CRAYFISH: SCAVENGER OR DEADLY PREDATOR? EXAMINING A POTENTIAL PREDATOR-PREY RELATIONSHIP BETWEEN CRAYFISH AND BENTHIC FISH IN AQUATIC FOOD WEBS

BY

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THESIS

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Abstract

Benthic food webs are complex and often poorly understood. Crayfish in particular play a key role in the transfer of energy to higher trophic levels, and can constitute the highest proportion of benthic invertebrate biomass in an aquatic system. Commonly referred to as a keystone species, crayfish are seen as ecologically important for their consumption of detritus and algal material, as well as for their role as a common prey item for over 200 North American animal species. More recently, crayfish have been recognized as obligatory carnivores, but studying crayfish as a potential predator of benthic fish in lotic systems, where they co-occur, has yet to be addressed. Competition exists between crayfish and Percid benthic fish in Illinois streams, for food resources and refuge from larger predators. Previous research, though, has ignored a potential predator-prey interaction between these two groups. I examined a possible relationship between crayfish and benthic fish populations, by both quantifying natural densities of both taxa and using enclosure/exclosure experiments. Data obtained from sampling Illinois streams showed that increased crayfish density correlates with decreased fish density, and this relationship is more pronounced in sites that have established populations of invasive crayfish. Additional data from manipulated in-stream enclosure/exclosure experiments examining the relationship between crayfish density and fish mortality from predation showed an occurrence of predation in all crayfish treatments, with the highest overall fish mortality occurring in the low density crayfish treatment. Finally, controlled tank experiments were performed to provide formal evidence that crayfish are capable of actively killing and consuming benthic fish. The results of this research provide novel insight into the complex and vital role of crayfish in aquatic food webs. This research also has important management and conservation implications, suggesting that streams that contain invasive crayfish might see a decline in native benthic fish biodiversity.

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INTRODUCTION

Ecological communities are complex and dynamic. Species composition and behavioral interactions can play a major role in shaping the structure of the community. Competition, in general, is a basic tenet of modern ecology and population biology which tries to explain community and species composition in terrestrial and aquatic ecosystems (Schoener, 1974). Groups of organisms that occupy the same ecological niche and vie for a limited resource, such as food, water, or mates, will be influenced by their interactions with members of their own species, e.g. intraspecific competition, and amongst members of different species, e.g., interspecific competition (Alley, 1982). Competition can be exploitative, where one group uses the limited resource, reducing the availability of that resource to other groups. Competition can also be interference, and refers to a group's ability to block access of a shared resource to others (Steinwasher, 1978). Competition can help to describe the equilibrium of species that is seen in nature, although competition alone is an oversimplified explanation when other complex interactions and evolutionary forces contribute as well (Fellers, 1987).

A second prominent selective force in ecological communities that also looks to explain phenomena such as microhabitat partitioning and behavior is predation, the act of one organism consuming another (Connell, 1980; Mercurio et al. 1985). Almost every animal is affected in their lifetime by the threat of predation. Predation can shape a species over evolutionary time, but individuals can also respond in ecological time to the pressures of predation, therefore predation has historical importance but also has effects on communities that can be observed in a generational time frame (Lima and Dill, 1990). Thus, as competition is a force affecting groups who require a shared and limited resource, predation plays an arguably stronger role in determining the interactions between co-occurring organisms in an ecosystem (Mercurio et al., 1985).

Aquatic ecosystems contain invertrebrates that play important ecological roles. Crayfish are an example of one important group of aquatic or semi-aquatic macroinvertebrates. They account for a substantial amount of biomass in both lotic and lentic ecosystems, and in some aquatic systems they comprise up to 50% of all benthic invertebrate biomass (Momot, 1995). Crayfish have commonly been referred to as a keystone species in lotic ecosystems (Lodge, 2001), because they have the ability to process organic matter and transfer energy between trophic levels. This is especially important in aquatic systems with low phytoplankton abundance, where crayfish are responsible for moving energy up the food chain through their consumption of detritus and algae (Momot et al., 1978).

Crayfish process substantial quantities of course particulate organic matter (CPOM) in streams. Whitledge and Rabeni (1997) found that crayfish processed more CPOM than all of the shredders combined in the White River basin system. Shredder is a term that refers to a functional feeding group of macroinvertebrates that process CPOM into fine particulate matter in stream ecosystems and are important for leaf decomposition (Merritt and Cummins, 1978).

Crayfish also alter physical habitats of the streams they inhabit. For this reason they are referred to as ecosystem engineers (Statzner et al. 2000). Crayfish move substrate and thus directly and indirectly influence macroinvertebrate communities. More specifically, crayfish increase habitat heterogeneity by perturbing sediment. This effect on habitat allows for an increase in abundance of smaller benthic invertebrates that can utilize the interstitial space being created by the crayfish (Brown and Lawson, 2010). Crayfish are also important in aquatic systems as they are prey for over 200 North American animal species, including a number of game fish, mammals, and birds (DiStefano, 2005).

The importance of crayfish in aquatic food webs has been rigorously studied, in regards to effects on macrophytes (Hanson and Chambers, 1995), algal growth (Charlebois and Lamberit, 1996) and other macroinvertebrates. Charlebois and Lamberti (1996) showed experimentally that the abundance of grazer invertebrates was decreased in the presence of crayfish, and periphyton levels were decreased by crayfish consumption. As a result, *chlorophyll a*, and thus primary production, was increased. This effect is consistent with a trophic cascade, although the total periphyton biomass did not show an overall decrease, which would be expected if a trophic cascade was occurring (Charlebois and Lamberti, 1996). Conversely, Dorn and Wojdak (2004) found that in the presence of crayfish, phytoplankton abundance, and thus primary production, was decreased. The authors attribute this to indirect and non-trophic related activities, such as bioturbation. By stirring up the water, crayfish hindered the ability of primary producers to photosynthesize, and overall levels of phytoplankton were reduced. This seemingly contradictory results support the complexity and sometimes unpredictability of trophic interactions in aquatic systems.

Crayfish can also decrease the abundance of detrital matter in stream ecosystems, and thus have a negative effect on primary consumers (Usio, 2000). Also, because crayfish are omnivorous, negative effects are seen at multiple trophic levels. Crayfish can decrease the amount of plant material in a system while simultaneously decreasing the abundance of predatory invertebrates (Usio and Townsend, 2002). Dorn and Wojdak (2004) discovered that zooplankton had a strong positive relationship with presence of crayfish, attributed to crayfish consumption of fish eggs, and thus overall reduction of fish predators.

Research has been conducted to look at interactions between crayfish, predatory fish, and small benthic fish in aquatic food webs. The virile crayfish, *Orconectes virilis,* is negatively affected by the common stoneroller (*Campostoma anomalum*), a grazing fish that was shown to monopolize resources and thus outcompete crayfish (Vaughn et. al, 1993). McNeely et al. (1990) showed experimentally that crayfish had a positive effect on mottled sculpin (*Cottus bairdi)* survival in predator-prey manipulations with smallmouth bass (*Micropterus dolomeiu)*. In the presence of crayfish, the mottled sculpin modified its behavior to use refugia more often, decreasing the rate of predation and increasing sculpin survival. From these two studies, one can see that crayfish can have differential effects on benthic fish that they co-occur with, and that competition is playing a role in determining species interactions.

Momot (1995), in a review, noted that crayfish had a relatively high proportion of animal matter in their diets, and proposed that crayfish are primarily carnivorous. It was also ascertained that the differing rates of digestion between plant and animal matter can skew results of gut content analyses in favor of plant material, because it takes longer to break down. Analyzing gut content is simple and inexpensive, but is only a short-term representation of what an organism was eating. This over-representation of plant matter in crayfish diet coupled with the hypothesis that crayfish cannot grow as quickly as they do while primarily consuming plant material led to a shift in the way crayfish are viewed in the food web. Instead of crayfish seen as opportunistic scavengers, it was hypothesized that they are obligate carnivores who facultatively consume plant matter (Momot, 1995).

Since 1995, other studies have given support to the concept of crayfish as carnivores. Parkyn et al. (2001) showed that crayfish preferably prey on benthic macroinvertebrates. At least 62% of crayfish analyzed by Parkyn et al. contained macroinvertebrates in their stomachs. Dorn and Wojdak (2004), in a pond experiment, documented crayfish consuming a large amount of Centrarchid sunfish eggs, and thus negatively affecting Centrarchid recruitment to year 1. Also, bluegill (*Lepomis macrochirus)* had unsuccessful nests until crayfish-free enclosures were built. Crayfish also reduced the number of bullfrog (*Rana catesbeina)* tadpoles in the experimental ponds, with no tadpoles found in crayfish ponds (Dorn and Wodjak, 2004). In a long-term field study, Wilson et al. (2004) showed a negative correlation between rusty crayfish (*O. rusticus)* density and density of smaller centrarchid species in lake experiments, and concluded this effect to be due to crayfish predation on centrarchid eggs, and direct competition for alternate invertebrate food sources.

Gherardi et al. (2001) showed experimentally that a satiated invasive red swamp crayfish (*Procambarus clarkii)* consumed tadpoles in half of the time it took for the satiated native European species in the study. Crayfish were shown to be opportunistic feeders that can switch quickly between prey items, but also, more generally, that crayfish have the ability to catch, kill, and consume tadpoles, which are similar in mobility and size to many North American benthic fishes.

Experimental manipulations between the introduced *O.virilis* and the federally threatened White Sands pupfish (*Cyprinodon tularosa)* yielded results demonstrating that the virile crayfish negatively affected reproduction and survival of *C. tularosa* (Rogowski and Stockwell, 2006). When *C. tularosa* was in the high density crayfish treatment, growth, reproduction, and survival decreased. There was no difference in these variables between the low density crayfish treatment and the crayfish-free controls. In addition crayfish were observed actively consuming adult pupfish in the high density treatment, though direct mortality was not quantified.

While crayfish may be viewed as capable consumers of fish, most studies have failed to empirically incorporate fish as a possible food source for crayfish. In their analyses, Guan and Wiles (1998) presented data that suggested that fish were a significant portion of crayfish diet; however, they failed to further quantify the relationship. Parkyn et al. (2001) included fish as a food source when looking at crayfish diet, but did not test this empirically, e.g., with a mixing model. Taylor and Soucek (2010) provide the first empirical evidence that fish are a significant portion of crayfish diet, shaping the view of their role in the aquatic food web.

Though fish are still ignored from many crayfish diet analyses, Taylor and Soucek (2010) utilized stable isotope analyses to determine that fish comprised approximately 12% of a native and non-native Illinois crayfish's diet. Crayfish consistently consumed fish matter in their diets, as the proportion of fish in the diet was less variable than other less nutritious (though still abundant) food sources such as detritus and algal material (Taylor and Soucek, 2010). With their diet analysis confirming that crayfish consume fish, Taylor and Soucek (2010) were not able to determine if crayfish were solely scavenging on fish or whether they were capable predators of fish.

Crayfish share specific riffle habitats with small benthic fish species (families: Ictaluridae, Percidae, Cottidae).In central Illinois, crayfish and Percid benthic fish commonly co-occur. Competition exists both for cover (Rahel and Stein, 1988) and for food resources (Becker, 1983). Diehl (1992) suggested a relationship between benthic fish and predatory macroinvertebrates, but only in terms of their relative predation by predatory fish. Guan and Wiles (1996) found that crayfish had an effect on benthic fish survival in British lowland streams. Lower abundance of two native benthic fish was correlated with higher abundance of crayfish. In artificial streams they also found fish survival decreased in the presence of crayfish and crayfish actively consumed the fish, as personally witnessed by researchers and verified by gut content analysis (Guan and Wiles, 1996). This suggests a complex relationship between crayfish and benthic fish that involves not only competition, but predation as well as an important selective pressure guiding the interactions of these two groups.

To better elucidate the relationship between crayfishes and stream benthic fishes, I employed a three-pronged approach to determine if crayfish predation affects benthic fish, particularly Percid benthic fish commonly found in central Illinois streams. Although Ictalurid and Cottid benthic fish also occur in Illinois, Percid benthic fish are the most abundant small benthic fish in Illinois, so they were used in this research. In the first phase, I sampled crayfish and benthic fish using a 1-m^2 quadrat sampling method to determine densities of both taxa in the environment. In the second phase, I used an enclosure/exclosure method to test if fish mortality increased as crayfish density increased. Finally, controlled tank experiments were performed to verify that crayfish are capable predators of benthic fish. These three aspects provide insight into the effect that crayfish have on populations of benthic fish in Illinois streams.

MATERIALS AND METHODS

Measuring the relationship between crayfish density and benthic fish density

Density Sampling: To test for a relationship between crayfish and benthic fish densities in central Illinois streams, a 1-m^2 quadrat sampler, a common sampling tool in stream ecology (Larson et al. 2008; design modified from Riggert and DiStefano, 1999 and Peterson and Rabeni, 1995; Fig. A.1) was applied May-August 2010. Thirty-one separate streams spanning five drainages in Illinois were sampled (Fig A.2). Streams were chosen that were wadeable and had gravel substrate, as this habitat is more conducive for Percid benthic fish and stream-dwelling crayfish. One riffle area site was identified in each stream, and length and width measurements of the riffle were taken. The area of the riffle was treated as an X-Y coordinate system. A random number generator was used to pick X and Y coordinates to randomly select square meter areas to sample with the quadrat within the selected riffle stretch. For the majority of streams (n=27), a total of five quadrats were sampled. For four streams, only three quadrats were sampled due to overall size of the riffle. Once a random square meter was chosen, the quadrat sampler was thrown over the area so that the bag opening faced upstream, to collect the dislodged organisms carried downstream. This method of placing the quadrat was deemed the most efficient means of collecting of animals through preliminary sampling, because fewer animals could escape. Substrate within the 1-m^2 quadrat was disturbed by vigorous kicking for a period of no less than one minute. Crayfish and fish were collected, identified, sexed (crayfish only), and measured with dial calipers. Velocity, temperature, and depth at each quadrat were then measured and recorded. For velocity, a digital flow meter measurement was taken just upstream of each quadrat. Depth was measured with a meter stick to the nearest centimeter.

For gravel, a five-point cross bar was placed in each quadrat after fishes and crayfishes were collected. The gravel piece at each of the five tips was then measured with a gravelometer, placed in a size class based on a modified Udden-Wentworth scale (Table A.1), and averaged per square meter and subsequently per site.

Statistical Analyses: The densities from the quadrat samples were averaged per stream to give a mean crayfish and benthic fish density per square meter per stream. Non-benthic fish densities were not included in analyses. An analaysis of covariance (ANCOVA) was run to test for a relationship between crayfish density and benthic fish density per square meter using JMP V.9. To normalize data to meet the assumptions of an ANCOVA, fish density data and crayfish density data were square root transformed (Dytham, 2007). Abiotic factors were also tested as covariates to determine their effect on crayfish and benthic fish densities using JMP V.9.

Measuring the effect of crayfish density on fish mortality through predation

Study System: A field experiment was performed August through September 2011 to test for an effect of crayfish density on fish mortality. An enclosure/exclosure method was employed, with a design replicated from Rabalais and Magoulick (2006) and Larson and Magoulick (2009). Trials consisted of three treatments and a control, and were run in a 14 m stretch of Jordan Creek, a second-order warm water tributary of the Salt Fork in Vermilion County, IL. Organisms involved were *O. propinquus* and *O. virilis* crayfish, and five species of *Etheostoma* darters, which co-occur in the riffles in this stream.

Crayfish manipulations: Enclosures measuring 71 cm long by 40 cm wide by 21 cm high and consisting of a plastic frame surrounded by 6 mm stainless steel woven mesh (Fig. A.3) were secured in the stream segment that was 11 m long and 7 m wide. The enclosures were set into the sediment and tethered to secure tree roots on land, to prevent them from washing downstream. Enclosures were placed into the stream segment at similar depth and current velocity, and in such a way that no two cages were aligned parallel to flow (Fig. A.4). Cages were placed at an average depth of 29 cm. Treatments were randomly assigned to enclosures. Seven rocks (average longest side 14.8 cm +/- 1 SD of 4.6 cm) from within the stream reach were placed in the enclosures and 1 piece of black hard plastic mesh (36 cm l by 30 cm w) was placed inside each enclosure to act as cover for crayfish and fish.

Crayfish for treatments were collected from Jordan Creek by kick-seining in nearby (<500 m) riffles (Fig. A.5). This involved setting a 3m seine (mesh size=6mm) downstream of a riffle, and vigorously disturbing substrate by kicking gravel. Crayfish flowed downstream into the seine, and the seine was brought on shore so that animals could be identified before placing them in a 3.8 L bucket. Although some studies suggest a sex difference in aggression and thus feeding habits (Abrahamsson, 1966), more recent work has found sex differences between crayfish in aquatic food webs to be insignificant (Usio and Townsend, 2002), thus both sexes were included in the experiment. Crayfish were identified and sexed, and carapace length (CL) was measured to the nearest millimeter with a dial caliper. The two species used were *O. propinquus* and *O. virilis.* Crayfish were then added to enclosures based on the randomly assigned treatment. Crayfish were assigned to low- (n=1), medium- (n=2), and high- (n=3) density treatments (Table 1). These densities correspond to scaled down densities that were obtained in Illinois streams with the quadrat sampler during Summer 2010, with the low density treatment corresponding to the average square meter density of crayfish obtained in central

Illinois streams, and the high density treatment corresponding to a density threshold apparently exhibited by crayfish in streams that contain invasive crayfish. A two-day acclimation period was allowed for crayfish to clear their guts. After the two-day acclimation, darters (Family: Percidae Genus: *Etheostoma*) were collected using the same kick-seine method in nearby riffles. Darters were then identified and standard length was measured to the nearest millimeter with a dial caliper. Two darters were placed in each enclosure. In the control trials, fish were allowed to live in enclosures for the five-day trial with no crayfish predators. After a five-day period, enclosures were removed from the stream and occurrence of fish mortality was recorded. If at least one fish was missing from the enclosure, the crayfish were vouchered, to verify through stomach content analysis that the fish did not simply escape the enclosure. Trials were run 12 at a time from May to September 2011.

Statistical Analyses: A one-way Analysis of Variance (ANOVA) was used to assess treatment effect on occurrence of predation events. The predation occurrence rate per treatment was treated as a binary response, where 1=all fish survived trial and 0= 1 or 2 fish consumed in trial. An ANOVA was performed to test if the rate of predation events differed by treatment. Predation occurrence data was arcsine square root transformed so that the data could be treated as a continuous variable.

A second ANOVA was run to determine the effect of treatment on total fish mortality using JMP V.9. For each ANOVA, a Tukey-Kramer HSD pairwise comparison was employed to compare differences between pairs of treatment means and between each treatment mean and the control. Sampling date was tested as a covariate in the ANOVA to account for temporal site differences.

Table 1: Experimental design of crayfish and benthic fish enclosures

Treatment	Number of Crayfish	Number of fish	Replicates
Fish only	0	2	19
Crayfish-Low	1	2	20
Crayfish-Medium	2	2	20
Crayfish-High	3	2	20

Measuring predation rate by crayfish on fish in controlled tank experiments

Tank set-up: Aquaria experiments were performed March through September 2011. Four 37.7 L aquaria were set up. Each tank contained a divider, which subsequently brought the available bottom area of the tank to 508 cm^2 and total available volume to from 37.7 L to 4.06 L. Sand and small gravel substrate were put equally into each tank to a height of 1.5 cm, along with one rock to act as cover for the animals (average size longest side= 16.6 cm). An airstone was added to each tank, along with dechlorinated tap water.

Aquarium trials: Crayfish (*Orconectes propinquus*, and *O. virilis;* Fig. A.6) and benthic fish (darters in the genus *Etheostoma)* were collected using a kick seine method from streams in east central Illinois. The animals were brought to the tank room in 3.8 L buckets. Crayfish CL was measured to the nearest millimeter using dial calipers, and individual crayfish were placed in experimental tanks to acclimate, whereas darters were acclimated in a 37.9 L holding tank with a filter and an airstone.

After the two-day acclimation period, one darter was captured from the holding tank and identified to species. Standard length was measured with dial calipers to the nearest millimeter. The fish was then placed in an experimental aquarium with a crayfish. The status of the fish was observed every 4 hours during daylight, and every 8 hours over-night for a total of 96 hours per trial. If the fish was not observed, the crayfish was preserved in 70% EtOH and stomach contents were identified to confirm the fish was consumed and did not escape. After 96 hours, remaining crayfish and unconsumed fish were preserved. 37 total trials were run. Frequency of fish mortality was calculated.

RESULTS

Measuring the relationship between crayfish density and benthic fish density

Crayfish and benthic fish density per square meter were quantified for 31 streams, and frequency distributions for crayfish and benthic fish densities were determined and averaged per site (Fig. 1; Fig. 2). The highest density of crayfish per quadrat was 11 per square meter; the highest density of benthic fish was five per square meter. (Table 2; Table 3; Fig. 3) Across the 31 sites there was a negative relationship (Fig. 4).

The correlation coefficient (*r)* for all sites combined was -0.4139. For sites with native crayfish only (n=23), *r* was -0.4081. At sites which included the invasive rusty crayfish, *O. rusticus* (n=8), *r* was slightly higher, at -0.5416. It was also observed that at average crayfish densities higher than 3 per square meter, benthic fish density leveled off, and no benthic fish density higher than 0.5 was observed. No abiotic factors were found to significantly explain crayfish and benthic fish density when tested as covariates in an ANOVA (Table 4; JMP V.9).

Table 2: Summary statistics for crayfish and benthic fish density sampling data of 31 streams.

Taxa	Min/Max densities per square meter	Mean density per square meter
Crayfish	0-11	2.67
Benthic fish	0-5	0.41

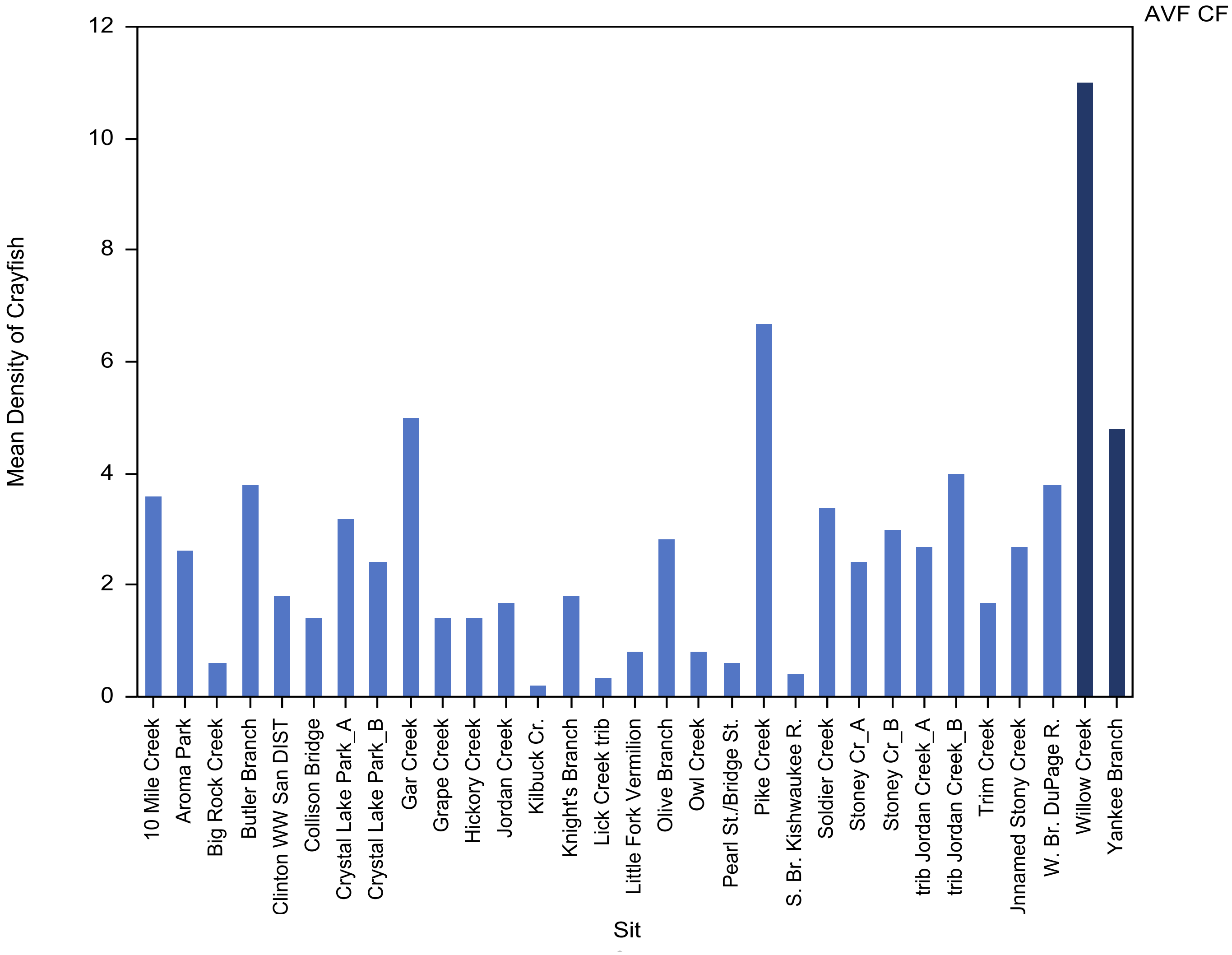


Figure 1: Mean density of crayfish per site of 31 streams sampled for density data.

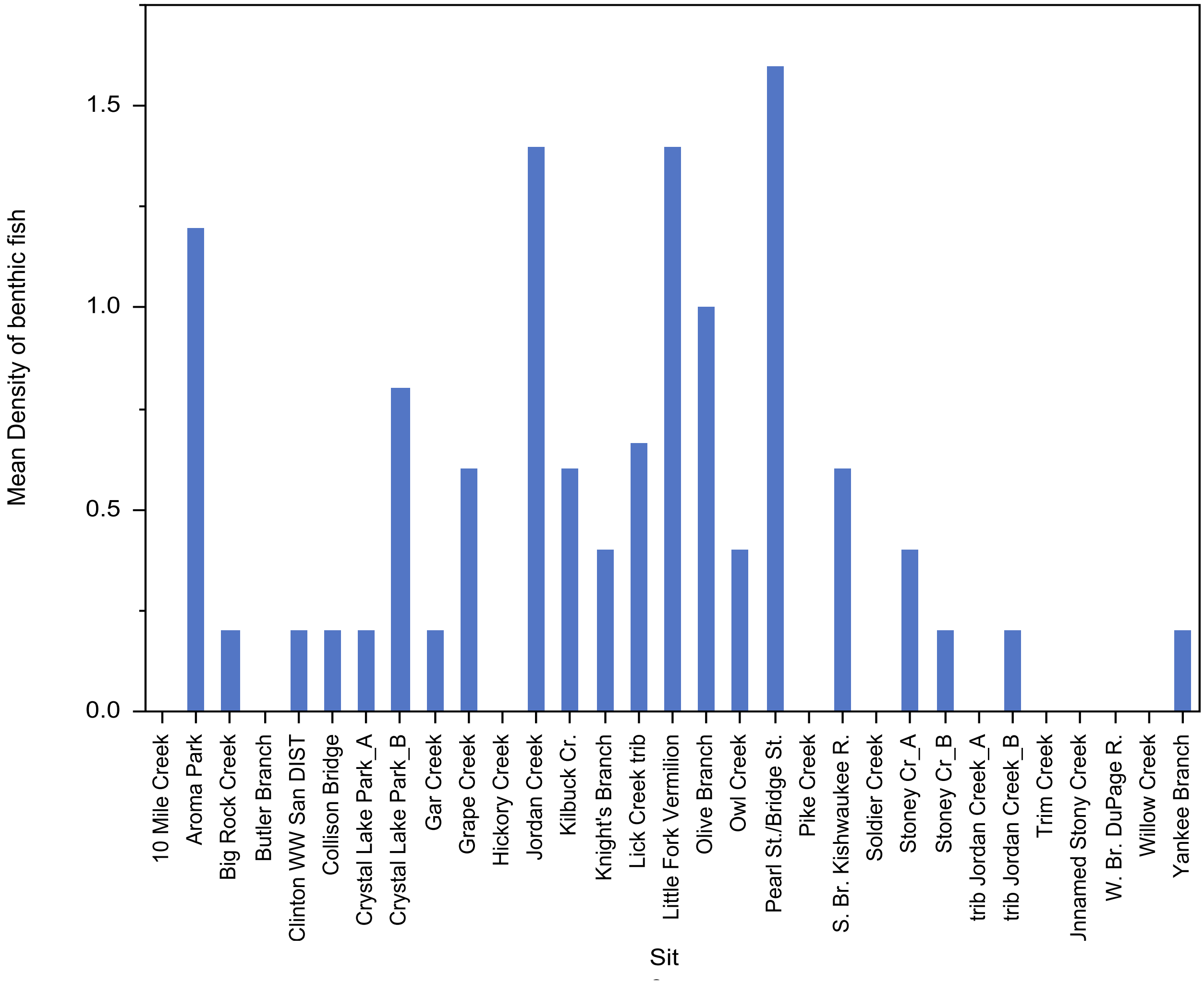
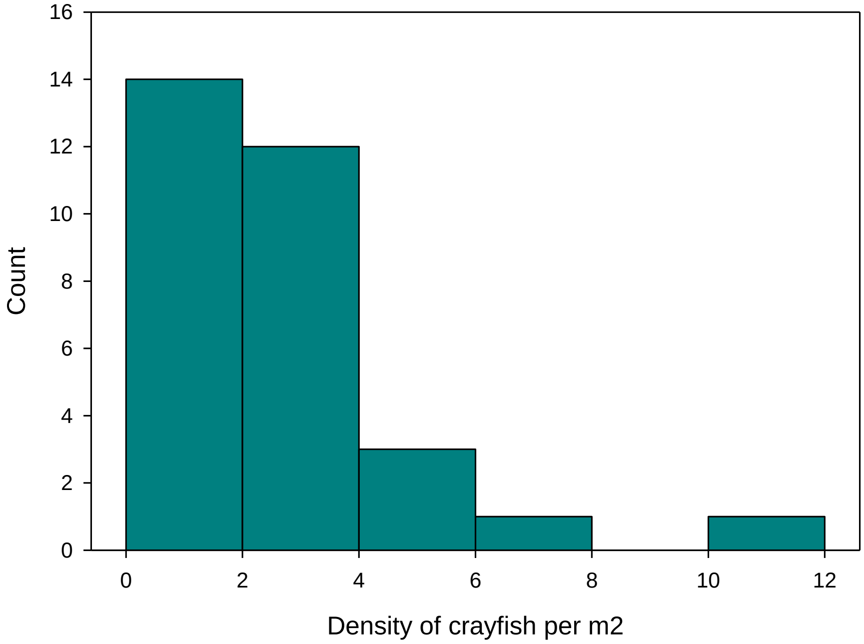


Figure 2: Mean density of crayfish per site of 31 streams sampled for density data.

Table 3: List of average crayfish and benthic fish densities per square meter for all 31 streams sampled with crayfish species present (*OP= O. propinquus, OV= O. virilis, OR= O. rusticus)* and geographic information (latitude/longitude).

Stream	Lat. (°N)	Long. (°W)	Mean Crayfish/ m^2	Crayfish species present	Mean benthic fish/ m^2	No. of quadrats per site
Stoney Creek A	40.09928	87.82677	2.4	*OP*	0.4	5
Saline Ditch A	40.12412	88.20944	3.2	*OP*	0.2	5
Saline Ditch B	40.13422	88.21034	2.4	*OP, OV*	0.8	5
Owl Creek	40.31089	88.34206	0.8	*OV*	0.4	5
Jordan Creek	40.07308	87.82439	1.67	*OP*	1.4	3
Butler Branch	40.07868	87.71389	3.8	*OP*	0	5
Knight Branch	40.25594	87.82453	1.8	*OP*	0.4	5
Stoney Creek B	40.19431	87.58418	3	*OP*	0.2	5
Lick Creek	40.08711	87.62308	0.33	*OV*	0.67	3
Grape Creek	40.06480	87.60843	1.4	*OP*	0.6	5
Middle Fork	40.22486	87.87524	1.4	*OP*	0.2	5
Soldier Creek	41.11247	87.97852	3.4	*OP, OR*	0	5
Trim Creek	41.20018	87.64513	1.67	*OP*	0	3
Pike Creek	42.21567	87.61537	6.67	*OP, OV,* OR	0	3
Kankakee River at Aroma Park	41.07431	87.80378	2.6	*OP, OR*	1	5
Unnamed trib of Jordan Creek A	40.09317	87.81347	2.67	*OP*	0	5
Olive Branch	39.92652	87.59082	2.8	*OP, OV*	1	5
Yankee Branch	39.94120	87.55151	4.8	*OP*	0.2	5
Little Fork Vermilion	39.95607	87.64618	0.8	*OP, OV*	1.4	5
Gar Creek	41.11247	87.97852	5	*OP*	0.2	5
10 Mile Creek	40.75002	89.49115	3.6	*OV*	0	5
Hickory Creek	41.55025	88.00563	1.4	*OP*	0	5
West Branch of DuPage River	41.93779	88.17421	3.8	*OR*	0	5
Big Rock Creek	41.67394	88.38158	0.6	*OP*	0.2	5
Willow Creek	42.32048	89.03065	11	*OR*	0	5
Pearl Creek	42.41205	89.01690	0.6	*OR*	1.6	5
South Branch Kishwaukee River	42.22601	88.50801	0.4	*OR*	0.6	5
Kilbuck Creek	42.28120	89.10324	0.2	*OR*	0.6	5
Unnamed trib of Jordan Creek B	40.10017	87.72244	4	*OP*	0.2	5
Unnamed trib of Stoney Creek	40.45573	88.16284	2.67	*OP*	0	5
Clinton WW Sanitation District	40.13880	88.96522	1.8	*OV*	0.2	5



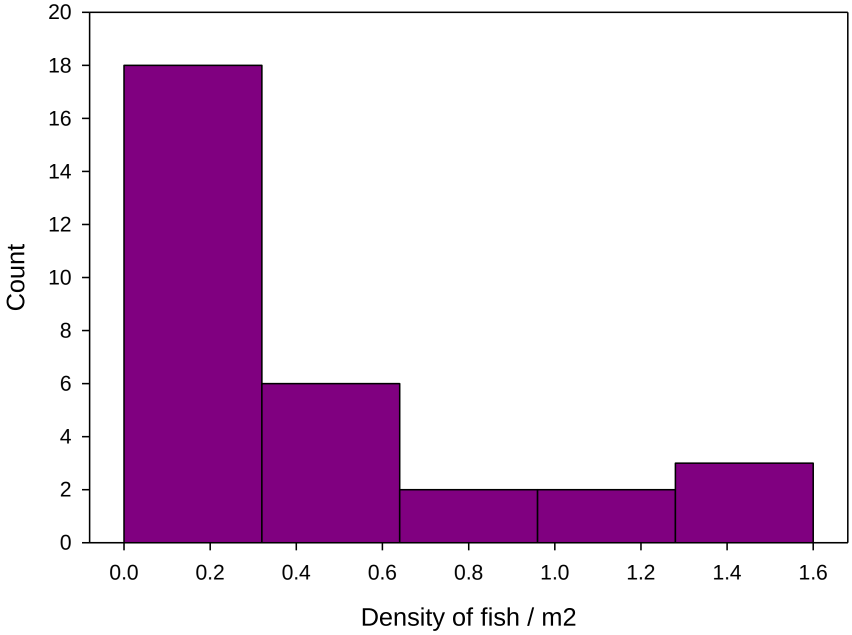


Figure 3: Histograms containing frequencies of average density of crayfish (top) and benthic fish (bottom) for the 31 sampled streams

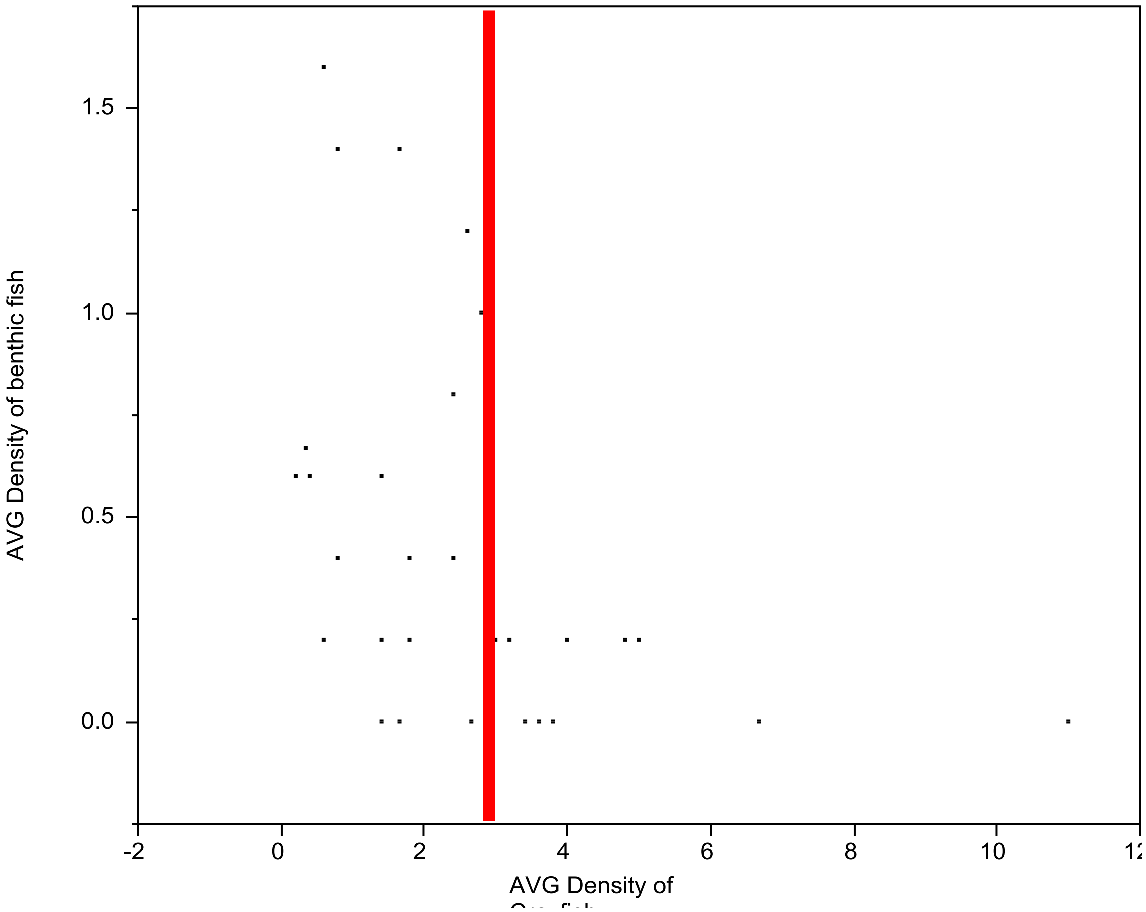


Figure 4: A correlation of crayfish density and benthic fish density, (n=31, r= -0.4319; JMP V.9). Red line indicates threshold where at higher crayfish densities benthic fish densities level out and remain below 0.5 per square meter.

Table 4: List of abiotic variables tested as covariates in an ANOVA to explain crayfish and benthic fish densities obtained with quadrat sampler (at α=0.05)

Variable	Effect on benthic fish density (p-value)	Effect on crayfish density (p-value)
Depth	.3741	.1727
Flow	.7523	.6455
Average Gravel Size	.7917	.3875
Temperature	.9001	.1439

Measuring the effect of crayfish density on fish mortality through predation

In control treatments, no fish suffered mortality in the enclosures or escaped. The size of crayfish used in enclosures ranged from 20 mm CL to 41 mm CL. Summary statistics for total fish mortality and number of predation events per treatment described the trends (Table 5).

Occurrence of predation did differ among treatments (*F*= 3.5718, p <0.0180; Fig. 5). A Tukey-Kramer HSD comparison revealed that rate of predation events for the low density treatment was found to significantly differ from the control, but no other treatment means differed (JMP V.9). Across treatments, the highest total fish mortality occurred in the low density treatment (Fig. 6). Results showed that fish mortality did differ significantly among treatments (*F*=3.2952, p< 0.0251). Crayfish injury and death was noted in three separate high density trials, but did not occur in any of the low or medium density trials.

Table 5: Summary statistics for enclosure experiment. Total fish mortality is out of a possible 40 (2 fish per trial per 20 trials). Number of predation events is out of 20 trials.

Treatment	Total fish mortality (%)	No. of trials with predation event (%)
Low density	20	35
Medium density	5	10
High density	17.5	20

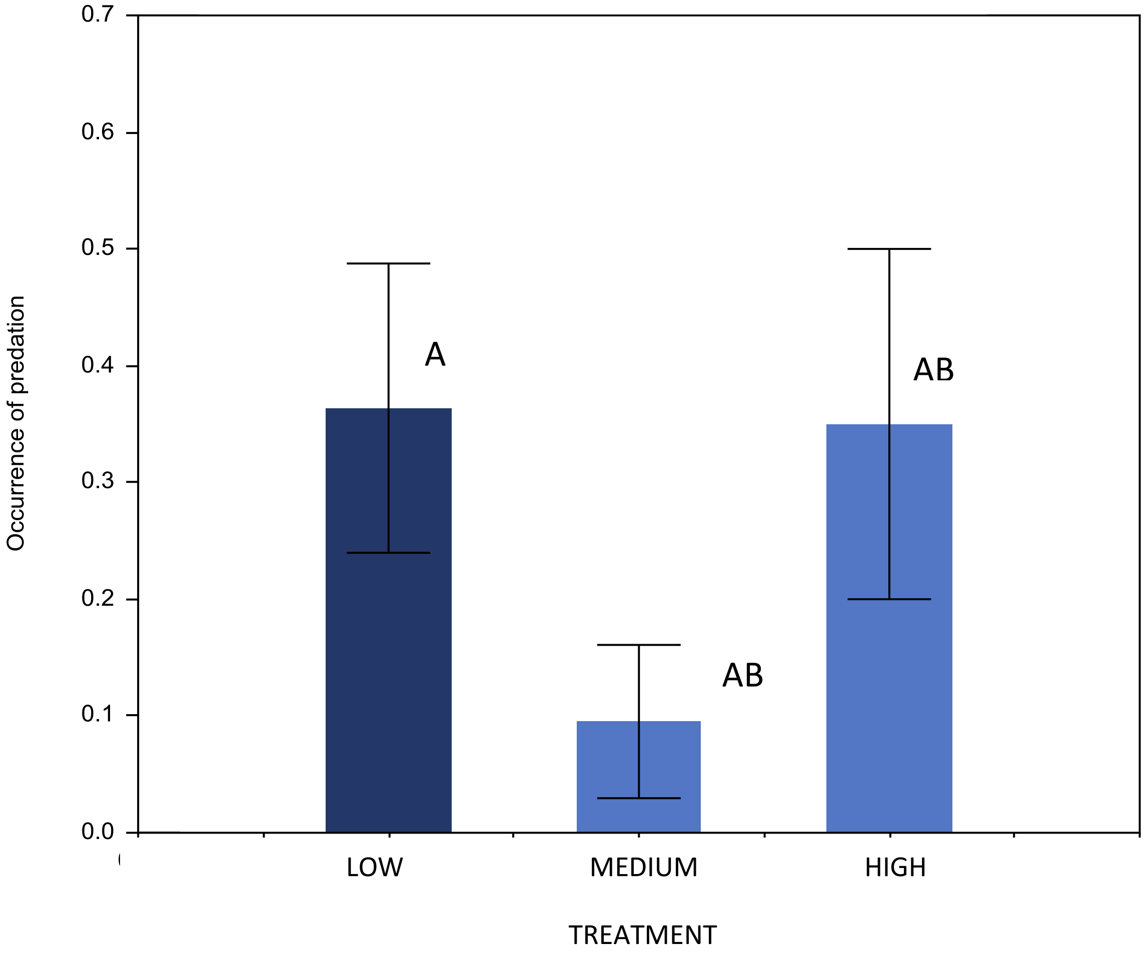


Figure 5: Results from an ANOVA (Tukey-Kramer HSD comparison) comparing mean rate of predation events by treatment. Means not connected by the same letter are significantly different from each other, CONTROL treatment mean was B (at α=0.05 p=0.0207). Each error bar represents 1 standard error from the mean.

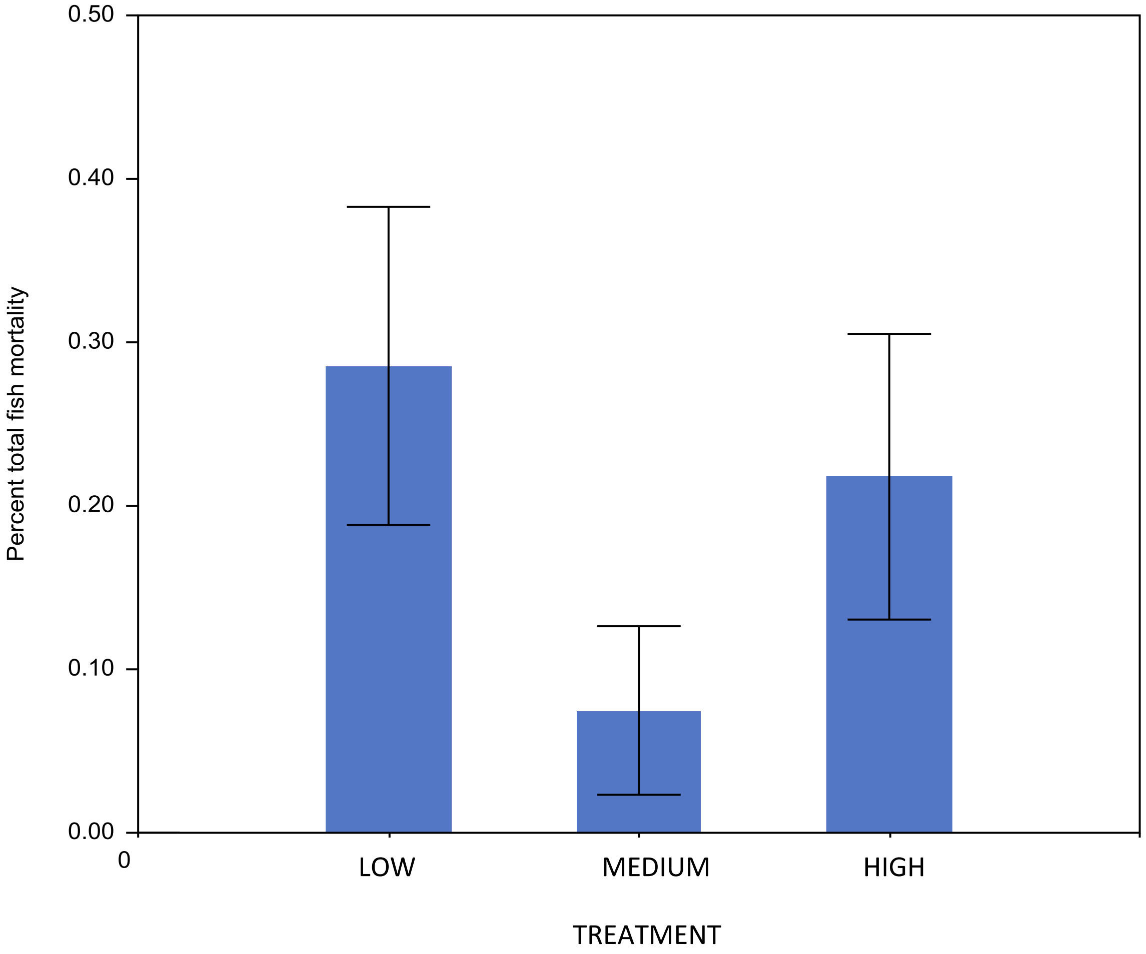


Figure 6: Mean fish mortality by treatment. Means were not significantly different. Each error bar represents 1 standard error from the mean.

Measuring predation rate by crayfish on fish in controlled tank experiments

Fish were consumed in six of the 37 trials (Fig. 7). The average size of fish used in the tank experiments was 43.3 +/- 6.5 mm SL The average size of consumed fish was 44.3 mm. The mean CL of crayfish used in the tank experiments was 38.1 +/- 10.5 mm. The average size of crayfish in trials where predation occurred was 41.6 mm. Crayfish and fish were paired at random and neither species nor size determined the outcome of a trial.

Three of the consumption events involved *O. virilis* (1 female, 2 male), and 3 of the events involved *O. propinquus* (1 female and 2 male. Crayfish were witnessed actively capturing and consuming fish in two of the trials (personal obs.). In all six instances of fish consumption, fish parts were found in crayfish stomachs. The fish parts can be attributed to consumption of fish from the trial; the two day acclimation period allowed time for previous crayfish gut contents to be cleared.

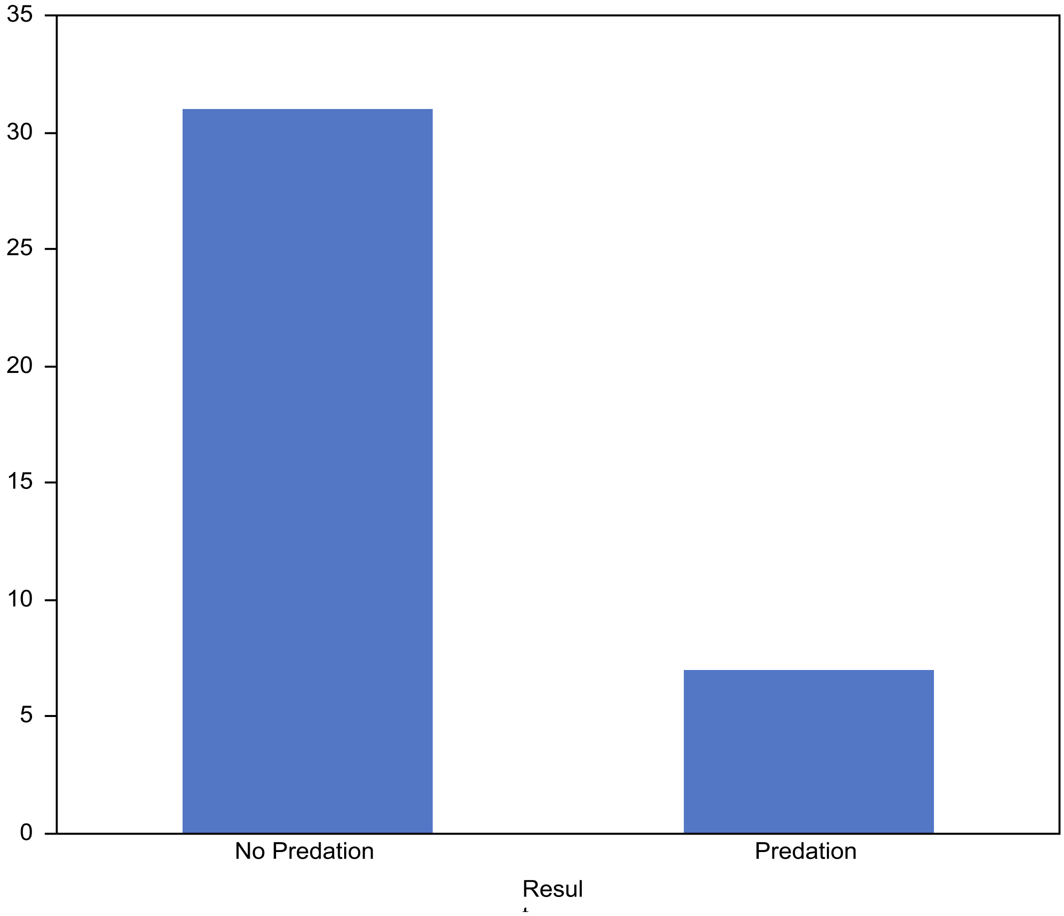


Figure 7: Results from controlled tank predation experiments. Predation result indicated the fish was either seen killed and consumed or fish was not present when tanks were checked, and fish consumption was confirmed in stomach content analyses.

DISCUSSION

Measuring the relationship between crayfish density and benthic fish density

My results indicate that at high benthic fish densities, lower densities of crayfish were encountered. More specifically, there is a threshold whereby if crayfish density is greater than three, we do not see a benthic fish density greater than the median density observed, 0.41 per square meter, and average benthic fish density levels off. Therefore, the relationship is not linear. It is interesting to note that in the streams that I sampled in the Kankakee area in northeast central Illinois where *O. rusticus* is currently invading (n=8, refer to Table 3), crayfish densities were much higher than densities observed in streams where only native crayfish occur. Some quadrat densities were as high as 11 per square meter, the high end of the range of *O. rusticus* density. *O. rusticus* generally grow faster and larger than our native crayfish, and they are more fecund, increasing their ability to displace natives (Wilson et al. 2004). The absolute value of *r* for the densities at sites with *O. rusticus* was higher than at sites without, providing initial evidence that sites with invasive crayfish might see a greater decline in native benthic fish biodiversity.

Crayfish and benthic fish compete for refuge amongst substrate (Rahel and Stein, 1988) and for food resources (Becker, 1983). The inverse correlation might have been exhibited because the spatial distribution of these two taxa is influenced by their direct competition for resources. But along with competing for food and shelter, crayfish may also be actively preying on benthic fish. A predatory relationship could account for the results of Taylor and Soucek (2010) who found that fish comprised 12% of crayfish diet when utilizing a stable isotope analysis.

Since my work was observational, not experimental, I cannot ascertain any causation behind the correlation, though I would argue that because the benthic fishes collected at sampling sites are gape-limited and because juvenile crayfish still possess chelae and a hardened carapace that crayfish are not a preferred food source in darter diet. Diet studies on other *Etheostoma* species have not identified crayfish as a component of darter diets (Barton and Powers, 2010; Carney and Burr, 1989; Cummings et al. 1984; Burr and Page, 1978), and instead there is a general trend that smaller *Etheostoma* species prefer microcrustaceans such as copepods, while larger *Etheostoma* darters prefer aquatic insects, especially those in the family Chironimidae (Cordes and Page, 1980). While crayfish might compete for food resources with darters, they themselves are not being consumed by fish; therefore the inverse correlation is better explained as an effect of crayfish on benthic fish.

The determination of crayfish density per square meter is important to report, and similar data are available and commonly used in stream experiments in the Midwest. In Missouri, where the quadrat sampling method for crayfish densities is commonly used, densities have been found as high as 30 per square meter, and average around 20 crayfish per square meter, one of the highest average densities of crayfish in the world (Flinders and Magoulick, 2007). The average crayfish density I obtained was 2.67 per square meter. One reason that accounts for my results being markedly less is that I restricted my sampling to riffle segments of streams, where crayfish co-occur with benthic fish. Flinders and Magoulick (2007) found their highest crayfish densities in backwaters and highly vegetated stream margins, presumably because amount of cover is greater and predation is reduced. Also, the densities that Flinders and Magoulick (2007) obtained were from streams in the Ozark region of south-central Missouri, where at least 26 of Missouri's 35 crayfish species co-occur. The streams I sampled mainly contained *O. propinquus* and *O. virilis*, two common species out of the 23 species found in Illinois. Other studies have found similar densities of stream-dwelling crayfish. For example, densities have been calculated of 2.51 crayfish per square meter (Rabeni et al. 1997), and 4.2 crayfish per square meter (Vlach et al. 2009). *O. virilis*, while native in Illinois, has been found in much higher densities when it establishes in non-native habitat, with an average density of 9.3 per square meter in the Yampa River in northwestern Colorada, a drainage which initially did not experience crayfish (Martinez, 2011).

Measuring the effect of crayfish density on fish mortality through predation

No fish in control treatments died or escaped, so it is not expected that fish would suffer mortality in the treatments with crayfish solely due to confinement in the enclosure. Thus, fish mortality can be attributed to presence of crayfish. It is still possible that direct competition for food in the enclosures caused the fish to decline in condition, making them easier prey for a crayfish. If this were the case, one would expect an increase in fish mortality in the high crayfish treatment. This was not found. The total number of fish consumed was highest in the low density crayfish treatment (Fig. 6). Competition influences these groups, but if it was the sole mechanism involved in the interactions between crayfish and darters, one would expect a decrease in fish condition and survival with an increase in crayfish density, which did not occur. Thus, competition alone cannot explain these findings, and it is likely that predation plays a key role in determining the overlap and interactions between the groups.

It is important to note that fish consumption was occurring in the three crayfish treatments. This result has important conservation implications. Many different aquatic organisms are in need of protected status for reasons ranging from competition with invasive species, to habitat loss and degradation. Fish are the most imperiled vertebrate group after amphibians, with 39% of species on the continent facing conservation threats (Jelks et al., 2008). Better conservation of aquatic organisms requires current information on species interactions. Clarifying the effect of crayfish on benthic fish provides a higher level of detail pertaining to aquatic food webs and can inform management decisions. Increasing crayfish densities could cause a decrease in benthic fish densities; this would be important information for resource managers who work in systems with threatened or endangered benthic fish.

When the data are modified to represent fish survival in terms of occurrence of predation events, there are significant differences. The rate of predation is significantly greater in the low density treatment than the control. Since predation did not occur in control trials, one might expect a significant difference between each treatment and the control, but only the low density treatment was significantly different. One reason for this might be crayfish conspecific aggression. Predation occurrence rates in the medium and high density treatments were not statistically different. But, crayfish injury and death was noted in three separate high density crayfish trials, and absent from low and medium density trials. This suggests that in higher densities, crayfish will show aggression towards their conspecifics over aggression towards fish.

Crayfish have differing levels of aggression, while invasive species tend to be more aggressive than the native crayfish (Pintor et al. 2008). Therefore, the biggest impact on benthic fish might occur in streams with species of more aggressive crayfish. Invasive crayfish such as the rusty crayfish, *O. rusticus,* are currently spreading throughout most of the Midwest, including Illinois (Fig. A.7), via bait bucket introductions (DiStefano, 2009). Crayfish, as a group, are severely imperiled. As of 2006, 48% of crayfish species in North America (174 out of 363 species) are of conservation concern (Taylor, et al., 2007), and invasive crayfish species are the greatest threat to native crayfish biodiversity (Lodge et al. 2000). The two species used in my manipulations, *O. propinquus* and *O. virilis*, are known to be less aggressive than *O. rusticus* (Wilson et al. 2004), so fish survival would be expected to decrease in the presence of a more aggressive predator.

Measuring predation rate by crayfish on fish in controlled tank experiments

Predation in the controlled aquaria experiments occurred in six out of 37 trials, or 16%, which was similar to the average fish predation of 23% measured in the enclosure experiment. Both of these rates are slightly higher than the 12% of crayfish diet that consisted of fish, as determined by Taylor and Soucek (2010). This could be explained by the rationale that fish will avoid crayfish when possible in nature, and would not have boundaries preventing their escape, such as the walls of the aquaria or the sides of the enclosures. So, while crayfish might prey on fish, the occurrence of predation in controlled settings could be higher than the actual rate of consumption of fish seen in the wild.

On two occasions the capture and consumption of a live fish was witnessed. These results unambiguously demonstrated that crayfish do have the ability to actively prey on live fish. Also, the fish component of crayfish diet does not have to solely originate from crayfish scavenging dead fish parts.

Except for the two trials where crayfish capturing and eating fish was witnessed, all predation events occurred at night. This may be explained by the fact that most darters are visual predators (Cordes and Page, 1980). They rely heavily on vision during foraging, but also arguably for predator avoidance. Since light levels diminish at night, it could be expected that most fish predation by crayfish occurs at night when darters are at a visual disadvantage.

The tank trials were performed to determine if crayfish can actively prey on fish. Differences among crayfish species was not quantified, but would be of interest since different crayfish from the same genus have been found to have differing aggression levels (Garvey et al. 1994) and feeding behavior (Olson et al. 1991).

SUMMARY AND CONCLUSION

Crayfish and benthic fish co-occur in riffles in gravel-bottom streams in Illinois and across North America. They compete for food and shelter. In Midwest streams where crayfish occur in higher densities, benthic fish occur in lower densities, and there appears to be a threshold where crayfish densities higher than 3 per square meter results in a sharp decline in Percid fish densities. This density corresponds to densities of crayfish sampled in Illinois streams which contained the rusty crayfish. When crayfish and benthic fish are placed in enclosures and allowed to live together for 5 days, crayfish had predation at 23%. Finally, crayfish killed and consumed fish in 16% of controlled aquaria experiments.

Crayfish and benthic fish do compete for food, and I have found that fish is ultimately a component of crayfish diet, so predation, as well as competition is affecting crayfish and benthic fish. If crayfish are actively preying on benthic fish, one would expect to see the inverse relationship in their natural densities that were recorded with the quadrat sampling method. This also is a cause for concern when quantifying the impacts of invasive crayfish species, which occur in higher densities, on native fish fauna. Crayfish predation is also supported by the finding that predation events occurred in each crayfish density treatment. Crayfish are affecting benthic fish that co-occur in stream riffles, and influencing their densities. Also, my controlled tank experiments provided verification that crayfish can actively kill and consume benthic fish.

I would argue that crayfish predation on benthic fish can potentially have a large impact on benthic fish populations, especially Percid darters, in Illinois. This impact will most likely be more pronounced in systems that contain invasive crayfish. My findings help to emphasize the importance of determining the circumstances surrounding fish consumption by crayfish, and provide quantitative evidence that crayfish predation on benthic fish is occurring in Illinois streams.

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APPENDIX A: Supplemental Tables and Figures

Table A.1: Scale used for classifying substrate by size, modified from Udden-Wentworth scale.

Classification	Size (mm)
Sand	0-2
Granule	2.1-16
Pebble	16.1-64
Cobble	64.1-256
Boulder	>256

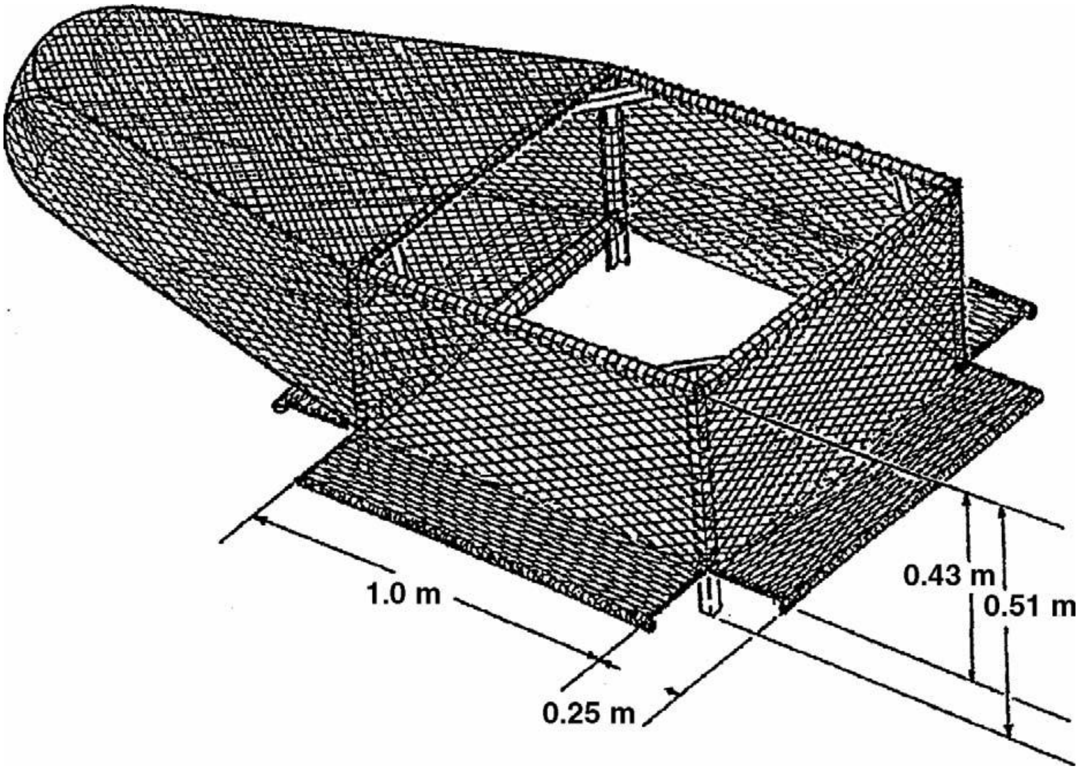

Figure A.1. Drawing of quadrat sampler taken from Larson et al. 2008. Slight height modifications were made with quadrat sampler used in this study.

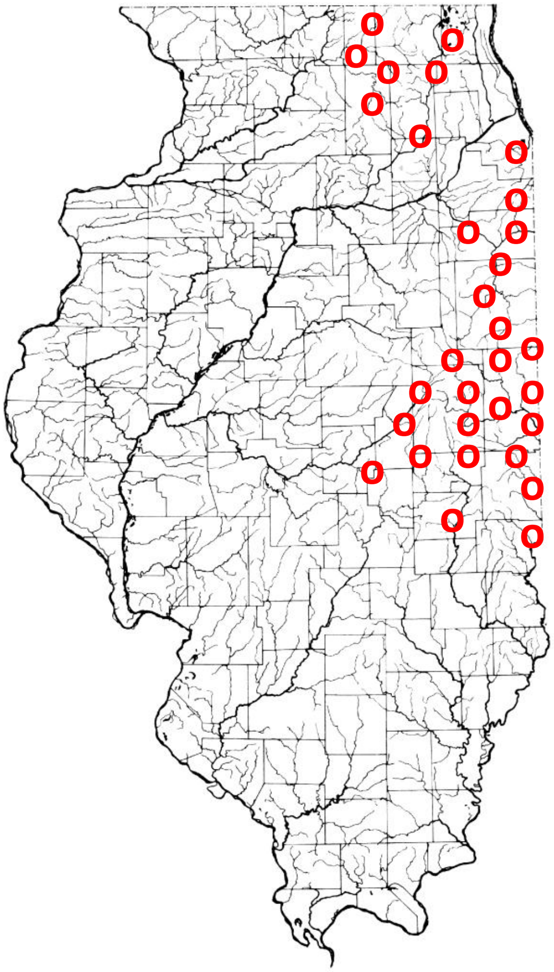

Figure A.2: Map of Illinois, with 31 sites sampled with quadrat shown with open circles.

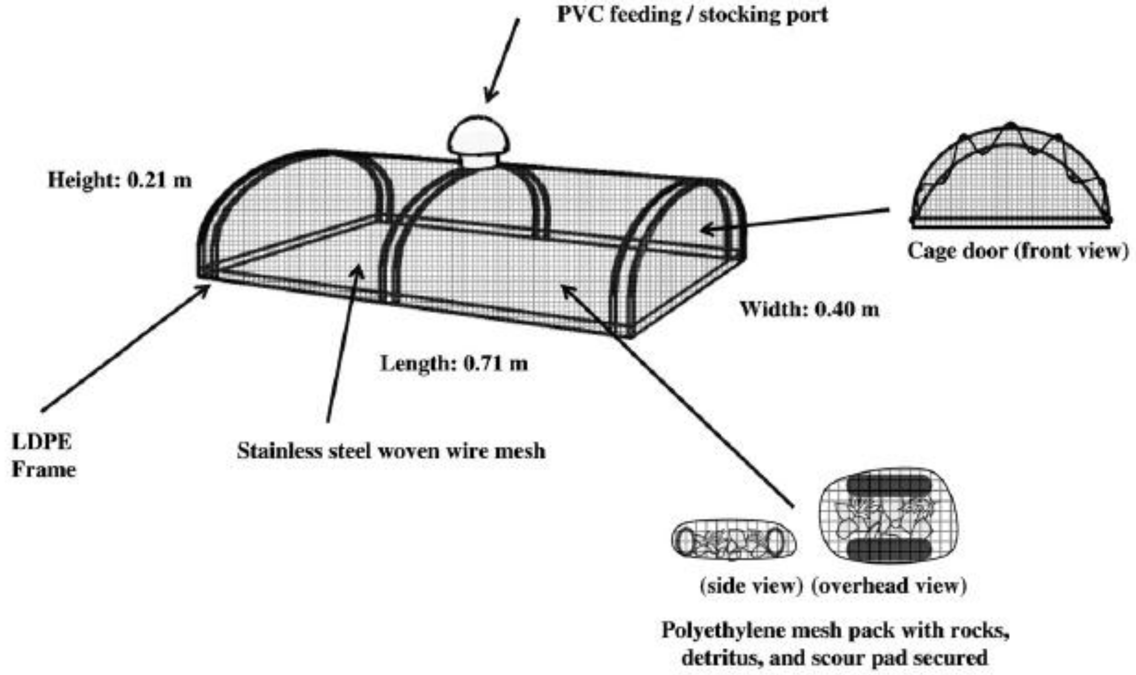

Figure A.3: Schematic of enclosure used in Jordan Creek manipulations, taken from Alert et al., 2009.

Figure A.4. Top: Photo of mesh enclosure in use in Jordan Creek, Vermilion Co., IL. Bottom: A set of enclosures in Jordan Creek.

Figure A.5: Demonstration of kick-seining, a common sampling method in wadeable stream ecosystems.

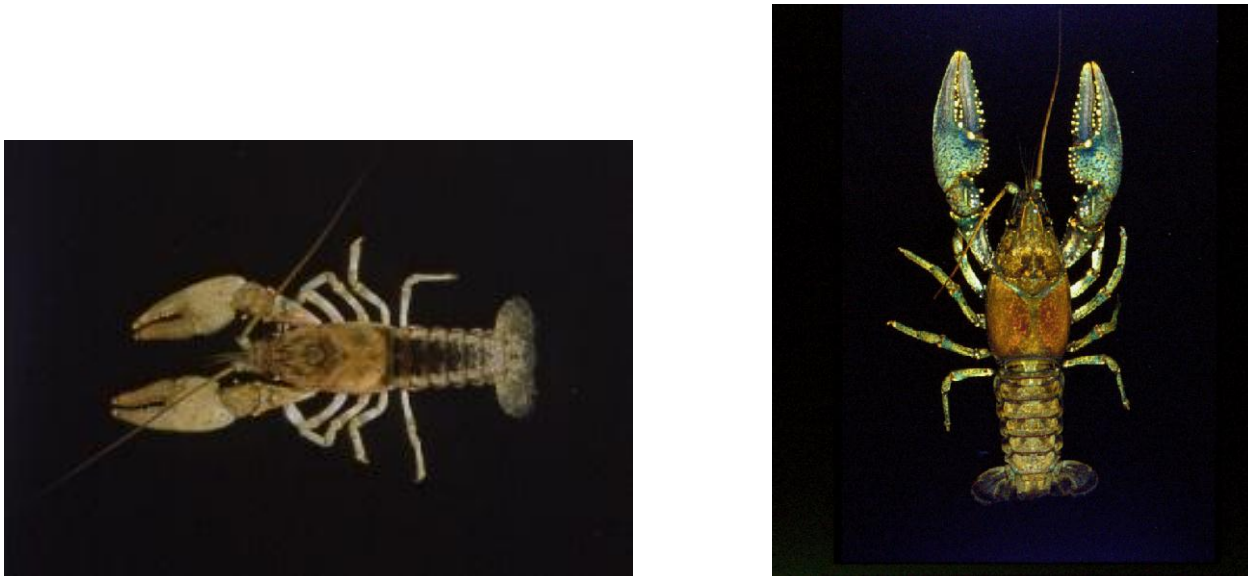

Figure A.6. Photographs of *Orconectes propinquus*(left) and *O. virilis* (right), two common stream-dwelling crayfish in central Illinois, and the most frequently encountered crayfish in my field sampling (Photographs courtesy of C.A.Taylor).

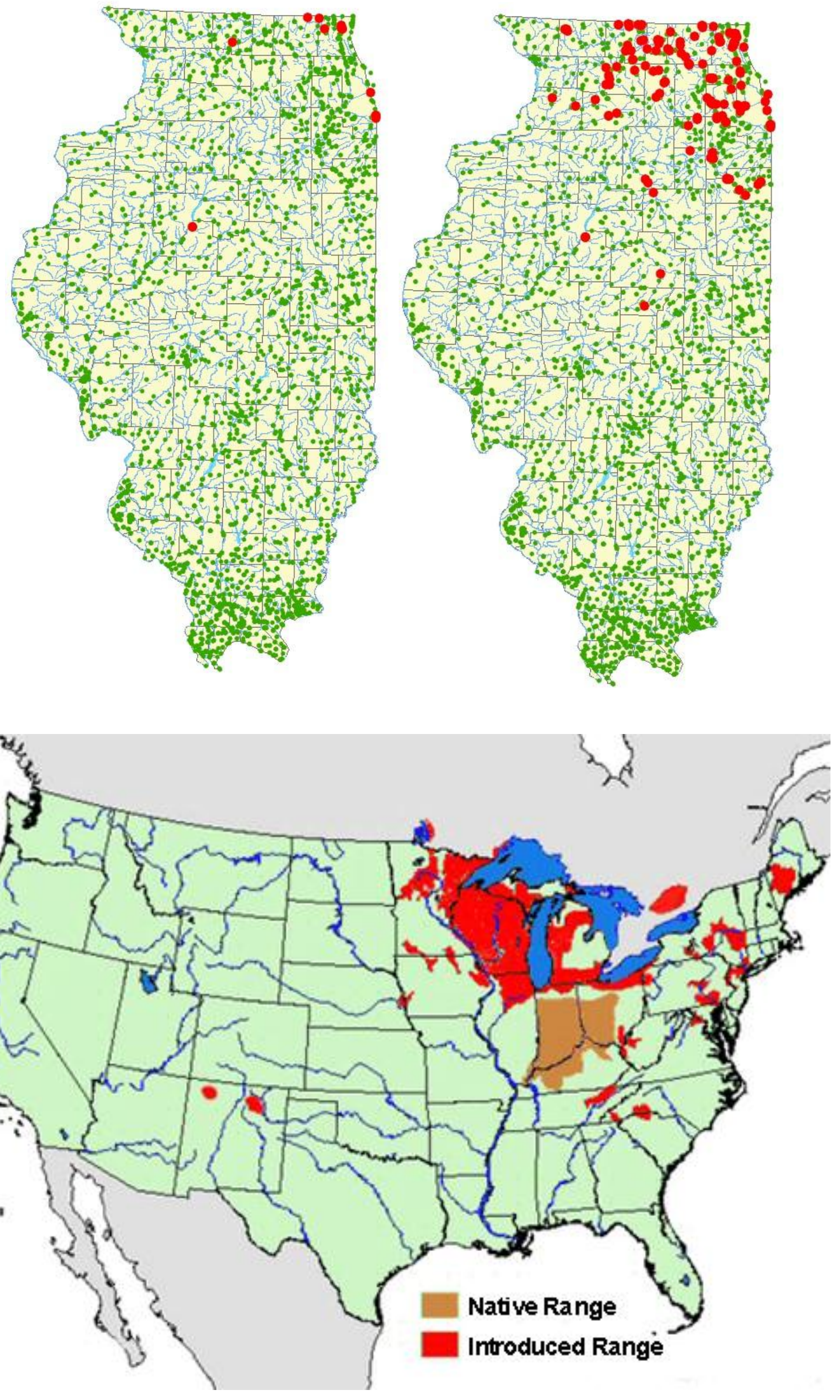

Figure A.7: Distribution maps of established populations of *Orconectes rusticus* in Illinois*.* Map on the top left is from 1985. Map on the top right is from 2009. There has been a dramatic increase in established populations of *O. rusticus* in northeast Illinois in the past 25 years. Bottom map depicts native versus introduced range of *O. rusticus* (Adapted from USGS Rusty Crayfish Fact Sheet (2007)).

APPENDIX B: Density Sampling Data

The stream density sampling data presented in this thesis may be found in its entirety in a supplemental file named **DensityDataSummer2010.xlsx**.

APPENDIX C: Experimental Cage Manipulation Data

The experimental cage manipulation data presented in this thesis may be found in its entirety in two supplemental files named **CrayfishDataFromCages.xlsx** and **CageDataSummer2011.xlsx**.

APPENDIX D: Aquarium Experiment Data

The aquarium experiment data presented in this thesis may be found in its entirety in a supplemental file named **2011TankExperimentData.xlsx**.