Combined analyses of O_2 and CO_2 for studying the coupling of photosynthesis and respiration in aquatic systems

Björn Wissel, Zoraida J. Quiñones-Rivera, and Brian Fry

Abstract: To simultaneously elucidate patterns of photosynthesis and respiration in aquatic systems, we developed a new gas-switching system for coupled measurements of dissolved metabolic gases. The methodology involves two gas chromatography columns to perform multiple gas separations. The first example using 24 h bottle incubations in estuarine waters showed a 1:1 molar relationship for the coupling between CO_2 and O_2 in closed systems during photosynthesis and respiration. In a second, open system application using depth-stratified sampling on the Louisiana continental shelf, deviations from this 1:1 relationship between CO_2 and O_2 were common. In surface waters, depletion of CO_2 exceeded excess O_2, likely owing to different gas-exchange rates with the atmosphere. In bottom waters, CO_2 accumulation could surpass O_2 losses, indicating anaerobic respiration. At intermediate depths, CO_2 and O_2 dynamics followed the 1:1 relationship that was observed in the closed incubations. This approach clearly showed that CO_2 and O_2 dynamics were tightly coupled on short-time scales, but anaerobic respiration and physical processes such as gas exchange can lead to strong divergence of CO_2 and O_2 stoichiometries. This combined analysis of respiratory gases that is readily achievable with isotope ratio mass spectrometer systems illustrates how oxygen and carbon cycles are coupled and decoupled in aquatic systems.

Résumé : Afin d'élucider simultanément les patrons de photosynthèse et de respiration dans les systèmes aquatiques, nous avons mis au point un nouveau dispositif de commutation des gaz qui permet des mesures appariées des gaz métaboliques dissous. La méthodologie utilise deux colonnes de chromatographie en phase gazeuse afin de réaliser des séparations multiples des gaz. Un premier exemple utilisant des incubations en bouteilles de 24 h dans des eaux estuariennes montre une relation molaire de 1:1 pour le couplage de CO_2 et de O_2 dans des systèmes fermés durant la photosynthèse et la respiration. Dans une seconde utilisation en système ouvert par échantillonnage stratifié en fonction de la profondeur sur la plateforme continentale de la Louisiane, les déviations de la relation 1:1 entre CO_2 et O_2 sont fréquentes. Dans les eaux superficielles, la baisse de CO_2 est plus importante que le surplus de O_2, vraisemblablement à cause des taux différents d'échanges gazeux avec l'atmosphère. Dans les eaux du fond, l'accumulation de CO_2 peut dépasser les pertes de O_2, ce qui signale une respiration anaérobie. Aux profondeurs intermédiaires, la dynamique de CO_2 et de O_2 suit la relation 1:1 observée dans les incubations en milieu fermé. Notre approche montre clairement que les dynamiques de CO_2 et de O_2 sont étroitement couplées sur de courtes échelles temporelles, mais que la respiration anaérobie et des processus physiques tels que les échanges gazeux peuvent entraîner d'importantes divergences dans la stoechiométrie de CO_2 et de O_2. Cette analyse combinée des gaz respiratoires, qui est facilement réalisable avec des systèmes de spectrométrie de masse de rapports isotopiques, illustre comment les cycles d'oxygène et de carbone sont ou non couplés dans les systèmes aquatiques.

[Traduit par la Rédaction]

Introduction

Productivity patterns in aquatic systems are commonly exhibited in the dynamics of dissolved oxygen (DO) and dissolved inorganic carbon (DIC). Concentrations of these two metabolic gases fluctuate depending on the relative importance of photosynthesis and respiration but also are strongly influenced by physical processes, such as gas exchange with the atmosphere and mixing. The individual analyses of DO and DIC have contributed substantially to the understanding of productivity patterns in aquatic systems. Early on, Odum (1956) measured primary production (PP) and respiration (R) in streams based on diurnal fluctuations of DO concentrations, an approach that is now considered a standard technique across many aquatic systems (Wetzel 2001; Mulholland et al. 2005). DIC measurements also have been conducted routinely to estimate productivity in marine systems (Hood 1981; Cai and Wang 1998; Wang et al. 2005) and freshwater systems (Cole et al. 1994; Duarte and Agusti 1998; Jonsson et al. 2003). Interestingly, diurnal patterns of DIC concentration are usually weak, unless total alkalinity is very low, e.g., in softwater lakes as shown by Schindler and Fee (1973) and Sellers et al. (1995). Compared with DO, fluctuations in DIC concentrations are much more pronounced on a seasonal basis, likely owing to the large DIC pool size and slow CO_2 exchange rate with the atmosphere (Weiss 1970, 1974).

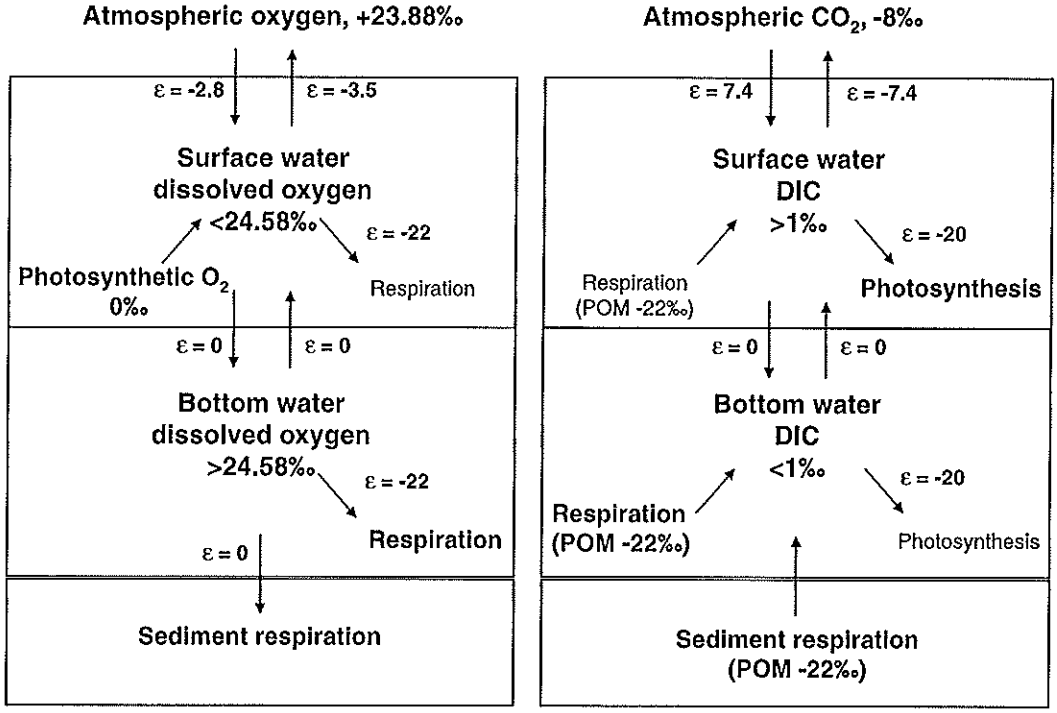
Received 3 December 2007. Accepted 27 March 2008.
Published on the NRC Research Press Web site at cjfas.nrc.ca on 18 October 2008.
J20296

B. Wissel,[1,2] Z.J. Quiñones-Rivera, and B. Fry. Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803, USA.

[1]Corresponding author (e-mail: bjoern.wissel@uregina.ca).
[2]Present address: Department of Biology, University of Regina, Regina, SK S4S 0A2, Canada.

 doi:10.1139/F08-146

Fig. 1. Conceptual model of oxygen and carbon isotopic values during stratified summer conditions. The ε symbols accompanying arrows are per mil fractionation factors (changes in $\delta^{18}O$ or $\delta^{13}C$ at 30 °C) expected during reactions and transfers. Isotopic values are representative values taken from this study and literature sources.



While concentration measurements of DO and DIC have helped to understand system metabolism in a number of cases, this approach is limited in its ability to separate effects of biological (primary production and respiration) and physical processes driving DO and DIC dynamics. Consequently, concentration measurements are now often complemented with the analyses of stable oxygen and carbon isotopes, as they correspond to various biological and physical processes in aquatic systems. In equilibrium with the atmosphere, DO has a stable isotope value of 23.88‰ (Barkan and Luz 2005), but photosynthesis and respiration can lead to significantly lower and higher $\delta^{18}O$ values, respectively (Fig. 1). Decreasing $\delta^{18}O$ values in response to photosynthesis are due to the addition of isotopically depleted (light) oxygen that is derived from ambient water to the existing DO pool (Guy et al. 1986, 1993), whereby $\delta^{18}O$ of the source water can range from 0‰ (seawater) to as low as –25‰ for freshwater at high latitudes (International Atomic Energy Agency 2006). In contrast, respiration preferentially removes light oxygen with a large fractionation factor (ε) of –15‰ to –25‰ (Kroopnick 1975; Quay et al. 1995; Hendricks et al. 2004), which results in increased $\delta^{18}O$ values of the residual DO pool.

Stable carbon isotopes respond to metabolic processes in a similar, but inverse fashion (Fig. 1; Parker et al. 2005). Respiration adds CO_2 from ambient organic matter to the DIC pool without fractionation (Farquhar et al. 1982; Lin and Ehleringer 1997), and therefore, $\delta^{13}C$ values of respired CO_2 are equivalent to those of the organic matter source. Generally, $\delta^{13}C$ of organic matter ranges from –20‰ to –35‰, whereby values in marine systems usually are closer to –22‰ (Peterson and Fry 1987). Atmospheric CO_2 has a $\delta^{13}C$ value of –8‰ (Keeling et al. 2005), and dissolved in water, CO_2 of atmospheric origin has a slightly lower value owing to an equilibrium fractionation of about –1‰ (Vogel et al. 1970). Further fractionation between CO_2 and bicarbonate (HCO_3^-) is temperature dependent and ranges from –9.6‰ to –7.4‰ between 10 and 30 °C, respectively (Mook et al. 1974). In natural systems, this carbon speciation is most strongly pronounced at pH values exceeding 8 (e.g., marine systems and hypertrophic or alkaline lakes), where free CO_2 is almost completely absent (Wetzel 2001). The actual CO_2 flux to and from the atmosphere depends on wind speed, mixing depth, and the partial pressure of CO_2 (pCO_2) in surface waters, which is a function of pH and DIC concentration (Wanninkhof and McGillis 1999; Wetzel 2001). Besides flux to the atmosphere, photosynthesis is the main sink for DIC. Generally, algal uptake of DIC is associated with a fractionation of about –20‰, which increases the $\delta^{13}C$ values of the remaining DIC pool.

For most metabolic processes, oxygen and carbon dynamics are coupled, as respiration consumes O_2 while producing CO_2, and conversely, photosynthesis generates O_2 while consuming CO_2. During photosynthesis, the molar ratio of O_2 production to CO_2 consumption (photosynthetic quotient (PQ)) is typically between 1.0 and 1.4, with larger values indicating the use of nitrate or proteins instead of ammonium as nitrogen source (Williams and Robertson 1991). Likewise, the respiratory quotient (RQ) (mol CO_2 produced / mol

O_2 consumed) is between 0.8 and 1.0, whereby lower values indicate that proteins or fatty acids are respired as opposed to carbohydrates (RQ = 1.0) (Robinson and Williams 1991).

To analyze the coupling of oxygen and carbon dynamics during metabolic processes, it is inherently appealing to simultaneously measure both O_2 and CO_2 from the same sample. Furthermore, under anaerobic conditions where denitrification is producing N_2, nitrogen dynamics could become important as well. Also, in these metabolic studies, it is desirable to have a reference gas that is inert to metabolism, and argon (Ar) (and in many cases nitrogen as well) can provide that reference (Benson and Parker 1961; Craig and Hayward 1987; Emerson et al. 1993). To accommodate the combined gas analysis, we implemented a new method for coupled gas analyses of O_2, CO_2, and N_2 using two gas chromatography (GC) columns for gas separation and an isotope ratio mass spectrometer to detect gas quantities and isotope compositions. The mass spectrometer is equipped with an extra collector that also allows determination of Ar amounts, even though Ar and oxygen are not separated in the gas handling system. Using two GC columns and switching valves, multiple gases can be routinely separated and analyzed from a single sample. Information for each sample includes up to eight parameters: amounts of O_2, CO_2, N_2, and Ar and the isotope values $\delta^{18}O_{O_2}$, $\delta^{13}C_{DIC}$, $\delta^{15}N_{N_2}$, and $\delta^{18}O_{H_2O}$.

Here, we present two examples of combined DO and DIC analyses to demonstrate how this new methodology increases not only the understanding of metabolic processes in aquatic systems but also the quality and quantity of the generated data in addition to decreasing analytical time and cost. One study was conducted in a productive wetland in southeast Louisiana and focused on DO–DIC dynamics in closed-bottle incubations over a 24 h period. The other study investigated open-system dynamics based on vertical DO and DIC profiles that were acquired in situ during summertime conditions on the Louisiana continental shelf at water depths of 30 and 40 m.

Materials and methods

Sample collection

Water samples were collected by overflowing 160 mL glass bottles three times and 1 mL of 6 mol·L^{-1} HCl was added as a poison to stop biological activity in the sample (Miyajima et al. 1995; Atekwana and Krishnamurthy 1998; Salata et al. 2000). Subsequently, bottles were immediately sealed without bubbles using a heavy rubber stopper (Bellco Glass, 20 mm; Wassenaar and Koehler 1999) and a syringe needle that allowed excess water to escape. The needle was then removed and the stopper was sealed with a crimped aluminum cap. For the incubation study (Louisiana wetland), the acid was added after the sample bottles were sealed. This change was done to avoid the potential loss of CO_2 to the atmosphere from acidified samples and significantly reduced the measurement error (standard deviation of 15 and 45 mmol·m^{-3} DIC for the wetland and offshore study, respectively).

Laboratory analyses

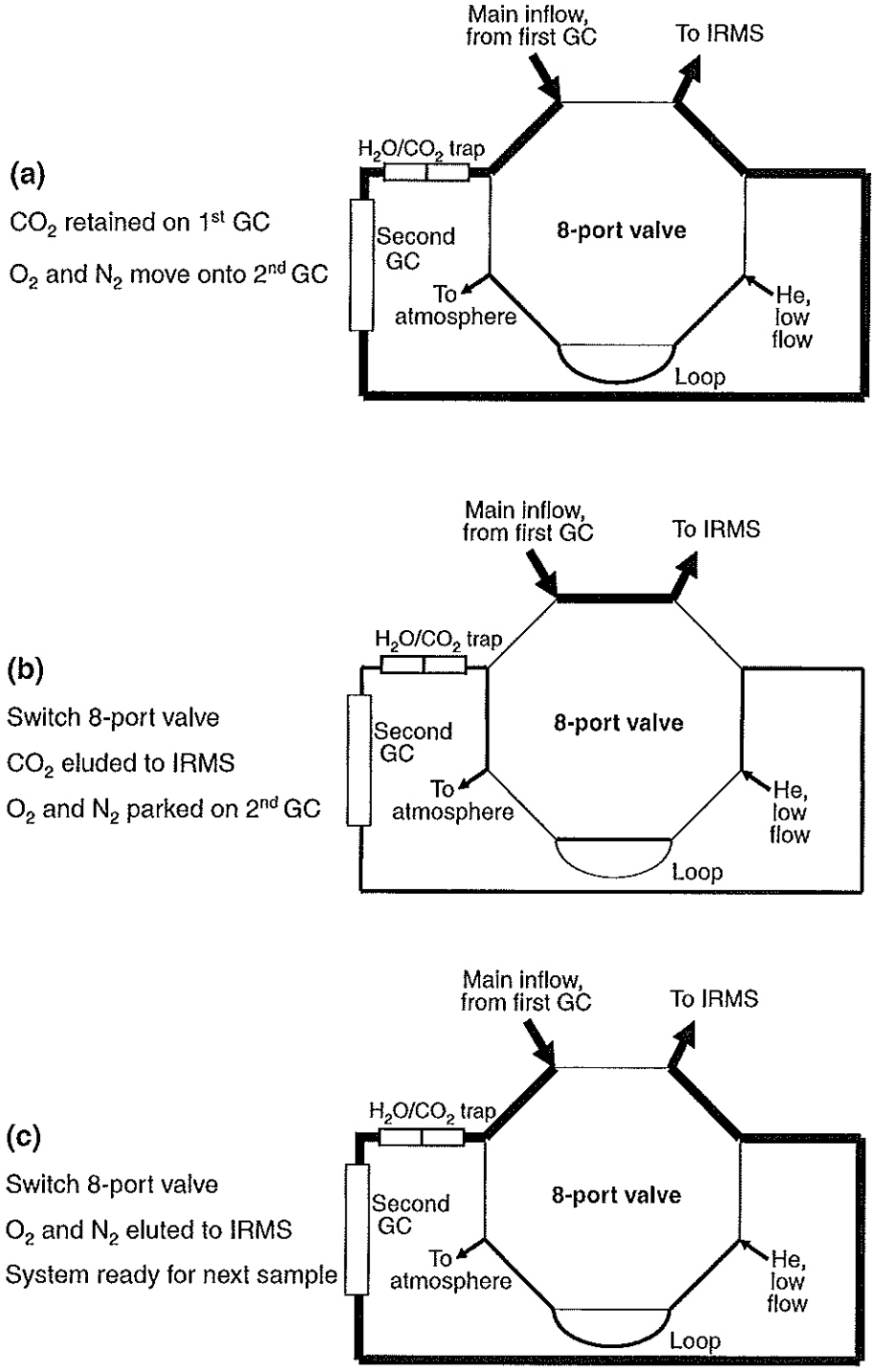
After return to the laboratory, samples were prepared for analysis by means of headspace equilibration (Kampbell et al. 1989; Miyajima et al. 1995; Wassenaar and Koehler 1999). A headspace was created by injecting 10 mL of ultrapure helium into inverted bottles while allowing 10 mL of sample water to drain out a small needle (BD brand precision glide 23G1). The helium was injected at the bottom of the inverted sample bottle using a 10 cm long stainless steel needle that was attached to a 20 mL syringe (BD brand general use). Before injection, the syringe was flushed with ultrapure helium five times to avoid contamination with atmospheric gases. Subsequently, samples were stored in the dark at 5 °C for up to 4 weeks. Before isotopic analyses, samples were placed in a shaker (100 r·min^{-1} (1 r = 2π rad) at room temperature) for at least 12 h to ensure equilibration of the dissolved gases with the headspace.

We used an eight-port Valco switching valve to accommodate the two-GC system (Fig. 2). The eight-port valve and the second GC were part of a computer-controlled gas handling device (GasBench II, Thermo Finnigan). The first GC was upstream of the switching valve and the second GC column was in a loop that could be switched in and out of the main flow. While out of the main flow, the second GC was flushed by a slow flow of helium. CO_2 was retained on the first, upstream column, while the other gases (Ar, O_2, and N_2) passed onto the second, downstream column. Flow paths were then changed to achieve optimal elution of all three gases (Fig. 2).

To start the actual analysis, a 3 mL sample was obtained from the headspace by first injecting an equal amount of helium into the headspace, flushing the syringe (5 mL BD brand general use, Luer-Lok tip; BD brand needle, precision glide 23G1) five times to mix the headspace, and then withdrawing the sample. This headspace sample gas was then immediately injected through a Supelco septum (6 mm, Thermogreen LB-2, 20651) into the sample train consisting of a water removal trap filled with magnesium perchlorate, a 2 m GC column (stainless steel, Costech No. 051080) for CO_2 separation from Ar, O_2, and N_2, and a downstream 2 m GC column (stainless steel, 5 Å mesh size, Costech No. 051088) for separating N_2 from Ar and O_2. An additional second trap filled 50% with ascarite and 50% with magnesium perchlorate was placed before the second GC column to remove traces of CO_2 and water, respectively, that otherwise could interfere with oxygen and nitrogen isotope measurements. (In case of water contamination, the 5 Å GC column could be regenerated within 12 h by slowly flushing it with helium at 250 °C inside a muffle furnace.) During the analysis, both GC columns were kept at room temperature.

CO_2 slowly passed onto the first GC column, while Ar, O_2, and N_2 quickly eluted onto the second GC column, which was then switched from a main flow rate (120 mL·min^{-1}) to a slow flow of 5 mL·min^{-1}. The Ar, O_2, and N_2 gases were effectively parked onto this second, slow-flow column while measurement continued with the CO_2 analysis. Switching out the second GC column also greatly increased the flow through the first GC column to 200 mL·min^{-1} and rapidly eluted the CO_2 for isotopic measurement. After completion of the CO_2 measurement, the second column was switched back in-line and oxygen and then nitrogen isotope measurements were made sequentially.

Fig. 2. Schematics for alternate sample trains to analyze three gases from a single sample. Only the module between the first gas chromatography (GC) and the isotope ratio mass spectrometer (IRMS) is shown. (*a*) Initially, a slow flow goes through both GC columns until O_2 and N_2 are parked onto the second GC. (*b*) After switching to the fast flow, the third gas (CO_2) is eluted from the first GC and carried directly to the mass spectrometer. (*c*) Subsequently, the valve switches back to the initial position, and the two gases parked on the second GC are eluted successively and carried to the mass spectrometer.



Argon, which has almost identical retention times as O_2, was measured simultaneously with O_2 but in a separate detection cup at the mass spectrometer.

Between injections, the 5 mL syringe was flushed five times with ultrapure helium and then pressurized (150 kPa) using a three-way stopcock. After a new needle (BD brand precision glide 23G1) was attached, the syringe was opened and quickly adjusted to an injection volume of 3 mL, which instantaneously released pressurized helium and displaced any air left in the needle and stopcock. In later work, injections sometimes failed to transfer all gas from the syringe through the septum to the GC system. To ensure complete transfer, a procedure was followed to inject through the septum and then the syringe was allowed to refill with pressurized helium flowing through the GC system without removing the syringe. The sample was then reinjected from the full syringe, the syringe allowed to fill again with helium, and a third, final injection performed. The repeated three injections of the same sample routinely transferred more than 99% of the sample and were performed in less than 20 s to prevent peak spreading.

Data analysis

The concentrations (millimoles per cubic metre) of dissolved O_2, CO_2, N_2, and Ar were calculated based on the isotope ratio mass spectrometer chromatograms of masses 32, 44, 28, and 40, respectively. The relationship between peak areas measured in volt seconds (Vs) and gas amounts were calibrated using air-equilibrated water (see below). Based on these laboratory measurement procedures, gas amounts (millimoles per cubic metre) in field samples could be calculated, and gas saturation levels were then determined according to temperature and salinity measured in the field.

Laboratory standards were prepared in the same way as individual samples, except that the water source was deionized laboratory water that was equilibrated with the atmosphere. Standards were stirred overnight for 16 h at moderate speed that produced a slight vortex in a 4 L beaker. No bubbles were observed during stirring and standards were assumed to be at saturation after this stirring. The saturation concentrations for O_2, N_2, and Ar at ambient laboratory temperature were calculated according to Weiss (1970). The deionized water contained little background CO_2, and the concentration for CO_2 in the standards was set to 2000 mmol·m^{-3} by adding 0.848 kg·m^{-3} Na_2CO_3 to the deionized water. Acidification (with 6 mol·L^{-1} HCl) of individual standards right before sealing of bottles transformed Na_2CO_3 into free CO_2.

To test for potential contamination of samples with atmospheric gases owing to sample handling, we prepared procedural O_2 blanks. These O_2 blanks were prepared in the same way as laboratory standards, but to obtain zero-O_2 water, 50 g·L^{-1} Na_2SO_3 was added (Kampbell et al. 1989). The O_2 concentration of procedural blanks was negligible (0.2 ± 0.06 (SD) mmol·m^{-3}, n = 17), so that no contamination with any atmospheric gases occurred during sample handling. Some dissolved gas was introduced with the added 1 mL of acid and could contribute a minor amount of ±0.06 SD, Ar, and N_2 to the sample. No correction for these minor additions was made in this study.

However, some uncertainty of concentration measurements was possible, which was related to the accuracy of headspace volume injected. Injection uncertainties of ±0.1 mL were corrected using Ar measurements. Any over- or under-saturations of Ar in a sample was assumed to be due to inaccuracy of the sample injection. The ratio of measured to expected Ar saturation (100%) was applied to correct O_2, CO_2, and N_2 concentrations as follows:

$$[Y]_{Ar_{corrected}} = [Y]_{measured}/(Ar_{measured}/Ar_{expected})$$

where Y represents saturation of either O_2, CO_2, or N_2. The Ar-corrected saturation levels for O_2 and N_2 in air-equilibrated laboratory standards were 100% ± 0.5% (SD). The calculated DIC concentration in the standards was 1995 mmol·m^{-3} (compared with 2000 mmol·m^{-3} that was added to the standards) with a standard deviation of 45 mmol·m^{-3} or a relative error of 2.2%.

The isotope values of oxygen, carbon, and nitrogen from headspace samples and standards were determined using a Finnigan Thermoquest Delta plus XP isotope ratio mass spectrometer. The headspace analyses involve equilibrations between liquid and gas phases, and both concentrations and isotopes reflect details of the equilibrations (e.g., Miyajima et al. 1995). For example, 15% higher CO_2 concentrations were measured in saltwater standards than in freshwater standards when both were prepared with equal amounts of added carbonate; O_2, N_2, and Ar concentrations also were about 10% higher than input values owing to reduced solubility at higher salinities. Normalizing to Ar removed most of these salinity-dependent effects, although a small 5% effect still remained for CO_2. This effect should be taken into account when waters of different salinities are compared, but in this study, salinities were uniform in each experiment, either all freshwater (field study 1) or all high salinity (field study 2).

We also measured equilibrium isotope effects in the dissolved gas standards, finding results similar to Miyajima et al. (1995) for CO_2 but smaller fractionation effects of approximately 0‰–0.3‰ for O_2 and N_2. These smaller fractionations differ from previously published estimates of approximately 0.7‰ for both O_2 and N_2 (Benson and Krause 1984; Knox et al. 1992) and likely reflect subtle differences in laboratory equilibration conditions, e.g., our equilibrations were done at pressures exceeding 1 atmosphere inside the bottles in a predominantly helium atmosphere. For such reasons, we used the prepared laboratory standards as our primary reference materials, assigning them the published isotope values (Benson and Krause 1984; Knox et al. 1992; Barkan and Luz 2005) of 24.58‰ and 0.7‰, respectively, for O_2 and N_2 in air-equilibrated water. With this calibration, our analyses of air (n = 19) resulted in values of 24.0‰ ± 0.1‰ (SD) $\delta^{18}O$ and 0.2‰ ± 0.2‰ (SD) $\delta^{15}N$, in good agreement with the known values of 23.88‰ and 0.0‰, respectively (Kroopnick and Craig 1976). The carbon isotope value of the added Na_2CO_3 was 1.8‰ ± 0.2‰ (SD) as determined by combustion. Carbon isotope values are expressed relative to VPDB. Precision (expressed as standard deviation) of the measurements of oxygen, nitrogen, and carbon isotope values for laboratory standards was 0.08‰, 0.07‰, and 0.02‰, respectively (n = 18).

The headspace equilibration technique also generates the $\delta^{18}O$ value of the oxygen in CO_2. Generally, the oxygen in CO_2 and H_2O molecules in water is in equilibrium in the field, and the $\delta^{18}O$ of CO_2 corresponds to the $\delta^{18}O$ value of water (Friedman and O'Neil 1977; Horita et al. 1989), which can be used as an important tracer for salinity (Coplen and Kendall 2000; Bastow et al. 2002) and geographic origin of the ambient water (Killingley 1980; Gao and Beamish 2003). Salinity and geographic variations were not the focus of this study, so the $\delta^{18}O_{H_2O}$ values measured along with $\delta^{13}C$ in CO_2 generally are not reported but are available from the authors. The current method provides a $\delta^{18}O_{H_2O}$ value at the time of sampling, since adding acid converts all DIC to CO_2 and prevents further water–CO_2 equilibration that is possible only in the presence of bicarbonate.

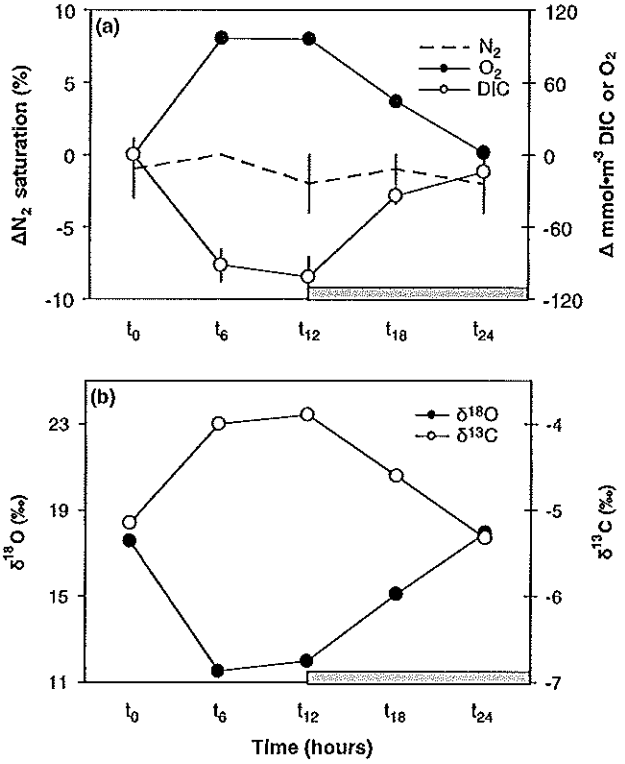
Field study 1

The goals of this study were to assess the coupling of O_2 and CO_2 dynamics under controlled, closed-system conditions where gas exchange with the atmosphere did not occur. On 24 August 2004, we collected surface water in the upper part of Breton Sound, an estuary located just southeast of New Orleans, Louisiana. The actual sampling site was at the intersection of Manuel's Canal and Reggio Canal (29°46′07″N, 89°56′07″W), an area that is heavily influenced by local runoff from the surrounding marshes (Wissel and Fry 2005; Wissel et al. 2005). Just before sunrise, we filled fifteen 160 mL bottles with surface water and immediately preserved three time-zero bottles. The remaining bottles were incubated in situ at 20 cm water depth (clear and sunny skies, water temperature between 30.5 and 31.7 °C, salinity 0.2 psu). Every 6 h, three more bottles were preserved until the experiment was terminated 24 h later the following morning.

Field study 2

Field study 2 was conducted to explore the coupling of O_2 and CO_2 dynamics in open systems where additional processes such as anaerobic respiration in bottom waters and air–sea gas exchange in surface waters may differently impact O_2 and CO_2 dynamics. In July 2002, we collected water samples across the water column at two stations located on the Louisiana continental shelf. Station A5 (29°04′20″N, 89°45′00″W) had a water depth of 30 m and was approximately 50 km west of the mouth of the Mississippi River, while station F6 (28°35′00″N, 91°37′00″W) had a water depth of 40 m and was about 200 km west of the Mississippi River. Both sites were visited during calm conditions within 3 h after sunset. Salinity, temperature, and pH were similar at both stations, with surface values of approximately 27.0 psu, 31.1 °C, and 8.3, respectively; bottom water salinity, temperature, and pH were approximately 27.0 psu, 25.0 °C, and 8.1, respectively. Surface water was collected with a polyvinylchloride bucket, and we used a 5 L polyvinylchloride Niskin sampler for subsurface water samples. For surface samples, the bucket was placed sideways onto the water surface. Once it started sinking, the bucket filled passively with water, avoiding intrusion of atmospheric O_2 into the sample water owing to turbulent mixing. Surface water samples were collected approximately

Fig. 3. (*a*) Evolution of O_2, dissolved inorganic carbon (DIC), and N_2 concentrations during the 24 h incubation experiment in Breton Sound (nighttime shown by the shaded bar). O_2 and DIC concentrations are presented as change relative to the initial sampling conditions (±SD). Absolute amounts of O_2 and DIC concentrations did not differ significantly ($p < 0.05$) for any of the sampling events. N_2 concentration is given as percent saturation, which was not significantly different from 100% at any of the sampling events. (*b*) Evolution of O_2 and carbon stable isotope values (±SD) during the 24 h incubation experiment in Breton Sound. Initial (t_0) and final (t_{24}) stable isotope values were not significantly different from each other for both O_2 and carbon ($p > 0.1$).



10 cm below the surface, while subsurface water samples were collected in 5 m depth intervals. Bottom water samples were taken within 1 m of the bottom sediments. Sample water was transferred to 160 mL Wheaton glass bottles using plastic Tygon tubing. The tubing was either attached to the Niskin sampler (bottom samples) or water was siphoned directly from the bucket into the sample bottles (surface samples).

Results and discussion

Field study 1

In these bottle incubations, dissolved N_2 was not affected by any biological processes. N_2 saturation levels were not significantly different from 100% throughout the incubation period ($p < 0.001$), and there were no significant changes in $\delta^{15}N$ values ($p < 0.001$) (Fig. 3). Consequently, N_2 could serve as a second "inert" reference gas besides Ar, whose saturation levels were at 101% ± 2% throughout the experiment.

Table 1. Results of the 24 h incubation experiment for dissolved oxygen (DO), CO_2, and N_2 concentrations and isotopic values that was performed in upper Breton Sound, Louisiana.

Time (h)	O_2 (mmol·m^{-3})	O_2 saturation (%)	CO_2 (mmol·m^{-3})	N_2 saturation (%)	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
t_0	147 (2.8)	63 (0.3)	2114 (13)	99 (1.9)	17.6 (0.2)	–5.2 (0.07)	0.85 (0.08)
t_6	244 (3.4)	102 (1.0)	2022 (14)	100 (0.3)	11.5 (0.2)	–4.0 (0.00)	0.83 (0.04)
t_{12}	244 (5.3)	103 (0.3)	2012 (17)	98 (2.2)	12.0 (0.1)	–3.9 (0.04)	0.82 (0.05)
t_{18}	191 (1.6)	81 (0.1)	2080 (7)	99 (1.1)	15.1 (0.1)	–4.6 (0.03)	0.79 (0.08)
t_{24}	147 (0.9)	62 (0.1)	2100 (19)	98 (2.1)	7.9 (0.0)	–5.3 (0.04)	0.83 (0.04)
p	4.9^{-12}	4.9^{-12}	0.0001	0.67	4.9^{-14}	0.0017	0.82

Note: Standard deviations of the three replicates are shown in parentheses; p values indicate significant differences between individual sampling times (one-way analysis of variance).

The O_2 concentration for the initial sampling just before sunrise was 147 mmol·m^{-3}, which corresponded to 63% saturation (Table 1). After 6 h, the O_2 concentration reached 244 mmol·m^{-3} (102% saturation) and remained largely unchanged until sunset. Alternatively, O_2 concentration could have further increased after 1300 but then returned to a value of 244 mmol·m^{-3} at sunset. At night, we observed a gradual decrease in O_2 concentration towards the initial value of 147 mmol·m^{-3} at dawn the next morning. Accordingly, there was no net O_2 production or consumption over the 24 h period and the ratio of production to respiration was 1. DIC concentrations followed a related but inverse pattern. Expressed as the difference from initial values (Δ) in millimoles per cubic metre, O_2 and DIC concentrations tracked each other almost perfectly in an inverse manner throughout the experiment (Fig. 3), and also, absolute amounts of ΔO_2 and ΔDIC were not significantly different throughout the incubations ($p > 0.1$ for all sampling times). The slopes of O_2 versus DIC (millimoles per cubic metre) during day and night (–0.94 and –1.09, respectively) were not significantly different from –1 ($p > 0.1$). Accordingly, both PQ and RQ were close to 1.0, consistent with carbohydrate metabolism throughout this incubation study.

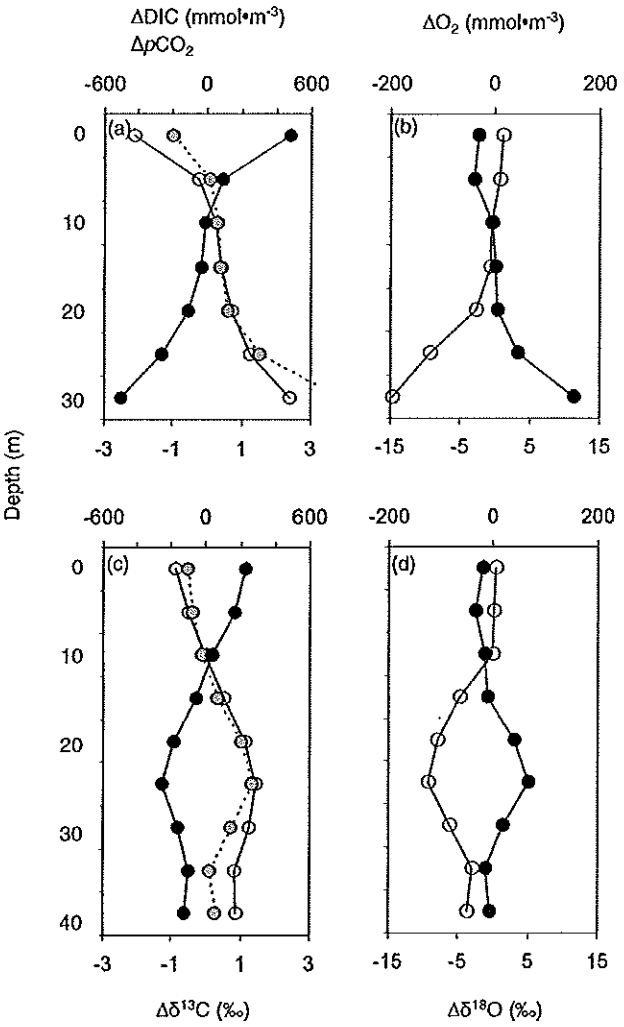
Similar to concentrations, oxygen and carbon isotope values tracked each other in an inverse fashion (Table 1; Fig. 3), further denoting very strong coupling between O_2 and DIC dynamics in these closed-system incubations. O_2 produced during photosynthesis originates from water, which is generally depleted in ^{18}O relative to atmospheric O_2 (Guy et al. 1986, 1993). Hence, the addition of photosynthetic O_2 reduces $\delta^{18}O$ values of the DO pool in the water, in our case from 17.6‰ to 12.0‰. A mass balance calculation of the water $\delta^{18}O$ using data from time 0 and 12 h yielded a value of about –4‰, which was identical to our measured value of –4‰ ($O_{2,t_{12}} \times \delta^{18}O_{t_{12}} = O_{2,t_0} \times \delta^{18}O_{t_0} + \Delta O_2 \times \delta^{18}O_{H_2O}$, solved for $\delta^{18}O_{H_2O}$). At night, respiration is dominant and isotopically lighter O_2 is taken up faster (Guy et al. 1993), a process that increased $\delta^{18}O$ values of the remaining DO from 12‰ to almost 18‰. The actual fractionation factor (ε) for respiration in closed systems (such as our incubation bottles) is described by a logarithmic distillation equation (Mariotti et al. 1981). For our incubations, we obtained an ε value of –12‰, which is at the low end of the range reported in the literature (Lane and Dole 1956; Kroopnick 1975; Kiddon et al. 1993). The measured respiration rate of 8 mmol·m^{-3}·h^{-1} in our incubations was very high and strongly exceeded values that are reported for a variety of estuarine systems (0.06–2.9 mmol·m^{-3}·h^{-1}; Dortch et al. 1994). This high respiration rate and very high O_2 demand might explain the reduced fractionation factor because low ε values are commonly observed in situations when either demand is very high or supply is limited (Fry 2006).

Stable isotope values of DIC (DI^{13}C) increased during the day owing to faster uptake of ^{12}C by photosynthetic algae and the calculated (Mariotti et al. 1981) ε was –25.1‰. At night, ^{12}C was replenished during respiration, as observed in other studies (Zhang and Quay 1997; Salata et al. 2000), and DI^{13}C values decreased again. Using a mass balance approach, we calculated the $\delta^{13}C$ values of the respired material, which were –30.2‰ and –30.8‰ for the time periods t_{12} to t_{18} and t_{12} to t_{24}, respectively ($DIC_{t_{18}} \times \delta^{13}C_{t_{18}} = DIC_{t_{12}} \times \delta^{13}C_{t_{12}} + \Delta DIC \times \delta^{13}C_{POM}$, solved for $\delta^{13}C_{POM}$, where POM is particulate organic matter). Both respiration and fractionation during algal DIC uptake (ambient DI^{13}C of –5‰ plus ε of about –25‰) identified particulate organic carbon (PO^{13}C) values of about –30‰. This result is in very good agreement with PO^{13}C values of samples that were taken at this location in the previous 2 months of June and July 2004 (–30.5‰ and –31.2‰, respectively).

Field study 2

The Louisiana continental shelf is heavily influenced by the discharge of the Mississippi River whereby riverine freshwater markedly reduces salinities in surface waters (Rabalais and Turner 2001). Average measured DIC concentrations, $\delta^{13}C$ values, and salinities differed between the two endmembers (Mississippi River: DIC = 2500 ± 30 mmol·m^{-3}, $\delta^{13}C$ = –10.2‰ ± 0.1‰, salinity = 0.0 ± 0.0 psu versus offshore: DIC = 2070 ± 110 mmol·m^{-3}, $\delta^{13}C$ = 1.5‰ ± 0.5‰, salinity = 34.9 ± 0.4 psu). For the Mississippi River, five replicate samples were collected in the mouth of the river 24 h prior to the study. To estimate the offshore endmember values, samples were taken during monthly sampling trips between June 2002 and July 2003 whereby only values from samples that had salinities above 34 psu and O_2 saturations between 98% and 102% were included to avoid any effects of the Mississippi River or primary production or respiration, respectively. To address the differences in endmember values, we calculated expected DIC concentrations and $\delta^{13}C$ values based on conservative mixing dynamics (weighted average) between

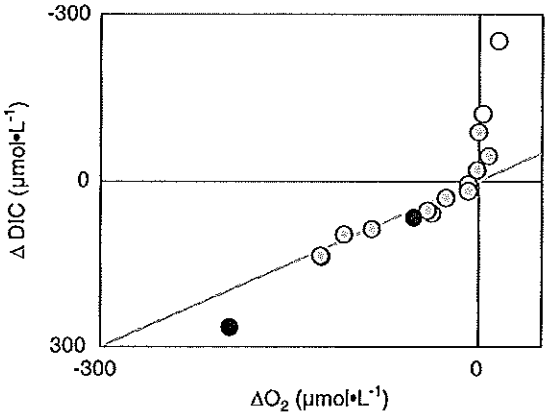
Fig. 4. (*a* and *c*) Vertical profiles of changes in dissolved inorganic carbon (DIC) concentrations, pCO_2, and $\delta^{13}C$ relative to conservative mixing predictions; (*b* and *d*): vertical profiles of dissolved oxygen (DO) concentrations and $\delta^{18}O$ relative to equilibrium with the atmosphere. Concentrations and stable isotope values are represented by open and solid circles, respectively; grey circles depict pCO_2. (*a* nad *b*) Station A5 in the vicinity of the mouth of the Mississippi River; (*c* and *d*) station F6 approximately 200 km west of the mouth of the Mississippi River.



Mississippi River and offshore waters (Fry 2002). Subsequently, we present DIC concentrations and $\delta^{13}C$ as deviations from our conservative mixing predictions. For reasons of consistency, we also express DO concentrations and $\delta^{18}O$ as deviations from equilibrium with the atmosphere.

DO and $\delta^{18}O$ dynamics can be very helpful, not only to model O_2 dynamics in surface waters (Quiñones-Rivera et al. 2007; Tobias et al. 2007; Venkiteswaran et al. 2007) but also to analyze the relative importance of water column versus benthic respiration in bottom waters, which has been shown for rivers (Quay et al. 1995), lakes (Russ et al. 2004; Ostrom et al. 2005), and coastal systems (Quiñones-Rivera et al. 2007). Nevertheless, for this study, we will focus on

Fig. 5. Relationship between measured change in dissolved oxygen (DO) and calculated change in dissolved inorganic carbon (DIC) (change of 104 mmol·m^{-3} per 1.0‰ change in $\delta^{13}C$) for stations A5 and F6 (surface, intermediate, and bottom samples are represented by open, shaded, and solid circles, respectively). The grey line represents the 1:1 relationship between DO and DIC.



the coupling of DO and DIC dynamics, as this is the unique advancement of our approach of combined analysis of DO and DIC.

Station A5, which is located closer to the mouth of the Mississippi River, had strongly depleted DIC concentrations in surface waters, exceeding a deficit of 400 mmol·m^{-3} (Fig. 4). In contrast, below 10 m depth, DIC concentrations were up to 500 mmol·m^{-3} higher than expected from conservative mixing. The pCO_2 values (microatmospheres calculated from measured DIC concentration and pH; Lewis and Wallace 1998) closely followed DIC concentrations but were slightly less depleted than DIC in surface waters (Fig. 4). In bottom waters, pCO_2 was always elevated, but relative to DIC concentrations, pCO_2 covaried with pH. At station A5, a relatively lower pH of 7.7 resulted in pCO_2 surpassing DIC excess, while at station F6, a higher pH of 8.1 reduced the excess of pCO_2 relative to DIC concentration.

The dynamics of DIC concentrations were clearly reflected in the $\delta^{13}C$ values (Fig. 4), whereby enriched $\delta^{13}C$ in surface waters indicated the uptake of depleted DIC owing to strong photosynthetic activity, and depleted $\delta^{13}C$ at greater depths pointed towards the addition of respired CO_2 from isotopically light organic matter. Yet, the magnitude of change in DIC relative to the change in $\delta^{13}C$ for offshore samples (186 mmol·m^{-3} per 1.0‰ change in $\delta^{13}C$) was about twice as large as measured in the closed-system incubations above (96 mmol·m^{-3} per 1.0‰ change in $\delta^{13}C$) and three shipboard respiration incubations with closed bottles held in the dark (106 mmol·m^{-3} per 1.0‰ change in $\delta^{13}C$). This larger CO_2 deficit observed in the offshore surface samples compared with the biology-only incubation reference experiments could be due to several factors, including carbonate mineral formation and dissolution or gas exchange. Carbonate formation is possible but is usually associated with blooms of coccolithophores that have not been documented for the Louisiana shelf ecosystem (Dortch et al. 2001). Also, carbonate formation should result in strong alkalinity losses that have not been observed in recent

surveys of the Louisiana shelf ecosystem (W.-J. Cai, Department of Marine Science, University of Georgia, Athens, GA 30602, USA, personal communication). An alternative to carbonate dynamics is that physical gas exchange in surface waters strongly affects the observed CO_2/O_2 stoichiometries. This is reasonable given the relatively fast equilibration of O_2 with the atmosphere and the relatively slow equilibration of CO_2 so that plankton productivity signals are preserved much longer in the DIC pool than in the O_2 pool (Sambrotto and Langdon 1994). This faster equilibration of O_2 than CO_2 after plankton blooms will lead to an apparent stronger DIC deficit, with isotope values of DIC more slowly returning to equilibrium (Sarmiento and Gruber 2006). At the current time, these exchange dynamics seem to be the most likely explanation for the decoupling of CO_2/O_2 stoichiometries from about 1:1 expected for metabolic cycling to the approximately 7:1 signals observed for surface waters (Fig. 5).

With respect to the coupling of CO_2 and O_2 dynamics on the Louisiana continental shelf, the combined analysis of DO and DIC indicated several different patterns. At intermediate water depths, DO and DIC were tightly coupled, confirming the PQ and RQ values close to 1.0 that we observed in the closed-system incubation experiments above. In one bottom water sample (Fig. 5), DIC surplus exceeded DO depletion, likely owing to additional anaerobic respiration processes that relied on nitrate, sulfate, or methane instead of DO as electron donor. Accordingly, we observed slightly elevated N_2 saturation levels (106%) in bottom waters of station A5 where DIC analysis had indicated anaerobic respiration. Yet, this increase was not associated with a significant decrease in $\delta^{15}N$ values that would be expected during intense denitrification. Lastly, in surface waters, DIC depletion greatly exceeded DO supersaturation. CO_2 dynamics were recording longer term productivity patterns owing to the slower re-equilibration of seawater pools for CO_2 than for O_2. Less than 1 week is usually required for reequilibration of surface O_2 pools, but reequilibration of CO_2 pools can take several months. The average surface net primary production across the continental shelf in July 2001 was estimated to be 0.06 g $C \cdot m^{-3} \cdot day^{-1}$ (Quiñones-Rivera et al. 2007). Assuming a mixing depth of 5 m, it would take approximately 22 and 40 days to reach the observed DIC depletions of 110 and 202 $mmol \cdot m^{-3}$ at stations F6 and A5, respectively (which is a conservative estimate, as it does not consider CO_2 influx from the atmosphere). In contrast, CO_2 influx from the atmosphere across the Louisiana continental shelf during comparable summer conditions in June 2003 was estimated to be 3–5 mmol $C \cdot m^{-2} \cdot day^{-1}$ (Lohrenz and Cai 2006), and at this rate, it would take several months to replenish a deficit of 200 $mmol \cdot m^{-3}$. Obviously, seasonal high-wind events are important to redistribute DIC across the water column, as they not only increase the air–sea gas exchange (Wanninkhof and McGillis 1999) but also bring DIC-rich bottom waters in contact with DIC-depleted surface waters.

Future work

Our new approach of coupled gas analysis made it possible to routinely study how O_2 and CO_2 dynamics are coupled and decoupled in aquatic systems. This methodology provides very good insight into the dynamics of PQ and RQ in coastal systems. Furthermore, the productivity and respiration rate measurements and associated fractionation factors from bottle incubations could be very helpful for subsequent modeling of metabolic processes, as shown by Tobias et al. (2007), Venkiteswaran et al. (2007), and Quiñones-Rivera et al. (2007). Finally, in situ sampling showed strongly divergent O_2 and CO_2 processes for both surface and bottom waters, and only the simultaneous analysis of concentrations and stable isotopes could separate physical and biological processes of DIC.

Lastly, uncertainties related to measuring O_2 and DIC concentrations using our injection technique may constrain the usefulness of this method to more productive waters where DO and DIC changes are strong. For low-productivity waters, either more elaborate gas handling techniques would be necessary where samples bottles are preevacuated and preserved with HgCl (Quay et al. 1993; Bender et al. 1999; Emerson et al. 1999) or concentration measurements should be performed independently using high-precision techniques to obtain these estimates.

Acknowledgements

This work was funded by an NOAA Coastal Ocean Program grant for hypoxic studies and EPA/STAR grant No. R828009. We thank W. Brand for suggesting these multiple GC systems for triple isotope measurements more than 10 years ago. B. Lavettri provided valuable advice during construction of the two-GC systems. Delacroix Corporation provided access to the marsh field site, and N. Rabalais organized and led the cruises during which offshore samples were obtained. We are grateful to K. Finlay and two anonymous reviewers for helpful comments on an earlier version of this manuscript.

References

Atekwana, E.A., and Krishnamurthy, R.V. 1998. Seasonal variations of dissolved inorganic carbon and $\delta^{13}C$ of surface waters: application of a modified gas evolution technique. J. Hydrol. (Amst.), **205**: 265–278. doi:10.1016/S0022-1694(98)00080-8.

Barkan, B., and Luz, E. 2005. High precision measurements of $^{17}O/^{16}O$ and $^{18}O/^{16}O$ ratios in H_2O. Rapid Commun. Mass Spectrom. **19**: 3737–3742. doi:10.1002/rcm.2250.

Bastow, T.P., Jackson, G., and Edmonds, J.S. 2002. Elevated salinity and isotopic composition of fish otolith carbonate: stock delineation of pink snapper, *Pagras auratus*, in Shark Bay, Western Australia. Mar. Biol. (Berl.), **141**: 801–806. doi:10.1007/s00227-002-0884-8.

Bender, M.L., Orchardo, J., Dickson, M.L., Barber, R., and Lindley, S. 1999. In vitro O_2 fluxes compared with ^{14}C production and other rate terms during the JGOFS Equatorial Pacific experiment. Deep-Sea Res. Part I Oceanogr. Res. Pap. **46**: 637–654. doi:10.1016/S0967-0637(98)00080-6.

Benson, B.B., and Krause, D. 1984. The concentration and isotopic fractionation of oxygen dissolved in fresh water and seawater in equilibrium with the atmosphere. Limnol. Oceanogr. **29**: 620–632.

Benson, B.B., and Parker, P.D.M. 1961. Nitrogen/argon and nitrogen isotope ratios in aerobic seawater. Deep-Sea Res. Part I Oceanogr. Res. Pap. **7**: 237–253.

Cai, W.J., and Wang, Y. 1998. The chemistry, and sources of car-

bon dioxide in the estuarine waters of the Satilla and Altamaha rivers, Georgia. Limnol. Oceanogr. **43**: 657–668.

Cole, J.J., Caraco, N.F., Kling, G.W., and Kratz, T.K. 1994. Carbon dioxide supersaturation in the surface waters of lakes. Science (Washington, D.C.), **265**: 1568–1570. doi:10.1126/science.265.5178.1568. PMID:17801536.

Coplen, T.B., and Kendall, C. 2000. Stable hydrogen and oxygen isotope ratios for selected sites of the U.S. Geological Survey's NASQAN and Benchmark Surface-water Networks. Open-file Rep. 00-160. US Geological Survey, Reston, Va.

Craig, H., and Hayward, T. 1987. Oxygen supersaturation in the ocean: biological versus physical contributions. Science (Washington, D.C.), **235**: 199–202. doi:10.1126/science.235.4785.199. PMID:17778634.

Dortch, Q., Turner, R.E., and Rowe, G.T. 1994. Respiration rates and hypoxia on the Louisiana shelf. Estuaries, **17**: 862–872. doi:10.2307/1352754.

Dortch, Q., Rabalais, N.N., Truner, R.E., and Qureshi, N.A. 2001. Impacts of changing Si/N ratios and phytoplankton species composition. *In* Coastal hypoxia: consequences for living resources and ecosystems. *Edited by* N.N. Rabalais and R.E. Turner. American Geophysical Union, Washington, D.C. pp. 37–48.

Duarte, C.M., and Agusti, S. 1998. The CO_2 balance of unproductive aquatic ecosystems. Science (Washington, D.C.), **281**: 234–236. doi:10.1126/science.281.5374.234. PMID:9657712.

Emerson, S., Quay, P.D., and Wheeler, P.A. 1993. Biological productivity determined from oxygen mass balance and incubation experiments. Deep-Sea Res. Part I Oceanogr. Res. Pap. **40**: 2351–2358. doi:10.1016/0967-0637(93)90109-G.

Emerson, S., Stump, C., Wilbur, D.O., and Quay, P.D. 1999. Accurate measurement of O_2, N_2, and Ar gases in water and the solubility of N_2. Mar. Chem. **64**: 337–347. doi:10.1016/S0304-4203(98)00090-5.

Farquhar, G.D., O'Leary, M.H., and Berry, J.A. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol. **9**: 121–137.

Friedman, I., and O'Neil, J.R. 1977. Compilation of stable isotope fractionation factors of geochemical interest. 6th ed. *In* Data of geochemistry. US Geol. Surv. Prof. Pap. 440-KK. US Geological Survey, Reston, Va. pp. 1–12.

Fry, B. 2002. Conservative mixing of stable isotopes across estuarine salinity gradients: a conceptual framework for monitoring watershed influences on downstream fisheries production. Estuaries, **25**: 264–271.

Fry, B. 2006. Stable isotope ecology. Springer, New York.

Gao, Y., and Beamish, R.J. 2003. Stable isotopic composition of otoliths from tagged Pacific halibut, *Hippoglossus stenolepis*. Environ. Biol. Fishes, **67**: 253–261. doi:10.1023/A:1025874110893.

Guy, R.D., Fogel, M.L., Berry, J.A., and Hoering, T.H. 1986. Isotope fractionation during oxygen production and consumption by plants. *In* Progress in photosynthesis research. Vol. III. *Edited by* J. Biggins. Martinus Nijhoff, Dodrecht, the Netherlands. pp. 597–600.

Guy, R.D., Fogel, M.L., and Berry, J.A. 1993. Photosynthetic fractionation of stable isotopes of oxygen and carbon. Plant Physiol. **101**: 37–47. PMID:12231663.

Hendricks, M.B., Bender, M.L., and Barnett, B.A. 2004. Net and gross O_2 production in the Southern Ocean from measurements of biological O_2 saturation and its triple isotope composition. Deep-Sea Res. Part I Oceanogr. Res. Pap. **51**: 1541–1561.

Hood, D.W. 1981. Preliminary observations of the carbon budget of the eastern Bering Sea shelf. *In* The Eastern Bering Sea Shelf: oceanography and resources. Vol. 1. *Edited by* D.W. Hood and J.A. Caldor. University of Washington Press, Seattle, Wash. pp. 347–358.

Horita, J., Ueda, A., Mizukami, K., and Takatori, I. 1989. Automatic delta-D and delta-O-18 analyses of multi-water samples using H_2 water and CO_2 equilibration methods with a common equilibration set-up. Appl. Radiat. Isot. **40**: 801–805.

International Atomic Energy Agency. 2006. Isotope hydrology information system. The ISOHIS Database. Available from isohis.iaea.org [accessed 29 September 2008].

Jonsson, A., Karlsson, J., and Jansson, M. 2003. Sources of carbon dioxide supersaturation in clearwater and humic lakes in northern Sweden. Ecosystems, **6**: 224–235. doi:10.1007/s10021-002-0200-y.

Kampbell, D.H., Wilson, J.T., and Vendegrift, S.A. 1989. Dissolved oxygen and methane in water by a GC headspace equilibration technique. Int. J. Environ. Anal. Chem. **36**: 249–257. doi:10.1080/03067318908026878.

Keeling, C.D., Bollenbacher, A.F., and Whorf, T.P. 2005. Monthly atmospheric $^{13}C/^{12}C$ isotopic ratios for 10 SIO stations. *In* Trends: a compendium of data on global change. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tenn. Available from cdiac.ornl.gov/trends/co2/iso-sio/iso-sio.html [accessed 29 September 2008].

Kiddon, J., Bender, M.L., Orchado, J., Caron, D.A., Goldman, J.C., and Dennett, M. 1993. Isotopic fractionation of oxygen by respiring marine organisms. Global Biogeochem. Cycles, **7**: 679–694. doi:10.1029/93GB01444.

Killingley, J.S. 1980. Migrations of California gray whales tracked by oxygen-18 variations in their epizoic barnacles. Science (Washington, D.C.), **207**: 759–760. doi:10.1126/science.207.4432.759. PMID:17796007.

Knox, M., Quay, P.D., and Wilbur, D.O. 1992. Kinetic isotope fractionation during air-water gas transfer of O_2, N_2, CH_4, and H_2. J. Geophys. Res. **97**: 20335–20343. doi:10.1029/92JC00949.

Kroopnick, P.M. 1975. Respiration, photosynthesis, and oxygen isotope fractionation in oceanic surface water. Limnol. Oceanogr. **20**: 988–992.

Kroopnick, P.M., and Craig, H. 1976. Oxygen isotope fractionation in dissolved oxygen in the deep sea. Earth Planet. Sci. Lett. **32**: 375–388. doi:10.1016/0012-821X(76)90078-9.

Lane, G.A., and Dole, M. 1956. Fractionation of oxygen isotopes during respiration. Science (Washington, D.C.), **123**: 574–576. doi:10.1126/science.123.3197.574. PMID:13311503.

Lewis, E., and Wallace, D.W.R. 1998. Program developed for CO_2 system calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tenn.

Lin, G., and Ehleringer, J.R. 1997. Carbon isotope respiration does not occur during dark respiration in C3 and C4 plants. Plant Physiol. **114**: 391–394. PMID:12223712.

Lohrenz, S.E., and Cai, W.J. 2006. Satellite ocean color assessment of air–sea gas fluxes of CO_2 in a river-dominated coastal margin. Geophys. Res. Lett. **33**: . doi:10.1029/2005GL023942.

Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., and Tardieux, P. 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. Plant Soil, **62**: 413–430. doi:10.1007/BF02374138.

Miyajima, T., Yamada, Y., Hanba, Y.T., Yoshii, K., Koitabashi, T., and Wada, E. 1995. Determining the stable isotope ratio of total

dissolved inorganic carbon in lake water by GC/C/IRMS. Limnol. Oceanogr. 40: 994–1000.

Mook, W.G., Bommerson, J.C., and Staverman, W.H. 1974. Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. Earth Planet. Sci. Lett. 22: 169–176. doi:10.1016/0012-821X(74)90078-8.

Mulholland, P.J., Houser, J.N., and Hayden, M.G. 2005. Stream diurnal dissolved oxygen profiles as indicators of stream metabolism and disturbance effects: Fort Bennings as a case study. Ecol. Indicators, 5: 243–252. doi:10.1016/j.ecolind.2005.03.004.

Odum, H.T. 1956. Primary production in flowing waters. Limnol. Oceanogr. 1: 102–117.

Ostrom, N.E., Carrick, H.J., Twiss, M.R., and Piwinski, L. 2005. Evaluation of primary production in Lake Erie by multiple proxies. Oecologia (Berl.), 144: 115–124. doi:10.1007/s00442-005-0032-5.

Parker, S.R., Poulson, S.R., Gammons, C.H., and Degrandpre, M.D. 2005. Biogeochemical controls on diel cycling of stable isotopes of dissolved O-2 and dissolved inorganic carbon in the Big Hole River, Montana. Environ. Sci. Technol. 39: 7134–7140. doi:10.1021/es0505595.

Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 18: 293–320. doi:10.1146/annurev.es.18.110187.001453.

Quay, P.D., Emerson, S., Wilbur, D.O., and Stump, C. 1993. The $\delta^{18}O$ of dissolved O_2 in the surface waters of the subarctic pacific: a tracer of biological productivity. J. Geophys. Res. 98: 8447–8458. doi:10.1029/92JC03017.

Quay, P.D., Wilbur, D.O., Richey, J.E., Devol, A.H., Benner, R., and Forsberg, B.R. 1995. The ^{18}O:^{16}O of dissolved oxygen in rivers and lakes in the Amazon basin: determining the ratio of respiration to photosynthesis rates in freshwaters. Limnol. Oceanogr. 40: 718–729.

Quiñones-Rivera, Z.J., Wissel, B., Justic, D., and Fry, B. 2007. Partitioning oxygen sources and sinks in a stratified, eutrophic coastal ecosystem using stable oxygen isotopes. Mar. Ecol. Prog. Ser. 342: 69–83. doi:10.3354/meps342069.

Rabalais, N.N., and Turner, R.E. 2001. Coastal hypoxia: consequences for living resources and ecosystems. Coastal and Estuarine Studies 58. American Geophysical Union, Washington, D.C.

Robinson, C., and Williams, P.J.leB. 1991. Development and assessment of an analytical system for the accurate and continual measurement of total dissolved inorganic carbon. Mar. Chem. 34: 157–175. doi:10.1016/0304-4203(91)90001-D.

Russ, M.E., Ostrom, N.E., Gandhi, H., Ostrom, P.H., and Urban, N.R. 2004. Temporal and spatial variation in *R:P* ratios in Lake Superior, an oligotrophic freshwater environment. J. Geophys. Res. 109: C10S12. doi:10.1029/2003JC001890.

Salata, G.G., Roelke, A., and Cifuentes, L.A. 2000. A rapid and precise method for measuring stable carbon isotope ratios of dissolved inorganic carbon. Mar. Chem. 69: 153–169. doi:10.1016/S0304-4203(99)00102-4.

Sambrotto, R.N., and Langdon, C. 1994. Water column dynamics of dissolved inorganic carbon (DIC), nitrogen and O_2 on Georges Bank during April 1990. Cont. Shelf Res. 14: 765–789. doi:10.1016/0278-4343(94)90072-8.

Sarmiento, J.L., and Gruber, N. 2006. Ocean biogeochemical dynamics. Princeton University Press, Princeton, N.J.

Schindler, D.W., and Fee, E.J. 1973. Diurnal variation of dissolved inorganic carbon and its use in estimating primary production and CO_2 invasion in Lake 227. J. Fish. Res. Board Can. 30: 1501–1509.

Sellers, P., Hesslein, R.H., and Kelly, C.A. 1995. Continuous measurement of CO_2 for estimation of air–water fluxes in lakes: an in situ technique. Limnol. Oceanogr. 40: 575–581.

Tobias, C.R., Böhlke, J.K., and Harvey, J.W. 2007. The oxygen-18 isotope approach for measuring aquatic metabolism in high-productivity waters. Limnol. Oceanogr. 52: 1439–1453.

Venkiteswaran, J.J., Wassenaar, L.I., and Schiff, S.L. 2007. Dynamics of dissolved oxygen isotopic ratios: a transient model to quantify primary production, community respiration, and air–water exchange in aquatic ecosystems. Oecologia (Berl.), 153: 385–398. doi:10.1007/s00442-007-0744-9.

Vogel, J.C., Grootes, P.M., and Mook, W.G. 1970. Isotope fractionation between gaseous and dissolved carbon dioxide. Z. Phys. 230: 225–238. doi:10.1007/BF01394688.

Wang, Z.A., Cai, W.J., Wang, Y., and Ji, H. 2005. The southeastern continental shelf of the United States as an atmospheric CO_2 source and an exporter of inorganic carbon to the ocean. Cont. Shelf Res. 25: 1917–1941. doi:10.1016/j.csr.2004.10.013.

Wanninkhof, R., and McGillis, W.R. 1999. A cubic relationship between air–sea gas exchange and wind speed. Geophys. Res. Lett. 26: 1889–1892. doi:10.1029/1999GL900363.

Wassenaar, L.I., and Koehler, G. 1999. An on-line technique for the determination of the $\delta^{18}O$ and $\delta^{17}O$ of gaseous and dissolved oxygen. Anal. Chem. 71: 4965–4968. doi:10.1021/ac9903961.

Weiss, R. 1970. The solubility of nitrogen, oxygen and argon in water and seawater. Deep Sea Res. Part I Oceanogr. Res. Pap. 17: 721–735. doi:10.1016/0011-7471(70)90037-9.

Weiss, R. 1974. Carbon dioxide in water and seawater: the solubility of a non-ideal gas. Mar. Chem. 2: 203–215. doi:10.1016/0304-4203(74)90015-2.

Wetzel, R.G. 2001. Limnology — lake and river ecosystems. Academic Press, San Diego, Calif.

Williams, P.J.leB., and Robertson, J.E. 1991. Overall planktonic oxygen and carbon metabolism: problem of reconciling observations and calculations of photosynthetic quotients. J. Plankton Res. 13: 153–169.

Wissel, B., and Fry, B. 2005. Tracing Mississippi River influences in estuarine food webs of coastal Louisiana. Oecologia (Berl.), 144: 659–683. doi:10.1007/s00442-005-0119-z.

Wissel, B., Gaçe, A., and Fry, B. 2005. Tracing river influences on phytoplankton dynamics in two Louisiana estuaries. Ecology, 86: 2751–2762. doi:10.1890/04-1714.

Zhang, J., and Quay, P.D. 1997. The total organic carbon export rate based on ^{13}C and ^{12}C of DIC budgets in the equatorial Pacific region. Deep Sea Res. Part II Top. Stud. Oceanogr. 44: 2163–2190. doi:10.1016/S0967-0645(97)00032-5.