April 2010

Crayfish Behavior and Differential Response to Environmental Conditions

Jerrod P. Oltmann
Worcester Polytechnic Institute

Repository Citation
Oltmann, J. P. (2010). *Crayfish Behavior and Differential Response to Environmental Conditions*. Retrieved from https://digitalcommons.wpi.edu/mqp-all/2005

Crayfish Behavior and differential response to Environmental Conditions

A Major Qualifying Project Report

Submitted to the Faculty

of the

WORCESTER POLYTECHNIC INSITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

By

Jerrod Oltmann

Date: April 29, 2010

Approved:

Abstract

The East Brimfield Damn provides a useful location to study the behavioral characteristics of *O. Limosus.* Samples of *O. Limosus* were collected and studied *in situ* as well as in the lab for different behavioral responses related to crayfish movement within the East Brimfield damn area. In situ crayfish movement within the stream was observed to determine general movement within the stream. Lab work was used to study the more basic crayfish response to differential coloring of their environment and burrow preference.

Acknowledgements

The author sincerely thanks his advisors Professors Mike Buckholt and Professor Lauran Mathews for their time, continued guidance and infinite patience during this project.

1. Introduction

The study of the habitat and movements of animals within their environment are of vital importance to ecology. Knowledge of microhabitat selection and movement preferences aids in understanding the place of organisms in their larger ecological context (Bencenuto et al 2008) (Morales et al 2008.) Without such knowledge misunderstandings of their behavior and how they interact with their environment can easily appear. Research, specifically in the area of habitat selection, aids in the understanding of not only the individual organisms but how they can potentially interact with other organisms (Bencenuto et al 2008.) This is particularly true for crayfish as they can be a keystone predator in many ecosystems and the presence of keystone predators can have a large affect on ecosystems (Crandal and Buhay 2007)(Smith 2006) For example one of the mechanisms through which the shell color of crayfish can change is related to their diet(Ghidalia 1985.), this has also been documented in other arthropods(Shmalhofer 200). Crayfish will consume organisms ranging from plant matter to macro-invertebrates and fish, scavenging whatever they find (Paloma et al 2004.) This fact coupled with being geographical wide spread makes their study important for understanding the water systems that they populate (Crandal and Buhay 2007.)

1.1 Microhabitat Selection

Microhabitat preference can be of key importance to species because it can a direct bearing on the survivability of individuals in a population (Morales et al 2008) (Collins et al 2009). Furthermore, the selections that individuals make show the criteria they prefer (Collins et all 2009.) Does natural selection select for individuals in a population that prefer a hot or cold environment or does natural selection select for a trait that conveys the ability to detect water content in soil? These types of selections made on an individual basis can have a significant bearing on an individual’s ability to gather resources find suitable mates, and produce fit offspring.

Microhabitat preference and its related factors are a particularly difficult area of scientific study. Attempting to quantify the actions of even some of the simplest organisms can be particularly challenging because of the scope of the uncontrollable variables in such research. Furthermore attempting to discern by what criteria an organism prefers one area or another is even more abstract and challenging. One of the ways to narrow down and focus such research is to look at the physical capabilities of the organism. Where an organism preferentially resides given the choice of distinct microhabitats is determined by its physical capabilities and sensory modes. If an organism cannot navigate or survive its surroundings then it is not likely to reside in that area. On the same note, if an organism’s particular set of adaptations does not suit it well for a certain area, over time a population of organisms may evolve a negative preference for that area or perhaps evolve new adaptations to the environment. It is because of the potential for naturally selective forces to change the physical makeup of an organism over time that these traits can be useful for their study and for inferring microhabitat preference.

An example of just such an occurrence can be found in recent research into crayfish by Stein and Magnuson (1997). Two distinct groups of individuals emerge under predation by bass in a river. A small malnourished group lives on the edges of a river and remains hidden in the local flora and a larger better fed group congregates in the center of the stream (Stein and Magnuson 1997). Each of these groups represents a different selection for microhabitat that has direct bearing on food and mate availability. Stein and Magnuson(1997) hypothesized, given their analysis of the gut contents, that the quality of food on the edges of the stream was much poorer however the protective cover present on there was superior. They came to this conclusion because the gut content on crayfish living there primarily consisted of plant mater as opposed to the gut content of crayfish in the center of the stream which had a higher density of animal mater. Furthermore the crayfish with the plant matter gut content appeared smaller and sicklier. In this case the preference for a safer environment had a direct consequence on the fitness of an organism. Research has also shown a correlation between female size and egg productivity in crayfish. This research combined with that of size differences between center and outer stream crayfish suggesting a potential for a direct link between microhabitat preference and the fitness of an organism (Ivanova, M.B. and Vassilenko 1987.) In this case larger well fed female crayfish have the potential to be more productive.

1.2 Environmental Factors and Physical Adaptations

Organisms in their natural environment are subject to many different environmental stimuli and restrictions. Furthermore overtime they can become adapted to their specific conditions. Research has also shown that not only do physical adaptations and heritable traits allow for better survival in their native environments but old traits can be provide increased fitness in new environments in unexpected ways. Gherardi and Barbaresi (2000) showed in their research that crayfish can move over long distances to new and unpopulated areas allowing them to better utilize clustered resources that are geographically separated. Furthermore, crayfish who survive low mortality floods can be displaced both up and downstream to areas that may have been previously inaccessible to them (Momot 1966.) Other studies have shown increased movement in similar high water flow events though not necessarily related to passive movement by the running water (Damient et al. 2000). Furthermore crayfish caught in seasonal dry spells can use hyporheic zones, areas of sediment through which a local body of water readily exchanges, to stay alive until the next wet season (Distefano et al. 2008)

Morphologically and phylogenetically similar organisms to crayfish show interesting adaptations to their environment that can affect range and microhabitat selection. Lobsters have been shown to orient downstream with the current and are morphologically streamlined for downstream movement (Neil and Chapman 1988.) In addition when exposed to running water they also have a tendency to orient downstream (Neil and Chaplman1988.) Adaptations such as those of the crustaceans in Neil and Chapman's research are likely indicative of specific sensory modes as the Lobsters were blinded during the orientation trials. In this case those modes are relevant to detecting the movement of water and an organism's orientation to it. Wiese expanded this understanding by showing specifically that crayfish have movement plane restricted hair follicles that detect water movement in specific directions(see Figure 1) (Wiese and Kennedy 1976.)

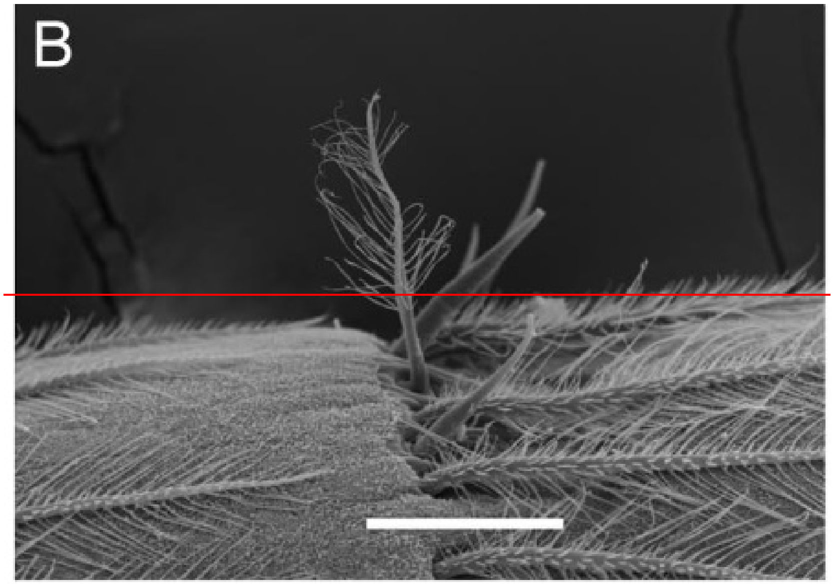

Figure 1: A Plane Restricted Hair Follicle of Procambarus clarkii. (Mellon and Christison-Lagay 2008)

1.3 Color Morphology

In addition to sensory adaptations organisms often show adaptations such as camouflage, fight or flight mechanism or increased mobility. Lobsters, for example, have been shown to exist in very different color morphs (Herrick 1895) and individual crayfish have been seen in different color morphs (Newcombe 1929) (Penn 1951)(Smiley and Miller, 1971) as well as in specifically black and white morphs (Dunham et al., 1979.) These color morphs are important to understanding animal behavior because they can be specific adaptations to local conditions or the result of some other processes such genetic recombination. Crayfish, for example, have a wide variety of color combinations depending on species and the local environmental conditions of the population (Thacker et al. 1993) (Ghidalia 1985.)

The color of other hermit crabs has even been shown to be dependent on diet (Ghidalia 1985.) In addition differences in crayfish color in the field based local environmental pressures (Kent 1901). Furthermore Hermit crabs have been shown to exhibit similar environmental color changes (Hazlett, 1966.) To understand these types of variations research needs to include an understanding of the root causes of color variation. Given research such as Ghidalia's (1985) the assumption cannot be made without evidence that crustacean color morphology is purely genetic, in that the exact color is inherited and solely expressed as single color that is acted on by natural selection. Furthermore research has shown that color can also vary with age (Bessinger and Copp 1985.) Significantly different findings included in both Ghidalia (1985) and Bessinger and Copp (1985) about the root cause of color variation require careful study of organismal behavior control for many different factors to prevent incorrect conclusions from being drawn about color morphs. The genetic findings of Ghidalia(1985) and the findings relating color to age in Bessinger and Copp(1985) could potentially both act on organism or perhaps a single or it could be a completely unknown factor. Further obscuring understanding of color morphology are the results of research relating to encounters between crustaceans. Van der Velden(2008) showed that crayfish encounters between individual crayfish *(Cherax destructor)* can be affected by prior encounters depending on color configuration. It is hypothesized that this is related to pattern recognition in crustaceans and other similar organisms. This hypothesis is furthered by the research on *Calcinus laevimanus* that showed that visual cues in decapods can be important for initial posturing before encounters. *Calcinus laevimus* a species of crab has evolved white spots on their chelae which based on relative size between two individuals affect agonistic encounters between crabs. The white spots themselves were shown to be the deciding factor since when they were covered the organisms no longer seemed to have the advantage in encounters and when painted on the crabs benefited as if they had been their naturally (Dunham D 1978). In addition Fiddler crabs show preference for approaching cospecifics with unfamiliar markings (Detto et al 2006) and *Potamon fluviatile* interactions with others can be modified by using the identity tag of (subject two) on a different individual(subject three) in later encounter with the same initial viewing individual(subject one) (Vannini and Gheradi 1981.) Related research has also shown that fiddler crabs can change their color on socially significant time scales when they molt (Detto et all 2008) These types of research indicate that color patterns can be very important not only as camouflage but also as a means to establish dominance and social interactions between organisms. See figure two on the next page for an example of degree of difference that can exist between crayfish different morphs.

Figure 2: Blue Color Morph

An example of blue color morph of *Pacifastacus fortis* juxtaposed next to a more normal variant (Doug Jensen 2009)

1.4 Visual Preferences

In particular the study of responses to visual cues can be of particular interest considering the abundance and the complexity of the sensory modes that have evolved in organisms to process them. The eye for example is present all across the animal kingdom and is thought to have independently evolved somewhere on the order of 50-100 times (Haszprunar 1995.) These analogous developments coupled with the early homologies from common animal ancestry make the study of visual stimuli and animal responses to it interesting for comparisons across species.

Crayfish eyes seem to be the result of an earlier radiation as they show little variation in sensitivity or lambda max among different species or different environments. Most crayfish spectral sensitivity lies between 350-620nm (Crandal and Cronin 1997.)

Research has also shown that less complex nature of the crayfish eye a useful model research system. This same fact however requires precise research into its exact functioning before hypotheses about behavioral patterns can reasonably be drawn. Some basic research has been done in the area of prey detection and the reflex responses related to visual stimuli. Crayfish have been shown to react towards visual stimuli of prey fish swimming in front of them (Hernández et al. 1999.) Furthermore the visual abilities of a crayfish also involve adaptations to polarized light that involve defense mechanisms and optokentetic responses (Glantz 2008.)

1.5 Crayfish, *Orconectes,* and *O. Limosus*

The use of crayfish for studying microhabitat selection can be particularly convenient due to their ready availability, ease of care, and limited regulation of their use as compared to larger vertebrates and their diversity. There are currently at least 540 species of crayfish that have been identified throughout the world. Each of these 540 crayfish can be divided into one of three families within the taxon Decapoda. The three families are *Astacidae*, *parastacidae*, and *Cambaridea*. All three are families of freshwater crayfish. *Parastacidae* is present in the southern hemisphere, while *Astacidae* are native to western North America and Europe. The last family *Cambaridae* lives east of the continental divide and contains around 390 crayfish species which breaks down to 75% of all crayfish species(Taylor et. al., 2002), included in that family is the Orconectes Genus and O. *Limosus*(see figure 3).

The *O. limosus* is found in freshwater bodies such as lakes, reservoirs, and streams. They also tend to burrow or hide under rocks or other obstructions to avoid their natural predators (Rhoades 1962.) Sexually mature males change between two forms: breeding (form I) and non-breeding (form II) (Rhoades 1962.) Form 1 is primarily adopted during mating season and form two is adopted after the season is complete (Hobbs 1991.) Male maturity can be recognized by presence of gonopods which prior to form I would have been the first pleopods (Hamr 2002.) Female maturity may be identified by the presence of eggs affixed to the pleopods and the existence of glair glands on the uropods (Hamr 2002.)

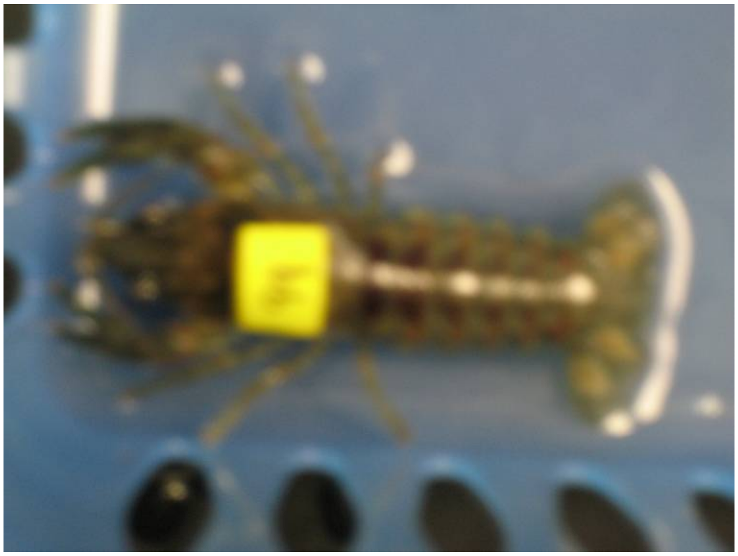

Figure 3: Dorsal View of Tagged O. Limosus

Orconectes limosus is particularly interesting for such investigations because it is currently all through the eastern seaboard from Maine to Virginia (Fetzner, J. 2006.) *Orconectes limosus* in particular has also been introduced to Europe as invasive species and is currently wreaking havoc with local populations (Lodge et al, 2000) Studying its movement patterns and the habitats it selects provides ample opportunities for studying the criteria that are used for selection and how they change as new factors are presented. The spread of a single invasive species allows for a greater understanding of its preferences. Not only can its current preferences be studied but also the new habitats it may have been previously adapted to and the range of habitats it is capable of surviving in. Lastly their relative simplicity also makes them an ideal model organism for study because the similarities in behavior between crayfish, crustaceans and some arthropods provide the potential for experimentation with one species to have broader significance than just the specific actions of *O. limosus*.

1.6 Experimentation

In this study microhabitat and movement characteristics of *Orconectes limosus* were explored. These characteristics were studied through the application of both field and lab experiments. The lab experiments consisted of testing the substrate color preferences of *Orconectes limosus* and the field experiments attempted to track its movement throughout a stream in its natural habitat.

Several discreet hypothesis were be tested in the lab experiments, that *Orconectes limosus* has a statistically significant preference for either a black or white colored bottom, that there is a statistically significant correlation between the physical characteristics (sex, length. color of *Orconectes limosus*) and its behavior(percent of total time spent on white substrate), and that there is no statistically significant change between *Orconectes limosus* shell color before and after the experiments. The field experiment tested the movements of Orconectes *limosus* it natural habitat and particularly where it moves within the stream. Does it tend to move up or downstream or stay in the same general area? This will be primarily explored through radio tracking but baited traps will be used to collect further evidence. I hypothesized that crayfish would preferentially move dove stream due to the current.

2. Materials and Methods

2.1 Collection of Animals for Laboratory Experiment

A total of 60 *Orconectes Limosus* were analyzed over the course of the laboratory experiments. All the organisms were gathered over the course of the prior year and half at the East Brimfield Dam, a United States Army Corps of Engineers site in Sturbridge, Ma (see figure 4) located 42.109175, -72.126274.

Figure 4: Figure 4 is an aerial view of the East Brimfield Dam and the river that flows below it where all crayfish field experiments were performed (Google Earth *42.109175, -72.126274* 2010.)

Crayfish were caught by hand by and transported back to the lab in ~20 liter buckets filled with ~10 liters of water for further study. Once back at the laboratory they were separated by sex and size to prevent mating and harmful agonistic encounters. All specimens caught were in Form I (reproductive) morphology. All individuals were kept either in ~15.30x30.50 centimeter plastic or glass enclosures or ~46x61 centimeter plastic bins filled with tap water and fed a continuous air supply through electric air pumps. The in ~15.30x30.50 centimeter plastic housed up to two crayfish of the same sex and ~15.30x30.50 centimeter enclosures housed up to six. All species were separated by sex in their enclosures. Water was changed at minimum on a weekly basis and crayfish were fed a compacted shrimp pellet diet of ~3-4 pellets per crayfish after water changes.

2.2 Substrate Preference Experiments

O. Limosus were placed in ~35 Liter tanks filled to approximately ~17.5 liters with water from the unfiltered lab tap. Each tank setup consisted of a tank and pieces of laminated black and white construction paper of equal size splitting the tank (see Figure 5). Hitachi DVD Cams (product code QL31212) were placed overlooking the tanks approximately 198 cm above the bottoms of the tanks. Crayfish were allowed to roam the tank for one hour of recording and then were removed. The tanks were cleaned and the water was changed between trails.

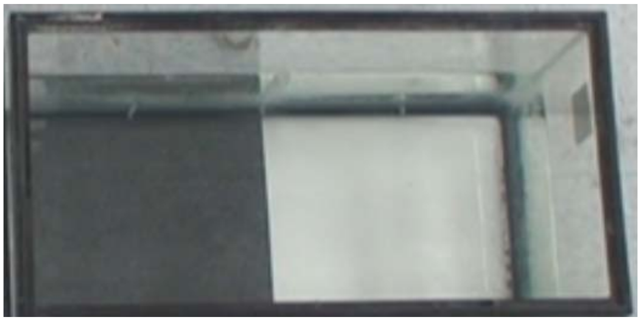

Figure 5: Substrate Preference Experiment

This is a picture of the setup of the substrate preference experiment. The setup consists of a ~ 35L tank filled to ~17.5 liters. The black and white substrates were placed underneath the tank to provide the visual cues for the experiment.

Calipers with an electronic digital display (see figure 7) were used to determine carapace length (+/- .5 cm) (see figure 6). In addition photographs were taken in triplicate before and after the experiments to ensure consistency in shell color before and after the trials and to quantify shell color. Photographs were taken at a distance of 30.48 cm above the crayfish under standard light conditions and auto zoom was used as necessary to ensure clear pictures were taken.

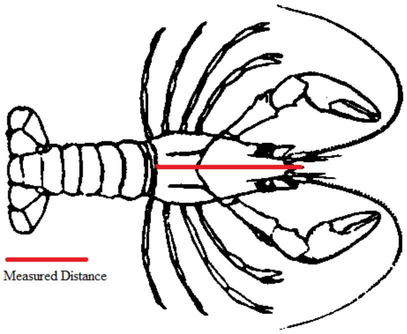

Figure 6: Measured Section of Crayfish Shell

Figure 7: Electronic Calipers

The photographs of the crayfish were then cropped to a ~1.5x1.5cm square section posterior to the eyes (see Figures 8 and 9). Pictures were then analyzed with a color averager in the imagemagick tool set to quantize their color characteristics (The imagemagick software set is available on most operating systems.) The function of the color average is to take the HTML color values of each pixel of a picture and average them together. This conceptually produces the same effect as if each pixel has been overlaid over every other pixel. The average color of the cropped areas was determined. The data was then converted to grayscale. This made the data a representation of relative whiteness or darkness of the total picture expressed as a percentile white, where 0% equals white and 100% equals black.

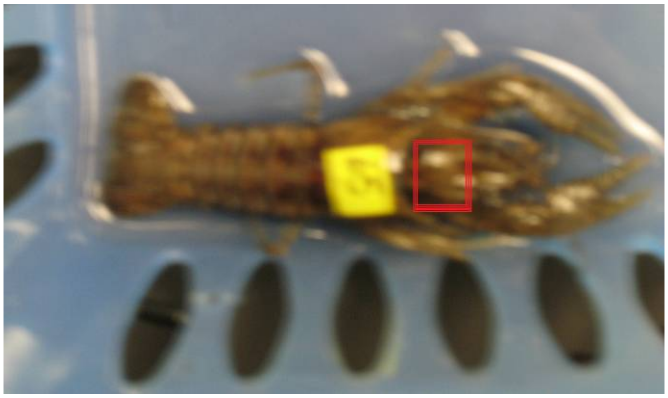

Figure 8: Analyzed Section

The area boxed in red outlines the area taken for analysis from each picture.

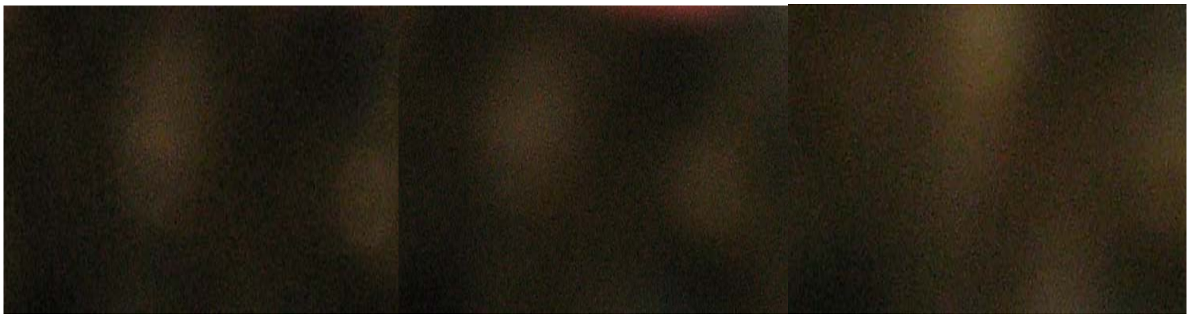

Figure 9: Cropped Crayfish Pictures

An example of three cropped pictures used for the analysis of crayfish color. Each picture is separate shot of the same crayfish and shows some the changes that can occur from lighting changes or other subtle shift in the work area.

2.3 Baited Trap Experiments

Trapping Sites

Traps were set along the river directly below the East Brimfield Dam at regular twenty meter intervals (see figure 10 below).

Figure 10: Aerial view of *trap locations at the East Brimfield Site(Google Earth* 42.109175, -72.126274 *2010*)

ID Tag Setup

Each was baited with raw chicken purchased from local stores. The traps were checked on a weekly basis and any crayfish caught were taken out and tagged with super glue and electrical tape. A small section of electric tape ~1.5x1.5cm was prepared for each crayfish with a unique identifier written with a Sharpie marker (see figure 11). Each tag was then cut from the role and then a small amount of superglue was dropped on the back of the tape and then it was placed carefully on the carapace of the crayfish.

Figure 11: Example Crayfish Electric Tape Tag

Careful attention was payed to make sure not too much superglue was applied. Liberal application has the potential to drip and can potentially inhibit the subject's ability to move. Each crayfish was given a unique code on their tag for future identification consisting unique number to identify the crayfish as well as a symbol such as a star or square for easy trap recognition. The crayfish were released back into the same spot they were caught once the glue set (~20 minutes) but outside the trap.

Traps

Traps were then reset an hour after the crayfish were released into the stream with fresh bait and were left again for a week. Each trap was weighted down with several stones so that the current would not displace the traps (~2 kg.) The traps functioned by providing a ramp up to a hole where the crayfish could enter but not climb back out (see figures 12-14).

Figure 12: End View of Trap

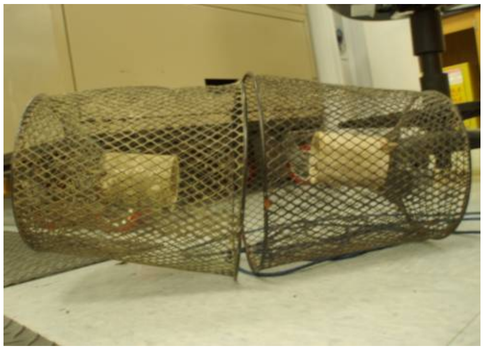

Figure 13: Side View of Trap

Figure 14: Securing Clip for Both Ends of the Trap

2.4 Radio Tracking

Crayfish were caught at the East Brimfield Dam starting in early November and radio tracking tags were attached to the dorsal carapace (see figure 15.) The glue was given a half hour to cure and then the crayfish were released back into the stream at the same location they were trapped. The body of each tag was ~1.75cm long by ~1.25cm wide. The antenna on the back of the tag extended approximately ~7.5cm behind the body of the tag. The tags transmitted on channel 93 tune 7, channel 95 tune 6, and channel 94 tune 8. Their location was recorded over several weeks with the aid of the radio antenna equipment designed for the tags (see figures 15-19). Their location was taken on Tuesdays and Thursdays for four weeks.

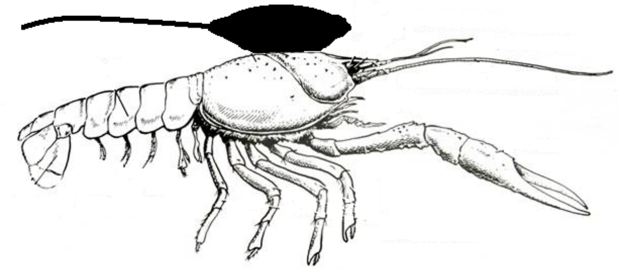

Figure 15: Diagram of Radio Tagged Crayfish

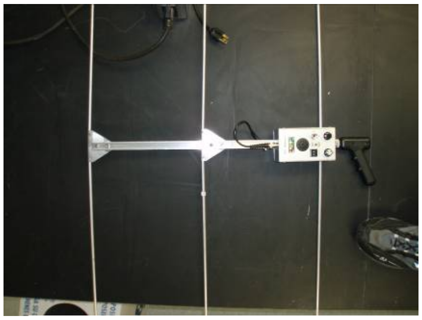

Figure 16: Radio Receiver

Figure 18: Close Up of Signal Strength Output Meter

Figure 17: Close Up of Radio Control Box

Figure 19: Close Up of Control Panel

2.7 Data Analysis

Microsoft Excel 2007, the Open Office Spread Sheet program and SPSS were used to organize data and run all statistical tests. SPSS 15 was used for the more advanced statistical procedures such as the paired T test and the regression model. A paired T test was used to determine if there was consistency between the before and after pictures of the crayfish. The average color values of three before and three after pictures for each of the 61 samples in the group were compared for this analysis. In addition a multiple regression model was constructed to model the data for the experiments. The percent time spent on the white substrate, sex, length, and percent white of the cropped crayfish pictures were all included in the model as well as all interaction effects. After the full model was run, non-significant factors ($p>0.05$) were removed. One by one to determine which factors yielded the model with the best possible fit to the data. Initially a linear model was used but the Pearson correlation indicated that an exponential model would be better suited to fit the data. No analysis of the radio tracking data or baited traps experiments was possible as not enough positive data was collected to provide the power needed to run any statistical tests.

3. Results

3.1 Substrate Preference experiment

The first step in the analysis was to determine if the data could be compared in a statistical manner. All of the subjects before and after photos were averaged together to output a single color value from the six individual color values. Those two populations of new values were then compared by a paired T test to ensure that there were no changes in the shell of the subjects between the beginning and end of the experiments. The paired T test yielded a p value of .431 indicating that there was no statistical reason to reject the null hypothesis that the before and after colors were statistically similar populations (t=.818182; DF=60; p=0.431.)

The color of each crayfish was determined by averaging the two populations of before and after photos into a single value representing the best estimate of the crayfishes' color averaged over six photos. The results could be pooled in this manner because of the results of the paired T test. The T test indicated that the populations were statistically similar and there for there is no reason not to average all the results to acquire a more precise color value.

Furthermore a T test was performed to compare male and female populations of crayfish to identify if there was a significant difference between male and female populations. The T test yielded indicated that there was no statistical reason to reject the null hypothesis that the male and female populations were similar (t=.818182; DF=60; p>.05)

Substrate preference was found to correlate inversely with color. The larger the percentage of white in the cropped pictures of the crayfish shell the more likely that crayfish was to spend more time on the black substrate(see figure 18).

The initial model that was developed used sex, shell color, and length as possible predictors of the percent of time spent on the black side of the tank. Possible non-linear interactions between these variables were also considered by incorporating all possible pairwise products and the product of all three independent variables into the model, which yielded a total of 7 independent variables in the model. Because sex was a categorical variable it was coded as -1for males and 1 for females. By recursively removing the least significant factor from the model only significant factors remained. The final model at this point was a linear model where only shell color was a significant predictor for the percent of time spent on the black side of the tank. Neither sex nor length was found to significantly affect this behavior, therefore separate predictive models based on sex was considered unnecessary.

At this point it was concluded that there was insignificant evidence to conclude that the data fit a linear model. The model that best fit the data was found to be an exponential model rather than a linear one due to the Pearson coefficient (-.064)

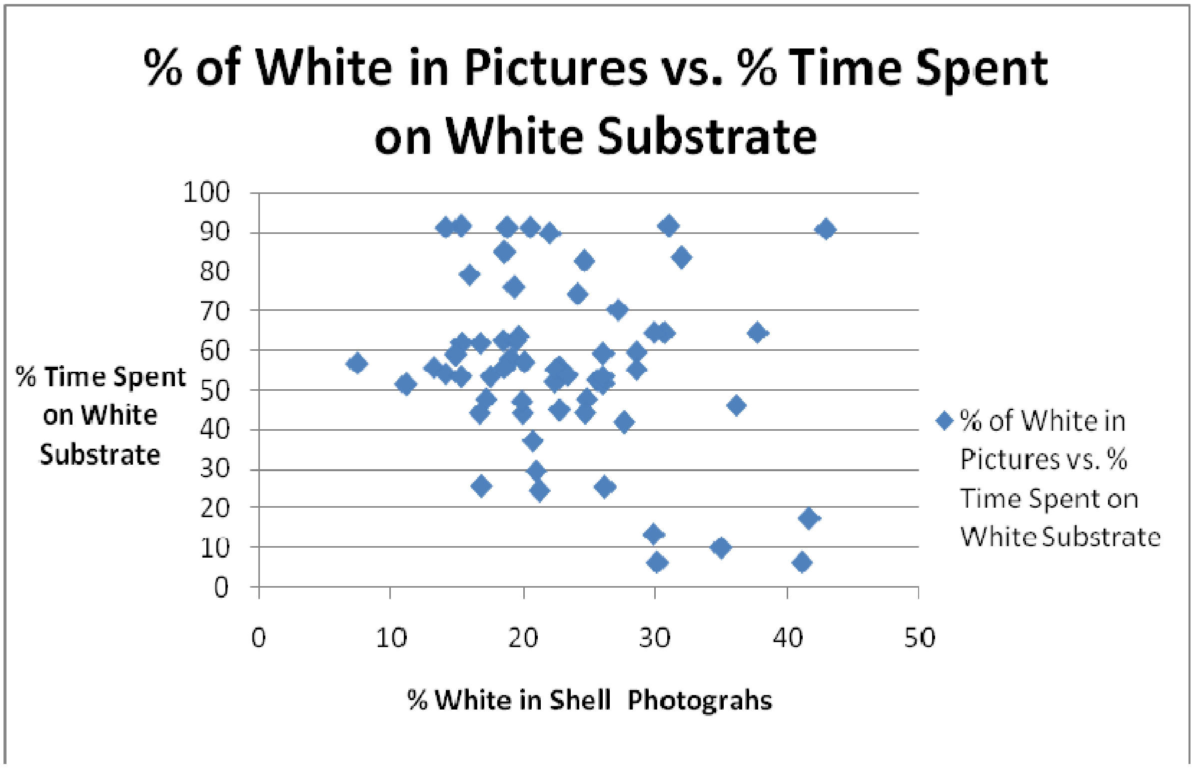

Figure 18: Graph of %Time Spent on White Substrate versus Percent White in Photographs. This graph depicts all of the data from the crayfish substrate preferences experiments. The %Time Spent on White Substrate is a measure of the percentage of the trial that the crayfish spent on the white substrate. The Percent White in Photographs is the averaged color value of the photos taken of a crayfish. There is both a best fit linear and exponential line to model the data. The exponential curve had the highest significance for the model (p =.004 as compared to .064 for the linear model)

3.2 Baited Traps

The baited trap experiment yielded no statistically significant data. Three Crayfish were found in the baited traps further downstream than they were initially trapped but no evidence for upstream movement was recorded. The number of crayfish caught did not yield the power required to detect any statistically significant difference.

3.3 Radio Tracking

The radio tracking yielded some very simple results. The subjects did not move for the first two weeks of the trial. After the first two week the stream began to build up ice which interfered with radio receiving equipment in addition the crayfish retreated into a large artificial rock berm that bordered the stream preventing a precise identification of their location due to the signal interference likely created by the large flat faces of the rocks used to construct the berm. (See figure 17) As with the baited trap experiment experimental difficulties in data collection percent statistical analysis due to statistical power issue and collection issues.

Figure 19: Berm at the East Brimfield Dam (Google Earth *42.109175, -72.126274* 2010*)*

The Berm at the East Brimfield Dam is outlined in red.

4. Discussion

This project posed an interesting question: How does one go about studying large and small scale movement of wild aquatic arthropods in such a way as to generate robust inferences using a combination of approaches. During the initial planning and research of this project it was decided that investigating substrate color preferences might yield interesting insights into microhabitat selection in *O. limosus*. Other Approaches have been used to gain insight into how individuals perceive and respond to environmental cues.

Visual cues can be a very important part of an organism's interactions with the world. The vision systems of what are traditionally viewed as less advanced organism can sometimes yield much more information than human visual systems. Honey bees, for example, like crayfish rely on polarized light to help navigate through their surroundings (Zolotov and Frantsevich. 1973.) These types of biological relationships have been documented in other organisms including fish (Hawryshyn and McFarland 1986.) However for visual cues to be relevant there must be some sort of adaption, perhaps over long evolutionary time, to utilize them. The crayfish behavior in this study is likely due to a convergence of factors over time rather than a single change in their biology. For example, the ability to discern certain shapes and colors is an ancient evolutionary development coming from a point in natural history before the animal kingdom had evolved enough to produce crayfish (Haszprunar 1995.) However given those abilities, over time crayfish were able to adapt preferences for and aversions to certain visual stimuli that helped them survive.

Examples of such preferences and aversions can vary with different crayfish species and different points in the life cycle of the crayfish. Crayfish sometimes seek out dark open area or sometimes burrow depending on whether the crayfish is adult of juvenile respectively (Alberstadt 1995.) It is precisely in this area of inquiry that I believe that my results fall.

To test these preferences a substrate preference experiment was designed with white and black substrates placed underneath the bottom of a water filled tank. Crayfish placed in the tank were allowed to roam for one hour while they were videotaped and then amount of time they spent on the white side of the tank was tallied from the video. Pictures of each crayfish were taken before and after the experiment and were then analyzed to determine an average color value for the crayfish in question.

Background research suggests the possibility that the light from the lab may have induced a sheltering response in the crayfish (Mason 1970.) This fits the current research because crayfish of different colorings reacted to the substrates in distinct manners at the appropriate light intensity of between 20-60 lumens (Mason 1970.) The results fell along a continuum with a highly significant correlation between color and substrate preference. The darker a crayfish was the more likely it was to spend more of its time on the white substrate. I hypothesize that the more whitely colored crayfish preferentially entered the darker substrate because the light levels in the lab (20-60 lumens) induced a sheltering response in the lighter crayfish similar in line with the results of Mason (1970.)

These results do however raise interesting questions since the white present in many crustaceans shells are also related to agonistic encounters and social hierarchy. In the wild could their white color offer a benefit to the type of agonistic encounters they tend to encounter in the center of the stream? Further research would be required to verify this. If this were true crayfish that had shells that contained more black relative to white could not rely on this benefit and as such despite high illumination preferentially remained on the white substrate. The results show an interesting line of potential evidence for the role of coloring in agonistic encounters linked with microhabitat selection. Different color morphs in various species such as pinnipeds (Bartholomew 1970), birds (Stacey and Chiszar 1975), and fish (Rhijn 1973) have been linked to aggression. Crayfish on also have other factors which affect the aggression such as increased chelae and body size (Garvy et al. 1994) but could whiter crayfish gain a social advantage in agonistic encounters like other crustaceans (Dunham DW 1978) over fights to secure burrows (Bergman and Moore 2003) and gain the greater resource benefits hypothesized to be at the center of the stream (Stein and Magnuson 1997) and therefore have a greater propensity to go into darker areas? Furthering this is the research of Bergman and Moore(2003) which showed the crayfish were more likely to fight more intensely and longer for a burrow than even food.

Further research in these areas could shed more light on the exact mechanisms and stimuli that produced these results. More research into the area of polarized versus non polarized light needs to be done to verify results of this laboratory experiment. Ideally experiments such as this should be done with actually sun to control for the possibility that crayfish may use their polarized light sense for navigation as other species are known to do (Zolotov and Leonid Frantsevich 1973.) Furthermore understanding the developmental variables in crayfish biology would be important for precise understanding. One of the drawbacks of harvesting crayfish indiscriminately from the river was there was no way to know the age of a crayfish which has been shown to affect color (Bessinger and Copp 1985.) Differences in sheltering behavior have also been noted between adults and juveniles so this begs the question could the results of these experiments be more specifically linked to some other factor related to maturity (Antonelli et al. 1999) (Alberstadt 1995)? Do crayfish become whiter as they age? Some crustaceans have also been shown to molt and change color on socially significant time scales so this adds another potential factor for further research (Detto et all 2008.) Is the white present in a crayfish shell indicative of its developmental status(Bessinger and Copp 1985.) If this is the case research do agonistic encounters in crustaceans related to overall shell color need to be revisited in greater depth? This could be explored through taking periodic pictures of lab grown crayfish in tandem with comparing populations of recently hatched crayfish with more developed adults. This would need to be done ensure that there were no environmental variables not effecting the growth of crayfish in the lab (Ghidalia 1985.) If done carefully and at the right time of year the age of crayfish could be reasonably inferred from form and size. In addition a test to see if white coloration as determined through the tests used in this project does have an effect on agonistic encounters between crayfish. Lastly to explore the results of these experiments more thoroughly a more realistic model of a stream as well as field work would be required. Ideally the environment of the tank would vary in light intensity and color to simulate the deepening of a stream and shade. This would allow for more thorough research of the findings of Mason (1970). This could not only potentially help to answer the question immediately relevant to these results but also whether or not variation in seasonal water level can affect crayfish preference. As for the field work crayfish would have to collected and photographed on site to see if there was a significant difference in color between those at the center of the stream an on the edges. Lastly the tagging experiments present some interesting results. While in early exploratory research for this project crayfish appeared to avoid artificial burrows they did however crawl far into the berm during the winter. Studying change in microhabitat selection as temperature varies could provide a useful predictive model for crayfish activity during the year given the research of Bubb et. al (2002.) Furthermore the baited traps experiments suggest that crayfish only move down stream but personal visual observation during this research suggests otherwise. Further research with radio tags checked on a daily basis over longer periods (several months) during warmer seasons could potentially provide more conclusive evidence.

References-

Antonelli, J; Steele, C; and Skinner, C (1999) Cover-Seeking Behavior and Shelter Use by Juvenile and Adult Crayfish, Procambarus clarkii: Potential Importance in Species Invasion , *Journal of Crustacean Biology*, Vol. 19, No. 2, pp. 293-300

"East Brimfield Dam" "42.109175" N, "-72.126274" E Google Earth April 27, 2010

Benvenuto, C; Gherardi, F; Ilhéu, M (2008). "Microhabitat use by the white-clawed crayfish in a Tuscan stream", *Journal of Natural History*. Vol. 42 Issue.1 pp. 27 < http://www.informaworld.com/10.1080/00222930701799090 >

Bergman, D and Moore, P. (2003) Field Observations of Intraspecific Agonistic Behavior of Two Crayfish Species, *Orconectes rusticus* and *Orconectes virilis*, in Different Habitats, *Biol. Bull. Vol* 205, pp. 26-35

Bessinger, K. and Copp N. (1985) Differential diurnal distribution of Procambarus clarkii (Girard) juveniles and adults and possible adaptive value of color differences between then (Decapoda, Astacieda), *Crustaceana*, Vol. 49, pp. 164-172

Hawryshyn, C and McFarland, W. (1986). Cone photoreceptor mechanisms and the detection of polarized light in fish. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*. Vol. 60, No. 4, pp 459-465

Collins. B.;Williams C.;and. Castelli, P. (2009) Reproduction and Microhabitat Selection in a Sharply Declining Northern Bobwhite Population, *The Wilson Journal of Ornithology* Vol. 121, Issue 1, pp. 688-695.

Crandal, K. and Buhay, J. (2007) Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae—Decapoda) in freshwater, *Hydrobiologia*.Vol. 595, No. 1

Crandall, K. and Cronin (1997) T "The molecular evolution of visual pigments of freshwater crayfishes (Decapoda: Cambaridae).", *Journal of Molecular Evolution*. Vol. 45, No. 5, pp. 524-534.

Bubb, D; Lucas, M; and Thom, T (2002) Winter movements and activity of signal crayfish Pacifastacus leniusculus in an upland river, determined by radio telemetry, *Hydrobiologia* Vol. 483, No. 1-3, pp 111–119

Detto, T; Backwell, P; Hemmi, J; and Zeil, J (2006) Visually mediated species and neighbor recognition in fiddler crabs (*Uca mjoebergi* and *Uca capricomis*). *Proc R Soc B* Vol. 273, pp. 1661–1666.

Detto T, Hemmi JM, Backwell PRY (2008) Colouration and Colour Changes of the Fiddler Crab, *Uca capricornis*: A Descriptive Study. PLoS ONE 3(2): e1629. doi:10.1371/journal.pone.0001629

Bauwens, D and Thoen, C (1981). Escape Tactics and Vulnerability to Predation Associated with Reproduction in the Lizard Lacerta vivipara, *Journal of Animal Ecology*, Vol. 50, No. 3, pp. 733-743

Disttefano et al., (2009) Imperiled Crayfishes use hyporheic zone during seasonal drying of an intermittent stream, *J.N Am. Benthol. Soc*., Vol. 28, No. 1, pp. 142-152

Jensen, D. (2009) Figure 4 http://www.seagrant.umn.edu/ais/rustycrayfish_invader

Dunham, D. (1978) Effect of chela white on agonistic success in a diogenid hermit crab (*Calcinus laevimanus*). *Mar Behav Physiol Vol*. 5, pp. 137–144

Dunham, D; Jordan, S; and Niglas, M. (1979). A new color morph of the crayfish *Orconectes propinquus*(Girard). *Am. Midl. Nat*., Vol. 102 pp.384-387

Fetzner, J. (2006). "*Orconectes* (*Faxonius*) *limosus* (Rafinesque, 1817)". *Crayfish Taxon Browser*. Carnegie Museum of Natural History.

Figure 1 Mellon, D. & Christison-Lagay, K. (2008). A mechanism for neuronal coincidence revealed in the crayfish antennule. *Proc. Nat. Acad. Sci.* Vol. 105

Figure 6 Kentucky Water Watch *Accessed* April 27, 2009 Modified http://www.state.ky.us/nrepc/water/crayfish.gif

Flood, M and Wilens A. (1978) Directional Sensitivity in a Directional Sensitivity in a Crayfish Mechanoreceptive Interneurons: Analysis by Root Ablation. *J. exp. Biol*. Vol. 77, pp. 89-106

Ghidalia, W. (1985). Structural and biological aspects of pigments. Pp. 301–394 *in* D. E. Bliss and L. H. Mantel, eds. *The Biology of Crustacea*: Integument, Pigments and Hormonal Processes. Vol. 9. Academic Press, New York

Glantz R. (2001) Polarization analysis in the crayfish visual system. *The Journal of Experimental Biology* Vol 204, pp. 2383-2390

Hernandez-Falcon J.; Serrato J.; Ramon F. (1999) Evoked Potentials Elicited by Natural Stimuli in the Brain of Unanesthetized Crayfish, *Physiology and Behavior*, Vol 66, No. 3, pp 397-407(11)

Hobbs H.H., III, Jass J.P. & Huner J.V. (1989) A review of global crayfish introductions with particular emphasison two North American species (Decapoda, Cambaridae). Crustaceana, 56, 299±316.

Hobbs H.H., III (1991) Adaptations and convergence in North American crayfishes. Freshwater Crayfish

Lodge, D; Taylor, C;Holdich, D; Skurdal, J. (2000). Nonindigenous crayfishes threaten North American freshwater biodiversity: Lessons from Europe. Fisheries Vol. 25(8) pp.7-20.

Mantel(eds.). The Biology of crustaceans, Vol. 9. Integuments, pigments and hormonal processes. Academic Press, New York

Mason, John C. (1970) Maternal-Offspring Behavior of the Crayfish, Pacifastacus trowbridgi (Stimpson) *American Midland Naturalist*, Vol. 84, No. 2, pp. 463-473 Stable URL: http://www.jstor.org/stable/2423861

Matthews S. & Brandt K. (2004) Africa Invaded: The growing danger of invasive alien species Global Invasive Species Programme

Momot W (1996) Upstream Movement of Crayfish in an Intermittent Oklahoma Stream, *American Midland Naturalist*, Vol. 75, No. 1, pp. 150-159

Morales, M; Traba, J; Carriles, M; Delgado, E; and Garcia de la Morena(2008)Sexual differences in microhabitat selection of breeding little bustards Tetrax tetrax: Ecological segregation based on vegetation structure, Acta *Oecologica*, Vol. 34, Issue 3, , pp345-353, ISSN 1146-609X, DOI: 10.1016/j.actao.2008.06.009. (http://www.sciencedirect.com/science/article/B6VR3-4T72K4C-1/2/d21ced066f200d9d7cc2ce5cabd1422c)

Newcombe., C. L. 1929. A new blue crayfish. Science, Vol. 70 pp.217

Newland, P ; Neil, D ; Chapman, C.(1988) The reactions of the Norway lobster, *Nephrops norvegicus* (L.), to water currents *Marine and Freshwater Behaviour and Physiology*, 1029-0362, Vol. 13, Issue 3, pp. 301 – 313

Garvey. J; Stein, R; Heather M; and Source, T(1994) Assessing How Fish Predation and Interspecific Prey Competition Influence a Crayfish Assemblage Author(s): *Ecology*, Vol. 75, No. 2 (Mar., 1994), pp. 532-547 Published by: Ecological Society of America Stable URL: http://www.jstor.org/stable/1939556 Accessed: 29/04/2010 00:05

Hamr P., (2002). Orconectes. In: D.M. HOLDICH (ed), Biology of Freshwater Crayfish.

Hamr, P. (2002). Orconectes. In: Holdich, D.M. (Ed.), Biology of Freshwater Crayfish. Blackwell Science, Oxford, pp. 585–608.

Hazlett, B. (1966). Social Behavior of the Paguridae and Digenidae of Curacao. Stud. Curacao Other Caribb. Isl., Vol. 23, pp. 1-143

Herrick F. (1895) The American Lobster. A study of its habits and development. Bull. U.S. Fush Com., 15:1-252.

Ivanova, Mand Vassilenko, S. (1987) Relationship between number of eggs, brood weight, and female body weight in Crustacea. *Internationale Revue der Gesamten Hydrobiologie.* Vol. 72, pp. 147–169.

Main, K.(1987) Predator Avoidance in Seagrass Meadows: Prey Behavior, Microhabitat Selection, and Cryptic Coloration. *Ecology*, Vol. 68, No. 1, pp. 170-180

Kent, V. (1901). The Colors of the crayfish. *Am. Nat*., Vol. 35 pp. 933-936

Paloma, A, Alcorlo P, Geiger W and Otero M (in press) (2004) Feeding preferences and food selection of the red swamp crayfish (Pro-cambarus clarkii) in habitats differing in food item diversity. *Crustaceana*

Penn, G. (1951). A color anomaly of the Lousiana red crawfish, Procambarus clarkii (Girard). *Proc. La. Acad. Sci*., Vol. 14 pp. 66-67

Newland, P; Neil, D; and Chapman, C (1988) The reactions of the Norway lobster, *Nephrops norvegicus* (L.), to water currents. *Marine and Freshwater Behaviour and Physiology,* 1029-0362, Volume 13, Issue 3, pp. 301 – 313

Alberstadt, P; Steele, C; and Skinner, C. (1995)Cover-Seeking Behavior in Juvenile and Adult Crayfish, *Orconectes rusticus*: Effects of Darkness and Thigmotactic Cues. *Journal of Crustacean Biology*, Vol. 15, No. 3, pp. 537-541 Stable URL: http://www.jstor.org/stable/1548774

Ra'Anana, Z and Sagi, A . (1985) Alternative mating strategies in males of the freshwater prawn Macrobrachium rosenbergii (de Man). *Biol. Bull*. Vol. 169 pp.592-601

Rhoades, Rendell (1962) The evolution of crayfishes of the Genus Orconectes Section Limosus (CRUSTACEA: DECOPODA). *The Ohio Journal of Science.* Vol 62 No. 2 pp1-32

Taylor, R (1975) Physical and physiological properties of the crayfish antennal flagellum, Journal of Neurobiology, Vol. 6, Issue 5, pp 501-519

Taylor, R (1968) Water-vibration reception: A neurophysiological study in unrestrained crayfish, *Comparative Biochemistry and Physiology*, Vol. 27, Issue 3, pp 795-805, ISSN 0010-406X, DOI: 10.1016/0010-406X(68)90619-1

Thacker, R; Hazlett, B; Esman, L; Stafford, C; and Keller, T(1993) Color Morphs of the Crayfish *Orconectes virilis* *American Midland Naturalist*, Vol. 129, No. 1, pp. 182-199

Smiley, J. and Miller, W (1971). The Occurrence of blue specimens of the crayfish Procambrus actus actus(Girard)(Decapoda, Astacidae). *Crustaceana*. Vol. 20, pp. 221

Smith, K (2006) Keystone predators (eastern newts, *Notophthalmus viridescens*) reduce the impacts of an aquatic invasive species *Oecologia* Vol .148, No. 2, pp 342-349

Schmalhofer , V. (2000) Diet-Induced and Morphological Color Changes in Juvenile Crab Spiders (Araneae, Thomisidae) *Journal of Arachnology*, Vol. 28, No. 1, pp. 56-60 Stable URL: http://www.jstor.org/stable/3706359

Stein and Magnuson (1976) Behavioral Response of Crayfish to a Fish Predator. *Ecology*. Vol. 57 No. 4, pp. 751-761

Taylor, C. (2002) Taxonomy and conservation of native crayfish stocks. pp 236–257 in D. M. Holdich, ed. *Biology of freshwater crayfish*. Blackwell Science, Oxford,UK.

Van der Velden, J; Zheng, Y; Patullo B; Macmillan, D (2008) Crayfish Recognize the Faces of Fight Opponents. PLoS ONE 3(2): e1695. doi:10.1371/journal.pone.0001695

Wiese, K.; Calabrese, R; and Kennedy, D (1976). Integration of directional mechanosensory input by crayfish interneurons. *J. Neuropltytiol.*Vol. 39, pp. 834-843.

Vannini, M. and Gherardi, F. (1981). Dominance and individual recognition in Potamon fluviatile (Decapoda, Brachyura): Possible role of visual cues. - *Mar. Behav. & Phys* Vol.8, Issue 1, pp. 13-20

Zolotov, V; and Frantsevich, L. (1973) Orientation of bees by the polarized light of a limited area of the sky . *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* Volume 85, Number 1 pp. 25-36