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This page certifies that the thesis prepared by Susan B. Gifford entitled “NOVEL METHODOLOGY FOR ASSESSING PHYTOPLANKTON RESPONSE TO PCO₂ ENRICHMENT IN FRESH AND SALTWATER” has been approved by her committee as satisfactory completion of the thesis requirement for the degree of Master of Science in Biology.

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**NOVEL METHODOLOGY FOR ASSESSING PHYTOPLANKTON RESPONSE
TO PCO₂ ENRICHMENT IN FRESH AND SALTWATER**

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

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Abstract

NOVEL METHODOLOGY FOR ASSESSING PHYTOPLANKTON RESPONSE TO PCO₂ ENRICHMENT IN FRESH AND SALTWATER

By Susan B Gifford, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology at Virginia Commonwealth University.

Virginia Commonwealth University, 2010

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Atmospheric CO₂ emissions are on the rise and are expected to reach 780 parts per million by the year 2100. Research investigating the impacts of increasing CO₂ is a relatively new field and the response of phytoplankton communities is largely unknown, especially in coastal and freshwater ecosystems where no CO₂ manipulation studies have completed. The present study attempts to encourage uniformity in methods utilized in CO₂ perturbation studies and identifies changes in phytoplankton abundance in freshwater (James River) and coastal ocean (Atlantic, Cape Hatteras) sites. A novel bubbling method to manipulate pCO₂ was compared with the classic method of acid addition in conjunction with laboratory and *in situ* experiments. The novel and classic

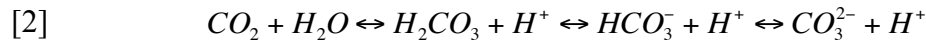
methods were equally effective at manipulating carbonate chemistry to predicted levels. However, the laboratory experiment saw greater variation in both pCO₂ levels and chlorophyll-a concentrations throughout the four-day incubation period. The results from the present study encourage use of the novel methodology in combination with *in situ* experimental setup to assess changes in phytoplankton communities as a result of pCO₂ enrichment. This pairing will allow greater replication of small volume incubations without introducing new abiotic conditions such as temperature and light. Additionally this study found no significant treatment effect on phytoplankton communities in either freshwater James River or coastal Atlantic.

INTRODUCTION

Carbon dioxide (CO₂) emissions have been the focal point of the contentious issue of climate change over the past few decades. With over 50 years of direct measurements, Keeling et al. (1995) showed that the concentrations of CO₂ in the atmosphere have been increasing over time. In 1958, the average atmospheric CO₂ concentration was approximately 312 ppm (Keeling 1960), whereas current levels are approximately 392 ppm (Tans 2010). It has been demonstrated that CO₂ levels are increasing and the elevated concentrations of CO₂ may impact ecosystem function in marine and aquatic communities (Hughes 2000). Indirect impacts of rising CO₂ such as global temperature change, sea level rise and coral bleaching (Hoegh-Guldberg 1999, Overpeck et al. 1997) have been well documented. Direct impacts such as changes in primary production may also be significantly altered and remain relatively unexplored, especially in freshwater and coastal systems.

Air-sea gas transfer and Fick's Law of Diffusion suggest that atmospheric CO₂ will diffuse into surface seawater until equilibrium with dissolved CO₂ is reached. Furthermore, Henry's Gas Law states that dissolved CO₂ concentrations are equal to the partial pressure of CO₂ (pCO₂) in surface waters. Increased CO₂ emissions may directly impact the chemical composition of the ocean more drastically than was initially anticipated (Hutchins et al. 2009; Doney et al. 2009b). Oceans were classically thought to act as a large carbon sink which kept relatively constant pace with increasing atmospheric and consequent dissolved CO₂ (equation 1). Seawater acid-base chemistry buffers excursions in pCO₂ and pH due to dissociation of carbonate species such as carbonic acid (H₂CO₃^{*}), bicarbonate (HCO₃⁻), and carbonate (CO₃²⁻) (equation 2) with no change in the acid base equilibria (Stumm and Morgan 1996).

$$[1] \quad pCO_{2(water)} = pCO_{2(atmosphere)}$$



However, the surplus in CO₂ emissions causes concomitant increases in dissolved CO₂ resulting in the release of H⁺, eventually overcoming oceanic buffering capacity. A small change in pH of 0.1 units equates to a 30% increase in hydrogen ion concentration and acidity. The elevation in pH as a result of H⁺ dissociation is referred to as ocean acidification. The average pH of the oceans is predicted to decrease 0.3 units by the year 2100 (Pachauri and Reisinger 2010). While oceanic pH and pCO₂ levels are influenced by atmospheric CO₂ concentrations, seasonal variability can be caused by microbial activity. Even in oligotrophic regions in the Central Pacific, microbial communities can alter levels through photosynthesis and respiration by 0.06 pH units on a yearly scale (Joint et al. 2011). Although phytoplankton may be able to acclimate to seasonal fluctuation, future pH conditions in seawater will be drastically lower, resulting in variable change of primary production (Doney et al. 2009a). Increased levels of primary production are caused by autotrophic phytoplankton utilization of the increased pCO₂, which is a function of decreased pH. The changes in pCO₂ and pH have led to predicted shifts in community composition and increased primary production in temperate ocean regions (Hallegraeff 2010), including areas off the northeastern U.S. coastline.

A pressing question is how increased concentrations of CO₂ may alter freshwater and coastal oceanic ecosystems as these systems commonly have elevated pCO₂ concentrations (Teodoru et al. 2009). The pCO₂ and associated pH of freshwater systems is highly variable between seasons and even daily cycles; Maberly (1996) saw excursions of 2-3 pH units on a diel cycle. Consequently, the species living in these communities may be more tolerant to abiotic changes (Cole and Prairie 2009). However, the elevated concentrations of atmospheric CO₂

could alter pH concentrations causing phytoplankton blooms, potentially having unknown positive or negative feedbacks on primary production in complex freshwater systems (Joint et al. 2011). Unlike marine systems, atmospheric CO₂ is not the only major source of inorganic carbon in freshwater systems. External inputs, such as bedrock weathering and ultraviolet (UV) mediated release of dissolved inorganic carbon from terrestrial sources, in addition to internal respiratory sources (Talling 1976, Cole and Prairie 2009) regulate ecosystem pCO₂ levels. The carbonate chemistry of freshwater ecosystems is more variable than marine systems; this is due to the lower total alkalinity (equation 3) (Dodds 2002, Andrews et. al 2004). The presence of bicarbonates causes the reaction to saturate more quickly with heightened concentrations of CO₂ and inhibits the ability of H⁺ to combine with the dissolved inorganic carbon, thus decreasing pH more quickly.

$$\begin{aligned}
 [3] \quad \text{Total Alkalinity} = & \quad [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] + [HPO_4^{2-}] \\
 & + 2[PO_4^{3-}] + [SiO(OH)_3] + [NH_3] + [HS^-] + \dots \\
 & - [H^+]_F - [HSO_4^-] - [HF] - [H_3PO_4] - \dots
 \end{aligned}$$

The current scope of acidification research is limited to open ocean systems and the inclusion of investigations on natural assemblages of phytoplankton communities in both coastal and freshwater systems is important to understand the regional impacts of increasing CO₂. Although the number of studies investigating the biological impacts of ocean acidification has been increasing, most research has primarily focused on productivity of phytoplankton that produce or utilize calcium carbonate. Saturation levels for calcium carbonate minerals are expected to decrease linearly in acidified environments (Figure 1), making calcifying organisms more sensitive to pH changes than other types of microbes (Orr et al. 2005; Joint et al. 2011).

Furthermore, much of the earlier research has concentrated on single species cultures, which have been manipulated in the laboratory (Delille et al. 2005, Orr et al. 2005, Meseck et al. 2009). Previous research on cultured strains of coccolithophores, phytoplankton with calcium carbonate plates, has shown negative responses of survival rates with decreasing pH (Iglesias-Rodriguez et al. 2008). Although research has been conducted on natural phytoplankton communities, most studies were carried out shipboard on open ocean research cruises with limited space, resources and time constraints (Tortell et al. 2002, Hare et al. 2007, Tortell et al. 2008, Feng et al. 2009). The complex carbonate chemistry has made research investigating future implications for increased carbon emissions logistically challenging.

Due to the complexity of the chemistry, many methods have been utilized to simulate future aquatic conditions including bubbling pure CO₂ or CO₂ air mixtures into mesocosms, adding acids and bases, altering carbonate ion concentrations, and a combination of addition acid, bases and carbonate ions (Riebesell et al. 2010). Only two of the methods, bubbling of CO₂ and addition of a combination of carbonate ions and acid, alter the water chemistry in the exact manner as will happen naturally. Acidification research is in an exploratory phase, and thus method development is currently a part of all phytoplankton and microbial response studies.

The need for a standardized method has been identified as one of the priority questions for microbial ocean acidification research (Joint et al, 2010). Without a standardized method, changes found in phytoplankton community response cannot be attributed solely to natural variation in communities, but rather may be attributed to method inconsistencies (Table 1). Another priority question is researching impacts of CO₂ in freshwater ecosystems. An extensive literature search shows no published investigations into the consequences of a high CO₂ world on a regional scale across both aquatic and marine ecosystems. Therefore, this study serves two

purposes: (1) to determine which of two methods of CO₂ perturbation, CO₂ bubbling and carbonate/acid additions, will work best in both fresh and saltwater ecosystems and (2) to investigate potential changes in phytoplankton abundance as a result of elevated CO₂ concentrations in both fresh and saltwater environments.

METHODS

Methods

The study took place within a two-week period from February 27th to March 12th, 2011, and utilized two methods of perturbation, acid/bicarbonate addition and CO₂ bubbling, in a freshwater and saltwater site to investigate the shifts in abundance and stoichiometry of natural phytoplankton communities in high CO₂ environments. The study was replicated in both laboratory and *in situ* settings. Initial phytoplankton populations started at ambient site concentrations and pH for the control conditions. To mimic elevated CO₂ concentrations, as modeled based on estimates by the Intergovernmental Panel on Climate Change (IPCC) for global CO₂ emissions by the year 2100, CO₂ was elevated to 780 parts per million (ppm) and the pH was lowered to 7.79 (Pachauri and Reisinger 2010). The sites were chosen for their accessibility to collect samples and ability to deploy an enclosure for a period of four days to conduct an *in situ* experiment. Sites were used for a preliminary study of methods and biomass in November 2010. Photosynthetically active radiation (PAR) was measured with a LI-COR model LI-1400 at each site during sampling as well as periodic sampling during the experiment in both the laboratory and *in situ* settings to ensure 50% irradiance, or 50% incident surface PAR, levels remained constant in the incubations (Hawley 2011).

Site Description

James River, Rice Center, Hopewell, Virginia (37° 19' N, 77° 12' W)

The freshwater site was located on the tidal James River (river-kilometer 110), adjacent to Virginia Commonwealth University's biological field station, Rice Center. Preliminary analysis from November 2010 and February 2011 showed variation in carbonate chemistry at the site.

Total alkalinity fluctuated between 880 to 940 mol/kg, pCO₂ concentrations fluctuated between 295 ppm to 550 ppm and pH fluctuated from 7.57 to 8.32. Surface water pH averaged 8.3 during the sampling period. Early March surface water temperatures were measured at 13.1 °Celsius, and surface light conditions during water collection were at PAR of 900 (μmoles per m² second), pressure in surface water was assumed to be zero, and salinity was measured at 0 ppt. Natural autotrophic communities in March are typically composed of *Chlorophytes*, cyanobacteria, diatoms, and dinoflagellates with average abundance of 19 x10⁶ cells L⁻¹ (Marshall et al. 2009, Marshall 1967). Rainfall in February and March 2011 was slightly greater than average, with discharge within normal range. Departure from the mean average rainfall was 2.29 mm, indicating microbial community composition was typical for March averages. The *in situ* portion of the study took place in the tidal surface waters adjacent to the Rice Center property.

Coastal Atlantic Ocean, Duck, North Carolina (36°10' N, 75°44' W)

The saltwater site was located at the United States Army Corps of Engineers Research Facility in Duck, North Carolina. Preliminary analysis from November 2010 and February 2011 showed fluctuations in alkalinity between 1750 and 2138 umol/kg, pCO₂ concentrations between 380 ppm and 450 ppm, and natural pH fluctuations between 8.05 and 8.13. The site is tidal with a salinity of 29 ppt, pressure was assumed to be zero, March water temperatures were 8.3 °Celsius, and PAR was measured at 750 (μmoles per m² second). Typical surface water primary producers include *Cryptomonas*, *Calicomonas*, other photosynthetic nanoplankton and cyanobacteria with average abundances around 6-9 x10⁶ cells L⁻¹ (Verity 1996). The *in situ* portion of the study took place in the surface waters adjacent to the Army Corps Pier.

Experimental Setup

For both laboratory and *in situ* incubations, surface water was collected in nine acid-leached (10% HCl) 10 L low-density polyethylene cubitainers using a bucket-grab method. The incubations were subsampled for pH, inorganic nutrients, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and chlorophyll-a daily for a period of four days in the field. *The laboratory incubations* were housed in a flow-through water system designed to keep site water temperatures constant at ambient conditions. The closed system incubations (10 L cubitainers) were submerged in transparent bins connected by hoses that were open for constant water flow. Temperatures were monitored throughout the experiment to ensure close to ambient conditions remained constant. Screening was placed over the flow-through system to mimic ambient light conditions, which were kept at approximately 50% irradiance of natural light. The system was set up in the greenhouse at Virginia Commonwealth University (VCU).

For the in situ experiments, a floating enclosure was created to house the nine, 10 L cubitainers in the water column at both sites (Figure 2). The enclosure was tied down in about one meter of water and allowed to move with the tides at both sites. The enclosure was covered with screening to ensure close to 50% irradiance of natural light in the incubations.

CO₂ Perturbation Methods

Bicarbonate/Acid Addition Method: Concentrations of bicarbonate and hydrogen ions for addition in three of the cubitainers of site water was calculated using the CO2Calc program (Robbins et al. 2010). pH was measured by a Fisher Scientific Accumet AB15 pH meter, calibrated to three pH standards (4.0, 7.0, 10.0) with slope greater than 93 percent. pCO₂ was measured using an environmental gas monitor (EGM-4, ppsystems) that displays direct readings of CO₂. Running CO2Calc for both the initial and target pCO₂ and pH as inputs, the differences in the total concentrations of bicarbonates and hydrogen ions needed for addition were calculated. Sodium bicarbonate (NaHCO₃ - Sigma Aldrich #5651) amounts were weighed out,

based on CO₂calc outputs, and added to the respective cubitainers of site water. 0.1N hydrochloric acid (HCl) was added to each cubitainer until the target pCO₂ concentration of 780 ppm was reached.

CO₂ Bubbling Method: A novel CO₂ injection system was designed (Figure 3), to increase pCO₂ concentrations in controlled conditions using pure CO₂ (Richmond Oxygen Company). A syringe was filled with pure CO₂ directly from the tank using a low flow regulator and a flow meter. The CO₂ was injected in small increments into tubing connected a sparge submerged in the incubation cubitainers. Air was pumped through the tubing to gently pump CO₂ into the system; the pump was only operating when CO₂ was in the system to avoid excessive bubbles. Three incubation cubitainers were separately injected with CO₂ as replicates. CO₂ concentrations were measured concomitantly with the EGM. Injections of CO₂ were added until the target pCO₂ concentration of 780 ppm was reached.

Analyses

Carbonate Chemistry Calculations: CO₂Calc is a program designed to calculate the entirety of the carbonate chemistry parameters based on two carbonate measurements in addition to other inputs including pressure, temperature, and salinity. The inputs used for both the freshwater and saltwater sites were pCO₂ and total alkalinity (Table 2). pCO₂ concentration measurements were taken directly from the incubations with an Environmental Gas Monitor, which allows instant readings of pCO₂. Measurements were taken initially to identify the ambient CO₂ levels in the site water as well as throughout the perturbation process for both methods. The second measurement used to run CO₂Calc was total alkalinity. Daily, 40 mL amber vials, pre-combusted (525°C, 4 hours), were overfilled with filtrate (0.7 µm nominal pore size, GF/F) and immediately capped, ensuring no exchange of CO₂ with the atmosphere would occur during preservation. Total alkalinity was found through a pH based titration method. 20 mL of the

sample was added to a 50 mL, acid-leached (10% HCl) beaker. 0.02N sulfuric acid (H₂SO₄) was titrated into the sample water with a 10 mL cuvette until the equilibrium state was reached at a pH of 4.3. CO2Calc requires temperature, salinity and pressure to calculate the carbonate chemistry. Temperature of the water in the enclosure and flow-through system was monitored multiple times daily and incubation temperatures were taken with a digital thermometer during daily subsampling periods. Salinity was measured with refractometer and pressure was assumed to be zero at both sites based on CO2Calc input recommendations. The outputs of the program included concentrations of HCO₃⁻, H₂CO₃, CO₃²⁻ and pH.

Dissolved Nutrient Analysis: Inorganic nutrients were sampled on a daily basis, 45 mL of the filtrate (0.7 µm nominal pore size, GF/F) were collected and analyzed for ammonia (NH₃), nitrates and nitrites (NO_x) and orthophosphates (PO₄³⁻). NH₃ concentrations were based on the blue color formation of phenol and hypochlorite and analyzed on a GENESYS 6 spectrophotometer (Thermo Electron Corporation). NO_x concentrations were analyzed using an automated system on a SKALAR in the Environmental Analysis Laboratory at VCU. PO₄³⁻ concentrations were quantified using color formation methods and analyzed on a GENESYS 6 spectrophotometer (Thermo Electron Corporation) (Grasshoff et al. 1983). DOC was measured daily by collecting 35mL of filtrate (0.7 µm nominal pore size, GF/F), in 40mL amber vials, pre-combusted (525°C, 4 hours) and acidified to a pH of 2.0 with 200 µl of 100% HCl. Samples were run on a DOC analyzer, utilizing combustion oxidation methods, at William and Mary's Virginia Institute of Marine Sciences.

Phytoplankton Abundance Analysis: Biomass was measured by analysis chlorophyll-a concentrations. 80 mL and 120 mL of water was filtered through 25 mm (0.7 µm nominal pore size) Whatman GF/F glass-fiber filters from the Rice Center and Duck Pier samples,

respectively. The filters were stored in the -80°C freezer until analysis, when the filters were placed in 10 mL of buffered 90% acetone for a period of 24 hours and analyzed on a Turner Designs Fluorometer (TD-700). Analysis was completed within 2 weeks of sample collection.

Statistical Analysis: One-way ANOVA, Tukey's Honestly Significant Different Post Hoc Test and Student's t-tests were run on JMP 9.0 to test method effectiveness and comparison of mean values of parameters measured between sites, experiment types and methods. Repeated measures one-way ANOVA comparisons were run on Prism to compare both pCO₂ and chlorophyll-a concentration changes over the four-day incubation period. Multivariate Principal Component Analysis (PCA) and Pairwise comparisons were run on JMP 9.0 to correlate biomass (chlorophyll-a) with nutrient concentrations ($\alpha = 0.05$).

RESULTS

Effectiveness of Method and Experiment Type

Significant differences in pCO₂ were seen between sites, treatments and experiment type. All analyses for method effectiveness were run with data from day two, where the greatest change in both pCO₂ and chlorophyll-a was seen. The overall ambient levels of pCO₂ on day two averaged 322 ± 23 ppm across all sites and experiments, while the acid addition and bubbling method had significantly different averages of 723 ± 27 ppm and 921 ± 26 ppm (p < 0.0001), respectively. Both methods were effective at achieving significantly higher pCO₂ levels; however, they were not equally effective across sites and experiments. Both sites had significantly higher pCO₂ levels in laboratory experiments than *in situ* (p < 0.0001) experiments with respective averages of 556 ± 60 ppm and 408 ± 59 ppm at the Rice Center and 1091 ± 117 ppm and 567 ± 97 ppm at Duck Pier. In three of the four site and experimental type manipulations (Rice Center greenhouse and *in situ* and Duck Pier greenhouse), the pCO₂ concentrations in the acid addition and CO₂ bubbling methods reacted in similar patterns to each other and to ambient conditions (Figures 4a-c). This indicates that the biological processes can respond to changes in water chemistry, regardless of method type. The day two change in pCO₂ concentrations between methods during the Duck Pier *in situ* experiment were significantly different (p < 0.0001), the pCO₂ in the acid addition incubations increased 38% by the second day, while the bubbled CO₂ incubation concentrations had decreased by 25%. Ambient levels decreased slightly, but remained close to unchanged (Figure 4d).

Freshwater Site (Rice Center): The day two ambient pCO₂ levels at the Rice Center averaged 220 ± 33 ppm and were significantly different (p < 0.0001) than both the acid addition and CO₂ bubbling method which had averages of 581 ± 38 ppm and 646 ± 53 ppm, respectively. The

differences in the average pCO₂ levels of the elevated treatments were not significantly different indicating that both methods were effective at changing the water chemistry to mimic future levels of CO₂. Differences in ambient and elevated treatments lessened over time period of day zero (initial) to day two, indicating the need to adjust pCO₂ levels to treatment levels at least every two days (Table 3). Note that changes in almost all parameters were more drastic from initial levels to day two than the remaining incubation period.

Greenhouse pCO₂ concentrations in ambient conditions were constant from day zero to day one, decreased significantly ($p < 0.0001$) from day one to two and then remained constant from day two to three. The elevated treatments had significant decreases ($p < 0.0001$) in pCO₂ from day zero to two, after which concentrations stabilized (Figure 4a). *In situ* pCO₂ concentrations in ambient conditions decreased ($p = 0.03$) from day zero to day two and then significantly increased ($p < 0.001$) from day two to day three. The acid addition treatment followed a similar pattern with decreased pCO₂ ($p < 0.001$) from day zero to day two and significant increase ($p < 0.001$) on the final day. The CO₂ bubbling treatment had significant decreases from day zero to day two, with pCO₂ concentrations remaining constant from day two to day three (Figure 4b).

Saltwater Site (Duck Pier): The average ambient pCO₂ levels at Duck Pier were 425.4 ± 32.9 . The ambient pCO₂ values were significantly lower than the elevated values ($p < 0.0001$). The average pCO₂ levels in the acid addition incubations were 866 ± 38 ppm and 1196 ± 38 ppm in the CO₂ bubbling incubations. Duck Pier had higher pCO₂ values than Rice Center across all treatments and experiments. Average pCO₂ concentrations in the greenhouse followed similar patterns across all treatments, with significantly higher levels ($p < 0.0001$) on day one compared to all other days. Differences in pCO₂ levels between treatments declined over the four days,

(repeated measures ANOVA) with generally decreasing pCO₂ levels from days one to three (Figure 4c). *In situ* ambient conditions showed no significant difference in pCO₂ concentrations over the four-day period. The acid addition method had a significant increase in pCO₂ concentrations from day zero to day two (p<0.001), with a decrease from day two to day three. The CO₂ bubbling treatment showed an increase in pCO₂ from day zero to day one (p<0.01), with steady concentrations from day one to day three (Figure 4d).

Phytoplankton Response to elevated CO₂

Initial conditions for all parameters measured are shown in Figure 5 (panels a-g). Chlorophyll-a concentrations were used as an approximation for phytoplankton biomass. Initial chlorophyll concentrations were significantly different between site and experiment type (Figure 5h). Student's t-test showed that chlorophyll-a concentrations responded to experiment type in both sites, greenhouse biomass was significantly greater than *in situ* biomass (p = 0.0359). Further ANOVA and Tukey's HSD tests showed that significant differences were found in chlorophyll-a concentrations between sites. Chlorophyll-a concentrations at the Rice Center averaged 15.40 µg/L in the greenhouse (Figure 6a) and 23.34 µg/L (p=0.003) *in situ* (Figure 6b). Similar trends were found at Duck Pier where chlorophyll-a concentrations averaged 1.90 µg/L in the greenhouse (Figure 6c), and the *in situ* averaged 1.27 µg/L (Figure 6d). While significant differences in biomass were seen between sites and experiment types, no treatment effect on biomass was seen (r²=0.0145), regardless of site or experiment. Consequently, the correlation between biomass and pCO₂ was not strong enough to explain chlorophyll-a concentrations. Chlorophyll-a was further analyzed for the entire incubation period in order to assess the impact of elevated CO₂ conditions on the inorganic nutrients and the potential impact on biomass.

A principal component analysis was run and loading plots were created to assess the correlation strength of other factors that may impact biomass at each site and in each experiment type. The strongest correlations for the Rice Center during the greenhouse experiment, were pH ($r^2=0.6300$), pCO_2 ($r^2=0.6142$), and NH_3 ($r^2=0.6103$) (Table 4; Figure 7a). The biomass in the *in situ* experiment at the Rice Center was correlated with pH ($r^2=0.2749$), pCO_2 ($r^2=0.3795$), and NH_3 ($r^2=0.3454$), in addition to DOC ($r^2=0.1388$) (Figure 7b). While phytoplankton at the Rice Center had strong correlation with carbonate chemistry parameters, pCO_2 and pH, Duck Pier experiments had very weak correlations ($r^2 < 0.06$) (table 4). The Duck Pier greenhouse experiments showed strongest correlation with PO_4^{3-} ($r^2 = 0.0527$), NO_x ($r^2=0.0937$) and DOC ($r^2=0.0955$) (Figure 7c). The *in situ* experiments at Duck Pier chlorophyll-a were weakly correlated with PO_4^{3-} ($r^2 = 0.0305$) and NO_x ($r^2=0.0254$) (Figure 7d).

DISCUSSION

This study investigates the effectiveness of the two most commonly utilized methods of CO₂ perturbation, CO₂ bubbling and addition of bicarbonates and hydrochloric acid in both fresh and saltwater. Acid addition method utilizes CO₂Calc to calculate amounts of acid and bicarbonate needed to be added to the system to change pCO₂ levels to predicted amounts. Whereas there is only one way to perturb CO₂ concentrations with acid addition, there have been multiple methods of CO₂ bubbling. The most common bubbling procedure utilizes long periods of bubbling pre-mixed air with elevated CO₂ concentrations. Two logistical issues with previous bubbling methods include the immobility and expense of pre-mixed gases and more importantly the increase of Transparent Exopolymer Particles (TEP) with excessive bubbling (Riebesell et al. 2010). TEP is a naturally occurring accumulation of organic matter consisting of gel-like, polysaccharide conglomerations that are typically found in times of increased microbial production due to phytoplankton exudates (Surosz et al. 2006). TEP has been shown to drastically change microbial carbon cycling and thus, increased TEP could alter phytoplankton community structure within the incubations. This study attempts to avoid the TEP issue by creating a novel CO₂ enrichment injection system (Figure 1) that utilizes minimal bubbling disturbance. Additionally, pure CO₂ tanks are affordable and more mobile which makes them easier to use in the field.

Method Selection: The methods of CO₂ perturbation utilized in the current study should have the same effect on the carbonate system and initially both were successful at achieving initial target pCO₂ levels. Differences in pCO₂ levels may be attributed to variation within the replicates of the acid addition method. The amounts of HCl and NaHCO₃ that needed to be added must be

calculated in the field using CO2Calc. They are based on actual pCO₂ and total alkalinity measurements. Access to electricity is required for the use of a scale that has the accuracy needed to measure out minute amounts of bicarbonate. The increase in pCO₂ in the *in situ* experiment at Duck Pier after initial setup may be the result of inconsistencies between replicates, seen in the large standard error. This effect was not seen during the Rice Center *in situ* experiment, which is likely a result of better facilities adjacent to the river.

Recommendation of method choice to be used depends on facilities available; however the CO₂ novel bubbling system outlined is designed for ease of use in the field and laboratory and provided less inconsistency across both sites.

Experiment Type: Greenhouse conditions elicited a greater response in chlorophyll-a concentrations than *in situ* conditions. Change in biomass indicated that a bloom of phytoplankton production was stimulated regardless of treatment. Although biomass was higher, the greenhouse incubations experienced a lag in biological response to elevated CO₂ treatments. This suggests that transportation of the site water to the laboratory had an effect on biological response. Factors such as light conditions, temperature and physical movement of water during transport may have impacted phytoplankton function initially and perhaps even in longer term. The greenhouse lag time was seen in both the Rice Center (30 miles travel distance) and Duck Pier (175 miles) sampling, indicating that even short time periods and distances traveled impacted chlorophyll-a concentrations. Greenhouse experiments may have also introduced new conditions to the phytoplankton and therefore potential response may not be attributed solely to natural function, but instead could be a response to different conditions. Although more variable conditions may exist in greenhouse experiments, there is the possibility to have more replicates, which are usually minimal in acidification research due to financial and physical space

constraints. Additionally, greenhouse conditions can be better controlled and more easily measured than *in situ* conditions; therefore a tradeoff of replication versus response to natural condition exists. The current study proposes use of an alternative experiment type to greenhouse experiments without loss of replication.

Mesocosms are an effective way to study phytoplankton communities, but they are expensive to build and relatively permanent. Therefore, very few laboratories have invested in mesocosms and new ways to study these systems are needed. The current study uses a temporary, relatively inexpensive, *in situ* floating enclosure to house the incubation containers for the duration of the experiment. The enclosure (Figure 2) provides *in situ* conditions such as water temperature, light conditions (photosynthetically active radiation (PAR)), and diel cycles. Concurrently, the enclosure ensures closed-system incubations and easy access for daily sub-sampling. An *in situ* study decreases the amount of time that the site water is handled and may decrease damage to microbial function. As stated previously, physical movement of site water to the laboratory could introduce factors to the study that may impact the overall results.

Incubation time: After initial setup, significant changes in pCO₂ were seen within two days for all elevated treatments, while ambient pCO₂ levels remained constant (Figure 4a-d). This suggests constant delivery of CO₂ into the system to ensure that treatment levels remain elevated. This is more difficult *in situ*, where such systems tend to be expensive and maintenance is time consuming. The current study suggests re-treating the incubations on a daily basis, preferably during periods of subsampling to minimize atmospheric contact with samples. The total length of incubation period for the study depends on the study organism. This study showed biological changes within the four-day period. Rice Center pCO₂ levels decreased significantly over the four days in the greenhouse, while *in situ* levels decreased for three days and then spiked in the

ambient and acid addition treatments (Figure 4b), which indicates the presence of an entire phytoplankton (autotrophic) bloom cycle and the beginning of a consequent heterotrophic microbial bloom. The greenhouse experiments experienced a lag or slowing of bloom conditions, and consequently the four-day period was not long enough to capture the entire bloom phase. Incubation period could be longer, however nutrients would have to be added to the system to prevent phytoplankton growth.

Study Organism: The current study quantified changes in phytoplankton biomass of naturally occurring communities from each site. Many previous studies utilize cultured phytoplankton communities to research species-specific alterations in biology and function due to increased pCO₂ (Leonardos and Geider 2005, Meseck et al. 2009, Wang et al. 2010). While culture experiments are important to investigate changes of production rates per species, they could lead to incomplete conclusions about community function. More studies on natural communities are needed in both coastal and freshwater systems.

Biological Response to CO₂ treatments

Saltwater: No significant changes in biomass were seen as a result of elevated CO₂ treatments in the coastal systems ($r^2=0.0004$). This is an indication that coastal phytoplankton is not generally pCO₂ limited and therefore an increase of pCO₂ may not stimulate a phytoplankton bloom. The principal component analysis showed that coastal phytoplankton biomass was most correlated (albeit not significantly) to DOC, PO₄³⁻, and NO_x in both greenhouse and *in situ* experiments. These results are similar to findings in open ocean studies where phytoplankton abundance was not impacted by elevated CO₂ environments (Burkhardt et al. 1999; Tortell et al. 2002; Leonardos and Geider 2005). Coastal ecosystems have high nutrient inputs and well-mixed surface waters, causing highly variable conditions for phytoplankton to survive in. This study

indicated that although pCO₂ was not significantly impacting chlorophyll -a, the loading plots indicated that nitrogen could be an additional limiting factor (Figure 6c and 6d).

Freshwater: Rice Center had significantly lower pCO₂ concentrations ($p < 0.0001$) than Duck Pier, most likely due to the ability of freshwater to act as a stronger buffer to changes in carbonate chemistry (Dodds 2002, Andrews et. al 2004). Unlike Duck Pier, pCO₂ concentrations in both *in situ* and greenhouse experiments at the Rice Center had significant Pairwise correlation with chlorophyll-a concentrations ($r^2=0.3795$ and $r^2=0.6142$, respectively) (Table 4). The significant effect of pCO₂ treatment on biomass indicates that the blooms were a response to pCO₂ in both experiment types. The effect is similar to fertilizer effect with phytoplankton blooms, the pCO₂ stimulated production and then biomass declined as pCO₂ was utilized (Joint et al. 2011). There was also a relationship with NH₃ and chlorophyll-a with $r^2=0.3454$ *in situ* and $r^2=0.6103$ in the greenhouse. Higher initial biomass in the James River could account for the utilization of the pCO₂ for autotrophic production. Further analysis of size structure changes with elevated CO₂ treatments using a flow cytometer will elucidate if changes in chlorophyll-a was same species production or a shift in community composition to larger organisms.

Broader Impacts

The need for a uniform method has been identified. This study shows that replication does not need to decrease at the expense of mesocosm investigations. The CO₂ injection system is mobile to ensure ease and accuracy of *in situ* experiments and the floating enclosure prevents other abiotic changes such as light and temperature, allowing changes in phytoplankton communities to be attributed solely to elevated CO₂ treatments. Many scientific resources have been put into the investigation of ocean acidification, most likely because the impacts are presumed to be greater than in freshwater acidification. It is important, however, to begin studying regional systems instead of isolated marine areas. The pH tolerance of the diversity and abundance of

species that live in freshwater systems should not be assumed. This study showed significant treatment effects on overall biomass, and may elucidate additional community structure shifts with flow cytometry analysis. While no significant treatment effects were seen in coastal system biomass, additional resources should be put towards research in these systems. The James River, Chesapeake Bay and adjacent coasts could be a model system for regional effects of elevated CO₂ on phytoplankton biomass and community structure. This study can be extrapolated to many systems in the Eastern United States and perhaps help influence decision making processes related to effective management of the waterways and their watersheds.

Future Directions

The novel CO₂ injection system outlined in the current study can be utilized in both laboratory and *in situ* experiments. Factorial experiments are needed to show combined and individual impacts of variables such as CO₂, temperature, nutrient additions, and irradiance as outlined by Joint et al. (2010). The design of factorial experiments in traditional *in situ* mesocosm studies is not feasible, mainly due to space and financial constraints. Therefore, the use of smaller *in situ* incubations will circumvent these limitations and allow increased replication and ability to study natural phytoplankton communities instead of cultured individual species. Use of the CO₂ injection system opens up research opportunities including the investigation of biochemical changes in phytoplankton. These include total lipid and fatty acid analysis in addition to stable isotope analysis to aid in the investigation phytoplankton's ability to uptake various species of DIC. Furthermore, stable isotopes could elucidate the ability of each community to withstand changes in carbon availability with use of carbon compensation mechanisms (Joint et al., 2011).

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Table 1. Summary of previous acidification research investigating phytoplankton responses to elevated CO₂. Methods outlined and commented on, if specified in the respective paper.

System/Species	Methods	Treatments	Results	Authors	Comments
Noncalcareous phytoplankton cultures	HCl/NaOH and nutrient additions	6 CO ₂ concentration levels	No effect of CO ₂ treatment seen	Burkhardt et al. (1999)	No clear method written
Pacific natural assemblages cultured	Bubbling CO ₂ , 30% Irradiance, ambient temperatures, natural nutrients	pCO ₂ = 150ppm and 750ppm	Primary production (¹⁴ C) showed no significant changes in high CO ₂	Tortell et al. (2002)	Bubbling method unspecified
Cultured strains noncalcareous <i>Emiliana huxleyi</i>	Bubbling CO ₂ , irradiance and nutrient treatments	pCO ₂ = 360ppm and 2,000ppm	Increased growth at high CO ₂ but no increase in abundance	Leonardos and Geider (2005)	Bubbling method unspecified
Ross Sea natural assemblages	Shipboard bubbling CO ₂ , 30% irradiance, 0°C, ambient nutrients	pCO ₂ = 100, 380, and 800 ppm	Linear increases in growth rates from low to high pCO ₂ in 2 seasons	Tortell et al. (2008)	Unspecified bubbling of air- CO ₂ mixtures
North Atlantic natural bloom assemblages cultured	Shipboard bubbling CO ₂ , temperature treatments	pCO ₂ = 390 and 690ppm temp = 12°C and 16°C	Increase in POC and photosynthesis, due to increased temp. not pCO ₂ treatments	Feng et al. (2009)	Unspecified bubbling of air- CO ₂ mixtures
<i>Phaeocystis globosa</i> culture, Harmful Algal Bloom species	Bubbling of CO ₂ in growth chamber, constant irradiance, 20°C	pCO ₂ = 380 and 750ppm	Shift of preference to colony cells from solitary cells under high pCO ₂	Wang et al. (2010)	Continuous bubbling of air- CO ₂ mixtures

Table 2. Inputs for program CO2Calc to calculate all carbonate chemistry parameters. The two measured parameters used as inputs in this study were pCO₂ and total alkalinity (TA).

Site	Inputs	Outputs	Constants	pH Scale
Rice Center	pCO ₂ (ppm) TA (μmol/kg) Temperature = 13.1 °C Salinity = 0 ppt Pressure = 0 decibars	H ₂ CO ₃ [*] , HCO ₃ ⁻ , CO ₃ ²⁻ , H ⁺ (μmol/kg)	Millero 1979	NBS scale (mol/kg-H ₂ O)
Duck Pier	pCO ₂ (ppm) TA (μmol/kg-SW) Temperature = 8.3 °C Salinity = 29 ppt Pressure = 0 decibars	H ₂ CO ₃ [*] , HCO ₃ ⁻ , CO ₃ ²⁻ , H ⁺ (μmol/kg-SW)	Lueker et al. 2000	Total scale (mol/kg-SW)

Site	Metric	Day 1			Day 2			Day 3			Day 4		
		amb	acid	bubble	amb	acid	bubble	amb	acid	bubble	amb	acid	bubble
Rice Center <i>in situ</i>	pH	8.05±0.04	7.75±0.05	7.73±0.03	8.44± 0.10	8.02±0.07	7.99±0.02	8.67±0.18	8.71±0.02	8.51±0.12	7.94±0.12	8.17±0.03	8.70±0.04
		ns ns ns	‡ § §	‡ § §	ns ns ns	‡ § *	‡ § §	ns ns ns	§ § §	§ § *	n,ns ns	§ * §	§ § *
	pCO ₂	470.6±4	957.3±11	1012.5±7	192.2±47	517.8±78	549.8±26	117.1±56	100.2±5	166.1±44	631.1±169	357.7±26	104.2±9
		* * ns	‡ § §	§ § §	* ns ‡	‡ ‡ ns	§ § §	* ns ‡	§ ‡ *	§ § ns	ns ‡ ‡	§ ns *	§ § ns
	NH ₃	15.4±4	15.6±5	13.7±2	8.7±0.7	9.1±0.5	7.0±0.7	4.6±0.1	5.4±0.1	4.7±0.5	2.0±0.5	1.9±0.2	0.8±0.3
		* ‡ ‡	ns ‡ ‡	‡ § §	* ns ns	ns ns *	‡ ns ‡	‡ ns ns	‡ ns ns	§ ns *	‡ ns ns	‡ * ns	* ‡ *
	PO ₄	0.08±0.01	0.08±0.02	0.09±0.02	0.07±0.01	0.06±0.01	0.07±0.01	0.06±0.01	0.08±0.06	0.06±0.01	0.06±0.0	0.06±0.01	0.06±0.02
		ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns	ns ns ns
Chl-a	15.05±1.3	14.00±2.3	15.90±1.4	25.68±3.8	28.73±1.2	29.87±2.3	23.36±2.1	28.01±0.4	27.08±2.6	22.52±1.8	25.29±0.5	24.43±1.8	
	‡ * *	§ § §	§ § §	‡ ns ns	§ ns *	§ * §	‡ ns ns	§ ns ns	§ * *	* ns ns	§ * ns	§ § *	
pH	8.11±0.0§	7.73±0.01	7.73±0.08	8.16±0.03	7.89±0.03	7.83±0.12	9.07±0.03	8.84±0.04	8.44±0.05	8.97±0.01	8.87±0.08	8.75±0.04	
	ns ns ns	* § §	ns § §	ns ns ns	* § §	ns § §	ns ns ns	§ § ns	§ § ‡	* ns ns	§ § ns	§ § ‡	
Rice Center Greenhouse	pCO ₂	396.5±24	920.3±67	928.5±63	352.6±8	644.3±9	742.9±61	40.8±4	69.9±14	181.1±33	53.3±14	64.6±7	86.2±8
		* * ns	§ § §	‡ § §	* ns ‡	§ § §	‡ § §	* ns ‡	§ § ns	§ § ns	ns ‡ ‡	§ § ns	§ § ns
	7.1±1.4	6.7±1.1	6.5±1.0	3.2±0.4	3.9±0.8	2.7±0.3	1.3±0.1	0.2±0.2	0.7±0.5	-0.1±0.3	-0.9±0.1	-0.6±0.2	
	* ‡ ‡	* § §	‡ § §	* ns ns	* ‡ §	‡ * ‡	‡ ns ns	§ ‡ ns	§ * ns	‡ ns ns	§ § ns	§ ‡ ns	
	0.05±0.01	0.05±0.01	0.05±0.0	0.3±1.9	0.7±0.6	2.3±2.5	0.07±0.03	0.05±0.0	0.06±0.01	0.05±0.01	0.06±0.01	0.05±0.0	
	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	
Chl-a	12.61±2.7	8.82±0.5	10.47±5.1	10.68±2.7	12.37±1.6	12.25±1.2	17.23±3.4	18.47±0.4	18.89±1.6	20.53±0.5	20.07±1.3	21.4±2.6	
	‡ * *	ns ‡ ‡	‡ ns *	‡ ns ns	ns * *	ns ns ns	* ns ns	‡ * ns	ns ns ns	* ns ns	‡ * ns	* ns ns	
Duck Pier <i>in situ</i>	pH	8.20±0.06	7.76±0.02	7.7±0.02	8.20±0.06	7.89±0.02	7.64±0.04	8.15±0.1	7.82±0.07	7.68±0.11	8.21±0.05	7.95±0.07	7.82±0.14
		ns ns ns	* ns ‡	ns ns ns	ns ns ns	* ns ns	ns ns ns	ns ns ns	ns ns *	ns ns ns	ns ns ns	‡ ns *	ns ns ns
	227.9±34	320.6±19	244.8±89	224.1±36	576.6±30	883.1±87	258.6±8	686.8±120	832.1±87	219.7±30	508.1±84	588.6±210	
	ns ns ns	* ‡ *	* * ns	ns ns ns	* ‡ ns	* ns ns	ns ns ns	‡ ns ns	* ns ns	ns ns ns	* ns ns	ns ns ns	
	1.0±0.1	1.1±0.2	1.0±0.1	1.0±0.1	0.9±0.1	1.3±0.2	1.3±0.6	0.6±0.4	0.9±0.2	0.9±0.2	2.5±2.2	2.1±2.0	
	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	
NH ₃	0.1±0.0	0.1±0.01	0.1±0.0 *	0.09±0.01	0.08±0.03	0.09±0.01	0.07±0.01	0.08±0.0	0.08±0.01	0.07±0.01	0.07±0.01	0.1±0.1	
	ns ns *	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	* ns ns	ns ns ns	ns ns ns	
PO ₄	2.82±1. *	2.27±0.8	2.26±0.2	1.74±1.5	1.77±0.9	2.24±0.6	1.39±0.9	1.08±0.8	2.00±0.2	1.31±0.2	1.70±0.2	2.27±0.3	
	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	
Chl-a	8.05±0.02	7.75±0.02	7.72±0.02	8.44±0.03	8.02±0.02	7.99±0.03	8.67±0.03	8.71±0.02	8.51±0.02	7.94±0.02	8.17±0.01	8.70±0.05	
	§ ns ‡	§ § §	§ ns *	§ § §	§ § §	§ § §	n,§ ns	§ § ns	ns § ns	* § ns	§ § ns	* § ns	
Duck Pier Greenhouse	pCO ₂	381.3±20	865.2±57	715.5±47	705.9±35	1156.6±49	1508.7±72	335.2±13	591.8±8	637.5±24	307.8±21	612.2±30	570.6±71
		§ ns *	§ § ‡	§ ns ns	§ § §	§ § §	§ § §	ns § ns	§ § ns	ns § ns	* § ns	‡ § ns	ns § ns
	0.4±0.2	0.5±0.1	1.2±0.7	0.7±0.3	0.7±0.1	0.7±0.1	0.8±0.2	0.6±0.3	0.5±0.1	0.5±0.1	0.5±0.3	0.5±0.3	
	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	
	0.1±0.01	0.1±0.02	0.09±0.0	0.08±0.01	0.1±0.02	0.09±0.02	0.09±0.01	0.08±0.01	0.09±0.02	0.09±0.02	0.08±0.02	0.07±0.01	
	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	
PO ₄	0.60±0.4	0.48±0.4	0.73±0.2	1.40±0.3	1.80±0.4	1.30±0.4	1.67±0.2	1.41±0.7	1.41±0.3	1.71±0.9	1.21±0.2	1.50±0.2	
	ns ns ns	* ns ns	ns ns *	ns ns ns	* ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	ns ns ns	* ns ns	

Table 3. Mean ± SE of each parameter from day zero (initial) to day three. The symbols below each mean represent significance levels of mean comparison per day. ns = no significant difference between mean values per day for each treatment and parameter. § = p < 0.0001, ‡ = p < 0.001, * = p < 0.01. Repeated measures one-way ANOVA was run on Prism.

Table 4. Correlation table for chlorophyll-a concentration response. r^2 values are shown for all measured parameters run with Pairwise comparison on JMP. Bold type indicates the three most significant r^2 values per site and experiment type.

Parameter	Duck Pier		Rice Center	
	<i>in situ</i>	greenhouse	<i>in situ</i>	greenhouse
pH	0.0000	0.0044	0.2749	0.6300
pCO ₂	0.0004	0.0003	0.3795	0.6142
DOC	0.0018	0.0955	0.1388	0.0489
NH ₃	0.0005	0.0021	0.3454	0.6103
PO ₄ ⁻	0.0305	0.0527	0.0718	0.0412
NO _x	0.0254	0.0937	0.0001	0.0084

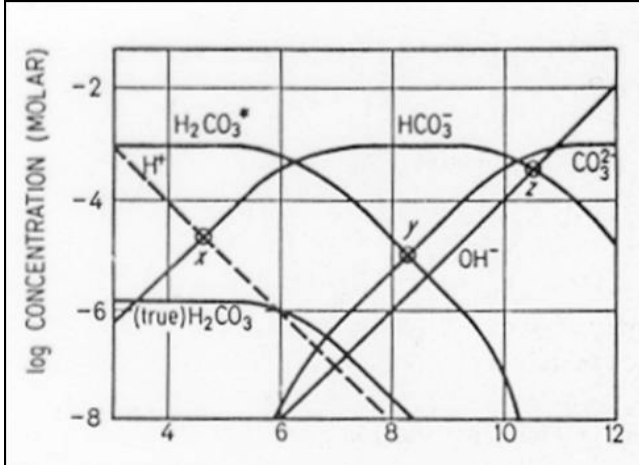


Figure 1. Speciation Diagram for Carbonate Ions in Seawater (Morgan and Stumm 1996). pH is on the X-axis, log concentration is on the y-axis. Note that as pH decreases carbonate ion concentrations decrease exponentially.

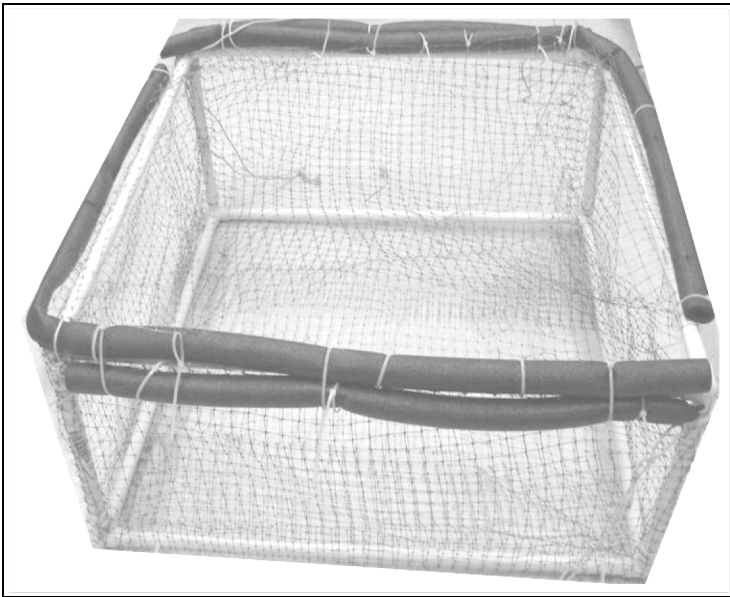


Figure 2: Floating incubation enclosure (1m x 1m x .33m) designed to house all 9 *in situ* incubations.

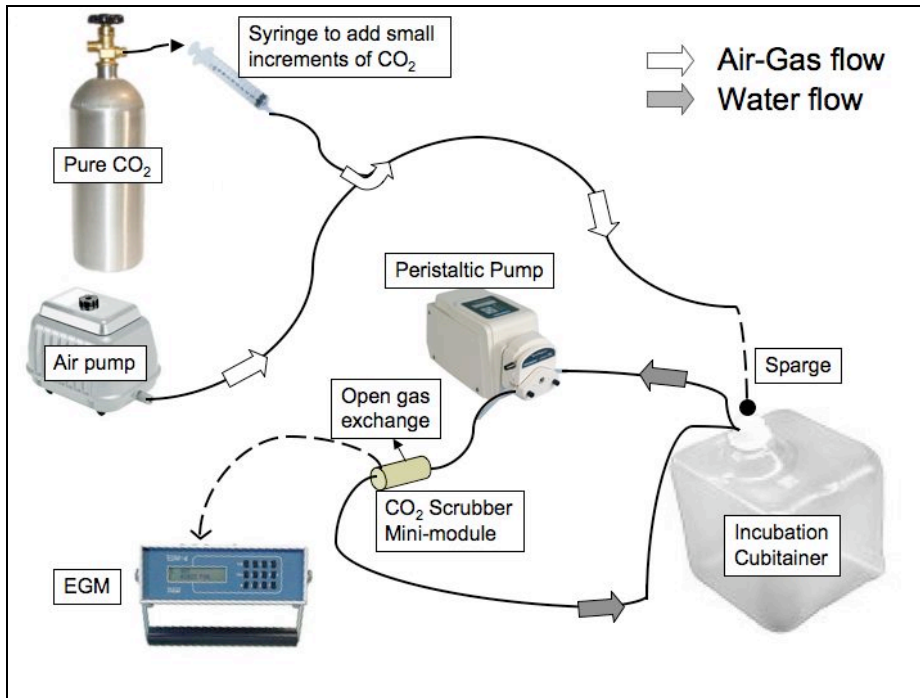
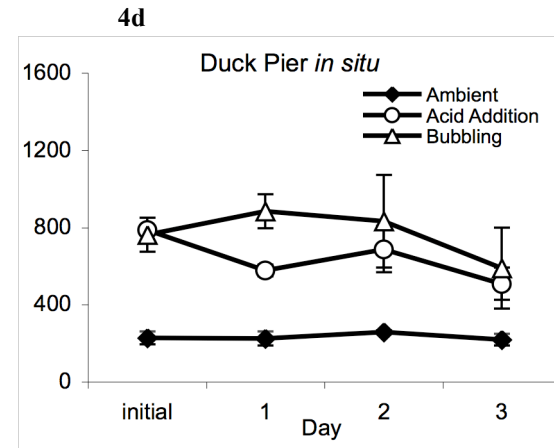
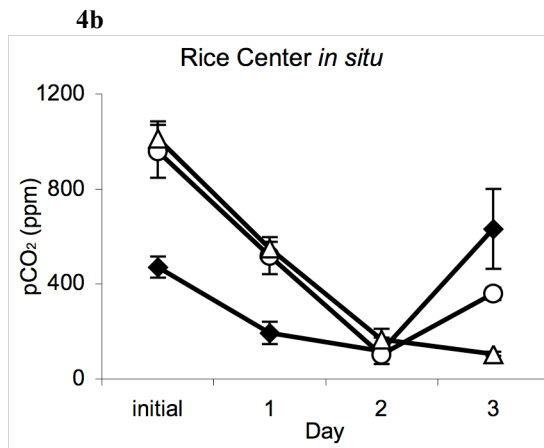
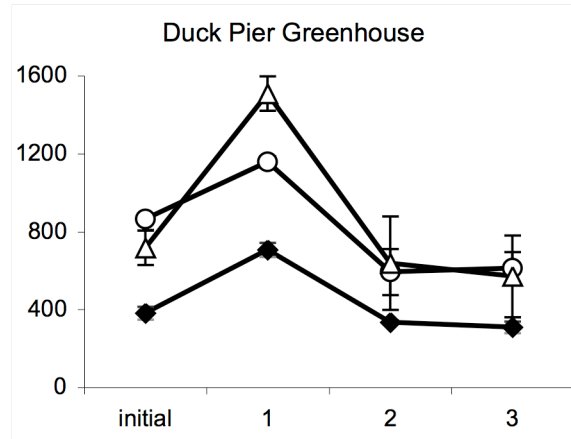
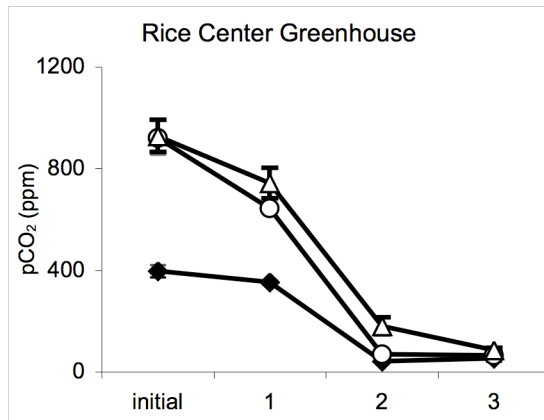
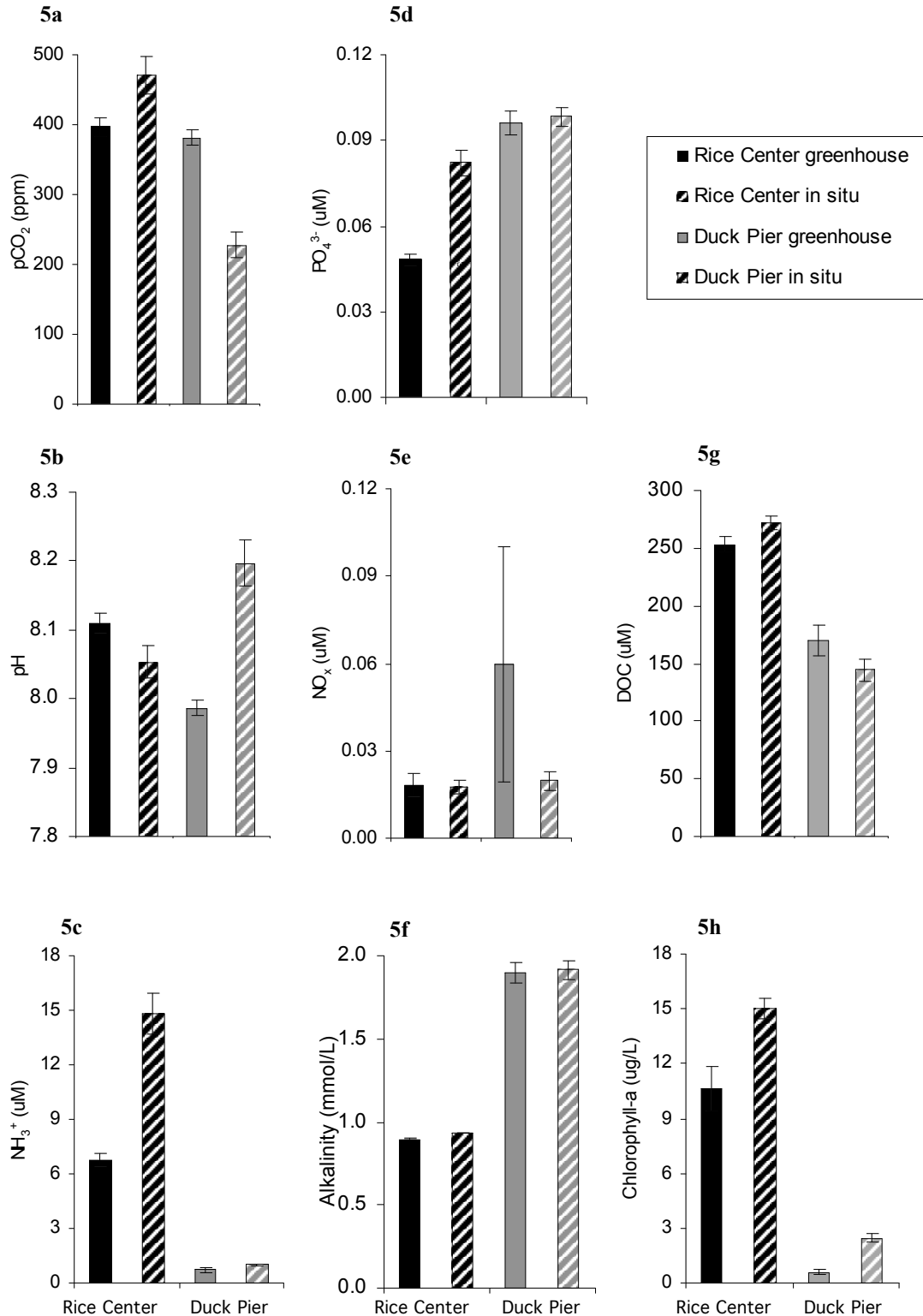


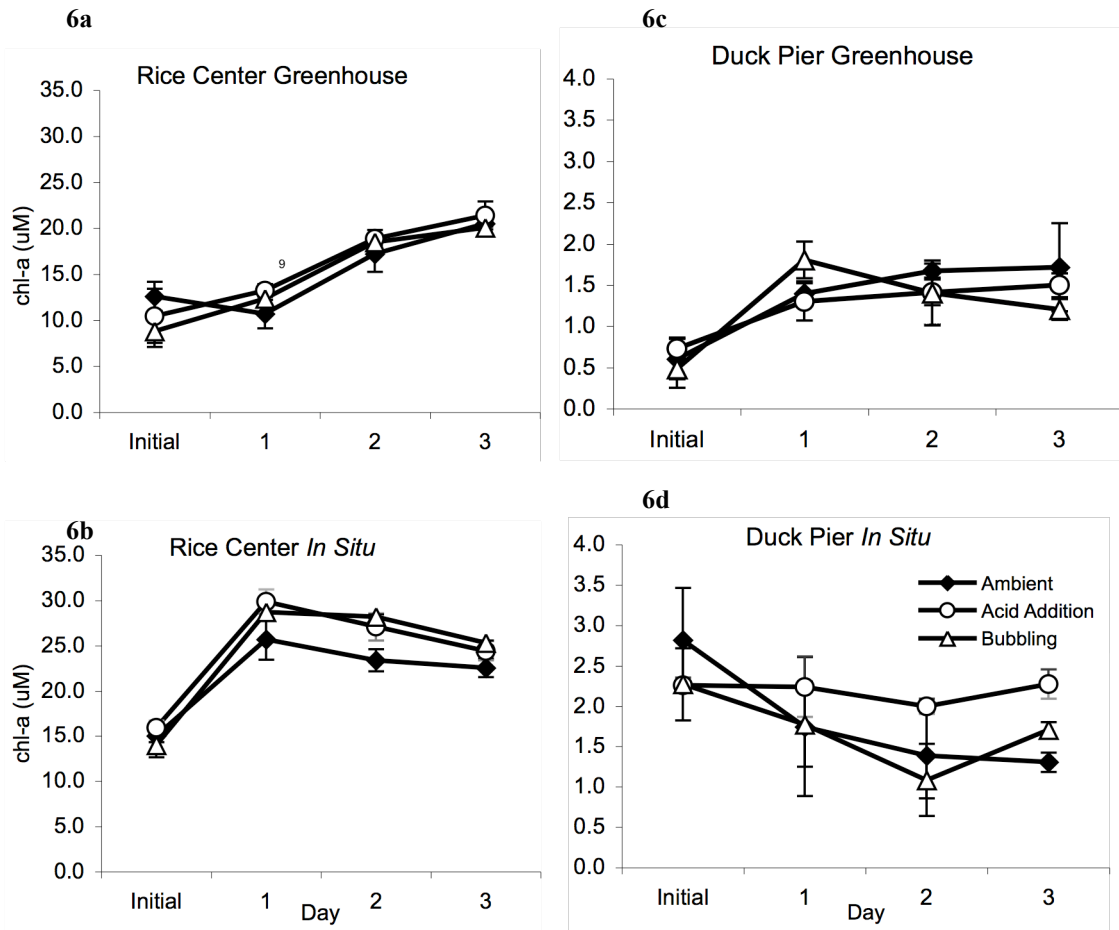
Figure 3. Diagram of novel CO₂ injection system. A syringe was filled with pure CO₂, which was injected into tubing connected to a sparge in the incubation cubitainers. Air was pumped through the tubing to gently pump CO₂ into the system. The environmental gas monitor was constantly measuring pCO₂ to ensure CO₂ levels reached treatment targets.



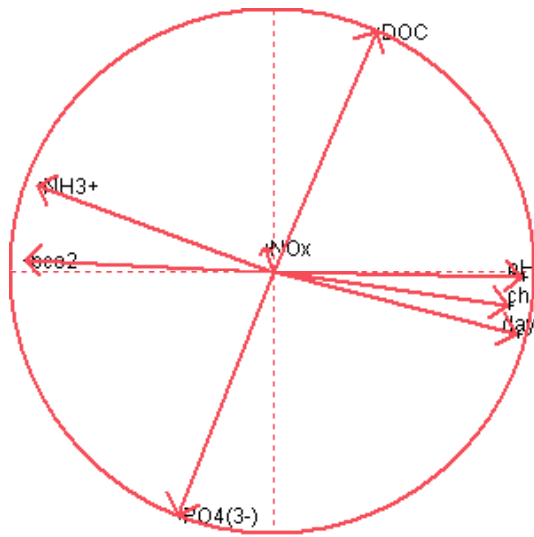
Figures 4a-4d. Mean \pm SE of pCO₂ concentrations (ppm) for each treatment at each site and experiment type over the four-day incubation period. Ambient pCO₂ (control) is solid black diamonds, elevated CO₂ through acid addition treatment is hollow triangles, and elevated CO₂ through bubbling method is hollow circles.



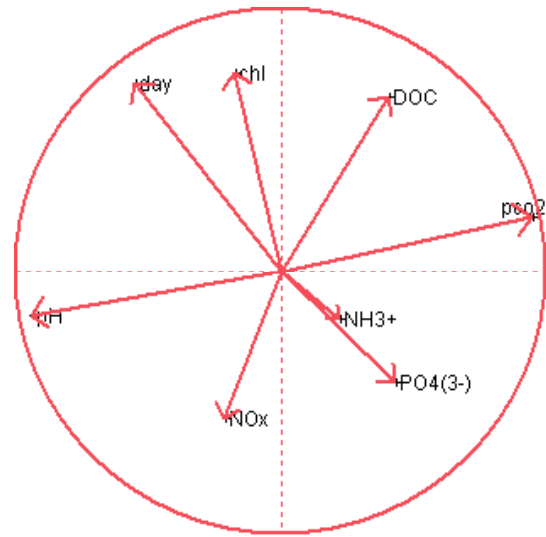
Figures 5a-5i: Initial conditions (mean ± SE) for each site and experiment type. 5a-5c show carbonate chemistry parameters, 5d-5f show inorganic nutrient concentrations, 5g shows dissolved organic carbon concentrations, and 5h shows biomass. Black=Rice Center, Grey=Duck Pier, solid=greenhouse, hatched=*in situ*.



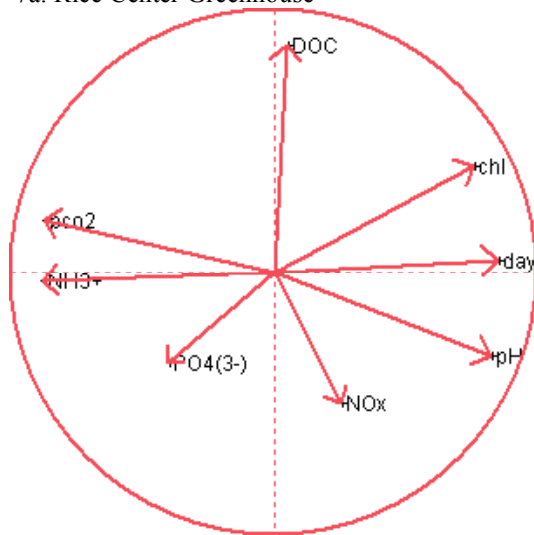
Figures 6a-6d. Mean \pm SE of chlorophyll-a concentrations over the 4 day incubation. Ambient pCO_2 (control) is solid black diamonds, elevated CO_2 through acid addition treatment is hollow triangles, and elevated CO_2 through bubbling method is hollow circles.



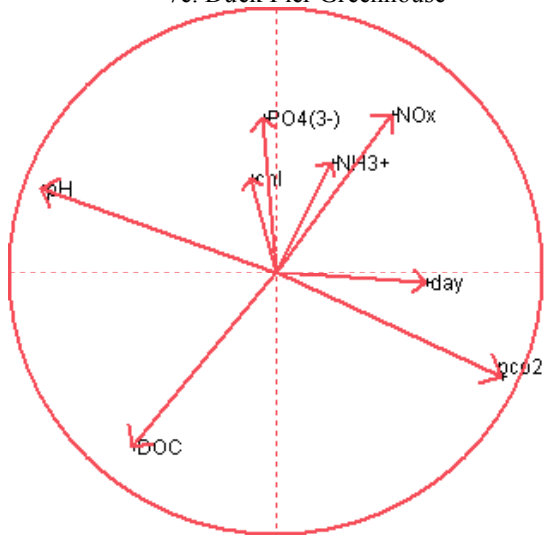
7a. Rice Center Greenhouse



7c. Duck Pier Greenhouse



7b. Rice Center *in situ*



7d. Duck Pier *in situ*

Figures 7a-7d. Loading plots for the multivariate principal component analysis (PCA) showing data for entire incubation period. Arrows pointed in the same or opposite direction as chlorophyll-a ($\mu\text{g/L}$) will be more significantly related than arrows at angles around 90 degrees.

Vita

Susan (Susie) B. Gifford was born on January 29, 1985 in Valley Falls, New York where her family has an operational farm and small family business. She graduated from Hoosic Valley High School in 2003 and moved to Ithaca, New York to attend Cornell University from which she graduated in 2007 with a B.S. in Science of Earth Systems. Susie lived in seven states between 2006 and 2009, New York, Hawai'i, Oregon, Massachusetts, Florida, Colorado and Virginia, working for a variety of agencies including National Park Service and U.S. Fish and Wildlife Service by way of Student Conservation Association. Susie matriculated at Virginia Commonwealth University in August 2009 to pursue a M.S. degree in Biology and immediately began her research in the McCallister Lab. After graduation, Susie plans to remain in Richmond to teach college level biology before pursuing a career in federal government.