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NORTHERN ILLINOIS UNIVERSITY

Symbiodinium Reactive Oxygen Species (ROS) Production and Migration in the Octocorals Sympodium sp. and Sarcothelia sp.

A Capstone Submitted to the

University Honors Program

In Partial Fulfillment of the

Requirements of the Baccalaureate Degree

With Honors

Department Of

Biological Sciences

By

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DeKalb, Illinois

Summer 2019
University Honors Program
Capstone Approval Page

Capstone Title (print or type)  
*Symbiodinium* Reactive Oxygen Species (ROS) Production and Migration in the Octocorals *Symposium* sp. and *Sarcothelis* sp.

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Department of (print or type)  **Biological Sciences**

Date of Approval (print or type)  **6/24/19**

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HONORS CAPSTONE ABSTRACT

Guidelines

Your abstract should begin with a definitive statement of the problem of project. Its purpose, scope and limit should be clearly delineated. Then, as concisely as possible, describe research methods and design, major findings, including the significance of the work, if appropriate, and conclusions.

Students whose thesis involves “creative” work (original, fine art, music, writing, theatre or film production, dance, etc.) should describe process and production. Indicating the forms of documentation on file as “thesis” materials.

Please have your advisor review your abstract for organization, content, grammar and spelling before submission.

Climate change may be increasingly causing corals to bleach, as bleaching may be triggered by elevated temperatures and light. Corals bleach due to their symbionts’ overproduction of reactive oxygen species (ROS). This mechanism breaks down the syntrophic relationship between corals and symbionts, as the symbionts either migrate or die (Parrin et al., 2012).

We primarily aimed to identify the relationship between stress, ROS production, and symbiont migration in the octocorals, *Sympodium* sp. and *Sarcothelia* sp. The experimental groups were stressed with 140 µmol photons sec⁻¹m⁻² for 4 hours (Table 1), while the control groups were kept in a culture tank with minimal light. The groups were put in finger bowls in an incubator with a probe that detects ROS for 1 hour. Through Image Pro 6.3, we were able to quantify background and colony luminance that represents ROS production. We also used the manual tag feature in the software to count the migrating and non-migrating symbionts. Our data is shown in graphs (Figs. 3-6) and paired comparisons (Tables 2-3).

In general, there was a positive correlation between stress and colony luminance and moving symbionts on both *Sympodium* sp. and *Sarcothelia* sp. (Figures 3-6). There was a greater correlation between relative luminance and stress in *Sympodium* as compared to stressed *Sarcothelia* (Tables 2-3). This implies that in the presence of ROS, symbionts are more likely to exit the cells of *Sarcothelia*. We had good control groups for both octocorals, as there wasn’t a significant difference between nonmoving symbionts in the control and stressed groups in either (Figure 7). To form more conclusive results, we could have done more trials, while limiting our errors, on both species.

Because coral bleaching is detrimental to corals and other marine organisms, many sought to find ways to prevent or alleviate it. Environmental conditions that may prevent or aid in the recovery of bleached corals include shade and deeper waters, as they lack in temperature and illumination (Graham et al., 2015). Symbionts may avoid stress by migrating to the coenenchyme lumen, then back to the stolons of the coral tissues, once the stress ceases (Parrin et al., 2012). Parrin and his team, also found that because *Sarcothelia* and *Sympodium* had higher surface area coenenchyme than *Phenganax parrini*, they had more symbiont recovery. *Acropora millepora* increased their tolerance to stress by changing from type C symbionts to type D symbionts (Berkelmans and van Oppen, 2006). Further studies on the syntrophic associations between corals and symbionts, may help increase knowledge of the coping mechanisms that may mitigate coral bleaching.
Symbiodinium Reactive Oxygen Species (ROS) Production and Migration in the Octocorals Sympodium sp. and Sarcothelia sp.

**Introduction**

Corals reefs harbor the most diverse communities of marine organisms in the world. They are also important for nutrient cycling and nitrogen and carbon fixation (Johannes et al., 1972). For many, their syntrophic associations with the dinoflagellates, *Symbiodinium* spp, is central to their survival (Blackstone & Golladay, 2018). Syntrophic associations describe symbiotic relationships in which symbionts use each other for energy conversion in order to carry of photosynthesis and cellular respiration. In this case, the corals carry out cellular respiration, while the *Symbiodinium* carry out photosynthesis and cellular respiration. For simplicity sake, *Symbiodinium* spp. will be referred to as symbiont(s).

The presence of cooperators, symbionts that translocate their photosynthate to the corals, would favor selection at the level of the coral. However, selection at the level of the symbionts would favor defectors, symbionts that use their own photosynthates for replication (Blackstone & Golladay, 2018). This is a common biological conflict, as higher-level beings should be prioritized and conserved. This conflict may be mediated by the structure of the population the symbiont lives in, as less dense population and decreased migration may decrease harmful symbiosis. Reactive oxygen species (ROS) production by the symbionts is a mechanism of conflict mediation in coral communities (Blackstone & Golladay, 2018). ROS may act as a signal that leads to the programmed cell death or expulsion of defectors.

Environmental stress is increasingly causing coral bleaching, a breakdown in the syntrophic association between the corals and symbionts (Parrin et al., 2012). This is because bleaching is often triggered by elevated temperatures and light. Production of ROS by symbionts has been shown to be a cause for the bleaching of corals. ROS forms when photosynthetic apparatus of symbionts shifts in the direction of reduction of molecular oxygen. ROS may trigger programmed cell death in the symbionts (Weis, 2008). Stress may cause symbionts to leave their intracellular locations (polyps) and migrate into the gastrovascular (coenenchyme) lumen in several species of octocorals (Parrin et al., 2012). Here the symbionts are safer, and they may return to the polyps once the environment stabilizes. This behavior exhibited by the symbionts, would be favored by higher and lower level selection, because it would aid the survival of both the corals and the symbionts.

Hexacorals are the most prominent types of corals, they feature calcium carbonate exoskeletons and are reef building. Octocorals lack such exoskeletons and are non-reef building, but they also harbor diverse communities. Both types of corals may form symbiotic relationships with *Symbiodinium* spp. and are at risk of bleaching in stressful environments (Parrin et al., 2012). Due to their similarities, octocorals are good model organisms for studying hexacorals. *Sympodium* sp. and *Sarcothelia* sp. (Fig 1-2) are octocorals that have Clade C1 and clade D4-5/D4-5-9 symbionts respectively. These different clades of symbionts are differently adapted to thermal stress. It has been shown that clade D symbionts may be more resistant to these stressors than clade C symbionts (Berkelmans and van Oppen, 2006).

Experiments were conducted on the octocorals colonies of *Sympodium* sp. and *Sarcothelia* sp. to determine how stress (4 hours of 140 µmol photons sec\(^{-1}\) m\(^{-2}\) exposure) affects their symbiont ROS production and migration. We hypothesize that this stress on the octocorals *Sympodium* sp. of *Sarcothelia* sp will lead to greater ROS production and symbiont migration in both.

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Figure 1: Image of a *Sympodium* sp. colony

Figure 2: Image of a *Sarcothelia* sp. colony
Methods

Determining the optimal amount of light stress for our symbionts

Colonies of *Sympodium* sp. (clade C symbionts) and *Sarcothelia* sp. (clade D symbionts) were explanted and grown on 15 mm cover glass for two weeks. Experiments to determine the optimal amount of stress towards symbionts were carried out on *Sympodium* sp. (table 1). Subsequent experimentation on *Sympodium* sp. And *Sarcothelia* sp. featured 4 hours of stress.

<table>
<thead>
<tr>
<th>Experiment Date</th>
<th>Stress (140 µmol photons sec⁻¹m⁻²)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>06/19/2018</td>
<td>1 h by the light</td>
<td>Too little stress</td>
</tr>
<tr>
<td>07/15/2018</td>
<td>1.5 h by the light</td>
<td>Too little stress</td>
</tr>
<tr>
<td>07/19/2018</td>
<td>2.5 h by the light</td>
<td>Too little stress</td>
</tr>
<tr>
<td>07/24/2018</td>
<td>3.5 h by the light</td>
<td>Slightly too little stress</td>
</tr>
<tr>
<td>06/26/2018</td>
<td>12 h in the dark, 4 hours by the light</td>
<td>Too much stress</td>
</tr>
</tbody>
</table>

Preparing *Sympodium* sp. And *Sarcothelia* sp experimental and control groups

The colonies were kept in tanks at 27 C with light supplied by fluorescent and metal halide lights at a maximum illumination of 110 µmol photons m⁻²s⁻¹. After the colonies matured, the experimental colonies were placed in finger bowls with their tank water and stressed by the light at 140 µmol photons sec⁻¹m⁻² for 4 h. The control colonies were covered away from light sources in their tanks. The control colonies were moved to the finger bowl with the seawater and the experimental colonies.

Preparing the fluorescent probe

A stock solution of 10 mmoll⁻¹ H₂DCFDA (2’,7’-dichlorodihydrofluorescein diacetate) was prepared in anhydrous dimethylsulfoxide (DMSO). The solution was added to finger bowls with the control and stressed colonies in a concentration of 10 µmol l⁻¹ (Blackstone, 2001). The finger bowls with the colonies and the fluorescent probe (H₂DCFDA) were placed in a dark incubator for an hour.

Fluorescent probe

The fluorescent probe (H₂DCFDA) is nonreactive and nonfluorescent and easily taken up by cells (Blackstone, 2001). After H₂DCFDA passes through a cell’s membrane, it is deacetylated and forms the reactive species, H₂DCF (Blackstone, 2001). H₂DCF may interact with ROS and create fluorescent DCF that can be detected with fluorescent microscopy. (DCF may freely diffuse out of the cell. ROS such as H₂O₂ also freely diffuse out of cells.

Imaging and analyzing the colonies

Each colony was imaged at 3 stolon locations. At each location, a series of 20 bright-field images and a single image using the fluorescent probe were taken. Alternation of the imaging of the control and experimental colonies helped ensure the colonies were exposed to similar amounts of stress. Through Image Pro Plus 6.3, the images of the colonies had their ROS production (foreground/background luminance) and moving/nonmoving symbiont values quantified. These data are displayed in graphs (Fig. 3-6) and paired comparison tables (tables 2-3).
Data and Results

Figure 2: Means ± standard error of *Sympodium* and *Sarcothelia* colony luminance

The *Sympodium* and *Sarcothelia* stressed groups were treated with 140 µmol photons sec\(^{-1}\) m\(^{-2}\) for 4 hours (28°C), while the control groups were kept in a culture tank with minimal light (27°C). Both groups were put in finger bowls in an incubator with a probe for 1 hour (27°C), then imaged and had their colony luminance calculated using Image pro 6.3. In each experiment, 3 stressed and 3 control colonies were imaged and quantified in 3 stolon areas. There were 11 experiments done on each octocoral, represented by the replicates in figures. Thus, each replicate features control and stressed bars, that represent an average value of 9 stolon areas. Luminance is depicted in arbitrary units on a 12-bit scale (0-4095). (A) *Sympodium* colony luminance data with mean ± SE. (B) *Sarcothelia* colony luminance data with mean ± SE.
Figure 4: Means ± standard error of *Sympodium* and *Sarcothelia* background luminance

*Sympodium* and *Sarcothelia* stressed groups were treated with 140 µmol photons sec\(^{-1}\) m\(^{-2}\) for 4 hours (28C), while the control groups were kept in a culture tank with minimal light (27C). Both groups were put in finger bowls in an incubator with a probe for 1 hour (27C), then imaged and had their background luminance calculated using Image pro 6.3. In each experiment, 3 stressed and 3 control colonies were imaged and analyzed in 3 stolon areas. There were 11 experiments done on each octocoral, represented by the replicates in figures. Thus, each replicate features control and stressed bars, that represent an average value of 9 stolon areas. Luminance is depicted in arbitrary units on a 12-bit scale (0-4095). (A) *Sympodium* background luminance data with mean ± SE. (B) *Sarcothelia* background luminance data with mean ± SE.

Figure 5: Means ± standard error of *Sympodium* and *Sarcothelia* relative luminance

*Sympodium* and *Sarcothelia* stressed groups were treated with 140 µmol photons sec\(^{-1}\) m\(^{-2}\) for 4 hours (28C), while the control groups were kept in a culture tank with minimal light (27C). Both groups were put in finger bowls in an incubator with a probe for 1 hour (27C), then imaged and had their relative luminance calculated using Image pro 6.3. The difference in the means of colony (foreground) and background luminance is the relative luminance. Luminance is depicted in arbitrary units on a 12-bit scale (0-4095). In each experiment, 3 stressed and 3 control colonies were imaged and analyzed in 3 stolon areas. There were 11 experiments done on each octocoral, represented by the replicates in figures. Thus, each replicate features control and stressed bars, that represent average value of 9 stolon areas. (A) *Sympodium* relative luminance data with mean ± SE. (B) *Sarcothelia* relative luminance data with mean ± SE.
Figure 6: Means ± standard error of *Sympodium* and *Sarcothelia* moving symbionts

*Sympodium* and *Sarcothelia* stressed groups were treated with 140 µmol photons sec\(^{-1}\) m\(^{-2}\) for 4 hours (28°C), while the control groups were kept in a culture tank with minimal light (27°C). Both groups were put in finger bowls in an incubator with a probe for 1 hour (27°C), then imaged and had their moving symbiont values calculated using Image pro 6.3. In each experiment, 3 stressed and 3 control colonies were imaged and analyzed in 3 stolon areas. There were 11 experiments done on each octocoral, represented by the replicates in figures. Thus, each replicate features control and stressed bars, that represent an average value of 9 stolon areas. (A) *Sympodium* moving symbiont data with mean ± SE. (B) *Sarcothelia* moving symbiont data with mean ± SE.
Figure 7: Means ± standard error of *Sympodium* and *Sarcothelia* non-moving symbionts

*Sympodium* and *Sarcothelia* stressed groups were treated with 140 µmol photons sec$^{-1}$m$^{-2}$ for 4 hours (28°C), while the control groups were kept in a culture tank with minimal light (27°C). Both groups were put in finger bowls in an incubator with a probe for 1 hour (27°C), then imaged and had their nonmoving symbiont values calculated using Image pro 6.3. In each experiment, 3 stressed and 3 control colonies were imaged and analyzed in 3 stolon areas. There were 11 experiments done on each octocoral, represented by the replicates in figures. Thus, each replicate features control and stressed bars, that represent an average value of 9 stolon areas. (A) *Sympodium* nonmoving symbiont data with mean ± SE. (B) *Sarcothelia* nonmoving symbiont data with mean ± SE.
In tables 2-3, we aimed to identify the significance of the stress on our measured values on both octocorals. Our measured values were colony luminance, background luminance, relative luminance, moving symbionts, and non-moving symbionts. To do this we subtracted the experimental value for each treatment, paired by time. In other words, control replicate 1, colony 1, area 1 was subtracted from stressed replicate 1, colony 1, area 1, then control replicate 1, colony 1, area 2 was subtracted from stressed replicate 1, colony 1, area 2, and so on. Then for all of these values we calculated minimum, maximum, mean, standard dev, standard error, t values, and p values for each paired (subtracted) values. The equations used to obtain our paired comparison statistics are shown below. The paired comparison values are lum1d (colony luminance), lum2d (background luminance), rellumd (relative luminance), movingd (moving symbionts), and nonmovingd (non-moving symbionts). These values may show the significance our stress had for their corresponding categories. For instance, a lower p value in lum1 may indicate more positive correlation between stress and colony luminance.

**Standard Deviation**

\[ \text{Standard Deviation} = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}} \]

**Standard Error**

\[ \text{Standard Error} = \frac{\text{Standard Deviation}}{\sqrt{n}} \]

**T value**

\[ T \text{ value} = \frac{\bar{d}}{\text{Standard Error} (\bar{d})} \]

p values are determined by t-test tables, where n-1 = degrees of freedom.
Discussion

Some general insight of the results can be gained by inspecting the figures. For instance, there was a positive correlation between stress and colony luminance on Sympodium and Sarcothelia (Fig. 3). The 1\textsuperscript{st}, 3\textsuperscript{rd}, 5\textsuperscript{th}, 8\textsuperscript{th}, 10\textsuperscript{th} and 11\textsuperscript{th} stressed Sympodium replicates exhibited higher colony luminance than their controls (A). However, the 2\textsuperscript{nd}, 4\textsuperscript{th}, and 9\textsuperscript{th} control Sympodium replicates had higher colony luminance. All the Sarcothelia replicates, except the 3\textsuperscript{rd}, 5\textsuperscript{th}, and 8\textsuperscript{th} show that stress is linked to higher colony luminance (B). Colony luminance represents ROS production in the colonies. These data suggest that stress was correlated to increased levels of ROS production in both colonies.

While the experiments were not designed to directly compare the two species, it is interesting that Sarcothelia stressed replicates had more background luminance than stressed Sympodium replicates (Fig 4). The 1\textsuperscript{st}, 3\textsuperscript{rd}, 5\textsuperscript{th}, 8\textsuperscript{th}, 10\textsuperscript{th} and 11\textsuperscript{th} stressed Sympodium replicates exhibited higher background luminance than their controls (A). There was a mixed correlation between background luminance and stressed replicates, as the 2\textsuperscript{nd}, 4\textsuperscript{th} and 9\textsuperscript{th} control replicates had higher background luminance. All the Sarcothelia stressed replicates, except the 3\textsuperscript{rd} had higher background luminance values than their controls (B). Background luminance represents ROS production from inside the colonies that subsequently exited the colony. These data suggest that stress is linked to increased levels of ROS production outside the colonies of Sympodium and more decisively in Sarcothelia.

Sympodium stressed replicates had more relative luminance than stressed Sarcothelia replicates (Fig 5). The difference in the means of colony (foreground) and background luminance is the relative luminance. All stressed Sympodium replicates except the 2\textsuperscript{nd} and 4\textsuperscript{th}, had a higher background luminance than their controls (A). The 1\textsuperscript{st}, 2\textsuperscript{nd}, 4\textsuperscript{th}, 9\textsuperscript{th} and 10\textsuperscript{th} stressed Sarcothelia replicates exhibited higher relative luminance, while the 3\textsuperscript{rd}, 5\textsuperscript{th}, 8\textsuperscript{th}, and 11\textsuperscript{th} control replicates had higher relative luminance (B). These data suggest that ROS more readily diffused out of Sarcothelia cells.

Blackstone and Golladay (2018) predicted that Sarcothelia should emit more ROS in control colonies. The clade D symbionts, of Sarcothelia, may have mutations that lead to electron buildup and ROS production in their electron transport chain. This may make these types of symbionts more adaptable to living in stressful environments. While this experiment was not designed to directly test this hypothesis, the data (Fig. 3-5) suggests support.

There was a clear positive correlation between stress and symbiont migration on Sympodium and Sarcothelia (Fig. 6). All Sympodium and Sarcothelia stressed replicates exhibited higher moving symbiont values than their controls (A, B). It has been shown that the clade D symbionts of Sarcothelia are more resistant to these stressors than the clade C symbionts of Sympodium (Berkelmans and van Oppen, 2006). This may imply that Sarcothelia should have more moving symbionts, as the migration is likely coping mechanism to stress. Parrin and his team’s experimentation showed that stressed Sarcothelia had more moving symbionts than Sympodium (Parrin et al., 2012). Our data, however, doesn’t decisively support this claim.

There was no clear correlation between stress and symbiont nonmigration on Sympodium and Sarcothelia (Fig 7). The 2\textsuperscript{nd} - 4\textsuperscript{th}, 7\textsuperscript{th}, 8\textsuperscript{th} Stressed Sympodium replicates had more non-moving symbionts, while the 1\textsuperscript{st}, 5\textsuperscript{th}, 6\textsuperscript{th}, 9\textsuperscript{th}, 10\textsuperscript{th}, and 11\textsuperscript{th} controls had more non-moving symbionts (A). All control Sarcothelia replicates except 7\textsuperscript{th} - 9\textsuperscript{th}, had more non-moving symbionts as their corresponding experimental groups (B). While it was more severe for Sarcothelia, both octocoral controls had more nonmoving symbionts. This may be because there was more symbiont death/migration in the stressed groups.

While inspection of the figures can provide some insight into the results, a statistical comparison is much more effective. Because the measures of control and stressed colonies were paired in time, paired comparison analysis can negate the time effect and reveal the treatment effect more clearly (tables 2-3). There was strong positive correlation between stress and colony luminance on both octocorals, as their respective paired comparison p values were <.0001. There was strong positive correlation between stress and moving symbionts on both octocorals, as their respective paired comparison p values were <.0001. These two pieces of data directly support my hypothesis. While the experiment was not designed for between-species comparison, there was a greater positive correlation between stress and background luminance in Sarcothelia than Sympodium, as their respective paired comparison p values were <.0001 and .0150 respectively. There was a greater positive correlation between stress and relative luminance on Sympodium than Sarcothelia, as their respective paired comparison p values were <.0001 and 7806 respectively. These data imply that in the presence of ROS, symbionts are more likely to exit the cells of Sarcothelia. Analyzing the structures of the octocorals with transmission electron microscopy may help explain this.
Conclusion

In general, our data and findings align with our hypothesis. There was a positive correlation between stress, ROS production, and moving symbionts on *Sympodium* sp. and *Sarcothelia* sp. (figures 3-6). This may imply that light induces symbiont ROS production, while migration is a mitigating mechanism. While our experiment was not designed to compare the species, our data shows a greater correlation between relative luminance and stress in *Sympodium* as compared to stressed *Sarcothelia* (tables 2-3). This implies that in the presence of ROS, symbionts more readily exit the cells of *Sarcothelia*.

Differences in *Sympodium* and *Sarcothelia*’s perturbation responses could be due to their symbiont-host relationship (Parrin et al., 2012). Blackstone and Golladay (2018) predict that *Sarcothelia* should emit more ROS in control colonies. The clade D symbionts, of *Sarcothelia*, may have mutations that lead to electron buildup and ROS production in their electron transport chain. While this experiment was not designed to directly test this hypothesis, the data (Fig. 3-5) do suggest support. Unlike our study, Parrin and his team, concluded that there was more symbiont migration in *Sarcothelia* than *Sympodium*. It has been shown that clade D symbionts may be more resistant to stressors than clade C symbionts (Berkelmans and van Oppen, 2006). The clade D symbionts of *Sarcothelia* may more readily migrate to the coenenchyme of the coral, than clade C symbionts of *Sympodium*, in order to avoid heat damage and exclusion from the colony. Further studies on how stress effects different combinations of corals and symbionts, may help us gain knowledge and regulate mechanisms that mitigate coral bleaching.
References