PERGAMON

Deep-Sea Research II 48 (2001) 4179–4197

DEEP-SEA RESEARCH
PART II

www.elsevier.com/locate/dsr2

Glucose fluxes and concentrations of dissolved combined neutral sugars (polysaccharides) in the Ross Sea and Polar Front Zone, Antarctica

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Received 21 March 2000; received in revised form 12 December 2000; accepted 3 January 2001

Abstract

We hypothesized that dissolved carbohydrates would be large components of the labile dissolved organic carbon (DOC) pool and would support much bacterial growth in Antarctic waters, especially the Ross Sea, since previous work had observed extensive phytoplankton blooms with potentially high production rates of carbohydrates in Antarctic seas. These hypotheses were tested on cruises in the Ross Sea and Antarctic Polar Front Zone as part of the US JGOFS program. Concentrations and fluxes of free glucose (the only free sugar detected) were very low, but dissolved polysaccharides appeared to be important components of the DOC pool. Concentrations of dissolved combined neutral sugars increased >3-fold during the phytoplankton bloom in the Ross Sea and were a large fraction (ca. 50%) of the semi-labile fraction of DOC. The relatively high concentrations of dissolved combined neutral sugars, which are thought to be quite labile, appear to explain why DOC accumulated during the phytoplankton bloom was degraded so quickly once the bloom ended. Some of the polysaccharides appeared to be more refractory, however, since dissolved combined neutral sugars were observed in deep waters (>550 m) and in early spring (October) in the Ross Sea, apparently having survived degradation for >8 months. The molecular composition of these refractory polysaccharides differed from that of polysaccharides sampled during the phytoplankton bloom. Fluxes of DOC were low in the Ross Sea compared to standing stocks and fluxes of particulate material,

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0967-0645/01/$ - see front matter
PII: S0967-0645(01)00085-6

but the DOC that did accumulate during the phytoplankton bloom appeared to be sugar-rich and relatively labile.

1. Introduction

Dissolved organic carbon (DOC) can be divided into three fractions as defined by turnover times (Kirchman et al., 1993; Carlson and Ducklow, 1995). The largest fraction is the refractory pool with concentrations about 40 μM-C (Hansell and Carlson, 1998) and turnover times exceeding 1000 years (Williams and Druffel, 1987; Bauer et al., 1992). The labile pool is the smallest fraction with concentrations usually in the nanomolar range. Although concentrations are low, fluxes through the labile pool can be very high because of fast turnover times, approaching minutes for some components (e.g. Fuhrman and Ferguson, 1986). The third fraction of DOC, the semi-labile pool, can be a large fraction of total DOC in surface waters (roughly 50%). Since this pool is not used as rapidly by bacteria as the labile pool, concentrations potentially can increase greatly on month to season time scales (Carlson et al., 1994; Williams, 1995). This accumulated semi-labile DOC is available for export or utilization by heterotrophic bacteria when inputs of more labile DOC are low.

Carbohydrates are potentially a large component of the semi-labile pool because they are the largest identified component of DOC, constituting as much as 40% of DOC in surface waters (Benner et al., 1992; Børsheim et al., 1999; Pakulski and Benner, 1994). Polysaccharides are thought to be abundant especially during or following phytoplankton blooms, as demonstrated in the North Sea (Ittekkot et al., 1981; Eberlein et al., 1985) and in a Norwegian fjord (Børsheim et al., 1999). Several studies have concluded that dissolved polysaccharides are produced directly by phytoplankton (e.g. Biersmith and Benner, 1998; Aluwihare and Repeta, 1999) as extracellular polymers secreted often in response to nutrient depletion at the end of blooms (e.g. Mopper et al., 1995; Obernosterer and Herndl, 1995; Fajon et al., 1999).

In addition to polysaccharides, another potentially important type of carbohydrate is the dissolved pool of free monomers. Neutral monosaccharides (aldoses) such as glucose have been examined most extensively since the introduction of techniques to measure concentrations in seawater (Mopper et al., 1992). Concentrations of free neutral sugars are usually very low (<50 nM), much lower (>10-fold) than dissolved combined sugars (Borch and Kirchman, 1997; Skoog and Benner, 1997), i.e. the sugars released from polysaccharides and other polymers by acid hydrolysis. Although concentrations are low, turnover rate constants and thus uptake rates of free sugars can be high (Rich et al., 1996; Rich et al., 1997; Skoog et al., 1999). In fact, a large fraction ($>30\%$) of bacterial growth can be supported by one monosaccharide, glucose, in some oceans (Rich et al., 1996, 1997) and in lakes (Jørgensen, 1990; Jørgensen and Jensen, 1994; Bunte and Simon, 1999), although that is not the case in the Gulf of Mexico (Skoog et al., 1999).

Carbohydrates may be particularly important components of the DOC pool and flux in the Ross Sea because of its large phytoplankton bloom consisting of *Phaeocystis antarctica* and diatoms (DiTullio and Smith, 1996; Asper and Smith, 1999) known to produce copious carbohydrates (Eberlein et al., 1985; Biersmith and Benner, 1998; Janse et al., 1996, 1999; Aluwihare and Repeta, 1999). These phytoplankton groups and others often have high amounts

of glucose in their exopolymers and storage polysaccharides (Biersmith and Benner, 1998; Aluwihare and Repeta, 1999; Janse et al., 1999). High concentrations of dissolved carbohydrates and glucose-rich polymers have been found in particulate material suspended in Antarctic seas (Liebezeit and Bolter, 1991; Pakulski and Benner, 1994), although the Ross Sea has not been examined.

The goals of this study were to estimate the contribution of carbohydrates to the DOC pool and to supporting bacterial growth in the Ross Sea and in the Antarctic Polar Front region. Data on the DOC components supporting bacterial growth should provide insights into controls of bacterial standing stocks and production (Ducklow et al., 2000, 2001) since DOC fluxes often limit bacterial growth (Church et al., 2000; Kirchman et al., 2000) even in the iron-poor waters of Antarctic seas (Martin et al., 1990). In turn, understanding bacterial growth is important for deciphering fluxes of labile DOC since DOC consumption is mainly due to bacterial activity. We hypothesized that glucose-containing polymers would be a large fraction of the bulk DOC pool and thus may help explain the apparent lability of DOC in the Ross Sea; DOC concentrations increase rapidly during the short growing season of the Ross Sea, but then as quickly disappear from the surface layer, unlike in the Sargasso Sea where surface-layer DOC can be exported to deeper waters (Carlson et al., 1998). Since we thought that glucose-containing polymers would be common, we also hypothesized that uptake of free glucose would support substantial bacterial growth. We found that although glucose uptake was low relative to bacterial production, dissolved glucose-containing polymers were a large fraction of the semi-labile pool of DOC at the height of the phytoplankton bloom in the Ross Sea.

2. Methods and materials

The data reported here were collected during six cruises of the RVIB *Nathaniel B. Palmer* (Ross Sea) and the R.V. *Revelle* (Antarctic Polar Front region) in 1996–1998 as part of the US-JGOFS AESOPS program (Table 1). During the Ross Sea cruises, most samples were collected on a transect along 76.5°S, between 178°W (Station Orca) and 169°E (Station Minke) (see map in Carlson et al., 2000). The Antarctic Polar Front samples were collected on a transect at 170°W

Table 1
Summary of AESOPS cruises for this study

Region	Cruise name	Cruise number	Dates	Deep samples (m)[a]
Ross Sea	Process 1	NBP96-4A	October 2–November 8, 1996	563–578
	Process 2	NBP97-1	January 13–February 11, 1997	550–747
	Process 3	NBP97-3	April 4–May 12, 1997	NA
	Process 4	NBP97-8	November 4–December 13, 1997	NA
Polar Front	Process 1	RR-KIWI-7	December 2, 1997–January 3, 1998	NA
	Process 2	RR-KIWI-9	February 13–March 19, 1998	1000–3399

[a] Depth of samples collected to determine concentrations of sugars in deep waters. "NA", sugar concentration data not available.

from 53°S to 72°S. Activity measurements and sugar concentrations were determined in samples taken on CTD casts in the upper 100 m (three to four depths, usually 0, 20, 40 and 60 m) and at selected deeper depths (concentrations only; Table 1). To preserve water for measuring sugar concentrations, surface water was filtered through Acrodisc syringe filters (mixed esters of cellulose, pore size of 0.22 μm) that had been rinsed twice with deionized water and then once with sample water before the sample was collected into combusted (500°C for 24 h) glass vials. Previous work demonstrated that the rinsed filters do not leach sugars (Rich et al., 1996; Borch and Kirchman, 1997).

Except for the October Ross Sea cruise, samples from deep waters (> 100 m) were not filtered in order to minimize contamination. Neutral sugar concentrations in filtered deep water were either the same or higher than in unfiltered samples (data not shown). The direct contribution of sugars from particulate organic material (POM) in the unfiltered samples was probably quite low. Sugars from POM would be about 0.25 μM-C or 17% of the combined neutral sugar concentrations in deep waters during the January–February cruise in the Ross Sea, given that POM concentrations at 200 m were about 5 μM-C as determined by filtration (Gardner et al., 2000) and assuming a neutral sugar yield from POM of about 5% (Skoog and Benner, 1997).

2.1. Analysis of neutral sugars

Dissolved free monosaccharides were analyzed by anion-exchange high performance chromatography (AE-HPLC) with pulsed amperometric detection (PAD) as described previously (Mopper et al., 1992; Rich et al., 1996). Since seawater samples must be desalted before AE-HPLC, all charged sugars (e.g. amino sugars) are removed and are not analyzed. Recovery during the desalting step was measured by spiking seawater with the sugars commonly found in seawater (see results) and subjecting the spiked sample to the desalting step. Reproducibility during desalting and during AE-HPLC was followed by using rhamnose as an internal standard for examining free sugar concentrations; 2-deoxyribose was the standard when "combined" neutral sugars were examined (see below). Some samples were analyzed with 15 mM NaOH, which gives the best separation, to determine the composition of free neutral sugars. Since glucose was the only free neutral sugar detected (others were < 3 nM) in these samples (see results), 30 mM NaOH was usually used for AE-HPLC to maximize sensitivity.

Water samples were subjected to acid hydrolysis in order to estimate concentrations of dissolved "combined" neutral sugars, i.e. polysaccharides, other sugar-containing polymers, and otherwise free neutral sugars adsorbed to materials (e.g. colloids) that pass a 0.22-μm filter. The hydrolysis procedure and analysis followed the methods described by Borch and Kirchman (1997). Briefly, sulfuric acid was added to seawater samples (0.85 M, final concentration) which then are heated for 24 h at 100°C. After hydrolysis the samples were partially neutralized by addition of $CaCO_3$ and 2-deoxyribose was added as an internal standard (Borch and Kirchman, 1997). Rhamnose is a better internal standard than 2-deoxyribose but rhamnose occurs in the combined pool although it is undetectable in the free pool. Naturally occurring 2-deoxyribose is destroyed by hydrolysis. The samples then were desalted and the monomers were analyzed by HPLC-PAD as described above, but using 12 mM NaOH in order to maximize separation of the various sugars. Mannose and xylose were not separated in our analysis, but Skoog and Benner (1997) report that the yield of these two sugars is about equal. The recovery of free neutral sugars

was about 60–70%, similar to the yields reported previously (Borch and Kirchman, 1997). Yields of individual sugars were calculated for each HPLC run by subjecting standard sugar mixtures to the same hydrolysis and desalting procedure as used for the samples. The concentrations reported here were corrected for the yield. As with free sugars, the pool of combined sugars measured by this method does not include any charged sugars.

The data reported here are averages from an entire cruise, during which several depth profiles were taken with three or more depth samples per profile. When possible, duplicate samples from a single depth were also analyzed. The variability in the dissolved combined pool between these duplicate samples ranged from 3 nM for fucose to 30 nM for glucose or about 17% to 25% of average concentrations. The variability between replicate samples for dissolved free glucose was 4 nM or 30% of the average concentration. The limit of detection was 3 nM.

2.2. Rate measurements

All rate measurements were determined by the microcentrifuge method as originally described by Smith and Azam (1992). Briefly, triplicate water samples were incubated in 2.0 ml microcentrifuge tubes at the in situ temperature for various times, ranging from a few hours (Ross Sea, January 1997) to a day (Ross Sea, November 1997), depending on the activity. After incubation, trichloroacetic acid (TCA) was added to a final concentration of 5% and the samples were centrifuged in a microcentrifuge at maximum speed (ca. 14,000 RPM). The pellet was washed once each with 5% TCA and then 80% ethanol. After the pellet was dry, scintillation cocktail (Optima Gold, Packard) was added directly to the microfuge tube and the entire tube was radioassayed. Incorporation into the TCA-insoluble fraction was measured for all compounds, including glucose and free amino acids. The concentrations of added tritiated compounds were 20 nM (thymidine), 20 nM (leucine), and 0.5 nM of glucose ([6-^{3}H] glucose from NEN) or a mixture of amino acids that mimics the composition of algal protein (Amersham). Details on measuring bacterial production using leucine and thymidine in the Ross Sea are given by Ducklow et al. (2000, 2001).

Turnover rate constants (d^{-1}) were calculated for glucose and the amino acid mixture by dividing the incorporated radioactivity by the added radioactivity and the incubation time. Uptake rates ($pM\,d^{-1}$) were calculated by multiplying the rate constants by the measured concentrations. To calculate bacterial production, we used only the leucine data and assumed a conversion factor of 1.5 kg C per mole of incorporated leucine. This conversion factor was demonstrated to be valid for the Ross Sea (Ducklow et al., 1999; Ducklow et al., 2000, 2001).

3. Results and discussion

The purpose of this study was to evaluate the contribution of dissolved carbohydrates to supporting bacterial production and to standing stocks of DOC in the Ross Sea and the Antarctic Polar Front region during several AESOPS cruises in 1996–1998. To simplify analysis, we averaged data over an entire cruise (about a month) in a given region using data collected over three to four depths (0–60 m) at several stations (usually one depth profile per station).

3.1. Turnover of free glucose and support of bacterial production

Glucose was the only detectable dissolved neutral monosaccharide during our study, as has been found for other marine systems when concentrations are low (e.g. Skoog et al., 1999). Dissolved free glucose concentrations during all cruises were very low and ranged from undetectable during October–November 1996 in the Ross Sea to 14 nM following the phytoplankton bloom in the Ross Sea in January–February 1997 (Table 2). These concentrations are among the lowest that have been reported for oceanic waters and in particular they are much lower than the >40nM levels observed in the Central Arctic Ocean (Rich et al., 1997) (Table 3).

Glucose turnover was also low compared to previous studies. Rate constants ranged from 0.003 d^{-1} in November 1997 to 0.06 d^{-1} in January 1997 in the Ross Sea; turnover in the Polar Front zone was generally at the high end of this range, 0.02–0.07 d^{-1} (Table 2), but still low compared to other oceanic regimes. In particular, glucose turnover was much higher in the Central Arctic (0.2–0.6 d^{-1}) (Rich et al., 1997) than what we observed during this Antarctic study (Table 3).

Uptake of dissolved free glucose was calculated from data on glucose turnover and concentrations and then the uptake rates were compared to bacterial production in order to estimate the contribution of glucose to supporting bacterial growth. Glucose uptake was highly correlated ($r = 0.818$; $p < 0.01$) with bacterial production when data from all cruises were analyzed together in a log–log plot (Fig. 1). However, all uptake rates were substantially below the 1 : 1 line on this plot; that is, glucose uptake was usually a small fraction of bacterial production. The highest percentages of 6% and 11% were observed in the Ross Sea in January–February and in the Antarctic Polar Front region in December 1997, respectively (Table 2).

Glucose supports a much higher fraction of bacterial production in two of the three other oceanic systems for which similar data are available. In the Central Arctic up to 100% of bacterial production could be accounted for by uptake of glucose alone (Rich et al., 1997), and in the equatorial Pacific glucose uptake was 15–47% of bacterial production (Rich et al., 1996). Studies

Table 2
Summary of dissolved free glucose concentrations, glucose turnover, and fraction of bacterial production (BP) supported by glucose in surface waters (0–70 m). Means ± SD are given for N samples. To compare with production, glucose uptake was converted to C units

Region	Time period[a]	Glucose (nM)	Glucose turnover (d^{-1})	Glucose uptake/BP (%)	N
Ross Sea	October–November 1996	<3	0.00084 ± 0.00054	2 ± 1	9
	January–February 1997	14 ± 14	0.058 ± 0.042	6 ± 9	24
	April–May 1997	NA[b]	0.00027 ± 0.00013	0.2 ± 0.2	15
	November–December 1997	5.9 ± 4.9	0.0041 ± 0.0025	3 ± 3	20
Polar Front	December 1997–January 1998	10 ± 24	0.078 ± 0.075	11 ± 8	29
	February–March 1998	2.0 ± 0.9	0.016 ± 0.016	4 ± 4	30

[a] For complete dates and names of cruises, see Table 1.
[b] Free glucose concentrations are not available. For calculating uptake, we assumed the detection limit (3 nM). For calculating average glucose concentrations, a zero was used when concentrations were undetectable.

Table 3
Summary of studies of dissolved neutral sugar concentrations and fluxes in oceanic surface waters. Only studies reporting sugar concentrations determined by HPLC-PAD are given

Location	Free glucose conc. (nM)	Glucose turnover (d^{-1})	%gluc uptake/BP[a]	Dissolved combined sugars (μM-C)	% of DOC in combined sugars[b]	Reference
Equatorial Pacific February–March 1992	30–110	0.02–0.2	30	1–8	2–17	Rich et al. (1996); Kirchman and Borch (unpublished data)
Equatorial Pacific August–October 1992	5–20	0.4–0.6	15–30	1–4	1–7	Rich et al. (1996); Kirchman and Borch (unpublished data)
Equatorial Pacific	NA	NA	NA	1–2[c]	1–7	Skoog and Benner (1997)
Gulf of Mexico	3–7	0.2–0.4	1–10	NA	NA	Skoog et al. (1999)
Central Arctic	31–68	0.2–0.5	10–97	3–8	2–20	Rich et al. (1997)
Ross Sea	0–14	0.0008–0.06	0.2–6	0.3–3	1–11	This study
Antarctica Polar Front Zone	0–10	0.02–0.08	4–11	1.3–1.7	NA	This study

[a] Glucose uptake (nM-C d^{-1}) divided by bacterial production X 100.
[b] Dissolved combined sugar concentrations divided by total DOC concentrations X 100.
[c] Concentrations in unfiltered samples. Since concentrations of particulate neutral sugars were low related to the unfiltered sample, values for unfiltered samples are close to the dissolved concentrations. Data from 2°S and 12°S.

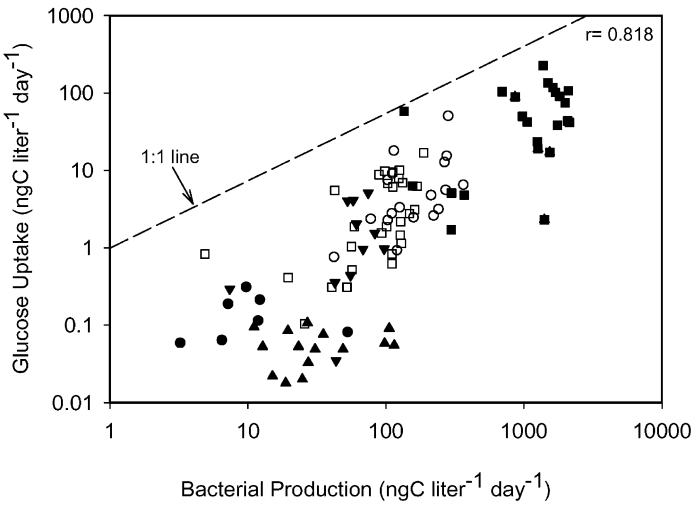

Fig. 1. Glucose uptake versus bacterial production (leucine incorporation) for all cruises listed in Table 2. Ross Sea October–November 1996 (●); Ross Sea January–February (■); Ross Sea April–May (▲); Ross Sea November–December 1997 (▼); Antarctic Polar Front October–November (○); and Antarctic Polar Front February-March (□). The dashed line is the 1 : 1 line.

of lakes have also generally found that glucose supports a large fraction of bacterial production (Jørgensen, 1990; Jørgensen and Jensen, 1994). In contrast, glucose uptake was low in the Gulf of Mexico where it supported $<10\%$ of bacterial production (Skoog et al., 1999). Uptake rates of glucose was relatively low in the Gulf in spite of high turnover rate constants because concentrations were low (<10 nM) (Skoog et al., 1999). In general, however, variation in uptake rates is driven by variation in rate constants because these vary >100-fold, whereas average glucose concentrations vary about 10-fold among the oceanic waters examined to date (Table 3).

We measured incorporation of glucose into the cold TCA insoluble fraction, not total uptake, which must be remembered when comparing our numbers with previous studies. However, using total uptake rates does not change our conclusions. Our rates are probably about half of total glucose uptake, if experiments in the Delaware estuary and the equatorial Pacific (Kirchman, unpublished data) are any guide. Even given this large correction, we still conclude that little bacterial production appears to be supported by glucose and hence glucose was probably a small fraction of the labile DOC flux. If the percentages reported in Table 2 are doubled to correct for lost during TCA extraction, then glucose may have supported up to 20% of bacterial production in the Polar Front regime in November 1997. But this and our other percentages are still low compared to most other studies (Table 3).

Like other studies, we have compared glucose uptake into biomass with bacterial production, i.e. neither estimate includes respiration. We can estimate how much bacterial respiration is potentially supported by glucose by calculating the ratio of glucose uptake to total uptake (both uptake and respiration) which can be termed the ''glucose utilization efficiency''. These efficiencies are generally on the order of 40–60% (e.g., Rich et al., 1996), i.e. higher than estimates of overall carbon growth efficiencies (12–38%) in the Ross Sea (Carlson et al., 1999). Extrapolations from these percentages suggest that relatively little bacterial respiration appears to be supported by glucose catabolism, except when glucose uptake into biomass exceeds 75% of bacterial production. Glucose does not support much respiration in nearly all oceanic environments examined to date, but especially in the waters we studied and in the Gulf of Mexico (Skoog et al., 1999).

3.2. Glucose versus amino acid turnover: why low rates?

Turnover of free glucose was significantly correlated ($r = 0.781$; $p<0.01$) with turnover of dissolved free amino acids (DFAA) when data from all cruises were analyzed together (Fig. 2). Glucose turnover was substantially lower (nearly 7-fold, on average) than amino acid turnover during every AESOPS cruise (Fig. 2). This difference may be misleading because amino acid turnover was measured with a mixture of amino acids. However, turnover of a free neutral sugar mixture is likely to be even slower than turnover of free glucose alone because the few available data indicate that uptake of free neutral sugars other than glucose is slower than glucose (Rich, 1993; Rich et al., 1996). In the most extensive study of this issue, Bunte and Simon (1999) found that turnover rate constants for glucose were much higher than for galactose, fucose, mannose, and glucosamine over a year in Lake Constance.

The relatively high turnover suggests that free amino acids may be important substrates for bacterial growth. Since we do not have data about DFAA concentrations, we need to assume that concentrations are roughly equivalent to glucose concentrations to evaluate how much bacterial

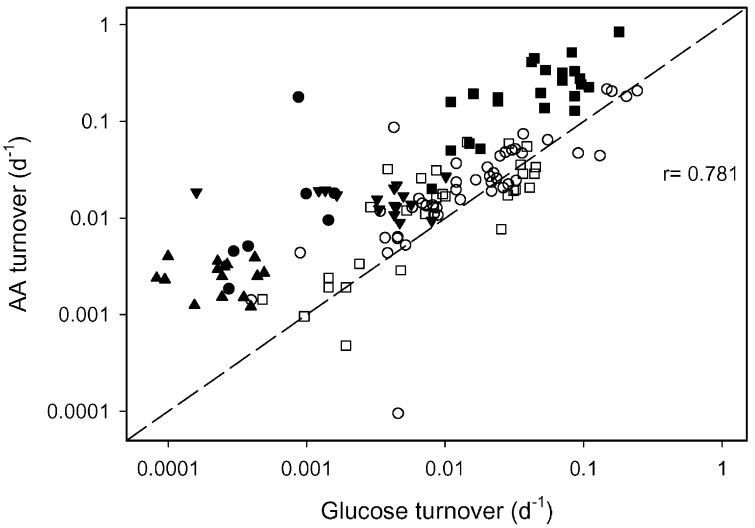

Fig. 2. Turnover of dissolved free amino acids (AA) versus turnover of glucose, both determined by incorporation of ^{3}H-compounds into the TCA-insoluble fraction for all cruises listed in Table 2. Ross Sea October–November 1996 (●); Ross Sea January–February (■); Ross Sea April–May (▲); Ross Sea November–December 1997 (▼); Antarctic Polar Front October–November (○); and Antarctic Polar Front February–March (□). The dashed line is the 1 : 1 line.

growth can be supported by DFAA. This assumption probably leads to conservative estimates of DFAA uptake because in the Central Arctic DFAA concentrations were much higher than dissolved free neutral sugar concentrations (Rich et al., 1997). Even given equal concentrations, the relatively high turnover rate constants imply that DFAA potentially support a large fraction (at least 50%) of bacterial growth in the surface waters we studied.

Turnover of glucose was lower in these Antarctic waters than in other oceanic regimes where this parameter has been measured (Table 3). Rate constants for glucose uptake were low even when bacterial activity was high during our study. For example, glucose turnover was low in the Ross Sea during January–February 1997, but leucine incorporation then was as high as seen in the equatorial Pacific (Ducklow et al., 2000, 2001). One explanation is temperature limitation of glucose uptake. But this explanation would require that temperature somehow affects glucose uptake more so than leucine incorporation and by implication the uptake of other DOC compounds supporting bacterial growth. This seems unlikely. Another, perhaps better argument against temperature is that glucose and amino acid turnover were orders of magnitude faster in the Central Arctic (Rich et al., 1997) where water temperatures were as cold as during our study. What seems most likely is that glucose turnover was low during our study because low concentrations had not induced rapid uptake systems in bacteria. The problem with this hypothesis is that the Gulf of Mexico also has low glucose concentrations, but quite high glucose rate constants (Skoog et al., 1999).

The low turnover and uptake of glucose is consistent with the observation that bacterial production is a small fraction of primary production in the Ross Sea (Ducklow et al., 2000, 2001). Ducklow et al. (2000, 2001) argue that the relatively low bacterial production is not due to temperature limitation because per cell leucine incorporation rates were as high in the austral

summer in the Ross Sea as in the equatorial Pacific. Rather, they concluded that the DOC supply limited bacterial growth, a hypothesis supported by organic and inorganic addition experiments (Kirchman and Steward, unpublished data). The flux of labile DOC is low in the Ross Sea probably because grazing pressure is also low, allowing the development of large phytoplankton and bacterial blooms (Arrigo and McClain, 1994; Ducklow et al., 2000, 2001).

3.3. Contribution of dissolved combined neutral sugars (polysaccharides) to DOC

Dissolved combined neutral sugars measured by our method are probably mostly from polysaccharides, but our estimates also would include other sugar-containing polymers (e.g. glycoproteins) and adsorbed sugars. There is NMR evidence that polysaccharides dominate the combined neutral sugars of high molecular weight DOC (Aluwihare et al., 1997; Aluwihare and Repeta, 1999), but the case is less clear for the low molecular weight fraction. The high molecular weight fraction contains about 50–75% of all dissolved combined neutral sugars (Skoog and Benner, 1997).

In the Ross Sea, concentrations of dissolved combined neutral sugars in surface waters were very low (50 nM monomer equivalents or 0.3 μM-C) in October–November 1996 and increased nearly 10-fold following the phytoplankton bloom in January 1997 (Fig. 3A). The concentrations were about 1.5 μM-C in surface waters (0–60 m) of the Polar Front Zone, i.e. two-fold lower than the highest concentrations in the Ross Sea (Table 3). The 10-fold increase in dissolved combined neutral sugar concentrations from October to January in the Ross Sea is associated with the phytoplankton bloom that develops over this time period.

What is surprising is that the relative increase in DOC concentrations during this same period is much smaller, only about 20%, from 42 to about 48 μM-C in January–February 1997 (Fig. 3B). The average DOC concentration in January–February was higher (55 ± 5 μM-C) when the entire data set is analyzed (Carlson et al., 2000), but the percent increase (31%) was still much smaller than that in sugars. (The DOC data given in Fig. 3B correspond to only samples with sugar concentration data. The DOC plus sugar data set is smaller than the entire DOC data set.) Dissolved combined neutral sugars accounted for usually less than 10% of DOC concentrations in the Ross Sea (Table 3).

Combined neutral sugars appear to be a substantial fraction of the semi-labile fraction of DOC in the Ross Sea. This semi-labile fraction can be calculated by subtracting the concentrations of refractory DOC, i.e. the deep-water value (42 μM-C; Carlson et al., 1998, 2000), from the surface-layer concentrations. DOC concentrations in October–November 1996 were essentially at this deep-water value (i.e. the semi-labile fraction was negligible), but then increased by 6 ± 0.6 μM-C in January–February 1997 in samples with complementary sugar data. During the same period, the dissolved combined neutral sugar pool increased by 2.8 ± 0.3 μM-C or about 45% of the semi-labile DOC pool. (The standard "errors" cited here reflect spatial and temporal variability over the entire cruise, including variation among 3–4 depths.)

The fraction of semi-labile DOC recovered as neutral sugars in the Ross Sea is among the highest observed to date (Fig. 4). Combined neutral sugars also comprised a high fraction of DOC in some stations occupied in the Central Arctic (Rich et al., 1997), but generally sugars were $<20\%$ of semi-labile DOC (Fig. 4). Likewise, dissolved combined neutral sugars sometimes were over 20% of semi-labile DOC in surface waters of the equatorial Pacific, but again overall the

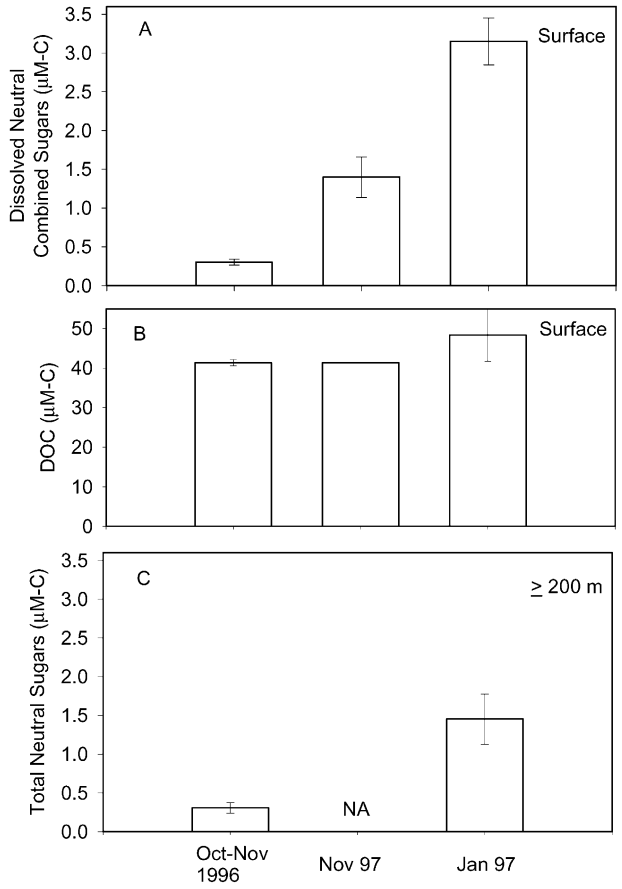

Fig. 3. Average concentrations in surface and deep waters during three Ross Sea cruises. (A) Dissolved combined neutral sugars; (B) DOC concentrations; and (C) Total neutral sugars which are mostly dissolved (see text). Error bars are standard errors. "NA" indicates that data are not available.

levels were much lower than what we observed in the Ross Sea. As discussed below, some of the dissolved combined neutral sugars may be rather refractory and should not be included in the semi-labile pool. However, the error in including the refractory sugars is rather small ($<10\%$), because of the large difference in concentrations between deep and surface waters (0.3 versus 3.0 μM-C).

Dissolved combined neutral sugars are a large fraction of the semi-labile pool in the Ross Sea mainly because the accumulation of non-sugar DOC components was low, not because combined sugars were exceptionally high. Except for the October–November 1996 Ross Sea cruise, the combined neutral sugar concentrations observed here were similar to concentrations found in other oceanic regimes (Table 3). In contrast, DOC concentrations were lower than observed in other oceans (Carlson et al., 1998; Carlson et al., 2000). The average DOC concentration in samples with comparable sugar data was 48 ± 2 μM-C in January–February, much lower than concentrations observed in other oceans (>65 μM-C; Fig. 3). For the entire DOC data set, DOC

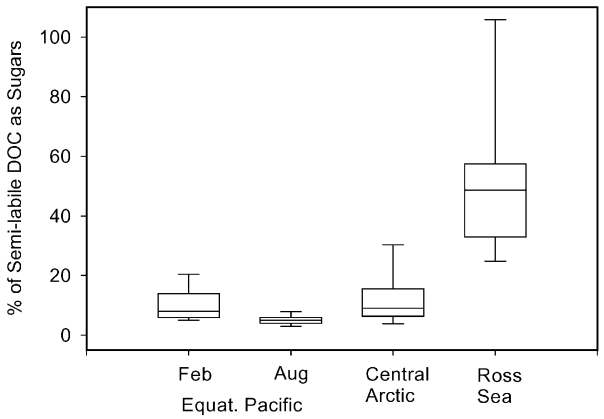

Fig. 4. Dissolved combined neutral sugars as a fraction of semi-labile DOC in surface waters of various oceanic systems. Data from the equatorial Pacific are from Kirchman and Borch (unpublished data) and those from the Central Arctic are from Rich et al. (1997). The box indicates 10th and the 90th percentiles and the error bars are 5th and 95th percentiles.

was 55 ± 5 μM-C on average in January–February 1997. At times and at selected depths and locations within the Ross Sea, concentrations were as high as observed elsewhere (Carlson et al., 2000), but the overall average was still low. The net result is that dissolved combined neutral sugars make up a large fraction of the semi-labile pool in the Ross Sea.

In other environments, neutral sugars are a small fraction of the total carbohydrate pool (ca. 7–20%; Skoog and Benner, 1997) measured by other approaches (e.g. the MBTH method); the other carbohydrates have not been identified at the molecular level (Skoog and Benner, 1997). In contrast, neutral sugars are probably a much larger fraction of total carbohydrates in the Ross Sea, since neutral sugars alone are nearly 50% of semi-labile DOC. Even if carbohydrates made up the other half of semi-labile DOC, neutral sugars would roughly equal the carbohydrates unidentified at the molecular level in the Ross Sea, much higher than observed in other oceanic regimes. The unidentified carbohydrate pool appears to be much smaller (factor of two or more) in the Ross Sea than elsewhere. The molecular composition of dissolved carbohydrates has implications for understanding the age of selected DOC pools and its susceptibility to degradation, as discussed below in greater detail.

3.4. Degradation of dissolved sugars and semi-labile DOC

Changes in DOC concentrations suggest that the semi-labile DOC pool in the Ross Sea is actually quite labile. Concentrations of DOC reached 55 ± 5 μM-C in January–February 1997, but then decreased to nearly background levels less than two months later (43 ± 3 μM-C; Carlson et al., 2000). The lability of this DOC pool is consistent with the sugar data. As discussed above, neutral sugars make up a large fraction of the semi-labile DOC and of the total carbohydrate pool. This relatively high contribution by neutral sugars is similar to freshly produced DOC; neutral sugars are roughly 50% of carbohydrates produced by phytoplankton cultures (Biersmith and Benner, 1998; Aluwihare and Repeta, 1999). Although not large when integrated over time and space, what DOC accumulated in the Ross Sea apparently was sugar-rich and labile.

We can obtain a crude estimate of the degradation rate of dissolved combined neutral sugars by examining how concentrations decreased from February to October in the Ross Sea. The calculation assumes that production of sugars is negligible (i.e. this is a net degradation rate), that advection can be ignored, and that degradation occurs uniformly over the entire time period. Using these assumptions, the rate constant is $0.01\,d^{-1}$ (ln(0.3/3.2)/240 d). This rate constant for combined neutral sugars is the same order of magnitude as the rate constants for semi-labile DOC. From data on the decrease in DOC from February to April, a rate constant of $0.03\,d^{-1}$ can be calculated (ln{(43–40)/(55–40)}/60 d). Rate constants for DOC utilization in two bag experiments, 0.01 and $0.03\,d^{-1}$ in January–February 1997 (Carlson et al., 1999), were remarkably similar to the in situ-based rate constants. In contrast, analogous rate constants for the Sargasso Sea are much lower ($0.0002–0.003\,d^{-1}$) (Hansell and Carlson, 2001).

To compare degradation with bacterial production, we need to calculate incorporation into biomass; the rate constant calculated above includes losses due to respiration in addition to incorporation into biomass. Assuming a 50% utilization efficiency, the $0.01\,d^{-1}$ estimate for sugars should be reduced to $0.005\,d^{-1}$ in order to estimate how much bacterial biomass production can be supported by combined neutral sugars. If the uptake-only constant was $0.005\,d^{-1}$ and given a concentration of 3 μM-C in January in the Ross Sea, then about 25% of bacterial production could have been supported by use of dissolved combined neutral sugars. Of course these combined sugars may support an even higher fraction of bacterial growth in the austral fall and winter when DOC inputs have ceased.

Unfortunately, there are very few estimates of degradation rates or rate constants for dissolved combined sugars in the oceans. Using the MBTH method for total carbohydrates, Burney (1986) calculated degradation rates from the decrease in concentrations in dark incubations with water from the Sargasso Sea and Gulf of Maine. Using his data, we calculate rate constants ranging from 0.3 to $0.4\,d^{-1}$, orders of magnitude higher than our estimate. For comparison, a rate constant of $0.06\,d^{-1}$ can be calculated from the data of Janse et al. (1999) who examined degradation of purified extracellular polymers from a *Phaeocystis* strain. In addition to the obvious difference in environments, the degradation of the carbohydrates measured by the MBTH method may differ from that of the neutral sugars. Borch and Kirchman (1997) used the range of concentrations found in different environments to argue that the turnover of neutral sugars has to be more rapid than MBTH-positive carbohydrates. This hypothesis is consistent with the observation that neutral sugars appear to decrease more rapidly than other carbohydrates as DOC ages (Biersmith and Benner, 1998; Aluwihare and Repeta, 1999).

3.5. Molecular composition of dissolved combined neutral sugars

Previous work had suggested that the molecular composition of sugars released by acid hydrolysis is rather uniform among diverse oceanic regimes (McCarthy et al., 1993; Aluwihare et al., 1997; Borch and Kirchman, 1997), although relatively few studies using the HPLC-PAD method have been published (Table 4). This uniform composition has been observed for the high molecular weight fraction of DOM (McCarthy et al., 1993; McCarthy et al., 1996; Aluwihare et al., 1997; Skoog and Benner, 1997), total DOM (Borch and Kirchman, 1997) and POM and DOM measured together (Skoog and Benner, 1997). In studies to date, glucose is usually the most abundant combined neutral sugar, but other sugars such as galactose can be nearly as common.

Table 4
Molecular composition of the dissolved combined neutral sugar pool in various oceanic waters. Only studies reporting sugar concentrations determined by HPLC-PAD are given[a]

	Depth (m)	% Of total (molar)						Reference
		Fuc	Rha	Ara	Gal	Glc	Man + Xyl	
Oregon, inshore	10–40	12	2	9	17	46	15	Borch and Kirchman (1997)
Oregon, offshore	15	13	8	8	31	17	24	Borch and Kirchman (1997)
Sargasso Sea	0	17	10	9	15	35	15	Borch and Kirchman (1997)
Equatorial Pacific	10–80	18	9	12	23	21	18	Borch and Kirchman (1997)
Equatorial Pacific	2–100	11	8	9	16	28	27	Skoog and Benner (1997)[b]
Central Arctic	0–100	13	7	5	9	39	27	Rich et al. (1997)
Mean (SD)		15(3)	7(3)	10(2)	18(7)	29(11)	22(6)	
Ross Sea (January)	0–60	6	7	14	17	37	20	This study
Polar Front	0–60	11	9	11	20	35	17	This study

[a] McCarthy et al. (1993) and Aluwihare et al. (1997) reported results for high molecular weight fractions (> 1000 Da), whereas the data given here are for the entire DOC pool.

[b] Values are from unfiltered samples. Since concentrations of particulate neutral sugars were low related to the unfiltered sample, values for unfiltered samples are close to the dissolved concentrations. Skoog and Benner (1997) reported separate concentrations for mannose and xylose, but these two sugars are added together here.

The molecular composition of dissolved combined neutral sugars during our study was similar to the "global" average only during cruises with relatively high biological production, i.e. the austral summer Ross Sea cruise and both Polar Front cruises.

Fig. 5 compares the Ross Sea to the global average by plotting the percentages for the Ross Sea sugars versus the average percent composition. The molecular composition of neutral sugars during the austral summer (January–February) is similar to the global average and follows the 1 : 1 line within experimental errors (Fig 5). In contrast, the molecular composition during the austral spring cruises (October) differed substantially from the global average and the summer values. All sugars from the spring are substantially off the 1 : 1 line with the exception of fucose. The glucose and arabinose percentages are clearly much higher than the 1 : 1 line, indicating that these two sugars, most notably glucose, was substantially enriched in the dissolved combined pool during early spring compared to the austral summer and the global average. Rhamnose was not detected during the austral spring cruise, whereas it was 7% of dissolved combined neutral sugars during the other cruises and in other oceanic regimes (Table 4). Galactose and mannose + xylose are below the line, indicating that these sugars were also depleted during the austral spring cruises.

We think that the sugars observed in the early austral spring cruises (and deep water—see below) are from a refractory pool that has survived degradation by bacteria for > 8 months. Degradation has removed the more labile components, and this diagenetic impact is observable in the sugar composition. The relatively high glucose concentrations found in this refractory pool is consistent with the observation of high glucose amounts in low molecular weight DOC (Skoog and Benner, 1997); combined glucose in the low molecular weight fraction is about 50% of total neutral sugars, similar to what we observed for the apparently refractory sugars and higher than

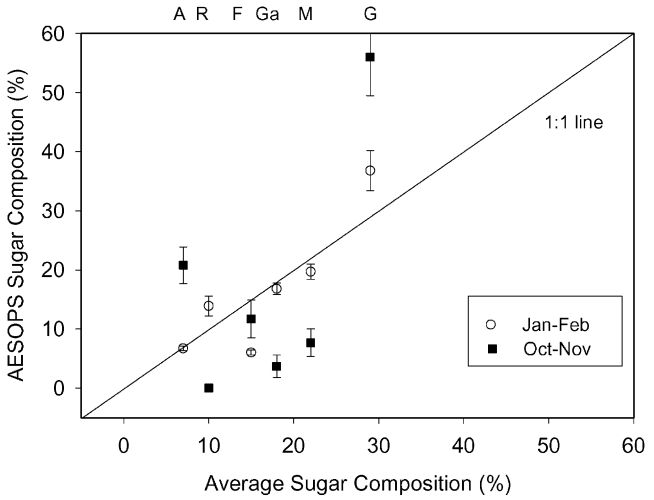

Fig. 5. Composition of dissolved combined aldoses (% of total on a molar basis) during two Ross Sea cruises compared with the global average given in Table 4. Error bars are standard errors. The letters refer to: glucose (G), mannose + xylose (M), galactose (Ga), fucose (F), rhamnose (R) and arabinose (A).

the global average of about 30% in surface waters with fresh inputs from the plankton (Skoog and Benner, 1997). With the exception of free monomers such as glucose and DFAA, the low molecular weight fraction is thought to be the most refractory component of DOC (Amon and Benner, 1994). Glucose yields have been suggested to indicate labile POM (Hernes et al., 1996), but for the dissolved pools, it may indicate the opposite.

We had expected that the phytoplankton bloom in the Ross Sea would have a large impact on sugar concentrations and composition. The effect of the bloom on concentrations is clear, but its effect on the composition of dissolved sugars is more subtle. The composition of bloom-associated sugars did not differ from that of sugars found in low-latitude oceans with greatly different phytoplankton assemblages that probably produce different polysaccharides (Biersmith and Benner, 1998; Aluwihare and Repeta, 1999). In general, phytoplankton blooms may have some impact on sugar composition and by implication the composition of the entire DOC pool, but the effect seems to be quite transient, lasting only days once the bloom begins to decline (Meon and Kirchman, 2001). The implication is that the composition of the observed DOC pool depends heavily on selective removal of DOC components by heterotrophic bacteria and less so on differential production of various organic compounds.

3.6. Sugars and DOC in deep waters

Dissolved neutral sugar concentrations in deep waters (> 200 m) were very low (0.3 μM-C) in the Ross Sea in October–November 1996 (Fig. 3). By January–February 1997, deep-water concentrations had increased by more than 3-fold to about 1.5 μM-C, although they were still substantially less than surface concentrations (> 3 μM-C). In contrast, total DOC in deep waters did not change significantly from October 1997 to January–February 1997; it averaged

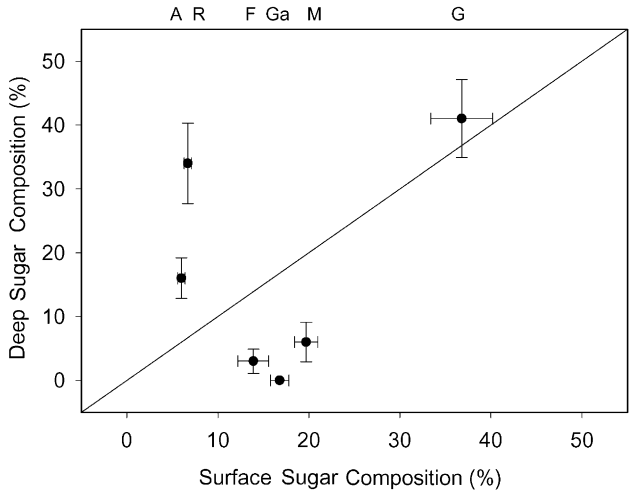

Fig. 6. Composition of dissolved combined aldoses (% of total on a molar basis) in surface waters of the Ross Sea in the austral summer (January–February 1997) versus deep waters in spring (October–November 1996). Error bars are standard errors. The letters refer to: glucose (G), mannose + xylose (M), galactose (Ga), fucose (F), rhamnose (R) and arabinose (A).

$42.2 \pm 0.8\,\mu$M-C (Carlson et al., 2000). Although the $1\,\mu$M-C increase in neutral sugar concentrations is relatively large for deep waters, it is indistinguishable from the natural variation in deep-water DOC concentrations ($0.8\,\mu$M-C).

The overall sugar composition of the deep-water dissolved pool was quite different from the surface pool in summer (Fig. 6). Arabinose and rhamnose were both enriched several-fold in the deep pool whereas fucose, galactose and mannose + xylose were all substantially depleted and were nearly undetectable, unlike the sugars in summer. The composition of this deep sugar pool, on the other hand, was similar (with the exception of glucose) to the other presumed refractory pool, the sugars observed in surface waters in the spring. Unexpectedly, the glucose content of deep combined neutral sugars was about equal to that observed in combined sugars presumed to be labile, i.e. the surface pool in summer. This was unexpected because the refractory spring sugar pool was enriched in glucose compared to the surface pool in summer. This difference in glucose yields probably reflects the different sources for dissolved sugars in the surface layer (suspended POM) versus the deep layer (sinking POM).

4. Conclusions

Molecular analysis of DOC components can provide insights into fluxes of labile DOC and the production and fate of less labile DOC that accumulates during a growing season. In our study, the data showing on low glucose uptake help us understand why bacterial production is so low relative to primary production in the Ross Sea (Ducklow et al., 2000, 2001) and why fluxes of DOC are small compared to POM dynamics (Carlson et al., 1998). About half of the semi-labile

DOC in the Ross Sea is dissolved combined neutral sugars, which helps to explain why the DOC accumulated during the phytoplankton bloom was degraded so quickly once the austral winter began to set in. The accumulated pool of dissolved polysaccharides and other semi-labile DOC components potentially could support much bacterial production and in turn other microbial loop organisms after the bloom ends. The impact of degradation on the molecular composition of the dissolved combined neutral sugar pool becomes apparent when we examine sugars from the early spring and deep waters, two pools removed in time and space from production processes. With the exception of these two pools, however, the composition of the dissolved neutral sugars was similar to that observed in low latitude oceans. This constancy in composition suggests that production and degradation processes have the same impact on DOC composition in the world's oceans in spite of great variation in many biogeochemical parameters.

Acknowledgements

We thank the following people who helped with various aspects of the project and made this study possible: Matt Church, Laura Fandino, Dawn Ward, Helen Quinby, Alison Sanford and Flynn Cunningham. The input of W. Gardner and Walker Smith is also greatly appreciated. The comments by Ron Benner, Annelie Skoog and two anonymous reviewers improved the paper. This work was supported by National Science Foundation grants OPP-9531977 (DLK), OPP-9730734 (HWD), OPP-9530851 (Farooq Azam), OPP-9530609 (CAC), and OPP-9530845 (DAH).

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