Development of Ocean Acidification Flow-Thru Experimental Raceway Units (OAFTERU)

Emily R. Hall[1], David Vaughan[2], Michael P. Crosby[1]

[1]Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236 USA
[2]Mote Tropical Research Laboratory, 24244 Overseas Highway, Summerland Key, FL 33042 USA
Corresponding author: emily8@mote.org

Abstract. Ocean acidification, whether from anthropogenically-induced CO_2 production or natural causes, is an ecological threat to marine organisms. In order to calculate potential impacts of ocean acidification upon ecologically keystone species such as coral, it is essential to employ forecasted pH levels in manipulative experiments to determine physiological indices of such species. The Mote Marine Tropical Research Laboratory (Mote TRL) in Summerland Key, Florida has an established deep well from which its supply of seawater is obtained. This unique source of seawater is 80 feet deep, "fossil" marine water that has a pH that is relatively acidic (pH around 7.6, *p*CO2 ranging from 200 to 2000 μatm). Manipulation of this water by aeration adjusts the pH to varying levels between 7.6 and present day values (>8.0-8.4). We are currently testing methods for utilizing this unique seawater system as the foundation for manipulative ocean acidification studies with Florida Keys corals and other reef ecosystem species in both flow-through and large mesocosm-based designs. Advance knowledge of potential climate-driven trends in coral growth and health will permit improved modeling for prediction and more effectively guide policy decisions for how financial resources should be directed to protection and restoration of coral reef ecosystems. Developing such long term research infrastructure at the existing Mote TRL facility will provide an optimum global research center for examining and modeling effects of ocean acidification on corals as well as other important estuarine and marine species.

Key words: Ocean Acidification, Corals, Mesocosm, Facility.

Introduction

Ocean acidification, whether a consequence of natural variability or anthropogenic CO_2 production, has been referred to as "the other CO_2 problem" (Doney et al., 2009) and is receiving much attention in the marine scientific community. Currently, the ocean absorbs approximately one-third of the excess CO_2 from the atmosphere, acting as a sink for atmospheric CO_2, which leads to a reduction in pH and dramatic shifts in seawater carbonate chemistry (Doney et al., 2009). Compared to preindustrial times, atmospheric CO_2 concentrations are expected to triple within the next century (IPCC, 2007). It is estimated that present day ocean pH has decreased from historic levels of approximately 8.3 to 8.1 (Jacobson, 2005), and current pH levels in the ocean surface will approach 7.8, a value that has not been experienced for several millions of years (Caldiera and Wickett, 2003; Feely et al., 2004; Feely et al., 2009; Hoegh-Guldberg et al., 2007; Riebesell et al., 2000; Wolf-Gladrow et al., 1999). It has been well established that this ocean acidification will likely directly impact marine organisms that build shells or skeletons from calcium carbonate (Doney et al., 2009; Fabry et al., 2008; Guinotte and Fabry et al., 2008; Hoegh-Guldberg et al., 2007; Iglesias-Rodriguez et al., 2008; Langdon and Atktinson, 2005; Riebesell et al., 2000; Riebesell, 2004). Critical needs that have been identified by top climate change and marine scientists include using projected pCO_2 (partial pressure of CO_2 in seawater) levels in manipulative experiments to determine physiological indices of ecologically important species, such as corals (Doney et al., 2009; Fabry et al., 2008; Kleypas et al., 2006).

Coral reefs were one of the first ecosystems to be documented as susceptible to ocean acidification (Kleypas and Yates, 2009). The Florida Keys reef system has already experienced a long-term deterioration in ecosystem health, as evidenced by Porter (1992), Porter et al. (2001), and Lapointe et al. (2004). It has been speculated that this decline in reef ecosystem health could be exacerbated by increasing atmospheric CO_2 levels and resulting ocean acidification (Feely 2009; Kleypas and Yates, 2009). Therefore, reef resilience or reef behavior to ocean acidification in the Florida Keys is of great concern. Many experimental setups for testing effects of ocean acidification on corals have already been established and tested (Riebesell et al, 2010), however it would be beneficial to develop and maintain an ocean acidification testing system specific to organisms and ecosystems indigenous to the Florida Keys reef tract.

The Mote Marine Tropical Research Laboratory (Mote TRL) in Summerland Key, FL already has an established deep well where the facility gets its supply of seawater for lab use. This 80 feet deep well is unique in that it pumps out fossil marine water that contains naturally high concentrations of CO_2. The water is pumped from the local aquifer and is then treated via aeration (to reduce H_2S and ammonia), biofiltration, and clarification through sand filters. Other experiments that have taken place at these facilities have noted that the pH of the water coming out has been shown to be comparatively acidic (pH around 7.6, pCO_2 ranging from 1000 to 2000 µatm) and that further aeration will adjust the pH of the water, by driving off more CO_2, back up to present day values (>8.0-8.4). Existing data on selected water chemistry parameters are presented in Table 1.

We are developing this unique water system so that it can be used as an ocean acidification testing location for Florida Keys corals and other reef species. Once stability of the system is demonstrated, we will determine if water with different pH has an effect on different coral reef species. This will continue to be developed and tested providing results that will establish the Mote TRL facility as a permanent ocean acidification testing facility.

Locale	Salinity	TCO_2 (µM)	TA (µM)	pCO_2 (µatm)	pH	Ω_{Arag}
Raw well	37.84	4136.6	2165.9	2105	7.66	3.16
1st stage of degassing	37.72	3952.1	2147.0	1237	7.86	4.68
Entering coral tank	37.68	3928.4	2143.9	1165	7.88	4.87
Following incubation with coral and aeration	37.71	3328.3	2133.0	250	8.4	11.67

Table 1: Previous measurements (from March, 2009) of the well system at Mote TRL.

Material and Methods

All assays were conducted at the Mote TRL field station in Summerland Key, Florida (Fig. 1). This was a two part study where part I was primarily for establishing the system as a stable ocean acidification testing system and part II was for testing a variety of corals in the system. Specific water chemistry parameters that were measured regularly for parts I and II include pH, temperature, and salinity. Ammonia, nitrate/nitrite, phosphate, and alkalinity were measured regularly for part I, and prior to, throughout, and following each experiment for part II.

Part I

Two future climate scenarios predicted by the Intergovernmental Panel on Climate Change (IPCC) for various planning horizons (IPCC, 2007) were simulated. Deep well water was collected in two storage tanks after aeration and biofiltration to drive off H_2S and NH_4, and manipulated to achieve different pH scenarios (based on modern day and IPCC predictions up to year 2100). The high pH tank was bubbled with air until a pH of 8.1 was maintained wile the low pH tank had no bubbling and was maintained at a pH of 7.5. The two pH treatments were controlled with pH controllers (to turn bubbling on or off). Water from the two storage tanks was continuously transferred to eight testing aquaria (4 for each pH) (Fig. 2). Salinity, temperature, and pH were monitored regularly and kept stable. Nutrients and alkalinity were also monitored regularly.

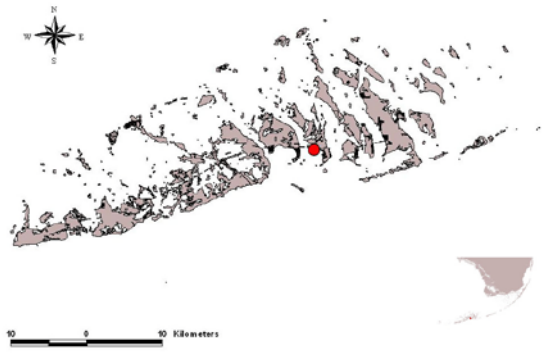

Figure 1: Location of Mote TRL and OAFTERU.

Part II

Part II of the experimental design is still in progress. During part II, the system is maintained identically to part I with the addition of three different coral species (*Montastraea cavernosa, Montastraea annularis,* and *Oculina diffusa*) and one other calcifying mollusk species (worm-snail). Water from the two storage tanks is continuously transferred to eight testing aquaria (4 for each pH). Three tanks from each pH treatment contain three replicates of each organism. One tank from each pH treatment is kept as a control and contains no organisms. The organisms are being exposed to the two pH conditions for a total of 90 to 120 days to observe health and growth rate changes (following methods by Davies, 1989). Light is being maintained at 60 to 80 µE/m^2/sec. All tanks are being kept in a water bath to keep temperature uniform at 25ºC. Salinity, temperature, and pH will be monitored regularly and kept stable. Nutrients and alkalinity are being measured prior to, throughout, and following each experiment.

Finally, to help determine carbon chemistry in the deep well system, the Excel Macro CO2Sys9 (Perriot,

2007 using code from Lewis and Wallace, 1998) was used to calculate concentrations of the inorganic carbon system in each tank. Input data from our experiment included total alkalinity, pH, temperature, total phosphate, and salinity. Output for this program provided CO_2 parameters (total CO_2 and partial pressure of CO_2) and degree of saturation for aragonite (Ω_{Arag}).

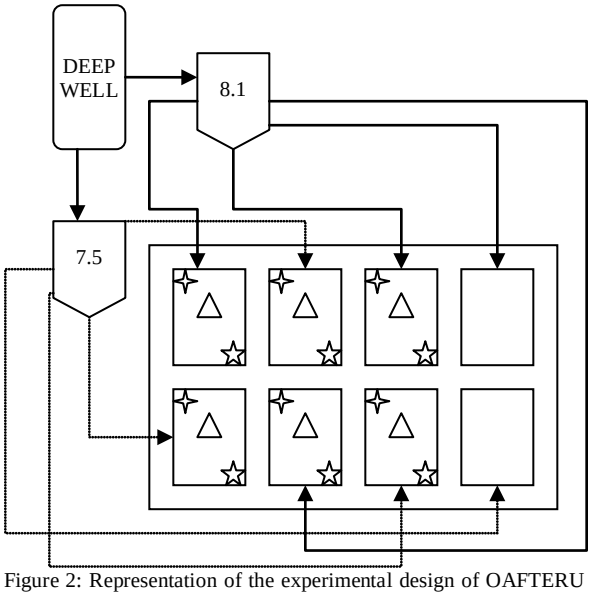

Figure 2: Representation of the experimental design of OAFTERU at Mote TRL.

Results

Setup of the indoor storage tanks and raceways has been completed and multiple experiments with system design have occurred including flow and pH tests with different size tanks (for the low and high pH seawater bulk tanks). At this time, we have determined that the experimental design will allow us to manipulate and control two pH treatments. We have been able to stabilize the low pH tank at a pH of 7.5, and the high pH tank at a pH of 8.1. Physical analyses from part I of the study are presented in Table 2 and chemical analyses are presented in Tables 3 and 4. Salinity remained constant in all water sources. Total alkalinity was comparable to average tropical surface ocean alkalinity in all three source tanks. TCO_2, pCO_2, and Ω_{Arag} calculations were highest in the raw well source and the low pH source water. Dissolved oxygen ranged from 6.4 to 6.9 mg/L in the raw well as well as the high and low pH source water. All nutrient concentrations remained relatively constant throughout the experiment. Concentrations of NO_2+NO_3-N remained were lowest in the raw well water and were comparable in the high and low pH sources of water. Concentrations of NH_4-N were highest in the raw well water and were comparable in the high and low pH sources of water. Concentrations of PO_4-P were comparable in all three sources of water. Concentrations of several major and minor ionic species and typical seawater values are presented in Table 4.

Currently, three coral species and one other calcifying mollusk are being tested at the two different pH levels (Fig. 3). Preliminary results show that all three species of coral in the high pH (lower pCO_2) tanks have more open polyps and extended tentacles (visual observations), while corals in the low pH (high pCO_2) tanks appear more stressed. The mollusk species show no apparent differences between the pH treatments so far. Long term wet buoyancy weight will be used to determine growth measurements of all four test species. This part of the experimental design will continue for up to 90 more days to determine if there are any effects on the corals and mollusks.

Source	Sal (‰)	TCO_2 (µM)	TA (µM)	pCO_2 (µatm)	pH	Ω_{Arag}	DO mg/L
Raw Well	37.0 ± 0.01	2104.7	2175.5 ± 47.5	1920.9	7.43 ± 0.04	0.93	6.92
High pH	36.9 ± 0.4	1786.2 ± 67.1	2127.5 ± 37.4	295.1 ± 78.1	8.10 ± 0.09	3.68 ± 0.60	6.71± 0.60
Low pH	36.8 ± 0.6	2048.7 ± 25.8	2135.8 ± 45.5	1389.0 ± 226.2	7.53 ± 0.07	1.22 ± 0.22	6.44± 0.81

Table 2: Summary of physical and chemical conditions in the OAFTERU system at Mote TRL. Standard deviation is presented unless multiple sample analyses were unavailable.

Source	NO_2+NO_3-N (µM)	NH_4-N (µM)	PO_4-P (µM)
Raw Well	4.79 ± 0.60	14.68 ± 0.90	1.43 ± 0.46
High pH	21.50 ± 1.96	0.50 ± 0.65	1.37 ± 0.10
Low pH	20.56 ± 1.86	0.47 ± 0.34	1.38 ± 0.11

Table 3: Summary of nutrient analyses in the OAFTERU system at Mote TRL.

Analytes	Raw Well	Filtered Well	Typical Seawater *
	mg/L	mg/L	mg/L
Na	12000	12000	10781
Cl	22000	22000	19400
Mg	1600	1500	1284
SO4	3600	3200	2710
Ca	570	530	412
K	550	530	399
Sr	9.9	9.8	8.1
B	5.3	5.2	4.5
SiO_2	3.7	3.8	2.9

Table 4: Summary of chemical analyses in the OAFTERU system at Mote TRL and of typical seawater (*Pilson, 1998).

Figure 3: Experimental design of OAFTERU with test organisms (*Montastraea cavernosa*, *Montastraea annularis*, *Oculina diffusa* and worm-snails) at Mote TRL.

Discussion

The IPCC carbon models are predicting atmospheric CO_2 concentrations to triple within the next century, thus reducing the pH (ocean acidification) of marine ecosystems (IPCC, 2007). Decreasing pH levels in oceans have been shown to produce weaker coral skeletons, reduce growth rates of corals, and increase erosion of calcifying species (Fabry et al., 2008; Hoegh-Guldberg et al., 2007; Kleypas and Yates, 2009; Guinotte and Fabry, 2008). Critical needs that have been identified by top climate change and marine scientists include using projected pCO_2 levels in manipulative experiments to determine physiological indices of ecologically important species like corals (Doney et al., 2009; Fabry et al., 2008). Ocean acidification is predicted to have more immediate effects in colder climates (Steinacher et al., 2009; Feely et al., 2009), however, studies have also shown declines in calcification rates, growth rates, etc. in tropical ecosystems (Doney et al., 2009; Kleypas and Yates, 2009; Hoegh-Guldberg and Bruno, 2010).

Establishment of a permanent testing facility adjacent to reef ecosystems of concern provides many advantages. For example, the Climate Change Mesocosm (CCM) project at the Heron Island Research Station (HIRS) is the first coral reef research station for studying the predicted effects to the adjacent Great Barrier Reef. This unique system provides infrastructure for multidisciplinary ocean acidification experiments on the local coral reef community with capabilities to control temperature and pCO_2 conditions with daily and seasonal changes that occur locally on the Great Barrier Reef (Marker et al., 2010).

The OAFTERU system strives to replicate the CCM system at a smaller scale, using naturally CO_2 enriched seawater, and in proximity to the Florida Keys reef ecosystem. Preliminary results demonstrate the capabilities of this unique seawater system to function as a foundation for manipulative ocean acidification studies with Florida Keys corals and other reef ecosystem species in both flow-through and large mesocosm-based designs. This system allows us to manipulate and store large volumes of CO_2 enriched seawater. Currently, indoor tanks allow control of pH (and pCO_2) with pH controllers while maintaining steady temperature and salinity levels. We are continuing to build the system to include outdoor tanks, temperature controls (to combine projected CO_2 and temperature changes), and larger storage tanks.

Our early results clearly indicate an ability to determine responses of corals to altered states of ocean acidification, when nutrients are controlled and at elevated levels. The amount of NO_2+NO_3-N and PO_4-P in the high pH tank, low pH tank, and all raceways in the OAFTERU system is comparable, suggesting that the preliminary differences seen in the corals in the high pH tanks and low pH tanks could be attributed to the difference in CO_2 concentrations. Marubini and Atkinson (1999) have demonstrated that changes in calcification rates of corals were greater from decreased pH than from elevated nutrients. However, Langdon and Atkinson (2005) and Holcomb et al. (2010) have demonstrated that elevated levels of NO_3, NH_4 and PO_4 rendered some species of coral less sensitive to the effects of elevated CO_2. Hence, elevated nutrients, even when consistent between pH treatments as in the OAFTERU system, presents a confounding affect that can influence coral response to altered state of acidification and lead to ambiguous interpretations by natural resource managers and policy-makers. It is important to note that the well-water nutrient level parameters at Mote TRL have been growing multiple species of hard and soft corals in the laboratory for over 10 years, with comparable growth rates to south Florida inshore and offshore reef environments. However, as we continue to further fine-tune the OAFTERU system, we are taking steps to reduce

nutrient (NO_2+NO_3-N and PO_4-P) concentrations to that found in "typical" coral reef ecosystems in order to more definitively ascertain impact due to changing pH, without the added influence of elevated nutrient concentrations. We also plan to run analyses of iron concentrations, where elevated levels have been shown in other well systems (Langdon, pers. comm.).

Developing such long term research infrastructure at the existing Mote TRL facility will provide an optimum global research center for examining and modeling effects of ocean acidification on corals as well as other important estuarine and marine species in the Florida Keys.

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