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POSTER ABSTRACTS

Alkalinity enrichment enhances net calcification of a coral reef flat

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Ocean acidification is projected to shift reefs from a state of net accretion to one of net dissolution sometime this century. While retrospective studies show large-scale changes in coral calcification over the last several decades, determining the contribution of ocean acidification to these declines is difficult due to confounding factors of temperature and other environmental parameters. Here, we quantified the calcification response of a coral reef flat to alkalinity enrichment to test whether reef net calcification increases when ocean chemistry is restored to near pre-industrial conditions. We used sodium hydroxide (NaOH) to increase the total alkalinity of seawater flowing over a reef flat, with the aim of increasing carbonate ion concentrations [CO_3^{2-}] and the aragonite saturation state (Ω_{arag}) to values that would have been attained under pre-industrial atmospheric pCO_2 levels. We developed a dual tracer regression method to estimate alkalinity uptake (i.e., net calcification) in response to alkalinity enrichment. This approach uses the change in ratios between a non-conservative tracer (alkalinity) and a conservative tracer (a non-reactive dye, Rhodamine WT) to assess the fraction of added alkalinity that is taken up by the reef as a result of an induced increase in calcification rate. Using this method, we estimate that an average of 17.3% ± 2.3% of the added alkalinity was taken up by the reef community. In providing results from the first seawater chemistry manipulation experiment performed on a natural coral reef community (without artificial confinement), we demonstrate that, upon increase of [CO_3^{2-}] and Ω_{arag} to near pre-industrial values, net reef calcification increases. Thus, we conclude that, the impacts of ocean acidification are already being felt by coral reefs. This work is the culmination of years of work in the Caldeira lab at the Carnegie Institution for Science, involving many people including Jack Silverman, Kenny Schneider, and Jana Maclaren.

Changes in coral reef communities across a natural gradient in seawater pH

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Ocean acidification threatens the survival of coral reef ecosystems worldwide. The negative effects of ocean acidification observed in many laboratory experiments have been borne out in studies of naturally low-pH reefs, with little evidence to date for adaptation. Recently, we

reported initial data suggesting that low-pH coral communities of the Palau Rock Islands appear healthy despite the extreme conditions in which they live. Here we build on that observation with a comprehensive statistical analysis of benthic communities across Palau's natural acidification gradient. Our analysis revealed a shift in coral community composition but no impacts of acidification on coral richness, coralline algae abundance, macroalgae cover, coral calcification, or skeletal density. However, coral bioerosion increased eleven-fold as pH decreased from the barrier reefs to the Rock Island bays. Indeed, a comparison among all naturally low-pH coral reef systems studied so far revealed increased bioerosion to be the only consistent feature across them, as responses varied across other indices of ecosystem health. Our results imply that while community responses may vary, escalation of coral reef bioerosion and acceleration of a shift from net accreting to net eroding reef structures will likely be a global signature of ocean acidification.

The impact of increased CO_2 on the sinking and swimming of *Limacina retroversa* pteropods

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Shelled pteropods, or thecosomes, are considered a sentinel species of the impact of ocean acidification on pelagic ecosystems due to their production of an aragonite shell. In laboratory exposure experiments and *in situ*, pteropod shells have consistently been observed to become degraded at low aragonite saturation states, but the consequences of this dissolution to a pteropod's fitness are mostly unclear. To answer this question, the pteropod *Limacina retroversa* from the Gulf of Maine was caught during multiple cruises and brought back to the lab to be reared under three different CO_2 exposures: ambient (400 ppm), medium (800 ppm), and high (1200 ppm). Over the course of 2-4 week exposures to CO_2, the live animals were observed in a mirrored tank using high speed video, which allowed for 3D analysis of an animal's movements as they repeatedly sank and swam. In conjunction, shell condition analysis revealed changes to the appearance of the shell. Sinking velocities standardized by shell length were significantly slower when animals were exposed to medium and high CO_2 and the effect was more severe with increased duration of exposure. Upward swimming velocities also differed and indicate that enhanced CO_2 may reduce swimming ability. Changes to the shell condition of wild populations of pteropods could affect their fitness by reducing their vertical mobility in the pelagic zone, decreasing their ability to use sinking as a predator avoidance technique.

Malformation in nannoplankton species from the Paleocene-Eocene Thermal Maximum, a potential response to ocean acidification

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A ~170,000-year-long transient warming event 56 milion years before present, the Paleocene-Eocene thermal maximum (PETM) provides a unique opportunity to elucidate the impact of future global warming and associated environmental changes, including ocean acidification. PETM warming was driven by input of a massive quantity of carbon, as suggested by a prominent carbon isotope excursion (CIE). A significant fraction of this carbon was sequestered in the deep ocean, causing a lengthy period of acidification and chemical erosion of deep-sea sedimentary archives. Our funded project focuses on PETM sections in Maryland and New Jersey deposited on the continental shelf which preserve a highly expanded record of the earliest part of the event largely missing in the deep sea.

The PETM is characterized by a transient group of nannoplankton whose range is restricted to the interval of the CIE. Many of these so-called excursion taxa belong to the genus *Discoaster,* a large group characterized by multi-rayed specimens. The origin of the *Discoaster* excursion taxa is debated, and, since their phylogeny is unknown, these taxa have previously been classified as discrete species. Our observation of excursion *Discoaster* taxa in the early part of the PETM from shelf sections provides a unique and refined view of their morphology, phylogeny and relationship to environmental change. *Discoaster* shows three progressive stages of morphological response, starting with inter-ray irregularity of spacing, followed by ray-tip deformation and ray malformation, and, ultimately, ray loss. Tip deformation and ray malformation and loss characterize the dominant excursion taxa, *Discoaster anartios* and *D. araneus*. We observe signficant morphological variation among *D. anartios* and *D. araneus,* and our data suggests that these two taxa derived from long-standing "normal" *Discoaster* species, *D. multiradiatus* and *D. salisburgensis.* Finally, detailed observations suggest that *D. anartios* morphed into *D. araneus* via progressive malformation and ray loss. We find inter-ray irregularity in specimens from near the onset of the PETM, but observe ray-tip deformation and ray malformation and loss beginning close to the peak of the CIE and extending through much of the recovery.

Our observations illustrate a much larger extent of morphological variation in excursion *Discoaster* than observed in deep sea sites where the fossil record of the earliest part of the PETM has been compromised by dissolution. Our results indicate that the excursion taxa are not separate species, but rather morphological variants of existing species of *Discoaster*. The extent of malformation in PETM *Discoaster* is considerably greater than observed in living and cultured coccolithophores, where such a response has been proposed to result from decreased Ca concentration, elevated CO_2, decreased salinity and upwelling. The extent of malformation is indicative of decreased calcification, potentially as a result of ocean acidification. Like modern coccolithophores, PETM malformation is highly taxon-selective; excursion *Discoaster* constitute a minor component of the nannoplankton assemblage, with a broad range of species showing no apparent morphological change during the PETM.

Coral interior pH and carbonate chemistry as revealed by microelectrode profiles

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Reliably predicting how corals may respond to ocean acidification due to atmospheric carbon dioxide (CO_2) increase depends on our understanding of the internal carbonate chemistry. The speciation, sources, and dynamics of dissolved inorganic carbon (DIC) in corals are controversial in part because previously only pH has been measured and a critical second parameter to characterize carbonate chemistry was missing. Here we report the first set of complete depth profiles of pH and carbonate ion concentrations ($[CO_3^{2-}]$) measured inside coral polyps including the calcifying fluid where carbonate minerals form. Observed sharp increases in pH and $[CO_3^{2-}]$ inside the calcifying fluid and very low pH and $[CO_3^{2-}]$ above it support the existence of an active process that pumps proton (H^+) out of the calcifying fluid into the coelenteron solution above. A very sharp gradient from the coelenteron to the calcifying fluid confirms that molecular CO_2 diffusion into the calcifying fluid alone can support calcification. In contrast to a popular recent view, our data also suggest that DIC inside the calcifying fluid is lower than or similar to the background seawater value due to consumption by calcification whereas total alkalinity (TAlk) is only moderately higher than that in seawater due to H^+ pumping. These findings suggest that corals can heavily regulate the carbonate chemistry of the calcifying fluid and that any effects of ocean acidification on calcification are independent of the internal carbonate chemistry but may be a result of pH sensitivity of gene regulation that controls calcification.

Oxidation of reduced chemicals leads to strong acidification in Chesapeake Bay waters

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It has been known for over a decade that anthropogenic CO_2 uptake has acidified the ocean and caused detrimental effects on marine organisms and ecosystems. More recently, it has also been reported that eutrophication and its subsequent respiration can lead to further acidification in coastal oceans. It is however not known how the production and consumption of reduced chemicals would do to the acidification in very low oxygen or anoxic environments. We report here an extreme acidification caused by the oxidation of reduced chemicals (H_2S and NH_3) in waters with an already weakening buffer capacity in Chesapeake Bay. A simple model quantitatively links the acidification to redox reactions at the oxic-anoxic boundary and suggests this could be a common phenomenon in many coastal water bodies.

Ocean acidification effects in the early life-stages of summer flounder *Paralichthys dentatus*

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Early life stage (ELS) responses of summer flounder (*Paralichthys dentatus*) were evaluated for responses to ocean acidification (OA). Survival of embryos was reduced by 50% below survival at local ambient conditions when maintained at the intermediate conditions (7.4 pH, 1860 ppm pCO_2), and by 75% below local ambient survival when maintained at the most acidic conditions tested (7.1 pH, 4,715 ppm pCO_2). Reduced embryo survival was consistent among three females used as sources of embryos. Sizes and shapes of larvae were altered by elevated CO_2 levels. Larvae were longer at hatching (but with less energy reserves) to midway through the larval period. Larvae from the most acidic conditions initiated metamorphosis earlier and at smaller sizes than those from more moderate and ambient conditions. Tissue damage and altered cranial-facial (CF) features were evident in older larvae (> 14-d posthatching) from both elevated CO_2 levels. Effects in CF features changed with larval ages: CF elements of larvae from ambient CO_2 environments were comparable or smaller than those from elevated CO_2 environments at 7 and 14-d post-hatching but larger at older ages. The degree of impairment in the ELS of summer flounder due to elevated CO_2 levels suggests that this species will be challenged by OA in the near future. Further experimental comparative studies on marine fish are encouraged in order to identify the species, life-stages, ecologies, and responses that are most sensitive to increased levels of CO_2 and acidity in near-future ocean waters.

Implications of multiple stressors on abalone population dynamics within the California Current large marine ecosystem

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Acidification, hypoxia, and ocean warming are escalating threats in the world's coastal waters, with potentially severe isolated and or chronic consequences for marine life and ocean-based economies. In particular, eastern boundary current ecosystems, including the California Current Large Marine Ecosystem (CCLME), are experiencing large-scale declines in pH and dissolved oxygen (DO)—with the latter linked to changes in thermal stratification and shoaling of the oxygen minimum zone. To examine the consequences of ocean acidification and other climate-related changes in oceanographic conditions on nearshore marine communities within the CCLME, we are exploring the potential effects of current and future upwelling-type conditions on the population dynamics of abalone populations, with a focus on sensitive early life history phases (e.g., fertilization, larval development, and juvenile growth and survival) expected to be important determinants of abalone population dynamics. Our approach focuses on three objectives, including: 1) measure and characterize the temporal variability of pH, DO and temperature in nearshore abalone habitat in Baja California and in the Monterey Bay, 2) experimentally measure the effects of low pH, low DO conditions on the reproductive success, growth, calcification, and survival of juvenile abalone, and 3) estimate the impacts of environmental and local anthropogenic stressors on the resilience of abalone dynamics using demographic and bio-economic modeling by scaling up from individual to population level effects. Here we present our framework and initial experimental results.

Seawater CO_2 system thermodynamics under high-pCO_2 conditions

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Robust thermodynamic models are required for assessments of the marine CO_2 system under elevated pCO_2 conditions. A variety of studies have shown that measurements of keystone CO_2 system variables (i.e., dissolved inorganic carbon (DIC), total alkalinity (TA), pH and CO_2 fugacity (f_{CO2})) are not fully consistent with the thermodynamic models that are widely used to relate the parameters. For example, direct measurements of a parameter such as TA are, on average, significantly different from TA values that are calculated from different combinations of the other keystone parameters (i.e., DIC-pH, DIC- f_{CO2} and f_{CO2}-pH). Due to the lack of internal consistency between calculations and direct measurements, it follows as well that aragonite saturation states (Ω_A) calculated from different parameter combinations, but never directly measured, also show substantial variation. Investigations underway at the University of South Florida College of Marine Science (USF) and SRI International (SRI) are assessing the internal consistency of existing CO_2 system models through (a) laboratory measurements of all four keystone CO_2 system parameters DIC, TA, pH and f_{CO2} and (b) evaluation of CO_2 system parameters measured under field conditions at state-of-the-art accuracy and precision. The overall goal of this this work is the development of models for internally consistent descriptions of state-of-the-art CO_2 system measurements in the laboratory and at sea. We describe the initial results of those studies and discuss a number of new laboratory procedures that have been developed to facilitate our work.

What's energetics got to do with it? Coral calcification response to OA under light, feeding and nutrients

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Calcium carbonate production by reef building corals is thought to be a metabolically costly process, with corals actively investing energetic resources to modify the chemistry (i.e., increase the saturation state) of the calcifying environment. Ocean acidification would inherently exacerbate the metabolic cost of maintaining calcification, which several studies hypothesize could be mitigated by enhancement of coral nutritional status.

To test this hypothesis, we conducted a series of CO_2 and nutrition (i.e., feeding and light) manipulation experiments using juveniles of the Atlantic coral *Favia fragum*. We found that different forms of nutrition elicited different coral calcification and tissue responses: Fed corals are larger than unfed corals while corals subjected to elevated light conditions exhibit higher lipid (i.e., energetic) reserves. However, regardless of nutritional enhancement, coral calcification remained sensitive to elevated CO_2 conditions. This contrasts with published results from nutrient-enrichment experiments that show enhanced symbiont photosynthesis and reduced CO_2 sensitivity under high levels of dissolved inorganic nutrients. Additionally, we found in our studies that CO_2 did not significantly affect tissue lipid content. These results highlight two main findings: 1) The role of nutrition in coral calcification response to OA varies based on the type of nutritional enhancement (e.g., heterotrophy vs. photosynthesis), and 2) it appears these responses may be driven by factors other than availability of energetic reserves.

Combined study of environment, genetics, and physiology as a framework for assessing biological resilience to ocean acidification

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Assessing the impact of ocean acidification requires a robust analysis of carbonate chemistry and biological adaptive potential. Assessment of $p\text{CO}_2$ and saturation state conditions along the U.S. coastlines is under rapid progression, and there are extensive habitat- and regional-specific $p\text{CO}_2$ features. For example, instrumentation at the University of Southern California Wrigley Marine Science Center, located on Santa Catalina Island, has revealed dampened $p\text{CO}_2$ variability relative to the regional $p\text{CO}_2$ environment. Such local environments could be considered as stable study sites for biological studies, for example as in-situ controls. While the chemical environment is under extensive study, the mechanisms of biological resilience and adaptive potential to ocean acidification remain elusive. Such studies will be greatly improved by an enhanced understanding of the genetic basis of physiological variance. As such, we utilize crosses of pedigreed lines of the Pacific oyster, *Crassostrea gigas*, to understand the metabolic bases of homeostatic maintenance under ocean acidification. Revealing genetic and physiological biological variance will enhance predictive power for the identification of potential resilience to environmental change.

Tracking short and long-term changes in marine phytoplankton physiology in response to increased CO_2

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The carbon dioxide concentration of Earth's atmosphere ($p\text{CO}_2$) is rising faster than at any point in the last several hundred thousand years and is expected to reach 800 ppm by 2100. With the continued increase in $p\text{CO}_2$, ocean pH is predicted to drop in that same timeframe potentially resulting in large-scale shifts in phytoplankton community structure or the evolution of species to prevent displacement. These events could change biogeochemical cycles as well as alter trophic relationships and ecosystem structure and function. Our research is focused on the following overarching goals: 1) examine how CO_2 increase affects major functional groups of marine phytoplankton; 2) explore fitness changes in a high-CO_2 ocean; and 3) predict how these changes might affect community structure and biogeochemistry. To address these goals, we are using experimental laboratory evolution, coupled with genetic/genomic approaches, to observe and track adaptation in model representative phytoplankton functional groups and assess the response of these model organisms to changes in CO_2. Long-term evolution experiments and modeling work are ongoing. As a test bed for the analytical pipeline required for the genomic analyses of the long-term experiments, we examined the transcriptomic response of a model raphidophyte and dinoflagellate to short-term changes in CO_2. Transcriptomes of ~30M paired-end 50-bp reads were sequenced for several model species grown under present (400 ppm; control) and future (800 ppm; +CO_2) $p\text{CO}_2$. Over 2 Gbp of sequence data was generated per library. Reads

were mapped to reference databases and significance in differential expression in the +CO_2 condition relative to the control was determined with Analysis of Sequence Counts (post-p <0.05, fold change = 2). Analyses to date have detected a suite of carbon metabolism genes (e.g., rubisco, carbonic anhydrase, HCO_3 co-transporters) for both species. These genes were significantly upregulated in the raphidophyte +CO_2 transcriptome relative to the control, and present, but not regulated, in the dinoflagellate +CO_2 transcriptome. Querying the transcriptomes from these two test species has yielded a host of target genes to focus on for further work and are providing a valuable template for the development of bioinformatics/analytical approaches as we move into investigations with evolved clones. Ultimately, these data will be used to inform a model of global ocean dynamics to incorporate both differential response to CO_2 and evolution to predict how an acidifying ocean will influence phytoplankton community structure and biogeochemical dynamics in the next century.

Abrupt onset and prolongation of aragonite undersaturation events in the Southern Ocean

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Rapid progression of ocean acidification is a threat to key organisms of the Southern Ocean ecosystem. While the severity of ocean acidification impacts is mainly determined by the duration, intensity, and spatial extent of aragonite undersaturation events, little is known about the nature of these events, their evolving attributes, and the timing of their onset. Using output of historical and RCP 8.5 simulations from ten Earth System Models from CMIP5, we found that aragonite undersaturation will spread rapidly after 2035, covering 70% of the Southern Ocean surface waters by 2095. Surface aragonite undersaturation events will last for about 5 months in areas south of 60°S by 2055, and for more than 8 months by the end of the century. Overall, the duration of events increases from 1 month to more than 6 months within fewer than 20 years in >75% of the affected area. This abrupt change in exposure duration to unfavorable conditions may be too fast for organisms to adapt, as these changes will occur within just a few generations. The threat of ocean acidification to the Southern Ocean ecosystem may be more imminent than previously thought, and may spread quickly to the southern tips of New Zealand, South America, and South Africa, with potentially far-reaching consequences to fisheries, local economies, and livelihoods.

Effects of ocean acidification and seawater temperature on larval development, settlement success and predation vulnerability in the blue mussel (*Mytilus edulis)*

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Important processes governing intertidal bivalve population dynamics may be greatly affected by the anticipated effects of marine climate change, including increased sea surface temperature, dissolved carbon dioxide and reduced aragonite saturation state. Such stress may require greater energetic investment during calcification of the blue mussel, *Mytilus edulis*, potentially shifting life-history characteristics within affected populations. While recent studies have documented increased larval developmental abnormalities and mortality, and reduced settlement success and shell growth in experiments with low pH, the combined effects of warming and acidification have been largely unexplored, particularly over entire life-spans. Further, the majority of acidification studies focus on single-variable effects in isolated life-stages, precluding comprehensive understanding of how interacting shifts in marine chemistry anticipated by ongoing climate change may impact ecologically relevant species throughout successive life stages.

To assess the potential effects of climate change on population dynamics, proposed research will test the hypotheses that increased temperatures and pCO_2 (aq), and decreased $[CO_3^{2-}]$ and Ω aragonite (1) increase larval developmental abnormalities and mortality rates, (2) reduce settler growth rates and increased mortality rates, (3) slow shell growth rates in juveniles and (4) increase vulnerability to predation during direct exposure. The effects of climate change on successive life stages in the blue mussel, *Mytilus edulis*, will be tested by spawning and rearing larvae, recording settlement and rearing juvenile mussels in triplicate at factorially combined temperature and pCO_2 (Temp: 12, 16, 20 °C; pCO_2: 380, 480, 760, 1000 ppm). Given kinetic relationships between temperature, pCO_2 (aq), pH, HCO_3^-, CO_3^{2-}, and $\Omega_{aragonite}$, and metabolic costs of amorphous calcium carbonate (ACC), calcite, and aragonite deposition during shell growth, it is hypothesized that increased temperatures will ameliorate high rates of larval developmental abnormality, increased settler and juvenile mortality and increased predation vulnerability observed at high pCO_2. This research will provide important contributions to our understanding of the potential impacts of climate change on mussel physiology through several successive life-stages and will serve as an important model that can be further applied to the diverse assemblage of calcifying invertebrates found in the northern Gulf of Maine.

Development of a compact instrument for field measurements of pH, total dissolved inorganic carbon and total alkalinity

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We are developing a third generation multi-parameter inorganic carbon analyzer (MICA III) that will allow facile measurements of DIC, TA and pH in the field. This multi-parameter sensor will be easy to operate, highly portable, and have virtually no supporting infrastructure requirements. This will have a positive impact on the ocean acidification community by making measurements of the marine carbon system readily accessible to a much wider range of researchers than current technology permits. Our overarching objective in this project is to make carbon system measurements as routine as determinations of salinity.

Evidence of a light-induced H^+ pump and photosynthesis-calcification coupling/uncoupling in a tropical crustose coralline algae (CCA)

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Presently, there is a limited mechanistic understanding of tropical reef macroalgae photosynthesis and calcification, which undermines our ability to predict how this important group of autotrophs will respond to ocean acidification (OA). The goal of our present research is to provide new insights into the fundamental biochemistry and physiology of photosynthesis and calcification that drive tropical reef macroalgal growth responses to increasing pCO_2, OA and warming. To examine the hypothesis that photosynthesis-calcification processes become uncoupled under OA, photosynthesis, pH and calcification were simultaneously measured with microsensors at the thalli surface of a tropical crustose coralline algae (CCA; *Porolithon* sp.) at ambient (8.1) and low (7.8) pH levels at in situ temperature (27^{o}C). Profiles of O_2, pH and Ca^{2+} were established from 800 to 0 mm from the thalli surface at 50 mm intervals using automated microprofilers. The diffusive boundary layer (DBL) gradients of O_2, H^+ and Ca^{2+} were established across a range of irradiance (0-1400 μmol photons m^{-2} s^{-1}) and fluxes calculated. To determine how Ci uptake pathways for photosynthesis affect conditions for calcification at the DBL and calcification rates, inhibitors (acetazolamide [AZ] and 4,4′ diisothiocyanatostilbene-2,2′-disulphonate [DIDS]) were used to block extracellular carbonic anhydrase (CA) mediated hydrolysis of bicarbonate at the plasma membrane and direct HCO_3^- uptake by anion exchange transport. In addition to establishing profiles across the DBL, light-dark dynamic studies were conducted at the thalli surface to determine the immediate response of O_2, H^+ and Ca^{2+} flux to irradiance and darkness.

The diffusive boundary layer where CCA had a significant influence on seawater chemistry was approximately 0-300 mm. Net photosynthesis calculated from the DBL indicated diffusive uptake of CO_2 and HCO_3^- use through hydrolysis. Regardless of the initial pH (8.1 or 7.8) or CA inhibitor, surface thalli pH was raised to approximately 8.3 to 8.4 at saturating irradiance ≥ 280 μmol photons m^{-2} s^{-1}. Dynamic studies also showed a strong correspondence between the onset of light and rapid O_2 and H^+ flux without a lag phase. These data suggest a light-mediated proton pump is present that corresponds to, but is likely not driven by, photosynthesis. While O_2 and H^+ profiles and dynamics were consistent amongst trials and replicate CCA fragments, the Ca^{2+} results were less lucid and mechanisms apparently more complex. Based on profile data, Ca^{2+} uptake rates were always positive, ranging from 0.5 to 10 μmol m^{-2} s^{-1}, but without a clear response to irradiance or pH, and interestingly, appeared to be upregulated with CA inhibition by AZ. Calcium dynamics at the thalli surface showed extremely high mM efflux or uptake with light, perhaps related to spatial or temporal dominance by either rapid calcification or dissolution required to slough surface cells, a known strategy of CCA to remove epiphytes. Thus, the study indicates the potential to elevate surface pH regardless of bulk seawater pH through a light-induced H^+ pump, but specific linkages of this mechanisms and photosynthesis to calcification require further research.

Exploring the seasonal response of the thecosome pteropod *Limacina retroversa* to CO_2 in the Gulf of Maine

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Limacina retroversa is a thecosomatous pteropod found year round in the Gulf of Maine. Because carbonate chemistry within this shelf system is spatially variable and exhibits seasonal cycles, pteropods in this region may already be exposed to under-saturated, and hence corrosive, waters during certain seasons. To understand the implications of this variability, we have explored the physiological responses of *L. retroversa* at four time points over the course of a year to determine whether pteropods vary seasonally in their sensitivity to CO_2 exposure on time-scales relevant to acclimation responses. In the laboratory, these animals were exposed to CO_2 (ambient, 800, 1200 ppm) for 7-14 days and their response was assessed using an integrated set of metabolic, gene-expression and shell quality metrics. Similar to previous work with this species and others, pronounced changes in shell quality of exposed adults were discernible after less than 3 days of exposure, while changes to respiration rate were not consistently apparent. There were, however, seasonal variations in respiration rate indicative of an acclimation response. Differential expression analysis and directed studies of biomineralization-associated gene families are underway. These gene expression studies, together with both respiration rate and shell quality metrics will provide a complete picture of the seasonal effect of CO_2 on this sentinel species.

Assessing the effect of environment on barnacle adhesion and biomineralization

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Barnacles are dominant members of marine biofouling communities throughout much of the world's oceans. The barnacle's base attaches to surfaces with secreted proteinaceous adhesives that bond with surfaces and cure. Although the biochemical mechanisms involved in barnacle adhesion are starting to be understood, relatively little is known about how the environment affects barnacle adhesion and base plate mineralization. This becomes increasingly important to understand in a changing environment with the onset of climate change and ocean acidification. Here, the impact of variations in salinity on adhesion strength and biomineralization in the barnacle *Amphibalanus amphitrite* was assessed. Barnacle larvae were cultured from adults collected from the Beaufort Inlet, NC, where salinity is typically within the range of 30 – 36. Larval cultures were maintained at a salinity of approximately 35. Barnacles were settled on T2 silicone coated glass panels. Starting at 11 days post-settlement, juveniles were gradually acclimated to eight target levels of salinity, ranging from 10 – 45 in steps of 5. After 4 months growth at target salinities mortality was low on all panels and not significantly affected by salinity. Growth, measured as area of the barnacle base plate, was also not affected by salinity. Sexual maturity determined by the presence of eggs within the shell, was not significantly different between treatments. Shell mass was not significantly different between treatments, however, tissue mass was with salinities 15 and 35 showing the highest tissue mass. Adhesion strength differed significantly between treatments with salinity of 15 showing the strongest adhesion while adhesion was progressively weakened to a low at 35. Interestingly, there was an inverse relationship between adhesion strength (decreasing) and tissue mass (increasing) at salinities from 20 – 35. Initial results suggest that *A. amphitrite* is well-adapted to a broad range of environmental salinities. In addition to salinity exposures, we have begun to assess the effects of ocean acidification on *A. amphitrite* by exposing barnacles to various levels of pH from a very low of 7.50, low 7.80 and ambient of 8.10. Future studies include a multivariable approach combing variations of salinity, pH, and temperature.

Net ecosystem calcification by a coral reef community under natural acidification

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Net Ecosystem Calcification (NEC) is a measure of the balance between calcium carbonate production (calcification) and loss (dissolution) within a coral reef system. Establishing baseline NEC estimates for a broad range of coral reef systems today provides much needed information to constrain spatial and temporal variability within and amongst different systems, investigate the sensitivity of reef scale calcification to environmental forcing, and improve projections of coral reef futures under 21st century ocean acidification. Previous NEC studies focused on coral reefs with unidirectional (Lagrangian and flow respirometry studies) or negligible (slack water Eulerian studies) water flow across the reef for at least part of the day, usually on the order of hours. Here, we present NEC rates in a naturally low pH, semi-enclosed coral reef lagoon with tidally driven flow within the Palau Rock Islands. NEC was determined from data collected over the full diel cycle for four consecutive days, during two successive years and different seasons, using total alkalinity (TA), salinity, and volume budgets. Two different methods used to calculate NEC are in good agreement and show that the coral community is net calcifying despite high rates of bioerosion and pH (mean pH = 7.88 ± 0.02) and aragonite saturation state (Ω_{ar} = 2.66 ± 0.11) levels close to those projected for the end of this century. Critically, NEC rates in year 1 (17.0 – 23.7 mmol m^{-2} d^{-1}) were half those of year 2 (42.0 – 48.1 mmol m^{-2} d^{-1}) in the absence of changes in the carbonate chemistry of the source water. This suggests that single occupations and short term measurements do not adequately capture the full range of NEC variability within a system and that factors other than ocean acidification play an important role in modulating NEC rates.

Coastal CO_2 climatology of Oahu, Hawaii: Six years of high-resolution time-series data

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This poster presents a statistical evaluation and interpretation of six years of high-resolution CO_2 time-series data, collected in coastal waters off the Island of Oahu, Hawai'i. Surface water and atmospheric CO_2, along with various water quality parameters, were measured *in situ* at three hour intervals using Moored Autonomous CO_2 ($MAPCO_2$) buoys as part of a collaboration with the NOAA/PMEL CO_2 monitoring program. The continuous monitoring program for CO_2 on Oahu, Hawaii, represents the longest running coral reef time-series in the world. A description of CO_2 variability is presented for three coastal coral reef sites, and compared to CO_2 dynamics (offshore and) in the open ocean using data from $MAPCO_2$ buoys deployed at the Hawaii Ocean Time Series (HOT) site in the North Pacific Subtropical gyre and outside Kaneohe Bay. Fluxes of CO_2 between seawater and the atmosphere were calculated over various time scales were calculated to determine whether the individual locations were sources or sinks of CO_2 and to evaluate the extent of variability between different reef sites. Principal component analysis (PCA) was performed on the data to examine correlations and covariance among biogeochemical and physical parameters at each site. Climatological monthly statistics for pCO_2, air-sea CO_2 fluxes and various water quality parameters are discussed. These climatological data can serve as a baseline for CO_2 and water quality in Oahu's coastal waters, which will be particularly crucial in

the face of changing conditions due to rising atmospheric CO_2 concentrations and consequent increase in the pCO_2 of seawater, which leads to ocean acidification.

A novel refuge from climate change for reef-building corals

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Risk analyses indicate more than 90% of the world's reefs will be threatened by climate change and local anthropogenic impacts by the year 2030 under "business as usual" climate scenarios. Increasing temperatures and solar radiation cause coral bleaching that has resulted in extensive coral mortality. Increasing carbon dioxide reduces seawater pH, slows coral growth, and may cause loss of reef structure. Management strategies include establishment of marine protected areas where environmental conditions promote reef resiliency. However, few resilient reefs have been identified, and resiliency factors are poorly defined. We characterized the first natural, non-reef coral refuge from thermal stress and ocean acidification, and identified resiliency factors for mangrove–coral habitats. We measured diurnal and seasonal variations in temperature, salinity, photosynthetically available radiation, and seawater chemistry; characterized substrate parameters; and examined water circulation patterns in mangrove communities where scleractinian corals are growing attached to and under mangrove prop roots in Hurricane Hole, St. John, US Virgin Islands. We inventoried coral species and quantified coral bleaching, mortality and recovery for two reef-building corals, *Colpophyllia natans* and *Diploria labyrinthiformis*, growing in mangrove-shaded and exposed (unshaded) areas. Over 30 species of scleractinian corals were growing in association with mangroves. Corals were thriving in low-light conditions from mangrove shading and at higher temperatures than nearby reef tract corals. A higher percentage of *C. natans* colonies were living shaded by mangroves, and no shaded colonies were bleached. Fewer *D. labyrinthiformis* colonies were shaded by mangroves, however more unshaded colonies were bleached. A combination of substrate and habitat heterogeneity, proximity of different habitat types, hydrographic conditions, and biological influences on seawater chemistry generate chemical conditions that buffer against ocean acidification. This previously undocumented refuge for corals provides evidence for potential adaptation of coastal organisms and ecosystem transition due to recent climate change.

CO_2 dose response of developing squid *Doryteuthis pealeii*: Growth, energetics, and behavior

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Ocean acidification (OA) has become an increasingly relevant threat to coastal environments. Squid, many of which lay their eggs in nearshore waters, are a key trophic organism in the ocean, acting as both an active predator and a major prey stock. This study looked at the potential effects of a range of CO_2 levels on the early life history of a local squid species: the Atlantic longfin squid, *Doryteuthis pealeii*. Eggs were raised in a flow-through system at CO_2 levels ranging from ambient (400ppm) to 2200ppm. Time to hatching, embryonic survival, and hatchling mantle length, yolk sac size, statolith size, and oxygen consumption rate were all examined. Paralarvae from several CO_2 levels were also recorded in 3D for analysis of swimming behavior. Delays in hatching time, reduced mantle lengths, and smaller, lower quality statoliths were seen at exposures of 1600ppm and above. Yolk sac sizes varied, but did not demonstrate a trend with CO_2, and may instead be linked to seasonality. Paralarval oxygen consumption rates did not show a trend across OA treatments. *Doryteuthis pealeii* appears to be able to withstand acidosis stress without effect until at least 1600ppm; a surprising resilience given monthly CO_2 concentrations measured in Vineyard Sound rarely went much above 400ppm. Even with reduced, poorer quality statoliths, CO_2-exposed paralarvae currently show no impact on orientation ability or swimming behaviors. It is likely that during embryonic development, *D. pealeii* reallocates energy from growth processes rather than tapping energy reserves in order to mitigate the stress of acidosis.