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ANALYSIS OF THE STRESS RESPONSE OF CORALS EXPOSED TO WHITE PLAGUE DISEASE

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ANALYSIS OF THE STRESS RESPONSE OF CORALS EXPOSED TO WHITE PLAGUE DISEASE

by

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ABSTRACT

ANALYSIS OF THE STRESS RESPONSE OF CORALS

EXPOSED TO WHITE PLAGUE DISEASE

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The University of Texas at Arlington, 2019

Faculty Mentor: Laura Mydlarz

Coral reefs are important ecosystems that sustain marine biodiversity and serve as a critical economic resource. Unfortunately, the increased prevalence of coral disease is decimating reef populations. Disease exposure triggers an intracellular stress response within corals, involving antioxidants and melanin synthesis, that is mitigated by their innate immunity. The overarching purpose of this project is to investigate why some coral species survive better than others when exposed to a disease. To do this, the variability in stress response between seven Caribbean coral species—*Orbicella faveolata, Orbicella annularis, Colpophyllia natans, Montastraea cavernosa, Siderastrea siderea, Porites porites,* and *Porites astreoides*—after exposure to white plague disease was quantified using immunoassays. The assays measured the activity levels of two antioxidants (catalase and peroxidase) and a melanin synthesis cascade precursor (prophenoloxidase). This data

will provide insight into why some coral species are resistant to disease, while others are susceptible.

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CHAPTER 1

INTRODUCTION

1.1 Background

Increased ocean temperatures driven by climate change have caused corals to lose their symbionts and pigmentation, a phenomenon known as bleaching (Martin et al. 2010). The loss of symbionts weakens the corals' immune systems, leaving them more susceptible to diseases (Martin et al. 2010). The prevalence of coral disease is increasing (Fuess et al. 2017). Currently, there are over 20 disease syndromes, including yellow band, red band, black band, and white plague diseases, named for the noticeable markings that are symptoms of the disease (Martin et al. 2010). Coral mortality from major bleaching events and post-bleaching disease outbreaks is severe (Martin et al. 2010). As populations diminish for these crucial animals, an understanding of the underlying defense mechanisms of their immunity is critical in developing conservation and restoration programs to combat stressors and ensure their survival in the future (Mydlarz et al. 2010).

Marine invertebrates live in environments with high pathogenic loads, pollutants, and changing temperatures, which require them to have efficient immune systems (Mydlarz et al. 2006). The effectiveness of the immune response of corals is based upon their ability to respond to intruders and to identify and destroy damaged or infected self-cells (Mydlarz et al. 2006). The best studied mechanisms of the innate immune systems of corals are the melanin synthesis cascade, antioxidants, and antimicrobial compounds (Pinzón et al. 2014). These components allow the coral to fight pathogens, repair tissues,

and reduce the amount of reactive oxygen species (ROS) generated while under stress or during infection (Pinzón et al. 2014).

The immune system of Caribbean corals has been molded by evolutionary history (Pinzón et al. 2014). The different evolutionary pressures on each species have likely led to the development of species-specific immune defenses (Pinzón et al. 2014). Older Caribbean coral species, like *Porites astreoides* and *Siderastrea radians* that diverged more than 200 million years ago, have lower disease prevalence, and have higher levels of melanin and antibacterial compounds, allowing them to maintain normal physiological function under stress (Pinzón et al. 2014). More recently diverged Caribbean species, like *Orbicella faveolata* and *Orbicella annularis*, showed higher disease prevalence and have lower levels of immune defense components, which has led to significant population losses (Pinzón et al. 2014). Eventually, selection pressure will lead to an abundance of the coral species with higher resistance to disease (Mydlarz et al. 2010).

1.2 Significance of Research

Coral reefs are very important ecosystems that sustain marine biodiversity by supporting the life stages for 25% of marine organisms (Ruckelshaus et al. 2013). This biodiversity can serve as an important source of food and also attracts many tourists, which makes reefs an important economic resource for both the food and ecotourism industries (Ruckelshaus et al. 2013). Unfortunately, corals are facing mortality due to the increasing prevalence of coral diseases (Fuess et al. 2017). The purpose of this project is to determine what allows some species to survive better than others in the face of disease. More specifically, this study aims to uncover differences in immunity between disease-resistant and disease-susceptible species. Once infected by a disease, each coral species attempts to

fight pathogens by initiating a unique intracellular stress response mitigated by its innate immunity (Fuess et al. 2017). This project will quantify the intracellular stress responses of seven different Caribbean coral species—*Orbicella faveolata, Orbicella annularis, Colpophyllia natans, Montastraea cavernosa, Siderastrea siderea, Porites porites,* and *Porites astreoides*—after exposure to white plague disease. This requires measuring the activity levels of several proteins involved in coral immunity. Variabilities in protein activity between species can provide insight into why some coral species are resistant to marine diseases, while others are susceptible.

This study focused on two main components of the intercellular stress response-melanin synthesis and antioxidant activity (Figure 1.1). Melanin is used within corals to create barriers employed by the immune system for defense against pathogens (Pinzón et al. 2014). The melanin is synthesized via a cascade that is triggered by the enzyme phenoloxidase (PO), which in its inactive form is called prophenoloxidase (PPO) (Mydlarz et al. 2010). The activity of the enzymes involved in the cascade (PPO and PO) is inversely related to the amount of melanin synthesized (Pinzón et al. 2014). This study measured the activity levels of PPO in each of the seven coral species. It was hypothesized that coral fragments infected with white plague disease would show lower levels of PPO activity because they would need to synthesize more melanin when actively fighting pathogens. Similarly, it was hypothesized that disease-susceptible species would have lower rates of PPO activity. This is because susceptible species have less melanin than resistant species (Pinzón et al. 2014), so higher levels of synthesis are required for efficient pathogen defense.

The other immune system component quantified in this study was antioxidant activity. This was done by measuring the activity levels of catalase (CAT) and peroxidase (POX). When infected, corals release reactive oxygen species (ROS) to kill pathogens (Pinzón et al. 2014). Unfortunately, ROS can also be dangerous to corals because they have the capacity to denature proteins and damage nucleic acids (Weis 2008). In order to mitigate self-damage, corals will upregulate antioxidants to neutralize ROS. Both CAT and POX are antioxidants employed to scavenge and remove excess hydrogen peroxide (a type of ROS) (Couch 2008). Because corals upregulate antioxidants during infections (Pinzón et al. 2014), it was hypothesized that infected coral fragments would have higher CAT and POX activity. It was also hypothesized that, because resistant species are less likely to become infected, they would have lower levels of CAT and POX activity than susceptible species.

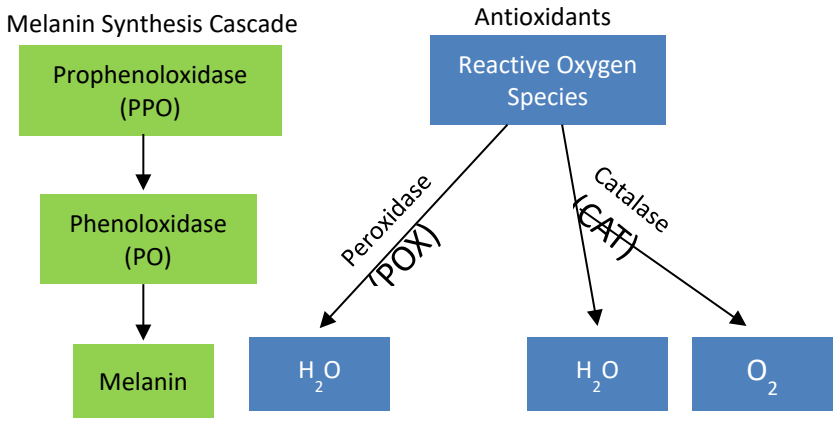

Figure 1.1: Components of the innate immune system of corals involved in the intracellular stress response

CHAPTER 2

MATERIALS AND METHODS

2.1 Coral Collection & Disease Transmission

Five parental colonies for each of the seven Caribbean coral species—*C. natans, O. faveolata, O. annularis, S. siderea, M. cavernosa, P. astreoides,* and *P. porites*—were collected from Brewers Bay in St Thomas, USVI. Colonies were then transported to The University of the Virgin Islands Marine Science Station in Brewers Bay where white plague disease transmission was conducted. Coral colonies were fragmented for paired design in a controlled setting. Colonies were either placed in the experimental group where they were exposed to a piece of *Orbicella franksi* infected with white plague disease or they were placed in a control group (Figure 2.1).

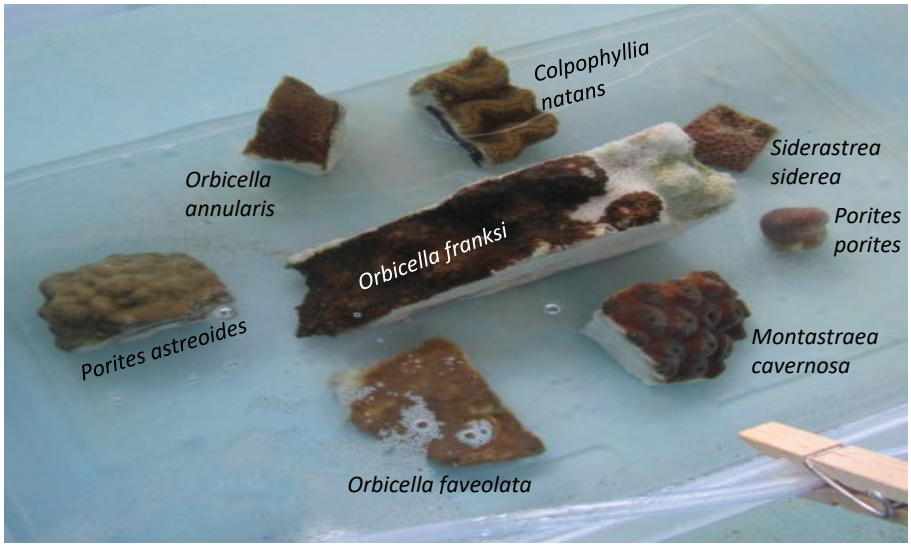

Figure 2.1: Fragments of seven Caribbean coral species exposed to white plague disease infected *O. franksi*

Disease phenotypes including days to infection, lesion growth rate, and percent tissue loss were recorded for each fragment during a transmission period of seven days. Corals remained untouched after infection to allow the disease to spread. Infected corals were identified by the tissue loss and white lesion growth symptomatic of white plague disease. After approximately thirty percent of the fragment became infected, corals were removed from exposure to keep enough living tissue available for protein extractions and to allow for sufficient lesion growth calculations. Once the fragments were culled, tissue samples were broken off with a bone cutter and flash frozen in liquid nitrogen. Samples were then shipped to the University of Texas at Arlington.

2.2 Sample Preparation

To assess stress response, the tissue of each collected coral fragment was removed and processed into protein samples for use in immunoassays. To remove tissue, coral samples were airbrushed with extraction buffer (100mM TRIS buffer + 0.05mM dithiothreitol) until 6 ml of tissue slurry was obtained. Tissue slurry was kept on ice and homogenized with a hand-held tissue homogenizer (Powergen 125, Fisher Scientific, Waltham, Massachusetts) for 60 seconds. Glass beads were then added to the tissue slurry and vortexed for 60 seconds. The tissue slurry was centrifuged at 3500 RPM at 4 °C for 5 minutes to pellet cellular debris. Next, 4 ml of supernatant from the centrifuged tissue slurry was pipetted into two microcentrifuge tubes (2 ml per tube) to be used as the crude protein extract for measuring protein concentration and activity. All samples were stored in a freezer at -80 °C.

2.3 Immunoassays

Protein concentration and activity were measured using various assays with unique protocols and spectrophotometer parameters. The methodologies used to perform each assay are detailed below.

2.3.1 Protein Concentration (Red 660)

The protein concentration (measured in mg protein per ml sample) was quantified using the Red 660 assay. Each well of a 96 flat bottom well plate (Greiner) was loaded with protein sample (10µl) and Red 660 working solution (20µl) (G Biosciences, Saint Louis, Missouri). The protein concentration was estimated by comparison of sample absorbance against absorbance of a standard curve. The standard curve was made by serial diluting bovine serum albumin (BSA) with extraction buffer (100mM TRIS buffer + 0.05mM dithiothreitol). Absorbance was read at 660 nm for 60 seconds.

2.3.2 Prophenoloxidase (PPO) Activity

PPO activity (measured in absorbance per mg protein per minute) was measured by loading the wells of a 96 flat bottom well plate (Greiner) with protein sample (20µL), trypsin (20µL of 0.1 mg/mL), and phosphate (PBS) buffer (20µL of 100mM, pH7). The plate was incubated at room temperature for 30 minutes. Immediately before placing the plate into the spectrophotometer, L-1,3-dihydroxphenylalanine (30µL of 10mM) (L-dopa, Sigma-Aldrich) was added to the wells. Absorbance was read every 60 seconds for 20 minutes at 490 nm.

2.3.3 Peroxidase (POX) Activity

POX activity (measured in absorbance per mg protein per minute) was measured by loading the wells of a 96 flat bottom well plate (Greiner) with protein sample (25µL),

guaiacol (25µL of 25mM), and PBS buffer (20µL of 10mM, pH7). Immediately before placing the plate into the spectrophotometer, hydrogen peroxide (25µL of 25 mM) was added. Absorbance was read every 31 seconds for 45 minutes at 470 nm.

2.3.4 Catalase (CAT) Activity

CAT activity (measured in absorbance per mg protein per minute) was measured by loading the wells of a 96 well UV plate (Greiner) with protein sample (15µL), PBS buffer (45µL of 50mM, pH 7), and hydrogen peroxide (75µL of 25mM). CAT activity levels were calculated by comparing the absorbance of each sample against the absorbance of a standard curve of hydrogen peroxide serial-diluted with PBS buffer (50mM, pH 7). Absorbance was read every 31 seconds for 15 minutes at 240 nm.

2.4 Statistical Analysis

A total of ten fragments were collected per species. The paired design of this experiment evenly divided fragments from each species into two groups- exposed (n = 5) and control (n = 5). *O. annularis* only had eight fragments, so the exposed and control groups for that species both had an n value of 4. Each assay was conducted in duplicate, so average absorbance between plates was calculated for each sample. Activity for POX, CAT, and PPO was normalized using the protein concentrations calculated from Red 660.

The mean protein activities of each sample were then used to calculate the overall protein activity means for each infection status (Table 3.1). There were three infection statuses. Fragments in the control group were designated “control.” Fragments in the exposed group that were unaffected by white plague disease were designated “exposed.” Fragments in the exposed group that developed symptoms of white plague disease were designated “infected”. A one-way analysis of variance test (ANOVA) was run with R-

Studio, R software version 3.5.3 from the R Project for Statistical Computing to test for significance in protein activity for each infection status. The alpha value was 0.05. Significance was determined by a p-value of less than 0.05 (p-values are in Table 3.1).

CHAPTER 3

RESULTS

3.1 Level of Susceptibility

A total of 68 coral fragments were collected from Brewer's Bay (St. Thomas, USVI). The fragments were designated with three infection statuses (control, exposed, or infected). A breakdown of the number of fragments in each infection status per species is shown in Figure 3.1.

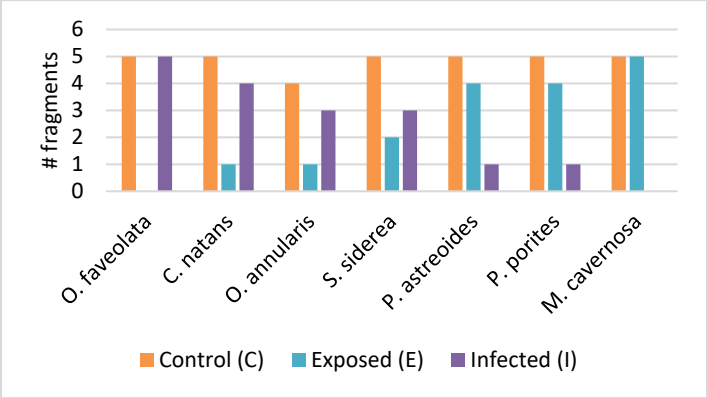

Figure 3.1: Number of fragments in each infection status per species

Each species was given a percentage of susceptibility by dividing the number of infected fragments by the total number of disease-exposed fragments for that species. For example, all exposed *O. faveolata* fragments became infected with white plague disease, giving that species a susceptibility percentage of 100%. Conversely, none of the exposed fragments of *M. cavernosa* became infected, which gave that species a susceptibility percentage of 0%. The susceptibility percentage for each species is shown in Figure 3.2.

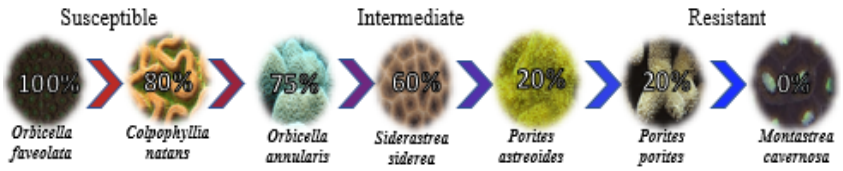

Figure 3.2: Percentage of susceptibility for each species

3.2 Infection Status Comparison

The activity of each immune protein was first compared by infection status (control, exposed, or infected). The mean protein activities (measured in absorbance per mg protein per ml) and the associated p-values for each infection status are recorded in Table 3.1. Overall, CAT and POX activity was upregulated in infected individuals, while PPO activity was downregulated in infected individuals.

Table 3.1: Means (± SE) of protein activity for each infection status

Test	Control	Exposed	Infected	p-value
Catalase	18.3245 ± 7.224	11.2848 ± 2.682	43.1631 ± 15.781	0.0864
Peroxidase	1.0203 ± 0.239	0.5576 ± 0.1726	1.7107 ± 0.5438	0.09374
Prophenoloxidase	1.7709 ± 0.329	2.5636 ± 0.449	1.1083 ± 0.251	0.05531

3.3 Species Comparison

3.3.1 Catalase

The activity of each immune protein was then compared between species. The average CAT activity of each infection status per species is shown in Figure 3.3. An analysis of CAT activity versus relative risk of disease is shown in Figure 3.4. The relative risk analysis was done by Dr. Erinn Muller, a collaborator at Mote Marine Laboratory. The analysis assigned each species a number based on their relative risk of developing white plague disease. The graph of CAT activity versus relative risk was generated using a Spearman, nonparametric correlation. Overall, the trend showed that CAT activity was highest in species with a high percentage of susceptibility.

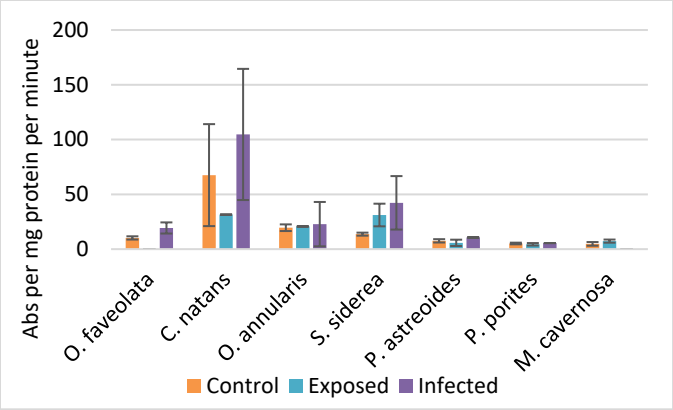

Figure 3.3: Comparison of CAT activity by species

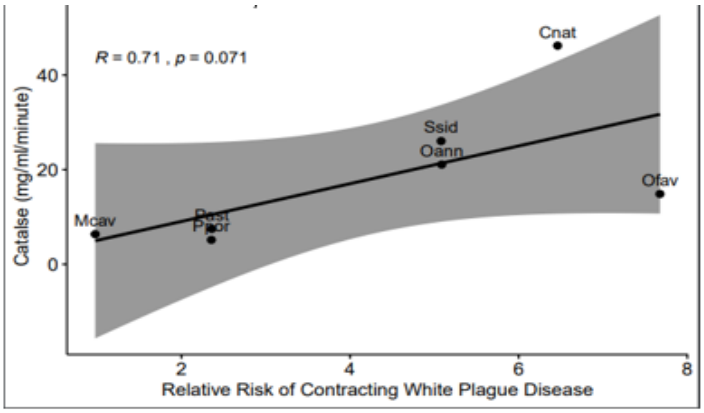

Figure 3.4: CAT Activity versus Relative Risk of Disease (Spearman, nonparametric correlation)

3.3.2 Peroxidase

The average POX activity of each infection status per species is shown in Figure 3.5. An analysis of POX activity versus relative risk of disease using a Spearman, nonparametric correlation is shown in Figure 3.6. Overall, POX activity was highest in species with a high percentage of susceptibility. Interestingly, *O. faveolata* and *S. siderea* downregulated POX activity in infected individuals.

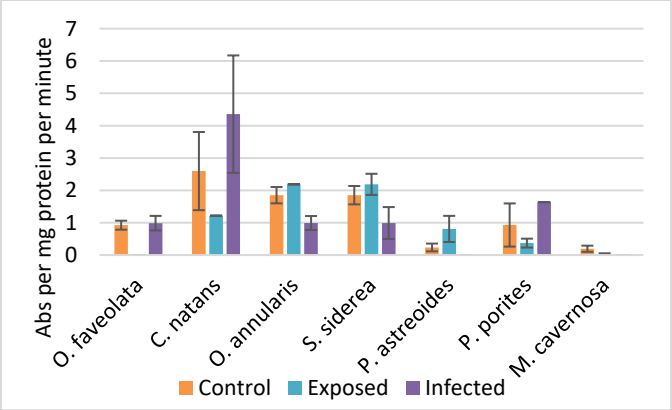

Figure 3.5: Comparison of POX activity by species

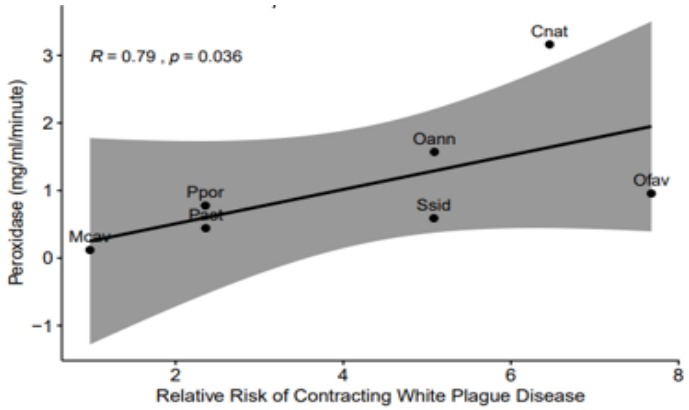

Figure 3.6: POX Activity versus Relative Risk of Disease (Spearman, nonparametric correlation)

3.3.3 Prophenoloxidase

The average PPO activity of each infection status per species is shown in Figure 3.7. An analysis of PPO activity versus the relative risk of disease using a Spearman, nonparametric correlation is shown in Figure 3.8. The overall trend showed that PPO activity was lowest in species with a high percentage of susceptibility.

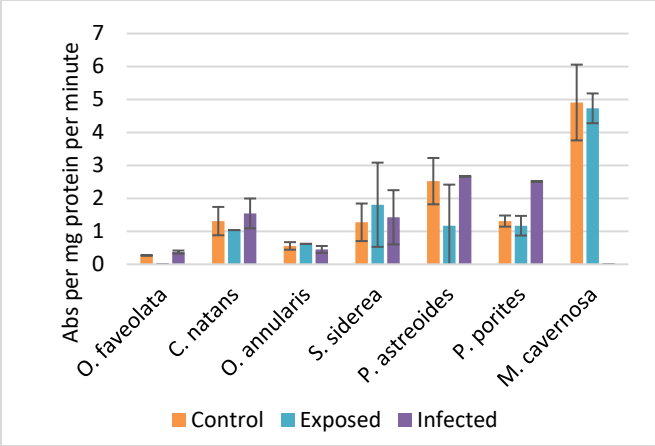

Figure 3.7: Comparison of PPO activity by species

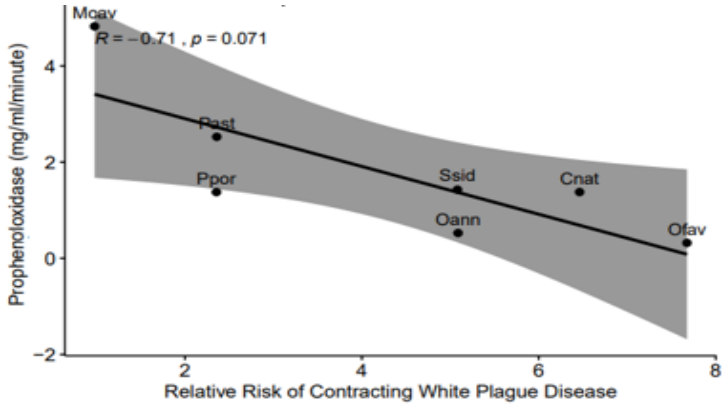

Figure 3.8: PPO Activity versus Relative Risk of Disease (Spearman, nonparametric correlation)

3.3.4 NMDS Plot

A Non-metric Multidimensional Scaling (NMDS) plot was created in R-Studio, R software version 3.5.3 from the R Project for Statistical Computing (Figure 4.1). This plot groups together data points that are similar in magnitude. Each color represents a different species. The symbols (circle, triangle, and square) represent infection status (control, exposed, and infected, respectively). This plot reiterates the previous results. The resistant species (*P. porites, P. astreoides,* and *M. cavernosa*) are clustered together because they

showed the most similar data. Similarly, the susceptible species (*O. faveolata, O. annularis,* and *C. natans*) are also clustered together.

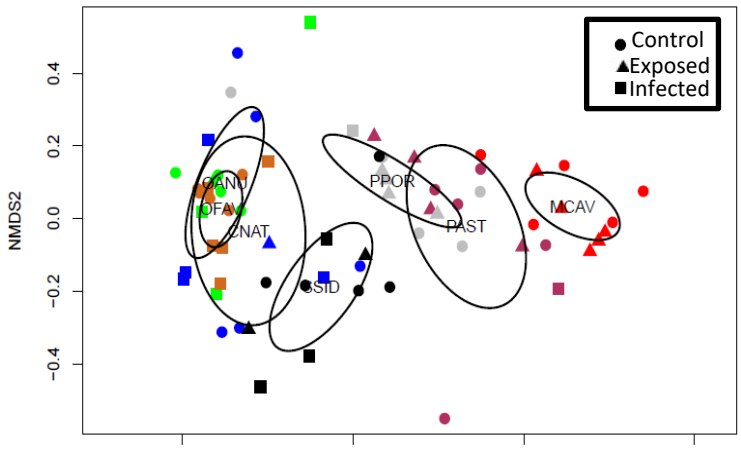

Figure 3.9: NMDS plot of protein activity data points in each species

CHAPTER 4

DISCUSSION

4.1 Variance between Infection Statuses

Overall, antioxidant (CAT and POX) activity was upregulated in infected fragments (Table 3.1). Melanin synthesis was also upregulated in infected fragments (reflected by lower PPO activity) (Table 3.1). This is because infected coral fragments were actively fighting invading pathogens. Melanin is synthesized for better defense and ROS is released to kill pathogens (Pinzón et al. 2014). ROS release coincides with the upregulation of antioxidants to mitigate self-damage. Interestingly, *O. faveolata* and *S. siderea* showed a decrease in POX activity in their infected fragments (Figure 3.5). This may be due to a preference in those species to use CAT for scavenging and removing hydrogen peroxide, as opposed to POX.

4.2 Variance between Species

Based on the percent susceptibilities shown in Figure 3.2, *M. cavernosa* was the most resistant to white plague disease. Accordingly, *M. cavernosa* had the highest PPO activity and the lowest CAT and POX activity. Similarly, *P. porites* and *P. astreoides*, the other species with high disease resistance, showed the lowest antioxidant and highest PPO activities (Figures 3.4, 3.6, and 3.8). This correlates with the predictions made for species with a higher resistance to disease. Resistant species have lower infection levels, so they will not need to synthesize as much melanin nor release as many ROS as susceptible

species. Lower ROS levels are correlated with the lower antioxidant (CAT and POX) activity seen in the resistant species.

Conversely, *O. faveolata, O. annularis*, and *C. natans* were the most susceptible to white plague disease. These species showed the highest CAT and POX activities and the lowest PPO activities (Figure 3.4, 3.6, and 3.8). Higher infection rates in these species causes the release of more ROS, which in turn necessitates the upregulation of antioxidants. These species also synthesize higher rates of melanin in order to build a more sufficient barrier against pathogens.

4.3 Conclusion

This study aimed to determine why some coral species survive better than others after exposure to disease by quantifying the intracellular stress response of seven Caribbean coral species exposed to white plague disease. Variance in the immune protein activity of disease-exposed and disease-infected fragments of different species was measured.

Overall, the results showed that infected corals upregulate antioxidants and melanin synthesis. This was reflected in the increase of average CAT and POX activity and the decrease in average PPO activity that infected fragments had when compared to exposed fragments (Table 3.1). This trend was also seen when comparing the intracellular stress response of disease-susceptible species to disease-resistant species.

In summary, resistant species showed lower levels of antioxidant activity and melanin synthesis than susceptible species. This is because resistant species tend to have higher levels of melanin and antibacterial compounds (Pinzón et al. 2014), which allow for better pathogen defense and lower infection rates. Because resistant species are less likely to develop infections, they experience less disease-associated stress and therefore have less

extreme intracellular stress responses. The results of this study support these conclusions. Species found to be more resistant to white plague disease (*P. porites, P. astreoides,* and *M. cavernosa*) showed lower levels of antioxidant (CAT and POX) activity and lower levels of melanin synthesis (higher PPO activity) than disease-susceptible species (*O. faveolata, O. annularis,* and *C. natans*) (Figure 3.4, 3.6, and 3.8). These properties are what make resistant species better equipped for survival than susceptible species in the face of increasing marine disease prevalence.

REFERENCES

Couch, Courtney S., et al. “Variation in Measures of Immunocompetence of Sea Fan Coral, Gorgonia Ventalina, in the Florida Keys.” Marine Biology, vol. 155, no. 3, 10 Sept. 2008, pp. 281–292., doi:10.1007/s00227-008-1024-x.

Fuess, Lauren E., et al. “Life or Death: Disease-Tolerant Coral Species Activate Autophagy Following Immune Challenge.” Proceedings of the Royal Society B: Biological Sciences, vol. 284, no. 1856, 7 June 2017, doi:10.1098/rspb.2017.0771.

Martin, Lynn B., et al. “The Effects of Anthropogenic Global Changes on Immune Functions and Disease Resistance.” Annals of the New York Academy of Sciences, vol. 1195, no. 1, May 2010, pp. 129–148., doi:10.1111/j.1749-6632.2010.05454.x.

Mydlarz, Laura D., et al. “Innate Immunity, Environmental Drivers, and Disease Ecology of Marine and Freshwater Invertebrates.” Annual Review of Ecology, Evolution, and Systematics, vol. 37, no. 1, 12 Dec. 2006, pp. 251–288., doi:10.1146/annurev.ecolsys.37.091305.110103.

Mydlarz, Laura D., et al. “What Are the Physiological and Immunological Responses of Coral to Climate Warming and Disease?” Journal of Experimental Biology, vol. 213, no. 6, 26 Feb. 2010, pp. 934–945., doi:10.1242/jeb.037580.

Pinzón, Jorge H., et al. “Relationship between Phylogeny and Immunity Suggests Older Caribbean Coral Lineages Are More Resistant to Disease.” PLoS ONE, vol. 9, no. 8, 18 Aug. 2014, doi:10.1371/journal.pone.0104787.

Ruckelshaus, M., et al. “Securing Ocean Benefits for Society in the Face of Climate Change.” Marine Policy, vol. 40, 2013, pp. 154–59, doi:10.1016/j.marpol.2013.01.009.

Weis, V. M. “Cellular Mechanisms of Cnidarian Bleaching: Stress Causes the Collapse of Symbiosis.” Journal of Experimental Biology, vol. 211, no. 19, 19 Oct. 2008, pp. 3059–3066., doi:10.1242/jeb.009597.

BIOGRAPHICAL INFORMATION

Kirsten is an undergraduate student at the University of Texas at Arlington pursuing an Honors Bachelor of Science in Biology. She will graduate in May 2019. During her time at UTA, Kirsten was an undergraduate research assistant in the Mydlarz Lab where she helped conduct research on the immunology of corals.