A short-term in situ CO₂ enrichment experiment on Heron Island (GBR)

David I. Kline1*, Lida Teneva2, Kenneth Schneider3, Thomas Miard1, Aaron Chai1, Malcolm Marker4, Kent Headley5, Brad Opdyke6, Merinda Nash6, Matthew Valetich6, Jeremy K. Caves2,6, Bayden D. Russell7, Sean D. Connell2,6, Bill J. Kirkwood5, Peter Brewer5, Edward Peltzer5, Jack Silverman8, Ken Caldeira3, Robert B. Dunbar2, Jeffrey R. Koseff9, Stephen G. Monismith9, B. Greg Mitchell10, Sophie Dove1 & Ove Hoegh-Guldberg1

Correspondence and requests for materials should be addressed to D.I.K. (dkline@ucsd.edu)

* Current address: Scripps Institution of Oceanography, Integrative Oceanography Division, University of California, San Diego, 9500 Gilman Drive, MC 0218, La Jolla, CA, 92093–0218.

Ocean acidification poses multiple challenges for coral reefs on molecular to ecological scales, yet previous experimental studies of the impact of projected CO₂ concentrations have mostly been done in aquarium systems with corals removed from their natural ecosystem and placed under artificial light and seawater conditions. The Coral–Proto Free Ocean Carbon Enrichment System (CP-FOCE) uses a network of sensors to monitor conditions within each flume and maintain experimental pH as an offset from environmental pH using feedback control on the injection of low pH seawater. Carbonate chemistry conditions maintained in the −0.06 and −0.22 pH offset treatments were significantly different than environmental conditions. The results from this short-term experiment suggest that the CP-FOCE is an important new experimental system to study in situ impacts of ocean acidification on coral reef ecosystems.

Atmospheric CO₂ levels are rising rapidly due to increasing fossil fuel combustion, deforestation, and construction1,2. The current CO₂ concentration of 389 ppm is the highest seen during the last 800,000 years, if not since the middle Pliocene (~3 million years ago), with a rate of increase ~2 orders of magnitude faster than during any time in the last 300 million years, with the possible exception of major mass-extinction events3,4. The world’s oceans have absorbed over 25% of the CO₂ emitted to the atmosphere since the beginning of the industrial revolution1,5 resulting in a decline in oceanic pH, termed ocean acidification (OA)6,7. Since pre-industrial times the ocean’s pH has declined by ~0.1 pH units8, representing a ~30% increase in hydrogen ion concentrations, and a rate of decline ~2 orders of magnitude faster than during glacial-interglacial transitions4,9. Oceanic pH in the year 2100 has been predicted to decline by up to an additional 0.2 to 0.5 pH units under the United Nations International Panel on Climate Change (IPCC) scenarios for CO₂ emissions4,10, levels more extreme than those seen in the last 40 million years8. Coral reefs are likely to be particularly vulnerable to ocean acidification; as reductions in calcification rates may influence the delicate balance between growth rates and erosional forces.

There is abundant evidence from laboratory experiments that OA causes reduced calcification in marine organisms11–13, however, the physiological impacts are often complex and life-stage dependent14,15. OA is likely to have synergistic impacts on the resilience of coral reefs to other disturbances including global warming (i.e. bleaching and disease events), local human stressors (e.g. declining water quality, over-fishing), and increased storm frequency and intensity16,17. It has also been suggested that the combined effects of hypoxia and OA may lead to the expansion of marine dead zones as increasing CO₂ concentrations further lowers the respiration index18. However, as most coral reefs are in the mixed layer and presumably in equilibrium with the atmosphere, hypoxia will likely not be a major factor. Yet, for studies below the mixed layer or in regions where the upwelling of low oxygen water onto the continental shelf is common or predicted as possible in the near future, the non-linear effects of combined hypoxia and hypercapnia should be considered. In addition, coral coring studies have
revealed a 20% decline in coral calcification rates over the last 20 years across the Great Barrier Reef, which is consistent with the impacts of increasing sea temperature and possibly ocean acidification\textsuperscript{14–16}. It has even been predicted that one third of coral species face extinction associated with global warming, OA and local stressors\textsuperscript{17}.

Marine ecosystems have been shown to have large regional and diel variability in pH\textsuperscript{23}, and recently variability in pCO\textsubscript{2} and pH within reef systems has been demonstrated. pCO\textsubscript{2} levels measured from the Lady Elliot Island reef flat, Southern Great Barrier Reef, showed variations as extreme as 200 ppm–1300 ppm in a single diel cycle\textsuperscript{21}. In Ningaloo reef (Western Australia) and Lizard and Magnetic Islands (GBR) pH fluctuations of up to 0.42 pH units were measured, with the variations dependent on sampling locality rather than diel cycles\textsuperscript{22}. Similarly, diel pH changes as high as 0.75 have been recorded in the Heron Island reef lagoon\textsuperscript{21}. In Palmyra, Northern Line Islands a pH range of 0.253 was measured on the reef terrace and 0.121 on the fore reef, while a fringing reef in Moorea, French Polynesia had a pH range of 0.101 and both locations showed clear diel cycles in pH\textsuperscript{22}. Incorporating regional and diel variability in pH into OA experiments will be critical for better understanding the future impacts of OA on marine ecosystems\textsuperscript{22}.

Studies of natural systems with high CO\textsubscript{2} levels such as vent sites and upwelling areas are beginning to provide critical data about the long-term impacts of ocean acidification on organisms, communities and ecosystems\textsuperscript{24–26}. Studies from CO\textsubscript{2} vent sites reveal a decline in reef rugosity, diversity, and the prevalence of calcareous organisms with declining pH levels\textsuperscript{27,28,29}. Similarly, coral reefs near coastal margins with upwelling of deep lower pH waters have revealed that coral reefs in a high CO\textsubscript{2} world will likely be poorly cemented\textsuperscript{30}. Furthermore, biogeography studies reveal that cold-water corals\textsuperscript{31,32} and scleractinian corals\textsuperscript{33} tend to grow in areas with higher aragonite saturation states (\(\Omega_{\text{arag}} > 1\) and >3.5 respectively). Additionally, in situ studies conducted with the Submersible Habitat for Analyzing Reef Quality (SHARQ), a clear incubation tent to monitor in situ calcification rates without CO\textsubscript{2} manipulation, revealed that a reef flat in Hawaii begins dissolving between 467–1003 \(\mu\text{mEq}\) of CO\textsubscript{2} depending on the substrate type\textsuperscript{44,45}. Further support for these measurements was provided by a long coral reef metabolism record from the Gulf of Eilat that demonstrated a positive relation between calcification and \(\Omega_{\text{arag}}\), temperature and nutrients\textsuperscript{46}. This empirical relation was applied to reefs globally using model results of \(\Omega_{\text{arag}}\) and sea surface temperature\textsuperscript{47} indicating that reef calcification rates will decline in the future and will eventually start to dissolve when atmospheric CO\textsubscript{2} levels reach twice their preindustrial levels (560 ppm). However, it has been suggested that coral acclimation may be a mechanism to reduce OA impacts on coral calcification and research into this topic is ongoing\textsuperscript{48–50}.

While important insights have been gained from natural in situ experiments, these studies have not allowed for experimental control over treatment conditions. In these studies, organisms are often exposed to pH levels that are more extreme and variable than those that are predicted to occur in the next century. Furthermore, confounding environmental factors often make the results difficult to interpret\textsuperscript{51}. Additionally, these often extreme in situ conditions frequently occur as a pulse, with brief extreme conditions, rather than as a chronic stress as would occur if pH were delta offset from controls as will occur with OA. In situ carbon dioxide perturbation experiments allow for greater replication and better control over treatment conditions. In 2007, the Monterey Bay Aquarium Research Institute (MBARI) developed the FOCE (Free Ocean Carbon Enrichment) experimental system, which allowed for highly controlled semi-enclosed experiments in the deep sea within a \(1 \times 1 \times 0.5\) m enclosure within a flume\textsuperscript{48,52}. We modified the MBARI FOCE system for application to the challenging conditions found on coral reefs including warm temperatures, high flow rates, high UV, and high wave energy\textsuperscript{53}. The Coral Proto – Free Ocean Carbon Enrichment system (CP-FOCE) was developed to test the impact of ocean acidification on coral reef organisms as in natural a reef setting as possible. In situ experiments make it possible to study how organisms and ecosystems respond to lowered pH and \(\Omega_{\text{arag}}\) with natural conditions including the associated reef biodiversity, ecosystem interaction, ambient seawater chemistry, natural sediment communities, as well as diel and seasonal changes in environmental conditions\textsuperscript{54}. The CP-FOCE provides an important experimental platform to study the complex, interactive impacts of ocean acidification on coral reef organisms. It was designed to produce predicted future pH conditions as an offset from environmental conditions within discrete limits in situ, to test the effects of future conditions on the coral reef community. Here we present results from a short-term deployment on the Heron Island reef flat with three pH levels and experimental flumes containing key ecological elements of the reef community, corals (Acropora millepora), crustose coralline algae (CCA, Porolithon onkodes), and reef sediments.

**Results**

**Carbonate Chemistry Measurements.** Carbonate chemistry measurements taken in the three CP-FOCE flumes and the environment reveal typical diel variability in all of the measured parameters (Fig. 1). Dissolved Inorganic Carbon (DIC) in all flumes decreased during the day and increased during the night. The difference between the \(-0.06\) pH treatment and the environment had consistently higher DIC values than the control minus the environment, and the difference between \(-0.22\) pH treatment and the environment was consistently higher than the \(-0.06\) pH and the control treatments, except during the low tide slack water periods when there was slower water movement. When the DIC measurements were compared across a four-day period (Fig. 2A), the average values as offset from the environment were 3.98 ± 19.39 (±SD) for the controls, 48.68 ± 56.41 for the \(-0.06\) pH treatment and 101.04 ± 53.64 for the \(-0.22\) treatment. The \(-0.06\) pH treatment had significantly greater offsets compared to the control offsets with respect to the ambient DIC (Table 1, n=23, p=0.00082, df=44, F=12.91, Single Factor ANOVA). The \(-0.22\) pH treatment produced even greater DIC excursions from ambient conditions compared to the control (Table 1, n=23, p=2.4x10\textsuperscript{–10}, df=44, F=66.61, Single Factor ANOVA). Additionally, the DIC offset was significantly higher in the \(-0.22\) pH treatment than at the \(-0.06\) pH treatment (Table 1, n=23, p=0.0024, df=44, F=10.41, Single Factor ANOVA).

Total alkalinity (\(A_{\text{T}}\)) measurements were almost identical across the experimental period except for some variability during slack low tides (Fig. 1B,D). There was no significant difference in \(A_{\text{T}}\) values across the 3 CP-FOCE flumes during the course of the four-day experiment (n=23, p=0.923, df=66, F=0.08, Single Factor ANOVA).

The pH measurements were almost identical between the environment and the control treatments, except during the low tide slack water periods. The pH measurements in the environment showed a diel variation, with a maximum pH during the day of over 8.4 (around 16:30 local time) and a minimum pH of 8.0 at night (around 03:30 local time; Fig. 1C). The control CP-FOCE flume also showed extreme diel cycles with a maximum pH of over 8.3 during the day and a minimum pH below 7.9 at night, values very similar to those measured by Santos et al. (2011) on the same reef flat the week before this experiment was conducted\textsuperscript{44}. The pH in the \(-0.06\) pH unit treatment was lower than the control treatment by an average value of 0.055±0.11 (±SD). Similarly, the pH in the \(-0.22\) pH treatment was consistently lower than the \(-0.06\) pH by an average of 0.17±0.18 and from the control treatments by an average of 0.23±0.15. The recorded pH offsets from the environment in the \(-0.06\) pH treatment was significantly lower than the control (Table 1, n=23, p=0.014, df=44, F=6.55, Single Factor ANOVA) as were those in the \(-0.22\) pH treatment (Table 1, n=23,
Figure 1 | Diel fluctuations in seawater chemistry in the environment and the three CP-FOCE flumes for the short-term experiment on the Heron Island reef flat. Discrete carbonate chemistry measurements (DIC, ALK, pH, O₂) were made every 3–6 hours throughout the experiment. The system was able to manipulate pH at environmental flow rates up to 0.1 m²s⁻¹, which could explain why pH and DIC control was not as controlled during transitions from low to high tides and vice versa as these were periods of maximum flow rates. Grey shading indicates night time periods with no light.
p = 2.4 × 10⁻⁷, df = 42, F = 39.72, Single Factor ANOVA). Additionally, the −0.22 pH treatment had significantly lower pH than the −0.06 pH treatment (Table 1, Fig. 2B, n = 23, p = 5.6 × 10⁻⁵, df = 44, F = 20.47, Single Factor ANOVA).

Dissolved oxygen measurements were similar during the control and the environment except during low tide period during the day when they were higher in the environment (Fig 1E). The dissolved oxygen measured in the −0.06 pH treatment was consistently similar to the control dissolved oxygen except during low tide during the day when it was higher in the −0.06 treatment. The dissolved oxygen measurements in the −0.22 treatments were consistently lower than the other treatments during the day and higher at night although these trends became less pronounced from the first to the fourth day of the experiment.

The average calculated value of Ω_{arag} in the control flume was 3.76 ± 0.70 (±SD), in the −0.06 flume it was 3.28 ± 0.98, and in the −0.22 flume it was 2.80 ± 0.88. Thus, the average decline in the Ω_{arag} for the −0.06 pH and the −0.22 pH treatments relative to the control were 0.48 ± 0.64 and 0.96 ± 0.50, respectively (Fig. 2C). Ω_{arag} values in the −0.06 pH and −0.22 pH treatments were significantly lower than the control (Table 1, n = 23, p = 0.0029, df = 44, F = 10.59; and n = 23, p = 4.2 × 10⁻⁵, df = 44, F = 53.22, respectively) as well as significantly different from each other (Table 1, n = 23, p = 0.012, df = 44, F = 6.90). The change in Ω_{arag} between the experimental and control flumes indicates that the CO₂ enrichment in the CP-FOCE was effective.

Sediment and coralline algae dissolution and mineralogy. Surface sediment samples were taken from each of the flumes and the environment at the end of the experiment to assess if any pH related change in the amount of Mg-calcite present or the average mol % MgCO₃ of the Mg-calcite⁴ occurred (Fig. 3). These samples were collected within meters of each other and present a range of sediment mineralogy with average mol % MgCO₃ varying from 15.1–15.7 mol %, quantity of aragonite from 36–41%, and the calcite Mg-calcite group 64–59%. There was no significant difference between the control and environmental mol % MgCO₃ or Mg-calcite quantity, compared to the −0.06 pH or −0.22 pH treatments at the end of the experimental period. However, approximately 20% of the Mg-calcite quantity would need to dissolve for there to be a measurable change in sediment Mg-calcite quantity that could be attributable to the treatments.

CCA samples showed weight losses (dissolution) of 1.22 %, 1.76 % and 2.12 % for the control, −0.06 pH and −0.22 pH treatments, respectively (Table 2, Fig. 4). For the control and −0.1 pH treatments the % aragonite was lower in the CCA samples with least weight loss, but there was only a weak correlation for the −0.22 pH treatment (Fig. 4A). Weight loss was lower in CCA samples with higher mole %MgCO₃ as measured by ICP (Fig. 4B).

**Discussion**

The CP-FOCE as an *in situ* system avoids many of the problems associated with tank or mesocosm experiments such as unnatural light, isolation from key local hydrodynamics, controls on food energetics, disturbed microbial populations, lack of sediment and associated animal communities. Importantly, it allows for well-controlled *in situ* OA experiments with realistic future pH maintained as an offset from environmental conditions, as opposed to being limited by the often extreme pH variations often associated with natural experiments such as at CO₂ vents²⁶⁻²⁸.

The carbonate chemistry measurements from the environment reveal diel variations in DIC, pH, alkalinity, and dissolved oxygen that roughly followed the light intensity/dark cycle with the tidal cycle superimposed on it (Fig. 1). The carbonate chemistry clearly reveal diel variations in DIC, pH, alkalinity, and dissolved oxygen that roughly followed the light intensity/dark cycle with the tidal cycle superimposed on it (Fig. 1). The carbonate chemistry clearly had an association with the tidal cycles, but the metabolic signal is likely stronger and controls the diel swings in carbon chemistry, which is exaggerated at low tide when the seawater is stagnant. Daytime decline in DIC and increase in pH and O₂ (Fig. 1) were the result of net photosynthesis by the reef community. At night, the trend was reversed as respiration by reef organisms increased the DIC and decreased the pH and O₂. A_{T} maxima near sunrise are attributed to low precipitation and/or dissolution of CaCO₃ during the night and A_{T} minima near sunset would be due to the cumulative effect of daytime calcification. While our sampling resolution does not allow for an estimation of rates of change in DIC, pH, and O₂ on an hourly basis, the data suggest that the fastest rates of change occur shortly after sunrise and shortly after sunset. This is probably caused by the physiological shifts on the reef from day to night metabolism. A longer dataset will be necessary to decouple the contributions of light-driven reef metabolism and tidal hydrodynamics on the trends in the carbon system parameters, but in shallow reef flats due to a

**Table 1 | P-values for ANOVA on carbonate chemistry measurements from the 3 CP-FOCE flumes.** All treatments were significantly different from each other and the control flume for pH, Dissolved Inorganic Carbon (DIC) and Aragonite saturation state (Ω_{arag}).

|        | Control | −0.06 pH Treatment | −0.22 pH Treatment |
|--------|---------|---------------------|--------------------|
| pH     | 0.014   | 2.4 × 10⁻⁷          |                    |
| DIC    | 0.00082 | 4.5 × 10⁻⁵          | 2.4 × 10⁻¹⁰        |
| Ω_{arag} | 0.0029  | 4.2 × 10⁻⁸          | 0.012              |

|        | Control | −0.06 pH Treatment |
|--------|---------|---------------------|
| Surface |         |                     |

Figure 2 | Changes in carbonate chemistry measurements in the 3 CP-FOCE flumes. Average offsets from ambient carbonate chemistry, recorded in the control, −0.06 and −0.22 treatment flumes including A) DIC and B) pH and C) calculated Ω_{arag} in the three CP-FOCE flumes throughout the course of the 4 day experiment. ** Denote that the treatments were significantly different from the controls and each other at p<0.05.
smaller water volume and slower exchange with the water beyond the reef, the biological signal of reef community metabolism tends to be the dominant modulator of carbon and oxygen budgets on diurnal and seasonal time-scales.

The data presented here clearly demonstrate that the CP-FOCE approach successfully produced and maintained experimental conditions in the treatment flumes closely and consistently tracking the environmental conditions with an offset in the DIC and pH levels with the $-0.06$ pH treatment consistently higher than the control, and the $-0.22$ pH treatment consistently higher than the $-0.06$ pH treatment (Fig. 2). The demonstration from this prototype system has significant implications, as it indicates that similar manipulative experiments can be conducted on other reefs and in other coastal ecosystems with valuable scientific outcomes.

XRD results showed that most CCA samples (16 of 19) had aragonite present, six displayed asymmetrical Mg-calcite curves which represented the presence of dolomite ($\text{Ca}_{0.5}\text{Mg}_{0.5}\text{CO}_3$) and magnesite ($\text{MgCO}_3$) within the CCA crust$^{46}$ and seven displayed asymmetry potentially indicative of dolomite (Table 2). There are trends of increasing weight loss with increasing aragonite across all treatments (Figure 4A), correlation is strong for control and $-0.06$ pH with a $R^2$ of 0.58 ($n = 8$) and 0.89 ($n=6$) respectively, however poor for $-0.22$ pH 0.08 with a $R^2$ of (n=8). There was no clear trend with mol % MgCO$_3$ and dissolution, however, 3 of the 4 highest mol % were in the lower weight loss grouping. Notably, samples with the smallest weight loss typically had very asymmetrical XRD Mg-calcite curves. The ICP-AES bulk magnesium mol % MgCO$_3$ shows a clear trend. The two CCA samples with the lowest bulk magnesium [22.61 (control) and 15.64 (2.22 pH) mol % MgCO$_3$] lost more than 7 times the weight than those with higher magnesium [27.01 (control) and 23.8 (2.22 pH) mol % MgCO$_3$] (Figure 4B). Changes in mineralogy from a weight loss of 2–3% are likely below the detection level of the XRD method and could not account for the 8% difference in ICP in the 2.22 pH treatment. Magnesium enriched CCAs (Fig 4B) demonstrated dolomite ($\text{Mg}_{0.50}\text{CaCO}_3$) and magnesite ($\text{MgCO}_3$) features in XRD. These results suggest that magnesium enrichment by dolomite and magnesite lead to reduced dissolution relative to corallines with magnesium enriched phases. Although there was not

![Figure 3](image.jpg) X-Ray Diffraction (XRD) patterns for surface sediment samples taken after 4 days of experimental treatment. Scans for control, $-0.06$ pH and $-0.22$ pH treatments have been stacked for ease of viewing and their scale is the same as for the environment scan. In this figure: A = aragonite peaks with % aragonite, F= fluorite standard, C = calcite, Mg-C = Mg calcite with mole % Mg calcite.
sufficient replication in the bulk-magnesium samples for statistical tests, these preliminary data warrant further investigation as they are clearly in contrast with existing theories that predict increased dissolution with increasing magnesium content and support the proposition that magnesium content is only a minor factor in biogenic Mg-calcite solubility. The discovery of aragonite, dolomite and magnesite in the CCA samples together with these results will necessarily require a reconsideration of applicability of the $\Omega_{\text{Mg-calcite}}$ for predicting impacts of OA on CCAs.

This is the first in situ study to demonstrate the ability to simulate ocean acidification conditions in a shallow coastal ocean environment. Our results show that it can accurately change the carbonate chemistry of ambient seawater, simulating future predicted conditions of ocean acidification. This is the first study to document the predicted pH conditions simulated as an offset from the pH conditions that change naturally throughout the day cycle on the reef flat especially at low tide where a relation between photosynthesis, respiration, calcification and CaCO$_3$ dissolution control the ambient pH. This capability is extremely valuable and highlights the importance of running experiments as an offset from environmental conditions rather than as a constant pH.

The simulation of future pH changes, however is not linear because changes in DIC associated with respiration, photosynthesis and OA will lead to non-linear changes in pCO$_2$ and pH influencing diel carbonate chemistry fluctuations. The CP-FOCE could be modified to control for other carbon system parameters such as $\Omega_{\text{arag}}$, but pH is a convenient control variable from an engineering perspective as it can be measured directly with an electrode, while controlling for other parameters would require either making assumptions about or measurements of other carbon system parameters.

Both monitoring large natural diel variations in pH as well as simulating large diel pH variations with an offset towards more acidic conditions can provide valuable insight into ocean acidification sensitivity of various reef organisms and environments. As these reef flat communities regularly experience pH changes equivalent to conditions ranging from pre-industrial levels (280 ppm) to those predicted for 2100 (600–1000 ppm) in one diel cycle, perhaps they are less vulnerable to ocean acidification than other reef sub-environments.

Methods

The CP-FOCE uses replicate experimental flumes to enclose sections of the reef and dose them with CO$_2$ enriched seawater using peristaltic pumps with computer controlled feedback dosing (Fig. 5). Each flume is connected to a waterproof computer pod (National Instruments, CompacRIO, TX, USA) for pH control and to log its instruments including MBARI digital pH sensors (Nido Instruments, CA, USA), acoustic velocimeters (Nortek AS, Vectrinos, Rud, Norway), and a Conductivity, Temperature, Depth instrument (SBE-16plusV2 SEACAT, Sea-Bird Electronics, WA, USA). In addition, an identical set of sensors was deployed in ambient conditions on the reef flat to monitor environmental conditions and to determine a baseline for pH offsets (Fig. 5A).

pH Dosing. Seawater from the Heron Island Research Station aquarium system is pumped from the reef flat near the experiment and is treated onshore with CO$_2$ in a 1000 L sealed tank to reduce its pH to around 5.0. The water then passes through 1/2” polyethylene white plastic tubing to the 6 peristaltic pumps (2 per flume, Watson-Marlow Inc., 520 FDM, Wilmington, MA, USA) that are mounted in a waterproof housing on the instrumentation float. A parallel tank with seawater without CO$_2$ addition is also connected to the control flume. Feedback control of the pH is provided by the pH, temperature, and current velocity and speed measurements to determine whether the forward or reverse peristaltic pump should be activated and at what rate (Fig. 5E). The pH treatments are produced as an offset from the environment pH levels in order to have treatments reflect the diel variation in reef flat pH. The difference from the environment pH and the experimental offset determines the flume pH set point that is compared to the pH measured in the flume. This produces an error value, which is processed by a simple Proportional and Integral (PI) control algorithm. The PI algorithm parameters are tuned to optimally operate over...
the measured range of flow rates, and produces a demand signal that is converted to pulse width modulation (PWM) to control the power going to the dosing pump motors. The flow direction and speed is determined by the current measurements and the direction of the flow is used to determine which dosing pump motor is turned on so that the low pH water is added in the direction of the ambient flow (Fig. 5E). pH dosing did not work effectively at flow speeds above $0.1 \text{ m/s}$ in the chambers because of the pumping rates required to sufficiently lower the pH within the flumes at these high flow rates. The cross-sectional area of each flume is $0.0625 \text{ m}^2$, and for a 0.3 pH offset the approximate required flow rate for the pH 5.0 water addition at a chamber flow speed of $0.1 \text{ m/s}$ would be approximately $1.5 \text{ L/s}$ and for a

---

**Figure 5 | Photographs and schematics of the CP-FOCE as deployed on the Heron island reef flat in December 2009.** A) Shows a wide view of the experimental system including the 3 CP-FOCE flumes and the wind generator. B) Photograph of the experimental float that has the 4, 12 V deep cycle batteries as ballast, and contains all of the control computers, peristaltic pumps, the solar panel, and the radio antennae to transmit the data real-time back to the laboratory. C) Schematic of a CP-FOCE flume showing the 3 experimental sections and the flow conditioners with CO2 enriched seawater injection sites. D) Photograph of a section of a CP-FOCE flume containing a rack with *Acropora millepora* corals and the pump that is turned on when the doors are closed to provide water movement. E) Schematic of the feedback control between the pH sensors, flow sensor and the National Instrument control computers which decide which peristaltic pump to turn on and at what rate for the dosing.
Disolved oxygen values were determined using the modified Winkles method\(^\text{49}\) on samples preserved in 50 mL BO D bottles using a Metrohm titrator. Results obtained from duplicates samples were all within the acceptable range of analytical error (± 1 mL L\(^{-1}\)).

Measured \(A_t\), DIC, and \(T\) along with average Heron reef flat salinity of 34.7 psu were used to derive other carbonate system parameters (pCO\(_2\), CO\(_2\)\(^\text{eq}\)) using CO\(_2\)SYS\(^\text{25}\). Dissociation constants K1 and K2 were calculated according to Merhbach et al. (1973)\(^\text{32}\) refit by Dickson and Millero (1987)\(^\text{19}\), and KSO\(_4\) was from Dickson (1990)\(^\text{30}\).

Dissolution Experiments. To obtain an estimate of Mg-calcite dissolution rates, samples of dead CCA's were placed in the control and each of the treatment flumes. These samples were collected fresh and sun dried for several days until there was no measurable photosynthetic activity, as measured by pulse amplitude modulated fluorometer (PAM, Waltz, Germany). At the beginning and end of the experiment, samples were oven dried at 40 °C and weighed to the nearest 0.00001 g. The mo\(\text{mol}\) of \(\text{g CO}_2\) of the individual CCA samples was measured using X-ray Diffraction (XRD), following Nash 2011\(^\text{25}\). Carbonate mineral phases were quantified by XRD using the area under the curve method\(^\text{30}\) and bulk magnesite was measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) on a subset of four of the samples that had the highest and lowest weight loss\(^\text{25}\).

Statistics. All tests for statistically significant difference between populations were performed with single factor, one-way ANOVAs.

Data Management. Data used in the analyses for this paper have been deposited with the NO-AA National Oceanographic Data Center (NODC). Please contact NODC directly to obtain a copy of these data.

1. Raupach, M. R. et al. Global and regional drivers of accelerating CO\(_2\) emissions. Proc Nat Acad Sci USA 104, 10288–10293 (2007).
2. Sabine, C. L. et al. The oceanic sink for anthropogenic CO\(_2\). Science 305, 367–371 (2004).
3. Pagani, M., Liu, Z. H., LaRiviere, J. & Ravelo, A. C. High Earth-system climate sensitivity determined from Pliocene carbon dioxide concentrations. Nat Geosci 3, 27–30 (2010).
4. Honisch, B. et al. The geological record of ocean acidification. Science 335, 1058–1063 (2012).
5. Millero, F. J. The marine inorganic carbon cycle. Chem Rev 107, 308–341 (2007).
6. Caldeira, K. & Wickett, M. E. Anthropogenic ocean and carbon pH. Nature 425, 365–365 (2003).
7. Feely, R. A. et al. Impact of anthropogenic CO\(_2\) on the CaCO\(_3\) system in the oceans. Science 305, 362–366 (2004).
8. Pelejero, C., Calvo, E. & Hoegh-Guldberg, O. Paleo-perspectives on ocean acidification. Trends Ecol Evol 25, 332–344 (2010).
9. Caldeira, K. & Wickett, M. E. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. J Geophys Res-Oceans 110, C09S04 (2005).
10. Solomon, S. Intergovernmental Panel on Climate Change. Working Group I. Climate change 2007: the physical science basis: contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. (Cambridge University Press, 2007).
11. Doney, S. C., Fabry, V. J., Feely, R. A. & Kleypas, J. A. Ocean acidification: the other CO\(_2\) problem. Annu Rev Mar Sci 1, 169–192 (2009).
12. Kleypas, J. A. & Yates, K. K. Coral reefs and ocean acidification. Oceanography 22, 108–117 (2009).
13. Schneider, K. & Erez, J. The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral Acropora eurystoma. Limnol Oceanogr 51, 1284–1293 (2006).
14. Hendriks, I. E., Duarte, C. M. & Alvarez, M. Vulnerability of marine biodiversity to ocean acidification: A meta-analysis. Estuar Coast Shelf Sci 86, 157–164 (2010).
15. Dupont, S., Dorey, N. & Thorndyke, M. What meta-analysis can tell us about vulnerability of marine biodiversity to ocean acidification? Estuar Coast Shelf Sci 89, 182–185 (2010).
16. Anthony, K. R. N., Kline, D. I., Diaz-Pulido, G., Dove, S. & Hoegh-Guldberg, O. Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc Nat Acad Sci USA 105, 14422–14427 (2008).
17. Morse, J. W., Arvidson, R. S. & Latrue, A. Calcium carbonate formation and dissolution. Chem Rev 107, 342–381 (2007).
18. Brewer, P. G. & Peltzer, E. T. Limits to marine life. Science 324, 347–348 (2009).
19. De ath, G., Lough, J. M. & Fabricius, K. E. Declining coral calcification on the great barrier reef. Science 322, 116–119 (2009).
20. Cooper, T. E., De Ath, G., Fabricius, K. E. & Lough, J. M. Declining coral calcification in massive Porites in two nearshore regions of the northern Great Barrier Reef. Glob Change Biol 14, 529–538 (2008).
21. Carpenter, K. E. et al. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science 326, 560–563 (2009).
22. Hofmann, G. E. et al. High-frequency dynamics of CO\(_2\) pH: a multisystem comparison. PLoS One 6, e28933 (2011).
23. Shaw, E. C., McNeil, B. I. & Tilbrook, B. Impacts of ocean acidification in naturally variable coral reef flat ecosystems. J Geophys Res-Oceans 117, C03038 (2012).
24. Gagliano, M., McCormick, M., Moore, J. & Depczynski, M. The basics of acidification: baseline variability of pH on Australian coral reefs. *Mar Biol* **157**, 1849–1856 (2010).
25. Santos, I. R., Glad, R. N., Maher, D. & Eyre, B. D. Diel coral reef acidification driven by pore water advection in permeable carbonate sands, Heron Island, Great Barrier Reef. *Geophys Res Lett* **38**, L03604 (2011).
26. Fabricius, K. E. *et al.* Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nat Clim Change* **1**, 165–169 (2011).
27. Barry, J. P. From extreme CO2 venting to ocean acidification - Experimental approaches to assess the fate of deep sea ecosystems. *Geochim Cosmochim Acta* **73**, A93–A93 (2009).
28. Hall-Spencer, J. M. *et al.* Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* **454**, 96–99 (2008).
29. Munro, D. R., Dunbar, R. B., Mucciaronne, D. A. & Arrigo, K. R. Stable isotope composition of dissolved inorganic carbon and particulate organic carbon in sea ice from the Ross Sea, Antarctica. *J Geophys Res* **115**, C12 (2010).
30. Manzello, D. P. *et al.* Poorly cemented coral reefs of the eastern tropical Pacific: Possible insights into reef development in a high-CO2 world. *Proc Nat Acad Sci USA* **105**, 10450–10458 (2008).
31. Guinotte, J. M. *et al.* Will human-induced changes in seawater chemistry alter the distribution of deep-sea scleractinian corals? *Front Environ 4*, 141–146 (2006).
32. Turley, C. M., Roberts, J. M. & Guinotte, J. M. Corals in deep-water: will the unseen hand of ocean acidification destroy cold-water ecosystems? *Coral reefs* **26**, 445–488 (2007).
33. Kleypas, J. A. *et al.* Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science* **284**, 118–120 (1999).
34. Yates, K. K. & Halley, R. B. Measuring coral reef community metabolism using new benthic chamber technology. *Coral reefs* **22**, 247–255 (2003).
35. Yates, K. K. & Halley, R. B. CO2 and pCO2 thresholds for calcification and dissolution on the Molokai reef flat, Hawaii. *Biogeochemistry* **3**, 357–369 (2006).
36. Silverman, J., Lazar, B. & Erez, I. Effect of aragonite saturation, temperature, and nutrients on the community calcification rate of a coral reef. *J Geophys Res-Oceans* **112**, C05004 (2007).
37. Silverman, J., Lazar, B., Cao, L., Caldeira, K. & Erez, I. Coral reefs may start dissolving when atmospheric CO2 doubles. *Geophys Res Lett* **36**, L05606 (2009).
38. Form, A. U. & Riebesell, U. Acclimation to ocean acidification during long-term CO2 exposure in the cold-water coral Lophelia pertusa. *Glob Change Biol* **18**, 843–853 (2012).
39. McCulloch, M., Falter, J., Trotter, J. & Montagna, P. Coral resilience to ocean acidification and global warming through pH up-regulation. *Nat Clim Change*, In press (2012).
40. McCulloch, M. *et al.* Resilience of cold-water scleractinian corals to ocean acidification: Boron isotopic systematics of pH and saturation state up-regulation. *Geochimica et Cosmochimica Acta*, In press (2012).
41. Barry, J., Hall-Spencer, J. & Tyrrell, T. in *Guide to best practices for ocean acidification research and data reporting* (eds Riebesell, U., Fabry, V. J., Hansson, L. & Gattuso, J. P.) 123–136 (Publications Office of the European Union, 2010).
42. Walz, P. M., Kirkwood, W. J., Peltzer, E. T., Hester, K. C. & Brewer, P. G. Creating *Mar Biol*, In press (2012).
43. Santos, I. R., Glad, R. N., Maher, D., Erez, I. B. Die coral reef acidification driven by porewater advection in permeable carbonate sands, Heron Island, Great Barrier Reef. *Geophys Res Lett* **38**, L03604 (2011).
44. Andersson, A. J., Mackenzie, F. T. & Bates, N. R. Life on the margin: implications for coral reefs. *Coral reef flat, Hawaii. Biogeochemistry* **3**, 357–369 (2006).
45. Andersson, A. J., Mackenzie, F. T. & Bates, N. R. Life on the margin: implications for coral reefs. *Coral reef flat, Hawaii. Biogeochemistry* **3**, 357–369 (2006).
46. Andersson, A. J., Mackenzie, F. T. & Bates, N. R. Life on the margin: implications for coral reefs. *Coral reef flat, Hawaii. Biogeochemistry* **3**, 357–369 (2006).
47. Andersson, A. J., Mackenzie, F. T. & Bates, N. R. Life on the margin: implications for coral reefs. *Coral reef flat, Hawaii. Biogeochemistry* **3**, 357–369 (2006).
48. Tribollet, A., Godinot, C., Atkinson, M. & Langdon, C. Effects of elevated pCO2 on dissolution of coral carbonates by microbial endoliths. *Global Biogeochem Cycles* **23**, GB3008 (2009).
49. Orliordan, C. A., Monismith, S. G. & Koseff, J. R. A Study of Concentration Boundary-Layer Formation over a Bed of Model Bivalves. *Limnol Oceanogr* **38**, 1712–1729 (1993).
50. Randall, D. A. *et al.* in Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (eds Solomon, S. D. *et al.*) (Cambridge University Press, 2007).
51. Marker, M. *et al.* The Coral Proto Free Ocean Carbon Enrichment System (CP-FOCE): Engineering and Development. *OCEANS IEEE 2010*, 1–10 (2010).
52. Dickson, A. G., Afghan, J. D. & Anderson, G. C. Reference materials for oceanic CO2 analysis: a method for the certification of total alkalinity. *Mar Chem* **80**, 185–197 (2003).
53. Strickland, J. D. H. & Parsons, T. R. A practical handbook of seawater analysis. *Ball Fish Res Bd Canada*, Bull 167, 311 p. (1972).
54. Lewis, E. & Wallace, W. R. *CO2SYS-Program developed for the CO2 system calculations*. (Carbon Dioxide Information Analysis Center, Oak Ridge, Tennessee, 1998).
55. Mehrbach, C., Culberson, C. H., Hawley, J. E. & Pytkowicz, R. M. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol Oceanogr* **18**, 897–907 (1973).
56. Dickson, A. G. & Millero, F. J. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Res 34*, 1733–1743 (1987).
57. Dickson, A. G. Standard potential of the reaction: AgCl(s) + 1/2 H2(g) = Ag(s) + HCl(aq), and the standard acidity constant of the ion HSO− in synthetic seawater from 273.15 to 318.15 K. *J Chem Thermodyn* **22**, 113–127 (1990).
58. Sepulcre, S., Durand, N. & Bard, E. Mineralogical determination of reef and periplatform carbonates: Calibration and implications for paleoceanography and radiochronology. *Global Planet Change* **66**, 1–9 (2009).
59. Ricker, W. E. Linear regressions in fishery research. *Bull Fish Res Bd Canada*, Bull **30**, 409–443 (1973).
60. Kuffner, I. B., Andersson, A. J., Jokiel, P. L., Rodgers, K. S. & Mackenzie, F. T. Decreased abundance of crustose coralline algae due to ocean acidification. *Nat Geosc* **1**, 114–117 (2008).

**Acknowledgments**

The authors wish to acknowledge the Australian Research Council (ARC) Linkage Infrastructure, Equipment and Facilities grant #LE0989068, ARC Linkage grant #LP0775303, ARC Centre of Excellence grant #CE1056145, a Queensland Government Smart State Premier’s Fellowship to OHG, National Science Foundation grants (NSF OCE-0729236, to RBD and JRK; and NSF-ATM-0941760 to BGM), and the Pacific Blue Foundation for supporting this research. We also wish to thank the staff of the Heron Island Research station especially Matt Roy, Steve Venn, Kyra Hay, and Liz Perkins for all of their support throughout this project. Permits from the Department of Environment and Resource Management (#CSC80874010) and the Great Barrier Reef Marine Park Authority (GC09/29996.1) were provided to conduct this research.

**Author Contributions**

DIK, LT, and KS wrote the main manuscript text. DIK, LT, and MN prepared the figures, MN and BO did the XRD and ICP measurements, DIK, LT, KS, TM, AC, MM, KH, BO, MN, MV, JC, BDR, SDC, SD and OHG participated in field work and carbonate chemistry measurements. All authors reviewed the manuscript.

**Additional information**

**Competing financial interests:** The authors declare no competing financial interests.

**License:** This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-sa/3.0/

**How to cite this article:** Kline, D.I. *et al.* A short-term in situ CO2 enrichment experiment on Heron Island (GBR). *Sci. Rep.* **2**, 413; DOI:10.1038/srep00413 (2012).