

# **Student Support for Studies of the Covariance of Fluorescent Coralline Pigments Under Changing Environmental Conditions**

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## **LONG-TERM GOALS**

The long-term goal of the CoBOP program, of which this project is a part, is to gain a better understanding of the sources, nature, and variability of upwelling radiance in shallow water. This will include both magnitude and spectral characteristics of the radiance. An additional goal is to understand the relationship of coral fluorescence to environmental stress and the possibility for using *in-situ* remote sensing techniques to monitor these events.

## **OBJECTIVES**

The objective of this project is to investigate the response of fluorescent pigments in corals subjected to thermal stress. Images of fluorescent emissions at 515 nm, 575 nm, and 680 nm were captured with our Low-light-level Underwater Multispectral Imaging System (LUMIS). The results of the study led to a better understanding of the nature of upwelling radiance and its variability in optically clear, shallow waters.

## **APPROACH**

Specimens of the Caribbean coral species *Montastraea faveolata* were collected using a paired-sampling technique from White Horse Reef, near Lee Stocking Island (LSI), the Bahamas. The specimens were transported back to Scripps for use in a controlled thermal stress experiment. Half of the corals were induced to bleach by elevating the water temperature to 4 °C above the average annual maximum at LSI. After one week of exposure to bleaching conditions, the temperature was returned to normal so that the recovery was monitored. Throughout the experiment, LUMIS images of 515, 575, and 680 pigment fluorescence for all of the coral samples were collected twice daily.

## **WORK COMPLETED**

The period covered by the funding of this project during 2001 was 3 months. During this time Dave Zawada (the student supported under this proposal) finished his Ph.D. Thesis and successfully defended. As such, the work accomplished consisted of some refinement of the data processing schemes of past years coupled with the writing and review of the thesis.

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Here, we present some of the previous results, as this work was being carried on into the period covered by this report.

In the first year of the program, the field operations were completed and the bleaching experiment produced a high-quality data set. Custom software was developed to correct for systematic errors introduced by the camera system. The second year of the project focused on analyzing the imagery. Inspection of the corrected images revealed that both the 515 and 575 nm signals emanated from the polyp centers and that fluorescence values rapidly decreased toward zero in the space between polyps.

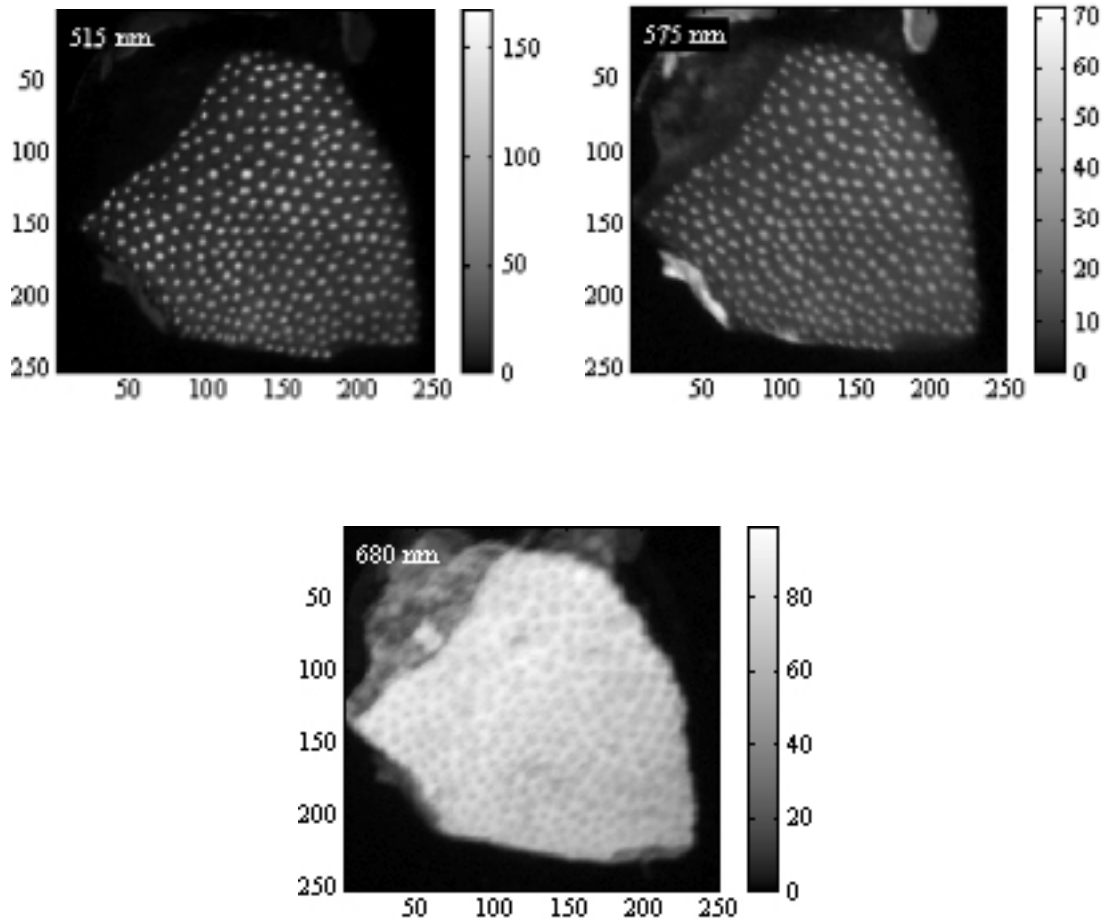
## RESULTS

The data set for this experiment consists of over 15,000 LUMIS images. Since one LUMIS image is composed of four distinct images, one for each spectral band, the total number of images that need to be processed is over 60,000. LUMIS was configured to capture fluorescent emissions from corals at narrow wavebands centered at 515, 575, and 680 nm. Figure 1 depicts a set of images corresponding to each of these wavebands. Pixel values are in digital numbers proportional to the number of photons collected by the CCD at a given pixel. The stronger the fluorescent emission, the higher the recorded digital number. This particular set of images is from the beginning of the experiment.

In this study, high-resolution (400  $\mu\text{m}/\text{pixel}$ ) multispectral imagery was employed to investigate the fluorescent responses of two host-based coralline pigments, plus chlorophyll, throughout the bleaching process. Analysis of the data disclosed both spatial and temporal patterns. The imagery revealed that fluorescent emissions of both the 515 and 575 host pigments were concentrated at polyp centers and declined by 70–90% in regions between polyps. In contrast to these pigments, chlorophyll fluorescence was more uniform, decreasing by only 10–30% around polyp centers. This distribution of the host-based pigments is consistent with the theory that these compounds may provide some measure of photoprotection to the coral reproductive organs and suggests a localization of these pigments so as to minimize interference with zooxanthellar photosynthesis. However, the results do not support the proposition that these pigments function as a sunscreen for the zooxanthellae.

A surprising outcome of this experiment was the resilience exhibited by these particular specimens of *Montastraea faveolata*. No indications of stress were detected for three of the seven specimens. One coral showed a dramatic bleaching pattern, but recovered, and the remaining three corals died.

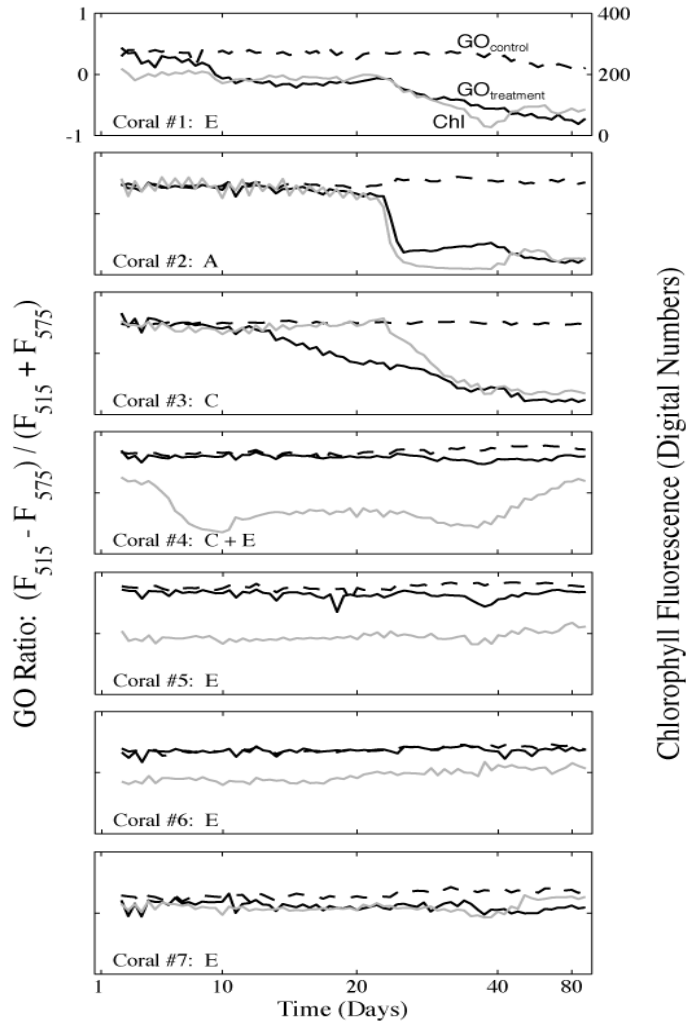
This variable response to the thermal stress prompted an interest in the genotypic nature of the zooxanthellae harbored by the coral specimens. These symbiotic algae were extracted from tissue samples collected at the onset of the experiment. DNA analysis revealed an odd mixture of zooxanthellar types or clades (Table 1). Interestingly, all of the treatment corals that survived the heat stress contained *Symbiodinium* E, while two of the dead corals did not contain any *Symbiodinium* E. The one outlier is treatment coral #1, which primarily harbored *Symbiodinium* E, but nevertheless died. What is especially intriguing is that this clade is locally abundant in coastal regions off Panama near river outflows. We therefore hypothesize that this clade contributes to the heartiness of the corals living in these habitats by tolerating environmental conditions inhospitable to other taxa of zooxanthellae. *Symbiodinium* E is uncommon in offshore reefs and in the Bahamas (N. Knowlton, Scripps Institution of Oceanography, pers. comm.). Perhaps the presence of this clade is responsible for the thermal tolerance exhibited by treatment specimens #4–7.



**Figure 1:** Images of coral fluorescence images from the 515 nm, 575 nm, and 680 nm spectral bands of LUMIS. Each image is 250 x 250 pixels in size. The color bar scales are in digital numbers recorded by the CCD camera.

Specimen	Clade	State
Coral 1	E	Died
Coral 2	A	Died
Coral 3	C	Died
Coral 4	C + E	Survived
Coral 5	E	Survived
Coral 6	E	Survived
Coral 7	E	Survived

**Table 1:** Zooxanthellar clades identified in the coral specimens from this experiment. The “State” column only pertains to treatment corals. All of the controls survived.



**Figure 2: The GO ratio. The normalized difference ratio of green (515 nm) to orange (575 nm) fluorescence is plotted for each of the seven coral pairs. The dashed line corresponds to the control and the black line to the paired treatment coral. For comparison purposes, the chlorophyll fluorescence of the treatment coral is plotted as a gray line. Letters in each plot designate zooxanthellae clades.**

The results of temporal analysis are summarized by the GO ratio: the normalized difference ratio of green (515nm) to orange (575 nm) fluorescence (Figure 2). The first noteworthy point is the correspondence to chlorophyll fluorescence. For both the controls and those treatments that survived the heat stress, the GO ratio remains relatively flat; for those corals that died, it drops and remains between -1 and 0.

While the end results of the GO ratio and chlorophyll fluorescence are the same, *i.e.* both clearly indicate coral death or survival, there are some discrepancies. For example, in corals #1 and 3 the GO ratio declines about 10 days prior to chlorophyll fluorescence. For coral #4, the GO ratio remains stable throughout the experiment, while chlorophyll fluorescence experiences several declines and recoveries. We speculate that such inconsistencies are caused by the different origins of the pigments.

The 515 and 575 pigments are believed to be animal-based, while chlorophyll is plant-based. It is possible that the particular zooxanthellae in coral #4 were more thermally sensitive.

## **IMPACT/APPLICATIONS**

As evidenced by the examples presented above, LUMIS imagery is capable of revealing some interesting characteristics about fluorescent coralline pigments and their response to thermal fluctuations. Especially significant is the discovery of the GO ratio. Besides being an additional indicator of coral bleaching, a sustained GO ratio of less than zero seems to signal impending coral death. It also has the advantage of being resistant to contamination from other sources of chlorophyll fluorescence, such as filamentous algae. Finally, the results of this study show a decline in the GO ratio prior to that of chlorophyll fluorescence, implying that this ratio can be used as an early warning indicator of imminent coral bleaching.

## **RELATED PROJECTS**

This work is part of the COBOP program, sponsored by the environmental optics program.

## **PUBLICATIONS**

Zawada, D.G. and J. Jaffe. Changes in the fluorescence of the Caribbean coral *Montastraea faveolata* during heat-induced bleaching. Limnol. & Oceanogr (In Press), 2001.

Zawada, D. G., Imaging Processing of Underwater Images, Journal of Ocean Engineering (submitted).