Phototaxis vs. Shelter in *Hemigrapsus sanguineus*

Advanced Marine Biology (331)
Independent Research Project
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Introduction:

The Asian shore crab *Hemigrapsus sanguineus* was first found to invade the Eastern coast of the U.S. in 1988 and has since spanned the rocky intertidal from Maine to South Carolina (Benson, 2005). It is a brown and green colored crab with stripes on the legs and a carapace width of approximately 20mm (Benson, 2005). These crabs are scavengers and can consume a broad range of food (Castro and Huber, 2005) (McDermott, 1998). It has been shown that these crabs usually forage at night and tend to stay hidden during the day (Okano, Suzuki and Horie, 2002). The hypothesis of this study is that given a choice between dwelling in a covered environment and an open bright environment, *H. sanguineus* will dwell in the covered environment, however negative phototaxis will be more influential in determining position than coverage. The purpose of this experiment is to try to study the chosen position of these crabs in the rocky intertidal habitat to infer how that adaptation has allowed them to survive and evade predation. Being totally exposed on a rocky intertidal would leave *H. sanguineus* vulnerable to predation, so I hypothesis that given the choice between being totally exposed in light or being in the shelter of a transparent tube, *H. sanguineus* will choose to take the coverage of the tube as shelter. However given the choice between taking coverage in a clear tube or hiding in total darkness *H. sanguineus* will chose to take cover in total darkness. This preference of darkness is supported by Okano, Suzuki and Horie's study of habitat use and activity pattern of three Japanese fresh water crabs which showed that the crabs usually came out at night and were infrequently found during the day (2002).

This study was conducted in collaboration with Serena Strydom, Alex Adams, Jenny Fisher and Cassie MacDonald as a research project for biology 331 at Wheaton College in the spring of 2005. The research in this study will relate to Strydom's research on the Effects of Changing Light Intensity on Reorientation of *Aiptasia palladia* (2005) because both topics deal with marine intertidal species reactions to light although Strydom's research will be dealing with the photosynthetic algae of the anemones and their positive phototaxis whereas this paper will deal with negative phototaxis. This research will relate to Adam's research on olfaction and antennule flicking rates in the American lobster (*Homarus americanus*) in response to contact with an odor plume because the American lobster used in Adam's research and *H. sanguineus* are both decapods and they both detect food using chemoreception (Diehl, Feeley and Gibson, 1971) (Morris, 2005). The use of chemoreception connects these two test subjects because crabs forage at night as shown in research done by Okano, Suzuki and Horie, 2002 so they would need the chemoreception to locate prey because they couldn't see them at night. Adam's research would further show how it is possible for these crabs to find their food in the dark. Fisher's research on the size ratio for predatory interactions of *Asterias forbesi* and velocities of locomotion per weight of *Asterias forbesi* will relate to the research on *H. sanguineus* because both studies will address negative phototaxis in marine benthic species and how this negative phototaxis helps both species to evade predation. This research will relate to MacDonald's study of the effects of light on the foraging habits of periwinkles because both experiments deal with the effects of light on different marine species. In each experiment it will be seen how light effects the location of the organism on the rocky intertidal and from there infer the threat of predation in the light on either organism depending on their response to the light source.

Materials and Methods:

In the execution of this experiment, one twenty gallon tank was used to conduct all testing, one timer, black plastic was used to cover half of the tank to make it dark, 0.5 liter plastic bottles cleaned and cut in half the long way with either end cut off were used as tubes, wooden dowels were used to hold down the tubes, plastic string was used to hold up the dowels, tape was used to hold the string and keep the black plastic on the outside of the tank, salt water was used to conduct experiments in as well as fill a holding tank for the crabs, 10 *Hemigrapsus sanguineus* crabs were used, a net was used to get the crabs out of the tank, a large bucket was used to hold the crabs in, a bubbler was used in the large bucket that the crabs were kept in when they weren't being studied to keep the water aerated and rocks were used to create a habitat for the crabs in holding.

This experiment was preformed in the 37 degree C cold room on the second floor of the science center at Wheaton College in Norton Massachusetts. To begin this experiment a 20 gallon tank was set up with three inches of water covering the bottom of the tank. The salt water was prepared using Instant Ocean and mixed to 35 ppt. A bubbler was placed in the bucket of salt water for a few hours and then the crabs were placed in the bucket with rocks; this bucket served as the holding place for the crabs that were not being tested at the time. It was determined in the procedure that timing the crab for one minute was just as effective for determining the crab's final location as two minutes so one minute was used to establish the crabs preferred location. It was after a one minute time period that the end position of the crab was recorded. Each part of every trial was executed using a ten crab sample pool so each crab was tested once per part of the situation and the results were stated as the number of crabs out of ten that were finally located in the specified position. If a crab was not fully in a specified location but only half way in it was not counted in the data.

<u>Trial 1: Light vs. Dark Trial</u>

In the light dark trial half of the tank was covered with black plastic on the outside and a panel of black plastic was taped up just above the water line on half of the tank. Thus half of the tank was very dark and there was a distinct line where the dark began and the light ended. Ten crabs were used in each trial; each crab was in the tank by itself for the duration of the trial. In this trial each crab was placed in the middle of the light side of the tank with its back facing the dark half of the tank, after one minute its ending position was recorded in a table in a lab notebook. Next each crab was placed in the middle of the dark half of the tank with its back facing the light half of the tank, after one minute the position of the crab was recorded in a lab notebook.

<u>Trial 2: Light with Tubes vs. Dark</u>

In trial 2 half of the tank was dark, as in trial 1, and in the light half two half plastic bottles cut lengthwise and open at either end were added, this created a tube like structure. To keep these tubes on the bottom, a dowel was placed on each of them, the dowels were tied together with string and the string was taped to the side of the tank to keep the dowels vertical. The tubes covered half the lighted space so there was fifty percent open light space and fifty percent tube-covered light space. Each of the ten crabs was started in the dark with their backs facing the light side of the tank and was then timed for one minute, then each of the crabs was started in the light out of the tube and timed for one minute, and then each of the crabs was stared in the tube and timed for one minute.

Trial 3: Light with Dowels vs. Dark

To perform a control for trial 2 the tubes were taken out and the dowels left hanging. Ten individuals were started in the dark with their back facing the light side of the tank and timed for one minute and then ten individuals were started in the light with the dowels hanging over them with their backs facing the dark and then timed for one minute. This set up tested to see if the dowels holding the tubes in place had an effect on the crab's probability of going into the light.

Trial 4: Light with Rocks vs. Dark

In trial 4 half of the tank was dark and the other half was light with rocks covering half of the lit area. This was tried to see if the type of shelter/coverage present had an effect on the crab's preference. In this trial ten crabs were started in the light individually with their backs facing the dark and timed for one minute and then each crab was started in the dark with its back facing the light and timed for one minute, these times were recorded in a lab notebook.

Trial 5: Light vs. Tubes

In trial 5 the entire tank was light and there were tubes covering half of the available tank space, these tubes were held in place using dowels. These tubes were only open at one end. In trial 5 ten crabs were started in the middle of the tank individually out of a tube and timed for one minute. Then each of the ten crabs were started individually in the tube and timed for one minute. These times were recorded in a lab notebook.

Trial 6: Right (light) vs. Left (Dark)

In trial 6 the entire tank was open and each of the ten crabs was started on the left side with their backs facing the right side and then each of the ten crabs was started on the right side with their backs facing the left side and they were timed for one minute. This trial was a positive control for trials 1, 2, 3 and 4, the right side of the tank was a control set up for the light half of the tank and the left side was a control for the dark side of the tank. Each of the times was recorded in a lab notebook.

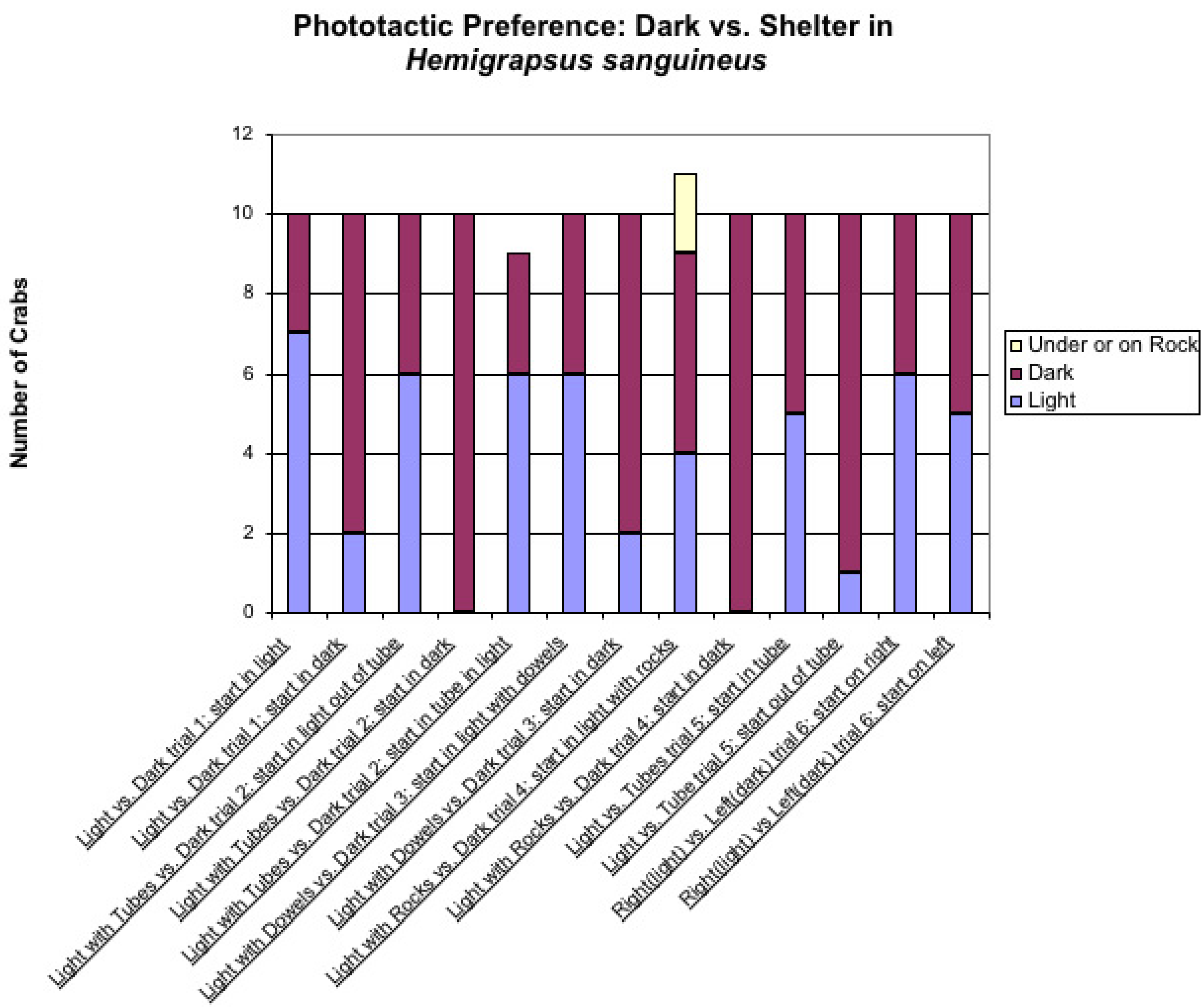
Results:

In this research six trials were designed to test the hypotheses. In each trial the crabs starting and ending position was recorded. Each crab's final position was considered its ÒpreferredÓ location. In trial 6, right vs. left, 60% of the time the crabs stayed on the right when started on the right and went to the right 50% of the time when started on the left. These data would suggest that it is equally likely that the crab will choose either side when there are no other influences in the tank. Thus it is possible to compare trials to this control to see if the crabs are choosing a preferred location and that it is not just random distribution.

In trial 1 the crabs stayed in the light 70% of the time when started in the light and stayed in the dark 80% of the time when started in the dark. This shows that the crabs preferred the dark over the light but only slightly. In trial 2, light with tubes vs. dark there were three options for the crab; it could either be in the light, in the dark or in the light in the tube. When the crab was started in the light out of the tube it stayed in the light 60% of the time, ended in the dark 40% of the time and never ended in the tube. When the crab was started in the dark, it ended in the dark every time and when the crab was started in the tube in the light, it ended in the light 60% of the time, ended in the dark 30% of the time and one time it ended half in the dark and half in the light so it was not counted in the results. This shows that when started in the light the crabs preferred the light, when started in the dark the crabs preferred the dark and when started in the tube the crabs preferred the open light. Dowels were used to hold these tubes in place so a control was run to test if the dowels had an effect on the end positioning of the crabs which was trial 3, light with dowels vs. dark. When the crabs were started in the light with the dowels 60% of the time they stayed in the light, 30% of the time they stayed in the dark and once the crab was half in the light and half in the dark, in this study half in and half out of the light was considered neutral so that crabs positioning was not counted in these results. When the crabs were started in the dark they ended in the dark 80% of the time. This trial shows that when the crabs started in the light they preferred the light but when started in the dark the crabs preferred the dark and when comparing the two starting positions the crabs preferred the dark more often than they preferred the light. In trial 4, light with rocks vs. dark, when the crabs were started in the light with the rocks they stayed in the light but not under a rock 36% of the time, they went into the dark 45% of the time and they hid behind or on a rock 18% of the time and when started in the dark the crabs preferred the dark 100% of the time. This trial shows that the crabs preferred the dark over the light and over the rock shelter. In trial 5, light vs. tubes, when the crab was started in the tube it stayed in the tube 50% of the time and when the crab was started out of the tube 90% of the time it stayed out of the tube. This trial shows that the crabs preferred to stay in the light out of the tubes. The table below describes each of these scenarios and displays the number of crabs that ended in each place for each setting.

Figure 1:

Results:



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This figure shows each setting and is described under each column by where the crab started. The lighter color indicates when the crab ended in the light, the darker color indicates when the crab ended in the dark and the lightest color represents when the crab ended in the tube. In the setting where only in the tube or out of the tube could be chosen staying in the tube is indicated with the lighter color and ending out of the tube is indicated by the darker color. In a situation where there is only light as in the control for the light and dark ending on the right is indicated by the lighter color and ending on the left is indicated by the darker color.

Conclusion:

The results of this study are preliminary; to obtain results where actual definitive answers could be reached and proper statistical information calculated would take at least ten times the repetition of this study according to biological statistician Dr. S. Shawn McCafferty. The result of trial 1, light vs. dark, show that 70% of the time the crab stayed in the light when started in the light and stayed in the dark 80% of the time when started in the dark. These results would indicate that *H. sanguineus* preferred the dark 10% more often than the light. When started in the light they usually stayed in the light and when started in the dark they usually stayed in the dark. This could also be interpreted as response to being picked up and placed in the tank. In the procedure being in the light was defined as being anywhere in the light, being in the dark was defined as being anywhere in the dark and being in shelter was defined as being in a tube or under a rock. After research began it was observed that when in the light, *H. sanguineus* often went to the corner of the tank and never stayed out in the open exposed light nor in the open exposed dark, each crab always had a solid surface on one side of it. Since being in the corner or having a solid surface on one side of it was not considered shelter it was not recorded as such but in a future study the protective effects of the corner of the tank could be taken into consideration.

To test the probability of the crab choosing the light vs. the dark side of the tank a control was set up where the entire tank was open and the crabs were placed in the tank starting on either side with their backs to the appropriate side. In trial 6, right vs. left, *H. sanguineus* when started on the right stayed on the right six 60% of the time and when started on the left stayed on the left 50% of the time. Since this test indicated that *H. sanguineus* is about as likely to choose either side these results would indicate that *H. sanguineus* is choosing the light or the dark in trial 1, light vs. dark, where the results are further from fifty-fifty. Although when started in the light they chose the light more frequently, in comparison to trial 6 they are making some sort of choice. It was expected that *H. sanguineus* would prefer to stay in the dark when given the choice between light and dark.

In trial 2, light with tubes vs. dark, when started in the light *H. sanguineus* chose the light 60% of the time and the dark 40% of the time where as the tube was never chosen. When started in the dark *H. sanguineus* stayed in the dark every time and when started in the tube stayed in the light 60% of the time, in the dark 30% of the time and never in the tube. One time the crab was half in the tube and half in the light so that trial was not counted because it could not be determined as either in the light or in the tube. It was expected that in this situation *H. sanguineus* would prefer the dark.

When started in the light the hypothesis of this research states that the crab will usually choose the tube over the light and the dark over the tube. It was not expected that the crab would choose the light more often than the dark and it was not expected that the crab would choose the light over the tube. Observations recorded during this trial show that when in the light *H. sanguineus* went to the corner every time, this would indicate that since the crabs did move and they moved to the corner that there is something special about the corner of the tank that attracts them to it such as a protective quality.

When started in the tube *H. sanguineus* usually ended in the light, sometimes ended in the dark and never ended in the tube. This is not the hypothesized result; it was hypothesized that when started in the tube the crab would prefer to stay in the tube or go into the dark but not leave the tube and go into the light.

To test to see if the dowels holding the tubes in place had an effect on the results of this test a situation designed where half of the tank was dark, and half was light with the dowels hanging over the light. The results of this showed that when started in the light *H. sanguineus* usually stayed in the light and when started in the dark usually stayed in the dark. Since these results are similar to the results of the situation where half of the tank was dark and half was light with no dowels in the light it can be assumed that the dowels did not have an effect on the light vs. dark preference of the crab.

When *H. sanguineus* was put in a situation where half of the tank was dark and half was light with half of the light area covered with rocks it was found that when started in the light *H. sanguineus* was about as likely to choose the light as the dark and went under or on the rock twice. This experiment was done to see if it was possible that *H. sanguineus* did not view the plastic tube as a shelter and would instead view a rock, which would be a known shelter to them, as a place to hide. Although the crabs did choose the rock twice they did not choose the rock more often than open light which would mean that the hypothesis that shelter would be chosen over open light was not supported.

There is also a flaw with using rocks because they provide both coverage and some darkness so it is hard to determine which characteristic they are choosing. Trial 5, light vs. tubes, was preformed to distinguish between the choice between open light and shelter. When started in the tube *H. sanguineus* stayed in the tube 50% of the time and when started outside of the tube stayed outside of the tube 90% of the time. Marked observations during this experimentation were that every time the crab did not stay in the tube or go into the tube it went to one of the corners of the tank. Though this was not recorded as coverage because it was not defined as such it is still interesting to note that when staying in the light the crabs were not fully exposed, sitting in the middle of the tank but rather crouching or reaching their legs up the side of the tank in a corner. The data from this trial indicates that although when started in the tube the crabs were as likely to stay in the tubes as leave they were not likely to choose to enter the tube if they weren't placed there.

In regard to the hypothesis, from the data collected it would seem that when put in a situation where they are already in the dark *H. sanguineus* will stay in the dark, however when started in the light they are slightly more likely to stay in the light than go to the dark. This could mean a few things; either the crabs are already scared from being picked up and escapes to the closest corner of the tank. This could be true because it is known that crabs locomote laterally, however in this study it was observed that they often moved front and backward which is a predatory response (McDermott, 1998). It could also mean that the crabs are finding some sort of shelter or relief in the corner of the tank and the attraction to the corner is greater than the attraction to the dark. If the corner was considered shelter or was shown to have shelter like qualities it would be possible to determine that shelter was more influential in determining the crabs positioning rather than darkness.

When put in a situation where there was a choice between being in the light or being in a tube shelter in the light *H. sanguineus* was as likely to stay in the shelter as leave when put there but hardly ever voluntarily went to the shelter. This goes against the hypothesis because it was thought that when having the choice between open light and shelter the shelter would be chosen. Again in this study the crab always went to the corner when in the light. This preference of the dark over the light can also be supported by Okano, Suzuki and Horie's study of habitat use of Japanese freshwater crabs where they found that the crabs were somewhat nocturnal meaning that in this study most of the crabs were found out at night foraging for food but were not present during the day (Okano, Suzuki and Horie, 2002).

This research can imply that in the rocky intertidal habitat of *H. sanguineus* it is likely that they will stay hidden in the dark when there is a choice between light and dark. In the real life situation of the rocky intertidal the tendency to hide against the corner of the tank in the lab would mean that crab would have less available surface area from which to be grabbed in the intertidal and this would be a helpful adaptation to predator evasion.

In Strydom's research with the photosynthetic algae in the tubes and tentacles of *Aiptasia palladia* she found that there was a maximum saturation point at which the photosynthetic algae had absorbed enough light and started to shrink away from it at the maximum saturation point. In Strydom's research and the research in this paper both hypothesis looked at the effects of light on marine species. Strydom's research deals with a marine species that needs the light to feed and therefore reaches toward the light. The research on *H. sanguineus* deals with a species that needs to evade the light to escape predation by animals such as gulls and other larger animals that might randomly be on the rocky beach habitat that wouldn't usually be found there (McDermott, 1998). Both are marine species and both respond to the stimulus of light in different adaptive ways that have allowed each of them to survive.

The results of Adam's research with the olfaction and antennule flicking rates in the American lobster (*Homarus americanus*) in response to contact with an odor plume can be helpful in learning some things about the feeding strategies of decapod crustaceans. *H. sanguineus* also feeds by chemoreception (Diehl, Feeley and Gibson, 1971) (Morris, 2005) and they are scavengers (Castro and Huber, 2005), Adam's findings that the rate of antennule flicking increased when in contact with an odor plume helps to understand how *H. sanguineus* forages on the rocky intertidal especially at night because they are mostly nocturnal scavengers and wouldn't have the light of day to help them locate their prey (Okano, Suzuki and Horie, 2002). The increased antennule flicking shows how chemoreception works and provides data that would show how it would be possible for crabs to feed with out the light of day to guide them to their prey which would make it possible for them to stay hidden during the day.

Fisher's research on the size ratio for predatory interactions of *Asterias forbesi* and velocities of locomotion per weight of *Asterias forbesi* is helpful to see the similarities between two marine species negative phototaxis. In Fisher's study it was shown that as light decreases the risk of predation decreases meaning that it would be safer to be in the dark. This would be supported by Okano, Suzuki and Horie's study which shows that the crabs were more likely to be out at night and hidden during the day. The risk of predation by gulls would decrease at night, and since *H. sanguineus* uses chemoreception to find food (Diehl, Feeley and Gibson, 1971) (Morris, 2005) they wouldn't need to be able to see well in the dark.

MacDonald's research with the phototactic and feeding strategies of periwinkles is helpful in understanding the relationship of periwinkles to *H. sanguineus* and each of their responses to light. MacDonald's research dealt with how light effects the feeding strategies of periwinkles, the study on *H. sanguineus* studied how light effected the preferred positioning of the crab in a tank in simulation of how a crab would prefer to position itself in the rocky intertidal habitat. It was found that periwinkles did venture into the light for food which may happen with *H. sanguineus* but according to Okano, Suzuki and Horie's study, crabs are more likely to feed in the dark.

With more data available, statistical comparisons between situations would be possible. Using other types of crabs might be helpful in a future experiment because different types of crabs may respond differently to situations. In a future experiment research could be done to test the protective qualities of the corner of the tank, it would seem from the observations in this experiment that the corner has some unique quality that attracts the crabs to it. In a future experiment it might also be helpful to see how crabs respond when placed in the tank with other crabs as far as where they go in the tank and what they will use as shelter when the corners are taken by another crab. Such a study would tell us about the competitive nature of these crabs and also give a more realistic portrayal of life in the rocky intertidal.

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