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Calcification in Caribbean reef-building corals at high pCO_2 levels in a recirculating ocean acidification exposure system

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ABSTRACT

Projected increases in ocean pCO_2 levels are anticipated to affect calcifying organisms more rapidly and to a greater extent than other marine organisms. The effects of ocean acidification (OA) have been documented in numerous species of corals in laboratory studies, largely tested using flow-through exposure systems. We developed a recirculating ocean acidification exposure system that allows precise pCO_2 control using a combination of off-gassing measures including aeration, water retention devices, venturi injectors, and CO_2 scrubbing. We evaluated the recirculating system performance in off-gassing effectiveness and maintenance of target pCO_2 levels over an 84-day experiment. The system was used to identify changes in calcification and tissue growth in response to elevated pCO_2 (1000 µatm) in three reef-building corals of the Caribbean: *Pseudodiploria clivosa*, *Montastraea cavernosa*, and *Orbicella faveolata*. All three species displayed an overall increase in net calcification over the 84-day exposure period regardless of pCO_2 level (control + 0.28–1.12 g, elevated pCO_2 + 0.18–1.16 g), and the system was effective at both off-gassing acidified water to ambient pCO_2 levels, and maintaining target elevated pCO_2 levels over the 3-month experiment.

1. Introduction

Over the past two decades, there have been numerous studies examining the effects of ocean acidification (OA), a term used to describe the reduction of seawater pH in response to oceanic absorption of atmospheric carbon dioxide (CO_2) from anthropogenic sources (Caldeira and Wickett, 2003). The general consensus is that OA has detrimental effects on calcifying organisms; the decrease in seawater pH reduces aragonite saturation (Ω_{arag}) which causes dissolution of calcium carbonate ($CaCO_3$) skeletons. The majority of studies have accomplished this research utilizing a flow-through water design, and those studies which utilize a recirculating or static system have produced differing results. Additionally, differences such as methodology of generating projected pCO_2 levels and length of exposure can seemingly all have effects on the experimental outcome.

The majority of ocean acidification studies have utilized a flow-through water regime, whereby acidified treatment water is continuously created and replaced, eliminating any issue of compounding acidification effects. Few studies have used a static or recirculating exposure system, due to challenges such as frequent water changes, organismal fouling, and how to effectively restore pCO_2 to ambient levels in recirculated waters. Studies that have measured coral calcification rates in response to elevated pCO_2 without a flow-through water regime include the Biosphere 2 coral reef biome (recirculating system with water volume of 2650-m^3; Langdon et al., 2003), 8-L static mesocosms requiring frequent water changes (Renegar and Riegl, 2005), and an experimental system consisting of three, independent recirculating aquaria (Lunden et al., 2014). Interestingly, the experimental design of a study can seemingly influence the observed response to acidification. Langdon et al. (2003) explored the effects of elevated pCO_2 for 30–60 d in Biosphere 2 and reported a detrimental effect in multiple species including *Acropora cervicornis*, several *Porites* species, and *Pocillopora damicornis*. The 16-week exposure of *A. cervicornis* by Renegar and Riegl (2005) in a closed system indicated increased pCO_2 had a negative effect on coral growth. However, other studies have shown that *Porites* spp., and *Pocillopora damicornis* are minimally sensitive to OA in flow-through systems (Comeau et al., 2013; Edmunds, 2011), and an experiment using a flow-through regime on *Acropora cervicornis* indicated this species could maintain calcification rates under elevated pCO_2 if provided proper supplemental nutrition (Towle et al., 2015). Kurman et al. (2017) demonstrated a net decline in calcification in the cold water coral *Lophelia pertusa* over a 6-month exposure using a recirculating water regime. However, a 6-month exposure of *L. pertusa* using a flow-through system showed acclimation to

Abbreviations: BWT, buoyant weight; EPA, Environmental Protection Agency; GED, Gulf Ecology Division; SAD, surface area density; TSA, tissue surface area

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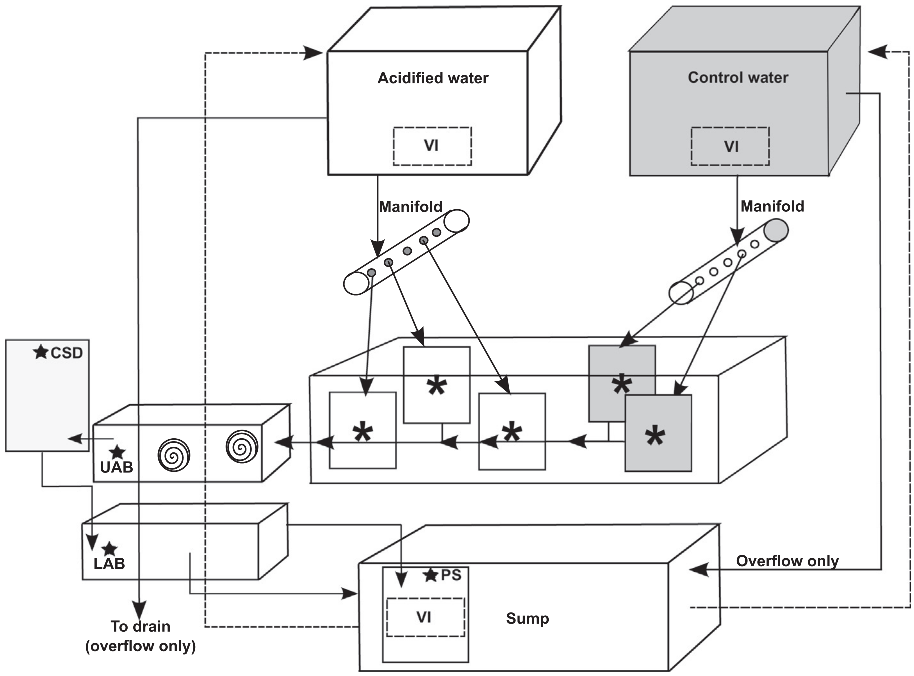

Fig. 1. Diagram of the recirculating OA exposure system. Arrows indicate direction of water flow with dashed lines indicating return water from the sump to each headbox. Asterisks denote experimental chambers (n = 5 for control and n = 5 for elevated pCO_2 chambers, only five chambers shown), and spiraled circles denote CO_2 scrubbers. UAB: Upper acrylic box housing CO_2 scrubbers, LAB: lower acrylic box, CSD: Carlson surge devise, VI: Venturi injector, PS: Protein skimmer. Control water headbox and chambers indicted by grey shading, and a star denotes an off-gassing measure of the system.

elevated pCO_2 and a net calcification increase (Form and Riebesell, 2012).

Laboratory studies that simulate projected pCO_2 levels have used two primary methods to acidify treatment waters, both of which have direct implications on carbonate chemistry. First, a strong acid can be added in order to decrease pH levels, which changes total alkalinity (TA) while keeping dissolved inorganic carbon (DIC) levels constant. Alternatively, pure CO_2 or a mixture of CO_2 and air can be bubbled into treatment waters, keeping TA constant and increasing DIC concentration. Studies have shown different carbonate chemistry results depending on the methodology used, most notably that adding an acid generated pH values well below those anticipated in the year 2100 (Gattuso and Lavigne, 2009). Two studies exposing the coral *Stylophora pistillata* to increased pCO_2 levels showed differing results depending on whether an acid or CO_2 was used to acidify treatment waters. Marubini et al. (2008) used a strong acid and found a marked decline in calcification rate; however, Reynaud et al. (2003) found no discernable change in calcification rate when using carbon dioxide to acidify treatment water. Bubbling CO_2 or a blend of CO_2 and atmospheric air is the preferred method in most OA studies as it more accurately mimics anticipated changes due to anthropogenic ocean acidification, and is easily used over long (> 30 d) experimental time periods (Atkinson and Cuet, 2008; Gattuso and Lavigne, 2009).

Finally, the length of time an organism is exposed to acidified waters can influence measured calcification rates. Calcification rates of the coral *S. pistillata* were shown to decline more in a 2.5 h exposure to elevated pCO_2 by Gattuso et al. (1998) when compared to a 5-week exposure by Reynaud et al. (2003), and *Porites compressa* calcification rates were two times higher in a 10-week study compared to a 1.5 h exposure (Langdon and Atkinson, 2005; Marubini et al., 2001). More recently, a 95 d study on *Siderastrea siderea* to extreme pCO_2 elevations (2553 µatm) showed an increase in calcification rate up to 60 d, followed by a decline between 61 and 95 d (Castillo et al., 2014).

The purpose of the current study was to develop and test an experimental ocean acidification exposure system that could accurately and precisely create projected pCO_2 levels using CO_2 and air to acidify treatment water, utilize a recirculating water flow regime, and have the capability of running longer-term experiments (30 d or more). The U.S. Environmental Protection Agency (EPA) Coral Research Laboratory at Gulf Ecology Division (GED) resides on a barrier island; yet, the sound-side location of the facility makes pumping water directly from the Gulf of Mexico impractical. Therefore, all coral cultures at the laboratory function as indoor recirculating systems. As in nature, the culture systems experience drastic changes in pH and pCO_2 levels over the course of the day due to photosynthesis and respiration (Manzello et al., 2012; Yates et al., 2007). To complete our experiments, we needed to address this fluctuation in the exposure system, as well as effectively off-gassing treated water before it was returned back into the system. Here, we describe a recirculating OA exposure system that has the ability to maintain projected pCO_2 levels indefinitely. A recirculating system capable of off-gassing acidified water will allow other laboratories with limited or no flow-through water capability the opportunity to conduct acidification experiments without building independent treatment systems, as well as eliminate some of the challenges of a static system or microcosm (such as frequent water changes, organismal fouling, and alterations to carbonate chemistry from nutrient build-up). The recirculating system was used to investigate the calcification response of three scleractinian corals species found in the Caribbean Sea and Gulf of Mexico to projected pCO_2 levels using the Intergovernmental Panel on Climate Change A1F1 scenario (1000 µatm; IPCC, 2007) over an 84 d exposure period.

2. Materials and methods

2.1. Exposure system

The exposure system consisted of three levels; headboxes which gravity-feed the system, a top water table and a bottom sump (Fig. 1). The fiberglass sump (58.5″L × 30″W; approximately 375-L) maintained water quality through biological filtration (invertebrates, live rock and sand), mechanical filtration (in-line canister with 1 µm filter and ETSS-600 series protein skimmer), and chemical filtration (locally built calcium reactor filled with CaribSea Oolite® aragonite media and a Two Little Fishes PhosBan reactor® filled with a PhosBan media and activated charcoal mixture). Temperature in the sump was maintained within 1.5 °C of target levels by an Aqualogic 1/3 hp. chiller (Model CY-4) and a titanium heater (GLO-Quartz model # SLTX-11010R19-FB), controlled by an Aqua Logic NEMA 4 × thermostat. An Iwasaki 400 W, 6500 Kelvin metal halide light illuminated the sump on a 10.5/13.5 light/dark cycle. The top portion of the system consisted of a water