

AUTOMATED ANALYSIS OF DIC AND DOC IN WATER SAMPLES FOR STABLE ISOTOPES – A NEW TECHNIQUE

R.R. DOUCETT AND B.A. HUNGATE

COLORADO PLATEAU STABLE ISOTOPE LABORATORY, DEPARTMENT OF BIOLOGY, NORTHERN ARIZONA UNIVERSITY, FLAGSTAFF, AZ, 86011-5640
TEL: 928-523-0967, EMAIL: RICHARD.DOUCETT@NAU.EDU, WEBSITE: WWW4.NAU.EDU/CPSIL

ABSTRACT

We present a new technique to measure stable isotope ($\delta^{13}\text{C}$) and concentration data (ppm C) on dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) simultaneously from the same water sample using a commercially-available TOC analyzer interfaced to an isotope-ratio mass spectrometer (TOC-IRMS). Water samples from two streams in northern Arizona were used to demonstrate the utility of the technique. Ease of sample collection and analysis using TOC-IRMS should encourage researchers to include isotope data in future studies of DIC and DOC cycling in freshwaters.

INTRODUCTION

Stable isotopes are fast becoming routine tools to describe food-web relations and understand nutrient cycling in aquatic ecosystems^{1,2}. Stable isotopes ($\delta^{13}\text{C}$) of dissolved inorganic carbon (DIC) are used primarily in the study of aquatic carbon cycles^{3,4}, but are also used to interpret constraints on aquatic photosynthesis^{5,7}. The $\delta^{13}\text{C}$ values of dissolved organic carbon (DOC) can be used to identify inputs derived from terrestrial and aquatic sources^{8,9}, and also to understand microbial processes impacting DOC in surface and ground waters^{10,11}.

Unfortunately, relatively few food-web studies include isotope data on DIC or DOC because of the increased effort needed to prepare and analyze these samples. In the past, isotope analysis of DIC involved extracting CO_2 off-line and manually analyzing CO_2 using dual-inlet mass spectrometry¹². Isotope analysis of DOC also involved off-line preparation and, due to low DOC concentrations, usually required large quantities of water (10-120 L)¹³. DOC is also difficult to analyze due to the insolubility of high molecular-weight compounds (e.g., humic and fulvic acids) at low pH¹⁴.

Recently, a new technique (TOC-IRMS) has been described which measures isotope ($\delta^{13}\text{C}$) and concentration (ppm C) data on both DIC and DOC from the same water sample¹⁵. The technique utilizes pre-existing instruments to obtain data sequentially on DIC and DOC that has been converted to CO_2 using heated wet-oxidation methods (Fig. 1). Analysis is automated, requires small volumes (1-25 ml), and is accomplished in < 12 min per replicate.

In this study, we demonstrate the utility of the new TOC-IRMS technique using water samples collected from two streams in northern Arizona.

METHODS

In the field, water samples were collected in duplicate from various locations along Fossil Creek and Oak Creek, Arizona, in March, April, and May, 2003 (Fig. 2). Samples were field-filtered using pre-combusted Whatman GFF series 0.7- μm glass-fiber filters, spiked with 250 μl of a saturated HgCl_2 solution, and stored (without airspace) in 40-ml glass vials with open-top 0.125" septum-lined caps (ICHEM 200 series).

In the laboratory, stock solutions of Sigma-Aldrich reagent-grade sodium carbonate (DIC) and sucrose (DOC) were used to generate concentration curves for ppm C correction (not shown). These solutions, along with other standards, were also analyzed via conventional EA-IRMS to obtain known isotope values for $\delta^{13}\text{C}$ correction using TOC-IRMS (Table 1).

Preliminary analyses suggested that samples with > 60 ppm DIC might interfere with DOC results (Table 2). For this reason, samples were analyzed separately for DIC and DOC. The first sample was analyzed for DIC without treatment. The second sample was pre-treated with 20 μl of 85% H_2PO_4 and sparged with helium for 10 min to remove DIC before analysis of DOC. Concentration data are reported in parts-per-million carbon (ppm C). Isotope data are reported in per mil (‰) using conventional delta (δ) notation. Precision is ± 0.2 ppm C on the TOC, and $\pm 0.2\text{‰}$ on the IRMS. Optimal sample sizes of 5-120 μg C were obtained using 1-4 ml of water.

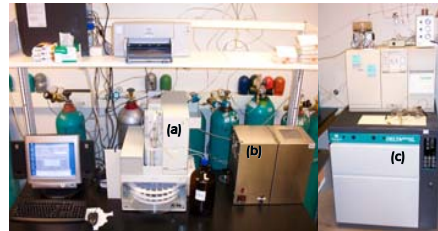


Figure 1. TOC-IRMS setup showing (a) TOC analyzer, (b) interface, and (c) IRMS.

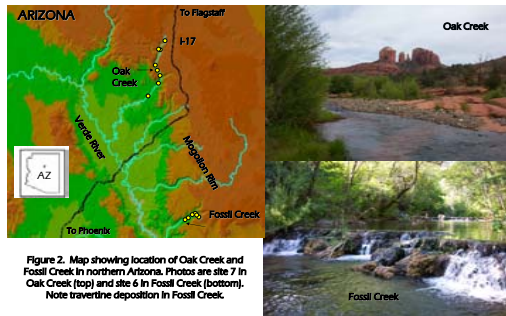


Figure 2. Map showing location of Oak Creek and Fossil Creek in northern Arizona. Photos are site 7 in Oak Creek (top) and site 6 in Fossil Creek (bottom). Note travertine deposition in Fossil Creek.

Table 1. Comparison of $\delta^{13}\text{C}$ results obtained using conventional EA-IRMS and the new TOC-IRMS.

	$\delta^{13}\text{C}$ (‰)	
	EA	TOC
Standard		
Acetanilide	-25.87	-25.97
Nicotinamide	-32.53	-32.76
Sucrose	-11.16	-11.32
Humic acid	-25.70	-25.51
Citric acid	-16.74	-16.79
IAEA-CH6	-10.39	-10.56
Sodium carbonate	-26.38	-26.50

Table 2. Effects of pre-treatment to remove DIC on $\delta^{13}\text{C}$ results of DOC. Data are from Fossil Creek.

DIC ppm C	DOC $\delta^{13}\text{C}$	
	Before	After
60.27	-17.81	-25.31
65.11	-5.64	-24.92
65.74	-5.36	-23.95
62.27	-6.33	-24.12
56.35	-14.34	-24.01
63.69	-5.89	-24.19

DISSOLVED INORGANIC CARBON

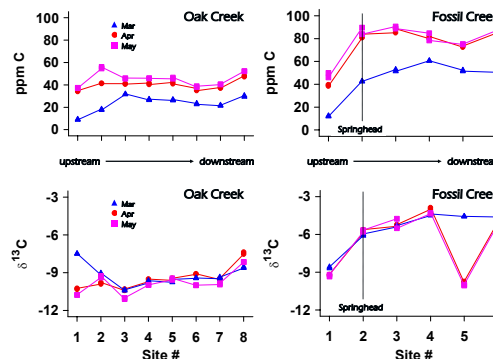


Figure 3. ppm C and $\delta^{13}\text{C}$ data for DIC in Oak Creek and Fossil Creek, Arizona.

DISSOLVED ORGANIC CARBON

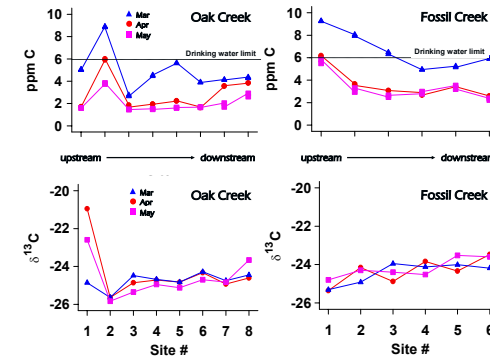


Figure 4. ppm C and $\delta^{13}\text{C}$ data for DOC in Oak Creek and Fossil Creek, Arizona.

RESULTS AND DISCUSSION

DIC levels in Oak Ck and Fossil Ck ranged from 9.4 to 90.7 ppm C during the 3 months that sampling occurred (Fig. 3). In general, DIC levels were higher in Fossil Ck (66 ± 22 ppm C) than in Oak Ck (36 ± 11 ppm C). Fossil Ck is a spring-fed stream that flows through a travertine deposition zone. High DIC levels are often found in spring water super-saturated with CO_2 ^{16,17}. Lower DIC levels in March were likely due to the influence of snow melt.

DIC $\delta^{13}\text{C}$ values ranged from -11.1‰ to -4.0‰, and were more enriched than expected for stream water in equilibrium with soil CO_2 (-21‰)¹⁸. $\delta^{13}\text{C}$ -enrichment of DIC in Fossil Ck and Oak Ck may be related to exchange with atmospheric CO_2 (-8‰) or exposure to carbonate weathering (-1‰ to +1‰). Due to the persistence of travertine and high DIC levels along the length of Fossil Creek, carbonate dissolution probably controlled $\delta^{13}\text{C}$ values in this system. Loss of isotopically light CO_2 during out-gassing may have contributed to more enriched $\delta^{13}\text{C}$ values downstream^{18,19}.

DOC concentrations in Oak Ck and Fossil Ck ranged from 0.9 to 9.6 ppm C during the sampling period (Fig. 4). DOC levels in both streams were generally below drinking-water standards, except during snow melt. Higher DOC levels are expected during snow melt when soils saturated with melt water are flushed into nearby lakes and streams⁹.

DOC $\delta^{13}\text{C}$ values ranged from -25.8‰ to -20.9‰ and were typical for streams draining catchments dominated by C_3 plants. $\delta^{13}\text{C}$ in both systems exhibited slightly higher values downstream. Peculiar values at site 1 in Oak Ck may have been due to the presence of a trout hatchery upstream, where waste products derived from fish raised on commercial marine feeds are $\delta^{13}\text{C}$ -enriched (-20‰ to 18‰). We will continue monitoring this site and others in Oak Ck throughout the year in attempts to investigate pollution sources using $\delta^{13}\text{C}$ and ppm C data²⁰.

SUMMARY

TOC-IRMS successfully measured stable isotope ($\delta^{13}\text{C}$) and concentration (ppm C) data on DIC and DOC in Fossil Ck and Oak Ck, Arizona. Ease of sample collection, speed of analysis, small volumes, and automation should make this technique desirable to researchers investigating DIC and DOC dynamics in aquatic ecosystems. The technique is currently being modified to deal with saline waters, microbial extracts in K_2SO_4 isotope tracer studies, and DOC fractions separated by HPLC.

ACKNOWLEDGEMENTS

The authors would like to thank Gilles St. Jean for his ingenuity, Jaina Moan and Arista Lee assisted with sample collection.

LITERATURE CITED

- (1) Hering, A.L., and Peterson, B.J. 1996. Methods in Stream Ecology, pp. 511-530. (2) Peterson, B.J. et al. 2001. Science 292: 84-89. (3) Leggett, W.C. et al. 1999. Can. J. Fish. Aquat. Sci. 56: 221-227. (4) Pridmore, J.C. 2000. Microchem. J. 62: 20-25. (5) Hollander, D.L. and Johnson, J.A. 1991. Geology 19: 829-832. (6) Goussot, G. et al. 1994. Stable isotope in ecology and environmental science, pp. 103-121. (7) Pridmore, J.C. et al. 1999. Journal of Great Lakes Research 25: 103-115. (8) Seitz, G. et al. 1997. Biogeochemistry 38: 45-63. (9) Seitzinger, S.P. et al. 1998. Journal of Great Lakes Research 24: 428-434. (10) Peterson, B.J. et al. 1991. Can. J. Fish. Aquat. Sci. 48: 20-25. (11) Hall, G.D. and Meyer, A.L. 1998. Biology 26: 1998-2011. (12) Chai, L.G. and Rees, P. 1997. Environmental Impact on Hydrology, 113. (13) Peterson, B.J. et al. 1999. Can. J. Fish. Aquat. Sci. 56: 221-227. (14) Hering, A.L. and Johnson, J.A. 1991. Geology 19: 829-832. (15) Peterson, B.J. et al. 2001. Science 292: 84-89. (16) Goussot, G. et al. 1994. Stable isotope in ecology and environmental science, pp. 103-121. (17) Pridmore, J.C. et al. 1999. Journal of Great Lakes Research 25: 103-115. (18) Seitz, G. et al. 1997. Biogeochemistry 38: 45-63. (19) Seitzinger, S.P. et al. 1998. Journal of Great Lakes Research 24: 428-434. (20) Peterson, B.J. et al. 1991. Can. J. Fish. Aquat. Sci. 48: 20-25.

