

## Automated analyses of $^{18}\text{O}/^{16}\text{O}$ ratios in dissolved oxygen from 12-mL water samples

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### Abstract

We introduce a new technique to routinely determine the  $^{18}\text{O}/^{16}\text{O}$  ratio of  $\text{O}_2(\text{aq})$  from 12-mL Exetainer® vials. Results were expressed in a  $\delta$ -notation versus air and the Vienna Standard Mean Ocean Water (VSMOW). Samples were prepared by creating a He-headspace and stripping  $\text{O}_2(\text{aq})$  from solution by shaking for 30 min on a wrist shaker. Subsequent isotope analysis of the extracted  $\text{O}_2(\text{g})$  was achieved by converting the entire headspace into a large sampling loop using a double-hole needle. This enabled admission of sufficient  $\text{O}_2(\text{g})$  into a packed A5-Å-molecular sieve column, where it was separated from  $\text{N}_2$  before admission to the isotope ratio mass spectrometer. The latter was tuned to an  $m/z$  ratio of 32, thus enabling direct determination of molecular  $\text{O}_2(\text{g})$  without conversion to  $\text{CO}_2$ . External standards consisted of dry air samples in helium-flushed vials and had between 1.5 and 16.8 parts per thousand  $\text{O}_2(\text{g})$  in a He matrix and a known isotopic composition of 0‰ air (+23.8‰ VSMOW). The method allows automated analyses of up to ~180 samples in one single batch and will provide new quantitative information about oxygen turnover in aqueous systems, including rates of gas transfer, redox processes, respiration, and photosynthesis. Repeat  $\delta^{18}\text{O}_{\text{O}_2(\text{aq})}$  measurements on samples with concentrations between  $15.6 \mu\text{mol L}^{-1}$  and saturation revealed standard deviations of 0.3‰. This is a typical precision encountered in continuous flow applications, and the method is available for studies using either  $^{18}\text{O}$ -labeled water to evaluate  $\text{O}_2$  gross production by incubation experiments or for natural abundance studies when isotope shifts are larger than 0.8‰. It may also become useful in microbiological and medical applications and can serve to quantify plant-gas exchange and soil gas processes.

The concentration of  $\text{O}_2(\text{aq})$  is one of the most commonly measured parameters in aqueous sciences and is routinely applied in marine and freshwater systems. Nevertheless, only a few studies have investigated its isotopic composition, although this additional information can provide detailed insight into the dissolution of atmospheric gases, respiration, and photosynthesis in aqueous systems. Previous applications revealed new aspects about oxygen turnover and the carbon cycle in the

deep sea (Kroopnick et al. 1972, 1976, 1980), surface ocean waters (Bender et al. 1992; Quay et al. 1993; Luz and Barkan 2000), ice cores (Sowers et al. 1989), groundwaters (Aggarwal and Dillon 1998), and fresh waters (Quay et al. 1995; Wassenaar 1999; Wang and Veizer 2000). Other workers have investigated oxygen isotope effects and their associated physical aspects, such as equilibrium dissolution (Kroopnick and Craig 1972; Benson and Krause 1980; Knox et al. 1992; Aregbe et al. 2002).

In the above studies, the sample volume represents the main analytical challenge. For instance, in one of the more recent studies by Wassenaar et al. (1999), the volume of water samples was 125 mL from which 10 mL of water were displaced with He to form a headspace. The oxygen was then extracted by shaking and subsequently transferred manually by syringe to a gas chromatograph that was linked to an isotope ratio mass spectrometer. Here we present an expansion of this technique that allows determination of the isotopic composition of  $\text{O}_2(\text{aq})$  ( $\delta^{18}\text{O}_{\text{O}_2(\text{aq})}$ ) on an automated basis and on much smaller sample volumes of less than 12 mL.

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This enables analyses of more samples with less effort in a single batch, thus providing more detailed biogeochemical information about  $O_2(aq)$  turnover with a better time resolution. For instance, diurnal variations of the aqueous oxygen cycle can be better investigated in this manner. Such quantitative knowledge can also reveal new indirect information about other essential elements of life (i.e., C and N) in aqueous systems because oxygen has strong influences on the aqueous carbon and nitrogen cycles through respiration, photosynthesis, and exchange with the atmosphere. With the  $^{18}O/^{16}O$  ratio of  $O_2(aq)$  responding systematically to these processes, such oxygen isotope information therefore helps to increase knowledge about fundamentally important parameters in aqueous sciences and the global carbon cycle. The method can also be applied in incubation experiments in which  $^{18}O$ -labeled water is used to evaluate gross primary production in aqueous systems. Furthermore, the method holds promise to reveal new information about rates and turnover in microbiological and medical applications.

The aims of this work were therefore (a) to test the validity of the new method on 12-mL samples and (b) to automate the technique to enable direct  $^{18}O/^{16}O$  determination in continuous-flow mode.

### Materials and procedures

**Preparation of samples**—Twenty liters of tap water were equilibrated with air at a temperature of 11.9°C for more than 5 h. During this time, the water was stirred at regular time intervals to enhance oxygen dissolution and equilibration. The quality of the experiments with tap water was also verified by control experiments using distilled and boiled water that revealed the same results.

By rinsing a 20-L container and the butyl caps of the Exetainers®, we ensured sterile laboratory sample preparation. The dry glass 12-mL Exetainers were wrapped in aluminum foil and heated for a minimum of 2 h at 200°C. These procedures killed any microbes that could have caused secondary isotope effects on the  $O_2(aq)$ .

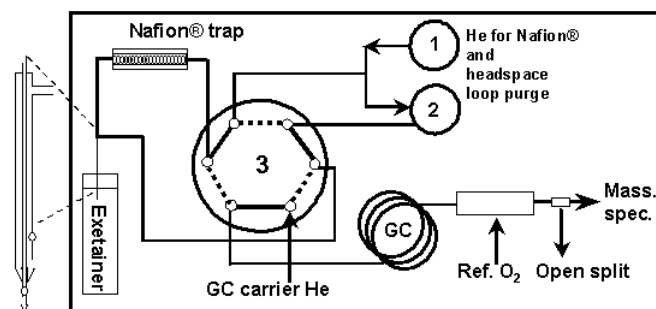
After cooling to room temperature, the 12-mL Exetainers (exact volume 12.1 mL) were completely filled with the equilibrated water and immediately capped with a butyl rubber septum of 3-mm thickness that was held in place with a plastic screw cap. For generation of the headspace, the filled vials were then loaded on a Gilson® auto sampler (model liquid handler XL222) with a capacity of 220 positions. The autosampler needle (GV instruments GV 30020058) had an outer diameter of 1.5 mm, a total length of 140 mm, and two side holes that were located 3 and 15 mm from the tip. A schematic outline of this needle is displayed in Fig. 1, to the left of the flow diagram. The tubing used in the entire flow path had inner and outer diameters of 0.6 and 1.6 mm, respectively (1/16 inch).

In headspace generation mode, the higher side hole was connected to a constant helium flow of 11 mL/min that was

fed through the side arm of the needle. The lower side hole was connected to a plastic tube that channeled the water that was replaced with He to waste. In sampling mode, the lower side hole was connected to a flow line that channeled the mixture of He and  $O_2(g)$  via the Nafion® trap to the isotope ratio mass spectrometer. For creation of the headspace, the needle was automatically lowered and inserted through the septum with the He flow on. This displaced the water in a nonturbulent manner. It also minimized the loss of  $O_2(aq)$  during headspace creation, which is important so as to avoid any secondary fractionation effects. After creation of the desired headspace, the needle was raised from the vial and, with the He flow still on, automatically moved to the next vial. This procedure ensured He headspaces of equal sizes for all samples in the batch. Headspace volumes ranged between 2.6 and 7.6 mL, depending on the penetration depth of the double-hole needle in headspace creation mode. The smaller headspaces were necessary to produce higher concentrations of the extracted  $O_2(g)$  for processing undersaturated oxygen samples.

To mobilize the  $O_2(aq)$  into the headspace, the vials were shaken for 30 min with a rate of ~250 strokes per min on a wrist shaker (model Stuart Flask shaker) that could hold up to 200 vials on an attached platform. When shaking for this time period, two effects have to be considered: (a) Most of the dissolved oxygen was stripped into the headspace, and (b) fractionations between the remaining  $O_2(aq)$  and  $O_2(g)$  within the vial were too small to be detected with the present continuous flow method; any expected isotope shifts due to headspace variations were well within the analytical error of the method.

The latter point will also be demonstrated in a later section of the manuscript and seen in Fig. 4, in which we show that expected values for air-equilibrated water were achieved with good accuracy and repeatability. We also tested heating the vials between temperatures of 50°C and 85°C as an alternative



**Fig. 1.** Diagram of sample flow on the AP2003 mass spectrometer and preparation unit with Gilson autosampler (model XL222 liquid handler) to hold 220 Exetainers not included. The circled numbers (1) and (2) represent electronic valves that permit helium flow to be switched on or off and the circled number (3) represents a six-position Valco valve that permits purging of the GC column or flow of  $O_2(g)$  from the headspace in the Exetainer to the mass spectrometer. A detailed explanation of the analyses sequence can be found in the section *Modification of the flow path*.

method to partition  $O_2(aq)$  into the headspace. However, this method yielded less consistent results and added the difficulty of keeping a constant temperature on a heating block, which also necessitated raising the Gilson autosampler. We therefore discarded this option and conducted the analyses at room temperature.

Undersaturated water samples of known concentrations were established in the laboratory by mixing oxygen-free and saturated waters to reach the desired concentration. First, a known aliquot of water was pipetted into a 12-mL Exetainer, which was then capped. Water and headspace were then purged with He. For this, a capillary with an inner diameter of 320  $\mu\text{m}$  was connected to a He flow  $> 60 \text{ mL min}^{-1}$  and fed through a needle with 1.1 mm inner diameter. Both were inserted through the septum. The He-dispensing capillary was then lowered to the bottom of the vial under the water surface. By flushing for 1 min, all gases in the water and headspace were driven out through the space between the capillary and the needle inside wall, thus generating a blank sample. In order to create solutions with 15.6, 31.2, 78.1, and 156.3  $\mu\text{mol L}^{-1}$  (i.e., 0.5, 1.0, 2.5, and 5  $\text{mg L}^{-1}$ ), the oxygen-free water was then topped up with a known amount of saturated water. The latter was added directly through the septum with a gas tight syringe. According to Henry's Law, air-equilibrated water at a temperature of 11.9°C has a concentration of 343  $\mu\text{mol L}^{-1}$  ( $\sim 11 \text{ mg L}^{-1}$ )  $O_2$  (Rettich et al. 2000). This solution was mixed with the oxygen-free water to reach the desired concentration with a total volume of 9.5 mL (i.e., a headspace of 2.6 mL). This is the minimum allowable size of headspace to avoid uptake of water into the flow path. For example, 4.3 mL of  $O_2$ -saturated water was added to a volume of 5.2 mL oxygen-free water to create a standard of 156.3  $\mu\text{mol L}^{-1}$   $O_2$ .

Flushing dry 6.9-mL Exetainers in the same manner as generating a headspace on an aqueous sample was used to generate dry blank samples. The flushing time for each vial was 10 min, and in order to ensure better quality of the blanks, a small amount of vacuum grease was added to the rim of the Exetainers. The dry blank samples were also used to produce oxygen standards of known concentrations and isotope values by adding various amounts of air via syringe. For this, fresh outside air was let into the syringe barrel by completely removing the plunger. This avoided any diffusional isotope effects that might result from the air partitioning inside the syringe needle. For the same reason the syringe was completely emptied during injection. All dry air standards had an isotopic composition of 0‰ air ( $+23.8\text{‰ VSMOW}$ )  $\pm 0.3\text{‰}$ , the ubiquitous value for air. The latter had a previously accepted value of 23.5‰ (i.e., Kroopnick and Craig 1972) but the most recent internationally accepted value for air has been adjusted to 23.8‰ (Coplen et al. 2002). By adding air aliquots of 50, 100, 200, 400, and 600 mL to He-flushed 6.9-mL Exetainers,  $O_2$  standards with concentrations of 1.5, 3.0, 5.9, 11.5, and 16.8 parts per thousand  $O_2(g)$  were established.

For testing the method on water with different  $\delta O_{2(aq)}$  values, field samples were collected from Lochan Dubh, a small oligotrophic lake near the Glasgow University lake research station at Loch Lomond. They originated from depths of 7.0, 3.5, and 0.1 m below the surface and were filled into 12-mL Exetainers, to which 200  $\mu\text{L}$  of a saturated  $\text{HgCl}_2$  solution had been added in order to avoid any secondary biological activity. Samples were kept at a temperature of 4°C and prepared in the same manner as the laboratory samples.

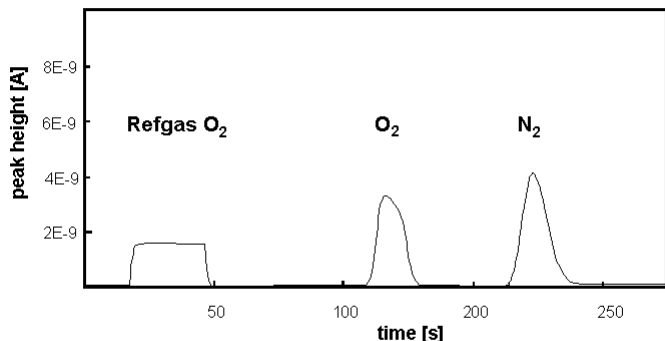
*Modification of the flow path*—Fig. 1 shows the flow path of the Analytical Precision® (model AP2003) preparation unit including Gilson autosampler. We modified the conventional  $\text{CO}_2$  setup for carbonate and water analyses by converting the headspace, including the flow path, with the Nafion trap into a large-volume sample loop. This was achieved by connecting the He carrier gas to the side arm of the double-hole needle that connected to the upper hole at 15 mm distance from the needle tip. The lower needle hole was connected to the outlet that carried the extracted  $O_2$  via the gas chromatography (GC) column to the isotope ratio mass spectrometer with a flow rate of 11  $\text{mL min}^{-1}$ . The analysis succession was as follows: The needle and the Nafion trap were purged with the needle still outside the Exetainer. During this step, the electronic valves (1) and (2) in Fig. 1 were open for 30 s while the central Valco® valve (3) was switched to the flow path, marked by solid lines in the figure.

Then the needle pierced the Exetainer septum, with the flow still on, until both side holes were underneath the septum. The He flow from valves (1) and (2) was maintained for 20 s to pressurize the headspace and to ensure backflow in the direction of the Nafion trap. During this step, a reference gas pulse was channeled to the mass spectrometer for a time period of 30 s.

After this, the Valco valve (3) automatically rotated by 60° to open the flow path (indicated by the thick dashed lines in Fig. 1) for 30 s while the flow from valves (1) and (2) stopped. The Valco valve rotation allowed flow of the  $O_2(g)$  from the headspace to the isotope ratio mass spectrometer via the Nafion trap and GC column. The latter was an A5-Å-molecular sieve (80/100 mesh size) packed column, with an inner diameter of 2 mm, an outer diameter of 3 mm, and a length of 77 cm, that enabled separation of  $O_2$  from  $N_2$ . The column was held at ambient temperature and the He flow rate was 11  $\text{mL/min}$ .

Subsequently, the Valco valve (3) rotated back to its original position with the solid line flow path, the needle was retrieved from the Exetainer and valves (1) and (2) opened again to flush for the next analyses.

Fig. 2 shows the good separation of  $O_2$  from  $N_2$ . Even though the mass spectrometer was specifically tuned for oxygen, this separation was necessary to avoid peak overlapping that would admit two gases with different ionization properties to the mass spectrometer source. Associated changes in the  $O_2/N_2$  ratio of samples and standards with different con-



**Fig. 2.** Separation of reference gas, oxygen, and nitrogen peaks

concentrations would result in inconsistent <sup>18</sup>O/<sup>16</sup>O measurements. The gas chromatographic column was not able to separate Ar from O<sub>2</sub>(g) at ambient temperatures, thus giving rise to similar concerns. Nevertheless, due to small Ar concentrations we achieved good repeatability of standards and field samples of various O<sub>2</sub>(aq) contents, thus rendering this influence insignificant at this scale.

*Analyses with quality assurance and control*—For headspace analyses, the piercing of the septum always took place while He was bleeding from both side holes of the needle, thus avoiding any outside air leaking into the sample headspace. For direct isotope determination, the mass spectrometer was tuned to an m/z ratio of 32. All <sup>18</sup>O/<sup>16</sup>O isotope ratios were expressed in ‰ with

$$\delta^{18}\text{O} = \left[ \frac{(^{18}\text{O}/^{16}\text{O})_{\text{SAMPLE}} - ^{18}\text{O}/^{16}\text{O}_{\text{STANDARD}}}{^{18}\text{O}/^{16}\text{O}_{\text{STANDARD}}} \right] \times 1000(\text{‰})$$

Although air was used as the external standard, samples can either be referred to by either the international Vienna Standard Mean Ocean Water (SMOW) or air. With the delta scale being a relative scale, the conversion from  $\delta^{18}\text{O}_{\text{air}}$  versus air to  $\delta^{18}\text{O}_{\text{VSMOW}}$  is

$$\delta^{18}\text{O}_{\text{VSMOW}} = \delta^{18}\text{O}_{\text{air}} + 23.8 + (\delta^{18}\text{O}_{\text{air}} \times 23.8/1000).$$

Conversely the VSMOW values are converted back to air by

$$\delta^{18}\text{O}_{\text{air}} = \delta^{18}\text{O}_{\text{VSMOW}} + A + (\delta^{18}\text{O}_{\text{VSMOW}} \times A/1000)$$

where  $A = -23.8/(23.8/1000 + 1)$ .

Air standards of different concentrations were analyzed to prove the absence of any dependency between peak size and isotope ratio (linearity effects). For undersaturated water samples of less than 156 μmol L<sup>-1</sup> O<sub>2</sub>, the trap current in the tuning file was raised to a value of 300 μA in order to yield higher sensitivity. This caused linearity effects for standards that should be isotopically equal. These secondary effects were cor-

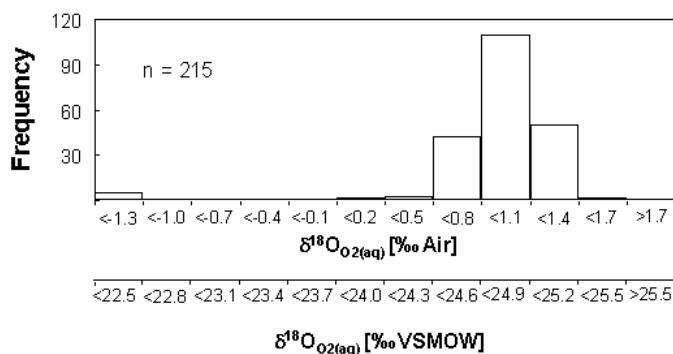
rected for with air standards of different concentrations. For measurements of the undersaturated water samples, the size of the headspace was decreased to 2.6 mL, thus ensuring a higher concentration of O<sub>2</sub>(g) in the headspace.

Sample analysis was started immediately after O<sub>2</sub> extraction on the wrist shaker. However, the same results were still obtained after a time period of more than 106 h. For setting up longer analyses, a series of air standards at various concentrations and blanks were run every 10 samples. This allowed a total of ~180 water samples to be analyzed in one run. The reference gas was oxygen of 99.995% purity that had an isotopic composition of -22.1‰ air (1.2‰ VSMOW). We also measured several samples of molecular oxygen from a different cylinder as a control that had an isotopic composition of +4.6‰ air (+28.5 SMOW) ± 0.3‰. Blanks revealed oxygen peak heights of less than 0.03 nanoA, which, after a period of > 24 h, were close to the base line. This corresponds to less than 0.12 parts per thousand O<sub>2</sub>(g).

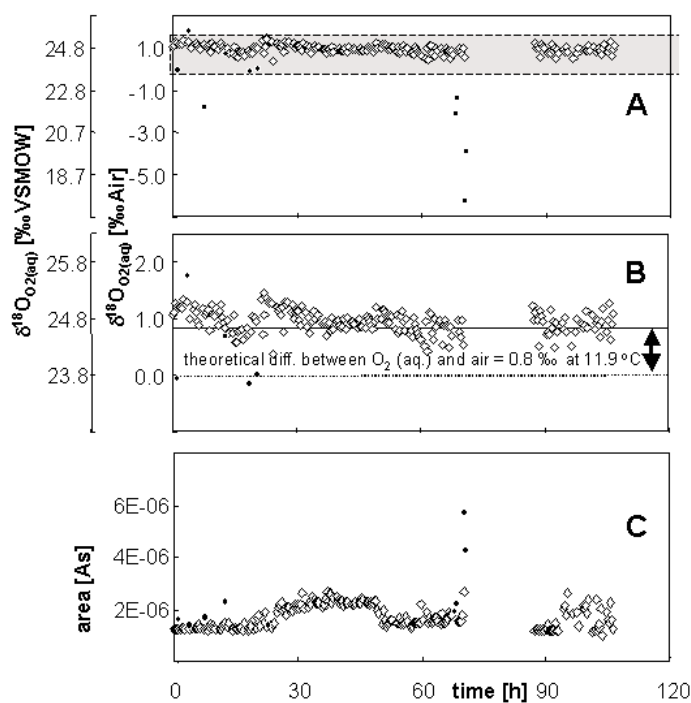
### Assessment

A multitude of repeat measurements revealed a standard deviation of better than 0.3‰ for the entire procedure including oxygen extraction and analysis. Fig. 3 shows the frequency distribution for an experiment with water samples that were run up to 106 h after extraction. Of 215 measurements, 206 (95.8%) fell between values of 0.2‰ and 1.4‰ air (24.0‰ and 25.2 ‰ VSMOW), with the majority of data averaging around a value of 0.8‰ air (24.6 ‰ VSMOW). This matches the expected value for air-equilibrated O<sub>2</sub>(aq). Literature values for <sup>18</sup>O equilibrium enrichment in O<sub>2</sub>(aq) when compared to O<sub>2</sub>(g) range between 0.7‰ and 0.8‰ for an equilibration temperature of 11.9°C (Kroopnick and Craig 1972; Benson and Krause 1980). Here we used the latter value of 0.8‰.

Fig. 4A shows all measurements of atmospherically equilibrated laboratory waters including outliers (black symbols). Fig. 4B shows how the majority of measurements are scattered around the solid line that represents the expected value for O<sub>2</sub>(aq) that was equilibrated with air oxygen at 11.9°C, i.e.,



**Fig. 3.** Histogram showing the distribution of all data points during a 105-h experiment with saturated O<sub>2</sub>(aq) samples that were shaken for 30 min.



**Fig. 4.**  $\delta^{18}O_{O_2(aq)}$  composition for a 106-h experiment using air saturated water with all data including outliers as black markers (A), a zoom-in of the gray zone (B), and peak areas (C).

+0.8‰ air (+24.6‰ VSMOW). Fig. 4C shows variations of peak areas resulting mainly from different size headspaces, which originated from different flow rates during flushing.

Increasing the mass spectrometer trap current from 200  $\mu A$  to a value of 300  $\mu A$  increased the sensitivity and enabled analyses of undersaturated water samples. However, it resulted in linearity effects that increased the  $\delta^{18}O_{O_2(aq)}$  values with increasing concentration. Fig. 5A shows this effect on the uncorrected isotope values of air standards with different concentrations. The regression equation was established with dry air standards and then applied to the measurements from undersaturated aqueous samples. Results were corrected by adjusting the determined measurement to an ideal value that matched the height of the reference gas peak (point of reference, Fig. 5). Performing this correction with respect to the peak height was preferred because the areas between reference gas and sample peaks were not comparable, due to different shapes (cf. Fig. 2). The linearity correction led to increases in isotope values with smaller peak heights and decreases of those with larger peak heights (Fig. 5A). After correction, samples with  $O_2(aq)$  concentrations of 15.6, 31.2, 78.1, and 156.3  $\mu mol L^{-1}$  again scattered around the expected value for air-equilibrated water (Fig. 5B).

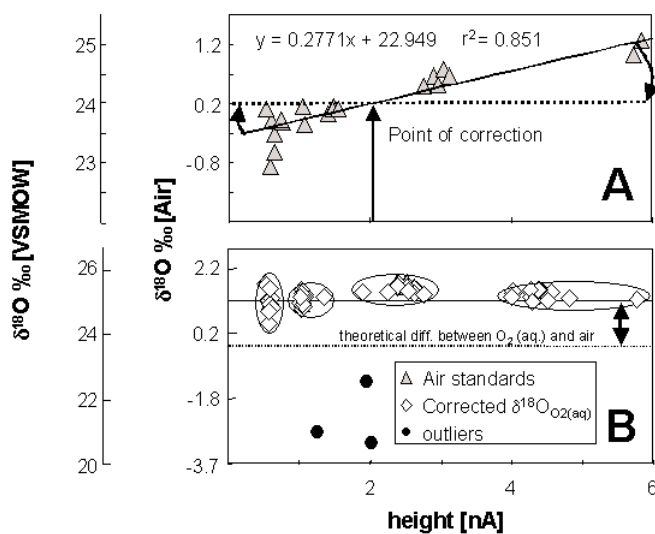
Fig. 6 shows samples from the field that are up to 8.9‰ enriched when compared to air-equilibrated water. This enrichment results from preferential uptake of  $^{16}O$  during predominant respiration, which enriches the remaining  $O_2(aq)$  in

$^{18}O$ . Conversely, the lake-surface samples were influenced by dominance of photosynthesis, thus depleting the  $O_2(aq)$  in  $^{18}O$  by up to 2‰ (Fig. 6). This results from splitting the water molecule during photosynthesis and imparting its usually more negative isotopic composition into the  $O_2(aq)$ . Field samples were run within 40 h of sampling.

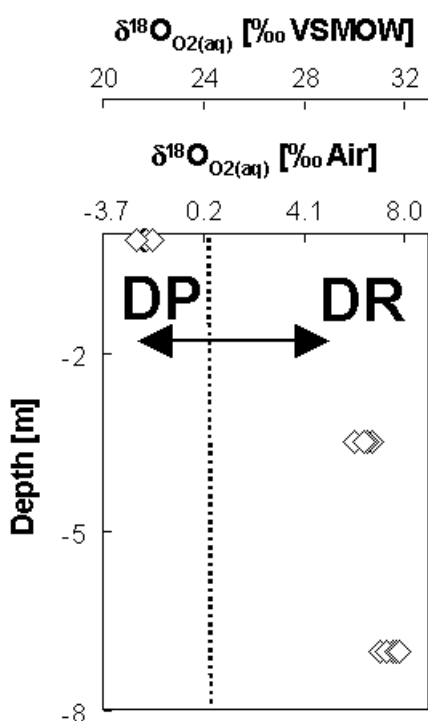
### Comments and recommendations

Our experiments showed that direct online oxygen isotope measurements without conversion to  $CO_2$  are possible, accurate, and precise with this type of continuous flow mass spectrometer. Converting the entire headspace into a sample loop also allows much smaller sample volumes of water. This modification of the flow path enables extraction of sufficient  $O_2$  to produce reasonable peak sizes for isotope measurements of undersaturated samples. The significant decrease of sample volume improves field logistics and allows more routine analyses and replicates in longer batch runs, which can be automatically analyzed.

Samples with undersaturated oxygen, particularly, are prone to in-gassing of atmospheric  $O_2(g)$ . This danger of contamination was realized a long time ago during water sampling for oxygen concentration measurements in the laboratory and led to the increasing use of field equipment to allow in situ  $O_2(aq)$  concentration measurements. With mass spectrometers being difficult to transport to the field, samples will still have to be brought



**Fig. 5.** Air standards (A) and undersaturated samples (B) that were run at different concentrations at a high sensitivity tuning with a trap current of 300  $\mu A$ . (A) The triangles show the uncorrected  $\delta^{18}O_{O_2}$  values of air standards. Linearity corrections were carried out via the peak height with the arrow marking the point of correction that is the same height as the reference gas peak. (B) The encircled diamonds are the corrected  $\delta^{18}O_{O_2(aq)}$  values for water samples with concentrations of 15.6, 31.2, 78.1, and 156.3  $\mu mol L^{-1}$  (i.e., 0.5, 1, 2.5, and 5.0  $mg L^{-1}$ ), increasing from left to right. The corresponding standard deviations for these groups were 0.4‰, 0.2‰, 0.1‰, and 0.1‰, respectively.



**Fig. 6.** Field samples from Lochan Dubh run within 40 h after collection showing enrichment in  $^{18}O$  with increasing depth and depletion at the surface. The vertical dashed line represents the isotope value expected for air-oxygen saturated water (+0.8‰ or +24.6‰ VSMOW) with slight variations due to different temperatures) whereas “DP” and “DR” signify dominance of photosynthesis and respiration respectively.

back to the laboratory for  $\delta^{18}O_{O_2(aq)}$  determinations. Associated longer storage demands leak-tight bottles and good preservation of samples. The long-term sealing quality of Exetainers still has to be established, and we recommend rapid analyses of the  $\delta^{18}O_{O_2(aq)}$  after sampling. For field samples with longer storage times, sample vessels with a better seal such as Winkler titration bottles with fritted and greased glass stoppers or Wheaton bottles with thicker crimp septa may be more suitable. Greasing of Exetainer rims or dipping the top of the vials in hot wax after sampling as an additional seal may also be an option.

The presented method is readily available for detailed studies of  $O_2(aq)$  and the aqueous carbon cycle in marine and freshwater systems. The oxygen isotope trend to be measured should be either larger than 0.8‰ in natural abundance applications or the method may be used in experiments with  $^{18}O$  labels to evaluate aqueous gross productivity. Determination of the isotopic composition of molecular oxygen in gas samples is also possible with this method and thus provides a new tool to monitor the partitioning of  $O_2$  between aqueous and gaseous phases and the study of macrophyte production of  $O_2$ .

Running repeats of air, laboratory, and field samples as well cylinder  $O_2$  gas that were all isotopically different to the reference gas produced good repeatability of less than 0.3‰. It also

proved the absence of any memory effects between samples. Furthermore, the fact that blanks were always close to the baseline is proof that contamination from the outside air during analysis is negligible during analyses. With the vials being slightly overpressurized during preparation, it seems plausible that negligible amounts of outside air entered the Exetainers. This also ensured good quality of analyses up to 106 h after preparation of the vials, thus allowing long run times and preparation of larger amounts of samples at the same time. Nevertheless vials should be stored without He headspace after sampling.

For preparation of the air-saturated laboratory samples, humidity, and temperature fluctuations as well as insufficient equilibration time may have caused variations in the  $\delta^{18}O_{O_2(aq)}$ . However, these uncertainties were not further investigated here because our results indicated that the expected  $\delta^{18}O_{O_2(aq)}$  values for air-equilibrated water were achieved with good accuracy. Some of the outliers not shown in Fig. 4 had incorrectly integrated sample peaks that were caused by electronic inconsistencies, which may, in turn, have been a result of the molecular oxygen attacking the tungsten filament. These results were discarded from the sample population, however we recommend the use of more robust thorium or yttrium filaments for longer oxygen isotope experiments. The other outliers shown in Figs. 4A and 4B result from either incompletely closed vials that leaked air or were caused by poor peak centering. The fact that only small peak area changes can cause drastic outliers shows that small amounts of air leaking in are sufficient to alter the isotopic composition of samples. Usually, these outliers are drastically different in their isotopic composition and can easily be singled out when running multiple samples.

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