

Workshop Report:

New Instrumentation for Assessment of Ocean Acidification in Coral Ecosystems, and Modeling of Coral Calcification



SRI International, Marine Technology Division, St. Petersburg FL; March 8-11, 2010

Sponsored By: NOAA Cooperative Institute for Ocean Exploration, Research, and Technology (CIOERT)

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CONTENTS:

SECTION	Page
EXECUTIVE SUMMARY	1
SITUATION AND NEED	2
APPROACH	3
SUMMARY: Coral Reef OA Network Instrumentation	4
SUMMARY: Observations Needed for Coral Physiology and Calcification	7
RECOMMENDATIONS	9
LITERATURE CITED	13
APPENDIX A: Participants	14
APPENDIX B: Workshop Agenda	15
APPENDIX C: Instrumentation Working Group Notes	16
APPENDIX D: Coral Physiology/Calcification Working Group Notes	28

EXECUTIVE SUMMARY

The goals of a three day workshop held March 8-11, 2010, were to examine the state of readiness of the various methods and instruments currently in use for measuring the four major chemical parameters needed for research and monitoring of ocean acidification effects on coral reef calcification ($p\text{CO}_2$, pH, DIC and total alkalinity (T-Alk)), and to determine where new funding efforts should be directed. Thirty seven participants included lead investigators from academia, NOAA and private industry involved in the development and use of these methods and instruments. The methods used to measure each of the four chemical parameters were discussed in detail, and the state of readiness of automated instruments evaluated using a scale developed by the Ocean Observing Initiative (OOI; <http://www.oceanleadership.org/programs-and-partnerships/ocean-observing/ooi/>). There are

commercially available instruments (MapCO₂; Caricoca) to measure pCO₂ of atmospheric and near-sea surface samples. Instruments to measure pCO₂ of deeper and reef surface waters are available commercially (Sami-CO₂; ProOceanus), but there are still reliability issues for longer term deployments. For pH, there is one commercially available instrument (Sami-pH) and two research prototypes (SEASII; SEAFET) but these still have problems with longer-term reliability. The above instruments have been designed for deployment on moorings, AUVs or for stationary benthic measurements, but none of the above instruments are suitable for divers making more detailed benthic measurements because of size and power requirements. For DIC, two techniques for *in situ* autonomous measurements are at the research prototype stage but further work is required to increase availability, decrease complexity and reduce costs. For T-Alk, there is no instrument currently available for *in situ* automated analyses, but this parameter is of major importance for measuring calcification rates, and thus further development should be encouraged with new funding.

Concurrent with the instrument development efforts, a small group of coral physiologists met to discuss the major unknown questions with regard to understanding ocean acidification effects on coral calcification. While new molecular, cell biological and geochemical approaches have greatly increased our knowledge of some of the details of coral calcification, we still have a rudimentary understanding of the proximal sources, mechanisms of uptake and pathways of the ions used by coral calicodermis to secrete the skeleton, micro-spatial patterns of skeletogenesis, pH regulation in coral tissues and fluids, and of differences among coral species in their level of biological control over these mechanisms. A simple conceptual model was developed to guide further research.

As a final note, the group re-iterated that the types and capabilities of instruments needed for ocean acidification research on coral reefs should consider the specific research objectives: longer term monitoring at a few specific sites will have different needs than a more detailed process oriented study, or studies of the metabolic responses of the benthic community.

SITUATION AND NEED

Ocean acidification refers to the reduction in seawater pH associated with the global oceanic uptake of increasing atmospheric CO₂. After dissolution, CO₂ reacts with water to form several dissolved species. In order to fully characterize the chemical changes caused by increasing CO₂, at least two of the four measurable inorganic carbon parameters and the dissociation constants must be known. Thus, instruments to measure dissolved inorganic carbon (DIC), total alkalinity (T-Alk), CO₂ partial pressure (pCO₂) and pH are of prime importance to OA research. Much of the effort to date to develop instruments to study the process and progression of OA has been aimed towards high precision and sensitivity instruments designed to measure small changes in seawater chemistry in low variability oceanic waters (e.g., MAP-CO₂, MICA, SAMI, SEAS, several mass spectrometers). These efforts have been accomplished with coordination by investigators at academic, R&D and NOAA institutions, and with funding from NSF, NOAA and other federal agencies. In spite of such effort, much remains to be done to produce instruments that are reliable and robust enough for prolonged deployments with the appropriate pairing of parameters to constrain the carbon chemistry.

Coral reef ecosystems are one of the most vulnerable to OA but also the most complex to understand in terms of biotic/seawater interactions. Large biomass of organisms on and within coral reefs respire (adding to localized OA), photosynthesize (potentially counteracting OA), and calcify at various rates in various sub-habitats creating a complex patchwork of interactions between metabolism and bulk ocean water chemistry that the calcifying organisms live within and have to deal with on a daily basis.

As a result of this biotic metabolism, coral reef seawater chemistry is highly variable over short time and spatial scales, and thus the instruments needed to study effects of OA on coral reef systems may have different specifications than those needed for oceanic studies. Even within coral reef systems, a distinction may be necessary for instrument packages needed to monitor diurnal or seasonal chemistry changes versus instruments needed to estimate fluxes (upstream/downstream, benthic concentration gradients) in order to determine local *in situ* productivity/calcification. Kleypas and Langdon (2006) reviewed the state of knowledge between seawater carbonate chemistry and coral calcification, and proposed a strategy that recommended many more measurements of carbonate chemistry, and coral calcification and dissolution, and asked “can we design ways to monitor calcification and dissolution rates within reef-building corals and within coral communities as a whole?” The Kleypas et al. (2006) report from a 2005 OA workshop co-sponsored by NOAA called for OA research on coral reefs, including the need to “Characterize the diurnal and seasonal cycles of the carbonate system on coral reefs, including commitment to long-term monitoring of the system response to continued increases in CO₂.” In recent years, instrumentation innovations put us closer to these goals. During the 3 day workshop OA workshop in 2010, experts (engineers, scientists and managers; Appendix A) reviewed the instrumentation needs and specifications of various types of OA research especially for coral reefs, capabilities and limitations of existing technologies, and on-going and future developments, including integration of sensors into instrument packages for both short-term, *in situ* application by divers or vehicles, and mid- to long-term deployments on coral reefs.

Concurrent with the instrument development efforts, a group of coral physiologists and cell biologists met to review our current understanding of the mechanisms of coral calcification to identify the major information needs in order to predict responses to OA. Discussions focused on what is known about ion transport processes, the locations within coral tissues of calcification, the unknowns with regard to exchange of ions between seawater and sites of calcification, and development of a conceptual model that can inform required OA measurements at all required space and time scales.

Expected workshop outcomes included:

- Strategy for development of an OA instrument suite for coral ecosystems;
- Input for CIOERT Science Plans for 2011 and beyond;
- Input for NOAA’s OA Program and future funding initiatives; and
- Team building for multi-investigator collaborations.

APPROACH

Participants (Appendix A) were by invitation and intended to cover the range of OA issues, from technical, scientific and management perspectives. The agenda (Appendix B) included plenary presentations to summarize and review key issues, including NOAA’s OA plans and objectives, followed by working group discussions on OA instrumentation needs and coral physiology and calcification processes. Discussions were moderated and served to address all the workshop goals and outcomes. This report is available on cioert.org/oa-workshop.

SUMMARY

CORAL REEF OA NETWORK INSTRUMENTATION:

The 2008 Second International Symposium on “*The Ocean in a High CO₂ World*” in Monaco (Orr et al. 2008) observed that “surface ocean pH has already dropped by 0.1 units since the beginning of the Industrial Revolution, which is equivalent to a 30% increase in hydrogen ion concentration (referred to here as acidity). This rate of acidification has not been experienced by marine organisms, including reef-building corals, for many millions of years.” The Monaco symposium resulted in science and outreach recommendations including several related to improving CO₂-system observations:

- Develop **new instrumentation for autonomous measurements of CO₂ system parameters**, particulate inorganic (PIC) and particulate organic carbon (POC), and other indicators of impacts on organisms and ecosystems;
- Maintain, enhance, and extend existing long-term time series that are relevant for ocean acidification;
- Establish new monitoring sites and repeat surveys in key areas that are likely to be vulnerable to ocean acidification;
- **Develop relaxed carbon measurement methods and appropriate instrumentation** that are cheaper and easier, if possible, for high-variability areas that may not need the highest measurement precision;
- Establish a high-quality ocean carbon measurement service for those unable to develop their own measurement capabilities.

According to Dickson et al. (2007), although the equipment and methods to make discrete carbon measurements are established, they are not necessarily cheap or easy (Table 2). Current instrumentation for measuring CO₂-system parameters using *in situ* underwater sensors (Table 3), versus discrete samples analyzed *in vitro*, holds promise for increasing the speed and density of observations at lower cost per measurement. These approaches vary in their technology readiness level, reliability (especially for long-term deployments), maintenance needs, and degree of difficulty for further development. The concept of readiness levels was adopted from the Ocean Observing Initiative to categorize status of sensors:

- Level 1- Proof of Concept: earliest stage, engineering drawings
- Level 2- research prototype: basic components integrated, prototype collecting data
- Level 3- research proven: not commercialized, but beyond prototype
- Level 4- commercial application: proven to work in environment as expected, commercial production
- Level 5- operational: final stage, working under sustained operational conditions.

Table 2. CO₂-system parameters and estimated difficulty, cost and sample stability for analysis of discrete water samples (Dickson et al. 2007).

Parameter	Difficulty	Cost	Storage Stability
DIC	high	High	<3mo.
ALK	mod.	Low	<6mo.
pH	low	mod.	<2h
pCO ₂	very high	mod/very high	<24h

Table 3. Seawater CO₂-system sensors capable of *in situ* measurements.

Parameter	Methods
pCO ₂	- Gas equilibration with NDIR detection - Membrane separation with dye + spectrophotometric detection - Membrane separation with mass spectrometer detection - Fluorescent optodes (Severinghaus based)
pH	- Dye addition with spectrophotometric detection - ISFET electrodes - Optodes using fluorescence lifetimes of embedded dyes
DIC	- Acidification with membrane separation and conductimetric detection - Acidification with membrane separation and dye + spectrophotometric detection
T-Alk	- Titration with spectrophotometric detection - ISFET titration-on-chip (Orion FLASH titrator)
CO ₃ ²⁻	- Complexation with spectrophotometric detection - Ion selective electrode - Direct spectrophotometric detection

NOAA is working on an OA Strategic Plan for the next decade. An identified component is an OA Coral Ecosystem Observing Network that includes “advanced technology development and standardization for carbonate chemistry measurements on moorings and autonomous floats” (NOAA 2008 OA Fact Sheet, http://oceanservice.noaa.gov/education/yos/resource/01state_of_science.pdf). This workshop focused on coral reef ecosystems; NOAA managers stated the additional need for portable/mobile sensing packages that could be diver or vehicle deployed to assess CO₂-system conditions at varying scales (cms to kms), in conjunction with ecological assessments.

The goal of this workshop was to identify challenges, hurdles and solutions to this NOAA strategy, including:

- Science requirements (possible relaxed criteria) for CO₂ system measurements in highly variable coral reef systems
- Current state of performance for *in situ* CO₂ system instrumentation
- Developments to advance current technologies and develop new instrumentation to meet coral reef science needs.

The group discussions on target uncertainties for CO₂ system measurements in coral reef ecosystems centered on the three questions posed in Dickson’s presentation: What do I need to know? How well do I need to know it? What resources do I need to achieve this?

The group reiterated the well-known consensus that we need to know at least two (and preferably three) of the four measurable CO₂ system parameters. For coral reef systems, T-Alk is a particularly desirable parameter as it is the most direct proxy for elucidating calcification. In addition, pairing with T-Alk with pH or pCO₂ minimizes the relative uncertainties (~ 4%) for calculation of carbonate ion concentration (i.e. saturation state if [Ca²⁺] is constant). DIC paired with pH or pCO₂ gives similar

relative uncertainties for calculated carbonate ion concentration. The most robust pairing for calculating carbonate ion concentration is DIC + T-Alk with relative uncertainties ~1%. Thus, development of robust and reliable instruments for *in situ* T-Alk and DIC measurements is highly recommended for coral reef OA studies.

The variability in precision and accuracy (combined as the uncertainty) of the measurements (and associated derived values) was also discussed. The stated relative uncertainties stated above come from Dickson's estimates for measurements with discrete reference methods (http://cdiac.ornl.gov/oceans/Handbook_2007.html) and thus represent the state of the art scenario. It is likely that other techniques, particularly autonomous versions, will, at least initially, have greater uncertainties. It has been suggested that relaxed measurement criteria might be acceptable for coral reef environments where temporal variations in CO₂ system parameters are often many fold greater than oceanic sites. However, recent studies (Gledhill et al. 2009; Bates, 2007) indicate that the mean decadal change in saturation state for reef waters may be on the order of only a few percent. Measurements for longer time series must be able to capture considerable variation in concentration, but have minimal drift. In contrast, process studies directed at measuring *in situ* metabolism (upstream/downstream; eddy covariance in the benthic boundary layer [BBL]) must be able to detect relatively small (1-10s $\mu\text{mol kg}^{-1}$) differences in concentration over a short time scale. Target uncertainties must be suitable to needs of the study and should be thoroughly vetted using CRMs and intercomparisons with reference methods.

Plenary presentations and discussions further focused on the current OA technologies that are suitable for *in situ* and/or autonomous operation.

The pCO₂ instruments are the most mature group in terms of technical readiness (level 4-5). There are at least four commercially available autonomous pCO₂ instruments, but only two are currently configured to go subsurface. The buoy-based MAPCO₂ and CARIOCA instruments have a proven track record for long term deployments. The subsurface-capable pCO₂ instruments such as SAMI-CO₂ and Pro-Oceanus are less proven in terms of reliability and have biofouling issues that need to be resolved. Several intercomparison evaluations of *in situ* pCO₂ instruments have been conducted.

Autonomous pH instruments are progressing in technical readiness (level 3-4). The commercial available SAMI-pH and research prototype SEAS II both use the well-established, calibration-free spectrophotometric technique. Both have been successfully deployed for short durations, but longer term reliability is still hampered by biofouling. A second ISFET electrode-based pH technique shows great promise due to its low power requirements and rapid response times, but calibration issues have not been resolved.

Two techniques for *in situ* autonomous DIC measurements are at the research prototype stage (level 2-3). Both rely on acidifying the seawater and allowing the evolved CO₂ gas to equilibrate across a permeable membrane into an indicator solution. The instruments demonstrate that autonomous DIC is feasible with current technology, but further work is required to increase availability, decrease complexity and reduce costs.

Measurement of T-Alk is typically done by titrimetric techniques that are difficult to adapt to autonomous instruments, but some early efforts (Martz et al. 2006; Watanabe et al. 2004) indicate that it is possible. The tracer monitored titration technique of Martz et al. (2006) is compelling due to the

elimination of the need for precise sample volume calibration and control. However, there has been no further work to improve the techniques and the readiness level remains at 1-2.

Related discussions are included in Appendix C, and resulting recommendations follow below.

OBSERVATIONS NEEDED FOR CORAL METABOLISM AND CALCIFICATION

(Participants: D. Allemand, A. Cohen, S. Edge, P. Furla, J. Ries, L. Jewet, D. Manzello, P. Medina-Rosas, A. Szmant, C. Woodley):

Many but not all marine calcifiers tested exhibit reduced calcification with increased OA (Fabry et al., 2008). As noted by Orr et al. (2008), however, “marine calcifiers differ because they have different mechanisms that control their internal microenvironment where calcification takes place.” Also, different life stages of marine calcifiers respond differently. These differences need to be taken into account when designing experiments to evaluate likely future changes in calcification rates due to OA. Several studies have shown reduced calcification in corals due to OA, however, results are not always consistent and vary due to many physiological and environmental factors (Atkinson and Cuet, 2008; Jury et al., 2009; Ries et al. 2009). At a seawater aragonite saturation state (Ω_{Arag}) of about 4, close to the mean for Caribbean reefs, corals and coral reefs produce calcium carbonate about 1000 times faster than it can be produced abiogenically. Understanding how corals and other marine calcifiers do this is key to understanding how, why and when OA impacts calcification.

The major physiological issue tackled by the group was the question of how ions reach the sites of calcification within the coral, and how much control the animal exerts over the rates and pathways of transport. The aragonite saturation state hypothesis (ASH) generally invoked as the mechanisms for why/how OA affects coral calcification is based on the premise that the reductions in $[\text{CO}_3^{2-}]$ and Ω_{Arag} that result from CO_2 dissolution in seawater make it more difficult for the corals to take up CO_3^{2-} from seawater, thus reducing calcification rates. Several lines of evidence suggest that corals use either metabolic CO_2 or HCO_3^- for calcification, thus casting doubt that a reduction in CO_3^{2-} should affect coral calcification rates. Alternatively, the decrease in seawater pH that results from CO_2 dissolution could directly affect calcification because of the need by the coral to excrete protons (H^+) during the uptake of Ca^{+2} as well as from the conversion of HCO_3^- to CO_3^{2-} at some step during CaCO_3 precipitation.

Cohen and Ries proposed, based on the results of recent laboratory studies in which experimentally precipitated abiotic aragonite crystals had similar characteristics to those secreted by corals (Holcomb et al 2009), that coral calcification is driven by the Ω_{Arag} , and that the experimental results from this study could be used to estimate the Ω_{Arag} within coral calcifying fluids. They also believed that there is a thin, semi-continuous layer of fluid between the calicoderm and the coral skeleton, and that there must be continuous “flow” of seawater from the exterior of the coral to the calcifying fluids of the coral.

Allemand and Furla proposed, based on ultra-structural and cellular work from the Monaco research group, that calcification occurs in small discrete pockets rather than in continuous sheets disputing the concept of a semi-continuous calcifying fluid, and that the calicodermis is structurally differentiated between areas active in calcification vs adjacent sites. Furthermore, their work shows that ion transport into and out of sites of calcification is highly biologically regulated by various ion transport channels and pumps. Earlier work by Furla suggests that up to 70 % of the C in skeletal CaCO_3 is

derived from metabolic CO_2 (Furla et al 2000) and thus calcification rates should not depend on seawater $[\text{CO}_3^{2-}]$. The most recent physiological work is summarized in Allemand et al (2010).

The group recognized that coral calcification response to OA has been found to be influenced by feeding, temperature and nutrients (e.g. McNeil et al 2004; Langdon and Atkinson, 2005; Holcomb et al., 2010; Manzello 2010; Ries et al., 2010). The role of zooxanthellae photosynthesis in both coral calcification in general, as well as how symbiont metabolism could ameliorate the effects of OA were also discussed.

Figure 1 and accompanying tables were developed to provide a simplistic overview of our current state of understanding of interactions between coral polyps and the seawater surrounding it with regard to calcification, and a ‘straw man’ for where research efforts were needed. Figure 1 is a simple, stylized representation of a coral polyp soft tissues, skeleton and the three fluid compartments of interest: the external seawater surrounding the coral, the internal gastrovascular cavity (GVC), and the hypothesized and debated extracellular calcifying medium (ECM) from which CaCO_3 is precipitated. Also represented (letters and arrows) are the potential pathways by which Ca^{+2} and seawater HCO_3^- and CO_3^{2-} can be taken up from the outside seawater (through the mouth (a) and across tissue layers (b), and exchanged among the various tissues and internal compartments (c, d, e). Within the tissues and compartments, carbonate chemistry, pH, and other chemical characteristics are constantly changing depending on diel cycles in metabolism and physiology (e.g. photosynthesis dominant during the day, respiration at night; level of feeding and digestion; rates of water exchange between the surrounding seawater and the GVC depending on pumping of water into/out of the polyp mouth). Best estimates for the concentrations in each of the 3 compartments are summarized in Table 1. In order to understand the effects of OA on coral calcification we will need a better understanding of the actual concentrations of parameters of interest within the three compartments, the fluxes across the various pathways, and the mechanisms that determine these concentrations and fluxes. This will require new approaches for micro-scale sampling of all these compartments, and for determining the extent and structure of the ECM. If we were able to parameterize all of the letters and arrows in this diagram, and how they respond to changes in light, temperature and nutrients, nutritional state, and external seawater chemistry, we would be able to predict responses of corals to OA.

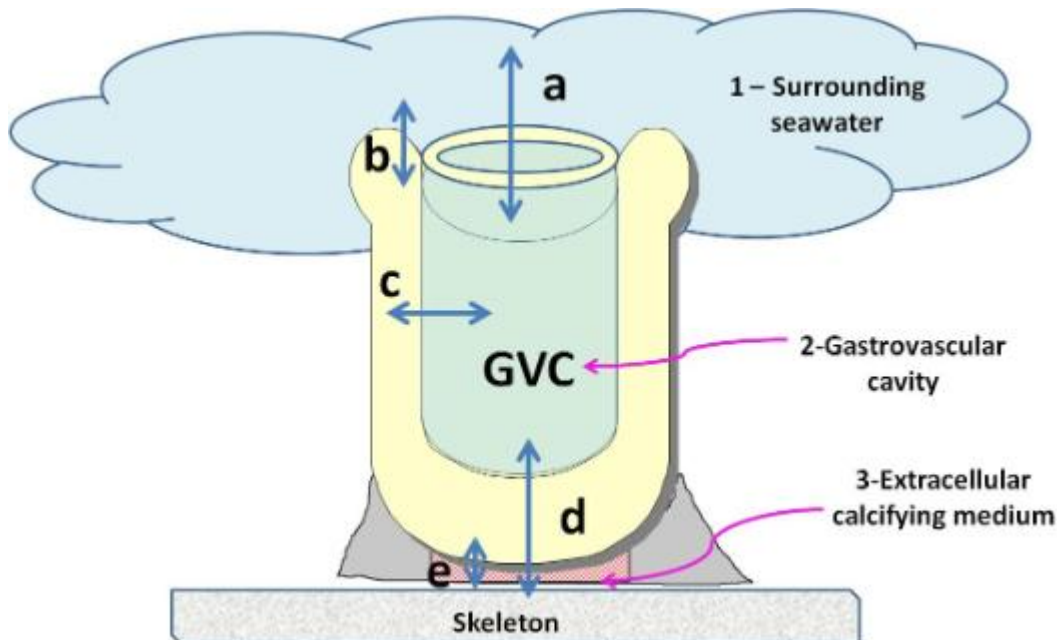


Figure 1. Coral anatomy and pathways that control coral calcification and physiology: a) Physical exchange between seawater & GVC (gastrovascular cavity); b) Active, passive or paracellular uptake and flux across tissues; c) Exchange between GVC & surrounding gastrodermal tissues; d) Exchange between GVC & calcifying media; e) Active/passive transport between GVC to ECM by calciblastic layers (Ca^{2+} -ATPase; Ca^{2+} channels; proton pumps).

Table 1. Estimates of various parameters relevant to the calcification (See figure 1 for compartments).

Compartment	pH	O ₂	DIC	Ca ⁺⁺	Ω _{Arag}	Notes
Reef Seawater (SW)	7.5-8.4	200 – 500 uM			4*	
GVC	7 – 9 (diel variation)		1.2 – 2.5 mM (diel variation)	9.5 – 10.2 mM		
3 Extracellular calcifying medium (ECM)	8.1-9.2			10.6-11.1 mM	14- 30	Concentration unknown; elemental ratios ~ seawater

*- estimate for Caribbean region based on NOAA Coral Reef Watch data (http://coralreefwatch.noaa.gov/satellite/oa/saturationState_GCR.html)

Given the variables affecting coral calcification and detection of relevant chemistry, several questions need to be addressed in order to understand how corals will respond to OA:

- What/where/how is the connection between conditions in the seawater environment and conditions in the coral's calcifying environment?
- How leaky is the tissue with regard to the ions of interest, and how much direct communication occurs between the calcification environment and the outside? If leaky, does leakiness change with changing seawater pH and or saturation state?
- How well can corals modulate conditions within the calcifying environment when seawater conditions change – is there a threshold?
- Is coral nutritional status a key component of the coral response and do we need to monitor nutrients on reefs and fat content of corals as much as we need to monitor temperature and saturation state?

Related discussions are included in Appendix D, and resulting recommendations follow below.

OVERALL WORKSHOP RECOMMENDATIONS:

Recommendations are presented in the following categories:

- CO₂-system measurements;
- Instrument innovations;
- Ecosystem approach;
- Standards;
- Outreach.

CO₂-SYSTEM MEASUREMENTS:

Recommendation: Understanding CO₂-system over coral reefs minimally requires measurement of at least two (preferably three) of four of the following parameters at the following target uncertainties: pH - 0.001; DIC - < 5 μmol kg⁻¹; T-Alk - 5 μequiv kg⁻¹; pCO₂ - 2 μatm; Carbonate ion - unspecified.

Discussion:

CO₂-system sensors need to operate down to at least 100m, with preferred measurement rate of seconds, but there will be trade-offs. Figure of merit of sample frequency x sample/joules must be high. Sensor requirements include: Precision/accuracy, response times, power (joules/data moment), and length of deployment.

pH and pCO₂ sensors are pressure sensitive, so, data will need to be corrected, but these sensors are closest to readiness level 5. DIC and T-Alk are not pressure dependent, but are at lower readiness levels. DIC is closer to being the next readiness level 4 sensor, but T-Alk is most useful based on fitness for purpose for coral ecosystems because Δ T-Alk is the most direct proxy for calcification. DIC and pH are robust pairing for calculation of other parameters. NOAA should consider funding development of higher risk T-Alk and lower risk DIC sensors.

Why not measure DIC versus T-Alk? It is more difficult to infer calcification rates from changes in DIC. Change in carbonate ion concentration is proxy for calcification, but does not have the property of pseudo-conservative measure like T-Alk. Thus, both DIC and carbonate ion measurements must be coupled with an additional parameter for use in estimating calcification. In addition, carbonate ion measurement is at low readiness level, high risk stage. pH/pCO₂ is the parameter pair at the highest readiness levels, but show strong co-variance and their use for assessing T-Alk is MOST sensitive to errors. For any calculation of T-Alk from other parameter pairs, measurements must be synchronized to measure the exact same water; random error needs to be reduced by filtering.

Recommendation: In order to interpret the carbonate chemistry on reef ecosystems, CO₂-system measurements need to be accompanied by other synoptic measurements including: Standard—temperature, salinity, oxygen, light/PAR; Nutrients- nitrate, phosphate, ammonia.

Recommendation: RFP should provide specific requirements for target sensors. Example requirements include: autonomous for > 1 month; Cost (within budget); IEEE standards for integration of sensors; response (measurement rate) time enables precisely located (± 0.1 m) measurements, appropriate for sampling platform and parameter; on-board data logging that stores and time synchs all input; Transition plan that spells out NOAA partners, readiness level and how project will move tech to level 5; Benthic Boundary Layer (BBL) package must sample synoptically from seafloor to at least 1 m above bottom.

INSTRUMENT INNOVATIONS:

Recommendation: NOAA OA should consider two topics for its initial RFP: 1) incremental innovations and transition to NOAA programs, and 2) new ideas (transformative innovations). More detailed suggestions follow.

Recommendation: To assist with site characterizations for NOAA's OA network, and serve the broader science community need for assessment of coral reef health, an incremental innovation that can be largely built based on existing capabilities is a small (diver-carried), self-contained, user-friendly sensor package for use down to 100 m depth, which can be left on a reef for up to one month and includes sensors for T, P, S, dissolved oxygen, nutrients, light (PAR), time-lapse camera, and the required CO₂-system observations. Sampling rate must be high enough to enable geo-referenced observations during swimming transects (1 knot). All observations need to be recorded and time-synched and stored in the package, or capable of being cabled for real-time transmission to shore.

Discussion:

NOAA may need to develop separate systems for mobile platform and stationary node; fixed instruments may have different figures of merit. Mobile packages may be more difficult, however,

NOAA cannot achieve OA Network goals without making synchronized measurements on same water as ecological observations, thus, need for mobile package early. Other requirements include: simultaneous data logging from multiple sensors in the integrated package; cost must be reasonable (affordable based on RFP budget and duplication of package); meet IEEE standards for data management and sensor integration. Other package capabilities may include imaging and paired sampling ports to quantify differences between <10 cm from benthos and <1m from bottom in order to estimate flux across the BBL. Proposals must include a validation program that demonstrates the utility, reliability, durability, and accuracy of the entire instrument package.

Recommendation: NOAA OA funding should support phased development of the proposed package. This should begin with instrument development that improves the size, power needs and reliability of individual CO₂-system parameter sensors. Phase two is integration of instruments into an *in situ* package. Phase three is test, evaluation, validation, and application of the sensor suite in both fixed and mobile configurations. More development may be required (fourth phase) to support longer-term (year) deployments.

Discussion:

NOAA OA initial focus should be on instrument package development versus experimental design; RFP should state that package will be broadly used in standardized scientific investigations and monitoring with global/ international applications. System architecture and modularity should facilitate maintenance, new additions, and ongoing improvements to the integrated measurement system. OANet should also start with at least one pilot study site to provide inter-comparison of technologies, validation of new technology; partner with established real-time observing system if possible-- technology development should also serve IOOS needs.

Recommendation: Any proposal funded by the NOAA OA program should require integration of NOAA stakeholder/customers during all phases of development, and a technology transition plan agreed to from the outset by developers and customers, with details of the schedule and deliverables.

Recommendation: If funding allows, NOAA OA funding should also consider investing in transformative innovations that are now at very early Readiness Levels, for example, micro-technology solutions for CO₂-system parameters, or the next generation of total alkalinity sensor.

ECOSYSTEM APPROACH:

Recommendation: NOAA's proposed OA Network should base its deployment on knowledge of the health of the ecosystem, assessed by water quality (including OA parameters) and biological observations.

Recommendation: OANet must implement a validation program that includes: work in known areas; synoptic physical, chemical and biological measurements; inter-comparisons with proven techniques; mesocosm/tank test facility for pre-field studies; healthy "control" versus impacted sites; and use of accepted standards (more below).

Recommendation: OANet should be deployed based on a strategy that includes: baseline (preferably long-term) characterization of reefs that assesses ecosystem health; first need mobile package for baseline characterizations; once candidate reefs are characterized and hypotheses formed, then

establish network with moorings; final goal is ability to detect net dissolution across a reef over time (minimum of 10 years of observations).

Recommendation: Coral calcification processes are affected by processes at multiple time and spatial scales, including reef-wide, across the benthic boundary layer BBL (< 1 m above bottom), to water chemistry and processes inside corals.

Discussion:

Broader NOAA objective is ecosystem approach; plans to develop OA observing network (OANet) that functions at reef to ecosystem scales; needs to enable monitoring of real-time coral metabolic performance as an indicator of health, including growth, respiration, and reproduction
CRCP does not develop advanced technologies, but OER (CIOERT) does. OANet target sites also need to consider net productivity and community dynamics. CO₂-system measurements must be in context of research needs and conceptual calcification model developed by coral physiologists and ecologists.

STANDARDS:

Recommendation: Any competitive proposal must utilize appropriate standards and include a plan for central data and metadata archiving at a publically available repository.

Discussion:

A critical issue is availability of appropriate standards and reference materials for CO₂-system measurements, e.g., Dickson Lab standards and as presented in Best Practices for Ocean CO₂ Measurements (http://cdiac.ornl.gov/oceans/Handbook_2007.html); need to be more systematic in these areas, for example, regarding CRMs, buffers, sterilized seawater, and synthetic seawater for pH; need to consider for measurements, related constants, and derivative products. OANet needs to meet and exceed national standards including providing access to data sets and use of FGDC-compliant metadata; work with IOOS; highlight and promote use of European Ocean Carbon EPOCA Web site for best practices.

OUTREACH:

Recommendation: NOAA OA program must find mechanisms to promote sharing of data, knowledge, experience and resources. When possible build on and promote existing efforts, such as PMEL's OA Web pages, CoRIS, NODC and NCDDC.

Recommendation: NOAA OA program should include broader impacts activities including education activities to train the future workforce and promote public literacy. Example activities include:

- Train experts: need to enhance college preparation for OA science. Centers of Excellence should be created to serve this role, as well as promote science community communications and training.
- OA literacy: ocean acidification is good topic for teaching; need to separate fact from speculation and sensationalism; create NOAA OA Web site-- use workshop presentations and related publications to tell Web-served stories, rely on links, e.g., PMEL site (<http://www.pmel.noaa.gov/co2/OA/>) and dedicated Oceanography issue on OA (e.g., http://www.tos.org/oceanography/issues/issue_archive/22_4.html).

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Appendix A: Participants

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Appendix B: Agenda

March 8 -- Introductions and Plenary Presentations

12:00 – 1:00 pm Lunch in SRI conference room.

1:00 – 2:00 pm:	Alina Szmant, UNCW CIOERT- Welcome, Introductions, and Comments Workshop logistics, conflicts of interest, intellectual property
2:00 -2:30 pm:	Felipe Arzayus, NOAA OER- New funding opportunities Richard Feely, NOAA PMEL Overview of NOAA Integrated Ocean Acidification Research Implementation Plan
2:30 – 3:15 pm:	Denis Allemand, Background/review of calcification mechanisms and responses related to OA
3:15 – 3:30 pm	Coffee break
3:30 – 4:15 pm	Andrew Dickson, UCSD – Measurement requirements for carbonate chemistry parameters for different ecosystems and applications
4:15 – 4:45 pm	Rob Whitehead, UNCW – Current efforts in OA measurements
4:45 – 5:30 pm	Group discussion to outline needs for coral reef and coastal zone processes related to OA (parameters, accuracy, precision, time scales for measurements and deployments)
6:00 – 8:00 pm	Welcoming Reception at SRI

Day 2

Technology Status and Physiological Model (two concurrent sessions)

8:15 – 9:00 am	Continental breakfast at SRI Physiological Group: Work on physiological model as a breakout group Instrumentation Group: 30 minute presentations and 15 minute discussion
9:00 – 10:00 am	Chris Sabine, NOAA PMEL, MAPCO2 case study of successful OA technology transition and other pCO2 instruments
10:00 – 10:15 am	Coffee break
10:15 – 11:00 am	Mike DeGrandpre, U. Montana, Spectrophotometric pH systems
11:00 – 11:45 am	Ken Johnson, MBARI, ISFET pH systems
11:45 – 12:30 pm	Lunch at SRI
12:30 – 2:00 pm	Tour of SRI
2:00 – 2:45 pm	Todd Martz, UCSD, Flow through total alkalinity
2:45 – 3:30 pm	Bob Byrne, UF, Flow through DIC
3:30 – 3:45 pm	Coffee break
3:45 – 4:30 pm	Tim Short, SRI, Underwater mass spec.
4:30 – 5:30 pm	Group discussion on additional new technologies and integrating instruments into a package. Update on progress on physiological model

Day 3

Break out groups, Roll Up Your Sleeves

8:15 – 9:00 am	Continental breakfast at SRI
9:00 – 10:45 am	Break-out groups to discuss Technology Readiness Levels for each of the 4 carbonate chemistry parameters and how to close the gaps in capabilities. Physiology group reconvene.
10:45 – 11:00 am	Coffee break
11:00 – 12:30 pm	Continue break-out groups, re-mix if necessary
12:30 – 1:30	Lunch at SRI
1:30 – 3:00 pm	Reports from break-out groups
3:00 – 3:15 pm	Coffee Break
3:15 – 5:30 pm	General Discussion <ul style="list-style-type: none">• Physiology group report to entire group• Brainstorming the roadmap to development of an integrated OA instrument suite• If we make it, will they come? Who are the potential users and how do we train them to use and maintain the instruments?

Day 4

Farewells, core group outline workshop report

8:00 – 8:45 am	Continental breakfast at SRI
9:00 – 11:00 am	Wrap up discussion and preparation of draft report; some participants leaving early.

Appendix C: Instrumentation Group Notes

Disclaimer: These notes are the result of one recorder and subsequent post-meeting edits by participants, and not meant to be recorded minutes.

Day 1 Plenaries

Day 1 Group Discussion

Day 2 Plenaries

Day 2 Group Discussion

Day 3 Group Discussion

Day 4 Wrap-up, Group Discussion: Report Recommendations

Day 1 (3/8) Plenary Presentations: Overview of NOAA OA Needs and Key Issues

NOTE- pdfs of a few of the ppts released by presenters will be posted at cioert.org/oa-workshop.

Alina Szmant, UNCW CIOERT- Welcome, introductions, and Comments Workshop logistics, conflicts of interest, intellectual property

Q/A/Comments:

Why is physiology group separate? Need to hear what they have to say and then have close discussion?
There are too many unknowns regarding coral physiology/calcification processes and the invited participants offer a rare opportunity to identify these unknowns.

Felipe Arzayus, NOAA OER- New funding opportunities (NO PPT)

Q/A/Comments:

How will this workshop help NOAA? Results will be considered in development of NOAA OA implementation plan
What will NOAA OA project office do? See Feely presentation next.

Richard Feely, NOAA PMEL-- Overview of NOAA Integrated Ocean Acidification Research Implementation Plan (SEE PPT)

Q/A/Comments:

- Plan development should use international input (e.g., see <http://www.epoca-project.eu/>)
- Currently, CREWS buoys do not have OA sensors; need to get 2-3 components of C-system on existing buoys
- Also need to identify micro-sensors for use around and inside corals
- Are we seeing impacts on corals? Debatable. Temperature has certainly compromised Caribbean corals. In Pacific, secondary calcification has been compromised by OA. Galapagos has always had low cement formation.
- Reef community response is what is more important—how are ecological services compromised?
- OA changes and dissolution processes happen naturally; CO₂ varies on diel cycle.
- Critical issue is availability of appropriate standards and reference materials, such as Dickson Lab standards; this is critical gap; need to be more systematic in measuring parameters; CRMs, buffers, sterilized seawater, synthetic SW for pH

Denis Allemand, Background/review of calcification mechanisms and responses related to OA (SEE PPT)

Andrew Dickson, UCSD – Measurement requirements for carbonate chemistry parameters for different ecosystems and applications (SEE PPT)

Q/A/Comments:

- See Best Practices for Ocean CO₂ Measurements document (http://cdiac.ornl.gov/oceans/Handbook_2007.html)
- Requirements-- What do I need to know? How well do I need to know it? What resources do I need?
- Carbonate chemistry parameters including measurements, related constants, and derivative products
- How do they change by ecosystem? Are parameters different?
- Do parameters differ with respect to the level of uncertainty tolerated?
- Resources needed will vary—some are more challenging and expensive.
- Precision versus accuracy- repeatability (variance/SD) versus is the number right?
- Alk, pH, pCO₂—Dickson favors Alk, pH and TC
- DOC buffer against OA in estuaries; very little work in these areas

Rob Whitehead, UNCW – Current efforts in OA measurements (SEE PPT)

Q/A/Comments:

Major issue is determining what is a bad measurement?

Day 1 Group discussion: outline needs for coral reef and coastal zone processes related to OA (parameters, accuracy, precision, time scales for measurements and deployments)

- In situ instruments that deal with fluxes, coupled with physics; forest canopy approach
- Need to add calcification—calcium measurements?
- OA labs needed to handle samples; can be expensive operations; establish centers of excellence
- Cost per sample is high now (\$1000/sample)
- Instruments to measure processes versus concentrations?
- Accuracy and precision are linked—both can get relaxed.
- 0.002 pH units is accuracy we seek for CO₂ cycle.
- SCALE ISSUES:
 - o We want to work less than meter from bottom, and measure inside reefs (microhabitats, crevices, holes).
 - o Requirements change over space/time scales, from km to mm and seconds to seasons. One instrument package may not serve all scales and apps.
 - o NOAA needs to focus on applied research to serve ecosystem management. We struggle with this level of research, e.g., community calcification rates. Managers want to model human impacts. Is CO₂ going to impact ecosystem?
 - o Need both scales (ecosystem to extracellular) and figure out how they relate.
 - o Some regional larger scale questions can be answered now.
 - o Seasonal change, daily change at microscopic scale, large scale, e.g., aragonite saturation state
 - o To assess organismal responses need suite of high resolution probes/sensors on both organismal and tissue level?
- SBIR program should be considered for technology developments. Need to get topic into announcements.

Day 2 (3/9) Plenary Presentations: OA Technology Status

Chris Sabine, NOAA PMEL, MAPCO2 case study of successful OA technology transition and other pCO₂ instruments (SEE PPT)

Q/A/Comments:

- Licor820 sensor and equilibrator (see <http://www.licor.com/env/Products/GasAnalyzers/li820/820.jsp>) now in use on Battelle autonomous buoy pCO₂ systems
- Tech transfer (MBARI to PMEL and from PMEL to Batelle), lessons learned:
 - o Need stable, reliable, well-documented system;
 - o Need commercial partner with capital; cannot get paid for technology, but can for time/effort spent; commercial partner must make profit!! They need to sell 1200 systems @ \$39,000 per system.
 - o lots of communication and continued verification;
 - o anything manufactured by hand had to be re-engineered for factory production;
 - o Special Studies Agreement (FOI Act);
 - o Moving from proven prototype to commercial production took years.
 - o Alliance for Coastal Technology (ACT) demos were useful.
- Now working on DIC sensor
- How good is good enough (precision)? Would like 2ppm but seeing variability of 5ppm.
- How was intercomparison in Japan done? IOPCC and Japanese Carbon program.
- Sabine is working with Liquid Robotics to create Wave glider PCO₂ sensor.

Mike DeGrandpre, U. Montana, Spectrophotometric pH systems (SEE PPT)

Q/A/Comments:

- Builds on USF Byrnes Lab spectrophotometric technology
- SAMI-CO₂: measurement takes about 6 sec. (mins. to equilibrate); commercial end is Sunburst Sensors (<http://www.sunburstsensors.com/>)

Ken Johnson, MBARI, ISFET pH systems (SEE PPT)

Q/A/Comments:

- Ion sensitive field effect transistor tech (ISFET) (look at Bergveld, 2003); challenge is to operate electrical device underwater/pressure; durafet limited to 70 m; Satlantic looking to produce; want to put on Argos floats (2000 m)
- Precision 0.001 pH units
- biofouling challenge, causing longer term drift

Larry Langebrake, SRI, Maritime Domain Awareness (NO PPT)

Q/A/Comments:

- system approach designed for homeland security applications; service-oriented architecture; SIMON- Smart Integration Manager Ontologically Networked; looking for environmental applications
- services include data archive/recall
- legacy data can be incorporated, if it fits data model

Todd Martz, UCSD, Flow through total alkalinity (SEE PPT)

Q/A/Comments:

- bottom line—will there ever be a moored Alk sensor? On edge of spectrophotometric detection/precision; many felt development has been hampered by lack of investment; may be more wanted by coral reef scientists as more direct measurement of calcification conditions

Bob Byrne, UF, Flow through DIC (SEE PPT)

Q/A/Comments:

- DIC with pH or pCO₂ provides accurate account of marine co₂-sys
- high freq mmts useful for variable environments,
- commercialization required to reduce cost/inc. availability
- SEAS precision 0.001, accuracy depends on pK, perhaps .003 to .005
- For use of SEAS on moorings long-term, biofouling is big challenge.
- Will carbonate ion mmt be integrated with MICA? First will be in situ; need refinements in molar and equilibrium constants.

Tim Short, SRI, Underwater mass spec (SEE PPT).

Q/A/Comments:

- Need more standards!
- Mass spec miniaturization needed to reduce power needs.

Day 2 Group discussion: new technologies and integrating instruments into *in situ* package

OOI TRL*	DESCRIPTION
1. Proof of Concept or developmental	Lowest level, speculative (none on OOI)
2. Research Prototype	Basic components integrated, prototype sensors used to collect data
3. Research Proven	Not commercialized, but clearly beyond prototype, collected data
4. Commercial	Proven to work in environment as expected, commercial production
5. Operational	In final form, proven to work under sustained operational conditions

*after DOD

Technology Readiness Levels adopted from OOI. From Whitehead ppt.

Following are notes transcribed from E-sheets recorded (written) during facilitated discussions:

1. pH Sensors
 - a. SEAS pH
 - i. ± 0.002 units
 - ii. TRL 2
 - iii. Good precision
 - iv. Not commercially ready
 - v. Cost N.A.
 - vi. High power requirements/ cable
 - b. SAMI pH

- i. ± 0.002 units
 - ii. TRL 3
 - iii. Can be deployed for 7 months with samples collected every 3 hours
 - iv. \$17.5K per unit
 - v. Available now
 - vi. Needs some “simplification steps” to get to more rapid sampling/ longer-term deployment
 - c. ISFET pH
 - i. \$4K \pm 50% (K.J.)
 - ii. TRL 4, 3 for Deep ISFET
 - 1. Martz Lab builds now for around \$5K
 - a. Martz prototype combines with Aanderaa oxygen optode, and conductivity (in development) for around \$10K
 - 2. all ISFET prices are for uncalibrated instrument
 - iii. Deep ISFET in development with Honeywell
 - iv. Can integrate with LOBO system
 - 1. \$70 K in benthic or float configuration
2. CO₂
 - a. MAPCO2
 - i. \$40K
 - ii. TRL 5
 - 1. but large and must be on surface buoy
 - iii. $\pm 2-5$ umol
 - iv. Satellite telecom
 - 1. SAMI pH compatible with MAPCO2 coms
 - v. System is “fixed” and may not work for small mobile package; Sabine- “not really,” just issue of reducing scale
 - b. SAMI pCO₂
 - i. \$18 K
 - ii. TRL 5
 - iii. ± 5 umol
 - c. MIMS
 - i. TRL 3
 - ii. $\pm 2-5$ umol
 - d. CONTROS
 - i. TRL 4
 - ii. Precision ?
 - e. Proceanus
 - i. TRL 4
 - ii. Precision ?
 - f. Carioca
 - i. TRL 5
 - ii. Large buoy
 - iii. $\pm 2-5$ umol
 - g. Potential of using pH + pCO₂ to calculate Total Alk
 - i. Near 20 μ mol precision, can be better with data filtering
 - ii. Approach is sensitive to errors and flux
3. DIC
 - a. SEAS DIC
 - i. Cost unknown
 - ii. TRL 1
 - iii. ± 5 umol
 - iv. Nearly ready

- b. Ammonia sensor in development with ± 10 μmol precision
 - i. This precision is good considering diel range of DIC
 - c. DIC approximately 2000 ± 10 μmol
 - i. But there are gradients and we may need the lower range due to prevalence of low coral cover reefs
 - d. Can improve precision of DIC by increasing equilibration time
 - i. Trade off with less frequent measures
 - e. Should be considering joules per data point
 - f. Feedback loop between technology/ equipment driving research and research driving technology development
4. Total Alk
 - a. SAMI Alk
 - i. TRL 1
 - b. Minimal work on this front in general
 5. DO
 - a. Optodes are good
 - i. TRL 5
 - ii. $\pm 1-2$ μmol
 6. Nutrients
 - a. NO_x
 - i. TRL 5
 - ii. $\pm 1-10$ μmol
 - b. PO_x
 - i. TRL 3-4
 7. General comments
 - a. Any integrated suite should be modular to allow switching to the newest and best sensors
 - b. Should we include technology transition?
 - i. maybe not for this RFP
 - ii. But definitely intercomparison studies
 - c. In prioritizing any testbed site, we should know that system very well and the data from that system
 - i. including the physics (flux models) at fine scales
 1. measurement capable now at mm scales
 - d. power issues will need to be addressed, batteries? Boats?
 - i. Repeatability at a site is useful not only for data, but also for logistics
 - e. want FAST measures of net performance
 - f. just pick an “ok reef” but make sure it is well characterized
 - i. may be relevant and useful in variable habitat types
 - g. we are dealing with a “sensors now” demand
 - h. imaging technology including 3D reconstruction at cm scales is now available, currently implemented on gliders, etc.
 8. General recommendations
 - a. What to measure?
 - i. Temp, salinity, oxygen, light, carbonate system parameters (pH, DIC, pCO_2 , Total alkalinity), nutrients, calcium
 - b. Balance between high risk versus transitional innovation
 - c. Initial goal of autonomous for 1 month
 - d. Quick response time
 - e. Simultaneous data logging from multiple sensors in the integrated package
 - f. Cost must be reasonable
 - g. May including imaging
 - h. Paired sampling ports to quantify differences between <10 cm from benthos and $<1\text{m}$ from bottom need to characterize flux

- i. IEEE standards for data management and sensor integration
- j. Eventually transition to commercially available, goal TRL of 4-5

Day 3 (3/10) Plenaries:

Alina Szmant, progress report from coral physiology/calcification group (SEE PPT)

- Corals can control where and when they calcify; live coral skeletons not in contact with water; calcium needs to get through cells to skeleton without killing cells; all bicarbonate once inside coral; calcification is light-mediated.
- Algae provide energy, remove CO₂, waste, phosphate, and add organic matrix component
- Recommendation—in order to be successful, NOAA OA program must find mechanisms to promote sharing of knowledge, experience and resources.
- Relative merits of understanding integrated reef system; need to balance intracellular versus water chemistry; need further discussion with physiologists.

QA/Comments:

- o What is evidence for GVC being important to calcification, versus open water exchange? How is SW or constituents getting into calcification channel?

Dwight Gledhill, Recap of NOAA Priority Needs—an OA Observing Network (OANet) [NO PPT]

- NOAA is interested in development of a portable package for assessing coral health and OA conditions; including oxygen, alkalinity, and other C-sys parameters; even if Alk is primary, need to know other parameters to confirm changes in Alk are due to calcification
- Need to know net productivity
- Community dynamics versus strictly OA
- Need to put OA measurements in context of research needs and conceptual calcification model developed by coral physiologists and ecologists
- Broader NOAA objective is ecosystem approach; plans to develop OA observing network (OANet) that functions at reef to ecosystem scales; needs to enable monitoring of real-time coral metabolic performance as an indicator of health, including growth, respiration, and reproduction
- CRCP does not develop advanced technologies, but OER (CIOERT) does
- OANet should not be deployed until it knows exactly where to set up; step one is baseline characterization of reefs that must assess health; once candidate reefs identified and hypotheses formed, establish network with moorings; so, first need mobile package for baseline characterizations; final goal is ability to detect net dissolution across a reef over time (minimum of 10 years of observations)

Q/A/Comments:

- What would test-bed OANet look like?
- OAN nodes should not cost more than \$100k/y to operate
- Step-wise strategy to define size/scope; first few years need to tell us where and what OANet will be (footprint)
- We have interim tools and measurements, but don't always know what info tells us
- We have buoy-based sensors, but still need rapid assessment at many reefs; we need more info at variety of sites within and between reef ecosystems
- Should focus on at least one pilot study site to provide inter-comparison of technologies, validation of new technology, partner with established real-time observing system if possible; technology development should also serve IOOS needs
- can now do Alk, but is it operational for proposed applications?

Wade McGillis: BBL measurements related to coral productivity and calcification (SEE PPT):

Q/A/Comments:

- collaborations critical
- Automated samplers would be helpful approach
- Footprint based on expected currents and reef patch; 4-10 m² suggested for fluxes that impact corals

Chris Martens—Aquarius Reef Based Studies using SEAS [NO PPT]:

- SEAS *in situ* spectrophotometric sensor capable of nanomolar detection limits through use of a long path length optical cell; modular and can be fitted to an AUV or ROV; enables collection of 3-D nutrient or carbon system parameters, e.g., relative to microhabitats.
- Combined resolution and sensitivity provided by SEAS “unparalleled in an *in situ* nutrient sensor”; capability allows measurement of low concentrations often found in reef domains.

Q/A/Comments:

- Measures pH, pCO₂, with MICA backup, DIC coming on-line soon
- Power required is ~200 w

ANN Cohen—Corals and OA [NO PPT]:

- Corals do have direct and rapid exchange with seawater, also crystals vary with aragonite saturation state; they can form crystals in waters that are very low; may supplement in their growth spaces.
- How do they elevate sat state?
- Phosphate—is it crystal poison? May be important role of zooxanthellae.
- Ambient water sat state similar to coral water; coral water chemistry affected by many processes, and has to be actively maintained (e.g., proton pumps).

Bob Byrne—C-sys instrument suite [SEE PPT]:

- Productivity and calcification assessment requires measuring range of parameters:
 - o Temp, Salinity, Oxygen, Light, water flow
 - o C-sys including DIC, Alk, pH, pCO₂,
 - o turbidity
 - o nitrates
 - o phosphates
- Other requirements:
 - o Development of portable measurement system
 - o series of sensors to assess community scale metabolism
 - o validation program
 - o Automated bag samplers for discrete sampling.

Day 3 Group Discussion: Technology Readiness Levels for each of 4 carbonate chemistry parameters and how to close the gaps in capabilities. Facilitated by R. Whitehead.

What should NOAA support right away in terms of technology innovation?

- pH—7 months, power consumption issue, SAMI similar to SEAS; improvements can be made, but get good measurements now; combination of pH and pCO₂ should be combined; SAMI is \$17k; SEAS not commercially available and requires SRI support; need to verify on use of SAMI to assess alk on reefs
- pCO₂—\$30-40k for MAP-POC2, working on reducing so it could be dragged around in float
- pH/pCO₂ for assessing Alk is MOST sensitive to errors; pH and pCO₂ must measure exactly same water; random error needs to be reduced by filtering
- DIC and pH are robust pairing; ammonia sensor for DIC
- Whole reef metabolism will require buoy array over reef habitats
- Sensor requirements include: Precision/accuracy, response times, power (joules/data moment), length of time/deployment

Priority Carbonate System Chemistry measurements include:

- Standard—T, S, oxygen, light/PAR
- C-Sys: pH, DIC, PCO₂, Alk, carbonate
- Nutrients- nitrate phosphate
- Calcium
- Any chemical sampling program should be accompanied by biological program, e.g., Imaging

Example Requirements:

- o High risk versus transition
- o Autonomous, > 1 month
- o Cost (within budget)
- o IEEE standards for integration of sensors
- o Response time appropriate for sampling platform and parameter
- o On-board data logging that store and time synchs all input
- o Transition plan that spells out readiness level and how project will move tech to level 5
- o BBL package must sample from seafloor to at least 1 m above bottom

Sensors, Readiness Levels and Specifications:

Sensor	TRL	Accur.	Power (joules/mmt)	Other mmts
pH- ISFET	4	0.006 units		
pH-SAMI	4	0.002		
DIC	1	5 um		
PCO ₂ - MAP	5	2-5 um		T
PCO ₂ - SAMI	5	5 um		
PCO ₂ -MIMS	3	2-5 um		Dissolved gasesd
PCO ₂ -Contros	4	?		
PCO ₂ -Pro-oceanus	4	?		
PCO ₂ - Carioca buoy	5	2-5 um		Wind speed, T
PCO ₂ - Vaisala	4	?		
Alk- SAMI	1	5 um		
O ₂ optodes	5	1-2 um		T
CO ₃	0			
CA	0	10 um		
Total Alk	0			
NO _x - ISIIS	5	10 um		
NO _x - SEAS	3	10 um		
PO ₄	3-4			

What type of validation experiments are needed?

- Plan one big experiment—learning something new; begin with McGillis approach and add new methods and technologies; can autonomous measurement be used to achieve spatial resolution desired; want to look at different places on reef
- NOAA draft OA Plan includes 40 sites of interest to community, with no prioritization; what makes a good site?; What are criteria for test-bed and elements of test bed experiment?
- We need agencies to collaborate on test-bed experiment; there is effort through ORRAP to get specific advice

- Need fast discrete observations of reef performance/health; then based on observations, decide on test-bed
- Need to understand ecosystem first; better to go to test bed vetted by group like this
- One reef will be inadequate; health scan is worthy but need to know what disease looks like
- We have urgent mandate to develop new instruments
- Both approaches required—1) develop basic package, and 2) set up relevant experiment.
- Must know physical processes at reef with chemical data.
- Need to move from discrete to autonomous.
- Biological imaging technologies should be part of big experiment.
- Experiment must have appropriate disciplines and observations
- Utilize historic study sites when possible (e.g., LTERs, Morea, Tahiti, NWHWI)
- Forereefs are major sites of calcification.
- RFP coming is strictly for instrumentation; what does it look like?
- Developing things fast takes more money.
- No single measurement adequate; need suite
- Start with package of stuff that work now; later add TRL levels that are closer to operational.
- What do we need to assess coral community metabolism? Do we have instruments? If not, what's needed?
- Need to be more explicit concerning relationship between chemistry and biological processes
- Technology developed with intent of application to broader system
- Measuring not just carbonate on reef, but reef processes
- In order to reduce uncertainty and concern that OA will impact coral ecosystems, need to determine what the reef ecosystems are currently experiencing
- Need to quantify carbonate processes at reef scale
- Need to clearly state that C-sys measures will not alone quantify overall coral reef health; rather, carbonate chemistry dynamics is one important component of assessing coral reef processes; clear understanding of what C-sys does mean for reef health falls outside the scope of this workshop, this development will take into account the ecological components as well.

Day 4 Wrap-up Plenary Discussion: Recommendations

Recommendation: Consider two topics for RFP: 1) new ideas (transformative innovation) and 2) incremental innovations and transition

DON'T USE TRLS TO CHOOSE PARAMETERS

Recommendation: Must implement validation program including: work in known areas; synoptic physical, chemical and biological measurements; inter-comparisons with proven techniques; mesocosm/tank facility; healthy “control” versus impacted sites; use and develop accepted standards

Recommendation: Coral calcification processes are closely tied to both benthic boundary layer BBL (< 1 m above bottom) water chemistry and processes in corals; OANet requires both types of measurements. BBL water chemistry is variable on scales of < minute and to decimeter, so sensors must be able to measure and respond at these scales.

Recommendation: Understanding C-Sys over coral reefs minimally requires the following parameters at the following precision/accuracy:

- pH- 0.001
- DIC- < 5 μ m
- Total Alk- 5 μ m
- pCO₂- 2 uatm
- Need 3 of 4!!
- Carbonate ion- not specified

Discussion:

- Sensors to 100m; pH and pCO₂ pressure sensitive; need to correct for this; closest to level 5
- DIC/Alk not P independent; earlier readiness level
- DIC is closer to being next available; Alk is most useful
- Based on fitness for purpose, delta total alk is most direct proxy for calcification.
- Fund high risk Alk and low risk DIC?
- Why not total carbon versus T-alk? Harder to know calcification inference from total carbon.
- Carbonate Alk is proxy of P and T. Does not have property of conservative measure.
- Carbonate ion? High risk development.
- We would like a measurement rate of seconds, but will be trade-offs.
- Only one pH sensor does not rely on discrete water samples.
- Figure of merit- sample freq x joules/sample must be high.
- May need to develop two systems—mobile platform and stationary node?; Fixed instrument may have different figures of merit.
- Mobile package is more difficult; begin with sensor development for fixed deployment
- Movable versus mobile.
- We cannot achieve OA Network goal without making synchronized measurements on same water.

Recommendation: NOAA's proposed OA Network should base its deployment on knowledge of the health of the ecosystem, assessed by water quality (including OA parameters) and biological observations.

Recommendation: To assist with site characterizations for NOAA's OA network, and serve the broader science community need for assessment of coral reef health, there is need for a small (diver-carried), self-contained, user-friendly sensor package for use down to 100 m depth, which can be left on a reef for up to one month and includes T, D, S, O₂, light (PAR), time-lapse camera, and the required C-sys observations. All observations need to be recorded and time-synched and stored in the package, or capable of being connected to cable for real-time transmission.

- Add nutrients to the package
- Central data archiving IEEE standard node will be REQUIRED

Recommendation: Any system proposed must include a validation program that demonstrates the utility, reliability, durability, and accuracy of the entire instrument package.

Recommendation: NOAA OA funding should support phased development of the proposed package, beginning with instrument development that improves the size, power needs and reliability of pH and other C-sys sensors. Phase two is validation program. Phase three should be integration with the complete proposed sensor suite, again followed by demonstration and validation.

Discussion:

- There are “more phases” leading up to a longer term deployable devices
- Initial focus of NOAA OA funding will be instrument package development versus experimental design
- Resulting package will be broadly used in standardized scientific investigation and monitoring across globe
- Modularity will facilitate developments and ongoing improvements in the integrated system
- Integrated software and data management:
 - o Seabird has protocols, IEEE sets standard for communications; Use non-proprietary IEEE comms standards
 - o Design instrumentation to meet standard protocols; controlling node will be required on the package; explicitly separate software design and integration
 - o Real-time telemetry ultimately desired
- Phase 1 – develop new sensors; P2 integration; P3 deploy/validate

Recommendation: Any proposal should include NOAA partners who are interested in transition of the instrument package into their NOAA operations and science programs.

Recommendation: If funding allows, NOAA OA funding should also consider investing in transformative innovations that are now at very early Readiness Levels, for example, microtechnology solutions for C-sys parameters, or next generation of total alkalinity.

Recommendation: NOAA OA program should include outreach program to improve public literacy. Example activities include:

- Train experts: need to enhance college preparation for OA science. Centers of Excellence should be created to serve this role, as well as promote science community communications and training.
- OA literacy: ocean acidification is good topic for teaching; need to separate fact from speculation and sensationalism; create NOAA OA Web site-- use workshop presentations and related publications to tell Web-served stories, rely on links, e.g., PMEL site (<http://www.pmel.noaa.gov/co2/OA/>) and dedicated Oceanography issue on OA (http://www.tos.org/oceanography/issues/issue_archive/22_4.html)
- Data Management: OANet needs to meet and exceed national standards including providing access to data sets and use of FGDC-compliant metadata; work with IOOS; highlight and promote use of European Ocean Carbon EPOCA Web site for best practices.

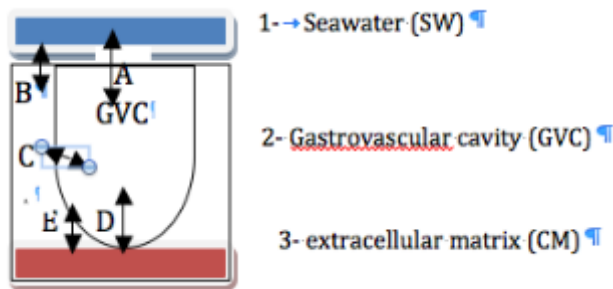
Appendix D: Coral Physiology Group Notes

Disclaimer: These notes are the result of one recorder and subsequent post-meeting edits by participants, and not meant to be recorded minutes.

Overall Vision: Understand Mechanisms of Calcification

Questions:

We want to know how the calcifying environment, e.g., fluid in the GVC, responds to changes in the environment.



- Physical exchange between SW & GVC
- Active, passive or paracellular flux across tissues
- Exchange between GVC & surrounding tissues
- Exchange between GVC & calcifying media (physical pathways)
- Active/passive transport between GVC to CM by calciblastic layers.

Related research questions include:

- How do corals create an environment in which crystals can grow?
- What are the boundaries of the coral's ability to control its internal chemistry with regard to changes in seawater chemistry?
- How do they manage their internal chemistry with regard to changes in seawater chemistry with regard to ocean acidification?
- How much exchange is there between the external calciblastic epithelium and outside space?

Extracellular space..

- What is the nature of the biological control? What is the role of the GVC in this control?
- How are ion fluxes into and through the tissue controlled? Perhaps the mesoglea has an involvement in the movement of ions; Dextran experiment – water can come in and out of the coelomic cavity, but there is a selectivity in what they let in/out of cells
- Do corals irrigate their calciblastic spaces/cavities?
- Are corals going to respond to expected seawater chemistry changes and how does that response vary between species?
- What are mechanisms of calcification, including 'buffering' bounds?
- How does functional microbial community associated with corals and other reef microenvironments affect calcification rates?
- How does speciation of metals and other micronutrients affect calcification rates?

What we know regarding coral calcification:

1. Skeleton is aragonite; consistently 2 crystal types: Granular or fusiform crystals that form at night; Fibrous needles that form in daylight
2. Centers of calcification occupied by granular crystals
3. Not all corals respond similarly to acidification, and thus the implication is that control mechanisms must differ.
4. Calcification of shallow water zooxanthellate corals responds to light with formation of different types of crystals likely mediated by diel cycles of photosynthesis
5. There is an organic matrix that serves as a framework for calcification (2% by weight estimate) specifically secreted by the calicodermis. Inhibition of organic matrix secretion results in inhibition of calcification.
6. Mapped sulfate (SO₄) polysaccharides has a pattern of deposition; high concentration of polysaccharides during early mineralization phases; coral producing highly sulfated ribbons to determine where nucleation is happening (idea – hypothesis); determining meso-architecture required to know where/how products of calcification are delivered to the calicoblastic layer
7. Quantum Dots (Molecular Devices) may be useful for tracking movement through tissue/cellular compartments from seawater into the sites of calcification.
8. Calcification process is spatially regulated process to create detailed and specialized skeletal structures; hypothesize that centers of calcification are where the highest control is located, however, these are only 1 cell thick; controlled at the micro and macro scale
9. Calcification process in corals is much faster process than growing crystals abiogenically from seawater, indicating biological control – about 1000 times faster under the same saturation conditions; abiogenic aragonite ~2000 umoles/ml
10. $[Ca] \gg [C_T]$ in seawater – corals must actively bring carbon in somehow but there is also metabolic CO₂ used in calcification
11. Differentiation of calicoblastic epithelia related to where calcification is occurring; crystals are formed outside of the cell; secretes the organic matrix
12. Junction shapes similar to tight junctions and septal junctions in insects, so can conclude they are tight, and probably restrict paracellular transport; diameter of cells about 10 um – wide space suggests ion transport; increases surface area for ion transport (peri-cellular transport); only one cell touching the skeleton at any one place – Ushing chamber used at ectoderm, mesoderm and mesoglea may play a role in filtering; perhaps calcine permeates the space, but only a small amount needed (20umolar); more seawater through junctions, but don't know for sure
13. Paradox is that some chemicals get across coral membranes while others are blocked. There are spatially variable areas of calcification, similar to otoliths that have differential distribution of ions on the growing edge vs the back edge
14. Calcification C is coming from respiration, as evidenced by pH going down during dark and up in the light; zooxanthellae mainly in tentacles and oral disk, scattered through the gastric cavity; cilia in GVC keep things moving to keep it from going anoxic
15. Zooxanthellate corals have faster calcification than non-zooxanthellate corals, but too many zooxanthellae may reduce calcification; we don't know a lot about fine-scale respiration and the contribution of respiratory CO₂ to coral calcification.

What we need to know:

1. Respiration rates and differences within colonies and between species
2. Volume of the gastrovascular cavity; Stylophora exhibit trophophilia and have very large polyp volume, about 5mls (range 10ul to 5mls)
3. Role of carbonic anhydrase to make bicarbonate from CO₂ which can diffuse across membranes; $Ca^{++} + 2HCO_3 \rightleftharpoons CaCO_3 + CO_2 + 2H$ – during light zooxanthellae take up CO₂ for calcification; role of Calcium ATPase pump described by Allemand et al. (Furla, P., D. Allemand and M. Orsenigo. 2000. Involvement of

H⁺-ATPase and carbonic anhydrase in inorganic carbon uptake for endosymbiont photosynthesis. Am J Physiol Regul Integr Comp Physiol 278: R870-R881)

4. Concentrations across time and space dimensions for Ca and O₂

5. Chemistry (composition) of the calcifying fluid, e.g., saturation state, Ca concentration, pH, various ion concentrations

6. pH throughout coral structure; trying to get at pH with dye sensitive dyes under the polyp; problem getting into the corals to measure; trying to get some to grow out in thin layers

7. Fluid dynamics in calicoblastic space, e.g., exchange rates between calicoblastic layer, GVC and CM.