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Behaviors of a Captive *Coenobita clypeatus* in the Presence of Varying Light Stimuli

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Coenobita clypeatus, or the purple pincher, is a terrestrial hermit crab and common pet in the United States. Modern behavioral studies have waned since the early 2000s; we therefore sought to characterize the behavior of a single captive C. clypeatus under varying light conditions. Frequency and duration of behaviors were studied in the presence of different light stimuli, along with weight monitoring. The crab was recorded for a set period of time every night with all conditions standardized except for light. The light stimulus alternated between no light, blue light, and red light. The case study revealed a significant increase in feeding behavior duration under blue light and a return to baseline conditions under red light. A non-significant trend of greater frequency of activity under red light was observed. These data support further research on C. clypeatus that might have implications for visual learning and improving captive conditions.

Keywords: crustacean, light discrimination, case study, animal behavior, hermit crab, Coenobita clypeatus

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The anomuran crustacean *Coenobita clypeatus*, also known as the purple pincher, is a nocturnal species of hermit crab native to the West Atlantic (de Wilde, 1973). They are often used as pets in the United States and can be found for sale in beach hubs. The commercial uses of this species affect population density and can have ecological consequences (Calado, Lin, Rhyne, Araujo, & Narciso, 2003). Over the years, research regarding this crustacean's behavior has been limited. However, there remains much more about these creatures to be studied, given their wide ranging abilities in visual perception, and importance to general ecological health (Biagi, Meireles, & Mantelatto, 2006). Captive breeding has a very low success rate in repopulating *C. clypeatus,* and might be due to the insufficient availability of certain environmental stimuli outside of its normal habitat (Summers, Symula, Clough, & Cronin, 1999). Reproduction in many crustacean species relies on a number of environmental cues, one of the most important being visual stimuli of specific wavelengths of light (Summers, et al., 1999). It is not clear if this is the case for *C. clypeatus,* however, very few studies have reported its general behavioral responses to varying wavelengths or colors of light when in captivity. Thus, the current work sought to categorize C. *clypeatus* behaviors under varying light stimuli

Previous research has found that the visual spectrum of some species of crustaceans has a maximal range of 565 nm, and therefore would be unable to perceive longer wavelengths like red light (Goldsmith & Fernandez, 1986). The hypothesis is supported by the findings of Goldsmith and Fernandez's 1986 study of decapod and isopod photoreceptor response to varying wavelength of light stimuli. For example, in the decapod species *Porcellio scaber* the researchers found maximal sensitivity at 515 nm, or blue/green. In contrast to the idea that crustaceans are unable to perceive red light, Cronin and Harivama's 2002 findings suggest that the maximal range of visual perception in this taxa extends to 725 nm, that is well in the red part of spectrum. Cronin and Harivama demonstrated this range by employing a microspectrosotometry technique. Unlike the past studies that reported a narrower range of visual processing, this technique uses molecular genetics to determine the structure of opsin proteins that respond to light stimuli of specific wavelength ranges. Cronin and Hariyama reached this conclusion through suggesting that the previous works used a misleading technique in retinal extracts and that microspectrophotometry gave a more accurate depiction of the range of the crustacean visual spectrum.

Interestingly in the anomuran crustacean *C. clypeatus*, Ping, Sun Lee, Garlick, Jiang, and Blaisdell (2015) were able to elicit a hiding response suggestive of discrimination between shorter (blue) and other wavelengths of light. Furthermore, the researchers concluded that *C. clypeatus* is most likely more sensitive to color features and not spatial features. Ping et al states that most crustaceans possess photoreceptors that are maximally sensitive to blue/green light (480nm-540nm). Additional studies suggest that among the variety of crustaceans, crabs are ideal for physiological and behavioral studies involving light stimuli (Maldonado et al, 2002; Glantz & Miller; Cronin & Feller, 2014). Maldonado for example, studied habituation as memory (stimulus recall) and the context in which the recall occurred. The types of memory were respectively termed signal memory (SM) and context-signal memory (CSM). This study was done using a semi-terrestrial crab named *Chasmagnathus granulatus,* that is similar to similar to *C. clypeatus.* Habituation is a type of memory demonstrated by decreased responding to repeated presentation of a stimulus, in this case a specific parameter of the stimulus, which indicates memory of stimulus features (Maldonado, 2002). SM was defined as the behavioral response or lack of response to subsequent presentations of a stimulus sometime after its initial presentation. CSM occurs when there is a large gap of time, or rest between stimulus presentations. CSM learning is associative and long lasting, whereas SM learning is non-associative and intermediate. Maldonado altered light stimulus to create a shadow of a natural predator in order to test memory in terms of CSM and SM. The CSM is sensitive to a chemical mediated by the cyclic adenosine mono-phosphate (cAMP) signal pathway, necessary for learning and memory in both crustaceans and mammals. Maldonado (2002) and a similar study by Tomsic, Berón de Astrada, and Sztarker (2003) demonstrate that crabs can be a model for the type of neural and behavioral studies often conducted in mammals. However, in the case of *C. clypeatus*, characterization of behaviors under varying light conditions would provide a basis for testing additional hypotheses related to the neurobiology of vision.

Using light as a visual stimulus is of further interest because crustaceans in general have blue-light sensitive photoreceptors, a feature that lends itself to various types of behavioral studies. Findings by Glantz and Miller (2002), show that blue-sensitive receptors are part of a structure involved in the coding of moving versus stationary stimuli (2002). Light, for example, activates a specific set of crustacean photoreceptors to enable the appropriate behavioral responses. Consequently, crustaceans are best suited for visual ecological research because they possess one of the most diverse visual systems of any other animal group on the planet (Cronin & Feller, 2014). More specifically, as suggested by Cronin and Feller (2014), terrestrial crabs often use vision as a primary sense and are highly adaptable to the environment. The current work aimed to use a case study design to observe and categorize the hermit crab's behavioral responses to different light stimuli.

A case study design was used because past studies have shown it useful for expanding knowledge of animal behavior and conservation ecology. For example, a case study design used by Sherman, Semel, and Byers (1986) provided more efficient means for reducing brood parasitism in wood duck populations. By observing and measuring the effects of altering nest-box placement in wood duck, new methods were implemented that resulted in decreased predation and the removal of wood ducks from the endangered species list (Sherman, et al, 1986). Furthermore, classical conditioning and its effects on conservation efforts are highlighted by another ecological case study. McLean, Holzer, and Studholme (1999) conditioned predator avoidance in a species of robin that had no natural predators. The findings of this case study has led to better conservation efforts for some bird species. For example, conditioning predator avoidance in naïve species is essential to releasing a captive animal back into the wild. Previous case studies on crustacean have proven to be effective in ascertaining new knowledge on novel species. Gherardi's 2006 case study on *Procambarus clarkii* allowed for further insight on a species that was invading bodies of water in Spain. The case study found specific behaviors of that species that made it a successful invader.

Information from both Hazlett (1981) and Ping et al. (2015) in combination with pilot observations spurred a crude ethogram and designation of certain light conditions in the experiment. Hazlett identified *C. clypeatus* as a slow moving, nocturnal crustacean that mostly hid during the day (1981). Ping et al. found that blue light triggered more hiding behaviors (2015), and our pilot observations revealed that *C. clypeatus* tended to eat longer during blue light exposure. Given these observations, not only did we expect to see these findings, we expected to see other behaviors that were indicative of nocturnal behaviors. Given that *C. clypeatus* is nocturnal, it would make sense for the blue light to act as a cue for sleep because of this we included red light and absence of artificial light as testable conditions. Absence of artificial light allows the crab to habituate back to baseline behaviors where it was not exposed to any artificial light in its environment. Red light acted as a condition on the other side of the electromagnetic spectrum. Furthermore, these observations led to a categorization of four types of behaviors for the ethogram: active, exploratory, stationary, and feeding. These were umbrella categories that contained sub-behaviors. For example, feeding was comprised of eating food and drinking water, as they were recorded as separate behaviors, but under the same category. This current study serves as a preliminary work to support future studies of light stimuli on crustacean behavior, specifically *C. clypeatus*.

Method

Animal care and use

In order to test the hypothesis that blue light increases nocturnal behavior in *C. clypeatus*, one male crab of indeterminate age was obtained from the Mount Airy carnival ground in Maryland. The crab was housed at room temperature, roughly 17-22.2 °C in the Cuvilly Faculty Exchange at Stevenson University. Following two weeks of habituation to the environment, observations of one male crab's response to altering light stimuli were conducted over the course of eight days. Crab habitat consisted of a 12.3L x 12.3 W x 10.25 H plastic/wire terrarium with approximately 3 inches of coconut fiber substrate. Sponges within the habitat were moistened daily with a spray bottle containing de-ionized double filtered water. Food was available *ad libitum* and restocked daily. Daily food consisted of 0.5 grams of All Living Things © hermit crab food pellet, one mealworm, and 3.5 grams of fresh apple slices. Distilled water was also provided in a dish that was filled every day at the same time as the food. The crab was also weighed daily as a measure of health monitoring using an American Weigh Scales © (USA) black blade digital pocket scale. Humidity was kept between 95 and 99 percent with temperatures ranging between 17-22.2 °C and monitored using an Acurite © (Wisconsin, USA) indoor temperature and humidity monitor. Data collection began immediately after the two-week habituation period.

Video recordings and stimuli

Recordings of behavior were done using a Canon © Vixia HFR20 (Tokyo, Japan) camcorder overnight that was positioned directly in front of the terrarium allowing the entirety of the circular habitat to be in view. Behavior measurements consisted of three and a half hours of continuous sampling of video recordings of the crab under three different conditions of light stimuli. White incandescent lighting during daylight hours and no lights after 6 p.m. served as the control stimuli (baseline). Baseline behaviors were measured to track possible order effects of the colored lights. Two consecutive days were assigned for observation in the presence of blue light, which consisted of 25-watt incandescent blue colored light (450-495 nm) after 6 p.m. Following the two days of blue light, the crab was exposed to two consecutive days of no light to allow for a return to baseline between stimuli. The two day period with each condition was chosen to ensure that a behavior was not unique for one full circadian cycle (24 hours). Following that return to baseline, the crabs experienced two consecutive days in the presence of a 25-watt incandescent red light bulb (620-750 nm). These were also recorded after 6 p.m. and measured after observation of the second baseline behaviors. A general ethogram, time budget and frequency of behaviors were composed based on the behaviors observed between 6 and 9:30 p.m. under each light condition.

Data analysis

Categories of crab behavior were constructed based on pilot observations, Ping et al.'s findings, and Hazlett's findings. Frequency of behavior was calculated through marking on paper each time the crab engaged in a new behavior in the video recording then summed for a total frequency per behavior category. To determine how long the crab engaged in each behavior a stopwatch was engaged at the initiation of a given behavior, and stopped when the behavior ended each time it occurred. A total duration in seconds for each type of behavior was calculated by summing the duration of each instance of a given behavior per light condition. The sums for each frequency and duration measure was averaged per light condition. These methods allowed us to prepare an ethogram that shows frequency of occurrence, and duration of each behavior under the various light conditions. The data consisted of frequency and duration before blue light exposure (baseline blue), before red light exposure (baseline red), during blue light exposure (blue light) and during red light (red light). We tested for differences between each baseline measure to check for order effects of the colored light exposure.

The individual measures of behavior did not differ significantly over the no-colored light or baseline days (data not shown). Frequency and duration of each behavior were listed in an ethogram and calculated before blue light exposure (baseline blue), before red light exposure (baseline red), during blue light exposure (blue light), and during red light exposure (red light). Average of the baseline measures were collapsed into an overall baseline because the individual measures did not differ significantly over the no-colored light or baseline days (data not shown). The average occurrence of each category of behavior (frequency) under the different light conditions and the overall duration of active behaviors (all behaviors excluding stationary) under each light condition were calculated. Stationary behavior was a category excluded from the calculation of overall active behaviors to avoid misrepresenting data by double counting. For example, *C. clypeatus* remaining stationary while feeding would not count as both stationary activity and feeding activity. For the purpose of this study, the behavior would be designated as feeding. As the ethogram shows (Table 1), during the first 3.5 hours after 6 p.m. the crab engaged primarily in four main categories of behavior: exploratory, active, feeding, and stationary. These categories were further broken down into more specific behaviors but they did not differ significantly in frequency. For example, exploration of shell and exploration of substrate were both categorized as exploratory behavior. All statistical analyses were conducted using SPSS version 23 by IBM Analytics (Armonk, New York). One-way ANOVAs were used to determine whether the average frequency of occurrence in each category of behavior differed under each light condition. One-Way ANOVAs were also used to determine the differences in average duration for each category of behavior. Statistically significant ANOVAs were followed-up with LSD post-hoc tests.

Results

The duration of each category of behaviors had a slightly different pattern. Mean duration of exploratory behaviors during baseline was 9 seconds (*SD*=18), 31 seconds during blue light (*SD*= 43.84), and 275 seconds during red light (*SD*=388.91). Duration of stationary behaviors was greatest under red light: the baseline mean was 4228.75 seconds (*SD*=3343.61); the blue light mean was 4090 seconds (*SD*=2390.02) and duration of stationary behaviors under red light revealed a mean of 4844.50 seconds (*SD*=3331.18). These behaviors, however, varied non-significantly. For example, the duration of active behaviors revealed a baseline mean of 1592.75 seconds (*SD*= 2045.34), a blue light mean of 1058.50 seconds (*SD*=959.54), and a red light mean of 1830.50 (*SD*=2588.72).

Although the smallest percentage of overall time recorded was spent feeding (data not shown), the duration of feeding behavior varied significantly [F(2,5)= 15.13, p= .008)] (see Figure 1). Except for this feeding behavior, all other categories of behavior did not differ significantly in duration. Weight was also recorded to determine whether some behaviors were a result of changes in feeding or illness resulting in weight change. Despite a small variability in weight over the course of eight days, the day-to-day differences were not statistically greater than chance (see Figure 2).

Discussion

In support of our hypothesis, feeding behavior increased significantly from baseline under blue light, but did not differ from baseline when red light was present. One explanation for these data is that that perhaps *C. clypeatus* does not possess a visual spectrum that expands beyond 620nm as proposed by Goldsmith and Fernandez (1986). If this is the case, red light and baseline behaviors would not differ significantly and perhaps *C. clypeatus* would not be able to detect longer wavelengths. Perhaps the need to detect ocean water using both visual and olfactory systems makes detection of wavelengths of the blue/green range adaptive (De Wilde, 1973; Greenaway, 2003; Hazlett, 1981). Wavelengths that extend into the blue/green range could reasonably be expected to serve as salient signals for survival behavior; for example, nocturnal feeding. Thus evolution would favor blue photoreceptors and nocturnal behaviors in this species. Additionally, Hazlett's 1981 report suggested *C. clypeatus* tends to be more active at night, remaining mostly buried in the sand except when eating or to evade predators during the day, supporting the adaptive value of blue light discrimination.

Because there were no other behavioral differences during exposure to blue and red light it is possible that *C. clypeatus* could detect red light as proposed by Cronin and Hariyama (2002). Perhaps *C. clypeatus* eats less in red light because it can distinguish between a signal related to survival (blue) and one that has no adaptive or maladaptive effect (red). Consequently, the behaviors under red light would not differ from baseline. In order to test this idea, researchers should condition *C. clypeatus* to associate red light with food. Afterwards, a discrimination task could identify whether they retain the association through detection of red light.

A limitation of the study included using a single male crab which does not allow us to generalize the findings as being typical of the species. Using a single crab also did not allow us to study social behaviors which are essential to *C. clypeatus*' functioning in the natural world (De Wilde, 1973; Hazlet, 1981). Furthermore, using a small terrarium that is not typical of their habitat could have confined the crab to certain behaviors. Because of the study utilizing an ethogram which was tested for the first time in a case study design we were not able to test the ethogram's validity to the fullest extent. In order to address these limitations, future studies should use more crabs in a larger habitat. Additionally, these studies should observe social behaviors in relation to varying light stimuli.

The current finding in relation to blue light feeding behavior confirms research by Ping et al. (2015), which suggests blue light stimuli increases survival behaviors, such as hiding, in *C. clypeatus*. These data support the need for further research on *C. clypeatus*, particularly because their social behaviors are a function of environmental conditions (Goldsmith & Fernandez, 1968). Because we examined a single individual in captivity under the varying light conditions, studies should continue to examine the relationship between *C. clypeatus*' behavior and blue light stimulus. A careful study of the relationship between light exposure and behaviors in a larger sample will help us to determine whether the behaviors observed under the varying light stimuli are unique to this individual or is typical of the species. This follow up study will allow the current work and Hazlett's 1966 work on social behaviors to be expanded, specifically on visual discrimination in adaptive behaviors. Furthermore, methods could be developed on standardizing measures to gauge variables related to *C. clypeatus*' behavioral ecology. This could also have implications for understanding visual perception in anomuran crustaceans.

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Appendix

Table 1

Descriptive Ethogram of C. Clypeatus

Behavior	Operational Definition	Baseline Occurrence (Frequency)	Blue Light Occurrence (Frequency)	Red Light Occurrence (Frequency)	Category Statistical Analysis
Exploratory Behavior					
Explored Shell	The crab approached various shells and touched them with its antennae and scraped it with its claws.	3	2	2	NS
Explored Substrate	The crab stood in the substrate and alternatively stamped its claws into the ground.	-	-	-	NS
Explore Sponge	The crab approaches a moist sponge and feels it with its antennae.	1	1	3	NS
Active Behavior					
Carried Sponge	The crab placed its claws into a moist sponge and moved it.	-	-	-	NS
Climbed Cage	The crab grasped onto the cage wiring and walked around it.	7	5	6	NS
Changed Shell	The crab latches onto another shell, moving its body in and out of the new shell, resulting in swapping of the shells.	-	-	1	NS
Feeding Behavior*					
Eat	The crab grasps food in its claw and brings it towards its mouth.	1	3	3	NS
Entered Water	The crab walked on top of its water dish and walked into the water.	-	2	-	
Stationary Behavior	The crab remains in one place		5		NS

The asterisk (*) represents overall occurrence of feeding behaviors, which is the sum of eating and drinking. There was a non-significant trend toward increased feeding behaviors (*p*=.19).

Figure 1

Mean Duration of Feeding Behavior in Seconds

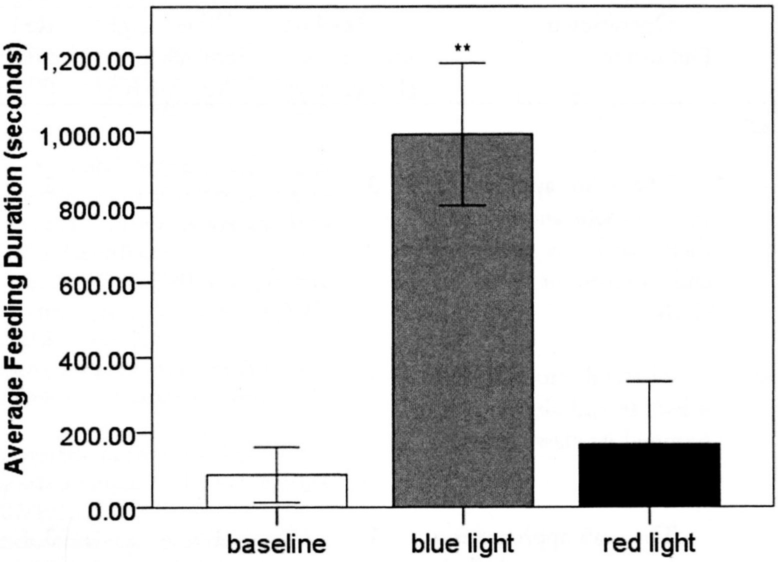

Figure 1 Analysis of the average duration of feeding behavior under three different light conditions. The error bars represent 2 +/- standard error of the mean. The double asterisks (**) represents the probability results are likely due to random errors (*p*=.008).

Figure 2

Weight of Coenobita clypeatus Throughout the Study in Grams

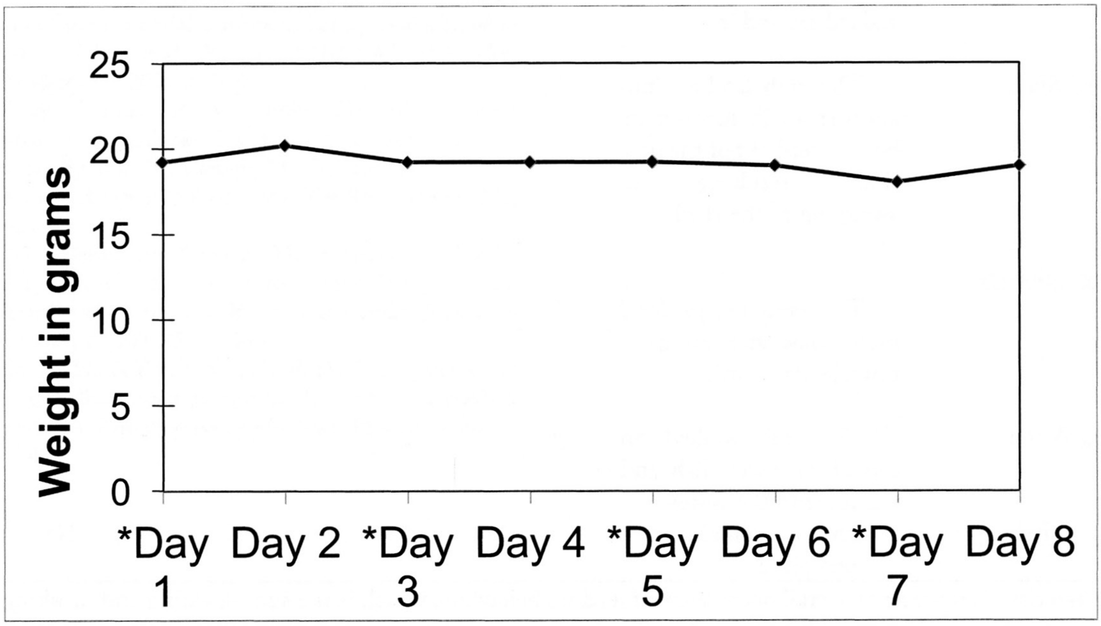

Figure 2 Daily weight outside of the shell over the 8-day course of the study. The single asterisk (*) represents days in which C. clypeatus was exposed to either blue or red light. Day 1 and Day 3 consisted of blue light; Day 5 and 7 consisted of red light. Unmarked days represent baselines days where the crab was not directly exposed to any colored light. The slight variability of day-to-day weight did not differ significantly (p>.05).

Figure 3

Coenobita clypeatus during various light recordings

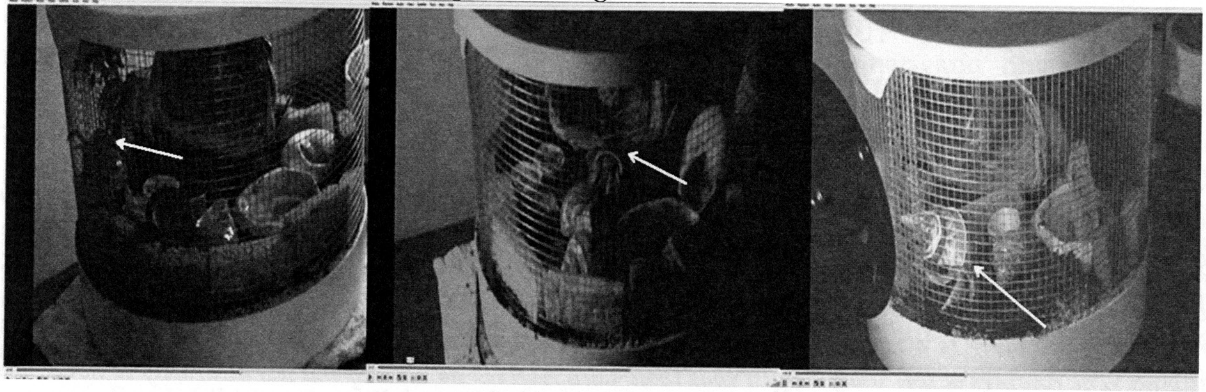

Figure 3 Snapshots from video recordings of *C. clypeatus* under different light conditions. The left panel points to the crab under no artificial light, the middle panel points to the crab under red light, and the right panel points to the crab under blue light.