Effects of Changing Light Intensity and Wavelength on Re-Orientation of *Aiptasia palladia*

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I. Introduction

Sea anemones are carnivorous invertebrates in the phylum Cnidaria and the class Anthozoa (Castro & Huber, 2005). They are in the same class as corals, also remaining in the polyp body form exclusively throughout their life cycle, but do not have a hard exoskeleton (Pechenik, 2000). All sea anemones are benthic and attach to rocks or the sea floor, often indefinitely (Pechenik, 2000). This renders the organisms relatively incapable of rapid locomotion as a method for prey capture, so anemones have several adaptations that aid in this process. Their mouth and oral surface is on the top of their tube shaped bodies and is surrounded my many tentacles which are studded with nematocysts- stinging cells which serve as a means for capturing and stunning or killing prey. The ability of the nematocysts to serve as a defense mechanism, also allows the anemone to be devoid of a hard covering or exoskeleton for protection from possible predators (Pechenik, 2000).

The digestive system of sea anemones is very simple. The Anthazoan mouth opens into a tubular pharynx that has some infoldings of the gastroderm and mesoglea, which increase the amount of surface area available for digestive enzyme secretion and nutrient absorption. All undigested food and waste material exit through the oral opening because these organisms lack an anus (Pechenik, 2000). Sea anemones tissues also contain circular and longitudinal muscles, which can be used to inflate the body, extend tentacles, locomote or burrow depending on the synchrony of contraction. These muscle layers are composed of cells that have long contractile bases called epitheliomuscular cells which can be contracted, provided the mouth is closed to pressurize the water inside the organism. Thereby, the seawater in its gastrovascular cavity can act as a hydrostatic skeleton (Pechenik, 2000). For example if only the circular musculature is contracted with the mouth closed (relaxing the longitudinal musculature), then the anemone will become tall and thin. However if the reverse occurs and only the longitudinal musculature is contracted with the mouth open, then the organism would flatten as the fluid in the gastrovascular cavity is released (Pechenik, 2000). This maneuverability can allow for control of location and direction to some degree to maximizing optimal feeding conditions.

Sea anemones and corals in the subclass Hexacorallia (Zoantharia) also have an endosymbiotic relationship with unicellular photosynthetic organisms called zooxanthellae in addition to being, themselves, carnivores. The zooxanthellae provide their host with organic compounds that are energy-rich such as fatty acids, amino acids or glucose and research has discovered that anywhere from 20% to 90% of the fixed material is transferred to the host (Pechenik, 2000). In return, the zooxanthellae have access to the hostÕs metabolic wastes such as nitrogen and carbon dioxide, which are used for photosynthesis and algal growth. Also the host provides a home that keeps the zooxanthellae somewhat safe from herbivores.

The zooxanthellae must be exposed to a light source to form energy-rich compounds by photosynthetic reactions. Therefore, the anemone must be able to maximize the amount of light that can reach the zooxanthellae. Clusters of photosensitive cells in localized areas in the anemone serve as light detectors that can measure the intensity of the light and can signal a behavioral change in the organism (Martin, 2002) to benefit the zooxanthellae and in turn the anemone as well.

Different species of coral known to have zooxanthellae have been studied to test for phototaxis to varying wavelengths of light. The changes measured were tentacle expansion and contraction. That study yielded results suggesting that species containing large algal populations would be more positively responsive to the stronger intensities of light (Levy et al., 2003). Conversely, those species that harbor minimal algal populations would not be as positively responsive to the light. It was also suggested that in zooxanthellate sea anemones there is a direct relationship between the amount of photosynthesis and the degree of expansion or contraction (Levy et al., 2003). This research is the stepping-stone to the present study. It was hypothesized that an increase in light intensity will increase the amount of positive orientation, towards the light source, to maximize the amount of light that reaches the algae. Positive reorientation would support the claim that morphological changes have a direct relationship with the amount of photosynthesis occurring and would have evolutionary implications to ensure the propagation of the species. In addition to light intensity, two colored bulbs in red and blue wavelengths will be tested as well. It is hypothesized that the anemones will display mostly positive orientation when exposed to blue wavelengths and mostly negative orientation when exposed to red wavelengths because these organisms are not accustomed to being exposed to large amounts of red light. The long blue and green wavelengths of the spectrum penetrate deepest into the ocean and the shorter red wavelengths do not penetrate as well (Castro & Huber, 2005). However in shallow waters, organisms are exposed to small amounts of red as well as ultra-violet wavelengths (Levy et al., 2003), therefore the anemones may be somewhat responsive to it.

The relationship between a symbiont and its host is interesting to study because both organisms rely on one another in a delicate balance for survival. Therefore having the ability influence one another can optimize the relationship for both organisms. The anemone and the zooxanthellae have successfully adapted to the marine environment by fostering a symbiotic relationship. The anemone has a greater chance of survival because it is not solely dependent on capturing prey to subsist. As long as the anemone is exposed to some sunlight, it will receive nutrients, which it would not be able to obtain without the zooxanthellae. The aggregation of zooxanthellae within the tentacles and tube-shaped body of the anemone protects them from disruption by the waves and currents so they can divide and propagate as well (Pechenik, 2000).

The sea anemones used in this study are the species *Aiptasia pallida*, which can often be found affixed to substrata in shallow water in the southeastern United States (Hauter, 2005). The color of the organism can vary from white to light yellow and a dark brown, depending on the amount of *Symbiodinium bermudense,* the symbiotic dinoflagellate algae, present in the gastrodermal cells (Trapido-Rosenthal et al., 2001). To test the hypothesis, light of two different intensities; from a 25 watt bulb and from a 60 watt bulb, were independently directed at one side of a clear plastic tank containing 10 to 12 sea anemones, serving as the only light source for the specimens. Three trials of each light intensity were carried out and recorded with a time-lapse camera, which took a photo every ten seconds for 120 minutes. Ambient sunlight was used as the control and trials with blue and red light at their respective wavelengths were also tested.

This study will be collaborative with other studies done by Ali Roca, Cassie MacDonald and Maris Madeira, all students in the Advanced Marine Biology course in the spring semester of 2005 at Wheaton College. Ali Roca examined the chemoattraction of the sea star *Asterias forbesi* to an odorant laced with the scent of prey (Roca, 2005). This study relates to hers because both involve manipulation of a food source in a sensory fashion to elicit a positive response from the specimen. This would indicate that though these organisms do not have complex nervous systems, the ability of their sensory systems, for photoreception or chemoreception, is crucial for locating a food source.

Cassie MacDonald tested the degree of attraction and recognition of a food source by Periwinkle snails (*Littorina littorea*) as well as what colored light they are most willing to feed under (MacDonald, 2005). She also studied whether it was light color or algae abundance that influenced the snailsÕ desire to feed (MacDonald, 2005). Her study relates to this one because both examine the effects of a food source on the behavior an organism as well as if changing the conditions under which the food source is presented, namely the color of the light, will affect behavior. Different levels of discernment of an organism towards food depending on surrounding conditions are crucial to marine species because they must avoid predators and can be vulnerable when feeding. Selectively choosing one environment over another can decrease the amount of vulnerability for the organism.

Maris Madeira studied negative phototaxis in *Hemigrapsus sanguineus*, by examining if the crabs preferred to dwell in a dark covered area or in full light (Madeira, 2005). Her study relates to this one because both organisms must move or be oriented towards the amount of light that is most beneficial for their survival. The crabs will most likely remain in the dark, as it affords them protection from predators and the anemones will orient themselves towards the light as much as possible to maximize the photosynthetic reactions carried out by the zooxanthellae.

II. Materials and Methods

Materials

- 10 to 13 photosynthetic *Aiptasia palladium* sea anemones from the Carolina Biological Supply Company
- Epson Photo PC time-lapse camera (850 or 200)
- Plastic tank (that holds approximately 2.5 Liters of water)
- Seawater with a salinity of 35ppt (salt and filtered reverse osmosis water)
- Hydrometer to check water salinity
- A lamp with a swiveling arm and a place to affix the camera directly over the tank
- Light bulbs with different intensities (25 watt and 60 watt) and wavelengths (blue light and red light)
- Small plastic pipette to stimulate the anemones to contract and close at the beginning of each test.
- 1000mL plastic beaker (for changing the tank water periodically)
- Notebook and pen
- Metric ruler
- White paper (for backdrop, to be placed directly underneath the tank)

Methods

This experiment was conducted in the Wheaton College Science Center, specifically in the Urchinology Laboratory (referred to as the Room 220 laboratory) on the second floor.

Aquarium Preparation

1. Seawater was prepared by mixing Instant Ocean with reverse osmosis water until a salinity of 35 ppt was reached (Morris, 2005). This water was transferred into a transparent 2-liter plastic tank.
2. Ten anemones were carefully removed from a large existing tank after measuring the salinity of the water from their current habitat with a hydrometer as not to tax them biologically during transplantation. The researchers washed their hands thoroughly and then slid their thumbnail underneath the pedal disc and pushed forward to encourage its release from the substrate. The freed anemones were then transferred to the 4-liter plastic tank, which sat on the base of a lamp stand that has a rotating arm attached to the light bulb.
3. The specimens were given 7 days to adjust to the new environment before beginning experimentation. Half of the tank water was removed every 2 to 3 days and replaced with freshly aerated water.

Setup of Lamp Apparatus and Time Lapse Camera

1. The rotating arm of the lamp apparatus was turned so that the face of the bulb was at a 75^0 angle towards the right side of the tank and a distance of 20 cm from the tank and the Epson Photo PC 850Z time-lapse camera was positioned so that the camera faced down onto the top of the tank. A metric ruler was also placed underneath the tank as to be clearly seen by the camera during testing.
2. The settings on the camera were as follows: Interval mode was chosen and the time interval was set to one image per ten seconds for a duration of 120 minutes. The flash setting was turned off and the resolution of the images was set to the lowest possible setting.
3. Extraneous lighting was kept to a minimum by placing a screen of folded cloth diagonally across the window. This allowed enough sunlight to enter the lab for the camera to function but would not interfere with the tests. No lighting other than the source being used for testing was illuminated during the 120-minute period and all trials were completed during the day (from the hours of 6:00 a.m. to 7:30 p.m.)
4. The specimens were prodded with a pipette to encourage complete retraction of the tentacles as well as flattening of the tubular body cavity and then the photographic recording was begun. After 5 frames with ambient light had been taken, the light source was illuminated and a solid panel slightly taller than the tank was placed between the window and the tank to minimize the amount of ambient light the specimens were exposed to.
5. After the 120-minute trial was completed, the camera and light source were switched off and the specimens were returned to stable conditions for at least 60 minutes before performing another trial.

6. Three trials were done in this manner for each light condition; ambient light, a 25 Watt bulb and a 60 watt bulb.

Quantitation of Data

Three trials of 120-minute time-lapse video were recorded for each light intensity and wavelength that was tested, as well as for the control in ambient light. Of these three trials, one of each was used as the source of individual frames for analysis. Nine frames were selected at fifteen minute increments beginning at time zero and finishing at 120-minutes. The distance from the center of the oral surface to the most distant edge of the pedal disc on each anemone was measured in millimeters and recorded. Distances that were directed towards the light source were scored as positive and distances that were directed away from the light source were scored as negative. This was done for each of the twelve anemones in the tank.

A line graph of the measurements over time of all twelve anemones, as separate series, was constructed for each light intensity and wavelength tested. However due to the complexity of these graphs, only one is retained to demonstrate the wide range of fluctuation in length for individual specimens. To best represent the trends of the different light intensities and wavelengths tested, an average length for each time interval was calculated from data of the 12 anemones tested. These results are represented in a graph of average lengths for all different light intensities and wavelengths tested. Notes on particular types of movement were also recorded to supplement the quantifiable data.

III. Results

Several trends are visible when examining Figure 2, which represents the average orientation of all twelve sea anemone specimens at each 15-minute interval from time zero to 120 minutes for all five experimental conditions. The control trial in ambient light and the 25 Watt trial both show a relatively steady increase in length over time, peaking at 120 minutes. Both also decrease after 45 minutes and then peak again at 120 minutes. The 60 Watt trial shows a rapid increase in length, peaking at 75 minutes with the longest average length of all the conditions tested, and then drops off as it nears 120 minutes, resulting is a parabolic curve. The blue wavelength trial depicts a decrease in length at 45 and 75 minutes with two peaks at 60 and 90 minutes, finally decreasing at 120 minutes, though the general trend is positive. The most striking trend is that visible in the red wavelength trial, which immediately shows a drastic decrease in length. The line peaks at 90 minutes after which the length continues to decrease. The curve exists as a near mirror image to the ambient light control, both have a peak or drop at 60 minutes, but the control progresses in a positive direction at a relatively minimal incline, the opposite is true for the red wavelength data.

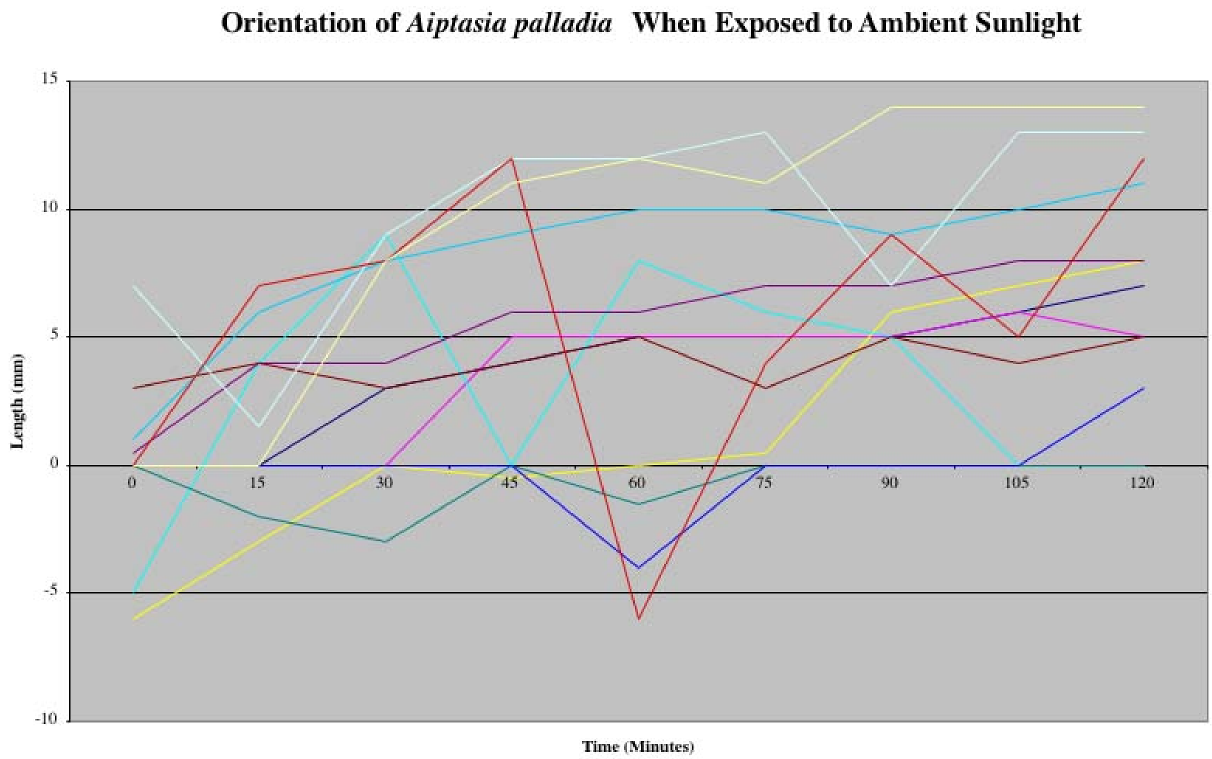

Figure 1. This graph represents the orientation of the sea anemone specimens relative to an ambient light source over a time period of 120 minutes. Each series in the chart above represents the progression of reorientation for an individual specimen over the 120 minute time period. There is some fluctuation in the graph, however all but one of the 12 specimens increased their length and subsequent proximity to the light source by 120 minutes. The large amount of variation among individual anemones is also strikingly visible throughout the 120-minute time period. This graph represents a trend of drastic amounts of variation across all light conditions tested.

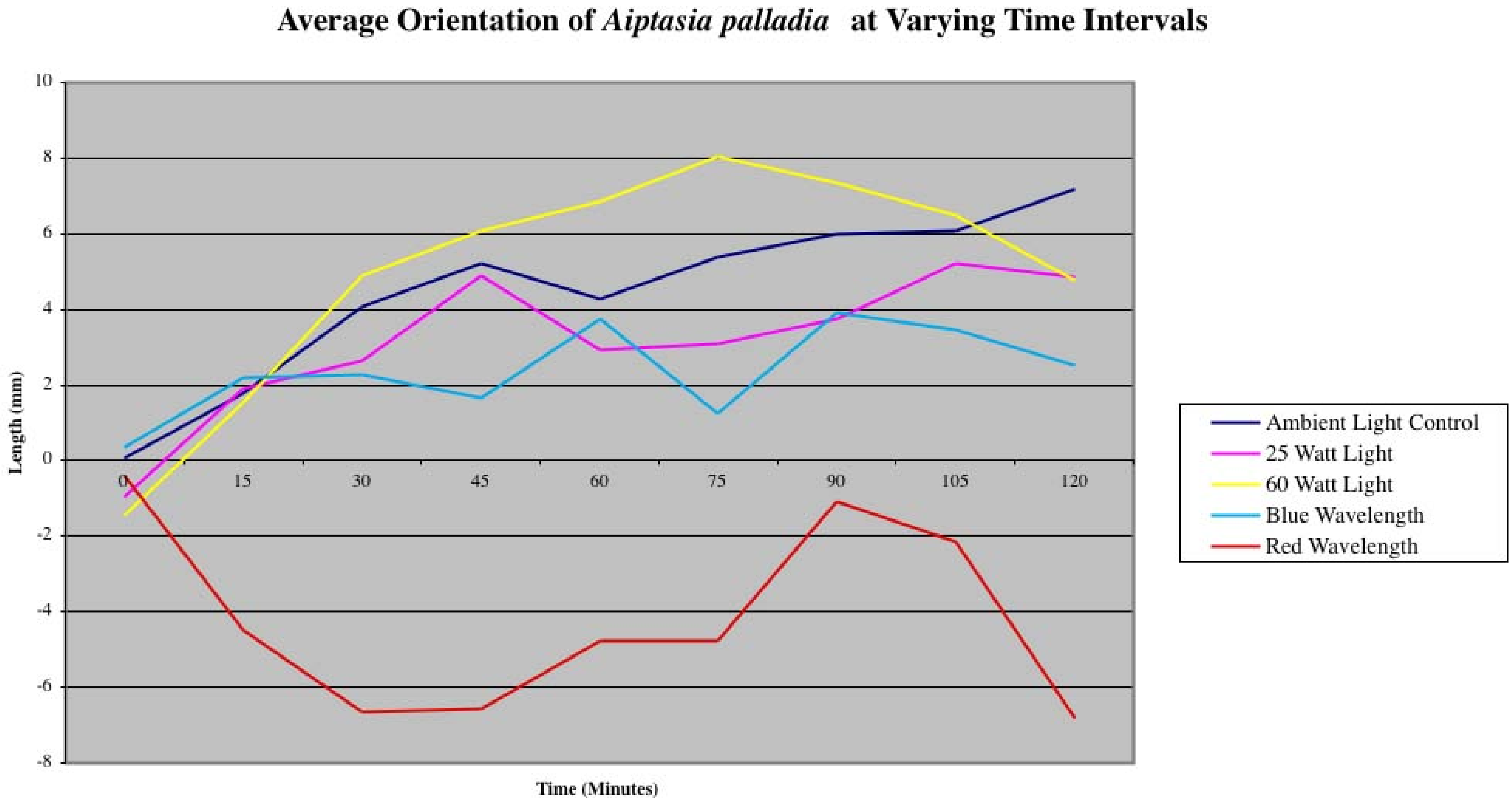

Figure 2. This graph represents the average orientation of all the sea anemone specimens (n =12 for each type of light source tested) relative to the five experimental conditions tested over a time period of 120 minutes in 15 minute incraments. The control trial in ambient light shows a relatively steady increase in length over time, corresponding to similar results from the 25 watt trial. The 60 watt trial shows a rapid increase in length, which then drops off as it nears 120 minutes. The blue wavelength trial depicts a decrease in length at first, with fluctuating lengths for the duration of the test, though the general trend is positive. Contrary to the other four trials, the red wavelenth trial shows a drastic decrease beginning at time 0. There is a peak present at 90 minutes however the length then contiues to drastically decrease towards the end of the trial.

IV. Discussion and Conclusions

From the data analysis we can conclude that light intensity does have an effect on reorientation of *Aiptasia palladia*, however the data collected does not support the proposed hypothesis that an increase in light intensity will increase the amount of positive orientation. When comparing the average length of the anemones at different time increments during the trial (Figure 2), we can see that the results of the ambient sunlight control and the 25-watt light are similar. Both show a relatively steady increase in body length over time, peaking at 120 minutes and both also show a decrease in body length from 45 to 60 minutes. A possible explanation for this is that the anemones have reached a point of momentary saturation with light and must allow the zooxanthellae to carry out photosynthetic reactions with the light energy they have already retained. We can see in both trials that the rest period lasts for 15 minutes and then the length begins to increase once more. The 60-watt trial exhibits this phenomenon in a more drastic way. There is first a rapid increase in length, peaking at 75 minutes and then decreases steadily to the 120-minute mark. It is speculated that because the intensity of the light was so strong, the anemones became saturated with light at 75 minutes, the algae were already rapidly producing glycerol and after a certain point, they cannot continue to increase their rate of photosynthetic reaction. It could also be that enough product was made so the anemones did not need to be constantly facing the light or perhaps that certain biochemical processes within the algae are unable to proceed at a faster rate, which would lead to saturation. More tests would need to be done to speculate as to why the point of saturation occurs 30 minutes sooner for the lower intensity light than for the 60-watt light as well as if there are underlying biochemical processes that cause the point of saturation and what they are. As this was a particularly rich data set, further analysis in the future may suggest a reason for this discrepancy, however molecular tools could prove most useful to discover why the saturation effect occurs. Another possibility is that the saturation effect observed is particular to the laboratory conditions or the specific specimens used and it does not reflect a greater natural trend.

The results of the blue and red wavelength trials support the hypothesis that the anemones will display mostly positive orientation when exposed to blue wavelengths and mostly negative orientation when exposed to red wavelengths. In Figure 2, we can see that there was fluctuation in the average length for the blue wavelength trial, but it continued to steadily increase for the duration of the trial. This data is similar to the 25 Watt and ambient light control trials. In comparison, the red wavelength data, shows a drastic decrease in positive length which continued for the majority of the trial. The positive length peaked at 90 minutes and then continued to rapidly decrease for the rest of the trial. Previous studies with corals have shown that the response of the polyps to different wavelengths of light correlates to the specific absorption spectrum of the zooxanthellae for the species (Levy et al., 2003). Also the spectra at which photosynthetic reactions occur in corals are very near to the spectra at which the light is absorbed (Levy et al., 2003). Therefore we can speculate that the spectra at which the photosynthetic reactions occur for the specific symbionts of *Aiptasia palladia* do not favor and may not include red wavelengths. This may be as a result of the fact that anemones are benthic organisms, therefore large amounts of red wavelengths do not adequately penetrate the water column, so the anemones do not need to foster zooxanthellae that are able to readily take up red wavelengths.

Possible sources of error in this experiment include the varying amount of ambient light present during the trials, as they were done at different times during the day and the possible presence of heat buildup on the side of the tank from the light source. Both of these errors could have affected the trials. If this experiment were to be repeated, the amount of daylight at any given time should be strictly monitored to eliminate that source of error. Also testing for the amount of heat buildup if any from the light source would be ideal to minimize its effect on the organisms.

A shortcoming of the quantitative analysis performed on the data is that average lengths of all anemones at certain time intervals were taken, disregarding the physiological characteristics that are unique to each specimen. The location of zooxanthellae concentration can affect how the individual organism reacts to a light source. Tests have shown that those specimens that concentrate the majority of symbionts in the pedal disc and lower column of the body tend to contract when under a light source (Day, 1994). Those specimens that have equal distribution of zooxanthellae in the lower column and tentacles expand when exposed to a light source (Day, 1994). The scoring system for positive or negative re-orientation is also limited because it does not account for the location of the tentacles and relative exposure of the oral surface to the light source. Often individual specimens were scored as positively re-oriented but their tentacles were not extended. Also when individual specimens were directly perpendicular to the camera, which was positioned to look down onto the tank, they were scored as 0 mm for length because that is the distance from the pedal disk to the center of the oral surface at that moment. However this does not account for the amount of extension or contraction of the tube-shaped body at that time in the perpendicular plane, nor does it account for the amount of tentacular extension. Again, more detailed analysis of the data may lead to further conclusions, but I believe that the general important trends were analyzed well with the methods used.

The results of the collaborators with this study can offer more insight into general adaptationd for marine organisms for their environment. Cassie MacDonald discovered that the snails she used are able to distinguish between areas of high and low algae concentration (MacDonald, 2005). This relates to the current study because the sea anemones can exert the same discretion when locating a light source. This is evolutionarily vital for both organisms because obtaining or synthesizing food can be a difficult task in their natural intertidal habitat. Researcher MacDonald also tested the snailÕs preference for what colored light they are most willing to feed under (MacDonald, 2005). The anemones are also more receptive to light of different intensities. Finally, she studied whether it was light color or algae abundance that influenced the snailsÕ desire to feed. She discovered that regardless of the light present, the snails always would eat the algae, therefore food concentration is a greater influence (MacDonald, 2005). This is not true of the sea anemones, they would readily open their tentacles and/or extend their bodies towards the light, but because they are dependent on the light as a food source, and their zooxanthellae do not have the capacity to process red wavelengths, they are limited. For the snails, the threat of not obtaining food is greater than the atmosphere in which the food is, so they make a conscious choice to eat a food source as self-preservation because they may not be able to find food in the near future and missing the opportunity to nourish themselves could be detrimental to their survival.

Maris Madeira discovered that the crabs would most often stay hidden in the dark and when they were placed in the light they immediately would move towards the corner of the tank, presumably for a sense of security (Madeira, 2005). Her study compares to this one in that both organisms actively choose an environment to better their chance of survival. It is crucial for the zooxanthellae containing sea anemones to be in the presence of light and they will seek out this optimal situation, exhibiting positive photo behavior. The crabs on the other hand rely on being hidden from view to minimize the possibility of being preyed upon and will subsequently look for dark places to hide.

The results of this study are important to the scientific community because they display how symbionts can influence host behavior and the mechanics and importance of photoreception to marine organisms. Future experiments in the biochemistry of photoreception and photosynthetic reactions by zooxanthellae may shed light on the saturation effect seen in these results as well as applying the same methods to other types of organisms who are also dependent on photosynthetic reactions. Another interesting study would be to use molecular phylogenetics to attempt to construct an evolutionary tree for varying Cnidaria species based on the spectra at which the zooxanthellae are photosynthetically active. This may offer insight into the evolution and divergence of different species.

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