THE APPLICATION OF
PUMPING INDUCED EBULLITION
BASED CARBON DIOXIDE MEASUREMENTS
IN NORTHERN WISCONSIN LAKE STUDIES

By

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ABSTRACT

Few lake studies have used a method that directly measures dissolved carbon dioxide (CO₂). Direct measurements are dissolved carbon dioxide estimates obtained with an analyzer or sensor, such as the headspace analysis method that uses gas collection syringes and an ion chromatography CO₂ analyzer (Cole and Caraco, 1998). In lake studies summarized in Sobek et al. (2005) for the period 1968 to 2005, the vast majority of estimates of dissolved carbon dioxide were indirectly derived from measurements of pH and dissolved inorganic carbon (DIC) or pH and acid neutralizing capacity (ANC). Traditional direct measurements are difficult to obtain. However, indirect measurements can under or overestimate the carbon dioxide concentration due to uncertainties associated with pH, DIC and ANC.

Recently, a new field based technology called pumping induced ebullition (PIE) was developed for the direct measurement of carbon dioxide (Browne, 2004a). This approach simplifies direct carbon dioxide measurements by using a process that mechanically induces ebullition of a dissolved gas in a water sample, traps the harvested gas within a collection chamber, and measures this harvested gas with an infrared CO₂ gas analyzer (IRGA).

This study explored the potential of this new technology for the collection of field based carbon dioxide data. The accuracy and precision of PIE based measurements of carbon dioxide were characterized using solutions of defined composition. PIE based carbon dioxide measurements were also made in lakes across three trophic classes (eutrophic, mesotrophic, oligotrophic). These results were
compared to traditional direct (headspace) and indirect (pH, ANC; pH, DIC) carbon dioxide measurements.

Carbon dioxide measurements made using PIE showed high precision and accuracy for defined carbon dioxide solutions from 0 to 100 μmol/liter DIC and 0 to 2610 μatm $P_{CO2}$. Linear regressions between PIE based $[H_2CO_3]^{-}$ and the sodium bicarbonate concentration added to the solutions showed a close relationship with $r^2$ values of 1.00, RMSE ranging from 0.57 to 1.99 μmol L$^{-1}$ $[H_2CO_3]^{-}$, and coefficients of variation ranging from 1.29 to 4.32 %. PIE’s trend with the sodium bicarbonate concentration was also close to the one-to-one line, with slope values ranging from 0.94 to 0.98. PIE based $P_{CO2}$ also proved to be a precise, accurate, and reliable measurement for surface analyses of lake bodies ranging from zero to 2314 μatm $P_{CO2}$ and for depth analyses of lake bodies ranging from 450 to 4250 μatm $P_{CO2}$.

The PIE method substantially increases the ability to measure carbon dioxide variations in time and space. In a continuous twenty-four hour diurnal pattern, PIE based $P_{CO2}$ and DIC measurements showed the actual respiration and photosynthesis of the lake from day to night and back to day at temporal resolutions inaccessible via traditional indirect and other direct sampling methods. PIE based $P_{CO2}$ and DIC whole lake maps also showed spatial variation of dynamic lake systems at rarely documented spatial resolution. PIE gives researchers direct measurement methods of known quality (accuracy and precision), spatial resolution, and temporal resolution for studying dissolved carbon dioxide, which were inaccessible via traditional sampling methods (indirect and other direct carbon dioxide measurement methods).
ACKNOWLEDGEMENTS

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The rate at which a dissolved gas (i) is pumped into the PIE device.

The rate of bubble formation.

The rate at which the remaining dissolved gas exhausts from the water outlet.

The partial pressure ($P_i$) of an individual dissolved gas within the gas collection chamber is related to its partial pressure in the original solution ($P_0$) by:

PIE based $P_{CO2}$.

PIE based DIC.

$[H_2CO_3^*]_{calc}$ is the calculated concentration of dissolved carbon dioxide.

Expected mole fraction response ($X_{E1}$) of the LI-COR model LI-840 for the sample for PIE based $P_{CO2}$.

$[DIC]_{calc}$ is the calculated DIC concentration.

Expected mole fraction response ($X_{E2}$) of the LI-COR model LI-840 for the sample for PIE based DIC.

Headspace based $[H_2CO_3^*]$.

$n_w$ is the total moles of $CO_2$ present in $V_w$ after headspace equilibration.

$n_{HS}$ is the total moles of $CO_2$ present in the 60-ml of headspace gas ($V_{HS}$) after headspace equilibration.
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INTRODUCTION

Dissolved carbon dioxide is an important biogeochemical component in lakes. Dissolved carbon dioxide from ground water, the atmosphere, surface water, and in-lake respiration of dissolved organic matter are important sources of carbon for within lake primary production (Portielje and Lijklema, 1995; Striegl and Michmerhuizen, 1998). Changes in dissolved carbon dioxide concentration can increase or decrease lake pH. These pH changes can lead to the precipitation (higher pH) or dissolution (lower pH) of calcium carbonate (Wetzel, 2001). The biogeochemistry of dissolved carbon dioxide in lakes may also have important consequences for greenhouse gases in the atmosphere. Recent studies of lake metabolism have shown that most of the world’s lakes surface waters are supersaturated with dissolved carbon dioxide, suggesting that lakes may be a net source of carbon dioxide emission into the atmosphere (Sobek et al., 2005).

Despite carbon dioxide’s central biogeochemical role, few detailed lake studies have used a method to directly measure dissolved carbon dioxide. Direct measurements are defined here as dissolved carbon dioxide estimates obtained by direct measurement with an analyzer or sensor. Direct measurements include headspace analysis that uses gas collection syringes and an ion chromatography CO₂ analyzer (Cole and Caraco, 1998), submersible autonomous moored instrument for CO₂ (SAMI-CO₂) that uses reagent and membrane based optical chemical sensors (Baehr and Degrandpre, 2004), in situ equilibration chamber (ISEC) that uses a gas-permeable membrane, equilibration chamber, and an infrared gas analyzer (IRGA)
(Sellers et al., 1995), or an automated system in Carignan (1998) that uses an equilibration chamber and IRGA. In contrast, indirect measurements are defined here as dissolved carbon dioxide estimates indirectly derived from measurements of pH and dissolved inorganic carbon (DIC) or pH and acid neutralizing capacity (ANC) as used in lake studies of Cole et al. (1994) and Kling et al. (1992).

In supersaturation studies and other lake studies, the vast majority of estimates of dissolved carbon dioxide have been indirect measurements based on pH and DIC or pH and ANC. Of 4902 lakes summarized in Sobek et al. (2005), carbon dioxide was calculated indirectly using thermodynamic equilibrium relationships for at least 1658 lakes (best estimate) based on pH and DIC measurements and for 2876 lakes (best estimate) based on pH and alkalinity or ANC measurements. In less than 439 lakes of 4902 lakes (best estimate) reported within Sobek et al. (2005) was carbon dioxide measured directly. This also holds true with studies by Anderson et al. (1997), Baehr and Degrandpré (2004), Baehr and Degrandpré (2002), Cole and Caraco (1998), Cole et al. (1994), Hope et al. (1996), Jonsson et al. (2003), Kling et al. (1992), Neumann et al. (2001), Sellers et al. (1995), Striegl and Michmerhuizen (1998), and SLU (2000) summarized in Table 1, where out of a total of 4732 lakes studied only 150 lakes had direct carbon dioxide measurements.

Indirect measurements of carbon dioxide can overestimate or underestimate dissolved carbon dioxide due to random or systematic errors in pH, DIC, or ANC (e.g., Cole et al., 1994; Herczeg et al., 1985; Lahav et al., 2001; Neumann et al., 2001; Raymond et al., 1997; Rosich, 2002). However, the nature and effect of these errors
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<table>
<thead>
<tr>
<th>Data Source</th>
<th>Indirect Measurement by pH &amp; DIC (number of lakes)</th>
<th>Indirect Measurement by pH &amp; ANC or pH &amp; Alkalinity (number of lakes)</th>
<th>Direct Measurement by headspace, SAMI-CO₂, or ISEC (number of lakes)</th>
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<tr>
<td>total directly measured lakes</td>
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</tr>
<tr>
<td>total lakes studied</td>
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</tr>
</tbody>
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*lake measured both directly and indirectly, therefore included in both counts.

Indirect measurements of dissolved carbon dioxide have been more numerous in the literature. This is mainly due to logistical impediments associated with traditional direct in-field measurements. Other factors that affect direct measurements include systematic errors and sensor drift resulting from quality of the colorimetric
indicator reagent, light input to sensor, sensor equilibration, or biological fouling (Degrandpre and Baehr, 1999; Degrandpre et al., 1995; Sellers et al., 1995).

Recently, a new field based technology called pumping induced ebullition (PIE) was developed for the direct measurement of carbon dioxide (Browne, 2004a). This approach simplifies direct carbon dioxide measurements by using a process that mechanically induces ebullition of a dissolved gas in a water sample, traps the harvested gas within a collection chamber, and measures this harvested gas with an infrared CO$_2$ gas analyzer (IRGA).

This new approach can directly measure carbon dioxide in real time while on a lake, stream, or river. Carbon dioxide data is recorded in real time at one record per second and stored on a data logger for archival purposes or for immediate review of the concentration on a computer display. PIE based measurements can be made continuously or in rapid succession throughout a water body at temporal and spatial resolutions inaccessible via traditional direct (headspace) and indirect (pH and DIC; pH and ANC) dissolved CO$_2$ measurement methods.

Additionally, this PIE based approach offers quality, time, and labor savings. This new approach avoids the systematic error associated with use of colorimetric indicator reagents, membranes, and equilibration chambers. The PIE based approach also avoids the time involved with the collection, storage, transport, and analysis of a bottle sample. The PIE based approach uses no sample analytical measurement time in the laboratory, whereas traditional direct (headspace) and indirect (pH and DIC; pH and ANC) dissolved CO$_2$ measurement methods involve many measurement hours.
This study explored the potential of this new technology for the collection of field based carbon dioxide data. The specific objectives were:

1.) Characterize the accuracy and precision of PIE based direct measurements of carbon dioxide using solutions of defined composition.

2.) Apply PIE based direct carbon dioxide measurements in a limited study of lakes across three trophic classes (eutrophic, mesotrophic, oligotrophic) and compare results to traditional direct and indirect carbon dioxide measurements.

3.) Explore the use of PIE measurements for developing whole lake maps of carbon dioxide concentration.

This research is presented in the four chapters that follow. The first chapter is a methods chapter. The METHODS chapter starts with the definitions and ideas for the main components of carbonic chemistry. The subsequent sections contain explanations of the direct (PIE; headspace) and indirect (pH and ANC; pH and DIC) dissolved carbon dioxide measurement methods used in this study. This is followed by subsections on Other Measurement Procedures and Data Management, Statistics, and Graphing.

In the ACCURACY and PRECISION of PIE chapter, the accuracy and precision of PIE based measurements were characterized using solutions of defined
composition. PIE based $P_{CO_2}$ and $[H_2CO_3^+]$, headspace, DIC, and ANC measurements were compared to known sodium bicarbonate concentrations. PIE based dissolved carbon dioxide measurements were also compared against headspace measurements. Subsections include the methods for the laboratory experiments, results achieved, and conclusions found.

The TRADITIONAL LAKE STUDIES with PIE chapter is where PIE based direct carbon dioxide measurements were applied in a limited study of lakes across three trophic classes (eutrophic, mesotrophic, oligotrophic) and compared to traditional direct and indirect carbon dioxide measurements. This is where researchers will learn the importance of how PIE based dissolved carbon dioxide measurements perform in natural systems and traditional lake studies. The first chapter section contains the methods used for the traditional lake studies. In the following chapter sections, (1) PIE based measurements were taken to have a range of carbon dioxide measurements similar to the laboratory experiments; (2) a set of replicate carbon dioxide measurements within a lake were made to investigate variations between PIE and traditional direct and indirect carbon dioxide measurements; (3) PIE based carbon dioxide measurements were compared in a traditional fifty-two lake surface water survey (one surface water sample measurement with each method at each lake); and (4) PIE based carbon dioxide measurements were compared in traditional depth profiles from two lakes. PIE based measurements were also compared against direct (headspace) and indirect (pH and ANC; pH and DIC) measurements. The last section contains the conclusions for the traditional lake studies.
The TEMPORAL and SPATIAL RESOLUTION of PIE chapter contains the methods, and results for the temporal and spatial lake experiments. This is where the PIE method was shown to substantially increase the ability to measure carbon dioxide variations in time and space. In a continuous twenty-four hour diurnal pattern, PIE based partial pressure of carbon dioxide (P$_{CO2}$) and DIC measurements showed the actual respiration and photosynthesis of the lake from day to night and back to day at temporal resolutions inaccessible via traditional indirect and other direct sampling methods. PIE based P$_{CO2}$ and DIC whole lake maps also showed spatial variation of dynamic lake systems at spatial resolution inaccessible via traditional indirect and other direct sampling methods. Several locations on these maps indicated possible ground and/or surface water entry of dissolved carbon dioxide into the lake. These maps also showed the spatial variation of dissolved carbon dioxide over time.
METHODS

This chapter starts with the definitions and ideas for the main components of carbonic chemistry. The subsequent sections contain explanations of the direct (PIE; headspace) and indirect (pH and ANC; pH and DIC) dissolved carbon dioxide measurement methods used in this study. This is followed by subsections on Other Measurement Procedures and Data Management, Statistics, and Graphing.

Carbonate Chemistry Background

The inorganic carbon in water consists mainly of carbonic acid (H$_2$CO$_3$), aqueous carbon dioxide (CO$_2$(aq)), bicarbonate (HCO$_3^-$), and carbonate (CO$_3^{2-}$). Aqueous carbon dioxide (CO$_2$(aq)) solutions (lakes or open systems) in the presence of atmospheric gaseous carbon dioxide (CO$_2$(g)) will exchange by the following reversible relationship:

\[ CO_2(g) + H_2O(aq) \rightleftharpoons CO_2(aq) + H_2O(aq) \]

(Stumm and Morgan, 1981). Once dissolved, carbon dioxide (CO$_2$(aq)) can dissociate in the following manner:

\[ CO_2(aq) + H_2O(aq) \rightleftharpoons H_2CO_3(aq) \]

where H$_2$CO$_3$ is carbonic acid. The total analytical concentration of dissolved carbon dioxide ([H$_2$CO$_3^*$]) in mol L$^{-1}$ is defined from the following relationship:

\[ [H_2CO_3^*(aq)] = [H_2CO_3(aq)] + [CO_2(aq)] \]
where $[\text{H}_2\text{CO}_3]$ is the concentration of carbonic acid and $[\text{CO}_2\text{aq}]$ is the concentration of aqueous carbon dioxide. This convention in Eq. [3] exists even though only about 0.1% of the solution actually forms as carbonic acid (Mook and de Vries, 1999).

Water has the ability to act as both an acid and a base at the same time. The dissociation of water or equilibrium equation for water (McMurrey and Fay, 2001) is given by:

$$[4] \quad 2\text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{OH}^-$$

where $\text{H}_2\text{O}$ is water, $\text{H}_3\text{O}^+$ is the hydronium ion, and $\text{OH}^-$ is hydroxyl. The dissociation constant or ion-product constant of water for concentrated solutions ($K_w$) is defined as:

$$[5] \quad K_w = \left\{\frac{\text{H}_3\text{O}^+}{\text{OH}^-}\right\}$$

(McMurrey and Fay, 2001), where the {} indicate activity. The temperature dependent values of $K_w$ used for these studies were obtained from the following relationship (Mook and de Vries, 1999):

$$[6] \quad K_w = e^{\left(148.9802 - 13847.26/T - 148.9802 \times \text{LnT}\right)}$$

where $T$ is the solution temperature in Kelvin.

Carbonic acid is a polyprotic acid that dissociates in this stepwise manner:

$$[7] \quad \text{H}_2\text{CO}_3 + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{HCO}_3^-$$

$$[8] \quad \text{HCO}_3^- + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{CO}_3^{2-}$$
(McMurrey and Fay, 2001). Each dissociation step for carbonic acid has its own acidity or dissociation constant. The first dissociation constant of carbonic acid ($K_1$, Eq. [9]) and the second dissociation constant of carbonic acid ($K_2$, Eq. [10]) are defined as:

\[
K_1 = \frac{[H_3O^+][HCO_3^-]}{[H_2CO_3^*]}
\]

\[
K_2 = \frac{[H_3O^+][CO_3^{2-}]}{[HCO_3^-]}
\]

(McMurrey and Fay, 2001), where the {} indicate activity. The temperature dependent values of $K_1$ and $K_2$ used for these studies were obtained from the following relationships (Eqs. [11] and [12], respectively; Mook and de Vries, 1999):

\[
K_1 = 10^{-(3404.71/T + 0.032786T - 14.8435)}
\]

\[
K_2 = 10^{-(2902.39/T + 0.02379T - 6.4980)}
\]

where $T$ is the solution temperature in Kelvin.

The total concentration of dissolved inorganic carbon (DIC; Stumm and Morgan, 1981; Mook and de Vries, 1999) in mol L$^{-1}$ is defined as:

\[
\text{DIC}_{(aq)} = [H_2CO_3^*_{(aq)}] + [HCO_3^-_{(aq)}] + [CO_3^{2-}_{(aq)}].
\]

The solubility of a gas in a liquid at a given temperature is directly proportional to the partial pressure of the gas over the solution (McMurrey and Fay, 2001). Increasing the pressure, (e.g. the atmospheric pressure) will increase the solubility of the gas. The concentration of carbon dioxide in the solution is a function
of the temperature of the solution and its partial pressure of carbon dioxide ($P_{CO_2}$) expressed here in atm. It follows that the total analytical concentration of dissolved carbon dioxide ($[H_2CO_3^*]$, Eq. [14]) in mol L$^{-1}$ can be calculated by Henry’s law:

$$[14] \quad [H_2CO_3^*] = K_H \times P_{CO_2}$$

where $K_H$ in mol L$^{-1}$ atm$^{-1}$ is the temperature dependent Henry’s law constant of the solution (Kling et al., 1992; Stumm and Morgan, 1981; Mook and de Vries, 1999). The temperature dependent values of $K_H$ in mol L$^{-1}$ atm$^{-1}$ used for these studies were obtained from the following relationship from Mook and de Vries (1999):

$$[15] \quad K_H = 10^{-((−2622.38/T )−(0.0178471 T )+15.5873 )}$$

where $T$ is the solution temperature in Kelvin.

The acid neutralizing capacity (ANC) concentration in mol L$^{-1}$ is defined here as the capacity of solutes in an aqueous system to neutralize strong acid. In a system with only carbonate species, ANC (Baker et al., 1990; Mook and de Vries, 1999) is defined as:

$$[16] \quad ANC = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+]$$

where [$H^+$] is the hydrogen ion concentration.

**Direct measurement of CO$_2$ using pumping induced ebullition (PIE)**

Ebullition of a dissolved gas in water is the act of a gas leaving the water in the form of a bubble. This process takes place when the partial pressure of the dissolved gas is in excess of the ambient pressure acting on the water. The spontaneous
formation of bubbles can be induced in a water sample simply by pumping sample water through a narrow-diameter tube to mechanically decrease the ambient pressure acting on the water. The frictional losses from the tube and the suction of the pump will mechanically decrease the ambient pressure acting on the water to a pressure near zero. The bubbles buoyancy can then be used to trap them within a gas collection chamber. Figure 1 shows the schematic of the pumping induced ebullition (PIE) device used in this research. After being trapped, the carbon dioxide gas in the PIE gas collection chamber can then be measured with an analyzer via the connected sampling port. A LI-COR LI-840 infrared CO₂ gas analyzer (IRGA) was used for this study. Note that the PIE device harvests all dissolved gases (e.g. N₂, O₂, Ar, H₂, N₂O, CH₄, CFC₁₁, CFC₁₂, CFC₁₁₃, SF₆) from the water sample and that any of these other gases in the gas collection chamber could also be measured with an analyzer, via this connected sampling port (Browne, 2004a). Because the mole fraction (Xᵢ) of an individual gas within the harvested gas reflects its Xᵢ within the solution, measurements of Xᵢ can be used to estimate the concentration of dissolved gases in solution via Henry’s law (Browne, 2004a).

The partial pressure (Pᵢ) of an individual dissolved gas within the gas collection chamber (Figure 1) can be lower or higher than in the original solution (P₀). This is a function of the solubility of the dissolved gas. The dilution of an individual gas within the gas collection chamber is defined as the fractionation of that gas. This can be adjusted for with a fractionation coefficient. The fractionation coefficient (fᵢ) for a gas is the ratio of the PIE based mole fraction (Xᵢ) to the original mole fraction.
These fractionation coefficient values are controlled by the sample temperature, the pumping rate, and the width and length of the narrow-diameter tube used in the ebullition process. A gas with no fractionation would have a fractionation coefficient value of one. Fractionation was assumed to be close to one for carbon dioxide ($f_i \approx 1.20$; Browne, 2004b) and therefore not included in this study.

Figure 1. Component diagram of PIE device with LI-COR LI-840 infrared CO$_2$ gas analyzer (IRGA).

After the PIE device reaches a stable reading (steady-state in the gas collection chamber) one to two minutes after initial start-up, the rate at which a dissolved gas (i) is pumped into the PIE device ($\frac{dn_i}{dt}$) is equal to the rate of bubble formation ($\frac{dn_i^g}{dt}$)
plus the rate at which the remaining dissolved gas exhausts from the water outlet

\[ \left( \frac{dn_i}{dt} \right)_{\text{outlet}} \] (Browne, 2004a and 2004b):

\[ \frac{dn_i}{dt} = \frac{dn_i^g}{dt} + \frac{dn_i^s}{dt} \]  \hspace{1cm} \text{[17]}

where \( n_i \) is the total moles of gas \( i \), \( n_i^g \) is the total moles in the gas phase, and \( n_i^s \) is the total moles in the solution phase. Using Henry’s law and the ideal gas law, these rates are defined as follows (Browne, 2004a and 2004b):

\[ \frac{dn_i}{dt} = P_{0,i} K_{H,i} \left( \frac{dV_w}{dt} \right) \]  \hspace{1cm} \text{[18]}

\[ \frac{dn_i^g}{dt} = \frac{P_i}{RT} \left( \frac{dV_g}{dt} \right) \]  \hspace{1cm} \text{[19]}

\[ \frac{dn_i^s}{dt} = P_i K_{H,i} \left( \frac{dV_w}{dt} \right) \]  \hspace{1cm} \text{[20]}

where \( \frac{dV_w}{dt} \) is the water pumping rate by volume, \( \frac{dV_g}{dt} \) is the rate of bubble accumulation by volume in the gas collection chamber (Figure 1), \( R \) is the universal gas constant in L atm mol\(^{-1}\) K\(^{-1}\), \( T \) is the temperature in Kelvin, and \( K_H \) is the Henry’s Law constant in mol L\(^{-1}\) atm\(^{-1}\). Therefore, the partial pressure \( (P_i) \) of an individual dissolved gas within the gas collection chamber is related to its partial pressure in the original solution \( (P_0) \) by the following relationship (Browne, 2004a and 2004b):
\[ P_i = P_{0,i} \left[ \frac{RT \prod K_{H,i}}{1 + RT \prod K_{H,i}} \right] \]

where \( \prod \) is the ratio of the water pumping rate \( \left( \frac{dV_w}{dt} \right) \) to the bubble accumulation rate \( \left( \frac{dV_g}{dt} \right) \) in the gas collection chamber.

Figures 1 and 2 illustrate the PIE device used in this study. The device was operated in two modes: carbon dioxide (CO\(_2\)) and dissolved inorganic carbon (DIC). In CO\(_2\) and DIC mode, the solution was pumped at a rate \( \left( \frac{dV_w}{dt} \right) \) of 1.78 L min\(^{-1}\) producing a bubble accumulation rate \( \left( \frac{dV_g}{dt} \right) \) of 5.24 ml min\(^{-1}\) in the gas collection chamber for the prototypes used in these studies (Browne, 2004a and 2004b). This gas streamed through a LI-COR model LI-840 infrared H\(_2\)O and CO\(_2\) gas analyzer (IRGA), where the response was recorded. The \( X_{co2} \) values in parts per million by volume (ppmv) were determined by comparing the instrument response to a standard curve (see standardization of the LI-COR model LI-840 with dry gases below).

In CO\(_2\) mode, the PIE based partial pressure of carbon dioxide (\( P_{CO2} \)) in \( \mu \)atm was estimated from the \( X_{co2} \) values in ppmv by:

\[ P_{CO2} = \frac{X_{co2}}{f_1} \times P \]

[22]
where P is the atmospheric barometric pressure in atm and \( f_i \) is the fractionation coefficient. As stated above, the fractionation coefficient was assumed to be close to one for carbon dioxide \( (f_i \approx 1.20) \) based on the work of Browne (2004b) and therefore not included in this study. The concentration of dissolved carbon dioxide \([H_2CO_3^+]\) was calculated using Henry’s law (Eqs. [14] and [15]) and Eq. [22].

The PIE device was easily switched to DIC mode by turning on the acid valve shown in Figure 1. In DIC mode, phosphoric acid was added to the solution upstream of the pump at a rate of 2 ml min\(^{-1}\). The acid addition causes inorganic carbon species (bicarbonate \([HCO_3^-]\) and carbonate \([CO_3^{2-}\]) to be converted into dissolved carbon dioxide \([H_2CO_3^+]\). The PIE based concentration of DIC in \( \mu \text{mol L}^{-1} \) was calculated by:
[23] \[ [\text{DIC}] = K_H \times P_{\text{CO}_2} \]

where the temperature dependent value of $K_H$ in mol L$^{-1}$ atm$^{-1}$ was from Eq. [15] and the $P_{\text{CO}_2}$ (Eq. [22]) in μatm was estimated from the DIC mode $X_{\text{CO}_2}$ value in ppmv.

PIE sample readings in CO$_2$ and/or DIC mode were recorded in succession using one PIE device (Figures 1 and 2) or simultaneously with two PIE devices. The PIE device was equipped with a LI-COR model LI-840 infrared H$_2$O and CO$_2$ gas analyzer (IRGA) with water vapor and pressure correction. Unless specifically stated differently in an experiment, a two-minute average measurement (after the PIE device stabilization) was used to estimate $P_{\text{CO}_2}$. This two-minute average was only chosen to set a sampling protocol. Therefore, any other protocol could have been used.

Readings were returned at 1-Hz. (one record/second) and stored on an ASUSTeK Computer Inc, ASUS R2H ultra mobile tablet personal computer. This computer was chosen for cost, compactness, and memory size. (Note that the PIE device has the ability to observe both numerical and graphical results in real time with this computer.)

Water temperature measurements for the PIE based measurements were made either with a Fluke® temperature probe (resolution = 0.0001 ± 0.0015 °C) in the gas collection chamber (Figure 1) or with a YSI sonde temperature probe (resolution = 0.01 ± 0.15 °C) at the water outlet (Figure 1) of the gas collection chamber. These water temperature measurements were based on factory calibrations of these probes.

The LI-COR model LI-840 was calibrated with NIST traceable dry gas standards of known mole fractions ($X_{\text{CO}_2}$), ranging from 0 to 79980 parts per million
volume (ppmv). Three calibration curve equations were developed for the PIE device in each experiment in $X_{CO_2}$ ranges of 0 to 2953, 2953 to 19970, and 19970 to 79980 ppmv. For most experiments, the PIE device response was fit with a linear function, third order polynomial, and fourth order polynomial for ranges of 0 to 2953, 2953 to 19970, and 19970 to 79980 ppmv, respectively. The atmospheric barometric pressures present during the calibration process may influence this fit.

The PIE device had problems with the non-linear portion (above 5000 ppmv) of the calibration curves obtained with dry gas standardization. Within this non-linear response range, the internal pressure compensation function of the LI-COR model LI-840 has failed as a reliable algorithm for mole fraction ($X_{CO_2}$) estimates (Figure 3; unpublished data from a Browne and Schueller (2007) UWSP and Seattle, WA experiment). With dry gas standardization, the PIE device was calibrated under the atmospheric pressure (cell pressure) present at the time of calibration. The internal pressure compensation function of the LI-COR model LI-840 was designed to compensate for shifts in the atmospheric pressure (cell pressure). However, under conditions of fluctuating atmospheric barometric pressures (Figure 3), shifts in response had lead to incorrect mole fraction ($X_{CO_2}$) estimates when using dry gas standardization. (See McDermitt et al.(1993a and 1993b) for further discussion on water vapor and pressure influence on infrared gas analyzer (IRGA) measurements of CO$_2$ gas and corrections for this by the LI-COR model LI-840.) To overcome this potential for incorrect determinations of $X_{CO_2}$ estimates using dry gas standardization, an aqueous standardization was used to develop empirical response relationships.
Figure 3. PIE based DIC calibration curves (dry gas standardization; unpublished data from a Browne and Schueller (2007) UWSP and Seattle, WA experiment) with response to shifts in the atmospheric barometric pressure. PIE based DIC LICOR 840 standard curves for 97 kPa (ambient conditions UWSP) and 103 kPa (ambient conditions Seattle, WA) cell pressures. Pressure compensation function of LICOR 840 breaks down at high mole fractions (a) Entire standard curve showing pressure compensation failure at high mole fractions. (b) Isolation of linear portion of curve shows no effect of pressure compensation. (c) Close-up of the pressure compensation departure in the upper curve. Notice that the curve shifts to the right (c) from the increase in atmospheric barometric pressure. Differences in 97 kPa curves may reflect effect of small atmospheric barometric pressure differences (e.g., 96.5 kPa v. 97.2 kPA) and shows need to standardize frequently for the upper end of the curve.
These aqueous standardizations were performed with water sample measurements (DIC, ANC, and \([\text{H}_2\text{CO}_3^*]\)) from the same location, depth, temperature, and time of the PIE based carbon dioxide measurements.

Laboratory ANC, laboratory DIC, and Henry’s law were used to develop an empirical response relationship for PIE based \(P_{\text{CO}_2}\). The expected mole fraction response (\(X_{E1}\)) of the LI-COR model LI-840 for the sample was calculated by:

\[
[H_2\text{CO}_3^*]_{\text{calc}} \approx [\text{DIC}] - [\text{ANC}]
\]

\[
X_{E1} = \frac{[H_2\text{CO}_3^*]_{\text{calc}}}{K_H \times P}
\]

where \([H_2\text{CO}_3^*]_{\text{calc}}\) is the calculated concentration of dissolved carbon dioxide, [DIC] is the laboratory DIC concentration in \(\mu\text{mol L}^{-1}\), [ANC] is the ANC concentration by Gran titration in \(\mu\text{mol L}^{-1}\), \(P\) is the atmospheric barometric pressure in atm, and \(K_H\) is the Henry’s law constant in mol L\(^{-1}\) atm\(^{-1}\) at the sample temperature. Note that the relationship in Eq. [24] is true when the carbonate, hydroxyl, and hydrogen ion species are small. The expected mole fraction response (\(X_{E1}\)) was then graphed versus the measured PIE based \(X_{\text{CO}_2}\) values for the sample to develop the empirical response relationship for PIE based \(P_{\text{CO}_2}\) and for each PIE device.

Laboratory ANC, PIE based \([H_2\text{CO}_3^*]\), and Henry’s law were also used to develop an empirical response relationship for PIE based DIC. The expected mole fraction response (\(X_{E2}\)) of the LI-COR model LI-840 for the sample was calculated by:

\[
[DIC]_{\text{calc}} \approx [\text{ANC}] + [H_2\text{CO}_3^*]
\]
\[ X_{E2} = \frac{[DIC]_{calc}}{K_H \times P} \]

where \([DIC]_{calc}\) is the calculated DIC concentration in \(\mu\text{mol L}^{-1}\), \([\text{ANC}]\) is the ANC concentration by Gran titration in \(\mu\text{mol L}^{-1}\), \([\text{H}_2\text{CO}_3^*]\) is the PIE based concentration of dissolved carbon dioxide in \(\mu\text{mol L}^{-1}\), \(P\) is the atmospheric barometric pressure in atm, and \(K_H\) is the Henry’s law constant in mol L\(^{-1}\) atm\(^{-1}\) at the sample temperature.

Note that the relationship in Eq. [26] is true when the carbonate, hydroxyl, and hydrogen ion species are small. The expected mole fraction response \((X_{E2})\) was then graphed versus the measured PIE based \(X_{\text{CO}_2}\) values for the sample to develop the empirical response relationship for PIE based DIC and for each PIE device.

**Direct measurement of CO\(_2\) using headspace analysis**

The headspace method used was based on a modified procedures of Cole and Caraco, (1998) and Loftfield et al. (1997). An insulated modified Supelco® 1000-ml gas collection headspace vessel (Figures 4 and 5) with two PTFE stopcocks and one Thermogreen® cylindrical half hole septum was placed vertical with both top and bottom valves open. A water sample was pumped through the sampling line and headspace vessel (Figures 4 and 5) until flushed with six times the combined volume of the sampling line and vessel. Both headspace vessel valves were then closed. Next, two gas tight 60-ml collection syringes (Monoject®) were filled with headspace gas (ambient air) (care was taken to avoid contamination with carbon dioxide from breath during collection). One of these syringes with the 60-ml of headspace gas (ambient air) was then introduced into the headspace vessel through septum in side of headspace.
vessel, replacing the 60-ml of sample water exiting through the now opened bottom valve. With both top and bottom valves now closed, the 940-ml of sample water was equilibrated with the 60-ml of headspace gas by vigorously shaking vessel for one
minute. This shaking is approximately twice the time required to equilibrate the air and water phases of the CO₂ (Cole and Caraco, 1998). The equilibrated headspace gas was then transferred to a gas tight 60-ml collection syringe (Monoject®). This

Figure 5. Component diagram of the insulated modified Supelco® 1000-ml gas collection vessel. Sample water entered through the bottom water inlet and valve and exited via a water outlet and valve at the top of the vessel. Gas collection was through septum visible on side of vessel.

equilibrated headspace gas and the gas of ambient air in the other syringe were injected into separate pre-evacuated 10-ml septum sample bottles sealed with Bellco® 20-mm stoppers. (Gas mole fractions within these septum sample bottles (Browne, 2004b) have been shown to be stable for greater than six weeks.) Mole fractions of
CO₂ in the headspace gas and ambient air were measured in the lab with a SRI 8610C gas chromatograph (SRI) equipped with a methanizer, flame ionization detector (FID), and a customized pressure controlled auto-sampler designed to accept 10-ml septum sample bottles sealed with Bellco® 20-mm stoppers. Temperatures for the ambient air and sample vessel water were measured with a Traceable® thermometer.

The concentration of dissolved carbon dioxide ([H₂CO₃*]) in μmol L⁻¹ was calculated using:

\[ [H₂CO₃*] = \frac{n_{TOTAL}}{V_w} = \frac{n_w + n_{HS} - n_A}{V_w} \]

where \( n_{TOTAL} \) is the total μmoles of CO₂ initially present in the 940-ml of water \( (V_w) \) added to the headspace analysis equilibrator, \( n_w \) is the total μmoles of CO₂ present in \( V_w \) after headspace equilibration, \( n_{HS} \) is the total μmoles of CO₂ present in the 60-ml of headspace gas \( (V_{HS}) \) after headspace equilibration, and \( n_A \) is the total μmoles of CO₂ present in the 60-ml of the initial headspace gas \( (V_A) \) before headspace equilibration.

The estimate for \( n_w \) (Eq. [29]) in μmol was obtained from the Henry’s law relationship (Mook and de Vries, 1999):

\[ n_w = K_H \times \frac{X^{HS}_{CO₂} \times P \times V_w}{P} \]

where temperature dependent values of \( K_H \) in mol L⁻¹ atm⁻¹ were from Eq. [15] at the headspace sample vessel water temperature after equilibration, \( X^{HS}_{CO₂} \) in ppmv is the measured mole fraction of CO₂ in the headspace gas after equilibration of the sample, and \( P \) is the atmospheric barometric pressure in atm.
The estimate for \( n_{\text{HS}} \) (Eq. [30]) in \( \mu \text{mol} \) was obtained from the ideal gas law:

\[
[30] \quad n_{\text{HS}} = \frac{X_{\text{CO}_2}^{\text{HS}} \times P \times V_{\text{HS}}}{R \times T_w}
\]

where \( V_{\text{HS}} \) is the volume of the headspace gas (60-ml) after equilibration of the sample, \( R \) is the universal gas constant (0.08206 L atm mol\(^{-1}\) K\(^{-1}\)), and \( T_w \) is the headspace sample vessel water temperature in Kelvin after equilibration.

The estimate for \( n_A \) (Eq. [31]) in \( \mu \text{mol} \) was also obtained from the ideal gas law:

\[
[31] \quad n_A = \frac{X_{\text{CO}_2}^{A} \times P \times V_A}{R \times T_A}
\]

where \( X_{\text{CO}_2}^{A} \) in ppmv is the measured mole fraction of \( \text{CO}_2 \) in the headspace gas before equilibration of the sample, \( V_A \) is the volume of the headspace gas (60-ml) before sample equilibration, and \( T_A \) is the headspace ambient air temperature in Kelvin before equilibration of the sample.

The headspace partial pressure of carbon dioxide (\( P_{\text{CO}_2} \)) in \( \mu \text{atm} \) was estimated using a rearrangement of Eq. [14], where \([\text{H}_2\text{CO}_3^*]\) in \( \mu \text{mol} \ \text{L}^{-1} \) was from Eq. [28] and the temperature dependent value of \( K_H \) in \( \text{mol} \ \text{L}^{-1} \ \text{atm}^{-1} \) was from Eq. [15].

**Indirect estimation of \( \text{CO}_2 \) using pH and ANC**

There are several traditional ways to indirectly measure carbon dioxide. Indirect measurements are defined here as dissolved carbon dioxide estimates indirectly derived from measurements of pH and dissolved inorganic carbon (DIC) or...
pH and acid neutralizing capacity (ANC). ANC and DIC were originally used for estimating carbon dioxide, because they were common measurements taken in lakes, streams, and rivers. To estimate carbon dioxide, all the indirect measurement methods require knowledge of appropriate equilibrium constants.

One indirect estimate of the partial pressure of carbon dioxide ($P_{CO2}$, Eq. [32]) in $\mu \text{atm}$ was obtained using thermodynamic equilibrium relationships based on pH and acid neutralizing capacity (ANC) measurements with corrections for ionic strength and activity coefficients (Baker et al., 1990; Stumm and Morgan, 1981; Mook and de Vries, 1999):

$$P_{CO2} = \frac{\text{ANC} - 10^{(\text{pH}-K_w)}}{K_1 K_H 10^{\text{pH}}} + \frac{2K_1 K_2 K_H 10^{2\text{pH}}}{\gamma_1} + \frac{K_1 K_2 K_H 10^{2\text{pH}}}{\gamma_2}$$

where ANC is in $\mu \text{mol L}^{-1}$, $K_w$ is the dissociation constant of water, $K_1$ is the first dissociation constant of carbonic acid, $K_2$ is the second dissociation constant of carbonic acid, $\gamma_1$ is the activity coefficient of bicarbonate, and $\gamma_2$ is the activity coefficient of carbonate. The temperature dependent values of $K_w$, $K_1$, $K_2$, and $K_H$ were obtained from Eqs. [6], [11], [12], and [15], respectively. The activity coefficients for bicarbonate ($\gamma_1$) and carbonate ($\gamma_2$) were based on the Lewis and Randall concept of ionic strength (Stumm and Morgan, 1981) and the extended Debye-Huckel limiting law (Stumm and Morgan, 1981).
The acid neutralizing capacity (ANC) was measured by Gran titration (Baker et al., 1990; Rounds, 2006). Water samples were collected in headspace-free 125-ml bottles and titrated to a pH around 3.0. The pH was measured with an Orion ROSS Sure-Flow glass electrode (resolution = 0.001 ± 0.010 units). The F1 Gran function (F1) was measured, calculated, and plotted versus the volume of the titrant (V1):

$$F_1 = (V_1 + V_2) \times (10^{-pH})$$

where $V_2$ is the volume of the sample in ml and pH is the sample pH at each $V_1$ addition. The upper right-most linear portion of the F1 curve was then extrapolated downward to determine the titrant volume ($V_E$) at the bicarbonate equivalence point (Baker et al., 1990). The ANC in $\mu$mol L$^{-1}$ was estimated from the following relationship (Baker et al., 1990; Rounds, 2006; Stumm and Morgan, 1981):

$$ANC = \frac{C_1 \times V_E}{V_2} \times (1 \times 10^6)$$

where $C_1$ is the normality of the titrant.

Acid neutralizing capacity (ANC) by $C_B$ and $C_A$ is another common method for measuring the ANC. The ANC by $C_B$ and $C_A$ samples were collected in headspace-free 125-ml bottles and transported to the laboratory for measurement of non-acidic cation concentrations ($C_B$, basic cations, Eq. [35]) and mineral acid anion concentrations ($C_A$, Eq. [36]) by Dionex ion chromatography (Baker et al., 1990; Neumann et al., 2001):

$$C_B = [Na^+] + [K^+] + 2[Ca^{2+}] + 2[Mg^{2+}] + [NH_4^+] + 3[Al^{3+}] + 2[Fe^{2+}] + 2[Mn^{2+}]$$

$$C_A = [Cl^-] + [NO_3^-] + 2[SO_4^{2-}] + [NO_2^-] + [F^-]$$
where [Na⁺] is sodium, [K⁺] is potassium, [Ca²⁺] is calcium, [Mg²⁺] is magnesium,
[NH₄⁺] is ammonium, [Al³⁺] is aluminum, [Fe²⁺] is iron, [Mn²⁺] is manganese, [Cl⁻] is
chloride, [NO₃⁻] is nitrate, [SO₄²⁻] is sulfate, [NO₂⁻] is nitrite, and [F⁻] is fluoride.

Using electroneutrality (Eqs. [37] and [38]) and ANC (Eq. [16]), the sum of
the non-acidic cation concentrations (CB, Eq. [35]) minus the sum of the mineral acid
anion concentrations (CA, Eq. [36]) is equal to the ANC (Eq. [39]; Baker et al., 1990;
Mook and de Vries, 1999; and Stumm and Morgan, 1981):

\[ \text{[37]} \quad [\text{Na}^+] + [\text{K}^+] + 2[\text{Ca}^{2+}] + 2[\text{Mg}^{2+}] + [\text{NH}_4^+] + 3[\text{Al}^{3+}] + 2[\text{Fe}^{2+}] + 2[\text{Mn}^{2+}] + [H^+] = [\text{Cl}^-] + [\text{NO}_3^-] + 2[\text{SO}_4^{2-}] + [\text{NO}_2^-] + [\text{F}^-] + [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{OH}^-] \]

\[ \text{[38]} \quad C_B + [H^+] = C_A + [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{OH}^-] \]

\[ \text{[39]} \quad \text{ANC} = C_B - C_A. \]

The ANC calculated above using \( C_B \) and \( C_A \) measurements is equivalent to the ANC
measured by Gran titration.

**Indirect estimation of CO₂ using pH and DIC**

A second indirect estimate of the partial pressure of carbon dioxide (\( P_{CO_2} \), Eq.
[40]) in \( \mu\text{atm} \) was obtained using thermodynamic equilibrium relationships based on
pH and laboratory DIC measurements with corrections for ionic strength and activity
coefficients (Stumm and Morgan, 1981; Mook and de Vries, 1999):

\[ \text{[40]} \quad P_{CO_2} = \frac{\text{DIC}}{K_{H} + \left( \frac{K_{1} K_{H} 10^{pH}}{\gamma_1} \right) + \left( \frac{K_{1} K_{2} K_{H} 10^{2pH}}{\gamma_2} \right)} \]
where DIC is in μmol L⁻¹ and the temperature dependent values of \( K_w, K_1, \) and \( K_HR \) were obtained from Eqs. [6], [11], [12], and [15], respectively. The activity coefficients for bicarbonate \( (\gamma_1) \) and carbonate \( (\gamma_2) \) were based on the Lewis and Randall concept of ionic strength (Stumm and Morgan, 1981) and the extended Debye-Huckel limiting law (Stumm and Morgan, 1981).

The laboratory dissolved inorganic carbon measurements were performed using a Shimadzu Corp. non-dispersive infrared gas analyzer (NDIR) model TOC-5000A (e.g., Baehr and DeGrandpre, 2002) in conjunction with the methods described in Shimadzu Corp. (1994). Headspace-free water samples were collected in 125-ml bottles as described above and stored for analysis. The water samples were then auto injected into an inorganic carbon (IC) reactor vessel and acidified. The acid converts inorganic carbon species (\([\text{HCO}_3^-] \) and \([\text{CO}_3^{2-}] \)) into dissolved carbon dioxide (\([\text{H}_2\text{CO}_3^*] \)), allowing the total DIC (Eq. [13]) in μmol L⁻¹ to be measured by the NDIR.

**Other Measurement Procedures.**

The water temperature, atmospheric barometric pressure, and specific conductivity measurements required for the carbon dioxide estimates were performed with an YSI 600 MDS equipped with a 600XL multi-parameter sonde. Additionally, pH measurements required for the indirect carbon dioxide estimates were performed with either a YSI 600 MDS equipped with a 600XL multi-parameter sonde pH probe (resolution = 0.01 ± 0.20 units) or an Orion ROSS electrode pH probe (resolution =
0.001 ± 0.010 units) using the methods described in Rosich (2002), and Wilde et al. (2006). The sonde and Orion electrode pH probe were calibrated with two buffers (e.g., 4 and 7 pH or 7 and 10 pH), thoroughly rinsed, and allowed to equilibrate with a representative sample of solution for 30 minutes before they were used for pH measurement (Rosich, 2002; Wilde et al., 2006). To measure pH, the probes were placed in a sample and then the first reasonably stable measurement of pH was taken (i.e. with its unsaturated partial pressure of carbon dioxide value intact).

Measurements of pH were recorded within the first minute to accurately reflect the true pH values (Rosich, 2002; Wilde et al., 2006). When the Orion electrode pH was used, the YSI sonde pH was also measured at the same time for comparison. The water temperature, atmospheric barometric pressure, and specific conductivity measurements from the YSI sonde were based on factory calibrations.

**Data Management, Statistics, and Graphing**

Several personal computer based programs were used to collect, download data from data loggers, store, compile, manipulate, and display the data. Ecowatch by InstallShield Software Corp. was used to download all YSI sonde data. LI-1400 by LI-COR Biosystems or LI-840 by LI-COR Biosciences was used to record and download all LI-COR model LI-840 infrared H₂O and CO₂ gas analyzer (IRGA) data. Data collection, storage, compilation, manipulation, statistics, and graphing were completed with Microsoft Excel (Microsoft Corporation) and SAS (SAS Institute Inc.).
Accuracy and precision were reported using box plots and analyses of regression with statistical parameters of root mean square error (RMSE), coefficient of variation (CV), mean (x-bar), median, square of the correlation (r²), standard deviation, slope, and p-values. The root mean square error (RMSE) is the standard deviation of the error term and defined as the square root of the mean square residual or error. The coefficient of variation (CV) is the relative standard deviation as a percent and defined as the standard deviation divided by the mean of the dependent variable. In analyses of regression using SAS, the coefficient of variation is defined as the RMSE divided by the mean of the dependent variable. The CV measures the relative scatter of two or more samples about their means. The CV is useful for making comparisons between distributions and/or regressions that have different means, standard deviations, RMSE’s, and/or units of measure. The square of the correlation (r²) is the fraction of variation in the values of y that is explained by the least squares regression of y on x. The r² can also be described as the proportion of variance in the dependent variable which can be predicted from the independent variable. The slope term for the regression is the parameter estimate of the independent variable. The square of the correlation and the slope term are a measure of the strength of association between the variables for the regression.
ACCURACY and PRECISION of PIE

In this chapter, the accuracy and precision of PIE based measurements were characterized using solutions of defined composition. PIE based $P_{\text{CO}_2}$ and $[\text{H}_2\text{CO}_3^*]$, headspace, DIC, and ANC measurements were compared to known sodium bicarbonate concentrations. PIE based dissolved carbon dioxide measurements were also compared against headspace measurements. Subsections include the methods for the laboratory experiments, results achieved, and conclusions found.

**Methods - Accuracy and Precision of PIE**

The accuracy and precision were assessed using solutions of known carbon dioxide composition. These solutions were prepared in 119.46 ± 0.46-liter Rubbermaid® containers by acidification of known concentrations of sodium bicarbonate with 1N sulfuric or 36N phosphoric acid. The sodium bicarbonate mass for these known concentrations were measured on an analytical scale (resolution = 0.0001 grams), after which the mass was used to calculate the known concentrations of sodium bicarbonate. The bicarbonate is converted to carbonic acid and dissolved carbon dioxide when acidified (Eqs. [2] and [7]). Because the experiments were of short duration (less than two days), solutions were closed (capped) to the atmosphere, and solutions had little headspace, the total analytical concentration of dissolved carbon dioxide ($[\text{H}_2\text{CO}_3^*]$) was assumed equal to the added bicarbonate concentration and initial carbon dioxide (present in the deionized water used to make the solutions) or equal to the quantity of bicarbonate concentration acidified plus any initial
[H$_2$CO$_3$]$. Table 2 shows the experimental range of dissolved carbon dioxide concentrations for these laboratory experiments.

Table 2. Summary of Experiments 1 through 7.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Solution prep. date</th>
<th>Sampling date</th>
<th>dissolved carbon dioxide conc. (μmol/L)</th>
<th>dissolved carbon dioxide conc. as PCO$_2$ (μatm)</th>
<th>Number of solutions measured</th>
<th>36N H$_3$PO$_4$ added (mL)</th>
<th>1N H$_2$SO$_4$ added (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/4/2008</td>
<td>1/5/2008</td>
<td>0.10-100</td>
<td></td>
<td>12</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1/2/2008</td>
<td>1/3/2008</td>
<td>0.1-10</td>
<td></td>
<td>12</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1/7/2008</td>
<td>1/8/2008</td>
<td>0.10-100</td>
<td></td>
<td>14</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1/9/2008</td>
<td>1/10/2008</td>
<td>0.10-100</td>
<td></td>
<td>14</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1/16/2008</td>
<td>1/17/2008</td>
<td>0.10-100</td>
<td></td>
<td>13</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2/9/2008</td>
<td>2/10/2008</td>
<td>0.100</td>
<td>0-2610</td>
<td>14</td>
<td>incrementally</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2/23/2008</td>
<td>2/24/2008</td>
<td>0.100</td>
<td>0-2610</td>
<td>14</td>
<td>incrementally</td>
<td></td>
</tr>
</tbody>
</table>

The first five experiments used bulk solutions prepared by addition of sodium bicarbonate to deionized water. The concentration for these sodium bicarbonate solutions ranged from one to one-hundred μmol L$^{-1}$ (Table 2). Blanks were prepared as bulk solutions of deionized water alone. Once prepared, blanks and sodium bicarbonate solutions were allowed to sit overnight. The next day the mole fraction of carbon dioxide was measured immediately before and immediately after solution acidification. The total solution acidification caused a conversion of all bicarbonate to dissolved carbon dioxide (Eqs. [2] and [7]). The amount of time between pre- and post-acidification measurements was around 10 to 17 minutes for Experiments 1 through 3 and around 22 to 33 minutes for Experiments 4 and 5.
The pre-acidification PIE based carbon dioxide measurement only measured the background $[\text{H}_2\text{CO}_3^+]$ present in the pre-acidified solution. The post-acidification PIE based carbon dioxide measurement contained the sum of all carbon species (Eq. [13]), including the sodium bicarbonate concentration added to the solution plus the background DIC.

Concurrent measurements included YSI sonde temperature, specific conductivity, and barometric pressure. Additionally, in Experiments 4 and 5, measurements for laboratory DIC, ANC, and headspace were compared against the sodium bicarbonate concentration; pH by YSI sonde was also measured.

Experiments 6 and 7 also used bulk solutions prepared by addition of sodium bicarbonate to deionized water. These solutions were prepared with one-hundred $\mu\text{mol L}^{-1} [\text{NaHCO}_3]$ (Table 2) and allowed to sit overnight. The next day they were acidified with sulfuric acid. Immediately after the pH stabilized, the mole fraction of carbon dioxide was measured. The amount of acid added would be equal to the amount of bicarbonate concentration that would be converted to dissolved carbon dioxide. Acid additions were used to obtain a known range spanning from three to one-hundred $\mu\text{mol L}^{-1} [\text{H}_2\text{CO}_3^+]$. Concurrent measurements include pH by Orion electrode and pH, water temperature, specific conductivity, and barometric pressure by YSI sonde.
Results and Discussion - Accuracy and Precision of PIE

The results from all of the experiments evaluating the accuracy and precision of PIE are summarized in Table 3. Results from Experiment 4 are presented in detail here to summarize the findings from this part of the study. In Experiment 4, PIE, headspace, DIC, and ANC measurements were compared against known sodium bicarbonate concentrations. PIE based dissolved carbon dioxide measurements were also compared against headspace measurements. Table 3 shows PIE based $P_{\text{CO}_2}$ and $[\text{H}_2\text{CO}_3^+]$ measurements taken immediately before and immediately after total solution acidification regressed against the sodium bicarbonate concentration added to the solutions. (Solution temperatures ranged from 19.89 to 22.15 °C.) Pre-acidification $P_{\text{CO}_2}$ did not have a statistically significant relationship to the sodium bicarbonate concentration ($p = 0.1921$, $r^2 = 0.1374$, slope $= 0.3501 \pm 0.2533$ atm L mol$^{-1}$, Table 3). Thus, similar to Experiments 1 and 3, pre-acidification $P_{\text{CO}_2}$ (Table 3) did not decrease systematically with the sodium bicarbonate concentration. Post-acidification $P_{\text{CO}_2}$ increased systematically with sodium bicarbonate concentration ($p < 0.0001$, $r^2 = 0.9979$, slope $= 27.0937 \pm 0.3574$ atm L mol$^{-1}$, Table 3).

Corresponding concentrations of $[\text{H}_2\text{CO}_3^+]$, estimated by Henry’s law, illustrate similar trends (Table 3). As in Experiments 1 and 3, pre-acidification PIE based $[\text{H}_2\text{CO}_3^+]$ did not exhibit a statistically significant trend with the sodium bicarbonate concentration ($p = 0.13$, $r^2 = 0.18$, slope $= 0.01 \pm 0.01$, Table 3). However, post-acidification PIE based $[\text{H}_2\text{CO}_3^+]$ increased systematically with sodium bicarbonate concentration ($p < 0.0001$, $r^2 = 1.00$, slope $= 0.98 \pm 0.01$, Table 3).
A comparison of PIE based $P_{CO_2}$ versus sodium bicarbonate concentration (Table 3) and $[H_2CO_3^*]$ versus sodium bicarbonate concentration (Table 3) showed a slight improvement in the $[H_2CO_3^*]$ versus sodium bicarbonate concentration relationship ($r^2 = 0.9979$ and $r^2 = 0.9994$, respectively). The use of Henry’s law to calculate $[H_2CO_3^*]$ adjusts for variation due to temperature (19.89 to 22.15 °C), effectively improving the measurements overall. This adjustment for temperature would have likely been more evident for a broader temperature range.

In Figure 6, the PIE based $[H_2CO_3^*]$ data were regressed against the sodium bicarbonate concentration after excluding the background $[H_2CO_3^*]$ present in the pre-acidified solutions:

$$[41] \quad [H_2CO_3^*]_{\text{adjusted}} = [H_2CO_3^*]_{\text{post}} - [H_2CO_3^*]_{\text{pre}}.$$

Adjustment of the post-acidification data for the background $[H_2CO_3^*]$ brought the association between $[H_2CO_3^*]$ and the sodium bicarbonate concentration to a slightly closer relationship. The association between $[H_2CO_3^*]$ and the sodium bicarbonate concentration was close to a one-to-one relationship ($r^2 = 1.00$, slope $= 0.97 \pm 0.01$, Table 3). Similar results were also found in Experiments 1 through 3 and Experiments 5 through 7. All results for Experiments 1 through 7 are reported in Table 3.

As in Experiments 1 through 3, the y-intercept of $1.14 \pm 0.39 \ \mu\text{mol L}^{-1}$ $[H_2CO_3^*]$ likely reflects the presence of background carbonic acid and bicarbonate above the amount of $HCO_3^-$ added to the solution.
There are several sources of background dissolved carbon dioxide. The deionized water used for the experiment always contained some dissolved carbon dioxide, regardless how long we ran the deionized water tap. The containers used in the experiments were closed. However, the covers did not completely seal the containers from atmospheric interaction. Although complete equilibration with the atmosphere would take time, some transfer with the atmosphere likely occurred. None
of the solutions in Experiments 1 through 7 had time to equilibrate completely with the atmosphere.

The inverse of the slope of the regression line of the post-acidification PIE data in Experiment 4, Table 3, was used to estimate the average Henry’s law constant ($K_H$). The inverse of the slope of the regression line ($0.0369 \pm 0.0005 \text{ mol L}^{-1} \text{ atm}^{-1}$) was not significantly different from the average Henry’s law constant ($K_H = 0.0379 \pm 0.0010 \text{ mol L}^{-1} \text{ atm}^{-1}$) at the $p = 0.4602$ significance level. This means that the inverse of the slope of the regression line was nearly equal to the average Henry’s law constant.

In Figure 7, Headspace based [H$_2$CO$_3^{\ast}$] measurements taken immediately after total solution acidification were regressed against the sodium bicarbonate concentration added to the solutions. There was a strong correlation between [H$_2$CO$_3^{\ast}$] and the sodium bicarbonate concentration ($r^2 = 0.99$, slope $= 0.97 \pm 0.03$, Table 3). However, the headspace measurements overestimate the sodium bicarbonate concentration. This overestimation and y-intercept of $8.55 \pm 1.75 \mu\text{mol L}^{-1}$ [H$_2$CO$_3^{\ast}$] likely reflect the presence of background carbonic acid and bicarbonate above the amount of HCO$_3^{-}$ added to the solution.
Figure 7. Experiment 4 (N=14), headspace based \([H_2CO_3^*]\) post-acidification values (Eq. [28]) versus sodium bicarbonate concentration.

In Figure 8, the PIE based \([H_2CO_3^*]\) post-acidification measurements were also compared against the headspace based \([H_2CO_3^*]\) post-acidification measurements. PIE based post-acidification \([H_2CO_3^*]\) increased systematically with headspace based \([H_2CO_3^*]\) measurements \((r^2 = 0.99, \text{ slope } = 1.00 \pm 0.03, \text{ Table 3})\). There was a close one-to-one relationship.
Figure 8. Experiment 4 (N=14), PIE based [H$_2$CO$_3$*] post-acidification values (Eq. [14]) versus headspace based [H$_2$CO$_3$*] post-acidification values (Eq. [28]).

Laboratory based DIC measurements taken immediately before total solution acidification were regressed against the sodium bicarbonate concentration added to the solutions. Figure 9 shows a strong correlation between the laboratory based DIC pre-acidification measurements and the sodium bicarbonate concentration ($r^2 = 1.00$, slope = 0.95 ± 0.02, Table 3). However, the laboratory based DIC clearly underestimated the sodium bicarbonate concentration.
Figure 9. Experiment 4 (N=14), laboratory based DIC pre-acidification values versus sodium bicarbonate concentration.

All the laboratory and lake experiments had problems with the laboratory based DIC measurements. Laboratory based DIC measurements (Experiments 4 and 5) were taken immediately before total solution acidification, in order reduce of loss of $[\text{H}_2\text{CO}_3]^-$ during sampling and/or analyses. However, the laboratory based DIC pre-acidification measurements y-intercepts (Experiments 4 and 5) are shifted below the origin, unlike the headspace based $[\text{H}_2\text{CO}_3]^-$ post-acidification measurements (Experiments 4 and 5) and the PIE based $[\text{H}_2\text{CO}_3]^-$ post-acidification measurements.
(Experiments 4 and 5). Therefore, we suspect that the y-intercepts of -1.0 ± 0.25 and -5.84 ± 0.85 μmol L⁻¹ DIC for Experiments 4 and 5 (Table 3), respectively, likely reflect the loss of [H₂CO₃⁺] during sampling and/or analyses. Other lake and laboratory studies have also reported this [H₂CO₃⁺] loss during sampling and/or analyses.

Results from replicate laboratory based DIC pre-acidification measurements, which had been performed in conjunction with the laboratory experiments, also showed this [H₂CO₃⁺] loss during sampling and/or analyses. Results showed large variations in the laboratory based DIC concentrations of the (triplicate) replicate samples. The first replicate set measured by the gas chromatograph had better results than the following sets. The variations had no specific pattern for the measured DIC range (0 to 100 μmol L⁻¹) other than a decrease in DIC concentrations (5 to 20 μmol L⁻¹) recorded over the time of measurement by the gas chromatograph. It was suspected that some samples had built up gas pressure, were supersaturated with respect to the atmosphere, and had subsequent loss of carbon dioxide to the atmosphere. Other studies also have found that built up gas pressure (Portielje and Lijklema, 1995), biotic activity (Neumann et al., 2001; Portielje and Lijklema, 1995; and Rounds, 2006), warming (Neumann et al., 2001), and leakage during sampling, storage, transport, and measurement (Rosich, 2002) can alter the bicarbonate and H₂CO₃⁺ concentration and subsequently the DIC concentration.
Figure 10. Experiment 4 (N=14), ANC (by Gran titration) pre-acidification values versus sodium bicarbonate concentration.

ANC measurements (by Gran titration) taken immediately before total solution acidification were regressed against the sodium bicarbonate concentration added to the solutions. As shown in Figure 10, the ANC pre-acidification measurements are strongly correlated with the sodium bicarbonate concentration ($r^2 = 0.998$, slope = $1.014 \pm 0.012$, Table 3). However, the ANC measurements y-intercept is shifted below the origin. The y-intercept of $-5.954 \pm 0.643 \mu$mol L$^{-1}$ ANC (Table 3) likely reflects the presence of background negative ANC in the pre-acidified solutions.
Table 3. Results for Experiments 1 through 7.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Figure</th>
<th>Dependent variable (y)</th>
<th>Indep. variable (x)</th>
<th>N</th>
<th>RMSE</th>
<th>dep. mean</th>
<th>coeff. of variation (CV, %)</th>
<th>$r^2$</th>
<th>Adj. $r^2$</th>
<th>slope</th>
<th>std error slope</th>
<th>y-int.</th>
<th>std error y-int.</th>
<th>p-value</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>PIE PCO₂ (pre-acidification)</td>
<td>NaHCO₃ conc.</td>
<td>12</td>
<td>22.030</td>
<td>188.02</td>
<td>11.7167</td>
<td>0.0786</td>
<td>0.0135</td>
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<td>0.1905</td>
<td>196.1156</td>
<td>10.823</td>
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<tr>
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<td>NaHCO₃ conc.</td>
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<td>1405.8</td>
<td>2.4163</td>
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<td>0.9985</td>
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<td>PIE [H₂CO₃] (pre-acidification)</td>
<td>NaHCO₃ conc.</td>
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<td>0.66</td>
<td>6.95</td>
<td>9.54</td>
<td>0.02</td>
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<td>0.006</td>
<td>7.06</td>
<td>0.33</td>
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<td>PIE [H₂CO₃] (post-acidification)</td>
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<td>0.5933</td>
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<tr>
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<td>NaHCO₃ conc.</td>
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<td>NaHCO₃ conc.</td>
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<td>6.43</td>
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<td>0.05</td>
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Conclusion – Accuracy and Precision of PIE

For Experiment 1 and Experiments 3 through 7, the PIE based measurements had high accuracy and precision. The PIE based $[\text{H}_2\text{CO}_3^\cdot]$ measurements are strongly correlated with the sodium bicarbonate concentration added to the solutions. The $r^2$ values were 1.00. The root mean square errors (RMSE) were low and ranged from 0.57 to 1.99 $\mu$mol L$^{-1}$ $[\text{H}_2\text{CO}_3^\cdot]$ and the mean RMSE was 1.04 $\mu$mol L$^{-1}$ $[\text{H}_2\text{CO}_3^\cdot]$. The coefficients of variation (CV) were also low and ranged from 1.29 to 4.32 % and the mean CV was 2.41 %. The PIE based $[\text{H}_2\text{CO}_3^\cdot]$ measurements trend with the sodium bicarbonate concentration added to the solutions was close to the one-to-one line. These slope values ranged from 0.94 to 0.98 with a mean slope of 0.96.

This chapter has characterized and examined the accuracy and precision of PIE based direct measurements of carbon dioxide using solutions of defined composition. With this verification, researchers now have a PIE based direct measurement with known precision and accuracy that can be applied to laboratory and lake studies.
TRADITIONAL LAKE STUDIES with PIE

In this chapter, PIE based direct carbon dioxide measurements were applied in a limited study of lakes across three trophic classes (eutrophic, mesotrophic, oligotrophic) and compared to traditional direct and indirect carbon dioxide measurements. This is where researchers will learn the importance of how PIE based dissolved carbon dioxide measurements perform in natural systems and traditional lake studies. The first chapter section contains the methods used for the traditional lake studies. In the following chapter sections, (1) PIE based measurements were taken to have a range of carbon dioxide measurements similar to the laboratory experiments; (2) a set of replicate carbon dioxide measurements within a lake were made to investigate variations between PIE and traditional direct and indirect carbon dioxide measurements; (3) PIE based carbon dioxide measurements were compared in a traditional fifty-two lake surface water survey (one surface water sample measurement with each method at each lake); and (4) PIE based carbon dioxide measurements were compared in traditional depth profiles from two lakes. PIE based measurements were also compared against direct (headspace) and indirect (pH and ANC; pH and DIC) measurements. The last section contains the conclusions for the traditional lake studies.
Methods – Traditional Lake Studies with PIE

The fifty-two study lakes (Figure 11; Appendices B and C) for the traditional lake studies were located in Vilas and Oneida Counties of Northern Wisconsin. These lakes had a wide range of geologic, hydrologic, watershed, and landscape position characteristics. See Appendices B and C for lake names, lake numbers, and other characteristics. Three broad trophic classes (oligotrophic, N = 17; mesotrophic, N = 18; and eutrophic, N = 17) were represented.

Since there was little or no available data for selecting these lakes, these study lakes were chosen using Remote Sensing (Landsat satellite) estimates of the water clarity (Secchi disk transparency) of these lakes (Chipman et al., 2004) to derive the Wisconsin trophic state index Secchi disk transparency (WTSI (SDT)) (Carlson, 1977; Chipman et al., 2004; Lillie et al., 1993). Secchi disk transparency (SDT) is an estimate of the amount of nutrients in the water, growth of algae and plankton, and distance you can see down into the water. The greater the Secchi disk transparency, the deeper you can see into the water (Tyler, 1968). The WTSI (SDT) was used because it was specifically designed for the morphology of Wisconsin lakes (Lillie et al., 1993). Trophic class breakpoints were above fifty for eutrophic lakes, between forty to fifty for mesotrophic lakes, and below forty for oligotrophic lakes (Figure 11). After selection by trophic class, lakes were additionally chosen by accessibility (navigable boat ramp) and logistics (route between lakes). The fifty-two study lakes in Figure 11 were mapped using ESRI ArcGIS ArcMAP. For proper registration, this map was geo-referenced to the Wisconsin Transverse Mercator (WTM83/91)
Figure 11. This ESRI ArcGIS ArcMAP lake clarity map of the 52 lakes (Appendices B and C) in this study was based on Secchi disk transparency and classified with the Wisconsin trophic state index Secchi disk transparency (Carlson, 1977; Chipman et al., 2004; Lillie et al., 1993).
geographic coordinate system (meters) and North American 1983 HARN geographic projection system.

Lake samples were collected from a 17-foot boat. The boat was secured with two anchors at each sampling point. For the lake study with a similar carbon dioxide range to the laboratory studies, thirty-five near-shore surface water, deep-water surface water, and depth profile samples were taken throughout one lake. (The surface water samples were taken at a one-half meter depth or at least 30 cm above the sediment. The depth profile samples were taken at one-half to one-meter intervals starting at less than one-half meter below the surface water-atmosphere interface down to just above the lake sediment.) For the replicate sample lake study, ten $P_{co2}$ replicate measurements were taken from one point and 0.5m depth during a six-hour period on one lake. For the fifty-two lake surface-water lake study, near-surface water lake samples (0.5 m depth) were taken near the deepest point of lakes with known bathymetry or otherwise at a point near the middle of the lake and at least 100 meters from shore. For the depth profile lake studies, depth profiles were taken near the deepest point of two lakes at one-half to one-meter intervals starting at less than one-half meter below the surface water-atmosphere interface down to just above the lake sediment. A Trimble GeoExplorer Geo (CE) XT Global Positioning System (GPS) was used to record each surface water sampling point on these lakes. These points were geo-referenced to the Wisconsin Transverse Mercator (WTM83/91) geographic coordinate system (meters) and North American 1983 HARN geographic projection system. See Appendices B and C for the sampling point coordinates for the fifty-two
lake study, carbon dioxide replicate study, diurnal pattern study, and depth profile studies.

Samples for PIE, headspace, laboratory DIC, and ANC were obtained at the same time by lowering three polyethylene-sampling lines attached to a YSI multi-parameter sonde to the sampling depth. All PIE based carbon dioxide measurements were obtained with one sampling line and peristaltic pump. Headspace based carbon dioxide measurements and Orion ROSS electrode pH were obtained with the second sampling line and peristaltic pump. This pH was measured using an Orion electrode pH probe in a modified beaker that had been flushed with sample via the sampling line. The flow was then stopped for this Orion electrode pH measurement. All the other water samples for ANC and DIC were taken through the same third sampling line and peristaltic pump. The YSI 600 MDS equipped with a 600XL multi-parameter sonde attached to these three sampling lines was used to record depth, temperature, oxygen, pH, atmospheric barometric pressure, and specific conductivity measurements for each sampling position. Air temperatures measurements were also made with a Traceable® thermometer.
Results and Discussion – Traditional Lake Studies with PIE

Comparison of PIE to traditional methods on Allequash Lake. PIE based measurements were compared against direct (headspace) and indirect ($P_{\text{CO}_2}$ by pH and ANC) measurements. These carbon dioxide measurements (0 to 2314 $\mu$atm; 0 to 93 $\mu$mol L$^{-1}$) were taken to have a range of carbon dioxide measurements similar to the laboratory experiments (0 to 2610 $\mu$atm; 0 to 100 $\mu$mol L$^{-1}$). These measurements were composed of near-shore surface water, deep-water surface water, and depth profile samples.

Thirty-five $P_{\text{CO}_2}$ measurements were made at Allequash Lake from 06/06/2008 to 06/10/2008 with PIE. They were regressed against the headspace measured $P_{\text{CO}_2}$. There was a strong correlation between PIE based $P_{\text{CO}_2}$ and headspace based $P_{\text{CO}_2}$ ($r^2 = 0.9778$, slope = 0.9067 ± 0.0238, Table 4). (These measurements were made at different locations and depths throughout the northern bay of Allequash Lake, where lake temperatures ranged from 15.47 to 20.21 °C.)

Corresponding concentrations of $[\text{H}_2\text{CO}_3^+]$, estimated by Henry’s law, illustrate similar trends. There was a strong correlation between PIE based $[\text{H}_2\text{CO}_3^+]$ (Figure 12) and headspace based $[\text{H}_2\text{CO}_3^+]$ ($r^2 = 0.98$, slope = 0.87 ± 0.02, Table 4).
Figure 12. Comparison of PIE to traditional methods on Allequash Lake (N = 35), PIE based $[\text{H}_2\text{CO}_3^*]$ versus headspace based $[\text{H}_2\text{CO}_3^*]$.

PIE based $\text{P}_{\text{CO}_2}$ (Figure 13) were also regressed against the $\text{P}_{\text{CO}_2}$ by pH and ANC (Gran titration) measurements. There was a strong correlation between PIE based $\text{P}_{\text{CO}_2}$ and $\text{P}_{\text{CO}_2}$ by pH and ANC ($r^2 = 0.9797$, slope $= 1.0882 \pm 0.0272$, Table 4).
Figure 13. Comparison of PIE to traditional methods on Allequash Lake (N = 35), PIE based $P_{CO2}$ versus $P_{CO2}$ by pH and ANC (Gran titration).
Table 4. Comparison of PIE to traditional methods on Allequash Lake.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Figure</th>
<th>Dependent variable (y)</th>
<th>Indep. variable (x)</th>
<th>N</th>
<th>RMSE</th>
<th>dep. mean</th>
<th>coeff. of variation (CV, %)</th>
<th>$r^2$</th>
<th>Adj. $r^2$</th>
<th>slope</th>
<th>std error slope</th>
<th>y-int.</th>
<th>std error y-int.</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Allequash's range</td>
<td>PIE $P_{CO_2}$</td>
<td>headspace $P_{CO_2}$</td>
<td>35</td>
<td>84.9812</td>
<td>827.6831</td>
<td>10.2674</td>
<td>0.9778</td>
<td>0.9771</td>
<td>0.9067</td>
<td>0.0238</td>
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<td>PIE $[H_2CO_3^*]$</td>
<td>headspace $[H_2CO_3^*]$</td>
<td>35</td>
<td>3.15</td>
<td>31.98</td>
<td>9.84</td>
<td>0.98</td>
<td>0.98</td>
<td>0.87</td>
<td>0.02</td>
<td>1.81</td>
<td>0.90</td>
<td>&lt;.0001</td>
<td></td>
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<tr>
<td>Allequash's range</td>
<td>headspace $[H_2CO_3^*]$</td>
<td>PIE $[H_2CO_3^*]$</td>
<td>35</td>
<td>3.57</td>
<td>34.50</td>
<td>10.33</td>
<td>0.98</td>
<td>0.98</td>
<td>1.12</td>
<td>0.03</td>
<td>-1.37</td>
<td>1.06</td>
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<td></td>
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<tr>
<td>Allequash's range</td>
<td>PIE $P_{CO_2}$</td>
<td>$P_{CO_2}$ by pH and ANC (Gran tit.)</td>
<td>35</td>
<td>81.1889</td>
<td>827.6831</td>
<td>9.8092</td>
<td>0.9797</td>
<td>0.9791</td>
<td>1.0882</td>
<td>0.0272</td>
<td>15.9401</td>
<td>24.5168</td>
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</tr>
</tbody>
</table>
**PIE based replicate measurements on Allequash Lake.** A set of replicate $P_{CO2}$ measurements within a lake were made to investigate variations between PIE and traditional direct and indirect carbon dioxide measurements. Ten measurements were taken at one surface water point and 0.5 m depth over a six hour period on Allequash Lake. These measurements were made from 1133 to 1738 hours (CST) on 06/10/2008 in the middle of summer stratification. Figure 14 compares PIE based $P_{CO2}$ measurements against direct (headspace based $P_{CO2}$) and indirect ($P_{CO2}$ by pH and ANC) measurements. A dashed line was drawn at the extreme minimum and maximum values of the PIE based $P_{CO2}$ method in order to show the spread of values in comparison to the other methods. (These Allequash Lake temperature measurements only ranged from 18.84 to 19.10 °C.)

PIE based $P_{CO2}$ (Figure 14) measurements more closely matched the spread, median, and mean of the direct (headspace based $P_{CO2}$) measurements than the indirect ($P_{CO2}$ by pH and ANC) measurements. The PIE based $P_{CO2}$ (mean = 479.1384 ± 14.4888 μatm, Table 5) was not significantly different from the headspace based $P_{CO2}$ (mean = 493.75 ± 18.37 μatm, Table 5) at the p = 0.21 significance level. However, the PIE based $P_{CO2}$ (mean = 479.1384 ± 14.4888 μatm, Table 5) was significantly different from the $P_{CO2}$ by pH and ANC (mean = 403.33 ± 19.70 μatm, Table 5) at the p = 0.0002 significance level. All the methods have very low coefficients of variation with PIE based $P_{CO2}$ having the lowest CV at 3.0239 %. The indirect $P_{CO2}$ by pH and ANC measurements (ranging from 379 to 431 μatm) were also different from the direct measurements for having some measurements close to the June 2008 ambient
The spread of values in comparison to the other methods, extreme minimum and maximum values of the PIE based PCO2 method in order to show PIE, and PCO2 by pH and ANC (Gran titration). A dashed line was drawn at the mean (+) and median with horizontal line median and plus close-up starts at 350 µatm, N = 10, PCO2 quartiles with horizontal line median and plus.

Figure 14. Box plots for PIE based replicate measurements on Allequash Lake (box plot PIE based PCO2, headspace based PIE based PCO2, headspace based PCO2, and PCO2 by pH and ANC (Gran titration). A dashed line was drawn at the atmosphere PCO2 = 362 µatm.

Loss of ANC, carbon species change during sampling, transport, or measurement, which lead to net indirect measurement seems to be going through some block of temperature induced atmospheric global average of 362 µatm PCO2 (385 ppmv PCO2; Tans, 2008).
Table 5. PIE based replicate measurements on Allequash Lake.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Figure</th>
<th>Method</th>
<th>N</th>
<th>dep. Mean (μatm)</th>
<th>Median (μatm)</th>
<th>Standard Deviation</th>
<th>coeff. of variation (CV, %)</th>
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</thead>
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<tr>
<td>Allequash's replicates</td>
<td>14</td>
<td>PIE $P_{CO2}$</td>
<td>10</td>
<td>479.1384</td>
<td>475.0387</td>
<td>14.4888</td>
<td>3.0239</td>
</tr>
<tr>
<td>Allequash's replicates</td>
<td>14</td>
<td>headspace $P_{CO2}$</td>
<td>10</td>
<td>493.75</td>
<td>497.10</td>
<td>18.37</td>
<td>3.72</td>
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<tr>
<td>Allequash's replicates</td>
<td>14</td>
<td>$P_{CO2}$ by pH and ANC (Gran tit.)</td>
<td>10</td>
<td>403.33</td>
<td>399.80</td>
<td>19.70</td>
<td>4.88</td>
</tr>
</tbody>
</table>
**PIE in a fifty-two lake surface water survey.** Figure 15 shows a comparison between fifty-two surface water PIE based and headspace based $P_{CO_2}$ measurements made on lakes throughout Vilas and Onieda Counties (07/09/2007 to 07/22/2007). See Appendices B and C for temperature, lake name, GPS position, and other characteristics of these lakes. There was a strong correlation between PIE based $P_{CO_2}$ (Figure 15) and headspace based $P_{CO_2}$ ($r^2 = 0.8984$, slope = $0.9093 \pm 0.0432$, Table 6).

![Figure 15. PIE in a fifty-two lake surface water survey (N = 52), PIE based $P_{CO_2}$ versus headspace based $P_{CO_2}$.](image)

\[ y = 0.9093 x + 7.6128 \]

\[ r^2 = 0.8984 \]
In Figure 16, PIE based $P_{\text{CO}_2}$ were regressed against the $P_{\text{CO}_2}$ by pH and DIC measurements. There was a strong correlation between PIE based $P_{\text{CO}_2}$ (Figure 16) and $P_{\text{CO}_2}$ by pH and DIC ($r^2 = 0.9335$, Table 6). However, the slope ($1.4850 \pm 0.0561$, Table 6) was significantly different from one ($p = 0.0002$).

![Graph showing regression between PIE based PCO2 and PCO2 by pH and DIC](image)

**Figure 16.** PIE in a fifty-two lake surface water survey ($N = 52$), PIE based $P_{\text{CO}_2}$ versus $P_{\text{CO}_2}$ by pH and laboratory DIC.
In Figure 17, PIE based $P_{CO2}$ were regressed against the $P_{CO2}$ by pH and ANC (Gran titration) measurements. Similar to the other indirect measurements, there was a strong correlation between PIE based $P_{CO2}$ (Figure 17) and $P_{CO2}$ by pH and ANC ($r^2 = 0.9521$, Table 6). However, the slope ($1.3953 \pm 0.0443$, Table 6) was significantly different from one ($p = 0.0002$).

Figure 17. PIE in a fifty-two lake surface water survey ($N = 52$), PIE based $P_{CO2}$ versus $P_{CO2}$ by pH and ANC (Gran titration).
In Figure 18, PIE based P$_{CO2}$ were also regressed against the P$_{CO2}$ by pH and ANC (C$_B$ and C$_A$) measurements. Similar to the other indirect measurements, there was a strong correlation between PIE based P$_{CO2}$ (Figure 18) and P$_{CO2}$ by pH and ANC ($r^2 = 0.9241$, Table 6). However, the slope ($1.5620 \pm 0.0633$, Table 6) was significantly different from one ($p = 0.0002$).

Figure 18. PIE in a fifty-two lake surface water survey (N = 52), PIE based P$_{CO2}$ versus P$_{CO2}$ by pH and ANC (C$_B$ and C$_A$).
The results from the fifty-two lakes were also used to evaluate the importance of lake trophic state on the correlation between PIE and more traditional methods. Here are the method to method comparisons under different trophic conditions. Figure 19 shows a comparison between PIE based and headspace based $P_{\text{CO}_2}$ measurements for eutrophic lakes. There was a strong correlation between PIE based $P_{\text{CO}_2}$ (Figure 19) and headspace based $P_{\text{CO}_2}$ ($r^2 = 0.9878$, slope = $1.0527 \pm 0.0302$, Table 6).

**Figure 19.** PIE in a fifty-two lake surface water survey (eutrophic lakes; $N = 17$), PIE based $P_{\text{CO}_2}$ versus headspace based $P_{\text{CO}_2}$.
Figure 20 shows a comparison between PIE based and headspace based $P_{CO2}$ measurements for mesotrophic lakes. There was a strong correlation between PIE based $P_{CO2}$ (Figure 20) and headspace based $P_{CO2}$ ($r^2 = 0.7989$, Table 6). However, the slope (0.6005 ± 0.0753, Table 6) was significantly different from one ($p = 0.0002$). Additionally, these mesotrophic lake results are more scattered than the eutrophic lakes above.

Figure 20. PIE in a fifty-two lake surface water survey (mesotrophic lakes; $N = 18$), PIE based $P_{CO2}$ versus headspace based $P_{CO2}$.
Figure 21 shows a comparison between PIE based and headspace based $P_{CO_2}$ measurements for oligotrophic lakes. There was a strong correlation between PIE based $P_{CO_2}$ (Figure 21) and headspace based $P_{CO_2}$ ($r^2 = 0.8514$, slope $= 0.9007 \pm 0.0972$, Table 6). However, these oligotrophic lake results are more scattered than the eutrophic lakes above.

![Figure 21. PIE in a fifty-two lake surface water survey (oligotrophic lakes; N = 17), PIE based $P_{CO_2}$ versus headspace based $P_{CO_2}$.](image)
As described in the *Introduction*, recent studies of lake metabolism have shown that most of the world’s lakes surface waters are supersaturated with dissolved carbon dioxide (Sobek et al., 2005), suggesting that lakes may be a net source of carbon dioxide emission into the atmosphere (Sobek et al., 2005). However, most of the lakes in the *PIE in a fifty-two lake surface water survey* were not supersaturated with dissolved carbon dioxide. According to PIE based dissolved carbon dioxide measurements in Figure 22, thirteen lake samples were oversaturated and thirty-nine lake samples were undersaturated with respect to the July 2007 ambient atmospheric global average of 362 μatm $P_{CO2}$ (381 ppmv $P_{CO2}$; Tans, 2008). Therefore, most of these lakes are a sink for atmospheric carbon dioxide.

These findings do not necessarily refute the supersaturation findings in Sobek et al. (2005). Considering that only 150 of the world’s lakes had direct measurements of carbon dioxide and 4582 of the world’s lakes have been indirectly measured (Table 1), more research is needed and many more lakes will have to be directly measured with PIE to build a better picture of why these particular lakes in this study were undersaturated with respect to atmospheric carbon dioxide.
Figure 22. PIE in a fifty-two lake surface water survey (eutrophic, N = 17; mesotrophic, N = 18; oligotrophic, N = 17), measured PIE based $P_{CO_2}$ across trophic state.
Table 6. Pie in a fifty-two lake surface water survey.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Fig.</th>
<th>Dependent variable (y)</th>
<th>Indep. variable (x)</th>
<th>N</th>
<th>RMSE</th>
<th>dep. mean</th>
<th>coeff. of variation (CV, %)</th>
<th>r²</th>
<th>Adj. r²</th>
<th>slope</th>
<th>std error slope</th>
<th>y-int.</th>
<th>std error y-int.</th>
<th>p-value</th>
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<td>52 Lakes</td>
<td>15</td>
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<tr>
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<td>16</td>
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<td>52</td>
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<td>8.75</td>
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<td>0.90</td>
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<td>0.04</td>
<td>0.22</td>
<td>0.53</td>
<td>&lt; 0.0001</td>
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<tr>
<td>52 Lakes</td>
<td>17</td>
<td>headspace [H&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;]&lt;sup&gt;<em>&lt;/sup&gt; PIE [H&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;]&lt;sup&gt;</em>&lt;/sup&gt;</td>
<td>52</td>
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<td>0.05</td>
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<tr>
<td>52 Lakes</td>
<td>16</td>
<td>PIE P&lt;sub&gt;CO2&lt;/sub&gt; P&lt;sub&gt;CO2&lt;/sub&gt; by pH and laboratory DIC</td>
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<tr>
<td>52 Lakes</td>
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<td>52</td>
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<tr>
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<tr>
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<td>0.8415</td>
<td>0.9007</td>
<td>0.0972</td>
<td>31.1267</td>
<td>33.6432</td>
<td>&lt; 0.0001</td>
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</tr>
</tbody>
</table>
**Depth profile lake measurements.** To evaluate how PIE and traditional direct and indirect measurements compared within lakes, experiments were designed to explore the spatial resolution of PIE based measurements with depth for a mesotrophic lake (Allequash Lake) and an oligotrophic lake (Long Lake). These lakes were selected from the fifty-two study lakes and chosen because of easy accessibility.

Figure 23 shows the ten $P_{CO2}$ depth profile measurements made for Allequash Lake’s first depth profile on 09/15/2007 with PIE compared against the headspace measured $P_{CO2}$. There was a strong correlation between PIE based $P_{CO2}$ (Figure 23) and headspace based $P_{CO2}$ ($r^2 = 0.9279$, slope = 0.9527 ± 0.0939, Table 7). (The measurements made on this mesotrophic lake ranged from 15.18 to 15.80 °C in temperature.)

In Figures 24, 25, 26, and 27, the carbon dioxide measurement results from these methods and the oxygen results were graphed by depth for Allequash Lake. Figure 24 displays the high spatial resolution of the PIE based $P_{CO2}$ measurements with depth. The $O_2$ and PIE based $P_{CO2}$ fluctuations seemed to be a different magnitude, but opposite in direction. The profile (Figure 24) shows the dissolved oxygen ($O_2$) level had peak maximums (8.65 and 8.67 mg L$^{-1}$) and minimums (7.82 and 7.99 mg L$^{-1}$) at the same times that the PIE based $P_{CO2}$ had at its minimums and maximums, respectively. Similar relationships were also found in diurnal studies of dissolved carbon dioxide and dissolved oxygen in Hanson et al., (2006). Among all the methods used, headspace based $P_{CO2}$ (Figure 24) measurements more closely
mirrored the PIE based $P_{CO_2}$ values. Overall, the methods used had only small
differences with depth for this shallow mesotrophic lake.

Figure 23. Allequash Lake’s first depth profile measurements ($N = 10$), PIE based $P_{CO_2}$
versus headspace based $P_{CO_2}$.
Figure 24. Allequash Lake’s first depth profile measurements, PIE based $P_{\text{CO}_2}$ ($N = 11$), headspace based $P_{\text{CO}_2}$ ($N = 10$), and oxygen ($N = 11$) by depth.

Figure 25. Allequash Lake’s first depth profile measurements ($N = 11$), PIE based $P_{\text{CO}_2}$, $P_{\text{CO}_2}$ by pH and laboratory DIC, and oxygen by depth.
Figure 26. Allequash Lake’s first depth profile measurements (N = 11), PIE based P$_{CO_2}$, P$_{CO_2}$ by pH and ANC (Gran titration), and oxygen by depth.

Figure 27. Allequash Lake’s first depth profile measurements (N = 11), PIE based P$_{CO_2}$, P$_{CO_2}$ by pH and ANC (C$_B$ and C$_A$), and oxygen by depth.
Table 7, Allequash Lake’s first depth profile.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Figure</th>
<th>Dependent variable (y)</th>
<th>Independ. variable (x)</th>
<th>N</th>
<th>RMSE</th>
<th>dep. mean</th>
<th>coeff. of variation (CV, %)</th>
<th>$r^2$</th>
<th>Adj. $r^2$</th>
<th>slope</th>
<th>std error</th>
<th>y-int.</th>
<th>std error</th>
<th>y-int.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allequash depth</td>
<td>23</td>
<td>PIE $P_{\text{CO}_2}$</td>
<td>headspace $P_{\text{CO}_2}$</td>
<td>10</td>
<td>36.255</td>
<td>665.30</td>
<td>20</td>
<td>5.4495</td>
<td>0.9279</td>
<td>0.9189</td>
<td>0.9527</td>
<td>0.0939</td>
<td>66.1182</td>
<td>60.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Allequash depth</td>
<td></td>
<td>PIE $P_{\text{CO}_2}$</td>
<td>$P_{\text{CO}_2}$ by pH and laboratory DIC</td>
<td>10</td>
<td>52.236</td>
<td>665.30</td>
<td>20</td>
<td>7.8516</td>
<td>0.8503</td>
<td>0.8316</td>
<td>0.8249</td>
<td>0.1224</td>
<td>123.3614</td>
<td>82.07</td>
<td>0.0001</td>
</tr>
<tr>
<td>Allequash depth</td>
<td></td>
<td>PIE $P_{\text{CO}_2}$</td>
<td>$P_{\text{CO}_2}$ by pH and ANC (Gran tit.)</td>
<td>10</td>
<td>52.222</td>
<td>665.30</td>
<td>20</td>
<td>7.8494</td>
<td>0.8504</td>
<td>0.8317</td>
<td>0.8212</td>
<td>0.1218</td>
<td>129.0584</td>
<td>81.22</td>
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</tr>
<tr>
<td>Allequash depth</td>
<td></td>
<td>PIE $P_{\text{CO}_2}$</td>
<td>$P_{\text{CO}_2}$ by pH and ANC (Ca and Ca)</td>
<td>10</td>
<td>53.094</td>
<td>665.30</td>
<td>20</td>
<td>7.9805</td>
<td>0.8453</td>
<td>0.8260</td>
<td>0.7489</td>
<td>0.1133</td>
<td>146.0255</td>
<td>80.30</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
Allequash Lake was sampled again the next year in the middle of summer stratification. Even though the second depth profile was measured at a different time during the year and the carbon dioxide concentrations and temperatures were over a broader range, the PIE based measurements showed similar results as in the first depth profile.

Figure 28 shows the twelve $P_{CO2}$ depth profile measurements made for Allequash Lake’s second depth profile on 06/09/2008 with PIE compared against the headspace measured $P_{CO2}$. There was a strong correlation between PIE based $P_{CO2}$ (Figure 28) and headspace based $P_{CO2}$ ($r^2 = 0.9880$, slope $= 1.1277 \pm 0.0393$, Table 8). However, the slope and the y-intercept of $-180.9898 \pm 64.4389$ both seem to be influenced heavily by the one measurement above 4000 $\mu$atm $P_{CO2}$. (The measurements made on this mesotrophic lake ranged from 15.47 to 18.31 °C in temperature.)

The carbon dioxide measurement results from these methods and the oxygen results were also graphed by depth for Allequash Lake. Figure 29 also displays the same high spatial resolution of the PIE based $P_{CO2}$ measurements with depth as in the first depth profile. The $O_2$ and PIE based $P_{CO2}$ fluctuations also seem to be a different magnitude, but opposite in direction. Among all the methods used, headspace based $P_{CO2}$ (Figure 29) measurements more closely mirrored the PIE based $P_{CO2}$ values.
Figure 28. Allequash Lake’s second depth profile measurements (N = 12), PIE based $P_{CO_2}$ versus headspace based $P_{CO_2}$.

$\mathbf{Y=X \ \text{Line}}$

$P_{CO_2} (\mu \text{atm})$

0 500 1000 1500 2000 2500 3000 3500 4000 4500

Headspace based $P_{CO_2} (\mu \text{atm})$

$0 500 1000 1500 2000 2500 3000 3500 4000 4500$

$P_{CO_2} (\mu \text{atm})$

$Y = 1.1277 \times - 180.9898$

$y = 1.1277 x - 180.9898$

$r^2 = 0.9880$

Figure 29. Allequash Lake’s second depth profile measurements (N = 12), PIE based $P_{CO_2}$, headspace based $P_{CO_2}$, and oxygen by depth.
There were some problems verifying the accuracy and precision of PIE based DIC measurements. Figure 30 shows a comparison of PIE based DIC measurements to an estimated DIC (Eq. [43]) that was based on [H$_2$CO$_3$\textsuperscript{*}] and ANC measurements:

\[\text{estimated DIC} \approx [H_2CO_3^*] + \text{ANC}\]

\[\text{estimated DIC} \approx [H_2CO_3^*] + [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+]\]

where [H$_2$CO$_3$\textsuperscript{*}] was the PIE based [H$_2$CO$_3$\textsuperscript{*}] (Eq. [14]) and ANC was the ANC by Gran titration (Eq. [34]). Note that when the carbonate, hydroxyl, and hydrogen ion concentrations are low, the estimated DIC (Eq. [43]) approximates the PIE based DIC (Eq. [23]).

However, the estimated DIC in Figure 30 does not closely mirror the PIE based DIC measurement. This is most likely a phenomenon associated with the nonlinear portion of the PIE based DIC calibration curve and shifts in the atmospheric pressure as described earlier in the Methods section. Ranging from 15,700 μatm to 17,600 μatm, the PIE based DIC measurements in Figure 30 are well into this nonlinear range.

To correct PIE based DIC measurements for this problem, an empirical response relationship (Eq. [27]) based on ANC, [H$_2$CO$_3$\textsuperscript{*}], and Henry’s law was used. Figure 31 shows a comparison of the corrected PIE based DIC measurements to an estimated DIC (Eq. [43]). There was a strong correlation between the corrected PIE based DIC (Figure 31) and the estimated DIC ($r^2 = 0.94$, slope $= 1.04 \pm 0.08$, Table 8). Figure 32 also shows that the estimated DIC does closely mirror the corrected PIE
Figure 30. Allequash Lake’s second depth profile measurements (N = 12), methods displayed by depth include PIE based $[\text{H}_2\text{CO}_3^*]$, headspace based $[\text{H}_2\text{CO}_3^*]$, PIE based DIC, ANC by Gran titration, and estimated DIC. The estimated DIC does not closely mirror the PIE based DIC.
based DIC measurements. The use of an empirical response relationship effectively improves the PIE based DIC measurements.

Figure 31. Allequash Lake’s second depth profile measurements \((N = 12)\), corrected PIE based DIC (Eq. [27]) versus estimated DIC (Eq. [43]).
Figure 32. Allequash Lake’s second depth profile measurements (N = 12), methods displayed by depth include PIE based [H$_2$CO$_3^*$], headspace based [H$_2$CO$_3^*$], corrected PIE based DIC, ANC by Gran titration, and estimated DIC. The estimated DIC mirrors the corrected PIE based DIC.
Table 8, Allequash Lake’s second depth profile.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Figure</th>
<th>Dependent variable</th>
<th>Indep. variable</th>
<th>N</th>
<th>RMS E</th>
<th>dep. mean</th>
<th>coeff. of variation (CV, %)</th>
<th>$r^2$</th>
<th>Adj. $r^2$</th>
<th>slope</th>
<th>std error slope</th>
<th>y-int.</th>
<th>std error y-int.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allequash depth PIE PCO$_2$</td>
<td>28</td>
<td>headspace PCO$_2$</td>
<td>12</td>
<td>127.0</td>
<td>389</td>
<td>1341.4</td>
<td>577</td>
<td>9.4702</td>
<td>0.9880</td>
<td>0.9868</td>
<td>1.1277</td>
<td>0.0393</td>
<td>180.9898</td>
<td>64.438</td>
</tr>
<tr>
<td>Allequash depth PIE PCO$_2$</td>
<td>28</td>
<td>$P_{CO_2}$ by pH and ANC (Gran tit.)</td>
<td>12</td>
<td>34.02</td>
<td>33</td>
<td>1341.4</td>
<td>577</td>
<td>2.5363</td>
<td>0.9991</td>
<td>0.9991</td>
<td>1.1892</td>
<td>0.0110</td>
<td>-28.1076</td>
<td>16.050</td>
</tr>
<tr>
<td>Allequash depth Corr. PIE DIC</td>
<td>31</td>
<td>Estimated DIC</td>
<td>12</td>
<td>15.69</td>
<td>924.06</td>
<td>1.70</td>
<td>0.94</td>
<td>0.94</td>
<td>1.04</td>
<td>0.08</td>
<td>-16.24</td>
<td>72.23</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>
The depth profile made on Long Lake differed from those on Allequash Lake. Figure 33 shows the twelve $P_{\text{CO}_2}$ depth profile measurements made for Long Lake’s depth profile on 09/16/2007 with PIE compared against the headspace measured $P_{\text{CO}_2}$. There was a strong correlation between PIE based $P_{\text{CO}_2}$ (Figure 33) and headspace based $P_{\text{CO}_2}$ ($r^2 = 0.9954$, Table 9). However, the slope (1.8436 ± 0.0398, Table 9) was significantly different from one ($p = 0.0002$). (The measurements made on this oligotrophic lake ranged from 6.38 to 17.02 °C in temperature.)

Figure 33. Long Lake’s depth profile measurements (N = 12), PIE based $P_{\text{CO}_2}$ versus headspace based $P_{\text{CO}_2}$. 
In Figures 34, 35, 36, and 37, the carbon dioxide measurement results from these methods and the oxygen results were also graphed by depth for Long Lake. At ten meters depth and above, Figure 34 also displays the same high spatial resolution of the PIE based P_{CO2} measurements with depth as in the Allequash Lake depth profiles. These O_{2} and PIE based P_{CO2} fluctuations also seem to be a different magnitude, but opposite in direction.

Among all the methods used at nine meters depth and above, headspace based P_{CO2} (Figure 34) measurements more closely mirrored the PIE based P_{CO2} values. However, at depths greater than nine meters, none of the methods had a close relationship with the PIE based P_{CO2} values. The PIE based measurements at depths greater than nine meters likely reflect a phenomenon associated with the nonlinear portion of the PIE based P_{CO2} calibration curves and shifts in the atmospheric pressure as described earlier in the Methods section. Ranging from 790 to 15,200 μatm, some of the PIE based P_{CO2} measurements are well into this nonlinear range.

To correct PIE based P_{CO2} measurements for this problem, an empirical response relationship (Eq. [25]) based on ANC by Gran titration, laboratory DIC, and Henry’s law was used. Figure 38 shows a comparison of the corrected PIE based P_{CO2} measurements to the headspace based P_{CO2} measurements. There was a strong correlation between the corrected PIE based P_{CO2} (Figure 38) and the headspace based P_{CO2} (r^2 = 0.9299, slope = 0.8368 ± 0.0727, Table 9). However, the slope and the y-intercept of 39.7045 ± 308.8456 continue to be influenced heavily by the P_{CO2}
Figure 34. Long Lake’s depth profile measurements (N = 12), PIE based $P_{CO_2}$, headspace based $P_{CO_2}$, and oxygen by depth.

Figure 35. Long Lake’s depth profile measurements (N = 12), PIE based $P_{CO_2}$, $P_{CO_2}$ by pH and laboratory DIC, and oxygen by depth.
Figure 36. Long Lake’s depth profile measurements (N = 12), PIE based $P_{CO2}$, $P_{CO2}$ by pH and ANC (Gran titration), and oxygen by depth.

Figure 37. Long Lake’s depth profile measurements (N = 12), PIE based $P_{CO2}$, $P_{CO2}$ by pH and ANC (C_B and C_A), and oxygen by depth.
Figure 38. Long Lake’s depth profile measurements (N = 12), corrected PIE based $P_{CO2}$ versus headspace based $P_{CO2}$. measurements above 4000 μatm. Further, Figure 39 shows that the headspace based $P_{CO2}$ measurements do not closely mirror the corrected PIE based $P_{CO2}$ values. In this case, the use of an empirical response relationship for PIE based $P_{CO2}$ is in need of more study.
Several observations do stand out for this depth profile analysis. Figure 34 shows differences as large as 6500 μatm $P_{\text{CO}_2}$ between PIE based and headspace based $P_{\text{CO}_2}$ measurements. Further, Figure 38 shows that the comparison of the corrected PIE based $P_{\text{CO}_2}$ to the headspace based $P_{\text{CO}_2}$ yields a y-intercept of 39.7045 ± 308.8456. The error term of this y-intercept is huge in comparison to the intercept itself, which gives question of whether to use a corrected PIE based $P_{\text{CO}_2}$ in this instance. Additionally, previous experiments have shown only small differences between PIE based $P_{\text{CO}_2}$ and headspace based $P_{\text{CO}_2}$ measurements. Therefore, it is
likely that the influence of the phenomenon associated with the nonlinear portion of
the PIE based $P_{CO2}$ calibration curves and shifts in the atmospheric pressure may be
small for this depth profile. Further, it could be concluded that there may be other
factors influencing these measurements.

PIE based $P_{CO2}$ measurements could have other issues that are in need of
further study. An improvement of the internal pressure compensation function of the
LI-COR model LI-840, that has failed as a reliable algorithm for mole fraction ($X_{CO2}$)
estimates, may be very helpful in correcting some of these issues. It is also unknown
if low oxygen concentrations, high hydrogen sulfide concentrations, high methane
concentrations, low temperatures, or other characteristics in the hypolimnion (Figure
40) of this deep oligotrophic lake have an influence on these PIE based $P_{CO2}$
measurements.
Figure 40. Long Lake’s depth profile measurements (N = 12), PIE based $P_{CO2}$ and temperature by depth. Epilimnion, metalimnion, and hypolimnion zones are also included for reference.
Table 9, Long Lake’s depth profile.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Fig.</th>
<th>Dependent variable (y)</th>
<th>Indep. variable (x)</th>
<th>N</th>
<th>RMSE</th>
<th>dep. mean</th>
<th>coeff. of variation (CV, %)</th>
<th>$r^2$</th>
<th>Adj. $r^2$</th>
<th>slope</th>
<th>std error slope</th>
<th>y-int.</th>
<th>std error y-int.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long depth</td>
<td>33</td>
<td>PIE $P_{\text{CO}_2}$</td>
<td>headspace $P_{\text{CO}_2}$</td>
<td>12</td>
<td>415.29</td>
<td>4735.25</td>
<td>92</td>
<td>8.7703</td>
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<td>0.9949</td>
<td>1.8436</td>
<td>0.0398</td>
<td>-786.5748</td>
<td>168.98</td>
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<tr>
<td>Long depth</td>
<td>33</td>
<td>PIE $P_{\text{CO}_2}$</td>
<td>$P_{\text{CO}_2}$ by pH and laboratory DIC</td>
<td>12</td>
<td>700.29</td>
<td>4735.25</td>
<td>92</td>
<td>14.7889</td>
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<td>Long depth</td>
<td>33</td>
<td>PIE $P_{\text{CO}_2}$</td>
<td>$P_{\text{CO}_2}$ by pH and ANC (Gran tit.)</td>
<td>12</td>
<td>5002.7</td>
<td>4735.25</td>
<td>92</td>
<td>105.648</td>
<td>0.3281</td>
<td>0.2609</td>
<td>2.6863</td>
<td>1.2156</td>
<td>565.6784</td>
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<td>Long depth</td>
<td>33</td>
<td>PIE $P_{\text{CO}_2}$</td>
<td>$P_{\text{CO}_2}$ by pH and ANC (Ca and Ca)</td>
<td>12</td>
<td>4360.9</td>
<td>4735.25</td>
<td>92</td>
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<td>Long depth</td>
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<td>headspace $P_{\text{CO}_2}$</td>
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<td>29.8137</td>
<td>0.9299</td>
<td>0.9228</td>
<td>0.8368</td>
<td>0.0727</td>
<td>39.7045</td>
<td>308.84</td>
</tr>
</tbody>
</table>

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Conclusion – Traditional Lake Studies with PIE

For the traditional lake studies, the PIE based measurements also had high accuracy and precision. For one particular lake study (PIE based replicate measurements on Allequash Lake), PIE based measurements had the lowest coefficient of variation (3.0239 %) amongst direct and indirect methods used (Table 5). This CV falls within the CV range of the laboratory experiments. However, a comparison of PIE based \([H_2CO_3^*]\) versus headspace based \([H_2CO_3^*]\) for Experiment 4 (Figure 8) and a lake study (Figure 12; Comparison of PIE to traditional methods on Allequash Lake) showed lower precision and accuracy \((r^2 = 0.99, \text{ slope } = 1.00 \pm 0.03 \text{ (Table 3)}\) and \(r^2 = 0.98, \text{ slope } = 0.87 \pm 0.02 \text{ (Table 4), respectively) for this lake study.}

The fifty-two lake surface water survey also showed high precision and accuracy of the PIE based measurements. However, a comparison of PIE based \([H_2CO_3^*]\) versus headspace based \([H_2CO_3^*]\) for Experiment 4 (Figure 8) and this lake study (Table 6) showed a decrease in the precision and accuracy \((r^2 = 0.99, \text{ slope } = 1.00 \pm 0.03 \text{ (Table 3)}\) and \(r^2 = 0.90, \text{ slope } = 0.92 \pm 0.04 \text{ (Table 6), respectively) for this lake study.}

PIE based \(P_{CO_2}\) measurements were highly correlated with the headspace based \(P_{CO_2}\) measurements for eutrophic and oligotrophic lakes, except not for mesotrophic lakes. Eutrophic lakes show the tightest correlation, where the majority of lakes interestingly have \(P_{CO_2}\) concentrations below 200 \(\mu\text{atm.}

As described in the Introduction, recent studies of lake metabolism have shown that most of the world’s lakes surface waters are supersaturated with dissolved
carbon dioxide (Sobek et al., 2005), suggesting that lakes may be a net source of carbon dioxide emission into the atmosphere (Sobek et al., 2005). However, most of the lakes in the PIE in a fifty-two lake surface water survey were not supersaturated with dissolved carbon dioxide. According to PIE based dissolved carbon dioxide measurements in Figure 22, most of the fifty-two lakes were undersaturated with respect to ambient atmospheric $P_{CO_2}$. Therefore, these lakes are a sink for atmospheric carbon dioxide. More lakes will have to be directly measured with PIE to build a better picture of why these particular lakes in this study were undersaturated with respect to atmospheric carbon dioxide. These findings were based on the traditional collection of one surface-water sample from each lake, which will be further examined in the next chapter with diurnal patterns from one surface-water point and whole lake surface-water mapping.

There was also high precision and accuracy of the PIE based measurements in the depth-profile lake studies. For the Allequash and Long Lake depth profiles, the PIE based $P_{CO_2}$ measurements were highly correlated with the headspace based $P_{CO_2}$ measurements. The $r^2$ values ranged from 0.9279 to 0.9954 and the mean $r^2$ value was 0.9704. However, the coefficients of variation (CV) were high and ranged from 5.4495 to 9.4702 % and the mean CV was 7.8966 %. The accuracy of the PIE based measurement was also high for both Allequash Lake depth profiles. The PIE based $P_{CO_2}$ measurements trend with the headspace based $P_{CO_2}$ measurements was close to the one-to-one line. These slope values ranged from 0.9527 to 1.1277. In contrast, the accuracy of the PIE based measurement was low for the Long Lake depth profile. The
PIE based $P_{\text{CO}_2}$ measurements trend with the headspace based $P_{\text{CO}_2}$ measurements was biased high with a slope of 1.8436. However, the corrected PIE based $P_{\text{CO}_2}$ measurements improved the relationship with the headspace based $P_{\text{CO}_2}$ measurements with a slope of 0.8368.

For the Allequash and Long Lake depth profiles, PIE based $P_{\text{CO}_2}$ and oxygen measurements were graphed by depth along side headspace based $P_{\text{CO}_2}$, $P_{\text{CO}_2}$ by pH and laboratory DIC, $P_{\text{CO}_2}$ by pH and ANC (Gran titration), and $P_{\text{CO}_2}$ by pH and ANC ($C_B$ and $C_A$) measurements. At ten meters depth and above, the PIE based measurements had high spatial resolution with the dissolved oxygen levels. The dissolved oxygen (O$_2$) level had a peak maximum at or near the same time and a minimum at the same time that the PIE based $P_{\text{CO}_2}$ had at its minimum and maximum, respectively. Our O$_2$ and PIE based $P_{\text{CO}_2}$ fluctuations seemed to be a different magnitude, but opposite in direction. Similar relationships were also found in diurnal studies of dissolved carbon dioxide and dissolved oxygen in Hanson et al., (2006). Among all the methods used at depths of nine meters and above, headspace based $P_{\text{CO}_2}$ measurements more closely mirrored the PIE based $P_{\text{CO}_2}$ values.

However, at depths greater than nine meters on Long Lake, none of the methods has a close relationship to the PIE based $P_{\text{CO}_2}$ values. It is unknown if low oxygen concentrations, high hydrogen sulfide concentrations, high methane concentrations, low temperatures, or other characteristics in the hypolimnion (Figure 40) of this deep oligotrophic lake have an influence on the PIE based $P_{\text{CO}_2}$
measurements or other direct and indirect measurements. This is an area in need of further research.

In summary, this chapter has shown how PIE based direct carbon dioxide measurements in a limited study of lakes across three trophic classes (eutrophic, mesotrophic, oligotrophic) compare to traditional direct and indirect carbon dioxide measurements. PIE based $P_{CO_2}$ have proved to be a precise, accurate, and reliable measurement for surface analyses of lake bodies ranging from zero to 2314 μatm $P_{CO_2}$ and for depth analyses of lake bodies ranging from 450 to 4250 μatm $P_{CO_2}$. Researchers now have a PIE based direct measurement with known precision and accuracy that can be applied to natural systems and traditional lake studies.
TEMPORAL and SPATIAL RESOLUTION of PIE

This chapter contains the methods, and results for the temporal and spatial lake experiments. This is where the PIE method was shown to substantially increase the ability to measure carbon dioxide variations in time and space. In a continuous twenty-four hour diurnal pattern, PIE based partial pressure of carbon dioxide ($P_{CO2}$) and DIC measurements showed the actual respiration and photosynthesis of the lake from day to night and back to day at temporal resolutions inaccessible via traditional indirect and other direct sampling methods. PIE based $P_{CO2}$ and DIC whole lake maps also showed spatial variation of dynamic lake systems at spatial resolution inaccessible via traditional indirect and other direct sampling methods. Several locations on these maps indicated possible ground and/or surface water entry of dissolved carbon dioxide into the lake. These maps were also used to show the spatial variation of dissolved carbon dioxide over time.

Methods - Temporal and Spatial Resolution of PIE

PIE based diurnal carbon dioxide measurements were used to develop graphs of temporal variation within Allequash Lake. To accomplish this, one continuous twenty-four hour diurnal pattern of PIE based $P_{CO2}$ and DIC measurements were taken from one point and 0.5m depth on Allequash Lake. The boat was secured with two anchors at this sampling point. Note that this is the same sampling point coordinate (Appendices B and C) as for the fifty-two lake study, carbon dioxide replicate study, and depth profile studies. Water temperature measurements for the PIE based
measurements were made with a Fluke® temperature probe (resolution = 0.0001 °C) in the gas collection chamber.

PIE based measurements of carbon dioxide were also used to develop whole lake maps for Allequash Lake. Development focused on surface-water lake maps of carbon dioxide concentration, more specifically PIE based $P_{CO2}$ and DIC. This was an exploratory use of this PIE device for literature searches had provided no known existing sources of accurate carbon dioxide lake maps.

Surface water carbon dioxide measurements for these whole lake maps were made at multiple discrete points collected over the daylight hours (from dawn to dusk) in as few days as possible (two to three days). Note that these experiments were extended for a day or two in order to collect traditional direct and indirect measurements of dissolved carbon dioxide at some of these sampling points. Samples were collected while the sampling boat was slowly moving under power, adrift, or anchored. Selection of which technique used depended on location, wave action, and wind condition.

Allequash Lake’s PIE based $P_{CO2}$ mapping measurements (07/01/2007 to 07/03/2007) were used to develop lake maps with $P_{CO2}$ concentration. These maps were used to show spatial variation of $P_{CO2}$ concentration in a mesotrophic lake. The sampling boat was secured with two anchors at each randomly selected sampling point. A combined ninety-six near-shore and deep-water surface water samples were taken at a one-half meter depth or at least 30 cm above the sediment throughout Allequash Lake. A Trimble GeoExplorer Geo (CE) XT Global Positioning System (GPS) was
used to record the location of each sampling point. After the PIE device stabilization, a two-minute average from these PIE records was used to estimate PIE based $P_{CO2}$ for each mapped sampling point. Allequash Lake’s PIE based $P_{CO2}$ maps were developed using ESRI ArcGIS ArcMAP. These images and points were geo-referenced to the Wisconsin Transverse Mercator (WTM83/91) geographic coordinate system (meters) and North American 1983 HARN geographic projection system.

Allequash Lake’s PIE based $P_{CO2}$ and DIC mapping measurements (06/06/2008 to 06/08/2008) were used to develop lake maps with $P_{CO2}$ or DIC concentration. These maps were used to illustrate spatial variation of $P_{CO2}$ and DIC concentration in a mesotrophic lake. PIE based $P_{CO2}$ and DIC surface water samples were measured simultaneously on different PIE devices. The sampling boat was secured with two anchors for each near-shore surface water sampling point. Deep-water surface water samples were obtained while the boat drifted on the calm lake. Near-shore and deep-water surface water samples were taken at a one-half meter depth or at least 30 cm above the sediment throughout Allequash Lake. After the PIE device stabilization, a two-minute average from these PIE records was used to estimate PIE based $P_{CO2}$ and PIE based DIC for each mapped sampling point. A continuous record of surface water samples were also taken at a one-half meter depth or at least 30 cm above the sediment throughout the perimeter of Allequash Lake. The continuous record perimeter measurements were taken as the boat moved at minimum trolling speed about 70 meters from shore. After the PIE device stabilization, a one-minute average from these PIE records was used to estimate PIE based $P_{CO2}$ and PIE based...
DIC for each mapped perimeter sampling point. A Trimble GeoExplorer Geo (CE) XT Global Positioning System (GPS) was used to record the location of each sampling point. A total of four hundred fifty-eight near-shore, deep-water, and perimeter surface water sampling points were recorded for each PIE based $P_{CO2}$ and DIC measurement. Allequash Lake’s PIE based $P_{CO2}$ and DIC maps were developed using ESRI ArcGIS ArcMAP. These images and points were geo-referenced to the Wisconsin Transverse Mercator (WTM83/91) geographic coordinate system (meters) and North American 1983 HARN geographic projection system.

**Results and Discussion – Temporal and Spatial Resolution of PIE**

The PIE based dissolved carbon dioxide method allows for measurements to be made efficiently and repeatedly in time and space. This method was used to explore carbon dioxide variations in detail for Allequash Lake. Allequash Lake’s continuous twenty-four hour diurnal pattern of PIE based $P_{CO2}$ measurements are shown in Figures 41 and 42. These measurements show the actual respiration of a mesotrophic lake from day to night and back to day at temporal resolutions inaccessible via traditional indirect and direct carbon dioxide sampling methods.

These diurnal PIE based $P_{CO2}$ measurement results might show a repeatable pattern on a daily scale. The PIE based $P_{CO2}$ concentration (Figure 41) decreased steadily throughout the daylight hours. At the midway between sunset and last twilight, the $P_{CO2}$ (Figure 41) abruptly dropped to a minimum value of 125 $\mu$atm. The $P_{CO2}$ then increased steadily throughout the night until it reached its peak of 230 $\mu$atm at 7:00 a.m. about two hours after sunrise on the second day (Figures 41 and 42).
Figure 41, Allequash Lake Diurnal O₂ and PIE based P\textsubscript{CO₂}
06/30/2008, noon to midnight.
Figure 42, Allequash Lake Diurnal O₂ and PIE based $P_{CO_2}$
06/30/2008 midnight to 07/01/2008 noon.

Atmospheric $P_{CO_2} = 362$
After which, the $P_{CO2}$ steadily decreased to a value at noon on the second day that was within 20 $\mu$atm of the value of the previous noon.

The PIE based $P_{CO2}$ pattern during the day is consistent with $CO_2$ consumption and oxygen production during plant photosynthesis. The pattern of $CO_2$ increase throughout the night time hours is also consistent with plant respiration, where the energy or sugar stored during the day during photosynthesis is consumed along with oxygen to produce carbon dioxide and water during the night.

The minimum value, maximum value, and general shape of the diurnal $O_2$ and PIE based $P_{CO2}$ fluctuations seemed to be a different magnitude, but opposite in direction. The dissolved oxygen (DO, $O_2$) level also had a peak 9.4 mg L$^{-1}$ maximum (Figure 41) and about an 8.7 mg L$^{-1}$ minimum (Figure 42) at the same time that the PIE based $P_{CO2}$ had at its minimum and maximum, respectively. Similar relationships were also found in the depth profile experiments discussed earlier and in diurnal studies of dissolved carbon dioxide and dissolved oxygen in Hanson et al. (2006).

These PIE based $P_{CO2}$ diurnal measurements were also reported and graphed at temporal resolutions inaccessible via traditional indirect and direct carbon dioxide sampling methods. These diurnal patterns closely matched what was found by Hanson et al. (2006) and Carignan (1998), where both Hanson’s and Carignan’s $CO_2$ and $O_2$ data sets were reported as a one record per half-hour time series. In contrast, these PIE based data sets were recorded at a temporal resolution of one record per second $P_{CO2}$. However, limitations on the oxygen data logger led to a one record per minute $O_2$ time series. Therefore, a one-minute average was produced from the $P_{CO2}$ record
and then both CO$_2$ and O$_2$ were graphed as a one record per minute time series (one open circle per record; Figures 41 and 42).

Similar temporal resolution as found above was shown with the PIE based DIC measurements. Figures 43 and 44 show Allequash Lake’s continuous twenty-four hour diurnal pattern of PIE based DIC measurements, which were also taken at the same sampling times and locations as the PIE based P$_{CO2}$ diurnal measurements. These PIE based DIC diurnal measurements were also recorded and reported at temporal resolutions (one record per minute time series with one open circle per record) inaccessible via traditional indirect and direct carbon dioxide sampling methods, as described in the PIE based P$_{CO2}$ diurnal measurements above.

These PIE based DIC measurement results also might show a repeatable pattern on a daily scale. After measurement had started at noon on the first day, the PIE based DIC concentration (Figure 43) remained steady at its maximum level around 870 μmol L$^{-1}$ throughout the daylight hours until it proceeded to decrease about one hour after last twilight. The DIC (Figures 43 and 44) fluctuated quite rapidly after this until it decreased to its minimum value of 670 μmol L$^{-1}$ on the second day at 1:00, 2:00, and finally at 5:15 a.m. sunrise. The DIC (Figure 44) then proceeded to fluctuate again quite erratically as it increased to a value at noon on the second day that was within 50 μmol L$^{-1}$ of the value of the previous noon (Figure 43).

The drop and fluctuations of PIE based DIC concentrations at night are also consistent with rising carbon dioxide levels from plant respiration, where the lake becomes more acidic and the pH drops. A decrease in pH could dissolve base cation
Figure 43, Allequash Lake Diurnal O$_2$ and PIE based DIC
06/30/2008, noon to midnight.

DIC and [H$_2$CO$_3^*$] (μmol L$^{-1}$)

Dissolved Oxygen (mg L$^{-1}$)

NOON 64800 86400

8:49 pm 9:26 pm

00 000 00

time (seconds)

PIE based DIC (black) PIE based [H$_2$CO$_3$] (gray) DO
Figure 44, Allequash Lake Diurnal O₂ and PIE based DIC
06/30/2008 midnight to 07/01/2008 noon.
or mineral acid anion into solution, which could drive increases or decreases in ANC. Figures 43 and 44 also show that the carbon dioxide concentration is only a small portion of the total DIC concentration. The DIC concentration consists mainly of bicarbonate. With a decrease in pH, this bicarbonate could transform into carbonic acid, which could also decrease the ANC concentration. As ANC concentration decreases or increases rapidly, DIC concentration would have decreased or increased, respectively.

Allequash Lake’s PIE based P\textsubscript{CO2} measurements (07/01/2007 to 07/03/2007) were used to develop lake maps (Figures 45, 46, and 47) with P\textsubscript{CO2} concentration. These maps show the variation of P\textsubscript{CO2} concentration in the northern bay of Allequash Lake, a mesotrophic lake, at spatial resolutions inaccessible via traditional indirect and direct carbon dioxide sampling methods. Lake bathymetry are indicated in shades of gray and labeled in black. The open circles represent the ninety-six sampling point locations and are color coded with the P\textsubscript{CO2} levels in μatm. Figure 45 shows that some of the near shore sampling locations overshot the lakeshore edge (1621 feet above mean sea level) as indicated by the bathymetry. At the time of these carbon dioxide measurements, the water levels were higher than the lakeshore edge datum. In Figures 46 and 47, the color shading for the P\textsubscript{CO2} between open circles were done in ESRI ArcGIS ArcMAP with a triangulated irregular network (TIN) model and displayed as shaded triangles. Note that some of the TIN model has overshot the outside the lakeshore edge (1621 feet above mean sea level). This resulted from the rough edging
Figure 45. PIE based $P_{CO2}$ for Allequash Lake, Vilas County, WI, July 1 – 3, 2007.
Figure 46. PIE based $P_{CO_2}$ (TIN model) for Allequash Lake, Vilas County, WI, July 1 – 3, 2007.
Figure 47. PIE based P\textsubscript{CO}_2 (TIN model) and a topographic map for Allequash Lake, July 1 – 3, 2007.
function of the TIN model (i.e. convex hull boundary). Map legends were created using quantile classification with twenty classes (min = 39 μatm P CO2; max = 378 μatm P CO2; mean = 131 μatm P CO2; and standard deviation = 30 μatm P CO2). Figure 47 was created by overlaying the map of the PIE based P CO2 measurements onto a digital raster graphic (DRG) map. The DRG is a scanned image of a U.S. Geological Survey (USGS) standard series 1:24,000 topographic map.

These maps show that most of the ninety-six measured near-shore and deep-water surface-water carbon dioxide sample values were undersaturated with respect to the July 2007 ambient atmospheric global average of 362 μatm P CO2 (381 ppmv; Tans, 2008). At this time, Allequash Lake would have been a sink for atmospheric carbon dioxide. Carbon dioxide would have been moving from the atmosphere into the lake.

Several locations displayed in red (Figures 45, 46, and 47) in the northern bay of Allequash Lake indicate possible ground and/or surface water entry of dissolved carbon dioxide. Figures 46 and 47 show a red plume on the eastern edge of Allequash Lake indicating possible surface water entry of carbon dioxide from Allequash Creek. This red plume starts at the Allequash Creek surface water entry into the northern bay and extends all the way to the surface water exit (channel) into the southern bay of Allequash Lake (see the blue arrows labeled as flow). (Note that Allequash Creek then proceeds to a surface water exit at the southern end of the southern bay of Allequash Lake, which is not shown.) Figure 47 shows wetland areas in the northern and western end of the northern bay of Allequash Lake. The plume on the western end of the lake shows a possible surface water entry of carbon dioxide into the lake.
There are two sampling points near the wetland on the northern end of the bay that show possible surface water or ground water entry of carbon dioxide into the lake.

These 96 samples in Figures 45, 46, and 47 are more representative of the whole lake than the traditional single surface water sampling location used in the fifty-two lake surface water survey (black X on map). Traditional lake studies involving collecting one sample from a lake only offer a very small glimpse of that sampling point in time and space.

PIE based carbon dioxide measurements offer a method of measuring multiple samples over a lake surface in a short time. PIE based carbon dioxide measurements also offer spatial resolution unavailable with traditional methods (e.g. bottle samples of headspace, ANC, DIC) of measuring carbon dioxide.

Allequash Lake’s PIE based $P_{CO_2}$ and DIC measurements (06/06/2008 to 06/08/2008) were used to develop lake maps with $P_{CO_2}$ (Figures 48, 49, and 50) and DIC (Figure 51, 52, and 53) concentration. These maps show the variation of $P_{CO_2}$ and DIC concentration in the northern bay of Allequash Lake at spatial resolutions inaccessible via traditional indirect and direct carbon dioxide sampling methods. The open circles represent the four hundred fifty-eight sampling point locations and are color coded with $P_{CO_2}$ or DIC concentration. Note that some of the near shore sampling locations, like the previous year, also overshot the lakeshore edge (1621 feet above mean sea level) as indicated by the bathymetry. At the time of these carbon dioxide and DIC measurements, the water levels were also higher than the lakeshore edge datum. The TIN models, as described earlier, also had similar issues with
Figure 48. PIE based $P_{CO2}$ for Allequash Lake, Vilas County, WI, June 6 - 8, 2008.
Figure 49. PIE based $P_{CO2}$ (TIN model) for Allequash Lake, Vilas County, WI, June 6 - 8, 2008.
Figure 50. PIE based $P_{CO_2}$ (TIN model) and a topographic map for Allequash Lake, June 6 - 8, 2008.
overshooting the lakeshore edge. Map legends for Figures 48, 49, and 50 were created using quantile classification with twenty classes (min = 83 \(\mu\text{atm } \text{PCO}_2\); max = 2006 \(\mu\text{atm } \text{PCO}_2\); mean = 486 \(\mu\text{atm } \text{PCO}_2\); and standard deviation = 120 \(\mu\text{atm } \text{PCO}_2\)). Map legends for Figure 51, 52, and 53 were created using quantile classification with twenty classes (min = 567 \(\mu\text{mol } \text{L}^{-1} \text{DIC}\); max = 943 \(\mu\text{mol } \text{L}^{-1} \text{DIC}\); mean = 899 \(\mu\text{mol } \text{L}^{-1} \text{DIC}\); and standard deviation = 16 \(\mu\text{mol } \text{L}^{-1} \text{DIC}\)).

Unlike the previous year, Figures 48, 49, and 50 show that three-fourths of the four hundred fifty-eight measured surface water carbon dioxide sample values were saturated with respect to the June 2008 ambient atmospheric global average of 362 \(\mu\text{atm } \text{PCO}_2\) (385 ppmv; Tans, 2008). Three-fourths of Allequash Lake would likely have been a source for atmospheric carbon dioxide. Carbon dioxide would have been releasing from the lake surface into the atmosphere.

Even though the \(\text{PCO}_2\) levels are higher than the previous year, some of the same locations had higher \(\text{PCO}_2\) levels. As in the previous year, a red plume (Figures 49 and 50) on the eastern edge of Allequash Lake indicates possible surface water entry of carbon dioxide from Allequash Creek. A red plume starts at the Allequash Creek surface water entry into the northern bay and extend in patches all the way to the surface water exit (channel) into the southern bay of Allequash Lake (see the blue arrows labeled as flow). Several point locations (Figure 50) in the channel between bays display possible flow of carbon dioxide from the northern bay through the channel to the southern bay. The wetland on the western end shows two sampling points (Figure 50) of possible surface water entry of carbon dioxide into the lake.
There is also a plume (Figure 50) near the wetland on the northern end of the bay that shows possible surface water and/or ground water entry of carbon dioxide into the lake. The entire northeastern shoreline (Figure 50) shows possible ground water entry of carbon dioxide into the lake, conforming to the known flow paths for the groundwater hydrology of this watershed (Kratz et al., 1997).

These PIE based $P_{\text{CO}_2}$ measurements continue to offer spatial resolution unavailable with traditional methods (e.g. bottle samples of headspace, ANC, DIC) of measuring carbon dioxide.

The dissolved inorganic carbon (DIC) concentrations in Figures 51, 52, and 53 show some interesting patterns. Several locations in the northern bay of Allequash Lake (Figure 53) indicate possible consumption and creation of DIC. Areas of red (Figure 53) could indicate these areas of DIC creation. The spatial pattern of DIC suggests a very dynamic system with high DIC concentrations next to low DIC concentrations. There are higher DIC concentrations for the deep-water surface water samples, than for the near-shore surface water samples. Some of the DIC concentrations are 100 $\mu$mol L$^{-1}$ higher than in the diurnal DIC study (Figures 43 and 44). Similar to the dissolved carbon dioxide maps, the traditional method of collecting one DIC sample is obviously not representative of the whole lake system. This lake has an intriguing dynamic pattern of dissolved inorganic carbon in definite need of further study.
Figure 51. PIE based DIC for Allequash Lake, Vilas County, WI, June 6 - 8, 2008.
Figure 52. PIE based DIC (TIN model) for Allequash Lake, Vilas County, WI, June 6 - 8, 2008.
Figure 53. PIE based DIC (TIN model) and a topographic map for Allequash Lake, June 6 - 8, 2008.
Comparing maps of DIC (Figure 53) and P_{CO2} (Figure 50) can also give clues to the carbonate chemistry of this lake. Areas of high DIC and low P_{CO2} concentrations will have high levels of bicarbonate concentration. These high bicarbonate concentrations may give detail on the acid neutralizing capacity available in each part of the lake.

PIE based DIC measurements offer a method of measuring multiple samples over a lake surface in a short time. PIE based DIC measurements also offer spatial resolution unavailable with traditional methods (e.g. bottle samples of DIC) of measuring dissolved inorganic carbon.

PIE based measurements were also used for change detection in the environment, specifically the spatial variation of dissolved carbon dioxide concentration over time. Allequash Lake’s PIE based P_{CO2} measurements taken from 07/01/2007 to 07/03/2007 and from 06/06/2008 to 06/08/2008 were used to develop lake maps (Figures 54 and 55) with P_{CO2} concentration. These maps were used to show the spatial variation of PIE based P_{CO2} measurements between these two years. The map legend for Figure 54 was created using manual classification with six classes and intervals of sixty-seven (min = 39 \ \muatm \ P_{CO2}; \ max = 378 \ \muatm \ P_{CO2}; \ mean = 131 \ \muatm \ P_{CO2}; \ and \ standard \ deviation = 30 \ \muatm \ P_{CO2}). The map legend for Figure 55 was created using manual classification with thirty-one classes and intervals of sixty-seven (min = 83 \ \muatm \ P_{CO2}; \ max = 2006 \ \muatm \ P_{CO2}; \ mean = 486 \ \muatm \ P_{CO2}; \ and \ standard \ deviation = 120 \ \muatm \ P_{CO2}). These maps were developed using the same
Sampling position for PIE in a fifty-two lake surface-water survey

Figure 54. PIE based $P_{\text{CO}_2}$ (TIN model) and a topographic map for Allequash Lake, July 1 – 3, 2007.
Figure 55. PIE based $P_{CO_2}$ (TIN model) and a topographic map for Allequash Lake, June 6 - 8, 2008.
legend interval, in order to show how much higher the carbon dioxide concentrations are from year 2007 to year 2008. The carbon dioxide concentrations in June 2008 (Figure 55) are many times higher than in July 2007 (Figure 54). The mean value for the June 2008 carbon dioxide concentrations is nearly four times that of the July 2007 mean value. The change in carbon dioxide concentration from year 2007 to year 2008 could be from external or internal sources. What percentage of the carbon input came from runoff, ground water, surface water, or internal processes is unknown. This lake has carbon dioxide spatial variations in definite need of further study.

The PIE method substantially increases the ability to measure the spatial variation of dissolved carbon dioxide concentration over time. PIE based measurements show the variation of P$_{CO2}$ concentration in the northern bay of Allequash Lake at spatial and temporal resolutions inaccessible via traditional indirect and direct carbon dioxide sampling methods.
Conclusion – Temporal and Spatial Resolution of PIE

The PIE method substantially increases the ability to measure carbon dioxide variations in time and space. The PIE method allows for rapid measurement of carbon dioxide and improved understanding of carbon dioxide dynamics.

In a continuous twenty-four hour diurnal pattern of one surface water point on Allequash Lake, PIE based $P_{\text{CO}_2}$ and DIC measurements showed the actual respiration and photosynthesis of a mesotrophic lake from day to night and back to day at temporal resolutions inaccessible via traditional indirect and other direct sampling methods. These diurnal PIE based $P_{\text{CO}_2}$ measurement results also might have shown a repeatable pattern on a daily scale. The PIE based $P_{\text{CO}_2}$ pattern during the day is consistent with CO$_2$ consumption and oxygen production during plant photosynthesis. The pattern of CO$_2$ increase throughout the night time hours is also consistent with plant respiration, where the energy or sugar stored during the day during photosynthesis is consumed along with oxygen to produce carbon dioxide and water during the night.

The minimum value, maximum value, and general shape of the diurnal O$_2$ and PIE based $P_{\text{CO}_2}$ fluctuations seemed to be a different magnitude, but opposite in direction. Similar relationships were also found in the depth profile experiments discussed earlier and in diurnal studies of dissolved carbon dioxide and dissolved oxygen in Hanson et al., (2006).

These PIE based $P_{\text{CO}_2}$ diurnal measurements were also recorded and reported at temporal resolutions inaccessible via traditional indirect and direct carbon dioxide
PIE based data sets were recorded at a temporal resolution of one record per second $P_{CO2}$ and both $CO_2$ and $O_2$ were graphed as a one record per minute time series. Similar temporal resolution was also found with the PIE based DIC measurements.

PIE based $P_{CO2}$ and DIC measurements showed lake maps with $P_{CO2}$ and DIC concentration at spatial and temporal resolutions inaccessible via traditional laboratory DIC and traditional indirect ($P_{CO2}$ by pH and ANC, $P_{CO2}$ by pH and DIC) and direct (headspace) dissolved carbon dioxide measurement methods. PIE based $P_{CO2}$ and DIC whole lake maps showed spatial variation of dynamic lake systems at rarely documented spatial resolution. PIE based $P_{CO2}$ whole lake maps displayed possible ground and/or surface water entry of dissolved carbon dioxide to a lake. PIE based $P_{CO2}$ maps also showed spatial variation of dissolved carbon dioxide concentration over time. PIE based DIC whole lake maps showed an intriguing dynamic pattern of DIC in definite need of further study. PIE based carbon dioxide measurements offer a method of measuring multiple samples ($P_{CO2}$, $[H_2CO_3^*]$, and DIC) over a lake surface in a short time. The PIE method offers one sample measured per second, which can add up to thousands (86400) of samples measured for a whole lake in just one day.

PIE gives researchers direct measurement methods of known quality (accuracy and precision), spatial resolution, and temporal resolution for studying dissolved carbon dioxide, which were inaccessible via traditional sampling methods (indirect and other direct carbon dioxide measurement methods).
APPENDIX A

Bibliography


Table B-1. PIE in a fifty-two lake surface water survey (07/09/2007 to 07/22/2007). These GPS sampling points were geo-referenced to the Wisconsin Transverse Mercator (WTM83/91) projection system (meters) and North American 1983 HARN geographic coordinate system.

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<th>GPS Easting (m)</th>
<th>GPS Northing (m)</th>
<th>Sampling Depth (m)</th>
<th>Landsat Average SDT (m) (Chipman et al., 2004)</th>
<th>WT ISI (SDT) (Lillie et al., 1993)</th>
<th>Sample Site Lake depth (m)</th>
<th>Lake Area (m²)</th>
<th>Lake Perimeter (m)</th>
<th>Specific Conductivity (μS)</th>
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APPENDIX C
Table C-1. PIE in a fifty-two lake surface water survey (07/09/2007 to 07/22/2007).

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<td>291.4401</td>
<td>154.5275</td>
<td>154.9100</td>
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<td>WDNR Lake ID</td>
<td>Lillie WTSI (SDT) Trophic Class</td>
<td>Lake Temp. (F)</td>
<td>YSI sonde Lake pH</td>
<td>PIE based ( P_{CO_2} ) (μatm)</td>
<td>Headspace based ( P_{CO_2} ) (μatm)</td>
<td>( P_{CO_2} ) by pH and laboratory DIC (μatm)</td>
<td>( P_{CO_2} ) by pH and ANC (Gran titration) (μatm)</td>
<td>ANC by Gran titration (μmol L(^{-1}))</td>
<td>ANC by ( C_a ) and ( C_A ) (μmol L(^{-1}))</td>
<td>Laboratory DIC (μmol L(^{-1}))</td>
<td></td>
</tr>
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</table>
**APPENDIX D**

Table D-1. A comparison of total sample analytical measurement time \((N = 52)\) in the laboratory for the direct and indirect dissolved carbon dioxide measurement methods used in the \textit{PIE in a fifty-two lake surface water survey}.

**Methods to Measure \(P_{CO_2}\)**  
\((N = 52 \text{ lake samples})\)

<table>
<thead>
<tr>
<th>Method</th>
<th>Collecting Sample for Laboratory Analytic Measurement</th>
<th>Sample Analytic Measurement Time in Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIE based (P_{CO_2})</td>
<td>None! (Real Time Continuous; 1 record/sec)</td>
<td>None!</td>
</tr>
<tr>
<td>Headspace based (P_{CO_2})</td>
<td>One bottle (gas) sample</td>
<td>6 Hours (automated)</td>
</tr>
<tr>
<td>(P_{CO_2}) by pH and DIC</td>
<td>One bottle (liquid) grab sample</td>
<td>12 Hours (automated)</td>
</tr>
<tr>
<td>(P_{CO_2}) by pH and ANC (Gran titration)</td>
<td>One bottle (liquid) grab sample</td>
<td>20 Hours (manual)</td>
</tr>
</tbody>
</table>
APPENDIX E

Accuracy and precision of PIE based measurements in estimating the pK_a of carbonic acid - Experiment 6.

This experiment was used to show the accuracy and precision of PIE based measurements in estimating the pK_a of an acid. The negative log of the first dissociation constant (pK_a) of an acid expresses the pH buffering characteristic of a particular acid and its base. This means that the acid-base system will resist change most effectively within a narrow pH range of 3.4 units wide that is centered on its pK_a value. When the concentrations of the acid and base forms are equal in solution, the value of the pH (Eq. [E1]) is equivalent to the negative log of the first dissociation constant of the acid (pK_a):

\[ pH = pK_a. \]

For example, when the concentrations of the acid and base forms of carbonic acid are equal in solution, the value of the pH is equivalent to the negative log of the first dissociation constant of carbonic acid (pK_1).

PIE based measurements can be used to estimate the known pK_1 or pK_a of carbonic acid at a given temperature. A theoretical construct in carbonic chemistry is used to estimate this pK_a. The theoretical construct is completed by using a ratio of PIE based measurements (ratio, Eq. [E2]) to graph the fraction of total DIC (carbonic acid) in base form versus the pH to estimate the pK_1 or pK_a of the acid:
By acidifying solutions of sodium bicarbonate with known additions of sulfuric acid, the amount of solution in the acid form can be measured. From this, the percent of solution in the base form can be estimated from Eq. [E2] and graphed against the pH (Figure E-1). A horizontal bisector line can then drawn through the 50 % acid and base point on the ratio s-curve. Next, a perpendicular bisector line can be dropped down at the point of intersection of the horizontal bisector line and the ratio s-curve, and the pH read. This pH (Eq. [E1]) constitutes the estimation for the pK₁ or pKₐ of carbonic acid.

\[ \text{[E2]} \quad \text{ratio} = 1 - \frac{[H_2CO_3^*]}{\text{DIC}}. \]

Figure E-1. Theoretical construct for estimating the pK₁ or pKₐ of carbonic acid. The theoretical construct was completed by using a ratio of PIE based measurements (ratio, Eq. [E2]) to graph the fraction of total carbonic acid in base form versus pH to estimate the pK₁ or pKₐ (Eq. [E1]) of the acid.

The theoretical construct (Figure E-2) was completed by using a ratio of PIE based measurements (ratio, Eq. [E2]) to graph the fraction of total DIC (carbonic acid)
in base form versus the pH to estimate the pH (Eq. [E1]). A pKₐ of 6.38 pH was estimated for the carbonic acid, which matches the theoretical value at this temperature (@ 20°C, Harned and Bonner, 1945).

![Graph showing the relationship between fraction of total acid in base form and pH](image)

**Figure E-2.** Experiment 6 (N = 11), theoretical construct for estimating the pKₐ or pKₐ of carbonic acid. The theoretical construct was completed by using a ratio of PIE based measurements (ratio, Eq. [E2]) to graph the fraction of total carbonic acid in base form versus pH to estimate the pKₐ or pKₐ (Eq. [E1]) of the acid (estimated pKₐ = 6.38 pH @ 20).
The majority of the Fifty-two lakes in this study had surface waters undersaturated with dissolved carbon dioxide.

As described in the Introduction, recent studies of lake metabolism have shown that most of the world’s lakes surface waters are supersaturated with dissolved carbon dioxide (Sobek et al., 2005), suggesting that lakes may be a net source of carbon dioxide emission into the atmosphere (Sobek et al., 2005). However, most of the lakes in the PIE in a fifty-two lake surface water survey were not supersaturated with dissolved carbon dioxide. According to PIE based dissolved carbon dioxide measurements in Figure F-1, thirteen lake samples were oversaturated and thirty-nine lake samples were undersaturated with respect to the July 2007 ambient atmospheric global average of 362 μatm P$_{CO_2}$ (381 ppmv P$_{CO_2}$; Tans, 2008). Therefore, most of these lakes are a sink for atmospheric carbon dioxide.

It is interesting to note that all the traditional direct and indirect dissolved carbon dioxide measurement methods used for the fifty-two lakes in Figure F-1, also showed that most of the fifty-two lakes were undersaturated with respect to ambient atmospheric P$_{CO_2}$. Specifically, headspace based P$_{CO_2}$, P$_{CO_2}$ by pH and laboratory DIC, P$_{CO_2}$ by pH and ANC (Gran titration), and P$_{CO_2}$ by pH and ANC (C$_B$ and C$_A$) methods only found sixteen, two, three, and one lake samples, respectfully, that were oversaturated with respect to ambient atmospheric P$_{CO_2}$.
These findings do not necessarily refute the supersaturation findings in Sobek et al. (2005). Considering that only 150 of the world’s lakes had direct measurements of carbon dioxide and 4582 of the world’s lakes have been indirectly measured (Table 1), more research is needed and many more lakes will have to be directly measured with PIE to build a better picture of why these particular lakes in this study were undersaturated with respect to atmospheric carbon dioxide.
Figure F-1. PIE in a fifty-two lake surface water survey (N = 52), measured P_{CO2} by method, including PIE based P_{CO2}, headspace based P_{CO2}, P_{CO2} by pH and laboratory DIC, P_{CO2} by pH and ANC (Gran titration), and P_{CO2} by pH and ANC (C_N and C_A).