

## The use of wet chemical oxidation with high-amplification isotope ratio mass spectrometry (WCO-IRMS) to measure stable isotope values of dissolved organic carbon in seawater

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### Abstract

Few measurements of the carbon stable isotope value ( $\delta^{13}\text{C}$ ) of marine dissolved organic carbon (DOC), the largest pool of reduced carbon in the ocean, have been made because of analytical obstacles due to the interference of halides and the low amount of DOC in seawater. By using concentrated persulfate in a wet chemical oxidation organic carbon analyzer coupled to an isotope ratio mass spectrometry (WCO-IRMS) the analytical obstacles are overcome. Key to this method is reducing the persulfate blank and increasing the IRMS signal with larger amplifier gain resistors. After these simple modifications, a 2 mL sample provides enough signal to make precise measurements of DOC concentration and  $\delta^{13}\text{C}$  value on up to 15 samples per day. Sodium persulfate ( $1.68 \text{ mol L}^{-1}$ ) is cleaned by pre-heating and sparging with ultrahigh purity helium. In the WCO analyzer, 6 mL cleaned persulfate is added to 2 mL sample at  $98^\circ\text{C}$  for 8.5 min to completely oxidize DOC to  $\text{CO}_2$ . After quantitative measurement by nondispersive IR, the gases contained in the exhaust are swept through a cleanup reactor, separated by a GC column and introduced to the IRMS for  $\delta^{13}\text{C}$  measurement. Complete recovery of the DOC and  $\delta^{13}\text{C}$  values was confirmed with two DOC standards added individually to seawater. IRMS precision was confirmed by measuring a range of sea water samples. On several coastal water samples measured using this system,  $\delta^{13}\text{C}$ -DOC values ranging from  $-22\text{‰}$  to  $-25\text{‰}$ . These results were consistent with published reports of seawater  $\delta^{13}\text{C}$ -DOC using other methods.

Marine dissolved organic carbon (DOC) is the largest pool of reduced carbon in seawater and is a C reservoir nearly equivalent in size to atmospheric  $\text{CO}_2$  (Siegenthaler and Sarmiento 1993), making it the largest exchangeable organic C reservoir on Earth (Druffel and Williams 1992). Despite its global biogeochemical importance, little is known about the molecular characterization of marine DOC, especially its isotopic composition. Carbon stable isotope measurements of DOC ( $\delta^{13}\text{C}$ -DOC) provide information on DOC source and

reactivity that complements information both from source-specific molecular biomarkers (e.g., lipids, lignin; Bauer 2002) and other bulk molecular measurements (e.g., C/N ratios, NMR; Benner et al. 1992). However, a paucity of  $\delta^{13}\text{C}$ -DOC data exists for seawater because of analytical difficulties using traditional methods of DOC oxidation due to the presence of halides, low DOC concentration, high analytical blanks, and procedures that reduce sample throughput. Thus,  $\delta^{13}\text{C}$ -DOC analyses were not suitable for ocean monitoring operations where tens to hundreds of samples are collected, and the existing data are limited to only a few studies. Here we report simple modifications to existing technology that overcome the analytical difficulties and provide rapid (20 min) analyses of both DOC concentration and stable isotope value—thus enabling high throughput (up to 15 samples per day).

Early measurements of marine DOC concentration used wet chemical oxidation (WCO) techniques (e.g., Menzel and Vaccaro 1964), with or without UV irradiation to completely oxidize DOC to  $\text{CO}_2$ . Early DOC measurements were later found to be inaccurate after the advent of high temperature catalytic oxidation (HTCO) techniques (Hedges 2002). While

### Acknowledgments

Special thanks to Dr. Alex Sessions for reviews and discussions that vastly improved this manuscript. Also, we thank Tom Boyd and Rebecca Plummer at NRL and James McKenna for technical discussions; Sean Fitzgerald, Tina Bell, and Cynthia Gonzales at OI Analytical for technical support and service; and Paul Middlestead from the U of Ottawa's G.G. Hatch Stable Isotope Laboratory. Warwick F. Vincent (Laval University, Canada) and his research group for supplied samples from the Beaufort Sea within the Canadian Arctic Shelf Exchange Study (CASES), funded by the Natural Sciences and Engineering Research Council of Canada. John Pohlman and an anonymous reviewer provided useful comments. This work was funded in part by ONR Work Unit N0001403WX20946. Mention of trademarks and product names does not imply endorsement by the US Navy.

analytical blanks were problematic in all methods of DOC oxidation and measurement (Hedges 2002), the issue was resolved when these blanks were accounted for, and has resulted in good agreement between many different DOC analysis methods, reviewed elsewhere (Aiken et al. 2002; Sharp et al. 2002).

Most methods for seawater  $\delta^{13}\text{C}$ -DOC measurement techniques based on DOC oxidation suffer from low sample throughput, long analysis times, and high blanks, similar to measurement of DOC concentration (Bauer 2002b). Fry et al. (1996) improved the sample throughput by lyophilizing successive aliquots of seawater, but the dried salt/organic crust that they measured still had high blanks. The removal of salts by ultrafiltration, or the extraction of humic substances, provided information only on fractions of DOC and thus lacked valuable information on bulk DOC. None of these techniques were amenable to continuous-flow isotope ratio mass spectrometry (IRMS) that would allow multiple  $\delta^{13}\text{C}$ -DOC measurements to be made immediately after sample oxidation.

The first continuous-flow techniques for  $\delta^{13}\text{C}$ -DOC measurement were developed for freshwaters and interfaced an elemental analyzer (EA-IRMS; Ghandi et al. 2004) or WCO analyzers (WCO-IRMS; St-Jean 2003; Bouillon et al. 2006) to an IRMS. However, the EA-IRMS technique would probably require multiple microliter aliquots of seawater to be evaporated or freeze-dried, potentially leading to large analytical blanks (cf. Fry et al. 1996). The WCO TOC analyzers (e.g., OI Instruments Model 1010 TOC) are better suited for continuous flow application because UHP He can be used as the carrier gas rather than UHP  $\text{O}_2$ , which is used for the HTOC TOC analyzers. The latter gas will destroy the filament in an IRMS.

Definitive source information is required to resolve the biogeochemical properties and reactivities of DOC in seawater, especially on continental margins near the coasts. Evidence suggests that these margins are large sources of DOC to the interior ocean (Bauer and Druffel 1998). Continental margins are also sites of multiple DOC transformations (e.g., microbial and photochemical degradation; Bauer et al. 2002). Obtaining specific information about rates of DOC flux and transformation processes is, therefore, important, and  $\delta^{13}\text{C}$ -DOC values can provide some of this information.

### Materials and procedures

**Sample collection and processing**—Seawater samples from the continental shelf and margin of the Middle Atlantic Bight (MAB) and from the Beaufort Sea were collected from Niskin bottles and filtered through 0.2  $\mu\text{m}$  into detergent-washed and triple-rinsed polycarbonate bottles in the field. Upon return to the laboratory, 35 mL sample was transferred to pre-cleaned 40 mL borosilicate vials (detergent-washed and profusely rinsed with MilliQ water, then baked at 500°C for 6 h minimum) with Teflon-lined closures. A few drops of HPLC grade 85%  $\text{H}_3\text{PO}_4$  (Fisher Scientific Co.) were used to acidify samples to a pH of  $\sim 3$ . Seawater samples were also collected from the

Atchafalaya River discharge into the Gulf of Mexico. For the Gulf of Mexico samples, 35 mL was collected directly into pre-cleaned 40 mL borosilicate vials, preserved with acid, then kept in the dark at 4°C.

Samples were then stored in the dark at 4°C until analysis time, at which point they were warmed to room temperature before analysis. Multiple DOC analyses of MilliQ field and laboratory blanks confirm that these cleaning and handling procedures do not produce measurable DOC contamination.

**Reagent and standard preparation**—For the wet chemical oxidation of DOC on the 1010 TOC instrument, 5%  $\text{H}_3\text{PO}_4$  (v/v; Fisher HPLC grade) and 1.69 M  $\text{Na}_2\text{S}_2\text{O}_8$  (Sigma-Aldrich) solutions were prepared in 18 M $\Omega$  MilliQ water. The persulfate must be cleaned prior to use (McKenna and Doering 1995). Once completely dissolved, the persulfate solution was placed in a 1200 W microwave oven and heated for 5 min or until the solution began boiling vigorously. It was then immediately removed to an ice bath and cooled to room temperature. Finally, the persulfate was sparged for at least 2 h with UHP He to remove any  $\text{CO}_2$  formed during the cleaning procedure.

Solutions of potassium hydrogen biphthalate (KHP, NIST-84K) were used as the DOC standards for calibration of the WCO analyzer. Calibration curves using a DOC concentration range of 42 to 833  $\mu\text{mol C L}^{-1}$  were performed when check standards deviated by greater than 5%. Otherwise, DOC concentrations were corrected post-analysis by linear scaling to check standards.

**Stable isotope data processing**—The standard convention for expressing stable isotope values obtained from an IRMS is the “delta” notation, where the ratio ( $R$ ) of  $^{13}\text{C}$  to  $^{12}\text{C}$  is expressed relative to a known standard (presently, one calibrated against the Pee Dee Belemnite [PDB] standard):

$$\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad (1)$$

The values are in the parts per thousand range and are expressed as ‘per mil’ (‰). Raw measurements from our Delta-Plus XP IRMS are normalized to values for the KHP standard ( $\delta^{13}\text{C} = -24.5 \pm 0.2\text{‰}$  [ $n = 3$ ], measured by EA-IRMS), which has been extensively calibrated against the known isotopic standards, IAEA-8 oxalic acid ( $\delta^{13}\text{C} = -18.3\text{‰}$ ) and USGS-40 L-glutamic acid ( $\delta^{13}\text{C} = -26.2\text{‰}$ ). The precision of  $\delta^{13}\text{C}$  measurements is limited to tenths per mil.  $\delta^{13}\text{C}$ -DOC normalization was made by analyzing several of the IAEA and USGS carbon isotope standards during each group of analyses. Typically, we analyzed solutions of glutamic acid and oxalic acid to bracket the expected range of  $\delta^{13}\text{C}$  values for seawater DOC. Post-analysis processing consisted of linearly scaling samples to standards to correct for instrument drift (typically less than 1‰).

The  $\delta^{13}\text{C}$  value of KHP solutions in 18 M $\Omega$  MilliQ water (over a concentration range of 83 to 833  $\mu\text{M C}$ ) produced a  $\delta^{13}\text{C}$  of  $-24.8 \pm 0.3\text{‰}$  ( $n = 14$ ). Thus, KHP is a suitable working standard for DOC stable isotope value and concentration. However, one drawback to wet chemical oxidation in the presence of chloride is possible inefficiency of oxidation, especially of com-

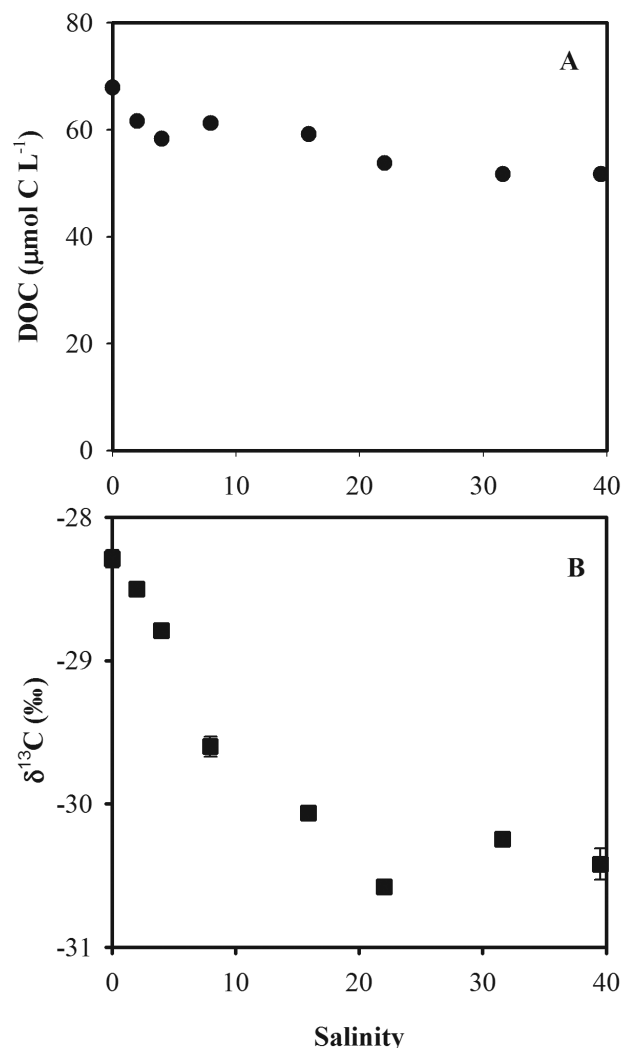
plex carbon compounds such as humic acids (Peyton 1993; Aiken 1992). Thus, Suwannee River humic acid (SRHA), available from the International Humic Substances Society (reported  $\delta^{13}\text{C}$  value =  $-27.7\text{‰}$ ), was also used to assess recovery of complex carbon in seawater. The SRHA solutions made with 18 M $\Omega$  Milli-Q water used in this study had a  $\delta^{13}\text{C}$  value of  $-27.9 \pm 0.3\text{‰}$  ( $n = 8$ ).

### Overview of persulfate-based TOC analysis

The use of photolysis or thermolysis of persulfate to generate the sulfate radical anion oxidant is the basis behind WCO. The measurement of DOC concentration in seawater is analytically challenging because of competition for sulfate radical anion between DOC and chloride (and bromide) ion. Aiken (1992), using an early generation WCO instrument (OI Analytical TOC 700), discussed problems of WCO measurement of  $417 \mu\text{mol C L}^{-1}$  concentrations of DOC from moderately salty samples (salinity  $\sim 15$  ppt) and offered suggestions for overcoming those problems. The addition of extra persulfate and extension of the reaction time provided quantitative recoveries. McKenna and Doering (1995)—also using an OI Analytical TOC 700—demonstrated quantitative recovery of DOC in Narragansett Bay by adding increasing amounts of  $0.84 \text{ mol L}^{-1} \text{ Na}_2\text{S}_2\text{O}_8$  until asymptotic recovery was achieved. They determined that a persulfate to chloride molar ratio of 8.75 would provide enough oxidant to completely oxidize DOC to  $\text{CO}_2$  in the presence of halides. A later generation OI Analytical TOC analyzer, the TOC 1010, was used in this study.

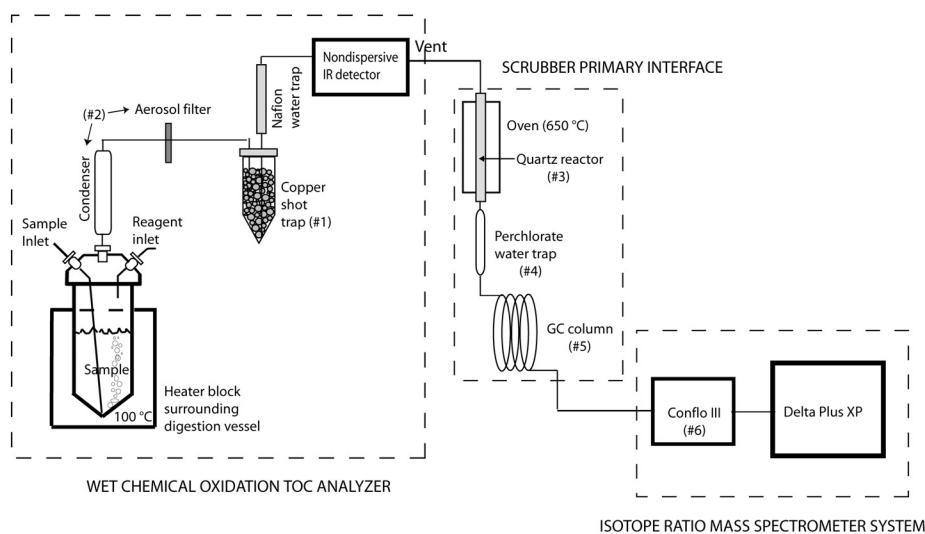
Any oxidizable species (e.g., transition metals with higher oxidation states, organics, bicarbonate, halides, and persulfate, itself) will compete for sulfate radical anion (House 1964) thus the radicals are quickly consumed. Halides are in such greater abundance in seawater than organics that the halide presence greatly reduces the chance that sulfate radical anion will react with carbon atoms. Rates of reaction are vastly greater for chloride and bromide ( $2 \times 10^8$  to  $3.5 \times 10^9$ , respectively) than for organics (estimated at  $1 \times 10^4$ ; Peyton 1993). Organohalides, such as trichloroacetic acid, were measured in the effluent and the gas stream of an OI TOC 700 by Aiken (1992), suggesting that these intermediates will also consume sulfate radical anion. However, Peyton (1993) concludes that for the aqueous phase, organohalides should not be important due to the overwhelming presence of chloride versus diatomic chlorine (which causes halogenation of organics). In seawater, the chlorine:DOC ratio is greater than 20,000:1. Because the halogenation of organics occurs in nonpolar solvents and in the gaseous phase, it is possible that Aiken (1992) was sampling gas phase condensate combined with the aqueous phase in the effluent. The reader is directed to Peyton (1993) and Goulden and Anthony (1978) for more information regarding the chemistry of persulfate oxidation of organic matter in natural waters.

*Overview of combined WCO-IRMS system*—St-Jean (2003) describes coupling the WCO analyzer in continuous flow with



**Fig. 1.** Effect of chloride on recovery of DOC concentration and  $\delta^{13}\text{C}$  value in 10 mL samples of NaCl solution spiked with SRHA to  $68 \mu\text{mol C L}^{-1}$  final concentration. Salinity was estimated from chloride concentrations.

an IRMS. The WCO analyzer used in this study (OI Analytical 1010 TOC analyzer) operates in three modes for both DIC and DOC analysis: Inject, React, and Detect. The Inject mode introduces the sample via either sample loops or septum syringe injection into the digestion vessel (Fig. 2). In DIC React mode, a metered amount of 5%  $\text{H}_3\text{PO}_4$  is pumped into the digestion vessel and allowed to react for a preset time. In DIC Detect mode, the digestion vessel is placed in-line with a nondispersive infrared detector (NDIR) and UHP He sparges  $\text{CO}_2$  and volatile organics, from the sample. In DOC React mode, a metered amount of  $\text{Na}_2\text{S}_2\text{O}_8$  is pumped into the digestion vessel, and the sample is heated to  $98^\circ\text{C}$  for a preset time. This step converts organic carbon to  $\text{CO}_2$ , which is then sparged from the sample during DOC Detect and measured by the NDIR. The 1010 TOC is controlled by WinTOC software provided by the manufacturer. WinTOC has a remote start



**Fig. 2.** Schematic diagram of WCO-IRMS system modified to analyze seawater samples. See text for description of numbered references.

option which allows a start pulse to be sent by the IRMS software (ISODAT NT) eliminating computer synchronization problems.

*The interference of chloride on DOC concentration and  $\delta^{13}\text{C}$  measurements*—The WCO reaction conditions for oxidation of DOC in seawater initially were set to comply with OI technical note #03520497, but later had to be modified. The DOC React time was set to 8.5 min, and the persulfate concentration was increased to  $0.84 \text{ mol L}^{-1}$ . The technical note was written for a 1 mL sample size, but we found that 10 mL of sample at DOC concentrations of seawater (50 to  $100 \mu\text{mol C L}^{-1}$ ) was necessary to get a measurable signal on the IRMS (without higher amplifier gain).

To investigate the chloride interference effect on  $\delta^{13}\text{C}$ -DOC values in seawater measured by WCO-IRMS, we analyzed several samples of a  $68 \mu\text{mol C L}^{-1}$  solution of SRHA in MilliQ water, to which increasing amounts of NaCl were added to approach seawater concentration. The NaCl was precombusted at  $450^\circ\text{C}$  for 6 h, yet we still measured  $13 \mu\text{mol C L}^{-1}$  when NaCl alone was added to Milli-Q water.

Both DOC concentration and  $\delta^{13}\text{C}$  value of SRHA solutions changed dramatically with increasing salinity, estimated from chloride concentration. The chloride effect is noticeable at an equivalent salinity of 2 ppt (Fig. 1A). DOC concentration decreased with salinity and became asymptotic at salinity  $\sim 22$  ppt. The effect on DOC concentration was similar to that reported by Aiken (1992) and indicated incomplete DOC oxidation. The  $\delta^{13}\text{C}$  value also decreased with salinity and became asymptotic at salinity  $> 22$  ppt (Fig. 1B). The results suggest either that the more easily oxidized fractions of SRHA are isotopically depleted relative to the bulk material, or that some isotopic fractionation in the DOC occurred.

It is clear that these operational parameters did not overcome the analytical problems of chloride interference, which put several constraints on WCO-IRMS analysis of 10 mL sam-

ples of seawater. First, 4.91 moles of persulfate would be required for complete DOC oxidation of seawater (at salinity = 36 ppt), based on the 8.75 molar ratio of persulfate to chloride (McKenna and Doering 1995). Second, we observed that persulfate solutions became supersaturated at concentrations  $> 2.00 \text{ mol L}^{-1}$ , causing re-crystallization, so we decided on a maximum concentration of  $1.69 \text{ mol Na}_2\text{S}_2\text{O}_8 \text{ L}^{-1}$ , double the concentration used previously. However, 29 mL of the  $1.69 \text{ mol Na}_2\text{S}_2\text{O}_8 \text{ L}^{-1}$  were required to deliver 4.91 moles of persulfate, but only 8 mL of persulfate can be added to the digestion vessel on the 1010 TOC by its control software. Moreover, the total volume of sample plus persulfate (39 mL) is greater than the 30 mL capacity of the digestion vessel. Fourth, the expected blanks associated with this amount of persulfate likely would overwhelm the  $\text{CO}_2$  signal from DOC. Fifth, the halide gases and aerosols generated by WCO from 10 mL seawater would quickly corrode the stainless steel components of the IRMS, foul the NDIR window on the TOC over time, and consume copper shot and the reagents in the Scrubber. Therefore, we had to reduce the required amount persulfate while still oxidizing enough DOC to  $\text{CO}_2$  in the presence of chloride for  $\delta^{13}\text{C}$ -DOC measurement. A few options were considered.

*Complex the chloride or reduce its concentration*—Bauer et al. (1991) used Hg salts to complex halides in a UV-persulfate reaction system. Mercury is highly toxic and the vapors generated by its heated persulfate oxidation would need to be removed from the 1010 TOC. Silver ion (or other transition metal ions) is another acceptable complexing agent. We added silver ion as  $\text{AgNO}_3$  to seawater, but achieved poor recovery of DOC (ca. 60%) and  $\delta^{13}\text{C}$  values that were unexpectedly depleted in  $^{13}\text{C}$  for seawater ( $-27\text{‰}$ ). The excess of  $\text{AgNO}_3$  required to complex a sufficient amount of chloride (roughly  $0.5 \text{ mol L}^{-1}$ ;  $\text{AgNO}_3$  reaches saturation at  $11.5 \text{ mol L}^{-1}$ ) may have resulted in trace organic contamination, as well as sample dilution. Moreover, the  $\text{AgCl}$  precipitate that forms

clogged the flow path at the digestion vessel on the 1010 TOC Analyser, requiring further filtration and centrifugation to remove.

A  $\text{Fe}^{2+}/\text{Fe}^{3+}$  redox couple, mediated with sodium thiosulfate, has been used to completely oxidize organohalogenes in natural waters (Liang et al. 2004). We tried this procedure and found it very cumbersome from the addition of more reagents, hence more sample dilution and an increase in the system blanks. While this latter technique worked well with organohalogenes for Liang et al. (2004), it did not appear to increase oxidation efficiency of halides in natural waters in our experiments, thus it was discarded.

*Increase the signal by trapping multiple small aliquots of  $\text{CO}_2$* —Successive WCO analyzer runs could be done so that the  $\text{CO}_2$  in the effluent UHP He stream from each run was trapped cryogenically or on a molecular sieve (Bauer et al. 1991, 1992; Bouillon et al. 2006). However, as Bauer et al. (1991) demonstrated, reagent blanks in the system are additive with trapping time (see their Fig. 2). Additional modifications would be required to vent  $\text{CO}_2$  generated from DIC reaction prior to DOC reaction. Moreover, even UHP He can have small traces of  $\text{CO}_2$ , which would accumulate with successive pulses of  $\text{CO}_2$  and trapping time. We did not explore this option, because we were concerned about the increase in system blank with trapping time.

*Increase the IRMS sensitivity and reduce sample size*—By increasing the gain amplification of the IRMS, the sample size can be reduced to the minimum volume required to produce enough  $\text{CO}_2$  from WCO of DOC, so that an accurate and precise isotopic measurement can be made. That is, the signal must be amplified into the normal range at which the IRMS software is capable of accurate integration. At a small sample size (e.g., 2 mL), the  $\text{Cl}^-$  effect can be overcome with a reasonable amount of cleaned persulfate. Given the volume constraints of the TOC Analyzer vessel described above, we determined that 2 mL sample sizes of seawater at salinity = 36 ppt were practical. With 2 mL sample sizes, only 6.05 mL of 1.6 mol  $\text{L}^{-1}$   $\text{Na}_2\text{S}_2\text{O}_8$  would be required to oxidize all species and the total volume of solution in the TOC Analyzer digestion vessel was manageable. These constraints were well within the capabilities of the TOC analyzer and the IRMS, so we chose this option.

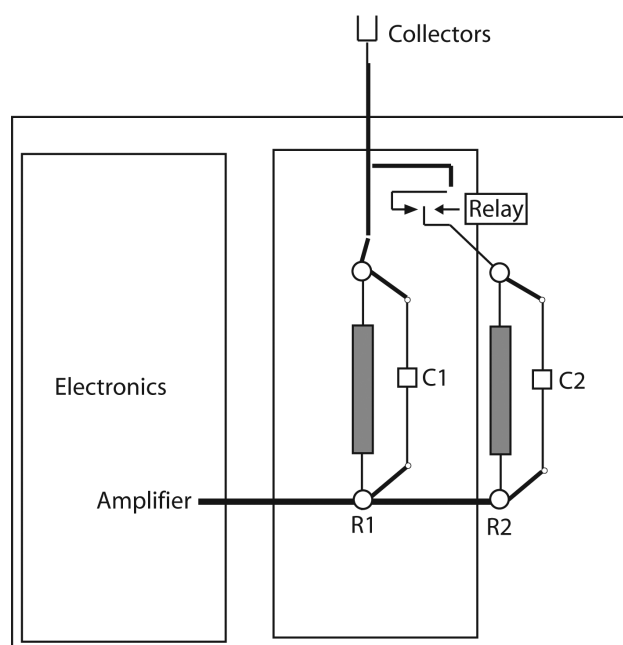
*Modifications to the WCO-IRMS system*—Three simple modifications were performed. First, we added a copper shot trap (#1 in Fig. 2), placed in the flow path between the Digestion vessel of the TOC Analyzer and its NDIR detector, as suggested by the manufacturer. Gases generated by the WCO reaction are sparged from the digestion vessel through a Condenser and Aerosol filter to the copper shot trap (#2 in Fig. 2). The copper shot (Fisher Scientific) adsorbs  $\text{Cl}_2$  gas and chloride in aerosols as reddish-brown copper chloride and green to black copper oxide (or copper sulfate) over time, at which point the spent Cu shot must be replaced. Further, salt and moisture tend to deposit at the bottom of the copper shot trap, likely

due to condensation of aerosols. Salt build-up can restrict flow over time, which was observed as an increase in system pressure monitored in the WinTOC software. To alleviate the aerosol problems, we placed copper wool in the glass condenser column attached to the digestion vessel in the 1010 TOC (Fig. 2). We also placed a 0.2  $\mu\text{m}$  Teflon in line filter (Gelman) to act as an additional trap for aerosols and to extend the lifetime of the copper shot trap.

Second, we packed the quartz reduction reactor column in the Scrubber primary interface (#3 in Fig. 2) with a layer of silvered cobaltous-cobaltic oxide (Costech Analytical) separated from a layer of reduced copper turnings by quartz wool. The  $\text{CO}_2$  generated by WCO and tapped at its Vent is swept in the UHP He stream through the quartz reactor column at 650°C, where  $\text{N}_x\text{O}_x$  are reduced to  $\text{N}_2$  and excess halogens are trapped. The gases then travel through a final water trap (packed with a magnesium perchlorate - quartz chip mix, #4 in Fig. 2) where  $\text{CO}_2$  is separated from  $\text{N}_2$  on a packed Poraplot column (#5 in Fig. 2). The Scrubber is the first of two devices that connects the WCO analyzer to the IRMS in continuous flow operation (#3 in Fig. 2). The second device is the ConFlo III (Thermo Electron Corp.; #6 in Fig. 2), which is an open split inlet interface to the IRMS and has separate capillaries for receiving sample gas and separately introducing reference gas ( $\text{CO}_2$  in this application) to the IRMS. The  $\text{CO}_2$  is ionized at the IRMS source and its isotopic measurement is made relative to several reference  $\text{CO}_2$  pulses. The WCO-IRMS set-up, including the Scrubber, is similar to EA-IRMS that also uses the ConFlo III inlet device. The UHP He flow rate from the 1010 TOC through both devices to the IRMS was set  $125 \pm 5$  mL/min to comply with the requirements of the ConFlo III.

Third, we increased the sensitivity of the IRMS by increasing the gain amplification. Modern IRMS instruments often allow users to switch to lower amplification for isotope-labeling work by changing the standard resistors to low-ohmic resistors. We used this feature to achieve higher amplification by installing higher gain resistors. Installation of the high-ohmic resistors to secondary channels on the amplifier board of the IRMS (Fig. 3) was quick (less than 1 h) and straightforward. Because the system did not need to be vented entirely, this modification allowed standards to be analyzed within the same day and samples to be run next day.

Typically, older, less sensitive dual-inlet IRMS instruments from the 1980s used higher amplification for  $\text{SO}_2$  analysis with good precision, thus this type of modification is not new. Modern IRMS instruments such as the Delta<sup>plus</sup> XP used in this study have a very high signal-to-noise ratio due to advances in electronics and the use of digital signal transfer. This instrument has the extra advantage that it can have two gain resistors in parallel on each collector amplifier board (Fig. 3) thus allowing on-time computer controlled switching between standard and high amplification arrangement. Without this option, the resistors would have to be changed manually. The rating of the resistors was entered in the IRMS control software



**Fig. 3.** A schematic of the amplifier board on the Thermo Finnigan DeltaPlus XP. The gain resistors (R1 & R2) are in parallel along with their matching capacitors (C1 & C2). The relay (RELAY) allows R1, the larger of the two gain resistors, to be alone or in parallel with R2. The value of R2 follows the law of parallel resistors where  $1/R_{\text{total}} = 1/R_1 + 1/R_2$ , and the capacitors in parallel follow  $C_{\text{total}} = C_1 + C_2$ .

in which the isotopic calculation routines adjust for the new resistor configuration.

### Assessment

*Effects of high amplification on IRMS baseline and signal*—The increase in amplification using larger high-ohmic gain resistors was the key to solving the sample size problem. This is because the  $\text{CO}_2$  signal for 2 mL sample size under standard amplification was too small for highly precise integration by the IRMS data system. Although larger resistors do not automatically translate into unusable baselines, it was still important to show that the increased baseline amplification would not be an issue for the precision needed. The larger resistors used here, for masses 44 and 45, are not unusual because they are often the default resistors on other manufacturers' instruments for  $\text{CO}_2$  analysis. The high amplification configuration resulted in roughly a 4-fold gain in signal for the  $\text{CO}_2$  masses 44 and 45 (Table 1), the ratio of which is used to calculate the  $\delta^{13}\text{C}$  value. The mass 46 amplifier gain is unmodified and the data from it is still used for interfering ion correction ( $^{17}\text{O}$ ; Santrock et al. 1985).

The integration problem was likely due to the resolution of the analog-to-digital conversion. The voltage at mass 44 in the standard IRMS configuration is calculated with a gain resistor rated at  $3.0 \times 10^8$  ohms where  $1 \text{ V} \approx 3.33 \text{ nA}$ . With the higher ohmic resistor ( $1.0 \times 10^9$  ohms) installed, the voltage at mass

44 is calculated where  $1 \text{ V} \approx 1.0 \text{ nA}$  (Table 1). The real signal though is a current (I) measured in amperes (A) but is converted to a voltage (V) through the amplifiers following Ohm's law (i.e.,  $V = R * I$ , where R is the resistance measured in ohms). The digitalization of the voltage is done by voltage to frequency converters (VFC), where the analog voltages are converted to proportional frequencies (cycles/sec). The precision of the frequency counting (used to calculate the peak area for the isotope ratio) is limited to five decimal places. Because we measure parts per million variations of the  $^{13}\text{C}$  isotope on natural abundance samples, the higher voltages result in > 5-6 digits counted for the area calculation and therefore a higher precision.

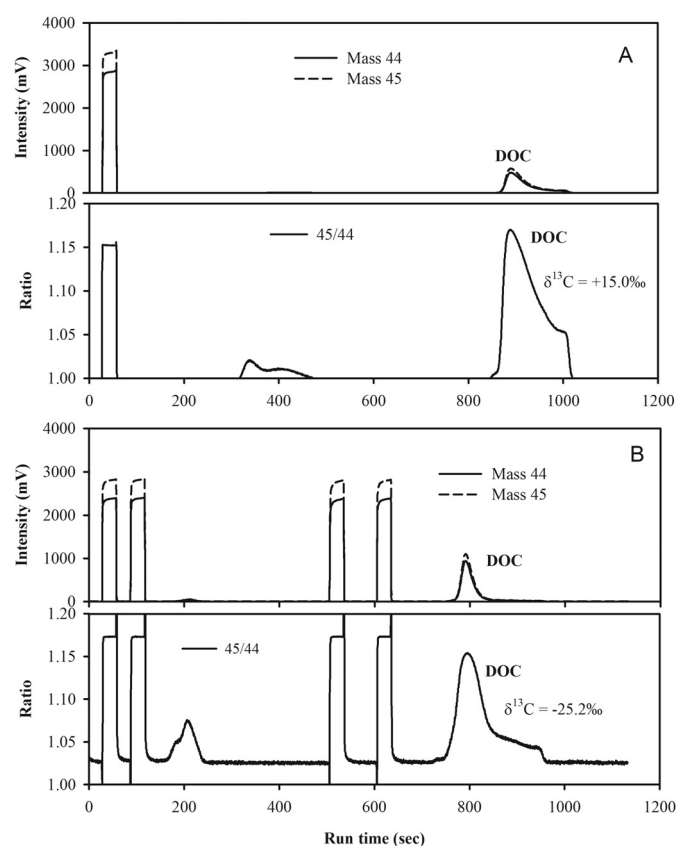
The background signal intensity at mass 44, representative of the instrument baseline under the standard amplification, was 2 mV, whereas in high amplification the baseline signal intensity at mass 44 was 8 mV. Note that these baseline values were recorded when the WCO analyzer was in Detect mode where the TOC Analyzer effluent was flushing through the reactor to the IRMS.

This baseline noise, even with the high ohmic resistors, is still 20 to 50 times lower relative to the previous generation of IRMS instruments, which had noise background intensities at about one hundred mV. The reason for better amplifier signal-to-noise is primarily due to advances in electronics. The collector amplifiers on the DeltaPlus XP IRMS are under vacuum, and the voltages are measured as a digital signal which helps with signal stability and eliminates noise from the wire transfer of analog voltage signals. Moreover, the electronic noise of this instrument is in the tens to hundreds of microvolts, so the signal is very stable and did not increase after high amplification. The end result was a much higher signal from a sample without any adverse effect on the amplifier noise or baseline.

**Table 1.** A comparison of WCO-IRMS operating conditions when measurements of 2 mL of  $90 \mu\text{mol C L}^{-1}$  oxalic acid were made with both normal and high amplification

Parameter (units)	Normal amplification WCO-IRMS	High amplification WCO-IRMS
Resistance at mass 44 ( $\Omega$ )	$2.31 \times 10^8$	$1.0 \times 10^9$
Resistance at mass 45 ( $\Omega$ )	$2.31 \times 10^{10}$	$1.0 \times 10^{11}$
Resistance at mass 46 ( $\Omega$ )	$1.0 \times 10^{11}$	$1.0 \times 10^{11}$
Background signal intensity (mV)	2	8
Sample signal intensity (mV)	94	409
Equivalent in current (nA)	0.31	0.41
Sample size ( $\mu\text{mol CO}_2$ )	0.18	0.18
Intensity (mV) per $\mu\text{mol CO}_2$	522	2272

A 4-fold increase both in background signal and in sample signal was calculated after installation of the high-ohmic resistors. Note that the normal resistors for masses 44 and 45 are actually  $3 \times 10^8$  and  $3 \times 10^{10} \Omega$ , respectively. The values in this table are the mathematical final values for resistors in parallel. Sample size indicates the micromoles of  $\text{CO}_2$  produced from oxidation of 2 mL of  $90 \mu\text{mol C L}^{-1}$  oxalic acid.



**Fig. 4.** The IRMS output of intensity and mass ratio traces versus time of a coastal seawater sample analyzed before and after the instrument modifications, and showing the salt plug effect. (A) The salt plug and chloride effects lead to clipped and tailing peaks. The effect caused an apparent isotopic fractionation leading to an enriched isotope value. (B) The intensity trace and mass ratio trace after the installation of the high-ohmic resistors lacked the tail seen with the salt plug.

Thus, the higher ohmic resistors enabled increasing the amplification of the IRMS, so that a smaller volume of sample gave a larger measurable response. For example, under standard amplification (standard resistor configuration), 2 mL of 90  $\mu\text{mol C L}^{-1}$  oxalic acid produced a 94 mV signal at mass 44 (the primary stable isotope mass for  $\text{CO}_2$ , Table 1). Under high amplification, 2 mL of 90  $\mu\text{mol C L}^{-1}$  standard solution of oxalic acid produced a 409 mV signal at mass 44. Thus, signal strength increased by a factor of about 4. Therefore, we determined that 2 mL would be an acceptable working volume for samples and adjusted the reagent volumes and react times for

the WCO analyzer accordingly (Table 2). The DOC React time on the WCO analyzer had to be extended to 10 minute to allow for 8.5 min of React time at 98°C after adding 6.05 mL of room temperature persulfate to the reaction vessel.

Stable and linear response of the IRMS configured for high amplification was confirmed for signal intensities less than 2 V. Each day, the reference  $\text{CO}_2$  gas was set at a signal intensity of 650 mV and a series of reference  $\text{CO}_2$  pulses were measured to confirm a stable and linear signal. Periodically, linearity tests were run by changing the reference  $\text{CO}_2$  intensity and measuring the  $\delta^{13}\text{C}$  of the reference  $\text{CO}_2$ . An acceptable linearity (0.07‰ per volt) was measured from 340 to 2370 mV on this system. Additionally, 80–90  $\mu\text{mol C L}^{-1}$  standards of glutamic acid and oxalic acid were run periodically and showed a variation of 0.3‰ to 0.6‰ from their known isotopic values. Thus the system was able to perform at an acceptable level for isotopic accuracy and precision at a signal intensity generated by 2 mL sample sizes near seawater DOC concentration.

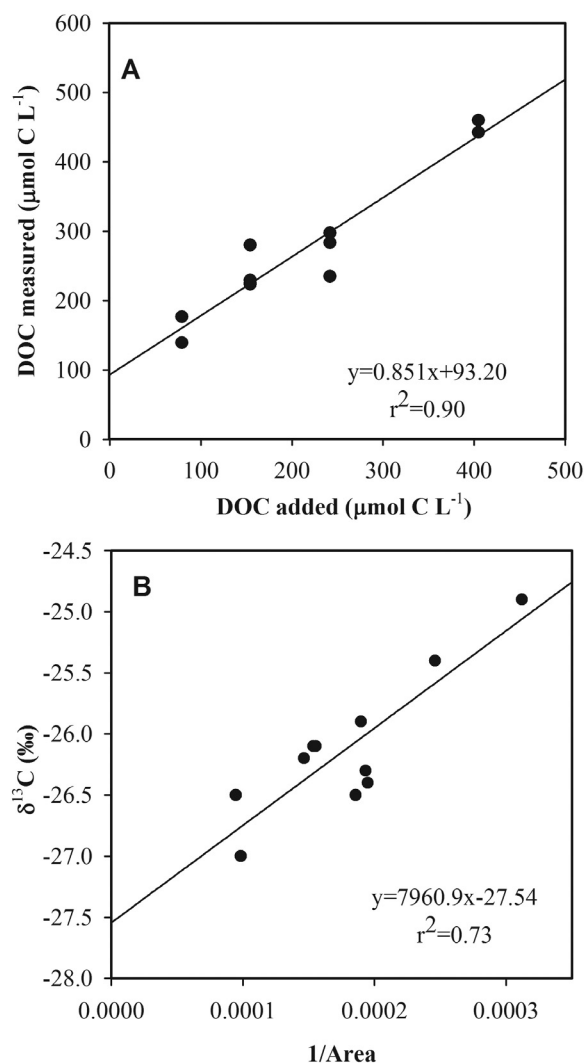
The improvements in IRMS performance are illustrated by a comparison of stable isotope value measurements of a surface water sample from the Beaufort Sea (salinity = 20 ppt) before and after the IRMS modifications (Figs. 4A and 4B). Before the modifications, 5 mL of this sample was measured and the chloride effect clearly decreased the peak intensity and altered the mass 45 to mass 44 ratio trace, such that the  $\delta^{13}\text{C}$  value was +15‰. Here, the chloride effects produced approximately a 30‰ difference in the stable isotope result from its true value. Further, a salt plug formed inhibiting gas flow. After the modifications, 2 mL of the same sample produced a peak and ratio trace similar to freshwater samples on a similar system (St-Jean 2003).

**Confirmation of DOC concentration and  $\delta^{13}\text{C}$  value after modifications**—We validated the recovery of DOC concentration and stable isotope value with two experiments in which DOC standards were added to seawater. In the first experiment, 417  $\mu\text{mol C L}^{-1}$  of glutamic acid stock solution ( $\delta^{13}\text{C}\text{-DOC} = -26.2\text{‰}$ ) was added to seawater that had been processed by tangential flow filtration, which removes molecules > 1 kDa. The DOC concentration of this filtrate was 67  $\mu\text{mol C L}^{-1}$ , so the final DOC concentration of the mixture was 484  $\mu\text{mol C L}^{-1}$ . The TFF filtrate had a salinity of 32.7 and a  $\delta^{13}\text{C}$  value of  $-31.1\text{‰}$ . Note that the spiked solution was roughly 5 times the DOC concentration normally found in seawater ( $\sim 50\text{--}100 \mu\text{mol C L}^{-1}$ ), but similar to the DOC concentration used by Aiken (1992).

The measured DOC concentration of the glutamic acid/sea-

**Table 2.** Wet chemical oxidation (WCO) analyzer operational conditions

DIC parameters (units)	Value	DOC parameters (units)	Value
Acid: 5% $\text{H}_3\text{PO}_4$ ( $\mu\text{L}$ )	800	Oxidant: 1.69 M $\text{Na}_2\text{S}_2\text{O}_8$ ( $\mu\text{L}$ )	6050
Reaction time (min:sec)	2:30	Reaction time (min:sec)	10:00
Detection time (min:sec)	2:05	Detection time (min:sec)	2:05
Reaction temperature ( $^\circ\text{C}$ )	85	Reaction temperature ( $^\circ\text{C}$ )	98



**Fig. 5.** The recovery of DOC concentration and  $\delta^{13}\text{C}$ -DOC value of various amounts of SRHA added to seawater (salinity = 36 ppt) using WCO-IRMS. (A) A standard addition approach where the y-intercept of the regression of DOC measured to DOC added is the DOC concentration of the seawater "blank." (B) The y-intercept of  $\delta^{13}\text{C}$ -DOC value regressed against the inverse of the DOC concentration ( $1/\text{DOC}$ ), at an infinitely large sample size, estimates the  $\delta^{13}\text{C}$  value for SRHA. See text for explanation.

water mixture was  $472 \pm 20 \mu\text{mol C L}^{-1}$ , which equaled a  $96 \pm 4\%$  recovery ( $n = 3$ ). Confirmation of isotopic recovery was done by mass balance after blank correction of the seawater permeate (discussed below). The average measured  $\delta^{13}\text{C}$  value of the glutamic acid/seawater mixture was  $-28.5 \pm 1.3\text{‰}$  ( $n = 3$ ). After blank correction, the  $\delta^{13}\text{C}$  value of the mix was  $-26.5 \pm 0.3\text{‰}$ , which was not statistically different from the  $\delta^{13}\text{C}$  value of the glutamic acid/MilliQ stock solution ( $-26.2\text{‰}$ ;  $P = 0.225$ ,  $df = 2$ ; two-tailed  $t$  test).

In the second experiment, we used another standard additions approach to confirm recovery of both DOC concentration and  $\delta^{13}\text{C}$  value. SRHA powder was dissolved in  $0.2 \mu\text{m}$  filtered seawater collected from the Gulf of Mexico (salinity = 36

ppt, DOC concentration =  $99 \pm 7 \mu\text{mol C L}^{-1}$ ;  $\delta^{13}\text{C} = -23.7\text{‰}$ ). SRHA was purchased from the International Humic Substances Society; the humic material was extracted on XAD resin and contains refractory carbon. Dissolved in seawater, this test solution probably represents the most difficult challenge for the WCO-IRMS system to recover DOC concentration and stable isotope composition in natural waters containing halides and complex carbon molecules.

Four concentrations of SRHA in seawater were used in the standard additions approach. Replicates at each concentration were measured for DOC concentration and  $\delta^{13}\text{C}$  value. A plot of DOC concentration measured versus DOC added to seawater was linear (Fig. 5A). The slope of this curve was not significantly different from unity ( $P = 0.545$ ; Welch corrected paired  $t$  test), indicating good recovery of the added DOC to the seawater matrix. The y-intercept of the regression,  $93 \pm 21 \mu\text{mol C L}^{-1}$ , serves as an estimate of the magnitude of the matrix blank (the Gulf of Mexico seawater in this case). This DOC estimate was not significantly different from the measured seawater DOC concentration (two-tailed  $P$  value = 0.615), so 100% DOC recovery was confirmed. Note that the minimum DOC concentration in this experiment was  $150 \mu\text{mol C L}^{-1}$ .

Recovery of carbon stable isotope value was also confirmed with the standard additions approach (Fig. 5B). When  $\delta^{13}\text{C}$ -DOC values (not blank corrected) were plotted versus the inverse of the DOC concentration, we could extrapolate to an infinitely large DOC concentration with no blank contribution. The y-intercept of a linear regression equation fit to these data then provides an estimate of the added SRHA without any interference from the blank. The  $\delta^{13}\text{C}$  value calculated from the regression was  $-27.5 \pm 0.3\text{‰}$ —which was not significantly different from the  $\delta^{13}\text{C}$  value for SRHA reported by IHSS ( $-27.7\text{‰}$ ). Thus, we also had 100% recovery of the  $\delta^{13}\text{C}$ -DOC value. These results clearly demonstrate that our modifications will allow the analysis of  $\delta^{13}\text{C}$ -DOC in seawater.

**Removal of reagent blanks and system sensitivity**—It was absolutely critical to correct for reagent blanks in the system. Using the concentrated persulfate ( $1.69 \text{ mol Na}_2\text{S}_2\text{O}_8 \text{ L}^{-1}$ ) produced reagent blanks that contributed up to 43% of  $\delta^{13}\text{C}$  values on 2 mL samples of seawater at DOC concentrations  $< 100 \mu\text{mol C L}^{-1}$  (Table 3). Further, Fig. 5B showed that  $\delta^{13}\text{C}$ -DOC for the SRHA/seawater mix deviated by 1.5‰, due to the influence of the seawater  $\delta^{13}\text{C}$ -DOC ( $-23.6\text{‰}$ ), which itself had to be blank-corrected.

Blank correction was most critical for measured  $\delta^{13}\text{C}$ -DOC values at DOC concentrations  $< 100 \mu\text{mol C L}^{-1}$  and was done by mass balance, based on peak areas (cf., Eq. 1 in Bouillon et al. 2006). After the seawater  $\delta^{13}\text{C}$ -DOC value was blank corrected, the mass balance equation was re-applied to calculate the actual  $\delta^{13}\text{C}$ -DOC value of each SRHA/seawater mixture. The results were within 0.3‰ of the y-intercept of the linear regression equation in Fig. 5B. Thus, both a standard addition and a mass balance approach (with blank corrections) confirmed 100% recovery of  $\delta^{13}\text{C}$  value of the

**Table 3.** Results of WCO-IRMS measurement of seawater samples collected from the Middle Atlantic Bight (MAB) and the Beaufort Sea

Sample	Date	Depth (m)	Salinity (ppt)	DOC ( $\mu\text{mol C L}^{-1}$ )	SD ( $\mu\text{mol C L}^{-1}$ )	Blank contribution		SD (%)	$N$
						(%)	$\delta^{13}\text{C}$ (‰)		
MAB Shelf	Mar-05	2	32.9	112	2	25	-23.4	0.1	3
MAB Shelf	Sep-03	5	32.2	96	12	29	-22.8	0.1	3
MAB Shelf	Sep-03	29	34	65	1	43	-23.4	0.2	2
MAB Shelf	Sep-03	90	36.3	73	5	38	-23.3	0.1	3
Franklin Bay, Beaufort Sea	Dec-03	3	25.2	90	4	31	-26.4	0.2	2
Sachs Harbor, Beaufort Sea	Nov-03	3	27.2	97	11	29	-24.3	0.4	2
Franklin Bay, Beaufort Sea	Mar-04	3	30.7	70	—	40	-24.5	—	1
Mackenzie Shelf, Beaufort Sea	Sep-03	1	21.1	203	1	14	-25.3	0.2	2

The contribution of the reagent blank to each DOC measurement is indicated and  $\delta^{13}\text{C}$  values were blank-corrected. SD = standard deviation.

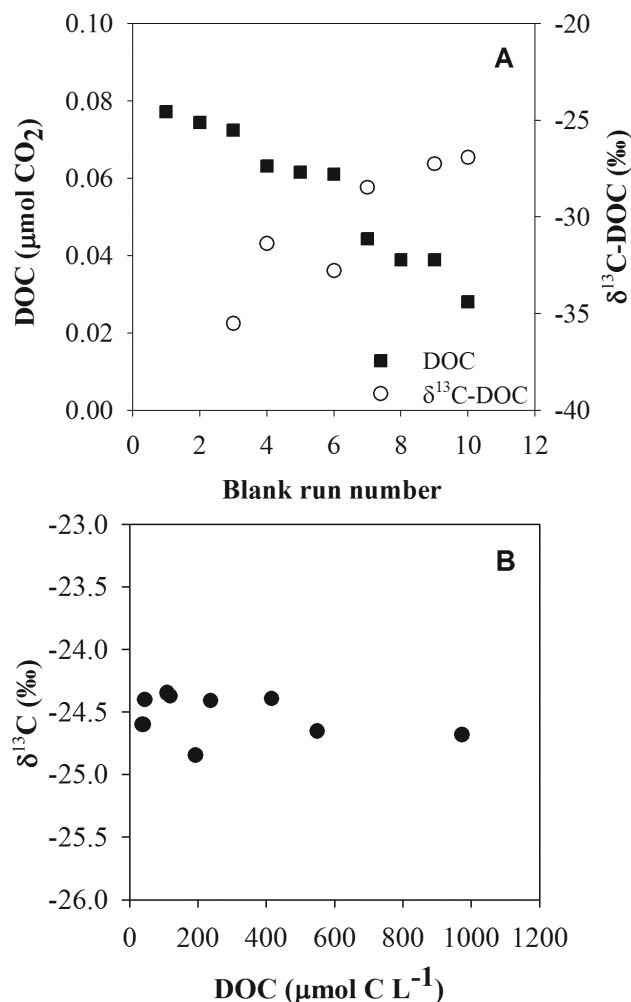
SRHA in seawater.

Given that blank correction was essential for seawater samples, the cleaning procedure (heating and sparging) assisted in reducing DOC background of the persulfate reagent. Blanks were higher in the morning and became stable after a series of 9 blanks were run (Fig. 6A). Each morning, 6 blanks were run with 5 mL of persulfate and a 5:30 React time to condition the system. Next, three reagent blanks were run using the conditions in Table 1. We measured typical reagent blanks (6.05 mL cleaned persulfate, no sample) of  $0.50 \pm 0.10 \mu\text{g C}$  or  $\sim 0.04 \mu\text{mol CO}_2$ —Fig. 6A). These blanks were then subtracted from the DOC concentration and  $\delta^{13}\text{C}$  value measurements, respectively. We determined that MilliQ water did not have a statistically significant DOC concentration from the reagent blank DOC concentrations (unpaired  $t$  test with Welch correction:  $P = 0.794$ ,  $n = 6$ ). Therefore, we periodically ran check standards of KHP with samples to confirm blank correction of  $\delta^{13}\text{C}$  values and to correct DOC concentration measurements.

The ultimate sensitivity and reproducibility of measurements was determined by measuring  $\delta^{13}\text{C}$ -DOC values of KHP solutions ranging in DOC concentration from  $37 \mu\text{mol C L}^{-1}$  to  $973 \mu\text{mol C L}^{-1}$  (Fig. 6B). For 11 measurements, the mean ( $\pm$  standard deviation)  $\delta^{13}\text{C}$ -DOC was  $-24.5 \pm 0.2\text{‰}$ , after blank correction, which was not different from the KHP  $\delta^{13}\text{C}$ -DOC measured in solid form by EA-IRMS ( $-24.5 \pm 0.2\text{‰}$ ,  $n = 3$ ). Thus, our system can measure  $\delta^{13}\text{C}$ -DOC values on DOC concentrations that approach those reported for the deep ocean,  $\sim 45 \mu\text{mol C L}^{-1}$  (Bauer 2002).

## Discussion

**Measurement of DOC in seawater samples**—DOC stable isotope values are determined initially from photosynthetic C fixation. Differences in the isotopic value of the inorganic C (DIC) being fixed,  $\text{CO}_2$  diffusion into seawater, as well as the biochemical pathway of fixation (e.g., C3, C4, or CAM), largely determines the  $\delta^{13}\text{C}$ -DOC value of the organic matter formed (Boutton 1991; Fogel and Cifuentes 1993; Lajtha and Marshall 1994). Most land plants and marine phytoplankton



**Fig. 6.** (A) Ten measurements of DOC concentration (as  $\mu\text{mol CO}_2$ ) and stable isotope value for morning reagent blanks show the decrease in the blank with analysis time. (B) Multiple analyses of KHP standard ( $\delta^{13}\text{C} = -24.5 \pm 0.2\text{‰}$ ) measured at DOC concentrations ranging from 37 to 973  $\mu\text{mol C L}^{-1}$  show the sensitivity of WCO-IRMS analysis.

use the C3 photosynthetic pathway which discriminates against the  $^{13}\text{C}$ , imparting an isotopic shift to fixed C of about 20‰. The difference in isotopic composition of  $\text{CO}_2$  in the atmosphere (−7.8‰, Mook 1986) versus DIC in seawater (−0‰) means that the expected isotope value for terrestrial DOC formed from C fixation is on the order of −27‰, whereas for marine DOC, it is about −22‰.

Of the few marine DOC stable isotope data that exist,  $\delta^{13}\text{C}$ -DOC values range from −23‰ to −18‰. The differences may reflect different DIC sources and other physicochemical and biogeochemical factors, or that multiple sources of marine DOC beside phytoplankton production exist (Bauer 2002 and references therein; Bauer et al. 2002; Boutton 1991). For example, geographical differences in  $\delta^{13}\text{C}$ -DOC values between the Atlantic Ocean (−22‰ to −23‰) and the Pacific Ocean (−19‰ to −22‰) might result from the contribution of DOC to the ocean interior from continental margins, in addition to contribution from terrestrial sources (Bauer et al. 1995, Bauer and Druffel 1998). In coastal wetlands, substantial DOC may originate from C4 grasses where, enzymatically, isotopic fractionation is much less than for C3 plants: −11‰ for *Z. marina* (Thayer et al. 1978) and −13‰ for *Spartina* (Coffin et al. 1994). Bianchi et al. (2004) have shown Mississippi River high molecular weight (HMW) DOC to be enriched in  $^{13}\text{C}$  (−16‰ to −21‰), which they attributed to DOC from river phytoplankton, possibly diatoms.

After confirming the recovery of DOC concentration and  $\delta^{13}\text{C}$  value of known C sources added to seawater by WCO-IRMS, a set of seawater samples from the Middle Atlantic Bight (MAB) were analyzed (Table 3). The samples were collected from coastal shelf waters near large terrestrial runoff. The samples from the MAB ranged in salinity from 32–36 ppt. DOC concentrations were higher at the surface than at depth and  $\delta^{13}\text{C}$ -DOC values ranged from −22.8‰ to −23.4‰. These results are similar to those reported by Bauer et al. (2002) for the MAB.

Several other samples were analyzed from the Mackenzie Shelf Estuary in the Beaufort Sea, at salinities of 20–30 ppt. These samples were depleted in  $^{13}\text{C}$  relative to the MAB samples, reflecting their proximity to the Mackenzie River (−26.4 ± 0.5‰; Osburn unpubl. data). Scant data exist in this region, but the strong riverine influence is comparable to DOC stable isotope measurements from other Arctic shelf regions (Benner et al. 2005). In shelf waters close to the large Arctic rivers, DOC should reflect the riverine isotopic signature as well as that of particulate organic matter (POM; Lobbes et al. 2000). Goni et al. (2000) have measured  $\delta^{13}\text{C}$  values for particulates in the Mackenzie River (−26.5 ± 0.3) and Shelf Estuary (−25.4 ± 0.5‰) similar to our  $\delta^{13}\text{C}$  values for DOC in the same region.

Carbon isotopic data for DOC can also be useful in mixing studies of marine and terrestrial DOC in estuaries and coastal waters (Coffin et al. 1994; Cifuentes and Eldrige 1998; Coffin and Cifuentes 1999; Raymond and Bauer 2001a). For example, coastal waters may diverge from conservative mixing between

river and seawater end members suggesting additional DOC inputs (Raymond and Bauer 2001b). We assembled two data sets of DOC concentration and stable isotope measurements made on samples from the Chesapeake Bay and MAB in September 2003 and from the Atchafalaya River estuary and Gulf of Mexico collected in January 2006 using our WCO-IRMS system (Fig. 7A and 7B). The conservative mixing line for  $\delta^{13}\text{C}$ -DOC values between the river and ocean end-members is also plotted, using the following equation from Raymond and Bauer (2001b):

$$\frac{I_s = f \times I_r \times \text{DOC}_r + (1-f) \times I_m \times \text{DOC}_m}{\text{DOC}_{\text{mix}}} \quad (2),$$

where  $I_s$  is the  $\delta^{13}\text{C}$  value of DOC at any salinity,  $f$  is the fraction of river DOC,  $I_r$  is the  $\delta^{13}\text{C}$  value of the riverine end member, and  $I_m$  is the  $\delta^{13}\text{C}$  value of the seawater end member.

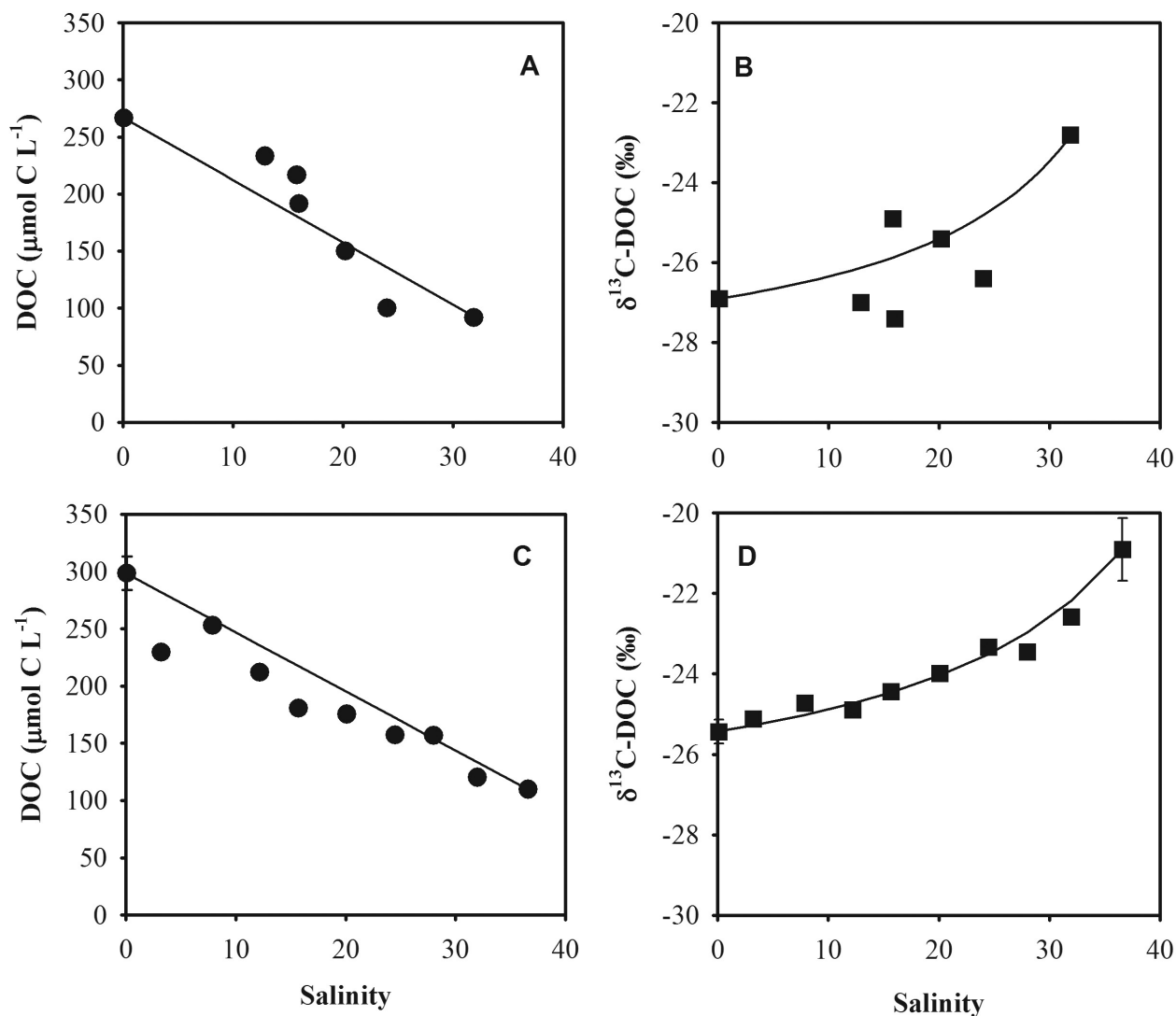
In the Chesapeake Bay, DOC concentration decreased with salinity (Fig. 7A). However, deviations occurred from the conservative mixing between riverine and marine DOC end-members occurred at salinity = 12 ppt and 22 ppt. At these salinities,  $\delta^{13}\text{C}$ -DOC values were more negative than predicted from conservative mixing between end-members (Fig. 7B). Several large rivers flow into the Chesapeake Bay below its main tributary (the Susquehanna River), which could explain these departures from conservative mixing.

Contrasting the deviation from conservative mixing in the Chesapeake Bay is an example of conservative mixing in an estuary connecting the Atchafalaya River to the Gulf of Mexico (Fig. 7C and 7D). Here, the data for both DOC concentration and  $\delta^{13}\text{C}$  value fall almost exactly on the conservative mixing lines calculated using Eq. 2. This effect is consistent with mixing between river water and seawater end-members. Further, these data compliment the carbon isotopic composition of macromolecular colloidal organic matter reported by Guo et al. (1999) for the Mississippi River plume.

We see no need to re-interpret existing data sets; rather, the DOC stable isotope data generated by WCO-IRMS complement previously published values and should contribute to the body of DOC stable isotope data that exists for the ocean. This study has demonstrated that WCO-IRMS is a viable methodology for DOC stable isotope measurement in seawater.

### Comments and recommendations

Our modifications to the WCO-IRMS system originally conceived and developed by St-Jean (2003) are effective at overcoming the problems for seawater analysis by WCO discussed by Aiken (1992) and Peyton (1993). The 100% recovery of DOC concentration and  $\delta^{13}\text{C}$  value in the SRHA/seawater mixtures was strong evidence that the extended reaction time and increased persulfate concentration quantitatively oxidized all DOC to  $\text{CO}_2$ . Further, high amplification enabled us to overcome the halide interference by analyzing 2 mL sample sizes with precision and accuracy for  $\delta^{13}\text{C}$ -DOC value. The range in



**Fig. 7.** Contrasting trends in  $\delta^{13}\text{C}$  value and DOC concentration plotted against salinity for two estuaries. (A) and (B) are from the Chesapeake Bay and Middle Atlantic Bight and show a nonconservative mixing trend. (C) and (D) are from the Atchafalaya River estuary and the continental shelf of the Gulf of Mexico and show a conservative mixing trend.

$\delta^{13}\text{C-DOC}$  values for coastal waters was  $-22.8\text{‰}$  to  $-26.4\text{‰}$ , and indicated some terrestrial or riverine influence on more negative values at salinities  $< 36$  ppt.

We have shown that rigorous measurement of the system blank prior to sample analysis is critical, but allows appropriate stable isotope value blank corrections to measured values. System conditioning with reagent blanks and/or MilliQ water is strongly suggested, as is periodic measurement of stable isotope standards. Further, we have shown that an IRMS instrument can be tuned to produce stable and reliable measurements at signal intensities from 0.4 to 2 V, covering an effective  $\text{CO}_2$  concentration of 0.2 to 2.0  $\mu\text{mol}$ . With careful attention to blanks, our system should be able to measure marine DOC concentrations down to  $\sim 45 \mu\text{mol C L}^{-1}$ .

Several aspects of this analysis will add cost and reduce

sample throughput. First, the continued measurement of salt-containing samples will, over time, corrode and destroy the thermocouple in the reaction vessel of the 1010 TOC and will require replacement. Second, WCO-IRMS will consume a great deal of copper shot in the halide scrubber and in the quartz reduction column. The copper shot can be regenerated by soaking in 30%  $\text{HNO}_3$  for about 20 min and then thoroughly rinsing with MilliQ water and drying overnight. Sample throughput was limited in seawater samples by the halogen trap (#1 and #3 in Fig. 2); about 10 seawater samples may be analyzed before this trap must be replaced.

Third, the quartz reduction column will require replacement after about 15-20 measurements. The development of a split peak will indicate when the quartz reduction column is spent. Similarly, the Nafion tube in the 1010 TOC will require

replacement after about 200 analyses. However, interspersing freshwater and estuarine samples with seawater samples in the autosampler should extend the sample throughput before chemicals require changing.

The system we have described here should allow the measurement of  $\delta^{13}\text{C}$  DOC values in most natural waters. The 1010 TOC has a septum port for manual sample injection, which was useful for measuring DOC concentration and  $\delta^{13}\text{C}$ -DOC values on surface waters from saline lakes, wetlands, and microbial mats. These systems can have DOC concentrations up to 125 mol C L<sup>-1</sup>, so we needed to inject as little as 100  $\mu\text{L}$  sample. We have also measured  $\delta^{13}\text{C}$ -DOC on porewaters collected from an IODP cruise leg (DOC range: 0.83 to 8.10 mol C L<sup>-1</sup>). Further, the high-amplification feature of the IRMS has been applied to solid C and N stable isotope analysis by EA-IRMS and to trace methane gas analysis on a gas chromatograph coupled to our IRMS. Thus, the applicability of these modifications extends well beyond the analysis of  $\delta^{13}\text{C}$ -DOC in seawater.

In conclusion, we have shown that straightforward modifications to an IRMS can enable 2 mL samples of seawater to be successfully analyzed for DOC concentration and stable isotope value using existing technology. A quick modification to IRMS instruments now in service will enable smaller sample sizes to be measured for isotope values than previously possible. Improvements in the sensitivity of next generation IRMS technology should decrease the sample size requirement even further.

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Submitted 17 July 2006

Revised 7 November 2006