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Novel methodology for in situ carbon dioxide enrichment of benthic ecosystems

Justin E. Campbell  
Department of Biological Sciences, Florida International University, jcamp013@fiu.edu

James W. Fourquarean  
Marine Science Program, Department of Biological Sciences, Florida International University, fourqure@fiu.edu

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Since the Industrial Revolution of the early 1800s, widespread fossil fuel combustion has contributed large quantities of carbon dioxide to both atmospheric and oceanic reservoirs around the globe. Present day atmospheric CO₂ concentrations of 387 ppm represent a near 30% increase over preindustrial values, with concentrations forecast to surpass 700 ppm by the end of the century (Meehl et al. 2007). Roughly 30% of this anthropogenically released CO₂ has been absorbed by the global oceans (Sabine et al. 2004) with important consequences for the carbonate chemistry of the surface waters (Feely et al. 2004). The carbonate equilibria of marine waters can be described by a series of dissociation reactions, whereby increases in dissolved CO₂ concentrations elevate the abundance of certain carbonate species (CO₂ and HCO₃⁻), while decreasing the abundance of others (CO₃²⁻):

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \rightleftharpoons 2\text{H}^+ + \text{CO}_3^{2-}
\]

Furthermore, CO₂-mediated increases in the abundance of H⁺ ions are expected to dramatically reduce oceanic pH, with forecasts of a 0.3-0.5 unit reduction by the year 2100 (Caldeira and Wickett 2003; Raven et al. 2005). These alterations in the pH and the carbonate equilibrium of the oceanic surface waters may have substantial implications for a variety of important biotic and ecosystem processes (e.g., photosynthesis and calcification), particularly when integrated over large spatial scales. Quantifying the responses of marine ecosystems to future climate change scenarios remains an important, albeit difficult task, as some biological responses can display considerable variation (Kroeker et al. 2010). Thus, calls have been made for additional empirical research that examines such impacts across a variety of taxonomic groups under realistic environmental conditions (Doney et al. 2009; Hendriks et al. 2010).

**Novel methodology for in situ carbon dioxide enrichment of benthic ecosystems**

Justin E. Campbell* and James W. Fourqurean

1Department of Biological Sciences, Florida International University, Marine Sciences Program, 3000 NE 151 Street, North Miami, FL 33181, USA

**Abstract**

Future climate change will likely represent a major stress to shallow aquatic and coastal marine communities around the world. Most climate change research, particularly in regards to increased pCO₂ and ocean acidification, relies on ex situ mesocosm experimentation, isolating target organisms from their environment. Such mesocosms allow for greater experimental control of some variables, but can often cause unrealistic changes in a variety of environmental factors, leading to “bottle effects.” Here we present an in situ technique of altering dissolved pCO₂ within nearshore benthic communities (e.g., macrophytes, algae, and/or corals) using submerged clear, open-top chambers. Our technique utilizes a flow-through design that replicates natural water flow conditions and minimizes caging effects. The clear, open-top design additionally ensures that adequate light reaches the benthic community. Our results show that CO₂ concentrations and pH can be successfully manipulated for long durations within the open-top chambers, continuously replicating forecasts for the year 2100. Enriched chambers displayed an average 0.46 unit reduction in pH as compared with ambient chambers over a 6-month period. Additionally, CO₂ and HCO₃⁻ concentrations were all significantly higher within the enriched chambers. We discuss the advantages and disadvantages of this technique in comparison to other ex situ mesocosm designs used for climate change research.

*Corresponding author: E-mail: jcamp013@fiu.edu

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Much of climate change research within marine systems has been directed toward understanding how long-term and pervasive changes in oceanic pCO₂ can serve as a stressor on both benthic and water column organisms, and the processes they regulate. For example, it is expected that increases in dissolved CO₂ concentrations are likely to reduce long-term calcification rates across many coral reef ecosystems (Kleypas et al. 1999; Langdon et al. 2000). A variety of calcifying marine organisms; microalgae (Riebesell et al. 2000; Zondervan et al. 2001), macroalgae (Jokiel et al. 2008; Kuffner et al. 2008; Gao and Zheng 2010), and corals/invertebrates (Langdon et al. 2000; Langdon and Atkinson 2005; Shirayama and Thornton 2005; Gazeau et al. 2007; Lombard et al. 2010) have all displayed declines in CaCO₃ production with experimentally elevated pCO₂. Additional studies have highlighted interactive effects between temperature and pCO₂ on the calcification rates of marine organisms (Martin and Gattuso 2009; Rodolfo-Metalpa et al. 2010). Such studies clearly demonstrate implications for the resilience of coral reefs under increasing anthropogenic and climatic pressures. However, a majority of these experiments have been conducted within artificial indoor mesocosms, which can isolate target organisms from realistic natural conditions, and can fail to account for environmental variation (Hall-Spencer et al. 2008; Kleypas and Yates 2009; Hendriks et al. 2010). Whereas some studies have begun using outdoor mesocosm facilities (Riebesell et al. 2007; Jokiel et al. 2008) and field manipulations along natural pH gradients (Cigiano et al. 2010; Días et al. 2010), we know relatively little in regards to in situ responses of organisms to altered pCO₂ and pH conditions.

Several studies have additionally suggested that altered pCO₂ within coastal environments may have the ability to impact the functioning of aquatic and marine plant communities (Zimmerman et al. 1997; Short and Neckles 1999; Palacios and Zimmerman 2007; Hall-Spencer et al. 2008; Martin et al. 2008; Kleypas and Yates 2009). External increases in CO₂ and HCO₃⁻ concentrations have the ability to increase the diffusive flux of dissolved inorganic carbon (DIC) across leaf boundary layers, and have been shown to increase seagrass production (Hall-Spencer et al. 2008), leaf photosynthetic rates (Durako 1993; Beer and Koch 1996; Invers et al. 1997; Zimmerman et al. 1997), and plant reproductive output (Palacios and Zimmerman 2007). Other studies have additionally demonstrated changes to the seagrass epiphyte community under various CO₂ enrichment scenarios, with large declines in biogenic carbonate production, and potential biogeochemical shifts within these shallow, coastal systems (Martin et al. 2008). Submerged macrophytes comprise much of the coastal benthic community around globe and are important contributors to the carbon sink capacity of the world’s oceans (Duarte et al. 2011). Thus, similar to declines in coral reef calcification, changes in oceanic pCO₂ may additionally have widespread implications for these productive and economically important ecosystems.

Experiments that address CO₂-mediated impacts on benthic plants have likewise mainly been restricted to mesocosm designs (Titus et al. 1990; Titus 1992; Zimmerman et al. 1997; Pagano and Titus 2007; Palacios and Zimmerman 2007). Aquarium and mesocosm facilities generally provide optimal conditions to encourage vigorous plant and/or algal growth, thus responses detected within the laboratory may not hold true for natural communities, where alternate resources may become increasingly limiting under elevated CO₂ loads. Several terrestrial studies have documented disparities between ex situ and in situ responses in regards to the impacts of CO₂ enrichment on plant community dynamics (Ainsworth and Long 2005). Within these systems, CO₂ mediated growth responses can be rapidly constrained by the availability of other essential resources, such as water and/or nutrients (Diaz et al. 1993).

Many mesocosm pCO₂ experiments control seawater carbonate equilibrium via either acid addition (which shifts the relative concentrations of the carbonate species with no increase in total DIC) or CO₂ bubbling (which simultaneously increases the abundance of CO₂, HCO₃⁻, and total DIC). Whereas it has been suggested that the latter technique of CO₂ enrichment best replicates forecasted changes in seawater carbonate parameters (Hurd et al. 2009; Schulz et al. 2009), few studies have attempted to transition this ex situ methodology toward an in situ design (Barry et al. 2010). Here we describe a novel technique of long-term CO₂ enrichment applicable to the study of changes in pCO₂ on the productivity and functioning of aquatic and marine benthic communities. Our methodology consists of a submerged array of clear, open-top, flow-through acrylic chambers, which allows for continuous CO₂ enrichment over long time periods. Our system utilizes an efficient technique of in situ CO₂ bubbling, which maximizes the dissolution and containment of CO₂ gas, while minimizing losses to the external water column and atmosphere. Furthermore, the open, flow-through design allows for ample light to reach target organisms (macrophytes, corals, and/or algae), while reducing caging effects. Easy access to the benthic community through the open tops of the enclosures additionally allows for accurate measurements of carbonate parameters (pH and alkalinity), and a variety of biotic processes (plant/algal growth rates, calcification rates, and photosynthetic fluorometric responses). This system provides an inexpensive and efficient technique of in situ pCO₂ manipulation around a wide variety of benthic communities to study various climate change/ocean acidification scenarios.

**Materials and procedures**

**Site description**

In situ pCO₂ manipulation was initiated on 1 Jul 2009 within a shallow, nearshore benthic plant community within the Florida Keys, Florida, USA (24.55° N, 81.75° W). The benthic community was dominated by the seagrass *Thalassia testudinum*, with lower abundances of the seagrasses *Syringodium*...
filiforme and Halodule wrightii. A variety of calcareous green algal species (Halimeda spp. and Penicillus spp.) were additionally present within the seagrass canopy, along with a substantial epiphyte community (dominated by filamentous turf and coralline encrusting algae) growing on the seagrass leaf surfaces. The sediments were composed of roughly 10% organic matter; with the remaining mineral fraction consisting of mud-sized biogenic calcium carbonates, and larger particles of the carbonate skeletal remains of a variety of calcifying organisms. The experimental seagrass bed was located 10 m offshore, within a shallow (1 m depth) embayment, which exchanged waters with the Gulf of Mexico to the northwest, and had an average salinity of 37. Noon light levels below the water surface averaged 1000 (µmol photons m$^{-2}$ s$^{-1}$), while light levels at the top of the seagrass canopy averaged 800 (µmol photons m$^{-2}$ s$^{-1}$) for the duration of the experiment. CO$_2$ enrichment was conducted continuously for a period of 6 months until 1 Jan 2010.

**Experimental design and chamber description**

The benthic CO$_2$ enrichment experiment consisted of three replicated ($n=5$) treatments, arranged in a complete randomized block design: 1) CO$_2$ enrichment within clear, open-top chambers; 2) no CO$_2$ enrichment within clear, open-top chambers; and 3) control plots lacking clear open top chambers. Treatments were arranged within a grid design (3 rows x 5 columns), with each column representing a separate complete block (Fig. 1). Treatments were randomly assigned within each block. This design was necessary to organize the plumbing system used to deliver CO$_2$-rich seawater to the enriched chambers. Each chamber was constructed of 4 optically clear, acrylic panels (0.8 m x 0.4 m x 0.4 m), producing a total chamber volume of 0.13 m$^3$ and enclosing a benthic area of 0.17 m$^2$. Each chamber was assembled using corrosion resistant (zinc plated) 3.8 cm corner braces, with adjacent acrylic panels additionally sealed at the corners with an adhesive. Each chamber was anchored to the benthos using a series of adjustable valves.

![Fig. 1. Schematic of chamber array. Solid boxes designate an acrylic chamber supplied with either CO$_2$ enriched seawater or CO$_2$ ambient seawater. Dashed boxes designate control plots with no chamber. Red and blue lines represent CO$_2$ enriched and unenriched seawater, respectively. Gray arrows indicate direction of water flow.](figure.png)
of plywood panels and cinder blocks. Each chamber received 4 plywood panels (0.4 m × 0.1 m) that were affixed to the base of each acrylic side using cable ties. Cinder blocks were then placed on top of each panel to provide a tight seal between the bottom of the chamber and the substrate (Fig. 2). Short, 2.5 cm diameter PVC segments were hammered into the sediment, down to the bedrock, underneath each plywood panel to provide support for each cinder block, and prevent compaction of the surrounding sediment. This system allowed the chambers to accommodate turbulent wave energy experienced in the field, while remaining firmly fixed in place. Chambers were cleaned of fouling organisms on a bi-monthly basis, and light measurements both within and outside several replicate chambers were conducted periodically with a submersible 2pi light sensor (WALZ Diving PAM).

**CO₂ enrichment system**

In situ carbon enrichment was achieved by rapidly mixing gaseous CO₂ with ambient seawater to achieve the desired pH. The rate of gas delivery was set to reproduce dissolved CO₂ concentration forecasts for the year 2100 (Caldeira and Wickett 2003) within our enriched chambers. These forecasts were selected as target values because they represent “business-as-usual” scenarios, whereby CO₂ emissions continue along current trends (Raven et al. 2005). To achieve enrichment, a 34-kg cylinder of compressed CO₂ (AirGas, beverage grade) was connected to a two-stage regulator with a fine control valve, which bled gas into a submerged water pump (120 V AC, 6800LPH, 145W). Once bled into the intake port of the pump, the CO₂ was rapidly mixed with ambient seawater by the impeller, and then delivered to the carbon-enriched chambers via a PVC plumbing network. Gas flow rates were incrementally increased until the pH within the enriched chambers was reduced by the target value of 0.4 units in comparison with the controls. Once established, CO₂ flow to the pumps remained constant, resulting in a constant, reduced pH within the enriched chambers compared with the surrounding environment. Gas pressure from the two-stage regulator was set at 15 psi, while the gas flow rate was set at 1.12 L/min. Flexible, CO₂-proof tubing (Cramer Decker, 150 m) was used to deliver CO₂ from the cylinder to the submerged pump. A second water pump was established without connection to a CO₂ line and delivered ambient seawater to the unenriched chambers via a secondary independent PVC plumbing network. Both submerged powerheads were caged within a mesh PVC frame (1 m × 1 m × 0.5 m) to exclude debris. Industrial garden hose (2 cm diameter, 10 m in length) was connected to the outflow of each water pump and used to deliver CO₂-enriched and ambient seawater to the PVC plumbing network.

**PVC plumbing network**

Seawater from each respective water pump was redirected to a series of independent submerged PVC pipes (one enriched line, one ambient line) that divided the main seawater flow amongst the replicates for each chamber treatment. Each garden hose from the powerheads was connected to a PVC pipe (3.8 cm diameter, 10 m in length) that ran lengthwise along the southern border of the chamber array (Fig. 1). Each PVC pipe contained 5 separate 3-cm hose bibs, which were evenly spaced at 1 m intervals (corresponding to the spacing between the grid columns). Industrial garden hose (2 cm diameter) was then used to deliver seawater from the hose bibb to each clear acrylic chamber, through a 1.9 cm diameter hole drilled through the base of the chamber. Each hose bibb along the PVC pipe could then be finely adjusted to equalize seawater flow between the chambers, which was measured at 340 L/h. Thus, the water volume within each chamber was replaced approximately 63 times daily.

**Measurement of seawater carbonate parameters**

Seawater carbonate parameters within each chamber were monitored periodically throughout the 6-month experiment. Temperature and pH inside the seagrass canopy within each chamber and control plot were recorded 53 separate times, across 18 distinct sampling dates. Six of the sampling dates represent diurnal measurements, when pH and temperature were recorded every 3 h from 900 to 2100. This sampling schedule produced over 800 pH measurements, allowing us to describe how carbonate parameters varied amongst treatments both throughout the day, and during the enrichment period. The pH was recorded using a handheld Thermo Scientific Orion 4-Star meter, calibrated with NBS standards (relative accuracy ± 0.002). Temperature was additionally recorded once every hour during the 6-month period by an underwater Onset Temp Logger, one within a randomly selected chamber, and a second within a randomly selected control plot. On a monthly basis, replicate diurnal (morning and evening) water samples (20 mL) were collected within each chamber and control plot, filtered through a 0.7 µm GF/F filters, and stored on ice within borosilicate glass containers until further processing. Upon return to the lab, total alkalinity was measured by automated, potentiometric titration with 0.1 N HCl. Salinity

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**Fig. 2.** Photograph of an enrichment chamber.
was recorded with an Orion conductivity meter. Carbonate parameters (CO$_2$aq, HCO$_3^-$, CO$_3^{2-}$, and calcite/aragonite saturation states) were calculated with the CO$_2$Sys Excel Macro (Lewis and Wallace 1998), using the dissociation constants of Mehrbach et al. (1973), refit by Dickson and Millero (1987).

**Stable isotope measurements**

Stable carbon isotope ($\delta^{13}C$) measurements were additionally used to further demonstrate the effectiveness of our CO$_2$ enrichment technique. If our carbon-enriched treatments were effective in increasing the diffusive flux of CO$_2$ to the benthic seagrass community, we hypothesized significant reductions in the $\delta^{13}C$ signature of the seagrass tissue growing within the enriched chambers, in accordance with previous correlative and experimental studies (Durako and Sackett 1993; Vizzini et al. 2010). Every month, aboveground leaf material from 3-5 shoots of *Thalassia testudinum* was collected within each chamber and control plot, scraped free of adhered epiphytes, separated according to leaf age, dried in the lab for 48 h at 60°C, and ground to a fine power. Isotope analyses were conducted on the youngest leaf material (leaf rank 1) using standard elemental analyzer isotope ratio mass spectrometer procedures. An elemental analyzer was used to combust all organic material and subsequently reduce the formed gases into N$_2$ and CO$_2$, which were measured on a Finnigan MAT Delta C IRMS in a continuous flow mode. The samples’ isotope ratios (R) are reported in the standard delta notation ($\delta$):

$$\delta(\text{‰}) = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right] \times 1000$$

These results are presented with respect to the international standards of atmospheric nitrogen (N$_2$) and Vienna Pee Dee belemnite (V-PDB) for carbon. Analytical reproducibility of the reported $\delta$ values, based on sample replicates, was better than ± 0.08‰ for carbon. Samples of the CO$_2$ source gas used for enrichment were additionally collected for stable isotopic analysis. Airtight, double-ended Swagelock sample cylinders were used to sample ten of the Airgas CO$_2$ cylinders during the enrichment period. The stable carbon isotopic composition of this source CO$_2$ was determined by directly injecting each gas sample into a dual inlet ratio mass spectrometer calibrated against a reference gas of a known isotopic value.

**Statistical analysis**

Seawater carbonate parameters were analyzed by comparing the 95% confidence intervals of the mean water quality measurements within the chambers and control plots throughout the enrichment period (Table 1). Due to diurnal variation, water quality measurements were compared within 5 distinct time segments (900-1200; 1200-1500; 1500-1800; 1800-2100; 2100-0000). Monthly measurements of seagrass stable carbon isotopes within each of the treatments were analyzed using a repeated-measures analysis of variance (ANOVA, $\alpha = 0.05$), with month as the within-subject factor, and CO$_2$ treatment as the between-subject factor. When significance was detected, a Bonferroni corrected post-hoc analysis was performed.

**Assessment**

For the duration of the 6-mo field enrichment period, our open-chamber, flow-through system functioned continuously without failure. The carbonate parameters within the enriched chambers were responsive to CO$_2$ addition from the gas cylinder, and displayed significantly reduced pH, and higher CO$_2$ and HCO$_3^-$ concentrations throughout the enrichment period as compared with the unenriched chambers and control plots (Fig. 3-6; Table 1). Conversely, CO$_3^{2-}$ concentrations, calcite saturation, and aragonite saturation states were all significantly reduced within the enriched chambers (Fig. 5,6; Table 1). There were no statistical differences in carbonate parameters between the unenriched chambers and control plots during any of the diurnal time segments.

Background carbonate parameters did vary throughout the day (reflecting water column and benthic processes of photosynthesis and respiration), however, the enriched chambers remained significantly enriched during each time segment throughout the 6-month period (Fig. 6). Temperature additionally varied both diurnally, and throughout the enrichment period, however during all sampling dates, there were no statistical differences between treatments. Total alkalinity ranged from an average of 2200 µmol kg$^{-1}$ in July, to an average of 2569 µmol kg$^{-1}$ in November, agreeing with other water quality measurements for Western regions of Florida Bay (Millero et al. 2001). Within each monthly sampling, total alkalinity was not impacted by CO$_2$ enrichment, nor were significant differences detected between the morning and afternoon alkalinity measurements, suggesting that the dissolution of carbonate sediments was not increased by CO$_2$ addition. Salinity displayed slight variation during the enrichment period, with values of 37.1 in July to 36.9 in November. Light measurements revealed that the chamber design did impose a slight reduction in light reaching the macrophyte canopy. Such reductions were highest (~5%), in the early morning and late afternoon when much of the irradiance was penetrating the water column at an oblique angle, and transmitted through the chamber panel. Light reductions were minimal during the noon hours when irradiance passed perpendicular to the water surface, and through the open top of the chamber. Overall, light reductions were lowest during the critical noon period when photosynthetic rates for benthic communities are generally at their highest (Yates et al. 2007).

Water quality measurements throughout the 6-month period reveal that our chamber design was effective in 1) reaching our target enrichment range forecasted for the year 2100, and 2) continuously holding those values within that range over long temporal periods. Median pH values within the CO$_2$ enriched chambers, CO$_2$ ambient chambers, and control plots were 7.75, 8.21, and 8.21, respectively (Fig. 3). On average, enriched chambers displayed a 0.46 unit reduction in pH values, as compared with the other unenriched treatments (Fig. 4). For 87% of the pH sampling dates, the enriched chambers were held within ± 0.2 pH
Table 1. Diurnal seawater carbonate parameters observed within the seagrass canopy in the CO₂ enriched chambers, CO₂ ambient chambers, and control plots during the 6-month enrichment period. Measurements are divided into 5 distinct 3-h time groups. Carbonate parameters (means plus 95% confidence intervals) represent seasonal averages of 7-10 distinct sampling dates. Total alkalinity averaged 2345 μmol kg⁻¹ while temperature averaged 29.9°C during the enrichment period.

<table>
<thead>
<tr>
<th>Time of day (observations)</th>
<th>CO₂ enriched</th>
<th>CO₂ ambient</th>
<th>Control plot</th>
<th>CO₂ enriched</th>
<th>CO₂ ambient</th>
<th>Control plot</th>
</tr>
</thead>
<tbody>
<tr>
<td>0900-1200 (8)</td>
<td>7.57 (7.49-7.65)</td>
<td>8.02 (7.96-8.08)</td>
<td>8.02 (7.96-8.08)</td>
<td>62.9 (51.5-74.4)</td>
<td>16.8 (14.2-19.4)</td>
<td>16.6 (14.1-19.1)</td>
</tr>
<tr>
<td>1200-1500 (9)</td>
<td>7.78 (7.72-7.84)</td>
<td>8.23 (8.17-8.29)</td>
<td>8.24 (8.18-8.3)</td>
<td>32.2 (25.5-38.9)</td>
<td>8.8 (6.9-10.7)</td>
<td>8.7 (6.8-10.7)</td>
</tr>
<tr>
<td>1500-1800 (10)</td>
<td>7.74 (7.62-7.86)</td>
<td>8.22 (8.14-8.30)</td>
<td>8.22 (8.14-8.30)</td>
<td>42.2 (25.8-58.6)</td>
<td>9.4 (7.4-11.4)</td>
<td>9.5 (7.5-11.5)</td>
</tr>
<tr>
<td>1800-2100 (7)</td>
<td>7.74 (7.6-7.88)</td>
<td>8.30 (8.26-8.34)</td>
<td>8.30 (8.26-8.34)</td>
<td>39.0 (22.4-55.6)</td>
<td>6.7 (5.7-7.7)</td>
<td>6.7 (5.7-7.7)</td>
</tr>
<tr>
<td>2100-0000 (7)</td>
<td>7.62 (7.44-7.80)</td>
<td>8.23 (8.17-8.29)</td>
<td>8.24 (8.18-8.3)</td>
<td>59.4 (6.8-9.6)</td>
<td>8.2 (6.8-9.6)</td>
<td>8.0 (6.6-9.4)</td>
</tr>
</tbody>
</table>

Stable carbon isotope measurements demonstrate that the benthic seagrass community growing within the enriched chambers did experience an increase in carbon flux to the photosynthetic tissues for the duration of the experiment (Fig. 7). Such measurements integrate information about the local carbon environment over long time periods and provide strong biological evidence of CO₂ enrichment. In accordance with our hypotheses, seagrass stable carbon isotope values were significantly reduced within the CO₂ enriched chambers compared with the unenriched treatments for all sampling periods during the experiment (F = 63.74, P < 0.01). There were no statistical differences in seagrass δ¹³C values between the unenriched chambers and the control plots. The within-subjects factor of time was significant during the experiment (F = 7.03, P < 0.01), strongly driven by the increasingly negative δ¹³C values within the enriched treatments over the course of the experiment. The interaction between time and treatment was additionally significant (F = 12.66, P < 0.01) because there was only a change in the δ¹³C of seagrass tissues from the CO₂-enriched chambers. The stable carbon isotope composition of the source CO₂ gas used for enrichment displayed slight variation, with a median value of ~4.32‰.

Discussion

In the current study, we explore the potential utility of open-top chambers (OTCs) as a means of in situ carbon enrichment within shallow aquatic or marine benthic environments. We submit that a number of factors must be met to make long-term carbon enrichment via this technique feasible: 1) significant increases in carbon supply must be detected within the CO₂-enriched chambers over long time periods, demonstrating the ability of this design to deliver gaseous CO₂ from an onshore location to a submerged benthic location, 2) unnecessary confounding effects such as light and/or water flow reductions must be minimized within all chambers, and 3) the OTC design must sufficiently constrain CO₂ parameters such that target enrichment values are achieved for the majority of the enrichment period.
Our carbonate parameter measurements show that the carbonate environment within the CO₂-enriched chambers was dramatically altered in comparison to the CO₂ ambient chambers and the control plots. The open top design allowed for sufficient containment of injected CO₂ and produced an acceptable rate of gas consumption for the size of the chamber array and our target enrichment level (approx. 68 kg CO₂/month). A larger array, with greater replication, could easily be implemented using this design, via the addition of extra chambers, or a complete duplication of the entire array. Overall, the possible level of carbon enrichment using this technique will depend upon the trade-off between chamber number, chamber size, and desired enrichment level. We submit that some pilot testing may be necessary to pinpoint the appropriate number of gas cylinders required, and the gas delivery rate from the CO₂ regulator to the submerged water pumps.

The arrangement of the PVC enrichment system additionally allows for careful adjustment of the water flow rates both within and between chamber treatments. Water flow rates can be individually adjusted to compensate for the loss of pressure head between chambers adjacent to the water pumps and those that are at the opposite end of the array. Utilizing larger pipe diameters to deliver seawater would reduce friction head within the system, and increase the efficiency of the submerged pumps. While restricted to nearshore environments, the use of larger, more powerful water pumps may alleviate such constrains, allowing for the placement of enrichment chambers at locations further offshore.

The flow-through, open-top chambers allowed for minimal caging confounds in regards to light and water flow conditions. Light reductions, on average, were no greater than 5%. Bi-monthly cleaning of both the internal and external surfaces of the chambers prevented algal fouling and maintained ade-
Fig. 5. Carbonate parameters (mean ± 1 SE) within CO₂ enriched chambers, CO₂ ambient chambers, and control plots during the 6-month enrichment period. Values represent treatment averages observed during the 12:00-15:00 time period for each respective date.
Fig. 6. Seasonal carbonate parameters (means ± 1 SE) within CO₂ enriched chambers, CO₂ ambient chambers, and control plots. Values within each time segment represent averages of 7-10 distinct sampling dates throughout the enrichment period.
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In situ benthic carbon dioxide enrichment

Stable carbon isotope values (means ± 1 SE) of aboveground leaf material of the seagrass *Thalassia testudinum* during the enrichment period. The June sampling date represents initial seagrass stable carbon material of the seagrass median isotopic signature of the source CO2 gas used for enrichment.

The dashed line represents the period. The June sampling date represents initial seagrass stable carbon material of the seagrass median isotopic signature of the source CO2 gas used for enrichment.

![Graph showing stable carbon isotope values](image)

**Fig. 7.** Stable carbon isotope values (means ± 1 SE) of aboveground leaf material of the seagrass *Thalassia testudinum* during the enrichment period. The June sampling date represents initial seagrass stable carbon isotope values prior to CO2 enrichment. The dashed line represents the median isotopic signature of the source CO2 gas used for enrichment.

Sufficient light levels for the duration of the experiment. Light reduction within the chambers at noon was minimal, as chamber light levels often displayed similar values to the unchambered control plots. The response of benthic communities to CO2 enrichment may strongly depend upon ambient light levels, thus the high levels displayed within our design (800 µmol photons m⁻² s⁻¹) ensure realistic values experienced by many shallow benthic communities. The continuous operation of the water pumps ensured that the chamber design did not strongly impact other water quality variables throughout the experiment. All water quality measurements and carbonate calculations (temperature, pH, alkalinity, salinity, CO2, HCO3, and CO3²⁻) were similar between the unenriched chambers and the unchambered control plots, suggesting that water flow was adequate to prevent biologically mediated shifts in water quality within each chamber. Thus, we feel that our OTC design served to only manipulate carbonate parameters within our enriched chambers, while not impacting alternate water chemistry variables.

The OTC design displayed moderate constraint of carbonate parameters within the CO2-enriched treatments for the duration of the experiment (Fig. 4). Over the 6-month period, an average difference of 0.47 pH units was maintained between the CO2 treatments throughout the day (Fig. 6). In comparison, mesocosm studies generally display a tighter constraint of CO2 parameters both within enriched and unenriched treatments due to the use of pH monitors coupled to solenoid gas valve regulators. For our in situ design, a number of complex environmental factors contributed to the variation in pH values across chamber treatments over time, attributable to both ambient background variation (which equally impacted all chambers), and turbulent weather conditions (which only impacted the CO2 enriched treatments). Back-ground variation caused both diurnal and seasonal variation amongst all treatments, and demonstrates the ability of biotic processes (photosynthesis and respiration) to control local carbonate parameters when water column mixing is relatively low, as previously documented for other regions of Florida Bay (Yates and Halley 2006). Turbulent weather from periodic storm events differentially increased carbonate parameter variation within our CO2-enriched treatments. High wind events increased the flushing rate of ambient seawater into the open-top chambers, and temporarily reduced the effectiveness of our enrichment treatment (pulling the pH and carbonate parameters toward background values). While impacting several of our sampling dates, these storm events were short-lived, and thus did not reduce the effectiveness of our CO2 treatments over the long term. Conversely, periods of low wind energy served to increase the effectiveness of our CO2 enrichment. In comparison, pH variation within our enriched treatments was of similar magnitude to other in situ observations that have examined the impacts of ocean acidification on benthic communities near volcanic vents, which naturally lower pH and increase CO2 concentrations (Hall-Spencer et al. 2008; Martin et al. 2008). We suggest that the overall precision of the control of carbonate parameters within the enriched treatments could be largely improved by incorporating a pH-dependent feedback loop, similar to many mesocosm studies.

Use of a variable-flow, magnetic solenoid valve (regulated by a pH monitor), would control gas delivery into the submerged water pumps and automatically regulate gas flow according to external wind conditions, providing greater constraint of carbonate parameters within the enriched treatments.

Stable carbon isotope measurements of the macrophyte community within the chambers and control plots suggests that over long time periods, vegetation within the enriched treatments was exposed to significantly elevated CO2 concentrations as compared with the unenriched treatments. The increasingly negative seagrass δ13C values with CO2 enrichment agree with our original hypotheses, and possibly reflect an effect of concentration-dependent isotope fractionation (Fig. 7). The δ13C value of plant material can be influenced by both the isotopic composition of the source DIC pool, and photosynthetic carbon isotope discrimination (Farquhar et al. 1989). Increases in the external supply of DIC impact plant δ13C values by allowing for increased discrimination against the heavier carbon isotope (δ13C) during photosynthetic carbon fixation (Smith and Walker 1980). Thus, our shifts in δ13C with carbon enrichment are consistent with these statements, and are in agreement with previous studies which demonstrate that an elevated CO2 supply results in increasingly negative δ13C values within both seagrass tissues and marine macroalgae (Durako and Sackett 1993; Kubler et al. 1999; Vizzini et al. 2010).

Shifts in the isotopic composition of the source DIC can additionally contribute to changes in the isotopic composition of plant material. Therefore, the calculation of isotopic fractionation factors would be the preferred technique of demon-
strating that isotopic shifts in plant material are related to changes in DIC concentration. While we did characterize the isotopic composition of the source CO₂ gas used for enrichment, we did not characterize the isotopic composition of the background DIC pool, thus could not conclusively determine if the change in δ¹³C we observed in seagrass leaves was caused by a change in fractionation. However, the source CO₂ used for enrichment had a median δ¹³C value of −4.32 %o, which is relatively heavy compared with dissolved atmospheric CO₂ (−9.0 %o) (Kroopnick 1985). Thus, despite adding CO₂ which displayed a relatively enriched δ¹³C carbon signal, we produced seagrass leaf tissue with progressively depleted δ¹³C values, suggesting an increase in the external supply of DIC. These data provide further evidence that our in situ chamber design was effective in achieving a long-term and persistent increase in carbon supply to our benthic community.

Throughout the 6-month period, our system was quite robust and amenable to future long-term carbon enrichment studies. While intense tropical storms did not impact South Florida during the 2009 summer season, our experimental array was subjected to a number of short-term local storms. Short periods of high wind/wave energy showed no short- or long-term impact on the integrity or positioning of the submerged chambers. We were additionally unable to detect any significant erosion or damage to the benthic community within any of the chambers after storm events.

Last, we submit that our technique of in situ carbon enrichment is feasible in regards to both overall cost and system maintenance. Acquisition of materials and construction/installation of the chamber array can be completed within a relatively short timeframe (2-3 weeks), with an approximate budget of US$ 5000. Once established, 1-2 h of bi-monthly maintenance is required to thoroughly clean the chambers of algal overgrowth and clear the pumps of any collected debris. For the size of our given array and level of enrichment, the CO₂ cylinder needed to be replaced on a bi-monthly basis. The onshore location of the gas cylinder, and nearshore location of the experimental chambers facilitates the ease of the maintenance schedule, and requires a minimal number of personnel. Continued costs for CO₂ gas and electricity for the submerged pumps totaled US$ 45/month.

Comments and recommendations

Our system of open-top, flow-through chambers was effective in conducting long-term in situ benthic CO₂ enrichment within nearshore coastal communities. Such a design allows calls for climate change research, which increasingly replicates realistic field conditions, and studies the impacts of altered pCO₂ over long time periods. Furthermore, this experimental design can be used to study climate change responses across multiple scales, ranging from organismal physiological responses, to ecosystem level responses such as primary production and nutrient cycling (particularly in regards to benthic plant/algal communities). This system was used to target enrichment levels forecasted for the year 2100, however alternate enrichment targets could easily be replicated by adjusting gas delivery to the submerged array. As currently designed, constraint of the carbonate parameters within the chamber array was less precise as compared with ex situ mesocosm studies. We suggest that higher constraint could be achieved by incorporating a pH dependent feedback, using a gas solenoid linked to a pH monitor. Another weakness in this design is its relative restriction to nearshore environments. We submit that there are a number of larger, more powerful water pumps that could be used to increase the number of potential locations accessible by this design. Larger pumps could additionally be used to increase chamber replication within the system. Overall, we feel that our in situ CO₂ enrichment system has potential for future aquatic and marine climate change studies, particularly those focused on addressing organismal, community, and ecological scale responses of benthic algal/plant assemblages to increased pCO₂ and ocean acidification scenarios.

References


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