FLUORESCENCE EMISSION AS AN EARLY INDICATOR OF CORAL HEALTH AND AS A VISUAL CUE FOR SYMBIOTIC ALGAE: THE EFFECT ON STEM INTEREST AFTER A FIELD TRIP TO A PUBLIC AQUARIUM

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Abstract: *Scleractinian* coral rely on the photosynthetic byproducts gained from their dinoflagellate endosymbiont. Bleaching events break down the symbiosis leaving the coral with reduced fecundity or dead. Examining the symbiotic relationship is crucial for continued coral survival. This research tested the change in coral fluorescence emission as an indicator of coral stress. The findings were variable and suggest fluorescent emission intensity could be an indicator of coral health, only in response to light and temperature stress. Emission measurement was not found to be a reliable indicator of coral health in response to salinity and pH manipulations. Once a coral has bleached, coral needs to repopulate their tissues with one or many species of endosymbionts. This research found a positive phototactic response of three dinoflagellates in response to a green fluorescing coral, suggesting an adaptive function of fluorescence as a mechanism to attract symbionts for repopulation after a coral has been subject to bleaching. Continued research today and in the future is key to understanding and sustaining coral reef systems. Unfortunately, the number of students who pursue STEM (science, technology, engineering, and math) fields will not meet the demands for future researchers. Informal science learning has been indicated as the necessary factor in inspiring student interest in science, facilitating increased interest to pursue a STEM field. The Oklahoma Aquarium was utilized to explore the impact of a field trip to a science center on a student's interest in STEM. Students who participated in a field trip believed that science was more "fun" after the field trip as compared to the commencement. Students who participated in an additional educational program indicated their increased desire to pursue a STEM field in addition to their change in attitude regarding science.

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CHAPTER I

GENERAL INTRODUCTION

INTRODUCTION

Five recorded global mass bleaching events have occurred in the previous twenty-year period. Three of these events occurred within the past five years. Bleaching is a phenomenon in which the endosymbiotic dinoflagellate (Symbiodiniaceae) either vacate or are evicted from the coral host. When corals bleach, they can lose 60 – 90% of their symbiont population (Glynn, 1996). The length and frequency of bleaching events is increasing due to a continued rise in sea surface temperature (SST) (Hoegh-Guldberg et al., 2017; Hughes et al., 2017; Sully et al., 2019). The bleaching events often coincided with El Niño Southern Oscillation which bring warmer waters to the Pacific Equatorial Zone. Warm sea surface temperatures initiate the breakdown of the coral-algae symbiosis (Baker et al., 2008; Heron et al., 2016; Hughes et al., 2017; Normile, 2016; Skirving et

al., 2019). Globally, coral reef systems have declined by 50% (Schuster, 2019). Coral reefs are one of the most biologically diverse ecosystems, with an estimated 30% of all identified fish species located on reef systems (Hixon & Randall, 2019). In addition to their role in maintaining ocean diversity, reef systems absorb up to 97% of wave energy from storms at sea providing protection from flooding and erosion of coastal regions (Elliff & Silva, 2017; Ferrario et al., 2014; Moberg & Folke, 1999). The economic importance of coral reef systems is valued between $1 – 20 trillion (Heron et al., 2016; Hoegh-Guldberg, 2015; Roth, 2014).

Within the coral tissue, there is an exchange of nutrients between symbiont and the coral host, which provides the coral with up to 90% of its energy (Muscatine & Porter, 1977; Weis, 2008; Yellowlees et al., 2008). The nutrient exchange between the coral host and symbiont are crucial for coral survival. Without the photosynthetic byproducts of the symbionts, the coral do not thrive (Matthews et al., 2017). The varied colors of coral within a reef system are provided primarily by the symbiotic algae along with fluorescent and non-fluorescent proteins (Dove et al., 2001). During a bleaching event when there is a breakdown in the symbiosis, the coral not only lose the symbiont, but also the color provided by the algae (Weis, 2008).

Fluorescent proteins (FPs) are pervasive within reef building coral, and their functionality is still being discovered (Alieva et al., 2008; Lapshin et al., 2015). Their functionality has been attributed to photoprotection and photo acclimatization (Salih et al., 2000; Smith et al., 2017). Previous research has explored the protection provided by FPs from stressful levels of light and temperature, as well as the pattern of change in emission intensity in response to the two stressors (Roth & Deheyn, 2013). The potential

protection FPs provide the coral holobiont, and the change in FP emission intensity in response to stress, has not been studied in relation to potential environmental stressors other than light and temperature. This research assesses the potential protection provided to coral by FPs in response to manipulated salinity and pH.

A proposed adaptive function for coral FP emission is known as the "Beacon Hypothesis," which states that coral utilize fluorescence to attract algae (Hollingsworth et al., 2005; Horiguchi et al., 1999). Dinoflagellate algae from the family *Symbiodiniaceae* possess two distinct morphologies (Horiguchi et al., 1999; Yamashita & Koike, 2015). When the algae are in symbiosis with the coral, they remain in a coccoid phase; when free-swimming, their morphology changes on a diel cycle. During the day, the algae have flagella and an eyespot; at night they return to a coccoid phase lacking both flagella and an eyespot (Horiguchi et al., 1999; Yamashita & Koike, 2015). Free-swimming dinoflagellates are known to utilize an eyespot to detect directionality of a light source to aid in photosynthetic efficiency (Horiguchi et al., 1999; Swafford & Oakley, 2018; Thompson et al., 2017). Recent research has supported a phototactic attraction of symbiotic algae to green fluorescence (Aihara et al., 2019). This research sought to identify additional species of symbiont that display phototactic attraction to green fluorescence. Recently, a phenomenon known as colorful bleaching has been observed on reef systems. As corals begin to bleach, they upregulate FP production, increasing the emission intensity from the coral (Bollati et al., 2020). The increased fluorescence may act as a beacon to attract other symbiont species.

Coral can host many different species of symbiont with some offering greater benefit than others to the coral (Baker et al., 2008; Berkelmans & van Oppen, 2006;

Cunning et al., 2015; Matthews et al., 2017). The adaptive bleaching hypothesis states that coral will eject less desirable algae to allow for recolonization of their tissues with symbionts that might provide greater protection from bleaching (Baker, 2003, 2004; Buddemeier & Fautin, 1993; Ware et al., 1996). In addition to photoprotection during a bleaching event, fluorescence utilized as a beacon is a possible explanation for colorful bleaching. There has been one study that examined the attraction of symbiotic algae to a fluorescing coral (Aihara et al., 2019). Additional phototaxis trials between motile dinoflagellates and fluorescing coral could support the beacon hypothesis and is a possible explanation for colorful bleaching. The attraction of hardier symbionts to replace the algae which are lost would describe additional adaptive mechanisms for coral bleaching, and fluorescence. I identified additional dinoflagellate species response to a green fluorescent coral, supporting the role of FPs in attracting dinoflagellates and an additional adaptive function of coral fluorescence.

Continued research involving actions such as supporting and conserving coral reef systems requires a new generation of scientists. The demand for professionals in the fields of science, technology, engineering, and math (STEM) is ever increasing (Sanders, 2009; Scott, 2012). Pursuit of a career in STEM starts with a student's interest and confidence in their scientific, technological, and mathematical abilities (Hinojosa et al., 2016; Mohd Shahali et al., 2019). A student's lack of confidence is a larger indicator than a student's lack of competency when considering whether to pursue a path in STEM (Lin & Schunn, 2016). Nurturing confidence and interest in STEM fields requires a combination of formal and informal learning (Dabney et al., 2012; Falk & Dierking, 2010) because the majority of a student's life is spent outside of a formal school

environment. Researchers have shown that informal science learning is crucial for students to develop an interest in STEM (Falk & Dierking, 2010; Lin & Schunn, 2016) and informal learning is the single biggest factor in determining future careers in science (Falk & Dierking, 2010). Informal science learning occurs in facilities such as nature centers, zoos, and aquariums (Bamberger & Tal, 2007; Falk, 2005). There are few studies that focus exclusively on informal science education within an aquarium, and the impact aquariums have on a student's interest in STEM. Exploring differences in attitude change based on structure of the field trip and toward STEM concepts following an aquarium visit provides an increased understanding and implementation of impactful teaching mechanisms for varied student populations. I sought to determine and the most effective methods of communication during an aquarium field trip to increase a student's interest in STEM fields.

OBJECTIVES

My objectives for the following three chapters of this dissertation were as follows:

Chapter II)

a. Identify changes in fluorescent protein (FP) emission intensity, symbiont density, and the relationship between the two within reef-building coral in response to four environmental stressors.
b. Analyze the relationship between initial FP emission intensity prior to the introduction of potential environmental stressors and the symbiont density of a coral after exposure to the environmental variable.

Chapter III)

a. Test the directionality of movement, speed, and displacement of two endosymbiotic dinoflagellates (*Symbiodiniaceae)* in relation to the green fluorescent emission of a reef-building coral.
b. Assess the movement of dinoflagellates in relation to green fluorescent emission of a live and a sealed coral. Also, assess the movement in relation to a live and sealed coral whose FPs have not been excited in order to ascertain attraction is due to phototaxis and not chemotaxis.
c. Compare the movement of endosymbiotic dinoflagellates and a non-symbiotic dinoflagellate in relation to the green fluorescent emission of a reef-building coral.

Chapter IV)

a. Assess the impact of a field trip to a public aquarium on student interest in the fields of science, technology, engineering, and math (STEM).
b. Compare student interest in STEM after a self-guided visit to a public aquarium as compared to a field trip which includes an educational component as well as the self-guided visit.

METHODOLOGICAL OVERVIEW

I measured the intensity of fluorescence emission for two species of hard coral, *M. capricornus* and *E. lamellosa*, at three time points over the course of two weeks of exposure to varying levels of four different environmental factors. I utilized an Ocean Optics spectrometer to capture fluorescence emission intensity at the start of the experiment, again after one week, and lastly after two weeks of environmental

manipulation. I arranged 15 independent recirculating saltwater research systems in three rows of five, with each row a replicate divided across three treatment groups. I placed coral samples within the research systems on a raised platform to allow for constant water flow, avoiding sedimentation and decomposition of settled organic material affecting the coral. After acclimatizing the 16 samples (8 of *M. capricornus* and 8 of *E. lamellosa*) per research system for 18 ± 4 days, I manipulated each system with four manipulated variables: light (μmol $m^{-2}s^{-1}$), temperature, salinity (ppt), and pH. I conducted two-week trials for each of the four environmental factors. For each variable I collected an initial fluorescence emission reading for 120 individuals of each species. At the conclusion of week one, I collected a measurement for 105 individuals of each species. At the conclusion of the trial, I collected emission measurements for 90 individuals of each species.

I removed one destructive sample (DS) per species per system at each time point for symbiont cell counts and surface area quantification to determine cell density (cells/cm^2). Following the protocol of (Kenkel et al., 2015), I separated coral tissue from the coral skeleton and homogenized the separated tissue to create a slurry of host tissue and symbiont cells. I used the coral skeleton to determine the surface area of the coral by wax method (Holmes, 2008; Stimson & Kinzie, 1991). I quantified cell counts again following the protocol of Kenkel *et al.* (2015).

Following the fluorescence emission trials, I acquired dinoflagellates from the family *Symbiodiniaceae* classified in separate clades from the Buffalo Undersea Reef Research (BURR) Culture Collection, University of Buffalo. I utilized *Cladocopium sp.*, Clade/cp type C180, *Breviolum psygomophilium*, with Clade/cp-type B224, and

Effrenium voratum Clade/cp type E202 for phototaxis trials to ascertain potential attraction to green fluorescence. I culled and fragmented *E. lamellosa* into approximately 8 x 10 mm sized fragments, then placed them in a holding vessel with outside measurements of 23 mm x 18 mm x 8 mm (L x W x H) and internal measurements of 20 mm x 15 mm x 5 mm under a dissection microscope at 50 x magnification. After dark adapting the three species of algae for 20 ±5 minutes to allow for even cell distribution (Swafford & Oakley, 2018), I gently swirled their culture vessel to further disperse the symbiont cells. I inoculated 1.5 ml of coral system water at 26 ° C in the holding vessel with 100 µl of algae in f/2 media suspension. After algae inoculation, I agitated the holding vessel to disperse the cells. I then placed the coral fragment in the vessel, acclimated the algae for one minute, and then filmed for 30 seconds independently for a blue light source at 440 - 460nm and a white light at 175 µmol $m^{-2}s^{-1}$. I used three conditions of coral to ascertain movement of the algae: a live coral, a coral which was sealed to control for potential chemotaxis, and a coral skeleton as a control. After filming I replaced the water within the vessel with new system water, re-inoculated with fresh algae, replaced the same coral fragment, and repeated the process 10 times. When capturing video under blue light, I utilized the same skeleton and sealed coral as the white light trial but replaced the live coral with a new specimen between algae species. I then quantified directional movement, speed, and displacement of the algae utilizing TrackMate, a plugin for Image J (Tinevez et al., 2017).

I quantified the impact of a field trip to a public aquarium on student interest in STEM by use of pre- and post-surveys. I contacted schools with students between grades three to six who requested a field trip to the Oklahoma Aquarium. I described the

research project and requested participation. Once a teacher agreed to participate in the study, I sent the teacher the pre/post survey form and parental permission form. The day prior to the visit, teachers administered the survey in the classroom to the students with obtained parental approval. Upon arrival to the aquarium, I collected completed survey forms. I separated the students into two sample groups—a control and an experimental. The control group participated in a self-guided tour of the aquarium. The experimental group participated in an educational program with an aquarium educator prior to the self-guided portion of the field trip. At the conclusion of the field trip, teachers again administered the survey, which I collected. I utilized the survey data to compare pre- and post-scores between the control and experimental groups based on; gender, whether they had previously met a STEM professional, if this was their first public aquarium visit, school of origin, and ethnicity.

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CHAPTER II

CHANGES IN FLUORESCENCE EMISSION INTENSITY AND SYMBIONT DENSITY AS CORAL ARE EXPOSED TO FOUR ENVIRONMENTAL FACTORS

INTRODUCTION

Anthropogenic effects on climate are having a particularly deleterious impact on coral reef systems (Hughes et al., 2017). Coral reefs are susceptible to high irradiance and temperature fluctuations (Glynn, 1996) with resilience further reduced by overfishing and pollution (Hoegh-Guldberg et al., 2017). There has been an approximate increase of 1°C in sea surface temperatures since the start of the Industrial Age (Heron et al., 2016). Coral health begins to deteriorate at a temperature increase of 1-2°C over the course of 5-10 weeks with the breakdown of the symbiosis between coral and coral's endosymbiotic dinoflagellates (Symbiodiniaceae) (Glynn, 1996). Reef corals are heterotrophs, capable of capturing prey, but are primarily dependent on their symbiont which provides up to 90% of the energy needed by the coral from the photosynthesis of the symbiont (Muscatine & Porter, 1977; Weis, 2008; Yellowlees et al., 2008). These corals will not survive without

the photosynthetic byproducts of the symbiont. Coral also face the challenge of acidification. One quarter of the carbon dioxide (CO_2) released by humans into the atmosphere is absorbed by the ocean, causing acidification (Albright et al., 2016; Hoegh-Guldberg et al., 2007). The anthropogenic CO_2 reacts with seawater to form carbonic acid, which has lowered the pH of the ocean by approximately 0.1 pH unit. In addition, carbonic acid breaks down into bicarbonate and protons, reacting with more carbonate ions, reducing the carbonate ion concentration and the availability for calcifiers. This makes acidification a major concern for marine organisms that form calcium carbonate shells or skeletons, such as corals (Albright et al., 2016; Hoegh-Guldberg et al., 2007).

Reef building corals obtain their bright colors from fluorescent proteins (FPs), which may also influence other aspects of the coral's biology, such as its response to stress. There are two primary sources of fluorescence within corals. The first source is the photosynthetic pigments of chlorophyll-a originating from a coral's symbiont with a peak emission at 685 nm. The second source is the FPs produced by the coral themselves. There are many different FPs within coral tissues and their emissions typically peak between 482 – 609 nm, from the shorter purple, blue, and green wavelengths to the longer yellow and red wavelengths (Alieva et al., 2008; Lapshin et al., 2015). The biological functions of the diverse animal FPs are still incompletely described (Alieva et al., 2008; Lapshin et al., 2015). The two most widely accepted hypotheses to explain the biological functions of FPs are photoprotection of the coral and the symbiont (Lapshin et al., 2015; Salih et al., 1998, 2000) or photo acclimatization for optimum photosynthesis by the symbiont in deep water (Smith et al. 2017). Coral inhabit a range of light habitats from shallow waters dominated by white light to deeper waters dominated by blue light and FPs may be important in the adaptation and acclimation of coral to these regimes

(Smith et al., 2017). FPs may serve as a "photobiological system" to control and regulate light, either by protecting the animal from harmful light levels at the surface, or by reflecting light in deeper waters (Salih et al., 2000; Smith et al., 2017). In addition to regulating light and protecting the coral from damaging levels of irradiance, FPs may also play a role in the protection of the coral holobiont from herbivorous fish, providing a visual barrier to the symbiont (Alieva et al., 2008). One objective of this research is to increase knowledge regarding the biological functionality of FPs within coral and their symbiont as a means of understanding their potential in sustaining reef systems under the increasing threat of anthropogenic effects.

At present, it is difficult to develop strategies to reverse the decline of reefs, as the physiological responses of corals to diverse stressors are not clearly understood (Hughes et al., 2010). Techniques that allow for the quick and non-invasive assessment of the impact of stressors on the symbiosis between coral and symbiont are urgently needed (Warner et al., 2010). GFPs are the most common FP in coral (Matz et al., 2006) and have been shown to be a potential indicator of coral health, with GFP concentration positively correlated with symbiont concentrations after exposure to both heat and cold stress (Roth & Deheyn, 2013). This suggests that GFP concentration and fluorescence intensity can be early proxies for coral health (Roth & Deheyn, 2013). Corals display an initial decrease in fluorescence intensity during heat and light stress, and then a significant increase just before bleaching (Roth & Deheyn, 2013). FPs have been shown to enhance resistance to bleaching in response to heat and light stress, with increased FP concentrations providing protection from harmful levels of irradiance and increased temperatures (Salih et al., 2000, 2006). Recent research suggests the significant increase in FP emission prior to bleaching is a final attempt of the coral to protect itself from the

harmful effects of over irradiance (Bollati et al., 2020). The association between fluorescence emission and abiotic influences such as temperature and light suggest that FPs may play an important role in mediating interactions between coral and their environment (Roth & Deheyn, 2013). The potential to provide an early warning system for coral bleaching, as well as the known contribution of FPs to coral health, require a greater understanding of the relationship between FPs, coral, and the coral symbiont. Change in FP emission, while documented in response to the stressors of increased heat and light, is not understood in the presence of other environmental stressors, such as salinity and pH outside of their natural range.

To provide insight into the relationship between FP emission and coral health, I observed the change in fluorescent emission intensity and symbiont density in response to known environmental stressors. I hypothesized that changes in FP emission would reflect underlying changes in symbiont density when coral were exposed to environmental stressors. I predicted that as symbiont density decreased in response to stress, there would be an initial increase in FP intensity, followed by an eventual decrease as the coral approached bleaching, potentially providing a proxy for coral health prior to visible paling of the coral due to bleaching. I also hypothesized that emission prior to environmental variable exposure could reflect resilience after exposure. I predicted that coral with higher emission levels prior to manipulated variable exposure would be more protected from bleaching after potential stress from exposure to variables by having higher symbiont densities.

Methods:

Model Species:

Due to their rapid growth after microfragmentation (Page et al., 2018) and predominately laminar growth pattern, I chose *Montipora capricornus* (Quoy and Gaimard, 1830) and *Echinopora lamellosa* (Lamarck, 1816) as model species. The flat morphology of the two species allows for ease of view, spectral measurement, and quantification of surface area. *M. capricornus* is native to the Indo-Pacific Ocean region, is often found in lagoons, and is a common species in these habitats. The species has closely arranged corallites with a rough coenosteum and no tuberculae or papilla, providing increased flat surface for ease of fluorescent measurement collection. The individual fragments used in the study were brown/green in appearance. *E. lamellosa* is native to the Indo-Pacific and eastern Pacific oceans. The species is common and is often a dominant species within shallow water habitats. The coralites are small with large spaces of coenosteum between coralites, providing ample room to collect fluorescent measurements. The individual fragments ranged from bright green to brown with bright green coralites (Veron & Stafford-Smith, 2000).

Coral acclimation and fragmentation:

Coral were obtained from the South Dakota Butterfly House and Aquarium. All coral within their individual species, *E, lamellosa and M. capricornus,* were fragmented from the same original colony, thus fragments are clonal. Coral were reared for two years in common garden conditions at the Oklahoma Aquarium within a 1135 L recirculating seawater system, under controlled conditions per Oklahoma Aquarium protocol (Appendix A). Coral were

fragmented from an original colony to provide samples for the trials, 8 individual *M. capricornus* samples and 8 *E. lamellosa* samples into each of the fifteen independent saltwater research systems within the Oklahoma Aquarium's Small Animal Holding Facility, for a total of sixteen individuals per research system. This was repeated for each environmental factor, PAR, temperature, salinity, and pH. Each treatment utilized 120 new samples from the original colony per species. The research systems were 19 L Fluval Aquariums, each with an independent recirculating saltwater system. Each system possessed a 25-watt Neo-therm submersible heater with an accurate electronic thermostat to +/-0.5° F. The lighting was provided by a 15-watt Kessil A80 Tuna Blue LED illuminator with adjustable intensity and color. Flow for each system was provided by a 3.9-watt, 120-volt circulation pump and filtration were provided by foam filter blocks and bio inserts, each of which were seeded with nitrifying bacteria from the 1136 L grow out system. I measured water chemistries biweekly (following Oklahoma Aquarium coral tank protocols) utilizing a portable HACH DR900 (Appendix A).

Experimental design for manipulation of environmental variables:

I arranged the 15 research systems in three rows of five, with each row a replicate divided across 3 treatment groups to control for effects of tank or position effect (left to right or top to bottom; Figure 1). Coral samples were placed within the research systems on a raised platform with a plastic nylon mesh surface attached to a plastic base. The structure of the platform allowed for constant water flow to avoid sedimentation and decomposition of settled organic material affecting the coral. After acclimatizing the 16 samples (8 of *M. capricornus* and 8 of *E. lamellosa*) per research system for 18 ± 4 days, I manipulated each

system based on the variable with three of the tanks held as controls. The four manipulated variables were: light (μmol $m^{-2}s^{-1}$), temperature, salinity (ppt), and pH. I conducted two-week trials for each of the four environmental variables.

Photosynthetically Active Radiation (μmol m^{-2} s^{-1}):

PAR levels on coral reef systems vary from 700 μmol m^{-2} s^{-1} to 1400 μmol $m^{-2}s^{-1}$ (Bainbridge, 2017), which is higher than what aquarium reared coral can typically withstand. Coral which have been reared in an aquarium are maintained at an overall lower PAR than coral in the field. This makes them more sensitive to a large increase in irradiance. I maintained lower PAR than those found in the field, to attain a stressful level of irradiance without immediately causing symbiont expulsion and subsequent bleaching of the coral samples. Throughout the trial, I controlled the PAR by manipulating a Kessel light source. I measured PAR with an Apogee MQ-500 Full Spectrum Cosine Quantum PAR Meter. Following the guidelines of Oklahoma Aquarium coral husbandry technique, I maintained an ambient PAR of 80 μmol $m^{-2}s^{-1}$ in three research systems; *E. lamellosa* (*n* = 24), *M. capricornus* (*n* = 24). Six systems were maintained at 100 μmol $m^{-2}s^{-1}$; *E. lamellosa* (*n* = 48), *M. capricornus* (*n* = 48), and six were maintained at 200 μmol $m^{-2}s^{-1}$; *E. lamellosa* (*n* = 48), *M. capricornus* (*n* = 48).

Temperature:

Coral are typically found in regions with water surface temperatures between 23° - 29° C, with the ability to survive short periods of increased temperature (Coffroth et al., 2010; Sheppard & Rioja-Nieto, 2005). Separate coral samples were maintained at three different temperatures across a two-week period. The ambient temperature was 26° C; *E.*

lamellosa (*n* = 24), *M. capricornus* (*n* = 24). There was a lower temperature of 21° C; *E. lamellosa* (*n* = 48), *M. capricornus* (*n* = 48), and an increased temperature of 31° C; *E. lamellosa* (*n* = 48), *M. capricornus* (*n* = 48).

Salinity (ppt):

In a natural ocean environment, coral inhabit regions with salinities from 25 to 42 ppt and are adapted to the salinity of their local environment (Ferrier-Pagès et al., 1999). I controlled salinity within each individual system through addition of Instant Ocean or Reverse Osmosis Deionized (RODI) fresh water provided by the Oklahoma Aquarium. The control salinity was 33 PPT; *E. lamellosa* (*n* = 24), *M. capricornus* (*n* = 24). The reduced salinity was 27 PPT; *E. lamellosa* (*n* = 48), *M. capricornus* (*n* = 48), and the elevated salinity was 36 PPT; *E. lamellosa* (*n* = 48), *M. capricornus* (*n* = 48) (Ferrier-Pagès et al., 1999; Hoegh-Guldberg & Smith, 1989; Kuanui et al., 2015). I maintained the high salinity at 36 PPT contrary to the protocol of Kuanui et al. (2015), which set salinity at 37 PPT, based on personal observation of rapid degradation of coral tissue at 37 PPT.

pH:

pH in the ocean were historically 8.2, but have dropped to 8.1 since the start of the Industrial Revolution (Liu & He, 2012). I achieved pH water chemistry manipulations utilizing sodium bicarbonate ($NaHCO_3$) to raise pH and hydrochloric acid (HCl) to lower pH. I dissolved $NaHCO_3$ in system water prior to dripping into the system on the opposite side from the filtration intake. I dripped HCl on the system side away from filtration. This location was to the side of the coral platform, not directly above the coral (Appendix A). The standard aquarium system pH was 8.1 ± 0.1; *E. lamellosa* (*n* = 24), *M. capricornus* (*n* = 24). The lower average pH was 6.72 ± 0.9 ; *E. lamellosa* (*n* = 48), *M. capricornus* (*n* = 48), and

the increased average pH was 8.9 ± 0.5 ; *E. lamellosa* (*n* = 48), *M. capricornus* (*n* = 48). Fluorescent proteins remain stable at pH 6 – 10, with intensity lowering at pH < 6, and increasing at pH 10-12 (Campbell & Francis, 2001).

Fluorescence Spectral Measurements:

I measured fluorescence with an Ocean Optics Flame-S-VIS-NIR-ES spectrometer with 600 μm reflectance probe terminated in a ¼" waterproof stainless ferrule. Reflectance emission was read by the OceanView software package (Ocean Optics). In order to reduce noise yet maintain high resolution, I set a low "boxcar width" of 3 and averaged 20 scans. Fluorescent proteins (FPs) in the green emission range were excited with lights provided by NightSea LLC (400-415 nm violet LED, 440-460 nm blue LED, 660 nm red LED). Cyan and green FPs possess the same chromophores, and both excite with blue LED (Mazel, Alieva *et al.*, 2008). The blue LED excited green (GFP) and cyan fluorescent proteins (CP), and the red LED was a control wavelength, which does not photoconvert GFP (A. Salih, personal communication). I took initial emission readings from *E. lamellosa* under violet excitation at 410 nm with emission at 502± 2 nm (*n* = 15). At all times, the CP emission peak was at a shorter wavelength with a peak of lower intensity than the GFP emission peak of 520 nm. After ascertaining that GFP was at a higher wavelength, I did not measure CPs in the remainder of the coral samples. I took initial emission readings from *E. lamellosa* under red excitation at 660 nm which provides no excitation for fluorescent proteins within the green range and has a peak emission at 635 ± 0.4 nm (*n* = 15). After ascertaining that red light did not excite the GFP within the coral samples, I did not measure emission from red excitation in the remainder of the samples.

I collected spectral measurements placing each individual sample within a [give polymer] weigh boat under a Leica MZ 9_5 dissection microscope illuminated with a blue excitation light at 440 – 460nm (NightSea LLC). The collected light emission was passed through a long pass filter within the spectrometer to block the blue reflected light leaving the emitted fluorescent light. I transferred each individual sample to the weigh boat containing 500 mL of system water from the corresponding research system. After taking an initial photograph against a fluorescent standard, I mapped five separate locations on the coenosarc between individual coralites (Figure 2) to standardize the location of spectral readings for each of the three time points. I placed the reflectance probe within a holding device for a fixed distance of 5 mm from the bottom of the weigh boat and a 60° angle to assure uniform readings per sample per time point. I took an initial fluorescence measurement against the white of the weigh boat under blue excitation and yellow barrier filter to set a baseline. I set the spectrometer to a baseline emission intensity per species; *E. lamellosa* – 2700 Arbitrary Units (AU), *M. capricornus* – 6000 AU. I re-calibrated to these initial AUs prior to removing samples from a new system to help prevent slow instrument drift (Mazel & Fuchs, 2003). I collected initial spectral measurements for each sample; *E. lamellosa* (n = 120), *M. capricornus* (n = 120). After one week of exposure to a manipulated variable, I took mid-trial readings of fluorescence spectral measurements; *E. lamellosa* (n = 105), *M. capricornus* (n = 105). I took post trial fluorescence spectral measurements; *E. lamellosa* (n = 90), *M. capricornus* (n = 90) (Table 1). Sample size differences represent the removal of destructive samples at each time point; one per species per research system. All fluorescent measurements were taken at locations marked on a map created on a photograph taken under the dissection scope at the commencement of the spectral measurements. I took fluorescent

measurements at five separate locations on the coenosarc. This process was repeated after one week at mid-trial and after two weeks at trial completion.

Symbiont Isolation and Quantification:

I removed one destructive sample (DS) per species per system for symbiont cell counts and surface area quantification to determine a starting cell density (cells/cm^2); *E. lamellosa* (*n* = 15), *M. capricornus* (*n* = 15). I again culled one DS per species, per tank; *E. lamellosa* (*n* = 15), *M. capricornus* (*n* = 15) at the mid-point of the trial. At the conclusion of two weeks of manipulated exposure, I again culled DS for cell counts and surface area quantification to determine a final cell density (cells/cm^2); *E. lamellosa* (*n* = 15), *M. capricornus* (*n* = 15). DS of each species from each of the 15 research systems at the start, midpoint and commencement of the four manipulation trials, consisted of total symbiont counts for 180 individuals of each species, and 360 total destructive samples.

Tissue Removal:

Following the protocol of (Kenkel et al., 2015), I separated coral tissue from the coral skeleton with an artist's airbrush powered by an air compressor set at 125 psi. After thawing the frozen destructive sample for one hour, I placed the coral in 5 mL of an extraction buffer (1 M Tris HCl, 1 M DTT) for 10 minutes before placing the coral in a 950 mL plastic bag to contain the tissue as it was removed from the skeleton. I added another 5 mL of extraction buffer to remoisten the coral during tissue removal until the white coral skeleton was completely exposed. I then added another 2 – 4 mL as needed to remove tissue from the sides of the bag. After recording the final volume, I transferred

the tissue from the bag to a 50 mL centrifuge tube and homogenized for 30 seconds with a Benchmark D1000 tissue homogenizer to create a slurry of host tissue and symbiont cells. I saved an aliquot of 100 μL of homogenized slurry in a 1.5 mL centrifuge tube with 100 μL of a 10% formaldehyde concentrate to fix the sample for future cell counts. Coral skeletons were then placed in a 10% bleach solution for 24-48 hours in preparation for surface area quantification by means of wax weight.

Wax Weights:

I determined the surface area of the coral by wax method (Holmes, 2008; Stimson & Kinzie, 1991). I weighed the skeletons of the destructive coral samples which had been treated in a 10% bleach solution and dried for 24 – 48 hours. The skeletons were dipped for 2 seconds in 65°C paraffin wax. After dipping, I rotated the skeleton to completely cover the surface of the skeleton and then I shook the skeleton six times to remove excess wax. I then took a single dip wax weight, repeated the dipping procedure and obtained a double dip wax weight. After plotting a linear regression from reference blocks of a known surface area ($r^2 = 0.9879$), I utilized the resulting regression equation to determine the surface area of each of the wax dipped corals. Cell count of each coral was expressed as number of cells per cm^2 of the coral surface area (Kenkel *et al.,* 2015).

$D = V_t (X_r * V_h * DF)/SA$

D = Symbiont density of coral sample (cells per cm^2)
V_t = Total volume of sample slurry
X_r = Mean of four replicate counts
V_h = Volume of 1.000 mm^2 grid of hemocytometer (10^4)
DF = Dilution factor (2)
SA = Surface Area (cm^2)

Symbiont Cell Counts:

All cell counts for coral samples were quantified by use of a Neubauer improved hemocytometer. Utilizing an OMAX 40X – 2500X compound LED microscope, I performed quadruple counts of 40 µL samples taken from the fixed slurry aliquots at 400x magnification. I dipped the edges of a glass cover slip in RODI fresh water and adhered to the hemocytometer. I vortexed the 1.5 mL aliquot of tissue slurry for sixty seconds, then pipetted 20 µL to both sides of the front edge of the cover slip of the hemocytometer. I utilized a thumb counter to count all cells within the 1.000 mm^2 grid located on the four corners of the hemocytometer grid, counting cells that fell on the bottom or right line of each grid and not the top or left line of the grid to avoid repeat counts. For each sample, I took an average of the four cell counts and multiplied by the dilution factor of 2 to account for the addition of 100 µL formaldehyde solution for fixation of tissue slurry.

Statistical Analysis:

Statistical analysis and modeling were performed in RStudio version 1.2.1335 (http://www.rstudio.com). In order to assess the change in fluorescent protein emission in response to symbiont count, I created linear models with emission as the response variable and symbiont count, species, and their interaction as explanatory variables. I first ran a global analysis which integrated all four environmental variables and both species, and then ran models for each individual variable and each of the two species independently. The combination of these analyses allowed me to test for general patterns across manipulated variables and species. I excluded random effects to assess only the relationship of FP

emission and symbiont density regardless of condition, and then the emission and density relationship evaluated by individual environmental factor and by individual species.

To assess the change in fluorescent protein emission intensity across timepoints and in response to the environmental factors of PAR, temperature, salinity, and pH, I ran linear mixed models (LMM) with the lme4 program in R (Bates et al., 2014) fit by restricted maximum likelihood estimation (REML) in the nlme program (Pinheiro et al., 2012). Due to a non-normal distribution determined by a Shapiro – Wilk's test for normality ($W = 0.941$, $P = 0.002$) (Wenger et al., 2016) of the change in symbiont density in response to the environmental manipulations across time, I fit a generalized linear mixed model (GLMM) (Bolker et al., 2009), fit by maximum likelihood estimation (ML) in the nlme program with a Poisson family distribution. I used mixed models to hold random effects constant in my analyses (Zuur, 2009). I generated degrees of freedom and p values using the Satterthwaite's method with the lmerTest package in R (Kuznetsova et al., 2017). For both response variables, I utilized the model with the lowest Akaike information criterion (AICc) score. For FP emission intensity, the environmental variable and sampling time point were included as fixed effects and individual sample and research system were included as random effects. For symbiont density, stressor and sampling time point were included as fixed effects and individual sample was included as a random effect. I did not include research system as a random effect because I collected only one measurement per tank at each time point. All plots were created in the ggplot2 program (Gómez-Rubio, 2017). I removed outliers when analyzing the relationship between FP emission and symbiont density (LM), when analyzing the change in fluorescence (LMM), and symbiont density (GLMM) over three time points to obtain a more normal distribution. I identified outliers utilizing the interquartile range rule

(Borovcnik, 2007). Changes in sample size are due to removal of outliers. Samples were also lost due to damage/spillage during wax weight measurements and cell counting process.

To assess the relationship between fluorescence emission at timepoint one and symbiont density at timepoint three, I ran linear models. The first model included the initial emission and final symbiont density for all the environmental stressors combined and both species combined. I then ran the same linear model for each stressor individually for both species combined, and each individual stressor for both species independently.

Results:

The relationship between fluorescence emission intensity and symbiont density in response to environmental conditions:

Global analysis of manipulated environmental variable levels

After first determining there was no significant effect on changes for either FP emission or symbiont density by individual research system, I analyzed the relationship between FP emission intensity and symbiont density in response to four environmental factors (light intensity, temperature, salinity, and pH). I first performed a global analysis of the relationship between fluorescence emission intensity and symbiont density for both species and all manipulated variables to determine a relationship between emission and intensity at all manipulated levels of the individual variables. The PAR condition produced a non-significant negative relationship between fluorescence emission intensity and symbiont density for *E. lamellose* and *M. capricornus* (Table 2; Figure 3).

During trials in which I manipulated temperature, there was a non-significant relationship between FP emission intensity and symbiont density for combined temperature

levels for *E. lamellosa* and *M. capricornus* (Table 2; Figure 3). The manipulated salinity trial produced a non-significant positive relationship between emission intensity and symbiont density for *E. lamellosa* and non-significant negative relationship for *M. capricornus* (Table 2; Figure 3).

The environmental variable of pH produced a significant positive relationship between emission and density for pH levels combined for *E. lamellosa,* and a non-significant negative emission and density relationship for *M. capricornus* exposed to manipulated pH (Table 2; Figure 3).

Effects of manipulated levels within each environmental variable on the relationship between fluorescence emission and symbiont density:

I analyzed the FP emission and symbiont density relationship by manipulated level of each environmental variable to determine if the relationship between emission and density differed across manipulated levels. FP emission intensity was not significantly related to symbiont density at any of the manipulated levels of PAR for the two species (Table 3; Figure 4).

For both *E. lamellosa* and *M. capricornus*, exposure to the increased manipulated temperature of 31° C there was a non-significant positive relationship (Table 3; Figure 5). Emission intensity and symbiont density were negatively related for both species at 21° C and 26° C. *M. Capricornus* displayed a significant negative relationship at 21° C.

There was a significant negative relationship between FP emission intensity and symbiont density when exposed to manipulated salinity for *M. capricornus* corals exposed to the control salinity of 33 ppt, but non-significant positive relationships at 27 and 36 ppt

(Table 3; Figure 6). *E. lamellosa* displayed a non-significant relationship across salinity between FP emission and symbiont density, with the salinity of 27 ppt showing a slightly positive relationship and salinities of 33 and 36 ppt displaying a slightly negative relationship.

Lastly, exposure to the environmental variable of pH produced a significant positive relationship between FP emission intensity and symbiont density for the species *M. capricornus* higher pH of 8.9 (Table 3; Figure 6). All emission and density relationships were non-significant and positive for *E. lamellosa. M. capricornus,* apart from the significant positive relationship at a pH of 8.9, were non-significant and negative.

Species comparison of effects for manipulated levels within each environmental factor on the relationship between fluorescence emission and symbiont density:

There were no significant differences between species in their relationship between fluorescence emission and symbiont density when exposed to PAR. Species responded significantly differently to temperature, salinity, and pH, but did not differ significantly by timepoint or the interaction of timepoint and species (Table 4).

Changes in fluorescence emission in response to environmental variables across time:

Given the limited and inconsistent relationships between fluorescence emission intensity and symbiont density, I next analyzed temporal changes in each of these variables independently in response to each of the four environmental variables. For both species combined, there was a non-significant trend at all levels of PAR for emission intensity to increase between time points 1 and 2, and to decrease between time points 2 and 3 (Table 5;

Figure 8a). Corals exposed to the increased PAR of 100 and 200 μmol $m^{-2}s^{-1}$ displayed greater fluctuations in emission than coral housed at the control level of 80 μmol $m^{-2}s^{-1}$. Fluorescence emission across the experimental period was not significantly affected by different levels of PAR in *E. lamellosa;* however, there was the same pattern of change as displayed by both species combined, with an increase in emission between time points 1 and 2, and a decrease between time points 2 and 3 for the increased light intensity of 200 μmol $m^{-2}s^{-1}$ (Table 5; Figure 8b). The levels of fluorescence emission for *M. capricornus* were significantly different over time, again with an increase in emission intensity between time points 1 and 2 and a decrease between time points 2 and 3 (Table 5; Figure 8c).

The coral exposed to differing temperatures displayed similar patterns in fluorescence emission across time for the three temperature levels and both species combined. There was very little change in FP emission from time point 1 to time point 2, and a decline from time point 2 to 3 (Table 6; Figure 9a). The interaction between temperature and time produced a significant change in emission for *E. lamellosa* exposed to the three temperature levels. For all the coral samples, there was a slight decrease in emission from time point 1 to 2, and a greater decrease from time point 2 to 3. The most significant change between time points 2 and 3 were for samples exposed to the highest temperature of 31° C, which had a much lower FP emission intensity (Table 6; Figure 9b). There was a significant difference in emission intensity over time for *M. capricornus* samples: increase in emission intensity between time points 1 and 2 and a decrease between time points 2 and 3 (Table 6; Figure 9c).

The interaction of manipulated salinity and time for both species combined significantly impacted fluorescence emission, with minimal change in emission between time points 1 and 2 and a significant decrease between time points 2 and 3 (Table 7; Figure 10a).

The fluorescence emission intensity of *E. lamellosa* samples was not significantly affected by time, salinity or their interaction. There was little change in fluorescence emission between time points 1 and 2 for samples exposed to the salinities of 27 and 33 ppt. Coral exposed to 36 ppt displayed a decrease in emission between the same two time points. All three levels of exposure produced a decrease in emission between time points 2 and 3 (Table 7; Figure 10b). There was a significant change in FP emission across time, salinity, and in response to the interaction between the two for *M. capricornus* samples. All three salinity level exposures produced a decrease in emission between time points 1 and 2 and again between 2 and 3 (Table 7; Figure 10c).

Fluorescence emission in both species combined was significantly impacted by time, pH, and the interaction between the two. Coral exposed to any one of the three pH levels displayed decreased emission between time points 1 and 2, and 2 and 3 (Table 8; Figure 11a). The interaction of time and pH produced a significant change in emission for *E. lamellosa* samples. As with both species combined, there was a decrease in emission over time (Table 8; Figure 11b). As with the species combined and *E. lamellosa,* there was a decrease in emission between each of the three time points for *M. capricornus*. FP emission intensity decline was significant as pH increased, over time, and was significant for the interaction of time and pH (Table 8; Figure 11c).

Species comparison for changes in fluorescence emission in response to environmental factors across time:

There was a predominantly significant difference in FP emission over time between species in response to manipulated environmental variables. Species differed significantly in response to PAR depending on timepoint; *E. lamellosa* and *M. capricornus* were not

significantly different by species alone. Fluorescence emission in response to manipulated temperature was significantly different between species, run and their interaction. Manipulated salinity had a different impact on emission between species and by run, but not their interaction. The same was true for emission in response to manipulated pH (Table 9).

Changes in symbiont density in response to environmental factors across time:

Symbiont density decreased significantly over time. The interaction between PAR exposure and time for both species combined significantly influenced symbiont density. Coral exposed to PAR of 80 and 100 μmol m^{-2} s^{-1} displayed a decline in symbiont density over time. Coral exposed to 200 μmol m^{-2} s^{-1} displayed a slight increase in density between time points 1 and 2 and then a significant decrease between times 2 and 3, with a final density less than the starting density (Table 10; Figure 12a).

E. lamellosa samples also displayed significant decreases in symbiont density by time and there was a significant interaction between PAR and time on symbiont density. Individuals exposed to 200 μmol $m^{-2}s^{-1}$ had little density change between times 1 and 2, but a significant decrease between 2 and 3. Individuals exposed to 80 μmol $m^{-2}s^{-1}$ or 100 μmol $m^{-2}s^{-1}$ had a decrease in density between time points 1 and 2, but then an increase between time points 2 and 3 (Table 10; Figure 12b). *M. capricornus* samples also displayed significant changes in emission by time, and the interaction effect between time and PAR significantly influenced symbiont density. Individuals exposed to any of the three PAR levels had an initial increase in symbiont density between time points 1 and 2 and then a decrease between time points 2 and 3 (Table 10; Figure 12c).

There was a non-significant change in symbiont density for both species combined across the three temperature levels; however, the change over time and the interaction between temperatures and time significantly impacted symbiont density. Coral exposed to any of the three temperatures decreased in density between the first two time points and increased from the middle to final time points. Individuals exposed to 26° C finished the trial with a higher density than at the start. Individuals exposed to 21and 31° C finished the trials with lower densities, with individuals exposed to 31° C displaying the greatest loss (Table 11; Figure 13a). *E. lamellosa* had a significant change in symbiont density in response to both time and the interaction between temperature and time. Samples exposed to any of the three temperatures had an initial decrease in density and then an increase. An exception was found in samples exposed to 31° C. They declined significantly from time point 1 and 2, and stayed consistent between the middle and final time points (Table 11; Figure 13b). *M. capricornus* had a significant change in symbiont density in response to temperature, time and the interaction of the two. Samples exposed to any of the three temperatures had a significant decline in symbiont density between time points 1 and 2, and an increase between time points 2 and 3. The most significant increase in the final density was for those exposed to the control temperature of 26° C (Table 11; Figure 13c).

Both species of coral displayed significant changes in symbiont density across time, salinity, and in response to the interaction between time and salinity. The symbiont densities of those exposed to 36 ppt remained consistent over time. Those exposed to 27 and 33 ppt increased symbiont density over time (Table 12; Figure 14a). *E. lamellosa* individuals displayed a significant change in density by time and the interaction between time and salinity also influenced symbiont density. Again, the high salinity group maintained similar

densities across time, and the control and lower groups increased symbiont density over time. The most significant increases in densities were from the control group (Table 12; Figure 14b). *M. capricornus* individuals had significant changes in symbiont density by salinity, time and the interaction between the two. Individuals exposed to 27 ppt possessed greater symbiont densities at each time point. The control group (33 ppt) initially decreased and then significantly increased in symbiont densities between the middle and final time points. (Table 12; Figure 14c).

Symbiont density changed significantly for both species combined due to pH, time, and the interaction between the two. At pH of 8.1 and 8.9, symbiont density decreased over time, with samples from both species individually and combined expressing an almost complete loss of symbionts by the third time point at pH 8.9. The *E. lamellosa* samples exposed to pH 7.4 initially increased in density, then significantly decreased between the second and third time points (Table 13; Figure 15a). *E. lamellosa* individuals also had significant changes in density in response to pH, time, and their interaction. Again, there was a consistent decline in densities across time for both the control and high pH groups, and an initial increase, and then, significant decrease for the individuals in the low pH group (Table 13; Figure 15b). *M. capricornus* samples also displayed significant changes in density across levels, time, and their interaction. At all pH, symbiont density declined over time (Table 13; Figure 15c).

Species comparison for changes in symbiont density in response to environmental variables across time:

Changes in symbiont density over time were significantly different between the two species of *E. lamellosa* and *M. capricornus* in response to four manipulated environmental factors; light, temperature, salinity, and pH. The significance in difference was between species, by run, and their interaction (Table 14).

Final symbiont density in relation to initial fluorescent emission intensity:

In order to ascertain if initial fluorescence emission might indicate the final symbiont density of the coral across the manipulated environmental variable trials, I ran a linear model incorporating both species and all the experimental manipulations. Across species and environmental conditions, there was a non-significant positive relationship between initial fluorescence level and final symbiont density for both species exposed to manipulated salinity. Unlike temperature and pH, which had negative relationships for both species, PAR produced a significant positive relationship for *M. capricornus*, and a non-significant negative relationship for *E. lamellosa.* The relationship between initial fluorescence emission and final symbiont density was not significant for *E. lamellosa* across environmental conditions. The relationship between initial fluorescence emission and final symbiont density for *M. capricornus* was significantly positive when manipulated by PAR and non-significant for the other three environmental variables (Table 15; Figure 16).

Species comparison for final symbiont density in relation to initial fluorescent emission intensity:

Of the four manipulated environmental variables, only PAR produced a significant difference between species on the impact of initial fluorescence emission on the final

symbiont density between species. Temperature, salinity, and pH differences did not significantly differ between species in the ability of a higher initial emission to provide protection from symbiont loss for the coral (Table 16).

Discussion:

This research examined changes in emission intensity as an indicator for coral health after exposure to four different ecologically relevant variables. My prediction that FP emission and symbiont density would be negatively related to one another was supported, in a few instances, most notably among *M. capricornus* samples (Table 3). My hypothesis that the pattern of FP emission intensity in response to exposure to the four environmental conditions over time would illustrate a significant change in response to stress, was only minimally supported among all factors except for pH, which displayed a significant change in FP emission intensity over time (Table 8). My results did not support the findings of previous research which illustrated an initial decrease in emission intensity and then significant increase in response to temperature stress (Roth & Deheyn, 2013). Instead, the results followed an overall pattern of a continual decrease as the exposure to manipulated temperature increased (Tables 6) . My prediction that the initial intensity of FP emission would be positively related to the final symbiont density was only partially supported. The overall pattern for both species and all stressors combined displayed a negative relationship that was not significant. *M. capricornus* exposed to manipulated levels of the environmental condition of PAR was an exception (Table 15), the significant positive relationship provides support for photoprotection of the symbiont provided by FPs.

The relationship between fluorescent emission intensity and symbiont density

The expected result of a negative relationship between fluorescent emission intensity and symbiont density due to the shading of the FPs by the symbiont (Roth & Deheyn, 2013) was supported only in the case of a significant negative relationship for *M. capricornus* exposed to 21°C and salinity at 33 ppt (Tables 11, 12). There was a trend for a negative relationship for both species at all manipulated levels, except for a positive relationship between emission and density in *E. lamellosa* in the pH experiment (Tables 10, 11, 12, 13). There was quite a bit of variability in the relationship between FP emission intensity and symbiont density within the individual manipulated levels of the four factors.

PAR

Coral of both species combined and individually did not have significant relationships between fluorescence emission intensity and symbiont density during the trials in which I manipulated light; however, when analyzing the relationship at all three PAR levels, there were similar patterns among the levels (Table 3). Coral exposed to the highest PAR, 200 μmol m^{-2} s^{-1}, displayed a slightly negative relationship with emission intensity increasing as density decreased. At 80 and 100 μmol m^{-2} s^{-1} intensity barely changed with density, unlike the positive or negative coral responses to changes in salinity, temperature and pH (Table 3). FPs are known to have photoprotective properties in symbiotic algae (Salih et al., 2000). Perhaps in the absence of other stressors, fluorescence intensity remains constant as the FPs continue to provide a protective barrier even with increased symbiont density, which would typically block the FPs, therefore, lowering intensity.

Temperature

Coral exposed to 26°C and 21°C followed the expected pattern of increased emission intensity in relation to decreased symbiont density due to shading by the symbiont (Roth & Deheyn, 2013). At 31° C, emission and density were positively related (Table 3). This could be a result of the higher temperature damaging the FPs while also facilitating bleaching, leaving a coral sample near bleaching with both reduced emission intensity and symbiont density. Previous research on GFPs supports thermal sensitivity of fluorescence: a steep decline in protein folding capacity at higher temperatures (Tsien, 1998; Zhang et al., 2009), and certain FPs downregulate emission in response to heat stress (Smith-Keune & Dove, 2008).

Salinity

Among the three levels of salinity, the emission intensity relationship to symbiont density followed the expected pattern of increased emission with reduced symbiont density due to blocking of the FPs by the symbiont. The exception was for *E. lamellosa* exposed to 27 and 36 ppt, in which there was a positive relationship between FP emission intensity and symbiont density (Table 3). The green fluorescent protein has been shown to be sensitive to ion concentrations (Morikawa et al., 2016). This suggests that as *E. lamellosa* approached bleaching while being exposed to either a high or low salinity, there was not only a loss of symbionts, but also potentially a decline in the efficiency of the FPs, causing a decrease in emission intensity. Despite the anomaly of the pattern within *E. lamellosa,* the overall relationship between emission intensity and symbiont density for both species combined followed the expected pattern of increased intensity with decreased symbiont density.

pH

During the pH manipulation trials, there was a significant positive relationship between emission and density when the species were combined or assessed separately at pH 8.9. There was rapid bleaching and subsequent death among the samples exposed to this pH (Table 3). FPs are known to be sensitive to intercellular conditions impacted by pH and ion levels (Morikawa et al., 2016). Although GFP is typically stable at a pH of 6-10 (Campbell & Francis, 2001), there were large fluctuations in pH daily in order to maintain a pH of 8.9. I was able to maintain consistency of pH more successfully at the control pH of 8.1 and the lower pH of 7.4. At these pH levels, the relationship between FP emission intensity and symbiont density followed the expected pattern of increased emission with decreased density.

Changes in fluorescence emission intensity in response to environmental conditions across time:

E. lamellosa, *M. capricornus*, and the species combined, displayed an increase in FP emission intensity between the start, and mid-point measurements during the PAR trial, and a decrease between the mid-point and final measurement time points. There was an exception for *E. lamellosa* when exposed to 80 and 100 μmol $m^{-2}s^{-1}$ (Table 5). FPs provide photoprotection (Salih et al., 1998, 2000), and an increase in fluorescence prior to bleaching would suggest that the coral are fluorescing more intensely in an attempt to protect themselves from irradiance and significant loss of symbiont. The change over time was significant only for *M. capricornus* exposed to manipulated light, but the pattern of an increase in emission intensity as a coral stresses and then decrease as they have continued stressful exposure to increased PAR, lends evidence to the need for further research to

potentially support the visual reports of an increase in emission intensity prior to bleaching. My findings do not support previous research illustrating an initial decrease in emission prior to an increase in emission while exposed to temperature stress. The discrepancy may be the result of this research collecting fluorescence readings at three time points across 14 days as compared with other research which collected six readings across 20 days.

Fluorescence emission intensity of coral exposed to temperature stress did not follow the same pattern of those exposed to PAR, initial increase and subsequent decrease of emission intensity. The one exception was *M. capricornus,* in which emission initially increased and then decreased by the third time point (Table 6). Increased temperature damages the photosynthetic apparatus of the symbiont leading to photoinhibition (Roth, 2014). Photoinhibition often initiates coral bleaching (Baird et al., 2009). Both increased temperature which damages the symbiont's ability to properly photosynthesize, and increased PAR which can inhibit the photosynthetic capability of the symbiont, could result in the same effects on emission intensity (Hill et al., 2011). For *E. lamellosa*, there was a significant effect of temperature on fluorescence emission intensity, with a steady decrease over time. Fluorescence emission intensity also decreased across the experimental period at all three temperature levels for both species combined.

Exposure to manipulated salinity caused decreased FP emission intensity at each time point for the control salinity of 33 ppt, the lower concentration of 27 ppt and the higher concentration of 36 ppt (Table 7). The stepwise decrease in emission intensity over time is most likely due to the relationship between FP emission intensity and symbiont density, as symbiont density increased over time. Increased symbiont density may have provided

shading of the FPs, causing a decline in the intensity of emission when measured externally with the spectrometer (Roth & Deheyn, 2013).

At all pH, each coral sample displayed a steady decline in FP emission intensity over time (Table 8). Exposure to pH 8.9 resulted in negligible emission intensity after two weeks and death of the coral, indicated by minimal symbiont density and the presence of external microalgae on the surface of the coral (Done, 1992)

Changes in symbiont density in response to environmental variables across time:

At all PAR, for each species individually and the species combined, symbiont density within coral significantly decreased after two weeks (Table 10). This would be expected due to the stress of increased PAR exposure and the loss of symbionts leading to eventual bleaching (Anderson et al., 2001; Shick et al., 1996).

Overall, symbiont density significantly decreased in corals across temperatures after two weeks, although there were notable differences in the patterns of change across species and temperatures. The most unexpected result was the high symbiont retention at 36°C. I was not able to identify the species of symbiont within each of the coral samples (Table 11). The level of protection from heat is often regulated by the species of symbiont within the holobiont (Howells et al., 2012) and differences in the species composition of the symbiont may have regulated the degree and pattern of symbiont loss.

Exposure to manipulated salinity resulted in different outcomes than the other three environmental treatments. There was an overall decrease in emission intensity over time as symbiont density increased (except at 36 ppt), which supports the prediction that as symbiont density increases, fluorescence emission intensity decreases (Table 12). Sudden changes in

salinity have a damaging effect on coral (Ferrier-Pagès et al., 1999), causing cellular damage due to the coral animal's stenohaline and osmoconforming nature (Seveso et al., 2013). Perhaps the unexpected result of increased symbiont density in response to salinity is explained by a change in salinity producing a less damaging effect on coral than either changes in light or temperature (Hoegh-Guldberg & Smith, 1989). Manipulation of pH produced a significant, profound effect on the fluorescence emission intensity and final symbiont density of the samples, especially at pH 8.9, which was lethal (Table 13).

Final symbiont density in relation to initial fluorescent emission intensity:

Overall, initial FP emission intensity was not significantly related to symbiont density at the completion of trials across species and environmental variables (Table 15). When PAR was manipulated, initial FP emission intensity was positively related to final symbiont density for *M. capricornus*. This research does not support an overall relationship between emission intensity and symbiont density, apart from those exposed to salinity and pH manipulations. The result of an increased protein emission resulting in a final higher symbiont density than at the start of the trial would warrant further exploration, considering previous research that supports that FPs provide photoprotection to the coral when exposed to light stress (Roth, 2014; Salih et al., 1998, 2000) with increased initial emission offering protection to the coral subjected to light stress. The lack of a significant relationship between initial emission and final density after exposure to temperature manipulations contradicts previous research hypothesizing a protective functionality of fluorescence emission on coral health in response to temperature stress (Table 15) (Roth & Deheyn, 2013).

In the salinity manipulation experiment, initial FP intensity and symbiont density exhibited a non-significant positive relationship. Coral with increased initial FP emission possessed higher symbiont densities after exposure to salinity manipulations (Table 15). Coral samples exposed to salinity changes at the ambient level had a significantly higher symbiont density by the third time point which is a result that is contrary to previous research illustrating damage to the stenohaline coral with changes in salinity (Seveso et al., 2013).

pH manipulations provided no support for a protective mechanism of fluorescence in the presence of stressful pH (Table 15). The results of this research do not support an overall protective function of initial fluorescence on the resulting symbiont density after exposure to the four environmental conditions of light, temperature, salinity and pH.

Comparison of species across trials:

Apart from PAR manipulations, species predominantly differed significantly when comparing the change in FP emission and symbiont density over time (Tables 9, 14), and final symbiont density in relation to initial FP emission (Table 16). While *E. lamellosa,* and *M. capricornus* responded differently to manipulated temperature, salinity and pH, they had the same relationship pattern between FP emission and symbiont density in response to PAR. The difference in response between species did not follow the same pattern regarding FP emission among the four manipulated environmental factors and illustrates the need for further exploration of fluorescence emission patterns from multiple species to develop a better understanding of fluorescence in response to stress.

Conclusion:

Exposing two species of Scleractinia coral to four known coral stressors at ecologically relevant levels, provided unique insight into the relationship between fluorescence and the coral symbiont in response to changes in environmental conditions. Except for coral exposed to manipulated pH, the overall pattern of decreased fluorescence emission with increased symbiont density would support the prediction that as symbiont density increases, the symbiont cells block FP emission. This pattern was only globally significant for the coral exposed to the ambient salinity, and for individuals exposed to higher and lower temperatures. The significant positive relationship between FP emission and symbiont density for *E. lamellosa* exposed to pH manipulations does not support the prediction of symbiont cells providing shading. Only *M. capricornus* in the PAR experiment supported the predicted increase in FP emission intensity upon initial change of conditions, which would suggest an increase in FP production as algae are nearing photoinhibition could be a warning mechanism for some, but not all species. There needs to be further exploration regarding a potential pattern of increased FP emission intensity with a decline in symbiont density. This result was significant for coral exposed to ambient salinity, with a non-significant pattern for coral exposed to temperature and PAR manipulations. Support for the pattern of FP emission shift while a coral is in the process of enduring a stress event could provide a mechanism for tracking symbiont loss over time based on an increase in FP intensity. The pattern of an initial increase of emission intensity as a coral begins to stress and then decline as stress continues in response to over irradiance could also provide a useful, non-invasive marker for coral health in response to light and temperature stress. Acquiring external fluorescent measurements requires techniques such as image scanning which require less precision than a

spectrometer. Controlling the necessary precise placement of a spectrometer probe is not realistic in a coral garden setting. An increase in fluorescence emission detectable prior to bleaching commencement would provide researchers with an earlier detection of declining coral health. A reef is often not recognized as stressed until the process of bleaching has begun (Marshall et al., 2012). It is crucial to explore techniques which allow for earlier assessments and monitoring. The prediction of increased initial FP emission providing protection from bleaching, with higher symbiont densities at the conclusion of the trial was marginally supported only for coral exposed to increased PAR. Further research examining environmental conditions in tandem would provide a more complete assessment of the relationship between intensity of coral fluorescence and health of the coral. An increased understanding of coral, and specifically the functionality and expression of FPs may offer valuable insight into reef systems by providing a visual representation of their rate of decline, and an earlier warning sign of an impending bleaching event.

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Table 1. Spectral measurements were collected pre/mid/post trial. One destructive sample per species, per research system was taken at each time point for symbiont density calculation.

Species	**Time point one Start**	**Time point two One week**	**Time point three Two weeks**
E. lamellosa	n = 120	n = 105	n = 90
M. capricornus	n = 120	n = 105	n = 90

Table 2 Fluorescent protein emission in relation to symbiont density (linear model) across all trials and species. Significant P values are shown in bold.

Environ-mental Condition	**Species**	**Estimate**	**SE**	**F**	**DF**	**Adjusted R^2**	**P**
PAR	*E. lamellosa*	-0.0002	0.0017	1.0840	1, 31	0.0026	0.3060
	M. capricornus	-0.0016	0.0026	0.3904	1, 40	-0.0151	0.5360
Temp	*E. lamellosa*	0.0002	0.0029	0.0033	1, 35	-0.0285	0.9540
	M. capricornus	-0.0031	0.0050	0.3826	1, 42	-0.0146	0.5395
Salinity	*E. lamellosa*	0.0004	0.0018	0.0507	1, 34	-0.0279	0.8232
	M. capricornus	-0.0023	0.0043	0.2903	1, 42	-0.0168	0.5929
pH	*E. lamellosa*	0.00424	0.0019	4.9570	1, 21	0.1524	**0.0371**
	M. capricornus	-0.0004	0.0040	0.0010	1, 38	-0.0261	0.9218

Table 3. Fluorescent protein emission in response to symbiont density across all trials and species by manipulated level of environmental conditions (linear model). Significant P values are shown in bold.

Environ-mental Condition	Species	Level	Estimate	SE	F	DF	Adj R^2	P
PAR	*E. lamellosa*	80	0.0009	0.0030	0.0984	1, 4	-0.22	0.7695
		100	-0.0009	0.0028	0.1080	1, 10	-0.0883	0.7497
		200	-0.0037	0.0032	1.3800	1, 13	0.0264	0.2611
	M. capricornus	80	-0.0008	0.0041	0.0371	1, 7	-0.1368	0.8528
		100	0.0006	0.0049	0.0144	1, 18	-0.0547	0.9059
		200	0.0041	0.0049	0.6905	1, 11	-0.0265	0.4237
Temp	*E. lamellosa*	26	-0.0172	0.0085	4.1220	1, 6	0.3084	0.0886
		21	-0.0024	0.0035	0.4824	1, 13	-0.0384	0.4996
		31	0.0063	0.0057	1.2300	1, 12	0.0174	0.2896
	M. capricornus	26	-0.0157	0.0115	1.843	1, 6	0.1075	0.2235
		21	-0.0191	0.0078	5.9720	1, 15	0.2372	**0.0273**
		31	0.0091	0.0069	1.749	1, 17	0.0400	0.2035
Salinity	*E. lamellosa*	33	-0.0008	0.0042	0.0337	1, 2	-0.4751	0.8713
		27	0.0016	0.0025	0.4057	1, 15	-0.0386	0.5337
		36	-0.0031	0.0044	0.5007	1, 13	-0.0370	0.4917
	M. capricornus	33	-0.0188	0.0046	17.1	1, 7	0.6680	**<0.01**
		27	0.0080	0.0070	1.2740	1, 16	0.0159	0.2757
		36	-0.0031	0.0044	0.5007	1, 13	-0.0370	0.4917
pH	*E. lamellosa*	8.1	0.0012	0.0048	0.0629	1, 2	-0.4543	0.8254
		7.4	0.0027	0.0035	0.5702	1, 8	-0.0502	0.4718
		8.9	0.0066	0.0036	3.4850	1, 7	0.2370	0.1042
	M. capricornus	8.1	-0.0070	0.0198	0.1199	1,7	-0.1236	0.7393
		7.4	-0.0044	0.0041	1.1790	1, 15	0.0111	0.2947
		8.9	0.0304	0.0099	9.4280	1, 12	0.3933	**<0.01**

Table 4. Comparison (linear model) of difference in fluorescent protein emission between *E. lamellosa* and *M. capricornus* by symbiont density and species across all trials. Significant P values are shown in bold.

Environ-mental Condition	Parameter	Estimate	SE	F	DF	P
PAR	Species	-2123	5069	0.1501	1, 71	0.6770
	Sym	-0.0017	0.0019	1.2689	1, 71	0.3740
	Species:Sym	0.0001	0.0031	0.0008	1, 71	0.9780
Temperature	Species	32040	5109	110.510	1, 77	**<0.01**
	Sym	0.0001	0.0026	0.0313	1, 77	0.9490
	Species:Sym	-0.0032	0.0061	0.2789	1, 77	0.5990
Salinity	Species	2759	8268	57.9016	1, 76	**<0.01**
	Sym	0.0004	0.0022	0.0169	1, 76	0.8503
	Species:Sym	0.0027	0.0044	0.3781	1, 76	0.5405
pH	Species	20120	8731	4.8037	1, 59	**0.0247**
	Sym	0.0042	0.0026	1.5017	1, 59	0.1127
	Species:Sym	-0.0046	0.0044		1, 59	0.2979

Table 5. Fluorescence emission for coral samples exposed to various light levels across three time points for either the species combined or separated with individual sample and research systems as random effects (linear mixed model). Significant P values are shown in bold.

Response	Species	Parameter	estimate	SE	F	DF	P
FP Emission	Both	PAR	15.072	27.512	1.258	230	0.586
		Run	1813.311	1530.728	15.705	374	0.237
		PAR: Run	3.034	10.988	0.0762	374	0.783
FP Emission	*E. lamellosa*	PAR	15.125	35.855	0.1780	112	0.675
		Run	-3311.914	1887.656	3.0783	183	0.081
		PAR: Run	6.109	13.391	0.2081	183	0.649
FP Emission	*M. capricornus*	PAR	3.778	34.486	0.0120	116	0.913
		Run	5871.586	2088.154	7.9065	189	**< 0.01**
		PAR:Run	5.894	15.162	0.1511	189	0.698

Table 6. Fluorescence emission for coral samples exposed to various temperature levels across three time points for either the species combined or separated with individual sample and research systems as random effects (linear mixed model). Significant P values are shown in bold.

Response	Species	Parameter	estimate	SE	F	DF	P
FP Emission	Both	Temp	-140.28	327.18	0.1838	236	0.672
		Run	-1853.17	2528.06	0.5373	373	0.464
		Temp:Run	-97.34	95.30	1.043	373	0.308
FP Emission	*E. lamellosa*	Temp	586.25	394.82	2.205	116	0.148
		Run	3234.4	3371.06	0.9206	182	0.339
		Temp:Run	-362.67	126.66	8.199	182	**< 0.01**
FP Emission	*M. capricornus*	Temp	-935.34	475.22	3.874	118	**<0.01**
		Run	-7771.13	3481.37	4.983	189	**0.030**
		Temp:Run	193.70	131.73	2.162	189	0.153

Table 7. Fluorescence emission for coral samples exposed to various salinity levels across three time points for either the species combined or separated with individual sample and research systems as random effects (linear mixed model). Significant P values are shown in bold.

Response	Species	Para-meter	estimate	SE	F	DF	P
FP Emission	Both	Salinity	116.06	383.29	0.0917	235	0.7645
		Run	5324.11	3710.58	2.0588	365	0.1521
		Salinity: Run	-333.69	115.41	8.3605	365	**< 0.01**
FP Emission	*E. lamellosa*	Salinity	-325.54	466.640	0.4867	116	0.4888
		Run	-5708.931	5696.259	1.0045	179	0.3175
		Salinity: Run	1.478	117.376	0.0001	179	0.9934
FP Emission	*M. capricornus*	Salinity	591.70	498	1.4117	117	0.2465
		Run	16703.11	4469.61	13.9655	184	**< 0.01**
		Salinity: Run	-675.07	138.96	23.5991	184	**< 0.01**

Table 8. Fluorescence emission for coral samples exposed to various pH levels across three time points for either the species combined or separated with individual sample and research systems as random effects (linear mixed model). Significant P values are shown in bold.

Response	Species	Para-meter	estimate	SE	F	DF	P
FP Emission	Both	pH	7566.85	2760.04	7.5162	235	**< 0.01**
		Run	31226.27	7513.57	17.2722	369	**< 0.01**
		pH:Run	-5130.14	920.86	31.0362	369	**< 0.01**
FP Emission	*E. lamellosa*	pH	3135.08	3561.7	0.7748	113	0.386
		Run	10129.22	8768.75	1.3344	187	0.249
		pH:Run	-2541.02	1074.83	5.589	187	**0.051**
FP Emission	*M. capricornus*	pH	12314.74	4113.63	8.9619	118	**< 0.01**
		Run	53621.56	11681.19	21.0720	180	**< 0.01**
		pH:Run	-7870.89	1430.90	30.2573	180	**< 0.01**

Table 9. Comparison (linear mixed model) of difference between *E. lamellosa* and *M. capricornus* when comparing fluorescence emission across three time points by manipulated environmental conditions. Significant P values are shown in bold.

Environ-mental Condition	Parameter	Estimate	SE	F	P
PAR	Species	-15558.9	2194.9	2.1614	0.1364
	Run	-2487.3	729.6	17.8840	**<0.01**
	Species:Run	9098.2	1021.1	79.3980	**<0.01**
Temperature	Species	21609.9	2109.7	361.672	**<0.01**
	Run	-6261.7	590.3	116.229	**<0.01**
	Species:Run	3544.8	825.7	18.432	**<0.01**
Salinity	Species	25123.3	2115	482.7994	**<0.01**
	Run	-5697.2	668.7	127.3125	**<0.01**
	Species:Run	751.5	943.2	0.6348	0.4256
pH	Species	5214.7	2903.4	13.3033	**<0.01**
	Run	-10566.5	905.9	266.923	**<0.01**
	Species:Run	160.5	1283.7	0.0156	0.9004

Table 10. Symbiont density for coral samples exposed to various light levels across three time points among both species combined and separate with research systems as a fixed variable (generalized linear mixed model). Significant P values are shown in bold.

Response	Species	Para-meter	estimate	SE	F	P
Symbiont Density	Both	PAR	-0.0025	0.0021	0.08	0.25
		Run	-0.2904	0.0004	1796885.8	**< 0.01**
		PAR: Run	0.0009	0.0000	129153.11	**< 0.01**
Symbiont Density	*E. lamellosa*	PAR	-0.0009	0.0027	0.6279	0.724
		Run	0.0159	0.0006	0.0000	**< 0.01**
		PAR: Run	-0.0008	0.0000	0.0004	**< 0.01**
Symbiont Density	*M. capricornus*	PAR	-0.0034	0.0025	0.1417	0.157
		Run	-0.5198	0.0005	0.0000	**< 0.01**
		PAR:Run	0.0024	0.0000	0.00010	**< 0.01**

Table 11. Symbiont density for coral samples exposed to various temperature across three time points among both species combined and separate with research systems as a fixed variable (generalized linear mixed model). Significant P values are shown in bold.

Response	Species	Parameter	estimate	SE	F	P
Symbiont Density	Both	Temp	0.0022	0.0173	1.743	0.899
		Run	0.2165	0.0010	5.982	**< 0.01**
		Temp: Run	-0.01336	0.0000	1210	**< 0.01**
Symbiont Density	*E. lamellosa*	Temp	-0.04107	0.03967	0.6299	0.3
		Run	-0.3135	0.0016	7455	**< 0.01**
		Temp: Run	0.04272	0.0001	4133	**< 0.01**
Symbiont Density	*M. capricornus*	Temp	0.03397	0.0143	0.8829	**< 0.05**
		Run	0.2234	0.0015	83070	**< 0.01**
		Temp:Run	-0.0114	0.00010	37335	**< 0.01**

Table 12. Symbiont density for coral samples exposed to various salinity levels across three time points among both species combined and separate with research systems as a fixed variable (generalized linear mixed model). Significant P values are shown in bold.

Response	Species	Parameter	estimate	SE	F	P
Symbiont Density	Both	Salinity	0.0459	0.0148	0.0359	**< 0.01**
		Run	0.8786	0.0007	2622300	**< 0.01**
		Salinity: Run	-0.0229	0.0000	949200	**< 0.01**
Symbiont Density	*E. lamellosa*	Salinity	0.0103	0.0125	6229	0.412
		Run	0.4606	0.0009	485980	**< 0.01**
		Salinity: Run	-0.0119	0.0000	164850	**< 0.01**
Symbiont Density	*M. capricornus*	Salinity	0.0756	0.0146	2846	**< 0.01**
		Run	0.8909	0.0013	1260000	**< 0.01**
		Salinity:Run	-0.0220	0.0000	286250	**< 0.01**

Table 13. Symbiont density for coral samples exposed to various pH levels across three time points among both species combined and separate with research systems as a fixed variable (generalized linear mixed model). Significant P values are shown in bold.

Response	Species	Parameter	estimate	SE	F	P
Symbiont Density	Both	pH	0.3778	0.0481	0.5878	**< 0.01**
		Run	2.3848	0.0016	19110000	**< 0.01**
		pH: Run	-0.3668	0.000	3.3051	**< 0.01**
Symbiont Density	*E. lamellosa*	pH	0.6785	0.0645	0.0249	**< 0.01**
		Run	3.3348	0.0023	11032000	**< 0.01**
		pH: Run	-0.4897	0.0003	2825000	**< 0.01**
Symbiont Density	*M.capricornus*	pH	-0.7356	0.1162	1.2818	**< 0.01**
		Run	-2.0726	0.0033	7577100	**< 0.01**
		pH:Run	0.1701	0.0004	163290	**< 0.01**

Table 14. Comparison (generalized linear mixed model) of difference between *E. lamellosa* and *M. capricornus* when comparing symbiont density across three time points by manipulated environmental conditions . Significant P values are shown in bold.

Environ-mental Condition	Parameter	Estimate	SE	F	P
PAR	Species	-0.3483	0.0005	4170216	**<.0.01**
	Run	-0.1966	0.0002	3003782	**<.0.01**
	Species:Run	-0.0451	0.0003	30882	**<.0.01**
Temperature	Species	-0.9105	0.0007	7767273	**<.0.01**
	Run	-0.1461	0.0002	490786	**<.0.01**
	Species:Run	0.0880	0.0003	71739	**<.0.01**
Salinity	Species	-0.8991	0.0005	16617589	**<.0.01**
	Run	0.1026	0.0001	1927435	**<.0.01**
	Species:Run	0.0954	0.0002	212318	**<.0.01**
pH	Species	0.6880	0.0006	1864777	**<.0.01**
	Run	0.4132	-2185.34	11815629	**<.0.01**
	Species:Run	0.3786	0.0003	1410009	**<.0.01**

Table 15. Comparison (linear model) of initial fluorescent emission intensity and final symbiont density by manipulated environmental conditions and individual species. Significant P values are shown in bold.

Environ-mental Condition	Species	Estimate	SE	F	DF	Adjusted R^2	P
PAR	*E. lamellosa*	-12.40	34.18	0.1316	1, 9	-0.0951	0.7252
	M. capricornus	69.31	16.95	16.73	1, 12	0.5475	**<0.01**
Temp	*E. lamellosa*	-0.0023	0.0040	0.3578	1, 2	-0.0519	0.5608
	M. capricornus	4.255	6.346	0.4495	1, 11	-0.0481	0.5164
Salinity	*E. lamellosa*	0.0023	0.0034	0.4398	1, 9	-0.0593	0.5238
	M. capricornus	7.520	22.82	0.1086	1, 10	-0.0882	0.7486
pH	*E. lamellosa*	-18.41	56.55	0.106	1, 7	-0.1258	0.7543
	M. capricornus	-5.60	4.55	1.515	1, 10	0.0447	0.2466

Table 16. Comparison (linear model) of difference between *E. lamellosa* and *M. capricornus* when comparing initial fluorescent emission intensity and final symbiont density by manipulated environmental conditions . Significant P values are shown in bold.

Environ-mental Condition	**Parameter**	**Estimate**	**SE**	**F**	**DF**	**P**
PAR	Species	-0.0002	5384	7.3469	1 21	**<0.01**
	GFP1	-0.0012	0.0026	3.950	1, 21	0.6617
	Species:GFP1	0.0096	0.0037	6.7316	1, 21	**0.0169**
Temperature	Species	18510	11790	26.1729	1, 23	0.130
	GFP1	-0.0024	0.0039	0.1759	1, 23	0.546
	Species:GFP1	0.0116	0.0147	0.6247	1, 23	0.437
Salinity	Species	25870	12570	32.778	1, 19	0.054
	GFP1	0.0023	0.0029	0.6676	1, 19	0.4416
	Species:GFP1	-0.0009	0.0062	0.0191	1, 19	0.8917
pH	Species	11940	10430	0.6212	1, 17	0.2682
	GFP1	-0.0008	0.0039	0.3023	1, 17	0.8393
	Species:GFP1	-0.0227	0.0165	1.8909	1, 17	0.1870

Figure 1. Research design schematic with repeating manipulated environmental variable level by row; three controls, six at first manipulated level, six at second manipulated level.

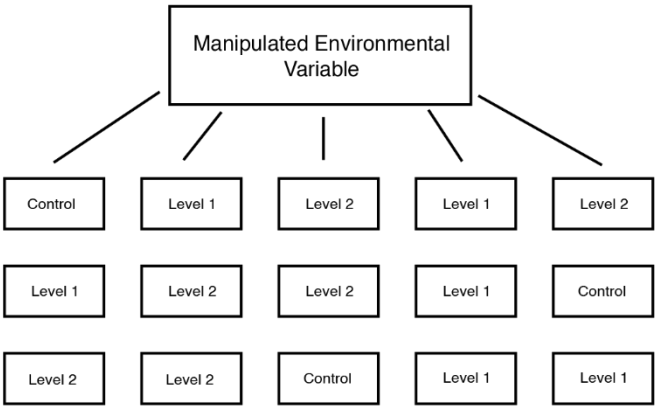


Figure 2. Photograph of coral sample with fluorescent standard and mapped locations for consistent spectrometer readings labeled on the coenosarc between corallites.

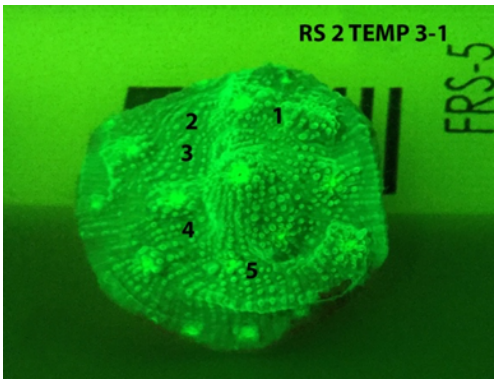


Figure 3. The relationship (linear model) between the density of symbionts within the coral sample and the intensity of fluorescence emission manipulated by environmental condition for *E. lamellosa* and *M. capricornus*.

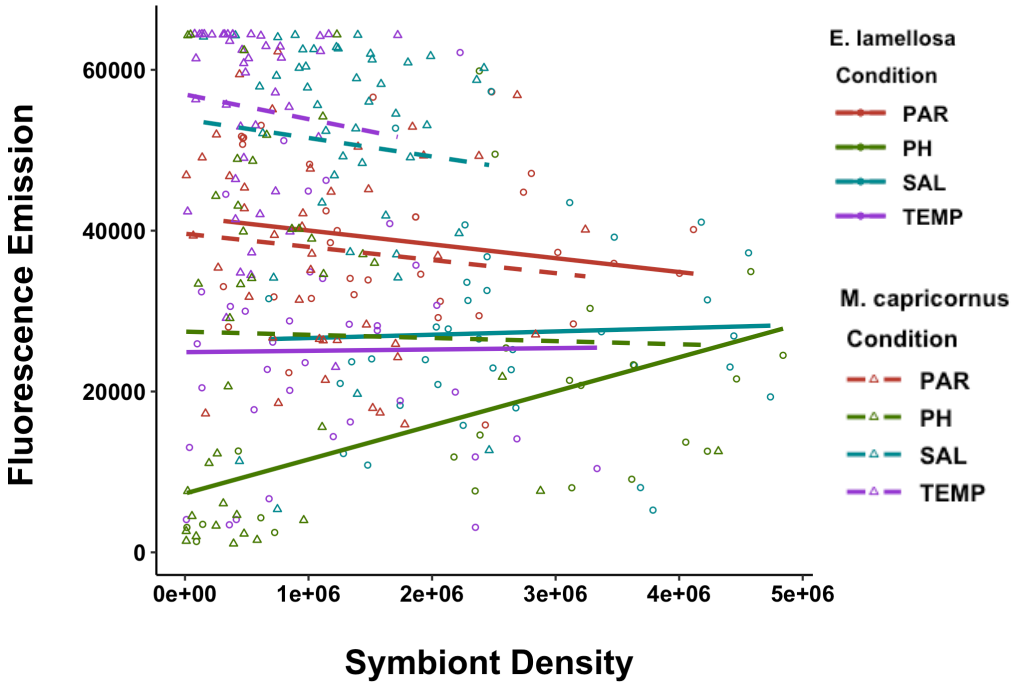


Figure 4. The relationship (linear model) between the density of symbionts within the coral sample and the intensity of fluorescence emission manipulated by the environmental condition of PAR for *E. lamellosa* and *M. capricornus*.

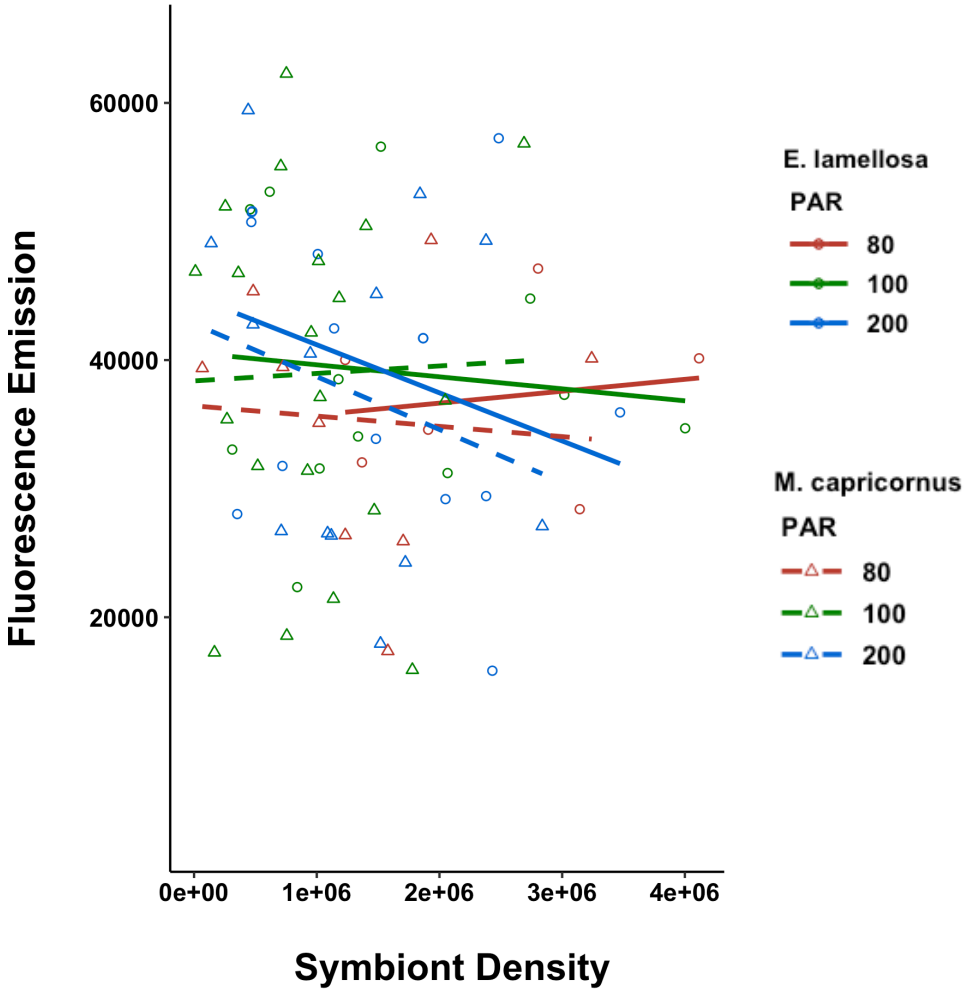


Figure 5. The relationship (linear model) between the density of symbionts within the coral sample and the intensity of fluorescence emission manipulated by the environmental condition of temperature for *E. lamellosa* and *M. capricornus*.

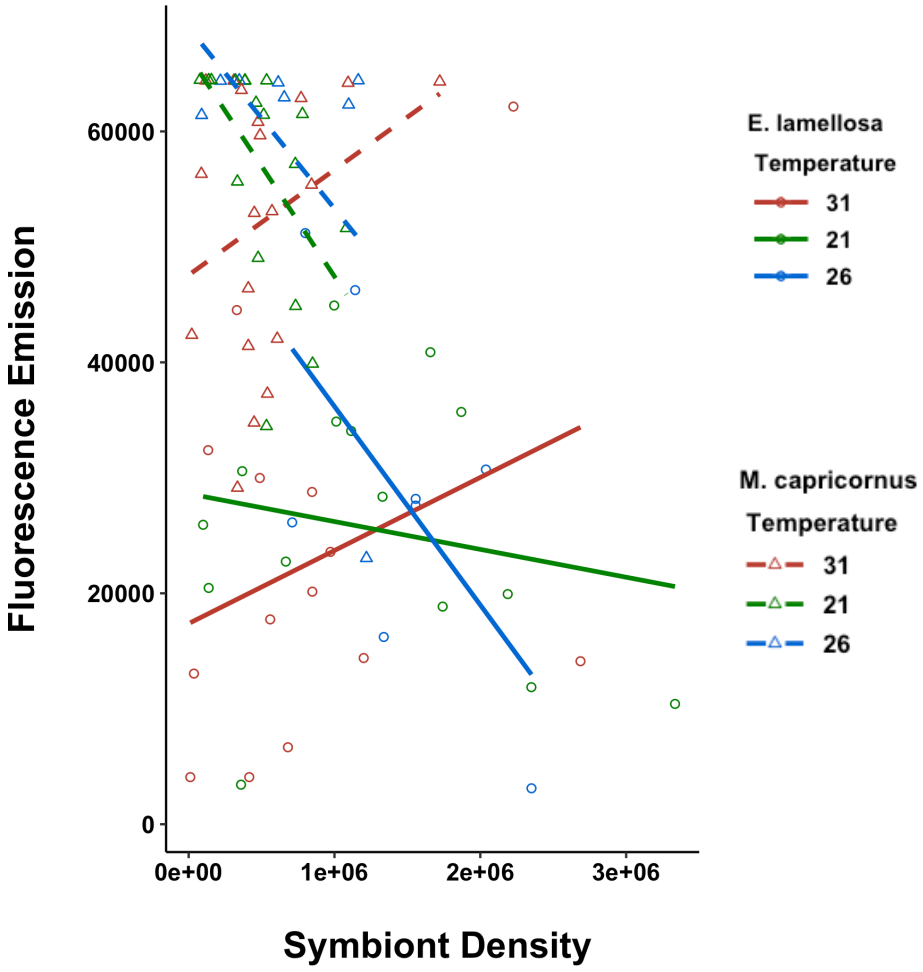


Figure 6. The relationship (linear model) between the density of symbionts within the coral sample and the intensity of fluorescence emission manipulated by the environmental condition of salinity for *E. lamellosa* and *M. capricornus*.

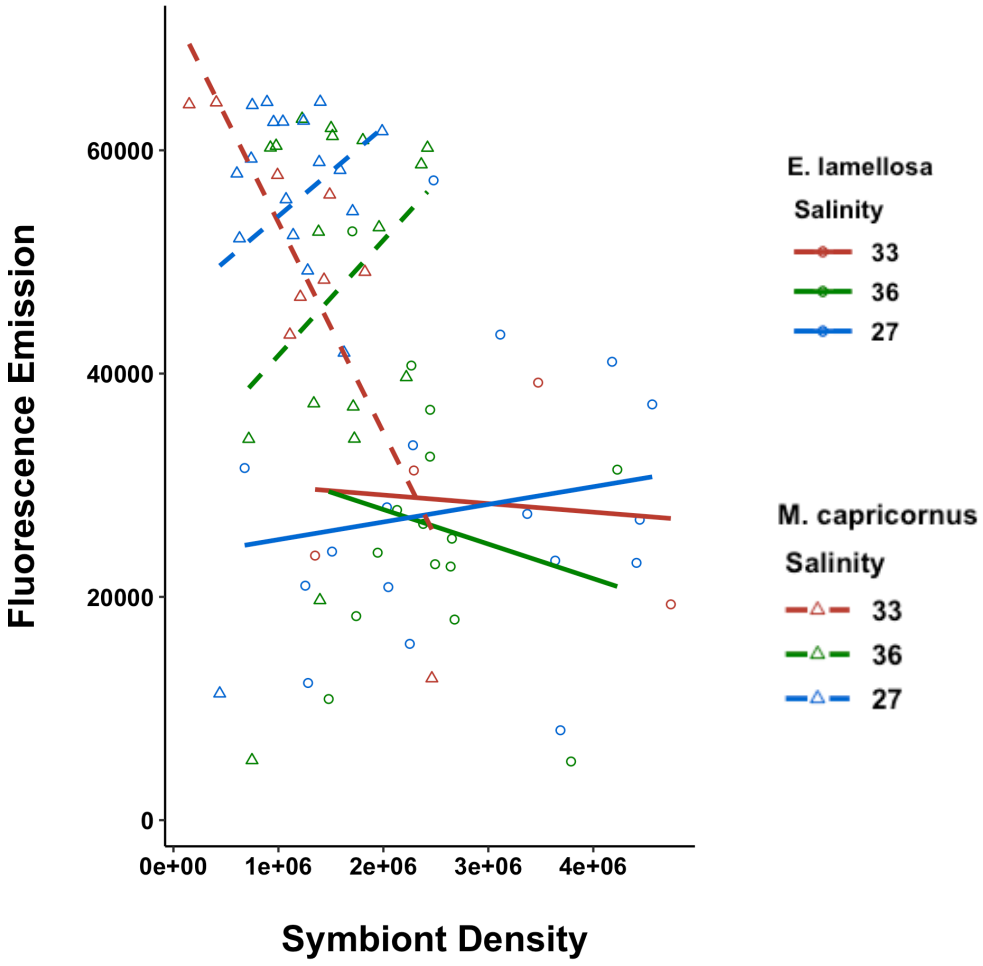


Figure 7. The relationship (linear model) between the density of symbionts within the coral sample and the intensity of fluorescence emission manipulated by the environmental condition of pH for *E. lamellosa* and *M. capricornus*.

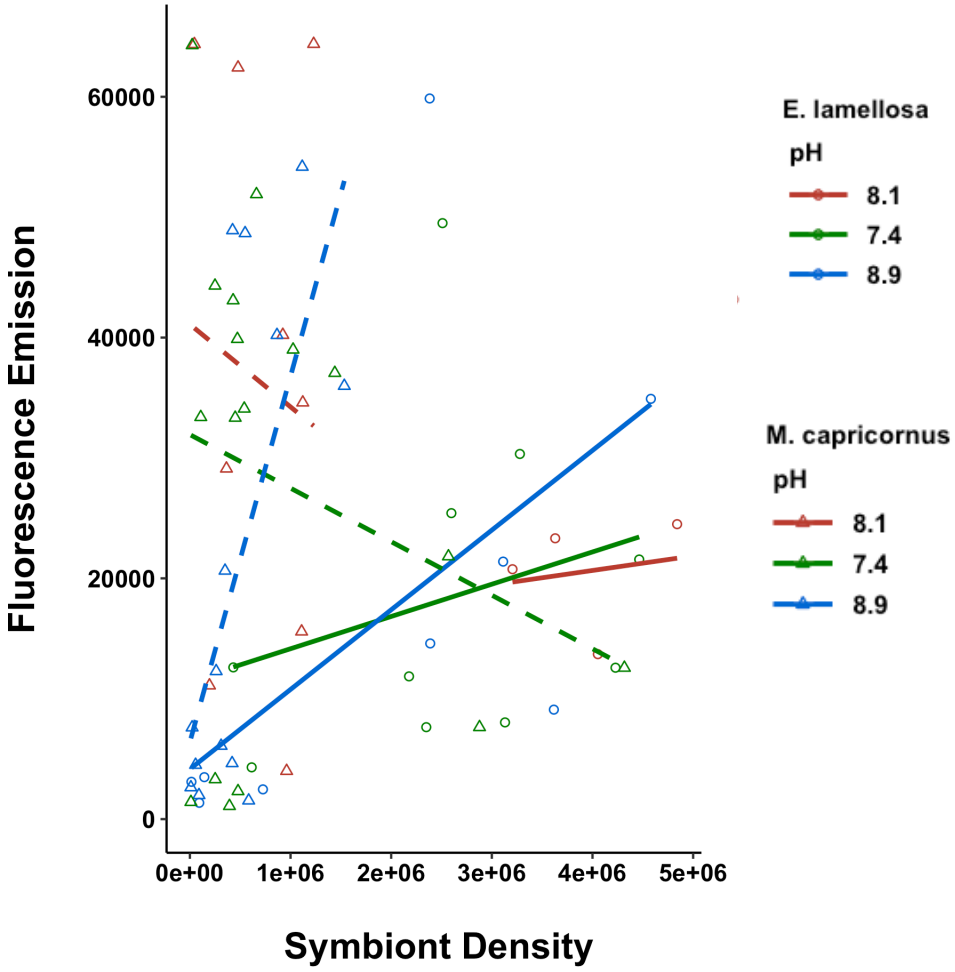


Figure 8. The change in fluorescence emission (linear mixed model) across three time points under the manipulated environmental condition of light for both species combined (a), *E. lamellosa* (b), and *M. capricornus* (c).

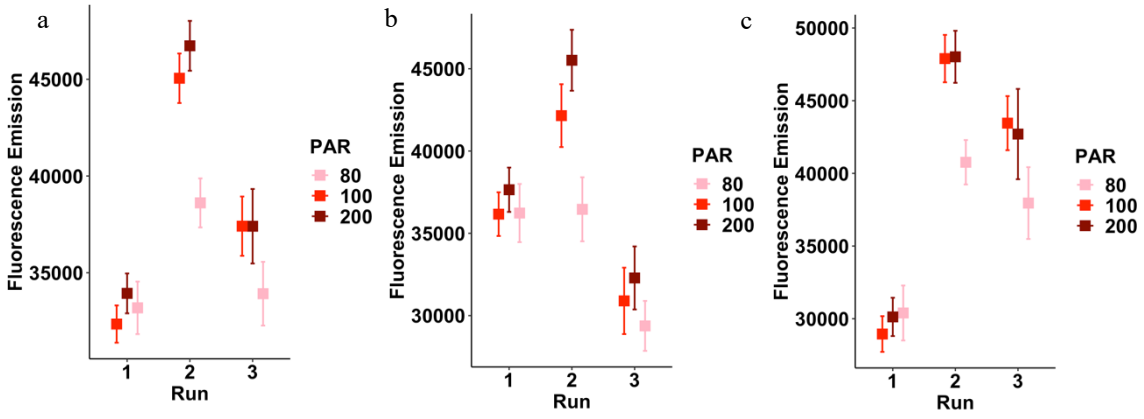


Figure 9. The change in fluorescence emission (linear mixed model) across three time points under the manipulated environmental condition of temperature for both species combined (a), *E. lamellosa* (b), and *M. capricornus* (c).

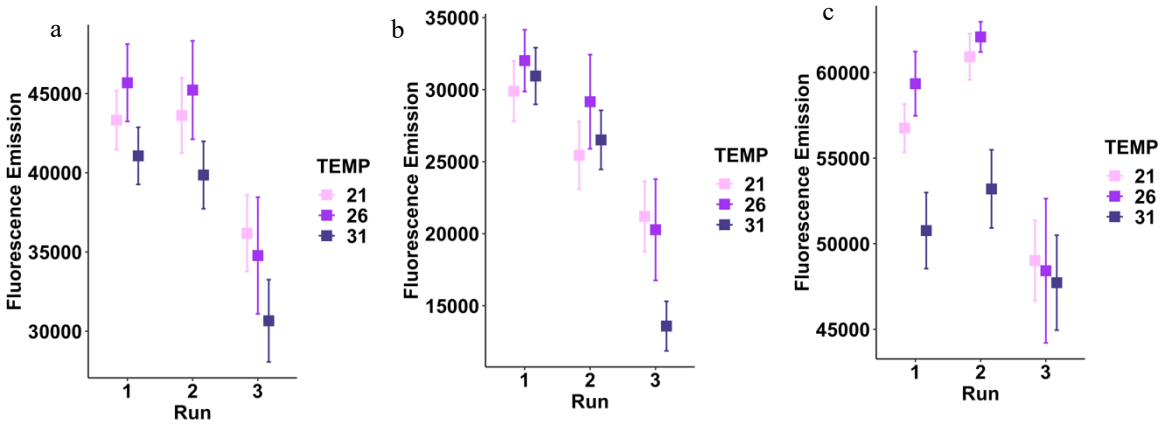


Figure 10. The change in fluorescence emission (linear mixed model) across three time points under the manipulated environmental condition of salinity for both species combined (a), *E. lamellosa* (b), and *M. capricornus* (c).

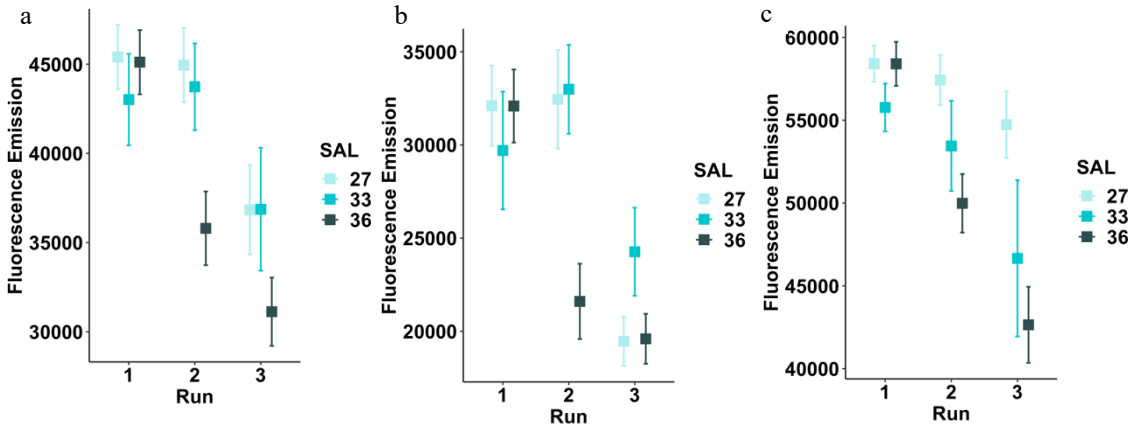


Figure 11. The change in fluorescence emission (linear mixed model) across three time points under the manipulated environmental condition of pH for both species combined (a), *E. lamellosa* (b), and *M. capricornus* (c).

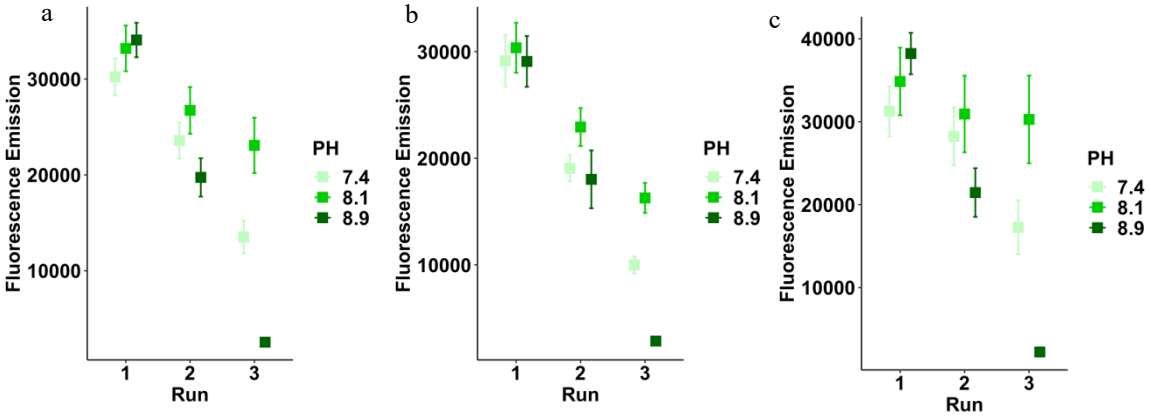


Figure 12. The change in symbiont density (generalized linear mixed model) across three time points under the manipulated environmental condition of light for both species combined (a), *E. lamellosa* (b), and *M. capricornus* (c).

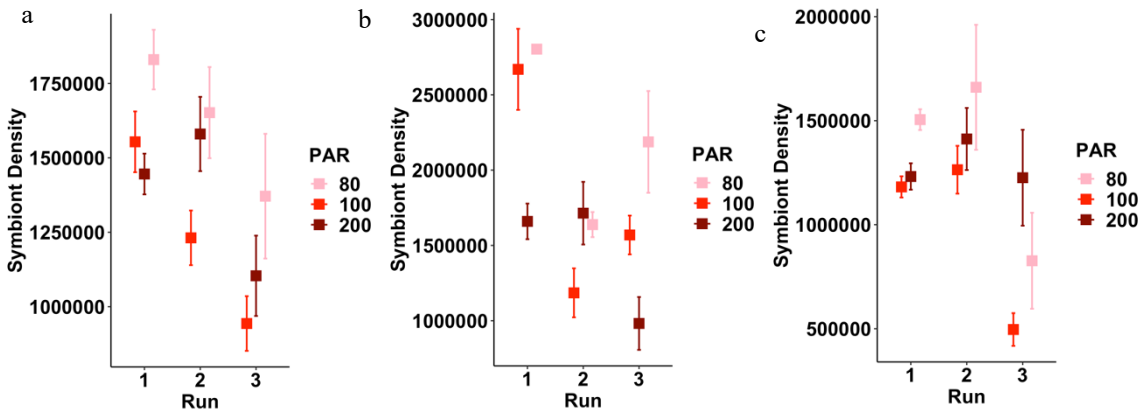


Figure 13. The change in symbiont density (generalized linear mixed model) across three time points under the manipulated environmental condition of temperature for both species combined (a), *E. lamellosa* (b), and *M. capricornus* (c).

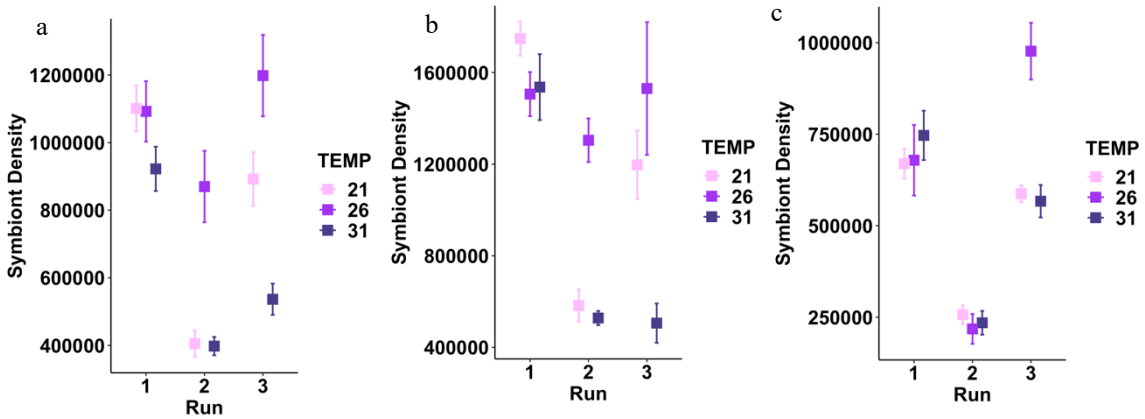


Figure 14. The change in symbiont density (generalized mixed model) across three time points under the manipulated environmental condition of salinity for both species combined (a), *E. lamellosa* (b), and *M. capricornus* (c).

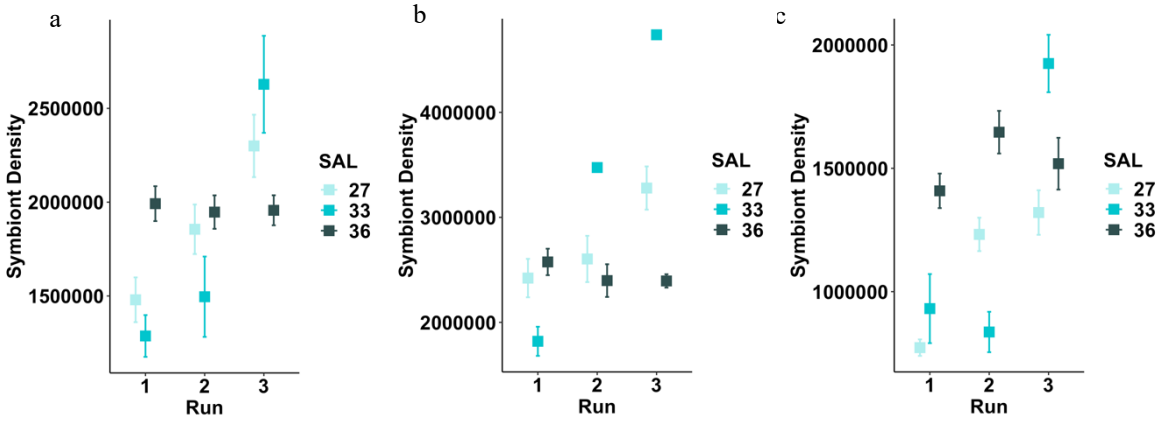


Figure 15. The change in symbiont density (generalized linear mixed model) across three time points under the manipulated environmental condition of pH for both species combined (a), *E. lamellosa* (b), and *M. capricornus* (c).

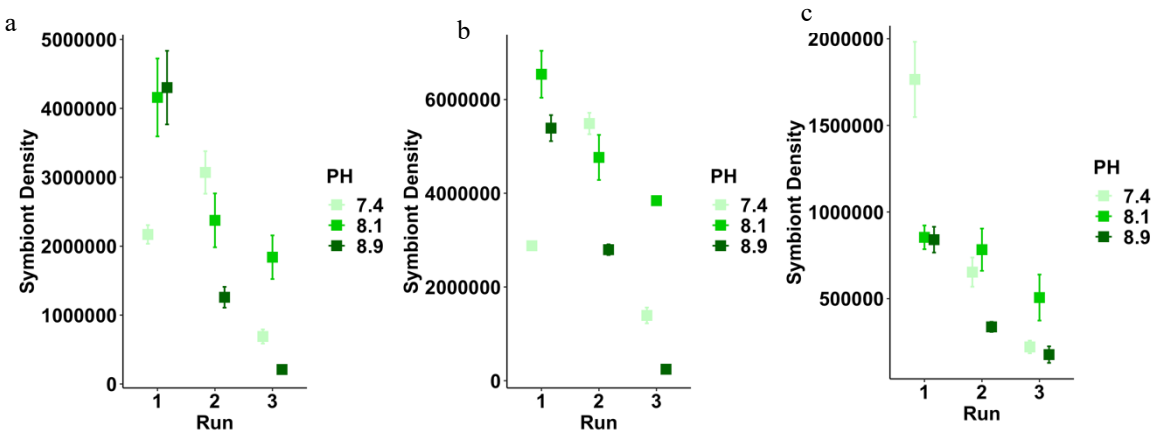
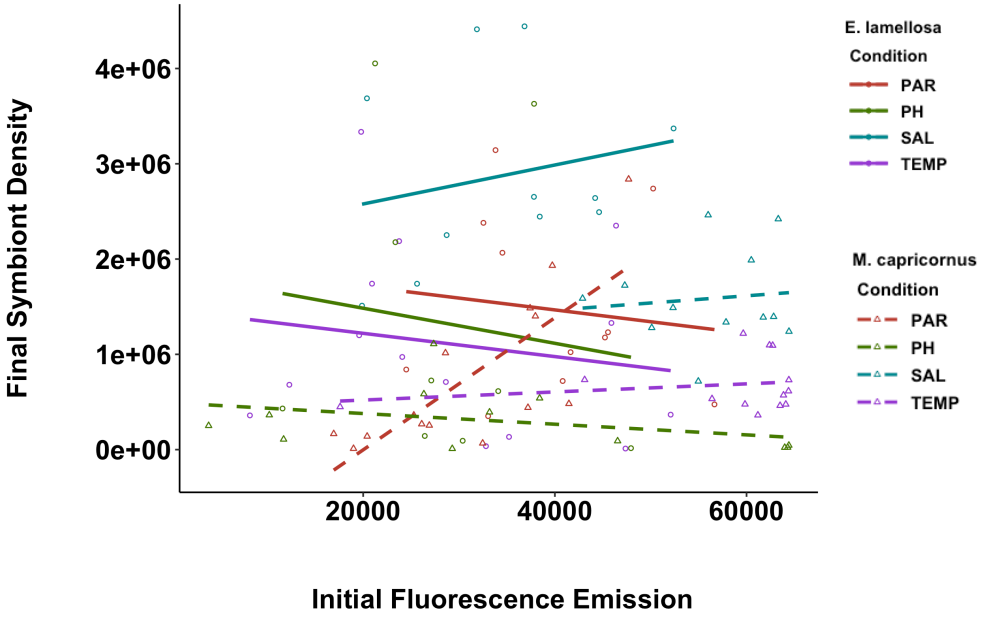


Figure 16. The relationship (linear model) between the initial fluorescence emission of the coral sample with its final symbiont density, for all manipulated environmental conditions and species combined (a) and combined manipulated environmental conditions for *E. lamellosa* and *M. capricornus* (b).



Appendix A: Water quality parameters of coral acclimation system following Oklahoma Aquarium protocol. PAR levels variable by vertical placement in water column.

PAR	44 – 190 μmol $m^{-2}s^{-1}$
Salinity	33 – 35 ppt
pH	8.1 – 8.2
Temperature ° C	25.6 – 26.1° C
ammonia (NH_3)	0.08 mg L^{-1} NH_3-N or below
NH_3-N, nitrite (NO_2)	0.1 mg L^{-1} NO_2–N or below
phosphate (PO_4)	0.1 mg L^{-1} PO_4^{3-} or below
iodine (I_2)	0.07 – 0.15 mg L^{-1}
Calcium (Ca)	400 – 500 mg L^{-1} (ppm)
Magnesium (Mg)	1300 – 1500 mg L^{-1} (ppm)
Alkalinity	<10dKH
Strontium (Sr)	1.85 g Sr L^{-1}

CHAPTER III

RESPONSE AND ATTRACTION OF ENDOSYMBIOTIC AND NON-SYMBIOTIC ALGAE TO GREEN FLUORESCENT CORAL

INTRODUCTION

Reef coral form a symbiosis with endosymbiotic dinoflagellates (Symbiodiniaceae). The symbiosis facilitates an exchange of nutrients within coral tissues between the symbiont and the host. The nutrient exchange provides the coral with up to 90% of its energy and is critical for coral survival (Muscatine & Porter, 1977; Weis, 2008; Yellowlees et al., 2008). Without the photosynthetic byproducts of the symbionts, the coral experience reduced fitness, fecundity, and growth (Matthews et al., 2017). A breakdown in the symbiosis occurs when the coral holobiont is subjected to stress, specifically heat stress (Glynn, 1996). Under stress conditions, algae travel through the tissues for expulsion from the coral mouth (Weis, 2008). Depending on the coral's level of thermal tolerance, many will not survive without the symbiont for extended periods of time (Claar & Baum, 2019; Coffroth et al., 2010; Pandolfi et al., 2011). In order for bleached coral to recover after a bleaching event, they must repopulate their endosymbiont community from existing resident species, or the coral must take up new symbionts from the water column

(Baker, 2003, 2004; Coffroth et al., 2010; Jones et al., 2008). As the symbiosis breaks down, the symbiont has been shown to be the weaker link in the symbiosis. The thermal tolerance of the symbiont indicates stress of the coral. This would suggest coral that repopulate their tissues with hardier symbionts are capable of increased survival (Baker, 2003, 2004; Douglas, 2003; Pandolfi et al., 2011). Symbiotic relationships are not static. There is the potential for coral to host many different species of symbiont as well as display flexibility in their hosted community (Baker, 2003; Berkelmans & van Oppen, 2006; Cunning et al., 2015; Kenkel & Bay, 2018; Matthews et al., 2017). The exchange of symbionts is somewhat limited by varying degrees of "host specificity", with some symbionts more flexible and some more specific between partners (Baker, 2003; Osman et al., 2020). Species specificity determines varying levels of benefit or detriment to the host, with a range of mutualistic to parasitic relationships within the symbiosis (Bayliss et al., 2019; Fabina et al., 2013; LaJeunesse et al., 2018; Matthews et al., 2017; Weis, 2008). Coral symbionts located in certain geographic regions display greater resistance to temperature increases that initiate bleaching. Coral symbionts in the northern Red Sea have been identified as hardier with increased plasticity, allowing for more rapid adaptation to warming temperatures (D'Angelo et al., 2015; Osman et al., 2020). An exchange of symbionts could allow for recolonization of coral tissue with algae possessing increased heat tolerance (Baker, 2003, 2004; Kinzie et al., 2001; Ware et al., 1996).

The adaptive bleaching hypothesis suggests that bleaching could have a beneficial effect on overall coral health with coral expelling less heat tolerant algae to allow for the introduction of new species of symbiont or a shuffling of existing species that could

provide increased fitness for the coral holobiont (Baker, 2003, 2004; Buddemeier & Fautin, 1993; Kinzie et al., 2001; Ware et al., 1996). An exchange of symbionts by horizontal uptake from the water column has been documented in adult anemones (Baker, 2003; Kinzie et al., 2001), and new symbioses have been formed between tridacnid clams and cultured algae (Belda-Baillie et al., 1999). Previous research has supported the uptake of novel symbiont species by coral during heat stress, but coral returned to their pre-stress symbiont species when the temperature stress passed (Coffroth et al., 2010). In addition to discoveries of naturally occurring heat tolerant symbionts, researchers have developed assisted evolutionary techniques in order to rear symbionts with increased thermal tolerance (Buerger et al., 2020; Chakravarti & van Oppen, 2018). Recent research utilized symbiont species *Cladocopium goreaui* to develop increased heat tolerance through assisted evolution over the course of four years (Buerger et al., 2020). Coral larva were able to uptake the lab assisted algae horizontally from the water column (Buerger et al., 2020), which lends support for further exploration of possible mechanisms adult coral utilize for horizontal uptake of novel species of endosymbiont (Coffroth et al., 2010).

Algal symbionts can survive outside of the coral host. They can not only be cultured ex-hospite, they are found free swimming (Yamashita & Koike, 2015). When ex-hospite they exhibit changes in morphology on a diel cycle. During the day, the algae have flagella and are motile. The algae also develop an eyespot located near the sulcus, a longitudinal groove that terminates in a flagellum (Colley & Nilsson, 2016; Yamashita & Koike, 2015). At night, algae enter a coccoid phase and are spherical losing their flagella and eyespot, (Yamashita et al., 2009; Yamashita & Koike, 2015). The phototactic ability

of dinoflagellates has been identified and is attributed to enhancement of photosynthetic efficiency. The eyespot allows the algae to detect and subsequently move toward a light source (Horiguchi et al., 1999; Thompson et al., 2017). Coral symbionts are attracted to light within the green wavelengths (Hollingsworth et al., 2005). More recently, three species of Symbiodiniaceae were identified that display attraction to fluorescent green light emitted by a coral (Aihara et al., 2019).

Fluorescence within the coral animal has two primary sources. The first is the photosynthetic pigments of chlorophyll-a from the symbiont with a primary emission at 685 nm. The second is the fluorescent proteins (FPs) found within the coral tissues, with emission ranging from 450 nm, in the shorter violet, blue, and green wavelengths to 600 nm, in the longer yellow and red wavelengths. FPs are diverse and plentiful within coral, but their functionality is still being discovered (Alieva et al., 2008; Lapshin et al., 2015). Fluctuations in fluorescence are not simply a physiological response but also serve an adaptive function for the animal, including photoprotection, and control and regulation of light through a "photobiological system" (Salih et al., 2000; Smith et al., 2017). FPs assist in depth dependent light regulation, allowing for either photoprotection or photo acclimatization to optimize symbiont photosynthesis at deeper depths (Salih et al., 2000; Smith et al., 2017). A central question is whether the coral FPs display additional adaptive functions. One proposed additional function, known as the "Beacon Function" (Hollingsworth et al., 2005; Horiguchi et al., 1999), is that dinoflagellates utilize their eyespot while in the motile phase to locate coral by means of fluorescence emission. The beacon hypothesis is supported by research illustrating attraction of algae to light in the green range (Aihara et al., 2019; Hollingsworth et al., 2005).

Certain bleaching events are termed colorful bleaching, meaning as the coral lose the algae and the color the algae provide, the coral animal upregulates production of FPs producing an increase in fluorescent emission (Bollati et al., 2020). There is no consensus on why these events happen, though recent research supports the upregulation of FPs minimizing the light stress within the tissues of the coral, facilitating re-colonization of the symbiont (Bollati et al., 2020). The increase in FP production provides protection to both the coral and symbiotic algae (Salih et al., 2000). I suggest that colorful bleaching may involve the loss of non-heat tolerant algae in pursuit of inoculation with hardier symbionts such as stated in the adaptive bleaching hypothesis (Baker, 2003; Buddemeier & Fautin, 1993; Kinzie et al., 2001; Ware et al., 1996). I further suggest that the upregulation of FPs during colorful bleaching (Bollati et al., 2020) could be support for the beacon hypothesis (Hollingsworth et al., 2005; Horiguchi et al., 1999) with fluorescence emission providing a source of bright fluorescent light to attract free swimming Symbiodiniaceae.

In order to test the attraction of motile dinoflagellates to a coral emitting green fluorescence, I utilized three species of algae and two light sources. I conducted trials under blue light at a wavelength known to excite fluorescent proteins, and white light, which is not at the optimal absorption rate for FPs within the green range (C. Mazel, 1995). I predicted that green fluorescing coral under blue light would attract algae more readily than coral under white light. Two species form symbioses with coral partners and one does not. Previous research studying the attraction of Symbiodiniaceae to green fluorescence utilized symbionts from clades A, B1, and C, which all form symbioses with coral (Aihara et al., 2019). The current study is the first to utilize a non-symbiotic

Symbiodiniaceae and one from clade E, *Effrenium voratum*. The two symbiotic species in the study: *Breviolum psygomophilium* (clade B) and *Cladocopium* sp. (clade C) are distinct algae species of the same clades utilized in previous research. I predicted that the attraction to fluorescence emission would be more pronounced for a symbiotic dinoflagellate as opposed to a non-symbiotic dinoflagellate. The current study is also the first to explore not only the attraction of a non-symbiotic species to a green fluorescing coral, but to quantify directional movement along the x and y-axes, speed, and displacement of algae in response to the coral. I predicted increased speed and displacement in response to exposure to a fluorescent coral.

As with the research of Aihara (2019), I sought to test if potential attraction was due to phototaxis or chemotaxis. Flagellated algae, bacteria, and viruses respond to chemotactic chemicals, which coral are known to emit (Meron et al., 2009; Takeuchi et al., 2017; Tout et al., 2015). I utilized a live coral and a coral which was sealed with a resin epoxy (1-Chloro-4 Trifluromethyl Bisphenol A) to prevent release of chemical compounds from the coral. I predicted similar movement toward the coral exposed to blue light under both sealed and unsealed conditions. I also predicted greater movement toward the coral during the sealed condition exposed to blue light as opposed to the unsealed coral in response to the white light condition. If that occurs, the predicted movement would support the attraction is due to phototaxis and not chemotaxis.

METHODS

Coral Model Species:

Due to their rapid growth after microfragmentation (Page et al., 2018) and predominantly laminar growth pattern, I chose *Echinopora lamellosa* (Lamarck, 1816) as model species (Figure 1). The flat morphology of the species allows for a uniform surface for algae attraction. *E. lamellosa* is native to the Indo-Pacific and eastern Pacific oceans. The species is common and is often a dominant species within shallow water habitats. The coralites are small with large spaces of coenosteum between coralites, providing ample space for collection of fluorescent measurements. The individual fragments range from bright green to brown with bright green coralites (Veron & Stafford-Smith, 2000).

Symbiont Model Species:

I acquired four symbiont species classified in separate clades from the Buffalo Undersea Reef Research (BURR) Culture Collection, University of Buffalo: *Cladocopium* sp., clade/cp type C180, *Breviolum psygomophilium*, with clade/cp-type B224, *Effrenium voratum* clade/cp type E202, and *Durusdinium trenchii*, clade D/. cp type D206. Symbiont strain was identified based on the fragment size in the hypervariable region of the chloroplast 23S rDNA (Santos, Gutierrez-Rodriguez, and Coffroth 2003). *Breviolum psygomophilium,* formally identified as *Symbiodinium psygomophilium* (LaJeunesse, 2001), is predominantly found in temperate and subtropical coral (Lee et al., 2014). Utilizing this symbiont as a model species allowed for phototaxis comparison of warmer and cooler water symbionts. Both *Cladocopium* sp. and *Durusdinium trenchii* have been identified as more heat tolerant symbionts, both

found in the rapidly thermally adaptive coral of the Northern Red Sea (Chakravarti et al., 2017; Osman et al., 2020). *Effrenium voratum* is the one species of the four that is non-symbiotic (Personal Communication, M. Coffroth, August 2019). It has been theorized that *E. voratum* evolved as a free-living dinoflagellate. They have an increased ability to capture prey and are the only Symbiodiniaceae representatives known to be capable of nighttime motility (Jeong et al., 2012, 2014; Yamashita & Koike, 2015). I did not utilize *Durusdinium trenchii* after finding no movement of the algae cells.

Coral acclimation and control conditions:

I reared *E. lamellosa* at the Oklahoma Aquarium for two years within a 1136 L recirculating seawater system, under controlled conditions. Lighting was provided by 400 watt, 14K halogen lights with a record of Photosynthetically Active Radiation (PAR) readings and stable water chemistries following Oklahoma Aquarium protocols (Appendix A)

Dinoflagellate cell culture:

I utilized the culture protocol of BURR lab for dinoflagellate propagation and growth (Appendix B). I isolated symbionts in a 1:500 solution of f/2 media from Algae Research Supply in filtered seawater from the Oklahoma Aquarium within 50 ml Erlenmeyer flasks. Unlike BURR labs, I did not make my own f/2 media but purchased nutrients from the Algae Research Laboratory. I added 100 µl of algae cells suspended in f/2 media solution to 30 ml of previously described media solution. I isolated a fresh culture every thirty days. Breaking with BURR lab protocol of a 14:10 hour light cycle, the algae

were on a 12:12 hour light cycle (Aihara et al., 2019) under a Kessel LED light source with PAR set at 80 µmol m^{-2} s^{-1} measured with an Apogee MQ-500 Full Spectrum Quantum PAR meter.

Tissue removal and sealing of coral:

Following the protocol of Kenkel, Almanza, and Matz (2015), I separated coral tissue from the coral skeleton with an artist's airbrush powered by an air compressor set at 125 psi. I placed the coral in 5 mL of an extraction buffer (1 M Tris HCl, 1 M DTT) for 10 minutes before placing the coral in a 950 mL plastic bag to contain the tissue as it was removed from the skeleton. I added another 5 mL of extraction buffer to remoisten the coral during tissue removal until the white coral skeleton was completely exposed. Coral skeletons were then placed in a 10% bleach solution for 24-48 hours. In order to ascertain that any potential attraction of the motile dinoflagellate to the coral was due to the fluorescence emission of the coral and not a chemotactic attraction, I utilized a living coral sample which I coated with an Art n' Glow clear casting and coating epoxy resin to seal the coral but maintain the same fluorescence intensity of the live coral

Fluorescence spectral measurements:

I collected fluorescence measurements of live, sealed, and skeletal coral with an Ocean Optics Flame-S-VIS-NIR-ES spectrometer with 600 µm reflectance probe terminated in a ¼" waterproof stainless ferrule. I placed the reflectance probe within a holding device for a fixed distance of 5 mm from the bottom of the coral holding vessel and a 60° angle to assure uniform readings of the coral. After taking five spectral measurements per coral condition, sealed, live, and skeletal, I determined the live

samples and the sealed samples with attached tissue had fluorescent measurements of 55,000 AU ± 1000 AU. The fluorescent emission of sample skeletons with all tissue removed was negligible. Reflectance emission was read by the OceanView software package from Ocean Optics. In order to reduce noise yet maintain high resolution, I set a low "boxcar width" of 3 and set the "scans to average" at 20. Fluorescent proteins (FPs) in the green emission range were excited with 440-460 nm blue LED lights provided by NightSea LLC. I took an initial fluorescence measurement against the white of the dissection scope base under blue excitation and yellow barrier filter to set a baseline. For blue excitation light trials, I set a baseline spectrometer emission intensity measurement of 2700 AU. For white light trials, I set the photosynthetically active radiation (PAR) level at 175 μmol $m^{-2}s^{-1}$. I took PAR and spectrometer measurements between each filmed run to prevent any changes in light intensity.

Experimental design for phototaxis trials:

The phototaxis trials followed a 3 x 2 factorial design, for three conditions: skeleton with removed tissue, sealed, and live (unsealed) coral, and two light sources. All phototaxis trials were conducted under a Nikon SMZ 745T dissection microscope at 50 x magnification. Videos were captured by NIS Elements software version 5.20.00, at a 1000 pixel per 1mm ratio (Video 1). All video captures were analyzed with the Image J TrackMate plugin (Tinevez et al., 2017) (Video 2). I used Adobe Premiere Pro version 2019 to convert the captured videos to a format that could be read by TrackMate, which identifies cells within a video frame, and then links cells through the frames of a video to form a track. Tracks consist of cells freeze-framed at different time points . I removed all

tracks with fewer than 10 links to eliminate tracks with cells possibly in the coccoid phase. TrackMate identifies the location on the x and y-axis for the first cell on the track and then identifies the location of the last cell on the track. I was able to measure direction and distance of movement from the difference between the start and end of the track. TrackMate also measures the speed of cells within a track and the total displacement along the x and y-axes.

I culled and fragmented *E. lamellosa* into approximately 8 x 10 mm sized fragments. I placed a holding vessel with outside measurements of 23 mm x 18 mm x 8 mm (L x W x H) and internal measurements of 20 mm x 15 mm x 5 mm under the dissection microscope at 50 x magnification (Figure 2). Phototaxis runs for each of the treatments were repeated 10 times per species, per light condition, for a total of 60 runs. I analyzed cells from each run by their number of tracks which consisted in total by species; *Cladocopium* sp. (n=33,8814), *B. psygomophilium* (n=31,426), *E. voratum* (n=29,689). Due to the necessary placement of the blue excitation light in front of the microscope, whereas the white light was placed directly overhead, I quantified algae movement parallel to the coral along the y-axis (Figure 3). Y-axis movement was quantified in the case of aversion to the blue light.

Phototaxis trials were conducted in a controlled laboratory environment. The light conditions during the laboratory trials were not comparable to light conditions on a wild reef system. PAR values on wild reef systems are approximately 700 – 1400 μmol $m^{-2}s^{-1}$ but can reach as high as 2000 μmol $m^{-2}s^{-1}$ (Bainbridge, 2017; Salih et al., 2006). The PAR measures of these trials were considerably lower at 175 μmol $m^{-2}s^{-1}$ The wavelength of available light in a reef habitat ranges from approximately 380 nm to 685 nm, with a

peak emission at approximately 450 nm, which is at peak excitation for green FPs (C. H. Mazel & Fuchs, 2003). The peak emission intensity increases with increased PAR (Eyal et al., 2015). The white light condition during laboratory trials did not produce a significant peak emission intensity with the ability to induce coral fluorescence in the *E. lamellosa* sample. The white light was a control to allow for comparison with the blue excitation light (440 – 460 nm) treatment. The research of Aihara et al. (2019) was conducted both in a laboratory setting and in the field. They were able to recreate attraction to fluorescence in a natural light setting utilizing a green fluorescent trap.

Algae and coral sample introduction and filming:

After first dark adapting the algae within the culture vessel for 20 ±5 minutes to allow for even cell distribution (Swafford & Oakley, 2018), I gently swirled the culture vessel to further disperse the symbiont cells. I inoculated 1.5 ml of coral system water at 26 °C in the holding vessel with 100 µl of algae in f/2 media suspension for the following densities; *Cladocopium* sp. (14 cells μl^{-1}), *B. psygomophilium* (8 cells μl^{-1}), *E. voratum* (8 cells μl^{-1}) . After algae inoculation, I agitated the holding vessel to disperse the cells. I then placed the skeletal coral fragment in the vessel, acclimated the algae for one minute and then filmed for thirty seconds independently for each light source and coral condition. After filming I replaced the water within the vessel with new system water, re-inoculated with fresh algae, replaced the same coral fragment, and repeated the process 10 times. When capturing video under blue light, I utilized the same skeleton and sealed coral as during the white light trial but replaced the live coral with a new specimen between algae species. All coral samples were placed on the far-right side of the vessel.

Statistical Analysis:

Statistical analysis and modeling were performed in Rstudio version 1.2.1335 (http://www.rstudio.com). Data were cube transformed to fit a normal distribution and to satisfy the assumptions of normality and homoscedasticity. All residuals were examined to ensure that assumptions were met. Data points consisted of tracks (previously described) that were calculated by the TrackMate plugin for Image J (Tinevez et al., 2017). In order to assess the movement of dinoflagellate cells, speed of movement, and displacement in relation to coral location and color of light source, I performed two-way ANOVAs with a 3 x 2 research design. I then performed a global one-way ANOVA to compare the above treatments by species. I measured the effect size with Cohens f at a 90% confidence interval, and significance with TukeyHSD at a 95% confidence level. To assess relationships between each of the measured variables, movement on the x and y-axes, speed, and displacement, I performed a Pearson Correlation Coefficient analysis.

RESULTS

Phototaxis, speed (100µm s^{-1}), and displacement of Symbiodiniaceae algae in response to E. lamellosa under blue excitation and white light:

Movement perpendicular to coral (x-axis):

Cladocopium sp.:

Movement of *Cladocopium* sp was furthest for both the sealed and unsealed coral under blue light. There was a significant difference in movement perpendicular to the coral among the three coral conditions ($F_{2, 33808} = 70.89$, Cohens f = 0.06, $p<0.01$), the two light

colors ($F_{1, 33808} = 359.62$, Cohens f = 0.10, $p<0.01$), and in relation to the interaction between coral condition and light ($F_{2, 33808} = 54.90$, Cohens f = 0.06, $p<0.01$). All movement along the x-axis was toward the coral sample (Figure 4a). The farthest movement of *Cladocopium* sp toward the coral occurred during trials with a sealed coral under blue light, followed closely by trials with an unsealed coral under blue light. During trials with a coral skeleton under blue light and trials with a sealed coral under white light, movement was similar and slower than movement during trials with either a sealed coral under blue light or unsealed coral under blue light. Algae moved the shortest distances along the x-axis during trials with either a coral skeleton or an unsealed coral under white light (Figure 4a). The color of light exposure produced the largest effect on x-axis movement. Most of the pairwise comparisons of movement along the x-axis were significantly different with a few exceptions. The non-significant differences were found when comparing sealed corals under white light to skeletons under blue light ($p=0.90$), and an unsealed coral to a skeleton under white light ($p=0.34$; Table 1; Figure 4a).

Breviolum psygomophilium:

Movement along the x-axis was significantly affected by coral condition ($F_{2, 31420} = 1583$, Cohens f = 0.32, $p<0.01$), light color ($F_{1, 31420} = 2122$, Cohens f = 0.26, $p<0.01$), and the interaction between the two factors ($F_{2, 31420} = 1866$, Cohens f = 0.34, $p<0.01$). *B. psygomophilium* displayed positive movement in relation to the coral sample with significantly more movement toward the coral while exposed to the blue sealed condition than movement under the remaining five conditions, in which there was very little movement (Figure 4b). None of the treatments were significantly different except

blue sealed compared to each of the others, which were statistically indistinguishable (Table 2).

Effrenium voratum:

There was a significant effect of coral condition ($F_{2, 29683} = 143.90$, Cohens f = 0.10, $p<0.01$), light color ($F_{2, 29683} = 1124.50$, Cohens f = 0.19, $p<0.01$), and the interaction between the two factors on movement ($F_{2, 29683} = 233.80$, Cohens f = 0.13, $p<0.01$). The movement of *E. voratum* exposed to a sealed, skeletal, or unsealed coral in response to blue or white light was positive in relation to the coral sample, except for algae exposed to a sealed coral under white light (Figure 4c). The farthest movement occurred during the algae exposure to blue light in the following order: unsealed, sealed, and coral skeleton. (Figure 4c). All the pairwise comparisons were significant apart from the comparison between trials with a coral skeleton either exposed to blue or white light ($p=0.18$; Table 3).

Species comparisons:

The three species displayed significantly different movement along the x-axis ($F_{2, 94926} = 425.00$, $p<0.01$). *B. psygomophilium* moved the farthest toward the coral, followed by *E. voratum,* and *Cladocopium* sp. displayed the least amount of movement (Figure 4d).

Movement parallel to coral (y-axis):

Cladocopium sp.:

Examining movement along the y-axis to determine a possible effect of the blue light source placed parallel to coral for *Cladocopium* sp*.,* there was a globally significant

difference in movement by coral condition ($F_{2, 33808} = 235$, Cohens f = 0.12, $p<0.01$), light color ($F_{2, 33808} = 225.10$, Cohens f = 0.08, $p<0.01$), and the interaction of the two ($F_{2, 33808} = 154.80$, Cohens f = 0.10, $p<0.01$). While exposed to the treatments of either a sealed or unsealed coral under blue light, *Cladocopium* sp. moved toward the blue light while those in the skeletal condition moved away from the blue light. There was little movement along the y-axis when under white light (Figure 5a). Coral condition was responsible for the largest effect. The interaction between treatment and color though globally significant, produced two non-significant pairwise comparisons: a coral skeleton under white light to a sealed coral under blue light (p=1.00), an unsealed coral under white light to a skeleton under blue light (p=0.11; Table 1).

Breviolum psygomophilium:

Coral condition ($F_{2, 31420} = 323.10$, Cohens f = 0.14, $p<0.01$), light color ($F_{2, 31420} = 215.10$, Cohens f = 0.08, $p<0.01$), and the interaction of the two ($F_{2, 31420} = 111$, Cohens f = 0.08, $p<0.01$) all significantly influenced movement of *B. psygomophilium* along the y-axis. In relation to the source of blue light, the algae in the sealed coral condition moved farthest away from the blue light, followed by the unsealed coral (Figure 5b). There was little movement along the y-axis for the three conditions under white light (Figure 5b). The pairwise comparisons were generally significant with the exception of a coral skeleton under white light to a skeleton under blue light (p=0.14), an unsealed coral under white light to a skeleton under blue light (p=0.80), and an unsealed and skeletal coral under white light (p=0.63; Table 2).

Effrenium voratum:

There were significant differences in movement along the y-axis by coral condition ($F_{2, 2968} = 22.87$, Cohens f = 0.04, $p<0.01$), light color ($F_{2, 29683} = 4.08$, Cohens f = 0.01, $p<0.01$), and the interaction of the two ($F_{2, 29683} = 38.86$, Cohens f = 0.05, $p<0.01$). *E. voratum* exposed to blue light had minimal movement away from the blue light only for a sealed coral (Figure 5c). Exposure to an unsealed and skeletal coral produced movement toward the blue light. The sealed and skeletal conditions under the white light displayed directional movement up the y-axis (Figure 5c). The pairwise comparisons supported the overall significance with the exception of the differences between an unsealed coral under white and sealed coral under blue light (p=0.43), an unsealed and skeletal coral under blue light (p=0.25), a sealed coral under white light and a skeleton under blue light (p=0.19), a skeleton under white light and blue light (p=0.88), a coral skeleton under white light and an unsealed coral under blue light (p=0.78), and an unsealed coral under white light and a sealed coral under white light (p=0.82; Table 3).

Species comparisons:

The three species differed significantly in movement along the y-axis ($F_{2, 94926} =$ 198.60, Cohens, $p<0.01$). *Cladocopium* sp. moved the farthest along the axis, followed by *E. voratum,* and then *B. psygomophilium* (Figure 5d).

Speed of movement by treatment:

Cladocopium sp.:

Speed (100 µm sec^{-1}) was significantly affected by coral condition ($F_{2, 33808}$ = 1741.40, Cohens f = 0.32, p<0.01), light color ($F_{2, 33808}$ = 18613.60, Cohens f = 0.74, p<0.01), and the interaction of the two ($F_{2, 33808}$ = 426.50, Cohens f = 0.16, p<0.01). The mean speed of movement was faster for all conditions under white light than blue light for *Cladocopium* sp. (Figure 6a). Light color had the most significant effect on the speed of movement. All the pairwise comparisons were significantly different (Table 1).

Breviolum psygomophilium:

For *B. psygomophilium*, speed of movement was significantly impacted by coral condition ($F_{2, 31420}$ = 2502.60, Cohens f = 0.40, p<0.01), light color ($F_{2, 31420}$ = 272.80, Cohens f = 0.09, p<0.01), and the interaction of the two $F_{2, 31420}$ = 2503.70, Cohens f = 0.40, p<0.01). Speed of movement was fastest for algae exposed to a sealed coral under blue light and slowest for algae exposed to an unsealed coral or a coral skeleton under blue light. Movement was slower in all white light conditions than when algae were exposed to sealed coral under blue light and faster than when tested with either a sealed or skeletal coral under blue light (Figure 6b). All the pairwise comparisons were significantly different (Table 2).

Effrenium voratum:

Speed was significantly affected by coral condition ($F_{2, 2968}$ = 1558.40, Cohens f = 0.32, p<0.01), light color ($F_{2, 29683}$ = 570.30, Cohens f = 0.14, p<0.01), and the interaction of the two ($F_{2, 29683}$ = 550.70, Cohens f = 0.19, p<0.01). Speed of movement was fastest for *E. voratum* exposed to the skeleton condition under white light. The three conditions

under blue light induced similar speeds which were faster than unsealed coral under white light and the slowest condition of sealed coral under white light (Figure 6c). All pairwise comparisons were significantly different except for the unsealed coral and skeleton under blue light (p=0.98; Table 3).

Species comparisons:

The three species differed significantly in their speed of movement ($F_{2, 94926}$ = 2760, p<0.01). The fastest moving species was *E. voratum,* followed by *B. psygomophilium,* the slowest moving algae were *Cladocopium* sp. (Figure 6d).

Displacement of dinoflagellates by treatment:

Cladocopium sp.:

Displacement of *Cladocopium* sp. was significantly affected by coral condition ($F_{2, 33808}$ = 547.70, Cohens f = 0.18, p<0.01), light color ($F_{2, 33808}$ = 7600.50, Cohens f = 0.47, p<0.01), and the interaction of the two ($F_{2, 33808}$ = 115.60, Cohens f = 0.08, p<0.01). Algae displacement displayed the same pattern as speed with the greatest movement for first the sealed, then unsealed and then skeletal coral under white light. There was less displacement for all three conditions under blue light (Figure 7a). All the pairwise comparisons were significantly different (Table 1).

Breviolum psygomophilium:

The displacement of *B. psygomophilium* cells was significantly affected by coral condition ($F_{2, 31420}$ = 357, Cohens f = 0.15, p<0.01), light color ($F_{2, 31420}$ = 313, Cohens f = 0.10, p<0.01), and the interaction of the two ($F_{2, 31420}$ = 343.70, Cohens f = 0.15, p<0.01). Displacement was greatest when algae were exposed to the sealed coral under blue light,

which was followed by all three conditions under white light. The skeleton condition under blue light produced less displacement than the three coral conditions under white light and greater than the unsealed coral under blue light (Figure 7b). Among the pairwise comparisons, one was not significant, the sealed and unsealed coral under white light ($p=0.09$; Table 2).

Effrenium voratum:

There were significant differences in displacement by coral condition ($F_{2, 29683} = 320.10$, Cohens $f = 0.15$, $p<0.01$), light color ($F_{2, 29683} = 261.20$, Cohens $f = 0.09$, $p<0.01$), and the interaction of the two ($F_{2, 29683} = 205.50$, Cohens $f = 0.12$, $p<0.01$). There was not a large difference in effect size among the three treatments. Displacement was much greater for *E. voratum* exposed to a skeletal coral under white light followed by an unsealed coral under white light. The treatments under blue light and the sealed coral under white light displayed little displacement (Figure 7c). The pairwise comparisons for displacement of *E. voratum* supported the global significance of the displacement of algae, with the exception of a skeletal and sealed coral under blue light ($p=0.47$), an unsealed and sealed coral under blue light ($p=0.72$), a skeletal and unsealed coral under blue light ($p=0.99$), a sealed coral under white light to a skeletal coral under blue light ($p=0.98$), and a sealed coral under white light to an unsealed coral under blue light ($p=0.78$; Table 3).

Species comparisons:

Dinoflagellate displacement differences among the three species were globally significant ($F_{2, 949.26} = 638.30$, $p<0.01$). *E. voratum* had the greatest displacement of the three species, followed by *B. psygomophilium,* and then *Cladocopium* sp. (Figure 7d)

Pearson Correlation Coefficient analysis of independent variables within and among species

Cladocopium sp.:

Correlation analysis among movement variables for *Cladocopium* sp. displayed significant relationships between the following variables: movement on the x-axis and y-axis ($r(33812) = 0.046$, $p < 0.01$), movement on the x-axis and speed ($r(33812) = -0.02$, $p < 0.01$), movement along the x-axis and displacement of algae ($r(33812) = -0.010$, $p = 0.06$), movement on the y-axis and speed ($r(33812) = 0.055$, $p < 0.01$), and movement on the y-axis and displacement ($r(33812) = 0.050$, $p < 0.01$). There was a significant positive relationship with a tighter correlation than the previous relationships between speed of movement and displacement ($r\ (33812) = 0.681$, $p < 0.01$).

Breviolum psygomophilium:

For *B. psygomophilium,* there was a statistically significant relationship between movement on the x-axis and movement on the y-axis ($r(31424) = 0.040$, $p < 0.01$), movement along the x-axis and displacement of algae ($r(31424) = 0.075$, $p < 0.01$), movement on the y-axis and speed ($r(31424) = 0.028$, $p < 0.01$), and movement along the y-axis and displacement ($r(31424) = -0.007$, $p = 0.244$). There were stronger, significant correlations between movement on the x-axis and speed ($r(31424) = 0.209$, $p < 0.01$) and speed of movement and displacement ($r(31424) = 0.524$, $p < 0.01$).

Effrenium voratum:

Correlation analysis among variables for *E. voratum* illustrated significant relationships between all variables. As with the two other algae species, there were significant relationships between: movement on the x-axis and y-axis ($r(29687) = 0.021$, $p < 0.01$), movement on the x-axis and speed ($r(29687) = 0.056$, $p < 0.01$), movement on the x-axis and displacement ($r(29687) = 0.018$, $p < 0.01$), movement on the y-axis and speed ($r(29687) = -0.027$, $p < 0.01$), and movement on the y-axis and displacement ($r(29687) = -0.015$, $p = 0.012$). There was a significant, positive correlation between speed of movement and displacement of *E. voratum* ($r(29687) = 0.439$, $p < 0.01$).

All Species:

Examination of correlations between variables for all species combined revealed significant relationship with weak correlation between movement on the x-axis and y-axis ($r(94927) = 0.036$, $p < 0.01$), movement on the x-axis and speed ($r(94927) = 0.085$, $p < 0.01$), movement on the x-axis and displacement ($r(94927) = 0.035$, $p < 0.01$), movement on the y-axis and speed ($r(94927) = 0.011$, $p < 0.01$), and movement on the y-axis and displacement *(*$r(94927) = 0.006$, $p = 0.071$). There was a significant positive relationship with a tighter correlation between speed of movement and displacement ($r(94927) = 0.578$, $p < 0.01$). I identified the positive or negative nature of the relationship of each of the four variables individually under either blue or white light. There was a significant negative relationship between speed along the x-axis under blue light ($r(39954) = 0.241$, $p < 0.01$) and displacement ($r(39954) = 0.163$, $p < 0.01$). The remaining comparisons under blue light all displayed positive relationships; speed of

movement on the y-axis under blue light ($r(39954) = 0.032$, $p < 0.01$), displacement along the y-axis under blue light ($r(39954) = 0.022$, $p < 0.01$), speed and displacement under blue light ($r(39954) = 0.608$, $p < 0.01$). As well, the relationships were all negative under white light; speed of movement along the x-axis ($r(54971) = 0.329$, $p < 0.01$). displacement along the x-axis ($r(54971) = 0.017$, $p < 0.01$), speed of movement along the y-axis ($r(54971) = -0.010$, $p = 0.025$), displacement along the y-axis ($r(54971) = -0.006$, $p = 0.182$), and between speed and distance under white light ($r(54971) = 0.510$, $p < 0.01$).

DISCUSSION

I found support for phototactic attraction of dinoflagellates to fluorescent coral for each of the three species due to their movement along the x-axis. This finding supports my prediction of greater attraction to a coral with excited FPs. Density of free-living symbionts is thought to be low in coral reef habitats (Muller-Parker et al., 2015; Takabayashi et al., 2012), and coral fluorescence may enhance recruitment of free-living symbionts. The non-symbiotic *E. voratum* also displayed significant phototaxis in response to a fluorescing coral. I predicted that without the need to form a symbiosis, *E. voratum* would be less attracted to the fluorescent coral than the two symbiotic species. The significant attraction of non-symbiotic *E. voratum* to a green fluorescent coral did not support my prediction of greater attraction for symbiotic than non-symbiotic algae. I predicted that the attraction to the fluorescent coral would cause the algae to move faster with greater displacement in the direction of the coral. In contrast to my prediction, exposure to white light induced the greatest displacement. There was not a predictable

pattern of change in speed in relation to fluorescence, except for in *Cladocopium* sp*.,* which displayed consistently faster movement under white light.

A known mechanism for attraction of symbiont to coral is the use of chemical cues by the coral (Muller-Parker et al., 2015; Takeuchi et al., 2017; Tout et al., 2015). My prediction of attraction to the coral caused by FP emission and not an attraction to a chemical cue was supported by similar movement by algae to both the sealed and unsealed coral under blue light. The greater attraction to the sealed coral with excited FPs than the unsealed coral without excited FPs, also supports that the attraction was a result of the green fluorescence and not due to a release of chemical compounds.

Movement toward and parallel to coral in response to white and blue excitation light:

The adaptive bleaching hypothesis proposes coral lose algal partners that are not thermally tolerant to allow recolonization with symbionts that provide greater protection during bleaching events (Buddemeier & Fautin, 1993; Kinzie et al., 2001; Ware et al., 1996). If true, coral would require mechanisms to attract symbiotic algae that are found in low densities within the water column (Muller-Parker et al., 2015). My finding of positive phototaxis in response to fluorescence also supports the "Beacon Hypothesis", which states that FP emission is an adaptation of coral to attract new and potentially hardier dinoflagellate species (Horiguchi et al., 1999). Colorful bleaching is a recently documented phenomenon linking coral bleaching and upregulation of FP expression (Bollati et al., 2020). The attraction of algae to fluorescing coral suggests a function in addition to the previously described function of photoprotection (Salih et al., 2000, 2006).

Symbiotic *Cladocopium* sp. and non-symbiotic *E. voratum* had significant

movement toward both a live coral and a sealed coral whose FPs were excited by blue light. *E. voratum* displayed greater attraction to the live coral than the sealed coral. Symbiotic *B. psygomophilium* displayed significant attraction toward the sealed fluorescent coral, but not the live fluorescent coral, in contrast to the response of either *Cladocopium* sp. or *E. voratum*. The significant positive taxis of *B. psygomophilium* toward the fluorescent, sealed coral and not the live, fluorescent coral could be due to a lack of a symbiotic relationship with *E. lamellosa* (Baker, 2003; Fabina et al., 2013). Non-symbiotic *E. voratum* displayed a greater attraction to both a sealed and live fluorescent coral with excited FPs than not; however, the attraction was greater toward the fluorescent, live coral. There was minimal movement toward the live coral under white light. *E. voratum* are free-swimming; and therefore, do not benefit from the nutrients provided by the coral's waste as do symbiotic algae (Muller-Parker et al., 2015). Perhaps the fluorescent emission was the initial attractant and as algae approached the coral, they were able to detect the bacteria within the coral holobiont (Krediet et al., 2013). Due to the heterotrophic nature of *E. voratum*, and their increased ability to capture and consume bacteria, the increased attraction could be an attempt to secure prey (Jeong et al., 2014).

Movement in response to the blue light and not the coral fluorescence displayed no predictable pattern of positive or negative taxis to the blue light. The one exception was significant, anomalously large negative phototaxis in *B. psygomophilium* exposed to the blue, sealed treatment. In contrast, there were non-significant differences in taxis between the blue and white light sources when *B. psygomophilium* was tested with either a live coral or a skeletal coral. Under the blue, sealed treatment, *B. psygomophilium* also

moved significantly closer to the fluorescent sealed coral than the other species exposed to all the manipulated conditions, supporting its attraction to green fluorescence. Both *Cladocopium* sp. and *E. voratum* displayed either non-significant differences in reaction under blue or white light or significant interactions with positive taxis in relation to the blue light. There was no repulsion of the algae from the blue light that could have affected the outcome of the phototaxis in relation to the fluorescent coral. I would not attribute repulsion as the motivation of movement away from the blue light as the same pattern was not seen when the algae were exposed to blue light with a live coral. The question of why there was an anomaly in the movement of *B. psygomophilium* while exposed to a sealed coral under blue light warrants further study.

Speed (100µl sec^{-1}) of dinoflagellate movement and displacement in response to white and blue excitation light:

The two symbiotic species of algae, *Cladocopium* sp. and *B. psygomophilium*, demonstrated greater speed during white light conditions. The one exception was the significantly increased speed of *B. psygomophilium* during the trials with a sealed coral under blue light. Non-symbiotic *E. voratum* was faster than the two symbiotic species in most instances while in the presence of both the fluorescing coral and the coral exposed to white light. The non-symbiotic algae without the benefit of the coral symbiosis has increased ability to hunt prey. I would surmise their higher mean speed has evolved to facilitate their prey capture (Jeong et al., 2012, 2014; Yamashita &

Koike, 2015). Speed of all threes species were within the range of dinoflagellate speed documented in previous research (Lewis et al., 2006)

Displacement was greatest in reaction to white light exposure across all species and treatments. The exception was *B. psygomophilium* during the sealed, blue trials, which displayed the greatest deviation across the three explanatory variables, coral sample, color of the light, and the interaction of the two. An analysis of the correlation between variables found a moderate positive correlation between displacement and speed for all species and a weak correlation between movement along the x-axis and speed for *B. psygomophilium.* I attributed the decreased displacement and speed of algae exposed to blue light to directed movement toward the fluorescing coral. A Pearson correlation analysis showed a significant, correlated negative relationship between both speed and displacement for combined algae movement exclusively under blue light and along the x-axis. Movement along the y-axis under both blue and white light displayed a positive relationship. The same is true for all movement influenced by white light.

Conclusion

The results of this research build upon previous phototaxis research of algae in response to FP excitation (Aihara et al., 2019; Hollingsworth et al., 2005). The novel components of this project are the study of two symbiotic algae, *Cladocopium* sp. and *B. psygomophilium* and one non-symbiotic algae, *E. voratum,* that have not previously been utilized in phototaxis trials. The attraction of three additional species of *Symbiodiniaceae* to green fluorescence illustrates the findings of previous research were not unique to the species previously utilized. The addition of a non-symbiotic species demonstrates that

attraction to green fluorescence is not confined to symbiotic species. The measurement of directional movement, speed, and displacement of algae in relation to a fluorescent coral provides the first study of how algae react to green fluorescence regarding their speed and displacement. In addition to supporting phototaxis toward green light, my study offers insight into how quickly algae move in response to two light sources and differences among species in response to fluorescence. Although all three species were attracted to fluorescence the degree of attraction toward the coral differed.

To date, there have been few studies exploring the phototactic relationship between endosymbiotic microalgae and coral. Results from the two symbiotic species and one non-symbiotic supported the hypothesis that the attraction of the algae to the coral is due to the emission from excitation of FPs within the coral and not a response to a chemical cue. The additional support this study provides for positive phototaxis of algae in relation to a fluorescing coral lends support to the hypothesis that coral utilize fluorescence emission to attract dinoflagellates from the surrounding environment (Hollingsworth et al., 2005; Horiguchi et al., 1999). The ability to attract new symbionts could be a means at attempted attraction of algae from the water column and recolonization of coral tissues with symbiotic dinoflagellates in possession of increased thermal tolerance. During a bleaching event, coral lose algae and must repopulate their endosymbionts for survival (Baker, 2003; Jones et al., 2008). The ability of coral fluorescence to attract algae could provide great benefits for a coral under stress.

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Table 1. TukeyHSD comparisons for directionality of movement, speed and displacement of *Cladocopium* sp. in relation to *E. lamellose.*

Treatment	Perpendicular to coral (x-axis)	Parallel to coral (y-axis)	Speed	Displacement
Skeleton blue - sealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Unsealed blue -sealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Sealed white - sealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Skeleton white – sealed blue	**P<0.01**	**P=0.01**	**P<0.01**	**P<0.01**
Unsealed white – sealed blue	**P<0.01**	**P=0.026**	**P<0.01**	**P<0.01**
Unsealed blue – skeleton blue	**P<0.01**	P=0.069	**P<0.01**	**P<0.01**
Sealed white - skeleton blue	P =0.895	P=0.219	**P<0.01**	**P<0.01**
Skeleton white - skeleton blue	**P<0.01**	P=0.052	**P<0.01**	**P<0.01**
Unsealed white - skeleton blue	**P<0.01**	P=0.112	**P<0.01**	**P<0.01**
Sealed white -unsealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Skeleton white - unsealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Unsealed white - unsealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Skeleton white -sealed white	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Unsealed white -sealed white	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Unsealed white - skeleton white	P=0.342	**P<0.01**	**P<0.01**	**P<0.01**

Table 2. TukeyHSD comparisons for directionality of movement, speed and displacement of *B. psygomophilium* in relation to *E. lamellose.*

Treatment	Perpendicular to coral (x-axis)	Parallel to coral (y-axis)	Speed	Displacement
Skeleton blue - sealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Unsealed blue -sealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Sealed white - sealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Skeleton white – sealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Unsealed white – sealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Unsealed blue – skeleton blue	P=0.069	**P<0.01**	**P<0.01**	**P<0.01**
Sealed white - skeleton blue	P=0.219	P=0.140	**P<0.01**	**P<0.01**
Skeleton white - skeleton blue	P=0.052	P=0.804	**P<0.01**	**P<0.01**
Unsealed white - skeleton blue	**P<0.01**	**P=0.040**	**P<0.01**	**P<0.01**
Sealed white - unsealed blue	P=0.985	**P<0.01**	**P<0.01**	**P<0.01**
Skeleton white - unsealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Unsealed white - unsealed blue	P=0.209	**P<0.01**	**P<0.01**	**P<0.01**
Skeleton white -sealed white	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Unsealed white - sealed white	P=0.028	**P<0.01**	**P<0.01**	P=0.094
Unsealed white - skeleton white	**P<0.01**	P=0.627	**P<0.01**	**P<0.01**

Table 3. TukeyHSD comparisons for directionality of movement, speed and displacement of *E. voratum.* in relation to *E. lamellose.*

Temperature	Perpendicular to coral (x-axis)	Parallel to coral (y-axis)	Speed	Displacement
Skeleton blue - sealed blue	**P<0.01**	**P<0.01**	**P<0.01**	P=0.470
Unsealed blue -sealed blue	**P<0.01**	**P<0.01**	**P<0.01**	P=0.072
Sealed white - sealed blue	**P<0.01**	**P<0.01**	**P<0.01**	P=0.05
Skeleton white – sealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Unsealed white – sealed blue	**P<0.01**	P=0.428	**P<0.01**	**P<0.01**
Unsealed blue – skeleton blue	**P<0.01**	P=0.248	P=0.952	P=0.992
Sealed white - skeleton blue	**P<0.01**	P=0.194	**P<0.01**	P=0.997
Skeleton white - skeleton blue	P=0.179	P=0.875	**P<0.01**	**P<0.01**
Unsealed white - skeleton blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Sealed white - unsealed blue	**P<0.01**	**P<0.01**	**P<0.01**	P=0.776
Skeleton white - unsealed blue	**P<0.01**	P=0.775	**P<0.01**	**P<0.01**
Unsealed white - unsealed blue	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Skeleton white - sealed white	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Unsealed white - sealed white	**P<0.01**	P=0.082	**P<0.01**	**P<0.01**
Unsealed white - skeleton white	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**

Figure 1: *Echinophyllia lamellosa,* the model coral species for green fluorescent phototaxis trials.

Figure 2. Holding vessel for phototaxis trials with coral sample placement. X and y-axes correspond to tracking of cell movement.

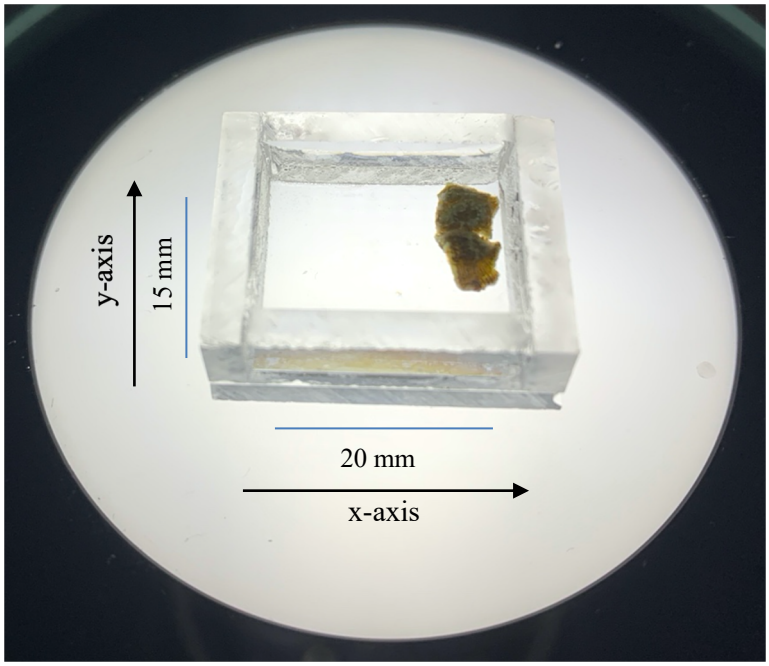


Figure 3. Blue excitation light in relation to holding vessel and coral sample during trials to quantify algal movement.

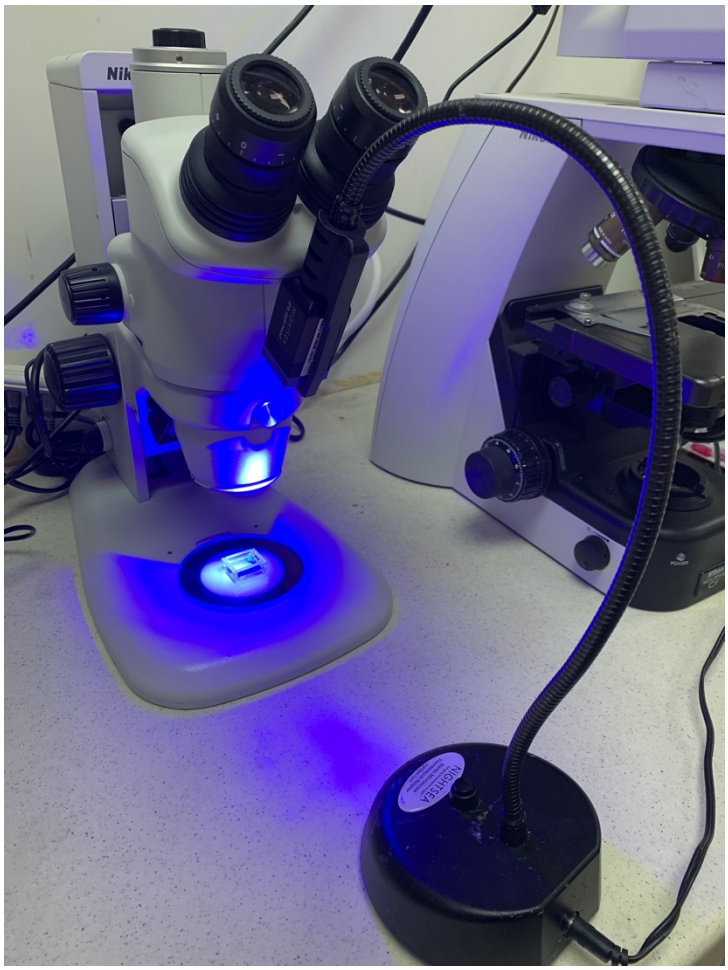
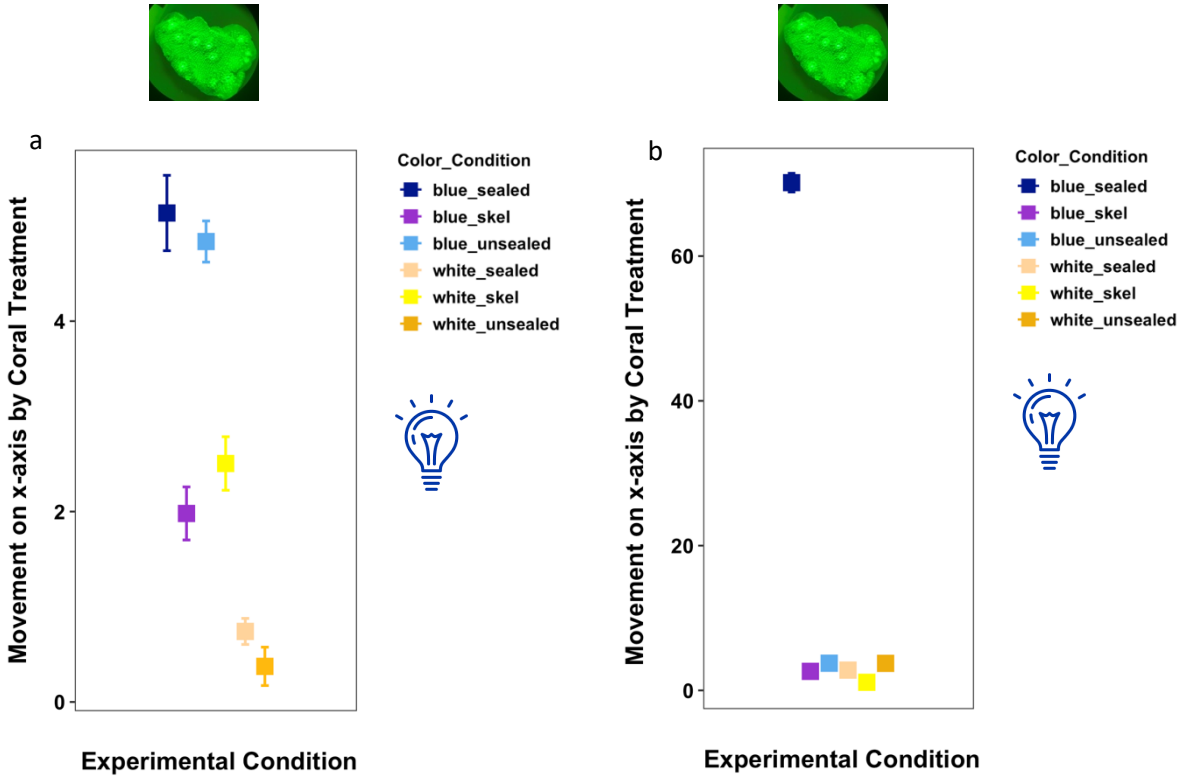
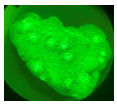
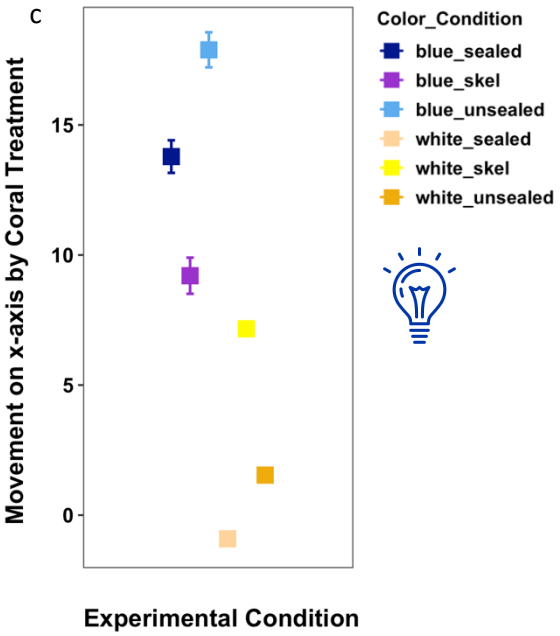
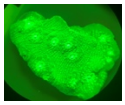


Figure 4. Movement of *Cladocopium* sp. (a), *B. psygomophilium* (b), *E. voratum* (c), and all species combined (d) in relation to three conditions of *E. lamellosa* (sealed, skeleton, unsealed) and two light sources, 440 – 460 nm blue light and natural light at 175 µmol $m^{-2}s^{-1}$ perpendicular (x-axis) to the coral sample. Error bars are ± se.






c
Movement on x-axis by Coral Treatment
15
10
5
0
Experimental Condition
Color_Condition
blue_sealed
blue_skel
blue_unsealed
white_sealed
white_skel
white_unsealed



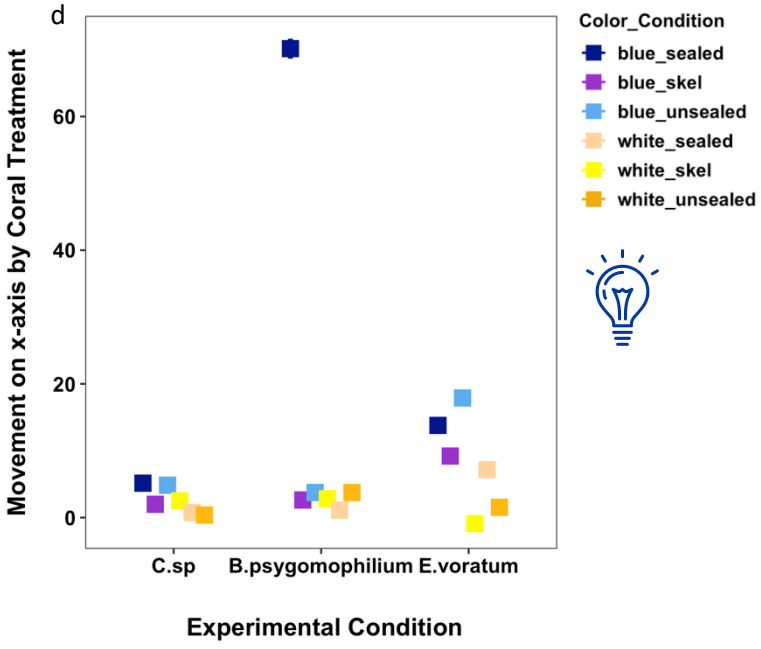
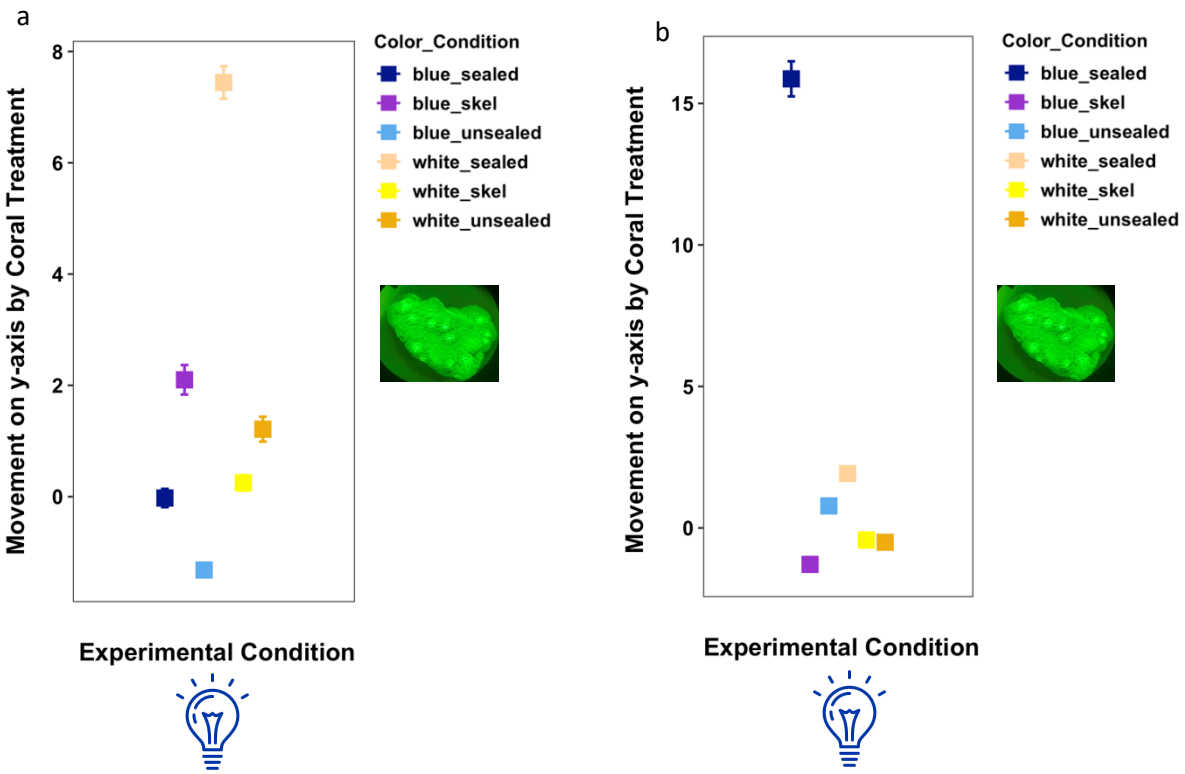
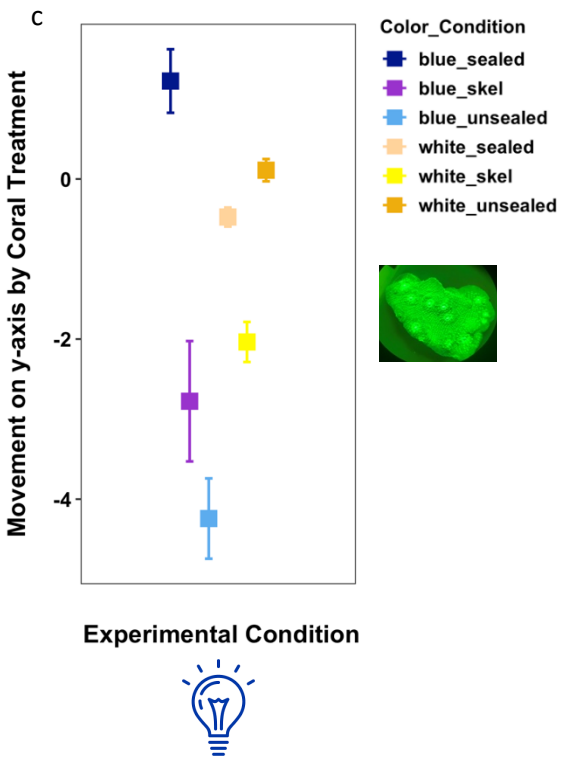

d
Movement on x-axis by Coral Treatment
60
40
20
0
C.sp
B.psygomophilium
E.voratum
Experimental Condition
Color_Condition
blue_sealed
blue_skel
blue_unsealed
white_sealed
white_skel
white_unsealed

Figure 5. Movement of *Cladocopium* sp. (a), *B. psygomophilium* (b), *E. voratum* (c), and all species combined (d) parallel to the coral in relation to three conditions of *E. lamellosa* (sealed, skeleton, unsealed) and two light sources, 440 – 460 nm blue light and natural light at 175 µmol $m^{-2}s^{-1}$. Parallel (y-axis) to the coral species. Error bars are ± se.



c


Color_Condition
blue_sealed
blue_skel
blue_unsealed
white_sealed
white_skel
white_unsealed
Movement on y-axis by Coral Treatment
0
-2
-4
Experimental Condition

d

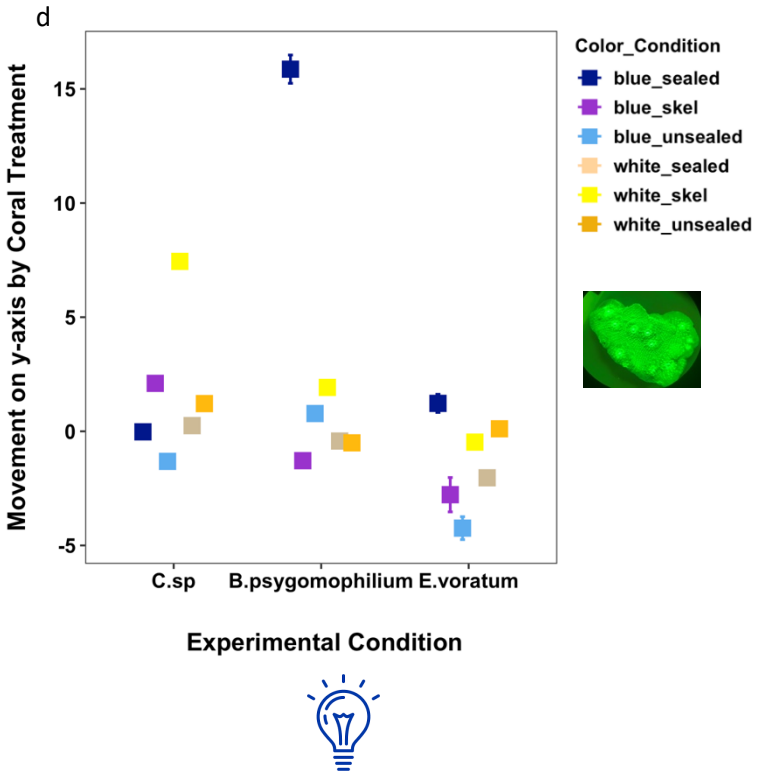
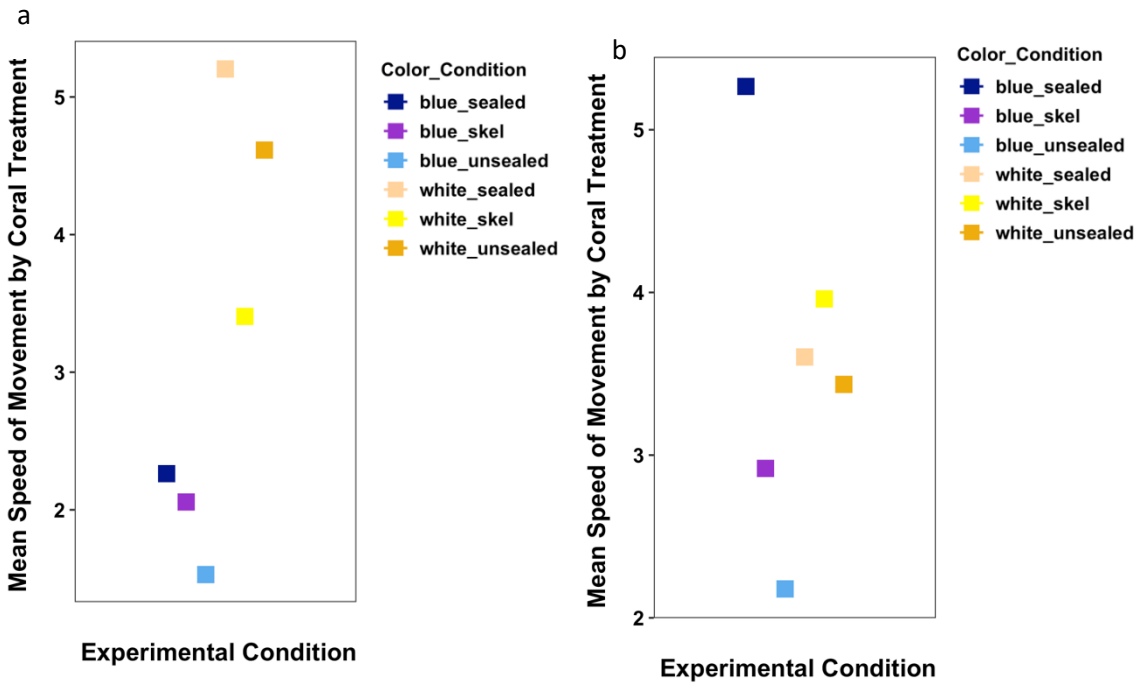
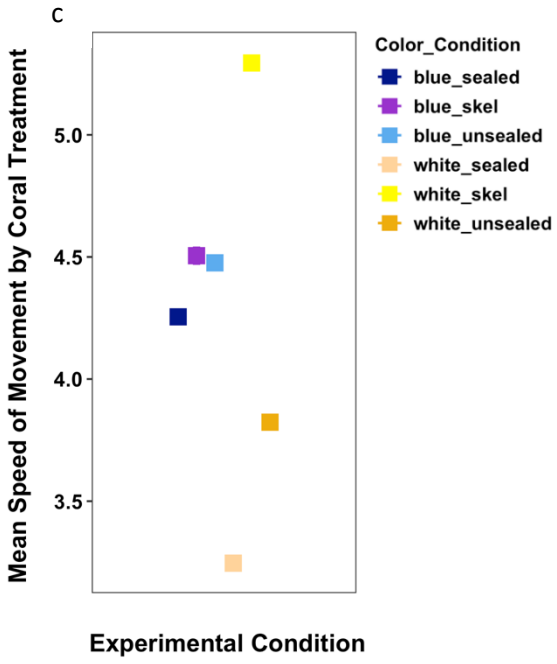

Color_Condition
blue_sealed
blue_skel
blue_unsealed
white_sealed
white_skel
white_unsealed
Movement on y-axis by Coral Treatment
15
10
5
0
-5
C.sp
B.psygomophilium
E.voratum
Experimental Condition

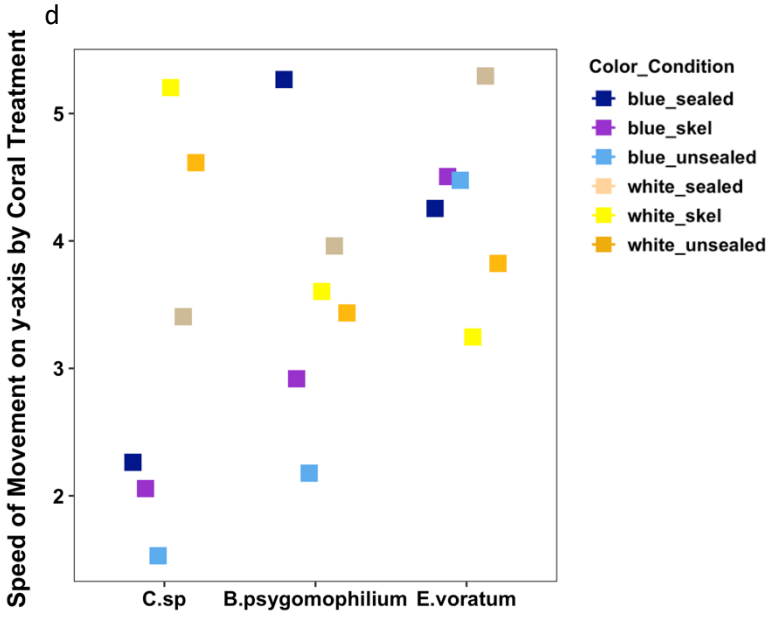
Figure 6. Mean speed of movement (100µm s^{-1}) for *Cladocopium* sp. (a), *B. psygomophilium* (b), *E. voratum* (c), and all species combined (d) in relation to three conditions of *E. lamellosa* (sealed, skeleton, unsealed) and two light sources, 440 – 460 nm blue light and natural light at 175 µmol $m^{-2}s^{-1}$. Error bars are ± se.



c

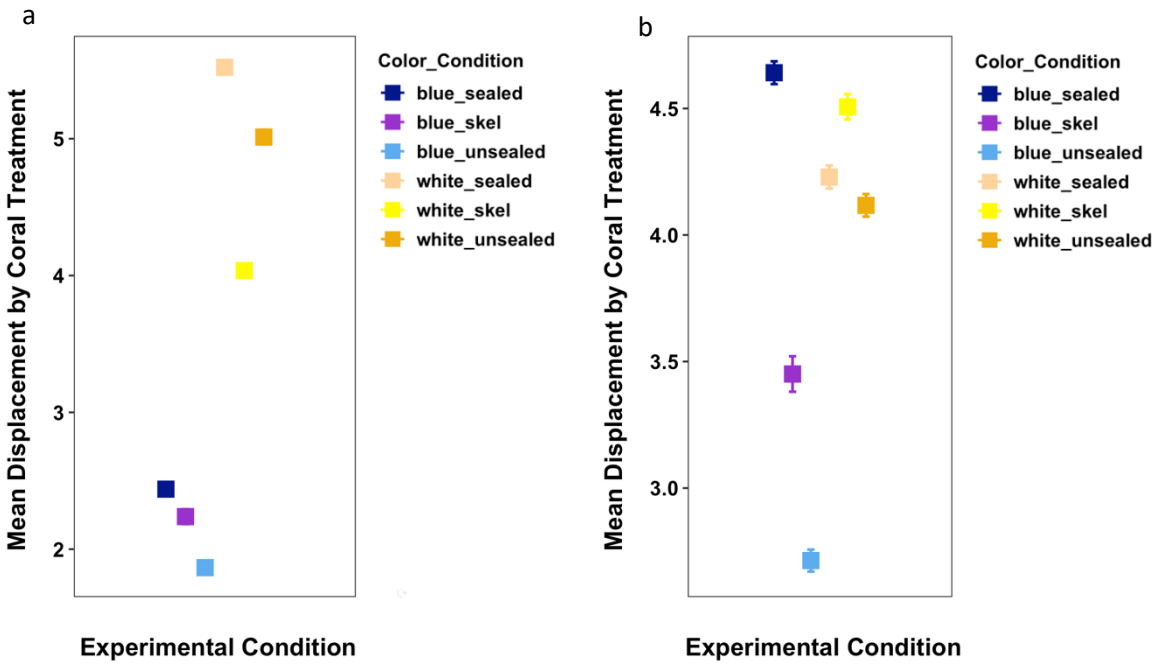

Color_Condition
blue_sealed
blue_skel
blue_unsealed
white_sealed
white_skel
white_unsealed
Mean Speed of Movement by Coral Treatment
5.0
4.5
4.0
3.5
Experimental Condition

d

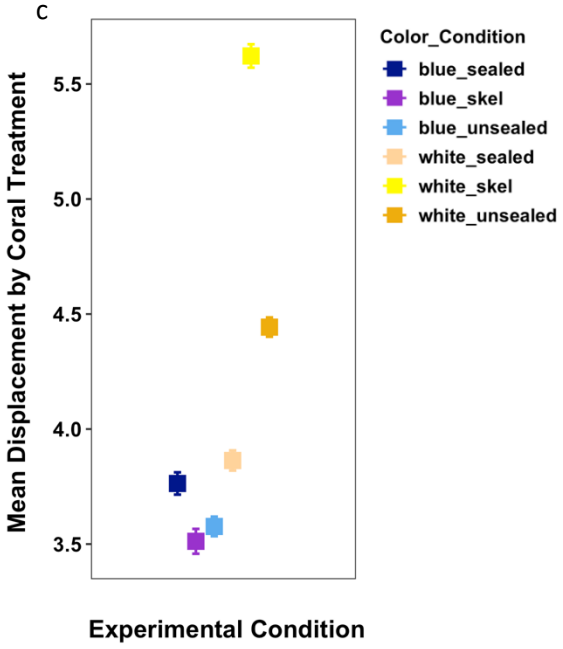

Color_Condition
blue_sealed
blue_skel
blue_unsealed
white_sealed
white_skel
white_unsealed
Speed of Movement on y-axis by Coral Treatment
5
4
3
2
C.sp
B.psygomophilium
E.voratum
Experimental Condition

b

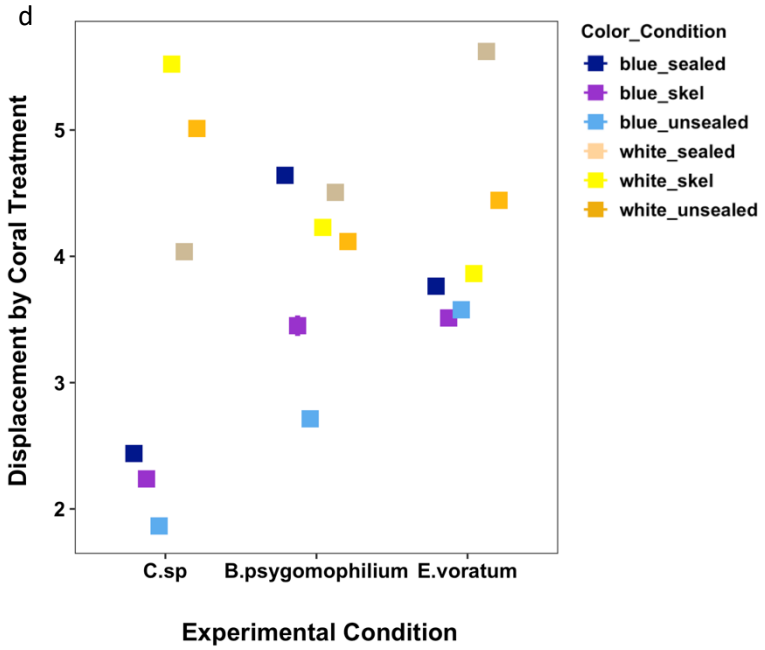
Figure 7. Mean displacement of *Cladocopium* sp. (a), *B. psygomophilium* (b), *E. voratum* (c), and all species combined (d) in relation to three conditions of *E. lamellosa* (sealed, skeleton, unsealed) and two light sources, 440 – 460 nm blue light and natural light at 175 µmol $m^{-2}s^{-1}$. Error bars are ± se.



c


Color_Condition
blue_sealed
blue_skel
blue_unsealed
white_sealed
white_skel
white_unsealed
Mean Displacement by Coral Treatment
5.5
5.0
4.5
4.0
3.5
Experimental Condition

d


Color_Condition
blue_sealed
blue_skel
blue_unsealed
white_sealed
white_skel
white_unsealed
Displacement by Coral Treatment
5
4
3
2
C.sp
B.psygomophilium
E.voratum
Experimental Condition

Appendix A: Water quality parameters of coral acclimation system following Oklahoma Aquarium protocol. PAR levels variable by vertical placement in water column.

PAR	44 – 190 μmol $m^{-2}s^{-1}$
Salinity	33 – 35 ppt
pH	8.1 – 8.2
Temperature ° C	25.6 – 26.1° C
ammonia (NH_3)	0.08 mg L^{-1} NH_3-N or below
NH_3-N, nitrite (NO_2)	0.1 mg L^{-1} NO_2–N or below
phosphate (PO_4)	0.1 mg L^{-1} PO_4^{3-} or below
iodine (I_2)	0.07 – 0.15 mg L^{-1}
Calcium (Ca)	400 – 500 mg L^{-1} (ppm)
Magnesium (Mg)	1300 – 1500 mg L^{-1} (ppm)
Alkalinity	<10dKH
Strontium (Sr)	1.85 g Sr L^{-1}

Appendix B: Culture protocol of BURR lab for dinoflagellate propagation and growth.

BURR Laboratory Culturing Protocols (http://burr.bio.buffalo.edu/index.php/cultures/)

- Mix f/2 media solution (the recipe is available on the website) to 500 ml filtered saltwater
- Filter the water again
- Transfer 100 µl of initial cell culture to 30 ml of f/2 media in 50 ml flask with foam stopper
- Place under grow lights on a 14h:10h light:dark cycle
- Transfer 100 µl of culture to 30 ml of fresh f/2 media monthly

Video 1: Movement capture of *Cladocopium* sp. exposed to 440 - 460 nm blue excitation light and an unsealed *E. lamellosa*. Video filmed in real-time, with dimensions of 1.4 mm x 1 mm. Blue light is located directly in the middle of the x-axis. The coral in on the right side out of frame in along the y-axis.

https://drive.google.com/file/d/1i2wpuFouI7B_z53jJ7lQPxInRiszWAUh/view?usp=sharing

Video 2: Tracking of *Cladocopium* sp. cells exposed to 440 - 460 nm blue excitation light and an unsealed *E. lamellosa* utilizing Image J plugin TrackMate. Dimensions of 1.4 mm x 1 mm.

https://drive.google.com/file/d/1l4HPeqHn71tAjgMCYlwfD0Mma62wFVmt/view?usp=sharing

CHAPTER IV

IMPACT OF A FIELD TRIP TO A PUBLIC AQUARIUM ON STUDENTS' INTEREST IN STEM

INTRODUCTION

The acronym STEM (Science, Technology, Engineering, and Math) was adopted by the National Science Foundation in the 1990s, and has since encompassed all education, policy, and involvement in; science, technology, engineering, and math (Bybee 2010). Since its inception, educators and schools have changed their perception and teaching of STEM concepts. There is an increased emphasis on inclusion of STEM concepts in all aspects of student learning (Kelley and Knowles 2016). Despite an increased emphasis on STEM education, there is an overall decline in interest in science after middle school (Fortus and Vedder-Weiss 2014). Only 28% of students leave high school with a desire to pursue an advanced degree in a STEM field, and of those students, approximately half switch to a non-STEM degree path (Shin, Levy, and London 2016; Chen 2013). The decrease of interest in STEM fields is contributing to a lack of needed STEM professionals. This has prompted an increase of funding in the US allocated to STEM education (Sanders 2009; Scott 2012; Shin, Levy, and London 2016). Federally funded programs have helped to identify that the inclusion of informal science learning is crucial

to a student's understanding of STEM concepts (Scott 2012; Falk and Dierking 2010; Bell et al. 2016).

Within the structure of STEM learning, there are two defining components, formal and informal education. Formal STEM learning occurs in the classroom, while informal learning occurs in facilities such as museums, science centers, zoos, and aquariums (Falk 2005; Ballantyne and Packer 2005). In combination, formal and informal STEM education are the backbone for scientific learning (Bell et al. 2016; Falk, Storksdieck, and Dierking 2007). Students who visit science centers, such as an aquarium, are more motivated to learn science (Rennie and McClafferty 1995).

Schooling occupies approximately 5% of a person's life, the other 95% is spent outside of the classroom (Falk and Dierking 2010). It has been the assumption that most science learning occurs in the classroom, so therefore, most education resources are committed to formal education. However, there is a growing body of evidence that the public learn science outside of the classroom in more informal settings such as aquariums (Falk and Dierking 2010). Learning outside of the classroom has been characterized as guided by self-exploration. The student can rely on their own experiences and interests to pursue topics of individual interest. The ability of the student to focus on topics of more interest promotes greater engagement than that found through formal science learning (Dierking et al. 2003).

Within the context of formal and informal science learning is free choice learning, which allows for self-determination to some degree or completely. The individual is able to follow their own interests rather than conforming to a structured learning experience (Falk 2005; Ballantyne and Packer 2005). Free choice learning allows students to mold their

experience, increasing interest and participation (Kola-Olusanya 2005). The concept of free choice learning can be adopted in the formal classroom setting, but is most often implemented in science centers, zoos, and aquariums (Bamberger and Tal 2007; Falk 2005). These facilities offer a free choice learning experience, fostering appreciation and interest in science, animals, and nature (Kola-Olusanya 2005). Nurturing an interest in science leads to increased likelihood of pursuing a career in the sciences (Dabney et al. 2012). Research conducted utilizing data from the National Educational Longitudinal Study found that adult attitudes toward science were formed primarily in an informal setting, and informal learning appeared to be the largest factor in determining future careers in science (Falk and Dierking 2010).

Approximately 700 million people visit zoos and aquariums worldwide every year (Gusset and Dick 2011). The number of visitors provides zoos and aquariums a unique opportunity to present science, nature, and research in a manner that appeals to and impacts many people. People often feel an emotional connection to animals in zoos and aquariums, which can increase their conservation awareness in relation to the animal (Myers, Saunders, and Birjulin 2004). Increased conservation awareness contributes to the desire to protect and conserve animals and their habitats (Myers, Saunders, and Birjulin 2004; Ballantyne et al. 2007; Ballantyne and Packer 2005).

The Oklahoma Aquarium (OKAQ) hosts approximately 30,000 students and 400,000 total guests annually. In addition to housing 10,000 animals for guests to encounter, the aquarium conducts behind the scenes research on coral reefs and bio-fluorescence. Coral reefs cover less than 0.1% of the planet, yet they are home to 25% of all marine life (Knowlton and Jackson 2008) and support the highest concentration of biodiversity of any

marine habitat (Polidoro and Carpenter 2013). Coral provide protection for shorelines from large storms at sea in addition to their value as tourism destinations, which places their economic importance between 1 – 20 trillion USD (Hughes, Kerry, et al. 2017; Hoegh-Guldberg 2015). These important habitats are failing in the wild due to climate change (Glynn 1996; Baker, Glynn, and Riegl 2008; Hughes, Barnes, et al. 2017). The OKAQ researches potential early warning systems of coral decline through the monitoring of bio-fluorescence within coral. Through its research activities and animal housing, the OKAQ provides a connection to animals, research, and habitats that can help promote positive conservation attitudes.

Research conducted at the OKAQ provides the opportunity for students to visit a coral research lab as well as observe coral fluorescing under a microscope. The visit provides an experiential research experience through a student's examination of a fluorescent coral. Experiential learning occurs through interactive experiences gained through hands on involvement of the student. When students are engaged in an "interactive learning process", they have greater motivation to learn (Falk, 2001). During a field trip to many aquariums including the OKAQ, students can play, crawl under exhibits, and touch animals. These opportunities for direct interaction with living animals and the exhibits support experiential learning. Interactions such as this are valuable in making the subject more interesting and increasing the opportunity to learn (Behrendt and Franklin 2013). Science activities that are experiential have a greater impact on the curiosity and excitement of a child; therefore, increasing interest and knowledge (Scarce 1997; Behrendt and Franklin 2013).

Interest and confidence in a subject are defining factors in the choice of a career (Mohd Shahali et al. 2019; Hinojosa et al. 2016). Lack of confidence in the ability to

understand scientific concepts is more impactful than actual abilities, and can hinder the pursuit of a career in a STEM field (Lin and Schunn 2016; Hinojosa et al. 2016). Students are often limited by what they believe is their capacity to understand science as opposed to their actual ability (Lin and Schunn 2016; Simpkins, Davis-Kean, and Eccles 2006). Supporting a student's confidence in their ability to understand science and conduct research positively impacts their belief in themselves, as well as their desire to pursue a career in science (Lin and Schunn 2016). There are fewer women and minorities who study for a career in STEM fields or remain in STEM fields once they begin their careers. In the US, women comprise 50% of the overall work force, yet only 25% within STEM fields (Beede et al. 2011). In the US, 11% of the workforce is black, and 9% of the STEM workforce is black. As well, 16% of the US workforce is Hispanic, yet only 7% of STEM jobs are held by Hispanics (Funk and Parker 2018). The gender gap within the sciences is related to differing attitudes and opinions regarding science and a lack of confidence in scientific ability (Guo 2019). The underrepresentation of minorities is sometimes attributed to a lack of relatability to the subject (Estrada et al. 2016). The decline of interest in science as students enter high school, is more prominent among women and minorities (Fortus and Vedder-Weiss 2014; Burns, Lesseig, and Staus 2016). In addition to the influence on STEM interest by gender and ethnicity, socioeconomic status (SES) has a significant impact on developing and maintaining an interest in STEM (Saw, Chang, and Chan 2018; Bianchini 2013). Schools that are categorized as having students with higher SES, have higher educational outcomes such as grades (Thomson 2018). Students with low SES in addition to having lower education outcomes, are half as likely to be interested in a career in STEM as those with high SES (Saw, Chang, and Chan 2018). SES of schools are assessed by their Title 1 status. The

National School Lunch Program offers free or reduced lunch to eligible students. Eligibility for free and reduced lunch is for students whose families are at or below 130% of the poverty line (Hoffman 2012). Schools are classified as Title 1 if 50% of their population is eligible for free or reduced lunch (Fritzberg 2004). For all students, involvement in out of school STEM activities increases interest in STEM (Burns, Lesseig, and Staus 2016).

In order to assess the impact of a field trip to the Oklahoma Aquarium on interest in STEM, I analyzed pre- and post-visit surveys from students participating in one of two different experiences within the aquarium. The first condition was a self-guided experience allowing for free choice exploration for the duration of the field trip. During the self-guided tour, students had the opportunity to attend feed shows where biologists talk directly to guests about the animals. Students also had the opportunity to touch animals in three separate touch tank areas and read interpretive graphics throughout the facility. Within the second condition, students first participated in a structured educational program before the self-guided experience. The education program was led by a female OKAQ educator and included a behind the scenes tour of the Small Animal Holding facility. During the behind the scenes tour, students listened to a lesson explaining the function of the facility, which is to quarantine new or sick animals and to conduct research. Students witnessed aquarium husbandry conducted by biologists, received a lesson on water filtration, learned the many responsibilities of animal care, and visited the coral research area. While at the coral research area, students learned about research design, the definition of a hypothesis, how everyone can be a researcher, and viewed a fluorescing coral under a microscope.

I hypothesized that a field trip to the OKAQ would have an impact on a student's interest in STEM. I predicted that offering students a free choice learning experience within

the aquarium would impart a sense of excitement, concern, and interest in oceans and ocean habitats. I also predicted students who first participated in an educational program would leave with a greater appreciation for STEM than students who had only the self-guided experience. Lastly, I predicted that a field trip to a research and education facility would increase student desire to pursue a career in a STEM discipline.

METHODS

Obtaining teacher agreement and parental approval:

Field trips to the Oklahoma Aquarium are reserved through an online reservation process. Upon receipt of the reservation, I contacted schools with students between grades three to six (n=11). I described the research project and requested participation. Once a teacher agreed to participate in the study (n=9), I sent them the pre/post survey form (Figure 1) and parental permission form (Figure 2). The survey was in a Likert format with five choices for attitude from strongly agree to strongly disagree. The attitude statements are as follows: I think science is fun, I would like a job in STEM, Research is something that I can do, I can make a difference for a healthy environment, I am excited to learn more about STEM, and I think oceans are important. The study was a blind study. I did not have knowledge of individual students other than by identification through unique student ids created from the participants' birthdate, school, grade, and experiment code. Teachers then sent the permission form to the parents and collected them from willing participants prior to the visit. Of the nine schools which agreed to participate, four did not bring their survey forms. The five remaining schools comprised a total of 216 students with 167 students who participated. Twenty-seven students were not included in the study due to missing birthdates.

After the creation of unique student IDs, the number of participants was 140. The removal of participants that did not have a pre-visit survey and a matching post-visit survey resulted in a final number of 84 students.

Completing the pre and post surveys:

The day prior to the visit, teachers administered the survey in the classroom to the students with obtained parental approval. Teachers did not discuss the items on the survey with the students prior to administering the survey. Upon arrival to the aquarium, I collected completed survey forms. At the conclusion of the field trip, teachers again administered the survey which I collected.

Student demographics:

Students from the five schools (A, B, C, D, E) comprised 57% females and 42% males. 80% of the population had previously visited an aquarium. When asked if they had ever met a professional from a STEM field, 32% of students responded in the affirmative. The racial composition was as follows: 64% White, 18% Native American, 8% Hispanic/Latino, 1.5% Asian, 1% Black, 3% identified as “other”, and 6% preferred not to answer. Schools B (n=9), C (n=20), D (n=17), and E (n=15), are public schools which all qualify for free or reduced lunch. School A (n=17), is a private school which is not a Title 1 school. 80% of students within this study were eligible for free or reduced lunch. I analyzed responses among schools, to compare Title 1 public schools and the private school for potential differences in attitude based on potential socioeconomic differences. I analyzed the survey responses to discover any potential differences between girls (n=44) and boys (n=34), and among ethnicities: White (n=57), Hispanic (n=3), Black (n=1), Native American (n=11),

Asian (n=2), other(n=2), and no answer (n=1). The survey questions “Have you ever met a STEM professional” and “Is this your first visit to an aquarium” were analyzed to ascertain if there was a difference in the degree of attitude change toward STEM based on the student’s acknowledgement of acquaintance with a STEM professional or previous exposure to an aquarium.

Control and experimental conditions:

The students were divided into two groups by school. Students from the same school were all assigned to the same condition. Students were assigned to a condition based on their teacher’s initial field trip request to receive an additional educational program or only a self-guided visit.

Control condition:

Students within the control condition (n=35) participated in a self – guided field trip. The students received an unstructured tour of the aquarium with their teacher or chaperone. Self-guided tours are typically 1 ½ hour in length. The field trip was a free-choice experience, with students free to tour the aquarium as they desired, or their teacher specified. Students in the control group had contact with volunteer staff of the aquarium at interactive touch tanks but did not interact with aquarium education staff. The level of structure within the self-guided group while touring the facility was variable based on the structure of the trip mandated by the teachers. Students were predominantly paired with adult chaperones who may or may not have followed the structure set by the teacher. Exhibit graphics provided information on animals including scientific name, range, diet, size, and a relevant fact about the animal and/or its habitat. In addition to the animal graphics, the aquarium posts “Think

Blue to Go Green" graphics throughout the facility, which is an initiative of the OKAQ to impress upon visitors that our planet is 71% water, so you must think blue to go green. The associated graphics address environmental issues such as climate change, overfishing, and contamination by plastics in the ocean.

Experimental condition:

Students within the experimental condition (n=49), in addition to the self–guided tour of the aquarium, received an additional program, "Swim into Science". The program was approximately 30 minutes long. A maximum of 25 students at a time were led to the aquarium's Small Animal Holding facility. They were then introduced to the concepts of water filtration, animal husbandry, and quarantine procedures during a 10-minute lesson. At the conclusion of the lesson, students were split into two groups, one toured the facility and the other was given a lesson regarding scientific research and the research specifically conducted at the OKAQ. Students learned about bio-fluorescence, protein excitation, and the coral – algae symbiosis. Students then viewed a coral sample under a light microscope by natural light and blue excitation light. The blue light excited the fluorescent proteins within the coral and the students utilized yellow barrier filters to block the reflected blue light and see the bio-fluorescence of the coral. The two divided groups then switched and the group that had been touring participating in the research portion of the program and the research group toured the facility. The self-guided portion for the experimental group lasted for approximately one hour instead of the 1 ½ hours of the control group. The time spent in the education program was split evenly among each section; initial lesson, tour of the facility, and the research portion, each was approximately ten minutes.

Statistical Analysis:

Statistical analyses were performed in RStudio version 1.3.959 (http://www.rstudio.com). I performed a paired t-test for students within the control condition and a paired t-test for students within the experimental condition to analyze changes in attitudes towards STEM from the pre- to post-survey sampling time points. To compare the difference in means between the pre to post surveys with both conditions combined, I performed a Welch's t-test to account for non-normal data distributions within the unpaired data between the control and experimental groups. I then performed Welch's t-tests for changes in mean comparisons by gender, whether a student had previously met a STEM professional, and whether they had previously visited a public aquarium. I performed one-way ANOVAs to test for any changes in mean response by school and ethnicity. Post hoc tests were performed using Tukey HSD at a 95% confidence level. I performed a Welch's t-test to determine if there were differences in pre-visit responses by gender, whether a student had previously met a STEM professional, and whether they had previously visited a public aquarium. Similarly, I performed one-way ANOVAs to determine if there were initial differences by school or ethnicity. Post hoc tests were performed using Tukey HSD at a 95% confidence level. Plots were created in ggplot2 (Gómez-Rubio 2017).

RESULTS

Variation in response from pre-visit to post-visit for students within the control condition:

In all cases, the students in the control condition were more likely to strongly agree with the six statements on the post-visit survey than on the pre-visit survey. Students were

3% more likely to believe that science was more fun after the completion of the field trip ($t(34) = 2.144$, $p=0.039$). Though not significant, the responses to the five other statements were encouraging in regard to support for the benefits of a field trip to an aquarium. Students were non-significantly more likely to report interest in pursuing a job in a STEM field ($t(34) = 1.384$, $p=0.175$). There was a non-significant increase in confidence regarding how they felt about research being something that they could do ($t(34) = 1.558$, $p=0.128$). There was a trend for students to report stronger agreement with the statement, "I can make a difference for a healthy environment" after the aquarium visit ($t(34) = 1.966$, $p=0.058$). There was little change in their excitement to learn more about STEM ($t(34) = 0.190$, $p=0.851$). Students concluded their field trip with no significant change in their belief that oceans are important ($t(34) = 0.915$, $p=0.367$; Figure 3, Table 1).

Variation in response from pre-visit to post-visit for students within the experimental condition:

Students within the experimental condition concluded their field trip with a 3% significant increase in their perception that science is fun ($t(48) = 2.617$, $p=0.012$). Student response to "I would like a job in STEM" was 4% significantly more positive after their visit ($t(48) = 2.424$, $p=0.019$). There was a trend for a more positive response to the statement "research is something that I can do" after the field trip ($t(48) = 1.783$, $p=0.081$). At the conclusion of the field trip, there was minimal change in how students felt about their ability to make a difference for a healthy environment ($t(48) = -0.573$, $p=0.569$), or their excitement to learn more about STEM ($t(48) = -0.044$, $p=0.758$). Student belief that oceans are important

had a negligible change between pre and post visit. (t(48) = 0.250, p=0.804; Figure 3; Table 2).

Differences in responses between control and experimental conditions pre and post-visit:

Students in the control and experimental conditions did not differ significantly in change of responses between the pre -to post survey for any of the statements: “I think science is fun” (t(81.641) = -0.512, p=0.610), “I would like a job in STEM” (t(79.914) = -1.175, p=0.243), “Research is something that I can do” (t(77.997) = -0.630, p=0.530), “I can make a difference for a healthy environment” (t(79.016) = 1.003, p=0.319), “I am excited to learn more about STEM” (t(77.976) = -0.351, p=0.726), or “I think oceans are important” (t(72.331) = 0.547, p=0.586; Figure 3; Table 3).

Differences in responses pre and post-visit by gender:

There were predominantly non-significant differences in the change in response to statements when examined pre and post visit by gender. Responses for “I think science is fun” (t(34.824) = -0.512, p=0.612), “I would like a job in STEM” (t(24.456) = -0.382, p=0.706), “research is something that I can do” (t(28.199) = -0.331, p=0.743), and “I think oceans are important” (t(37.800) = -0.452, p=0.654), were non-significantly different pre and post-visit between girls and boys. The one significant difference between girls and boys was that boys ended their field trip 6% more confident that they could make a difference for a healthy environment (t(35.082) = 2.503, p=0.017) than girls. Boys concluded the field trip with a trend for an increased desire to learn more about STEM relative to girls (t(27.789) = 1.701, p=0.099; Table 4; Figure 4). The pre-visit means for the following statements were

significantly different between genders: girls' attitudes toward pursuing a career in a STEM field were 16% lower than boys' ($t(237.51) = -3.703$, $p<0.001$), and girls' initial attitudes regarding " I can make a difference for a healthy environment ($t(194.55) = 2.149$, $p=0.033$) were 9% higher than boys'. After the field trip the differences were not significant between girls' attitude and boys regarding pursuing a STEM field ($t(120.07) = -1.158$, $p=0.116$. Post-visit, girls were only 6% less likely to pursue a job in STEM than boys. The difference in attitude post-visit between girls and boys was not significant regarding the environment $t(101.34) = 0.831$, $p=0.408$. Boys dropped from 9% to 2% less confident than girls in their ability to help the environment.

Differences in responses pre and post-visit dependent on whether the student had met a STEM professional:

Students that had previously met a STEM professional had significantly more negative changes in responses to the statements "Research is something that I can do" ($t(21.875) = -2.420$, $p=0.002$) and "I can make a difference for a healthy environment" ($t(26.617) = -2.402$, $p=0.024$), after the visit to the aquarium. Responses to the remaining statements were not significant in their changes from pre to post visit: "I think science is fun" ($t(22.192) = -1.082$, $p=0.291$), "I would like a job in STEM" ($t(21.75) = 1.682$, $p=0.107$), "I am excited to learn more about STEM" ($t(24.981) = 0.830$, $p=0.415$), and "I think oceans are important" ($t(29.669) = -0.120$, $p=0.905$; Table 5; Figure 5). There were no significant differences ($p>0.05$) in attitudes pre-visit for the six statements dependent on whether a student had met a STEM professional.

Differences in responses pre and post-visit dependent on whether the student had previously visited an aquarium:

Whether or not a student had previously visited an aquarium did not significantly impact their change of attitude in response to the statements: "I think science is fun" ($t(30.926) = -0.125$, $p=0.901$), "I would like a job in STEM" ($t(21.159) = 0.244$, $p=0.810$), "Research is something that I can do" ($t(20.844) = -0.914$, $p=0.371$), "I can make a difference for a healthy environment" ($t(18.697) = -0.073$, $p=0.943$), "I am excited to learn more about STEM" ($t(22.890) = -1.864$, $p=0.075$), or "I think oceans are important" ($t(17.098) = 0.005$, $p=0.996$; Figure 6; Table 6). There were no significant differences ($p>0.05$) in attitudes pre-visit for the six statements dependent on whether a student had previously visited an aquarium.

Differences in responses pre and post-visit dependent on school:

Student school of origin did not have a significant impact on the change in attitude pre- to post-visit for all statements: "I think science is fun" ($F_{4, 73} = 1.209$, $p=0.314$), "I would like a job in STEM" ($F_{4, 73} = 1.628$, $p=0.176$). "Research is something that I can do" ($F_{4, 73} = 1.625$, $p=0.177$), "I can make a difference for a healthy environment" ($F_{4, 73} = 1.809$, $p=0.136$), "I am excited to learn more about STEM" ($F_{4, 73} = 0.902$, $p=0.467$), or "I think oceans are important" ($F_{4, 73} = 0.341$, $p=0.849$), (Figure 7; Table 7). There were significant differences in pre-visit attitudes between the private school A and school E. E students had a 16% significantly lower desire to pursue a job in STEM ($t(108.93) = -2.795$, $p=0.01$), were 11% less to believe they could conduct research ($t(108.99) = -2.264$, $p=0.026$). They also believed 15% less in their ability to make a difference for a healthy environment

(t(108.49) = -4.058, p<0.001) than school A, and were 10% less excited to learn more about STEM (t(101.48) = -3.01, p<0.01) than school A. Students from school E felt 10% significantly less confident in their ability to help the environment than students from school D (t(79.07) = -2.983, p<0.01), and 14% less excitement to learn more about STEM than students from school B (t(102.55) = -3.406, p<0.001) prior to their field trip to the aquarium. There were fewer overall discrepancies in school E students post-visit attitudes. The difference in desire to pursue a job in STEM between schools E and A shifted from 16% higher for school A to 9% higher (t(48.452) = 1.677, p=0.1), as well, their belief in their ability to do research shifted from 11% to 7% less than school A (t(48.979) = 1.367, p=0.179). Students from school E felt 9% less confident in their ability to make a difference for the environment than school A, compared to 15% pre-visit (t(55.762) = 2.456, p=0.017). The shift in confidence regarding the environment was 10% different pre-visit between schools E and D compared to 12% difference post-visit (t(48.099) = 2.961, p=0.05). There was an increase in confidence for both schools. The difference in post-visit means increased by 1% between students from schools A and E (t(55.064) = -2.450, p=0.017), and B and E (t(52.833) = 2.562, p=0.013) regarding excitement to learn more about STEM.

Differences in responses pre and post-visit dependent on ethnicity:

Student ethnicity was not related to changes in attitude regarding the six statements pre and post visit: “I think science is fun” ($F_{6, 70} = 1.898$, p=0.093), “I would like a job in STEM” ($F_{6, 70} = 1.242$, p=0.296). “Research is something that I can do” ($F_{6, 70} = 0.304$, p=0.933), “I can make a difference for a healthy environment” ($F_{6, 70} = 0.357$, p=0.903), “I am excited to learn more about STEM” ($F_{6, 70} = 0.629$, p=0.706), and “I think oceans are

important" ($F_{6, 70} = 0.384$, p=0.887; Figure 8; Table 8). There were no significant differences (p>0.05) in attitudes pre-visit for the six statements dependent on ethnicity.

DISCUSSION

Impact of a visit to an aquarium on attitudes toward STEM:

Students who participated in this study concluded their field trip with a significant change in attitude, believing that science was more fun after their visit. This lends support to my hypothesis that an OKAQ field trip would provide increased STEM interest. The increased enjoyment of science supports previous research which found students who attend facilities such as aquariums are more likely to appreciate and have an interest in science (Dierking et al. 2003; Kola-Olusanya 2005; Scarce 1997). My prediction that offering students a free choice learning experience within the aquarium would impart a sense of excitement, concern, and interest in oceans and ocean habitats was partially supported. The level of excitement increased significantly, but not the students' feeling of concern or interest in oceans. The lack of support for increased concern for oceans after the field trip is contrary to previous research which illustrates an increase in appreciation and interest in the subject of the experience after a visit to an informal learning facility (Scarce 1997). However, I believe that the lack of a significant change in attitudes regarding the importance of oceans does not truly represent a lack of interest. The mean response on the pre survey was 94% with a score of 4.7 out of 5, and 96% on the post survey with a score of 4.8 out of 5. The pre visit mean for how students regarded oceans was significantly higher than the means of the five other statements. There was little opportunity for an increase in appreciation of oceans. My

prediction that students who first participated in the educational program would leave with a greater appreciation for STEM than students in the self-guided tour only group was only partially supported for most of the statements. Students who received a guided program, in addition to thinking science was more fun post visit, had a significant increase in their desire to pursue a STEM career. The previous result supports my final prediction of a field trip to a research and education facility increasing a student's desire to pursue a career in a STEM discipline, although only for the group who received an additional program.

Participants in both conditions participated in a free choice learning experience within the OKAQ. Free choice learning experiences are known to increase interest and enjoyment for students (Kola-Olusanya 2005; Falk 2005; Ballantyne and Packer 2005). Previous research has examined student responses regarding their interest in a field trip based on the structure of the experience from free choice to completely structured and the level of educator involvement. The most impactful experiences were free choice with some minimal structure (Bamberger and Tal 2007; Davidson, Passmore, and Anderson 2009). Many students expressed boredom when listening to an informal science center educator present on topics which did not interest them, preferring instead to have the time to explore in a more social setting. Students value the opportunity for social interaction with their peers and the level of social interaction affects the student's attitude regarding the field trip (Davidson, Passmore, and Anderson 2009). Perhaps the lack of more significant positive attitude changes for the experimental group is due to students within this group having less time for social interaction with their peers. The lessons provided within the educational program may not have been of interest to the students, which would decrease their enjoyment of the field trip.

Gender differences in attitudes toward STEM:

The difference in attitudes regarding the field trip experiences between girls and boys was most significant in attitudes regarding the ability to make a difference for a healthy environment. Boys had a significantly larger increase in confidence regarding their ability to make a change for a healthy environment after the field trip than girls. Girls are typically more engaged and interested in environmental issues than boys (Altunoğlu, Atav, and Sönmez 2017; Xiao and McCright 2015). The difference in attitudes between boys and girls, though significant, is more a reflection of an increase in engagement by boys, rather than a lack of interest by girls, because girls began the study with significantly higher confidence in their ability to help the environment than boys. Another significant difference in starting attitudes between genders was a significantly lower desire of girls to pursue a STEM field. Girls are more likely than boys to lack confidence in their scientific ability, and therefore are less likely to pursue a career in the sciences. The lower number of girls pursuing a career in a STEM field leaves a large gender gap (Beede et al. 2011; Guo 2019; Fortus and Vedder-Weiss 2014; Burns, Lesseig, and Staus 2016). Although the comparison of changes in attitudes regarding pursuing a STEM field was not significantly different between genders, there was a positive outcome regarding girls and STEM careers. Girls had a significant positive change in attitude regarding the desire to pursue a STEM career as a result of the field trip experience.

Differences in attitudes toward STEM based on having met a STEM professional or previously visiting a public aquarium:

Meeting a STEM professional has been shown to have a positive impact on students' interest in STEM (Shin, Levy, and London 2016); however, my results did not support these previous findings. Instead, there were significant negative changes in attitude regarding the ability to conduct research and make a difference for a healthy environment. The question "have you ever met a STEM professional" was meant as a marker of familiarity of the student with what constitutes STEM and the professions within STEM, which encompasses many disciplines (Bybee 2010). Students in this study were not asked the nature of their relationship or the field of study of their acquaintance. A student who is acquainted with a marine biologist could have knowledge and interests more aligned to learning within an aquarium than those who know an engineer (Falk and Adelman 2003). The negative change in attitude among those who had previously met a STEM professional could suggest a lack of interest in the subject. Having previously visited an aquarium did not influence the change in attitudes pre and post visit, although it is important to note that very few students had never previously visited an aquarium, which reduced my ability to detect an effect of previous experience.

Differences in attitudes toward STEM based on school attended and ethnicity:

There were no globally significant differences in attitude pre- and post-visit based on the school of origin. However, there were significant differences among the pre-visit means by school. In particular, School E had the biggest discrepancy in attitudes before the visit from the other four schools. Students from school E were significantly less likely to want a job in STEM, to be confident in their ability to conduct research, to make a difference for a healthy environment, or be excited to learn more about STEM than students from the private

school, A. E students were also less confident in their ability to help the environment than students from school D. As well, E students reported being less excited to learn more about STEM than students from school B. Researchers have concluded that socio-economic status has a large impact on a student's interest in STEM or pursuit of a STEM career, with students within the low SES category the least likely to show interest (Saw, Chang, and Chan 2018; Bianchini 2013). The percentage of students eligible for free or reduced lunch (Title 1 funding) within a school is a marker for the SES of students within the school (Fritzberg 2004). School A was a private school that does not receive Title 1 funding. The four public schools were eligible for Title 1 funding; however, there was a large disparity in the percentage of students eligible. According to the State Office of Educational Quality and Accountability, schools B, C, and D all have populations with 60 ±3% eligible students. School E students, who deviated the most from the other schools in their pre-visit attitudes, had 75% eligibility for free or reduced lunch. The increased occurrence of significantly negative attitudes before the visit between school E and the others supports previous research that illustrates a lowered interest in STEM is more prevalent within a lower SES population than in higher SES populations (Saw, Chang, and Chan 2018).

Though this study did not find significant differences between pre and post visit attitudes based on ethnicity, minorities are underrepresented in STEM fields (J. Mau 2016), with minority students often feeling disconnected from science learning. A decline in STEM interest has been attributed to believing pursuing a STEM field does not represent the reality of the student's future (Basu and Barton 2007). Building on existing knowledge and making STEM topics more relatable to minority students has a large impact on their desire to pursue STEM fields (Estrada et al. 2016). The free choice learning experience offered by an

aquarium provides an opportunity for students to pursue their individual interests within the aquarium, increasing the relatability of the subject to the student (Behrendt and Franklin 2013).

Conclusion:

Aquariums offer a platform for informal science learning that reaches 700 million people annually. The focus of most aquariums is to promote positive attitudes regarding animals, habitats, and conservation (Ballantyne and Packer 2005; Ballantyne et al. 2007). Exploring the impact of different learning experiences within informal science learning centers such as aquariums is important to maximize the benefit of the experience.

Students retain more information when they are provided an immersive learning experience outside of a formal classroom setting (Falk and Dierking 2010; Kola-Olusanya 2005). Students who participate in both formal and informal science learning, and more specifically free choice learning will be more excited about science (Falk 2005; Ballantyne and Packer 2005). They are also more likely to pursue a career in a STEM field, and have increased scientific literacy as adults (Bell et al. 2016; Ballantyne and Packer 2005; Bamberger and Tal 2007; Dabney et al. 2012). The Oklahoma Aquarium provides opportunities for free choice and experiential learning. Students can touch animals, watch sharks eat, and crawl through exhibits. A child will relate more to an animal that they have touched and had interactions with than one seen only in a book (Behrendt and Franklin 2013). Further research regarding how to maximize the aquarium field trip experience, specifically the level of structure provided from aquarium staff, will provide valuable information for properly utilizing the resources and the reach of a public aquarium.

Discovering what provides the largest positive impact on the attitudes of students after a field trip experience is important for structuring the time students spend in an informal science learning center.

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Table 1. Average response change in STEM perception for students grades 3 – 6 from pre to post visit at a public aquarium for students in the control condition (paired t-test). Significant ($p<0.05$) P values in bold.

Statement	Pre-Post Mean Difference	t	df	95% CI [lower, upper]	P
I think science is fun	0.286	2.144	34	[-0.015, -0.587]	**0.039**
I would like a job in STEM	0.190	1.384	34	[-0.089, 0.470]	0.175
Research is something that I can do	0.200	1.560	34	[-0.061 0.461]	0.128
I can make a difference for a healthy environment	0.286	1.966	34	[-0.010, 0.581]	0.058
I am excited to learn more about STEM	0.029	0.190	34	[-0.277, 0.335]	0.851
I think oceans are important	0.123	0.915	34	[-0.150, 0.397]	0.367

Table 2. Average response change in STEM perception for students grades 3 – 6 from pre to post visit at a public aquarium for students in the experimental condition (paired t-test). Significant ($p<0.05$) P values in bold.

Statement	Pre-Post Mean Difference	t	df	95% CI [lower, upper]	P
I think science is fun	0.388	2.6171	48	[-0.090, 0.686]	**0.012**
I would like a job in STEM	0.469	2.424	48	[-0.080, 0.859]	**0.019**
Research is something that I can do	0.347	1.783	48	[-0.044, 0.738]	0.081
I can make a difference for a healthy environment	0.082	0.573	48	[-0.205, 0.367]	0.569
I am excited to learn more about STEM	-0.044	0.310	48	[-0.331, 0.243]	0.758
I think oceans are important	0.028	0.250	48	[-0.196, 0.251]	0.804

Table 3. Average response change in STEM perception for students grades 3 – 6 from pre to post visit at a public aquarium between control and experimental conditions (Welch t-test).

Statement	Pre-Post Mean control	Pre-Post Mean experimental	t	df	95% CI [lower, upper]	P
I think science is fun	0.286	0.387	-0.512	81.641	[-0.499, 0.294]	0.61
I would like a job in STEM	0.190	0.469	-1.175	79.914	[-0.752, 0.193]	0.243
Research is something that I can do	0.200	0.347	-0.630	77.997	[-0.611, 0.317]	0.530
I can make a difference for a healthy environment	0.286	0.082	1.003	79.016	[-0.201, 0.609]	0.319
I am excited to learn more about STEM	0.029	-0.044	0.351	77.976	[-0.341, 0.486]	0.726
I think oceans are important	0.123	0.028	0.547	72.331	[-0.253, 0.443]	0.586

Table 4: Average response change in STEM perception between a pre and post visit to a public aquarium by gender (Welch t-test). Significant ($p<0.05$) P values in bold.

Statement	Female Mean	Male Mean	t	df	95% CI [lower, upper]	P
I think science is fun	0.227	0.353	0.623	75.999	[-0.277, 0.528]	0.535
I would like a job in STEM	0.386	0.284	-0.386	75.252	[-0.629, 0.425]	0.700
Research is something that I can do	0.364	0.206	-0.598	75.312	[-0.683, 0.367]	0.551
I can make a difference for a healthy environment	-0.045	0.470	2.422	74.485	[0.091, 0.941]	**0.01**
I am excited to learn more about STEM	-0.140	0.176	1.480	73.552	[-0.110, 0.743]	0.143
I think oceans are important	0.053	0.067	0.072	70.748	[-0.356, 0.383]	0.943

Table 5: Average response change in STEM perception between a pre and post visit to a public aquarium dependent on whether the student had previously met a STEM professional (Welch t-test). Significant ($p<0.05$) P values in bold.

Statement	Yes Mean	No Mean	t	df	95% CI [lower, upper]	P
I think science is fun	0.000	0.250	-1.082	22.192	[-0.729, 0.229]	0.291
I would like a job in STEM	0.589	0.136	1.682	21.75	[--0.106, 1.011]	0.107
Research is something that I can do	-0.231	0.500	-2.4236	21.857	[-1.356, 0.105]	**0.0241**
I can make a difference for a healthy environment	-0.077	0.455	-2.402	26.617	[-0.077, 0.455]	**0.024**
I am excited to learn more about STEM	-0.077	0.136	-0.830	24.981	[-0.077, 0.136]	0.415
I think oceans are important	0.077	0.098	-0.120	29.669	[-0.382, 0.340]	0.905

Table 6: Average response change in STEM perception between a pre and post visit to a public aquarium dependent on whether the student had previously visited an aquarium (Welch t-test).

Statement	Yes Mean	No Mean	t	df	95% CI [lower, upper]	P
I think science is fun	0.294	0.322	-0.125	30.926	[-0.484, 0.428]	0.901
I would like a job in STEM	0.412	0.316	0.244	21.159	[-0.718, 0.909]	0.810
Research is something that I can do	0.000	0.3559	-0.914	20.844	[-1.166, 0.454]	0.371
I can make a difference for a healthy environment	0.176	0.203	-0.073	18.697	[-0.803, 0.749]	0.943
I am excited to learn more about STEM	-0.412	0.116	-1.864	22.89	[-0.412, 0.116]	0.075
I think oceans are important	0.058	0.057	0.005	17.098	[-0767, 0.771]	0.996

Table 7: Average response change in STEM perception between a pre and post visit to a public aquarium dependent on the school attended (one-way ANOVA).

Statement	Type 1 Sum of Squares	Mean Square	F	df	P
I think science is fun	3.96	0.991	1.209	4, 73	0.314
I would like a job in STEM	8.73	2.183	1.628	4, 73	0.176
Research is something that I can do	8.69	23.171	1.625	4, 73	0.177
I can make a difference for a healthy environment	6.45	1.612	1.809	4, 73	0.136
I am excited to learn more about STEM	3.30	0.825	0.902	4, 73	0.467
I think oceans are important	0.92	0.229	0.341	4, 73	0.849

Table 8: Average response change in STEM perception between a pre and post visit to a public aquarium dependent on ethnicity (one-way ANOVA).

Statement	Type 1 Sum of Squares	Mean Square	F	df	P
I think science is fun	8.85	1.476	1.898	6, 70	0.093
I would like a job in STEM	9.73	1.621	1.242	6, 70	0.296
Research is something that I can do	2.62	0.437	0.304	6, 70	0.933
I can make a difference for a healthy environment	2.1	0.351	0.357	6, 70	0.903
I am excited to learn more about STEM	3.38	0.563	0.629	6, 70	0.706
I think oceans are important	1.59	0.265	0.384	6, 70	0.887

Figure 1: Pre/Post student survey administered to field trip participants at the Oklahoma Aquarium.

Oklahoma Aquarium Pre/Post Student Survey (Circle pre or post)

Date:_____ **School:**_____ **Grade:**_____ **Birthdate:** _____

PLEASE CIRCLE ONE ANSWER FOR EACH QUESTION AND REMEMBER THIS IS NOT FOR A GRADE!

Gender?

Male	Female	Prefer not to answer

Ethnicity or Race?

White	Hispanic/Latino	Black/African American	Native American/ American Indian	Asian/ Pacific Islander	Other:	Prefer not to answer

Is this your first trip to an aquarium?

Yes	No

Have you ever met a STEM professional (scientist, engineer, researcher)?

Yes	No

PLEASE CIRCLE THE NUMBER FROM 1 (NO, NOT AT ALL!) TO 5 (YES, ABSOLUTELY!) THAT BEST ANSWERS THE QUESTION FOR YOU

I THINK SCIENCE IS FUN

1 STRONGLY DISAGREE	**2** DISAGREE	**3** UNSURE	**4** AGREE	**5** STRONGLY AGREE

I WOULD LIKE A JOB IN SCIENCE, TECHNOLOGY, ENGINEERING OR MATH (STEM)

1 STRONGLY DISAGREE	**2** DISAGREE	**3** UNSURE	**4** AGREE	**5** STRONGLY AGREE

RESEARCH IS SOMETHING THAT I CAN DO

1 STRONGLY DISAGREE	**2** DISAGREE	**3** UNSURE	**4** AGREE	**5** STRONGLY AGREE

I CAN MAKE A DIFFERENCE FOR A HEALTHY ENVIRONMENT

1 STRONGLY DISAGREE	**2** DISAGREE	**3** UNSURE	**4** AGREE	**5** STRONGLY AGREE

I AM EXCITED TO LEARN MORE ABOUT STEM

1 STRONGLY DISAGREE	**2** DISAGREE	**3** UNSURE	**4** AGREE	**5** STRONGLY AGREE

I THINK OCEANS ARE IMPORTANT

1 STRONGLY DISAGREE	**2** DISAGREE	**3** UNSURE	**4** AGREE	**5** STRONGLY AGREE

Figure 2: Parental permission form for students to participate in the education research project conducted at the Oklahoma Aquarium.

PARENT/GUARDIAN PERMISSION FORM
OKLAHOMA STATE UNIVERSITY

Title: Student Interest in Science, Technology, Engineering and Mathematics (STEM) Before and After a Field Trip to the Oklahoma Aquarium

Investigators: Ann Money, PhD Candidate and Director of Education and Research at the Oklahoma Aquarium. Dr. Jennifer Grindstaff, Associate Professor, Department of Integrative Biology.

Purpose: To identify the potential benefit of informal science learning for students.

Procedures: Your child will complete an eleven-question survey, regarding their interest and knowledge of science, the oceans and environment, before and after their field trip to the Oklahoma Aquarium. Your student will be in one of two groups, one will receive a self-guided field trip and one a self-guided field trip with an additional tour. Groups will be selected at random. The aim of the study is to assess the impact on STEM (Science, Technology, Engineering and Math) interest following a field trip to an aquarium. Students will stay within their field trip group, no students will be separated from their group to participate in one study group or the other.

Risk of Participation: There are no risks above and beyond those encountered in daily life.

Benefits of Participation: The information gathered will be used to show the benefits of informal science education in partnership with formal classroom learning. The information will be used to benefit student learning in STEM fields.

Confidentiality: All information will be collected without identifying the individual student. The information will be collected based on field trip group with the surveys identified by birthdate and not by name.

Compensation: There is no compensation for participation in the project.

Contacts: For any questions regarding the project, you may contact the researchers: Ann Money @ ann.money@okstate.edu, Dr. Jennifer Grindstaff @ jen.grindstaff@okstate.edu, or the Oklahoma State University IRB (Institutional Review Board) @ 405.744.3377.

Participant Rights: I understand that my child's participation is voluntary; declining participation has no penalty or withholding of participation in the field trip.

Consent Documentation: I have been fully informed of the procedures listed here. I understand what my child will be asked to do and that my child or I can decline at any time.

I have read and fully understand this permission form. I give permission for my child _____ to participate in this study.

_____ _____
Signature of parent or guardian Date

Figure 3: Average response change in STEM perception for students grades 3 – 6 from pre to post visit at a public aquarium for students in the control and experimental conditions (paired t-test). The statements are as follows: 1. I think science is fun, 2. I would like a job in STEM, 3. Research is something that I can do, 4. I can make a difference for a healthy environment, 5. I am excited to learn more about STEM, and 6. I think oceans are important. Average response is the mean +/- 95% CI.

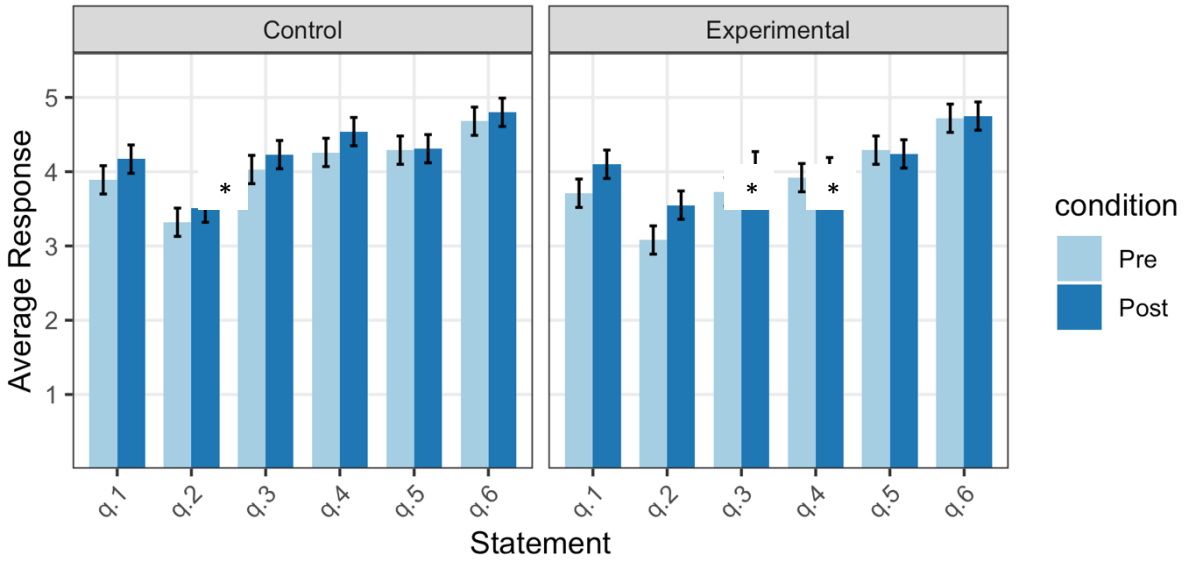


Figure 4: Average response change in STEM perception between a pre and post visit to a public aquarium by gender (Welch t-test). Average response is the mean +/- 95% CI.

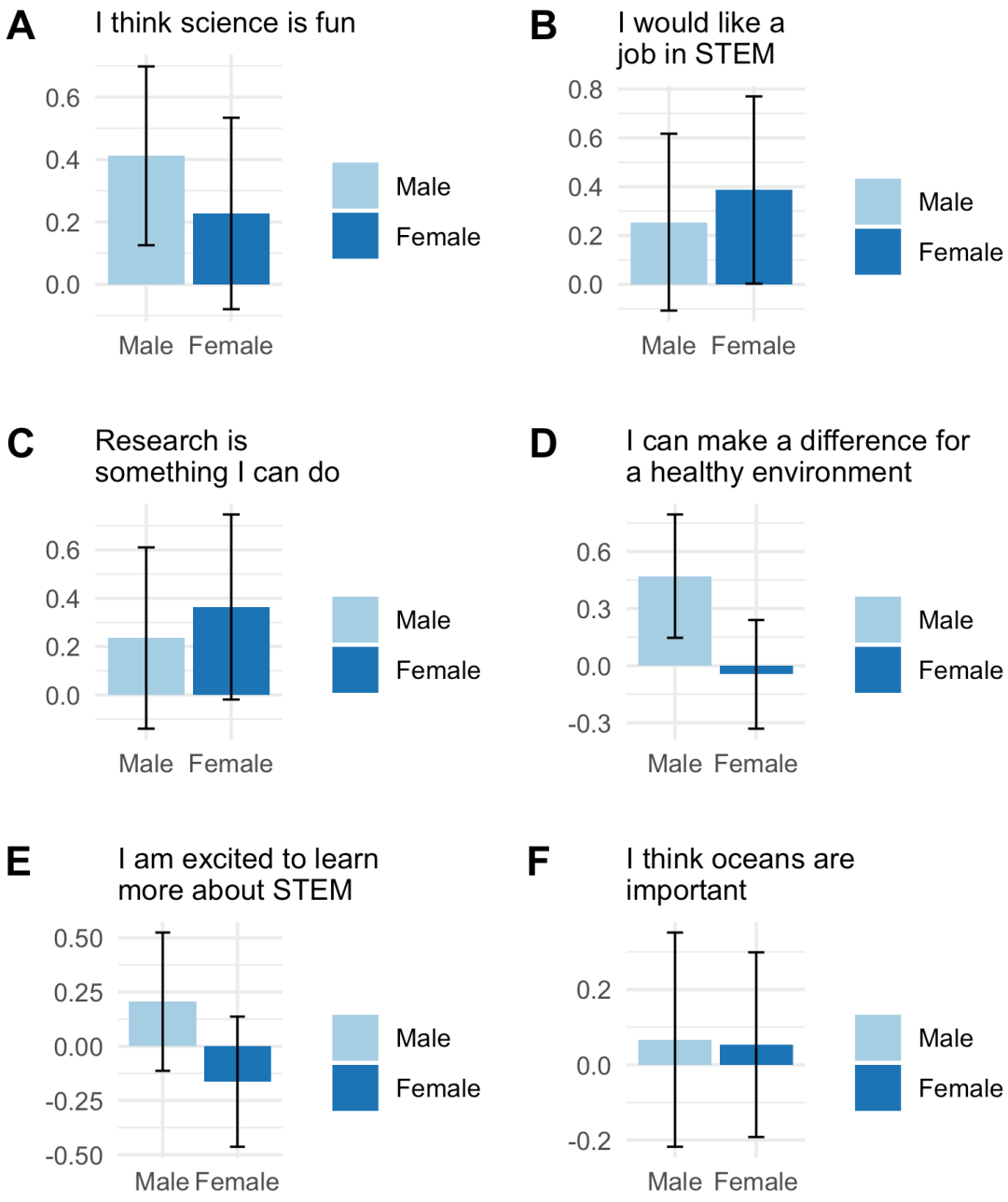


Figure 5: Average response change in STEM perception between a pre and post visit to a public aquarium dependent on whether the student had met a STEM professional (Welch t-test). Average response is the mean +/- 95% CI.

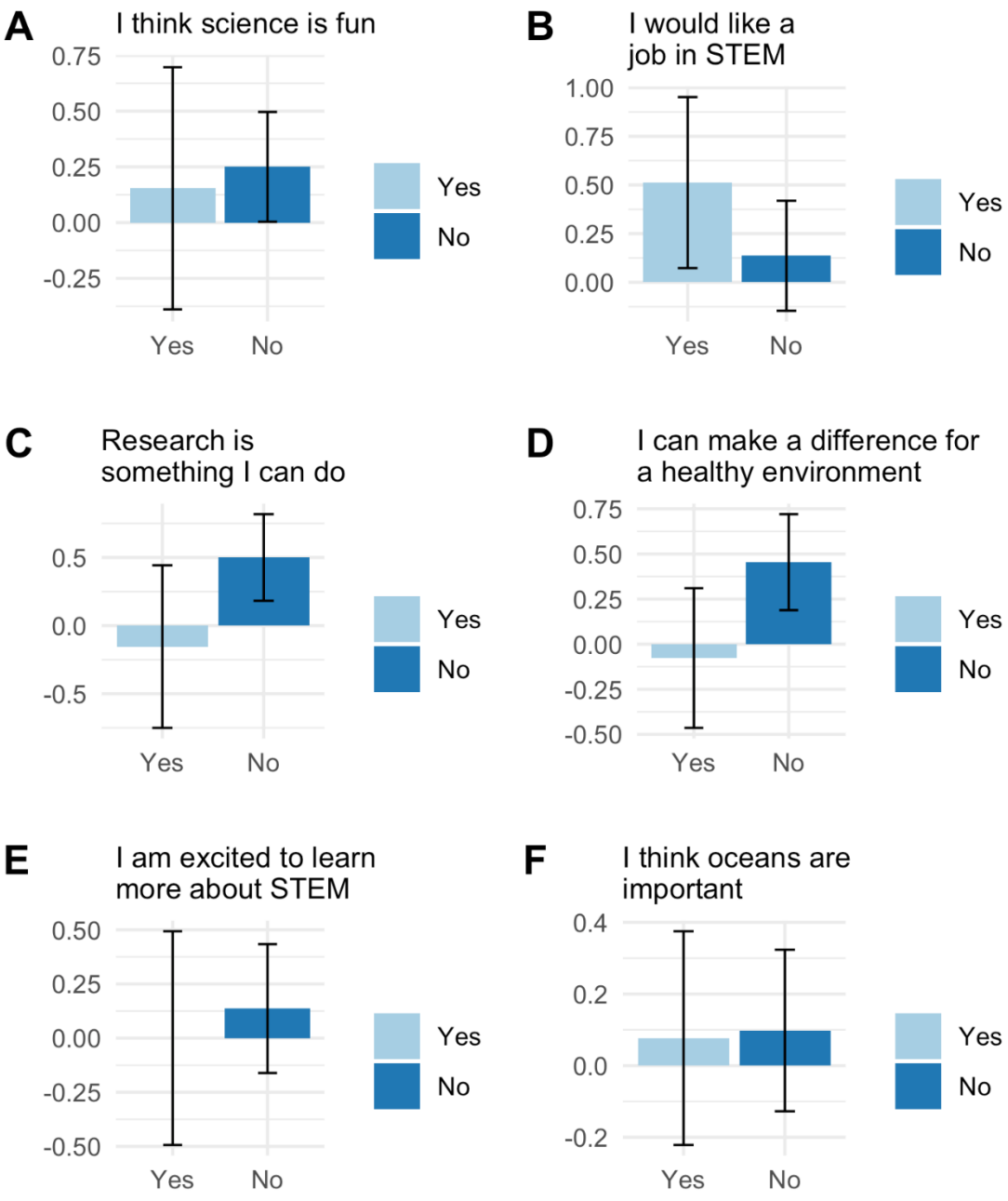


Figure 6: Average response change in STEM perception between a pre and post visit to a public aquarium dependent on whether the student had previously visited an Aquarium (Welch t-test). Average response is the mean +/- 95% CI

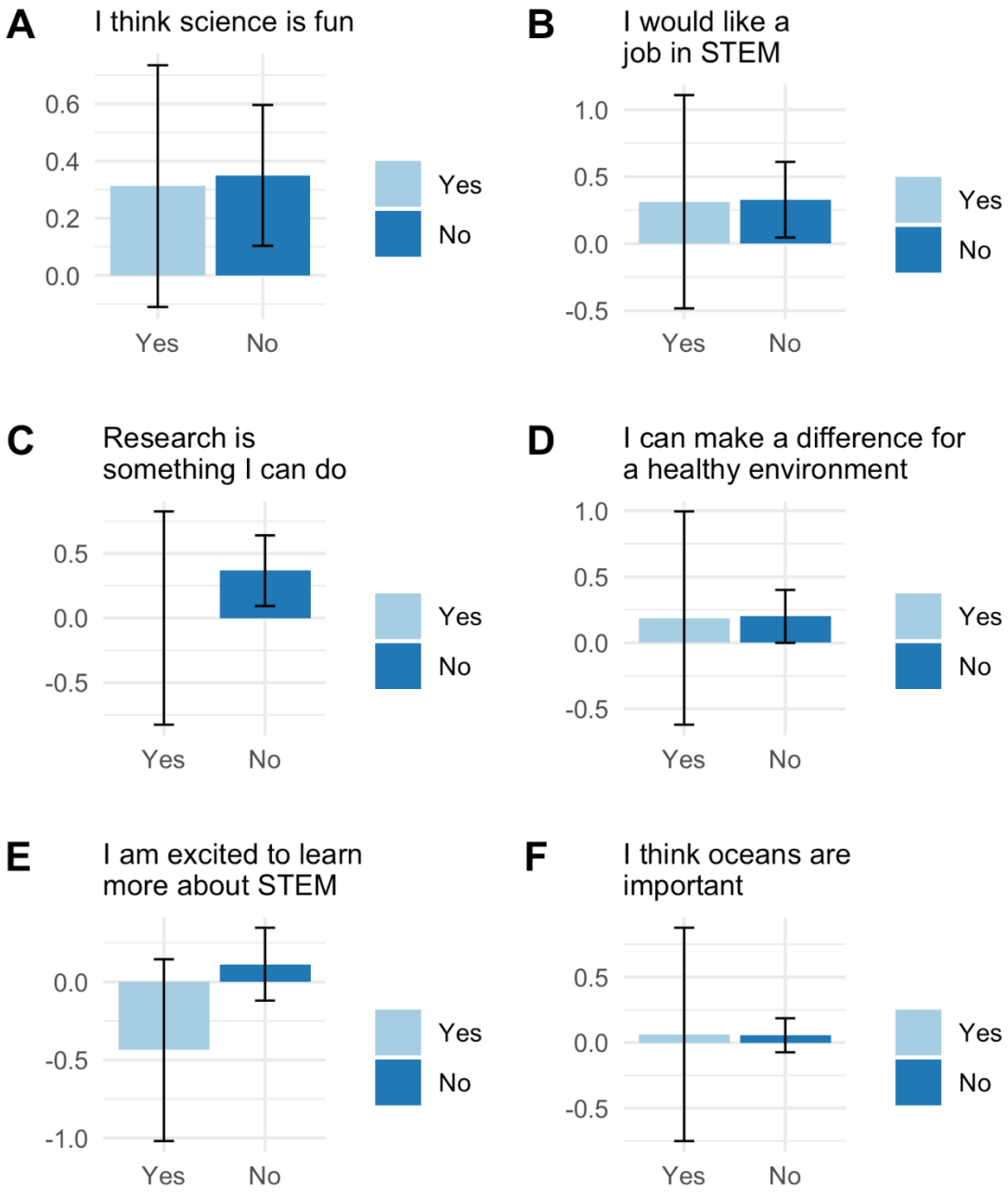


Figure 7: Average response change in STEM perception between a pre and post visit to a public aquarium dependent on school (one-way ANOVA). Average response is the mean +/- 95% CI.

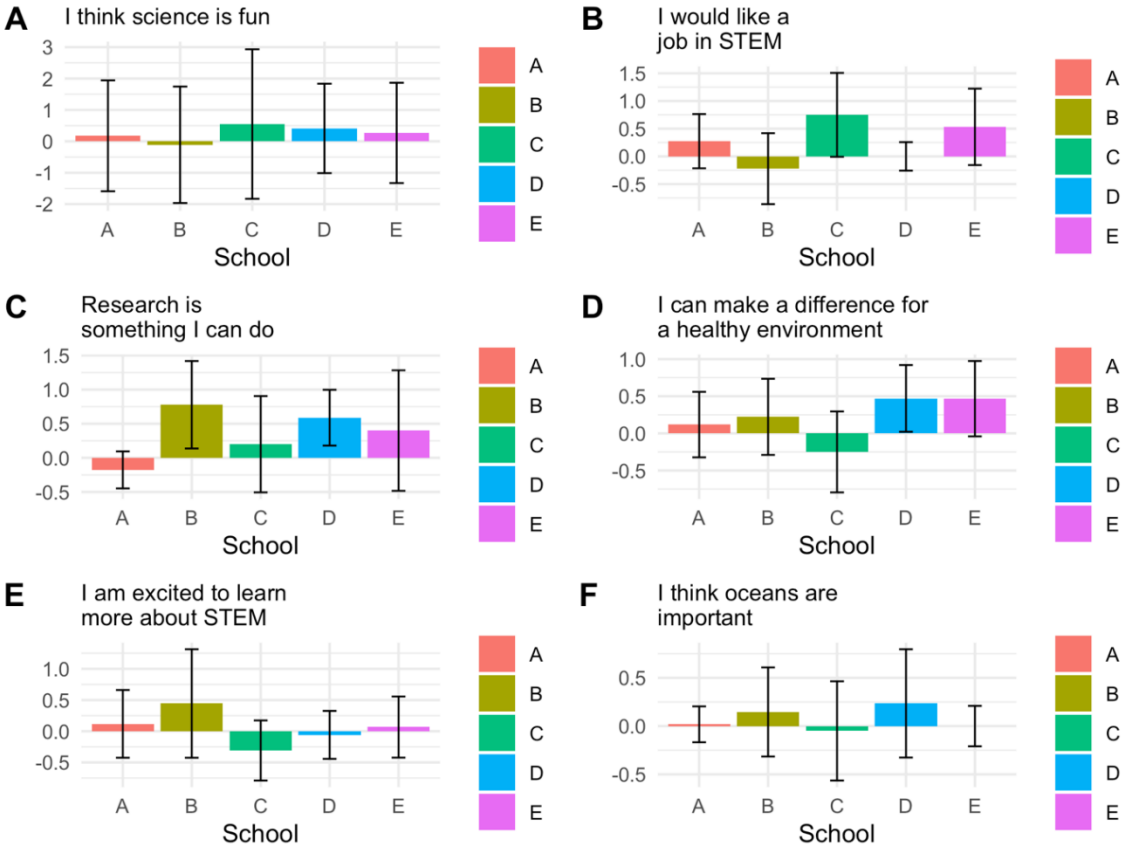
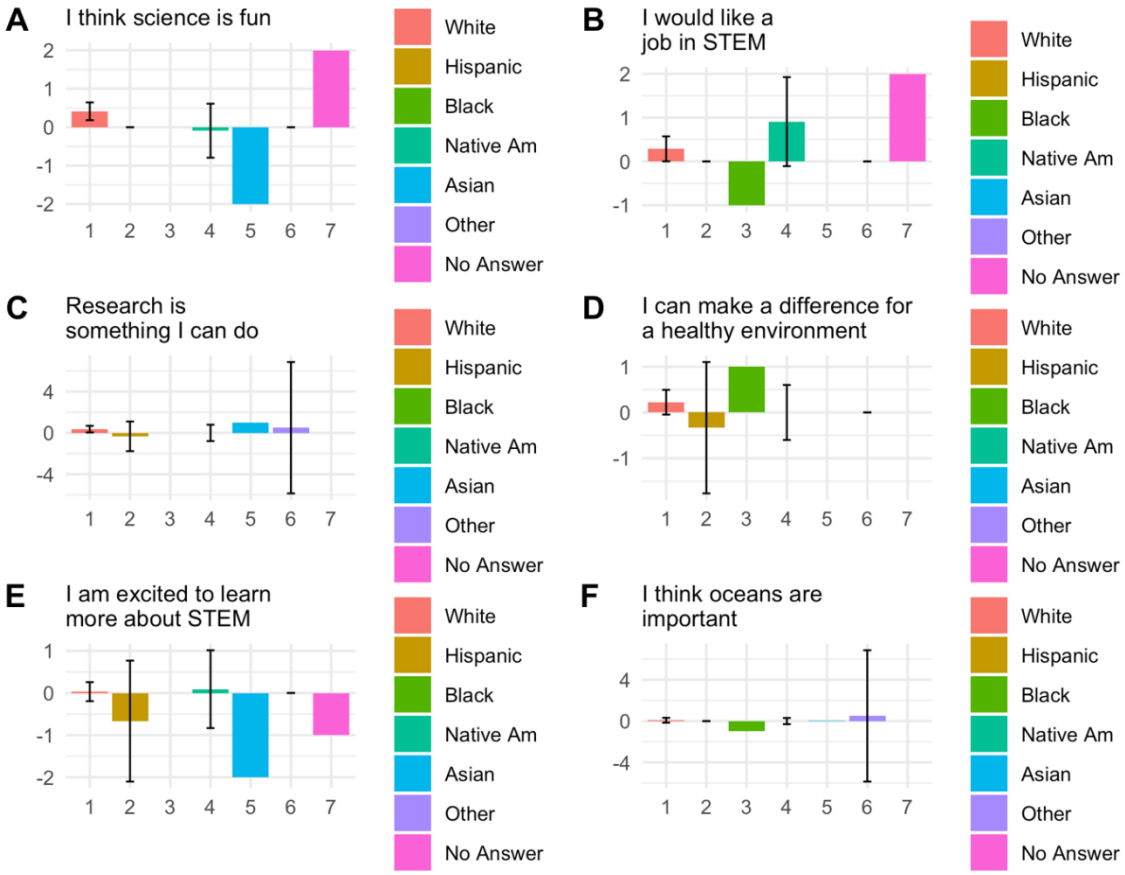


Figure 8: Average response change in STEM perception between a pre and post visit to a public aquarium dependent on ethnicity (one-way ANOVA). Average response is the mean +/- 95% CI.



CHAPTER V

SUMMARY

SUMMARY

In my dissertation, I answered questions regarding coral fluorescence as an indicator of stress, the attraction of coral symbionts to green fluorescence, and how a field trip to a public aquarium may impact a student's interest in science, technology, engineering, and math (STEM). In Chapter II, I explored the relationship between coral fluorescence and the health of the coral based upon the coral's symbiont density. I manipulated four environmental variables to assess their impact on a change in fluorescent protein (FP) emission and symbiont density for two species of coral: *E. lamellosa* and *M. capricornus*. In Chapter III, I tested the attraction of *Symbiodiniaceae* dinoflagellates to green fluorescent coral emission. In Chapter IV, I compared the attitudes of students regarding STEM before and after a trip to a public aquarium.

The relationship between fluorescent emission intensity and symbiont density was species-dependent and differed among environmental variables as well. Previous research has found a positive relationship between symbiont cell density and FP intensity from isolated proteins while exposed to manipulated PAR and temperature

(Roth & Deheyn, 2013; Salih et al., 2000). The emission intensity measurements for this study were collected externally, not from isolated proteins. Due to the physical barrier of symbiont cells, I expected to find a negative relationship between emission and density. My expectation of FP emission intensity decreasing as symbiont cell density increased and potentially shading FPs was not supported. I found predominantly non-significant relationships between the intensity of FP emission and symbiont density. Emission pattern followed my expectation of a significant decrease in external emission with increased cell density only for *M. capricornus* exposed to a salinity of 33 ppm and temperature of 21°C. The increased temperature of 31°C induced a positive relationship with emission intensity increase with symbiont density increase for both species. Manipulation of pH levels produced a significant positive relationship, which I believe was due to the loss of both FPs and symbiont cells and increased mortality of the samples. Samples exposed to manipulated PAR levels had consistent levels of FP emission across the experimental trial period, despite changes in cell density . PAR was the only factor that did not produce a significant difference in the relationship between emission and density between the two species. Maintenance of emission intensity, regardless of symbiont density may have been a product of regulation of emission for photoprotection.

As expected, symbiont density decreased over time when exposed to manipulated levels of PAR, temperature, and pH. Surprisingly, cell density increased over time for those coral exposed to salinity manipulations. Coral are osmoconformers (Seveso et al., 2013), and I expected to observe greater stress under hyper and hyposalinity conditions. Corals that are raised in aquariums are more sensitive to environmental changes (pers.

obs.), and I manipulated salinities at levels I believed would cause stress but not mortality. I would suggest future salinity stress research be conducted at more extreme levels of salinity.

My expected result of an initial increase and then decrease in FP emission intensity in response to stress was supported, but only for *M. capricornus* exposed to manipulated light and temperature. The increased intensity of emission in response to light stress lends support for a recently explained phenomenon, "Colorful Bleaching". During these events, coral upregulate fluorescence emission in the presence of thermal stress prior to bleaching, in an attempt of the coral to prevent further light damage (Bollati et al., 2020). By extension, my research supports the role of FPs providing photoprotection (Salih et al., 2000; Smith et al., 2013). The change in emission pattern during temperature manipulations for *M. capricornus* was consistent with the research of Roth (2013), which monitored FP emission differences of *Acropora yongei* in response to manipulated temperature. The pattern of increased emission intensity as the coral began to stress was not followed by *E. lamellosa* exposed to manipulated temperature. As of writing this summary, I know of no other studies that measured external FP emission intensity in coral exposed to manipulated salinity or pH. FP emission intensity did not change consistently between species in response to environmental manipulation, which would suggest external FP measurement is not a uniformly reliable measure of coral stress.

Hardier species of symbiont with a greater ability to withstand bleaching events have been, and are continuing to be discovered (D'Angelo et al., 2015; Osman et al., 2020). In addition, researchers have found success in utilizing assisted evolution to create

algae species with increased heat tolerance (Buerger et al., 2020; Chakravarti & van Oppen, 2018; van Oppen et al., 2015). In Chapter III, I provided evidence for a mechanism coral may use to attract potentially hardier symbionts. I conducted phototaxis trials utilizing three species of dinoflagellate, and all three species displayed significant phototaxis to the green fluorescence of the coral *E. lamellosa.* My research in combination with a previous study (Aihara et al., 2019) provides support for the "Beacon Function" of coral fluorescence which hypothesizes that coral utilize fluorescence to attract new, potentially more beneficial algae (Hollingsworth et al., 2005; Horiguchi, Kawai, Kubota,Takahashi,and Watanabe, 1999). During colorful bleaching events, coral upregulate fluorescence emission in the presence of thermal stress and could utilize fluorescence to attract potentially hardier symbiont species (Baker, 2003; Buddemeier & Fautin, 1993; Ware et al., 1996).

Coral bleaching is just one of the consequences of global climate change. As we witness an increase in climate induced events, we need a scientifically literate society with individuals pursuing fields in science, technology, engineering, and math (STEM) to sustain and protect our planet's habitats and inhabitants (McCright, 2012; Scarce, 1997). Interest in pursuing a STEM field is most impacted by a student's interest and confidence in STEM concepts (Dabney et al., 2012), and student interest is most impacted by learning outside of the classroom (Falk et al., 2007; Falk & Dierking, 2010). My study demonstrated that a field trip to the Oklahoma Aquarium increased student interest in STEM, regardless of the gender, socioeconomic status (SES), or ethnicity of students. All students concluded their field trip with a stronger belief that "science is fun" and were more excited to increase their STEM knowledge. Research that compares changes in

student attitude after a visit to informal science centers and accounts for the influence of demographic factors and field trip structure provides greater understanding of the impact of a public aquarium field trip on recruiting the next generation of STEM professional.

My research contributes knowledge regarding the applicability of utilizing external coral fluorescence measurement as a tool for monitoring health. Our understanding of the relationship between coral fluorescence and coral health would benefit from comparing differences in responses of additional coral and symbiont species when exposed to other environmental variables. The field of symbiont phototaxis research could be expanded by not only documenting attraction of additional algae species, but also by determining the ability of motile algae to access coral in wild reef systems. In order to further test ideas such as the adaptative bleaching hypothesis, we need to ascertain the ability of coral to uptake and retain novel symbiont species. Beneficial research cannot continue at a necessary pace unless we encourage students to pursue STEM fields. Motivating students to develop an interest in STEM and engage in research requires an understanding of what motivates individuals. Increased study of the impact of informal science learning on student populations from varied demographics would assist educators in understanding how to better engage students in science.

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VITA

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