The Relationship Between Fish Species, Fish Size, and Substrate on Predation of Crayfish

BIOS 35502: Practicum in Environmental Field Biology

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Abstract

Due to increasing problems with invasive rusty crayfish (*Orconectes rusticus*) in the region, I tested the role of fish predation on crayfish in two northern Wisconsin lakes. At each lake, I sampled three types of substrate (sand, cobble, and macrophyte beds), along three different 50-m transects (N = 9 per lake). Crayfish densities were estimated along each transect using 1m^2 quadrants. I electro-shocked the lakes in these areas for fish and conducted gastric lavage to determine which fish species were preying on crayfish. I found a significant trend as fish size increased and the mean size of crayfish carapace consumed also increased. The fish species that most often consumed crayfish were smallmouth bass, rock bass, and yellow perch. Fish (across all species) also were found to significantly consume more crayfish in cobble areas.

Introduction

Invasive species introductions can have dramatic effects on the surrounding ecosystem, such as species extinction via competition, predation, or habitat alteration (Hill and Lodge 1994). One introduction that has had a major impact on the northern regions of the U.S. is that of the rusty crayfish (*O. virilis*). Compared to native crayfish, these invaders are more aggressive, have larger claw sizes, and strong body armor, which help reduce predatory susceptibility and eliminate competitors (Garvey et al. 2003). These crayfish also have strong impacts on macrophytes and macro invertebrates present in its invading range (Lodge et al. 1994).

Rusty crayfish have especially become prevalent in recent years in northern Wisconsin and Michigan lakes, and have begun to replace the native *Orconectes virilis* and the previous invader *Orconectes propinquus* (Garvey et al. 1993). Both of the invading species were probably transported by fisherman using them as bait in these areas (DiDonato and Lodge 1993). These species invasions are affected by factors such as competition and predation, which are fundamental in structuring ecological communities (Garvey et al. 2003). DiDonato and Lodge (1993) state that predation by fish is an important factor affecting the abundance of crayfish in lakes. Garvey et al. (2003) found that largemouth bass (*Micropterus salmoides*), yellow perch (*Perca flavescens*), and rock bass (*Ambloplites rupestris*) frequently consumed crayfish while walleye (*Stizostedion vitreum*) consumed the least amount of crayfish. Stein (1977) also found that smallmouth bass (*Micropterus dolomieu*) were the main size-selective predators of crayfish. DiDonato and Lodge (1993) also found that *O. virilis* was eaten by fish at significantly higher rates than *O. propinquus*, and that predation by fish was a factor in the replacement of the native crayfish *O. virilis* by the invasive *O. propinquus*.

Fish predation also may be affected by differing substrates, because substrates such as cobble and macrophyte beds provide places for crayfish to use as cover (Kershner and Lodge 1995). Previous laboratory experiments supported Kershner and Lodge's (1995) study, and found that both cobble and macrophyte habitats provided refuge for crayfish to hide in and predation rates on crayfish are higher in sand substrates (Saiki and Tash 1979, Garvey et al. 1994). However, little is known about the exact consumption rates of fish and the factors that influence crayfish predation rates in different substrates (Grove and Seyhan 2001). These rates are important in determining the total effect that fish have on crayfish populations and are essential to understanding the long-term effects that fish have on the crayfish within the ecosystem (Hill and Lodge 1995).

The following study will focus on surveying fish size and their gut contents to assess how fish size, species, and substrate affects the frequency and size of crayfish consumed. The crayfish species present on UNDERC consists of *O. virilis* in only Plum, and in Tenderfoot both *O. propinquus* and *O. virilis* are present. I was also interested in how the species of crayfish consumed effects the above relationships. The goal of this study was to test the role of fish species and size in relation to predation on crayfish species and size, in case of a possible invasion by exotic crayfishes on UNDERC (University of Notre Dame Environmental Research Center) property.

Given previous studies, I predicted that 1) *O. propinquus* will be eaten less often by fish than that of *O. virilis,* because *O. propinquus* out competes the native *O. virilis* (Hill and Lodge 1994); 2) as fish size increases mean crayfish size eaten will increase; 3) in both lakes, smallmouth bass and yellow perch will consume the most crayfish, and walleye will consume the least (Garvey et al. 2003), 4) fish predation on crayfish will be highest relative to crayfish density in sand substrate compared to the other substrates (Saiki and Tash 1979, Garvey et al. 1994, and Kershner and Lodge 1995).

Methods

The study sites included Plum and Tenderfoot Lakes on UNDERC property. In both lakes I explored the relationships among substrates, fish abundance and diet, and crayfish abundance as well as size. At each lake, I sampled three types of substrate (sand, cobble, and macrophyte beds), each at three different locations, for a total of 9 sites per lake. My definitions of each substrate included, sand having less than 20 shoots of vegetation in 1-m^2, macrophyte having 20 or more shoots, and cobble having stones more than 10cm in diameter across the bottom of the transect. I sampled for fishes using an electro-shocking boat along approximately 50-m transects per site. Collected fishes were weighed and measured for total length. Stomach contents were also removed from selected fish using gastric lavage according to Light et al. (1983). Excess water was removed using a funnel filter, and prey items were placed into labeled plastic bags with 70% ethanol (Soupir et al. 2000). Fish were only lavaged if they were large enough to do so, for example minnows, young of year (YOY), and fish under about 10cm were not lavaged.

Stomach contents were sorted and marked if they contained crayfish (Soupir et al. 2000). Crayfish found in the stomach contents were measured directly if the carapace was intact or regressions were used to estimate carapace length based on other morphological measurements. Crayfish carapace length was used to estimate crayfish size, and if it was not found or in pieces, then chelae or other morphological traits (Table 1) were recovered and used to estimate crayfish length using chelae length vs. (CL) carapace length regressions (Kuhn unpublished data). As time was a limiting factor in this experiment, available rusty crayfish regressions (Table 1) were used to estimate our non-rusty crayfish size (Scott Kuhn, Lodge lab REU 2005). All crayfish were measured using vernier calipers and other morphological traits, such as chelae length, width, dactyl length, abdomen length, and abdomen width were measured using an ocular micrometer on a dissecting microscope. If there was more than one discernable crayfish in the lavage sample they were separated and counted as two or more data points for that fish. Data were categorized according to substrate to infer the different predation rates upon crayfish based on fish species and size.

Crayfish were sampled one day before shocking to estimate their relative abundance. They were sampled via snorkeling using $1m^2$ PVC quadrants to count the abundance of crayfish within each substrate (Garvey et al. 2003). Five quadrant sites midway along each approximate 50-m transects were randomly selected to determine the crayfish abundance. The 50-m transects and substrates were documented and recorded using a GPS device. I used the average (5) quadrant count for each site and date for my analysis.

I used regression analysis to assess how the size of crayfish consumed relates to fish size. For hypothesis one I planned on comparing regression lines for both species. For hypothesis two I pooled all crayfish species for my analyses. To assess differences in crayfish predation between species of fish in hypothesis three, I calculated the proportion of fish lavaged for each species which contained crayfish. I then tested for significant differences among species using ANOVA in SYSTAT 11.0 (Systat Software Inc., Point Richmond, CA). Only tenderfoot Lake data was used for this analysis and I replicated across sampling date. To assess how substrate affected fishes ability to consume crayfish in my fourth hypothesis, I calculated a new variable: Proportion of fish with crayfish in guts relative to crayfish density (PFWCGRCD). The formula for this variable is: (Proportion of fish with crayfish in guts +1)/ (Average crayfish density +1). I then used ANOVA to test for significant differences across substrates.

Results

I electro-shocked 706 fish and lavaged 364 of them (Table 2). Of the 364 fish lavaged, 23% (83 individuals) had crayfish present in their guts. Black crappie (*Pomoxis nigromaculatus*) had the smallest lavage sample size (6) because it was only caught 6 times, and walleye and yellow perch each had the largest lavage sample size (101; Table 3).

I was not able to test hypothesis 1 because I lacked samples of crayfish in fish guts from Plum Lake and was not able to differentiate crayfish species found in the guts of fish from Tenderfoot Lake. Plus only three *O. virilis* were observed while snorkeling quadrants in Tenderfoot, the rest were *O. propinquus*. However, a Pearson's chi-squared test found that Tenderfoot had significantly ($p=0.000002$, $X^2=22.89026$, $df=1$) more crayfish found in its gastric lavage, compared to Plum lake (Figure 3).

A regression comparing the length of crayfish carapace consumed against the length of fish consumer across all fish species showed a marginally significant positive relationship ($p=0.059$, $r^2=0.0928$, Figure 4). However, the r^2 value was low meaning that the relationship had a very large variance. When regressed individually, no species showed a significant relationship between fish size and size of crayfish eaten (Table 5). ANOVAs showed no statistical relationships between average mean crayfish carapace length and fish species ($p=0.264$, $F=1.385$, $df=3$), nor between carapace length and substrate type ($p=0.320$, $F=1.176$, $df=2$).

A one-way ANOVA was used to test for differences in the proportion of fish with crayfish in guts across fish species. The results showed that smallmouth bass guts were most likely to contain crayfish, followed by rock bass and then yellow perch (p=0.001970, F=5.895898, df=7; Figure 1). Black crappie had no crayfish found in its guts. Bluegill (*Lepomis macrochirus*), pumpkinseed (*Lepomis gibbosus*), largemouth bass, and walleye guts were least likely to contain crayfish. However, because the sample size for pumpkinseed and largemouth bass was low, the error was high, resulting in there being low confidence in the data for these fish. A Tukey's test revealed that smallmouth bass guts were significantly more likely to contain crayfish than black crappie (p=0.005900), bluegill (p=0.020445), largemouth bass (p=0.020178), and pumpkinseed (p=0.012779). Rock bass were more likely than black crappie to contain crayfish (p=0.033516).

An ANOVA determined that the crayfish in guts relative to crayfish density found in the quadrants (Table 4) was significantly (p=0.004, F=6.860, df=2) different among substrate types (Figure 2). A Tukey post hoc test revealed that cobble had significantly different predation rates from macrophyte (p=0.006) and sand (p=0.014). A two-way ANOVA showed significance (p<0.0001, F=3.986, df=22) between crayfish presence in diet against the species of fish and the substrate. However, there were not enough replicates of every species to get p-values for each individual species graph against substrate.

Discussion:

My first hypothesis stating *O. propinquus* will be eaten less often by fish than that of *O. virilis* could not be supported. Due to circumstances unrelated to this project, I was only able to conduct electro-shocking on Plum one time, which limited my data for *O. virilis*, (as it was the only crayfish species present in Plum and the main reason the lake was chosen). Another reason I could not test this hypothesis was due to the difficulty in distinguishing crayfish species in the fish lavage. Many of the crayfish present in the lavage consisted of tiny pieces estimated around (1 to 4mm) in length that could not be used to determine crayfish species or length regressions. I was not able to measure half of the lavaged crayfish samples, and as a result only presence and absence was reported. It was interesting to note that most crayfish were unidentifiable, because previous studies report high percentages of identifiable and measurable crayfish from guts contents (Garvey et al. 2003). My chi-square analysis did show that a significantly larger proportion of crayfish were consumed in Tenderfoot versus Plum Lake on the one sampling date (6/27/2006) where they could be compared. This suggests that predation rates are higher on crayfish in Tenderfoot (primarily *O. propinquus*) than Plum (*O. virilis* only) which is contrary to hypothesis 1. However the abundances of crayfish were much higher in Tenderfoot than in Plum lake (Table 4). This difference in crayfish abundance could account for the differences found in the proportion of fish with crayfish in their guts (Figure 3).

Further investigation is needed to determine whether species of crayfish is driving this pattern.

I was able to support my second hypothesis stating that as fish size increases, mean crayfish size eaten will increase. Regressions per individual species of fish did not show significant increases in crayfish size as fish size increased. However, there was a significant trend when all species were analyzed together, but the variance was still high. This occurred because each species of fish typically has an average mean size, and some species are bigger than others. For example, smallmouth bass are typically bigger than rock bass and therefore will eat slightly larger crayfish. When you graph all the species of fish together you fill in the holes of size difference between species and get a good regression, unlike the individual regressions. My regressions for each single species were not significant, but for yellow perch and rock bass a general trend can be seen as crayfish consumed size increases as fish size increases. I believe that more sampling would show a significant effect of the size of yellow perch and rock bass on the size crayfish they eat, because there were not many crayfish that were measurable for each fish species and the variance would even out after more replicates and show a significant trend. Plus other studies have shown that mean crayfish size increases with the size of fish (Garvey et al. 2003). I know there is some positive relationship between fish size and prey size because fish are gape-limited (Stein 1977). If I was able to sample a wider range of fish sizes, because the larger fishes were able to escape the electrical field when shocking and the smaller fishes could not be lavaged due to size and time limitations, for each species I believe I would get significant results. The results do suggest that smallmouth bass, rock bass, and yellow perch may be suitable crayfish control because they eat more crayfish than other fishes.

A factor influencing my crayfish size data, was estimating the size of the crayfish based on pieces that were found in the fish lavage. Many of the crayfish present did not have adequate parts to measure or were heavily digested. When pieces were found they consisted mainly of chelae, and regressions of rusty crayfish were used to get a good estimate of the crayfish size. If time were not an issue, I would create regressions for *O. virilis* and *O. propinquus* by trapping intensively for weeks or until I had several hundred of each. Then I would measure specific morphological characteristics, such as chelae and abdomen lengths and widths according to the crayfish species and sex in an effort to create a regression that would be more accurate for these crayfish.

My third hypothesis, that smallmouth bass and yellow perch would consume the most crayfish and walleye the least, is supported by my analyses. Figure 1 shows the likelihood of finding crayfish in guts is highest in smallmouth bass, followed by rock bass and yellow perch. This is not surprising because smallmouth bass, rock bass, and yellow perch are known to be the main predators of crayfish (Stein 1977 and Garvey et al. 2003). Bluegill, walleye, and black crappie all had low likelihood of crayfish in their diets and this was not unusual because they are not thought to be considerable consumers of crayfish (Garvey et al. 2003).

My results supported my fourth hypothesis stating that fish will have a higher crayfish predation rate in a sand substrate due to lack of cover. The results from the ANOVA conducted on the proportion of fish with crayfish in their guts relative to crayfish density, significantly differed among the substrates. There was lower predation level relative to density in the cobble substrate than both the macrophyte and sand substrates. This is likely due to cobble offering refuges for crayfish from predation (Saiki and Tash 1979, Garvey et al. 1994, Kershner and Lodge 1995).

I experienced difficulties during my research process with the substrates throughout the year. When I was mapping the three different substrates originally, it was difficult to find large transects exclusively containing sand, macrophyte, or cobble in both lakes. Therefore the size decreased to approx. 50-m transects instead of my intended 100-m transects. Plum was especially a problem in finding diverse substrates because there was little vegetation or cobble in the entire lake. Therefore, I ultimately selected sites at each lake that most closely resembled the type of substrate. However, as the summer progressed and the water warmed, more vegetation began to appear in all of my sand substrates that were not supposed to be vegetated. Therefore, there is some temporal variation in all of my sand substrate data that may have affected my analysis. If I were to conduct this research again, I would conduct intensive sampling in a shorter time period in early and late May, when the substrates were more distinct. Another option that would help improve the quality of my research would be to include more lakes, which would then provide me with more replicates and fish lavaged data that is not just specific to one are. I would also want to test a few lakes with just rusty crayfish in them to determine if the same fish species prey on them, or if the fish eat the same quantity of the crayfish that they did in the lakes I tested.

If *O. rusticus* were to invade UNDERC property smallmouth bass, rock bass, and yellow perch might become their primary predators. Perhaps as a control from a management standpoint UNDERC could stock more smallmouth bass, rock bass, and yellow perch in lakes where rusty crayfish invade, however if there is abundant cobble in the lake, predators may not be able to control crayfish.

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Tables:

Sex	Morphological Characteristics	Regression Used
Unidentifiable	Chelae Length	(x + 14.63038)/ 1.26041
Unidentifiable	Chelae Width	(x + 5.28744)/ .49620
Unidentifiable	Abdomen Length	(x - 3.17913)/ .5889
Unidentifiable	Abdomen Width	(x + .74899)/ .49620

Table 1: Morphological characteristics used to measure crayfish. Regressions were used to calculate the carapace length by morphological characteristics and sex. ("x"= measurement of morphological characteristic) However, all of my crayfish were unidentifiable by sex except for a few, so the average regressions of both males and females were used. Regressions were developed from measurements of crayfish collected in summer 2005 by Scott Kuhn (Lodge lab REU).

Lake	Date	Fish Shocked	Fish Lavaged
Tenderfoot	6/8/2006	119	78
Plum	6/27/2006	190	90
Tenderfoot	6/29/2006	241	124
Tenderfoot	7/17/2006	156	72
Total	-	706	364

Table 2: The total amount of fish shocked for each lake, and the amount of these fish that were actually lavaged.

Species	Lavage Sample Size
Black Crappie	6
Bluegill	34
Largemouth Bass	8
Pumpkinseed	18
Rock Bass	65
Smallmouth Bass	31
Walleye	101
Yellow Perch	101
Total	364

Table 3: Amount of fish lavaged according to species

Lake	Substrate	Average Crayfish Density M^2
Tenderfoot	Cobble	5.53
Plum	Cobble	0.133
Tenderfoot	Veg	0.022
Plum	Veg	0
Tenderfoot	Sand	0.289
Plum	Sand	0.033

Table 4: The average crayfish densities per m2 for each lake and substrate

Species	P	r^2
Rock Bass	0.216	0.2089
Smallmouth Bass	0.662	0.0122
Yellow Perch	0.219	0.1818

Table 5:Non-significant Regressions Comparing Carapace Length Consumed to Fish Length

Figures:

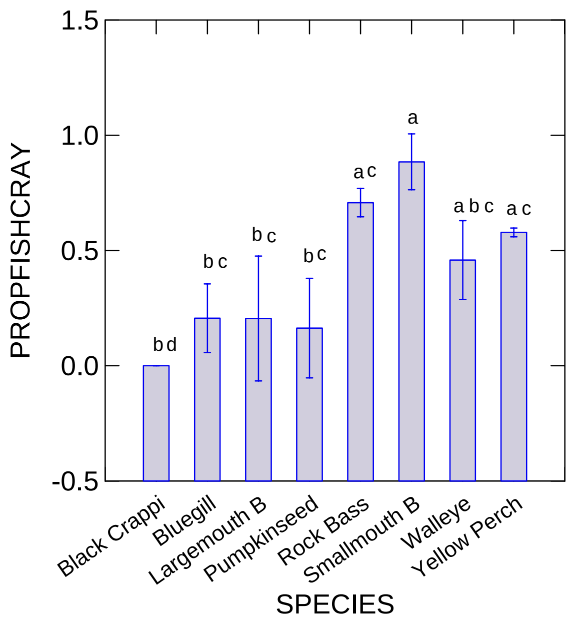

Figure 1: Percent of fish containing crayfish within each fish species. The data is significant with (p=0.001970, F=5.895898, df=7). Vertical error bars represent the standard error of each mean. Largemouth bass and pumpkinseed have high standard error because of low sampling size. A Tukey's test revealed that smallmouth bass guts were significantly more likely to contain crayfish than black crappie (p=0.005900), bluegill (p=0.020445), largemouth bass (p=0.020178), and pumpkinseed (p=0.012779). Rock bass were more likely than black crappie to contain crayfish (p=0.033516).

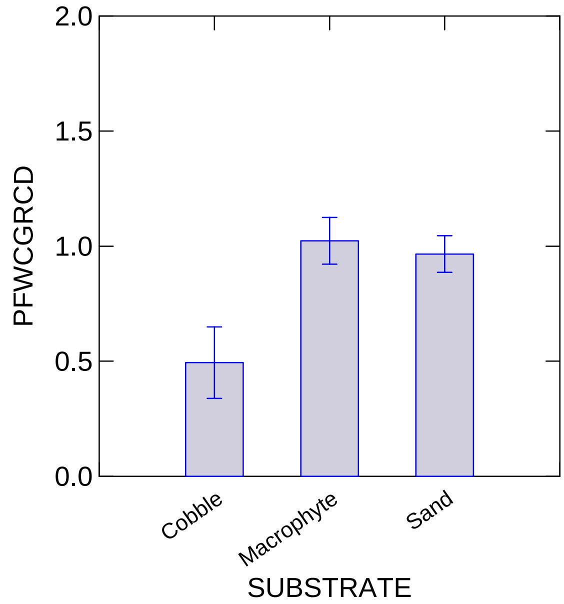

Figure 2: Proportion of fish with crayfish in guts relative to crayfish density (PFWCGRCD). was significantly (p=0.004, F=6.860, df=2) different among substrate types. A Tukey post hoc test revealed that cobble had significantly different predation rates from macrophyte (p=0.006) and sand (p=0.014).

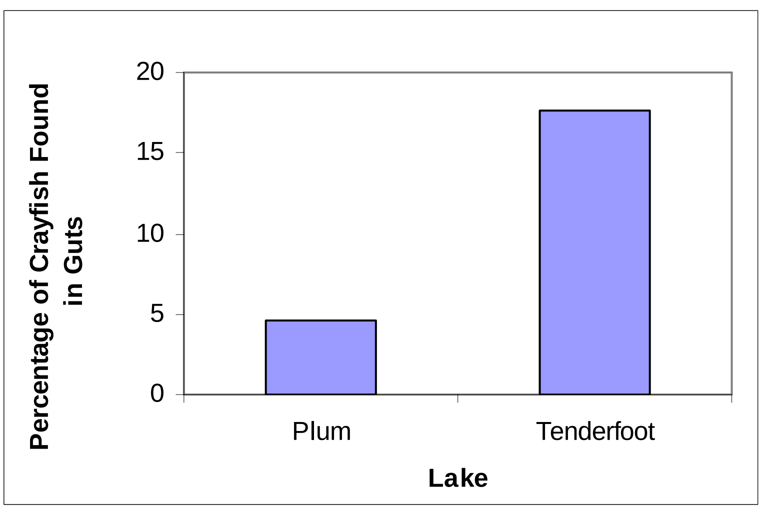

Figure 3: Percent of crayfish found in total amount of lavaged data per lake. A Pearsons chi-squared test found that Tenderfoot had significantly (p=0.000002, X^2=22.89026, df=1) more crayfish in the total amount of lavaged data.

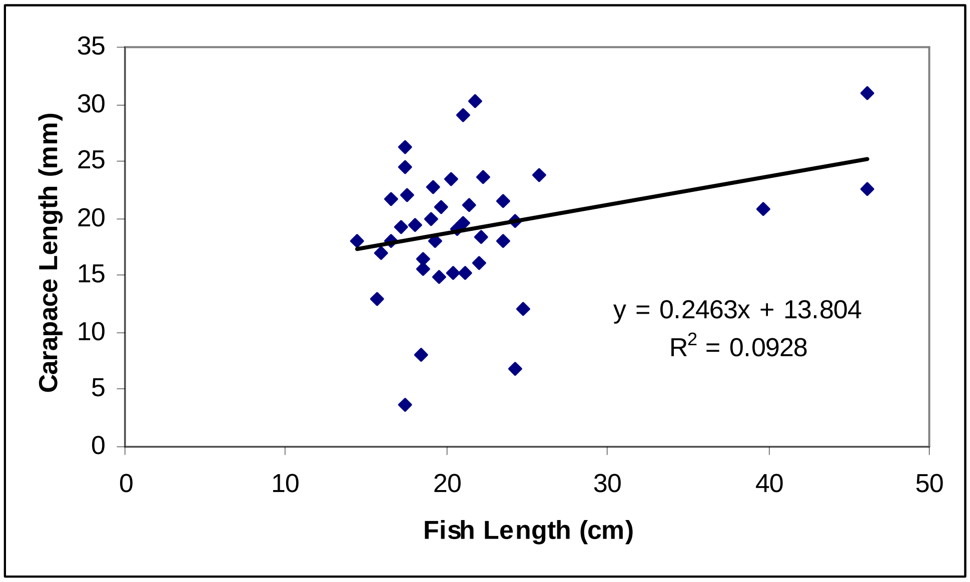

Figure 4: Length of crayfish carapace consumed vs. the length of fish consumer across all fish species. (p=0.059, r^2=0.0928)