Available online at www.sciencedirect.com
ScienceDirect
Geochimica et Cosmochimica Acta 75 (2011) 5187–5202
Geochimica et Cosmochimica Acta
www.elsevier.com/locate/gca

# Large-volume ultrafiltration for the study of radiocarbon signatures and size vs. age relationships in marine dissolved organic matter

B.D. Walker [a,*], S.R. Beaupré [b], T.P. Guilderson [a,c], E.R.M. Druffel [d], M.D. McCarthy [a]

[a] University of California – Santa Cruz, Department of Ocean Science, 1156 High St., CA 95064, USA
[b] Woods Hole Oceanographic Institution, Department of Geology and Geophysics, 266 Woods Hole Rd., Woods Hole, MA 02543-1050, USA
[c] Lawrence Livermore National Laboratory, Center for Accelerator Mass Spectrometry (CAMS), LLNL-L397, 7000 East Ave., Livermore, CA 94551, USA
[d] University of California – Irvine, Department of Earth System Science, 2212 Croul Hall, CA 92697-3100, USA

Received 13 January 2011; accepted in revised form 13 June 2011; available online 23 June 2011

**Abstract**

In recent decades, tangential-flow ultrafiltration (UF) technology has become a primary tool for isolating large amounts of "ultrafiltered" marine dissolved organic carbon (UDOC; 0.1 μm to ~1 nm) for the detailed characterization of DOC chemical composition and radiocarbon ($\Delta^{14}C$) signatures. However, while total DOC $\Delta^{14}C$ values are generally thought to be quite similar in the world ocean, previous studies have reported widely different $\Delta^{14}C$ values for UDOC, even from very similar ocean regions, raising questions about the relative "reactivity" of high molecular weight (HMW) DOC. Specifically, to what degree do variations in DOM molecular weight (MW) vs. composition alter its relative persistence, and therefore HMW DOC $\Delta^{14}C$ values?

In this study we evaluate the effects of varying proportions of HMW vs. low molecular weight (LMW) DOC on UDOC $\Delta^{14}C$ values. Using concentration factor (CF) as a proxy for MW distributions, we modeled the retention of both OC and $\Delta^{14}C$ in several very large CF experiments (CF >3000), from three depths (20, 670, and 915 m) in the North Pacific Subtropical Gyre (NPSG). The resulting DOC and $\Delta^{14}C$ UF permeation coefficients generally increase with depth, consistent with mass balance trends, indicating very significant permeation of LMW, $^{14}C$-depleted DOC at depth, and higher recoveries of $\Delta^{14}C$-enriched, HMW DOC in the surface. In addition, changes in CF during sample concentration and ionic strength during sample diafiltration had very large and predictable impacts on UDOC $\Delta^{14}C$ values.

Together these results suggest that previously reported disparities in UDOC $\Delta^{14}C$ values are reconciled by linked trends of $\Delta^{14}C$ content vs. MW. At low CFs, UDOC samples have similar $\Delta^{14}C$ values to total DOC. In contrast, UDOC samples collected at extremely high CFs (and after diafiltration) have more positive $\Delta^{14}C$ values. We demonstrate that the observed relationships between UDOC $\Delta^{14}C$ and CF derived from our data can directly explain offsets in all previously published UDOC $\Delta^{14}C$ values for the NPSG. While CF is not traditionally considered in UF studies, our results indicate it can substantially influence the interpretation of UDOC $^{14}C$ "age", and thus reactivity, in the marine environment. In addition, our results indicate that CF can in fact be used as a proxy for average MW. We suggest that a variable-CF-UF approach, coupled with molecular-level $\Delta^{14}C$ analyses, presents a new tool for studying relationships between molecular size, age, and "labile" DOC distributions in the ocean.


## 1. INTRODUCTION

At ~662 Pg C (Hansell et al., 2009), oceanic dissolved organic matter (DOM) represents one of the largest active

* Corresponding author. Tel.: +1 831 459 1533; fax: +1 831 459 4882.
*E-mail address:* bwalker@ucsc.edu (B.D. Walker).

0016-7037/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.gca.2011.06.015

pools of reduced carbon on Earth (Hedges, 1992), and the linkages between DOM production and remineralization are of primary importance to the ocean carbon cycle. Perhaps one of the most influential observations shaping our understanding of marine DOM cycling and reactivity has been the global distributions of dissolved organic carbon (DOC) and its radiocarbon ($\Delta^{14}C$) value (Williams and Druffel, 1987; Druffel et al., 1992). The strong $^{14}C$-depletion of deep ocean DOC with respect to dissolved inorganic carbon (DIC; by ~300‰) suggests that DOM in the deep ocean (at ~6000 $^{14}C$ ybp) is highly resistant to degradation and persists over multiple ocean mixing cycles. However, the low concentration of DOC relative to abundant seawater salts (~1 mg $l^{-1}$ DOC to ~35,000 mg $l^{-1}$ salt) has made more detailed molecular level and isotopic DOM analyses difficult. As a consequence, the role of specific DOM constituents, that combine to form these bulk $^{14}C$ "ages", and their individual cycling rates remain poorly understood.

In recent years, the application of tangential-flow ultrafiltration (UF) to the marine DOM pool has provided a highly effective tool for the chemical and isotopic characterization of marine DOM (Benner et al., 1992; McCarthy et al., 1996; Aluwihare et al., 2002), in particular the most reactive HMW components (Repeta et al., 2002). Together, the isolation of DOM collected by UF, coupled with $\Delta^{14}C$ measurements and molecular analysis, have provided a powerful new approach for understanding sources and cycling rates of individual DOM constituents in the carbon cycle (Loh et al., 2004, 2006; Repeta and Aluwihare, 2006). Because large-volume UF uses an open, continually recycling system (through which essentially unlimited seawater volumes can be processed), it allows for the isolation of >1 g of DOC. Typically, sample concentration is followed by diafiltration to remove sea salts. DOM isolated by UF represents organic material that passes through a 0.1–0.2 μm filter (to remove most particles and prokaryotic organisms) but is retained by a ~1 nm (1000 Da) nominal molecular weight cut-off (NMWCO) membrane. Some studies refer to material isolated by UF as "colloidal" based on this nominal size range (Buesseler et al., 1996; Guo and Santschi, 1996; Dai et al., 1998; Guo et al., 2000). However, work focused on the oceanic DOM pool has usually used "ultrafiltered" DOM (UDOM), a designation that makes no assumptions about its physiochemical form in the ocean. This definition also reflects the fact that while the isolated material is of higher average molecular size than total DOC, many bulk and compositional properties of UDOM (e.g. $C/N_a$ ratio and $\delta^{13}C$ composition) are generally similar to the total DOC pool (Benner et al., 1992, 1997; Amon and Benner, 1994, 1996; McCarthy et al., 1996, 1997; Loh et al., 2004).

In contrast, the radiocarbon isotopic ($\Delta^{14}C$) value of UDOM is one bulk compositional property that differs from total DOC. Published $\Delta^{14}C$ signatures of UDOM are generally more positive than total DO$^{14}C$ (McNichol and Aluwihare, 2007). This is consistent with the idea that HMW DOM predominately represents the "semi-labile" component of ocean DOM (Benner et al., 1992; Amon and Benner, 1994). Understanding the turnover of this pool is critical because this material advects carbon to the subsurface ocean, thereby closing key carbon budgets (Hansell et al., 2002). However, previous studies have also reported widely different $\Delta^{14}C$ values for UDOM from identical ocean regions. For example, previously reported $\Delta^{14}C$ values from contemporaneous UDOM isolations taken at the same location in the North Pacific Subtropical Gyre (NPSG), using the same membrane pore sizes, differ by ~130‰ (Loh et al., 2004 $\Delta^{14}C = -92$‰; Repeta and Aluwihare, 2006 $\Delta^{14}C = +42$‰). Even larger disparities (~240‰) have been reported from deep ocean UDOM samples, again with identical membrane pore sizes, taken at the same location and depth (670 m: Guo and Santschi, 1996 $\Delta^{14}C = -502$‰; Repeta and Aluwihare, 2006 $\Delta^{14}C = -262$‰). Because UDOM $\Delta^{14}C$ values are often used to interpret HMW DOC reactivity in the marine environment, these offsets in UDOM $\Delta^{14}C$ signatures suggest very significant temporal vs. spatial variability in semi-labile DOM formation processes and reactivity, even in similar ocean regions.

However, one alternate possibly is that variability in the distribution of sample molecular weight (MW) in recovered UDOM might alter measured $\Delta^{14}C$ UDOM values. Because UF represents a progressive "distillation" of a complex molecular mixture (based primarily on retention at a specified nominal MW cutoff), the molecular weight distribution within a specific UDOM sample might significantly affect its measured $\Delta^{14}C$ value. However, $^{14}C$ dating has not been done to directly evaluate this; the majority of studies investigating UF as a tool for isolating marine DOM have focused on establishing: (1) preliminary estimates of the molecular size distributions of marine DOM (Sharp, 1973), (2) rigorous cleaning, and operating procedures for evaluating the effects of membrane pore-size/manufacturer on the retention characteristics of UDOM (Buesseler et al., 1996; Guo and Santschi, 1996; Gustafsson et al., 1996; Chin et al., 1998; Dai et al., 1998), (3) the retention of trace metals complexed to DOM (Buffle et al., 1992a; Guo et al., 2000) and (4) evaluating the chemical and stable isotopic composition of UDOM (Benner et al., 1997). While these studies have provided invaluable guidelines for the collection of UDOC, most have used relatively low concentration factors of <100 (CF = sample volume/retentate volume), and also relatively small sample volumes (200 L or less). In contrast, recent interest in understanding individual DOM component cycling rates via $\Delta^{14}C$ measurements (Loh et al., 2004, 2006; Repeta and Aluwihare, 2006) requires that much larger sample volumes be processed to isolate sufficient material. However, no prior study has evaluated the recovery characteristics of DOM $\Delta^{14}C$ during UF, or how UF processing might alter the $\Delta^{14}C$ signature of isolated DOM, relative to that of the total DOM pool.

In this study, we model $\Delta^{14}C$ and DOC measurements from a series of UF experiments taken from three depths (surface and mesopelagic) in the NPSG, sampled from the Natural Energy Laboratory of Hawaii Authority (NELHA) site. In addition, we specifically examine how $\Delta^{14}C$ values for UDOM are influenced by varying CF and diafiltration. We show that both CF and diafiltration, by creat-

ing widely different effective MW distributions, have profound, yet predictable effects on the $\Delta^{14}C$ signature of UDOM, consistent with significant permeation of low molecular weight DOM. These models also reconcile $\Delta^{14}C$ offsets reported in all previously published UDOM samples in the Pacific Ocean, with important implications for relative reactivity of the ocean's semi-labile DOM pool.

## 2. METHODS

### 2.1. Study site and sample collection

Seawater samples were collected in December 2005 from 20, 670, and 915 m intake pipes at the Natural Energy Laboratory of Hawaii Authority (NELHA); located on the big island of Hawai'i just north of Kailua-Kona (19°69′N, 156°03′W). The station is located on a steep marine volcanic escarpment on the "desert" side of the big island of Hawai'i, and has no terrestrial freshwater sources. At NELHA, large diameter pipes bring seawater to the surface at very high flow rates (36,000–50,000 L $min^{-1}$). Results from previous studies suggest that particulate organic matter (POM) and DOM isolated from NELHA are similar to samples from the HOT-ALOHA site and representative of the NPSG (Ingalls et al., 2006; Repeta and Aluwihare, 2006; Roland et al., 2009).

Sample water from surface and mesopelagic depths were first pre-filtered through a 50 μm plankton net in order to remove larger marine particles, and subsequently through pre-cleaned (10% HCl) Whatman® 0.2 μm Polycap™ 75 TC polyethersulfone cartridge filters. Total dissolved organic matter samples (TDOC; <0.2 μm) were collected in precombusted (450 °C, 5 h) 2 L glass jugs with PTFE lined caps and were immediately frozen and stored until $\Delta^{14}C$ analysis at UCI. UDOM samples (<0.2 to ~1 nm) were obtained using two home-built UF systems. The structural system components, pumps, automation and plumbing used were analogous to those recently described by Roland et al. (2009). Prior to each use, UF membranes were rigorously cleaned using a series of detergent (0.01% Fisher FL-70), 0.01 N HCl, 0.01 N NaOH and were then rinsed thoroughly with >40 L of 18.2 MΩ Milli-Q water. Sub-sampling of the UDOM retentate along with measurements of permeate flow rates were used to monitor DOC mass balance. UDOM fraction recoveries were calculated using subsamples' DOC concentrations and sample volumes during each stage in the filtration (Table 1).

Briefly, the first "main filtration" system contained two large polyethersulfone (PES) UF membranes (GE Osmonics: GH 4040-C1072, NMWCO = 2.5 kDa) and a 100 L high-density polyethylene (HDPE) sample reservoir was used for the main sample concentration where sample feed solutions were continuously processed until a final sample throughput volume of ~5000–6000 L was obtained. Next, the sample feed was shut off and the remaining sample retentate was allowed to reduce from ~100 to ~20 L and was then collected and transferred to a second UF system for further sample reduction and diafiltration. This second "reduction/diafiltration" system contained a single, smaller PES UF membrane (GE Osmonics: GE 2540-F1072, NMWCO = 1 kDa) and 4 L glass funnel sample reservoir. The 20 L sample was further reduced to ~2 L prior to diafiltration. For the purposes of modeling UF behavior in the concentration mode later in the discussion, we make no distinction between these first filtration steps, and consider all sample concentration (i.e. 5000 to ~2 L) to represent the UF "concentration mode". Salty 2 L retentates were immediately frozen and later diafiltered in the laboratory at University of California, Santa Cruz. In order to conserve sample for future analysis, only ~200 ml splits of this salty 2 L retentate were diafiltered. Diafiltration of the salty UDOM 200 ml retentate splits was performed by bringing sample volumes up to ~2 L with 18.2 MΩ Milli-Q water and then gradually adding 20 L of Milli-Q water to the sample retentate at the same rate of fluid permeating the membrane (i.e. constant retentate volume). Final ~2 L diafiltered UDOM retentates were dried via centrifugal evaporation, homogenized with a mortar and pestle and subsequently stored in a desiccator cabinet in pre-combusted glass vials (450 °C, 5 h) prior to analyses.

In order to evaluate the permeation behavior of DOC and $\Delta^{14}C$ during UF, several discrete DOC retentate subfractions were collected throughout each UF experiment (following methods set forth by Kilduff and Weber, 1992). Each ultrafiltered UDOC fraction was collected at a defined CF, or in the case of the final UDOC isolate, after diafiltration. For clarity, a summary of UDOC sub-samples is provided in Fig. 1 (see Sections 3.3 and 3.4 for a more detailed discussion of concentration vs. diafiltration UF modes). UDOC retentates were first sub-sampled from the main 100 L filtration system tank at a low CF ($UDOC_{LCF}$; sampled at CF ~30–40, corresponding to ~3000 L total sample throughput) and were immediately stored frozen in the field. UDOC retentate sub-samples were also taken after ~4000–6000 L sample throughput at the end of the concentration mode ($UDOC_{HCF}$; CF ~3000). Finally, we define the aforementioned final diafiltered UDOC retentate splits as "D-$UDOC_{HCF}$".

### 2.2. Sample preparations and isotopic analysis

Total DOC (TDOC), $UDOC_{LCF}$, $UDOC_{HCF}$, and D-$UDOC_{HCF}$ concentrations (±1 μM) were determined via high temperature combustion using a Shimadzu TOC-V at the University of California, Santa Barbara (UCSB Carlson Lab), and also based on manometric measurements during offline combustion for isotopic analyses. TDOC concentrations reported in this study represent the average of all values determined by both UV oxidation/vacuum line purification at UC Irvine following the methods of Beaupre et al. (2007) and those determined by high temperature combustion at UCSB. Percent recoveries for each UDOC fraction are reported relative to TDOC concentrations (μM) and volume processed.

Natural abundance radiocarbon ($\Delta^{14}C$) determinations of all UDOC fractions were performed either at LLNL/CAMS or UC Irvine Keck Carbon Cycle AMS Laboratory following standard graphitization procedures (Vogel et al., 1987; Santos et al., 2007). Age-corrected $\Delta^{14}C$ values (‰) have been corrected for sampling year and year of analysis

Table 1
Summary of NELHA stable isotopic and radiocarbon data. All $\delta^{13}C$ and $\Delta^{14}C$ data for dissolved organic carbon (DOC) samples are reported in per mil (‰) notation and follow the conventions set forth by Stuiver and Polach (1977). For $UDOC_{HCF}$, $n = 2$ $\Delta^{14}C$ analyses were performed; in this case $\Delta^{14}C$ errors (±) represent the range in reported values. Percent recoveries for all UDOC fractions ($UDOC_{LCF}$, $UDOC_{HCF}$, D-$UDOC_{HCF}$) are calculated via determined molar DOC concentrations and total sample volume processed. Volume corrected and retentate DOC concentrations are reported in μM. Low molecular weight (LMW) DOC concentrations are calculated by difference with respect to TDOC, or UDOC sub-fractions, as specified in parenthesis.

| Sample fraction | Volume (*l*) | Concentration factor | Recovery %TDOC | Vol. Corr. DOC | Retentate DOC | $\delta^{13}C$ | ± | UC/ CAMS ID | $\Delta^{14}C$ | ± | Fm | ± | $^{14}C$ Age (ybp) | ± | LMW DOM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | DOC | $\delta^{13}C$ | $\Delta^{14}C$ | $^{14}C$ Age (ybp) |
| TDOC | | | | | | | | | | | | | | | | | | |
| 21 m | 2.0 | 1 | 100 | 73 | 73 | −20.4 | 0.2 | UC-9237 | −246 | 5 | 0.7596 | 0.0018 | 2210 | 5 | 63.5 | −20.2 | −281 | 2650 |
| 670 m | 2.0 | 1 | 100 | 40 | 40 | −21.7 | 0.2 | UC-9249 | −479 | 9 | 0.5249 | 0.0030 | 5180 | 9 | 37.2 | −21.8 | −492 | 5430 |
| 915 m | 2.0 | 1 | 100 | 43 | 43 | −23.2 | 0.2 | UC-9250 | −446 | 8 | 0.5583 | 0.0026 | 4680 | 8 | 39.6 | −23.4 | −454 | 4860 |
| $UDOC_{LCF}$ | | | | | | | | | | | | | | | | | | |
| 21 m | 2830 | 28 | 32 | 23.4 | 1162 | −21.2 | 0.3 | UC-10375 | −131 | 3 | 0.8747 | 0.0032 | 1080 | 30 | 49.6 | −18.9 | −299 | 2860 |
| 670 m | 3130 | 46 | 22 | 8.9 | 714 | −21.6 | 0.2 | UC-10291 | −424 | 3 | 0.5797 | 0.0028 | 4380 | 40 | 31.1 | −22.0 | −494 | 5480 |
| 915 m | 3010 | 38 | 22 | 9.3 | 351 | −22.3 | 0.2 | UC-10409 | −552 | 3 | 0.4515 | 0.0028 | 6390 | 60 | 33.7 | −26.6 | −416 | 4320 |
| $UDOC_{HCF}$ | | | | | | | | | | | | | | | | | | |
| 21 m ($n = 2$) | 5450 | 2725 | 21 | 15.1 | 43,242 | −21.5 | 0.2 | 125643/136977 | −80 | 1 | 0.9261 | 0.0013 | 620 | 20 | 8.3 | −21.1 | −223 | 2030 |
| 670 m ($n = 2$) | 6035 | 3018 | 12 | 4.7 | 12,414 | −21.7 | 0.1 | 136974/136975 | −393 | 2 | 0.6112 | 0.0018 | 3950 | 25 | 4.2 | −21.6 | −460 | 4940 |
| 915 m ($n = 2$) | 4990 | 2495 | 11 | 4.8 | 13,406 | −21.7 | 0.1 | 136978/136979 | −415 | 2 | 0.5887 | 0.0022 | 4260 | 30 | 4.5 | −22.4 | −699 | 9650 |
| D-$UDOC_{HCF}$ | | | | | | | | | | | | | | | | | | |
| 21 m | 5450 | 2725 | 13 | 9.5 | 22,487 | −22.1 | 0.2 | 129833 | −6 | 4 | 1.0008 | 0.0043 | >Modern | – | 5.6 | −21.4 | −206 | 1860 |
| 670 m | 6035 | 3018 | 7 | 2.8 | 8894 | −21.3 | 0.1 | 129437 | −306 | 2 | 0.6986 | 0.0022 | 2880 | 35 | 1.9 | −21.7 | −519 | 5870 |
| 915 m | 4990 | 2495 | 8 | 3.4 | 9536 | −21.4 | 0.2 | 129438 | −345 | 2 | 0.6594 | 0.0025 | 3350 | 35 | 1.4 | −21.7 | −588 | 7120 |

and are reported in accordance with conventions set forth by Stuiver and Polach (1977) using the Libby half-life of 5568 years. Reported values are given after subtracting sample preparation backgrounds based on a $^{14}C$-free calcite standard and have been corrected for isotopic fractionation of $\delta^{13}C$. Isotopic results are reported as Fraction Modern (FM), $\Delta^{14}C$, $\delta^{13}C$, and conventional radiocarbon age (ybp). For TDOC and $UDOC_{LCF}$ splits, $\Delta^{14}C$ and $\delta^{13}C$ were measured after UV-oxidation and vacuum line extraction following established protocols at UCI (Beaupre et al., 2007). $UDOC_{HCF}$ and D-$UDOC_{HCF}$ $\Delta^{14}C$ measurements were performed via closed tube combustion and graphitization at LLNL/CAMS. Because $UDOC_{HCF}$ fractions are inherently salty, 2.0 ml were pipetted into either precombusted quartz tubes for $\Delta^{14}C$ analyses, or to silver boats for CHN ($\delta^{13}C$) analyses. All $UDOC_{HCF}$ samples were acidified (0.5 N HCl) and dried prior to these analyses; for CHN analyses, $UDOC_{HCF}$ samples were oven-dried at 40 °C; CHN splits, for $\Delta^{14}C$ analyses of $UDOC_{HCF}$ samples were dried by lyophilization. $UDOC_{HCF}$ and D-$UDOC_{HCF}$ $\delta^{13}C$ values were determined by CHN analysis at the University of California, Santa Cruz – Stable Isotope Laboratory using a Carlo Erba CHNO-S EA-1108 Elemental Analyzer and Thermo-Finnigan Delta Plus XP isotope ratio mass spectrometer. Results are reported in standard per mil (‰) notation and relative to V-PDB; $\delta^{13}C$ values have an overall analytical error of ±0.1‰. Reported LMW DOM % recovery, $\delta^{13}C$, and $\Delta^{14}C$ values for all UDOC fractions were determined via isotopic mass balance where $\Delta^{14}C_{LMW} = [(TDOC)(\Delta^{14}C_{TDOC}) - (DOC_{HMW})(\Delta^{14}C_{HMW})]/(DOC_{LMW})$. Radiocarbon ages (ybp) are calculated using the relationship: $^{14}C$ ages (ybp) = $-8033^{*}\ln(Fm)$.

### 2.3. Permeation models and coefficients

We applied solute permeation models to the DOC and $\Delta^{14}C$ data presented in this study to examine the behavior of UF on the retention of DOC and $\Delta^{14}C$-content at extremely high CFs. Models used in this study are identical to those described by Kilduff and Weber (1992). Briefly, solute retention behavior during UF is generally characterized by the extent of solute "rejection" ($R$) by the membrane, which is defined as:

$$R = 1 - C_p/C_f \quad (1)$$

where $C_p$ is the solute concentration in the sample permeate (LMW DOC), and $C_f$ is the "feed" solute concentration in the sample retentate (both HMW and LMW DOC). This relationship can also be expressed in terms of the sol-

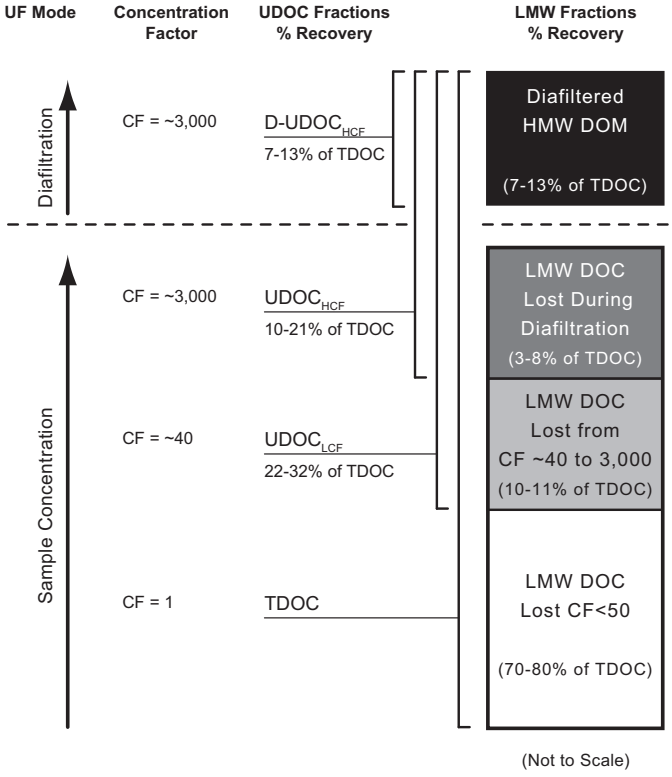


Fig. 1. Summary of isolated DOC fractions. Cartoon representing sampled UDOC sub-fractions isolated with increasing CF and after diafiltration, in terms of total DOC pool. Measured UDOC sub-fractions include: Total DOC (TDOC) collected at CF = 1, UDOC collected at low CF ($UDOC_{LCF}$; CF ~40), UDOC collected at high CF ($UDOC_{HCF}$; CF ~3000) and UDOC collected after diafiltration from the final 2 L sample retentate (D-$UDOM_{HCF}$: top black box). The total column composed of all non/shaded boxes represents the entire DOC pool. White and shaded boxes correspond to DOC progressively lost with increasing concentration factor (CF) and diafiltration, or LMW material lost during the UF process. Recoveries for each fraction are reported as percent of TDOC for determined ranges of surface (20 m) and mesopelagic samples (670 and 915 m). Figure is not to scale with respect to percent recovery or CF.

ute's ability to permeate the UF membrane, or permeation coefficient ($P_c$). The $P_c$ value of a given solution is related to membrane rejection factor in the concentration mode through the following relationship:

$$P_c = (1 - R) \quad (2)$$

During UF in the "sample concentration mode", the feed concentration of DOC ($C_{fDOC}$) is related to the concentration factor (CF) through the following relationship:

$$C_{fDOC} = C_{f0DOC}(CF)^{(1-P_c)} \quad (3)$$

where $C_{f0DOC}$ is the initial feed concentration (Total DOC), $P_c$ is the permeation coefficient and $C_{fDOC}$ is the sample retentate concentration (UDOC). Following Kilduff and Weber (1992) and Eq. (3), in the concentration mode a log-linearized plot of ln ($C_{fDOC}/C_{f0DOC}$) vs. ln (CF) throughout a UF experiment in the concentration mode will yield a slope ($m$) = $1 - P_c$, as indicated by the following expression:

$$\ln(C_{fDOC}/C_{f0DOC}) = (1 - P_c)\ln(CF) \quad (4)$$

Kilduff and Weber (1992) also demonstrated that a log-linearized plot of ln ($C_f$) vs. ln (CF) during the concentration mode yields a $y$-intercept equal to $C_{f0DOC}$. In this study and that of Kilduff and Weber (1992), $C_{f0DOC}$ is defined as the sample "feed" concentration at time = 0. In other words, $C_{f0DOC}$ is the DOC concentration of the sample fluid in the retentate reservoir just after it is filled (before any ultrafiltration takes place). Therefore, for the purposes of this study $C_{f0DOC}$ is considered equivalent to Total DOC. It is important to note that our definition of $C_{f0DOC}$ differs from that of Guo and Santschi (1996), where they measure the UDOC permeate (as opposed to retentate) fraction. Thus, in Guo and Santschi (1996), $C_{f0DOC}$ is not equivalent to Total DOC, but rather the initial concentration of LMW DOC in the sample fluid.

In this study, we use the same regression approach to evaluate the permeation behavior of DOC $\Delta^{14}C$-content during our UF experiments for the following reasons: (1) to evaluate whether or not DOC $^{14}C$-content permeates a UF membrane ideally with respect to permeation theory and (2) if so, to evaluate if this approach can reconcile the large offsets in previously reported UDOC $\Delta^{14}C$ signatures. If DOC $\Delta^{14}C$-content permeates a UF membrane ideally as a function of CF, a log-linearized plot of ln $\Delta^{14}C$ ($C_f/C_{f0}$) vs. log (CF) will demonstrate a statistically robust correlation and yield a slope ($m$) = $1 - P_c$. Similarly, a log-linearized plot of ln $\Delta^{14}C$ ($C_f$) vs. ln (CF) will yield a $y$-intercept of ln $C_{f0^{14}C}$ (or the $\Delta^{14}C$ signature of Total DOC). It is important to note that because "instantaneous" $P_c$ values (i.e. derived directly from Eqs. (1) and (2) at time = $t$) change significantly throughout UF, here we report "time/volume-integrated" $P_c$ values (in accordance with Kilduff and Weber, 1992). These $P_c$ values more accurately characterized the permeation behavior of the sample fluid over the entire experiment and are derived from the slopes of linear regression analyses described above. For our samples, we define DOC permeation coefficients as "$P_{cDOC}$" and $\Delta^{14}C$ permeation coefficients as "$P_{c^{14}C}$". Finally, a similar approach can be used to determine permeation behaviors during the diafiltration mode. In this case, as defined by Kilduff and Weber (1992), a plot of log ($C_f/C_{f0}$) vs. ($V_p/V_0$) will yield a slope ($m$) of $-P_c$. Where $C_{f0}$ is the initial retentate concentration before starting diafiltration, $V_0$ is the system volume and $V_p$ is the permeate volume.

### 2.4. Terminology and conventions for modeling DOC molecular weight fractions

As described above, our data represent discrete sub-samples taken from the retentate solution throughout several UF experiments (Fig. 1). These represent a continuum in the mixture of both high molecular weight (HMW, defined as material rejected by a membrane, nominally >1000 Da) and low molecular weight (LMW; defined as material which can pass membrane, nominally <1000 Da) DOM. In the following discussion, we refer to all of our retentate sub-samples as "ultrafiltered" DOC (UDOC). We also use the terms LMW and HMW as operational definitions based solely on membrane rejection. It is also possible to model DOM constituents of additional molecular weight categories (e.g. LMW, "intermediate" MW and HMW; Benner et al., 1997). However, while it may be true that individual components of "intermediate" molecular weight may permeate the system at different rates vs. "true" LMW material (e.g. Guo and Santschi, 1996; Benner et al., 1997), if there is no HMW membrane breakthrough, then ultimately a DOC mixture is defined by the mixture of these two basic operational components. This is particularly true in high CF experiments. Using only the HMW vs. LMW division thus provides an accurate, and also simplified, framework to interpret UF retention and permeation behavior.

## 3. RESULTS AND DISCUSSION

### 3.1. Recovery of ultrafiltered DOC

In the surface, UDOC fractions had overall higher recoveries at each stage in filtration than deep UDOC fractions at comparable CFs (Table 1). There was a consistent relationship at each depth between DOC recovery and CF. UDOC collected at low CFs had higher overall recoveries of TDOC (CF <50; $UDOC_{LCF}$ = 32% surface, 22% deep), and UDOC recoveries at high CFs had lower overall recoveries of TDOC (CF ~3000; $UDOC_{HCF}$ = 21% surface, 11–12% deep). Diafiltration also substantially decreased TDOC recoveries (D-$UDOC_{HCF}$ = 13% surface, 7–8% deep; Table 1). UDOC sub-sample recoveries at low CFs indicate that initial permeation of DOC is significant, with approximately 68% and 78% permeation of DOC at CF <50 for surface vs. deep, respectively. For the concentration mode, mass balance recoveries indicate that the permeation of "LMW" DOC accounts for 79% of TDOC in the surface and ~89% of TDOC at depth. Final DOC recoveries (D-$UDOC_{HCF}$) are slightly lower than recent work using UF membranes of similar NMWCO, but different manufacturer (Santschi et al., 1995; Benner et al., 1997; Aluwihare et al., 2002; Loh et al., 2004).

A dramatic increase in measured retentate DOC concentration is observed with increased CF at all depths (Fig. 2A). However, when normalized to TDOC and vol-

ume filtered, a progressively smaller fraction of the TDOC pool is in fact retained as the experiment progresses (Table 1). Put another way, continuing DOC loss is observed from the system during both sample concentration and diafiltration, but the *relative percentage* of DOC loss progressively decreases. For example, in the surface we observed a 68% decrease in total recovery from TDOC to $UDOC_{LCF}$ and subsequently smaller decreases in total recovery between $UDOC_{LCF}$ and $UDOC_{HCF}$ (11%).

During the diafiltration mode we observe large decreases in retentate DOC concentration for all depths. The proportional diafiltration losses are much greater in the surface vs. mesopelagic (Fig. 2A). At 20 m, diafiltration resulted in an additional 50% loss of the retained $UDOC_{HCF}$ (i.e. 43.2 mM of $UDOC_{HCF}$ dropped to 22.5 mM D-$UDOC_{HCF}$, after diafiltration; Table 1). At the 670 and 915 m depths, analogous losses were much lower and nearly equal ($28.5 \pm 0.3\%$, $n = 2$). These values are consistent with previous observations of DOC loss during sample diafiltration (Guo and Santschi, 1996; Benner et al., 1997; Guo et al., 2000), however, because we did not perform detailed sampling during the diafiltration mode, we do not further discuss the permeation behavior of DOC during diafiltration in this study. However, this comparison does illustrate

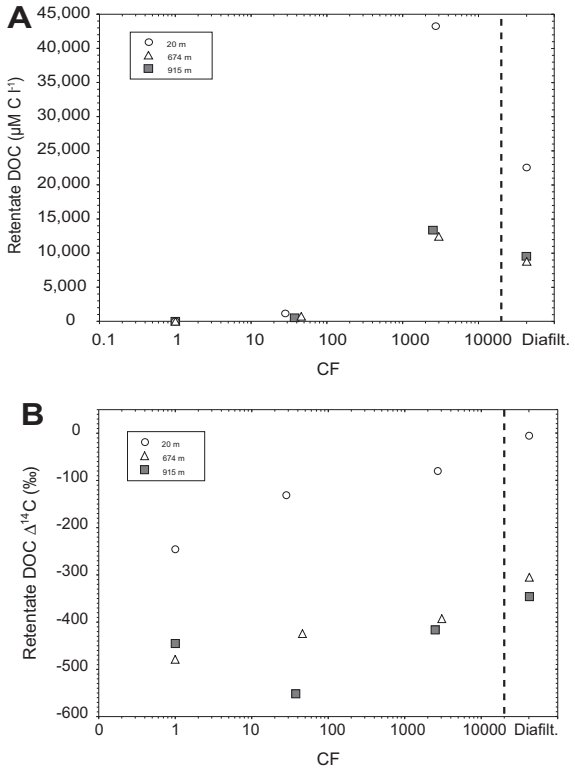


Fig. 2. UDOC retentate DOC concentration and $\Delta^{14}C$ vs. concentration factor. (A) DOC concentrations in UF retentate sub-samples (μM) at varying concentration factor (CF) and after diafiltration for the three sampled NELHA depths. For all data points, measured errors are smaller than the symbols used. (B) Measured retentate DOC $\Delta^{14}C$ values with CF and after diafiltration. Vertical dashed lines in A/B represent change from concentration to diafiltration mode (see Sections 2.1 and 2.2).

that for very large volume filtrations, CF has a larger cumulative effect on mass retention than does diafiltration.

The overall observation of DOC loss with increasing CF is consistent with previously reported permeation behaviors for seawater DOM, however an important difference is that our data indicate that much higher CFs are required to fully remove LMW material. Previous work has shown that at lower CFs (~20–100), HMW DOC concentrations can be overestimated by up to ~30% due to retention of LMW DOM (Guo and Santschi, 1996). Previous studies have also indicated that using CFs as low as 40 are generally sufficient to remove LMW material, and isolate a relatively "pure" HMW DOC sample (as defined by a membrane NMWCO, and assuming no breakthrough or concentration polarization; Guo et al., 2000). While in our study we observed no breakthrough of HMW DOC (discussed in Sections 3.2 and 3.3 below), our results indicate a large fraction of LMW DOC is retained at CF <40. In addition, from CF = ~40 to ~3000 we observed additional loss of LMW material equivalent to 10–11% of the TDOC pool. At CF = 40 in our experiments, apparent HMW DOC concentrations are overestimated by ~20% vs. recoveries at CF ~2500. These observations are consistent with other studies suggesting that much larger CFs are needed to fully remove LMW material and isolate a "pure" HMW sample. For example, Benner et al. (1997) found through modeling a mixture of LMW, "intermediate" and HMW DOC components (each with its own $P_c$ value), that CF ~100 removes 98% "LMW" and 86% of "intermediate MW" material, and modeled HMW concentration in the UDOC retentate for a large-volume isolation was greater than 95% (UDOC at CF = 1000). Our modeled results suggest that even after CF ~2500 in the concentration mode, roughly 5–8% LMW DOC remains in the UDOC retentate solution, which then permeates during diafiltration ($UDOC_{HCF}$ to D-$UDOC_{HCF}$). Thus, while previously modeled results for CF = 1000 are consistent with our observations, the precise amount of LMW DOC remaining in the UDOC solution during large-volume isolations is either (1) underestimated by these models or (2) dependent on the specific environment in which samples are taken (e.g. the nature of the HMW vs. LMW DOC mixture sampled). Later we invoke several permeation models to explain this behavior in the concentration mode (Sections 3.3 and 3.4). Together, these data indicate that low CFs are not adequate to fully remove LMW material (i.e. when UF is conducted at low CF, a much more representative sample of total DOC is isolated due to both LMW and HMW retention).

### 3.2. Carbon isotopic composition

A summary of carbon isotope data is provided in Table 1. Stable carbon $\delta^{13}C$ values for TDOC and all UDOC fractions fall within typical ranges for DOM from the NPSG (−20‰ to −22‰) with the possible exception of TDOC from 915 m ($\delta^{13}C = -23.2‰$), which was slightly lower than typical TDOC $\delta^{13}C$ values from the Central North Pacific (CNP; Druffel et al., 1992). These TDOC $\Delta^{14}C$ values are the first reported TDOC $\Delta^{14}C$ measurements for all water source depths available at the NELHA

site, and are $\Delta^{14}C = -246 \pm 5‰$, $-479 \pm 9‰$, and $-446 \pm 8‰$ for 20, 670, and 915 m depths, respectively. These values are consistent with ship-based measurements from the CNP (Druffel et al., 1992), further confirming isotopic and molecular-level data which suggests DOM from NELHA samples is representative of this general ocean region (Ingalls et al., 2006; Repeta and Aluwihare, 2006).

Even though all $\Delta^{14}C$ values are in expected ranges, the 33‰ TDOC $\Delta^{14}C$ *increase* observed between 670 and 915 m is the opposite of a typical depth profile. While it might be tempting to attribute this to a measurement error, we note a similar unexpected increase in $\Delta^{14}C$ values was observed in bacterial nucleic acids isolated from the same NELHA water sources (Hansman et al., 2009). In addition, the TDOC concentration at 915 m is slightly elevated vs. that at 670 m (43 vs. 40 μM) and its $\delta^{13}C$ value more negative than expected (−23.2‰). Together these observations would be consistent with an increase in DOC derived from surface-derived POC having nearly modern $\Delta^{14}C$ values. While we cannot fully explain these offsets from expected trends, it is important to emphasize that for the main purposes of this study they are inconsequential: i.e. water from 670 to 915 m are both clearly oceanic "deep" water in terms of their TDOC and $\Delta^{14}C$ values, so to first order, these samples will represent independent replicates of oceanic deep water for our tests of UF behavior. However, as discussed below, for some of the modeling approaches the offsets between depths do alter resulting regressions and other finer scale results.

All UDOC sub-fractions had more positive $\Delta^{14}C$ values with respect to TDOC, however, there was also a consistent trend of increasing $\Delta^{14}C$ value with higher CFs. $UDOC_{HCF}$ retentate subsamples were the most $^{14}C$-enriched ($UDOC_{HCF}$ $\Delta^{14}C = -80‰$, $-393‰$, and $-415‰$), while $UDOC_{LCF}$ retentate subsamples were less offset vs. TDOC at 20 and 670 m ($\Delta^{14}C = -131‰$ and $-424‰$, respectively). The 915 m $UDOC_{LCF}$ subsample was again slightly anomalous in terms of its $UDOC_{LCF}$ fraction, being $^{14}C$-depleted with respect to TDOC (−552‰ vs. −446‰ respectively). Results from an isotopic mass balance indicate this depleted $UDOC_{LCF}$ value can be accounted for by a ~34 μM loss of LMW DOC having a $\Delta^{14}C$ signature slightly more positive with respect to TDOC (−416‰ vs. −446‰ respectively: Table 1). This explanation would be consistent with a slightly more positive $\Delta^{14}C$ LMW contribution to the DOC pool from particle remineralization at this depth, perhaps from bottom accumulation of sinking material, or an intermediate nepheloid layer at this depth impinging on the steep volcanic escarpment near Keahole Point.

D-$UDOC_{HCF}$ retentates had the most positive $\Delta^{14}C$ values of all sampled UDOC sub-fractions. These D-$UDOC_{HCF}$ values ($\Delta^{14}C = -6‰$, $-306‰$, and $-345‰$ at 20, 670, and 915 m depths, respectively) are in agreement with previously reported "high CF" UDOC $\Delta^{14}C$ values from NELHA (Repeta and Aluwihare, 2006). The relative increase in $\Delta^{14}C$ observed with diafiltration for each depth was very similar (Fig. 2B; average $\Delta^{14}C$ enrichment $= +77 \pm 9‰$, $n = 3$). While this might initially suggest that similar LMW components are being permeated during diafiltration at all depths, isotopic mass balance results indicate clear differences between LMW material lost during diafiltration in the surface vs. mesopelagic. In the surface, LMW DOC lost during diafiltration is only slightly more positive with respect to TDOC (LMW $\Delta^{14}C = -206‰$ vs. TDOC $\Delta^{14}C = -246‰$), while at depth LMW material lost during diafiltration has far more negative $\Delta^{14}C$ values (LMW $\Delta^{14}C = -519‰$ and $-588‰$ for 670 and 915 m, respectively). This difference is also consistent with strong mass balance offsets between surface and deep LMW $\Delta^{14}C$ values determined for the entire UF experiment (i.e. material lost from TDOC to D-$UDOC_{HCF}$; $\Delta^{14}C = -281‰$ surface vs. $n = 2$ average $-473‰$ at depth). While in general these LMW DOC $\Delta^{14}C$ values are consistent with previously determined values by isotopic mass balance (Loh et al., 2004), the large change in LMW $\Delta^{14}C$ content which occurs during UF in this study suggests

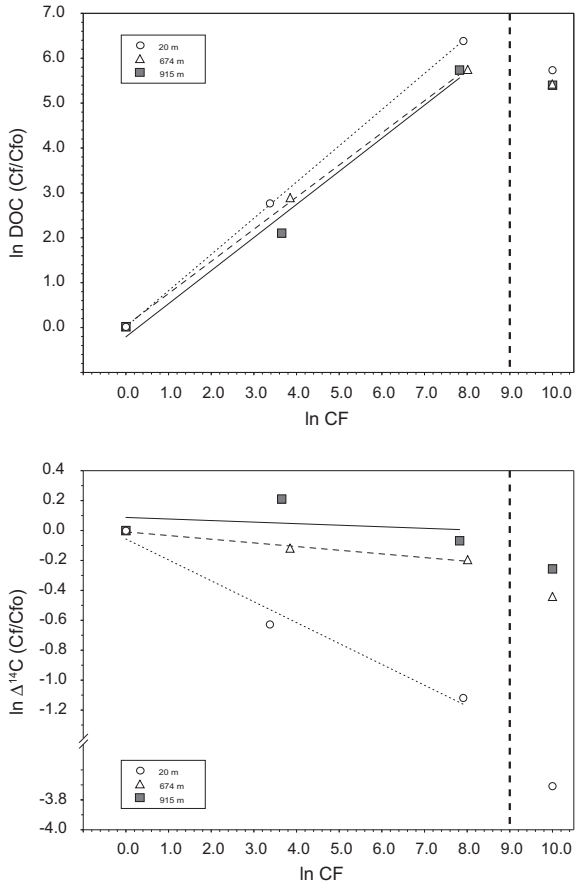


Fig. 3. Permeation models of DOC retention and $\Delta^{14}C$ content during ultrafiltration. (A) DOC permeation model (Kilduff and Weber, 1992). Model I regression lines for each depth are also shown with $R^2$ values and equations from which permeation coefficients were calculated (see Section 2.3). (B) Radiocarbon permeation model (see Section 2.3). NELHA depths (20, 670, and 915 m) are represented by open circles, open triangles, and gray squares, respectively. Vertical dashed lines represent a change in filtration parameters from concentration mode to diafiltration mode (see Section 2.3). All data shown represent the natural log transform of DOC and $\Delta^{14}C$ data reported in Table 1.

that UF and diafiltration can have an appreciable effect on resulting "HMW" $\Delta^{14}C$ values.

### 3.3. DOC and radiocarbon permeation models

In order to evaluate whether marine DOC retention at very high CF remains consistent with the ideal UF theory, as is observed at low CF in previous work (Buesseler et al., 1996; Guo and Santschi, 1996; Guo et al., 2000), we applied established UF permeation models to our UDOC fractions. Similar models are typically used to evaluate the permeation/retention behavior of organic macromolecules (Guo et al., 2000), and can be applied to both sample concentration and diafiltration mode. If a given solute performs according to UF theory, the HMW component will be rejected by the membrane at a constant rate throughout the experiment, no breakthrough of HMW component will occur, and there will be no significant macromolecular accumulation on the membrane surface (Buffle et al., 1992b). As described in the methods, under these conditions, a log–log plot of mass vs. CF should yield a straight line, and the *y*-intercept should correspond to the log of initial DOC concentration in the feed solution (i.e. UDOC at CF = 1, or $C_{f0DOC}$). Thus applying this approach in solutions containing a complex mixture of molecules, including oceanic DOC (Guo and Santschi, 1996; Guo et al., 2000), can be used to test these assumptions.

DOC permeation models demonstrate robust correlations for DOC at all depths, with $R^2$ values >0.98 (Fig. 3A and Table 2A). The model-derived *y*-intercepts also closely match our measured TDOC values for the 20 and 670 m depths, estimates of $C_{f0DOC}$ fall within ±2 μM of measured TDOC (Table 2), providing a robust verification of the application of UF models to these data. The 915 m $C_{f0DOC}$ value was lower than measured TDOC, yet is still within one standard deviation of the measured 915 m value ($C_{f0DOC} = 35 \pm 15$ μM vs. TDOC = 43 ± 2 μM). This is likely related to the unexpected higher TDOC concentration at this depth discussed earlier. However, we note that the model estimated value is actually closer to previously determined TDOC values from similar depths in the CNP (38 μM at 900 m; Druffel et al., 1992), suggesting that UF permeation models based on multiple measurements can essentially "dilute" the effect of a single uncharacteristic value. This indicates that even at extremely high CFs, concentration polarization and HMW breakthrough for marine DOC are negligible, such that theoretical UF behavior is maintained. This also supports a simple division (in terms of membrane rejection behavior) between HMW and LMW pools in ocean DOM. In other words, since there is no significant breakthrough of *any* HMW component (>1000 Da) during even very high CF experiments, the HMW mixture (>1000 Da) within seawater DOC also behaves ideally. Overall, this implies that all changes in DOC permeation (and also associated $\Delta^{14}C$ value) can be ascribed to LMW DOC that is being retained at lower CF, rather than to selective breakthrough of some HMW components.

Table 2
Summary of permeation model statistics for concentration and diafiltration mode. Regression coefficients ($R^2$) values represent correlation coefficients from Model I regression analysis, *p* represents the *p*-value for each correlation, m is the slope of the regression line, $P_c$ is the permeation coefficient as described in text (Section 2.3).

| Depth (m) | $R^2$ | *p* | *m* | $P_{cDOC}$ | ± | *y*-int† | ± | $C_{f0DOC}$† (μM) | ± |
|---|---|---|---|---|---|---|---|---|---|
| (A) DOC Permeation model results | | | | | | | | | |
| *Concentration mode* | | | | | | | | | |
| 20 | 0.9999 | 0.005 | 0.807 | 0.194 | 0.006 | 4.31 | 0.03 | 74 | 2 |
| 670 | 0.9994 | 0.016 | 0.716 | 0.284 | 0.018 | 3.73 | 0.09 | 42 | 4 |
| 915 | 0.9867 | 0.074 | 0.737 | 0.263 | 0.086 | 3.55 | 0.43 | 35 | 15 |
| *Diafiltration mode** | | | | | | | | | |
| 20 | 1.0 | – | −0.165 | 0.165 | – | 0.0 | – | 43,242 | – |
| 670 | 1.0 | – | −0.133 | 0.133 | – | 0.0 | – | 12,414 | – |
| 915 | 1.0 | – | −0.134 | 0.134 | – | 0.0 | – | 13,406 | – |
| Depth (m) | $R^2$ | *p* | \|*m*\| | $P_{c^{14}C}$ | ± | *y*-int† | ± | $C_{f0^{14}C}$† (‰) | ± |
| (B) \|DOC $\Delta^{14}C$\| Permeation model results | | | | | | | | | |
| *Concentration mode* | | | | | | | | | |
| 20 | 0.9765 | 0.098 | 0.140 | 0.860 | 0.022 | 5.45 | 0.11 | −232 | 25 |
| 670 | 0.9772 | 0.097 | 0.025 | 0.975 | 0.004 | 6.16 | 0.02 | −474 | 9 |
| 915 | 0.0754 | 0.823 | 0.010 | 0.990 | 0.036 | 6.19 | 0.18 | −486 | 88 |
| *Diafiltration mode** | | | | | | | | | |
| 20 | 1.0 | – | −0.360 | 0.360 | – | 0.0 | – | −80 | – |
| 670 | 1.0 | – | −0.125 | 0.125 | – | 0.0 | – | −393 | – |
| 915 | 1.0 | – | −0.119 | 0.119 | – | 0.0 | – | −415 | – |

† *y*-intercepts and $C_{f0}$ values derived from ln ($C_f$) vs. ln (CF) regressions (Kilduff and Weber, 1992). All other data are derived from ln ($C_f/C_{f0}$) vs. ln (CF) regressions.

* Diafiltration mode values were determined using relationships specified in Kilduff and Weber (1992): ln ($C_f/C_{f0}$) vs. $V_p/V_0$ because only $n = 2$ samples were available, $R^2$ and *p*-values were not determined. Measured UDOC-HCF values were used as $C_{f0DOC}$ and $C_{f0^{14}C}$ values in diafiltration mode models.

We also applied UF permeation models to our $\Delta^{14}C$ data. To our knowledge, this is the first study to examine the effects of UF on the $\Delta^{14}C$ content of DOC using this approach. As discussed in the methods, $\Delta^{14}C$ permeation model results can be interpreted in a similar manner to DOC: using $R^2$ and intercept results to evaluate if there is a consistent relationship between retained $\Delta^{14}C$ and HMW vs. LMW fractions. The regression results (Fig. 3B) demonstrate that the $\Delta^{14}C$ content of UDOC is also highly correlated to CF (Table 2B; $R^2 > 0.97$ for 20 and 670 m). This is similar to DOC models, indicating that retention and permeation of DOM $^{14}C$-content during concentration mode also follows theoretical UF behavior. The log $y$-intercepts ($C_{f0^{14}C}$) for the 20 and 670 m depths yield TDOC $\Delta^{14}C$ values of $C_{f0^{14}C} = -232‰$ for 20 m and $-474‰$ for 670 m. As in the case for $C_{f0DOC}$, if UF behavior of $\Delta^{14}C$ is ideal, then these values should match the $\Delta^{14}C$ content of the feed solution at CF = 1 (TDOC). Our modeled $C_{f0^{14}C}$ values are in fact indistinguishable from our measured TDOC $\Delta^{14}C$ values. However, again the results for 915 m are anomalous. The high $p$-value and lack of correlation (Table 2B) may be due to the lack of a significant slope ($m \sim 0.01$). However, despite the lack of significance and relatively large error (Table 2B), the 915 m modeled TDOC $\Delta^{14}C$ value nevertheless falls very close to the measured TDOC $\Delta^{14}C$ value ($C_{f0^{14}C} = -487‰$ vs. measured TDOC $= -446‰$). In addition, the intercept value is also very similar to the $\Delta^{14}C$ signature reported for 900 m NCP ($-470‰$, Druffel et al., 1992). Overall, despite the uncertainties at the 915 m depth, the data strongly indicates that $\Delta^{14}C$ permeation models can be used to directly evaluate a relationship between CF and retentate $\Delta^{14}C$ values at the surface and mesopelagic depths.

### 3.4. Permeation coefficients: an exploration of DOM molecular weight and $\Delta^{14}C$ distribution

UF model-derived permeation coefficients ($P_c$) represent a ratio of solutes permeating a UF membrane (LMW) to those retained by the UF membrane (HMW), and can be calculated either instantaneously or over the course of an entire UF experiment (see Section 2). Previous work has shown that $P_c$ and $C_{f0}$ values determined by permeation models (analogous to this study) can be used to more accurately determine both LMW solute permeation characteristics, and also solute molecular size distributions in natural waters (Logan and Qing, 1990). Because traditional DOC mass balance calculations inherently depend on running UF experiments to a high CF to fully remove LMW material, at lower CFs these mass balances can be misleading by underestimating the amount of LMW solutes that permeate the membrane. As a result (and as our own data confirms), this approach can potentially greatly overestimate HMW recoveries. In contrast, permeation models quantify membrane rejection and the initial concentration of the feed solution (in our case TDOC) independent of sample volume filtered or CF. Thus, using $P_c$ values determined from DOC measurements during UF have the potential to be a more accurate way to determine HMW vs. LMW abundance and molecular weight distributions of DOC within natural waters.

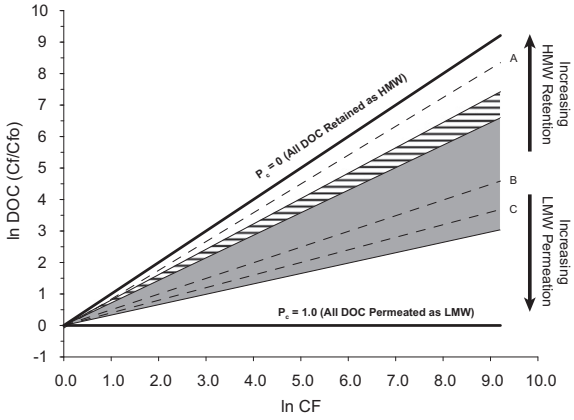


Fig. 4. Conceptual model of DOC permeation coefficients. Figure shows permeation coefficient ($P_c$) theoretical limits (thick lines) and the relationship between $P_c$ and solute mixtures of differing molecular weights. Gray shaded area represents the range of previously reported $P_c$ values for seawater DOC at low concentration factors (CF <100; Guo et al., 2000). Hatched area represents the range in $P_c$ values from surface and mesopelagic depths reported within this study ($P_c = 0.194$–$0.284$). Dashed lines A, B and C represent $P_c$ values of three DOC mixtures containing molecular probes of known MW and $P_c$ (Guo et al., 2000). Line (A) Sample feed solution contains an equal mixture (20%) of five molecular probes: Dextran 3 kDa ($P_c = 0.03$), Dextran 10 kDa ($P_c = 0.0$), Vitamin B12 1.33 kDa ($P_c = 0.15$), Glutathione 0.612 kDa ($P_c = 0.16$) and Rhodamine 0.495 kDa ($P_c = 0.60$), with resulting $P_c$ value for this equal mixture of $P_c = 0.085$. Line (B) Sample feed solution contains 1% HMW (Dextran 10 kDa) and 99% LMW (Rhodamine 0.495 kDa), resulting in $P_c = 0.471$. Line (C) Sample feed solution contains 100% LMW (Rhodamine 0.495 kDa), resulting in $P_c = 0.600$. All modeled regressions were determined assuming a feed DOC solution of $C_{f0} = 100$ μM.

To better illustrate the meaning of these coefficients, and the effect changing TDOC molecular weight distributions can have on $P_c$ values, a conceptual model summarizing both theoretical limits and prior measured $P_c$ values are presented in Fig. 4. The limits of $P_c$ values range from $P_{cDOC} = 0$ to $P_{cDOC} = 1.0$. If $P_{cDOC} = 0$, and a slope of $m = 1$, there is 100% sample retention, meaning that the TDOC mixture is comprised *only* of HMW DOC, and none of this HMW DOC permeates the system. In contrast, if $P_{cDOC} = 1.0$, there is 100% sample permeation from the system, meaning that TDOC is comprised *only* of LMW DOM, which is significantly smaller than the membrane NMWCO. Fig. 4 also illustrates the influence of MW diversity on $P_c$ values, using three modeled DOC mixtures made from five molecular probes of varying MW and membrane rejection properties (previously determined by Guo et al. 2000). These include: 10 kDa Dextran ($P_c = 0.0$), 3 kDa Dextran ($P_c = 0.03$), 1.33 kDa Vitamin B12 ($P_c = 0.15$), 0.612 kDa Glutathione ($P_c = 0.16$) and 0.495 kDa Rhodamine ($P_c = 0.60$). Line A in Fig. 4 represents an equal mixture of all five probes (20% each, resulting $P_c = 0.085$), whereas line B (1% 10 kDa Dextran, 99% 0.495 kDa Rhodamine) and C (100% 0.495 kDa Rhodamine) are mixtures dominated by LMW compounds. The TDOC solutions

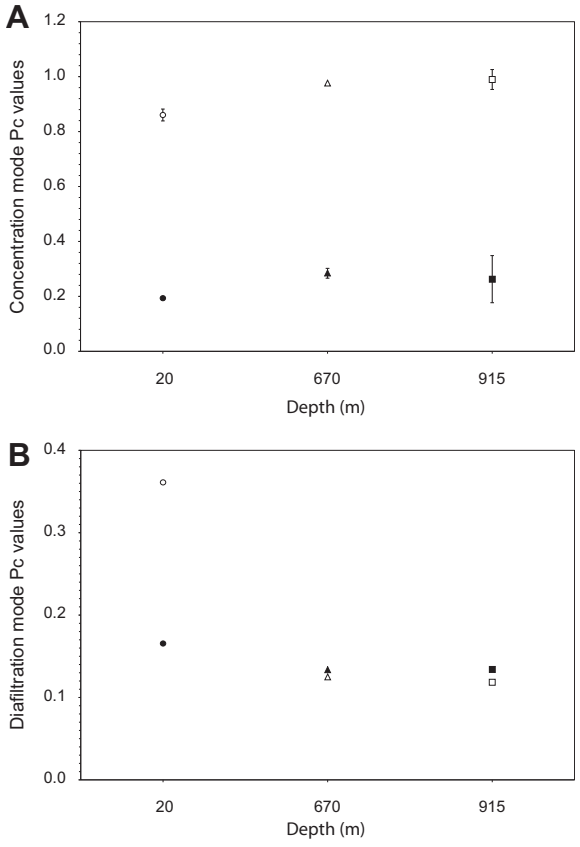


Fig. 5. Model-derived DOC and radiocarbon UF permeation coefficients. Solid symbols = $P_{cDOC}$ values; open symbols $P_{c^{14}C}$ values; circles (20 m), triangles (670 m) and squares (915 m) indicate different sampling depths at NELHA. (A) Shows concentration mode values. Error bars are extrapolated from the standard error of the regression slope (Table 2); if no error bars are shown, error is smaller than symbol. (B) Shows diafiltration mode $P_c$ values. As discussed in text (Section 3.4.2), because only $n = 2$ analyses were used, no errors were determined.

containing a higher abundance of LMW molecules will have $P_c$ approaching 1.0, whereas TDOC solutions rich in HMW molecules will have lower $P_c$ values, approaching zero. However, the additional influence of mixtures is clear in the relative positions of line B and C: addition of only 1% of a higher MW component causes a much larger logarithmic shift in Pc value (from line C, $P_c = 0.60$ to line B, $P_c = 0.47$).

This example illustrates how the relative proportion of LMW permeation vs. HMW retention can yield potentially more sensitive information regarding the general DOC MW distribution in a solution. Using this conceptual framework, and assuming ideal UF behavior, the proportion of HMW and LMW pools determined by UF permeation models may be more accurate than traditional mass balance determinations, and thus have the potential to act as proxies for relative changes in the molecular size distributions (LMW vs. HMW) of DOM at a given depth or location. We examined the relative changes in $P_{cDOC}$ and $P_{c^{14}C}$ values of our isolated UDOC retentates from the concentration and diafiltration modes in order to explore if changes in DOM molecular size and radiocarbon distributions are apparent with depth. As detailed in the methods, we defined DOC permeation coefficients as "$P_{cDOC}$" and $\Delta^{14}C$ permeation model coefficients as "$P_{c^{14}C}$".

### 3.4.1. Concentration mode permeation coefficients

In the concentration mode, $P_{cDOC}$ values increase with depth from 0.19 to 0.26–0.28 (Table 2A and Fig. 5A), reflecting overall higher recoveries of HMW DOM for surface vs. deep water (Table 1). These values are consistent with a modeled seawater DOC mixture by Benner et al. (1997) containing 20% HMW ($P_{cDOC} = 0$), 50% LMW ($P_{cDOC} = 1.0$) and 30% "intermediate" material ($P_{cDOC} = 0.5$). A regression of ln ($C_f/C_{f0}$) vs. ln (CF) applied to the solution over CF = 10,000 resulted in a $P_c = 0.16$ ($R^2 = 0.98$). The increasing $P_{cDOC}$ values with depth determined in this study, could derive from two end-member possibilities: (1) the increase in $P_{cDOC}$ reflects only a greater concentration of LMW DOM relative to HMW material at depth, or (2) the ratio of LMW to HMW DOM remains constant with depth, but a significant difference in LMW and/or HMW DOM chemical composition (i.e. molecular size, shape, flexibility, hydrodynamic radius and electrochemical properties) alters rates of HMW rejection and LMW permeation between the surface and deep. However, the latter would require substantial HMW breakthrough during concentration mode, inconsistent with our results and those from previous studies (e.g. Guo et al., 2000). Thus, observed increases in $P_{cDOC}$ values at depth (Fig. 5A) likely indicate a slightly more heterogeneous distribution of DOM molecular sizes in the surface ocean (i.e. more retainable HMW chemical species) and a more homogeneous molecular size distribution in the deep ocean (far fewer retainable HMW species).

While this interpretation has been inferred by previous studies based solely on HMW recovery, the specificity of $P_c$ values shows the potential for more sensitive (and CF-independent) $P_{cDOC}$ values to better quantify the distribution of HMW vs. LMW DOM in different environments. For example, Fig. 4 shows that the ranges of $P_c$ values reported for different marine environments are very large. The contrast between observed $P_{cDOC}$ values determined in this study from an oligotrophic gyre and those previously reported for the Gulf of Mexico and Galveston Bay estuary (TDOC = 241–245 μM and $P_{cDOC} = 0.45$–$0.67$ by Guo et al., 2000), may reflect distinctive DOM MW distributions between these distinct marine environments. It should be noted that UF membranes behave differently in solutions of different ionic strength, and therefore caution should be used when comparing $P_c$ values from environments of drastically different salinities (e.g. river vs. seawater). However, changes in UF behavior within seawater salinity ranges are much less likely. In general, decreasing $P_c$ values as a function of increasing solute molecular weight have also been reported in previous studies (Logan and Qing, 1990; Kilduff and Weber, 1992; Guo et al., 2000). While further investigation is needed, it seems likely that relative changes in measured $P_{cDOC}$ values could serve as proxies

for changes in HMW vs. LMW DOM spatial distributions in the ocean.

We also explored a similar approach using modeled $P_{c^{14}C}$ values to approximate the distribution of DOM $\Delta^{14}C$-content along depth profiles. In $\Delta^{14}C$ permeation models, $P_{c^{14}C}$ represents the ratio of LMW $^{14}C$-content (permeating the membrane) to HMW $^{14}C$-content "retained" by the membrane. As defined by our model (*see methods*), a $P_{c^{14}C} = 1.0$ (slope of zero) would indicate that TDOC is completely homogenous with respect to $\Delta^{14}C$-content and that the $\Delta^{14}C$ value of UDOC is independent of CF (and MW). In other words, a $P_{c^{14}C} = 1.0$ indicates that both LMW and HMW DOC have the same $\Delta^{14}C$ value. In contrast, large slopes in the model would correspond to very low $P_{c^{14}C}$ values, and would generally signify either a large amount of low-$\Delta^{14}C$ DOC (older carbon) permeating in the LMW fraction during the concentration mode, the continued retention of $\Delta^{14}C$-enriched (modern) HMW compounds during UF, or both. Our model-derived $P_{c^{14}C}$ values are lower in the surface (0.86) and increase in the mesopelagic (~0.98) (Table 2B and Fig. 5A). This is consistent with the relatively small offsets between TDOC $\Delta^{14}C$ and D-UDOC$_{HCF}$ $\Delta^{14}C$ at depth (~173‰ and ~101‰, at 670 and 915 m) vs. in the surface (~240‰ at 20 m; Table 1).

### 3.4.2. Diafiltration mode permeation coefficients

$P_{cDOC}$ and $P_{c^{14}C}$ values were also determined in diafiltration mode (Table 2A/B and Fig. 5B; see Section 2.3). We note that because only the starting concentration of UDOC isolates (UDOC$_{HCF}$) and the final concentration of UDOC after diafiltration (D-UDOC$_{HCF}$) were measured ($n = 2$ for each NELHA depth), it is not possible to assess correlation coefficients ($R^2$) or significance ($p$-values). However, we believe the trends in these $P_c$ values with depth can still provide meaningful information regarding permeation with MW and $\Delta^{14}C$ during diafiltration.

Estimated $P_{cDOC}$ values for diafiltration are similar to $P_{cDOC}$ values from the concentration mode (0.16–0.13). However, in contrast to concentration mode data, diafiltration $P_{cDOC}$ values *decrease* with depth, from $P_{cDOC} = 0.16$ in the surface to average $P_{cDOC} = 0.13$ in the mesopelagic (Fig. 5B). In the surface, the diafiltration $P_{cDOC}$ value is also higher relative to that determined for concentration mode (0.16 vs. 0.13), again reflecting the greater relative permeation of LMW DOC during the diafiltration step. In contrast, estimated mesopelagic $P_{cDOC}$ values are smaller during diafiltration vs. concentration mode (0.13 vs. ~0.27), suggesting that relatively *less* LMW DOC is permeated as a result of changing ionic strength (diafiltration) in deep water. These observations are consistent with mass balance results discussed above, indicating greater permeation of LMW material in surface vs. mesopelagic during diafiltration.

Estimated $P_{c^{14}C}$ values during diafiltration also display a clear offset between surface and depth: the estimated surface $P_{c^{14}C}$ value (~0.36) is much higher than mesopelagic $P_{c^{14}C}$ values (~0.12, $n = 2$). Here the relative overall change in UDOC $\Delta^{14}C$ content is highest in the surface (Table 1: ~93% change in $\Delta^{14}C$ from UDOC$_{HCF}$ to D-UDOC$_{HCF}$) and far lower at depth (Table 1: 22% and 17% for 670 and 915 m, respectively). Thus, these $P_{c^{14}C}$ values are consistent with the large overall change in UDOC $\Delta^{14}C$ content in the surface vs. relatively small change in $\Delta^{14}C$ HMW signatures at depth during diafiltration. In addition, these values are consistent with determined LMW $\Delta^{14}C$ permeation during diafiltration by isotopic mass balance, where LMW material permeating the system at depth was "old" (Table 1: $\Delta^{14}C = -553 \pm 35‰$, $n = 2$) in comparison to the permeation of more $^{14}C$-enriched LMW in the surface ($\Delta^{14}C = -206‰$).

While clearly not conclusive, to the best of our knowledge this exploration represents the first reported $P_c$ values used to describe the permeation of $\Delta^{14}C$ from marine DOM during a UF experiment. Our $\Delta^{14}C$ permeation models for the concentration mode demonstrate universally strong correlations between CF and retentate $\Delta^{14}C$ content. However, given the small range in $P_{c^{14}C}$ values determined here, we cannot unequivocally demonstrate that model-derived $P_{c^{14}C}$ values can be applied in an analogous way to evaluate relationships between both DOC MW and $\Delta^{14}C$. Nevertheless, it seems likely that $P_{c^{14}C}$ values (when placed into the context of HMW recoveries and $P_{cDOC}$ values) may provide LMW vs. HMW $^{14}C$-age information irrespective of the CF employed in a UF experiment, and would be relatively straight forward to determine. Given the dynamic range in reported $\Delta^{14}C$ values across marine environments, it is also possible that significant differences in $P_{c^{14}C}$ values may be potential indicators of DOM $^{14}C$-age heterogeneity in different environments.

## 3.5. Re-evaluation of open ocean HMW DOC $\Delta^{14}C$, reactivity and composition

If UF behaves ideally in terms of $\Delta^{14}C$ permeation and retention, then the basic trends we have identified should be universal and can be extended to other studies. Specifically, similar relationships between CF and $\Delta^{14}C$ would be predicted for UDOM isolated from at least comparable ocean regions. To test this idea, Fig. 6 summarizes all published surface and mesopelagic $\Delta^{14}C$ values for HMW DOC vs. corresponding CF data for the Pacific (including results from this study). The predicted effect of increasing CF on the enrichment of HMW DOC $^{14}C$-content is clear in both surface and deep water, and is remarkably consistent across all (diafiltered and non-diafiltered) published HMW data. Despite the fact that the compiled data comes from different membrane manufacturers (e.g. Amicon and GE Osmonics) and variable field operation conditions, statistically significant $y$ vs. $\log x$ regression correlations are obtained for both surface and mesopelagic data sets ($R^2 = 0.91$, $p = 0.0038$ and $R^2 = 0.81$, $p = 0.0149$, respectively). This comparison seems to confirm that our main conclusions regarding CF and $\Delta^{14}C$ are universal.

Together, these results indicate that when both variable-CF UF and diafiltration are used as key operational parameters, UF can become a highly versatile tool for isolation of the marine DOC pool for composition and $\Delta^{14}C$ studies. In general, using low CFs will effectively retain both HMW and LMW material, resulting in UDOC samples with $\Delta^{14}C$ values nearly representative of TDOC.

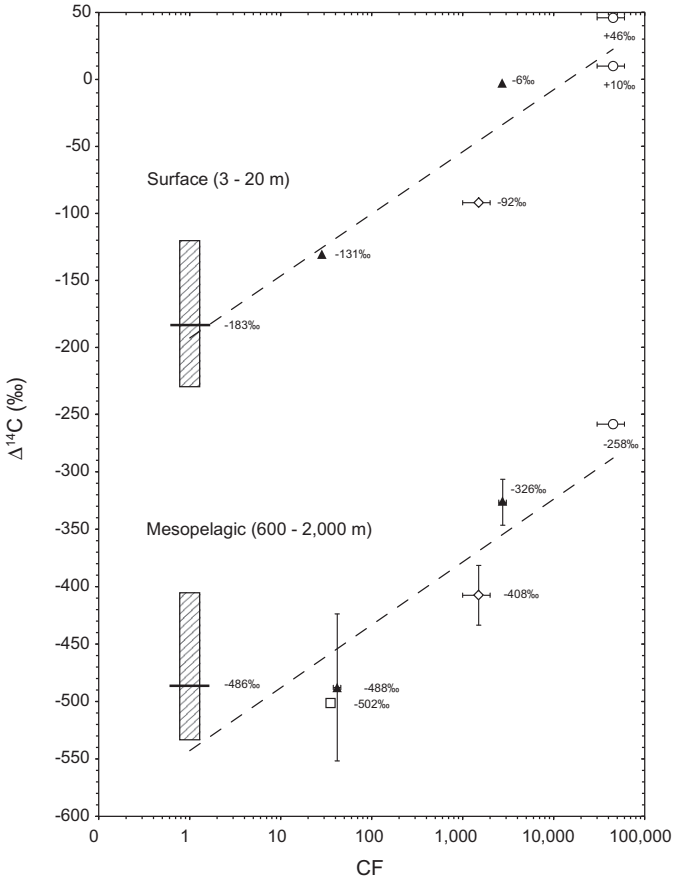


Fig. 6. Summary of published UDOC $\Delta^{14}C$ values and relationship to concentration factor: Central North Pacific Ocean. Surface (3–20 m) and mesopelagic (600–2000 m) ranges in known NPSG TDOC $\Delta^{14}C$ values (hatched rectangles). TDOC $\Delta^{14}C$ ranges are: surface = −137‰ to −246‰, deep = −405‰ to −533‰ (Druffel et al., 1992; Bauer et al., 1992, this study). Solid horizontal bars show average TDOC values from these ranges. With the exception of the low CF samples reported within this study, all other HMW DOC data points represent the $\Delta^{14}C$ content of diafiltered UDOC isolates. Solid triangles represent $\Delta^{14}C$ and CF data reported within this study ($n = 1$ surface and $n = 2$ average of 670 m and 915 m samples). Open diamonds, circles and squares represent values reported by Loh et al. (2004), Repeta and Aluwihare (2006), and Guo and Santschi (1996), respectively. The +10‰ surface and −258‰ deep values reported by Repeta and Aluwihare, in addition to the −502‰ value reported by Guo and Santschi (1996), represent samples taken from the same site (NELHA) as this study. Because only 1 or 2 samples are reported for each time/location, *y* error bars represent the total range in reported $\Delta^{14}C$ values. Similarly, *x*-error bars represent the total range of either reported CF values (this study) or possible CF values, when general ranges in literature sample volumes were reported in place of specific sample volumes (e.g. Loh et al., 2004; Repeta and Aluwihare, 2006).

For example, in the mesopelagic CNP, UDOC at low CFs have $\Delta^{14}C$ signatures very similar to the average TDOC $\Delta^{14}C$, and surface UDOC $\Delta^{14}C$ values are only moderately higher vs. surface TDOC $\Delta^{14}C$ (Fig. 6). Subsequent diafiltration will significantly alter $\Delta^{14}C$ values of UDOC collected at any CF. However, while UDOC isolates have traditionally been de-salted, diafiltration is actually not required for many molecular level analyses. For example, both total lipid extraction and acid hydrolysis (to liberate polar biopolymer constituents) can be readily performed in presence of some salt, and further desalting can be accomplished after hydrolysis by resin methods if required (Repeta and Aluwihare, 2006). In contrast, using high CFs coupled with diafiltration allows for the highly selective isolation of the most $^{14}C$-enriched DOC components from both surface and mesopelagic waters (Fig. 6). In deep waters, HMW DOC is still substantially $^{14}C$-depleted ($\Delta^{14}C \sim -250‰$) vs. surface sources, however in oligotrophic surface waters HMW DOC is typically fully modern (Fig. 6). While it might be tempting to conclude that more extensive diafiltration alone should be an easier way to remove all LMW DOC (and that all diafiltered UDOC samples would thus approach the same $\Delta^{14}C$ value), Fig. 6 clearly suggests this is not the case. If diafiltration did remove all LMW material, irrespective of CF, all diafiltered samples in Fig. 6 (each with different CFs) should have approximately the same $\Delta^{14}C$ content. However, they do not, and instead fall on a predictable linear regression with CF representing the principle driving variable. Overall, these results suggest that variable-CF UF experiments can be used as a new tool to target desired portions of the DOC pool, based on relative $\Delta^{14}C$ value and presumed reactivity, for isolation and study. By coupling molecular-level analyses with variable CF experiments, it is possible we can now gain insight into molecular-level variations within different DOC $^{14}C$-age classes.

The strong relationship between DOC and $\Delta^{14}C$ retention during UF also suggests that the chemical composition of a UDOC sample can be influenced by both CF and desalting. This observation may have important implications for assessing the overall "representativeness" of DOC that can be isolated by this method. UF typically isolates ~15% to 30% of the total DOC pool, and a key question has long been how "representative" such isolates are of the total dissolved material. Most studies have shown that despite relatively modest recoveries of TDOC, UDOC isolates are generally representative of total DOC in terms of their *bulk* composition (Benner et al., 1992, 1997; McCarthy et al., 1993). However, differences have also been reported in terms of both specific molecular-level composition (Skoog and Benner, 1997; Dittmar et al., 2001), and bulk $\Delta^{14}C$ signatures (Loh et al., 2004; McNichol and Aluwihare, 2007). Our results strongly suggest that CF is a central factor in the outcome of any such comparison, one that to our knowledge has not been explicitly considered.

We hypothesize that, as with $\Delta^{14}C$, the overall chemical composition of UDOC would be very similar to Total DOC at low CF, especially in the subsurface ocean. This view is supported by a growing body of data on both the major biochemical components of ocean DOC, and also how these vary in the surface vs. subsurface ocean. The $^{14}C$-depleted material in the subsurface ocean is dominated by aliphatic and carboxyl functions (Benner et al., 1992; McCarthy et al., 1993), now hypothesized to be predominantly composed of a family of carboxyl-rich alicyclic structures (CRAM; Hertkorn et al., 2006). In contrast, the $^{14}C$-modern "semi-labile" material added and remineralized in the upper ocean appears to be quantitatively dominated by HMW oligo- and polysaccharides (Benner et al., 1992; Pakulski and Benner, 1994; McCarthy et al., 1996; Aluwihare et al., 1997).

While clearly an important simplification, one can conceptualize major ocean DOC composition as a mixture of these two general components. This basic model is strongly supported by data from a new approach which allows nearly quantitative DOC recovery using electrodialysis/reverse osmosis (RO-ED; Koprivnjak et al., 2009). The overall solid-state NMR spectra of surface DOC isolated by RO-ED (representing up to ~75% of total DOC pool) are very similar to those for UDOC isolates; the only major difference being that additional carboxyl-rich alicyclic material (CRAM) is present in the RO-ED sample (Koprivnjak et al., 2009). Koprivnjak and coauthors averaged literature UDOC NMR spectra for this comparison, combining results from UDOC isolates with variable, but typically high, CF values. Based on our results, we hypothesize that a comparison of RO-ED material with low-CF UDOC should yield nearly identical NMR spectra, certainly for deep water. If proven, this would suggest that CRAM predominantly exists within the LMW DOC pool. In contrast, a comparison of RO-ED isolates with ultrahigh CF UDOC (>5000) would be hypothesized to show even greater compositional divergence. This thought experiment illustrates how variable CF could be used to target desired portions of the DOC pool for study: the most labile, polysaccharide-dominated HMW DOC fraction can be effectively isolated from most CRAM by using very high CFs and diafiltration in surface waters, while TDOC-representative samples of CRAM-enriched deep DOC can be isolated using low-CF experiments.

## 4. OVERVIEW AND IMPLICATIONS

Our results demonstrate that in UF isolations of oceanic DOC, CF can be used a proxy for MW distribution for a variety of experimental purposes. Even in large-volume experiments with extremely high CFs, oceanic DOC and its associated $\Delta^{14}C$ values still behave ideally in terms of theoretical UF permeation models. However, high CF isolations of oceanic DOC (including during diafiltration) also continued to result in the substantial permeation of LMW DOC – far beyond what might have been expected from lower CF ranges used in some prior studies – leading to large effects in DOC and $\Delta^{14}C$ recovery. As a consequence, changes in both TDOC and $\Delta^{14}C$ are closely linked, and can be explicitly predicted using UF permeation models. Finally, the $P_c$ values produced by these models also may provide a new approach for understanding DOC molecular size and $^{14}C$-age distributions in the ocean. Together these observations suggest that in practice the chemical and isotopic composition of a UDOC sample will strongly depend on the CF and diafiltration protocols used. The large range of $P_c$ values for seawater DOC suggests variability in MW distributions between ocean regions. This seemingly precludes the notion of an "optimal" CF, which can be universally applied for the complete removal of LMW DOC (e.g. Guo and Santschi, 1996 and Guo et al., 2000).

The strong relationship between DOC and $\Delta^{14}C$ permeation behavior with UF processing has implications for the study and interpretation of HMW DOC sources and reactivity in the global ocean. Without placing previously reported HMW DOC $\Delta^{14}C$ signatures into the context of CF, the large range in HMW $\Delta^{14}C$ values seemingly indicates large differences in surface HMW DOC reactivity, even in similar ocean regions. Our results suggest that this is not the case, instead suggesting that semi-labile DOC age and reactivity remain relatively constant in similar ocean regions. These results may also have implications for previously published compound-specific data. Published compound-specific $\Delta^{14}C$ results for oceanic DOC thus far have been derived mostly from high CF, diafiltered UDOC isolates, in some cases using extremely high CFs of ~10,000 (Aluwihare et al., 2002; Repeta and Aluwihare, 2006). Sugar monomers isolated from such UF samples have modern $^{14}C$-ages, however our results suggest these UF conditions should preferentially isolate only the most $^{14}C$-modern components. One possibility is that similar experiments conducted with low-CF UDOC (<100) would yield quite different results. However, this would not necessarily be the case, and should in fact depend on the relative distributions of hydrolyzable (and presumably more labile) biochemicals vs. their relative MW in the ocean's DOC pool. This is readily testable, and suggests that the use of variable-CF ultrafiltration, coupled with molecular-level analysis, can offer a new approach to testing fundamental

relationships among molecular size, $^{14}C$-age, composition and reactivity of oceanic DOC.

## ACKNOWLEDGMENTS

We acknowledge the Natural Energy Laboratory of Hawaii Authority (NELHA) and staff for providing facilities capable of large volume seawater DOM isolations. Jennifer Lehman and Leslie Roland (UC Santa Cruz) for help with sample collection and laboratory assistance. Rachel Porras (CSU Hayward/LLNL), Sheila Griffin and John Southon (UCI) for aid in $^{14}C$ sample preparation and analysis. We also acknowledge Dr. Carol Arnosti and three anonymous reviewers for their careful comments. This work was funded by the Campus Laboratory Collaboration (to M.D.M. and T.P.G.), NSF Chemical Oceanography program (OCE 0551940 to E.R.M.D.), and NSF Graduate Research Fellowship (to S.R.B.).

## REFERENCES

Aluwihare L. I., Repeta D. J. and Chen R. F. (1997) A major biopolymeric component to dissolved organic carbon in surface sea water. *Nature* **387**, 166–169.

Aluwihare L. I., Repeta D. J. and Chen R. F. (2002) Chemical composition and cycling of dissolved organic matter in the Mid-Atlantic Bight. *Deep-Sea Res. Part II* **49**, 4421–4437.

Amon R. M. W. and Benner R. (1994) Rapid-cycling of high-molecular-weight dissolved organic-matter in the ocean. *Nature* **369**, 549–552.

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

Bauer J. E., Williams P. M. and Druffel E. R. M. (1992) C-14 activity of Dissolved Organic-Carbon Fractions in the North-Central Pacific and Sargasso Sea. *Nature* **357**, 667–670.

Beaupre S. R., Druffel E. R. M. and Griffin S. (2007) A low-blank photochemical extraction system for concentration and isotopic analyses of marine dissolved organic carbon. *Limnol. Oceanogr. Methods* **5**, 174–184.

Benner R., Biddanda B., Black B. and McCarthy M. (1997) Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. *Mar. Chem.* **57**, 243–263.

Benner R., Pakulski J. D., McCarthy M., Hedges J. I. and Hatcher P. G. (1992) Bulk chemical characteristics of dissolved organic-matter in the ocean. *Science* **255**, 1561–1564.

Buesseler K. O., Bauer J. E., Chen R. F., Eglinton T. I., Gustafsson O., Landing W., Mopper K., Moran S. B., Santschi P. H., VernonClark R. and Wells M. L. (1996) An intercomparison of cross-flow filtration techniques used for sampling marine colloids: overview and organic carbon results. *Mar. Chem.* **55**, 1–31.

Buffle J., Perret D. and Newman M. (1992a) The use of filtration and ultrafiltration for size fractionation of aquatic particles, colloids and macromolecules. In *Environmental Particles* (eds. J. Buffle and H. P. v. Leeuwen). Lewis Publishers, Chelsea, MI, pp. 171–230.

Buffle J., Perret D. and Newman M. (1992b) The use of filtration and ultrafiltration for size fractionation of aquatic particles, colloids and macromolecules. In "*Environmental Particles,*" *IUPAC Series on Environmental Analytical and Physical Chemistry* (eds. J. Buffle and H. P. v. Leeuwen). Lewis Publishers, Chelsea, MI, pp. 171–230.

Chin W. C., Orellana M. V. and Verdugo P. (1998) Spontaneous assembly of marine dissolved organic matter into polymer gels. *Nature* **391**, 568–572.

Dai M. H., Buesseler K. O., Ripple P., Andrews J., Belastock R. A., Gustafsson O. and Moran S. B. (1998) Evaluation of two cross-flow ultrafiltration membranes for isolating marine organic colloids. *Mar. Chem.* **62**, 117–136.

Dittmar T., Fitznar H. P. and Kattner G. (2001) Origin and biogeochemical cycling of organic nitrogen in the eastern Arctic Ocean as evident from D- and L-amino acids. *Geochim. Cosmochim. Acta* **65**, 4103–4114.

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

Guo L. D. and Santschi P. H. (1996) A critical evaluation of the cross-flow ultrafiltration technique for sampling colloidal organic carbon in seawater. *Mar. Chem.* **55**, 113–127.

Guo L. D., Wen L. S., Tang D. G. and Santschi P. H. (2000) Re-examination of cross-flow ultrafiltration for sampling aquatic colloids: evidence from molecular probes. *Mar. Chem.* **69**, 75–90.

Gustafsson O., Buesseler K. O. and Gschwend P. M. (1996) On the integrity of cross-flow filtration for collecting marine organic colloids. *Mar. Chem.* **55**, 93–111.

Hansell D. A., Carlson C. A., Repeta D. J. and Schlitzer R. (2009) Dissolved organic matter in the ocean a controversy stimulates new insights. *Oceanography* **22**, 202–211.

Hansell D. A., Carlson C. A. and Suzuki Y. (2002) Dissolved organic carbon export with North Pacific Intermediate Water formation. *Global Biogeochem. Cycles* **16**.

Hansman R. L., Griffin S., Watson J. T., Druffel E. R. M., Ingalls A. E., Pearson A. and Aluwihare L. I. (2009) The radiocarbon signature of microorganisms in the mesopelagic ocean. *Proc. Natl. Acad. Sci. USA* **106**, 6513–6518.

Hedges J. I. (1992) Global biogeochemical cycles – progress and problems. *Mar. Chem.* **39**, 67–93.

Hertkorn N., Benner R., Frommberger M., Schmitt-Kopplin P., Witt M., Kaiser K., Kettrup A. and Hedges J. I. (2006) Characterization of a major refractory component of marine dissolved organic matter. *Geochim. Cosmochim. Acta* **70**, 2990–3010.

Ingalls A. E., Shah S. R., Hansman R. L., Aluwihare L. I., Santos G. M., Druffel E. R. M. and Pearson A. (2006) Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. *Proc. Natl. Acad. Sci. USA* **103**, 6442–6447.

Kilduff J. and Weber W. J. (1992) Transport and separation of organic macromolecules in ultrafiltration processes. *Environ. Sci. Technol.* **26**, 569–577.

Koprivnjak J. F., Pfromm P. H., Ingall E., Vetter T. A., Schmitt-Kopplin P., Hertkorn N., Frommberger M., Knicker H. and Perdue E. M. (2009) Chemical and spectroscopic characterization of marine dissolved organic matter isolated using coupled reverse osmosis–electrodialysis. *Geochim. Cosmochim. Acta* **73**, 4215–4231.

Logan B. E. and Qing J. (1990) Molecular-size distributions of dissolved organic-matter. *J. Environ. Eng. ASCE* **116**, 1046–1062.

Loh A. N., Bauer J. E. and Canuel E. A. (2006) Dissolved and particulate organic matter source-age characterization in the upper and lower Chesapeake Bay: a combined isotope and biochemical approach. *Limnol. Oceanogr.* **51**, 1421–1431.

Loh A. N., Bauer J. E. and Druffel E. R. M. (2004) Variable ageing and storage of dissolved organic components in the open ocean. *Nature* **430**, 877–881.

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

McCarthy M., Pratum T., Hedges J. and Benner R. (1997) Chemical composition of dissolved organic nitrogen in the ocean. *Nature* **390**, 150–154.

McCarthy M. D., Hedges J. I. and Benner R. (1993) The chemical-composition of dissolved organic-matter in seawater. *Chem. Geol.* **107**, 503–507.

McNichol A. P. and Aluwihare L. I. (2007) The power of radiocarbon in biogeochemical studies of the marine carbon cycle: Insights from studies of dissolved and particulate organic carbon (DOC and POC). *Chem. Rev.* **107**, 443–466.

Pakulski J. D. and Benner R. (1994) Abundance and distribution of carbohydrates in the ocean. *Limnol. Oceanogr.* **39**, 930–940.

Repeta D. J. and Aluwihare L. I. (2006) Radiocarbon analysis of neutral sugars in high-molecular-weight dissolved organic carbon: implications for organic carbon cycling. *Limnol. Oceanogr.* **51**, 1045–1053.

Repeta D. J., Quan T. M., Aluwihare L. I. and Accardi A. M. (2002) Chemical characterization of high molecular weight dissolved organic matter in fresh and marine waters. *Geochim. Cosmochim. Acta* **66**, 955–962.

Roland L. A., McCarthy M. D., Peterson T. D. and Walker B. D. (2009) A large-volume micro-filtration system for isolating suspended particulate organic matter: fabrication and assessment vs. GFF filters in central N. Pacific. *Limnol. Oceanogr. Methods* **7**.

Santos G. M., Southon J. R., Griffin S., Beaupre S. R. and Druffel E. R. M. (2007) Ultra small-mass AMS C-14 sample preparation and analyses at KCCAMS/UCI Facility. *Nucl. Instrum. Methods Phys. Res. Sect. B* **259**, 293–302.

Santschi P. H., Guo L. D., Baskaran M., Trumbore S., Southon J., Bianchi T. S., Honeyman B. and Cifuentes L. (1995) Isotopic evidence for the contemporary origin of high-molecular-weight organic-matter in oceanic environments. *Geochim. Cosmochim. Acta* **59**, 625–631.

Sharp J. H. (1973) Size classes of organic carbon in seawater. *Limnol. Oceanogr.* **18**, 441–447.

Skoog A. and Benner R. (1997) Aldoses in various size fractions of marine organic matter: Implications for carbon cycling. *Limnol. Oceanogr.* **42**, 1803–1813.

Stuiver M. and Polach H. A. (1977) Discussion: Reporting of $^{14}C$ data. *Radiocarbon* **19**, 355–363.

Vogel J. S., Southon J. R. and Nelson D. E. (1987) Catalyst and binder effects in the use of filamentous graphite for AMS. *Nucl. Instrum. Methods Phys. Res. Sect. B* **29**, 50–56.

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

*Associate editor:* Carol Arnosti