Overview of Ocean Acidification Experimental Design

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Major scientific issues

- Determine the calcification response to elevated CO_2 of planktonic (coccolithophorids, foraminifera, shelled pteropods) and benthic calcifiers (corals, coralline algae, molluscs and echinoderms)
- Discriminate the various mechanisms of calcification within calcifying groups to better understand the cross-taxa range of response to changing seawater chemistry
- Determine the interactive effects of multiple variables that affect calcification and dissolution in organisms (Ω, light, temperature, nutrients)
- Incorporate ecological questions into observations and experiments; e.g. How does a change in calcification rate affect the survivorship of an organism? How will ecosystems be affected by fewer calcifying species?

From Impacts of Ocean Acidification on Coral Reefs and Other Marine Calcifiers

Other important scientific issues

- There is growing evidence that OA can have impacts beyond just calcification
  - Reproduction
  - Larval settlement
  - Photosynthesis
  - N_2 fixation
  - Elemental ratios

Challenge is to expose organism to treatment chemistries while keeping all other aspects of their environment natural

- Provide natural light at intensity that is sufficient to saturate photosynthesis
- Fish larvae that are visual predators need light and a light colored background to see their prey
- Temperature - must be held constant and near optimum, some species have a narrow range of tolerance
- Nutrients - should try to provide natural concentrations , elevated levels have been shown to suppress calcification in coccolithophorids and corals
- Flow – many organisms require adequate flow to exchange gases and ions at optimal rates
- Food – for longer experiments heterotrophic organisms will need to be fed
- Handling – some small organisms and larvae are very delicate and require bubbling of separate volume of water. Some larvae require a large tank to minimize contact with the walls.
- Larger organisms with high metabolic rates may be best studied in a flow-thru chamber where the chemistry can be maintained constant by adjusting the flow and the rates can be obtained from the difference in chemistry between the inflow and outflow.

Things to think about in designing an OA experiment

- How to simulate the carbonate chemistry of seawater under past, present and future conditions?
- How many treatment levels?
- Duration of the experiments
- Type (open or closed top, flow-thru) and size of chamber
- How will the chemistry be monitored?

Manipulating the seawater carbonate system

- Vary DIC while holding TA constant (most like natural setting)
- Vary TA while holding DIC constant (often used in experiments)
  - this manipulation can precisely simulate the either the change in CO_3^{2-} or pH of the vary DIC/cnst TA method but not both at the same time

From Schulz et al. 2009 Biogeosciences 6: 2145-2153

Vary DIC/cnst TA

- This is the preferred method in most situations
- Bubbling with CO_2 enriched air
  - For small scale or short term experiments it may be most economical to purchase pre-mixed gases by the cylinder
  - For larger and/or longer experiments it will be more economical to make your own mixture of outside air and pure CO_2 gas using mass flow controllers
  - For very large experiments or underwater in-situ experiments it may be more practical to add $NaHCO_3$ as a salt or solution to increase the DIC to the desired level and then acid HCl to cancel the undesired increase in TA. The end result is exactly the same as bubbling.

Vary TA/cnst DIC

- OA conditions can be simulated by adding HCl to reduce the TA.
- Pre-anthropogenic conditions can be simulated by adding NaOH.
- The paper by Schulz et al. 2009 in Biogeosciences is a good reference on the differences between bubbling and acid addition manipulations in terms of CO_3^{2-}, HCO_3^-, and pH. They concluded that it is unlikely that biological responses will be different with the two approaches.
- There are some practical suggestions:
  - Adding conc. acid to seawater can result in loss of DIC due to gas exchange. Use dilute acid and mix it rapidly.
  - Adding conc. base to seawater will result in precipitation of $CaCO_3$. Use a dilute base solution.

How many CO_2 levels?

- The more the better to characterize the shape of the response function. Most experiments to date have used only two or three.
- If CO_2 treatments are crossed with one or more other factors (light, temperature, nutrients) it may be possible to only do two CO_2 levels.

Size of the experimental chambers or tanks

- Small if you want to have many replicate tanks
- Large so the organism under test does not alter the chemistry of the water significantly during the course of the experiment

Duration of the experiment

- For studies of physiological response the experiment may be just a few hours although there may be a lengthy pre-conditioning period
- Long term experiments to look at larval development, growth of adult organisms, acclimation, interactions with other species.

Monitoring chemistry is essential

- Gas exchange and metabolic activity of organisms under test mean that chemistry will change during the experiment.
- At a minimum treatment chemistry must be measured at the beginning and end of experiment.
- For longer experiments chemistry should be sampled on a daily or weekly basis.
  - For this purpose TA and DIC are generally the best because they can be preserved and are stable for months
- Continuous monitoring of pCO_2 is easy and informative
  - Less subject to drift and biofouling than pH
  - Autocalibration is easy to implement

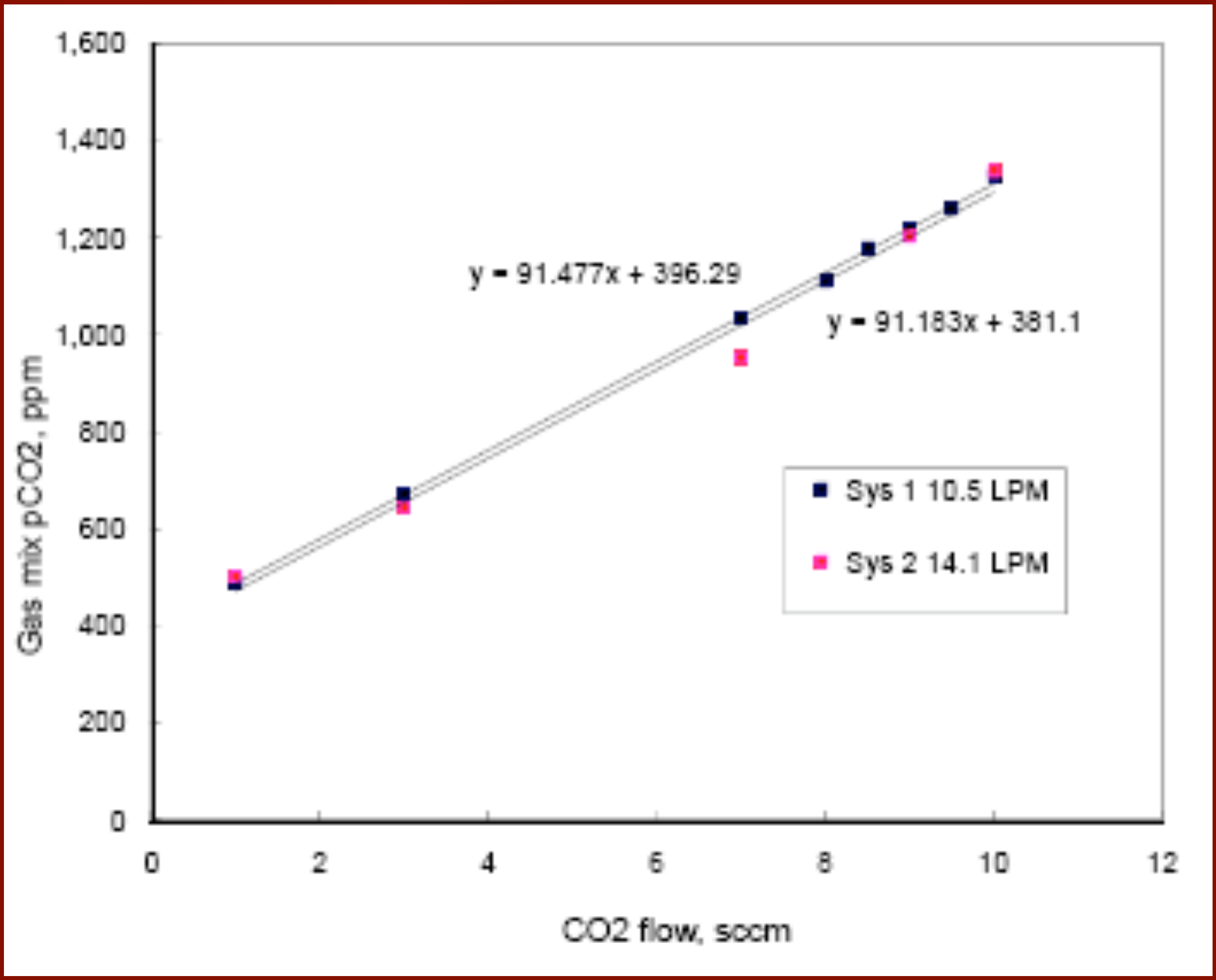
Sample pCO_2 data from the University of Miami Coral Culturing Facility

CO_2 control and monitoring system

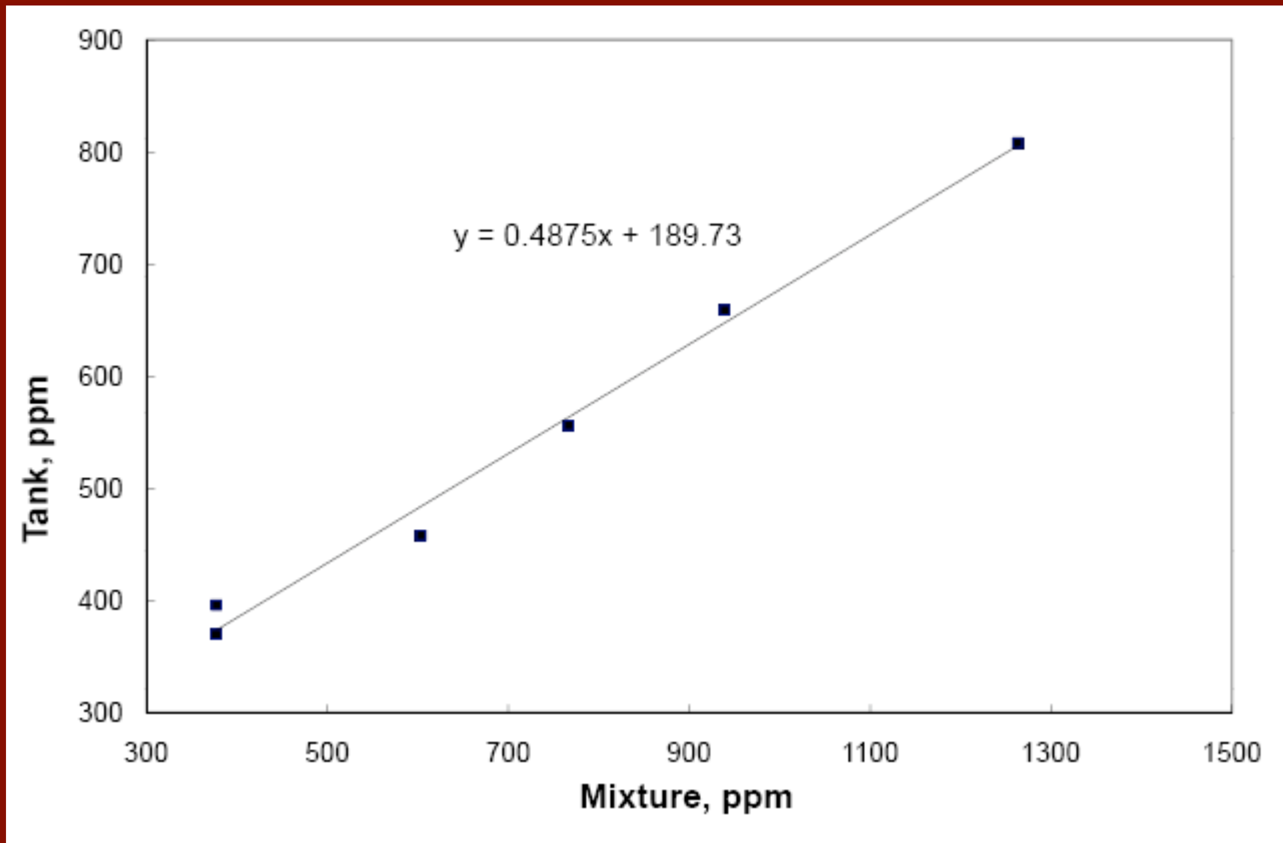
Mass flow controllers and Licor CO_2 analyzers

Equilibrator

Gas mix pCO_2 vs flow rate of CO_2 gas

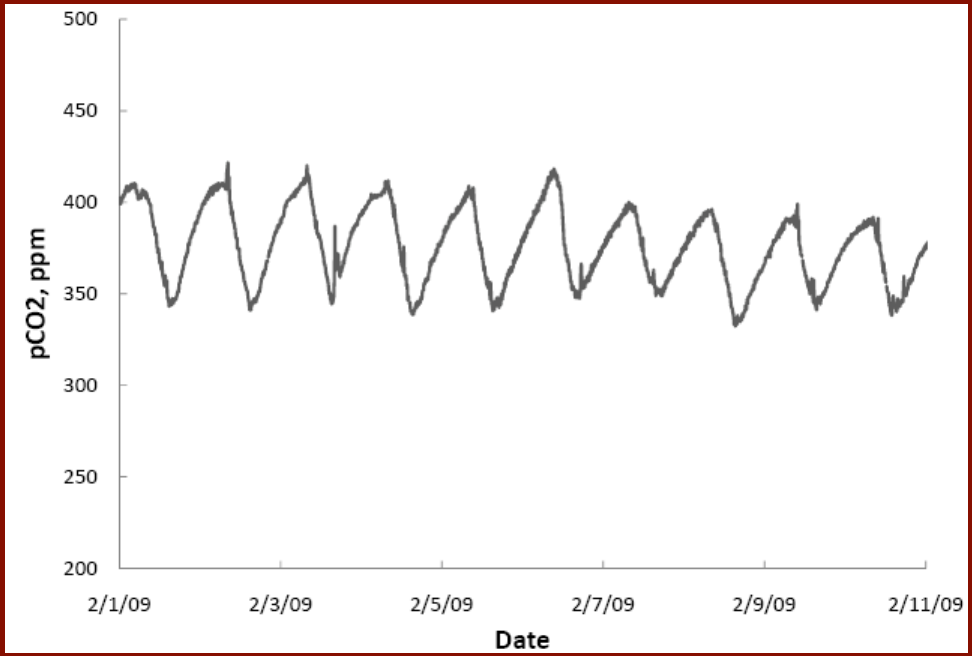


Tank pCO_2 vs Gas mix pCO_2

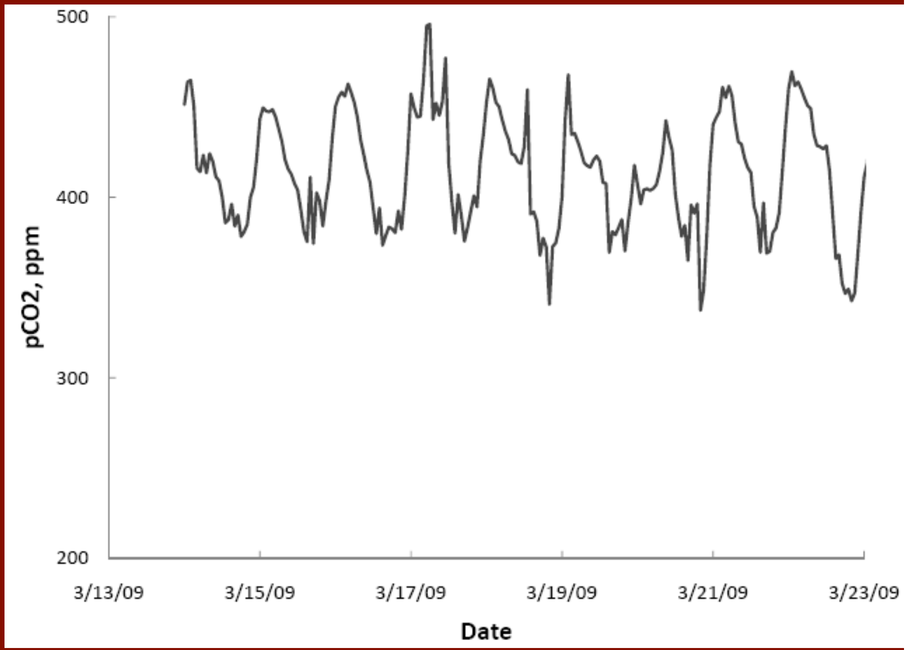


Typical pCO_2 variability in tank and on a coral reef

Tank bubbled with CO_2



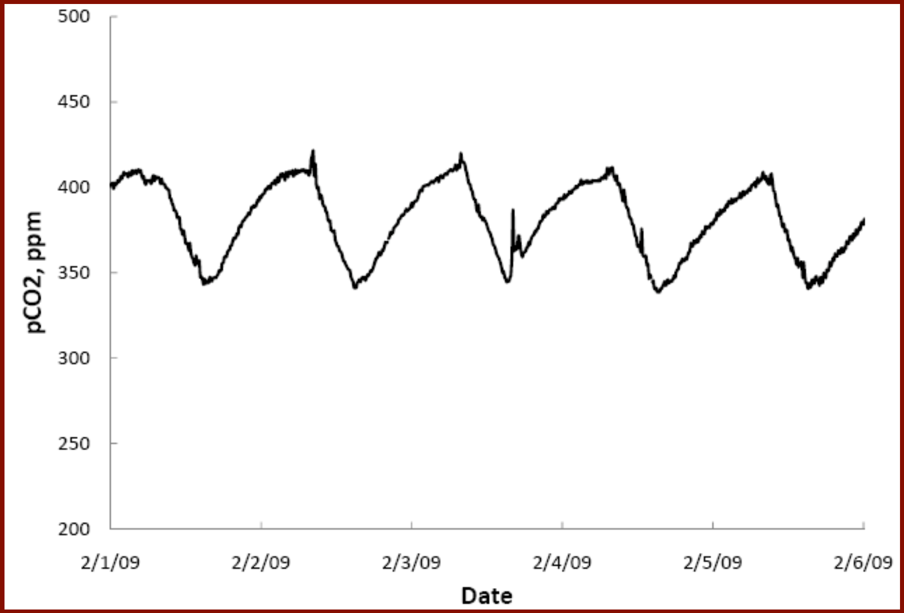
Molasses Reef, Florida Keys



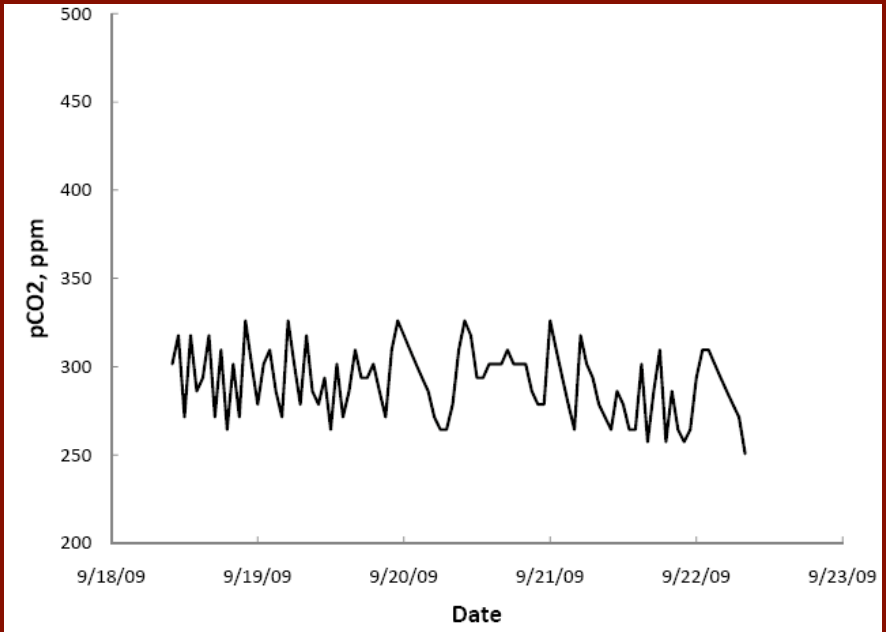
pCO_2 changes in continuously bubbled tank are similar to natural reef waters in terms of phase. Amplitude in the tank is a function of the amount of organisms placed in the tank.

Comparison of two CO_2 control systems

Continuously bubbled tank



pH stat system with CO_2 bubbling



Data kindly provided by Whitman Miller