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Low-cost, high-flow mesocosm system for simulating ocean acidification with CO_2 gas

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Abstract

The high cost of building and operating open-flow experimental systems for studies of biological response to ocean acidification (OA) has led to extensive use of short-term incubations in closed-flow systems that do not simulate natural conditions. An inexpensive, highly reliable, open-system that tracks natural diurnal and seasonal changes in water chemistry is described. This approach is based on a gravity-feed seawater supply system that uses a peristaltic pump to regulate CO_2 injection rate and a power head that cavitates the injected CO_2 into microscopic bubbles that are dissolved immediately. This report describes low-cost methods applicable to small or very large experimental systems. This methodology permits long-term experiments under full sunlight with rapid seawater turnover rate that assures realistic environmental conditions in the experimental chambers.

Controlled experiments designed to test the impact of ocean acidification (OA) have been limited by ability to create realistic continuous flow water chemistry conditions for cultured organisms (Riebesell et al. 2010), which has limited the applicability of the results to field situations. 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 predicted future global 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 seawater flow. Concentrations of CO_2 are controlled by mixing of CO_2-scrubbed air and pure CO_2 via mass flow controllers. 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 volume aquaria using closed systems or extremely low flow experiments that permit maintenance of the desired pH conditions (Table 1). Several reports describe closed-system incubations used to test the response of coral planula larvae or newly settled corals to various levels of pCO_2 (Anlauf et al. 2010; de Putron et al. 2010; Drenkard et al. 2013). Where there is high biological activity (such as occurs in corals and coral reef communities), the water chemistry in closed systems can be strongly influenced by the experimental subjects. Coral calcification can cause rapid changes in seawater total alkalinity (A_T) during the course of an incubation. Photosynthesis and respiration can result in extreme changes in pH and dissolved oxygen (DO). Closed 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 nonacidified 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). Closed system tests do not flush out accumulated metabolic wastes. Maintaining aquaria in full sunlight results in solar heating with high water temperature unless there is rapid flushing of the system. Therefore, most closed system tests are carried out under relatively low artificial irradiance regimes. 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), so experiments conducted without UVR can give unrealistic growth and mortality rates as reef organisms expend energy to mitigate and repair damage from UVR.

Reef community response to OA has also been studied by perturbation of a single, large, closed 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 h

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

Reference	Incubation time	Volume (L)	Open (Flow-through)	Closed (Recirculation)	Irradiance (μmol photons m^{-2} s^{-1})	Photoperiod	Seawater
Langdon et al. 2003	NA	2.65×10^6		BIOSPHERE-2 "Ocean," pump recirculation	Filtered sunlight	natural cycle	unfiltered
Reynaud et al. 2003	20 d	150		150 L aquaria with 4 h pump recycling through 150 L chamber	380	12 h light:12 h dark	filtered
Langdon and Atkinson 2005	1.5 h	2400		Flume with motor induced current, water replaced after each run	sunlight	natural cycle	unfiltered
Anthony et al. 2008	14 d	20	Continuous flow		sunlight	natural cycle	unfiltered
Jokiel et al. 2008	270 d	500	Continuous flow		sunlight	natural cycle	unfiltered
de Putron et al. 2010	14 d	30		Closed flow, aerated continuously	60	12 h light:12 h dark	filtered
Comeau et al. 2012	14 d	150		50% volume replaced every other day, stirred by pumps	500	12 h light:12 h dark	filtered
Edmunds et al. 2012	30 d	150		13% volume replaced daily, aerated continuously	600	12 h light:12 h dark	filtered
Drenkard et al. 2013	21 d	21		Volume replace every 7 d, aerated continuously	60	12 h light:12 h dark	filtered
Anthony et al. 2011, 2013	1-2 h	550		Motor driven current, water replaced after each run	~ 1000	12 hours light:12 h dark	Preconditioned

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 seawater carbonate chemistry on calcification in an assembled community of coral reef organisms consisting of corals, calcifying algae, and other typical reef biota. The seawater chemistry of the "ocean" was altered 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 $CaCl_2$. During 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 seawater.

In response to these limitations, open 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. Conditions were regulated in the experimental aquaria by custom-built CO_2 dosing (CO_2 bubbling) and temperature control systems 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 4-d test period. Obviously, such systems are beyond the reach of most investigators.

A continuous-flow, large-volume mesocosm system with high flushing rate (<1 h turnover) using "live" seawater directly from the ocean under full sunlight has been developed (Smith et al. 1977; 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 seawater 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 open system conditions (Andersson et al. 2009). OA experiments that use either the CO_2 bubbling method or the HCl addition method produce the same effects on coral growth (de Putron et al. 2010). The HCl method has been shown to be an appropriate method for OA studies (Andersson and Mackenzie 2012), but bubbling with CO_2 gas is preferred because it more accurately simulates future OA chemistry. The HCl method showed a clear advantage over the CO_2 bubbling method in large open systems with rapid turnover (Andersson and Mackenzie 2012). For example, use of the HCl method in a high flow, long term (seven-week) experiment (Kuffner et al. 2008) provided the first evidence that recruitment and growth of crustose coralline algae to OA is far more sensitive than recruitment and growth of corals under the same conditions. Nevertheless, the CO_2 bubbling method is more realistic in that it more precisely mimics the changes in seawater chemistry expected to occur in the future. Most investigators would prefer to acidify seawater using CO_2 gas rather than HCl. The main shortfall of the HCl method is that it doesn't produce the increase in DIC that results from anthropogenic OA. The DIC issue could be a problem for experiments including carbon-limited autotrophs that may show a CO_2 fertilization effect (e.g., seagrasses and others without carbon-concentrating mechanisms) under OA. Thus the bubbling CO_2 method presented here is well-suited for studies on macroalgae and seagrasses (Hurd et al. 2009; Koch et al. 2013) as well as corals.

Materials and procedures

The system consists of four major components.

(1) A reliable high-volume "live" seawater supply. Marine biological studies involving long-term aquarium culture under natural conditions require a reliable seawater system. The Hawaii Institute of Marine Biology (HIMB) seawater 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 seawater from Kaneohe Bay. Consequently, in the past 40 years of research in our laboratory (e.g., Jokiel and Coles 1977; Jokiel 1978; Jokiel et al. 1985, 1997, 2008) there have been no coral experiments that failed due to interruption of seawater supply.

(2) Stable flow rates of seawater. Maintaining the desired levels of pCO_2 enrichment requires the accurate measurement of water flow through the system. Stable flow is easily achieved using gravity feed and pipes that are free of biological fouling. A head box with constant overflow creates constant pressure in the line feeding the experimental system. The seawater supply pressure from the main laboratory seawater system will fluctuate with changes in tide and when other users open and close valves. The use of a head box with overflow will provide constant pressure to a particular experiment as long as the flow into the head box is always run at a higher flow rate than the demand from the head box.

(3) A continuous regulated stable supply of CO_2 gas. In this system the 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

Fig. 1. Precise 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.

two pump heads that can supply two treatments. Multiple pump heads can be run in tandem with different capacity heads and tubing 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.

(4) Efficient diffuser system. The key to the success of this system is the complete dissolution of the precisely metered CO_2 gas into the highly stable seawater flow. Increasing pCO_2 of seawater through use of bubbles of enriched air or pure CO_2 is dependent on the slow process of equalization between the water and bubbles. 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 aquaria. 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 seawater. 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.

Three examples are provided to show the applicability of this concept to a wide variety of experimental needs. The major features of these examples include rapid aeration (stable saturated DO), high levels of turbulent mixing, realistic carbonate chemistry, natural diurnal and seasonal cycles of chemistry and irradiance in addition to natural plankton and nutrient concentrations. In all three applications, a large pH offset was established between control and experimental treatments.

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 seawater.

Example 1

A simplified demonstration system was developed (Fig. 3) to demonstrate the low costs involved. The apparatus uses four 20-L glass aquaria and three 2-L 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 seawater to two aquaria and the second containing the diffuser system that provides high pCO_2 seawater 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 × 2 experimental design of temperature and ocean acidification. The 2 × 2 design seen in Fig. 3 could easily be replicated in both space and/or time for a randomized block design. The heated tanks are held at 2°C above ambient. The acidified tanks are held at 0.3 pH units below the nonacidified tanks. The residence time of seawater in the example was 15 min, 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. Seawater measurements were taken once daily between 10:00 and 13:00 h. Measurements of pH_{NBS} were made with an Accumet AP72 pH/mV/temperature meter verified spectrophotometrically using *m*-cresol purple dye according to SOP *7* (Dickson et al. 2007). Total alkalinity (A_T) was measured using a Titrino Model 877 titrator system. Alkalinity samples were equilibrated to 25°C and run within an hour of being taken. Accuracy and precision of the titrations was confirmed with certified reference materials (CRM Batch 129) from the Dickson Laboratory, Scripps Institution of Oceanography. All carbonate parameters were calculated using the program CO2SYS (Pierrot et al. 2006) and stoichiometric dissociation constants defined by Mehrbach et al. (1973) and refit by Dickson and Millero (1987).

All of the tanks tracked the ambient control (Fig. 4). Readings were taken only once per day in this example. Water parameters (i.e., temperature, pH, DO A_T) from the aquarium experiment are shown in Table 2. A one-way ANOVA of the comparison of mean A_T measurements show no difference between treatments ($P = 0.36$), with a significant difference ($P < 0.0001$) in DIC between acidified and nonacidified. There-

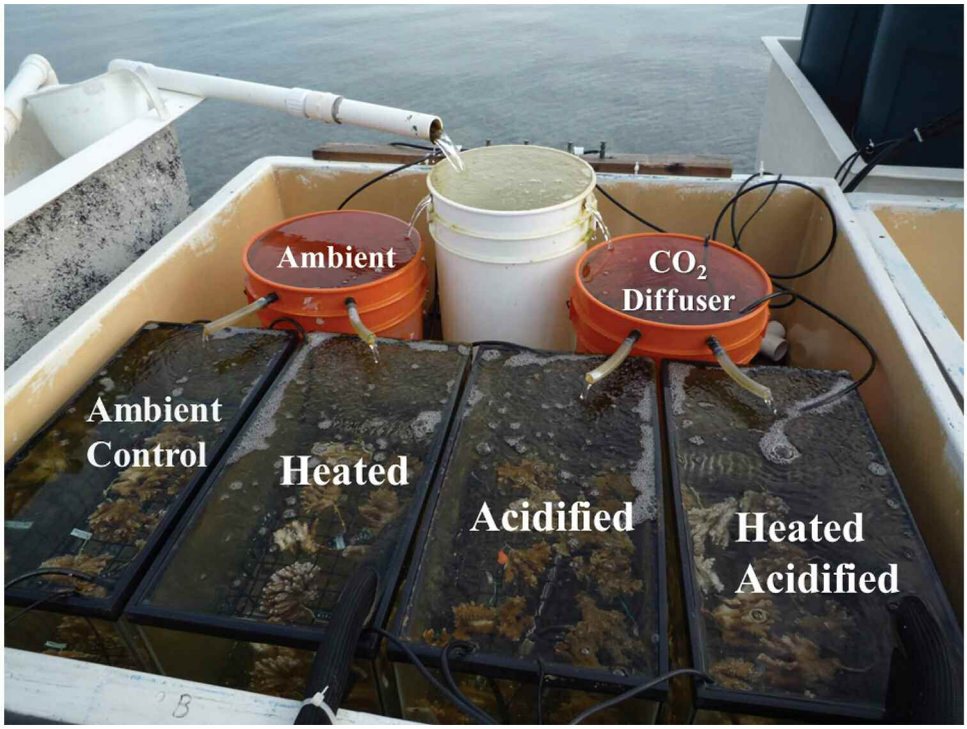

Fig. 3. Simplified low-cost flow through system using buckets and aquaria.

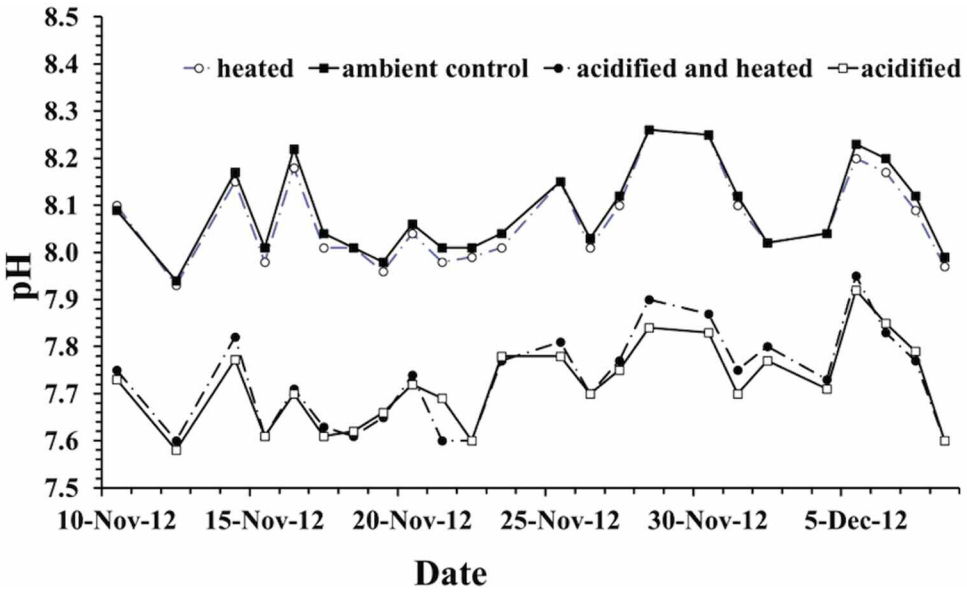

Fig. 4. Daily pH values taken between 10:00 and 13:00 in the simplified aquarium system shown in Fig. 3 over a four-week period with a 15 min residence time. Water pumped from Kaneohe Bay shows low pH early in the day and increases in the early afternoon. The tanks track each other very closely with a fixed offset for the acidified tanks.

fore, the acidification method did not alter A_T chemistry within the experimental treatments, but increased DIC.

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 m × 1 m × 0.5 m deep fiberglass mesocosm tanks are supplied with sea-

Table 2. Mean values ±SE for 30 d period of Example 1, Fig. 4.

Treatment	Temp. °C	pH_{NBS}	Salinity ppt	DO% Sat.	A_T μmol kg^{-1}	pCO_2 μatm	Ω_{arag}	DIC μmol kg^{-1}
Heated	27.9 ± 0.10	8.07 ± 0.02	35.3 ± 0.04	109 ± 2.2	2148.47 ± 4.9	588.7 ± 52.7	2.6 ± 0.2	1921.7 ± 17.1
Control	23.4 ± 0.12	8.09 ± 0.02	35.2 ± 0.06	100 ± 1.8	2158.17 ± 5.9	441.8 ± 45.4	2.8 ± 0.2	1909.6 ± 21.3
Acidified + Heated	28.0 ± 0.07	7.73 ± 0.02	35.4 ± 0.05	110 ± 2.2	2143.00 ± 8.7	1514.9 ± 195.2	1.3 ± 0.1	2064.9 ± 21.3
Acidified	23.7 ± 0.11	7.72 ± 0.02	35.3 ± 0.06	103 ± 1.7	2158.83 ± 7.3	1442.1 ± 204.2	1.2 ± 0.1	2090.5 ± 21.2

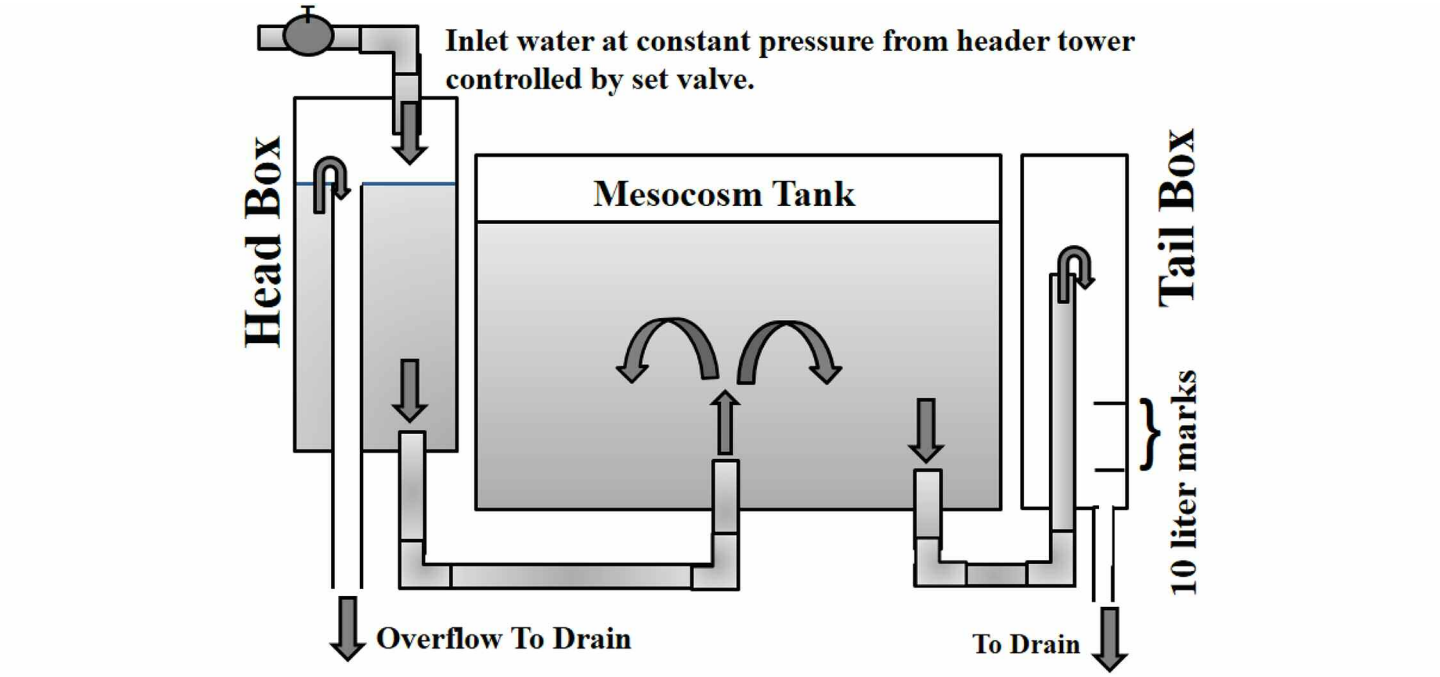

Fig. 5. The mesocosm system assures highly constant flow rates through a system of head box, tail box, and stand pipes.

water pumped directly from a depth of 2 m offshore of the adjacent Coconut Island Reef in Kaneohe Bay, Hawaii. All wetted surfaces are of inert plastic material. Piping is polyvinyl chloride (PVC) plastic with an inside diameter (ID) of 1.25-inch (3.17 cm ID), except for the overflow standpipe in the head box which is 2.5-inch I.D. (5.7 cm ID) to allow rapid overflow 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 2 weeks to ensure that buildup of fouling organisms does not impede flow. An adjustable head-box standpipe arrangement (Fig. 5) provides each mesocosm with an inflow of seawater at ~ l0 L min^{-1} resulting in a complete turnover rate of < 1 hour. Gravity flow and clean piping insures stable flow rates that hold within 1-2% over a 24-h 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 simply by tightening or loosening the threaded joints. 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 that it takes for the water level to rise between two marks that encompass a 10 L volume (Fig. 5). Elevated temperature is obtained by electrical resistance heaters run without thermostat system 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).

Example 3

The diffuser can be used to acidify a large number of tanks at high flow. During summer 2014, we completed an experi-

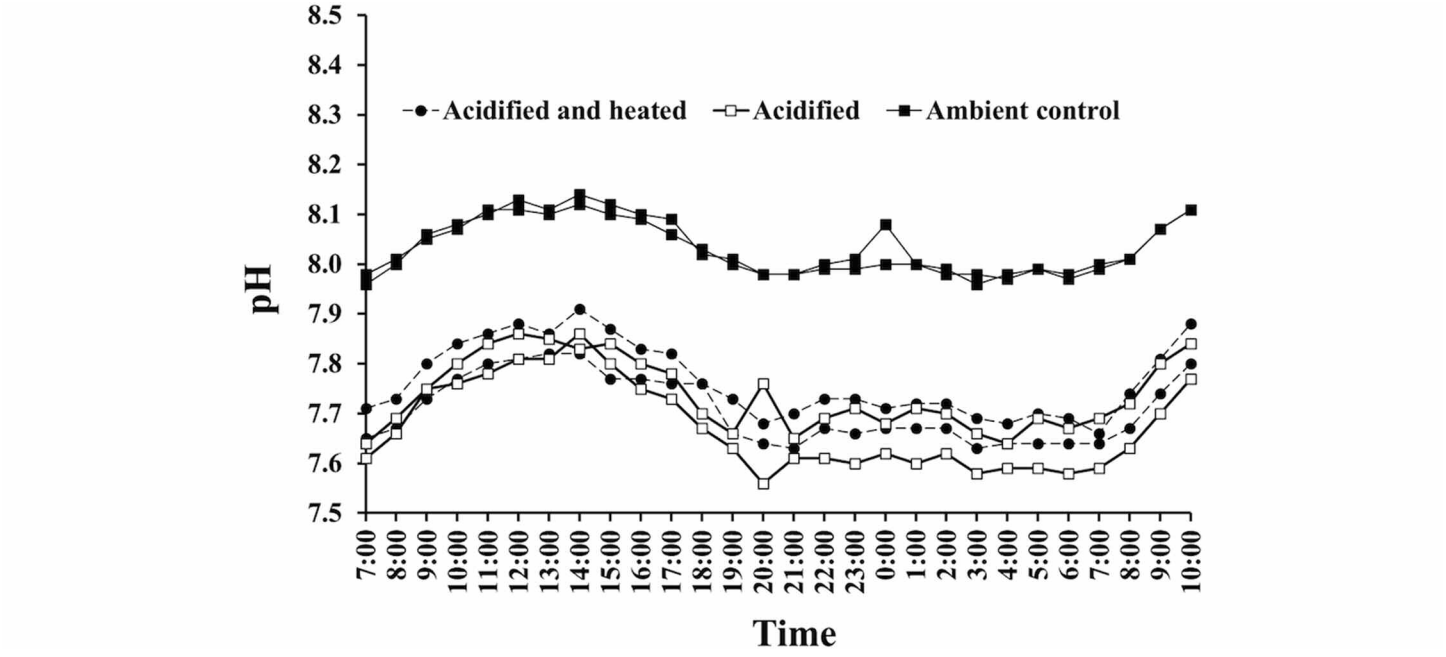

Fig. 6. Hourly pH data from a mesocosm run using 6 mesocosms (2 at ambient conditions, 2 acidified, plus 2 acidified and heated).

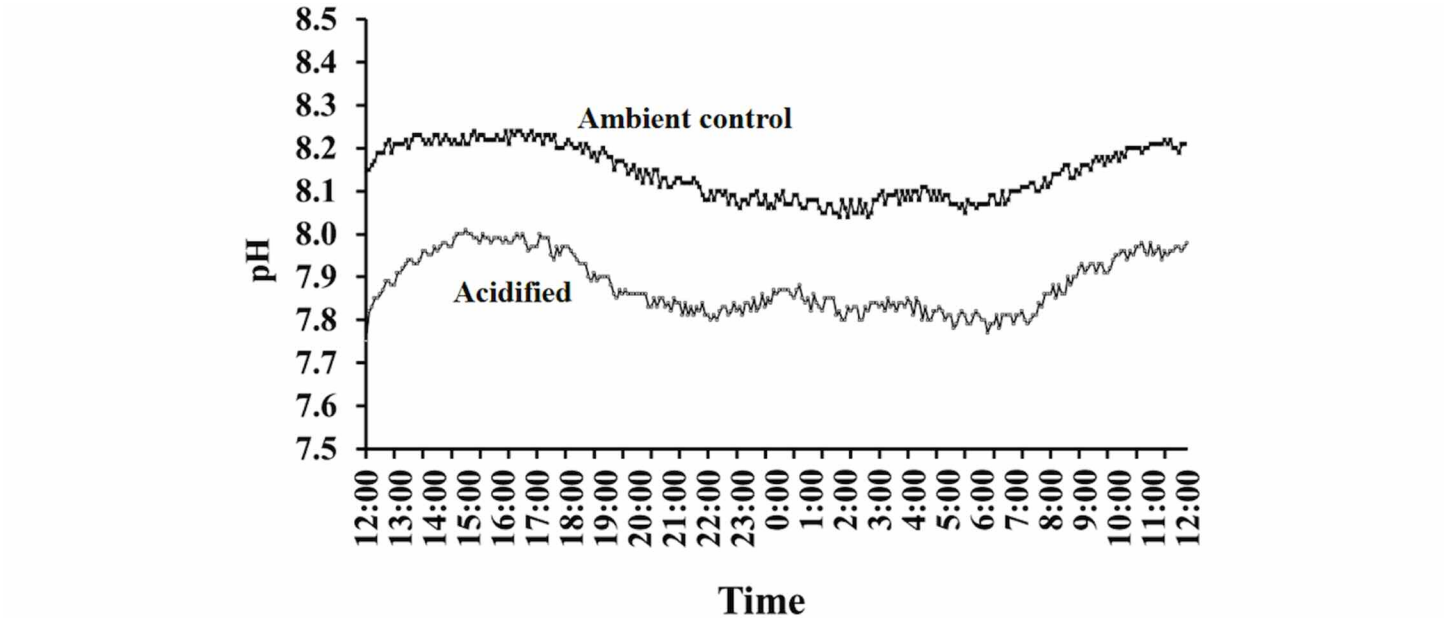

Fig. 7. Continuous fine-scale data taken in mesocosms (ambient and acidified mesocosms shown in Fig. 6) with YSI Incorporated 6920V2 multi parameter water quality sondes over a 24 h period.

ment that compared metabolic response of various benthic components that required monitoring a 24-h base-line metabolism at ambient conditions followed later by another 24-h 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–diffuser system. The diffuser (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 the desired pH offset. Inlets on all mesocosm tanks tracked each other in the manner shown in Figs. 6-7.

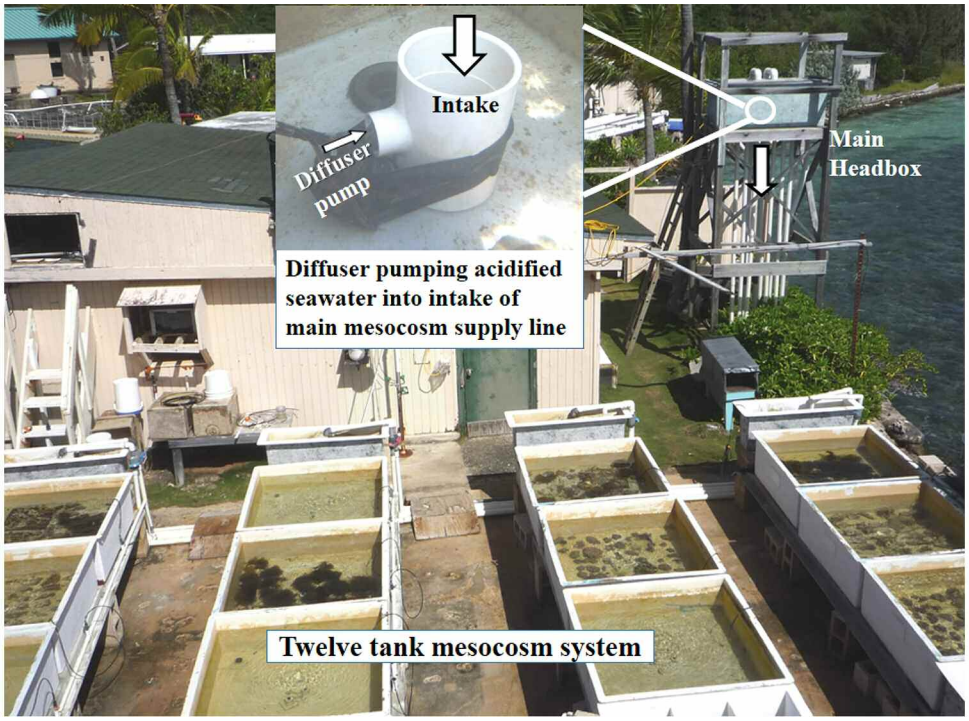

Fig. 8. System showing 12 mesocosms being supplied with highly acidified seawater (decreased by 0.4 pH units) at a high flow rate (10 L min^{-1} per mesocosm = 120 L min^{-1}) provided by a single power head (Fig. 2) pumping into the main supply line located in the elevated head box (inset). A second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

Assessment

The described pCO_2 mesocosm system has been in continuous use for over 1 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 always 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 to hold the desired offset between control and experimental mesocosms.

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, DO, 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 are influenced by irradiance and ultraviolet radiation (UVR) (Gleason and Wellington 1993; Lesser 1996). The UVR component is extremely important to coral physiology and bleaching rate (e.g., Gleason and Wellington 1993; Lesser 1996) and cannot simulate the conditions of high irradiance found on shallow tropical reefs. Thus, the system allows for long-term studies under natural conditions that cannot be realistically accomplished using indoor closed-flow experiments.

The power heads are widely used in small aquaria and have a useful service life of many years. Use of the diffuser system provides 100% efficiency of CO_2 transfer to seawater. This can readily be observed because when the motor is running no CO_2 bubbles can be seen in the water discharge from the pump. The fine bubbles created by cavitation are absorbed immediately by the seawater. The CO_2-carbonate system reactions are nearly instantaneous so equilibrium is quickly established as in the case of bubbling with larger bubbles. In these tests, we found that A_T is not affected, pH stabilizes immediately, and DIC is increased as expected (Table 2). This is a very advantageous method for experiments that require rapid acidification of large volumes of seawater. 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 interact to modify water chemistry (e.g., Murillo et al. 2014) can be conducted under various scenarios of OA. 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 (Hall-Spencer et al. 2008; Cigliano et al. 2010; Fabricius et al. 2011). This methodology has high potential value for ocean acidification studies on coral reefs. The low cost of replication for mesocosm systems enables complex experimental designs (e.g., randomized block and multi-variate) as well as regression approaches. The ready availability of the inexpensive components allows fabrication of systems that can be used in testing impacts of longer term ecological processes including recruitment, growth, and mortality. More complex interactions can be studied (e.g., OA versus temperature, nutrients, competition, bioerosion, etc). This approach brings CO_2 manipulation within the reach of tropical laboratories located in remote island locations throughout the world and to projects with limited resources.

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 6:1811-1823 [doi:10.5194/bg-6-1811-2009].

———, 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. 397:13-20 [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].

———, 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].

———, 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 [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 [doi:10.4319/lo.2002.47.1.0043].

———, 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 [doi:10.4319/lo.2001.46.4.0833].

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. Mar. Biol. 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 280:2012-2374 [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].

Dickson, A., and F. J. Millero. 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Res. 38:1733-1743 [doi:10.1016/0198-0149(87)90021-5].

Dickson, A. G., C. L. Sabine, and J. R. Christian (eds.) 2007. Guide to best practices for ocean CO_2 measurements. PICES Special Publication 3.

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] [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 Biol. 18:2173-2183 [doi:10.1111/j.1365-2486.2012.02695.x].

Fabricius, K. E., and others. 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., and others. 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454:96-99 [doi:10.1038/nature07051].

Hurd, C. L., C. D. Hepburn, K. I. Currie, J. A. Raven, and K. A. Hunter. 2009. Testing the effects of ocean acidification on algal metabolism: considerations for experimental designs. J. Phycol. 45:1236-1251 [doi:10.1111/j.1529-8817.2009.00768.x].

Jokiel, P. L. 1978. Effects of water motion on reef corals. J. Exp. Mar. Biol. Ecol. 35:87-91 [doi:10.1016/0022-0981(78)90092-8].

———, and S. L. Coles. 1977. Effects of temperature on the mortality and growth of Hawaiian reef corals. Mar. Biol. 43:201-208 [doi:10.1007/BF00402312].

———, R. Y. Ito, and P. M. Liu. 1985. Night irradiance and synchronization of lunar release of planula larvae in the reef coral *Pocillopora damicornis*. Mar. Biol. 88:167-174 [doi:10.1007/BF00397164].

———, M. P. Lesser, and M. E. Ondrusek. 1997. UV-absorbing compounds in the coral *Pocillopora damicornis*: interaction effects of light, water flow and UV radiation. Limnol. Oceanogr. 42(6):1468-1473 [doi:10.4319/lo.1997.42.6.1468].

———, 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, and H. Saito. 1995. Diurnal change in the partial pressure of carbon dioxide in coral reef water. Science 269:214-216 [doi:10.1126/science.269.5221.214].

Kline, D. I., and others. 2012. A short-term in situ CO_2 enrichment experiment on Heron Island (GBR). Sci. Rep. 2:413 [doi:10.1038/srep00413].

Koch, M., G. Bowes, C. Ross, and X.-H. Zhang. 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. Global Change Biol. 19:103-132 [doi:10.1111/j.1365-2486.2012.02791.x].

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 others. 2003. Effect of elevated CO_2 on the community metabolism of experimental coral reef. Global Biogeochem. Cyc. 17:1011 [doi:10.1029/2002GB001941].

———, 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].

Lesser, M. P. 1996. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. Limnol. Oceanogr. 41:271-283 [doi:10.4319/lo.1996.41.2.0271].

Mehrbach C., C. H. Culberson, J. E. Hawley, and R. M. Pytkowicz. 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18:897-907 [doi:10.4319/lo.1973.18.6.0897].

Murillo, L. J. A., P. L. Jokiel, and M. J. Atkinson. 2014. Alkalinity to calcium flux ratios for corals and coral reef communities: variances between isolated and community conditions. PeerJ 2:e249 [doi:10.7717/peerj.249].

Pierrot, D., E. Lewis, and D. W. R. Wallace. 2006. MS Excel program developed for CO_2 system calculations. Carbon Dioxide Information Analysis Center, Oak Ridge Nat. Lab., U.S. Dept. Energy, Oak Ridge, Tennessee [doi:10.3334/CDIAC/otg.CO2SYS_XLS_CDIAC105a].

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 [doi:10.3354/meps293069].

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.1365-2486.2003.00678.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/>.

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