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 was 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

In the first year of the program, the field operations were completed and the bleaching experiment produced a high-quality data set. The corals proved to be more resilient than expected. After three weeks of exposure to a water temperature 4 °C above the average annual maximum, the treatment corals exhibited few signs of stress. At that point, the temperature was increased another 3 °C and maintained for one week. Even after this extreme treatment, little sign of stress was manifested in 40% of the specimens. A three-week recovery period followed before the experiment was terminated.

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14. ABSTRACT
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.

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Custom software was developed to correct for systematic errors introduced by the camera system. These corrections involved removing CCD effects, such as bias, dark current, and differential pixel sensitivity. In addition, data processing revealed significant image warping in each of the spectral bands. A considerable effort was spent developing an automated technique to align the images with one another.

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. To improve the signal-to-noise ratio, pixels associated with polyp centers were extracted from all of the 515 and 575 nm images and used in further analysis. At each sample time, the relevant pixel values were averaged, yielding a representative fluorescence value for each LUMIS waveband. This procedure produced three time series (one per fluorescent pigment) for each of the control and treatment corals. The time series contain 72 data points, two for each sample day of the experiment.

In analyzing multispectral imagery, a frequent goal is to distill the multiple values per pixel into a single number that preserves the essence of the discrete spectral bands at each pixel. To investigate the interrelationship between 515 nm (green) and 575 nm (orange) fluorescence, a variant of the standard band ratio was used, known as the normalized difference, resulting in the GO ratio

$$GO(t) = \frac{F(t)_{515} - F(t)_{575}}{F(t)_{515} + F(t)_{575}}$$

where $F(t)_b$ represents fluorescence at sample point t in the time series ($-1 \bullet GO(t) \bullet 1$). The GO ratio accentuates the variance between the input signals. For highly correlated signals, this ratio will be relatively flat.

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 µm/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.

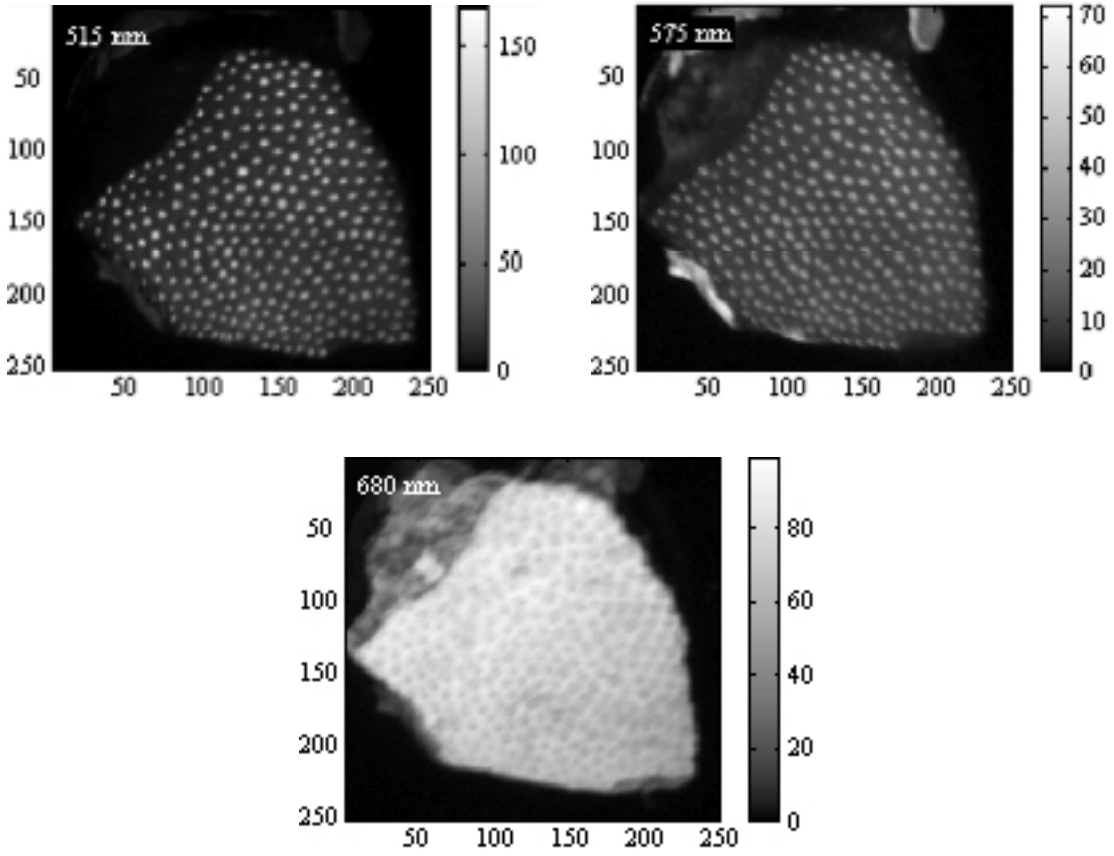

Figure 1: Images of coral fluorescence images from the 515 nm, 575 nm, and 680 nm spectral bands of LUMIS. Each image is 250 x 250 pixels in size. The color bar scales are in digital numbers recorded by the CCD camera.

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.

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.

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

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.

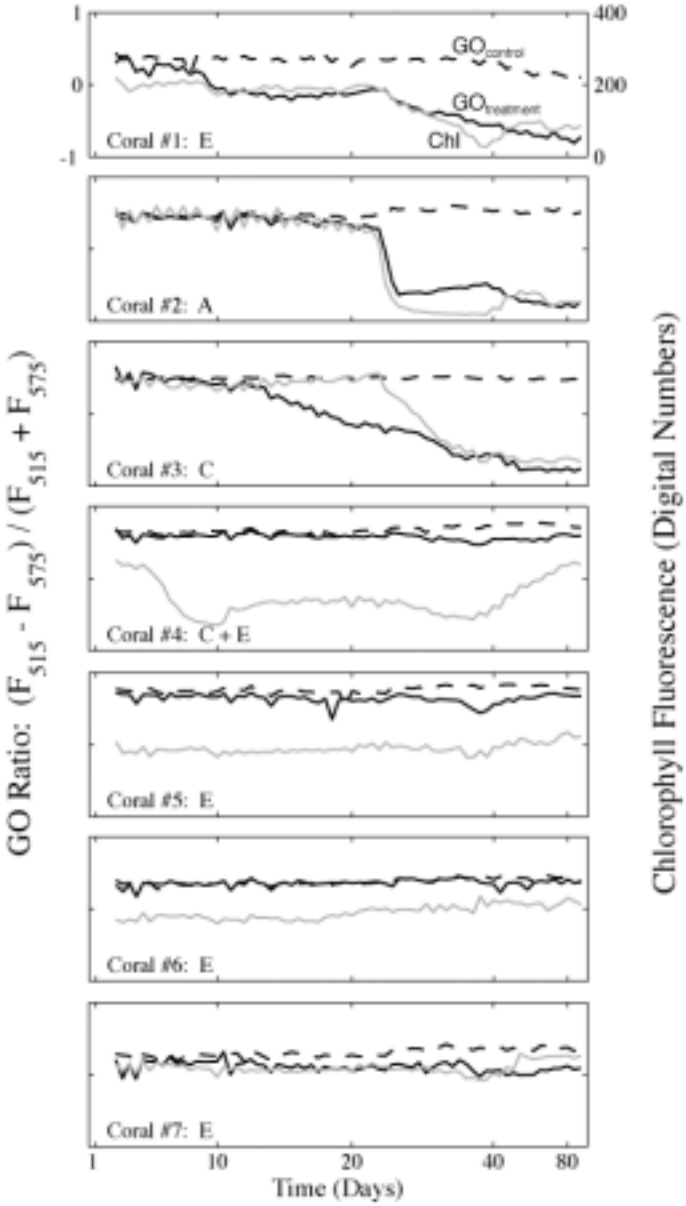

Figure 2: The GO ratio. The normalized difference ratio of green (515 nm) to orange (575 nm) fluorescence is plotted for each of the seven coral pairs. The dashed line corresponds to the control and the black line to the paired treatment coral. For comparison purposes, the chlorophyll fluorescence of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

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 review), 2001.