# Radiocarbon analysis of neutral sugars in high molecular weight dissolved organic carbon: implications for organic carbon cycling

Daniel J. Repeta[1], Lihini I. Aluwihare[2]

[1]Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA USA 02543

[2]Geosciences Research Division, Scripps Institution of Oceanography, La Jolla, CA USA 92093

## Abstract

We used compound specific natural abundance radiocarbon analyses of neutral sugars to study carbon cycling of high molecular weight (HMW) dissolved organic carbon (DOC) at two sites in the North Pacific Ocean. Sugars released from HMWDOC by acid hydrolysis were purified by high-pressure liquid chromatography and analyzed for radiocarbon content via accelerator mass spectrometry. We find the seven most abundant sugars recovered from HMWDOC to have similar radiocarbon values, supporting the hypothesis that these sugars are incorporated into a common family of polysaccharides. Neutral sugar $\Delta^{14}C$ values from surface waters collected in 1999 and 2001 are $89\pm13‰$ and $57\pm6‰$ respectively, which are much more enriched in radiocarbon than found in previous studies that used operationally defined carbohydrate fractions. Radiocarbon values for HMWDOC neutral sugars are the same as, or only slightly depleted relative to dissolved inorganic carbon (DIC), consistent with rapid cycling and a short (< 3 yr) residence time. In addition, we find the $\Delta^{14}C$ value of neutral sugars at 600m to be 20‰ enriched relative to DIC $\Delta^{14}C$, suggesting a fraction of dissolved neutral sugars at this depth are introduced by dissolution from large, rapidly sinking particles.

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## Introduction

Marine heterotrophic bacterial production is fueled by the metabolism of dissolved organic carbon (DOC) (Williams, 2000). Numerous studies show bacterial production is carbon limited, even though DOC concentrations are high throughout the water column (Kirchman, 1990; Carlson and Ducklow, 1995; Cherrier *et al*., 1996; Kirchman and Rich, 1997; Kirchman, 2000). The large reservoir of DOC that accumulates in seawater is therefore believed to be largely unavailable to meet bacterial carbon demand (Kirchman *et al*., 1993; Carlson and Ducklow, 1995). Radiocarbon measurements of deep sea DOC support the view that a large fraction of DOC is recalcitrant (Williams *et al*., 1969, Williams and Druffel, 1987, Druffel *et al*., 1992). Average radiocarbon values for DOC below 1000m at mid gyre sites in the North Pacific and North Atlantic Oceans are –525$\pm$20‰ and –390$\pm$10‰ respectively, consistent with a residence time of 4000-6000 yr, or several ocean-mixing cycles (Druffel *et al*., 1992). However, at depths < 1000 m DOC concentrations begin to rise, and surface ocean DOC values can be up to 50 µM higher than deep sea concentrations (Peltzer and Hayward, 1995; Hansell and Carlson, 1998). The elevated concentrations and the shorter average residence time (~2000 yr) of DOC in surface waters points to a large (30-50 GT C), recently synthesized DOC reservoir in the upper ocean. This reservoir is considered to be semi-reactive, turning over on timescales of upper-ocean mixing (months to decades). Due to the large size of the reservoir, semi-reactive DOC has the potential to satisfy a substantial fraction of annual bacterial carbon demand, but without a more exact estimate of its residence time, the role of semi-reactive DOC in bacterial metabolism cannot be fully evaluated.

Semi-reactive DOC is concentrated in the high molecular weight fraction of DOC (HMWDOC). Between 20-35% of total DOC is colloidal or HMWDOC and can be isolated by ultrafiltration for isotopic measurements (Buesseler *et al*., 1996). HMWDOC has higher (more modern) $\Delta^{14}C$ values than total DOC except in some deep-water samples influenced by the benthic boundary layer (Guo *et al*., 1994; 1996; Guo and Santschi, 1997, Aluwihare *et al*., 2002, Loh *et al*., 2004. Nuclear magnetic resonance spectra of HMWDOC show that most of the carbon, at least 50-70%, is carbohydrate. The remaining carbon is a mixture of proteins (3-5% HMWDOC), lipids (1% HMWDOC), and uncharacterized humic substances. Both the absolute and relative amounts of carbohydrate in HMWDOC decrease with depth (Aluwihare *et al*., 2002), in parallel with the loss of semi-reactive DOC, indicating that carbohydrates are a large part of the semi-reactive fraction of HMWDOC. Therefore, in order to obtain an estimate of semi-reactive DOC residence time without interference from the recalcitrant DOC fraction, several recent studies have reported radiocarbon measurements of carbohydrate fractions isolated from high molecular weight dissolved organic matter (HMWDOM).

Santschi *et al.* (1998) used ethanol precipitation to concentrate the carbohydrate fraction from surface HMWDOM sampled in the Middle Atlantic Bight and found this fraction was 138‰ enriched in $^{14}C$ relative to HMWDOC ($\Delta^{14}C$ of 26‰ *vs* -112‰). No enrichment was found for ethanol precipitates of deep sea HMWDOC samples, which are known to have much lower amounts of carbohydrate. More recently, Loh *et al*. (2004) used ion exchange chromatography and acid precipitation to isolate a "carbohydrate-like" fraction from HMWDOC. The isotopic value of carbohydrate-like fractions isolated from surface water HMWDOC ranged from 7‰ to 13‰ and were enriched in

radiocarbon by 18-97 ‰ over HMWDOC $\Delta^{14}C$ values, in agreement with the results of Santschi *et al*. (1998). Expected surface water DIC $\Delta^{14}C$ values at the study sites are significantly greater than the $\Delta^{14}C$ values reported for HMWDOC "carbohydrate-like" fractions, suggesting slow cycling and a long residence time for HMWDOC carbohydrates (> several decades). However, as carbohydrates were not directly identified or quantified in fractions that were analyzed for radiocarbon, the relative depletion in $\Delta^{14}C$ observed in both studies could be due to the inclusion of radiocarbon depleted, non-carbohydrate impurities in the carbohydrate-like fraction.

One potential solution for addressing the uncertainties inherent in radiocarbon measurements made on operationally defined HMWDOM fractions is to make compound specific radiocarbon measurements on neutral sugars that comprise a major fraction of dissolved carbohydrate. Nuclear magnetic resonance (NMR) and molecular level (gas chromatography-mass spectrometry, high pressure liquid chromatography (HPLC)) analyses reveal that 10-20% of HMWDOC is a suite of seven neutral sugars, and that the distribution of these sugars is conservative in samples from different geographic locations and depths in the ocean (McCarthy *et al*., 1996, Aluwihare *et al.*, 1997, Borch and Kirchman, 1997). HMWDOC with similar characteristics is produced by marine phytoplankton in culture, and Aluwihare *et al.* (1997) concluded that phytoplankton synthesize a specific family of closely related acylated polysaccharides (APS) that accumulate as semi-reactive DOC in seawater. APS is distinguished by a novel suite of seven neutral sugars and a high relative abundance of N-acetylated sugars (Aluwihare *et al*., 2005). In an earlier study of dissolved carbohydrates in the NW Atlantic Ocean, we measured the $\Delta^{14}C$ value of three neutral sugars (rhamnose, fucose, xylose) isolated from

HMWDOC by acid hydrolysis and purified by HPLC (Aluwihare *et al.* 2002). Neutral sugar values ranged from 49-92‰, with an average value of 71±30‰, comparable to the value of DIC $\Delta^{14}C$ of 59‰. Unlike other reports using operationally defined carbohydrate fractions, we found purified carbohydrates to have radiocarbon values that suggest very rapid, annual cycling of semi-reactive DOC. If additional measurements show that semi-reactive DOC is cycling on annual rather than decadal time scales, then this reservoir of carbon supports a significant fraction of annual bacterial carbon demand in the ocean.

## Methods

Ultrafiltration equipment, glassware and teflon products were purchased expressly for this study and had no history of exposure to tracer level radiocarbon contamination. Glassware was combusted at 450$^{o}$C overnight before use. Teflon products were cleaned by soaking in concentrated nitric acid for at least 24 hr. All manipulations were carried out in the Fye Organic Geochemical Laboratory, a radiocarbon-free building at the Woods Hole Oceanographic Institution. Water for cleaning and diafiltration was ultra-pure (Milli-Q) grade. Hydrochloric acid, $NH_4OH$ and NaOH were reagent grade ACS.

Large volume seawater samples were drawn from the 15m and 600 m in-take pipe at the Natural Energy Laboratory in Kona, Hawaii in February 2002 (Hawaii 15m, 600m). The samples were filtered to remove bacteria and small particles using a cleaned (10% HCl) Suporflow dual stage (0.8µm and 0.2 µm) Gelman polyether sulfone cartridge filter (Chisolm Corp., Lincoln, RI) fitted to an Advanta stainless steel housing. High molecular weight DOM samples were collected using a cross flow ultrafiltration system

consisting of a stainless steel centripetal pump and membrane housings and a fluorinated high density polyethylene reservoir. The system was plumbed with teflon tubing and fitted with PVDF valves. The ultrafiltration membrane (Separation Engineering, Escondido, CA) nominally retains organic matter with a molecular weight of greater than 1 kDa (> 99% rejection of vitamin $B_{12}$). Membranes were cleaned using isopropanol, detergent (0.01% micro), HCl (0.01N) and NaOH (0.01N), stored in sodium azide (0.55mM), and rinsed with water immediately before use. Between 30,000 and 60,000L of seawater were concentrated to approximately 20L, frozen in fluorinated HDPE containers, and returned to Woods Hole for further processing. Samples were desalted by diafiltration with water, reduced to 2L, and lyophilized to a fluffy white powder.

A sample of surface seawater (3-5 m) was also collected from the North Pacific Subtropical Gyre (NPSG) (31˚00' N, 159˚00' W) in June 1999. The sample was taken contemporaneously with the 20 m, Pacific Ocean HMWDOC sample reported in Loh *et al.* (2004). Seawater was collected using an air-driven diaphragm pump fitted with Teflon tubing deployed over the side of the ship and filtered (0.2 µm Criticap polycarbonate filter cartridges) before being ultrafiltered using a spiral wound 1 kDa nominal molecular weight cutoff filter (Amicon Corp.) mounted on an Amicon DC-10 pump (Aluwihare *et al.*, 2002). Sample concentrates (2-4 L) were stored in 2L Teflon bottles, frozen at -20$^{o}$C and returned to Woods Hole for processing as described above.

Monosaccharides were isolated from 0.5g HMWDOM after acid hydrolysis with 4M HCl (300 ml) at 108$^{o}$C for 4 hours. Following the hydrolysis, samples were cooled and frozen before the acid was removed by freeze drying. The residue was dissolved in approximately 4 ml of water and desalted using Biorex 5 anion exchange resin (4g, 100-

200 mesh, $OH^-$ form, Biorad Corp). The column was washed with 30 ml of ultra-high purity water to elute a carbohydrate fraction. A second fraction containing amino acids and molecularly uncharacterized carbon (MUC) was eluted next with 22 ml of 2M $NH_4OH$. The carbohydrate fraction was freeze dried, dissolved in 1-2 mL of water and further purified by HPLC with refractive index detection using two cation exchange columns (0.8 x 30 cm, sulphonated styrene-divinyl benzene in $Ag^{2+}$ form (Supelcogel$^{TM}$ Ag)) connected in series. Neutral sugars elute in approximately 30-45 minutes, using a flow rate of 0.5 ml/min of high purity water at 80$^o$C (Fig. 1A). Monosaccharides were collected in three fractions, F1 (glucose, rhamnose), F2, (galactose, mannose, xylose), and F3 (fucose, arabinose). Each fraction freeze-dried, was dissolved in 20-40 µL of water, diluted to 100-200 µL with acetonitrile, and further purified by reverse phase HPLC using two amino columns (Hamilton PXP-700) connected in series and eluted at 1 ml/min with 80/20 acetonitrile/water (Fig. 1B). Proton nuclear magnetic resonance ($^1$HNMR) spectra were collected on a Bruker 400 MHz spectrometer with water suppression (zgpr). All spectra were collected using $D_2O$ as a solvent and chemical shifts referenced to water at 4.8 ppm.

Purified sugars were collected, evaporated to dryness, dissolved in 1 ml of water, and transferred to a combusted (850$^o$C, 5 hr) 8" Vycor quartz tube (9mm OD, 7 mm ID) containing 300-500 mg copper oxide (Elemental Microanalyses, Manchester, MA). The samples were lyophilized overnight and sealed under vacuum for combustion. Sealed tubes were combusted at 850$^o$C for 5 hr, and the carbon dioxide generated during combustion was quantified, transferred to a 6 mm pyrex tube, and submitted to the

National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility in Woods Hole, MA for natural abundance radiocarbon analyses.

## Results and Discussion

*Compound specific radiocarbon analyses and DOC cycling in surface seawater.* To measure the residence time of dissolved carbohydrates , we used compound specific radiocarbon analyses of neutral sugars extracted from HMWDOC. Table 1 reports values for DIC $\Delta^{14}C$, HMWDOC $\Delta^{14}C$, and neutral sugar $\Delta^{14}C$ in samples collected at the study site near Kona, Hawaii. Our Hawaii surface seawater sample has a DIC $\Delta^{14}C$ value of 72 $\pm$ 7 ‰ and shows the incorporation of bomb radiocarbon into surface water DIC. Radiocarbon is supplied to the ocean by $CO_2$ exchange with the atmosphere, which is enriched in $^{14}C$ from natural and anthropogenic (above ground testing of nuclear weapons in the 1950's and 1960's) production. Bomb-produced radiocarbon raised marine DIC $\Delta^{14}C$ values from between –50‰ and –80 ‰ prior to 1945, to above 150 ‰ at some North Pacific Ocean sites in the mid 1970s (Druffel, 1987; Mahadevan, 2001). As a result of vertical mixing with radiocarbon depleted subsurface water, surface seawater DIC $\Delta^{14}C$ values are now decreasing and this is reflected in the DIC $\Delta^{14}C$ value of our surface water sample.

Hawaii surface water HMWDOC $\Delta^{14}C$ is 10 ‰, significantly depleted in radiocarbon relative to DIC. Purification of neutral sugars by HPLC yield mixed sugar fractions with $\Delta^{14}C$ values of 36‰ and 49‰, intermediate between DIC $\Delta^{14}C$ and HMWDOC $\Delta^{14}C$ (F1, F2; Table 1). Both HMWDOC and neutral sugar isolates show the incorporation of bomb radiocarbon, indicating that each fraction contains compounds

synthesized over the past fifty years. Further purification by reverse phase HPLC yields seven neutral sugars with radiocarbon values of 47 – 67‰. In an earlier paper, we suggested that the seven neutral sugars isolated from HMWDOM are incorporated into a common family of structurally related algal biopolymers that are selectively preserved as semi-reactive HMWDOM (Aluwihare et al., 1997). Proton and $^{13}$CNMR spectra of HMWDOM isolated from diverse locations in the North and South Pacific Ocean, and the North Atlantic Ocean, including coastal and open ocean sites, show remarkable similarity in the relative amounts of carbon and hydrogen in major functional groups (COOH, HCOH, $COCH_3$, $CH_x$). Likewise, hydrolysis of HMWDOM samples yields the same suite of seven neutral sugars in relatively fixed proportions (McCarthy et al., 1996; Aluwihare et al., 1997; Borch and Kirchman, 1997; Eglinton and Repeta, 2004). HMWDOM with similar NMR spectral characteristics and neutral sugar distributions has been recovered after bacterial degradation of DOM produced in laboratory algal cultures. The conservation of functional group and neutral sugar distributions in HMWDOM across many different oceanographic regimes in samples collected over a 10 year time span, along with the production of HMWDOM with similar characteristics in pure cultures, was taken as evidence for selective preservation of a novel biopolymer in HMWDOM

The similarity in radiocarbon values for different neutral sugars is consistent with our earlier suggestion that these sugars are incorporated into a common family of biopolymers (APS; Aluwihare *et al*., 1997). If neutral sugars were part of a common biopolymer, we would expect them to have the same carbon sources and diagenetic history, and therefore the same isotopic values within the range of normal cellular

isotopic heterogeneity for carbohydrates (3-5‰ for $^{13}C$, ), with an isotopic enrichment for C5 sugars (van Dongen et al., 2002). The radiocarbon data alone does not exclude the possibility that neutral sugars are derived from a mixture of different polysaccharides, however, along with previously published data on functional group (NMR) and monosaccharide distributions in HMWDOM, the similarity in radiocarbon values of neutral sugars does support the hypothesis that neutral sugars are bound together in a common family of biopolymers.

Assuming the radiocarbon determinations of individual sugars in HMWDOC serve as replicates, then the average $\Delta^{14}C$ for neutral sugars in HMWDOC is 57 $\pm$ 6 ‰(1 SD, n=11). This value is only slightly depleted in $^{14}C$ relative to DIC, indicating very recent synthesis of these sugars in surface waters. We believe that the small depletion in the neutral sugar $\Delta^{14}C$ value most likely results from incomplete HPLC purification of individual sugars. Our neutral sugar data is not corrected for blanks. Neutral sugar isolates typically contained 30-50 µmoles of carbon per sugar. Procedural blanks were found to have 0.5-1 µmole carbon for each sugar collected, representing 1-2% of the total carbon analyzed. Procedural blanks were too small to measure radiocarbon directly, so we made radiocarbon measurements on composite blanks by pooling the blanks of all individual sugars. Composite blanks were found to have radiocarbon values of approximately –700 to -800 ‰. If neutral sugars have the same radiocarbon value as DIC (72 ‰), we expect the value of 98-99% purified neutral sugars to have a radiocarbon value that is 7‰ to 16‰ depleted from DIC or about 56-65‰.

We measure the same trends in the radiocarbon distribution for DIC, HMWDOC, and neutral sugars collected in the NPSG (Table 2). Surface seawater DIC $\Delta^{14}C$ is 89$\pm$7

‰, (E. R. M. Druffel, pers. comm.) and is enriched relative to HMWDOC $\Delta^{14}C$ (46‰). Neutral sugars purified from HMWDOM have radiocarbon values between 67-103‰, and an average value of 89 $\pm$ 13‰, equal to DIC $\Delta^{14}C$. As with the Hawaii sample, we find very similar radiocarbon values for each neutral sugar, consistent with the hypothesis that these sugars are part of a common bioploymer.

Compound specific radiocarbon analyses provide a different view of carbon cycling than previous studies that measured radiocarbon in operationally defined fractions of HMWDOC. All previous measurements made on operationally defined "carbohydrate-like" fractions of HMWDOC show this fraction to be significantly depleted relative to DIC $\Delta^{14}C$, consistent with slow cycling and turnover times of several decades (Santschi *et al*., 1998, Loh *et al*. 2004. Using the compound specific approach we find carbohydrates to have radiocarbon values equal to or at most only slightly depleted from DIC, consistent with rapid cycling on annual rather than decadal time scales (see next section).

Williams and Druffel (1987) have modeled upper ocean DOC $\Delta^{14}C$ values as simple two component mixtures of old and new carbon. Their model assumes that old DOC has a concentration and radiocarbon value equal to deep sea DOC, while the new component includes DOC in excess of deep-sea concentrations and has a radiocarbon value equal to DIC $\Delta^{14}C$. Using this approach, Williams and Druffel (1987) showed that the measured isotopic value for DOC in North Pacific Ocean surface water (87 µM; -146 ‰) could be modeled as a mixture of recently synthesized DOC (49 µM C or 56% total DOC; $\Delta^{14}C$ = 150 ‰), and old DOC (38 µM C or 44% total DOC; $\Delta^{14}C$ = -525‰) up-welled from the deep ocean (model result = -147 ‰). A similar calculation for the North

Atlantic Ocean (Sargasso Sea) using the data of Druffel *et al* (1992) yields a value for upper-ocean DOC $\Delta^{14}C$ of -120‰, indistinguishable from the measured value of -127‰. These radiocarbon data support the conclusion that upper ocean DOC is a mixture of old, recalcitrant DOC and new, semi-reactive DOC. However, based on these measurements, the residence time of the semi-reactive fraction cannot be determined to better than a few decades.

The two component model used by Williams and Druffel (1987) predicts upper ocean DOC $\Delta^{14}C$ values quite well, but the major assumption of the model, that there is an old component of DOC with a radiocarbon value equal to deep sea DOC $\Delta^{14}C$, and a new component of DOC with a radiocarbon value equal to DIC $\Delta^{14}C$, has never been verified. Our data for HMWDOC neutral sugars substantiates the assumption that a fraction of upper ocean DOC has a radiocarbon value equal to DIC. In order to measure the radiocarbon value of the old component, we analyzed a molecularly uncharacterized carbon (MUC) fraction isolated by ion exchange chromatography from HMWDOC. Ten percent of HMWDOC from the NPSG site is retained by the Biorex anion ion exchange resin, but eluted by 2 M $NH_4OH$ (22 ml). This fraction has spectral characteristics nearly identical to molecularly uncharacterized carbon (MUC) found in deep sea HMWDOC (Fig. 2), and has a $\Delta^{14}C$ of –416‰. Our $\Delta^{14}C$ value for MUC in surface water is within the range of values for total HMWDOC isolated from 900-5200m at this site (-380 to –440‰, Repeta unpublished; Loh *et al*., 2004), and therefore verifies the existence of an old fraction of DOC in surface water with radiocarbon values similar to deep sea DOC.

The agreement between observed and modeled values for DOC $\Delta^{14}C$ reported by Williams and Druffel (1987) and Druffel *et al*., (1992) along with our measurements of

the radiocarbon values for neutral sugars and MUC suggest most upper ocean HMWDOC is a mixture of new ($\Delta^{14}C$ = DIC) and old ($\Delta^{14}C$ = deep sea HMWDOC) carbon. Our data imply that previous reports of DOC fractions with $\Delta^{14}C$ values intermediate between new (surface DIC) and old (deep sea DOC) likely reflect different proportions of old and new DOC within a particular fraction isolated for analyses. Although analyses of a much broader suite of organic HMWDOC components may ultimately yield some constituents with a range of radiocarbon values, to date we have not found evidence in support of a continuum of radiocarbon values for different components of DOC. Our data also show that both new (labile) and old (refractory) components exist within HMWDOM and as such,do not support a molecular-size dependant continuum of organic carbon diagenesis (Amon and Benner, 1996; Loh *et al.*, 2004). We suggest that the observed depletion in total DOC $\Delta^{14}C$ relative to HMWDOC $\Delta^{14}C$ results from the higher proportion of low molecular weight (LMW) humic substances in total DOC, as compared with the newly synthesized carbohydrate-rich APS fraction that is concentrated in HMWDOC. For example, marine humic substances isolated by adsorption onto XAD resins, have an average molecular weight < 1 kDa, and have been shown to be highly depleted in radiocarbon (Stuermer and Harvey, 1974; Druffel *et al.*, 1992). In contrast, APS is abundant in HMWDOC fractions from > 1 kDa to > 10 K Da (Eglinton and Repeta, 2004) and neutral sugars data provided here show APS to be modern with radiocarbon values similar to DIC. Therefore, differences in chemical composition (rather than molecular size) likely control differences in oceanic residence time, but chemically distinct components are unevenly distributed within HMW and LMWDOC.

*Bacterial production and the cycling of APS.* Our radiocarbon measurements can be used to estimate the turnover of dissolved neutral sugars in seawater, and evaluate their contribution to annual bacterial production. Marine bacterial production is thought to be supported by the respiration of highly reactive DOC that is consumed on timescales of hours to days, and maintained at low steady state concentrations (Carlson, 2002; Cherrier and Bauer, 2004). The global inventory of a semi-reactive fraction of DOC that accumulates at depths < 800-1000m is very large (30-50GT C), but is not thought to fuel a significant fraction of bacterial carbon demand (Kirchman *et al.*, 1993; Carlson and Ducklow, 1995). Loh *et al.* 2004 measured radiocarbon values for "protein-like" and "carbohydrate-like" fractions of HMWDOC in surface water and found both fractions to be considerably aged ($\Delta^{14}C$ values between 2‰ (protein-like HMWDOC) and 13 ‰ (carbohydrate–like HMWDOC) and –2‰ and 7 ‰ at their Pacific and Atlantic Ocean sites, respectively). Such values are consistent with a residence time of several decades (>50 yr) and slow cycling of semi-reactive HMWDOC, which implies that this fraction satisfies only a small fraction (< 1 GT C $yr^{-1}$) of annual bacterial carbon demand. The slow cycling of HMWDOM is further supported by the slow temporal evolution of semi-reactive DOC profiles in the upper ocean.

The value of DIC $\Delta^{14}C$ for surface waters in the NPSG is not currently at steady state, but is changing due to a decreasing atmospheric $\Delta^{14}C$ value, vertical mixing (convection and diffusion), and horizontal advection (Mahadevan, 2001). The history of radiocarbon changes in the NPSG are known from high-resolution $\Delta^{14}C$ measurements of corals, and from data collected as part of the GEOSECS, INDIGO, TTO, and WOCE programs. Coral-derived records show that for the century before 1952, NPSG surface

water was in steady state with respect to long-term mixing with atmospheric $\Delta^{14}C$, as shown by the 7‰ decrease in $\Delta^{14}C$ between 1893-1952 induced by the Seuss effect (dilution of atmospheric$^{14}C$ with dead carbon from fossil fuel combustion; Druffel, *et al*., 2001). High-resolution coral records additionally show seasonal changes in $\Delta^{14}C$ of approximately 7‰ near Hawaii due to wind driven changes in upper-ocean mixing. DIC$\Delta^{14}C$ data from INDIGO, GEOSECS, WOCE, and TTO have been modeled using the 3D MIT general circulation model to distinguish processes that affect the time series evolution of $\Delta^{14}C$ in the Pacific Ocean. These simulations suggest that near our NPSG study site, $\Delta^{14}C$ was decreasing at about 4‰ per year in the late 1990's and that the major process affecting surface water DIC $\Delta^{14}C$ (up to 1995) is convection within the mixed layer (Mahadevan, 2001). This agrees well with direct DIC $\Delta^{14}C$ measurements taken at the NPSG site in 1987 and 1999 that show an average annual decrease in $\Delta^{14}C$ of 4‰ between 1987 (DIC$\Delta^{14}C$ = 131‰; Druffel *et al.*, 1992) and 1999 (DIC $\Delta^{14}C$ = 89‰; E. Druffel, pers. comm).

To simulate the effect of a changing value of DIC$\Delta^{14}C$ on the semi-reactive fraction of HMWDOC as suggested by the Williams and Druffel model, we constructed a simple box model of semi-reactive $\Delta^{14}C$ in the upper ocean (Fig. 3). Our model assumes a pre-bomb (1959) value of DIC $\Delta^{14}C$ and semi-reactive HMWDOC $\Delta^{14}C$ of -80 ‰ in the NPSG (Mahadevan, 2001). After this date, as DIC $\Delta^{14}C$ values begin to rise, the isotopic enrichment is transferred into the semi-reactive HMWDOC at a rate determined by the residence time of the semi-reactive HMWDOC. Short residence times for semi-reactive HMWDOC result in rapid transfer of post bomb carbon into HMWDOC, while longer residence times result in slow transfer of bomb radiocarbon into HMWDOC (fig.

3A). By assuming different residence times for the semi-reactive HMWDOC fraction, we can compare the value of semi-reactive HMWDOC $\Delta^{14}C$ with DIC$\Delta^{14}C$. The model assumes that the concentration of HMWDOC is at steady state, that the same fractional amount of HMWDOC is replaced annually, and that there is no isotopic discrimination in the removal of semi-reactive HMWDOC. The $\Delta^{14}C$ value of semi-reactive HMWDOC ($HMWDOC_{SR}$) for any year (t) is therefore expressed as: $HMWDOC_{SR}$ $\Delta^{14}C$ (t) = DIC $\Delta^{14}C$ (t) (k) + $HMWDOC_{SR}$(t-1)(1-k), where t is the year, and k is the fraction of $HMWDOC_{SR}$ replaced annually (*e.g.* if has a residence time of 10 years, k = 1/10 or 0.1). If the residence time of $HMWDOC_{SR}$ is short (1-3 years), there is little difference between the isotopic value of DIC $\Delta^{14}C$ and $HMWDOC_{SR}$ $\Delta^{14}C$ ($\Delta\Delta^{14}C$; Fig. 3B ). As residence times for $HMWDOC_{SR}$ become longer, the $HMWDOC_{SR}$ fraction becomes progressively depleted in $\Delta^{14}C$ relative to DIC$\Delta^{14}C$ and $\Delta\Delta^{14}C$ increases (Fig. 3A).

DIC and neutral sugars at the NPSG site have the same radiocarbon value within the uncertainty of our measurements ($\pm$13 ‰), consistent with a residence time of < 1-3 yr or between 20-25 yr (Fig. 3). Because DIC had a $\Delta^{14}C$ value of 89‰ both before and after the ocean radiocarbon maximum in the late 1970's, our measurements at one time point do not allow us to determine a single residence time for neutral sugars. However, additional measurements of neutral sugars $\Delta^{14}C$ values on samples collected over the next several years would allow for a distinction between the <3 yr and 20-25 year residence times. If neutral sugars had residence times between 4- 20 years, they would have $\Delta^{14}C$ values measurably greater than present-day DIC $\Delta^{14}C$ because during this time period, DIC $\Delta^{14}C$ values in the surface ocean were higher due to the penetration of the atmospheric bomb $^{14}C$ signal. If neutral sugar residence times were > 20-25 yr, sugar

$\Delta^{14}C$ values in 1999 would have been significantly lower than DIC $\Delta^{14}C$. Our results show that neutral sugars have residence times that are at least a factor of 2-3, but perhaps over an order of magnitude shorter than suggested by previous radiocarbon measurements made on operationally defined carbohydrate fractions.

Our radiocarbon-derived residence time for semi-reactive HMWDOC can be compared with estimates of residence time derived from spatial and temporal changes in DOC inventory in the NPSG. Export of DOC either below the euphotic zone, or away from high productivity regions decouples DOC production from heterotrophic consumption thereby providing a complimentary means for measuring semi-reactive DOC residence time. Abell *et al*. (2000) followed the degradation of TOC along isopycnals that outcrop within the NPSG between 15-30$^{o}$N and 150-160$^{o}$W. They found that TOC at depths < 200m had a residence time of 5 yr while TOC at depths between 200-300m had a residence time of 13 yr. By comparing TOC and TON degradation, Abell *et al.* (2000) further showed that the remineralized organic matter was poor in nitrogen, with an a C:N of 30$\pm$10, suggesting a carbohydrate rich substrate. These estimates of DOC respiration provide similar residence times for TOC as we find for the neutral sugar fraction of HMWDOC and suggest our radiocarbon results may apply to a large fraction of the semi-reactive HMWDOC.

Neutral sugars represent 15-20% of the total HMWDOC, and at least 5-7% of total DOC. Although HMWDOC is more enriched in radiocarbon than total DOC, and therefore enriched in the semi-reactive component, our results apply to only a portion of the total semi-reactive DOC fraction. Further measurements are needed to determine if other fractions of semi-reactive DOC have the same radiocarbon values as neutral sugars,

and the extent to which semi-reactive DOC fuels bacterial production. If a large fraction of semi-reactive DOC cycles on timescales of < 1-3 years, semi-reactive DOC meets an important fraction of global marine bacterial carbon demand.

*Compounds specific radiocarbon analyses and DOC cycling in the mesopelagic ocean.* Neutral sugar concentrations decrease from 4-6 μM C or 13-21% of HMWDOC in surface samples, to 0.7μM C or 6% of HMWDOC at 600m. Despite the order-of-magnitude decrease in neutral sugar concentration, nuclear magnetic resonance spectra and molecular level sugar analyses show the APS portion of HMWDOC to be remarkably homogeneous in composition between all three samples (Fig. 4). HMWDOC samples collected in the NPSG all show the presence of carbohydrate, acetate, and alkyl carbon in the $^{1}$HNMR spectra, as well as the suite of seven neutral sugars characteristic of APS even to depths of 5200m (Fig. 4). The carbohydrate fraction of HMWDOC can be introduced into the mesopelagic ocean through two fundamentally different mechanisms. A small fraction of the reactive carbohydrate synthesized in the euphotic zone may escape degradation and be mixed into the mesopelagic ocean by advection (Hansell *et al.*, 2002). These relic sugars will age at the same rate as DIC, and have a radiocarbon value equal to DIC at depth. Alternatively, sugars could be introduced from the dissolution of rapidly sinking large particles (Smith *et al*., 1992; Engel *et al*. 2004). Reactive DOC injected by sinking particles will have radiocarbon values similar to surface water DIC.

To distinguish these two mechanisms, we compared radiocarbon values of DIC and neutral sugars in samples from 600m. The $\Delta^{14}$C of DIC and HMWDOC at 600m sample are –155 $\pm$ 7 ‰ and –258‰ respectively, and are typical of values at this depth in

the North Pacific Ocean. Neutral sugars at 600m have radiocarbon values between –108 and –133‰, and are enriched by up to 150 ‰ relative to HMWDOC. The average $\Delta^{14}C$ value obtained by treating glucose, galactose, xylose and mannose as replicates is –123 $\pm$ 10 ‰ (1SD, n=4), and is slightly enriched in radiocarbon relative to DIC (-155 $\pm$7‰). Our data suggest that some fraction of neutral sugars might be introduced by the dissolution of rapidly sinking particles. If we assume that neutral sugars at 600m are a simple mixture of newly synthesized sugars (introduced by sinking particles) with a $\Delta^{14}C$ value equal to surface water DIC, and relic sugars (introduced by advection) with a $\Delta^{14}C$ value equal to DIC $\Delta^{14}C$ at 600 m depth, then 15% of the neutral sugars at 600m are introduced by large, rapidly sinking particles. These sugars may be reactive and help support bacterial activity at depth (Arístegui *et al*., 2002). A more comprehensive set of radiocarbon measurements on neutral sugars from depth in the ocean is needed to fully establish the isotopic differences between DIC and reactive components of HMWDOC.

## Conclusions

Compound specific radiocarbon analysis offers a geochemical tracer approach to explore carbon cycling within the DOC reservoir. Using this approach we find that the neutral sugar fraction of HMWDOC has a residence time in the euphotic zone of < 25 yr and perhaps as short as < 3 yr. The similarity in NMR spectra, neutral sugar yields and distributions in HMWDOC in the upper ocean suggest that our measurements of neutral sugar $\Delta^{14}C$ values may be representative of a much larger fraction of HMWDOC. Our data also support other measurements that suggest semi-reactive DOC may play an important role in meeting bacterial carbon demand but radiocarbon analyses of other compound classes (amino acids, amino sugars) are needed to provide a more

comprehensive inventory of reactive DOC components and residence times. Finally, the data presented in this paper suggests that compound specific radiocarbon analyses may provide an avenue to distinguish delivery processes for reactive fractions of DOC in the meso- and bathy-pelagic ocean.

## References


Abell, J., S. Emerson, P. Renaud. 2000. Distributions of TOP, TON, and TOC in the North Pacific subtropical gyre: Implications for nutrient supply in the surface ocean and remineralization in the upper thermocline. J. Mar. Res. **58**, 203-222.

Aluwihare, L.I., D. J. Repeta. and R.F. Chen. 1997. A major biopolymeric component to dissolved organic carbon in seawater. Nature **387**, 166-167.

Aluwihare, L.I., D. J. Repeta, and R.F. Chen. 2002. Chemical characterization of high molecular weight dissolved organic matter isolated from the Mid Atlantic Bight. Deep Sea Res., **49**, 4273-4295.

Aluwihare, L. I., D.J. Repeta, S. Pantoja, and C. G. Johnson. 2005. Two chemically distinct pools of organic nitrogen accumulate in the ocean. Science *in press*.

Arístegui, J., C. M. Duarte, S. Agustí, M. Doval, X. A. Álvarez–Salgado, and D. A. Hansell. 2002. Dissolved organic carbon support of respiration in the dark ocean. Science **298**: 1967.

Amon, R.M.W. and R. Benner. 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnol. Oceanogr. **41**:41-51.

Borch, N.H. and D.L. Kirchman. 1997. Concentration and composition of dissolved combined neutral sugars (polysaccharides) in seawater determined by HPLC-PAD. Mar. Chem. **57**: 85-95.

Buesseler, K.O., J.E. Bauer, R.F. Chen, T.I. Eglinton, O. Gustafsson, W. Landing, K. Mopper, S.B. Morna, P.H. Santschi, R. VernonClark, and M.L. Wells. 1996. An intercomparison of cross-flow filtration techniques used for sampling marine colloids: Overview and organic carbon results. Mar. Chem. **55**: 1-32.

Carlson, C.A. 2002, Production and removal processes. pp. 91-151. *In* D.A. Hansell and C.A. Carlson [eds.] *Biogeochemistry of Dissolved Organic Matter*. Academic Press.

Carlson, C.A. and H.W. Ducklow. 1995. Dissolved organic carbon in the upper ocean of the central equatorial Pacific Ocean. Daily and fine scale vertical variations. Deep Sea Res. II. **42**: 639-656.

Carlson, C.A., H. W. Ducklow, and A. F. Michaels. 1994. Annual flux of dissolved organic carbon from the euphotic zone in the northwest Sargasso Sea. Nature **371**: 405-408.

Cherrier, J. and J.E. Bauer. 2004. Bacterial Utilization of transient plankton-derived dissolved organic carbon and nitrogen inputs in surface ocean waters. Aq. Micro. Ecol. **35**: 229-241.

Cherrier, J., J. E. Bauer, and E.R.M. Druffel. 1996. Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern North Pacific surface waters. Mar. Ecol. Prog. Ser. **139:** 267-279.

Druffel, E.R.M. 1987. Bomb radiocarbon in the Pacific: Annual and seasonal timescale variations. J. Mar. Res. **45**: 667-698.

Druffel, E.R.M., P. M. Williams, J. E. Bauer, and J.R. Ertel. 1992. Cycling of dissolved and particulate organic matter in the open ocean. J. Geophys. Res. **97**: 15639-15659.

Druffel, E.R.M., Griffin, S., Guilderson, T.P., Kashgarian, M., Southon, J., and D.P. Schrag. 2001. Changes in subtropical North Pacific radiocarbon and their correlation with climate variability. Radiocarbon **43**: 15-25.

Eglinton, T.I. and D.J. Repeta. 2004. Marine Organic Geochemistry. pp. 145-180. *In* H. Elderfield, [ed.], Treatise on Geochemistry, Volume 7. Elsevier.

Engel, A., U. Riebesell, E. Rochelle-Newall, and I. Zondorvan. 2004. Polysaccharide aggregation as a potential sink of marine dissolved organic carbon. Nature **428**, 929-932.

Guo. L., and P.H. Santschi. 1997. Isotopic and elemental characterization of colloidal organic matter from the Chesapeake Bay and Galvaston Bay. Mar. Chem. **59**: 1-15.

Guo, L., P. H. Santschi, L. A. Cifuentes, S. Trumbore, and S. Southon. 1996. Cycling of high molecular weight dissolved organic matter in the Middle Atlantic Bight as revealed by carbon isotopic (13C and 14C) signatures. Limnol. Oceanogr. **41**:1242-1252.

Guo, L., and P.H. Santschi. 1997. Composition and cycling of colloids in marine environments. Rev. Geophys, **35**: 17-40.

Hansell, D.A., and C.A. Carlson. 1998. Deep-ocean gradients in the concentration of dissolved organic carbon. Nature **395**: 263-266.

Hansell, D.A., C.A. Carlson and S. Suzuki. 2002. Dissolved organic carbon export with North Pacific Intermediate Water formation. Global Biogeochem. Cycles **16**: 77-84.

Kirchman, D.L. 1990. Limitation of bacterial growth by dissolved organic matter in the subarctic Pacific. Mar. Ecol. Prog. Ser. **62**: 47-54.

Kirchman, D.L. and J.H. Rich. 1997. Regulation of bacterial growth rates by dissolved organic carbon and temperature in the equatorial Pacific Ocean. Microb. Ecol. **33**:11-20.

Kirchman D.L., C. Lancelot, M.R. J. Fasham, L. Legendre, G. Radach, and M. Scott. 1993. Dissolved organic mater in biogeochemical models of the ocean. Pp. 209-225. *In*

G.T. Evans and M.J.R. Fasham, [eds.], Towards a model of Ocean Biogeochemical Processes. NATO ASI.

Kirchman, D.L., Meon, B., Cottrell, Mt.T., Hutchins, D.A., Weeks, D., and K.W. Bruland. 2000. Carbon versus iron limitation of bacterial growth in the California upwelling regime. Limnol. Oceanogr. **45**: 1681-1688.

Loh, A.N., Bauer, J.E., and E.R.M. Druffel. 2004. Variable aging and storage of dissolved organic components in the open ocean. Nature **430**: 877-880.

Mahadevan, A. 2001. Analyses of bomb radiocarbon trends in the Pacific. Mar. Chem. **73**: 273-290.

McCarthy, M.D., J. I. Hedges, and R. Benner. 1996. Major biochemical composition of dissolved high molecular weight organic matter in seawater. Mar. Chem., **55**: 281-297.

Pearson, A. 2000. Biogeochemical applications of compound specific radiocarbon ananlysis. Ph.D. thesis. Massachusetts Inst. Tech./Woods Hole Oceanogr. Inst.

Peltzer, E.T. and N.A. Hayward. 1996. Spatial and temporal variability of total organic carbon along 140˚ W in the equatorial Pacific Ocean in 1992. Deep Sea Res. II. **43**:1155-1180.

Quan, T.M. 2005. Chemical Characterization of Dissolved Organic Matter (DOM) in Seawater: Structure, Cycling, and the Role of Biology. Ph.D. thesis. MIT/WHOI Joint Program in Chemical Oceanography.

Santschi, P. H., E. Balnois, K. J. Wilkinson, J. Zhang, J. Buffle, and L. Guo. 1998. Fibrillar polysaccharides in marine macromolecular organic matter as imaged by atomic force microscopy and transmission electron microscopy. Limnol. Oceanogr. **43**: 896-908.

Smith, D.C., Simon, M., Alldredge, A.L., and F. Azam. 1992. Intense hydrolytic activity on marine aggregates and implications for rapid particle dissolution. Nature **359**: 139-142.

Steurmer D.H. and G.R. Harvey. 1974 Humic substances from seawater. Nature, **250**: 480-481.

Van Dongen, B.E., S. Schouten, and J.S. Sinnghe Damste 2002. Carbon isotope variability in monosaccharides and lipids in aquatic algae and terrestrial plants. Mar. Ecol. Prog. Ser., **232**: 83-92.

Williams, P. M., H. Oeschger, and P. Kinney. 1969. Natural radiocarbon activity of the dissolved organic carbon in the North-east Pacific Ocean. Nature **224**: 256-258.

Williams, P.M., and E.R.M. Druffel 1987. Radiocarbon in dissolved organic matter in the central North Pacific Ocean. Nature **330**: 246-248.

Williams, P.J.leB. 2000. Heterotrophic bacteria and the dynamics of dissolved organic material. pp. 153-200. *In* D.L. Kirchman, [ed.] *Microbial Ecology of the Oceans.* Wiley.

Table 1. Isotopic composition of bulk carbon fractions and neutral sugars from Hawaii. The $\delta^{13}C$ values are given relative to PDB. $\Delta^{14}C$ values were determined by accelerator mass spectrometry and are corrected for isotopic fractionation. We assumed a value of –20 ‰ for sugars with undetermined $\delta^{13}C$ values. The isotopic enrichment in the δ13C value of xylose relative to glucose is similar to that reported by van Dongen *et al*., 2002 for aquatic and terrestrial plants. Columns containing two values show the results of duplicate analyses of samples split immediately before combustion.

| Sample | Hawaii (15 m) | | Hawaii (600m) | |
|---|---|---|---|---|
| | $\delta^{13}C$ (‰) | $\Delta^{14}C$ (‰) | $\delta^{13}C$ (‰) | $\Delta^{14}C$ (‰) |
| DIC | 1.37 | 72 $\pm$7 (n=4) | -0.21 | -155 $\pm$ 7 (n=4) |
| HMWDOC | -21.9 | 10 | -20.9 | -262, -255 |
| F1 | | 49 | | |
| F2 | | 36 | | |
| Glucose | -19.9 | 47, 58 | | -133 |
| Galactose | | 67 | | -108 |
| Mannose | | 65 | | -121 |
| Xylose | -15.6 | 52, 58 | | -129 |
| Arabinose | | 63 | | |
| Fucose | -18.8 | 49, 52 | | |
| Rhamnose | | 40, 57 | | |

Table 2. Isotopic composition of bulk carbon fractions and neutral sugars at 3m in the North Pacific Subtropical Gyre. The data for DIC $\Delta^{14}C$ were kindly provided by Dr. Ellen Druffel.

| Sample | NPSG (3m) |
|---|---|
| | $\Delta^{14}C$ (‰) |
| DIC | 89±7 |
| HMWDOC | 46 |
| Glucose | 79 |
| Galactose | 103 |
| Mannose | 99 |
| Xylose | 94 |
| Arabinose | ND* |
| Fucose | 69 |
| Rhamnose | 57 |

* not determined due to insufficient sample.

## Figure Captions

Figure 1. (A) Separation of neutral sugars from HMWDOC by ion exchange HPLC after acid hydrolysis. Neutral sugars elute in three fractions (F1-F3; F1 glucose, rhamnose; F2 glactose, mannose, xylose; and F3 fucose, arabinose). The three fractions are collected separately, dried and further purified by reverse phase HPLC (B) using a polymeric amino column before radiocarbon analyses. The chromatogram shows the separation of fraction 1 (glucose, rhamnose) from the Hawaii surface water sample.

Figure 2. (Top) $^{1}$HNMR spectrum of HMWDOC isolated from the NPSG (3m) with a $\Delta^{14}$C value of 46‰. Major functional groups include OC**H**O (carbohydrate, 5-6 ppm), **H**COH (carbohydrate, 3-4.5 ppm), OCC$\mathbf{H}_x$ (acetate and other $\alpha$-functionalized alkyl carbon protons; 2 ppm), and alkyl carbon protons ( C$\mathbf{H}_x$,; 1.3 ppm). The integrated area of these functional groups is relatively fixed in HMWDOM from surface seawater at 60% (HCOH), 20% (acetate and other $\alpha$-functionalized alkyl carbon protons), and 20% (alkyl carbon), (Middle) The molecularly uncharacterized carbon (MUC) fraction of HMWDOC (HMW-MUC) isolated by ion exchange chromatography. Approximately 5-10% of the carbon in the MUC fraction is from amino acids released during the hydrolysis of dissolved proteins and which appear as sharp well-defined peaks in the $^{1}$HNMR spectrum (Quan, 2005). The HMW-MUC component surface seawater has spectral characteristics (29% HCOH, 34% $\alpha$-functionalized alkyl carbon protons; 37% alkyl carbon protons) similar to deep sea HMWDOC collected at 3600 m (Bottom; 32% HCOH, 37% $\alpha$-functionalized alkyl carbon protons; 31% alkyl carbon protons), as well

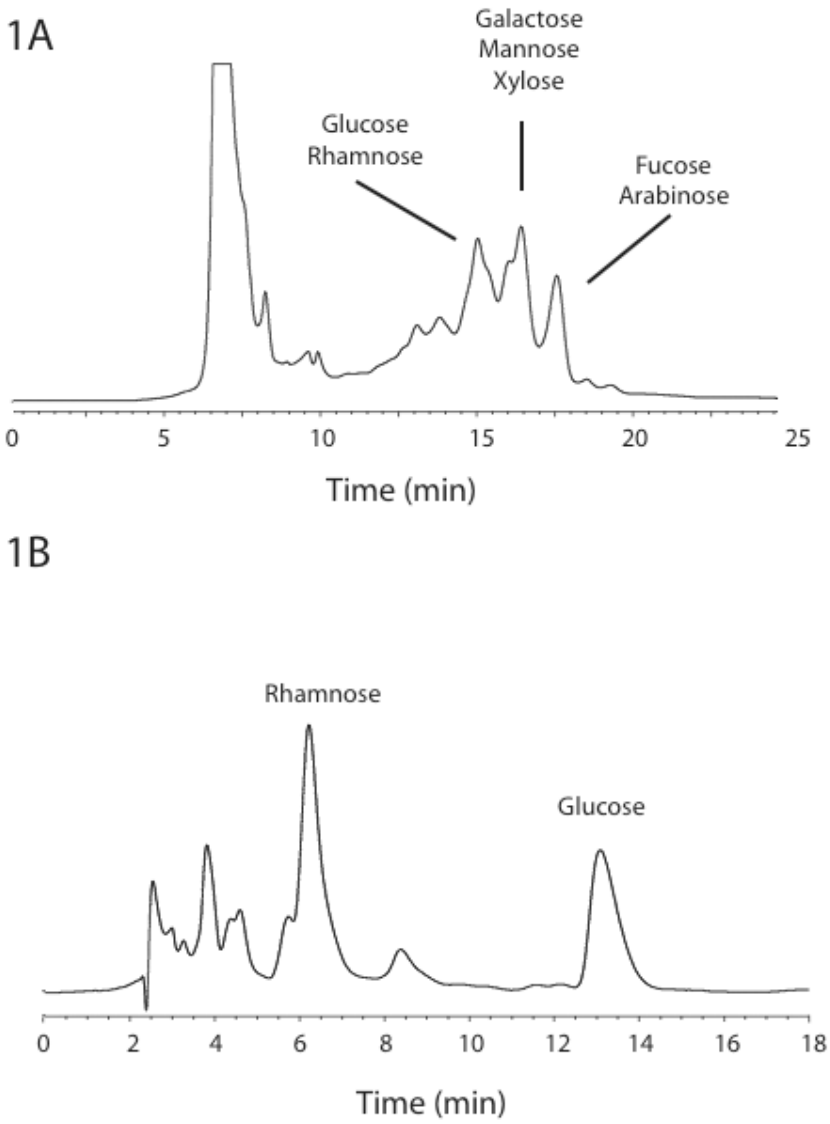
as a similar radiocarbon value (-416 ‰ for MUC isolated from surface seawater compared to –428‰ for HMWDOC at 3600m). The relative amounts of carbohydrate (3-4.5 ppm) α-functionalized alkyl (1.5-3 ppm) and alkyl carbon protons (1-1.5 ppm) are similar in the two spectra. NMR spectra were collected at 400 MHz in $D_2O$ using solvent suppression (zgpr) and referenced to water at 4.8 ppm.

Figure 3. (A) Results from a box model of semi-reactive HMWDOC in surface seawater of the NPSG between 1959 and 2002. The model assumes a pre-bomb DIC$\Delta^{14}$C value of -80 ‰. DIC$\Delta^{14}$C values then rise to their maximum in 1973-1974, and decrease thereafter. As bomb radiocarbon is transferred into semi-reactive HMWDOC, HMWDOC$\Delta^{14}$C values also begin to rise at a rate dependant on the assumed residence time of semi-reactive HMWDOC (1, 2, 3.3, and 10 years in the figure). The difference in DIC$\Delta^{14}$C and semi-reactive HMWDOC$\Delta^{14}$C ($\Delta\Delta^{14}$C ) is determined by the residence time of the semi-reactive HMWDOC fraction. (B) Expansion of the model output for 1990-2005. Radiocarbon values for DIC were taken from Pearson, 2000).

Figure 4. $^{1}$HNMR spectra and monosaccharide distribution of HMWDOC from surface (top) and 5200 m (bottom) seawater collected at the NPSG site. The $^{1}$HNMR spectra of HMWDOC at both depths display prominent peaks (*) for carbohydrate (3-4.5 ppm), acetate (2 ppm), and alkyl carbon (1.3 ppm). The sharp peak at 4.8 ppm is water, which was used as a solvent and reference. The monosaccharide distribution was determined by gas chromatography of alditol acetates according to methods described in Aluwihare et al. (2002). Acid hydrolysis of each sample releases seven major neutral sugars:

rhamnose (R), fucose (F), arabinose (A), xylose (X), glucose (Gl), mannose (M) and galactose (G). Numbers above each bar in the figure correspond to the percent of that sugar relative to the total seven neutral sugars. The 5200m sample is somewhat enriched in fucose relative to the surface sample, but otherwise contains the same suite of sugars in approximately the same relative amounts as surface seawater HMWDOC.

Figure 1.


1A
Galactose
Mannose
Xylose
Glucose
Rhamnose
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Time (min)
1B
Rhamnose
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Time (min)

Figure 2.

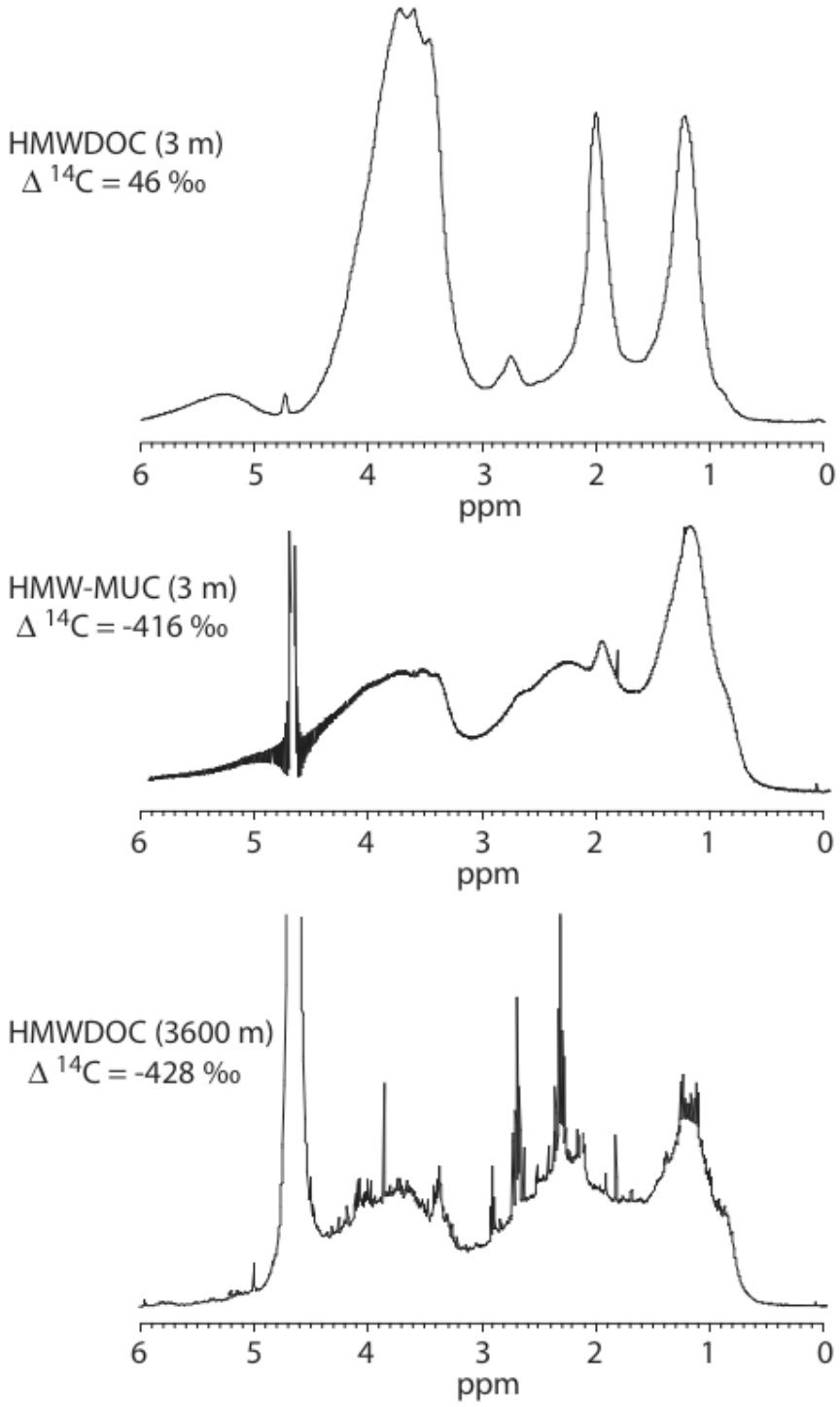
HMWDOC (3 m)
Δ 14C = 46 ‰
6 5 4 3 2 1 0
ppm
HMW-MUC (3 m)
Δ 14C = -416 ‰
6 5 4 3 2 1 0
ppm
HMWDOC (3600 m)
Δ 14C = -428 ‰
6 5 4 3 2 1 0
ppm

Figure 3.

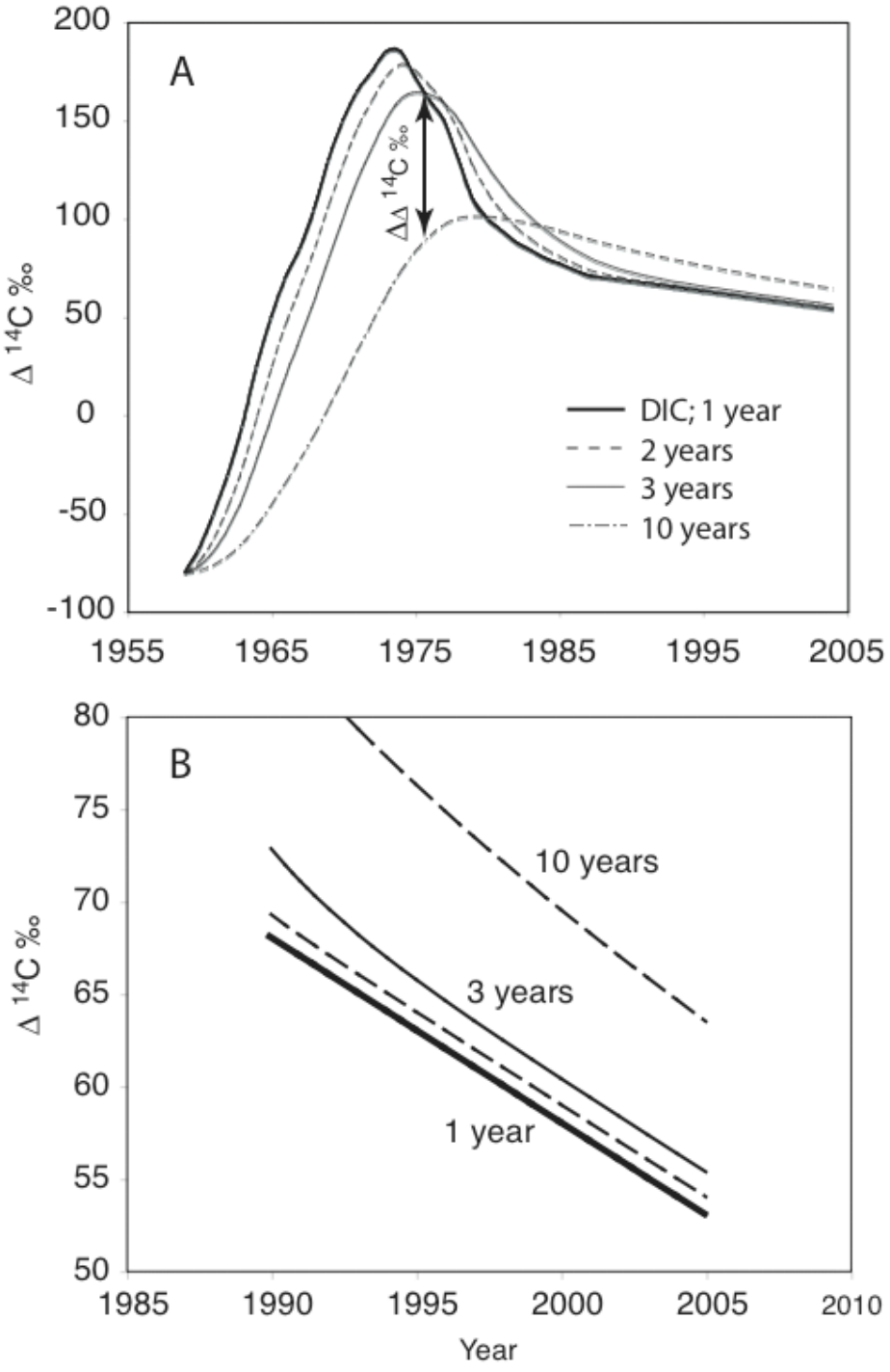
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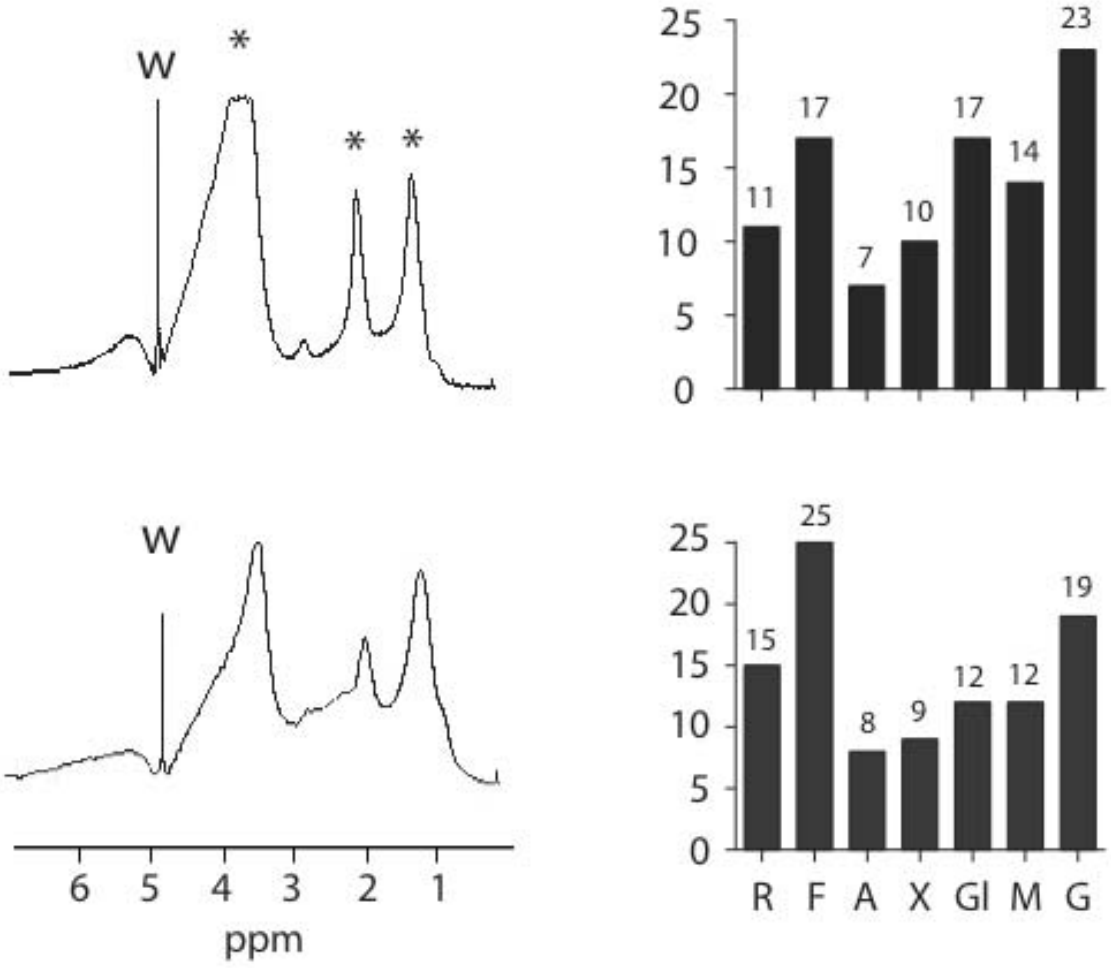
Figure 4.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