Use of novel optical technology, genetic analysis and physiological data to investigate the effect of light and temperature stress on coral bleaching

The recent increase in average global temperature to unprecedented levels has caused coral reef communities to experience severe damage, including chronic stress, disease epidemics, and mass bleaching episodes. With one-third of all identified marine species living in biodiverse coral reef ecosystems[1] and an annual net revenue of over $375 billion generated from reef-related industries,[2] the need for non-invasive and novel techniques for monitoring coral health is paramount. The purpose of this study is to observe the photosynthetic efficiency and physiological health of corals as they undergo light- and temperature-induced bleaching to better understand the effect of future climate conditions on coral reefs. The results would suggest non-invasive proxies for predicting at-risk corals in a warming climate, and offer a transition into an interdisciplinary senior year thesis in which I plan to monitor the protein content and composition of bleaching corals.

Coral bleaching is defined as the degradation of the fundamental mutualistic relationship between corals and their resident unicellular symbiotic algal organisms, zooxanthellae. The photosynthetic zooxanthellae satisfy the coral's carbon and energy needs for growth and calcification while living in the coral tissues. Corals and these symbionts exhibit different tolerances to temperature and light stressors depending on a variety of factors, including geography and skeletal structure. While the coral bleaching process is not entirely understood, we do know that increasing temperatures combined with the high light intensities of the tropics expedite the bleaching response.[3] It is hypothesized that high temperatures stress the zooxanthellae photosynthetic reaction centers, and simultaneous exposure to high irradiances compound this pressure, thereby accelerating the bleaching mechanism.[4] A study previously conducted by Dr. Marcelino demonstrated that bleaching susceptibility is related to the coral skeleton's light transport characteristics, particularly their ability to scatter light to zooxanthellae-containing tissues. High light scattering corals deflect photons back into the environment along the pathway of photon entry, thereby allocating more direct irradiance to a few zooxanthellae cells while cells located on other areas of the coral remain unexposed. Conversely, low light scattering skeletons deflect photons away from the point of entry, resulting in a diffusion of the light field throughout the coral and thus excess light exposure.[5] This redistribution of light in low light scattering corals is believed to be a risk factor in bleaching.

Corals initiate measurable defense responses to stress before presenting visible signs of bleaching, including: i) decrease in the overall zooxanthellae cell density,[6] ii) shifts in photosynthetic pigment production,[7] and iii) decrease in the overall photosynthetic efficiency.[8] This experiment will compare these measureable responses of both low light scattering and high light scattering corals undergoing temperature and light stress, thus testing the hypothesis that the optical characteristics of the coral skeleton predispose the coral to a particular defense response. While previous research in the field focuses on the zooxanthellar photo-protective defenses,[9] this study synthesizes information regarding the calcium carbonate skeletal structure and the combined effects of high temperatures compounded by high irradiances on the photosynthetic apparatus in a previously untested manner. The results of this study could propose the use of optical technology as a method for identifying at-risk coral species, which would allow for the localization of environmental protective policies on the most susceptible reef communities.

The preliminary experiment will begin with a selection of two coral species that exhibit low light scattering properties, and two with high light scattering properties. Because different clades of zooxanthellae exhibit different resistances to bleaching, the selected corals should contain the same clade of zooxanthellae to eliminate untested defense responses. Thus, the light scattering properties of the skeleton will be coupled with genetic analysis of the resident symbionts to ensure zooxanthellar cladal uniformity. The four corals will be selected for these characteristics from an approved collection of corals at the Shedd Aquarium, and all genetic analysis will be performed at the Pritzker Lab in the Field Museum. Dr. Marcelino's postdoctoral fellow Dr. Swain and I optimized genetic identification protocols for both coral and symbiont tissues last summer, including procedures for DNA extraction, PCR

amplification, DNA sequencing, and identification using GenBank in order to determine the genus and species of the corals and their symbionts provided by Shedd Aquarium. Prior to selection, I will verify the genetic identity of all experimental corals and symbiotic algae using these methods.

The preliminary experiment will test the two low light scattering skeletons and two high light scattering skeletons at different temperatures and irradiances in order to compare the responses of the two groups. The experiment will run for approximately ten days, allowing sufficient time for the experimental conditions to induce a bleaching response from the corals. The two low light scattering corals and two high light scattering corals will be fragmented, acclimated, and subjected to the following conditions: i) low temperature and low light, ii) low temperature and high light, iii) high temperature and low light, and iv) high temperature and high light. The low temperature low light condition acts as a control, which measures baseline stress response. The low temperature high light and high temperature low light conditions will test the independent effect of each stress condition on the health of the corals, and the high temperature high light condition will test the compound effect of both stressors. Because each condition will be tested on two low light scattering corals and two high light scattering corals, this experiment will allow for a detailed investigation into the impact of skeletal light scattering properties on bleaching susceptibility using several proxy tests.

The photosynthetic efficiency of the zooxanthellae will be observed daily using Pulse Amplitude Modulated fluorometry (PAM), a well-established technique that measures the difference in chlorophyll pigment fluorescence in dark- and light-acclimated conditions. These data map the rise and decay of fluorescence in the light-harvesting antennae in the chloroplast thylakoid membrane, providing information on the health of photosystem II (PSII) in the zooxanthellae, and therefore the overall health of the coral. Additionally, I will monitor oxygen production throughout the experiment as an indicator of photosynthetic health using a FireSting O2 probe. Both measurements are expected to decline as corals experience temperature- and light-induced stress. Dr. Marcelino's laboratory will provide the Junior PAM Chlorophyll Fluorometer probe and FireSting oxygen probe for these measurements.

In addition to daily photochemical measurements, I will also measure the zooxanthellae cell density using microscopic cell counts and surface area measurements. Zooxanthellae cells will be extracted from each coral fragment using a WaterPik, and visualized under a microscope on a hemacytometer grid. The surface area of the coral fragment will be estimated using the foil wrap method,[10] and verified with the wax dip method.[11] Zooxanthellae density has been observed to decline prior to observable bleaching, and therefore acts as an early indicator of stress. Additionally, samples will be transported to Northwestern University for pigment composition analysis using high-performance liquid chromatography (HPLC), a protocol that will be carried out by two other undergraduates in Dr. Marcelino's group. The purpose of this proxy is to observe any shifts in photosynthetic pigment composition as bleaching occurs. In the 9 weeks following the experiment proper, I will analyze these data for statistically significant trends, and adjust the protocols as needed. I will then present the results of this experiment to Dr. Marcelino to provide constructive input regarding coral selections and methods for her upcoming experiment.

Since the spring of 2011, I have worked closely with Dr. Marcelino and Dr. Swain on protocol optimization and overall experimental design. Due to my research experiences at Cenetron Diagnostics in Austin, Texas and with Dr. Marcelino's group, I am familiar with the biological laboratory techniques employed in this experiment. I further improved my relevant skill set by taking Environmental Microbiology at the Technical University of Denmark, which contained a microscope-intensive laboratory component. This research grant will provide me with the means to test my previously developed protocols in an experimental setting. Additionally, this experiment would allow me to continue my research into my senior year, in which I plan to supplement these results by monitoring the expression of heat-shock proteins in corals undergoing environmental stress. Working in Dr. Marcelino's group has inspired me to apply to molecular biology PhD programs this upcoming fall, and the opportunity to conduct an independent study this summer would support my transition into graduate-level research.

References

1. Veron, J.E.N. *et al.* The coral reef crisis: The critical importance of < 350 ppm CO(2). *Marine Pollution Bulletin* **58,** 1428-1436 (2009).
2. Costanza, R. *et al.* The value of the world's ecosystem services and natural capital (Reprinted from Nature, vol 387, pg 253, 1997). *Ecological Economics* **25,** 3-15 (1998).
3. Hill, R., Frankart, C. & Ralph, P.J. Impact of bleaching conditions on the components of non-photochemical quenching in the zooxanthellae of a coral. *Journal of Experimental Marine Biology and Ecology* **322,** 83-92 (2005).
4. Enriquez, S., Mendez, E.R. & Iglesias-Prieto, R. Multiple scattering on coral skeletons enhances light absorption by symbiotic algae. *Limnology and Oceanography* **50,** 1025-1032 (2005).
5. Marcelino, L. & al., e., presented at the American Society of Microbiology 110th General Meeting, San Diego, CA, 2010 (May 23-27) (unpublished).
6. Rodolfo-Metalpa, R., Richard, C., Allemand, D. & Ferrier-Pages, C. Growth and photosynthesis of two Mediterranean corals, Cladocora caespitosa and Oculina patagonica, under normal and elevated temperatures. *Journal of Experimental Biology* **209,** 4546-4556 (2006).
7. Apprill, A.M., Bidigare, R.R. & Gates, R.D. Visibly healthy corals exhibit variable pigment concentrations and symbiont phenotypes. *Coral Reefs* **26,** 387-397 (2007).
8. Jones, R.J., Hoegh-Guldberg, O., Larkum, A.W.D. & Schreiber, U. Temperature-induced bleaching of corals begins with impairment of the CO2 fixation mechanism in zooxanthellae. *Plant Cell and Environment* **21,** 1219-1230 (1998).
9. Brown, B.E. Coral bleaching: causes and consequences. *Coral Reefs* **16,** S129-S138 (1997).
10. Marsh, J.A. Primary Productivity of Reef-Building Calcareous Red Algae. *Ecology* **51,** 254-& (1970).
11. Veal, C.J., Carmi, M., Fine, M. & Hoegh-Guldberg, O. Increasing the accuracy of surface area estimation using single wax dipping of coral fragments. *Coral Reefs* **29,** 893-897 (2010).