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## Determining the stable isotope ratio of total dissolved inorganic carbon in lake water by GC/C/IRMS

**Abstract**—A simple, precise method for determining the carbon stable isotope ratio of total dissolved inorganic C ( $\Sigma\text{CO}_2$ ) in freshwater samples is described. Water samples are packed in airtight glass bottles of known inner volume (~70 ml) with no air bubbles. Subsequently, a headspace of 5.0 ml is created inside each bottle with pure helium gas, and each sample is acidified by adding 0.5 ml of a  $\text{CO}_2$ -free, 6.0 N HCl solution. After the original dissolved  $\text{CO}_2$  has equilibrated with the headspace gas, a portion of this headspace gas is subsampled and injected into the GC/C/IRMS (gas chromatograph/combustion furnace/isotope-ratio mass spectrometer) system to determine the carbon isotope ratio of the  $\text{CO}_2$ . The isotope ratio of  $\text{CO}_2$  remaining in the liquid phase is calculated by temperature-dependent isotope discrimination between gas and aqueous phases. The isotope ratio of  $\Sigma\text{CO}_2$  of the original sample is then derived assuming isotope mass balance. The analytical precision of this method is  $\pm 0.1\%$ . The method enables a single operator to determine the isotopic ratio in at least 60 lake-water samples within 3 d of sampling.

Dissolved inorganic carbon ( $\Sigma\text{CO}_2$ ) represents the largest reservoir of carbon in harmonic lakes. Bicarbonate is the predominant species of  $\Sigma\text{CO}_2$ , and it plays a role as the major source of alkalinity in these lake waters (Wetzel 1975). The concentration and stable carbon isotope ratio ( $\delta^{13}\text{C}$ ) of  $\Sigma\text{CO}_2$  in individual lakes are primarily determined by physical processes such as interaction with atmospheric  $\text{CO}_2$  and by the geological setting of the lake which governs the retention time of lake water and the supply of  $\Sigma\text{CO}_2$  from the watershed. However, the distribution of  $\Sigma\text{CO}_2$  and associated  $\delta^{13}\text{C}$  values within a lake are also strongly affected by metabolic processes in and around the lake (McKenzie 1985; Pearson and Coplen 1978). The concentration of  $\Sigma\text{CO}_2$  in the euphotic zone of a lake is reduced by photosynthetic assimilation of dissolved  $\text{CO}_2$  and  $\text{HCO}_3^-$ , and concomitantly, the  $\delta^{13}\text{C}$  of the  $\Sigma\text{CO}_2$  remaining in solution rises because  $^{12}\text{CO}_2$  is usually incorporated preferentially into phytoplankton biomass. The

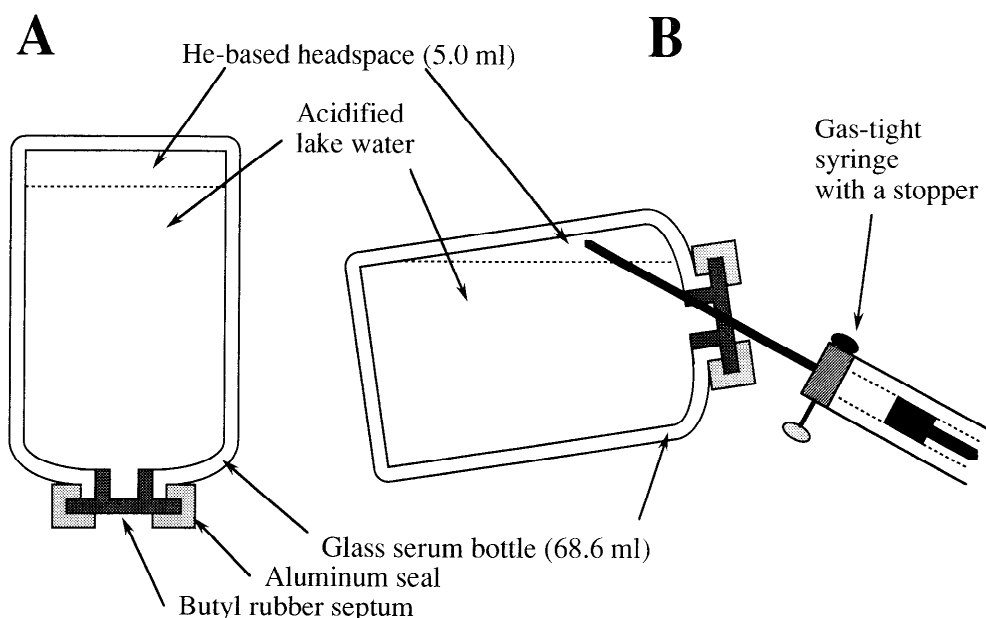


Fig. 1. Glass serum bottles containing acidified lake water and headspace. A. Equilibrating the distribution of  $\text{CO}_2$  between the headspace and the liquid phase. B. Withdrawing a portion of the headspace gas with a gas-tight syringe.

degree of discrimination due to photosynthesis may vary depending on ecophysiological conditions such as species composition of primary producers and the availability of  $\Sigma\text{CO}_2$  for them (Berry 1988; Farquhar et al. 1982; Fry and Sherr 1988). In the tropholytic zone, detrital organic material, which has lower  $\delta^{13}\text{C}$  than ambient  $\Sigma\text{CO}_2$ , undergoes mineralization that results in a gradual decrease in the average  $\delta^{13}\text{C}$  value of the  $\Sigma\text{CO}_2$  in this zone. Thus, the stable isotope ratio of the  $\Sigma\text{CO}_2$  reflects the internal biogeochemical structure of a lake. In addition, this value can be used to compare biogeochemical carbon cycling between lakes (e.g. Oana and Deevey 1960).

The activity of bicarbonate in normal lakes is at least  $10^{-4}$ . This concentration permits relatively accurate measurement of the  $\delta^{13}\text{C}$  by a simple headspace method combined with a GC/C/IRMS (gas chromatograph/combustion furnace/isotope-ratio mass spectrometer). The method enables a single operator to determine the isotopic ratio in at least 60 lake-water samples within 3 d of samplings, making it a superior method for routine analysis. Moreover, this method can be combined with  $\delta^{13}\text{C}$ -labeled tracers to estimate rate constants of metabolic reactions that yield  $\Sigma\text{CO}_2$  from these tracers.

We selected glass serum bottles as sample containers for isotopic analysis (Nichiden-Rika Glass Co., type V-50; inner volume,  $68.6 \pm 0.5$  ml); for simplification, we use ml ( $\mu\text{l}$ , liter) as the volumetric unit of both gases and aqueous solutions. Each bottle was fitted with a butyl rubber septum and an aluminum seal (Fig. 1). Lake-water samples were collected with a Van Dorn sampler (3.0-liter inner volume) at desired depths. Samples were dispensed from the sampler to the glass serum bottles through a rubber tube to minimize contact with the atmosphere. The bottles were carefully sealed so no air bubbles were

inside. If the samples need lengthy storage before analysis, 0.20 ml of a saturated  $\text{HgCl}_2$  solution should be injected into each bottle as a fixative, while the same volume of sample water is removed with another syringe. In addition, the top of each sample bottle should be covered with paraffin to prevent invasion of atmospheric  $\text{CO}_2$  through the septa. Otherwise, addition of  $\text{HgCl}_2$  and sealing with paraffin can be omitted.

Apart from the subsamples for  $\delta^{13}\text{C}$ , portions of each water sample were also placed in another serum bottle ( $\sim 30$  ml) and in two DO (dissolved oxygen) bottles ( $\sim 100$  ml) for later analyses of  $\Sigma\text{CO}_2$  (total organic C analyzer, Shimadzu TOC-500; analytical precision  $<1\%$  as C.V.) and DO (Winkler titration). Water temperature and pH were determined in situ with a thermistor thermometer and a combined glass electrode.

The following sample treatments and analysis were done in a room where temperature was kept constant at  $23^\circ\text{C}$ . We created a headspace inside each sample bottle by injecting 5.0 ml (at  $23^\circ\text{C}$ , 1 atm) of ultrapure He gas (Kyoto Teisan Co., nominally  $>99.9999\%$ ) into each bottle with a 10-ml gas-tight syringe (Dynatech Precision Sampling Corp.) through the septum. The same volume of sample water was removed with another plastic syringe. The sample was then acidified with 0.50 ml of  $\text{CO}_2$ -free HCl solution (6.0 N), which was likewise injected with a plastic syringe into the serum bottle and thoroughly mixed by vigorous hand shaking. This amount of HCl (final concn  $\sim 50$  mM) is sufficient to quantitatively convert all carbonate and bicarbonate anions into aqueous  $\text{CO}_2$ . The sample bottles were then left upside down (Fig. 1A) in the dark for at least 40 h, during which time the aqueous  $\text{CO}_2$  gradually equilibrated with the headspace gas.

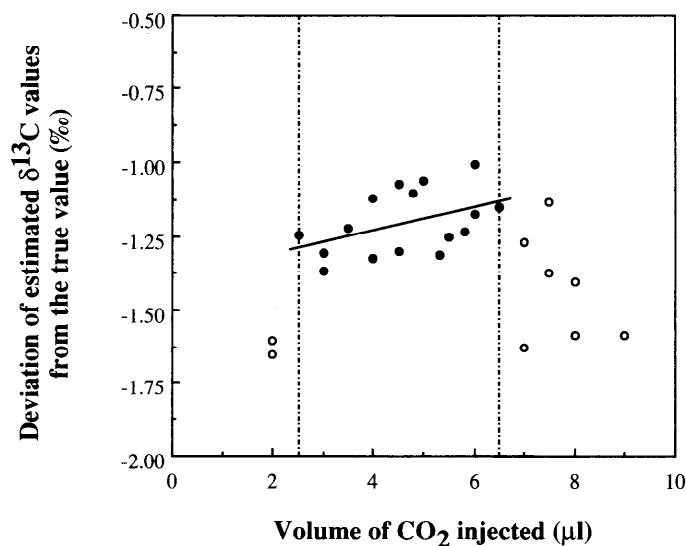


Fig. 2. Dependence of the  $\delta^{13}\text{C}$  value reported by the GC/C/IRMS on the quantity of injected  $\text{CO}_2$ . Ordinate shows the difference of reported values from the true  $\delta^{13}\text{C}$  value ( $-26.34\text{‰}$ ) of the injected  $\text{CO}_2$ .

To prepare the  $\text{CO}_2$ -free HCl solution, we stripped 6.0 N HCl of dissolved  $\text{CO}_2$  by bubbling with the same He gas as above for at least 3 h. Although a trace amount of  $\text{CO}_2$  seemed to remain in solution after bubbling, we confirmed by calculation that this residual  $\text{CO}_2$  could not give rise to any detectable errors in the later measurement of  $\delta^{13}\text{C}$  values.

After equilibration, a defined amount of the headspace gas was withdrawn with a 1-ml gas-tight syringe (Dyna-tech; Fig. 1B) and injected into the GC/C/IRMS (Finigan-Mat delta-S/GC, equipped with a Chromapack PLOT-Q column; see Sugimoto et al. 1991; Ricci et al. 1994). Conceptually, the GC/C/IRMS system consists of a gas chromatograph and an isotope-ratio mass spectrometer interfaced with each other through a combustion furnace. The injected headspace gas mixture is separated into constituents through the GC column, using an He carrier, and undesired constituents are excluded at the interface by backflushing the carrier. The combustion furnace operates at  $820^\circ\text{C}$ , so as to convert organic constituents into  $\text{CO}_2$ . Although no constituent had to be combusted for our purpose, we operated the furnace at this temperature to stabilize analytical conditions and reduce retention time of the gas flowing within the interface.

After a series of analyses, the GC/C/IRMS prints out calculated isotope ratios ( $\delta^{13}\text{C}$ ), along with some chromatographic parameters such as retention time, peak width, and peak area. These parameters are estimated from the temporal changes in the ion-current signal for M/e (molecular weight per electronic valence) of 44 ( $^{12}\text{C}^{16}\text{O}_2^+$ ). As the fraction of  $^{12}\text{C}^{16}\text{O}_2$  exceeds 98% in natural  $\text{CO}_2$  samples, the peak area for M/e 44 is closely related to the amount of  $\text{CO}_2$  injected into the GC. Thus, we calculated the concentration of  $\text{CO}_2$  in the analyzed headspace gas from the peak area for M/e 44 and the volume of the sample injected.

The  $\delta^{13}\text{C}$  values of the samples are determined in the GC/C/IRMS by comparison with a reference  $\text{CO}_2$  gas of known isotope ratio ( $\delta^{13}\text{C} = -26.34\text{‰}$ ) which is introduced from a reservoir via a mixing chamber to the IRMS. However, the reported  $\delta^{13}\text{C}$  values usually suffer a small bias, the degree of which depends on the amount of  $\text{CO}_2$  involved in the injected sample. Figure 2 shows the deviation of the estimated  $\delta^{13}\text{C}$  for a standard  $\text{CO}_2$  gas of known  $\delta^{13}\text{C}$  as a function of the amount of  $\text{CO}_2$  injected. The relationship between the degree of deviation and the injection volume is quasi-linear in the range between 2.5 and 6.5  $\mu\text{l}$  of injected  $\text{CO}_2$ , with the smallest deviation found around 6.0  $\mu\text{l}$   $\text{CO}_2$ . The injection volume of samples was controlled in such a way that the amount of  $\text{CO}_2$  injected would fall within this range. The reported  $\delta^{13}\text{C}$  values for the samples were then corrected using a best-fit line to the standard data (Fig. 2) and the amount of  $\text{CO}_2$  in the injected samples which was estimated from the peak area.

The response factor, which correlates the peak area of M/e 44 to the amount of  $\text{CO}_2$  injected as well as the best-fit line for the deviation of the estimated  $\delta^{13}\text{C}$  from the true value (Fig. 2), depends on several analytical conditions, including flow rate of the carrier gas, split ratio at the injection port, temperatures of column oven and combustion furnace, etc. Thus, it is necessary to recalibrate every day.

The isotope ratio of  $\text{CO}_2$  present in the headspace is somewhat higher than that of  $\text{CO}_2$  in aqueous solution at equilibrium with the headspace. Thus, it is necessary to discriminate  $\text{CO}_2$ -C isotopes between the gas and the aqueous phases to estimate the isotope ratio of  $\Sigma\text{CO}_2$  in the original samples. We used several methods to assess the distribution of  $\text{CO}_2$  between the headspace and the aqueous phase and the isotopic discrimination due to gas-solution exchange at the equilibrium.

First, we used a standard  $\text{CO}_2$  gas of known isotope ratio to evaluate the discrimination. The glass serum bottles were first filled with a  $\text{CO}_2$ -free HCl solution and sealed with no bubbles inside. The headspace was formed with ultrapure He, as described above. Various amounts (350–2,500  $\mu\text{l}$  at  $23^\circ\text{C}$ , 1 atm) of the standard  $\text{CO}_2$  gas ( $\delta^{13}\text{C} = -26.34\text{‰}$ ) were then added to the headspace with a gas-tight syringe. (Further acidification with 6.0 N HCl was omitted in this case.) The  $\text{CO}_2$ -free HCl solution used here was prepared by purging a 60 mM HCl solution with the ultrapure He gas for 3 h. The absence of dissolved  $\text{CO}_2$  was confirmed by injecting the solution into the TOC analyzer (detection limit,  $\sim 10 \mu\text{M}$ ).

The concentration of  $\text{CO}_2$  in the headspace at equilibrium was approximately proportional to the amount of initially loaded  $\text{CO}_2$  (Fig. 3A). Because the Ostwald solubility coefficient for  $\text{CO}_2$  at  $23^\circ\text{C}$  is 0.872, the amount of  $\text{CO}_2$  that remains in the headspace should be 8.27% of the amount of initially loaded  $\text{CO}_2$  if  $\text{CO}_2$  in the headspace (5.0 ml) is equilibrated with aqueous  $\text{CO}_2$  dissolved in the liquid phase (63.6 ml). However, the actual percentage of  $\text{CO}_2$  evolved into the headspace was slightly lower than the expected value, especially when the amount of initially loaded  $\text{CO}_2$  was relatively large. This deviation

is thought to be an effect of internal pressure, which would have been elevated due to the addition of  $\text{CO}_2$  itself.

The equilibrium isotopic difference between the gas and aqueous phases depends on temperature. When pure  $\text{CO}_2$  gas is equilibrated with aqueous  $\text{CO}_2$  at 1 atm and  $23^\circ\text{C}$ , the  $\text{CO}_2$  in the gas phase is  $1.07\text{‰}$  higher in  $\delta^{13}\text{C}$  than the  $\text{CO}_2$  in the aqueous phase (Mook et al. 1974; O'Leary 1984). The measured  $\delta^{13}\text{C}$  values of the headspace  $\text{CO}_2$  in our experiments are plotted in Fig. 3B. The  $\delta^{13}\text{C}$  values of the initially loaded  $\text{CO}_2$ , estimated from these data by isotope mass balance assuming the discrimination of  $1.07\text{‰}$ , are also plotted in Fig. 3B. These values are generally close to the expected value ( $-26.34\text{‰}$ ), especially when the amount of initially added  $\text{CO}_2$  exceeded  $1,500\text{ }\mu\text{l}$ . The smaller the amount of  $\text{CO}_2$  loaded initially, the greater the upward deviation of the estimated value. This deviation is thought to be due to small amounts of contamination by atmospheric  $\text{CO}_2$ , which may have remained in the 60 mM HCl solution used as the solvent in this experiment. This supposition would be consistent with the much higher isotope ratio of atmospheric  $\text{CO}_2$  ( $\sim -7\text{‰}$ ) than the standard  $\text{CO}_2$  used here. Therefore, the measurement should not be affected by this blank effect when lake water is analyzed.

Our second preliminary experiment was done with natural  $\Sigma\text{CO}_2$  dissolved in surface water of Lake Biwa, Japan, which has an unknown isotope composition. This water contains  $585\text{ }\mu\text{mol }\Sigma\text{CO}_2\text{ liter}^{-1}$ , as determined by the TOC analyzer. A number of sample bottles were filled with lake water and sealed. The samples were first acidified by injecting 0.50 ml of a  $\text{CO}_2$ -free, 6.0 N HCl solution, with the same volume of sample water removed. Headspaces of various volumes (2.5–30.0 ml) were then made within the bottles by replacing portions of acidified sample water with ultrapure He gas.

Both the  $\text{CO}_2$  concentration in the headspace and the  $\delta^{13}\text{C}$  of the headspace  $\text{CO}_2$  at equilibrium are plotted as a function of the volume of the headspace in Fig. 4. Theoretically, if the isotopic difference of  $\text{CO}_2$  between the gas and aqueous phases is essentially constant within the range of experimental conditions, the  $\delta^{13}\text{C}$  of the headspace  $\text{CO}_2$  should be a simple linear function of the headspace volume; furthermore, the difference between the  $\delta^{13}\text{C}$  values at a headspace volume of 0.0 ml and at 68.6 ml (the whole inner volume of the bottle), which are given by extrapolation of the regression line, should be equal to the equilibrium discrimination of  $\text{CO}_2$  between the gas and the aqueous phases. In the case of Fig. 4, the isotopic difference calculated in this way was  $1.06\text{‰}$  ( $=0.0154 \times 68.6$ ) which fits well with the value reported in the literature ( $1.07\text{‰}$ ). This result suggests that we can safely use the isotope discrimination reported in the literature to calculate  $\delta^{13}\text{C}$  values.

The open circles in Fig. 4B represent the  $\delta^{13}\text{C}$  values of  $\Sigma\text{CO}_2$  in lake water as calculated from the measured  $\delta^{13}\text{C}$  values of headspace  $\text{CO}_2$  (solid circles) under the assumption of isotope mass balance and an isotope discrimination of  $1.07\text{‰}$ . The values fall within a fairly narrow range with a standard deviation of  $0.09\text{‰}$ .

We analyzed the isotope ratio of  $\Sigma\text{CO}_2$  in Lake Biwa

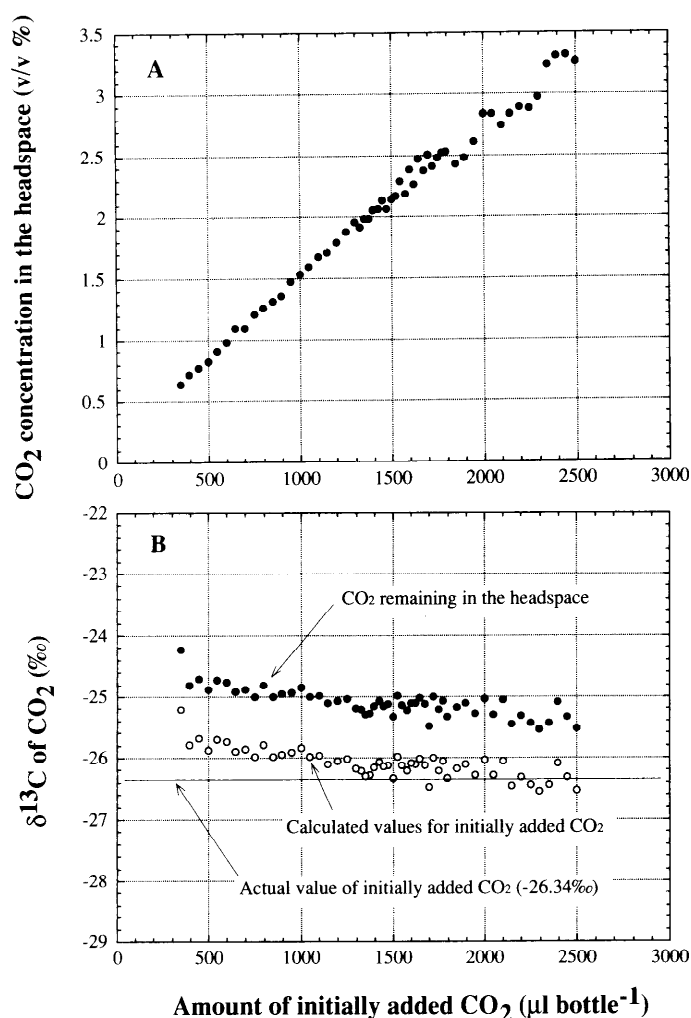


Fig. 3. Results of a preliminary experiment using standard  $\text{CO}_2$  of known isotope ratio ( $\delta^{13}\text{C} = -26.34$ ). A. Concentrations of  $\text{CO}_2$  in the headspace at equilibrium. B. The  $\delta^{13}\text{C}$  values of  $\text{CO}_2$  evolved into the headspace (●). The  $\delta^{13}\text{C}$  values of the initially added  $\text{CO}_2$  are calculated from those values of the headspace  $\text{CO}_2$  assuming the isotopic difference between the gas and aqueous phases to be  $1.07\text{‰}$  (○) and compared with the actual value.

water as follows. The concentration of  $\Sigma\text{CO}_2$  in this lake ranged from 500 to  $900\text{ }\mu\text{mol C liter}^{-1}$ . We found the concentration of  $\text{CO}_2$  evolved into the headspace (5.0 ml) of the sample bottle to range between 1.2 and 2.4% at equilibrium. These  $\text{CO}_2$  concentrations were not always identical to the values calculated from the dissolved  $\Sigma\text{CO}_2$  concentrations, assuming the Ostwald solubility coefficient of 0.872 (Fig. 5). We subsampled 250–400  $\mu\text{l}$  of the headspace gas with a gas-tight syringe and injected it into the GC/C/IRMS. Based on the reported peak area for the M/e 44 ion current, we obtained the amount of  $\text{CO}_2$  contained in the injected gas and calculated the concentration of  $\text{CO}_2$  in the headspace gas by dividing it by the injection volume. The amount of injected  $\text{CO}_2$  was also used to estimate the true  $\delta^{13}\text{C}$  value of  $\text{CO}_2$  in the headspace from the reported value by correcting for the concentration

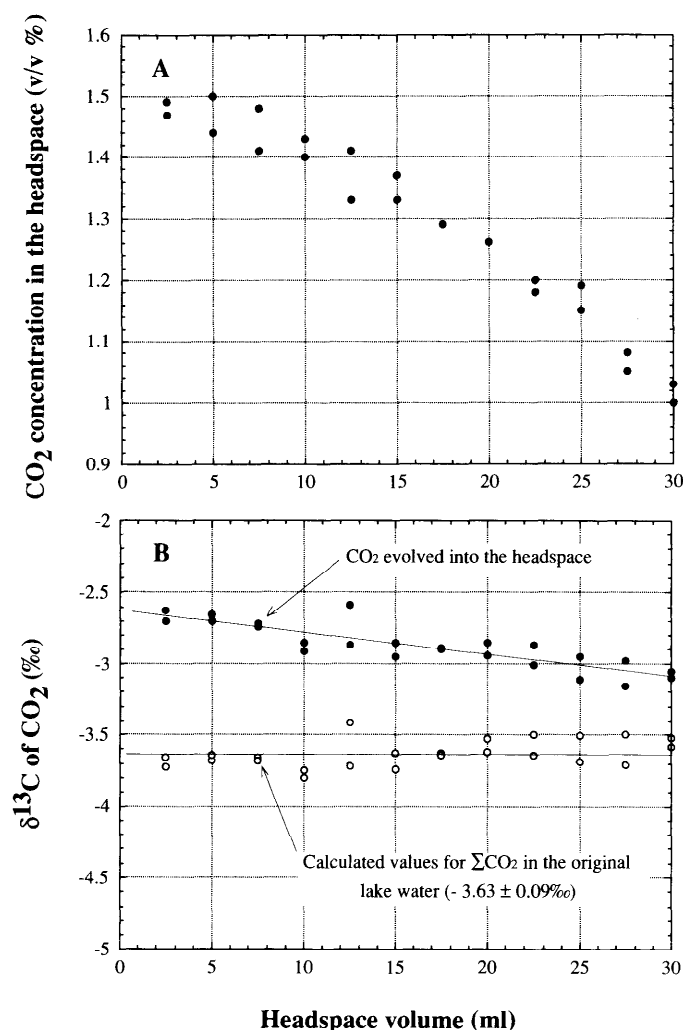


Fig. 4. Results of a preliminary experiment using a lake-water sample collected from the south basin of Lake Biwa. A. Concentration of CO<sub>2</sub> in the headspace is plotted against the volume of the headspace prepared. B. The observed  $\delta^{13}\text{C}$  values of CO<sub>2</sub> in the headspace (●) are plotted against the headspace volume. The best-fit line for these data is  $\delta^{13}\text{C} = -2.63 - 0.0154 \times (\text{headspace volume})$ . The  $\delta^{13}\text{C}$  values of  $\Sigma\text{CO}_2$  in the original lake-water sample are calculated from these data assuming the discrimination of 1.07‰ (○).

dependence of the GC/C/IRMS (cf. Fig. 2). The concentration of dissolved CO<sub>2</sub> remaining in the liquid phase was obtained by subtracting the amount of CO<sub>2</sub> in the headspace from the total amount of CO<sub>2</sub> present in the bottle, which was estimated from the concentration of  $\Sigma\text{CO}_2$  in the original lake water. The  $\delta^{13}\text{C}$  of CO<sub>2</sub> remaining in the aqueous phase was obtained by subtracting 1.07‰ from the  $\delta^{13}\text{C}$  value of the headspace CO<sub>2</sub>. The  $\delta^{13}\text{C}$  of the original lake water was then calculated as the weighted average of the  $\delta^{13}\text{C}$  values of CO<sub>2</sub> in the headspace and in the aqueous phase.

Because this method is based on isotope equilibrium between gas and liquid phases in a closed bottle with a relatively small inner volume, the headspace gas can be withdrawn only once. Withdrawing a subsample of head-

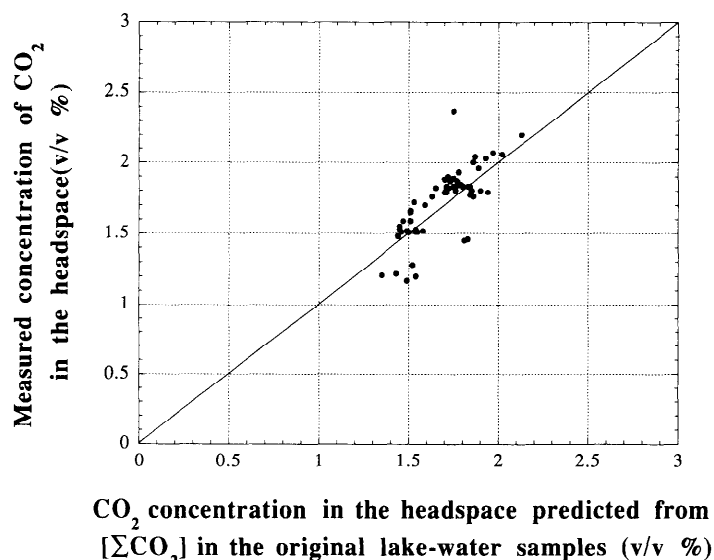


Fig. 5. Difference between the concentration of the headspace CO<sub>2</sub> predicted from the concentration of  $\Sigma\text{CO}_2$  originally present in lake-water samples assuming the Ostwald solubility coefficient to be 0.872 (abscissa) and the concentration of CO<sub>2</sub> actually evolved into the headspace at equilibrium (ordinate). Lake-water samples were collected from several stations in Lake Biwa.

space gas inevitably reduces the internal pressure and thus promotes the transfer of a part of dissolved CO<sub>2</sub> into the headspace. Because the CO<sub>2</sub> in the aqueous phase is depleted in  $^{13}\text{C}$  by 1.07‰ when compared with that in the headspace, exchange of aqueous-phase CO<sub>2</sub> reduces the  $\delta^{13}\text{C}$  value of CO<sub>2</sub> within the headspace. Therefore, analysis of subsequently withdrawn subsamples gives negative errors in  $\delta^{13}\text{C}$ . If multiple analyses are needed for statistical purposes, one must prepare replicate serum bottles containing samples of the same lake water.

Figure 6 shows vertical profiles of  $\delta^{13}\text{C}$  of  $\Sigma\text{CO}_2$  and several related variables at a 90-m deep station in the north basin of Lake Biwa. The standard error of multiple  $\delta^{13}\text{C}$  estimates for lake-water samples was  $\sim 0.1\text{‰}$  (see also Fig. 4). This value is precise enough to describe the vertical variation in this quantity.

The headspace method we have developed analyzes the isotope ratio of CO<sub>2</sub> in dissolution equilibrium. This aspect represents a major difference with the traditional purge-and-trap method, in which all the dissolved inorganic C must be stripped from water samples and trapped prior to the isotope ratio measurement (e.g. Kroopnick 1974). The headspace method requires no complex apparatus, as is needed for the purge-and-trap procedure, and greatly reduces labor and time necessary for the entire process.

However, the headspace method also has a drawback that originates in its use of dissolution equilibrium: the isotope discrimination of CO<sub>2</sub> between the gas and aqueous phases may differ depending on the nature of the water samples. Determining the dependence of the discrimination on salinity, ionic strength, and concentration

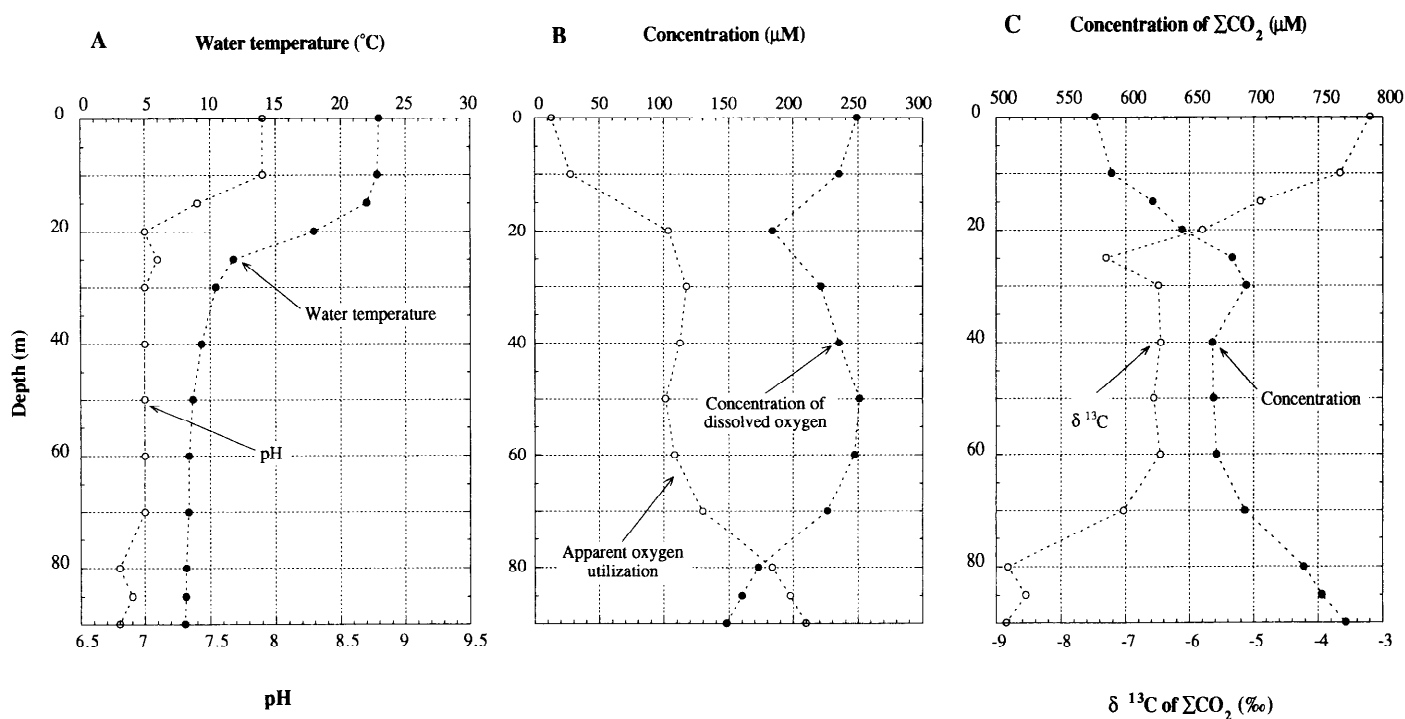


Fig. 6. Vertical profiles of water temperature and pH (A), dissolved oxygen concentration and the apparent oxygen utilization (B), and concentration and  $\delta^{13}\text{C}$  of  $\Sigma\text{CO}_2$  (C) observed at a 90-m-deep station in the north basin of Lake Biwa on 16 September 1993. The apparent oxygen utilization is defined as the difference between the actual oxygen concentration and the saturation concentration at the ambient temperature.

of humic substances would solve this problem. Alternatively, the discrimination factor can be determined in particular cases (as we did in Fig. 4) or by comparing with the data obtained by the purge-and-trap method.

The relatively large analytical errors inherent to the headspace method ( $\pm 0.1\%$ ) may also limit its applicability. Because modification of parameters such as headspace volume, equilibration time, and concentration of HCl could not drastically improve the analytical precision (unpubl. data), other factors are probably responsible for this error. One of the possible sources of error is water vapor in the injected gas. Although the water vapor is mostly removed by the water trap before inflowing to the IRMS, a trace vapor still inflows and disturbs the baseline of the M/e 45 ion current. Another possible interfering substance is nitrous oxide dissolved in the original samples, which has the same molecular weight as  $\text{CO}_2$  and cannot be perfectly separated from  $\text{CO}_2$  by the GC method we used.

The measurement of  $\delta^{13}\text{C}$  by the GC/C/IRMS also suffers a systematic error, as represented in Fig. 2. This error may be related to the ionization efficiency in the ion source of the IRMS, which depends on the concentration of  $\text{CO}_2$  in the gas stream. The  $\text{CO}_2$  contained in an injected sample is isotopically separated through the GC column in such a way that  $^{13}\text{CO}_2$  is eluted slightly faster than  $^{12}\text{CO}_2$ , whereas the standard  $\text{CO}_2$ , which is introduced via a mixing chamber, enters the IRMS as an isotopically homogeneous flow. This difference, together with the concentration dependence of the ionization efficiency, is thought

to have caused the systematic deviation (Fig. 2). An obvious way to circumvent such a complication is to inject the standard  $\text{CO}_2$  gas from the injection port of the GC/C/IRMS in an amount similar to the samples and use these data as the reference with which  $\delta^{13}\text{C}$  of the sample is calculated.

However, if the  $\text{CO}_2$  concentration in headspace gas is extremely small, the amount of  $\text{CO}_2$  to be injected into the GC/C/IRMS will also be small. This, in turn, may cause a large instrumental error in the determination of  $\delta^{13}\text{C}$  (Fig. 2). Consequently, the water samples must have sufficient dissolved inorganic C to generate  $\text{CO}_2$  in the headspace (at least 1% v/v). This limitation may be overcome by using an instrument with a higher acceleration voltage that provides a greater ionization efficiency. Although we used a relatively small headspace volume (5.0 ml) to keep the  $\text{CO}_2$  concentration in headspace gas above 1%, one may use a greater volume so as to reduce the effect of errors in the volume, if the  $\Sigma\text{CO}_2$  concentration in the sample is high enough.

In this study, the concentration of  $\Sigma\text{CO}_2$  in samples was measured separately and used to calculate the  $\delta^{13}\text{C}$  value of  $\Sigma\text{CO}_2$  from the  $\delta^{13}\text{C}$  value of the headspace  $\text{CO}_2$ . The estimate of  $\text{CO}_2$  concentration in headspace gas, based on the peak area of the M/e 44 ion current, is no less precise than the estimate made by the usual GC method with a TCD detector. However, the estimation of  $\Sigma\text{CO}_2$  concentration in lake water, based on the measured  $\text{CO}_2$  concentration in headspace gas and the Ostwald coefficient, is less reliable (cf. Fig. 5). The discrepancy between

$\Sigma\text{CO}_2$  calculated in this way and that measured separately by a TOC analyzer is presumably due to the dissolution equilibrium established in the sample bottles, which would not be the same as that in the ideal solution. Among factors that possibly affect the equilibrium is the internal pressure in the headspace, which depends on the concentrations of dissolved gases in the original samples. Another factor is the effect of solutes such as inorganic ions and dissolved organic matter that can alter both the solubility of dissolved gases and the water vapor pressure. In addition,  $\text{HgCl}_2$ , added as a fixative, interferes with transfer of dissolved  $\text{CO}_2$  into the headspace if the  $\text{HgCl}_2$  concentration is too high. The mechanism responsible for this effect has not been resolved.

Nevertheless, even when the  $\Sigma\text{CO}_2$  data are not available, the  $\delta^{13}\text{C}$  value of  $\Sigma\text{CO}_2$  can still be calculated from that of the headspace  $\text{CO}_2$  by using appropriate dissolution equilibrium constants, as in the following formula:

$$\delta^{13}\text{C}(\Sigma\text{CO}_2) = [V_h \times n + (V_b - V_h) \times \beta \times (n + \epsilon_g^a)] \div [V_h + (V_b - V_h) \times \beta]$$

where  $V_b$  is the inner volume of the serum bottle and  $V_h$  is the volume of the headspace,  $n$  is the  $\delta^{13}\text{C}$  value of the headspace  $\text{CO}_2$ ,  $\beta$  is the Ostwald solubility coefficient, and  $\epsilon_g^a$  is the isotopic difference between the gas and aqueous  $\text{CO}_2$ . Both  $\beta$  and  $\epsilon_g^a$  are temperature-dependent. In the case of our experimental conditions (23°C), the formula simplifies to

$$\delta^{13}\text{C}(\Sigma\text{CO}_2) = n - 0.98 (\text{‰}).$$

The difference between the estimate from this last formula and the value calculated with the separately measured  $\Sigma\text{CO}_2$  is always within 0.03‰; that is, the small unpredictability of  $\text{CO}_2$  concentration in headspace gas (Fig. 5) does not significantly affect  $\delta^{13}\text{C}$  calculated by this formula. This methodology eliminates the need for separately determined concentrations of  $\Sigma\text{CO}_2$ .

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