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Letter to the Editor

To the Editor-in-Chief
Sir,

Determination of $\delta^{18}O$ of water and $\delta^{13}C$ of dissolved inorganic carbon using a simple modification of an elemental analyzer/isotope ratio mass spectrometer: an evaluation

Both the stable carbon isotope ratio of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) and the oxygen isotope ratio of water ($\delta^{18}O_W$) can provide valuable information. For example, $\delta^{13}C_{DIC}$ values of estuarine waters can help decipher biogeochemical cycling,[1–3] whereas the $\delta^{18}O_W$ can be important for understanding $\delta^{18}O$ values of carbonates as a temperature proxy[4] or for tracing water masses.[5] Traditionally, samples for $\delta^{13}C_{DIC}$ and $\delta^{18}O_W$ determination have been measured as CO_2 purified off-line in glass extraction lines after acidification (in the case of DIC) or equilibration of CO_2 with the water to be analyzed, before being measured on a dual-inlet isotope ratio mass spectrometer (IRMS).[1,5] A variety of preparation techniques have since been developed for $\delta^{13}C_{DIC}$ (recently reviewed in Atekwana and Krishnamurthy[6]) and $\delta^{18}O_W$ determinations.[7,8] Although many of these newer techniques avoid the use of extensive glass extraction lines, they often require expensive peripherals in addition to the most common elemental analyzer (EA)-IRMS setup (e.g., St-Jean,[9] Torres *et al.*,[10] Spötl[11] and Seth *et al.*[12]) or require significant modifications to the IRMS such as sample loop systems (e.g. Prosser *et al.*[8] and Salata *et al.*[13]). We report herein the evaluation of a previously unpublished, but widely used,[2,3,14] adaptation of an EA-IRMS setup which enables the determination of $\delta^{13}C_{DIC}$ and $\delta^{18}O_W$. The approach differs slightly from those described by Prosser *et al.*[8] and Salata *et al.*,[13] but requires substantially less hardware modifications for most IRMS laboratories, and is therefore generally inexpensive to implement. These methods basically involve the injection of CO_2 from a sample headspace, obtained after acidification of water samples for $\delta^{13}C_{DIC}$ or equilibration of small water samples with CO_2 for $\delta^{18}O_W$, into the He flow of an EA-IRMS setup. The method is robust and standard deviations (1σ) better than $\pm 0.2‰$ can easily be achieved. We provide data from in-house standards as well as natural fresh and salt water samples.

The setup described here is a Flash 1112 Series EA coupled via a Conflo III to a ThermoFinnigan Delta+XL continuous flow (CF)-IRMS (Bremen, Germany). The only modification required is the installation of an injection port in the He carrier gas line, between the reduction column and the water trap. Since we inject the samples after the EA reactors, the oven temperatures for the reactors were set to lower temperatures than when operating for sample combustions (i.e. our standby settings are 800°C for the combustion column and 500°C for the reduction column). The gas chromatography (GC) column was held at 50°C, the water trap was filled with magnesium perchlorate, and the He flow was set at approximately 90 mL/min. The IRMS was run under the Isodat v2.0 software, and the method events consisted of three reference CO_2 pulses, up to four sample peaks (in principle, from different vials rather then multiple injections from a single sample headspace) at approximately 2–3 min intervals, followed by one or two final CO_2 reference pulses to correct for drift. Thus, four samples (generally two samples in duplicate) can be analyzed in approximately 15 min (for either $\delta^{18}O_W$ or $\delta^{13}C_{DIC}$).

Water samples for $\delta^{18}O$ analysis were collected by filling 100 mL polyethylene containers and adding 60 μL of a saturated $HgCl_2$ solution. Containers were capped tightly, the seal wrapped with Parafilm to avoid evaporation, and were stored at room temperature. Water samples for determination of $\delta^{13}C_{DIC}$ were sampled by gently over-filling headspace vials (25, 20, 10, 6 or 2 mL) with water. Vials were rinsed with sample water three times before sampling. A volume of 10 to 60 μL of a saturated $HgCl_2$ solution was added (depending on vial size) and the vials were capped and stored at room temperature until analysis. Alternatively, samples for $\delta^{13}C_{DIC}$ determination may be injected into He-flushed vials.

The procedure for measuring $\delta^{18}O_W$ is modified from Prosser *et al.*[8] and is both easier and faster than traditional off-line methods. The approach is similar to existing off-line methods, except that equilibration and gas extraction are done directly in a gas-tight headspace vial and only 0.5 mL of sample is required. Headspace vials (12 mL) are first flushed with He gas and are capped with a rubber septum and aluminum seal; alternatively, Exetainer vials (Labco, High Wycombe, UK) may be used. Approximately 500 μL of sample water is injected into the vial, and then 200 μL of pure CO_2 from a tank is injected using a gas-tight syringe. The samples are then placed in a shaker for 2 h and left to equilibrate for about 24 h (for freshwater samples) or about 48 h (for seawater samples) at ambient laboratory temperature (~23°C, in a climate-controlled room). This is more than enough time to compensate for the salt effect on the kinetics of the CO_2–H_2O isotopic exchange equilibrium, which has been determined to be three times longer in saltwater than in freshwater (or at least 24 h).[15] In each batch of samples described here, two in-house secondary standards (previously calibrated against the water standards: VSMOW, GISP and SLAP, see Table 1) were similarly processed: one seawater (SW1) and one tapwater standard (TAP0409, see Table 1). Table 1 also presents results on two other in-house seawater standards (NWS and NWSG) which had been prepared in a different context. After equilibration, 1000 μL of CO_2 is drawn from the headspace into a gas-tight syringe that has previously been flushed with He, and is injected into the injection port on the EA-IRMS setup. The

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Table 1. Uncorrected water isotopic standards analyzed using the method described here. All standard deviations (1σ) are better than 0.15‰. The data are compared with the accepted values for the IAEA standards. All environmental data are normalized so that SLAP is exactly −55.5‰[16]

Standard	Accepted $\delta^{18}O_{VSMOW}$ (‰)	Measured $\delta^{18}O_{VSMOW}$ (‰)	n
VSMOW[a]	0.00[16]	0.11 ± 0.09	5
GISP	−24.78 ± 0.09[18]	−24.70 ± 0.06	4
SLAP[a]	−55.5[16]	−54.76 ± 0.06	3
TAP0409[b]		−7.30 ± 0.14	18
NWS[b]		−7.36 ± 0.10	9
NWSG[b]		−7.66 ± 0.12	9
SW1[b]		0.01 ± 0.13	16

[a] By definition.[16]
[b] In-house standards.

uncorrected data, obtained using the $\delta^{18}O$ value of the tank CO_2 gas, are available in Table 1. All data are expressed in ‰ relative to VSMOW (0.0‰) on a scale normalized so that SLAP is exactly –55.5‰.[16] The precision was better than 0.15‰ (1σ), determined by repeated analyses of the seawater and tapwater standards and replicate sample analyses (Table 1). This precision is similar to or better than was obtained using traditional off-line methods (i.e. ≤0.2‰).

A modified version of the method described by Salata *et al.*[13] was used for $\delta^{13}C_{DIC}$ analysis. For vials filled to the top, a headspace was first created by inserting an empty, fully depressed, 10 mL syringe and needle through the septum, then inserting a needle attached to a He bottle at a pressure of 1–1.5 bar, until the required volume of water has been replaced (typically ~20% of the total volume of the vial, but with a minimum of at least 1.5 mL headspace for small vial types). After the He supply line had been removed, the pressure was equalized in the other syringe. Once the headspace had been created (or for vials where the sampling procedure already created a headspace), warm 85% phosphoric acid was added (typically ~500 μL for vials >10 mL and 250 μL for vials <10 mL). Samples were placed upside down in order to avoid contact between headspace and septum, thereby reducing the possibility of exchange with atmospheric CO_2,[17] and allowed to equilibrate for several hours in a sample shaker (generally overnight). Salata *et al.*[13] reported that the results were stable after 16 to 36 h of equilibration time. A similar experiment was carried out here with 24 replicate tapwater samples injected between 1 and 57 h after acidification and this showed that the samples were within 0.15‰ of the mean between 4 and 56.5 h (Fig. 1; Average = −12.41 ± 0.07‰, n = 20). In addition, 22 other tapwater samples were injected on four separate days and gave an average $\delta^{13}C_{DIC}$ of −12.45 ± 0.12‰.

We also tested the effect of using different vial sizes (2, 10, and 25 mL) with similar tapwater samples, each analyzed in duplicate or triplicate. The standard deviation on these seven samples was 0.05‰ (average $\delta^{13}C_{DIC}$ = −12.53‰) and no sample deviated by more than 0.1‰ from the overall mean. This indicates that vial size is not an important factor, but it should be noted

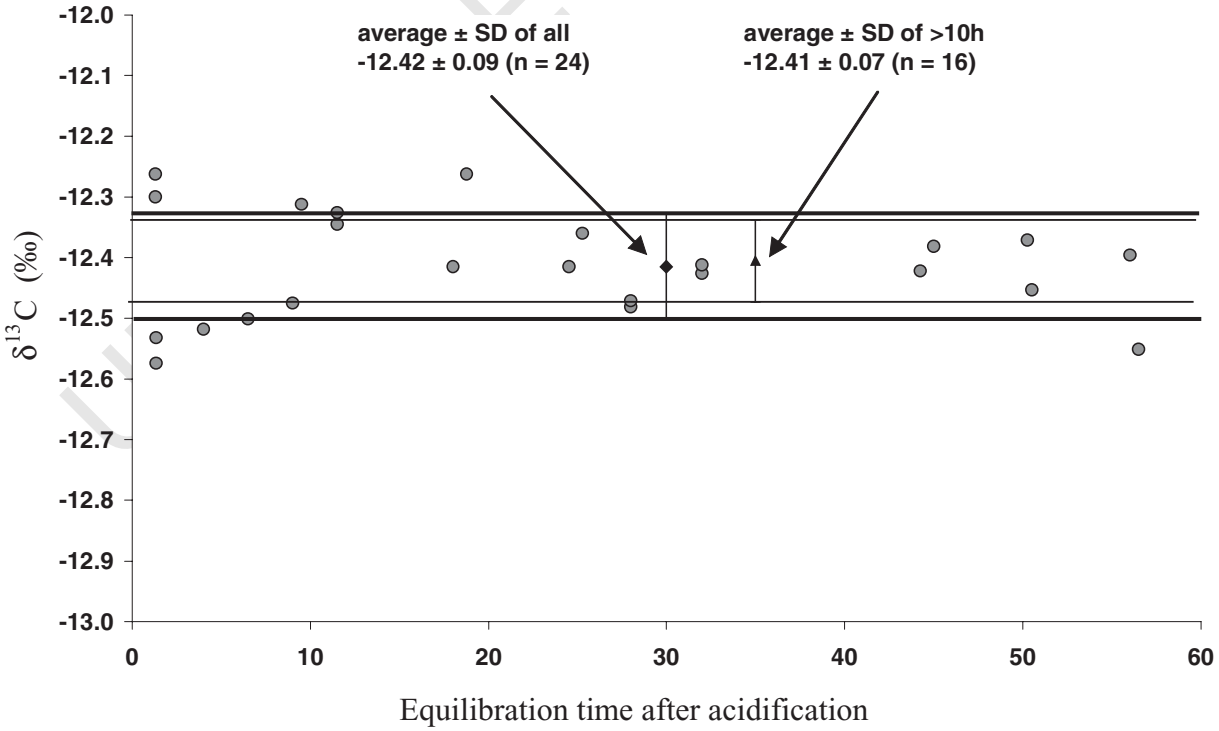

Figure 1. $\delta^{13}C_{DIC}$ (VPDB) of 24 replicate tapwater samples equilibrated for different periods of time (1.3–56.5 h) before injection into the EA-IRMS setup.

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that our tapwater had high DIC concentrations and therefore produced a lot of CO_2 after acidification. Samples with less DIC (e.g. seawater) will produce less CO_2 and therefore may not contain sufficient CO_2 for injection when using very small sample sizes (2 mL or less). A minimum of 8 μL of pure tank CO_2 was needed to obtain an acceptable signal in the IRMS (Table 2; or ~0.36 μmol of CO_2 assuming standard pressure and temperature). The $\delta^{13}C_{DIC}$ values obtained from the seawater standard with lower DIC concentrations were also very reproducible (+1.85 ± 0.08‰, n = 5).

Table 2. Injections of pure CO_2; CO_2 was drawn from the tank using a gas-tight syringe and injected into the EA-IRMS setup. Average $\delta^{13}C$ data (VPDB) from injections of 10–20 μL of CO_2 gas are −34.37 ± 0.07‰, n = 21

Injection volume (μL)	n	Area (vs.) ± 1σ	$\delta^{13}C$(‰) ± 1σ
2	1	0.49	−33.34
3	6	0.65 ± 0.16	−33.54 ± 0.33
5	3	1.85 ± 0.47	−33.87 ± 0.30
6	3	3.49 ± 0.52	−33.87 ± 0.37
8	3	5.33 ± 0.11	−34.25 ± 0.09
10	3	7.16 ± 0.71	−34.35 ± 0.15
13	2	9.59 ± 0.02	−34.37 ± 0.10
15	4	12.02 ± 0.24	−34.37 ± 0.09
17	4	14.05 ± 0.71	−34.38 ± 0.04
20	8	16.82 ± 0.55	−34.38 ± 0.04

To correct for the partitioning of CO_2 between headspace and the water phase and to calculate the $\delta^{13}C$ of the total DIC, the equation of Miyajima *et al.*[17] was applied:

$$\delta^{13}C_{DIC} = \frac{V_{headspace} * \delta^{13}C_{measured} + (V_{bottle} - V_{headspace}) * \beta * (\delta^{13}C_{measured} + \varepsilon_g^a)}{V_{headspace} + (V_{bottle} - V_{headspace}) * \beta}$$

where $\beta = 0.872$ at 23°C (Ostwald solubility coefficient); ε_g^a is calculated from $\varepsilon = -373/T(K) + 0.19$ (thus, $\varepsilon_g^a = -1.07$ at 23°C); and V_{bottle} and $V_{headspace}$ represent the internal volumes of the sampling vial and headspace, respectively.

These data were subsequently further corrected using the calibrated CO_2 gas (from a tank), which was injected periodically throughout the analysis sequence (~20 μL). The CO_2 used was calibrated using a dual-inlet IRMS (Delta+XL) against NBS-19 ($\delta^{13}C = +1.95$‰, $\delta^{18}O = -2.20$‰[16]). Typically, the standard deviations of the δ-values of this gas were less than 0.1‰ for repeated injections during a single day.

As there is no certified $\delta^{13}C_{DIC}$ standard, our in-house seawater standard (SW1) was used to evaluate the day-to-day variability of our method. Furthermore, to test the precision and accuracy of the method, a standard was produced by dissolving Na_2CO_3 in Ar-purged natural seawater from which all DIC had been previously removed. The Na_2CO_3 powder was also analyzed using an automated carbonate device (ThermoFinnigan

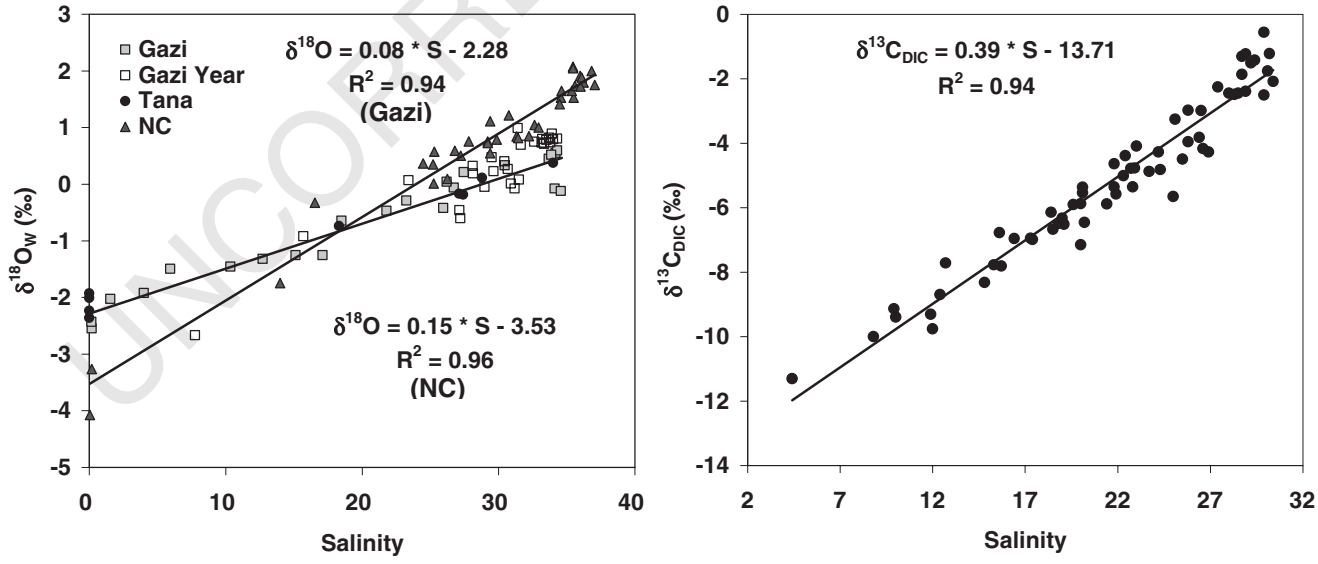

Figure 2. $\delta^{18}O_W$ (VSMOW) and $\delta^{13}C_{DIC}$ (VPDB) data from natural waters analyzed using the methods described here. Data in the left panel are from Gazi Bay (Kenya), the Tana River (Kenya), and from Wade Creek (NC, North Carolina, USA). Data labeled 'Gazi' were collected in July 2003 and data labeled 'Gazi Year' were collected monthly from June 2002 until July 2003. Data from the Tana River were collected in April 2004 and NC data are from various times between December 2001 and August 2003. Data in the right panel are from Gillikin *et al.*[2] and are taken from the Scheldt Estuary (The Netherlands) between November 2001 and November 2002.

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Kiel III) connected to a dual-inlet IRMS and calibrated versus NBS-19. We observed a difference of 0.22‰ between the $\delta^{13}C$ value of the Na_2CO_3 solid measured using the Kiel III (-1.10 ± 0.07‰, $n = 8$) and using the injection technique for the $\delta^{13}C$ value of the Na_2CO_3 dissolved in seawater (-1.32 ± 0.15‰, $n = 12$). This difference may be caused by exchange with the atmosphere during preparation of the standard, or could be due to an inhomogeneity in the Na_2CO_3 powder. Nevertheless, considering that these means are within 2σ of the analytical precision of the method ($1\sigma = 0.2$‰), the sets of data can be considered indistinguishable using this method.

Example data from estuarine waters analyzed using the methods described above are shown in Fig. 2. The expected linear relationship between salinity and $\delta^{18}O_W$[5] can be seen in samples from three sites [two in Kenya (Gazi Bay and Tana River) and one in North Carolina, USA (Wade Creek)]. The intercept of the higher latitude data are more negative as would be expected from Rayleigh distillation. For the samples collected in Gazi Bay (Gazi Year on Fig. 2), a single data point (at salinity ~8) plots off of the regression line; we interpret this as possibly resulting from an intense rain event, since these are known to produce precipitation which is more depleted in ^{18}O. The linear relationship between salinity and $\delta^{13}C_{DIC}$ as illustrated here is not always observed, as various processes may result in non-conservative behavior of DIC along estuarine mixing gradients[3] (see Gillikin *et al*.[2] for more discussion on these data).

In summary, this simple adaptation of an existing EA-IRMS setup allows for a fast, inexpensive and robust technique for the analysis of $\delta^{18}O_W$ and $\delta^{13}C_{DIC}$, with reproducibility consistently better than 0.2‰ for both parameters. Both the $\delta^{18}O_W$ and the $\delta^{13}C_{DIC}$ methods allow for the measurement of >50 samples per day, with very limited sample preparation time.

The approach described here is particularly suitable for estuarine research where large changes in both $\delta^{18}O_W$ and $\delta^{13}C_{DIC}$ can be expected (Fig. 2) and for laboratories for which an investment in dedicated, automated peripherals is not warranted by the amount of analyses performed.

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REFERENCES

1. Mook WG. In *Isotope Hydrology. Proceedings of the IAEA (International Atomic Energy Agency) Symposium*, IAEA: Vienna 1970; 163.
2. Gillikin DP, Lorrain A, Bouillon S, Willenz P, Dehairs F. *Org. Geochem.* 2006; **37**: 1371.
3. Bouillon S, Dehairs F, Schiettekatte LS, Borges AV. *Limnol. Oceanogr.* 2007; **52**: 46.
4. Epstein S, Buchsbaum R, Lowenstam HA, Urey HC. *Geol. Soc. Am. Bull.* 1953; **64**: 1315.
5. Epstein S, Mayeda T. *Geochim. Cosmochim. Acta* 1953; **4**: 213.
6. Atekwana EA, Krishnamurthy RV. In *Handbook of Stable Isotope Analytical Techniques*, vol. 1 de Groot PA (ed). Elsevier: Amsterdam, 2004; 203.
7. McMillan DC, Preston T, Taggart DP. *Biomed. Environ. Mass Spectrom.* 1989; **18**: 543.
8. Prosser SJ, Brookes ST, Linton A, Preston T. *Biol. Mass Spectrom.* 1991; **20**: 724.
9. St-Jean G. *Rapid Commun. Mass Spectrom.* 2003; **17**: 419.
10. Torres ME, Mix AC, Rugh WD. *Limnol. Oceanogr. -Methods* 2005; **3**: 349.
11. Spötl C. *Isot. Environ. Health Stud.* 2005; **41**: 217.
12. Seth B, Schneider C, Storck F. *Rapid Commun. Mass Spectrom.* 2006; **20**: 1049.
13. Salata GG, Roelke LA, Cifuentes LA. *Mar. Chem.* 2000; **69**: 153.
14. Moodley L, Boschker HTS, Middelburg JJ, Pel R, Herman PMJ, de Deckere E, Heip CHR. *Mar. Ecol. Prog. Ser.* 2000; **202**: 289.
15. Verma MP. In *Symposium on Quality Assurance for Analytical Methods in Isotope Hydrology*, IAEA: Vienna, 2004.
16. Coplen TB. *Geochim. Cosmochim. Acta* 1996; **60**: 3359.
17. Miyajima T, Yamada Y, Hanba YT, Yoshii K, Koitabashi T, Wada E. *Limnol. Oceanogr.* 1995; **40**: 994.
18. NIST (National Institute of Standards and Technology). *Report of Investigation – Reference Materials 8535, 8536, 8537 (VSMOW, GISP, SLAP)*, 2003.

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