Final Report
Part 1. ADMINISTRATIVE

Submitted To:
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Fish and Wildlife Agreement Number 12170-B-G101

Project Title**:** Synergistic impact of global warming and ocean acidification on calcification in shallow and deep coral reef environments

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Signature of Submitting Official

Part 2. PUBLIC SUMMARY:

One of the greatest ecological, social and economic issues of the day is the problem of climate change. Increasing levels of carbon dioxide (CO_2) in the atmosphere are increasing global temperatures. Much of the CO_2 dissolves in the ocean, creating more acidic conditions and leading to a process known as ocean acidification (OA). Higher temperatures and increased levels of CO_2 operating independently are known to be detrimental to corals, but little is known about their effect when operating in unison. Irradiance has a great influence on coral calcification rates and can interact with higher temperature and increased OA to an unknown extent. Therefore, experiments were performed in continuous flow mesocosms under various levels of solar radiation in order to describe the biological response of corals at the upper lethal temperature. Corals were grown under three conditions of irradiance (100%, 50% 8%), two pCO_2 levels (present day levels and twice present) and various temperature regimes ranging up to the high temperatures that will occur during this century due to global warming.

The results of this research indicate more rapid rates of bleaching in coral reefs under acidified conditions. During these experiments coral growth rates were suppressed under future climate change conditions, and high irradiance had the greatest effect on coral growth and bleaching. Temperature was more important than pCO_2 in controlling growth.

The interaction of temperature and irradiance had the strongest influence on coral growth rates. Therefore, low irradiance levels potentially provide a refuge for corals from thermal and irradiance stress in the deeper parts of their range. Coral health and mortality was observed to be influenced by the interaction of all three factors, while bleaching and mortality was accelerated under acidified conditions. Lastly, recovery rate of corals from bleaching was highest with lowest mortality rate in corals influenced by a single stressor. Recovery from bleaching was slowest and mortality highest in corals experiencing more than one climate change stressor.

3. PROJECT REPORT

A. TECHNICAL SUMMARY:

The aim of this project was to describe the interactions between the temperature, ocean acidification and solar irradiance on coral growth and mortality. These objectives were met. Upper lethal temperature threshold for corals at present day and future levels of ocean acidification were described in detail along with descriptions of the bleaching response and recovery rate. These data are necessary for the further development of models directed at evaluating the future impact of climate change on Pacific coral reef communities. In addition we made two major breakthroughs. The first was development of an inexpensive and very effective system for studying impact of OA on marine organisms that will enable scientists to conduct far more sophisticated experiments at low cost. The second was further development and validation of the Proton Flux Model which demonstrates that calcification is not controlled by the aragonite saturation state (Ω_{arag}), but rather by diffusion boundary conditions that limit the efflux of waste protons derived from the calcification reaction. The Proton Flux Model represents a major step forward in understanding coral reef metabolism.

B. PURPOSE AND OBJECTIVES:

Original Major Goals of this Project:

1. To determine the interactive effects of irradiance, temperature, and pCO_2 on coral growth, bleaching, and mortality.
2. To determine the relative importance of the three parameters to Hawaii reef corals.

This project met the original objectives as stated above. We described growth and mortality of reef corals in response to increased temperature and increased levels of ocean acidification (higher pCO_2) under conditions of high and low solar radiation (i.e. shallow vs. deep coral environments). There was special emphasis on defining interactions between the main components of increased temperature, ocean acidification and high solar irradiance. Upper lethal temperature threshold for corals at present day and future levels of ocean acidification were defined.

During the course of the project we made several very important breakthroughs. The first was the development of a new method of creating realistic conditions of OA as described in Jokiel et al. (Appendix 1). The high cost of building and operating continuous flow experimental systems for studies of biological response to ocean acidification (OA) has led to extensive use of static short-term incubations that do not simulate natural conditions. An inexpensive, highly reliable, continuous flow rate system that tracks natural diurnal and seasonal changes in water chemistry was developed. This approach is based on a gravity-feed seawater system that uses a peristaltic pump to regulate CO_2 injection rate and a power head that cavitates the injected CO_2 stream into microscopic bubbles that are dissolved immediately into the sea water. The approach has been proven as a low-cost method applicable to small or very large experimental systems. The method permits long-term experiments under full sunlight with rapid sea water turnover rate that assures realistic conditions. Measurement of inlet and outlet chemistry allows for calculation of net ecosystem calcification and net ecosystem photosynthesis of benthic communities throughout the diurnal cycle. The second major contribution is a fundamental revision of the existing paradigms of ocean acidification (Appendix 2). A widely held concept is that calcification rates (G_{net}) on coral reefs diminish under conditions of increasing OA due to the decreasing aragonite saturation state, Ω_{arag} (i.e. decreasing $[CO_3^{2-}]$). The recent "Proton Flux Hypothesis" states that decreased G_{net} can be attributed to decreasing net H^+ transport through the boundary layer caused by the increasing $[H^+]$ in the water column. The coral calcification equations show that G_{net} is directly proportional to the ratio of dissolved inorganic carbon (DIC) concentration to proton concentration ([DIC]:[H+] ratio). Plotting diurnal values of G_{net} against Ω_{arag} reveals a daily hysteresis pattern with Ω_{arag} lagging behind peak G_{net} by 2 or more hours, indicating that Ω_{arag} is the dependent variable. Results of a diurnal mesocosm study demonstrate that corals have limited ability to shed the waste protons generated during rapid calcification. High rates of H^+ efflux continued for several hours following peak $G_{net,}$ showing that maximum rate of proton efflux is limited. Measurements of DIC flux and H^+ flux are far more useful in describing coral metabolism dynamics than [DIC] and $[H^+]$. Likewise, Ω_{arag} is not a useful descriptor because it simply tracks pH. $[CO_3^{2-}]$ (and hence Ω_{arag}) shifts with changing $[H^+]$. DIC flux tracks P_{net} and G_{net} and drops off rapidly following peak P_{net} and peak $G_{net,}$ indicating

that rate of inorganic carbon uptake is rapid enough to meet the needs of rapidly calcifying corals.

C. ORGANIZATION AND APPROACH:

This research was conducted at the University of Hawaii's Hawaii Institute of Marine Biology (HIMB) at Moku O Loe (Coconut Island), Kaneohe Bay, Hawaii (21.4°N, 157.8°W). This study site provides a continuous-flow experimental facility that mimics the physical, chemical, and biological conditions on a reef under projected conditions of climate change (Jokiel *et al*, 2008; Kuffner *et al*., 2008). This continuous-flow experimental system duplicates the natural diurnal fluctuations in sea water chemistry that occur on the inshore reef of Coconut Island, where unfiltered seawater is pumped directly from (2 meters), and flushed through the fiberglass mesocosms (1m x 1m x 0.5m) at a rate sufficient to replace the volume every hour. Unfiltered seawater is pumped directly from 2 meters at the edge of Coconut Island into three 1m x 1m x 0.5m fiberglass mesocosms, located in full sunlight (Figure 1). The seawater inflow is located at the bottom center of each mesocosm, which allows for a uniform and well-mixed system that will be confirmed with measurements of the chemical and environmental parameters (Andersson *et al*., 2009).

The general experimental protocol was to acclimate the corals to the mesocosm environment for 1-2 weeks before initiating the treatments. The corals were then exposed to treatments differing in temperature, pCO_2, and irradiance operating independently and together, based on IPCC predictions for future conditions (IPCC 2007). Carbonate chemistry within the flow through mesocosm was manipulated by bubbling CO_2, while heaters and chillers were used to control temperature. Irradiance was controlled by neutral density screening, in which a series of shade cloths covered part of each temperature-*p*CO2 tank so that organisms under each shade regime experienced the same temperature and *p*CO2 but different irradiance levels.

Impact of OA and irradiance on upper lethal temperature:

This experiment defined the upper lethal temperature threshold of a common Hawaiian reef coral, *Montipora capitata*, in a split plot design experiment using the factors of temperature and CO_2 in plots of differing irradiance to test each factor independently, as well as a pairwise comparison of these variables, and interactions of all three. Coral fragments were placed into nine replicate experimental treatments composed of two pCO_2 levels (present day levels: 410µatm and 475µatm; and 2X present: 890µatm), two temperature regimes (summer ambient at 27.4^{o}C and heated to 29.1±2^{o}C and 29.3^{o}C), and three conditions of irradiance (470 $\mu E/\mu^2/d^{-1}$; 235 $\mu E/\mu^2/d^{-1}$; and 38 $\mu E/\mu^2/d^{-1}$) for 50 days (n=10)(Figure 3). Coral response (i.e., growth rates, health, and mortality) was measured at three time points 1) acclimatization period (days 0-23), 2) end of experiment (day 50) and 3) following recovery period (days 51-168). After the stress period, all treatment tanks were returned to ambient conditions, where the corals were assessed for recovery.

Carbonate chemistry within the mesocosms was manipulated through direct bubbling of CO_2. We have developed a low-cost innovative ocean acidification system that allows for natural diurnal fluctuations in seawater chemistry at present and future climate change projections (Appendix 1, Jokiel *et al*., in review). The temperature in the experimental mesocosms was controlled by heaters and chillers. Irradiance was controlled by neutral density screen covering, in which a series of shade cloths cover part of each temperature-pCO_2 tank so

that organisms under each shade regime are experiencing the same temperature and pCO_2 with different irradiance levels. Irradiance measurements were made using a quantum sensor and meter.

Daily mid-day water measurements included pH, salinity (‰), dissolved oxygen (%), and temperature (°C). pH measurements were verified spectrophotometrically using *m*-cresol purple dye according to SOP 7 (Dickson *et al*., 2007). TA was measured independently using a Titrino titrator (877). Lastly, accuracy and precision of the Titrino titrator was confirmed with certified reference materials (CRM) (Dickson Laboratory, Scripps Institution of Oceanography). All carbonate parameters were calculated using CO2SYS (sensu Jokiel, 2008).

Coral calcification rates were measured biweekly using the buoyant weighing method (Jokiel *et al*., 1978). To record the progression of the corals' health, the CoralWatch health chart was used as a proxy for symbiont density and/or chlorophyll (Siebeck *et al*., 2006), while chlorophyll *a* extractions validated the CoralWatch coral health chart to Hawaiian corals (Figure 2).

Impact of temperature pCO_2 and irradiance on coral growth, bleaching and mortality over and annual cycle:

In each of the experimental runs a total of 120 coral colonies of similar size and morphology (~8 cm in diameter) were photo-acclimated under each irradiance treatment for a period of one week. The corals were placed into three large mesocosms at two pCO_2 regimes (high and ambient) with neutral buoyancy screening at levels of 100%, 50% and 8% irradiance. Twenty corals were randomly assigned to each treatment. During the measurement period, temperature and pCO_2 were increased in tank one, tank two remained under ambient conditions of temperature and pCO_2 with temperature increased in tank three. The corals were exposed to these manipulative conditions for 4 weeks. Coral health (pigmentation) was assessed using a coral health chart and growth was measured directly by using buoyant weight (Jokiel *et al*, 2008). After the growth period, all treatment tanks were returned to ambient conditions. The recovery rate of bleached corals was monitored for several weeks.

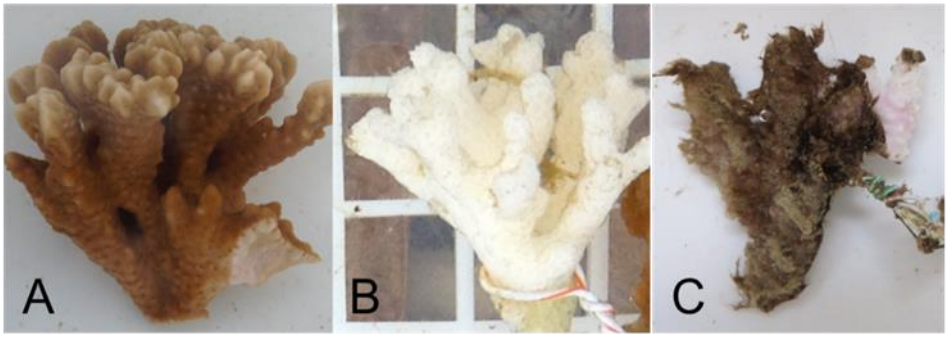

Figure 1. Images of *Montipora capitata* characterized as healthy (A), bleached (B), and/or dead (C) throughout the stress and recovery periods.

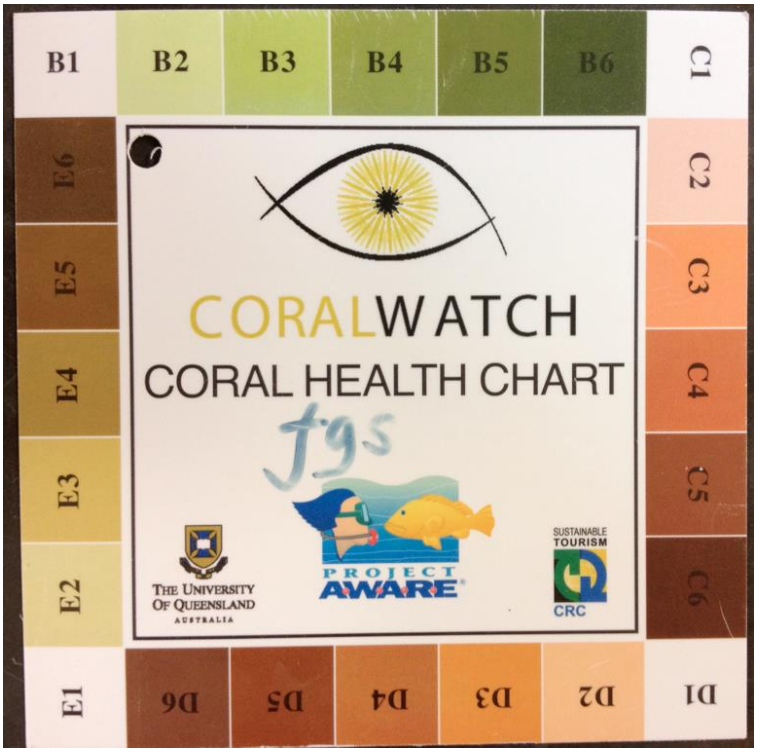

Figure 2. CoralWatch coral health chart used to assess coral health. The best color match for *Montipora capitata* was D1 – D6 (compare chart to Fig. 1)

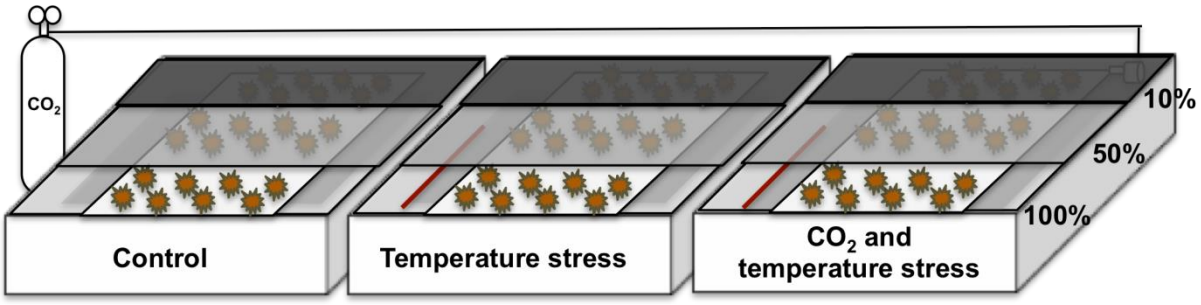

Figure 3a. Diagram of experimental setup at the Hawaii Institute of Marine Biology, Coconut Island in the Coral Reef Ecology Lab. Corals were exposed to high or ambient levels of CO_2 and temperature, while placed under three different irradiance levels (n=10).

Figure 3b. Photograph of continuous-flow mesocosm facility located at the Point Lab at the Hawaii Institute of Marine Biology, Coconut Island.

D. PROJECT RESULTS

Bleaching and Mortality:

Initial bleaching occurred 10 days sooner in corals grown at high temperature (29.1°C), high irradiance (470 μE m^{-2} d^{-1}) and high pCO_2 (890 μatm) (day 11) than corals in high temperature (29.3°C), high irradiance (470 μE m^{-2} d^{-1}), and ambient pCO_2 (475 μatm) (day 21) (Figure 4). Growth rates were suppressed in corals grown under future climate change conditions; however, no significant difference in growth rates was observed between corals exposed to high temperatures and corals grown in high temperatures and acidified conditions (Figure 6). Lastly, coral health and mortality responses were negatively affected by the interaction of irradiance, temperature, and OA, where corals were able to recover to thermal stress under reduced irradiances but fail to recover as fast in acidified conditions.

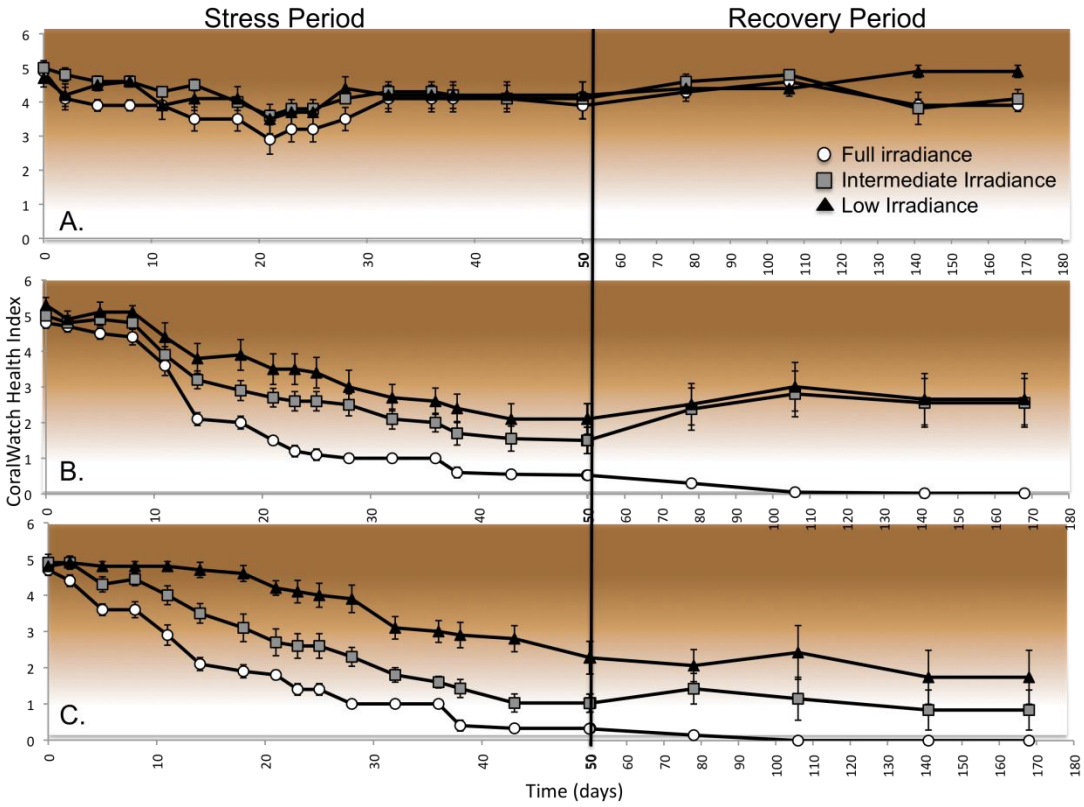

Figure 4. The coral health assessment of *Montipora capitata* during the 50-day stress period and following recovery period across three irradiance levels (470 µE m^{-2} d^{-1}, 50%: 235 µE m^{-2} d^{-1}, and 38 µE m^{-2} d^{-1}) within ambient (A) (27.4°C, 410µatm), temperature stress (B.) (29.1°C, 475µatm) and temperature and CO_2 (C.) (29.3°C, 890µatm) manipulative treatments. Stress period initiated on day 0, while growth rates were measured between day 23 and day 50. The manipulated conditions were terminated after day 50.

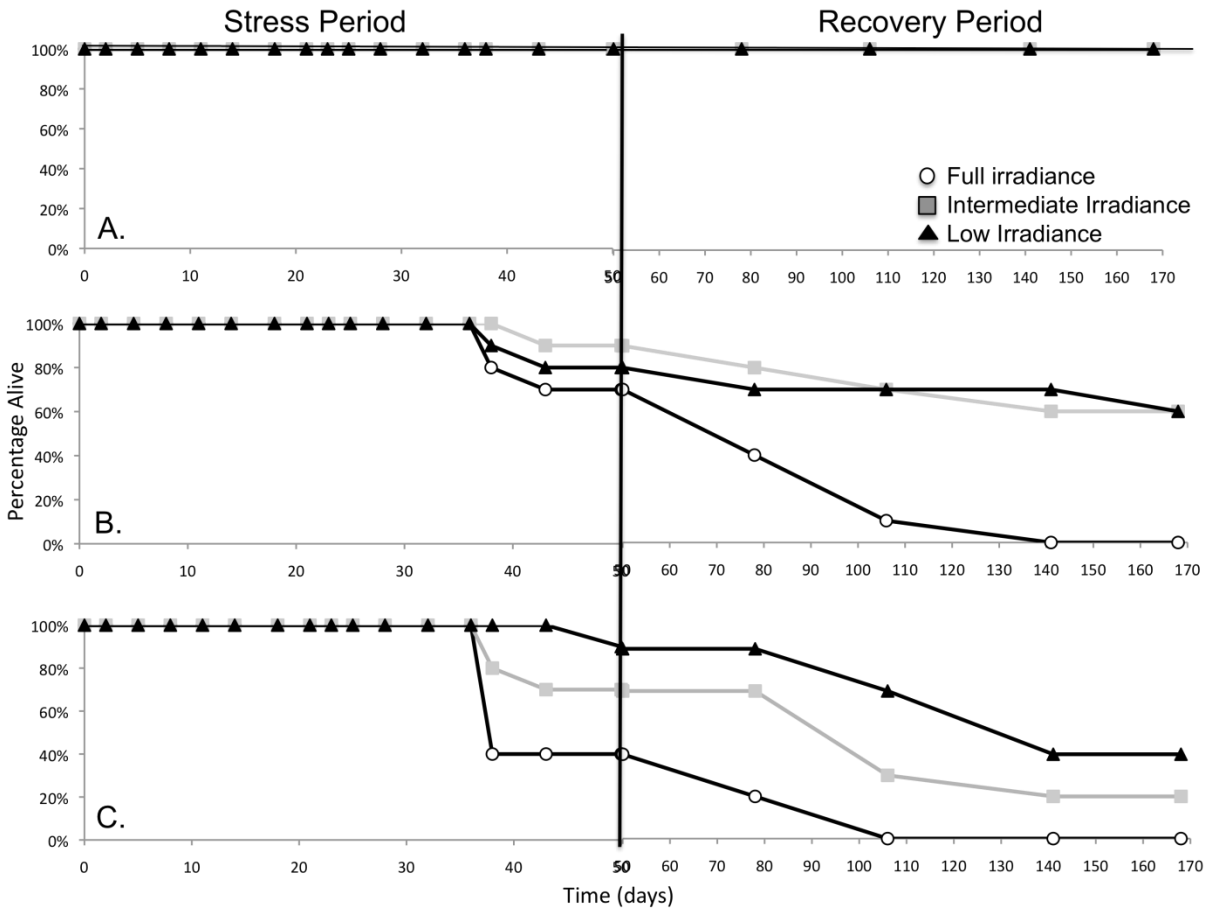

Figure 5. Survivorship curves of *Montipora capitata* during the 50 day stress period and following recovery period across three irradiance levels (470 µE m^{-2} d^{-1}, 50%: 235 µE m^{-2} d^{-1}, and 38 µE m^{-2} d^{-1}) within ambient (A) (27.4°C, 410µatm), temperature stress (B.) (29.1°C, 475µatm) and temperature and CO_2 (C.) (29.3°C, 890µatm) manipulative treatments.

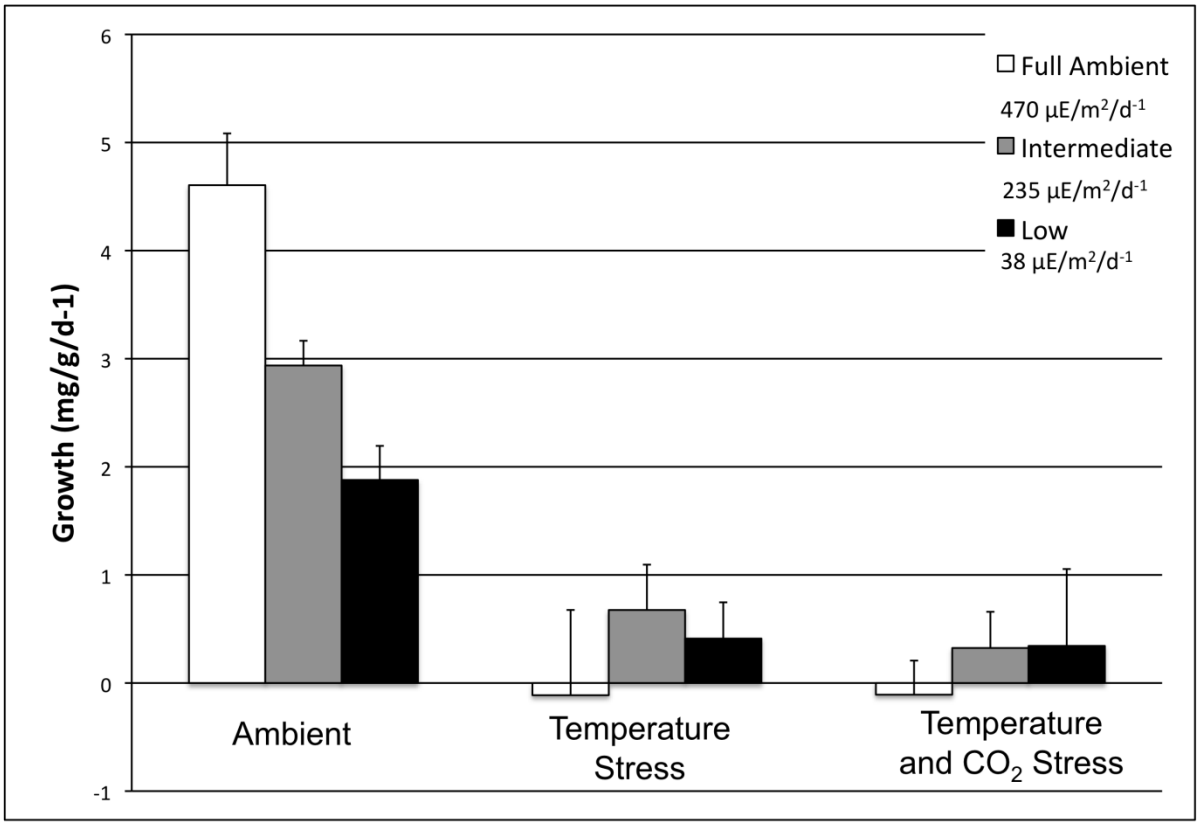

Figure 6. Coral growth rate of *Montipora capitata* at the end of the experiment (28 day growth period) exposed to three irradiance levels (470 µE m^{-2} d^{-1}, 50%: 235 µE m^{-2} d^{-1}, and 38 µE m^{-2} d^{-1}) within the manipulative treatments of CO_2 (high: 890 µatm and ambient: 410 µatm and 475 µatm) and temperature (high: 29.1°C and 29.3°C and ambient: 27.4°C). A Tukey post-hoc analysis showed a significant difference in coral growth rates that were exposed to high temperatures at high levels of irradiance (470 µE m^{-2} d^{-1}: $p<0.001$; 235 µE m^{-2} d^{-1}: $p=0.0249$) and in high temperatures with high pCO_2(470 µE m^{-2} d^{-1}: $p<0.001$; 235 µE m^{-2} d^{-1}: $p=0.0047$) in comparison to ambient conditions. However, no significant difference in growth rates was observed between corals exposed to high temperatures and corals grown in high temperatures in acidified conditions ($F(5,54) = 0.5292$, $p=0.7532$).

Impact of temperature pCO_2 and irradiance on coral growth, bleaching and mortality over and annual cycle:

These experiments were conducted to assess the impact of irradiance, temperature and OA on coral growth over an annual cycle. Experiments involved heating and chilling mesocosms relative to ambient temperature. Ten experiments were run. Figure 7 summarizes the data normalized to irradiance and plotted against temperature. Figure 8 shows data graphed separately for low, medium and high light treatments normalized to irradiance and plotted against temperature. It is apparent that OA has an impact under all temperature and irradiance regimes.

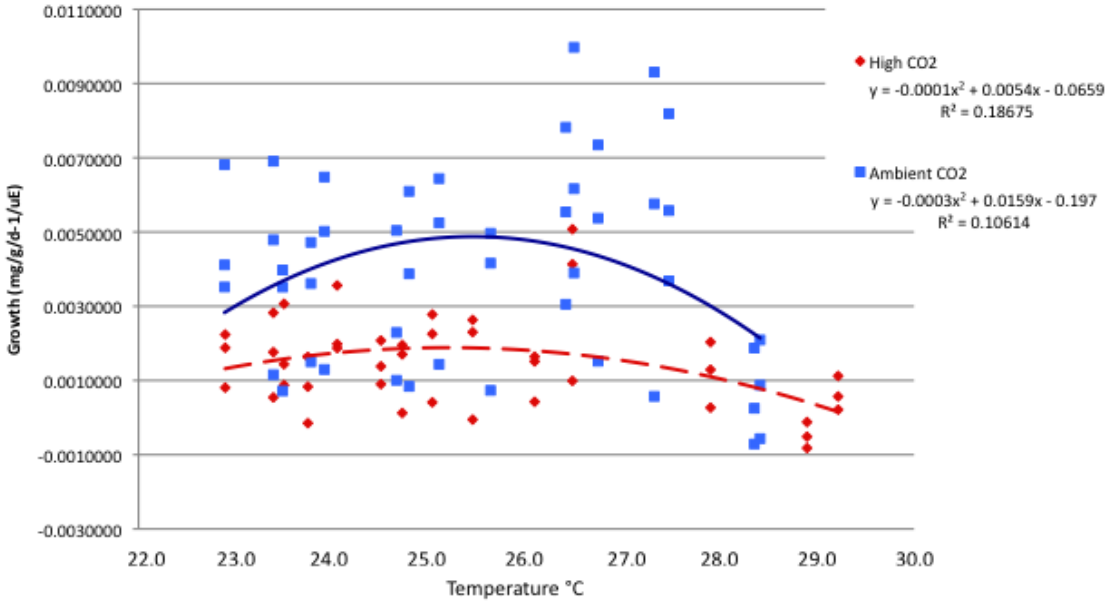

Figure 7. The effect of ocean acidification (red diamond markers) compared to present day CO_2 (blue square markers) over the full range of temperatures over an annual cycle.

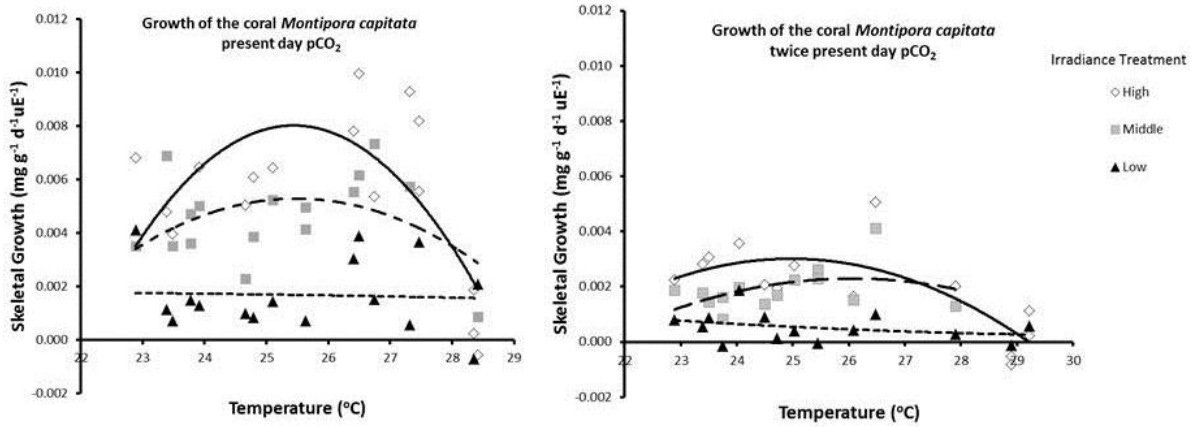

Figure 8. Growth of the coral *Montipora capitata* at three irradiance levels (100%, 50% and 10%) over a full range of temperatures over and annual cycle. The pCO_2 vs temp growth is normalized to irradiance.

Irradiance is a primary driver of calcification (Figure 7). Irradiance is a primary driver of calcification at all temperatures at present day levels of pCO_2 (Figure 8, left panel). At high pCO_2 there is a dramatic effect of OA at all levels of temperature and irradiance (right panel).

Two results that were not anticipated in the original proposal may prove to be the most important outcomes of this project:

The first is methodological. In order to conduct continuous flow experiments under highly realistic conditions of high pCO_2 we developed a system that injects bubbles of CO_2 at a constant rate. This method proved to be extremely reliable (Appendix 1). The high cost of building and operating continuous flow experimental systems for studies of biological response to ocean acidification (OA) has led to extensive use of static short-term incubations that do not simulate natural conditions. We developed an inexpensive, highly reliable, continuous flow rate system that tracks natural diurnal and seasonal changes in water chemistry. This approach is based on a gravity-feed seawater system that uses a peristaltic pump to regulate CO_2 injection rate and a power head that cavitates the injected CO_2 stream into microscopic bubbles that are dissolved immediately into the sea water. The method has been proven as a low-cost approach that is applicable to small or very large experimental systems. The method permits long-term experiments under full sunlight with rapid sea water turnover rate that assures realistic conditions. Measurement of inlet and outlet chemistry allows for calculation of net ecosystem calcification and net ecosystem photosynthesis of benthic communities throughout the diurnal cycle.

The second result is extremely important in that it revises the way OA will be studied in the future. A widely held paradigm is that calcification rates (G_{net}) on coral reefs diminish under conditions of increasing OA due to the decreasing aragonite saturation state, Ω_{arag} (i.e. decreasing $[CO_3^{2-}]$). The recent "Proton Flux Hypothesis" states that decreased G_{net} can be attributed to decreasing net H^+ transport through the boundary layer caused by the increasing $[H^+]$ in the water column. The coral calcification equations show that G_{net} is directly proportional to the ratio of dissolved inorganic carbon (DIC) concentration to proton concentration ([DIC]:[H+] ratio). Plotting diurnal values of G_{net} against Ω_{arag} reveals a daily hysteresis pattern with Ω_{arag} lagging behind peak G_{net} by 2 or more hours, indicating that Ω_{arag} is the dependent variable. Results of a diurnal mesocosm study demonstrate that corals have limited ability to shed the waste protons generated during rapid calcification. High rates of H^+ efflux continued for several hours following peak $G_{net,}$ showing that maximum rate of proton efflux is limited. Measurements of DIC flux and H^+ flux are far more useful in describing coral metabolism dynamics than [DIC] and $[H^+]$. Likewise, Ω_{arag} is not a useful descriptor because it simply tracks pH. $[CO_3^{2-}]$ (and hence Ω_{arag}) shifts with changing $[H^+]$. DIC flux tracks P_{net} and G_{net} and drops off rapidly following peak P_{net} and peak $G_{net,}$ indicating that rate of inorganic carbon uptake is rapid enough to meet the needs of rapidly calcifying corals.

E. ANALYSIS AND FINDINGS:

The results of this research indicate facilitation of bleaching in coral reefs under acidified conditions. Growth rates were suppressed under the future scenario of conditions where high irradiance and temperature had the greatest effect on coral growth and bleaching. Overall, the effect of the negative synergistic interaction of temperature and irradiance on coral growth rates

was not further aggravated in acidified conditions. However, coral bleaching was observed to be accelerated under acidified conditions with higher mortality during the recovery period.

Summary
Bleaching and Mortality:

1. Initial bleaching occurs sooner in corals grown at high temperature, high irradiance and high pCO_2 than corals in high temperature (29.3°C), high irradiance, and ambient pCO_2.
2. Growth rates were suppressed in corals grown under future climate conditions of OA and high temperature.
3. Coral growth, mortality, bleaching response and recovery rate were negatively affected by the interaction of irradiance, temperature, and OA. Corals that were able to recover from thermal stress under reduced irradiances failed to recover as fast in acidified conditions.

Coral Growth:

1. OA has an impact at all temperatures.
2. Irradiance the primary driver. Increasing depth and/or higher turbidity will lower impact of higher temperature.
3. Growth normalized to irradiance clearly shows change in growth relationship across all temperatures and irradiance regimes.
4. Incubations conducted to date by other investigators under artificial irradiance (generally only 20% of full solar irradiance) are not adequate for describing changes on reef growing in clear water at high irradiance such as occurs during bleaching events (Jokiel and Brown 2004). During bleaching events low wind stress, low cloud cover, and high water transparency result in extremely high levels of irradiance.

The results of this study provide basic data that will allow predictions of the impact of future conditions of global warming and ocean acidification on corals. These data will aid in the advancement of discovery and understanding of the importance of science in the management of coral reef ecosystems, while promoting teaching, training, and learning within the community and around the world.

F. CONCLUSIONS AND RECOMMENDATIONS:

This investigation proceeded according to the original plan and all goals were met. The results of this study provide basic data that will allow prediction of impact of future conditions of global warming and ocean acidification on corals. These data will aid in the advancement of discovery and understanding of the importance of science in the management of coral reef ecosystems.

The results of this research indicate that corals will bleach more rapidly under more acidified conditions. Growth rates were suppressed under the future climate change scenario, and high irradiance had the greatest effect on coral growth and bleaching. The interaction of temperature and irradiance stressors had the strongest influence on coral growth rates. Low irradiance levels potentially provide refuge for *M. capitata* from thermal and irradiance stress. Coral health and mortality was observed to be influence by the interaction of all three factors,

while bleaching and mortality was accelerated under acidified conditions. Lastly, recovery in coral health was observed in corals influenced by a single stressor, while mortality rates increased in corals experiencing more than one climate change stressor.

We conclude that:
1. Acidification accelerated bleaching and mortality.
2. Increased temperature, increase CO_2, and increased irradiance working in unison produced higher mortality rates.
3. Growth rates were suppressed under conditions of high temperature, high pCO_2.
4. Temperature and irradiance interaction had the greatest influence on coral growth rates
5. Increased recovery was observed in corals exposed only to a single stressor while those exposed to multiple stressors showed slow recovery and high mortality.
6. Deep reefs may provide refuge from thermal and high irradiance stress while shallow reefs will be the reef area most impacted by future climate change scenarios.

G. OUTREACH:

Opportunities for training and professional development provided:
Two undergraduate students (Dan Lager, Claire Sprecher) trained on marine carbonate chemistry and ocean acidification. One visiting undergraduate (Aaron Cevallos) completed an internship and wrote a research paper that was required for his BS degree. One graduate student (Keisha Bahr) conducted her PhD research requirements on this project. Additionally, this work supports the HIMB Education Program's ocean acidification lab and curriculum development, where thousands of students visit each year, by the development of new strategic initiatives of high school marine science modules that relate directly to research programs at the institute. These labs will improve the link between the development of science skills with the corresponding development of science content within broader societal issues that directly relate to environmental problems facing local communities. The knowledge gained from this project will provide accurate and current facts to facilitate curriculum development and revision in a manner that will help increase local Hawaiian students' interest in science, technology, engineering and mathematics (STEM). This research is a priority of the PICCC theme of "Ecological Responses to Climate Change" and EPSCoR.

Presentations:
July 1, 2011 Kamehameha Schools molecular biology Beth Kimokea ocean acidification lecture by Ku'ulei Rodgers.
July 6-August 6 Ku'ulei Rodgers and Paul Jokiel sponsored project of Aaron Cevallos undergrad Princeton on OA.
July 11, 2011 Undergraduate Research Mentoring-Rob Toonen 2 groups-climate change lecture by Ku'ulei Rodgers.
August 10, 2011 Ku'ulei Rodgers Presented information to DC staffers on Natural History of Kaneohe Bay and climate change.
Sept 9, 2011. Paul Jokiel interviewed by Beth-Ann Kozlovich on NPR (KIPH) talking about ocean acification.
Sept 15, 2011 Ocean acidification lecture for Topics in Geochemistry: Ocean 643 Ocean Acidification and Coral Reefs at HIMB by Paul Jokiel

Oct 4, 2011. Invited Sea Grant "Reef Talk" public presentation on ocean acidification and climate change at Kona Hawaii by Paul Jokiel.

Oct 21, 2011 Ku'ulei Rodgres met with Kati Slater-Szirom journalism student Western Washington University. Environmental magazine The Planet Ocean Acidification

Oct 28, 2011 Organism scale calcification lecture for Topics in Geochemistry: Ocean 643 Ocean Acidification and Coral Reefs at HIMB by Paul Jokiel

Nov 10, 2011. Invited public lecture on ocean acidification for the Windward community College Chemistry Forum by Paul Jokiel.

Jan 3, 2012 Hawaiian Islands Symposium, East West Center, UH. Is reduced coral growth due to ocean acidification controlled by aragonite saturation state or by hydrogen ion concentration? By Paul Jokiel and Ku'ulei Rodgers

January 17, 2012 Lecture on climate change to class "Ahupuaa 201", Windward Community College by Paul Jokiel.

January 26, 2012 Michelle Smith's Hawaii Community College Marine Biology Class HIMB lab visit and ocean acidification presentation conducted by Ku'ulei Rodgers

January 26, 2012 Jeremy Talbot Marynoll high school student, Responses to OA questions for project by Ku'ulei Rodgers

Feb 24, 2012. "Chemistry of Calcification" lecture to Zool 410 class "Corals and Coral Reefs" by Paul Jokiel

February 23, 2012 Washington DC Senate Appropriations Committee OA presentation by Ku'ulei Rogers at HIMB.

March 22, 2012 HIMB Windward Community College Ahupua'a class Clyde Tamaru Climate Change research presentation by Ku'ulei Rodgers

April 21, 2012 Ocean Expo Fair HIMB featured climate change researcher Ku'ulei Rodgers

August 28-29, 2012. Paul L. Jokiel participated in the Coral Reef Ocean Acidification Monitoring Portfolio Workshop which was hosted by the NOAA Ocean Acidification Program and the National Coral Reef Institute at the Nova Southeastern University Oceanographic Center Center of Excellence for Coral Reef Ecosystem Science, 8000 North Ocean Drive, Dania Beach, FL 33004. The mission statement of this project was to define a suite of metrics to include as part of long-term coral reef monitoring efforts most valuable towards discerning specific attribution of changes in coral reef ecosystems in response to ocean acidification (OA). This portfolio of observations is intended to leverage existing and proposed monitoring initiatives, and would be derived from a suite of biogeochemical and ecological measurements. The changing status of these metrics over time should aid in assigning specific attribution to OA, though it is recognized that there will likely be multiple synergistic factors causing the observed changes. The workshop outcomes will inform national and international long-term OA monitoring efforts within coral reef ecosystems. The outcomes of the workshop were as follows:

A. Recommendations for the most valuable long-term coral reef ecosystem monitoring <u>metrics</u> which would provide greatest capacity towards discerning changes in coral reef ecosystems in response to OA;

B. Recommendations for the most efficient and robust <u>monitoring approaches</u> for these metrics, as well as <u>gaps</u> in current capabilities;

C. Recommendations for <u>augmentations</u> to current OA monitoring, and/or <u>collaborations</u> using existing resources, that can serve to reduce the identified gaps;

D. A *Coral Reef Ocean Acidification Monitoring Portfolio* <u>technical memorandum:</u>

- A short review of the expected ecosystem responses to OA over the next century based upon current "state of the science," and an overview of existing or planned capabilities directed at monitoring OA and ecological impacts within coral reef ecosystems.
- A priority listing of core biogeochemical monitoring requirements for quantifying the rates and magnitude of acidification within coral reef ecosystems.
- Recommendations of key ecological monitoring requirements that would yield greatest insight into attribution of ecological changes in response to ocean acidification.
- A discussion of the expectations, uncertainty, and feasibility considerations with regards to incorporating these requirements into monitoring strategies.

Nov. 15, 2012. Webinar: PICCC webinar on Ocean Acidification

Dec. 11-12, 2012 NPS training, Drs Paul Jokiel and Ku'ulei Rodgers conducted a two day climate change training session of NPS staff at Koloko Hanakahou National Park

Oct. 3-6, 2013 Peterson A, Jokiel PL, Rodgers K, and KD Bahr. Spatial and temporal variation in water quality within major marine habitats of Kaneohe Bay, Hawaii. SACNAS National Conference. San Antonio, Texas.

April 19, 2013 Bahr KD, Rodgers, K, and PL Jokiel. Response of Hawaiian reef coral *Montipora capitata* to temperature, irradiance, and pCO_2. 38th Albert L. Tester Memorial Symposium. UH Manoa, Honolulu, Hawaii. (Best Graduate Poster)

July 16-18, 2013 Bahr KD, Rodgers K, and PL Jokiel. Response of Hawaiian reef coral *Montipora capitata* to temperature, irradiance, and pCO_2. Hawaii Conservation Conference. Honolulu, Hawaii

July, 2013 Bahr KD, Rodgers K, and PL Jokiel. Response of Hawaiian reef coral *Montipora capitata* to temperature, irradiance, and pCO_2. Pacific Island Climate Change Cooperation Science Symposium. Honolulu, Hawaii

Nov. 7-10, 2013 Bahr KD, Rodgers K, and PL Jokiel. Response of Hawaii reef coral, *Montipora capitata*, to multiple climate change stressors. Western Society of Naturalists Annual Meeting. Oxnard, California.

Feb. 24-28, 2014 Page HP, Yeakel K, Jokiel PJ, Rodgers KS, Bahr KD, and A Andersson. Diel trends in net ecosystem calcification and production rates for different benthic communities exposed to ambient and acidified seawater. Ocean Sciences Meeting. Honolulu, Hawaii

Feb. 24-28, 2014 Bahr KD, Rodgers K, and PL Jokiel. Response of Hawaii reef coral, *Montipora capitata*, to multiple climate change stressors. Ocean Sciences Meeting. Honolulu Hawaii.

H. SCIENCE OUTPUTS

Hoeke, Ron K., Paul L. Jokiel, Robert W. Buddemeier, Russell E. Brainard (2011) Projected changes to growth and mortality of Hawaiian corals over the next 100 Years. PLoS ONE 6(3): e18038. doi:10.1371/journal.pone.0018038

Jokiel, P. L. (2011a) Ocean acidification and control of reef coral calcification by boundary layer limitation of proton flux. Bulletin of Marine Science. 87(3):639–657. http://dx.doi.org/10.5343/bms.2010.1107(Acknowledgement of Federal support)

Jokiel, P.L. (2011b) The reef coral two compartment proton flux model: A new approach relating tissue-level physiological processes to gross corallum morphology. Journal of Experimental Marine Biology and Ecology 409 (2011) 1–12. doi:10.1016/j.jembe.2011.10.008. (Acknowledgement of Federal support)

Rodgers KS, Kido MH, Jokiel PL, Edmonds T, Brown EK. (2012) Use of Integrated Landscape Indicators to Evaluate the Health of Linked Watersheds and Coral Reef Environments in the Hawaiian Islands. Environmental Management. (DOI: 10.1007/s00267-012-9867-9).

Kuffner, Ilsa B., Paul L. Jokiel, Ku'ulei S. Rodgers, Andreas J. Andersson, Fred T. Mackenzie 2012. An apparent "vital effect" of calcification rate on the Sr/Ca temperature proxy in the reef coral *Montipora capitata*. Geochemistry Geophysics Geosystems 13(8): Q08004, doi:10.1029/2012GC004128

Jokiel PL. 2013 Coral reef calcification: carbonate, bicarbonate and proton flux under conditions of increasing ocean acidification. Proc R Soc B 20130031. http://dx.doi.org/10.1098/rspb.2013.0031

Jokiel, Paul L., Christopher P. Jury, and Ku'ulei S. Rodgers (in press) Resolution of conflicts concerning ocean acidification and coral calcification.

Jokiel, P. L., Ku'ulei S. Rodgers, Ilsa B. Kuffner, Andreas J. Andersson, and Fred T. Mackenzie. (in review) Warming amplifies negative effect of ocean acidification on coral calcification. Aquatic Geochemistry.Franklin EC, PL Jokiel, MJ Donahue (in press) Predictive modeling of coral distribution and abundance in the Hawaiian Islands. Marine Ecology Progress Series.

Murillo LJ, Jokiel PL, Atkinson MJ. (2014) Alkalinity to calcium flux ratios for corals and coral reef communities: variances between isolated and community conditions. PeerJ 2:e249 http://dx.doi.org/10.7717/peerj.249

Jokiel, PL, K. D. Bahr, and K. S. Rodgers (in review) Low cost ocean acidification system for high- flow mesocosm studies.

Jokiel, PL, Christopher P. Jury and Ku'ulei S. Rodgers (in review) Resolution of conflicts concerning ocean acidification and coral calcification

Bahr, Keisha, Ku'ulei S. Rodgers and Paul L. Jokiel (in preparation) Impact of irradiance and high pCO_2 on upper lethal temperature for the coral *Montipora capitata*

Bahr, Keisha, Ku'ulei S. Rodgers and Paul L. Jokiel (in preparation) Biological response of the Hawaiian reef coral *Montipora capitata* to multiple climate change stressors over an annual cycle.

Bahr, Keisha, Ku'ulei S. Rodgers and Paul L. Jokiel (in preparation) Relative sensitivity of Hawaiian corals to high tempertures and acidified conditions.

Bahr, Keisha, Ku'ulei S. Rodgers and Paul L. Jokiel (in preparation) Net ecosystem calcification and photosynthesis of Hawaiian reef communities to temperature and acidification.

Book Chapters:

Jokiel PL, Jury C, Kuffner IB and Rodgers KS (in press) Coral calcification and ocean acidification. In: Dennis Hubbard (ed) Coral Reefs at the Crossroads. Springer Books

Jury, C and Paul L. Jokiel (in press) Controls on reef-building over time. In: Dennis Hubbard (ed) Coral Reefs at the Crossroads. Springer Books.

References

Buddemeier, R. W., P.L. Jokiel, K.M. Zimmerman, D.R. Lane, J. M. Carey, G.C. Bohling, J.A. Martinich. (2008) A modeling tool to evaluate regional coral reef responses to changes in climate and ocean chemistry Limnol. Oceanogr.: Methods 6:395–411

Coles, S. L., P. L. Jokiel and C. R. Lewis. 1976. Thermal tolerance in tropical versus subtropical Pacific reef corals. Pac. Sci. 30:156-166.

Coles, S. L., and P. L. Jokiel. 1977. Effects of temperature on photosynthesis and respiration rates of reef corals. Mar. Biol. 43:209-216.

Coles, S. L., and P. L. Jokiel. 1978. Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa* (Lamarck). Mar. Biol. 49:187-195.

Hoeke, Ron K., Paul L. Jokiel, Robert W. Buddemeier, Russell E. Brainard (2011) Projected changes to growth and mortality of Hawaiian corals over the next 100 Years. PLoS ONE 6(3): e18038. doi:10.1371/journal.pone.0018038

Houck, J. E., R. W. Buddemeier, S. V. Smith and P. L. Jokiel. 1977. The response of coral growth rate and skeletal strontium content to light intensity and water temperature. Proc. Third Int. Coral Reef Symp., Miami, Florida. pp. 425-431.

IPCC. (2007). Climate Change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernment Panel on Climate Change. Cambridge University Press.

Jokiel, P. L. 2004. Temperature Stress and Coral Bleaching. 2004. pp. 401-425 *In* Coral Health and Disease (E. Rosenberg and Y. Loya Eds.). Springer-Verlag, Heidelberg.

Jokiel, P. L. and Eric K. Brown. 2004. Global warming, regional trends and inshore environmental conditions influence coral bleaching in Hawaii. Global Change Biology 10:1627-1641.

Jokiel, P. (2011). Ocean acidification and control of reef coral by boundary layer limitation of proton flux. Bulletin of Marine Science , 87 (3), 639-657.

Jokiel, P. L., and S. L. Coles. 1974. Effects of heated effluent on hermatypic corals at Kahe Point, Oahu. Pac. Sci. 28(1):1-18.

Jokiel, P. L., S. L. Coles, E. B. Guinther, G. S. Key, S. V. Smith and S. J. Townsley. 1974. Effects of thermal loading on Hawaiian reef corals, Final Report, Grant 18050DDN, Water Pollution Control Research Series, Water Quality Office, Environmental Protection Agency, 285 pp.

Jokiel, P. L., and S. L. Coles. 1977. Effects of temperature on the mortality and growth of Hawaiian reef corals. Mar. Biol. 43:201-208.

Jokiel, Paul L., Ku'ulei S. Rodgers, Ilsa B. Kuffner, Andreas J. Andersson, Evelyn F. Cox, Fred T. Mackenzie (2008) Ocean acidification and calcifying reef organisms: a mesocosm investigation. Coral Reefs 27:473-483

Smith, S. V., P. L. Jokiel, G. S. Key and E. B. Guinther. 1977. Metabolic responses of shallow tropical benthic microcosm communities to perturbation. Environmental Protection Agency, Narragansett, Rhode Island. Final report of contract R800906. 110 pp. Available for download at http://cramp.wcc.hawaii.edu/

Jokiel, P. L., J. E. Maragos and L. Franzisket. 1978. Coral growth: buoyant weight technique. In: Coral Reefs: Research Methods (D. R. Stoddart and R. E. Johannes, eds.). UNESCO Monographs on Oceanographic Methodology, Paris. pp. 529-542.

Jokiel, P. L., and E. B. Guinther. 1978. Effects of temperature on reproduction in the hermatypic coral *Pocillopora damicornis*. Bull. Mar. Sci. 28:786-789.

Kleypas, J. A., R. W. Buddemeier, D. Archer, J.-P. Gattuso, C. Langdon and B. N. Opdyke (1999). Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. Science **284**: 118–120.

Kuffner, I. B., Anderson, A. J., Jokiel, P. L., Rodgers, K., & Mackenzie, F. T. (2008). Decreased abundance of crustose coralline algae due to ocean acidification. Nature Geoscience , 114-117

Appendix 1. Jokiel et al. (submitted) draft of 10 Feb 2014

Low cost ocean acidification system for high- flow mesocosm studies

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Abstract

The high cost of building and operating continuous flow experimental systems for studies of biological response to ocean acidification (OA) has led to extensive use of static short-term incubations that do not simulate natural conditions. An inexpensive, highly reliable, continuous flow rate system that tracks natural diurnal and seasonal changes in water chemistry is described. This approach is based on a gravity-feed seawater system that uses a peristaltic pump to regulate CO_2 injection rate and a power head that cavitates the injected CO_2 stream into microscopic bubbles that are dissolved immediately into the sea water. The approach has been proven as a low-cost method applicable to small or very large experimental systems. The method permits long-term experiments under full sunlight with rapid sea water turnover rate that assures realistic conditions. Measurement of inlet and outlet chemistry allows for calculation of net ecosystem calcification and net ecosystem photosynthesis of benthic communities throughout the diurnal cycle.

Running Head*:* *Ocean Acidification System*

Introduction

Controlled experiments designed to test the impact of ocean acidification (OA) have been limited by ability to create realistic water chemistry and irradiance conditions for the cultured organisms (Riebesell et al. 2010). Bubbling with CO_2 gas or an air-CO_2 gas mixture is often used as the means of increasing pCO_2 in seawater to simulate future conditions of OA. The cost involved in the design, construction and operation of such systems is high due to the inherent complexity of the control system that must precisely regulate gas flow, gas mixing, and sea water flow. Transfer rate of CO_2 between bubbles and seawater is especially slow if CO_2 enriched air rather than pure CO_2 is used in the bubbling process. In either case, control of gas and water flow has been a major technical problem. Consequently, OA experiments have often been conducted in small water volume aquaria using static or extremely low turnover rate seawater systems that are easy to set up (Table 1). Several reports describe static incubation systems used to test the response of coral planula larvae or newly settled corals to various levels of pCO_2 (Anlauf et al. 2010, dePutron et al. 2010, Drenkard et al. 2013). In cases where there is high biological activity (such as occurs in corals and coral reef communities) the static incubation method the water chemistry can be strongly influenced by the experimental subjects. Coral calcification can cause rapid changes in seawater total alkalinity during the course of an incubation. Photosynthesis and respiration can result in extreme changes in pH and dissolved oxygen (D.O.). Static systems do not provide a continuous supply of plankton and nutrients, so the nutritional status of the experimental corals may be compromised. Coral growth under both acidified and non-acidified conditions is increased by planktonic feeding (Drenkard et al. 2013). Moreover, nutrient supply can influence the effects of OA on coral growth (Renegar and Riegl 2005, Chauvin et al. 2011). Static tests do not flush out accumulated metabolic wastes. Maintaining aquaria in full sunlight results in solar heating and resulting high water temperature unless there is rapid flushing of the system. Therefore, most static tests are carried out under relatively low artificial irradiance regimes. Artificial lighting lacks the ultraviolet radiation

Table 1. Comparison of systems previously developed for ocean acidification experiments on corals and coral reefs.

Reference	Incubation time	Volume (liters)	Turnover rate	Irradiance (μmol quanta m^{-2} s^{-1})	Photoperiod	Seawater
dePutron et al. 2010	14 d	30	Static	60	12 h light: 12 h dark	filtered
Reynaud et al. 2003	20 d	150	Static 150 L aquaria with 4 h recycling through small 150 chamber	380	12 h light: 12 h dark	filtered
Comeau et al. 2012	14 d	150	2 days (50% volume replaced every other day)	500	12 h light: 12 h dark	filtered
Edmunds et al. 2012	30 d	150	8 days(13% volume replaced daily)	600	12 h light: 12 h dark	filtered
Drenkard et al. 2013	21 d	21	7 days	60	12 h light: 12 h dark	filtered
Anthony et al 2008	14 d	20	continuous flow	sunlight	natural cycle	unfiltered
Jokiel et al. 2008	270 d	500	continuous flow, turnover 1 hour	sunlight	natural cycle	unfiltered
Anthony et al. 2011, 2013	1-2 h	550	Static flume with induced current, water replaced after each run	~1000	12 h light: 12 h dark	pre-conditioned in static holding tank
Langdon and Atkinson 2005	1.5 h	2400	Static flume with induced current, water replaced after each run	sunlight	natural cycle	unfiltered recirculating
Langdon et al. 2003	NA	2.65×10^6	Static BIOSPHERE-2 "Ocean"	filtered sunlight	natural cycle	unfiltered recirculating

(UVR) component that is extremely important to coral physiology and bleaching rate (e.g. Gleason and Wellington 1993, Lesser 1996).

Reef community response to OA has also been studied by perturbation of a single large static system while observing the metabolic response. For example, Langdon and Atkinson (2005) carried out a series of perturbation incubations measuring net photosynthesis, net calcification and nutrient uptake at different pCO_2 levels on a single coral community in a large flume. The pCO_2 level was altered using HCl or $NaHCO_3$. Between incubations the seawater was replaced. Metabolism was measured under various perturbed conditions relative to unperturbed conditions in a series of 1.5 hour incubations. The ultimate perturbation experiments were conducted by Langdon et al. (2003) in the 2650 m^3 "ocean" coral reef of BIOSPHERE-2 located near Tucson, Arizona. They studied the effects of sea water carbonate chemistry on calcification in an assembled community of coral reef organisms consisting of corals, calcifying algae, and other typical reef biota. The sea water chemistry of the "ocean" was perturbed between each of 42 experimental runs. The saturation state of the water was perturbed by adding various amounts of $NaHCO_3$, Na_2CO_3 and $CaCl_2$. In 1995-1998 additions of $NaHCO_3$ and Na_2CO_3 were made to keep the pH in a narrow range of 8.1±0.1 and isolate the effect of changing CO_3^{2-} concentration. During 1999 chemical additions were made to reproduce the carbonate chemistry of tropical seawater 18,000 years ago with $pCO_2 \approx 192$ μatm and pH ≈ 8.3. The major shortcomings of the perturbation approach include lack of replication, lack of a control and lack of the continuous replacement of natural sea water.

In response to these limitations, continuous flow systems have been developed. These systems are complex, expensive and time consuming to operate. For example, Anthony et al. (2011) used an automated system for producing various acidification and temperature regimes that was regulated by a custom-built CO_2 dosing (CO_2 bubbling) system and temperature control system with pH measured by 12 polarographic sensors connected to a logger/controller unit. A second complex system has been described by Kline et al. (2012), who report on the development of an *in situ* OA experimental system. The "Coral–Proto Free Ocean Carbon Enrichment System" (CP-FOCE) uses a network of sensors to monitor conditions within a flume to maintain experimental pH as an offset from environmental pH using feedback control on the injection of low pH seawater. The CP-FOCE uses experimental flumes to enclose sections of the reef and dose them with CO_2 enriched seawater. The system uses peristaltic pumps with computer-controlled feedback dosing. Each flume is connected to a waterproof computer pod, which controls pH and logs the output of instruments that include digital pH sensors, acoustic velocimeters, and a conductivity, temperature, depth (CTD) instrument. In addition, an identical set of sensors was deployed in ambient conditions on the reef flat to monitor environmental conditions and to determine the baseline for pH offsets. Carbonate chemistry offsets of -0.06 and -0.22 pH units were measured in the acidified treatments relative to the control and were shown to be significantly different from ambient conditions and from each other over a four day test period. Obviously such systems are beyond the reach of most investigators.

Natural experiments provide a realistic environment without the need for constructing and operating an experimental system. Observations have been conducted at volcanic CO_2 vents in the Mediterranean (Hall-Spencer et al. 2008, Cigliano et al. 2010) documenting major declines in many calcifying and non-calcifying organisms and increases in macroalgae and seagrasses at reduced seawater pH . Likewise, coral reef studies focused on shallow volcanic carbon dioxide seeps in Papua New Guinea (Fabricius et al. 2011) show reductions in coral diversity, recruitment and abundances of structurally complex framework builders, and shifts in

competitive interactions between taxa. Coral cover remained constant between pH 8.1 and 7.8, but with low rates of calcification. Reef development ceased below pH 7.7. These natural situations afford excellent opportunities to study OA, but unfortunately are limited in occurrence and do not allow for replication and alteration of other factors such as increased temperature or nutrients. Thus we must rely on controlled experimental systems.

A continuous-flow, large-volume mesocosm system with high flushing rate (< 1 hour turnover) using "live" sea water directly from the ocean under full sunlight has been developed (Smith et al. 1997, Jokiel et al. 2008). This system was used in the first long-term replicated experiment on impact of OA on coral reef calcifying communities (Kuffner et al. 2008, Jokiel et al. 2008, Andersson et al. 2009). The main features include a gravity feed sea water supply that enables precise flow control and a peristaltic pump system that fed dilute HCl into the inlet water to achieve desired levels of OA in each mesocosm. The system tracked normal diurnal and seasonal patterns of irradiance, temperature and water chemistry and was run without interruption for 10 months. Furthermore, this system allows measurement of net ecosystem calcification (NEC) under continuous flow conditions (Andersson et al. 2009). Coral growth OA experiments that utilize either the CO_2 bubbling method or the HCl addition method produce the same results on coral growth (de Putron et al. 2010). The HCl method has been shown to be an appropriate method for OA studies (Anderson and Mackenzie 2012), but bubbling with CO_2 gas is preferred because it more accurately simulates future OA chemistry. The HCl method shows a clear advantage over the CO_2 bubbling method in large continuous flow experimental systems with rapid turnover (Andersson and Mackenzie 2012). For example, use of the HCl method as a means of conducting a high flow, long term (seven-week) experiment (Kuffner et al. 2008) provided the first evidence that recruitment and growth of crustose coralline algae is far more sensitive to OA than coral growth. Nevertheless, the CO_2 bubbling method is thought to be more eloquent in that it more precisely mimics the changes in sea water chemistry expected to occur in the future. Most investigators would prefer to acidify sea water using CO_2 gas rather than HCl. Thus we undertook to develop a system for rapidly acidifying seawater using CO_2 gas as an alternative to the HCl method in our high volume continuous flow mesocosm experimental system.

Materials and Procedures

The system consists of four major components:

A reliable high-volume "live" sea water supply. Marine biological studies involving long-term aquarium culture under natural conditions require a reliable sea water system. The Hawaii Institute of Marine Biology (HIMB) sea water system is plumbed in duplicate with back-up pumps and emergency electrical generators that automatically come on line in the event of a power interruption. Having all parts of the system in duplicate with frequent change-overs between the two piping systems eliminates biological fouling of pipes and assures constant flow rates of unaltered sea water from Kaneohe Bay. Consequently, in the past 40 years of research in our laboratory there have been no coral experiments that failed due to interruption of sea water supply.

Stable flow rates of seawater. The ability to measure net calcification and net photosynthesis in an uninterrupted mode will produce more realistic results than long-term incubations in the static mode. A stable flow follows the diurnal and seasonal oscillations in water quality and temperature of the reef area that is the source of the water. Use of continuous flow mode of live sea water provides natural levels of inorganic nutrients, organic nutrients and planktonic food while flushing metabolic wastes from the system. Stable flow through larger mesocosms

or smaller aquariums is best assured though use of a precisely controlled gravity feed flow using a constant head source for the seawater supply.

A continuous regulated stable supply of CO_2 gas. In this system the continuous stable supply of CO_2 gas is supplied from a regulated gas cylinder and maintained at constant flow using a variable speed peristaltic pump. Flow can be accurately adjusted by simply changing the speed of the peristaltic pump or using different diameter pump tubes. Fig. 1 shows a peristaltic pump with two pump heads that can supply two experiments. Multiple pump heads can be run in tandem with different capacity heads and tubing so as to supply different levels of CO_2 supply at a constant delivery rate from a single peristaltic pump. Commercial mass flow controllers would provide an alternate method of CO_2 delivery.

Fig. 1. Precise continuous flow of CO_2 is controlled with a pressure regulator and peristaltic pump system. The system shown is running with two pump heads that feed different mesocosms. Size of tubing and pump heads allows simultaneous feeds at two or more levels of acidification.

Efficient diffuser system. The key to the success of this system is the complete dissolution of the metered CO_2 into the highly stable water flow. Increasing pCO_2 of sea water through use of bubbles of enriched air or pure CO_2 is dependent on the slow process of equalization between the water and bubbles. Control of the process is complicated. Much of the CO_2 is wasted in this process as bubbles reach the surface of the water without being fully absorbed. These problems are avoided by injecting the CO_2 directly into the intake of a small power head pump. Power heads are miniature submersible pumps with magnetically driven impellers that are widely used to circulate water in home aquariums. They are inexpensive and operate with low power consumption and high reliability. The CO_2 gas supply is bled directly into the intake (Fig. 2) and is broken by cavitation into miniscule bubbles. These bubbles have an extremely high surface to volume ratio and are absorbed immediately and completely by the

sea water. The power head system can be positioned upstream of the aquarium water supply or can be placed directly within a well-mixed mesocosm without creating localized variations in pH. Our mesocosm system was designed to produce rapid mixing without any stagnant areas (Smith et al. 1977), so this system can accommodate diffuser pumps located either within the mesocosms or upstream of the mesocosms.

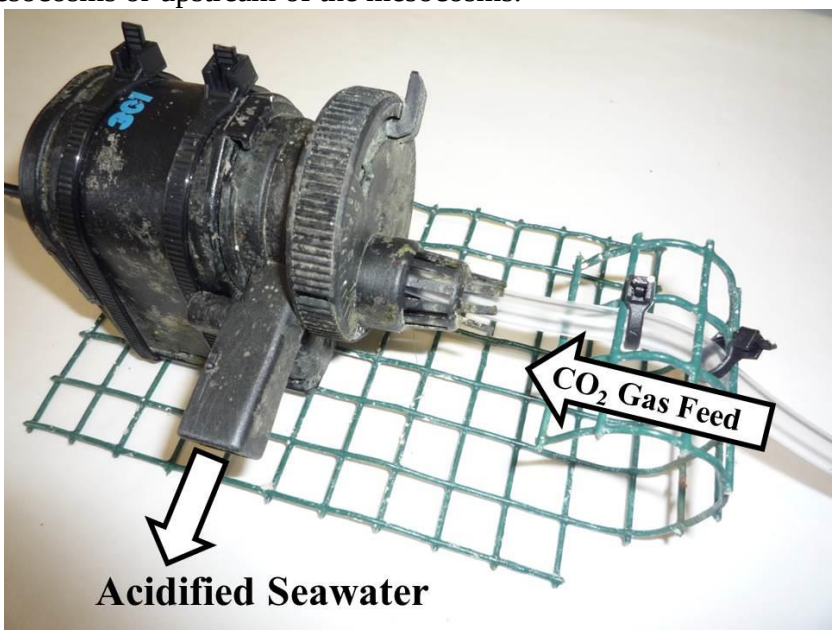

Fig. 2. The diffuser system is an aquarium power head fitted with the CO_2 gas supply into the inlet where the cavitation caused by the spinning impeller breaks the gas stream into microscopic bubbles that are absorbed instantly by the sea water.

Three examples are provided to show the applicability of this concept to a wide variety of experimental needs. All of the described systems are flexible and allow for rapid aeration (stable saturated O_2), high levels of turbulent mixing, realistic carbonate chemistry, natural diurnal and seasonal cycles of chemistry and irradiance as well as natural plankton and nutrient concentrations. In all three applications a large pH offset was established between control and experimental treatments. Total alkalinity (A_T) was checked regularly and was not altered by the addition of CO_2.

Example 1

A simplified demonstration system was developed (Fig. 3) to demonstrate the low costs involved. The apparatus uses four 20 liter glass aquaria and three 2 liter plastic buckets that are set up in a larger empty tank to collect the drain water. The spigots on the plastic buckets are commercially available tubing adaptors. The fittings are threaded into holes in the bucket walls that were made with a hot screwdriver to the proper diameter and threaded to receive the tubing adapters. The tubing adapters are available in a range of sizes. The elevated pail serves as a header tank and is allowed to overflow constantly. The tubing connectors deliver a constant stream to each of the two lower buckets. The lower buckets do not overflow with one bucket providing a constant flow of unaltered sea water to two aquaria and the second containing the

diffuser system that provides high pCO_2 sea water to the other two aquaria. Resistance heaters are placed in one of the unaltered pCO_2 aquaria and one of the high pCO_2 aquaria, giving a 2 X 2 experimental design of temperature and ocean acidification. The heated tanks are held at 2°C above ambient. The acidified tanks are held at 0.3 pH units below the non-acidified tanks. The residence time of sea water in the example was 15 minutes, providing for very high levels of water motion to the corals. Each aquarium contained 20 small colonies of the coral *Montipora capitata.* The system was located in full sunlight. Measurements of pH were taken once daily between 10:00 and 13:00 hours. All of the tanks tracked the ambient control (Fig. 4). Readings were taken only once per day in this example.

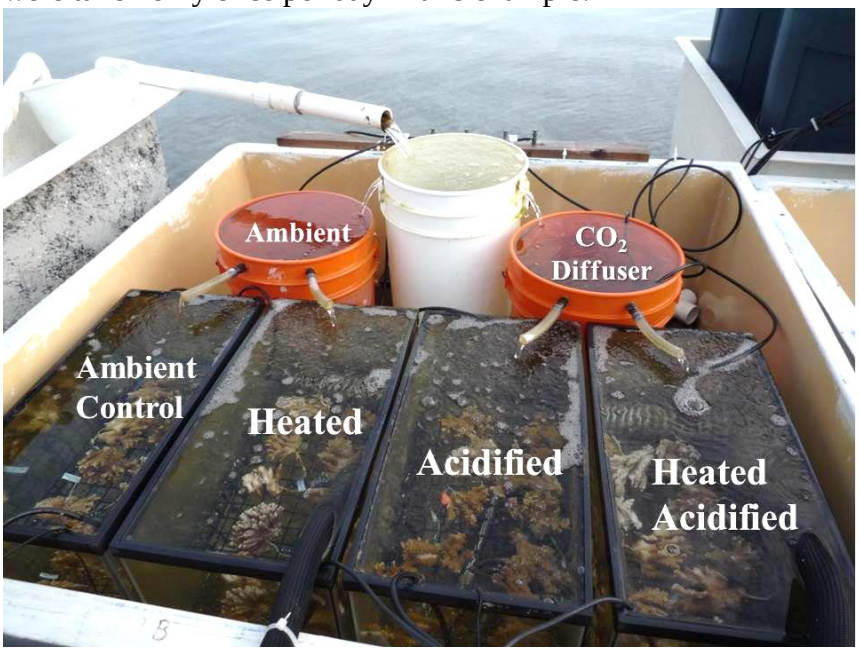

Fig. 3. Simplified low-cost flow through system using buckets and aquaria.

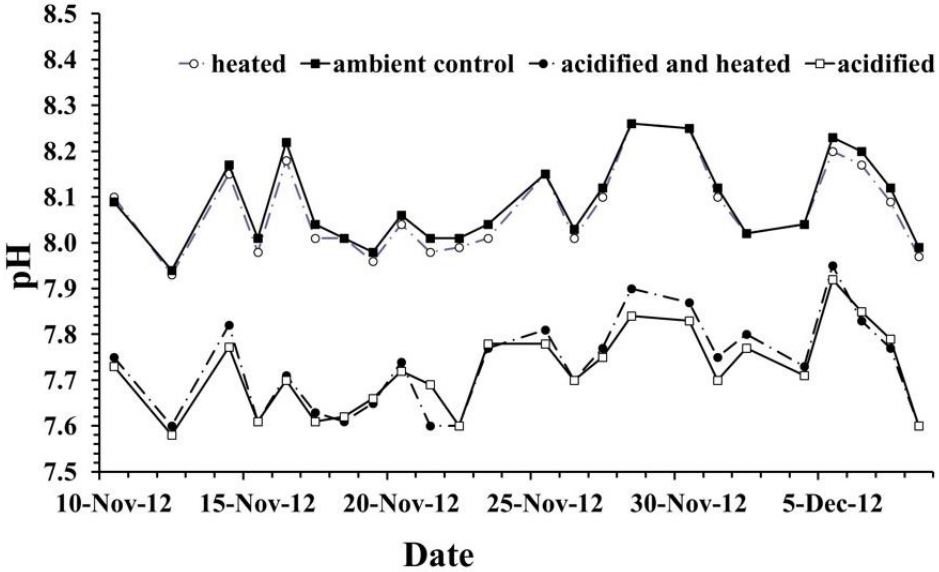

Fig. 4. Daily pH values taken between 10:00 and 13:00 in the simplified aquarium system shown in Fig. 3 over a four week period with a 15 minute residence time. Water pumped from

Kaneohe Bay shows low pH early in the day and increases in the early afternoon. The tanks track each other very closely with a fixed offset for the acidified tanks.

Example 2.

The main mesocosm system is more elaborate than the simplified demonstration described in Example 1 and has been in operation since the early 1970s. This is a highly flushed experimental system that tracks the diurnal and seasonal cycles of irradiance, temperature and water chemistry that characterize coral reef environments (Smith et al. 1977). Twelve 1 x 1 x 0.5 m deep fiberglass mesocosm tanks supplied with seawater pumped directly from a depth of 2 m offshore of the adjacent Coconut Island Reef in Kaneohe Bay, Hawai‘i. All wetted surfaces are of inert plastic material. Piping is polyvinyl chloride (PVC) plastic with an inside diameter (I.D.) of 1.25" (3.17 cm I.D.), except for the overflow standpipe in the head box which is 2.5" I.D. (5.7 cm I.D.) to allow rapid overflow in order to maintain a constant water height. Ninety degree cross fittings with threaded caps are used instead of ninety degree elbow fittings at each junction to allow for routine cleanout. The system is plumbed in duplicate to allow changeover every two weeks to insure that buildup of fouling organisms do not impede flow. An adjustable head-box standpipe arrangement (Fig. 5) provides each mesocosm with an inflow of sea water at ~ l0 liters min^{-1} resulting in a complete turnover rate of <1 h. Gravity flow and clean piping insures stable flow rates that hold within 1-2 % over a 24 hour period. The natural diurnal and seasonal fluctuations of pCO_2 that occur on inshore reef and coastal areas (Kayanne et al.1995; Bates et al. 2001; Bates 2002) are thereby retained in the mesocosms (Andersson et al. 2009). The major components of the mesocosm (Fig. 5) include a head box with an adjustable overflow standpipe consisting of several threaded nipples joined by threaded couplings to allow fine adjustments of standpipe height. Likewise the standpipe in the tail box consists of several threaded nipples connected by threaded couplings to allow fine adjustment of the standpipe height. A reducer fitting at the inlet within the mesocosm accelerates flow and creates a jet that enhances rapid mixing of inlet water with the mesocosm water. Flow rates are measured by temporarily plugging the drain in the tail box and using a stop watch to measure the time it takes for the water level to rise between two marks that encompass a 10 liter volume (Fig. 5). Increased temperature is obtained by electrical resistance heaters run without thermostatic controls to give a constant offset from the ambient temperature regime (Fig. 6). Injection of the CO_2 with the diffuser system within the head box or within the well-mixed mesocosm itself produces the same result. Fine scale continuous measurement of pH shows the system is very well mixed (Fig. 7).

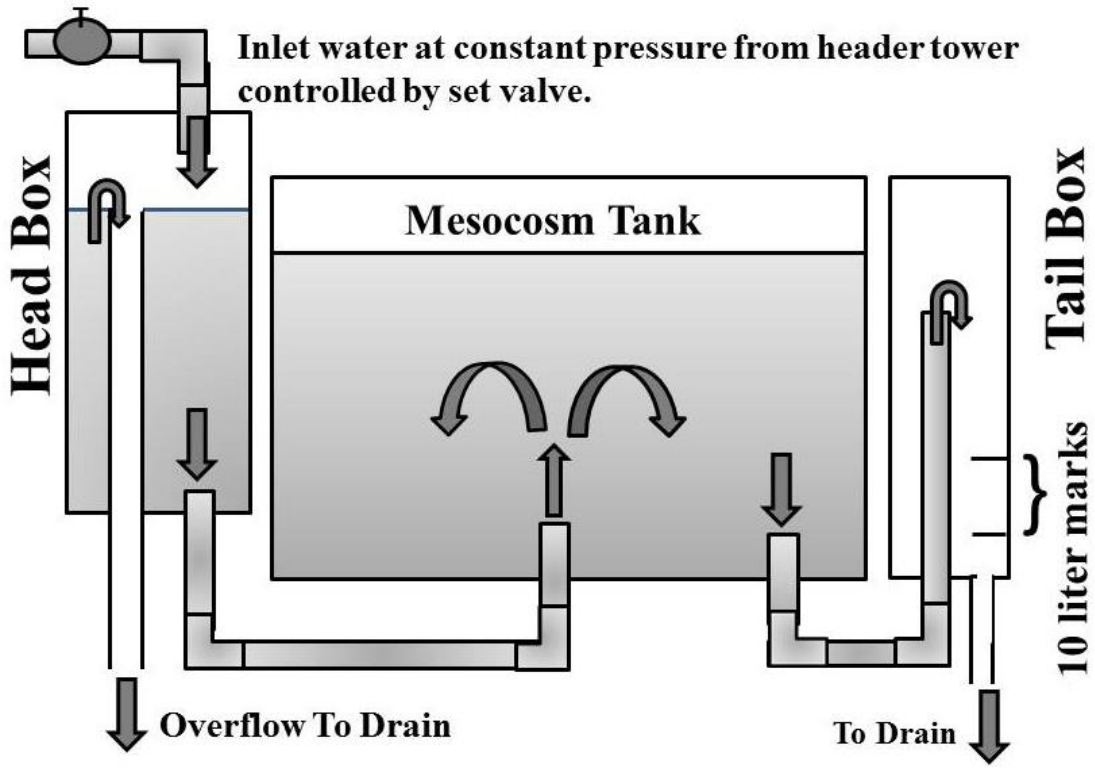

Fig. 5. The mesocosm system assures highly constant flow rates through a system of head box, tail box and stand pipes.

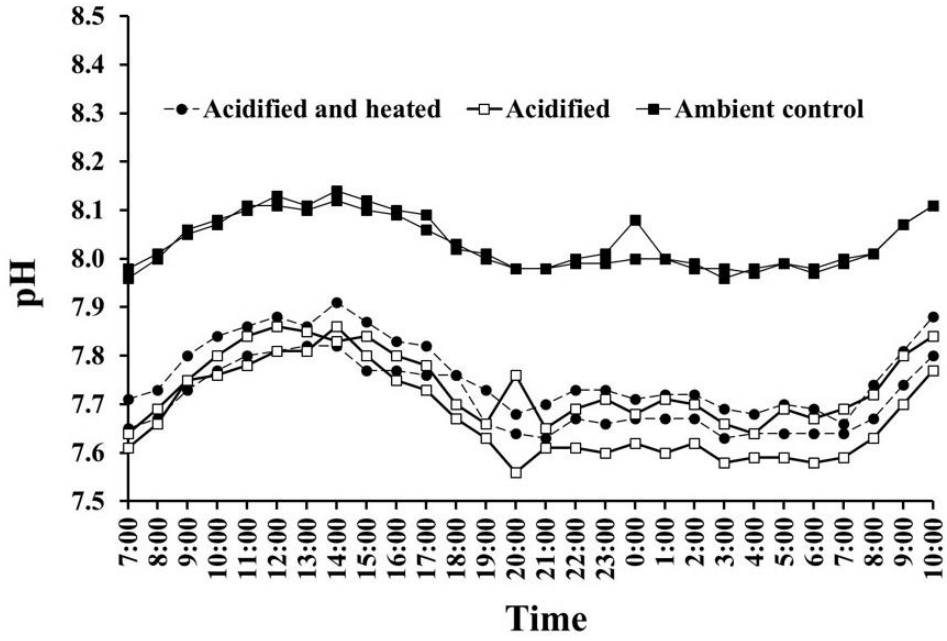

Fig. 6. Hourly pH data from a mesocosm run using 6 mesocosms (2 at ambient conditions, 2 acidified, plus 2 acidified and heated).

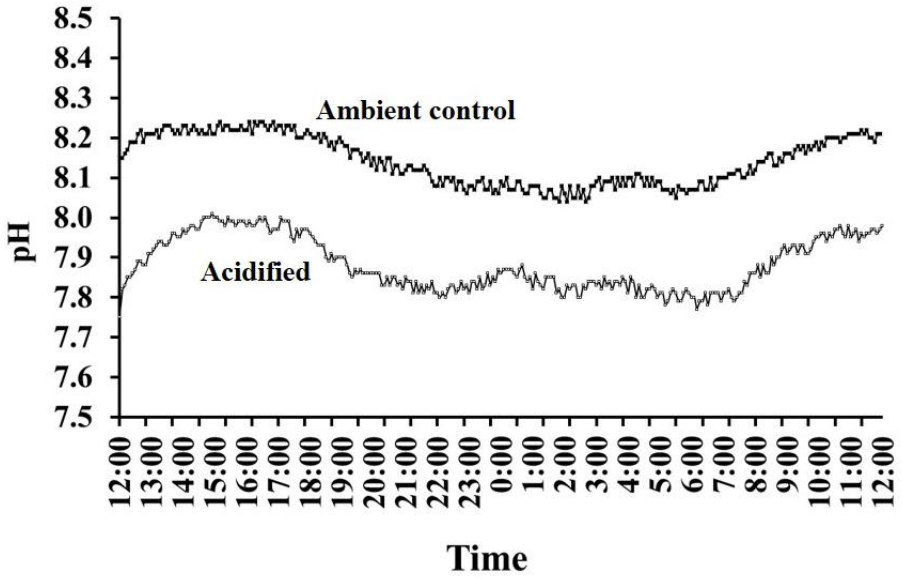

Fig. 7. Continuous fine-scale data taken in mesocosms (ambient and acidified mesocosms shown in Fig. 6) with YSI Incorporated 6920V2 multi parameter water quality sondes over a 24 hour period.

Example 3.

The diffuser can be used to acidify a large number of tanks at high flow. An experiment designed to compare metabolic response of various benthic components required monitoring 24 hour base-line metabolism followed later by another 24 hour series of measurements under acidification of 0.4 pH units in 12 mesocosms (Fig. 8). This project served as an excellent test of the peristaltic pump –injector system. The injector (Fig. 8, inset) was attached to a coupling attached to the intake for the main supply line feeding all 12 mesocosms. The flow rate of CO_2 was increased to a level that lowered the pH in the water supply by 0.4 pH units. The flow rate in each mesocosm was approximately 10 l min^{-1}, so the system was providing 120 l min^{-1} of acidified water at a 0.4 pH offset from the inlet pH. Inlets on all mesocosm tanks tracked each other in the manner shown in Fig. 6-7.

Assessment

The described pCO_2 mesocosm system has been in continuous use for over one year. Performance has been outstanding with the only interruption caused by a rupture of worn tubing in the peristaltic pump early in the testing phase. This was resolved by preventive maintenance with replacement of the tubes at regular intervals. Control instrumentation is not needed. Gravity flow of seawater has proven to be very reliable. Peristaltic pump control of the gas flow remains very constant. Once the system is running it has its own inertia. Minor adjustments in flow of seawater or of CO_2 can be made periodically in order to hold the desired offset between control and experimental mesocosms.

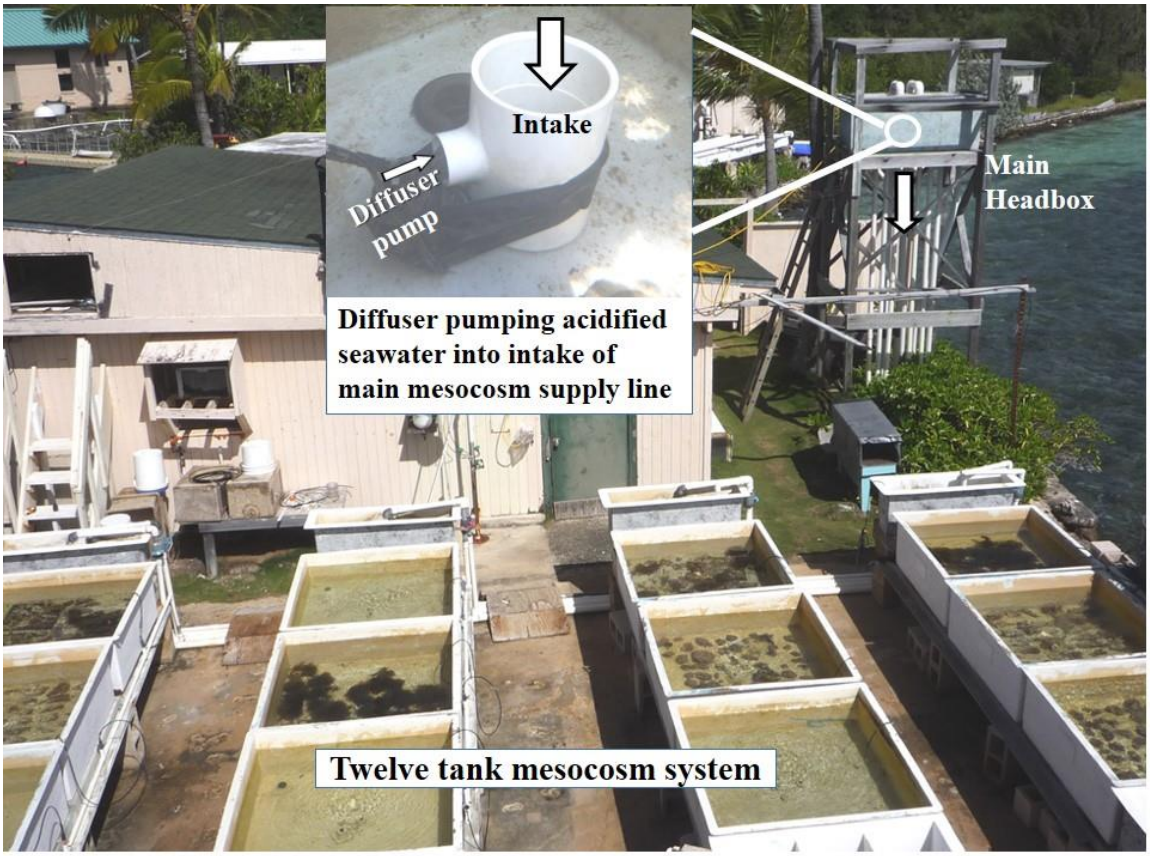

Fig. 8. System showing 12 mesocosms being supplied with highly acidified seawater (decreased by 0.4 pH units) at a high flow rate (10 liters min^{-1} per mesocosm = 120 liters min^{-1} total) provided by a single power head (Fig. 2) pumping into the main supply line located in the elevated head box (inset). A second supply line feeds tanks which are not to be acidified.

Discussion

This method meets all of the needs described in the introduction. Corals grown in the mesocosms experience natural diurnal and seasonal rhythms of pCO_2, irradiance, temperature, D.O., plankton food supply and inorganic nutrients. Dufault et al. (2012) found that coral recruits benefit from ecologically relevant fluctuations in pCO_2. Coral growth under acidified conditions is increased by planktonic feeding (Drenkard et al. 2013). Likewise nutrient supply can influence the effects of OA on coral growth (Renegar and Riegl 2005, Chauvin et al. 2011). Coral bleaching, growth and survival is influenced by irradiance and UVR (Gleason and Wellington 1993, Lesser 1996). Artificial lighting lacks the ultraviolet radiation (UVR) component that is extremely important to coral physiology and bleaching rate (e.g. Gleason and Wellington 1993, Lesser 1996) and cannot simulate the high irradiance found on a coral reef flat. Thus the system allows for long-term studies under natural conditions that cannot be realistically accomplished using static experiments.

The power heads used as part of this system are widely used in small aquariums and have a useful service life of many years. Use of the diffuser system provides 100% efficiency of CO_2 transfer to sea water, which would be a very advantageous method of acidifying experiments that require rapid acidification of large volumes of sea water. In other bubbling systems the CO_2

transfer rate is a function of bubble size, which is hard to regulate, and most of the bubbled CO_2 escapes to the atmosphere.

Comments and recommendations

This mesocosm system has operated reliably in all three configurations discussed above. These techniques will allow investigators to conduct experiments under more realistic conditions. Natural sand, rubble and macroalgae can be included in these large mesocosms to allow measures of community metabolism of "model reefs". A major advantage is that the mesocosm system described in Example 2 provides the means of measuring net ecosystem calcification and net ecosystem photosynthesis without interrupting the flow using a simple box model as demonstrated by Andersson et al. (2009). Studies of how ecosystem components such as coral, macroalgae, crustose coralline algae, sand bottom and coral rubble respond to pCO_2 independently and in combination are currently underway. Field application could involve efficient acidification of a large area of coral reef that would allow scientific investigations similar to those carried out in areas with natural CO_2 seeps (e.g. Fabricius et al. 2011).

References

Andersson, A. J., I. B. Kuffner, F. T. Mackenzie, P. L. Jokiel, K. S. Rodgers and A. Tan. 2009. Net loss of $CaCO_3$ from coral reef communities due to human induced seawater acidification. Biogeosciences Discuss. 6:1–20.

Andersson A. J. and F. T. Mackenzie. 2012. Revisiting four scientific debates in ocean acidification research. Biogeosciences 9:893–905 [doi:10.5194/bg-9-893-2012].

Anlauf, H., L. D'Croz, and A. O'Dea. 2010. A corrosive concoction: The combined effects of ocean warming and acidification on the early growth of a stony coral are multiplicative. J. Exp. Mar. Biol. Ecol. [doi:10.1016/j.jembe.2010.11.009].

Anthony K. R. N., D. I. Kline, G. Diaz-Pulido, S. Dove and O. Hoegh-Guldberg. 2008. Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc. Nat. Acad. Sci. 105:17442-17446. [doi:10.1073/pnas.0804478105]

Anthony, K. R. N., J. Kleypas, and J.-P. Gattuso. 2011. Coral reefs modify the carbon chemistry of their seawater: implications for the impacts of ocean acidification. Glob. Change Biol. 17: 3655–3666 [doi:10.1111/j.1365-2486.2011.02510.x].

Anthony, K. R. N., G. Diaz-Pulido, N. Verlinden, B. Tilbrook and A. J. Andersson. 2013. Benthic buffers and boosters of ocean acidification on coral reefs. Biogeosciences 10:4897–4909. www.biogeosciences.net/10/4897/2013/ [doi:10.5194/bg-10-4897-2013].

Bates, N. R. 2002. Seasonal variability of the effect of coral reefs on seawater CO_2 and air-sea CO_2 exchange. Limnol. Oceanogr. 47:43–52.

Bates, N. R., L. Samuels, and L. Merlivat. 2001. Biogeochemical and physical factors influencing seawater fCO_2 and air–sea CO_2 exchange on the Bermuda coral reef. Limnol. Oceanogr. 46:833–846.

Chauvin, A., D. Vianney, and C. Pascale. 2011. Is the response of coral calcification to seawater acidification related to nutrient loading? Coral Reefs 30:911–923 [doi:10.1007/s00338-011-0786-7].

Cigliano, M., M. Gambi, R. Rodolfo-Metalpa, F. Patti, and L. Hall-Spencer. 2010. Effects of ocean acidification on invertebrate settlement at volcanic CO_2 vents. Marine Biology 157:2489-2502 [doi:10.1007/s00227-010-1513-6].

Comeau, S., R. C. Carpenter, and P. J. Edmunds. 2012. Coral reef calcifiers buffer their response to ocean acidification using both bicarbonate and carbonate. Proc. R. Soc. B 280:20122374 [doi:10.1098/rspb.2012.2374].

de Putron, S. J., D. McCorkle, A. Cohen, and A. B. Dillon. 2010. The impact of seawater saturation state and bicarbonate ion concentration on calcification by new recruits of two Atlantic corals. Coral Reefs 30:321–328 [doi:10.1007/s00338-010-0697-z].

Drenkard, E. J., A. L. Cohen, D. C. McCorkle, S. J. de Putron, V. R. Starczak , and A. E. Zicht. 2013. Calcification by juvenile corals under heterotrophy and elevated CO_2. Coral Reefs 32: 727-735 [doi:10.1007/s00338-013-1021-5].

Dufault A. M., V. R. Cumbo, T.-Y. Fan, and P. J. Edmunds. 2012. Effects of diurnally oscillating pCO_2 on the calcification and survival of coral recruits. Proc. Roy. Soc. B: Biol Sci 279:2951–2958 [doi:10.1098/rspb.2011.2545].

Edmunds, P. J., D. Brown and V. Moriarty. 2012. Interactive effects of ocean acidification and temperature on two scleractinian corals from Moorea, French Polynesia. Global Change Biology 18:2173–2183, [doi:10.1111/j.1365-2486.2012.02695.x].

Fabricius, K.E., C. Langdon, S. Uthicke, C. Humphrey, S. Noonan, G. De'ath, R. Okazaki, N. Muehllehner, M. S. Glas, and J. M. Lough. 2011. Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nat. Clim. Change 1:165–169 [doi:10.1038/nclimate1122].

Gleason, D. F. and G. M. Wellington. 1993. Ultraviolet radiation and coral bleaching. Nature 365:836-838 [doi:10.1038/365836a0].

Hall-Spencer, J. M., R. Rodolfo-Metalpa, S. Martin, E. Ransome, M. Fine, S. M. Turner, S. J. Rowley, D. Tedesco and M.-C. Buia. 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454:96-99 [doi:10.1038/nature07051].

Jokiel, P. L., K. S. Rodgers, I. B. Kuffner, A. J. Andersson, F. T. Mackenzie and E. F. Cox. 2008. Ocean acidification and calcifying reef organisms: a mesocosm investigation. Coral Reefs, 27:473–483 [doi:10.1007/s00338-008-0380-9].

Kayanne, H., A. Suzuki, H. Saito. 1995. Diurnal change in the partial pressure of carbon dioxide in coral reef water. Science 269:214–216.

Kline, D. I., L. Teneva, K. Schneider, T. Miard, A. Chai, M. Marker, K. Headley, B. Opdyke, M. Nash, M. Valetich, J. K. Caves, B. D. Russell, S. D. Connell, B. J. Kirkwood, P. Brewer, E. Peltzer, J. Silverman, K. Caldeira, R. B. Dunbar, J. R. Koseff, S. G. Monismith, B. G. Mitchell, S. Dove and O. Hoegh-Guldberg. 2012. A short-term in situ CO_2 enrichment experiment on Heron Island (GBR). Scientific Reports 2:413 [doi: 10.1038/srep00413].

Kuffner, I. B., A. J. Andersson, P. L. Jokiel, K. S. Rodgers, and F. T. Mackenzie. 2008. Decreased abundance of crustose coralline algae due to ocean acidification. Nat. Geosci. 1: 114–117 [doi:10.1038/ngeo100].

Langdon, C. and M. J. Atkinson. 2005. Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. J. Geophys. Res. 110, C09S07 [doi:10.1029/2004JC002576].

Langdon, C., W. S. Broecker, D. E. Hammond, E. Glenn, K. Fitzsimmons, S. G. Nelson, T. H. Peng, I. Hajdas, and G. Bonani. 2003. Effect of elevated CO_2 on the community metabolism of experimental coral reef. Global Biogeochem. Cy. 17:1011 [doi:10.1029/2002GB001941].

Lesser, M. P. 1996. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. Limnol. Oceanogr. 41: 271-283.

Renegar, D. A. and B. M. Riegl. 2005. Effect of nutrient enrichment and elevated CO_2 partial pressure on growth rate of Atlantic scleractinian coral *Acropora cervicornis*. Mar. Ecol. Prog. Ser. 293:69–76.

Reynaud S., N. Leclercq, S. Romaine-Lioud, C. Ferrier-Pagès, J. Jaubert and J.-P. Gattuso. 2003. Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. Glob. Change Biol. 9:1660-1668 [doi: 10.1046/j.1529-8817.2003.00678.x].

Riebesell, U., V. J. Fabry, L. Hansson, and J.-P. Gattuso [eds.]. 2010. Guide to best practices for ocean acidification research and data reporting. Publications Office of the European Union. Luxembourg.

Smith, S. V., P. L. Jokiel, G. S. Key and E. B. Guinther. 1977. Metabolic responses of shallow tropical benthic microcosm communities to perturbation. Environmental Protection Agency, Narragansett, Rhode Island. Final report of contract R800906. 110 pp. Available for download at http://cramp.wcc.hawaii.edu/.

Appendix 2. Jokiel et al. (in press) draft of 10 Feb 2014

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Review / Research Article

Resolution of Conflicts Concerning Ocean Acidification and Coral Calcification

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Abstract: A widely held paradigm is that calcification rates (G_{net}) on coral reefs diminish under conditions of increasing OA due to the decreasing aragonite saturation state, Ω_{arag} (i.e. decreasing $[CO_3^{2-}]$). The recent "Proton Flux Hypothesis" states that decreased G_{net} can be attributed to decreasing net H^+ transport through the boundary layer caused by the increasing $[H^+]$ in the water column. The coral calcification equations show that G_{net} is directly proportional to the ratio of dissolved inorganic carbon (DIC) concentration to proton concentration ([DIC]:[H+] ratio). Plotting diurnal values of G_{net} against Ω_{arag} reveals a daily hysteresis pattern with Ω_{arag} lagging behind peak G_{net} by 2 or more hours, indicating that Ω_{arag} is the dependent variable. Results of a diurnal mesocosm study demonstrate that corals have limited ability to shed the waste protons generated during rapid calcification. High rates of H^+ efflux continued for several hours following peak $G_{net,}$ showing that maximum rate of proton efflux is limited. Measurements of DIC flux and H^+ flux are far more useful in describing coral metabolism dynamics than [DIC] and $[H^+]$. Likewise, Ω_{arag} is not a useful descriptor because it simply tracks pH. $[CO_3^{2-}]$ (and hence Ω_{arag}) shifts with changing $[H^+]$. DIC flux tracks P_{net} and G_{net} and drops off rapidly following peak P_{net} and peak $G_{net,}$ indicating that rate of inorganic carbon uptake is rapid enough to meet the needs of rapidly calcifying corals.

Keywords: calcification; corals; ocean acidification; seawater CO_2-carbonate system; aragonite saturation state, boundary layers, phase lag.

1. Introduction

Burning of fossil fuels continues to increase the partial pressure of carbon dioxide (pCO_2) in the atmosphere with consequent changes in ocean chemistry [1,2] that will result in a negative impact on coral and coral reef calcification [3].

The term Ω_{arag} is widely used to describe the CO_2-carbonate system aragonite saturation state of sea water and is defined as:

$$\Omega_{arag} = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{sp}} \quad (1)$$

where K_{sp} is the solubility constant of aragonite. The $[Ca^{2+}]$ in normal present-day oceanic seawater is essentially constant at 10.3 mmol kg^{-1} SW normalized to salinity. Likewise, K_{sp} is a constant, so in shallow oceanic waters Ω_{arag} is directly proportional to $[CO_3^{2-}]$. Burning of fossil fuels leads to an increase of CO_2 in seawater with a resulting decrease in Ω_{arag}. The Ω_{arag} concept has been used for decades by physical chemists to describe the seawater CO_2-carbonate system [4]. The discovery of changes in the ocean CO_2-carbonate system due to OA soon led to the question of whether or not the declining Ω_{arag} would impact coral reefs. Aragonite is the form of calcium carbonate laid down by reef corals. Smith and Buddemeier [5] reviewed the known literature in 1992 and suggested that OA would lead to decreasing net calcification (G_{net}) on coral reefs. Their conclusion was subsequently confirmed by laboratory studies showing that calcification rates of reef-building corals could decline by 20% to 40% under twice present day pCO_2 conditions [6,7]. These observations increased concern about the impact of OA on corals and coral reefs [8]. This combination of findings led to the widespread use of Ω_{arag} as an independent variable to describe changes in G_{net} resulting from OA [3].

1.1. *The physical chemist point of view of calcification*

Growing awareness of the negative impact of OA on G_{net} over the past decade led to a rapid expansion on research in this area [9]. Along with this expansion a number of apparent contradictions emerged as scientists approached the problem from different points of view. Research in this area is difficult because the acid-base chemistry of the CO_2-carbonate system of sea water is complex. The chemical process in which OA impacts calcification is generally described in the literature and in various presentations by a series of equations as follows:

When CO_2 dissolves in surface seawater it reacts with water to form a weak carbonic acid (H_2CO_3):

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \quad (2)$$

The carbonic acid then dissociates into bicarbonate ions (HCO_3^-) and hydrogen ions (H^+). In turn, the release of H^+ decreases the pH (i.e. increases the acidity) of the seawater:

$$H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \quad (3)$$

Excess hydrogen ions (H^+) react with carbonate ions (CO_3^{2-}) to form additional bicarbonate ions:

$$H^+ + CO_3^{2-} \Leftrightarrow HCO_3^- \quad (4)$$

Thus, the net effect of CO_2 dissolution in seawater is to increase $[H^+]$ and $[HCO_3^-]$, while decreasing $[CO_3^{2-}]$. This standard presentation of equations ends with the following:

$$Ca^{2+} + CO_3^{2-} \Leftrightarrow CaCO_3 \quad (5)$$

The above series of equations appears in many scientific papers and reviews of calcification (e.g. [2,4,8] and is also widely presented in talks on ocean acidification. Equation 5 can lead to the impression that G_{net} is driven by $[CO_3^{2-}]$ without any consideration of the possible role of H^+. A complete expression of the changes occurring in Equations 2-4 is shown as Equation 6. Equilibria between the various species is controlled by pH with higher pH driving the reactions to the right.

$$CO_2 + H_2O \Leftrightarrow H^+ + HCO_3^- \Leftrightarrow 2H^+ + CO_3^{2-} \quad (6)$$

Equation 5 is balanced but is not an accurate representation of calcification. The correct equation must show that the products of calcification are $CaCO_3 + 2H^+$. A number of combinations of reactants can be used to balance the calcification equation. Equation 6 shows that different species of dissolved inorganic carbon (DIC) can be involved and exchange with each other as follows:

$$Ca^{2+} + (CO_2 + H_2O) \Leftrightarrow CaCO_3 + 2H^+ \quad (7)$$

$$\updownarrow$$

$$Ca^{2+} + (H^+ + HCO_3^-) \Leftrightarrow CaCO_3 + 2H^+ \quad (8)$$

$$\updownarrow$$

$$Ca^{2+} + (2H^+ + CO_3^{2-}) \Leftrightarrow CaCO_3 + 2H^+ \quad (9)$$

Equations 7-9 are written in two dimensions with a red arrow showing the relationship between the carbonate species (shown in parentheses) that shift with changes in $[H^+]$ as shown in Equation 6. Dissolution is the reverse calcification reaction. Calcification inevitably produces an excess of two moles of H^+ and thus reduces total alkalinity (A_T) by two moles for every mole of $CaCO_3$ precipitated [10,11]. When the equations are written correctly in this manner the importance of protons becomes apparent with two moles of H^+ produced for every mole of $CaCO_3$ precipitated regardless of which form of dissolved inorganic carbon (DIC) is involved. [DIC] is the sum of inorganic carbon species in the seawater:

$$[DIC] = [HCO_3^-] + [CO_3^{2-}] + [CO_2] \quad (10)$$

The following equations describe carbohydrate formation from the various available CO_2 species and show no change in total alkalinity associated with organic carbon production:

$$(CO_2 + H_2O) \Leftrightarrow CH_2O + O_2 \quad (11)$$

$$\updownarrow$$

$$(H^+ + HCO_3^-) \Leftrightarrow CH_2O + O_2 \quad (12)$$

$$\updownarrow$$

$$(2H^+ + CO_3^{2-}) \Leftrightarrow CH_2O + O_2 \quad (13)$$

Note that photosynthesis results in a local increase in pH (lower $[H^+]$) while respiration results in a localized decrease in pH (higher $[H^+]$). Unlike calcification/dissolution, photosynthesis/respiration does not produce or consume protons and therefore does not alter total alkalinity (A_T). The photosynthesis equations are written in two dimensions with the red arrows showing changes in distribution of species that occurs (Equation 6) with shifts in pH. The absolute and relative distribution of inorganic carbon species that comprise DIC is controlled by pH, so photosynthesis and respiration have a powerful localized effect on inorganic carbon species distribution. The spatial arrangement of photosynthesis sites in relation to areas of rapid calcification can alter localized pH conditions and allow for biological facilitation and control of inorganic carbon flux.

In sum, photosynthesis and calcification both lower the seawater DIC, while respiration and $CaCO_3$ dissolution raise the DIC. Only the precipitation or dissolution of $CaCO_3$ significantly alters A_T; consequently, changes in $[A_T]$ can be used to calculate calcification and dissolution rates as G_{net}. However, photosynthesis and respiration can radically alter localized $[H^+]$ and thus can alter localized concentration of CO_3^{2-}, HCO_3^- and CO_2. Coral calcification is a biological process that is heavily influenced by the associated process of photosynthesis which modifies the local pH as well as by supplying energy to the calcification reaction. Protons can be considered a waste product of calcification and O_2 a waste product of photosynthesis.

A geochemical model of G_{net} based on enhanced kinetics of calcification has been proposed [12]. This model describes the effect of increased temperature on abiotic processes in the calcifying fluid located in the space between calicodermis and the skeleton. The model does not consider localized effects of photosynthesis, limiting processes at the tissue-seawater interface or acclimation limits imposed by biochemical processes. The authors concluded that an increase in calcification due to global warming will accelerate chemical reactions within the calcifying space which in turn will outweigh the negative effects of declining $[CO_3^{2-}]$. This conclusion runs counter to experimental evidence. McCulloch *et al*. [12] acknowledged that biological experimental and observational data do not support their model, and concluded that the fate of corals will ultimately depend on physiological adaptation. The McCulloch *et al*. model is reminiscent of the earlier geochemical model of McNeil *et al*. [13] that was based on the assumption that calcification increases linearly with increasing temperature above the present day temperature range. The McNeil *et al*. model predicted an increase in G_{net} of 35% by the year 2100, a conclusion that runs counter to all experimental observations which suggest a 15% to 30% decrease under future acidified conditions. This model did not consider various biological processes. In reality G_{net} declines sharply above peak summer temperature due to physiological limitations with bleaching and eventual death of corals under future temperature scenarios [14].

Obviously calcification is a biologically mediated geochemical process and the physiological dimension must be considered in calcification models.

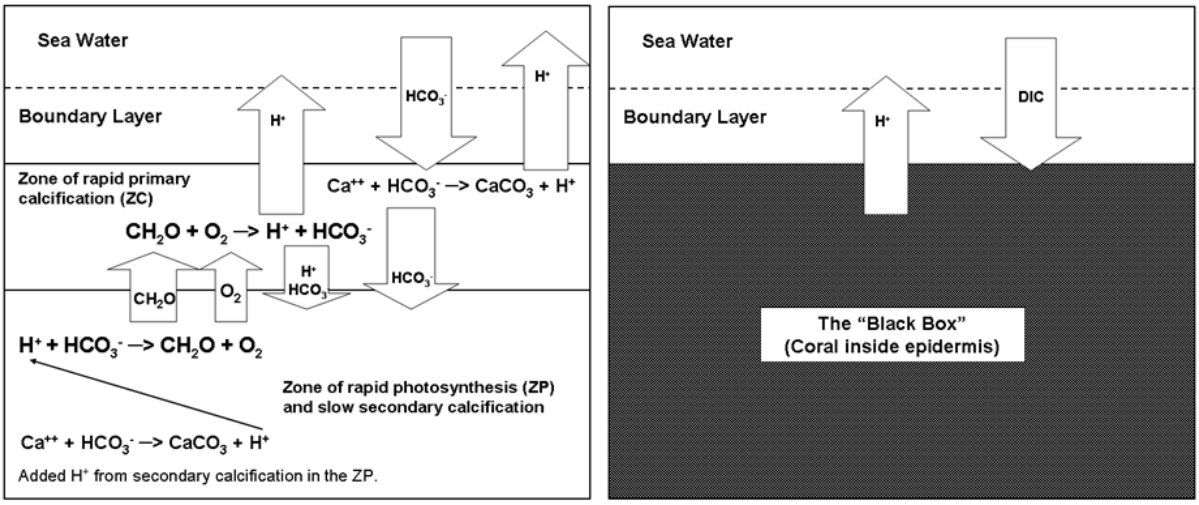
1.2. *The physiologist point of view*

Roleda *et al*. [9] noted that for several decades prior to our awareness of the "OA problem", the disciplines of carbonate physical chemistry and calcification physiology were largely unrelated fields. The dominant role that physical chemistry has played in OA research (i.e. decreasing $\Omega_{arag} \Rightarrow$ decreasing $CO_3^{2-} \Rightarrow$ decreasing calcification) resulted in a non-physiological based model of how OA will influence calcifiers [9]. Two disparate views evolved concerning mechanisms controlling G_{net}. The first was focused primarily on a physical chemistry view implying that CO_3^{2-} is the main inorganic source of carbon used for calcification. The second is the long held physiological view that calcifiers utilize HCO^- as a source for calcification and must rid H^+ as a waste product [9]. The correlation between Ω_{arag} and G_{net} in tropical reef building corals has been well documented [6,15]. However, a correlation between Ω_{arag} and G_{net} does not establish cause and effect because Ω_{arag} also correlates with other components of the seawater CO_2-carbonate system. Nevertheless, nearly all of the coral reef literature dealing with impact of OA on G_{net} use Ω_{arag} or its surrogate [CO_3^{2-}] as the independent variable with G_{net} as the dependent variable [7]. The possible role other variables such as of [HCO_3^-] and [H^+] are often left out of the discussion.

1.3. *Thinking outside of the box*

Most studies describing G_{net} involve incubation of corals in static containers under controlled conditions. Response of G_{net} to manipulation of the bulk water CO_2-carbonate chemistry has been used as an experimental method to gain insights into the processes occurring in the calcification space of the coral adjacent to the skeleton. Results from such incubations are useful, but must be viewed with caution because there is an organism located between the calcifying space and the bulk water being measured as well as a boundary layer (BL) between the organism and the water column. Complex carbon metabolism within the tissues (Figure 1, left panel) radically modifies the localized chemical environment inside of the coral epidermis, which can be termed the "black box" (Figure 1, right panel). The processes of photosynthesis and calcification compete for available inorganic carbon within the box, and corals have resolved this problem by spatially separating the primary sites of photosynthesis from the primary sites of calcification [16]. Corals elevate the pH of the fluid within the space between the skeleton and the coral calicodermis by removing protons. These protons ultimately must be transferred to the water column or acidosis of tissues within the box will slow and eventually stop further reactions. Coral incubation experiments can only measure the net change in A_T of the bulk seawater due to influx of various forms of DIC (Equations 7-9) and efflux of H^+ from the coral. Extrapolating changes in bulk water chemistry to hypothesized changes in the calcifying space involves a number of untested assumptions. Measurement of changes in bulk water chemistry can only describe net flux of H^+ and DIC between the coral and the water column through the boundary layer.

Figure 1. The biochemistry of reef corals is complex as presented in the Two Compartment Proton Flux Model [16] shown in the left panel. Measurements of coral calcification rate derived from changes in alkalinity and pH in the bulk seawater can only estimate flux of material (Equations 7-9) through the boundary layer shown in the right panel.



1.4. *Attempts to identify CO_2-carbonate system factors limiting calcification rate.*

The scientific literature contains numerous conflicting reports from experiments designed to determine the limiting factor in coral calcification due to changes in ocean carbonate chemistry. Schneider and Erez [15] conducted laboratory experiments designed specifically to separate the effects of Ω_{arag}, pH, CO_3^{2-} , dissolved CO_2 ($CO_{2(aq)}$), A_T, and dissolved inorganic carbon (DIC) on G_{net}. They showed that G_{net} (in both light and dark) was positively correlated with $[CO_3^{2-}]$. However, their data also show a statistically significant positive correlation between DIC and G_{net} and a negative correlation between G_{net} and $[H^+]$ under various conditions. Cohen and Holcomb [17] embraced the $[CO_3^{2-}]$ hypothesis with the explanation that under conditions of increasing OA corals must expend more energy and consequently calcify at a lower rate as seawater $[CO_3^{2-}]$ decreases. Jury *et al*. [18 (2010)] attempted to distinguish the effects of Ω_{arag}, pH, $[CO_3^{2-}]$ and $[HCO_3^-]$ on G_{net} by conducting incubations in six highly modified seawater chemistries. Coral calcification responded consistently to variation in $[HCO_3^-]$, but not consistently to $[CO_3^{2-}]$, Ω_{arag} or pH. Jury *et al*. [18] concluded that data from their study showed inconsistencies in the Ω_{arag} model and suggested that coral G_{net} is likely controlled by a combination of $[HCO_3^-]$ and $[H^+]$ in most coral species. Experiments designed to test the relative importance of $[HCO_3^-]$ versus $[CO_3^{2-}]$ in coral calcification by de Putron *et al*. [19] led to their conclusion that G_{net} was controlled by $[CO_3^{2-}]$ and not by $[HCO_3^-]$, though their data also show a positive correlation between DIC and G_{net} and a negative correlation between G_{net} and $[H^+]$. Edmunds *et al.* [20] conducted coral incubation studies and compiled existing information on coral calcification. Their analysis revealed a high degree of variation in calcification as a function of pH, $[HCO_3^-]$, and $[CO_3^{2-}]$.

This discussion was expanded when Jokiel [21] proposed that increase of $[H^+]$ in the water column can reduce G_{net} by increasing diffusion gradient strength between the coral epidermis and the water column with a consequent reduction of proton efflux. Further, he

proposed that G_{net} is proportional to [DIC] of sea water divided by the $[H^+]$ (i.e. [DIC]:$[H^+]$ ratio). In other words Jokiel [21] responded to the question "Is G_{net} controlled by H^+, HCO^{3-} or CO_3^{2-} ?" with the statement, "All of the above when expressed as the ratio of [DIC]:$[H^+]$". This was developed into the "Two Compartment Proton Flux Model" [16] for reef corals which is based on the spatial arrangement of the primary areas of calcification in relation to the primary areas of photosynthesis. Use of a two compartment model recognizes the spatial separation of primary photosynthetic areas of a coral in relation to primary calcification areas. The model recognizes different strengths of sea water boundary layers in areas of photosynthesis in the proximal areas of a colony versus thin boundary layers in areas of rapid calcification located on the distal portions of a colony. Hence the model allows different flux rates for various materials over different parts of a colony and resolves apparent contradictions in coral physiology while providing new insights into mechanisms controlling various aspects of coral biology.

Comeau *et al*. [22] measured growth of a coral and a crustose coralline alga under a wide range of sea water chemistries and concluded that both CO_3^{2-} and HCO_3^- were involved in calcification. Jokiel [23] used data on calcification rate of coral and crustose coralline algae from Comeau *et al*. [22] to test the Proton Flux Model of calcification. There was a significant correlation between calcification (G_{net}) and the ratio of dissolved inorganic carbon (DIC) to proton concentration ([DIC]:$[H^+]$ ratio). The ratio is tightly correlated with $[CO_3^{2-}]$ and with aragonite Ω_{arag}. Jokiel [23] noted that correlation does not prove cause and effect, and that Ω_{arag} and $[CO_3^{2-}]$ have no basic physiological meaning on corals other than a correlation with [DIC]:$[H^+]$ ratio, which was proposed as the driver of G_{net}. Comeau *et al*. [24] responded by describing the type of experiments that are needed to allow further evaluation of the Proton Flux Model in relation to their model. They conclude that their results do not challenge the paradigm that the control of coral calcification is mediated entirely by $[CO_3^{2-}]$. Obviously there is a need to reconcile these various observations.

1.5. *Conceptual stumbling blocks*

The widespread use of Equations 2-5, with emphasis on Equation 5 fails to communicate the importance of H^+ as a waste product as shown by Equations 7-9. The importance of Equations 7-9 cannot be overemphasized - calcification will always result in the production of two moles of H^+ for every mole of $CaCO_3$ precipitated. Otherwise the various studies using coral incubations could not calculate G_{net} from changes in the total alkalinity (A_T) with one mole of $CaCO_3$ precipitated for every two mole decrease in A_T [10, 11].

Coral calcification occurs in the space between the innermost tissue layer of the coral (calicodermis) and the skeleton. The real physiological question does not concern the Ω_{arag} at the site of calcification, which is under control of the coral animal. Rather, we need to know if protons produced by calcification are dissipating out of the organism at a rate sufficient to avoid acidosis of tissues (Figure 1). Measurements of CO_2-carbonate chemistry of the bulk water do not necessarily relate directly to chemistry of the calcification fluid. Furthermore, measurements made using changes in the chemistry of the bulk water during coral incubations cannot distinguish between whether the supply side or product side of Equations 7-9 is responsible for the decrease in G_{net}. The stoichiometry will be the same for both cases.

An addition conceptual problem traces its roots to the fact that $[H^+]$ is often reported as pH where:

$$pH = -\log[H^+] \quad (14)$$

The problem is that pH represents a double non-linear transformation of $[H^+]$ (i.e. the log of a reciprocal) that disguises the magnitude of change in $[H^+]$. The widespread use of pH compared to $[H^+]$ results from the fact that pH is easily measured with a pH electrode. However, the pH electrode measures activity of H^+ rather than $[H^+]$. Nevertheless, pH has a long history of use and is universally reported in research papers. This is all well and good because physiological systems generally respond to activity of H^+ (rather than concentration), so use of pH is a useful index of acid-base conditions. Free H^+ (i.e. bare protons) are not the actual form present in solution anyway. However, physical processes outside of the organism such as diffusion through a boundary layer respond to concentration. The use of pH (-log $[H^+]$) rather than $[H^+]$ clouds the fact that immense changes in $[H^+]$ across diffusion barriers outside of the tissues occur with increasing OA [21].

Until recently the focus on limiting factors for coral calcification rate has been on the reactants (left side of Equations 7-9). Failure to dissipate H^+ from the site of calcification, through the tissues and out through the seawater boundary layer (right side of Equations 7-9) will cause acidosis and disruption of normal biological processes. An excellent analogy is furnished by the companion process of photosynthesis in reef corals. Photosynthesis produces fixed carbon as a product while calcification produces calcium carbonate as a product. Photosynthesis produces the "waste product" O_2 while calcification produces the "waste product" H^+. Boundary layer thickness can control primary production by limiting efflux of O_2 from the coral. Increased water motion decreases boundary layer thickness, increases O_2 flux rate and increases primary production [25]. By analogy, boundary layer thickness presumably can control proton efflux and thereby control calcification rate. Corals have evolved a very sophisticated morphology that results in a highly effective means of dealing with the waste products of O_2 and H^+ [16].

Control of G_{net} by $[CO_3^{2-}]$ is not an attractive hypothesis. As pointed out by Hofmann and Todgham [26] CO_3^{2-} is rarely transported across membranes. Further, $[CO_3^{2-}]$ is an order of magnitude lower than $[HCO_3^-]$, which appears to be the preferred form of DIC utilized by reef corals [27,28]. The importance of HCO_3^- uptake to coral metabolism is shown by the abundance of the enzyme carbonic anhydrase (CA) in reef corals. The reaction described in Equation 4 is accelerated by CA which has a reaction rate that is among of the fastest of all enzymes. Coral tissues and zooxanthellae contain large amounts of CA [28,29] which play a major role in controlling transport of CO_2 throughout the coral colony. Al-Horani *et al*. [30] identified CA bound to the membranes of the epidermal cells of the surface body wall. Moya *et al*. [31] identified CA in the calicodermis, which controls the precipitation of skeletal material. Wherever the conversion between CO_2 and HCO_3^- is very fast (i.e. such as occurs in presence of CA) in comparison to the rate of diffusion a difference in HCO_3^- concentration corresponding to the CO_2 tension difference will be established [32].

2. Resolution of conflicts in OA studies

A major difficulty with the Ω_{arag} model is failure to explain why G_{net} decreases with increasing OA in the face of increasing [DIC] and increasing $[HCO_3^-]$. G_{net} increases under higher $[HCO_3^-]$ [18,33,34], so G_{net} should increase with increasing OA. The increase in G_{net} due to increased $[HCO_3^-]$ caused by a doubling of pCO_2 from pre-industrial levels is only 3.8% compared to the predicted decrease in G_{net} of 32% due to increased $[H^+]$ for a net decrease of

28.2% [21]. Thus in the next century any benefit to skeletal growth caused by higher $[HCO_3^-]$ will be overwhelmed by the order of magnitude greater negative impact due to increased $[H^+]$.

In an attempt to reconcile the other disparities in the literature we undertook an investigation to further demonstrate the relationship between G_{net} and the seawater CO_2-carbonate system parameters of A_T, Ω_{arag}, CO_3^{2-}, HCO_3^-, $CO_{2(aq)}$, H^+ and the $[DIC]:[H^+]$ ratio. Rather than following the equations used by physical chemists (Equations 1-5) we elected to use a physiological organism-centered approach based on documented metabolic processes. The major focus was on the fact that any protons generated by the calcification reaction must dissipate out of the coral. Also, there must be uptake of DIC if the coral is to calcify (Figure 1).

The chemistry of the CO_2 -carbonate system is complex, but only two parameters are needed to calculate the distribution of DIC species and Ω_{arag} in seawater at known salinity (S), temperature (T), and pressure (P). The relationship between the major parameters of the system can be demonstrated simply by varying pCO_2 at constant A_T, S, T and P. As pCO_2 increases, pH decreases (i.e. $[H^+]$ increases), [DIC] increases, $[CO_3^{2-}]$ decreases, and Ω_{arag} decreases. The opposite is true for decreasing pCO_2. In other words, $[H^+]$ varies directly with [DIC] and inversely with $[CO_3^{2-}]$. The rules of proportionality [35] allow us to state this relationship mathematically using proportionality constant (k_1) as follows:

$$[H^+] = \frac{[DIC]}{[CO_3^{2-}]} k_1 \tag{15}$$

These terms can be rearranged as follows:

$$[CO_3^{2-}] = \frac{[DIC]}{[H^+]} k_1 \tag{16}$$

In oceanic surface water, Ω_{arag} is proportional to $[CO_3^{2-}]$, so we can rewrite the equation with a different proportionality constant (k_2) as:

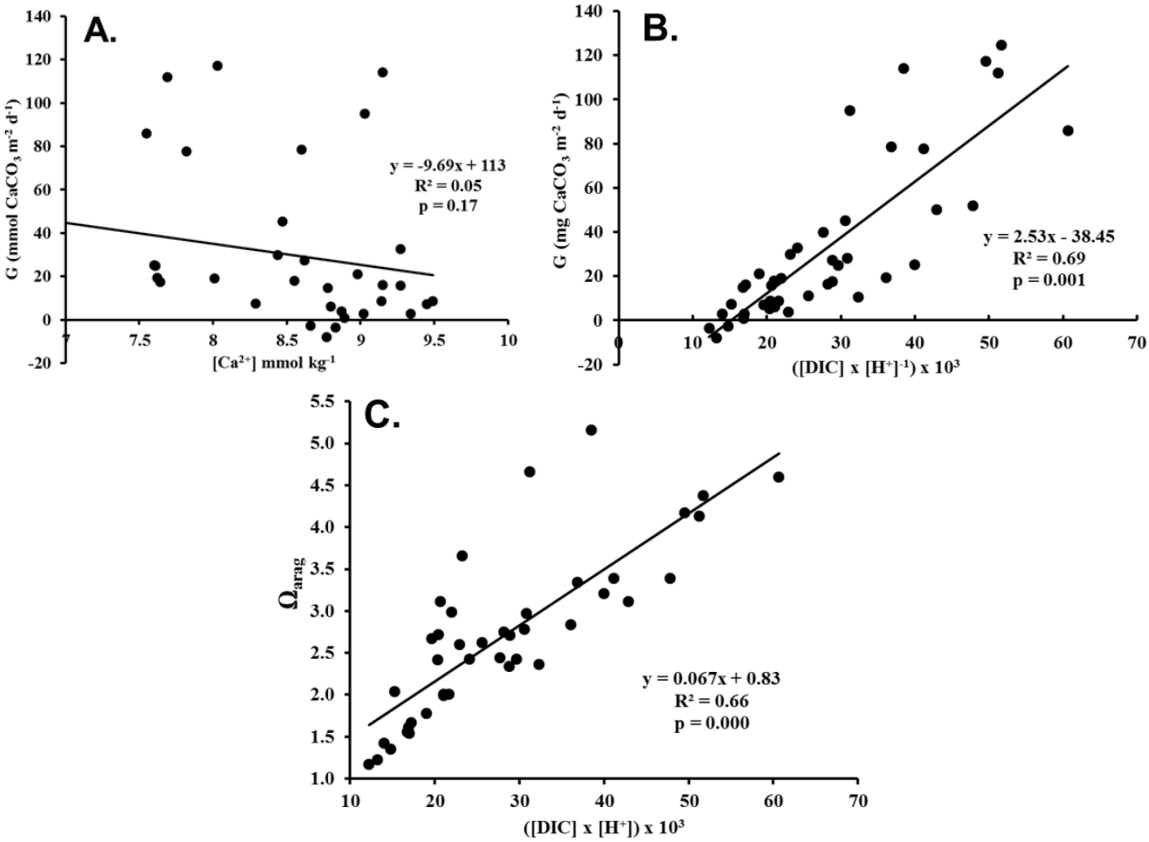
$$\Omega_{arag} = \frac{[DIC]}{[H^+]} k_2 \tag{17}$$

There is a large body of data showing that G_{net} is proportional to Ω_{arag}. Therefore we can rewrite Eq. 16 as:

$$G_{net} = \frac{[DIC]}{[H^+]} k_3 \tag{18}$$

Equation 18 could also be derived from the observation of Schneider and Erez [15] that G_{net} is directly proportional to [DIC] and inversely proportional to $[H^+]$. The plot of G_{net} versus Ω_{arag} (or G_{net} versus $[CO_3^{2-}]$) should be similar to the plot of G_{net} versus the $[DIC]:[H^+]$ ratio times the appropriate proportionality constant.

Figure 2. Biosphere-2 data from Langdon *et al*. [36] showing: A.) G_{net} as a function of Ca^{2+}, B.) G_{net} as a function of the [DIC]:[H^+] ratio and C.) Ω_{arag} vs. [DIC]:[H^+] ratio. Data and calculations are presented in Table S1.



3. Evaluating the [DIC]:[H^+] versus Ω_{arag} relationship

The [DIC]:[H^+] ratio concept is an alternate way of viewing net calcification. A high quality data set was produced by Langdon *et al*. [36] who conducted long term static tests in highly modified sea water chemistries. This work was conducted in the 2650 m^3 "ocean" coral reef mesocosm of Biosphere-2 located near Tucson, Arizona. Effects of sea water carbonate chemistry on G_{net} was determined under various sea water chemistries in an assembled community of coral reef organisms consisting of corals, calcifying algae, and other typical reef biota. The investigators manipulated the saturation state of the water by adding various amounts of $NaHCO_3$, Na_2CO_3 and $CaCl_2$. In 1995-1998 additions of $NaHCO_3$ and Na_2CO_3 were made to keep the pH in a narrow range of 8.1±0.1 and isolate the effect of changing CO_3^{2-} concentration. During 1999 chemical additions were made to simulate the carbonate chemistry of tropical seawater that existed 18,000 years ago with $pCO_2 \approx 192$ μatm and pH ≈ 8.3. They showed that G_{net} was a function of the product of [Ca^{2+}] and [CO_3^{2-}], leading to their conclusion that "saturation state (and not pH, pCO_2, or HCO_3^-) affects coral reef calcification". Data reported for A_T, Ca^{2+}, CO_3^{2-}, HCO_3^-, Ω_{arag}, pH and G_{net} during each of the experimental trials (Table S1) was used to calculate [DIC], [H^+] and the DIC:H^+ ratio. Analysis of these data shows a non-significant relationship between G_{net} and [Ca^{2+}] (Figure 2A), reflecting the superabundance of

Ca^{2+} (≈10 mmole kg^{-1}) in relation to CO_3^{2-}(≈0.2 mmol kg^{-1}) or DIC (≈2 mmol kg^{-1}). In the case of corals, Gagnon *et al*. [37] describe rapid exchange of Ca^{2+} and other cations between seawater and the calcifying fluid. The mechanism for this type of transport appears to be a voltage-dependent Ca^{2+} channel that accelerates the trans-epithelial transport of Ca^{2+} that is used for coral calcification [38], but has not been shown for anions such as CO_3^{2-}. Presumably then, [Ca^{2+}] is not a major driver of calcification (Figure 2A). In contrast, G_{net} shows a significant correlation with the DIC:H^+ ratio (Figure 2B). In retrospect, the significant relationship between G_{net} and [CO_3^{2-}] or its surrogate Ω_{arag} is due to correlation of Ω_{arag} with the DIC:H^+ ratio (Figure 2C).

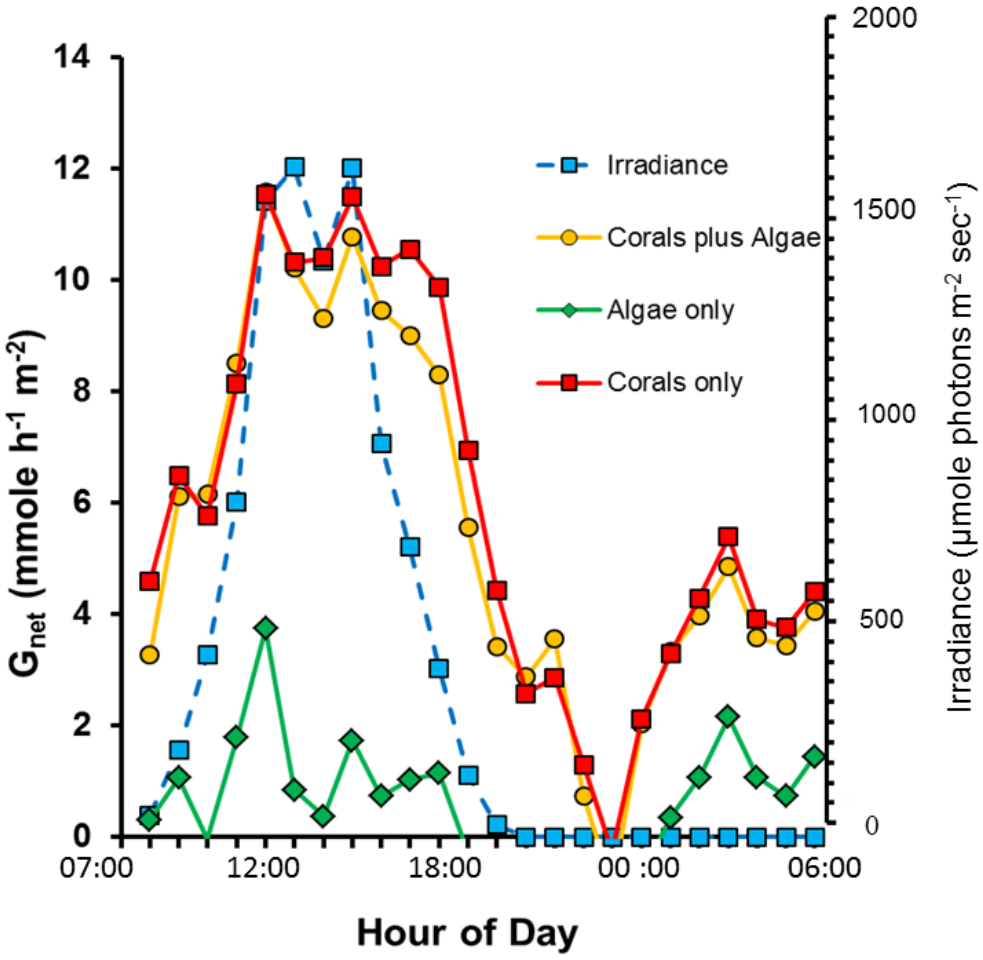
4. Evaluating the relationship between CO_2 chemistry and coral reef metabolism.

A mesocosm experiment was undertaken in order to demonstrate and amplify the concepts presented in the above sections. The relationship between calcification and the major parameters of the seawater carbonate system was investigated using the flow-through mesocosm system at the Hawaii Institute of Marine Biology, Kaneohe Bay, Oahu, Hawaii. The mesocosm system has been described previously in detail [39, 40]. Three mesocosms were used for this demonstration. Major parameters for the experiment are shown in Table S2. The first mesocosm ("Coral only") was loaded with healthy live corals having a total buoyant weight of 7.6 Kg buoyant weight (which equals 11.5 Kg dry skeletal weight [41] of the reef coral *Montipora capitata* for close to 100% coverage of the bottom. The second mesocosm ("Coral plus Algae") contained the same weight of live coral plus 3.1 Kg wet weight of the macroalgae *Gracillaria salicornia*. The third mesocosm ("Algae only") was loaded with the same weight of the macroalgae. A small biomass of calcifying organisms was present on the macroalgae in the form of epiphytes that were not removed. Dead skeletal material and sediment were excluded from all three mesocosms to reduce the effect of decalcification and other processes on G_{net}. The mesocosms were provided with a constant flow of seawater sufficient to flush them at approximately once per hour. The organisms in the mesocosms were allowed to acclimate for one week. Water chemistry was sampled hourly from 06:00 on 24 April to 07:00 on 25 April 2012 at the inlet and outlet and the flow rate recorded (Table S3). The same methods described in Andersson *et al*. [40] were used to measure T, S, pH and A_T. Precise hourly measurements of flow and A_T were used in a simple box model to calculate hourly net calcification G_{net} [40]. Likewise, precise measurements of dissolved oxygen (DO) and flow rate were used in the box model to calculate hourly rate of net photosynthesis (P_{net}). The various carbonate parameters were determined using the program CO2sys [42]. Solar input at the site was monitored with a LiCor Brand Quantameter (Li-Cor Inc., Lincoln, NE, USA), which measured photosynthetically active radiance (PAR) between 400 nm and 700 nm.

Calcification over the 24 hour period (Figure 3) shows a typical diurnal pattern related to light enhanced calcification and solar irradiance and dark calcification. Values for G_{net} are high due to the large biomass of live coral, high solar irradiance in the shallow mesocosms and absence of sediment or dead carbonate skeleton within the mesocosm. The "Corals only" and "Corals plus Algae" treatments track each other closely. Light saturation of calcification did not occur until irradiance exceeded 1,500 μmole photons m^{-2} sec^{-1}, which is three times the level of artificial light used is most laboratory studies of coral calcification. Calcification rate is very low in the "Algae only" treatment due to low biomass of calcifying organisms. Low-levels of dark calcification occur at night. There is a dip in calcification to zero around midnight with a return

to the dark calcification rate peak at approximately 03:00 hours. This night pattern is persistent and has been observed in 18 recent mesocosm runs and in a series of flume runs.

Figure 3. Diurnal net calcification rate (G_{net}) and irradiance for the three mesocosms (Table S3).



In this experiment the [DIC]:[H^+] ratio showed a near perfect correlation with Ω_{arag} (Figure 4) for all three treatments as predicted in Equation 18. Jokiel [23] showed the same tight correlation of the [DIC]:[H^+] ratio with [CO_3^{2-}] and with Ω_{arag} for the coral *Porites rus* and the crustose coralline algae *Hydrolithon onkodes* over a wide range of seawater carbonate chemistry conditions. In contrast, the relationship between the Ω_{arag} and DIC:H^+ ratio in the Biosphere-2 experiments (Figure 2) showed extremely high variance. Chemistry in the closed cycle Biosphere-2 ocean experiments was influenced by oxidation, reduction and remineralization of the inorganic components as well as by organic processes such as decomposition of organic matter and nitrogen fixation/reduction. These processes cause deviations from the expected relationship between G_{net} and the CO_2-carbonate system parameters when measured over long time scales in the closed Biosphere-2 system. In contrast, experimental data taken in rapidly flushed continuous flow mesocosms lacking sediment and non-living carbonate shows a very tight relationship (Figure 4).

Figure 4. Plot of Ω_{arag} versus the [DIC]:[H^+] ratio in the three mesocosms over 24 hours.

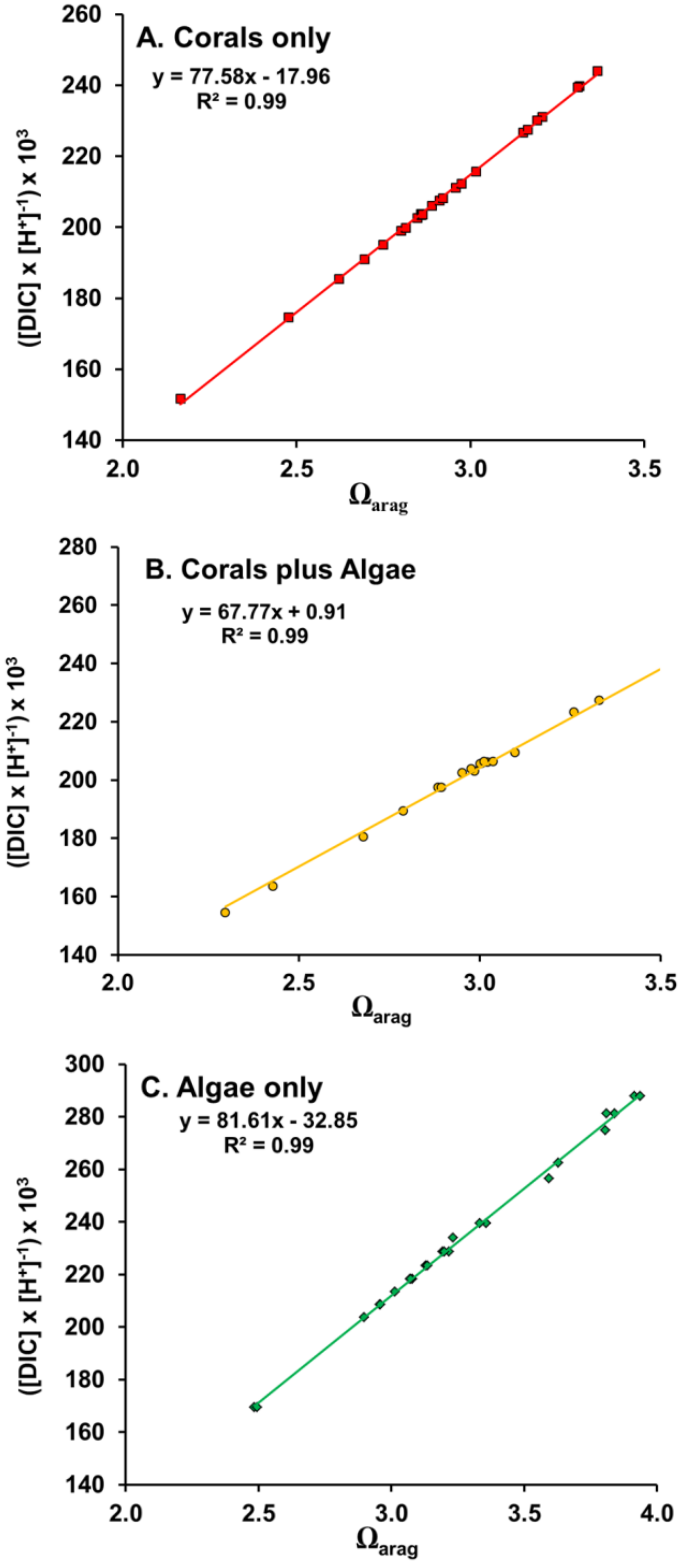
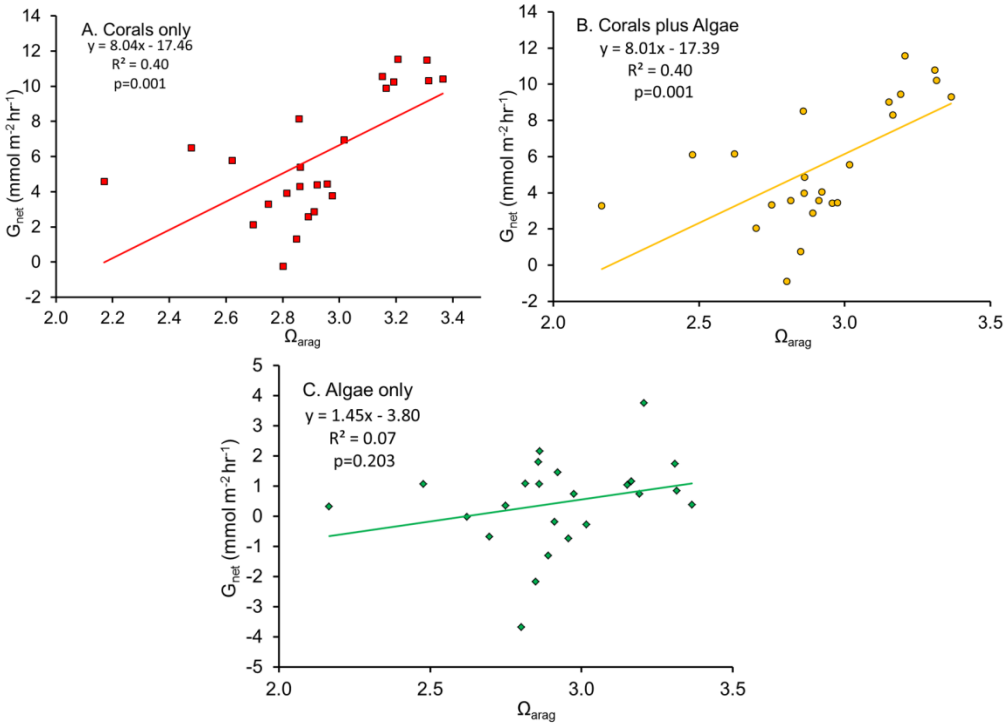


Figure 5. Net calcification rate (G_{net}) as a function of [DIC]:[H^+].

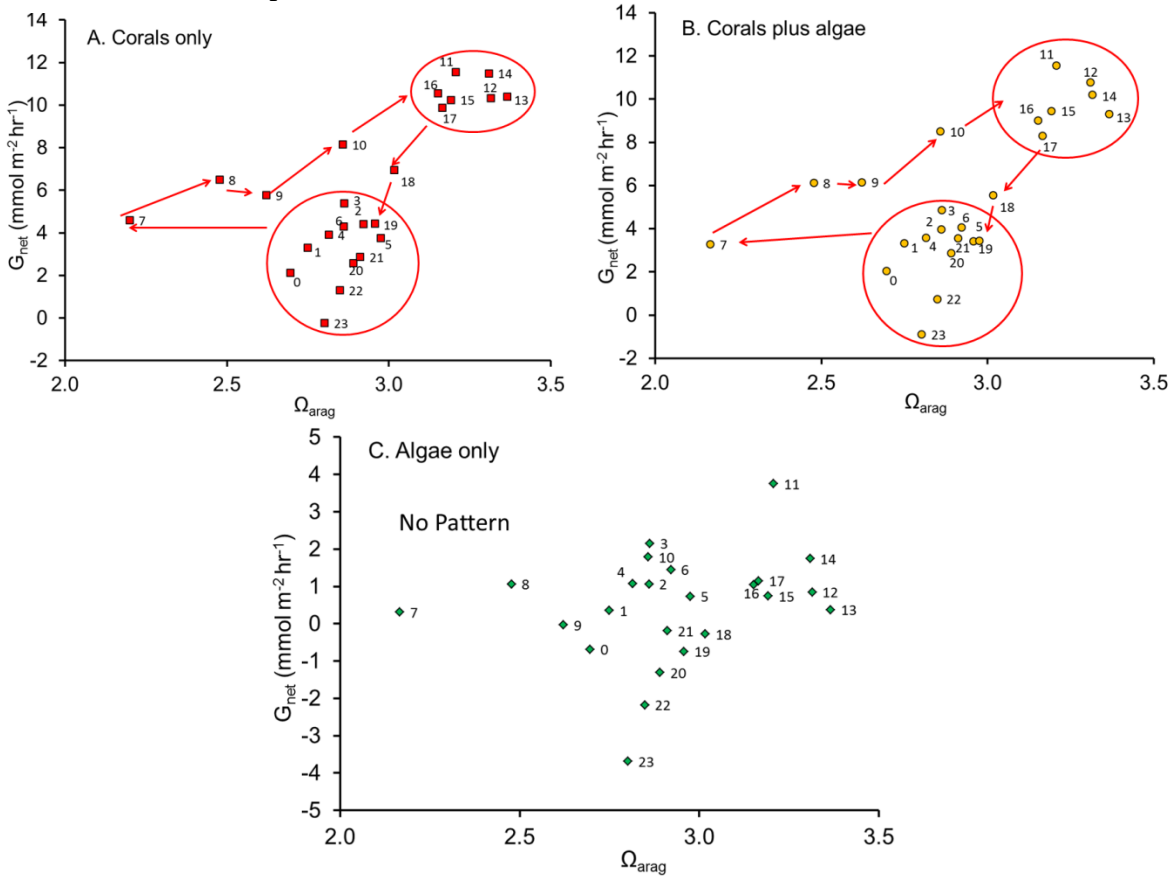


4. Diel hysteresis, phase lags and night calcification patterns.

4.1 Connecting the dots.

McMahon *et al*. [43] quantified G_{net} in a healthy coral reef lagoon in the Great Barrier Reef during different times of day. Their observations revealed a diel hysteresis pattern in the G_{net} versus Ω_{arag} relationship. This phenomenon can be demonstrated by labeling the points in Figure 2 with the hour of day as shown in Figure 6. The diel pattern moves from the lower left quadrant early in the day toward the upper right through mid-day and then back to the lower center during the night before returning to the lower left quadrant at first light. The pattern is nearly identical for the "Corals only" mesocosm (Figure 6A) and the "Corals plus Algae" (Figure 6B), which tracked each other closely (Figure 3) in terms of calcification. The "Algae only" mesocosm did not show this pattern. A linear regression for the G_{net} vs. Ω_{arag} data (Figure 5) accounted for part of the variance (R^2=0.40), but did not effectively describe the variance resulting from the diel pattern.

Figure 6. Net calcification rate versus Ω_{arag} (Table S3) with each point labeled with hour of the day which reveals the clockwise coral reef diel hysteresis pattern for: A.) "Coral only" mesocosm, B. "Coral plus Algae" mesocosm and the C.) "Algae only" mesocosm, which showed no pattern.



Cyronak *et al*. [44] used chambers to measure *in situ* benthic solute fluxes at three different advection rates at Heron Island lagoon, Australia and observed a strong diurnal hysteresis pattern similar to that in Figure 6. They suggested that diel hysteresis is caused by the interaction between photosynthesis and respiration (P_{net}). The data did not follow a trend consistent with the Ω_{arag} of the water column being the main driver of carbonate precipitation and dissolution. Instead, carbonate precipitation and dissolution in these sediment communities is linearly correlated to the rates of photosynthesis and respiration occurring over the same time period. Changes in flux rates over a diel cycle demonstrate the importance of taking into account the short-term variability of benthic metabolism when calculating net daily flux rates for coral reef communities.

4.2 Phase shifts.

Evaluation of phase relations for the various parameters listed in Table S3 can be facilitated by scaling each variable on a 0 to 1 scale. The normalized value of a_i for variable A in the i^{th} row was calculated using the equation:

$$\text{Normalized value } (a_i) = \frac{a_i - A_{min}}{A_{max} - A_{min}} \quad (19)$$

Where A_{min} = the minimum value for variable A and A_{max} = the maximum value for variable. Figure 7 summarizes the results for the variables most often considered in the literature (pH, Ω_{arag}, P_{net} and G_{net}).

Figure 7. Normalized pH, Ω_{arag}, P_{net} and G_{net} values versus time of day for the three mesocosms using data from Table S3 and Equation 19. Arrows point to relative maxima for each parameter on a 0 to 1 scale.

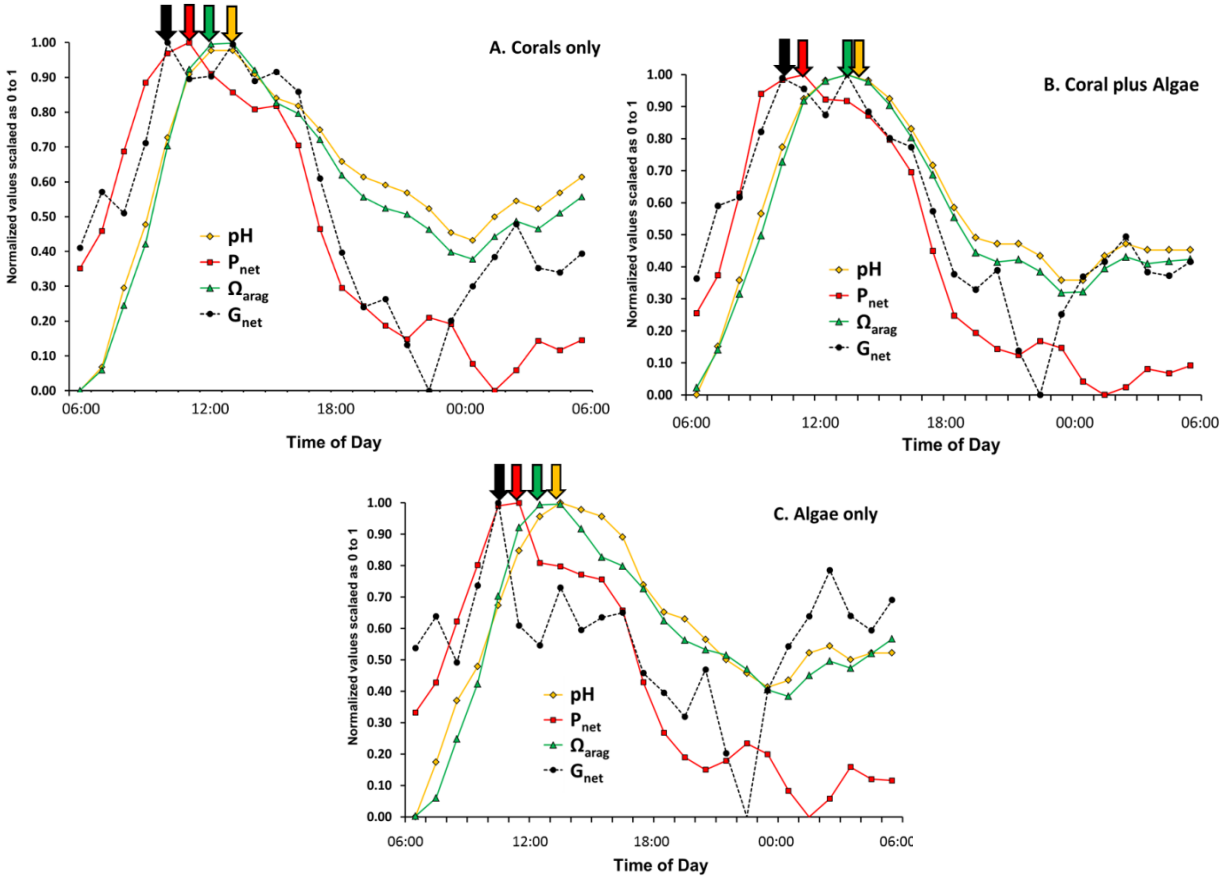


Figure 7 shows that peak pH and Ω_{arag} lag behind G_{net} throughout the daily cycle by two or more hours. The figure also shows that peak G_{net} follows P_{net} during daylight photosynthetic hours with a reverse during the nighttime hours. The reversal is the result of respiration increasing with night calcification (Figure 3). Shamberger *et al*. [45] reported that maximum Ω_{arag} lags behind G_{net} on the reefs of Kaneohe Bay, Hawaii. McMahon *et al*. [43] report that peak G_{net} rates occurred 2-3 hours before the Ω_{arag} maximum on a healthy coral reef on the Great Barrier Reef. Thus Ω_{arag} (along with closely correlated $[CO_3^{2-}]$, pH and [DIC]:$[H^+]$ ratio) is not the primary driver of coral calcification over a diurnal cycle. The paradigm that Ω_{arag} correlates with G_{net} on a global scale must be tempered with caveat that on smaller spatial and temporal scales other processes dominate. The data presented above show that diurnal irradiance drives P_{net}, which in turn drives G_{net}, which alters pH, which controls $[CO_3^{2-}]$ and Ω_{arag} as well as the

ratio of [DIC] to [H^+]. A better understanding of this hierarchy will resolve many of the contradictions in the literature on coral reef calcification.

4.3 Night calcification.

Laboratory studies show that coral calcification continues in darkness, but at a lower rate than observed in light enhanced calcification [15]. Night calcification rates have generally been assumed to be low and constant at night, although this assumption has largely gone untested. Figure 7 and Figure 9 show decreasing dark calcification following sunset, reaching zero near midnight followed by an increasing rate of dark calcification and an increase in respiration (Figures 8C and 8D) that rises to a peak at 03:00 just before dawn. This pattern has occurred consistently in our mesocosm experiments, with the same pattern observed in 30 separate mesocosm runs with different communities under various conditions. Barnes and Crossland [46] used time-lapse photography to measure diurnal growth in the staghorn coral *Acropora acuminata* and found that night-time extension rate was similar to or greater than day-time extension. They suggested that, "symbiotic association permits rapid growth because the coral can invest in flimsy scaffolding at night with the certainty that bricks and mortar will be available in the morning". Wooldridge [47] has proposed a new model for "dark" coral calcification, whereby O_2-limitation of aerobic respiration during the night initiates a homeostatic host response that forms the skeletal organic matrix. The matrix formed at night subsequently allows rapid growth of the aragonite fibers during the "light-enhanced" period of calcification, when abundant energy derived from photosynthesis is available. Perhaps the mid-night calcification minimum observed in Figure 7 at 00:00 reflects this period of organic matrix formation that precedes the 03:00 night calcification peak.

4.4 Diurnal changes in concentration of A_T, pH, Ω_{arg} and DO.

The variables of A_T, pH, Ω_{arg} DIC, and DO are concentrations while P_{net} and G_{net} are actually flux rates. Care must be taken when comparing concentrations to flux rates because flux rate can be high when concentration is high or low. Or flux rate can be low when concentration is high or low. Figure 7 shows patterns that are difficult to explain because the figure mixes flux rates with concentrations. This issue will be discussed in Section 4.5, but first we will compare differences in concentrations of key variables over the diurnal cycle.

Figure 8. Diurnal changes in seawater chemistry in the three mesocosms for: A.) total alkalinity A_T, B. aragonite saturation state Ω_{arag}, C. pH and D. dissolved oxygen.

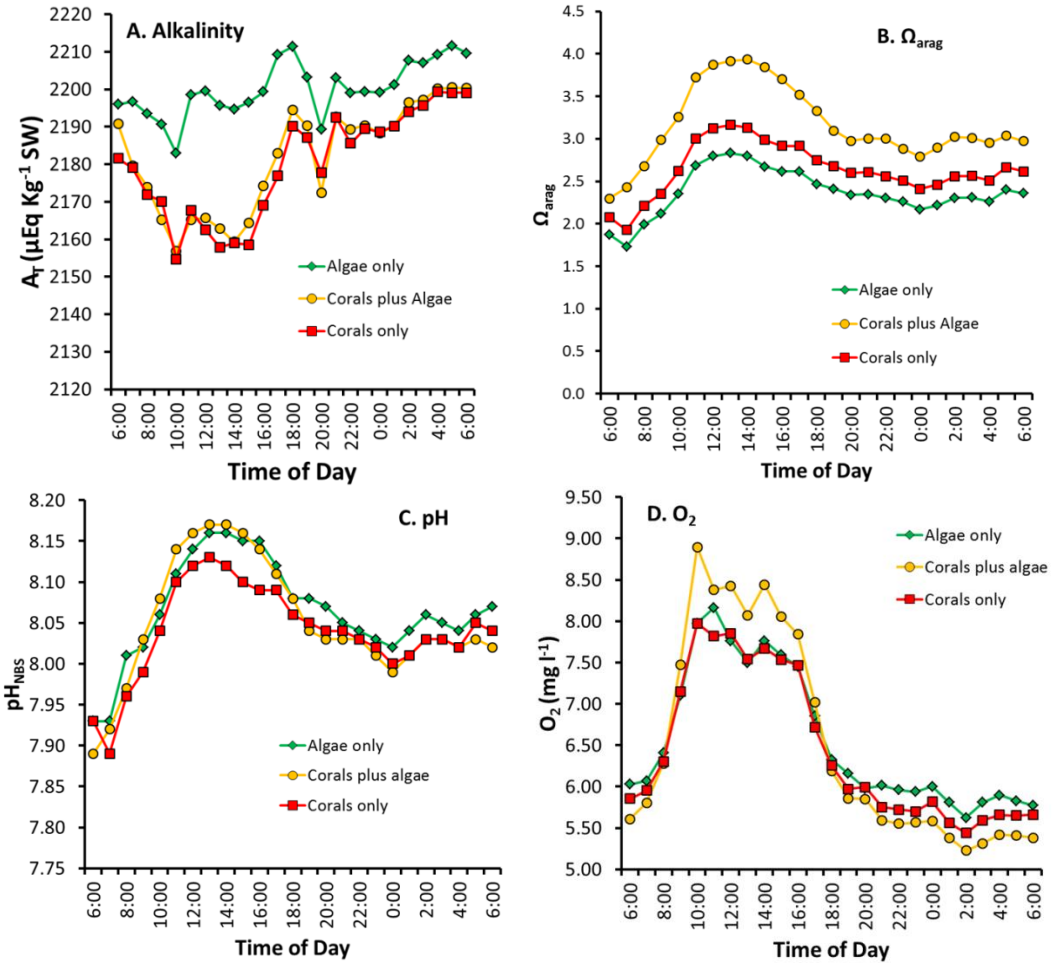


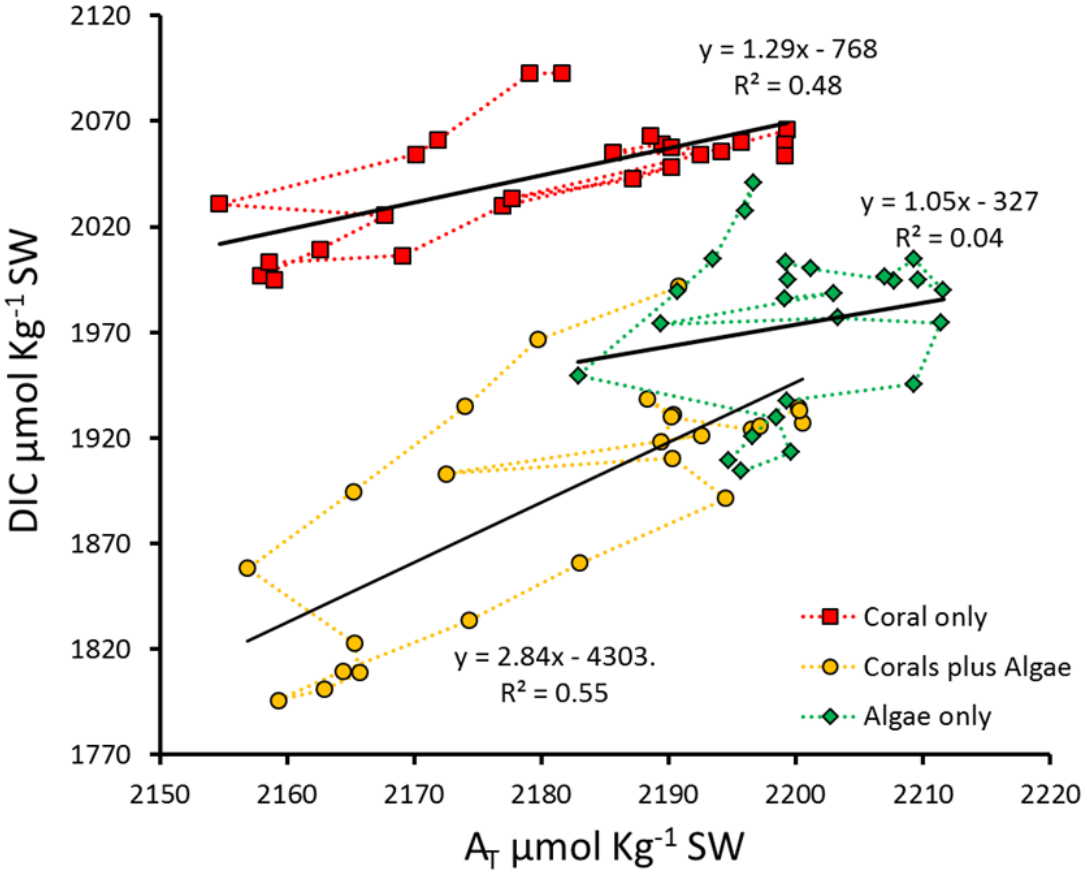
Figure 8 reveals several important patterns:

1. Alkalinity in the "Algae only" mesocosm remained high during the entire diurnal cycle. In contrast, the mesocosms containing corals showed lower A_T (Figure 8A) caused by rapid calcification. A_T reduction by the corals was greatest during the daylight hours when G_{net} was high (Figure 3) with the difference diminishing during nighttime hours.
2. The two mesocosms with algae maintained a higher Ω_{arag} throughout the mid-day portion of the diurnal cycle (Figure 8B) which can be attributed to higher pH resulting from rapid rates of algae photosynthesis and coral photosynthesis (Equations 11-13), with a less pronounced difference during the rest of the cycle.
3. The extreme difference in Ω_{arag} between the "Corals only" and the "Corals plus Algae" mesocosms (Figure 8B) did not produce a corresponding difference in G_{net} between the two mesocosms (Figure 3), which demonstrates that in this situation Ω_{arag} is uncoupled

from G_{net} which explains differences when comparing different systems with different diurnal P_{net} regimes.

4. Night-time pH and Ω_{arag} values (Figures 8B and 8C) show less variability than A_T (Figure 8A) and G_{net} (Figure 3).
5. The high biomass in the "Coral plus Algae" mesocosm (Figure 8D) resulted in the highest O_2 values during daylight hours (photosynthesis) and the lowest O_2 during the night (respiration). The "Algae only" treatment had the second highest daytime level O_2 due to algal photosynthesis and relatively high levels of O_2 at night.
6. The 03:00 calcification peak observed in Figure 3 show a decrease in O_2 concentration and a drop in pH due to accelerated respiration. The O_2 (Figure 8D) and the pH (Figure 8C) are measured independently and show this effect to corroborate the observation.

Figure 9. Plot of hourly DIC versus hourly A_T for the diurnal data measured in each of the three mesocosms. Successive hours are connected by the dotted lines to show the patterns of diurnal hysteresis.



Plotting DIC versus A_T (Figure 9) demonstrates the major influence of P_{net} on seawater Ω_{arag}. Calcification and dissolution shift A_T values horizontally along the abscissa in Figure 9. Calcification and dissolution also changes DIC value co-ordinates vertically along the ordinate. However, photosynthesis and respiration change DIC along the ordinate without changing A_T.

The observed hysteresis pattern is the result of P_{net} driving G_{net}. A linear relationship accounting for approximately half of the variance ($R^2 \approx 0.5$) can be observed between DIC and A_T for the two rapidly calcifying mesocosms containing corals. This relationship does not hold for the low-calcification "Algae only" mesocosm.

As pointed out by McMahon *et al*. [43], connecting the points on a graph of A_T vs. DIC reveals a circular hysteresis pattern over the diel cycle as shown for the G_{net} versus Ω_{arag} plot (Figure 6). G_{net} can account for changes in both the A_T and DIC concentrations. However, P_{net} can only account for changes DIC concentration. Therefore, Ω_{arag} is a function of the changes in carbonate chemistry due to both P_{net} and G_{net}, and any changes in DIC concentration relative to A_T will result in different influences on Ω_{arag}. For example, in systems with high organic production relative to calcification (Coral plus Algae mesocosm), Ω_{arag} will increase during daylight due to high pH caused by high uptake of CO_2 used for photosynthesis (Figure 8). Conversely, in systems with low organic production relative to calcification (Coral only mesocosm), Ω_{arag} will decrease due to the uptake of A_T. Any decrease in G_{net} associated with an increase in P_{net} will increase Ω_{arag} and change the way that G_{net} responds to OA. Therefore, any prediction of future global changes on coral reef G_{net} based on ambient seawater Ω_{arag} also needs to take into account the influence of future localized P_{net} on both G_{net} and Ω_{arag}.

Table 1. Hourly and daily G_{net} and P_{net} values for the three mesocosms.

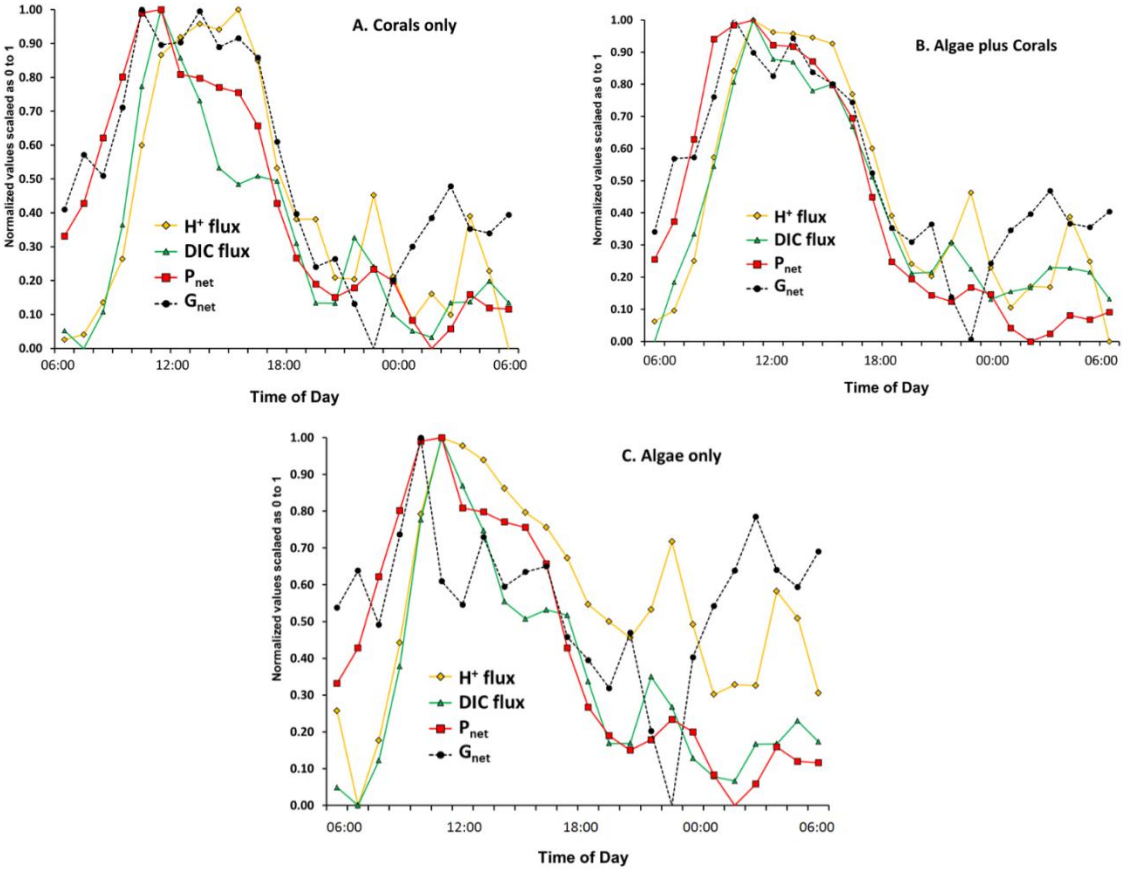
		G_{net} (mmol m^{-2} h^{-1})			P_{net} (mmol m^{-2} h^{-1})		
Time of Day	**Solar Irradiance** µmol photons m^{-2} sec^{-1}	Corals only	Corals plus Algae	Algae only	Corals only	Corals plus Algae	Algae only
07:00	56.3	4.6	3.3	0.3	-59	-264	22
08:00	221.6	6.5	6.1	1.0	65	-67	127
09:00	464.6	5.8	6.2	0.0	327	361	340
10:00	854.0	8.1	8.5	1.6	553	883	539
11:00	1619.0	11.5	11.6	3.4	650	956	746
12:00	1705.0	10.3	10.2	0.8	685	983	756
13:00	1468.0	10.4	9.3	0.3	582	853	546
14:00	1702.0	11.5	10.8	1.6	521	845	534
15:00	1001.0	10.2	9.5	0.7	466	768	504
16:00	739.0	10.5	9.0	1.0	477	644	488
17:00	429.0	9.9	8.3	1.1	347	472	379
18:00	159.0	6.9	5.6	-0.3	71	61	127
19:00	31.7	4.4	3.4	-0.7	-123	-277	-50
20:00	0.0	2.6	2.9	-1.2	-183	-366	-135
21:00	0.0	2.9	3.6	-0.2	-247	-450	-179
22:00	0.0	1.3	0.7	-2.1	-292	-484	-147
23:00	0.0	-0.2	-0.9	-3.5	-221	-410	-87
24:00	0.0	2.1	2.0	-0.6	-242	-446	-124
01:00	0.0	3.3	3.3	0.3	-373	-621	-252
02:00	0.0	4.3	4.0	1.0	-462	-691	-344
03:00	0.0	5.4	4.9	2.0	-394	-651	-280
04:00	0.0	3.9	3.6	1.0	-297	-556	-169
05:00	0.0	3.8	3.4	0.7	-328	-578	-212
06:00	0.0	4.4	4.0	1.3	-295	-538	-216
Daily (mmol m^{-2} d^{-1}) =		**144**	**133**	**10**	**1226**	**427**	**2913**

Comparisons between the hourly and daily G_{net} and P_{net} values (Table 1) show a similar calcification rate for both the "Coral only" mesocosm and the "Coral plus Algae" mesocosm in spite of the differences in dissolved oxygen, pH, A_T (Figure 8) and P_{net}. However, daily P_{net} for the "Coral plus Algae" mesocosm was only one third of the P_{net} of the "Coral only" mesocosm. Hourly production was much higher in the "Corals plus Algae" mesocosm during the daylight hours, but production was consumed by the extremely high respiration during nighttime hours. The "Algae only" mesocosm showed very low daily G_{net} and extremely high daily P_{net}.

4.5 Diurnal changes in material flux (P_{net}, G_{net}, H^+ flux and DIC flux).

G_{net} and P_{net} are measures of material flux. DO, pH and DIC are measures of concentration. The preceding discussion and numerous publications often compare concentrations of one material to flux rate of another material or vice versa. Much more can be learned by plotting DIC flux and H^+ flux rather than [DIC], [H^+] or pH in relation to P_{net} and G_{net}. DIC flux and H^+ flux were calculated using the box model and graphed on a 0 to 1 scale in the same manner as in Figure 8 with the result presented as Figure 10. This figure illustrates the dynamic physical and metabolic relationships involved in coral and coral reef metabolism.

Figure 10. Plot of normalized data for P_{net}, G_{net}, DIC flux and H^+ flux for the experiment.



The highly calcifying mesocosms containing coral **(**Figures 10A and 10B) show continuous high dissipation rates of H^+ (flux) for 2-3 hours following the peak rates of P_{net} and G_{net}. Proton efflux continues at a high rate for hours after peak calcification because the corals must rid themselves of the backlog of H^+ being generated during rapid calcification (Equations 7 thru 9). Thus the lag of pH (concentration) behind the peak flux rates of P_{net} and G_{net} (Figure 7A and 7B) is the result of high proton efflux from the corals. Ω_{arag} as a function of CO_3^{2-} concentration is simply the response of the CO_2-carbonate system to pH as [H^+] shifts the equilibria (Equations 6-9) and redistributes the [CO_3^{2-}] relative to the other DIC components of [HCO_3^-] and [CO_2] (Equations 6 and 10) . Therefore Ω_{arag} closely tracks pH as the result rather than the cause of calcification rate G_{net}. Hence the Ω_{arag} peak and the pH peak lag behind the P_{net} and G_{net} peaks (Figures 7A and 7B), but do not track with H^+ flux. This observation

demonstrates the importance of understanding the difference between H^+ concentration and H^+ flux.

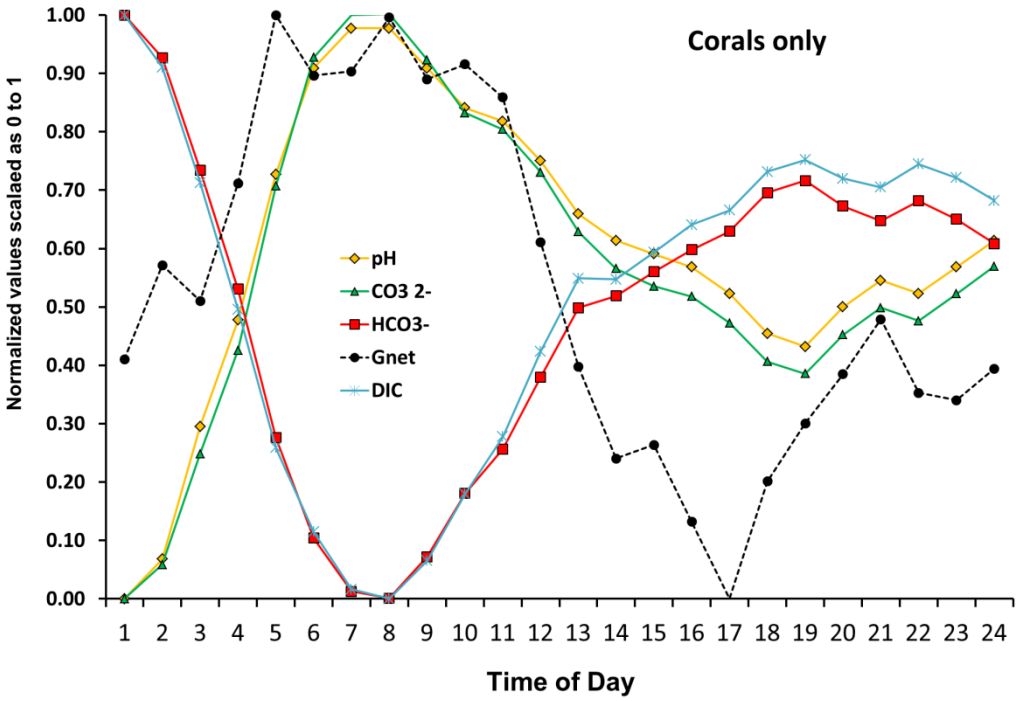
During the night the H^+ flux rate is very responsive to changes in G_{net} in the "Algae only" and "Coral plus Algae" due to large changes in respiration (Figure 10). The fluctuations of proton flux at night in the "Coral only" mesocosm are dampened considerably compared to the "Algae only" treatment. The "Coral plus Algae" mesocosm shows an intermediate response. This pattern could be explained if the coral skeleton acts as a buffer in a manner similar to that proposed by Suzuki et al. [48]. The macroalgae lack the large skeletal carbonate buffer of reef corals.

4.6 Back to the basics.

The preceding sections have established the importance of using flux rates rather than concentrations when we are describing a dynamic metabolic system such as a coral or coral reef. Most of the research in this area has focused on the relationship between G_{net}, $[CO_3^{2-}]$ (or its surrogate Ω_{arag}), $[HCO_3^-]$, and $[H^+]$ expressed as pH. Plotting these variables in exemplary Figure 11 is very informative and sheds light on results of previous studies.

A coral must uptake inorganic carbon in order to maintain photosynthesis and calcification. As a result [DIC] will decrease no matter which carbonate species (HCO_3^-, CO_3^{2-} or CO_2) is taken up by the coral (Equations 6-9). Thus we see a dramatic decline in [DIC] at high rates of G_{net} (Figure 11). $[HCO_3^-]$, which has been identified as the preferred substrate for photosynthesis and calcification [9,27,28] closely tracks [DIC] during daylight hours. In contrast, $[CO_3^{2-}]$ lags behind G_{net} and closely tracks pH during the day as shown for Ω_{arag} in Figure 7. If $[CO_3^{2-}]$ (or its surrogate Ω_{arag}) drives calcification, then how do we explain the lag behind G_{net}? And if $[CO_3^{2-}]$ is limiting, how do we explain the fact that $[CO_3^{2-}]$ is increasing rather than decreasing as the coral calcifies rapidly and takes up inorganic carbon? The answer is obvious, $[CO_3^{2-}]$ increases because of the increase in pH caused by through rapid photosynthesis, which shifts the equilibrium between $[HCO_3^-]$ and $[CO_3^{2-}]$ (Equations 6 thru 9). A basic physiological interpretation of the patterns shown in Figure 11 is that daytime coral metabolism rapidly removes DIC (primarily in the form of HCO_3^-) while photosynthesis provides the energy that drives G_{net} (Figure 7). Higher pH resulting from rapid photosynthesis pushes the equilibria toward higher $[CO_3^{2-}]$. This scenario results in a correlation between G_{net} and Ω_{arag}, with Ω_{arag} as the dependent variable.

Figure 11. The flux rate of calcification-dissolution (G_{net}) plotted against the concentrations of important variables in the CO_2-carbonate system for the "Corals only" mesocosm with all values normalized to a 0 to 1 scale as in Figures 7 and 10.



During the night [HCO_3^-], [DIC], [CO_3^{2-}] and pH mirror changes in G_{net}. However, note that [HCO_3^-] diverges from [DIC] and [CO_3^{2-}] diverges from pH in darkness. The night divergence can be attributed to respiration causing a decrease in pH. The decreasing pH shifts the equilibria so that [CO_3^{2-}] is converted to [HCO_3^-], thereby changing the offset between the points. This phenomenon is also reflected in the pattern of diurnal hysteresis show in Figure 6.

5. Conclusions

5.1 *Correlations do not establish cause and effect.*

Linear regression using Ω_{arag} as the independent variable may be useful as a first approximation, but is a poor descriptor of calcification dynamics on coral reefs. Most of the existing data on coral calcification was developed in static or low turnover incubation experiments under typical laboratory low irradiance artificial light sources on a 12 h light, 12 h dark cycle. This regime results in an unrealistic simulation of the actual diurnal cycle that occurs on coral reefs. The standard protocol has been to compare linear regressions between or among treatments. Linear regression provides a very limited description of the actual relationship between the key factors controlling organic and inorganic processes on coral reefs. The linear regression approach does not fully embrace natural diurnal calcification patterns because these processes are non-linear. The linear regression Ω_{arag} – G_{net} model wrongly assumes that Ω_{arag} is the independent variable driving the calcification reaction. Nevertheless, significant correlations can result because the two quantities track each other to some extent. Use of Ω_{arag} as an independent variable to describe G_{net} is known to create difficulties. For example, measured daily G_{net} of the Kaneohe Bay, Hawaii barrier reef is among the highest in the world compared to other coral reefs even though Ω_{arag} levels are among the lowest measured in coral reef

ecosystems [45]. Falter *et al*. [49] measured rates of P_{net} and G_{net} in a coral-dominated reef flat community on Ningaloo Reef in northwestern Australia. In summer G_{net} linearly correlated with Ω_{arag}, but not in the winter.

Equations 15-18 were developed to show that the [DIC]:[H^+] ratio correlates with Ω_{arag} in describing G_{net}. The ratio is informative and useful from a physiological point of view because it involves pH and all of the inorganic carbon species. However, the [DIC]:[H^+] ratio is simply another variable based on concentration (along with pH, Ω_{arag}, CO_3^{2-}, etc.) that shows a correlation with G_{net}. Nevertheless, the [DIC]:[H^+] ratio can be important in describing G_{net} in situations where Ω_{arag} is decoupled from [H^+] as occurs in the paleo-ocean over time scales greater than 10,000 years [50]. The [DIC]:[H^+] correlation with G_{net} has previously been verified for corals and crustose coralline algae [23] and now with mesocosm and Biosphere-2 artificial ocean results. A calcifying organism must uptake DIC in order to continue the calcification reaction and must rid itself of the waste protons. So G_{net} correlates directly to [DIC] and inversely to [H^+].

Results of this investigation (Figure 10) demonstrate the difficulty that corals encounter in shedding waste protons generated during calcification. High rates of H^+ flux continued for several hours following peak G_{net}. An important conclusion of this work is that measurements of DIC flux and H^+ flux are far more useful in describing coral metabolism dynamics than [DIC] and [H^+]. Likewise, Ω_{arag} is not a very useful variable in that it simply tracks pH (Figure 7). The reason should be obvious because [CO_3^{2-}] (and hence Ω_{arag}) both shift with changing [H^+] as described in Equations 6 thru 9. DIC flux follows P_{net} and G_{net} and drops off rapidly following peak P_{net} and peak G_{net} indicating that the high levels of inorganic carbon is sea water can readily meet the metabolic needs of rapidly calcifying corals.

5.2 *Always question the assumptions.*

The extensive literature on coral and coral reef calcification is based implicitly or explicitly on several largely untested assumptions: 1.) The rates of photosynthesis and calcification are proportional to changes in the concentrations of the bulk-water inorganic carbon source. 2.) Calcification and photosynthesis are not influenced by build-up of the calcification waste product H^+ in the tissues. 3.) The chemistry of the seawater contacting the coral tissue has the same chemistry of the bulk seawater. 4.) Boundary layers are of little or no relevance to the relationship between coral calcification and bulk water chemistry. 5.) Night calcification is zero or constant at a very low level. These assumptions are flawed. Corals exert significant control over conditions through mechanisms such as by storing photosynthetic energy for later use or through heterotrophic feeding. Biological activity modifies local conditions within the tissues and boundary layers which conceal the extent to which external seawater conditions control corals and corals control external conditions.

5.3 *Future research directions*

The time lag between G_{net} and Ω_{arag} reported previously in field studies [43,44,45] provided evidence that diffusion and advection of materials between the "black box" and the water column (Figure 2) involves a time delay. The reason for the time lag is that rapidly calcifying systems have difficulty dissipating waste protons as shown by continued rapid proton efflux for hours after peak calcification (Figure 10). What mechanism can account for the phase lag between Ω_{arag}? Boundary layers (BL) can slow the exchange of metabolic materials between the black box of the coral (Figure 1) and the water column. The results of Cyronak *et al*. [44]

revealed that stirring had a net stimulatory effect on A_T flux and on the diurnal cycle of hysteresis. Boundary layers slow exchange of metabolic materials, so this is the area of investigation that will provide the explanation.

5.4 *Boundary layers will provide the answers.*

Three hydrodynamic boundary layers have previously been defined and measured [51.52]. The Diffusion Boundary Layer (DBL), related to diffusion-limited processes such as respiration and photosynthesis. The Momentum Boundary Layer (MBL) controls water movement in the proximity of the sessile organisms and is thicker by an order of magnitude than the DBL. The Benthic Boundary Layer (BBL), which controls the interactions of the reef with the surrounding sea water, was typically found to be more than 1 m thick and characterized by a roughness height of 31 cm and a shear velocity of 0.42 cm s^{-1} in the studies by Shashar et al. [52, 53].

Frictional drag produces a thin layer of stagnant seawater adjacent to the calcifying or dissolving carbonate surface. This quiescent layer influences the flux of material between the benthic surface and the water column and is called the diffusive boundary layer (DBL). The transport of Ca^{2+}, CO_2, CO_3^{2-}, HCO_3^-, O_2, nutrients and H^+ through the DBL is limited by the physical processes of diffusion and advection [52-56]. Kühl et al.[57] found that zooxanthellae photosynthesis in the light resulted in a build-up of O_2 in the photosynthetic tissue of up to 250% saturation and a tissue pH of up to 8.6 (i.e. 0.7 pH units above the pH value of the overlying seawater). In darkness the O_2 within the coral tissue was depleted by respiration to near anoxic (<2 % air saturation) conditions, with tissue pH of 7.3 - 7.4. O_2 and pH profiles demonstrated the presence of a 200-300µ thick DBL that separated the coral tissue from the overlying flowing seawater. Various models invoke boundary layer controls on coral metabolism. Kaandorp et al. [55-56] addresses DBL limitation of total dissolved inorganic carbon (DIC) influx while Jokiel [16,21] focuses on DBL limitation of proton efflux.

The rapidly changing and extremely high diurnal rates of photosynthesis, respiration, calcification and dissolution on a coral reef represent a dynamic system that is in disequilibrium within and between the different boundary layers and the water column. This, in turn, has the potential to produce the observed spatial differences and temporal lags. Thus Jokiel [16,21,23] challenged the paradigm that calcification is limited by CO_3^{2-} supply on the reactant side of the calcification equation. Rather, he argued that rate of dissipation of H^+ on the product side due to boundary layer conditions can be the actual limiting factor. Boundary layer limitation of photosynthesis provides an analog to boundary layer limitation of calcification. Photosynthetic rate is limited by rate of waste O_2 dissipation through the boundary layer under certain conditions rather than being limited by supply of reactant CO_2. By analogy calcification may be limited by removal of waste protons rather than by availability of inorganic carbon. The importance of water motion in reducing boundary layer thickness and thereby increasing oxygen flux between the photosynthetic organisms and the water column has been demonstrated (58). By analogy, increased water motion can decrease boundary layer thickness and thereby increase removal of protons from the "black box" (Figure 1) and enhances flux of other materials between calcifying or dissolving surfaces and the bulk sea water.

Studies of reef metabolism beginning with the classic work of Odum and Odum [59] at Enewetak Reef flat and followed by others [45,49] were conducted in shallow water within the BBL in situations where unidirectional currents allowed calculation of flux rates. Substantial

boundary layers occur over all reefs. For example, Price *et al*. [60] investigated a range of sites from exposed coastal situations to lagoons and found that ambient variability in pH is substantial and oscillated over a diurnal cycle with diel fluctuations in pH exceeding 0.2. Daily pH maxima were identified as an important control on calcification. Net accretion among sites was positively related to the magnitude and duration of pH above the climatological seasonal low, despite myriad other ecological (e.g. local supply, species interactions, etc.) and physical oceanographic (e.g. temperature, current magnitude and direction, wave strength, latitudinal gradients, etc.) drivers. In general, accretion rates were higher at sites that experienced a greater number of hours at high pH values each day. Where daily pH within the BBL failed to exceed pelagic climatological seasonal lows, net accretion was slower and fleshy, non-calcifying benthic organisms dominated space. Thus, key aspects of coral reef ecosystem structure and function are clearly related to natural diurnal variability in pH, which is driven primarily by photosynthesis/respiration as P_{net}.

5.5. *The master variables.*

The practice of calculating and comparing linear regressions of G_{net} vs. Ω_{arag} to obtain a first approximation of calcification rates under different conditions will probably continue because it is convenient to relate a primary biological response (G_{net}) to a primary physical chemistry measurement (Ω_{arag}), especially in modeling the future changes on coral reefs. Unfortunately, the physical chemistry concept of Ω_{arag} has no basic physiological meaning in describing G_{net} other than a correlation with the [DIC]:[H^+] ratio as well as with other factors such as pH. So we must take care not to be led astray in our thinking about the variables that actually drive coral and coral reef metabolism. The correlation between G_{net} and other factors is a result of the way that P_{net} drives both G_{net} and Ω_{arag} in different situations [43]. The observed phenomena of diurnal hysteresis and diurnal phase lag show the importance of proton flux and emphasize the challenge in predicting the future effects of OA on coral reefs. The method of using linear extrapolations of Ω_{arag} to determine threshold levels that will shift coral reefs from net calcifying systems to a net dissolving state is questioned [43]. The master variable, however, is H^+ flux.

An explanation for the many paradoxes of coral calcification discussed herein has been presented as the "Two Compartment Proton Flux Model of Coral Metabolism" [16]. This model is focused on localized gradients that influence coral metabolism with a focus on proton flux, carbon pools and translocation of fixed carbon. A major feature of the model is the presence of boundary layers which control local pH gradients and inorganic carbon speciation in addition to proton flux. Results of the present investigation support this model.

Acknowledgments

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References and Notes

1. Z**eebe, R. E.**; Wolf-Gladrow, D. CO_2 in Seawater: Equilibrium, Kinetics, Isotopes. *Elsevier Oceanography Series*, 65, Amsterdam. **2001**.
2. Royal Society. Ocean acidification due to increasing atmospheric carbon dioxide. Policy document 12/05 Royal Society, London. The Clyvedon Press Ltd, Cardiff, **2005**.

3. Hoegh-Guldberg, O.; Mumby, P.J.; Hooten, A.J.; Steneck, R.S.; Greenfield, P.; Gomez, E.; Harvell, C.D.; Sale, P.F.; Edwards, A.J.; Caldeira, K.; Knowlton, N.; Eakin, C.M.; Iglesias-Prietor, R.; Muthiga, N.; Bradbury, R.H.; Dubi, A.; Hatziolos, M. E. Coral reefs under rapid climate change and ocean acidification. *Science* **2007**, *318*, 1737-1742.
4. Feely, R.A.; Sabine, C.L.; Lee, K.; Berelson, W.; Kleypas, J.; Fabry, V.J.; Millero, F.J. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science* **2004,** *305(5682)*, 362–366, doi:10.1126/science.1097329.
5. Smith, S. V.; Buddemeier, R.W. Global change and coral reef ecosystems. *Ann. Rev. Ecol. Syst.* **1992**, *23*, 89–118.
6. Gattuso, J.P.; Allemand, D.; Frankignoulle, M. Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: A review on interactions and control by carbonate chemistry. *Am. Zool.* **1999**, *39*, 160–183.
7. Erez, J.; Reynaud, S.; Silverman, J.; Schneider, K.; Allemand, D. Coral calcification under ocean acidification and global change, In *Coral Reefs: An Ecosystem in Transition*, Dubinsky, Z.; Stambler, N., Eds; Springer Press, **2011**; pp.151-176.
8. Kleypas, J.A.; Buddemeier R.W.; Archer D.; Gattuso J.-P.; Langdon C.; Opdyke B.N. Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science*, **1999**, *284*, 118–120.
9. Roleda, M. Y.; Boyd, P.W.; Hurd, C.L. Before ocean acidification: calcifier chemistry lessons. *J. Phycol.* **2012**, *48*, 840-843, doi:10.1111/j.1529-8817.2012.01195.x.
10. Kinsey, D. Alkalinity Changes and Coral Reef Calcification. *Limnol. Oceanog.* **1978**, *23(5)*, 989-991.
11. Smith, S.V.; Kinsey, D.W. Calcification and organic carbon metabolism as indicated by carbon dioxide. In *Coral Reefs: Research Methods*, Stoddart, D.R.; Johannes R.E., Eds. Monographs on Oceanographic Methodology Series, UNESCO, Paris, **1978**; Volume 5, pp. 469-484.
12. McCulloch, M.; Falter, J.; Trotter, J.; Montagna, P. Coral resilience to ocean acidification and global warming through pH up-regulation. *Nature Climate Change* **2012**, DOI:10.1038/NCLIMATE1473.
13. McNeil, B. I.; Matear, R. J.; Barnes, D. J. Coral reef calcification and climate change: The effect of ocean warming. *Geophys. Res. Lett.*, **2004**, 31: L22309, doi:10.1029/2004GL021541.
14. Kleypas, J.A.; Buddemeier, R.W.; Eakin, C.M.; Gattuso, J.-P.; Guinotte, J.; Hoegh-Guldberg, O.; Iglesias-Prieto, R.; Jokiel, P. L.; Langdon, C.; Skirving, W.; Strong, A. E. 2005. Comment on "Coral reef calcification and climate change: The effect of ocean warming", *Geophys. Res. Lett.* **2005**, *32*, L08601, doi:10.1029/2004GL0222329.
15. Schneider, K.; Erez, J. The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurystoma*. *Limnol. Oceanog*. **2006**, *51*, 1284–1293.
16. Jokiel, P.L. The reef coral two compartment proton flux model: A new approach relating tissue-level physiological processes to gross corallum morphology. *J. Exp. Mar. Biol. Ecol.* **2011**, *409*, 1–12, doi:10.1016/j.jembe.2011.10.008.
17. Cohen, A.; Holcomb, M. Why corals care about ocean acidification. *Oceanography* **2009**, 22, 118-127.
18. Jury, C.P.; Whitehead, R.F.; Szmant, A. Effects of variations in carbonate chemistry on the calcification rates of *Madracis auretenra* (= *Madracis mirabilis sensu* Wells, 1973):

bicarbonate concentrations best predict calcification rates. *Global Change Biol.* **2010**, *16*, 1632–1644, doi:10.1111/j.1365-2486.2009.02057.x.

19. de Putron, S. J.; McCorkle, D.C.; Cohen, A.L.; Dillon, A.B. The impact of seawater saturation state and bicarbonate ion concentration on calcification by new recruits of two Atlantic corals. *Coral Reefs* **2010**, *30(2)*, 321-328, doi:10.1007/s00338-010-0697-z.
20. Edmunds, P.J.; Brown, D.; Moriarity, V. Interactive effects of ocean acidification and temperature on two scleractinian corals from Moorea, French Polynesia. *Global Change Biol.* **2012**, *18*, 2173–2183, doi: 10.1111/j.1365-2486.2012.02695.x.
21. Jokiel, P.L. Ocean acidification and control of reef coral calcification by boundary layer limitation of proton flux. *Bull. Mar. Sci.* **2011**, *87(3)*, 639–657, doi:10.5343/bms.2010.1107
22. Comeau, S.; Carpenter, R.C.; Edmunds, P.J. Coral reef calcifiers buffer their response to ocean acidification using both bicarbonate and carbonate. *Proc. Royal Soc. London Ser. B* **2012**, *280*, 20122374, doi:10.1098/rspb.2012.2374
23. Jokiel, P.L. Coral reef calcification: carbonate, bicarbonate and proton flux under conditions of increasing ocean acidification. *Proc. Royal Soc. London Ser. B* **2013**, *280*, 20130031. doi:10.1098/rspb.2013.0031.
24. Comeau, S.; Carpenter, R.C.; Edmunds, P.J. Response to coral reef calcification: carbonate, bicarbonate and proton flux under conditions of increasing ocean acidification. *Proc. Royal Soc. London Ser. B* **2013**, 280, 20131153. doi 10.1098/rspb.2013.1153.
25. Mass, T.; Genin, A.; Shavit, U.; Grinstein, M.; Tchernov, D. Flow enhances photosynthesis in marine benthic autotrophs by increasing the efflux of oxygen from the organism to the water. *Proc. Nat. Acad. Sci. United States of America*, **2010**, 107(6), 2527–2531.
26. Hofmann, G. E.; Todgham, A.E. Living in the now: physiological mechanisms to tolerate a rapidly changing environment. *Ann. Rev. Physiol.* **2010**, *72*, 127–45, doi:10.1146/annurev-physiol-021909-135900.
27. Furla, P.; Galgani, I.; Durand, I.; Allemand, D. Sources and mechanisms of inorganic carbon transport for coral calcification and photosynthesis. *J. Exp. Mar. Biol. Ecol.* **2000**, *203*, 3445–3457.
28. Weis, V.M.; Smith, G.J.; Muscatine, L. A "CO_2 supply" mechanism in zooxanthellate cnidarians: role of carbonic anhydrase. *Mar. Biol.* **1989**, *100*, 195-202.
29. Graham, D.; Smillie, R.M. Carbonate dehydratase in marine organisms of the Great Barrier Reef. *Aust. J. Plant Physiol.* **1976**, *3*, 113-119.
30. Al-Horani, F.A.; Al-Moghrabi, S.M.; De Beer, D. Microsensor study of photosynthesis and calcification in the scleractinian coral, *Galaxea fascicularis*: active internal carbon cycle. *J. Exp. Mar. Biol. Ecol.* **2003,** *288*, 1-15.
31. Moya, A.; Tambutté, S.; Bertucci, A.; Tambutté, É.; Lotto, S.; Vullo, D.; Supuran, C.T.; Allemand, D.; Zoccola, D. Carbonic anhydrase in the scleractinian coral *Stylophora pistillata*: characterization, location and role in biomineralization. *J. Biol. Chem.* **2008**, 283, 25475-25484.
32. Enns, T. Facilitation by carbonic anhydrase of carbon dioxide transport. *Science* **1967**, *155*, 44-47.
33. Marubini, F.; Ferrier-Pagés, C.; Furla, P.; Allemand, D. Coral calcification responds to seawater acidification: a working hypothesis towards a physiological mechanism. *Coral Reefs* **2008**, *27*, 491–499, doi:10.1007/s00338-008-0375-6.

34. Herfort, L.; Thake, B.; Taubner, I. Bicarbonate stimulation of calcification and photosynthesis in two hermatypic corals. *J. Phycol.* **2008**, *44*, 91–98, doi:10.1111/j.1529-8817.2007.00445.x.
35. Tourniaire, F.; Pulos, S. Proportional reasoning: A review of the literature. *Educ. Stud. Math.* **1985**, *16(2)*, 181-204.
36. Langdon, C.; Takahashi, T.; Sweeney, C.; Chipman, D.; Goddard, J.; Marubini, R.; Aceves, H.; Barnett, H.; Atkinson, M.J. Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. *Global Biogeochem. Cycles* **2000**, *14*, 639-654.
37. Gagnon, A.C.; Adkins, J.F.; Erez, J. Seawater transport during coral biomineralization. *Earth Planet. Sci. Lett.* **2012**, *329-330*, 150-161. doi:10.1016/j.epsl.2012.03.005.
38. Zoccola, D.; Tambutté, É.; Sénégas-Balas, F.; Michiels, J.-F.; Failla, J.P.; Jaubert, J.; Allemand, D. Cloning of a calcium channel α1 subunit from the reef-building coral, *Stylophora pistillata*. *Gene* **1999**, *227(2)*, 157–167.
39. Jokiel, P.L.; Rodgers, K.S.; Kuffner, I.B.; Andersson, A.J.; Cox, E.F.; Mackenzie, F.T. Ocean acidification and calcifying reef organisms: a mesocosm investigation. *Coral Reefs* **2008**, *27*, 473-483.
40. Andersson, A. J.; Kuffner, I.B.; Mackenzie, F.T.; Jokiel, P.L.; Rodgers, K.S.; Tan, A. Net loss of $CaCO_3$ from coral reef communities due to human induced seawater acidification. *Biogeosci. Discuss.* **2009**, *6*, 1–20.
41. Jokiel, P.L.; Maragos, J.E.; Franzisket, L. Coral growth: buoyant weight technique. In *Coral Reefs: Research Methods*, Stoddart, D.R.; Johannes R.E., Eds. Monographs on Oceanographic Methodology Series, UNESCO, Paris, **1978**; Volume 5, pp. 529-542.
42. Pierrot, D.; Lewis, E.; Wallace, D.W.R. *MS Excel Program Developed for CO_2 System Calculations*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee, **2006**. doi:10.3334/CDIAC/otg.CO2SYS_XLS_CDIAC105a.
43. McMahon, A.; Santos, I. R.; Cyronak, T.; Eyre. B. D., Hysteresis between coral reef calcification and the seawater aragonite saturation state, *Geophys. Res. Lett.* **2013**, *40*, 4675–4679, doi:10.1002/grl.50802.
44. Cyronak, T.; Santos, I. R.; McMahon, A.; Eyre, B. D. Carbon cycling hysteresis in permeable carbonate sands over a diel cycle: Implications for ocean acidification. *Limnol. Oceanogr.*, **2013**, 58(1), *131–143*, doi:10.4319/lo.2013.58.1.0131.
45. Shamberger, K. E. F.; Feely, R. A.; Sabine, C. L.; Atkinson, M. J.; DeCarlo, E. H.; Mackenzie, F. T.; Drupp, P. S.; Butterfield, D. A. Calcification and organic production on a Hawaiian coral reef. *Mar. Chem*. **2011**, *1271–4*, 64–75, doi:10.1016/j.marchem.2011.08.003.
46. Barnes, D. J.; Crossland, C. J. Diurnal and seasonal variations in the growth of a staghorn coral measured by time-lapse photography. *Limnol. Oceanogr.* **1980**, *35(6)*, 1113-1117.
47. Wooldridge, S. A new conceptual model of coral biomineralisation: hypoxia as the physiological driver of skeletal extension. *Biogeosciences* **2013**, *10*, 2867–2884, doi:10.5194/bg-10-2867-2013.
48. Suzuki, A.; Nakamori, T.; Kayanne, H. The mechanism of production enhancement in coral reef carbonate systems: model and empirical results. *Sediment. Geol*. **1995**, *99,259-280.*

49. Falter, J. L.; Lowe, R. J.; Atkinson, M. J.; Cuet, P. Seasonal coupling and de-coupling of net calcification rates from coral reef metabolism and carbonate chemistry at Ningaloo Reef, Western Australia. *J. Geophys. Res.* **2012**, *117*, C05003, doi:10.1029/2011JC007268.
50. Hönisch, B.; Ridgwell, A.; Schmidt, D.N.; Thomas, E.; Gibbs, S.J.; Sluijs, A.; Zeebe, R.; Kump, L.; Martindale, R.C.; Greene, S.E.; Kiessling, W.; Ries, J.; Zachos, J.C.; Royer, D.L.; Barker, S.; Marchitto, Jr. T.M.; Moyer, R.; Pelejero, C.; Ziveri, P.; Foster, G.L.; Williams, B. The geological record of ocean acidification. *Science* **2012**, *335*, 1058-1063, doi:10.1126/science.1208277.
51. Shashar, N.; Cohen, Y,; Loya, Y. Extreme diel fluctuations of oxygen in diffusive boundary layers surrounding stony corals. *Bio. Bull*. **1993**,185:455-461.
52. Shashar, N.; Kinane, S.; Jokiel, P.L.; Patterson, M.R. Hydromechanical boundary layers over a coral reef. *J. Exp. Mar. Biol. Ecol.* **1996**, 199:17-28
53. Jokiel, P.L. Effects of water motion on reef corals. **1978**, *J. Exp. Mar. Biol. Ecol.* 35:87-97
54. Lesser, M.P.; Weis, V.M.; Patterson, M.R.; Jokiel, P.L., Effects of morphology and water motion on carbon delivery and productivity in the reef coral, *Pocillopora damicornis* (Linnaeus): Diffusion barriers, inorganic carbon limitation, and biochemical plasticity. *J. Exp. Mar. Biol. Ecol.* **1994**, *178*, 153-179.
55. Kaandorp, J. A.; Sloot, P. M. A.; Merks, R. M. H.; Bak, R. P. M.; Vermeij, M. J. A.; Maier, C. Morphogenesis of the branching reef coral *Madracis mirabilis*. *Proc. R. Soc. Lond. B* **2005**, 272:127–133.
56. Kaandorp, J.A., Filatov M., Chindapol N. (2011) Simulating and quantifying the environmental influence on coral colony growth form. In: Dubinsky Z, Stambler N (eds) Coral reefs: an ecosystem in transition. New York; Springer Press. pp177-185.
57. Kühl, M., Cohen Y., Dalsgaard T., Jørgensen B.B., Revsbech N.P. Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O_2, pH and light. *Mar. Ecol. Prog. Ser.* **1995** 117:159-172.
58. Mass T., Genin A., Shavit U., Grinstein M., Tchernov D. Flow enhances photosynthesis in marine benthic autotrophs by increasing the efflux of oxygen from the organism to the water. *Proc. Natl. Acad. Sci.* USA, **2010** 107:2527–2531.
59. Odum, H. T.; Odum, E. P. Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. *Ecol. Monogr*. **1955**, *25: 291-320*.
60. Price, N. N.; Martz, T. R.; Brainard, R. E.; Smith, J. E. Diel Variability in seawater pH relates to calcification and benthic community structure on coral reefs. *PLoS ONE* **2012** *7(8)*, e43843, doi:10.1371/journal.pone.0043843.