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Effects of Temperature stress on coral fluorescence and reflectance

Andrea Gomez
CUNY City College

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Effects of temperature stress on coral fluorescence and reflectance

A thesis by

Andrea Gomez

as part of the requirements for the degree of

Master of Science in Biology

The City University of New York

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Co-advisors: Dr. Ana Carnaval and Dr. Kyle McDonald
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Abstract.

Around the world, climate change, land-based pollution and fishing impacts are recognized as principal threats to coral reef ecosystems. Sea surface temperatures, in particular, are expected to change dramatically over the next decades and result in high coral mortality in some regions. Through controlled experiments, I examined changes in coral fluorescence and reflectance in response to water temperature alterations to assess the potential of employing these signatures as a diagnostic tool to measure coral health. At the NOAA Coral Culture and Collaborative Research Facility, I conducted controlled laboratory-based heat stress experiments on three Caribbean species of coral: *Acropora cervicornis*, *Orbicella annularis*, and *Porites furcata*. All species exhibited signs of stress and bleaching after the experiment, based on their chlorophyll fluorescence and reflectance responses. Upon exposure to water temperatures raised incrementally from 31°C to 34°C, no significant change was observed in host fluorescence for *A. cervicornis* and *O. annularis*; however, a change was detected for *P. furcata*. I nonetheless detected change in the algae’s (zooxanthellae) fluorescence in both *A. cervicornis* and *O. annularis*. Fluorometer analyses further demonstrated that the photosystems of the algae (PSII) were damaged by exposure to higher water temperatures, implying that the immediate effect of the heat stress was on them, as opposed to the hosts. These results support the use as algae fluorescence as an early indicator of change in coral health. While measurements of host fluorescence were not clear indicators of coral health and bleaching changes in my experiments, the detected changes in reflectance were consistent with declining coral health: due to the larger proportion of exposed skeletal material, stressed corals had higher reflectance values relative to controls. This study will be extended to examine possible linkages with satellite-based remote sensing measures utilized in NOAA’s Coral Reef Watch in an attempt to help managers and
conservation agencies to more efficiently monitor and preserve coral health at regional and
global scales.

**Introduction.**

Coral reefs cover less than 1% of the ocean floor yet contain a tremendous amount of
biodiversity, providing habitat for over 25% of all known marine species. In addition, they are
one of our planet’s most diverse and economically valuable biomes (Cesar et al. 2003). Globally,
the potential net economic benefit of healthy coral systems is estimated to be near $30 billion per
year, sustaining half a billion people worldwide (Wilkinson 2004). Reefs are found along the
coastline of more than one hundred countries, especially in tropical regions; millions of humans
depend on coral reefs for part of their livelihood or diet (Moberg and Folke, 1999). However,
approximately 60% of Earth’s coral reefs are currently threatened by natural and anthropogenic
impacts. Around the world, climate change, land-based pollution, and fishing impacts are
recognized as the most concerning risks to these ecosystems (Hughes et al. 2003).

Coral reefs are comprised of hundreds to thousands of individual animals called polyps
(phylum: Cnidaria), all of which are connected through living tissue and secrete a skeleton of
calcium carbonate. Hermatypic corals, also known as reef-building coral, are distributed in
shallow waters across the latitudes 35°S to 35°N, and are generally exposed to maximum
temperatures of circa 29°C, with an annual temperature flux of 4°C (Hume et al. 2013). Shallow
reef-building corals exhibit a unique symbiotic relationship with the unicellular *Symbiodinium*
algae. Maintaining this relationship is vital for coral health: these endosymbiotic dinoflagellates
are responsible for capturing solar energy, as well as nutrients, and provide the coral with more
than 95% of their metabolic requirements (Hoegh-Guldberg et al. 2007). This partnership with
the zooxanthellae is also thought to be essential to the coral’s resistance and resilience to bleaching. Bleaching occurs when the coral and algae experience stress, and their symbiotic relationship shifts from being a beneficial association to a harmful one; consequently the coral ends up expelling their symbiotic algae, leaving their calcium carbonate skeleton exposed, giving them their white, bleached appearance (Bhagooli and Hidaka, 2003; Baker et al. 2004). Different species of coral harbor distinct clades of zooxanthellae (recently identified as A through I; Pochon and Gates, 2010), with one of them recognized as the most thermally tolerant (clade D in Fabricius et al. 2004). Approximately 23% of all known coral are believed to be able to shift symbionts when stressed, obtaining temporary symbionts that are more tolerant to particular stressors (Goulet 2006; Goulet 2007).

Decreasing fluorescence and increasing reflectance levels are both seen as signs of declining coral health. Heat-stress experiments conducted on the common Indo-Pacific branching coral *Acropora yongei* demonstrated an initial decrease in the amount of green fluorescent proteins (GFP) and level of fluorescence prior to signs of bleaching, as coral health declined when exposed to warmer temperature conditions (Roth and Deheyn, 2013). *In-situ* spectral reflectance measurements collected off healthy and bleached Indo-Pacific scleractinian corals also revealed a spectral distinction between the two states (Holden and LeDrew, 1998). Building on those observations, I ask how elevated temperature affects the fluorescence and reflectance signatures of three Caribbean species of coral (*Acropora cervicornis, Orbicella annularis, and Porites furcata*). I use point-specific hyperspectral remote sensing measurements in a laboratory setting to observe the response of coral fluorescence and reflectance signatures to warmer waters. I hypothesize that heat-induced temperature stress will not only lead to declining coral health and overall decreased coral fluorescence, but also to an increase in coral reflectance.
as coral skeleton becomes more exposed when corals expel the zooxanthellae. My goal is to provide a foundation for future explorations of the use of satellite-based remote sensing to monitor coral reflectance to support improved management and preservation of coral reefs through non-invasive techniques.

The role and assessment of coral fluorescence, reflectance, and temperature

Fluorescence is caused by the absorption of light, and, along with reflectance, gives corals their perceived colors. Fluorophore pigments are those responsible for fluorescence in corals: they are able to absorb high energy and shorter-wavelength photons and reemit them at a lower energy and longer-wavelengths. There are two primary classes of fluorophores in reef-building corals. The first group includes the fluorescent proteins (Fps) that are found in the coral animal tissue (host). Four colors are associated with these corals Fps: cyan (CFP, emission peak 485-495 nm), green (GFP, emission maximum 500-524 nm), red (RFP, emission peak >560 nm), and blue/purple non-fluorescent chromoproteins (emission maximum normally at 595 nm; Palmer et al. 2009). The second group includes the photosynthetic pigments found in the unicellular algae (zooxanthellae) that live in the corals’ gastrodermal cells, with chlorophyll A (Chl. A) being the predominant pigment. Chl. A emits with a primary peak at ~685 nm, with a secondary peak at ~735 nm (Mazel et al. 2003). The intensity of the host fluorescence depends on the concentration of the zooxanthellae, which in high concentrations tends to obstruct and shade the corals’ Fps (Roth and Deheyn, 2013). Dead corals will not exhibit any host fluorescence, yet they still may weakly fluoresce red due to algal overgrowth (Treibitz et al. 2015). The ecological role of coral fluorescence derived from fluorescent protein families is still unclear. Two main hypotheses for the function of the green fluorescent protein family, for
instance, are photoprotection and improved photosynthesis for the symbiotic zooxanthellae (Johnsen 2012).

Coral reflectance is determined by the spectral absorption and fluorescent properties of many pigments. The zooxanthellae and the ectodermal and endodermal tissues of polyps contain many different pigments, including Chl. A, Chl. C, peridinin, GFP, and diatoxanthin; the composition and content of these pigments determine the optical characteristics of the coral (Hochberg et al. 2004; Torres-Perez et al. 2012; Xu and Zhao, 2014). Coral hyperspectral reflectance signatures can be very informative for coral identification, health assessment, and monitoring. Further, they may be taken from two classes of remote-sensing tools: hand-held instruments such as microscopes (which collect point-specific measurements), and sensors deployed aboard aircraft or satellites (which provide local to regional-scale measurements). Although spectral reflectance signatures can be difficult to obtain from underwater targets using devices observing from above the water surface due to effects of the seawater column and atmosphere, preliminary studies using point-specific measurements have found that hyperspectral signatures may be used to differentiate between coral disease states (e.g. *Orcella faveolata*; Anderson et al. 2013). Hyperspectral signatures of Caribbean corals have also been shown to enable discrimination between species through specific analyses of pigments and reflectance (Torres-Perez et al. 2012).

Complementing these point-scale data, aircraft and satellite remote sensing techniques have been used successfully to survey larger areas of the marine environment, offering continuous and more cost-effective surveys of the ocean (Mumby et al. 2004). Past research has shown that remote sensing can be used to monitor changes in corals: hyperspectral sensors potentially enable distinguishing between live corals, recently dead corals, and corals that have
been dead for an extended period of time, based on their reflectance signatures (Kutser et al. 2003). For instance, spectrometers with ten spectral bands have been shown to distinguish living *Porites* from partially dead, recently dead, and long dead individuals (Mumby et al. 2004). Also based on hyperspectral signatures, NASA’s Airborne Visible/Infrared Imaging Spectrometer (AVIRIS) hyperspectral sensor has been shown to discriminate between coral and algae (Hochberg and Atkinson, 2003).

The ability to utilize remote sensing techniques to survey corals regionally to globally is particularly attractive in the face of anthropogenic climate change. Already, coral bleaching outbreaks have increased in frequency and intensity over the past 30 years (Wellington et al. 2001). Sea surface temperatures are expected to further increase in multiple regions over the next few decades, resulting in biophysical stress and increased mortality in areas currently harboring corals (Jokiel and Coles, 1977; Clark et al. 2000; Ostrander et al. 2000; Sheppard 2003; Rowan 2004; McClanhan et al. 2007; Fitt et al. 2009). Despite involving multiple factors and changes to the marine environment, temperature is the only environmental variable that can cause coral bleaching at a regional or global scale. Because most corals already live near their thermal threshold, temperature increases of as little as 1°C above the current long-term summer maxima can potentially result in mass bleaching events (Lesser 1996).

The mechanism involved with coral bleaching continue to be documented as we advance experimental studies worldwide (Buddemeier et al. 2004). It is known that increased temperatures and light are responsible for preventing the coral’s algae from processing light (photoinhibition) by damaging the photosynthetic system of the zooxanthellae (Baird et al. 2009). Coral bleaching by heat stress involves the production of excess reactive oxygen species, which are toxic and contribute to oxidative damage, leading to metabolic dysfunction and expulsion of
the symbiotic zooxanthellae. Depending on the duration of the heat stress, it can ultimately cause death (Baird et al. 2009). Moreover, it has been reported that bleached corals experience a weakened immune system that can result in major shifts in the coral microbial assemblages, including an increased proportion of pathogenic microorganisms (Littman et al. 2011; Bourne et al. 2009). Yet, while heat temperature stress has been shown to negatively impact coral physiology, research suggests that the effects of temperature change may depend on the species and location of the coral (Fitt et al. 2009). By evaluating the impact of temperature stress on fluorescence and reflectance of three different species, I seek to advance the ability to monitor coral health across taxa and marine realms.

**Methods.**

I performed heat-temperature-stress experiments at the National Oceanographic and Atmospheric Administration (NOAA) National Ocean Service (NOS) Center for Coastal Environmental Health and Biomolecular Research, and the Hollings Marine Laboratory in Charleston, SC. Because less experimental work had been conducted on Caribbean corals compared to Indo-Pacific species, I selected three Caribbean species of coral for my study: *Acropora cervicornis*, *Orbicella annularis*, and *Porites furcata*. These taxa possess a wide range of fluorescence pigments and strong fluorescence signatures, and are thus appropriate for the questions asked. Importantly, two of them are considered under threat. Under the U.S. Endangered Species Act, *A. cervicornis* is listed as critically endangered, and *O. annularis* is considered endangered (Aronson et al. 2008).

Before being placed in their study vessels (250ml beakers), all coral fragments had their fluorescence and reflectance measurements taken with a BX-51 Olympus fluorescent microscope
(Olympus America, Inc., Center Valley, PA) outfitted with a Prism and Reflector Imaging Spectroscopy System (PARISS) hyperspectral spectrometer (Lightform, Inc., Asheville, NC). The system was equipped with custom long-pass filter cubes with 430 and 480 nm excitation filters (Chroma Technology Corp., Bellows Falls, VT), and a 10X submersible dipping scope since all measurements were taken underwater. The PARISS system supports simultaneous collection of data between 360 and 900 nm at 1 nm resolution by using an imaging spectrometer that is coupled with a spectrum detector (camera) which supports characterization of spectral signatures related to the fluorescence of corals. Fluorescent images of each specimen were taken with an Olympus MVX-10 fluorescent macroscope, which had its own custom long-pass filter cubes (430, 480, and 540 nm).

Individuals of all species were attached with glue to Teflon pegs to be exposed to progressively warmer conditions. The experimental setup consisted of two 20-gallon circulating water-bath aquaria, located side by side, under 156 watt AquaticLife lighting. The experiment was run for 17 days. Corals were exposed to a 08:16 h light-dark cycle to simulate that of the tropics. Each tank contained a heater, two circulation pumps, nine 250 ml beakers, and nine air lines (one for each beaker). A control aquarium was set at 26°C, and the test aquarium was initially set at 31°C. To test whether I could measure responses to rapid changes and fluctuations, the test aquarium temperature was increased incrementally to 32°C on day 12, 33°C on day 13, and lastly 34°C on day 15. The Photosynthetically Active Radiation (PAR) was initially set to 100 µmol photons m⁻²s⁻¹ but raised to 135 on day 10 to further increase the level of stress imposed on the system. There were three replicates of each of the three species (nine beakers in each temperature treatment), totaling 18 beakers. Temperature, salinity, and fluorescence and reflectance measurements were taken daily at noon. To record the reference down-welling
irradiance for the reflectance measurements, a National Institute of Standards and Technology (NIST) white-tile standard was used, with a reflectance value of 99.8%. Since the fluorescent and reflectance measurements were taken directly above the coral, and because the water column between the dipping scoop and coral was negligible, no correction for attenuation was needed. The water in the beaker was changed once a day to maintain the salinity level of approximately 35 ppm. A Walz Pulse Amplitude Modulated (PAM) Fluorometer was used to measure the effective quantum yield \((Y)\) of photosystem II (PSII) in the zooxanthellae, defined as Beer et al. (1998):

\[
Y = \frac{(F'_m - F)}{F'_m}
\]

where \(F'_m = \text{maximal fluorescence yield following a saturating light pulse}\),
\(F = \text{normal fluorescence in light}\).

These measurements were carried out on days 11, 14, and 17 for all three species. Effective quantum-yield data were recorded directly from the PAM fluorometer. The statistical computer language R was used to test for statistical significance between the control and heated samples on day 17 using the Mann-Whitney U Test (R Core Team 2014).

Fluorescence and reflectance graphs were used to visually inspect the data. Graphs of the averaged fluorescence data were produced for each species and treatment in Excel (Microsoft 2010), displaying relative intensity vs. wavelength (nm) for the fluorescent data. To graph the reflectance data, averages were first taken from the reflectance spectra of three controls and heat samples, per species, for the visible wavelengths (400 – 700 nm). The reflectance \((R)\), defined as the ratio of the reflected radiant flux to the incident radiant flux (Morel and Smith, 1993), was calculated as:
\[ R = \frac{L_c}{E_g} \]

where \( E_g = \pi \times L_p \)

\( L_c \) = coral’s radiance (upwelling)

\( L_p \) = radiance of the Lambertian surface

The reflectance value from this equation was multiplied by 100 to provide the value as a percentage. Next, the reflectance spectra of the control corals were combined and averaged for days 4, 6, 8, 10, 11, 12, 14, 16, and 17. Graphs were produced in Excel (Microsoft 2010) displaying reflectance (%) vs. wavelength (nm), displaying the average reflectance spectra of the control corals and of the heated sample corals for days 4, 6, 8, 10, 11, 12, 14, 16, and 17. Excel was also used to calculate the standard deviation for the averaged reflectance measurement of the control samples in the visible wavelengths (400 – 700 nm) from days 4, 6, 8, 10, 11, 12, 14, 16, and 17; this was graphed separately.

Using the statistical computer language R, a Mann-Whitney U Test (R Core Team 2014) was run for the control vs. heated samples on days 11 and 17 to test for statistical differences between treatments on those specific days. The same test was employed to compare values before and after the temperature increase from 31°C to 32°C on Day 12. Acropora cervicornis and O. annularis exhibit the following fluorescent proteins: cyan, GFP, and chlorophyll A. For P. furcata, an additional GFP protein is present, instead of a cyan protein, as well as a red protein. The test was thus run individually for each fluorescent peak related to the different proteins (A. cervicornis at 485 nm, 512 nm, 680 nm, and 730 nm; O. annularis at 481 nm, 505 nm, 682 nm, and 740 nm; P. furcata 507 nm, 536 nm, 590 nm, 684 nm, and 726 nm), and the five distinct reflectance peaks (A. cervicornis ~434 nm, 490 nm, 570 nm, 606 nm, and 635 nm; O. annularis ~438 nm, 504 nm, 576 nm, 606 nm, and 632 nm; P. furcata ~436 nm, 496 nm, 579 nm, 605 nm, and 632 nm). Due to the small sample size of these exploratory analyses (\( n = 3 \)), the threshold for
statistical significance was set at $\alpha = 0.10$.

Cluster analyses were performed on the reflectance data in R using the package “stats” to further document differences in reflectance spectra between the control and heated samples. These analyses included reflectance spectra for the visible wavelengths (400 - 700 nm) for the control and heat samples on days 4, 6, 8, 10, 11, 12, 14, 16, and 17, and were run for each species separately. Cluster dendrograms were produced for each species.

**Results.**

Temperature treatments adversely affected all three Caribbean species of coral and induced bleaching. As corals were warmed, the test sample corals became paler in color relative to the control corals (Figure 1). All polyps in the heated samples became retracted and tightly tucked in, whereas the polyps of the corals in the control setting remained open (Figures 1 and 2).

The fluorescent images taken on the last day of the experiment confirmed that the heated samples of *A. cervicornis* were severely bleached, whereas the *O. annularis* and *P. furcata* heated samples appeared less impacted. Heated samples of *A. cervicornis* exhibited lower fluorescence relative to control samples (one notices a more intense red color in *A. cervicornis* control given that the algae fluoresce red; Figure 2). Heated samples of *Orbicella annularis* showed slightly lower fluorescence than the control samples (green stripes of the heated sample are fainter than, or not as pronounced as, those in the control samples; Figure 2). However, heated samples of *P. furcata* were able to retain zooxanthellae and exhibited high levels of fluorescence (see intense red color in both *P. furcata* control and heat sample; Figure 2).
Figure 1: Pictures of the three target species of Caribbean coral (from left to right: *Acropora cervicornis*, *Orbicella annularis*, and *Porites furcata*) under controlled temperatures (26°C, top) and after heat experiment (31-34°C, bottom). All pictures were taken on the last day of the experiment.

Figure 2: Fluorescent pictures of experiment controls (top) vs. heat samples (bottom) for the three target species of coral: *Acropora cervicornis* (left), *Orbicella annularis* (middle), and *Porites furcata* (right). All pictures were taken on the last day of the experiment.
Results from the PAM fluorometer revealed that all species were adversely impacted by heat stress, showing a decrease in the efficiency of quantum yield for photosystem II (PSII). While all control samples exhibited a stable quantum yield of PSII, all heated samples experienced sharp declines in their photosystem efficiency between days 11 and 17 (Figure 3). For all species, the control and heated samples on day 17 exhibited statistically significant differences (all tests yielded the same p-value; p = 0.0636).

By day 11 (heated samples at 31 °C), high levels of variability in relative fluorescence were noticed across species and between the control and heated samples (Figure 4). Host fluorescence of *A. cervicornis* seemed to differ between control and heated samples (for cyan: 485 nm and GFP: 512 nm fluorescent peak wavelength), while algae fluorescence did not (chl. A: 680 nm, 730 nm). The same general trend was observed in *O. annularis*: host fluorescence differed between control and heated samples (for cyan fluorescence: 480 nm, though not GFP: 512 nm).
505 nm), but algae fluorescence did not (chl. A: 682 nm; only a small difference was observed for chl. A fluorescence at 740 nm). However, *P. furcata* followed a different trend on day 11, showing small differences in host fluorescence between the control and heated samples (at 506 nm and 536 nm; red protein at 590 nm) yet large differences in algae fluorescence (chl. A fluorescent peak at 730 nm, though not for algae fluorescence at 680 nm).

On day 17 (last day of the experiment, heated samples at 34°C), the patterns reversed for some of the host and algae fluorescence (Figure 4). Large differences were noticed in algae fluorescence between control and heated samples of *A. cervicornis* (680 nm and 730 nm) and *O. annularis* (682 nm and 730 nm), while only small differences were noticed in host fluorescence. The opposite occurred for *P. furcata*, where host fluorescence (506 nm, 536 nm and 590 nm) showed moderate to large changes between control and heated samples, but little to no change was detected in algae fluorescence (680 nm and 730 nm).
Figure 4: Graphs of average fluorescence of the three replicates under control and heated treatments for three species of coral: *Acropora cervicornis* (graphs 1-4), *Orbicella annularis* (graphs 5-8), and *Porites furcata* (graphs 9-13). Each graph represents a different fluorescent protein (cyan, GFP, red, chl. A or chl. C). “Before” represents the time when all coral fragments were exposed to the control temperature.
Consistent with the highly variable levels of coral fluorescence, statistical tests performed on the fluorescence data detected minor differences between the control and heated samples. Test results for *A. cervicornis* revealed a few statistically significant differences between the control and heated samples for day 11, and none for day 17 (p-value/fluorescent peak wavelength = 0.08/458 nm, 0.06/512 nm, and 0.10/730 nm; Table 1). In *A. cervicornis*, none of the control and heated samples exhibited statistically significant differences in their fluorescence signatures on day 17 (Table 1). *O. annularis* algal fluorescent peaks (682 nm and 740 nm) revealed statistically significant differences between control and heated samples on days 11 and 17 (p-values for algae fluorescent peaks at day 11 and 17 for 680 nm and 740 nm respectively = 0.10 and 0.27, 0.08 and 0.08; Table 1). The host fluorescence and chl. A peak at 682 nm had statistically significant differences on day 11 for *O. annularis* (Table 1). In *P. furcata*, only the algae fluorescent peak at 684 nm exhibited statistically significant differences on day 17 ((p-value = 0.10; Table 2).

Table 1: Fluorescent measurements for both *Acropora cervicornis* and *Orcibella annularis* taken on days 11 and 17. Results are given per species of coral and their associated fluorescent peaks (nm); p-values were obtained through a Mann-Whitney U Test based on three replicates (n=3). Asterisks indicate statistical significance with α = 0.10.

<table>
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<th>11</th>
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<td>0.70</td>
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Table 2: Fluorescent measurements for *Porites furcata* taken on days 11 and 17 in nm; p-values were obtained through a Mann-Whitney U Test based on three replicates (n=3). Asterisks indicate statistical significance with α = 0.10.

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<tr>
<td>Fluorescent peaks (nm)</td>
<td>507</td>
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<td>590</td>
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<td>507</td>
<td>536</td>
<td>590</td>
<td>684</td>
<td>726</td>
</tr>
<tr>
<td>p-value</td>
<td>0.27</td>
<td>0.16</td>
<td>0.70</td>
<td>0.40</td>
<td>0.12</td>
<td>0.12</td>
<td>0.2</td>
<td>1.0</td>
<td>0.10*</td>
<td>1.0</td>
</tr>
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</table>
A noticeable difference in reflectance was observed between control and heated samples for all species. Increased reflectance values were seen in heated samples, with days 16 and 17 yielding the highest values for all species (Figure 5). Five distinct reflectance peaks were seen for each species: *A. cervicornis* = 434 nm, 490 nm, 570 nm, 606 nm, and 635 nm; *O. annularis* = 438 nm, 504 nm, 576 nm, 606 nm, and 636 nm; and *P. furcata* = 436 nm, 596 nm, 579 nm, 605 nm, and 632 nm.

All species had different levels of variation in their reflectance measurements for the averaged control samples consisting of days 4, 6, 8, 10, 11, 12, 14, 16, and 17 (Figure 6). *A. cervicornis* showed the smallest amount of variation around its first reflectance peak (434 nm), and then relatively small deviation for the rest of the spectrum. *O. annularis* depicted roughly uniform small variation throughout the spectrum (Figure 6). *P. furcata* had the largest amount of variation throughout the entire spectrum (Figure 6).
Figure 5: Reflectance (%) vs. wavelength (nm) for all three species of coral throughout selected days of the experiment: AC=Acropora cervicornis, OA=Orbicella annularis, and PF=Porites furcata; C=control, H=heated samples. Control values consist of averaged measurements across days 4, 6, 8, 10, 11, 12, 14, 16, and 17.
Figure 6: Average reflectance (%) vs. wavelength (nm) for all three species under controlled temperature regimes. Values were collected for three replicates on days 4, 6, 8, 10, 11, 12, 14, 16, and 17. Standard deviation is plotted as error bar (C=control, AC=A. cervicornis, OA=O. annularis, and PF=P. furcata).
All species displayed statistically significant differences in reflectance levels between control and heated samples. For *A. cervicornis*, four out of five p-values (434 nm = 0.06, 570 nm = 0.10, 606 nm = 0.08, and 635 nm = 0.07) revealed statistically significant differences on day 11, but only two out of five p-values (434 nm = 0.10 and 490 nm = 0.10) exhibited statistically significant differences for day 17 (Table 3). *O. annularis* exhibited no statistically significant differences on day 11, but four out of five p-values (438 nm = 0.10, 576 nm = 0.10, 606 nm = 0.08, and 632 nm = 0.08) were significantly different on day 17 (Table 3). *P. furcata* also had no statistically significant differences on day 11, but all five p-values (436 nm = 0.10, 496 nm = 0.10, 579 nm = 0.10, 605 nm = 0.08, and 632 nm = 0.10) displayed statistical significant differences on day 17 (Table 3).

Table 3: Reflectance measurements taken on days 11 and 17 for each species of coral (*Acropora cervicornis*, *Orbicella annularis*, or *Porites furcata*) and their associated reflectance peaks (nm); p-values obtained through a Mann-Whitney U Test (n=3). Asterisks indicate statistical significance with α = 0.10.

<table>
<thead>
<tr>
<th>Day</th>
<th>11</th>
<th>11</th>
<th>11</th>
<th>11</th>
<th>11</th>
<th>17</th>
<th>17</th>
<th>17</th>
<th>17</th>
<th>17</th>
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<tbody>
<tr>
<td><strong>A. cervicornis</strong></td>
<td></td>
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<tr>
<td>Reflectance peaks (nm)</td>
<td>434</td>
<td>490</td>
<td>570</td>
<td>606</td>
<td>635</td>
<td>434</td>
<td>490</td>
<td>570</td>
<td>606</td>
<td>635</td>
</tr>
<tr>
<td>p-value</td>
<td>0.06*</td>
<td>0.30</td>
<td>0.10*</td>
<td>0.08*</td>
<td>0.07*</td>
<td>0.10*</td>
<td>0.10*</td>
<td>0.66</td>
<td>0.70</td>
<td>0.40</td>
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<tr>
<td><strong>O. annularis</strong></td>
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<tr>
<td>Reflectance peaks (nm)</td>
<td>438</td>
<td>504</td>
<td>576</td>
<td>606</td>
<td>632</td>
<td>438</td>
<td>504</td>
<td>576</td>
<td>606</td>
<td>632</td>
</tr>
<tr>
<td>p-value</td>
<td>0.37</td>
<td>1.0</td>
<td>0.40</td>
<td>0.40</td>
<td>0.12</td>
<td>0.10*</td>
<td>0.40</td>
<td>0.10*</td>
<td>0.08*</td>
<td>0.08*</td>
</tr>
<tr>
<td><strong>P. furcata</strong></td>
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<tr>
<td>Reflectance peaks (nm)</td>
<td>436</td>
<td>496</td>
<td>579</td>
<td>605</td>
<td>632</td>
<td>436</td>
<td>496</td>
<td>579</td>
<td>605</td>
<td>632</td>
</tr>
<tr>
<td>p-value</td>
<td>0.70</td>
<td>0.82</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.10*</td>
<td>0.10*</td>
<td>0.10*</td>
<td>0.08*</td>
<td>0.10*</td>
</tr>
</tbody>
</table>

Cluster analyses help to illustrate distinctions in the reflectance spectra of control vs. heated corals. For all species, two main groups were apparent in the cluster dendrograms (Figure 6) – one of them clustering the reflectance’s of heated samples on days 16 and 17. In all species, the reflectance spectra of the control group were clustered along with that of heated samples collected on earlier days of the experiment (right branch, Figure 7).
Figure 7: Cluster dendrograms reflectance amounts, per species (top, AC=Acropora cervicornis; middle, OA=Orcibella annularis; bottom: PF=Porites furcata; C=Control, H=Heat). Includes control and heated samples reflectance spectra for days 4, 6, 8, 10, 11, 12, 14, 16, and 17, for the wavelengths 400-700 nm.
Discussion.

Adding to the existing literature (Jones et al. 1998; Fitt et al. 2001; Downs et al. 2002; Eakin et al. 2010), this study demonstrates that heat stress negatively affects overall coral health and induces bleaching. In the beginning of the experiment, when samples were not experiencing stress, all corals had their polyps out and open, and were active. Near the middle and end of the heat treatment, all three species retracted their polyps, and became less active. A. cervicornis and O. annularis were both severely bleached, appearing much paler in color relative to the controls. P. furcata looked only slightly pale, yet its polyps were tightly tucked in after the heat treatment, as a sign of stress (Brown et al. 1994).

Fluorescence

The data suggest that algae fluorescence may be an indicator of coral health in some, but not all, species. Heated samples of P. furcata held on to most of its zooxanthellae (fluorescing in red), unlike those of A. cervicornis, which displayed a much fainter red. The red fluorescence of the algae of O. annularis is not detected in the control or heated sample because this species has very bright and intense host fluorescent proteins, which overpower the algae fluorescence (Dr. Sylvia Galloway, personal communication).

Along with the observations of polyp configuration, the results suggest that P. furcata is more resistant to bleaching relative to the other two species. Past research demonstrated that P. furcata has relatively high amounts of Fps compared to Acropora, and that such increased levels may be responsible for reducing bleaching in this species (Salih et al. 2000; Salih et al. 2006). Observations of a natural bleaching event in Australia, where bleached corals had lower fluorescence relative to unbleached corals, have been used to support the role of Fps as
photoprotective (Salih et al. 2000). Although algae that colonize dead coral do contain chlorophyll A and fluoresce red, they generally have a weaker signal and different texture relative to the symbiotic algae (Trebitz et al. 2015).

When heat stress is introduced, host fluorescence is first expected to decrease, then to become amplified due to expulsion of the algae, ultimately diminishing when corals die (Roth and Deheyn, 2013). By the last day of the heat experiment, however, no significant change was noticed in host fluorescence in either A. cervicornis or O. annularis. Both species nonetheless experienced a decrease in the algae fluorescence peaks. This suggests that the immediate effect of the heat stress was on the algae, not on the host, and that the algal fluorescence decreased as the coral began to bleach and expel them. These results support previous suggestions that chlorophyll fluorescence can be used as a reference for evaluating coral bleaching (Zawada and Jaffe, 2003). P. furcata, in contrast, showed decreased host fluorescence but little to no change in the algal fluorescence when control and heated samples were contrasted at the end of the experiment. These results may suggest that P. furcata possesses a more heat tolerant zooxanthellae clade. The lineage of zooxanthellae has been shown to depend on coral species, depth and location, and can be a key factor determining coral tolerance to heat stress (LaJeunesse 2002; Fabricius et al. 2004). A recent assessment proposed that A. cervicornis contains one symbiont type (either A3 or C12), that O. annularis may harbor one or two symbiont types (B1, C3, or D3), and that P. furcata has one to two symbiont types (A4, B1, or C4; LaJeunesse 2002). Clade B1, which both O. annularis and P. furcata may potentially harbor, is thought to photo-acclimate better to high and low irradiances compared with other clades, therefore increasing the likelihood of coral survival when exposed to higher temperatures (Iglesias-Prieto and Trench, 1997a). My results support this hypothesis: both O. annularis and P. furcata responded better to
heat than *A. cervicornis*, which is thought not to harbor the clade B1, and, in this experiment, was the first species to bleach.

The PAM results revealed that heat stress impacted and damaged algal PSII, lowering their overall efficiency in all species. This mechanism explains published observation of severe damage and inactivation of PSII, as documented in southern Florida during the summer of 1997, when coral colonies experienced seawater temperatures above 30°C for several weeks (Warner et al. 1999). I argue that this decrease in the efficiency of quantum yield for photosystem II may be used as an early indicator of change in coral health; this is a promising possibility for future *in-situ* and remote sensing studies of coral health.

Fluorescence level was not a reliable variable to assess coral health and bleaching. This was unexpected, and contradicts previous observations of changes in green fluorescence with declining coral health (Roth and Deheyn, 2013). Based on Roth and Deheyn’s (2013) study, I had expected that the last day of the experiment would yield the highest differences between control and heated samples. In my experiment, however, *A. cervicornis* had more statistically significant differences between control and heated samples on day 11, not day 17 (the last day of experiment). In fact, none of the *A. cervicornis* fluorescence results had statistically significant differences on day 17 despite the fact that samples of this species were clearly unhealthy and experiencing bleaching.

Fluorescence results for the other two species were better aligned with my previous expectations. *O. annularis* exhibited statistically significant differences for algae fluorescence on day 17, which supports the idea that heat stress negatively impacts algal fluorescence and correlates to decreased coral health. However, both host fluorescence peaks displayed statistically significant differences on day 11 and not on day 17. A possible explanation for this
observation may be that the *O. annularis* heat samples began to bleach early on, uncovering the host fluorescence pigments and thus amplifying their intensity, and then the coral died – at which point opportunistic algae may have started to colonize the coral skeleton, once again resulting in decreased host fluorescence (dead corals don’t exhibit host fluorescence). Conversely, on day 11, none of the fluorescence peaks had statistically significant differences in *P. furcata* (implying that the control and heated samples had similar levels of fluorescence). For this species, algae fluorescent peak at 684 nm exhibited the only statistically significant response, on day 17, again supporting the idea that the immediate effect of the heat stress is on the zooxanthellae. More experimental laboratory studies are needed to elucidate whether coral fluorescence is a practical variable to employ for assessing coral health effectively.

**Reflectance**

The reflectance results provide a more consistent assessment of coral overall health compared to the fluorescence results. Reflectance graphs for all species display obvious changes between control and heated samples with the latter yielding a higher magnitude of reflectance as the experiment continued. Heated samples had higher reflectance compared to the control samples because zooxanthellae are expelled as corals began to bleach, leaving the coral carbonate skeleton exposed (Kleppel et al. 1989). Because the skeleton is white, corals become optically more reflective as bleaching occurs. Even *P. furcata*, which appeared to be more resistant to heat stress, showed an increase in reflectance in heated samples compared to controls. These results validate previous claims that the reflectance spectra of healthy and bleached corals are optically distinct (Holden and LeDrew, 1998). This is important as it implies that the use of
hyperspectral remote sensing to monitor and assess coral health based on reflectance spectra is a true possibility for the future.

The first two peaks observed in the reflectance data of all three species have not been previously reported. It is likely that this observation was enabled because the hyperspectral instrument employed in this study obtained reflectance spectra at a 1 nm resolution, whereas most other studies to date have used instruments with lower spectral resolution. These newly reported reflectance peaks may be associated with microalgae or reflectance from the coral skeleton that have very low magnitudes of reflectance, and which can only be picked up at fine spectral resolutions. This being the case, and if these peaks in coral reflectance signature render the coral spectra more distinguishable from that of other benthic substrates (e.g. sand, algae), these peaks may potentially aid in remote sensing applications. However, it is also possible that the peaks are being caused by an element in the coral surroundings, as most fluorescent proteins emit either in the green or red wavelength regions (Gruber et al. 2008).

Despite the low number of replicates, my results suggest a correlation between coral skeletal density and variance in measured reflectance. Graphs of the standard deviation of reflectance values of specimens of *A. cervicornis* and *O. annularis* that were kept under the control treatment show all measurements to be very close to the mean of the three replicates. On the other hand, values measured from control samples of *P. furcata*, a species known to have higher skeletal density relative to the other two, showed much higher standard deviation. I hypothesize that the increased reflectance of organisms of higher skeletal density will result in more noise and variation detected by the hyperspectral instrument.

Although all species showed significant changes in reflectance spectra between control and heat samples at the end of the experiment (day 17), the data provide evidence that coral
response to heat stress varies widely by taxa (McClanahan et al. 2005). \textit{A. cervicornis}, for instance, also had statistically significant differences in reflectance on day 11. In fact, for this species, reflectance spectra of control and heated samples were more dissimilar on day 11 compared to 17. If this observation is confirmed by additional experiments, it may be that this species bleaches and dies faster than the other two, resulting in distinctive differences earlier in the experiment due to more exposed coral carbonate skeleton. Although speculative, it is possible that, by day 17, microfilament algae were already colonizing the dead coral framents of that species, reducing its reflectance.

Cluster analyses successfully grouped control and heated samples according to their reflectance spectra, generally assigning healthy and bleached fragments to distinct clusters. In all species, there were nonetheless instances where heated samples were clustered with control specimens. I hypothesize that this may be an effect of secondary colonization of dead corals by algae, which affects the magnitude of the reflectance spectra.

\textbf{Conclusions.}

In this study, coral fluorescence and reflectance signatures were analyzed to assess the possibility of using them as diagnostic tools to assess coral health. No significant change was noticed in the host fluorescence for \textit{A. cervicornis} and \textit{O. annularis}, however there was a decrease in chlorophyll fluorescence, suggesting that the immediate effect of the heat stress was on the algae. Conversely, \textit{P. furcata} experienced a decrease in host fluorescence but little to no change in the algae fluorescence, implying that this species might possess a more heat tolerant algae clade. This study supports the idea that coral response to heat stress varies widely by taxa. Statistical analyses suggest that fluorescence is not a reliable variable to assess coral health and
bleaching, whereas changes in reflectance spectra are consistent with declining coral health. There was a statistically significant difference between the reflectance spectra of healthy and bleached coral. The two novel peaks before 500 nm in the reflectance data have not been recorded before and could help make the coral reflectance signature more distinguishable from that of other benthic substrates (e.g. sand, algae) when using remote sensing tools. The data from this study supports the proposal for future use of hyperspectral remote sensing techniques to monitor and assess coral health based on their reflectance spectra. Future laboratory work should repeat the heat stress experiments with higher numbers of individuals and fixed temperature conditions (cold stress should also be examined) to assess whether these preliminary findings are further supported.

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References.


