Cryptic Substrate Preference in Crayfish

Melanie Florkowski, Erin Gregoire, Cailin McLean, Matthew Silbergleit

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Professor Joel Heinen

Abstract

Selective substrate choice is a form of crypsis commonly used as a predator avoidance strategy by crayfish. Substrate preference in crayfish of the genus *Orconectes* was tested and analyzed regarding cryptic behavior related to predator avoidance. Crayfish showed strong preferences for cryptic substrates such as rocks and woody debris over the less cryptic substrate sand. Overall, crayfish showed the strongest preference for big rocks than any other substrate choice, spending a significant majority of their time on this substrate. Crayfish also displayed more agitated behavior when placed on less cryptic substrates such as sand and small rocks and had more relaxed postures on big rocks and in woody debris.

Introduction

Crypsis is a phenomenon observed in nature in which an animal either actively seeks a substrate to hide in or changes its appearance to decrease the risk of predation (Heinen, 1994). Color matching, hiding, nocturnality and other behaviors for avoiding detection by visual predators are examples of this phenomenon. Many species have been documented to utilize crypsis including swallowtail butterfly larvae, eggs of ground-nesting birds, coho salmon, and a number of reptiles, amphibians and invertebrates (Tullberg et al., 2005; Solis & De Lope, 1995; Donnelly & Dill, 1983; Heinen, 1985; Heinen, 1993). This study focuses on crypsis in crayfish of the genus *Orconectes*, a small freshwater crustacean.

It is important to study crayfish for several reasons. One of these reasons is their economic importance as a commercially farmed product. A significant change in their population could have an impact on the economy of certain Southern states (New, 1995). Another reason it is important to study crayfish is because they are an indicator species. Due to their sensitivity to water pollution, their populations are an excellent measure of the overall health of an ecosystem. A large decrease in crayfish population could be indicative of an issue with water quality, or the recent release of aquatic pollutant (Markert et al., 2003). Common sources of pollution which affect crayfish include agriculture and logging, which create silt runoff. Other human activities near lakes and rivers also have the potential to negatively impact crayfish populations by creating pollution. This pollution, in turn, has a large impact on the ecosystem as a whole (Lagrue et al., 2014). Because Douglas Lake is located in an area that has a history of extensive logging and is subject to human activity along the lakeshore (Heinen & Vande Kopple 2003), these pollutants may be directly affecting the crayfish near and on the

property of the University of Michigan Biological Station. By knowing which substrates crayfish prefer, ecologists may be able to more effectively and accurately monitor their populations. Additionally, knowing that there is a difference in crayfish abundance between substrates could amplify the importance of consistency in location of collection when monitoring populations.

Crayfish live in shallow freshwater habitats such as lakes and streams. They occupy a key role in the food web by feeding on smaller invertebrates and serving as prey for larger vertebrates including fish, raccoons and herons (Englund & Krupa, 2000; Lagrue et al., 2014). Any change in crayfish population could greatly affect the populations of other animals higher up the food chain (Wolff et al., 2015). Crayfish are generalists, meaning they eat a variety of organisms including snails, insects and larvae, as well as dead and decaying plants and organic matter. Predation by crayfish helps control prey populations (Lodge, 1994) and their consumption of organic debris keeps streams and lakes clear (Usio & Townsend, 2002). Common substrates in crayfish habitats, such as rocks and woody debris, are used as camouflage and provide crevices in which they can hide.

Many species of crayfish rely on crypsis to avoid detection making hiding an important aspect of survival. One type of crypsis that crayfish use is color matching. Color matching in crayfish has been observed to be environmentally induced (Thacker, 1993; Hazlett, 1966), and to occur over a period of weeks, ultimately resulting in the crayfish matching its background color (Kent, 1901). Because this process occurs over a longer period of time, crayfish also rely on other forms of crypsis to avoid fast moving predators. Another method of crypsis that crayfish employ is nocturnality. Nocturnality allows crayfish to avoid exposure during peak predation

times by foraging primarily at night (Hirvonen, 2007). Finally, the aspect of crayfish crypsis that we were interested in studying was their ability to selectively choose the substrate that was most cryptic (Stein, 1976).

The purpose of this study was to investigate crayfish crypsis strategies related to substrate choice, as well as substrate specific behaviors. To answer the question of which substrate crayfish prefer, our experiment tested crayfish preference when given the choice between common materials found in their habitats: sand, big rocks, small rocks, and woody debris. We hypothesized that when given a choice between a cryptic substrate such as rocks or woody debris and a non-cryptic substrate such as sand, crayfish would spend more time on the cryptic substrate in order to be less visible to predators. Additionally, we predicted that the most preferred cryptic substrate would be woody debris, because this is where we most frequently observed wild crayfish and this substrate type may also serve as a food source (Usio & Townsend, 2002). We also observed crayfish behavior on the varying substrates and hypothesized that the crayfish would exhibit more agitated behavioral patterns when placed on sand than on any cryptic substrate.

Materials and Methods

Experimental Procedure

The crayfish used in our study were *Orconectes propinquus* and *Orconectes virilis*, the two most common species in Northern Michigan. For the purposes of this experiment we did not differentiate between crayfish species because the crayfish were approximately the same size and inhabited the same ecological niche as small stream bottom feeders. They should have exhibited similar behavior due to the fact that they have similar predators and eat similar food. We caught

crayfish in two locations in the East Branch of the Maple River, one on Riggsville Road and the other on Douglas Lake Road. The sites were located in a temperate climate near the University of Michigan Biological Station (Heinen & Vande Kopple, 2003). To obtain the specimens, we waded into the river with dip nets and placed them primarily near the banks. Along the banks there were large amounts of woody debris consisting of fallen branches and sticks lodged into the sand. With the nets downstream from where we were standing in the river, we disrupted the river bottom to startle the crayfish into our nets. We stored the crayfish individually in glass fish bowls separate from the experimental aquaria with a habitat of sand, wood and rocks until testing. All collection of crayfish occurred in late May and early June of 2016.

We tested substrate preference using four different substrates: sand, woody debris, small rocks and big rocks. All of the substrates were collected from the shore of Douglas Lake. Small rocks were defined as being between two and four centimeters in diameter, and big rocks were defined as being between ten and fifteen centimeters in diameter. We prepared six 38 liter aquaria (50.8 x 26.7 x 30.5 cm) with two substrates side by side, using all possible combinations of the four substrates (Figure 1). By measuring 25.4 centimeters from each end to the center, we ensured that each aquarium was split exactly in half. This eliminated any error that would result from a difference in substrate area cover. Each aquarium had eight centimeters of lake water covering the substrate. To avoid possible light, directional or edge biases, the aquaria were oriented to vary the position of each substrate. Additionally, paper was placed over every wall of the aquaria in order to prevent visual interference from crayfish in nearby aquaria and to limit other visual distractions.

We performed three trials with six crayfish in each trial, for a total of eighteen crayfish. Each crayfish was placed individually in an aquarium and given five minutes to acclimate to the new environment (Hazlett, 1998). After the acclimation period, the substrates the crayfish were on were recorded every minute for ten minutes. After ten minutes, the crayfish were rotated to new aquaria and the process was repeated until every crayfish had been in every aquarium.

After the crayfish were tested in each aquarium, all six were placed in a larger arena (41.6 x 55.9 x 30.5 cm), where each substrate covered one fourth of the area (Figure 2). The crayfish were again given five minutes to acclimate, and the substrate each crayfish was on was recorded every minute for ten minutes. Over the course of three trials 1,375 data points were collected between the two-substrate aquaria and the larger arena.

Additionally, we tested the behavior of the crayfish on each substrate. The goal of this aspect of the experiment was to provide further evidence that crayfish exhibit substrate preference. Observations of differences in crayfish behavior provided insight into their level of agitation on different substrates. We placed an individual in a single-substrate aquarium for five minutes. We observed the posture of the crayfish and noted whether the chelipeds were in a raised or lowered position and whether the abdomen was curled or extended. The relevant anatomy is highlighted in Figure 3. These positions were defined by Hazlett (1984) and indicate the level of agitation in a crayfish. Raising chelipeds is a protective behavior exhibited when crayfish face a predator, and therefore correlates with a high level of agitation (Stein, 1976). Similarly, extension of the abdomen is a sign of stress (Hazlett, 1984). After observing the posture and movement on one substrate, the crayfish was moved to a new substrate to repeat

observation until it had been in an aquarium with every substrate. Four crayfish were tested for behavioral differences among four different single-substrate aquaria.

Statistical Methods

A T-test was used to compare the substrate preferences of the crayfish in each of the six two-substrate aquaria. We chose to use a T-test because it tests the differences between the means of independent samples (Ambrose & Ambrose, 2007).

To compare the substrate preferences of the crayfish in the arena with all four substrates present we used an ANOVA. As an analysis of variance, the ANOVA allowed us to analyze differences among our four group means of big rocks, sand, woody debris and small rocks (Ambrose & Ambrose, 2007).

Results

Table 1. Breakdown of Statistical Analyses Run for Substrate-Divided Aquaria

Substrates	**Statistical Test**	**p-value**
Sticks v. Small Rocks	T-test	0.008
Sticks v. Sand	T-test	0.000
Small Rocks v. Big Rocks	T-test	0.020
Big Rocks v. Sand	T-test	0.001
Big Rocks v. Sticks	T-test	0.027
Small Rocks v. Sand	T-test	0.002
Small Rocks v. Big Rocks v. Sand v. Sticks	One-way ANOVA	0.002

The results we obtained were all significant (α=0.05 for all tests; Table 1). The results are reflective of the amount of time the crayfish spent on each substrate when given a choice of multiple substrates. Crayfish have a clear, statistically significant substrate preference when given the choice of small rocks and sticks (p-value: <0.05, Table 1). Crayfish are more likely to favor one substrate over another to a significant degree if they are placed in an aquarium with sticks and sand (p-value: <0.05, Table 1). There was also a significant difference in crayfish substrate preference when comparing small rocks and big rocks (p-value: <0.05, Table 1). The big rocks and sand comparison also produced a statistically significant crayfish substrate preference (p-value: <0.05, Table 1). Crayfish were observed to have a statistically significant substrate preference when comparing big rocks and sticks (p-value: <0.05, Table 1). For the final two-substrate test the crayfish showed a statistically significant preference for one substrate when given the choice between small rocks and sand (p-value: <0.05, Table 1). The last test consisted of placing six crayfish into a larger arena that contained all four substrates in equal proportion. Once again, statistical analysis showed that there was a significant difference in which substrate crayfish prefer (p-value: <0.05, Table 1).

Table 2. Crayfish Posture and Time Spent Moving in Single-Substrate Aquaria

Behavior (% of Time)	**Sand**	**Small Rocks**	**Big Rocks**	**Woody Debris**
Chelipeds Raised	Medium	Medium	Low	Low
Chelipeds Lowered	Medium	Medium	High	High
Abdomen Extended	High	High	Low	Low
Abdomen Curled	Low	Low	High	High
Moving	High	High	Low	Low

For the behavior observation portion of our experiment we coded the results into three categories. If the behavior was observed for more than 75% of the time the crayfish spent in the aquarium that behavior was coded as "high." If the behavior was observed between 25% and 75% of the time that behavior was coded as "medium." If the behavior was observed less than 25% of the time that behavior was coded as "low." Overall, we observed that crayfish spent a larger amount of time with chelipeds raised and abdomen extended when on the open substrates of small rocks and sand (Table 2). We also observed more movement when the crayfish were on open substrates (Table 2). When on the more cryptic substrates of big rocks and woody debris, the crayfish spent less time with chelipeds raised and abdomen extended and also remained in one place for longer (Table 2).

Discussion

We hypothesized that crayfish would prefer to spend more time on cryptic substrates rather than on non-cryptic substrates. Our data supported this hypothesis. Crayfish strongly preferred the cryptic substrates of woody debris, big rocks and small rocks over the non-cryptic sand (Figure 4, 5, 6). Because crayfish are a prey species it is important for them to hide from their visual predators (Englund & Krupa, 2000). Rocks and wood provide hiding places as well as camouflage that makes it difficult to see the crayfish. In the light sand, the dark crayfish are highly visible. We did not observe the crayfish exhibiting crypsis on the sand, as they did not burrow and could not color match in the short period of time.

Our hypothesis that the crayfish would prefer woody debris over big or small rocks was not supported; the most favored substrate was big rocks (Figure 10). When collecting our

specimens, we observed a larger number of crayfish in woody debris. Because of this, we predicted that it would be the preferred substrate. However, it is possible that the reason we caught more crayfish in the woody debris was because this was where they were easiest to catch. There may have been an equal or even a larger number of crayfish in the big rocks of the stream, but we were not as successful catching them there. We also hypothesized that the woody debris would be favored because it provided a food source of dead organic matter for crayfish, but because crayfish are generalists food is found in a large variety of substrates (Lodge et al., 1994). Rocks that provided better cover had algae and other organisms hiding under them that could also act as a food source, accounting for the preference of big rocks over woody debris. The overall preference for big rocks is consistent with the fact that crayfish, while often found in areas that do have woody debris, also inhabit areas that have little vegetation or sticks but are very rocky (Thorp, 2011).

The big rocks and small rocks used in the study were similar in color; however, crayfish strongly prefered big rocks over small rocks (Figure 8). We observed crayfish crawling under both of these substrates, but the crayfish were less visible in the large crevices between the big rocks. The preference for big rocks over small rocks of the same color provides evidence that hiding is an important cryptic mechanism for crayfish.

When placed in the arena with all four substrate choices at once, the substrate choice of the crayfish reflected the results of the individual trials (Figure 10). In the arena we tested six crayfish together in each trial, which could have introduced a social factor. Crayfish are known to establish dominance hierarchies (Bovbjerg, 1953). When in the arena, some of the crayfish would chase and displace each other from substrates suggesting the beginnings of a dominance

hierarchy forming. However the crayfish were only placed together for a total of fifteen minutes, so there was not adequate time for dominance to be established (Gherardi & Daniels, 2003). Qualitative observations indicated that the bigger, and presumably more dominant, crayfish spent nearly all of their time on the big rocks. It is possible that because they were the most dominant they controlled the preferred substrate, and that the smaller crayfish were relegated to less favorable substrate. It is unlikely that male-female interactions impacted the results. Although we did not sex our specimens, mating occurs in early spring, before the trials began (Arsenault & Berrill, 1984). Despite the potential confounding factors of social interactions between individuals that were not present in the two-substrate aquaria tests, the substrate preferences shown in the arena were consistent with the two-substrate aquaria results.

Although crayfish showed a strong preference for cryptic substrates, they did not always remain on these cryptic substrates. Avoiding predators is crucial to crayfish survival, yet they are also predatory themselves (Lodge et al., 1994). Crayfish often hunt for food, requiring them to periodically move to open areas. An example of crayfish predation on a non-cryptic surface was observed in a trial when one of our crayfish caught a small invertebrate from the sand in one of the aquaria. Predation is one explanation for crayfish presence on non-cryptic sand in our trials (Figures 4, 5, 6).

We observed the behavior of crayfish when placed on each of our four substrates based on findings by Hazlett (1984). We hypothesized that crayfish would exhibit more agitated behavior when on sand than on cryptic substrates. This hypothesis was partially supported, as the crayfish displayed much more agitated behavior on the sand. Agitated behavior was also displayed on small rocks, which we did not hypothesize (Table 2). On the open substrates of

sand and small rocks, the crayfish were observed moving frequently around the substrate with their chelipeds raised and their abdomens extended, indicating agitation (Table 2). On the woody debris and big rock substrates the crayfish moved around much less frequently, as they preferred to remain stationary under the cryptic substrates (Table 2). They also exhibited the relaxed behavior of a curled tail and lowered claws (Hazlett, 1984; Stein, 1976). The agitation we observed on sand and small rocks is likely because the crayfish were less able to hide and therefore remained out in the open. The crayfish were also constantly moving, possibly trying to find a more cryptic substrate on which to hide. On these substrates the crayfish had their chelipeds raised. Chelipeds are specifically adapted to serve a defensive purpose, meaning that in these trials the crayfish were more alert and ready to defend themselves (Guyer, 1941).

While the results of our experiment were significant, it is important to recognize the limitations of this study. There are several factors that may also play a role in substrate preference that we did not take into consideration. The size of the crayfish may have an impact on substrate choice, as shown by Rabeni (1985) and Englund & Krupa (2000). The majority of our specimens were relatively small, which may present a bias in the data. Smaller crayfish may have a different substrate preference than larger crayfish because of their ability to hide in smaller places, as well as their increased vulnerability. There is also possibility for error in the setup of our experimental aquaria, as we could not replicate all aspects of the natural crayfish habitat. It has been shown that water depth as well as current can influence habitat use in crayfish (Englund & Krupa, 2000; Rabeni, 1985). Our aquaria had relatively shallow water and no current, which was present in the stream where the crayfish were collected. The absence of these factors could have impacted the crayfish's behavior and substrate preference.

Our experiment relied on a functional approach to ecology to define a correlation between crayfish and substrate preference. To build on this study in the future we would examine the evolutionary consequences of the correlation we observed. To do this, we would execute live predation trials to observe if certain substrates provide an increase in fitness and therefore an evolutionary advantage (Stein, 1976). We would expect that crayfish on more cryptic substrates would be caught less often by predators. This result could provide an explanation for the correlation shown in our study.

Our results showed that crayfish preferred the substrate of big rocks the most, followed by woody debris, small rocks, and then sand. When given a choice, crayfish hid among big rocks the highest percentage of time. Additionally they exhibited more relaxed behavior on the big rocks. Big rocks were chosen the most often, implying that they provided the best hiding places and were the most cryptic substrate.

Acknowledgements

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Appendix

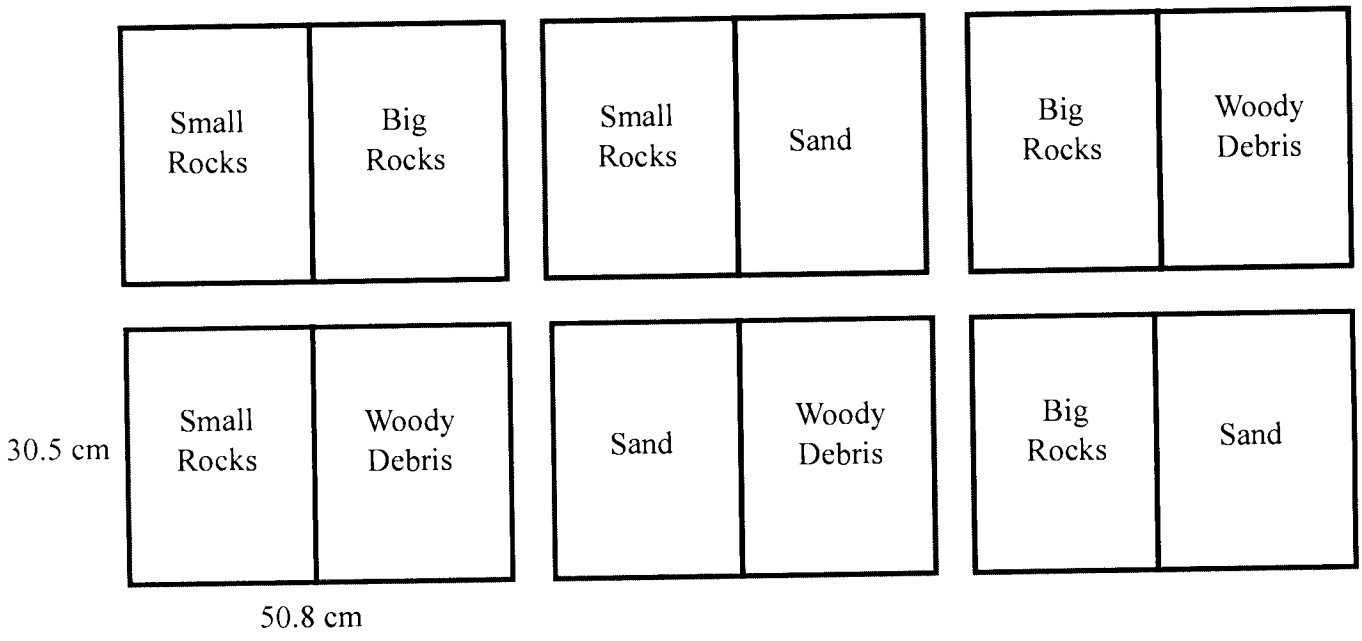

Figure 1. Six 38-Liter Aquaria with Combinations of Four Substrates

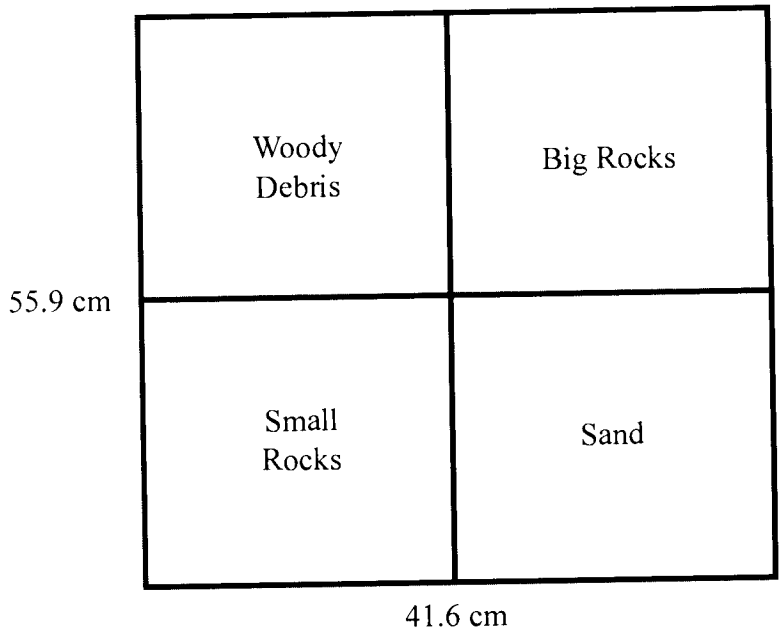

Figure 2. Large Arena Divided into Fourths with Each Substrate Choice

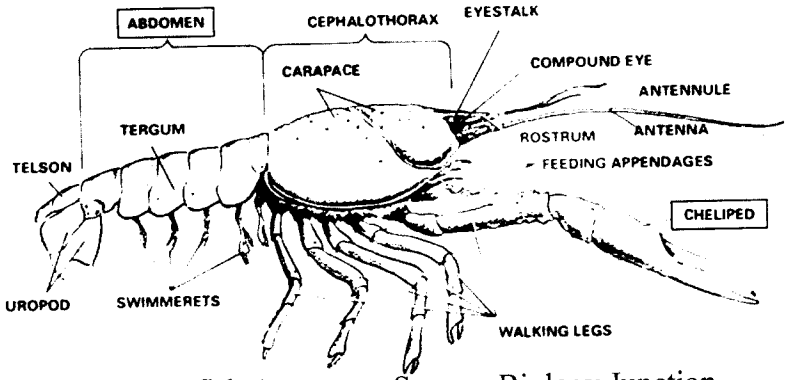

Figure 3. Crayfish Anatomy. Source: Biology Junction

Figure 4. Crayfish Substrate Preference; Big Rocks v. Sand

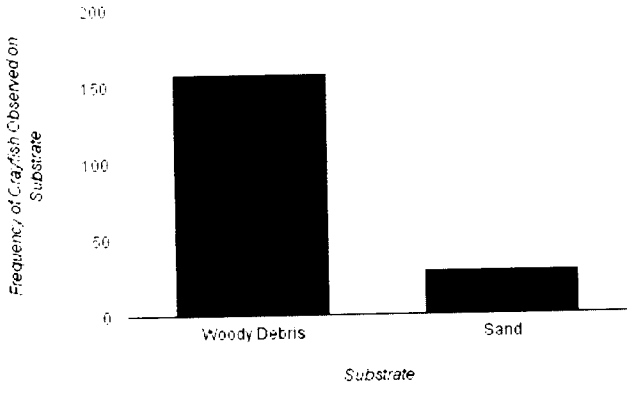

Figure 5. Crayfish Substrate Preference; Woody Debris v. Sand

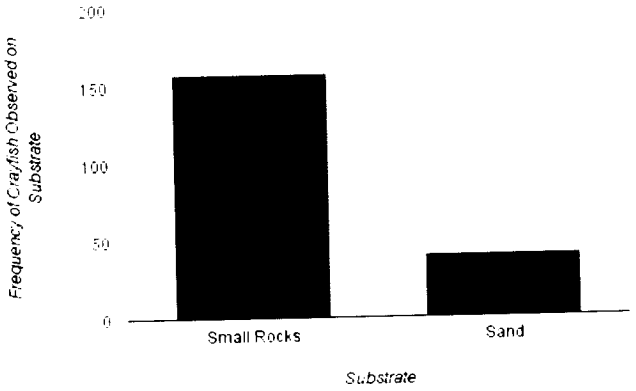

Figure 6. Crayfish Substrate Preference; Small Rocks v. Sand

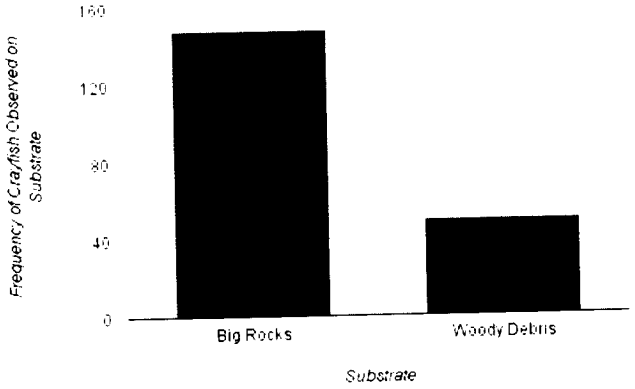

Figure 7. Crayfish Substrate Preference; Big Rocks v. Woody Debris

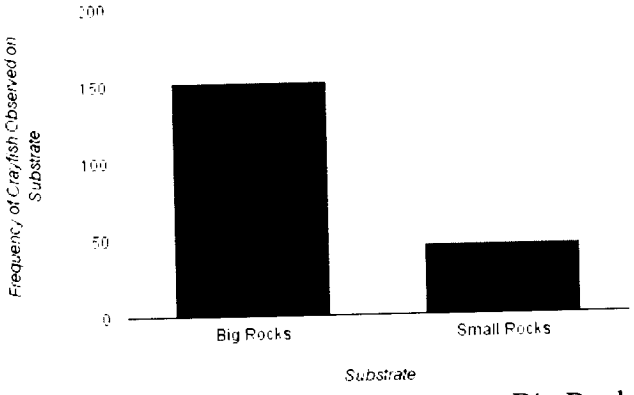

Figure 8. Crayfish Substrate Preference; Big Rocks v. Small Rocks

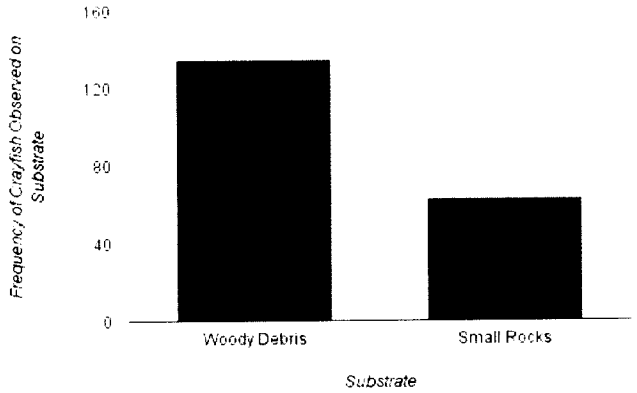

Figure 9. Crayfish Substrate Preference; Woody Debris v. Small Rocks

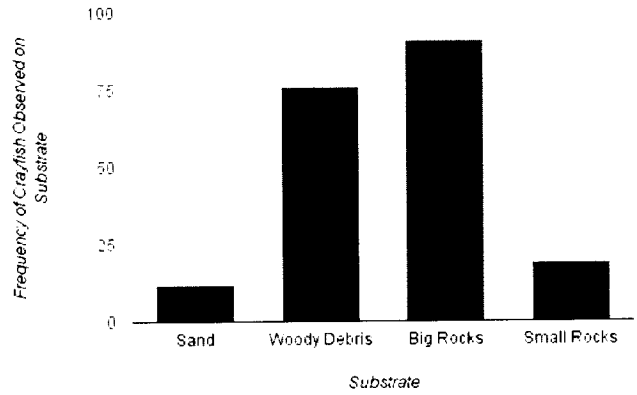

Figure 10. Crayfish Substrate Preference; Comparison of all Four Substrates