial DIN (μmol l^{-1})	Initial PO_4^{3-} (μmol l^{-1})	DOC consumption rate[a] (μmol^{-1} d^{-1})	DOC consumed (%)	Increase in bacteria (10^5 cells ml^{-1})	Increased bacterial C[b] (μmol l^{-1})	BGE[b] (%)	n
April	53.7–55.2	1.1–3.3	23.0–23.9	1.9	0.08–0.17	2.1–6.0	1.7–3.6	0.17–0.35	9.8–13.0	3
May	58.0–63.5	2.1–2.3	1.8–14.1	0.3–1.3	0.09–0.15	3.6–4.1	4.5–6.9	0.45–0.69	17.7–23.3	3
June	67.7–68.4	3.6–5.6	3.6–5.6	0.1–0.2	0.16–0.18	5.3–8.3	5.7–7.1	0.57–0.71	9.2–16.6	2

[a]Estimated from the slope of the linear regression of DOC concentrations against time during 15 to 17 d incubation
[b]Estimated from the assumption of a bacterial carbon content of 12 fg C cell^{-1} (Ducklow 2000)

of labile DOC; this was the DOC consumed during 15 to 17 d (within the period of exponential bacterial growth). The concentrations of labile DOC ranged from 1.1 to 5.6 µmol l^{-1} (Table 3, Fig. 5). As bacteria were only removed at the start of incubation, DOC consumption was underestimated according to the increased bacterial carbon.

During 15 to 17 d of incubation, increases in bacteria ranged from 1.7×10^5 to 7.1×10^5 cells ml^{-1} (Table 3, Fig. 5). Assuming an oceanic bacterial carbon content of 12 fg C $cell^{-1}$ (Ducklow 2000), the increases in bacterial carbon were 0.17 to 0.35 µmol l^{-1} in April, 0.45 to 0.69 µmol l^{-1} in May, and 0.57 to 0.71 µmol l^{-1} in June (Table 3). Total labile DOC concentrations (consumed DOC + increased bacterial carbon) ranged from 1.3 to 6.2 µmol l^{-1}. Also, by using an oceanic bacterial carbon content of 12 fg C $cell^{-1}$, the values of the bacterial growth efficiency (BGE) were estimated to be 9.8 to 13% in April, 18 to 23% in May, and 9.2 to 17% in June (Table 3), which are within the range of reported values for marine bacteria (del Giorgio & Cole 2000).

BGE is likely to increase with increasing primary production (del Giorgio & Cole 2000). Eichinger et al. (2006) showed that BGE is higher in surface layers (5 m) than in deep layers (200 and 400 m), and is lower in winter than in spring or summer. In the present study, chl *a* concentrations and BGE values, both of which were estimated for surface waters (0 m), showed a weak correlation (Fig. 7a; $p < 0.06$). These results are not inconsistent with the relationship derived by del Giorgio & Cole (2000).

It has been suggested that the C:N ratio of DOM is a controlling factor for the BGE (Cherrier et al. 1996). Amon & Benner (1996) showed that BGEs were higher when low-molecular-weight DOM was utilized by bacteria rather than high-molecular-weight DOM. They suggested that the fact that the C:N ratio of the high-molecular-weight DOM is higher than that of the low-molecular-weight DOM causes the former to contribute to a lower BGE. In the present study, however, the relationship between BGE and the C:N ratios of DOM was not statistically significant (Fig. 7b; $R^2 = 0.19$, $p > 0.28$). Cherrier et al. (1996) added model compounds such as glucose and amino acids to their culture so that the C:N ratios of the initial DOM were spread over a wide range (3 to 18). As no model compounds were added in the present study, the range of the C:N ratios was relatively narrow (13 to 18). The effect of the C:N ratio on BGE may, therefore, be uncertain because of the narrow range of C:N ratios employed.

Many studies have made use of seawater culture-type experiments; however, most of these studies assessed seawaters with DOC concentrations of more than 100 to 650 µmol l^{-1} (for reviews, see Søndergaard & Middelboe 1995, del Giorgio & Davis 2003), which were much higher than the typical DOC concentrations present in the surface ocean, i.e. 60 to 90 µmol l^{-1} (Benner 2003). Table 4 lists results of previous seawater culture experiments that were performed using surface seawaters with DOC concentrations of <100 µmol l^{-1}. The remarkably slow rates of DOC consumption in the present study (0.08 ± 0.05 to 0.18 ± 0.15 µmol l^{-1} d^{-1}) could have been caused by the relatively low DOC concentrations (54 to 68 µmol l^{-1}) and the low incubation temperature (5°C, Table 4). The results for the percentages of DOC consumed in the current study fell within the range of previously reported values. The

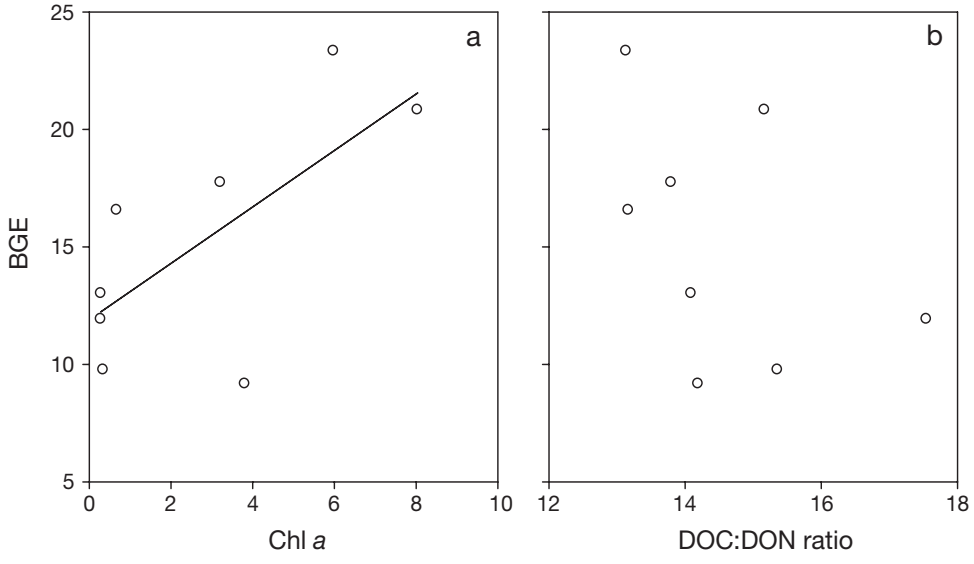

Fig. 7. Relationship between (a) the chlorophyll *a* (chl *a*) concentrations and the bacterial growth efficiency (BGE) and (b) the C:N ratios of the bulk dissolved organic matter (DOM) and the BGE. Solid line is the regression line ($y = 1.2x + 12$; $R^2 = 0.48$, $p < 0.06$)

Table 4. Comparisons of the results of seawater culture-type experiments using the surface seawaters with dissolved organic carbon (DOC) concentrations of <100 µmol l^{-1}

Site	Initial DOC (µmol l^{-1})	DOC consumed (%)	DOC consumption rate (µmol^{-1} d^{-1} ± SE)[a]	Duration of incubation (d)	Temperature (°C)	n	Source
Sargasso Sea	68–77	0–7.0	0–2.4 ± 0.24	1.7–7.5	20–26	4	Carlson & Ducklow (1996)
Middle Atlantic Bight	81–94	9.9–14[b]	0.54–0.83[b]	15	19–20	3	Hopkinson et al. (2002)
Eastern North Pacific	69–72	0–7.0	0–4.4±2.6	2.5–2.9	11–13	4	Cherrier et al. (1996)
Subarctic western North Pacific	54–68	2.1–8.4	0.08 ± 0.05–0.18 ± 0.15	14.7–16.6	5	8	This study

[a]Estimated from the slope of the linear regression of DOC concentrations against time during the incubation period
[b]Estimated from DOC concentrations on Days 0 and 15. DOC concentrations on Day 15 were recalculated from Hopkinson et al. (2002)

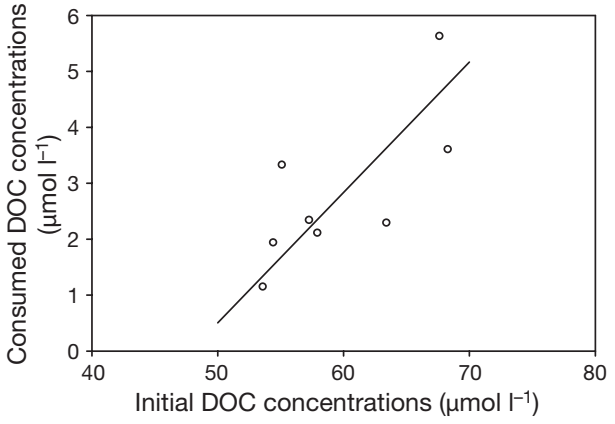

Fig. 8. Relationship between the initial and consumed dissolved organic carbon (DOC) concentrations. Solid line is the regression (Model II) line. Resulting slope (±95% confidence interval) is 0.23 ± 0.16 (n = 8, R^2 = 0.54, p < 0.05). Consumed DOC concentrations are the DOC concentrations that were consumed during 15 to 17 d incubations of seawater cultures

average percentage of DOC consumed (±SD) in these 4 seawater culture experiments (Table 4) was 5.1 ± 4.0%, which is significantly lower than the average percentage for marine environments of 22 ± 13% (recalculated from the review by Søndergaard & Middelboe 1995; Mann-Whitney *U*-test, p < 0.0001); most of the values used in calculating the latter percentage resulted from experiments performed at high DOC concentrations (100 to 650 µmol l^{-1}). The labile fraction of around 5% of the whole DOC might be common in the surface ocean, where DOC concentrations are <100 µmol l^{-1}.

A property–property plot of DOC versus DOC consumed is shown in Fig. 8. The regression line (Model II) of $y = 0.23x - 11$ predicts that labile DOC will become undetectable when the initial DOC concentration is 48 µmol l^{-1}. This value is close to the average (±SD) concentration at a depth of 500 m during the observation period (46 ± 2.5 µmol l^{-1}; Fig. 2), where most of the DOC consists of semi-labile and refractory fractions.

As the DOC concentrations increased, the DOC consumed also increased. However, the slope of the regression line of the initial DOC and the consumed DOC concentrations was only 0.23, suggesting that most of the increase in DOC could be attributed to the accumulation of semi-labile to refractory DOC that could be exported by advection and diffusion.

The refractory nature of DOC could explain its seasonal accumulation in surface waters (Carlson 2002). Also, the accumulation of DOC could be explained by a mechanism in which competition for mineral nutrients between bacteria and phytoplankton keeps the bacterial growth low, and bacterial predators keep the bacterial biomass low, so that degradable DOC can accumulate (Thingstad et al. 1997).

According to the latter hypothesis, bacterial utilization of accumulated DOC could be enhanced by reducing the grazing pressure or by addition of limiting nutrient(s) (Carlson 2002). In our seawater-culture experiments, the number of grazers might be negligible as a result of the filtration procedure (GF/F); this assumption is supported by the bacterial growth curves (Fig. 5). On the other hand, depletion of dissolved inorganic nitrogen (DIN) could restrict utilization of DOC. (In the June experiments, the DIN concentrations were <0.05 µmol l^{-1} and the PO_4^{3-} concentrations were >0.08 µmol l^{-1} during the incubation periods; Table 3.) However, the C:N ratios of newly accumulated DOM were around 7.3 (Fig. 6), which implies, at the very least, that the quantity of N did not limit DOC availability, because bacteria act as regenerators of NH_4^+ when the C:N ratio of available substrates is <10:1 (Goldman et al. 1987). Although nitrogenous nutrients were supplied as freshly released DON, this nitrogen did not have a marked effect on the rapid (days to weeks) bacterial consumption of DOC. In the Oyashio region, seasonal DOC accumulation in the surface water could be explained principally by the refractory nature of DOC and/or DON.

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