

## **Carbon Dioxide Efflux and Storage in Small, Drying Alpine Ponds**

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## Abstract

Climate change is expected to influence the drying regimes of small ponds, impacting their carbon cycling capabilities and potentially inducing unprecedented pond drying that could lead to higher greenhouse gas efflux rates. Small ponds are already known to release disproportionately high amounts of carbon dioxide and methane. Riverine, lake and large pond systems have all received increasing attention, both for their vulnerability to climate change and their ability to exacerbate it. Yet the role of small ponds, especially alpine ponds, has remained understudied. The goal of our work was to quantify aqueous CO<sub>2</sub> concentrations in small alpine ponds in northwestern Colorado and to explore the drivers of these concentrations. Additionally, we linked these concentrations to drying patterns and the unique characteristics of ponds with different permanence traits. We found that these ponds had smaller concentrations than comparable small ponds at temperate latitudes, and more closely resembled concentrations in boreal ponds. Our study ponds had aqueous CO<sub>2</sub> concentrations ranging from 33.6 to 77.6  $\mu\text{mol/L}$ , as compared to measurements as high as 360  $\mu\text{mol/L}$  in Connecticut ponds. We found that permanence traits had no significant relationship with CO<sub>2</sub> concentrations early in the season, and that dissolved oxygen was not a good predictor of these concentrations. Further work will be conducted into investigating CO<sub>2</sub> concentration drivers. Our work impacts understanding of the global carbon budget with respect to the role of small ponds.

## Introduction

Earth is experiencing unprecedented, rapidly evolving effects of climate change triggered by recent atmospheric influxes of greenhouse gases, especially carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>). Understanding the global carbon budget is essential to predict the extent of changes, but the contribution of inland waters to greenhouse gas emissions is known to be important but still faces major uncertainties (Raymond et al, 1997; Raymond et al 2013; Campeau and Giorgio, 2014; Selvam et al, 2014; Rantakari et al 2015; ). Small ponds with surface areas less than 0.001km<sup>2</sup> are particularly understudied (Holgerson, 2015) largely due to the difficulty of mapping and identifying them (Downing et al, 2006; Verpoorter et al, 2014). While they account for just 8.6% of global lentic surface area, these small ponds are responsible for 15.1% of CO<sub>2</sub> emissions and 40.6% of CH<sub>4</sub> emissions from lentic systems (Holgerson and Raymond, 2016). Given their disproportionately high greenhouse gas emissions, they warrant further study. Furthermore, alpine ponds have received little attention compared to boreal systems despite the similar climatic stressors alpine systems face. Climate change will likely alter carbon cycling and efflux in small alpine ponds as species compositions and historical pond hydroperiod shift along elevations (Holgerson and Raymond, 2016).

CO<sub>2</sub> concentrations are inversely related to dissolved oxygen (DO) concentrations. pH and chlorophyll-a are also strong predictors of CO<sub>2</sub> concentrations in both aquatic and forest systems (Vourlitis et al, 2005; Holgerson 2015). However, potential masking effect of DO diffusion was not considered, nor were particulate organic carbon (POC), dissolved organic carbon (DOC) or soil organic carbon (SOC), despite their importance in lake systems (Kankaala et al, 2006). Furthermore, Campeau and Giorgio (2014) showed that riverine metabolism did not play a strong role in CO<sub>2</sub> dynamics, suggesting that the link between respiration and CO<sub>2</sub> in ponds may be weak. Nevertheless, the relationship between DO and CO<sub>2</sub> deteriorates near the bed of a water body likely as friction prevents

thorough mixing of the boundary layer between water and sediment. Because ponds have a low volume to surface area ratio, a significant amount of water is in contact with the carbon-rich bottom (Holgerson 2015). Thus, carbon availability likely drives respiration. At this oxic-anoxic interface, we hypothesized that DOC and SOC are better predictors of dissolved CO<sub>2</sub> than DO, while DO remains the best predictor in the water column. We planned to measure DO and CO<sub>2</sub> levels at shallow and deep points in the water column ranging from just below the surface to approximately 7cm above the bed. We also measured DOC and SOC levels, and ran correlation analyses to determine which variables best predict CO<sub>2</sub> amounts in different locations in the water column.

The hydroperiod characteristics of a pond are likely to influence CO<sub>2</sub> concentrations. At the extremes of the hydroperiod gradient, permanent ponds are inundated all year long and temporary ponds completely dry annually, while semi-permanent ponds dry only in some years (Wissinger et al 1999). Permanent ponds tend to be deeper, with less thorough mixing and the possibility for stratification along the water column. We expect that permanent ponds have accumulated massive carbon stores over long timescales (up to thousands of years) that are unavailable for decomposition and assimilation because they are buried in anoxic conditions. In contrast, temporary ponds are shallower and short-lived, with biota adapted for quick lifecycles that take advantage of all available carbon stores. We hypothesize that temporary ponds have less SOC at any given depth since carbon stores are mineralized annually, and that they have higher decomposition rates and concentrations of aqueous CO<sub>2</sub>. Due to thorough mixing and frequent drying, the relationship between CO<sub>2</sub> and DOC will likely be less significant than in permanent ponds. We collected data from temporary, semi-permanent and permanent ponds at the sub-alpine Mexican Cut Nature Preserve in southwestern Colorado to investigate the effect of hydroperiod and respiration on the dynamics of CO<sub>2</sub> concentration.

Climate change is expected to raise temperatures in alpine systems, advancing pond thawing and raising the likelihood of drying in all ponds. Accordingly, ponds at the study site have experienced shifts in permanence (Greig and Wissinger 2010). In short, ponds are expected to become more temporary. Because temporary ponds have rapid lifecycles, dry annually and mineralize all available carbon, this drying effect will not unlock previously inaccessible carbon stores. However, permanent ponds have massive buried carbon stores in anoxic environments. As the margins dry, we hypothesize that these stores will become aerated and available for aerobic decomposition. Consequently, pond margins will release large, accumulated, untapped stores of carbon relatively quickly with respect to burial rates. We were interested in measuring CO<sub>2</sub> efflux from pond waters and from dry pond margins to explore the relationship between SOC and soil CO<sub>2</sub> efflux with respect to hydroperiod at drying pond margins. We expected that as pond hydroperiods become increasingly temporary previously inundated soils saturated with SOC will become aerated and higher rates of microbial and animal decomposition of accumulated carbon stores will prompt more CO<sub>2</sub> efflux from soils.

Diel variation of CO<sub>2</sub> efflux is observed from soil throughout the day (Barron-Gafford et al, 2011) and from water bodies including rivers, lakes and ponds, but has not been explored carefully in ponds. Among open wells, lakes, rivers, canals, ponds, springs and reservoirs, ponds had the largest range of flux values and the biggest margins of error (Selvam et al, 2014). Dijkstra et al (2013) found that the effects of climate change, primarily warming and increasing CO<sub>2</sub> levels, reduced the capabilities of a semi-arid grassland to act as a CH<sub>4</sub> and N<sub>2</sub>O sink. Similarly, ponds are predicted to release increasingly more CO<sub>2</sub> as the balance between carbon burial and emission shifts towards emission with heightened levels of decomposition (Kosten et al, 2010). Kankaala et al (2013) found that total organic carbon (TOC) was the best predictor of CO<sub>2</sub> efflux in larger lakes while physical factors including lake size, turbulence

and mixing best predicted CO<sub>2</sub> efflux in smaller ponds (less than 1km<sup>2</sup>). We will explore diel variation in CO<sub>2</sub> efflux with respect to hydroperiod. Quantifying CO<sub>2</sub> efflux from pond surfaces will further our understanding of small pond contributions to the global carbon budget and of the drivers of carbon fluxes, especially with respect to the time of day. Finally, we will explore the possibility of a positive feedback loop as warming causes ponds to dry and potentially release large amounts of greenhouse gases, which increases warming and furthers pond drying. This work advances our understanding of carbon dynamics in ponds in several fundamental ways.

## Methods

**Study Site.** Twelve ponds spanning the permanence gradient were selected -- three permanent, three semi-permanent, and three temporary -- at the Mexican Cut Nature Preserve in Gunnison County, southeastern Colorado (Figure 1). The ponds are sub-alpine at approximately 3400m above sea level and are biologically productive from late June to mid October.

**CO<sub>2</sub> Diel Efflux Measurements.** Eight static chambers were modeled after Helton et al (2014), with similar designs reported by other literature (Vachon et al, 2010; Morse et al, 2012; Campeau and Giorgio, 2014). Briefly, 10L Tedlar gas sampling bags (Supelco Analytical) were attached to ¾" PVC scaffolds. One end was cut open and the base was secured into a 15x15cm square with garden wire. The exact volume of each 10L Tedlar gas bag was determined by injecting 50mL SF<sub>6</sub> of a known concentration into a floating chamber, measuring the diluted concentration, and back calculating for volume. The chambers were rotated between the 12 ponds such that each the efflux from each pond was measured with the same chamber. Chambers were placed on ponds and anchored to shore with twine such that the open end of the gas bag was submerged at least one inch and turbulence from chamber movement was minimized. Diel measurements were taken once weekly from the southern edge of each pond during four sampling times; 3:00-6:00, 9:00-12:00, 15:00-18:00 and 21:00-0:00. Vinyl tubing (1/16" inner diameter) was attached to the gas bag valve and pumped into an Environmental Gas Monitor (EGM, PP Systems) capable of reporting CO<sub>2</sub> concentrations in the field. After approximately one hour, chambers were sampled and removed. We will convert gas concentrations (ppm) to flux (µg/m<sup>3</sup>) using the Ideal Gas Law.

**CO<sub>2</sub> Aqueous Measurements.** Efflux of CO<sub>2</sub> is dependent on the concentration gradient of CO<sub>2</sub> across water and air and on the gas transfer velocity (k). Henry's Law describes the proportionality of the partial pressure of a gas in air to its aqueous concentration. Consequently we can measure the partial pressure of CO<sub>2</sub> after equilibration with air to determine the concentration in ponds, following standard methods (Cole and Caraco, 1998; Jonsson et al 2003). A 0.63L bottle was filled with water after rinsing several times. Approximately 200mL of water was discarded to create a headspace of ambient air and the bottle was shaken vigorously for 2min to allow for equilibration. 15mL samples were injected into the EGM with a flow rate of 85% on site immediately after equilibration. The exact volume of water was measured with a graduated cylinder, and the concentration in ppm was recorded.

**Water Chemistry.** Dissolved organic carbon (DOC) was collected once weekly from each of the twelve study ponds. A 30mL Nalgene bottle was rinsed three times with filtered pond water, then filled

with 25mL filtered pond water. The samples were stored on ice on site then placed in the freezer. DOC samples will be shipped for analysis to a commercial lab. Other water properties were measured with a Dissolved Oxygen meter (YSI), including pH, temperature (°C), depth (mmHg), dissolved oxygen (% and mg/L), specific conductance (SPC) and oxidation-reduction potential (ORP, mV).

Soil Chemistry. Benthic organic matter (BOM) samples were collected once weekly from each pond. On the bottom of each pond at a depth of about 10cm, an area of approximately 0.04m<sup>2</sup> was thoroughly disturbed and contained within a metal cylindrical tube. 60mL falcon tubes were rinsed with the water-mud mixture once, then filled and stored on ice on site, then placed in the freezer. The soil will be air dried and massed, and subsequently heated in a furnace and weighed again to conduct loss on ignition analysis to obtain estimates of soil organic matter content following the methodology of Wang et al (2011).

Detrital Breakdown Rates and Chlorophyll-a. Three leaf packs were placed on the southern edges of each pond, and all ponds except 42 received a second set of three leaf packs in another location to capture variability. A loose mesh accessible to microbes and detritivores was pre-weighed and filled with 5g of dried grass, *Carex aquatilis*, and a weight. The packs were ironed shut at one end, secured with a zip tie at the other and massed. They were then submerged and left for one, two or three weeks. After removal, packs were placed on ice and stored in the freezer until they were dried in an oven at 55°C. The dry packs were again massed to calculate detritus loss and determine the linear breakdown rate. Productivity was measured via chlorophyll-a from the water column, sedge surface and benthos. Chlorophyll from the water column was collected by filtering 120mL of pond water and retaining the filter for analysis. Chlorophyll from biota growing on the sedge surface was collected by gently scraping all material from a sample of sedge into pond water. As much of the resulting water as possible was filtered and the filter was retained. For the benthic chlorophyll, 1mL of the BOM samples was filtered and the filter was retained. All filters were stored on ice as they were collected on site, then transferred to the freezer. Chlorophyll-a samples were then measured via the acidification method.

## Results

We conducted a survey of aqueous CO<sub>2</sub> concentrations in the 12 study ponds and two additional temporary ponds. Concentrations were measured twice over the span of two weeks and averaged (Figure 2). The range of concentrations fell between 33.6 µmol/L and 77.6 µmol/L (Table 1), with pond 1 exhibiting the lowest concentrations and pond 42 exhibiting the highest. The concentrations followed a normal distribution (Figure 3) (W=0.9839, p=0.9915)

We measured 12 ponds and represented the three classifications of hydroperiod -- permanent, semi-permanent, and temporary -- with four ponds each. Averaging measurements across two sampling dates and all ponds within a classification, permanent ponds had an average of 40.93±11.1 µmol/L aqueous CO<sub>2</sub>, semi-permanent ponds had 75.24±10.47 µmol/L, and temporary ponds had 33.95±12.8 µmol/L. There was a significant difference in aqueous CO<sub>2</sub> concentrations between ponds of different hydroperiods (F<sub>2,20</sub>=3.9512, p=0.0358) (Figure 4). However, post-hoc testing demonstrated that there was no difference between permanence types when making pairwise comparisons. Semi-permanent ponds had

concentrations more than twice as high as temporary ponds, but without a statistically significant difference ( $p=0.0536$ ).

Aqueous  $\text{CO}_2$  concentrations were measured at two depths, with a shallow measurement at about 10cm below the surface and a deep measurement at about 20cm below the surface. Shallow concentrations averaged  $47.16 \pm 10.8 \mu\text{mol/L}$  and deep concentrations averaged  $57.46 \pm 10.3 \mu\text{mol/L}$ . There was no significant difference in average  $\text{CO}_2$  concentrations at shallow locations compared to deep locations ( $F_{1,21}=0.475$ ,  $p=0.4982$ ) (Figure 5). Aqueous  $\text{CO}_2$  concentrations did not differ between depth locations within permanence types ( $F_{2,2}=1.0172$ ,  $p=.3826$ ).

Aqueous  $\text{CO}_2$  concentrations were measured at four different time periods throughout the day: early morning (3AM to 6AM), late morning (9AM to 12PM), afternoon (3PM to 6PM) and night (9PM to 12AM). There was a significant difference in concentrations with respect to time period ( $F_{3,3}=3.2404$ ,  $p=.0317$ ), although post-hoc testing found no statistically significant differences in means with pairwise comparisons.

Dissolved oxygen content was examined across six ponds (1, 3, 12, 13, 15, and 22). The ponds were well oxygenated, with DO content falling between 5.23 mg/L DO (pond 3) and 6.84 mg/L (pond 22). There was no significant relationship between aqueous  $\text{CO}_2$  and DO (Figure 6).

## Discussion

Studies of lake systems have already demonstrated that particulate organic carbon (POC), dissolved organic carbon (DOC), soil organic carbon (SOC) and allochthonous carbon loading are established predictors of aqueous carbon dioxide levels in lakes (Hope et al, 1996; Jonsson et al 2003; Kankaala et al, 2006). Furthermore, small ponds have a low volume to surface area ratio such that water contact with carbon-rich pond basins is high, and the comparatively low impact of physical forcings (ie wind, flow) results in little mixing throughout the water column. As the relationship between dissolved oxygen (DO) and carbon dioxide ( $\text{CO}_2$ ) degrades towards the bottom of the pond, Holgerson (2015) suggested that respiration drives carbon dioxide concentrations but did not include POC, DOC, or SOC in the model. Given the lack of relationship between aqueous  $\text{CO}_2$  and DO in our study ponds, these factors are likely to be very strong predictors. We measured pH, chlorophyll-a and detrital breakdown rates in conjunction with aqueous  $\text{CO}_2$  and DO to expand upon the drivers of aqueous  $\text{CO}_2$  concentrations and will include DOC and SOC in the model after sample processing. Diffusion of DO throughout the water column of these ponds may help to explain the insignificant relationship. In addition, the Mexican Cut study ponds had a more narrow range from 54% to 64.5% DO, possibly due to more thorough mixing. Small, temporary ponds in Connecticut, USA, had a broader range of DO contents, spanning from hypoxia (3.9% DO) to well oxygenated (74.7%) (Holgerson 2015), possibly allowing for better visualization of the relationship. While this preliminary data does not examine the relationship between aqueous  $\text{CO}_2$  and POC, DOC, SOC, pH or temperature, our future work will determine whether these variables are predictors for  $\text{CO}_2$  concentration.

If POC, DOC and SOC are better predictors of aqueous  $\text{CO}_2$  concentrations, we would expect concentrations to be different among ponds with different hydroperiods. Since permanent ponds have historically been completely inundated year-round without appreciable drying in the last 50 years (Wissinger et al, 1999), their sediments have had minimal exposure to aerobic respiration processes. Thus, organic matter that collects in these ponds accrues in the benthos. Temporary ponds, on the other

hand, often completely dry each season, allowing organic matter to be largely aerated and decomposed. Therefore, the two pond types have different carbon cycle histories and we expected aqueous CO<sub>2</sub> levels to be higher in permanent ponds. Because these ponds tend to be larger and do not dry, they are often deeper with less mixing and more stratification in the water column. Given their comparatively large carbon stores, some of which may have been accumulating since the glaciation of Mexican Cut, there is a potential for these ponds to be more rich in SOC, and in turn more concentrated for aqueous CO<sub>2</sub>. We found that, on average across two weeks, there was no significant difference in aqueous CO<sub>2</sub> concentrations in ponds with different hydroperiods. Sampling occurred during the beginning of the season before full drying changes could be observed. Since pond ecosystems with different permanence types have different lifecycles as dictated by drying regime, these ponds behave differently both physically and biologically as the summer season progresses. Thus the ponds were unable to undergo their complete drying schedule, potentially preventing us from detecting time-dependent differences in concentrations between ponds of different hydroperiods.

We compared aqueous CO<sub>2</sub> concentrations in the 12 study ponds to previously reported data. The study ponds were supersaturated in CO<sub>2</sub> similarly to small, permanent boreal ponds reported by Jonsson et al (2003), Kling et al (1991), Laurion et al (2010), and Pelletier et al (2014) in tundra environments in Sweden, Canada and the Alaskan US. They were less saturated than those studied by Holgerson (2015) at a more comparable, temperate latitude. However, the aqueous CO<sub>2</sub> concentrations were still considerably higher than those reported for lakes (Jonsson et al 2003). The Mexican Cut ponds may be more well mixed, explaining the lack of a relationship between aqueous CO<sub>2</sub> and DO as was observed by Holgerson (2015). Terrestrial growth at Mexican Cut is dominated by coniferous trees and seasonal vegetation which are likely to give lower loads of allochthonous terrestrial carbon. Given that high aqueous CO<sub>2</sub> concentrations in small temperate latitude ponds is linked to their relatively high carbon loads per unit volume (Hope 1996), this may partially explain the lower concentrations in our study ponds, which may more closely match boreal ponds due to similar weather, vegetation types and growth patterns. Despite concentrations that are lower than expected, because these ponds are supersaturated they likely have a net outward flux of CO<sub>2</sub> to the atmosphere, contributing to greenhouse gas emissions.

In conclusion, our work reports the first aqueous CO<sub>2</sub> concentrations in small alpine ponds, a system which is understudied in general. We demonstrated that the established relationship between CO<sub>2</sub> and DO does not hold true for these ponds, and laid the groundwork for exploring other potential drivers, especially SOC and DOC. We showed that hydroperiod does not play a role in CO<sub>2</sub> concentrations early in the season, but will continue collecting data to elucidate potential differences as drying continues throughout the fall. Finally, we were able to tie our work into the global carbon budget by beginning measurements of CO<sub>2</sub> efflux from pond surfaces and by working towards understanding a potential climate change positive feedback loop.

## Figures and Tables

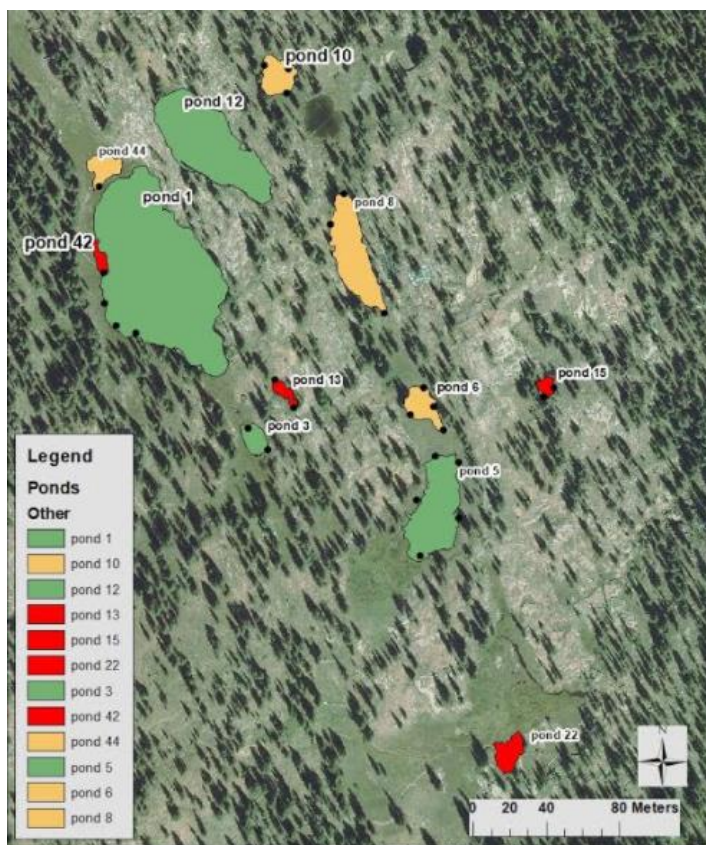


Figure 1. Mexican Cut study ponds. Green ponds are permanent (have not dried in at least the last 50 years; 1, 3, 5 and 12), yellow ponds are semi-permanent (dry in some years and not in others; 6, 8, 10 and 44) and red ponds are temporary (dry completely every year; 13, 15, 22 and 42). Four ponds of each permanence type were included in the study.

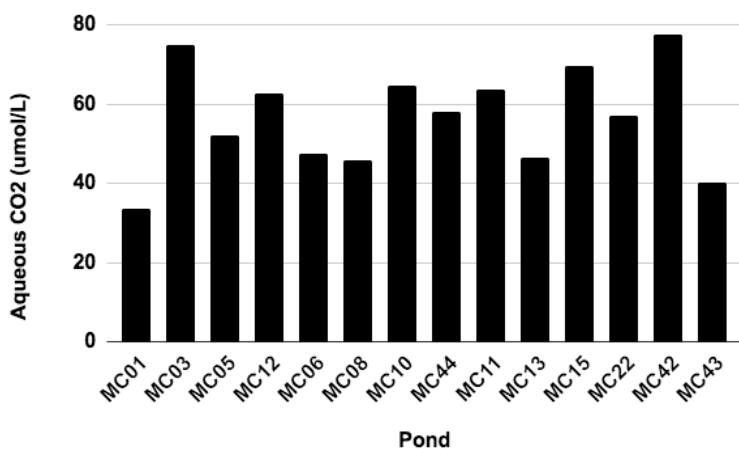


Figure 2. Aqueous CO<sub>2</sub> concentrations of 14 ponds, including the 12 study ponds and ponds 11 and 43. Concentrations were averaged over two collection periods and range from 33.6 to 77.6  $\mu\text{mol/L}$ .

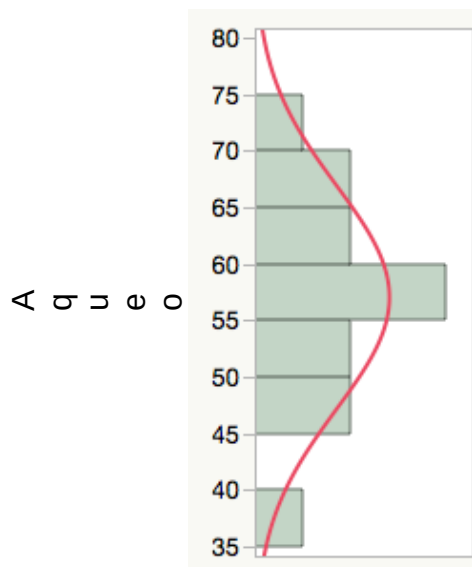


Figure 3. Distribution of aqueous CO<sub>2</sub> concentrations follows a normal distribution.

Table 1. Average aqueous CO<sub>2</sub> concentrations for 14 ponds with permanence type classification.

| Pond | Permanence Type | Aqueous CO <sub>2</sub> (uM) |
|------|-----------------|------------------------------|
| 1    | Perm            | 36.7                         |
| 3    | Perm            | 55.3                         |
| 5    | Perm            | 61.7                         |
| 12   | Perm            | 59.6                         |
| 6    | Semi-perm       | 56.9                         |
| 8    | Semi-perm       | 61.7                         |
| 10   | Semi-perm       | 59.5                         |
| 44   | Semi-perm       | 48.1                         |
| 11   | Temp            | 51.2                         |
| 13   | Temp            | 67.6                         |
| 15   | Temp            | 74.9                         |
| 22   | Temp            | 67.6                         |
| 42   | Temp            | 50.6                         |
| 43   | Temp            | 47.0                         |

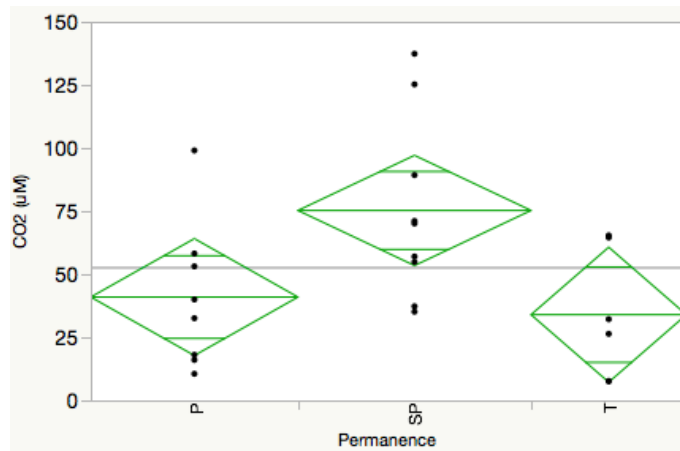


Figure 4. Average aqueous CO<sub>2</sub> concentrations of 12 ponds sampled twice over two weeks, separated by permanence type.

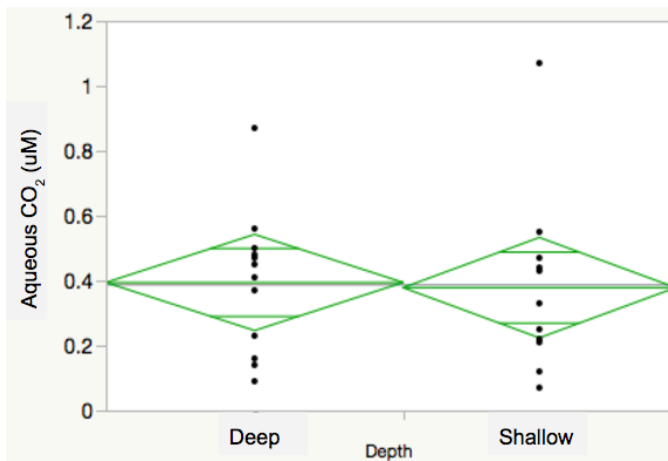


Figure 5. Average aqueous CO<sub>2</sub> concentrations of 12 ponds sampled twice over two weeks, separated by depth.

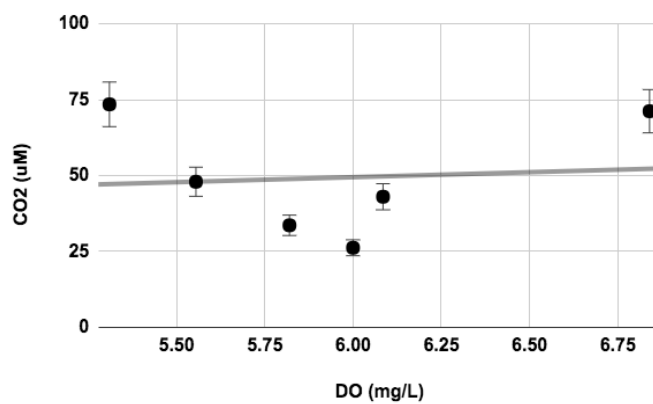


Figure 6. Relationship between aqueous CO<sub>2</sub> (µmol/L) and DO (mg/L) for six ponds. The line represents the linear best fit curve.

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