Spring 2016

Size-Selective Predation By Ringed Crayfish (Orconectes Neglectus) On Native And Invasive Snails

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SIZE-SELECTIVE PREDATION BY RINGED CRAYFISH (*ORCONECTES NEGLECTUS*) ON NATIVE AND INVASIVE SNAILS

A Masters Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Whitney Marie Kelley

May, 2016

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Biology

Missouri State University, May 2016

Master of Science

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ABSTRACT

As invasive species are introduced into new habitats, predator-prey interactions can be altered. *Cipangopaludina chinensis* is a large snail that has been introduced into numerous waterbodies throughout the United States, where it coexists with native snails and their crayfish consumers. This study examined the relative susceptibility of *C. chinensis* and two native snails (*Elimia potosiensis* and *Physella gyrina*) to predation by the ringed crayfish (*Orconectes neglectus*). I conducted field surveys of snails and crayfish in three Ozark streams, plus a series of four lab experiments to determine feeding rates and prey preference by *O. neglectus* on these three snail species. *Elimia potosiensis* was often observed living near crayfish, but was rarely consumed regardless of snail size. *Physella gyrina* was easily consumed by crayfish, but was rarely found living near crayfish and exhibited avoidance behavior by crawling out of the water. *Cipangopaludina chinensis* was readily consumed by crayfish and exhibited no avoidance behavior. Although *C. chinensis* was previously reported from the James River, subsequent visits to the original site failed to find a single living individual of *C. chinensis*. Together, these data suggest that predation by native crayfish may be an important limit to the spread of *C. chinensis* populations in Ozark streams.

KEYWORDS: avoidance behavior, *Cipangopaludina chinensis, Elimia potosiensis*, Ozark streams, *Physella gyrina*, prey selection

This abstract is approved as to form and content

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John E. Havel, PhD
Chairperson, Advisory Committee
Missouri State University

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ACKNOWLEDGEMENTS

I would like to thank the Missouri State University Graduate College and Biology Department for funding.

I would like to thank my advisor Dr. Havel and committee members Dr. Mathis and Dr. Barnhart for advice and guidance throughout the process of research and writing.

I would like to thank my husband, Justin Kelley, for endless love and support during my time at Missouri State University, accompanying me on every lake and stream collection trip, as well as lab and writing assistance. I would like to thank Beth Glidewell and Kari Wolken for their assistance in both the lab and field. Lastly, I would like to thank the many individuals that provided a helping hand, tip of advice, and support.

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INTRODUCTION

Antipredator adaptations are widespread in nature. Prey size and morphology, protective retreats, and behavior are just a few ways that prey animals reduce their chances of being eaten by predators. Many prey animals, such as crayfish and prairie dogs, can prevent an encounter with a predator by avoiding an interaction. Once an interaction is unavoidable, animals attempt to evade or retreat from the predator. Protective retreats are a first line of defense for many prey animals in the presence of predators and includes rabbits going into a hole, birds flying away from cats, a spider dropping on a thread when attacked, frogs jumping into the safety of water, and crayfish retreating into burrows or moving under rocks when predators approach (Edmunds 1974a, Edmunds 1974b). Armadillos rolling into a ball and turtles retreating into their shell are variations of this defense. In a similar way, snails protect their soft bodies from predation by withdrawing their visceral masses into their hard outer shells (Edmunds 1974a).

Snails (Mollusca: Gastropoda) are widely distributed in freshwater, marine, and terrestrial ecosystems throughout the world (Taylor 2003). Size varies greatly between snail species, with some hatchlings <1 mm, to the largest species, the marine *Syrinx aruanus*, with mature individuals up to 910 mm shell length and 18 kg in weight (Taylor and Glover 2003). Most snails eat periphyton and detritus (Brönmark 1989, Brown and Lydeard 2010), although some are predaceous (Meyer and Cowie 2010, Patel et al. 2014). Snails are also important intermediate hosts to many parasitic worm species, e.g; *Leucochloridium paradoxum* (Okulewicz and Sitko 2012). Snails serve as a food source

for many different species, including small mammals, flatworms, fish, turtles, crustaceans, snakes, and frogs (Brown and Lydeard 2010). Some animals, like predatory snails, consume snails whole (Meyer and Cowie 2010); others, such as flatworms, can invade the shell (Iwai et al. 2010). Pumpkinseed sunfish ingest the snails after crushing the shell (Mittelbach et al. 1992). Other predators, like crayfish, chip away at the shell (Hoverman and Relyea 2008).

Crayfish (Crustacea: Decapoda) have a worldwide distribution, with the exception of Antarctica and the Indian subcontinent (Crandall and Buhay 2008). More than 640 species have been identified worldwide (Crandall and Buhay 2008), with 320 species in North America (Pflieger 1996) and 32 species in Missouri (Pflieger 1996). Crayfish can be found living in freshwater lakes, streams, and wetlands, as well as caves. Crayfish are also found in terrestrial habitats, burrowing deep enough to access the water table (Crandall and Buhay 2008). The size of a crayfish increases markedly during its lifespan of 2-20 years, growing from 2 mm when the crayfish hatches from its egg to over 250 mm, including pincers, in species like the longpincered crayfish (*Orconectes longidigitus*) (Pflieger 1996). Crayfish are opportunists, capable of feeding on both plants and animals, as well as carrion and detritus (Pflieger 1996, Hobbs 2001, Crandall and Buhay 2008). Crayfish also serve as an important food source for many freshwater aquatic and terrestrial species, including raccoons, fish, birds, turtles, snakes, and other crayfish (Pflieger 1996).

The predator-prey relationship between crayfish and snails has been well studied (Brown and Lydeard 2010). Snails are a high energy food source for crayfish (Olden et al. 2009), abundant in many types of aquatic habitats, and because of their high

production, they are an important food source (Brown and Lydeard 2010). Crayfish possess powerful mandibles that are capable of crushing the shells of small snails and chipping away at the opening of larger or thicker-shelled snails (Alexander and Covich 1991a). Snails have a variety of adaptations that reduce their risk of mortality to crayfish. One method is to detect the presence of crayfish early in the encounter and then avoid them by crawling out of the area before a physical interaction occurs (Turner et al. 1999, Bernot and Turner 2001). Snails are also capable of growing very quickly when young, increasing shell size and thickness (DeWitt et al. 2000, Trussell and Smith 2000, Krist 2002, Lakowitz et al. 2008), which likely protects against consumption following capture by size-limited predators. Some snail species, especially those with an operculum, will retreat into their shell and cover the opening with their operculum, reducing the chance that a crayfish can gain access to their visceral mass (DeWitt et al. 2000, Olden et al. 2009).

The overall goal of this thesis is to examine the predator-prey interaction between crayfish and snails, as related to invasion by an exotic snail. For experiments, I used a crayfish species common in the region near Springfield, Missouri. *Orconectes neglectus* Faxon 1885 (Decapoda: Cambaridae, ringed crayfish) is a common, native crayfish found in the White River basin of southern Missouri, Oklahoma, Arkansas, with small populations also observed in Colorado, Nebraska, and Kansas (Pflieger 1996, Fetzner 2011). Ringed crayfish have also been introduced into the Spring River basin in southwestern Missouri, as well as some regions of Oregon and New York (Pflieger 1996, Daniels et al. 2001, Flinders and Magoulick 2005, Larson and Magoulick 2008, Fetzner

2011). Ringed crayfish are most commonly found in permanent streams and rivers that have rocky or cobble bottoms (Pflieger 1996).

The snails of interest in this study are *Physella gyrina* Say 1821 (Heterobranchia: Physidae), *Elimia potosiensis* Lea 1841 (Caenogastropoda: Pleuroceridae), and *Cipangopaludina chinensis* Gray 1934 (Caenogastropoda: Viviparidae). *Cipangopaludina chinensis* is sometimes referred to as *Bellamya chinensis* (Smith 2000); however the latter name does not have official recognition (ITIS 2016). Both *P. gyrina* and *E. potosiensis* are native to the study area (Angelo et al. 2002, Taylor 2003). *Physella gyrina* is common throughout North America, including numerous locations in Canada, the United States, and Mexico (Burch 1989, Taylor 2003). The species occurs in lakes, ponds, streams, marshes, and ditches (Taylor 2003). *Elimia potosiensis* has a considerably smaller range than that of *P. gyrina*, restricted to streams and rivers throughout Arkansas and Missouri, with populations also found in portions of Oklahoma and Kansas (Angelo et al. 2002). *Cipangopaludina chinensis* is native to Russia, China, Japan, and Taiwan (Chung and Jung 1999, Chiu et al. 2002) and was introduced into Canada and the United States over the past 125 years (Abbott 1950, Burch 1989, Distler 2003). *Cipangopaludina chinensis* is widespread in North America (36 states, and 4 provinces, USGS 2016) and was recently introduced in the study region (Duzan et al. 2007), with small, isolated populations (Clark 2009). This species is found mainly in lentic bodies of water, but can also be found living in slow moving areas of streams (Jokinen 1982, Stanczykowska et al. 1971).

After seeing that *O. neglectus* and all three snail species co-occurred in the same local body of water, the James River, I began to ask questions about their interactions with crayfish. In this thesis, I explore answers to the following questions:

1.) How are the snails distributed with respect to crayfish?

2.) Does predation rate depend on snail species?

3.) Does predation rate depend on shell thickness and does shell thickness depend on shell length?

4.) Do crayfish show a preference based on snail species?

5.) Do crayfish show a preference based on snail size?

MATERIALS AND METHODS

Study Sites

Animals for experiments and surveys were collected from three streams in southwest Missouri: Pearson Creek, Wilson's Creek, and the James River (Table 1). All of these stream sites are in the Ozark region of southwestern Missouri, characterized by its karst landscape and thin soils (Petersen et al. 1998). All three streams flow year round and have cobble, gravel, and bedrock as dominant substrate.

Pearson Creek drains an area of 60 km^2 in eastern Greene county. Pearson Creek flows 12 km through agricultural and residential areas before entering the James River east of Springfield, Missouri (MEC Water Resources 2007). At the site sampled, Pearson Creek had extensive cobble substrate and, on the date sampled, a width of 7 m (Table 1).

Wilson's Creek drains an area of 218 km^2 in western Greene and Christian counties. The Wilson's Creek collection area is 725 m downstream of the Southwest Clean Water Plant, which provides wastewater treatment to the City of Springfield and discharges its effluent into Wilson's Creek. Wilson's Creek then flows 12 km before entering the James River (MEC Water Resources 2007). At the site sampled, Wilson's Creek had extensive gravel and bedrock substrate and, on the date sampled, a width of 10.5 m (Table 1).

The James River drains an area of 3,767 km^2 in Webster, Greene, Christian, Stone, Barry, Lawrence, and Douglas counties, starting in eastern Webster county, flowing 249 km to the southwest, and ending as it flows into Table Rock Lake in Stone county (MEC Water Resources 2007). The James River has multiple tributaries that feed

into it, including the other two study streams, Wilson's Creek and Pearson Creek (Kiner and Vitello 1997). The sampling location used for the current study was at Crighton Access, located after the flow of Pearson Creek was added, but before the addition of the Wilson's Creek flow. At the site sampled, the James River had extensive cobble and bedrock substrate and, on the date sampled, a width of 24.5 m (Table 1). Although the majority (83%) of the James River basin consists of rural grassland and forested areas, my study area was in an area dominated by agricultural and residential use (MEC Water Resources 2007).

Spatial Distribution and Co-occurrence of Snails and Crayfish

To determine the distribution of common snails and their co-occurrence with crayfish, I collected during June-July 2012 from 100 randomly-selected quadrat locations (1 m^2 in area) from transects in each of three study streams. To determine the relative abundance of the common species of crayfish, I revisited the sites in September 2015 (Missouri Department of Conservation Wildlife Collector's Permit #15255).

In all three streams, I ran a transect in an upstream direction and randomly sampled quadrats, stratified to include different habitat types. In both Pearson Creek and Wilson's Creek, I sampled 50 locations that were adjacent to shore and 50 that were in open water. In the James River, I sampled 33 locations adjacent to shore, 33 in vegetated areas (dominated by water willow, *Justicia americana*), and 34 in open water.

At each of the 300 locations, I stood facing downstream and slowly placed a 1 m^2 square frame onto the bottom. Crayfish were counted first since they are easily startled. Because the position of my body was upstream of the frame, the crayfish tended to swim

downstream, which reduced the chance of double counting as I moved upstream to the next location. Snails were then counted on the surface and underneath rocks. Snails were left in place and not removed from the rocks. Moving upstream also prohibits duplicate counts of snails that became dislodged and swept downstream. Because crayfish were not individually captured, I could not identify them to species during this sampling period. Later (September 2015), I collected samples from each site with a D-frame net to assess crayfish species composition.

Test Animals

Study animals were collected for each experiment during summer 2011 and 2012 and maintained in the limnology laboratory at Missouri State University (water temperature range 25-27˚C). *Elimia potosiensis* was collected by hand from Pearson Creek and *Physella gyrina* from Wilson's Creek within 12 h of the start of the experiments. Juvenile Chinese mystery snails (*Cipangopaludina chinensis*) were laboratory-reared from adults collected at Alex George Lake (Jackson County, Missouri). The *E. potosiensis* and *P. gyrina* were held in plastic containers (30 x 17 x 10 cm; 5.7 L) in water from their collection location until use. All containers were aerated while in the laboratory and *C. chinensis* were fed flake fish food *ad libitum*.

These snail species are shown in Figure 1, which illustrates the large range in body size. All animals used in experiments were measured using digital calipers ($\pm$ 0.01 mm), with shell length measured from the apex to the posterior margin of the aperture (Figure 1).

Ringed crayfish for experiments were collected by hand with a D-frame net from Pearson Creek, where they were the dominant crayfish (Table 2). Ringed crayfish were isolated from other crayfish species and only visibly undamaged (all pincers and legs intact) ringed crayfish were brought to the lab, other crayfish were returned alive to the stream. Crayfish carapace length was measured from the tip of the rostrum to the end of the carapace (Figure 2) using digital calipers ($\pm$ 0.01 mm). Each crayfish was housed and later tested individually in a covered, aerated plastic container (30 x 17 x 10 cm) in 3 cm deep (1.3 L) of filtered well water (Figure 3). The source of the well water was a home in north Springfield (2857 N. Summit Ave.), and has a hardness (300 mg/L), comparable to the source streams (Table 1). In order to provide shelter and reduce potential stress, each enclosure was supplied with structure (rock and leaning unglazed ceramic tile) (Figure 3).

Preparing Crayfish for Experiments

Ringed crayfish were acclimated to the lab on a regulated diet for one week prior to experiments and held for one week after each experiment to ensure no molting occurred. Molting has been previously shown to interfere with crayfish feeding (Guan and Wiles 1998), so omitting animals that molted removed this potential confounding variable from the experiments. Crayfish that molted before the start of the experiment were excluded from the experiment and data collected from crayfish that molted within one week after the experiment were removed from analysis. Prior to experiments, each crayfish was fed 1 cm^3 of thawed bloodworms (larval Chironomidae) every other day, left undisturbed in darkness, and then starved in their enclosures for 48 h immediately prior

to experiments. Water was changed 24 h prior to the start of the experiment to remove any remaining food. To minimize disturbance, all experiments were conducted at night in the dark in a room with little human activity during the summer.

During summer 2011, I ran predation rate and prey preference experiments, and during summer 2012 two size preference experiments. The length of time for each experiment was determined based on both the total number of snails and number of each group (size and/or species) provided, preventing the crayfish from eating all snails of a group before the experiment time ended. All feeding experiments were conducted at night in a dark room.

Shell Thickness Versus Shell Length

I examined the relationship between shell length and shell thickness for the three snail species used in the experiments. Fifty individuals of each species were measured for shell length (as above), and shell thickness was measured at the distal margin of the aperture. Shell thicknesses were compared among snail species and across different sizes of snails using an Analysis of Covariance, with shell thickness regressed on shell length (the covariate) and classified by snail species, using Minitab 17 statistical software. In order to satisfy assumptions of regression analysis, I log transformed both shell thickness and shell length prior to analysis.

Experiment 1: Predation Rate by Snail Species Experiment

In experiment 1 (summer 2011), each ringed crayfish was offered 20 snails of a single species: *C. chinensis* (size range 6.4-8.6 mm), *E. potosiensis* (10.5-12.1 mm), or

P. gyrina (9.0-10.1 mm), and allowed to feed for 12 h overnight. A total of 33 ringed crayfish were included in this experiment; consumption of *C. chinensis* was tested with 10 crayfish, *E. potosiensis* with 10 crayfish, and *P. gyrina* with 13 crayfish. Size (carapace length, CL) of the crayfish ranged from 13-31 mm.

Surviving snails were counted at the end of the experiment and the number of snails consumed per crayfish was calculated. Predation rates were compared among snail species using a Kruskal-Wallis test, with number of snails eaten as the response and snail species as the factor, using Minitab 17 statistical software.

To explore dependence of predation rate on crayfish carapace length and snail species, I ran Analysis of Covariance on the square root of snails eaten, classified by snail species, with crayfish carapace length as covariate.

Experiment 2: Prey Preference by Snail Species Experiment

In experiment 2 (summer 2011), each ringed crayfish was simultaneously offered 10 snails of each snail species (*C. chinensis*, *E. potosiensis*, and *P. gyrina*) and allowed to feed for 4 h at night. Snails were of a similar size as used in experiment 1. A total of 9 crayfish (CL 20-29 mm) were included in experiment 2. Surviving snails were counted at the end of the experiment and the number of snails of each species consumed per crayfish was calculated.

Preference for or against each species was determined using Manly's Alpha electivity index (α_i) for variable prey population (Krebs, 1989: formula 11.27); α_i is the probability that an individual prey item is selected from a particular prey class (i) when

all prey species are equally available, p_i is the proportion of prey class i alive at the end of the experiment, and summation is across all prey classes:

$$\propto_i = \frac{\log_e p_i}{\Sigma \log_e p_i}$$

Since there were three prey classes (k), a neutral result (no selection for or against) would occur in this experiment if the electivity index was 0.33 ($^1/_k$). An electivity significantly index higher than 0.33 would indicate preference for the prey class and an electivity index below 0.33 would indicate preference against the prey class.

For each snail species Manly's Alpha electivity index values (α_i) were tested for significant departure from neutrality (H_o: Θ_α=0.33), using one sample Wilcoxon tests with Minitab 17 statistical software. Predation rate on snails was compared among snail species and individual crayfish using a Friedman test, with number of snails eaten as the response, snail species as the treatment, and blocked by crayfish individual, using Minitab 17 statistical software.

Experiments 3 & 4: Prey Preference by Size Experiments

Size preference was explored in two experiments (summer 2012). In experiment 3, each of 10 ringed crayfish (CL 20-28 mm) was simultaneously offered 10 snails of each of three size classes of *E. potosiensis* (small—4-6 mm, medium—9-11 mm, and large—14-16 mm) and allowed to feed for 12 h. Surviving snails were counted at the end of the experiment and the number of snails of each size consumed per crayfish was determined.

Preference for or against each size class was determined using Manly's Alpha electivity index as above. Since there were three prey classes, a neutral result (no selection for or against) would occur in this experiment if the electivity index was 0.33.

In experiment 4, each of 20 ringed crayfish (CL 17-23 mm) was offered five snails of each of two size classes (small—4-6 mm and medium—9-11 mm) from each of three species of snail (*C. chinensis*, *E. potosiensis*, and *P. gyrina*) and allowed to feed for 6 h. I counted surviving snails at the end of the experiment and determined the number of snails eaten from each size and species.

Preference for or against each size class and species was determined using Manly's Alpha electivity index as above. Since there were six prey classes (2 sizes X 3 species), a neutral result (no selection for or against) would occur in this experiment if the electivity index was 0.17. Manly's index values were tested for each snail species and size for significant departure from neutrality (H_o: Θ_α=0.17), using one sample Wilcoxon tests using Minitab 17 statistical software.

Following each of the experiments the crayfish were euthanized in hot water.

RESULTS

Spatial Distribution and Co-occurrence of Snails and Crayfish

During the June-July surveys, crayfish and *Elimia potosiensis* were found together in 100% of the locations at the Pearson Creek site (Tables 3 and 4). Although previously observed at this site when a vegetated backwater was present (J. Havel personal communication), *Physella gyrina* was not collected in the current study. In Wilson's Creek, crayfish and *P. gyrina* were found together in only 1% of the sample locations. During this 2012 survey, *E. potosiensis* were not observed. However, during a revisit in September 2015, *E. potosiensis* were also found at Wilson's Creek (Table 2). In the James River, where *P. gyrina*, *E. potosiensis*, and crayfish co-occur, crayfish and *P. gyrina* were found together in 0% of the locations and crayfish and *E. potosiensis* were found together in 8% of locations (Tables 3 and 4).

Densities of *E. potosiensis* in Pearson Creek ranged from 9-57 per m^2 among all sampling locations, with an average density of 35 snails per m^2 (Table 5). Here, *E. potosiensis* appeared to show no habitat preference and, when abundant, were readily found in all locations (Table 5), including along shore, in vegetated areas, in riffles, runs, and pools, and at all depths. Densities of ringed crayfish in Pearson Creek ranged from 1-10 per m^2 among all sampling locations with an average density of 5.12 crayfish per m^2 (Table 5). In both the James River and Wilson's Creek, *P. gyrina* were mainly found in very shallow areas (<10 cm) and along the shore (Table 5). In the James River, no *P. gyrina* were found in open water and only low densities were found in vegetation and near shore (0-3 snails per m^2), while ringed crayfish and *E. potosiensis* were found in all

locations at low densities (0-6 crayfish and 0-6 snails per m^2) (Table 5). In Wilson's Creek, low densities of crayfish were found at all depths (0-2 crayfish per m^2). High densities of *P. gyrina* were found near shore (0-20 snails per m^2), with much lower densities in open water (Table 5).

Cipangopaludina chinensis were not observed in any of the three sites on the 20 occasions I visited (May-August in 2011 and 2012). I also searched the James River at the Rivercut site, where *C. chinensis* was previously discovered (Duzan et al. 2007) and later observed in low densities (Clark 2009), but in a 3 hour search I found no live individuals, only two empty adult shells.

Experiment 1: Predation Rate by Snail Species Experiment

During experiment 1, shells were either fully crushed and soft tissues consumed or the snail remained alive; no snails were observed to be partially consumed. During this experiment, one *E. potosiensis* was eaten by one crayfish, but no other crayfish consumed this species. In contrast, the ringed crayfish readily consumed *C. chinensis* (mean 0.69 snails·crayfish^{-1}h^{-1}) and *P. gyrina* (0.35) (Figure 4). These results were confirmed statistically, with a significant difference in number of snails eaten among snail species (Kruskal-Wallace test, H=14.91, 2 df, p=0.001). The number of snails eaten also depended on crayfish carapace length (Table 6), with larger crayfish eating more snails than smaller crayfish (Figure 4). Larger crayfish ate as many as 20 *C. chinensis*, while the highest number of *P. gyrina* eaten was eight (Figure 4). Interestingly, the change in predation rate on *C. chinensis* with increasing crayfish size (slope = 1.35) is

much larger than that for *P. gyrina* (slope = 0.48). The difference in slopes among species is confirmed by the significant interaction effect with ANCOVA (Table 6).

During this experiment, I observed different behaviors for the three species of snails. *Physella gyrina* quickly moved to the top of the enclosure and nearly all surviving snails were found on the lid of the enclosure at the end of each experiment. In contrast, I never observed *E. potosiensis* or *C. chinensis* moving away from crayfish; instead they stayed in the water throughout experiments either attached to the enclosure or at the bottom of the enclosure with their opercula shut. Similar behaviors were observed in other experiments.

Shell Thickness Versus Shell Length

Part of the difference in susceptibility among species may also be from differences in shell thickness. For each species, shell thickness was strongly related to shell length (Figure 5, Table 7). After adjusting for shell length, *C. chinensis* (*Cc*), *E. potosiensis* (*Ep*), and *P. gyrina* (*Pg*) did not have significantly different shell thicknesses (Table 7). However, the significant interaction effect (Table 7) indicates that the increase in shell thickness with length depends on the species of the snail. *Physella gyrina* had the steepest slope, indicating that, as this species grew, its shell thickness increased faster than that for other snails. Nevertheless, since *C. chinensis* grows to a much larger size (maximum SL= 60 mm) than *P. gyrina* (22 mm) and *E. potosiensis* (40 mm), *C. chinensis* ended up with the thickest shell on average (Figure 5).

Experiment 2: Prey Preference by Snail Species Experiment

The prey preference experiment (experiment 2) confirmed the trends observed in experiment 1: *E. potosiensis* were never eaten by ringed crayfish and *C. chinensis* were eaten at equal or greater rates than *P. gyrina*. Of all the snails eaten, 75% were *C. chinensis*, 25% were *P. gyrina*, and 0% were *E. potosiensis*. The number of snails eaten was significantly different among snail species, when blocked by crayfish (Friedman test, S=16.22, 2 df, $p<0.001$). Among the 9 crayfish, mean Manly's Alpha selectivity index for *C. chinensis* was 0.749, for *P. gyrina* 0.251, and for *E. potosiensis* 0 (Figure 6). The Wilcoxon signed rank test on the Manly's Alpha index values indicated a significant difference from neutrality (0.33) for feeding on both *C. chinensis* (positive selection) and *E. potosiensis* (negative selection), but no significant difference for *P. gyrina* (Table 8).

Experiments 3 & 4: Prey Preference by Size Experiments

During experiment 3, 10 ringed crayfish (CL 20-28 mm) were offered a wide size range of *E. potosiensis* (4-16 mm). During this experiment, no snail of any size class was eaten.

During experiment 4, crayfish were offered two different sizes from each of the three snail species. During this experiment, *E. potosiensis* were rarely eaten (0.07 $\text{snails}\cdot\text{crayfish}^{-1}\text{h}^{-1}$), *P. gyrina* were eaten at a moderate rate (0.57 $\text{snails}\cdot\text{crayfish}^{-1}\text{h}^{-1}$), and *C. chinensis* were eaten at the highest rate (1.07 $\text{snails}\cdot\text{crayfish}^{-1}\text{h}^{-1}$). After blocking by crayfish, the number of snails eaten was significantly different among the 6 groups of snails (Friedman test, S=30.5, 5 df, $p<0.001$). Of all the snails eaten, 64% were *C. chinensis*, 31% were *P. gyrina*, and 4% were *E. potosiensis* (only the small size class).

Both sizes of *C. chinensis* were eaten at similar rates while smaller *P. gyrina* were more likely to be eaten than the larger size class (Figure 7). The Manly's Alpha index, as compared to neutrality (α_i=0.17), indicated positive selection for 5 and 10 mm *C. chinensis*, negative selection for 5 and 10 mm *E. potosiensis* and 10 mm *P. gyrina*, and no significant difference for 5 mm *P. gyrina* (Figure 7). The Wilcoxon signed rank test on the Manly's Alpha index values indicated a significant difference from neutrality for feeding on all snail species and sizes except 5 mm *P. gyrina* (Table 9).

DISCUSSION

Freshwater, marine, and land snails are susceptible to a variety of different predators including fish (Brönmark 1989, Bernot and Turner 2001, Dauwalter and Fisher 2008, Halwart et al. 2014), crustaceans (Edgell and Rochette 2008, Pascoal et al. 2012, Stanhope et al. 2015), birds (Sousa 1993, Cheverie et al. 2014), starfish (Paine 1969), and even other snail species (Curry and Yeung 2013). Crayfish tend to have omnivorous diets that include detritus, macrophytes, invertebrates, and vertebrates such as amphibians and fish (Momot 1995). Crayfish diets generally include snails when snails are available (Covich 1977, Lodge et al. 1994, Nyström et al. 1996, Parkyn et al. 2001, McCarthy et al. 2006).

Ringed crayfish (*Orconectes neglectus*) were common at all three sample sites, as they are in other streams in the regions (Pflieger 1996, Fetzner 2008). This species has also been introduced, perhaps through bait bucket introductions, into several locations bordering their native range of southern Missouri, Arkansas, and Oklahoma (Magoulick and DiStefano 2007). Ringed crayfish are effective competitors and may be responsible for displacing more narrowly endemic native crayfish (Magoulick and DiStefano 2007). In the current study, I found *O. neglectus* co-occurring in area streams with two common snail species, *Elimia potosiensis* and *Physella gyrina*. During a 1991-1992 survey of 56 stream sites near Springfield, *Elimia* and *Physella* species were very common, found in 80 and 29%, respectively, of the stream sites surveyed (K. Koontz unpublished data). In Pearson Creek, *E. potosiensis* was the only snail species found, although *P. gyrina* was

observed on earlier dates when macrophytes were present (J. Havel personal observation).

The region also has an invasive snail, *Cipangopaludina chinensis*, observed several times over the past decade (Duzan et al. 2007, Clark 2009). However, I was unable to find an extant population in the original site of first record, or at my specific study sites.

At the micro-habitat scale, crayfish and *E. potosiensis* frequently co-occurred with one another. Crayfish and *E. potosiensis* were abundant at the Pearson Creek site and co-occurred in 100% of the quadrats. The lower co-occurrence at the James River site (8%) may be due to lower densities than in Pearson Creek. In contrast to *E. potosiensis*, *P. gyrina* was rarely found with any species of crayfish (Table 4). My study has shown that small and thin-shelled snails are especially susceptible to ringed crayfish predation and that this crayfish shows preference toward consuming *C. chinensis* and *P. gyrina*, but rarely consumes *E. potosiensis*. Other studies have also indicated that *Physella* and *C. chinensis* are susceptible to predation by crayfish (Table 10; Alexander and Covich 1991a, Alexander and Covich 1991b, Bernot and Turner 2001, Dickey and McCarthy 2007). A literature search failed to find that *Elimia* species are prone to predation from any predator. Experiments with *C. chinensis* have shown that several crayfish species (both native and invasive) readily eat a range of size classes of the snail (Table 10; Olden et al. 2009). *Cipangopaludina chinensis* has successfully colonized hundreds of locations over a broad range of North America (USGS 2016), yet in some places, like Ozark streams, *C. chinensis* are rare or appear to have disappeared. Although there are many possible explainations for reduction or extinction of colonizing populations, a high

susceptibility to crayfish predation may have contributed to the disappearance of *C. chinensis* in the James River.

There are several possible reasons why there are differences in susceptibility to crayfish predation among snail species, including snail size, shell thickness, and defensive behaviors. These factors will be discussed below.

Size Selectivity

My experiments have shown that, of the snail sizes provided (5-10 mm), ringed crayfish readily consumed both size classes of *C. chinensis* and consumed smaller *P. gyrina* more readily than larger *P. gyrina*. In contrast, *E. potosiensis* were generally not consumed, regardless of snail size. *Cipangopaludina chinensis* grows to a much larger size (maximum 60 mm) than either *P. gyrina* (15 mm) or *E. potosiensis* (25 mm), suggesting adult *C. chinensis* are protected from crayfish predators by size. I observed that crayfish rarely consumed *C. chinensis* beyond the size of 20 mm. Similarly, Olden, et al. (2009) also found that smaller size classes of *C. chinensis* were preferred by crayfish over larger snails.

Using behavioral observations, Alexander and Covich (1991a) explored size selective predation by the crayfish *Procambarus simulans* on two pulmonate snails, *Physella virgata* and *Planorbella trivolvis* (Planorbidae). Alexander and Covich (1991a) used crayfish of slightly larger sizes (carapace length, CL = 28-40 mm) than the crayfish used in my experiments (CL = 13-31 mm). In their experiment, snails were grouped into five size classes, ranging from 4-16 mm, and introduced to an enclosure containing crayfish, which were then allowed to feed. They observed about 60% of small (4-8 mm)

P. trivolvis were released once captured and very few *P. trivolvis* larger than 8 mm were even picked up by the crayfish. *Physella virgata* were found to be more vulnerable at all size classes and were also more likely to be consumed once captured than *P. trivolvis* (Alexander and Covich 1991a). This observation was supported by higher numbers of *P. virgata* being eaten and shorter handling time (median T_h = 60 s) compared to similar sized *P. trivolvis* (median T_h = 180 s). They also found that handling time increased with both snail species as snail size increased. Vulnerability to predation was considerably different between snail species and sizes due to differences in shell architecture and predator avoidance behavior. Larger snails with thicker shells were less likely to exhibit predator avoidance behavior and were also less likely to be consumed (Alexander and Covich 1991a). Predator avoidance movements of the snails were also observed in their study and will be discussed below.

Olden et al. (2009) looked at the susceptibility of differently sized *Bellamya chinensis* (= *C. chinensis*) to three different crayfish species, *Pacifastacus leniusculus*, *Orconectes virilis*, and *Procambarus clarkii*. Since their distributions in the field did not overlap, none of the three crayfish species had been exposed to this prey species previously. Snails were grouped into four size classes (Table 10) and introduced to their predetermined crayfish predator. Crayfish used by Olden et al. (2009) were considerably larger (CL 42-67 mm, Table 10) than those used in my experiments (CL 13-31 mm). They found that the smaller *C. chinensis* were most susceptible to predation, but that even the largest snails could be consumed by some crayfish species. Only *P. leniusculus* consumed all four size classes, while *O. virilis* consumed the smaller three size classes, and *P. clarkii* consumed the two smallest snail size classes. As would be expected,

handling time of *C. chinensis* by crayfish increased as snail shell length increased (Olden et al., 2009).

Shell Thickness

Shell thickness varied considerably between the three different snail species used in my experiments. During my trials, I used small snails with thin shells, but as each of these snail species grew in length, their shell thickness increased dramatically (Figure 5). Furthermore, the slopes of each snail species were significantly different. For instance, a doubling of length led to a 2.17x increase in thickness in *P. gyrina* versus a 2.03x increase in thickness for *C. chinensis*.

Other studies have shown that when snail predators, such as fish and crayfish, are commonly found in an area, those snails with thicker shells have a selective advantage over those snails with thinner shells (DeWitt et al. 2000, Krist 2002, Lakowitz et al. 2008). DeWitt et al. (2000) explored changes in shell shape of *Physella* species when exposed to crayfish (*Orconectes obscurus*) and fish (pumpkinseed sunfish, *Lepomis gibbosus*; common carp, *Cyprinus carpio*; goldfish, *Carassius auratus*). During field surveys shell morphology depended on the most common predator in the area. They found that, when crayfish were the prevalent predator in the area, *Physella* tended to have a more elongated shell shape, which prevented shell entry by the crayfish. The reduction of susceptibility was due to an increase in handling time by the predator. In contrast, when fish were the major predator, *Physella* had a more rotund shell shape, which reduced the chance of shell crushing (DeWitt et al. 2000). Since *Physella* are only

capable of having one shell shape or the other, when the snail is resistant to one predator, they become more susceptible to predation by the other.

Lakowitz et al. (2008) examined shell changes in a freshwater pulmonate snail, *Radix balthica*, when exposed to fish (*Tinca tinca*), crayfish (*Pacifastacus leniusculus*), and a combination of the two. He found that changes in shell shape were induced by chemical cues when exposed to predation by fish, moving to a rounder shape and increasing shell thickness. This morphotype was less susceptible to predation by fish due to an increase in crushing resistance. When the same snail species was exposed to crayfish, such changes in shell shape and thickness were not observed, and thus the vulnerability to crayfish predation was not reduced (Lakowitz et al. 2008).

Predator Avoidance Behavior

Observations made during my experiments indicated that the three snail species behaved very differently from one another when exposed to the crayfish predator. *Physella gyrina* were frequently observed moving to the top of the enclosure and most surviving snails were found on the lid of the enclosure at the end of experiments. When *P. gyrina* were being kept in culture before use in each experiment, most individuals of this species moved up to the water line, with individuals rarely on the lid. As pulmonates, *Physella* are adapted to breathing air (Brown and Lydeard 2010), and are thus pre-adapted to use an existing behavior for defense: moving outside the range of crayfish attack. Smaller *P. gyrina* were more susceptible to crayfish predation than the larger size class, perhaps due to differences in crawling speed. In contrast, *E. potosiensis* or *C. chinensis* were never observed moving away from crayfish; instead they most often

stayed in the water throughout experiments, either attached to the enclosure or at the bottom of the enclosure with their opercula shut. These species are caenogastropods ("gilled snails"), unable to breathe air out of water (Brown and Lydeard 2010).

A considerable body of work is available for predator avoidance behavior exhibited by *Physella* species (Brönmark 1989, Alexander and Covich 1991a, Alexander and Covich 1991b, Covich et al. 1994, DeWitt et al. 1999, Turner et al. 1999, McCarthy and Fisher 2000, Turner et al. 2000, Bernot and Turner 2001, Lewis 2001, Dickey and McCarthy 2007, Turner 2008, Covich 2010).

Bernot and Turner (2001) showed that the behavior of *Physa integra* changed depending on what predator the snails were exposed to during field and mesocosm experiments. When *P. integra* in field experiments were exposed to caged pumpkinseed sunfish (*Lepomis gibbosus*) in separate 40 cm^3 mesh cages, the snails moved to covered locations (ceramic tiles covering the bottom of cages) within their habitat. Fish predation could occur in all depths in the environment, but predation in covered locations was limited by fish size. In contrast, when exposed to caged *Orconectes rusticus*, a large invasive crayfish, snails were observed moving to the surface of the water (Bernot and Turner 2001), as also observed for *P. gyrina* in my study. Crayfish predation is limited to the bottom of the mesh enclosures. This behavior suggests that these snails have the ability to recognize different predators by their chemical cues and react to reduce the risk of predation as related to the predator's hunting behavior. Very similar results were found for *Physella gyrina* (Turner et al. 1999) and *Physella virgata* (Alexander and Covich 1991a) when exposed to crayfish predation.

Alexander and Covich (1991a) exposed multiple size classes of the snail *Planorbella trivolvis* (another pulmonate) to the crayfish *Procambarus simulans*. Only small *P. trivolvis* exhibited the same crawling behavior. Larger *P. trivolvis* were less vulnerable to crayfish predation due to a strong shell and did not move up the water to avoid crayfish (Alexander and Covich 1991a).

Physella have been documented exhibiting predator avoidance behaviors not only when exposed to crayfish, but also when exposed to crushed snails. McCarthy and Fisher (2000) studied the behavior of *Physella heterostropha pomila* in 38 L aquaria with 2 cm muddy sediment as substrate when exposed to various levels of predation risk. The four treatments included water from tanks that contained no snails or crayfish (control), nonforaging crayfish, crushed conspecifics, or crayfish consuming conspecifics. Snails were observed exhibiting three main behaviors; burial into the substratum, movement to the water surface, and crawling out of the water. McCarthy and Fisher (2000) found that snails were most likely to bury into the substratum when exposed to crushed snails. When snails were exposed to crayfish or injured conspecifics they were more likely to move to the water surface or crawl completely out of the water.

In the current study, I observed *P. gyrina* moving both to the water surface and crawling out of the water. In this study, snails did not have access to a substrate and therefore burial was not an option. Similar behaviors were also observed in *Physa gyrina* when exposed to crayfish (DeWitt et al. 1999).

My experiments as well as several others cited in this thesis have shown that susceptibility of snails to crayfish predation is modified by variation in shell length, shell thickness, and behavior. Snails of a larger size tend to be eaten at a lower rate than those

of a smaller size, perhaps due in part to shell thickness increasing as snails grow in size. Some species are also capable of exhibiting predator avoidance behaviors (*P. gyrina* in my own study) that allow them to move out of reach of crayfish and therefore make them less vulnerable to predation. The current study, as well as Olden et al. (2009) illustrate the high susceptibility of *C. chinensis* to crayfish predation. These data suggest that crayfish may be an important factor limiting the spread of *C. chinensis* in freshwater habitats. Future surveys should focus on examining the occurrence and habitat preferences of different size classes of *C. chinensis*, as related to abundance of crayfish predators.

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APPENDICES

Appendix A. Driving directions to sampling locations.

A. James River – Crighton Access

Battlefield east from US Hwy 65, south onto FR 187, southeast onto Kinser Rd., and northeast onto FR 193 to Crighton Access.

B. Pearson Creek

Chestnut Expressway east from US Hwy 65. Sampling location is 200m east of FR 193.

C. Wilson's Creek

Hwy FF south from James River Freeway, west onto FR 168 to sampling location.

Appendix B. Spatial distribution and counts of *Elimia potosiensis* (*Ep*) and crayfish in Pearson Creek on June 18, 2012. Quadrats (1m^2 area) 1-50 were located near shore and quadrats 51-100 were located in open water. *Physella gyrina* were never observed even though previously common in vegetated areas (J. Havel personal observation). We have observed three crayfish species in Pearson Creek including *Orconectes neglectus*, *Orconectes luteus*, and *Orconectes ozarkae*, with *O. neglectus* being most abundant (73% by number).

Quad	Crayfish	*Ep*
1	5	38
2	8	12
3	3	19
4	1	57
5	4	25
6	7	42
7	4	21
8	5	9
9	3	31
10	6	29
11	7	35
12	4	26
13	10	41
14	5	15
15	3	22
16	8	34
17	4	27
18	6	40
19	3	32
20	5	30
21	9	38
22	8	40
23	2	19
24	4	22
25	5	33

Quad	Crayfish	*Ep*
26	5	38
27	5	42
28	6	55
29	4	27
30	8	25
31	3	33
32	5	37
33	7	42
34	6	35
35	4	29
36	5	44
37	2	53
38	8	39
39	9	44
40	6	47
41	7	23
42	5	38
43	5	31
44	4	46
45	6	39
46	7	43
47	3	28
48	5	21
49	7	34
50	8	32

Appendix B (cont.)

Quad	Crayfish	*Ep*
51	10	38
52	8	40
53	5	45
54	7	41
55	5	37
56	3	28
57	9	29
58	6	50
59	4	47
60	3	39
61	5	45
62	7	41
63	8	39
64	4	35
65	2	36
66	3	12
67	1	18
68	1	20
69	1	30
70	3	32
71	4	33
72	8	26
73	7	29
74	5	31
75	5	35

Quad	Crayfish	*Ep*
76	4	55
77	3	49
78	4	47
79	6	42
80	6	41
81	7	45
82	5	30
83	4	32
84	1	29
85	2	30
86	3	41
87	5	44
88	5	33
89	6	38
90	5	35
91	7	40
92	5	41
93	4	38
94	3	32
95	8	43
96	7	50
97	6	41
98	5	31
99	5	34
100	3	37
Frequency of Occurrence	100	100
Total Number of Individuals	512	3496

Appendix C. Spatial distribution and counts of *Physella gyrina* (*Pg*) and crayfish in Wilson's Creek on June 28, 2012. Quadrats 1-50 were located near shore and quadrats 51-100 were located in open water. *E. potosiensis* were not observed at this site during this sampling but were later observed in September 2015. We have observed two crayfish species in Wilson's Creek, including *Orconectes neglectus* and *Orconectes luteus*.

Quad	Crayfish	*Pg*	Quad	Crayfish	*Pg*
1	0	3	26	0	0
2	0	0	27	0	0
3	0	0	28	0	0
4	0	2	29	0	0
5	0	0	30	0	0
6	0	0	31	0	0
7	1	0	32	0	0
8	0	0	33	1	1
9	0	0	34	0	0
10	0	15	35	0	3
11	0	18	36	0	1
12	0	10	37	0	0
13	1	0	38	0	0
14	0	0	39	1	0
15	0	0	40	0	0
16	1	0	41	1	0
17	0	12	42	0	4
18	0	20	43	0	0
19	0	19	44	0	0
20	0	5	45	1	0
21	0	0	46	0	0
22	0	0	47	0	0
23	1	0	48	0	0
24	0	0	49	0	0
25	0	0	50	0	0

Appendix C (cont.)

Quad	Crayfish	*Pg*	Quad	Crayfish	*Pg*
51	0	0	76	1	0
52	0	0	77	0	0
53	0	0	78	0	0
54	0	0	79	0	0
55	0	0	80	1	0
56	0	0	81	0	0
57	0	0	82	0	0
58	0	0	83	0	0
59	2	0	84	0	0
60	0	0	85	0	0
61	0	1	86	0	1
62	0	0	87	0	0
63	0	0	88	0	0
64	0	0	89	0	0
65	0	0	90	0	0
66	1	0	91	0	0
67	0	0	92	1	0
68	0	0	93	0	0
69	0	1	94	0	0
70	0	0	95	1	0
71	0	0	96	0	0
72	0	0	97	0	0
73	0	0	98	0	0
74	0	0	99	0	0
75	0	0	100	0	0
			Frequency of Occurrence	14	16
			Total Number of Individuals	15	116

Appendix D. Spatial distribution and counts of *P. gyrina* (*Pg*), *E. potosiensis* (*Ep*), and crayfish in James River on July 10, 2012. Quadrats 1-33 were located near shore, quadrats 34-66 were located near vegetated areas, and quadrats 67-100 were located in open water. We have observed two crayfish species in James River including *Orconectes neglectus* and *Orconectes longidigitus*, with *O. neglectus* most abundant (75%).

Quad	Crayfish	*Pg*	*Ep*
1	0	1	0
2	0	0	0
3	0	0	3
4	2	0	0
5	0	0	0
6	0	0	0
7	0	2	0
8	3	0	0
9	0	0	0
10	0	1	1
11	1	0	0
12	0	0	0
13	0	0	0
14	0	0	6
15	0	0	0
16	1	0	0
17	0	0	0
18	0	0	0
19	0	2	0
20	0	0	4
21	0	0	0
22	3	0	0
23	0	0	0
24	0	0	0
25	0	0	0
26	1	0	0
27	0	0	1
28	0	3	0
29	0	0	0
30	0	0	0
31	1	0	0
32	0	0	0
33	0	0	0

Quad	Crayfish	*Pg*	*Ep*
34	3	0	2
35	1	0	0
36	2	0	0
37	5	0	0
38	3	0	4
39	3	0	3
40	0	0	4
41	0	0	0
42	2	0	0
43	1	0	0
44	0	1	0
45	2	0	2
46	2	0	1
47	3	0	5
48	2	0	0
49	0	0	0
50	0	0	0
51	5	0	0
52	6	0	0
53	3	0	0
54	2	0	3
55	2	0	1
56	0	0	2
57	0	0	1
58	0	0	0
59	0	0	0
60	5	0	0
61	4	0	0
62	4	0	0
63	5	0	0
64	3	0	0
65	2	0	0
66	2	0	0

Appendix D (cont.)

Quad	Crayfish	*Pg*	*Ep*
67	0	0	0
68	0	0	0
69	0	0	0
70	0	0	0
71	2	0	0
72	2	0	0
73	0	0	0
74	0	0	0
75	0	0	0
76	0	0	2
77	0	0	2
78	0	0	0
79	0	0	0
80	3	0	0
81	2	0	0
82	3	0	0
83	0	0	0
84	0	0	0
85	0	0	1
86	0	0	0
87	0	0	0
88	0	0	0
89	4	0	0
90	3	0	0
91	2	0	0
92	1	0	1
93	0	0	0
94	0	0	0
95	0	0	0
96	3	0	0
97	2	0	0
98	0	0	0
99	0	0	0
100	0	0	0
Frequency of Occurrence	42	6	20
Total Number of Individuals	111	10	49

Appendix E. Predation rate data from Experiment 1 showing *Orconectes neglectus* carapace length (mm), snail species fed (*C. chinensis* (*Cc*), *E. potosiensis* (*Ep*), and *P. gyrina* (*Pg*)), and number of that snail species eaten out of 20 snails placed in enclosure at the beginning of experiment.

Carapace Length (mm)	Snail Fed	Snails Eaten
27.71	*Cc*	20
30.96	*Cc*	18
22.34	*Cc*	16
25.70	*Cc*	14
18.62	*Cc*	8
29.05	*Pg*	8
22.51	*Pg*	8
24.81	*Pg*	8
25.09	*Pg*	7
29.13	*Pg*	7
20.16	*Pg*	6
20.80	*Cc*	3
15.64	*Pg*	3
20.54	*Pg*	3
22.80	*Cc*	2
19.34	*Pg*	2
21.34	*Pg*	2
18.91	*Cc*	1
15.27	*Cc*	1
22.69	*Ep*	1
13.03	*Pg*	1
20.13	*Cc*	0
21.00	*Ep*	0
14.19	*Ep*	0
28.90	*Ep*	0
25.08	*Ep*	0
24.97	*Ep*	0
17.93	*Ep*	0
26.46	*Ep*	0
18.14	*Ep*	0
17.39	*Ep*	0
20.91	*Pg*	0
20.03	*Pg*	0

Appendix F. Shell thickness (mm) versus shell length (mm) data for three species of snails, *C. chinensis*, *E. potosiensis*, and *P. gyrina*.

C. chinensis

Length	Thickness
5.38	0.04
5.46	0.09
5.52	0.06
5.93	0.07
5.99	0.09
6.25	0.09
6.41	0.08
7.04	0.13
7.64	0.08
7.84	0.09
8.00	0.14
8.66	0.12
8.86	0.14
8.98	0.11
9.19	0.13
9.41	0.10
9.53	0.09
9.59	0.11
9.69	0.15
9.69	0.11
9.77	0.08
9.78	0.13
9.88	0.16
10.48	0.08
10.62	0.13

Length	Thickness
10.75	0.14
11.06	0.09
11.16	0.12
11.59	0.10
12.13	0.14
12.49	0.12
12.49	0.12
12.73	0.16
14.43	0.16
18.04	0.20
18.90	0.22
25.96	0.29
39.60	0.61
40.80	0.37
41.58	0.27
43.18	0.73
43.53	0.43
49.09	0.50
49.10	0.55
50.09	0.84
51.13	0.65
52.83	0.67
54.50	0.43
58.66	0.82
58.74	0.75

Appendix F (cont.)

E. potosiensis

Length	Thickness	Length	Thickness
3.73	0.09	8.18	0.21
4.00	0.05	8.20	0.23
5.94	0.12	8.34	0.25
5.94	0.14	8.38	0.23
5.95	0.16	8.40	0.19
5.98	0.14	8.43	0.21
6.26	0.14	8.48	0.16
6.26	0.14	8.55	0.21
6.30	0.13	8.56	0.23
6.31	0.14	8.56	0.24
6.51	0.17	8.82	0.21
6.71	0.20	9.04	0.24
7.00	0.17	9.10	0.21
7.06	0.14	9.15	0.24
7.12	0.15	9.18	0.26
7.19	0.19	9.58	0.28
7.38	0.16	9.63	0.25
7.49	0.18	9.87	0.24
7.55	0.21	9.88	0.18
7.57	0.10	9.93	0.24
7.81	0.19	10.04	0.23
7.88	0.19	10.05	0.25
7.89	0.21	10.29	0.21
8.02	0.18	10.30	0.28
8.12	0.25	10.74	0.37

Appendix F (cont.)

P. gyrina

Length	Thickness	Length	Thickness
3.62	0.02	14.60	0.25
3.87	0.03	14.91	0.21
4.03	0.05	15.21	0.17
4.15	0.14	15.34	0.17
4.55	0.03	15.36	0.25
4.74	0.05	15.49	0.28
5.15	0.16	15.95	0.35
5.49	0.09	16.04	0.29
5.59	0.15	16.10	0.28
5.88	0.12	16.34	0.37
6.00	0.18	16.52	0.42
6.24	0.10	17.22	0.30
6.56	0.17	17.80	0.23
7.80	0.11	17.82	0.37
8.13	0.10	17.94	0.27
9.04	0.10	17.95	0.25
9.26	0.22	18.20	0.25
9.62	0.17	18.44	0.27
11.17	0.24	18.49	0.29
11.29	0.16	18.66	0.48
12.35	0.24	18.68	0.36
12.57	0.37	20.18	0.42
13.03	0.15	20.54	0.45
14.02	0.35	21.50	0.47
14.33	0.29	21.60	0.36

Appendix G. Prey preference data from Experiment 2. Nine snails of each species were placed in each enclosure at the beginning of the experiment.

Carapace Length (mm)	*Cc* eaten	*Pg* eaten	*Ep* eaten
19.83	4	2	0
19.85	1	1	0
21.46	3	1	0
24.08	4	3	0
24.32	4	4	0
24.39	9	2	0
27.47	8	2	0
28.38	9	1	0
28.96	7	2	0

Appendix H. Size preference data from Experiment 4. Chart shows crayfish carapace length (mm) and how many snails of each size per species were eaten by each crayfish out of five of each size that were placed in enclosure at beginning of experiment and were allowed to feed for 6h. Small 4-6mm and medium 9-11mm.

	C. chinensis		*E. potosiensis*		*P. gyrina*	
Carapace Length (mm)	Small	Medium	Small	Medium	Small	Medium
18.81	2	2	0	0	3	2
19.04	3	2	0	0	2	0
19.78	3	3	0	0	2	0
19.87	0	4	0	0	0	0
19.89	1	2	0	0	0	0
20.13	2	3	0	0	3	2
20.32	2	2	0	0	2	0
20.48	3	2	1	0	2	1
21.33	0	2	1	0	2	0
21.48	2	0	0	0	0	0
21.53	3	4	0	0	0	0
21.87	3	4	0	0	1	0
22.12	3	0	1	0	3	1
22.44	2	3	0	0	0	0
22.45	1	1	1	0	2	3

Table 1. Characteristics of stream sites sampled in summer 2012. All sites were downstream from the indicated road crossing and are located in Greene County, Missouri. Driving directions to sampling locations found in Appendix A. Historical discharges (median among years) on sampling day of year from USGS (2015a, b, c) and nutrient concentrations from MEC Water Resources (2007).

Stream	Pearson Creek	James River	Wilson's Creek
	FR 132	FR 193	FR 168
Site			
GPS coordinates	Lat: 37°10'40.6"	Lat: 37°08'59.9"	Lat: 37°08'49"
	Long: 93°11'54.2"	Long: 93°12'12.2"	Long: 93°22'26"
Stream width (m)	7.0	24.5	10.5
Mean depth (m)	0.15	0.23	0.40
Discharge historical (m^3s^{-1})	0.71	1.33	0.16
Dominant substrate	Cobble	Cobble, Bedrock	Gravel, Bedrock
Local land use	Agriculture, Residential	Agriculture, Residential	Wastewater Treatment, Agriculture
Conductivity (μScm^{-1})	415	518	800
Hardness (mg L^{-1})	200	300	310
Total nitrogen ($\mu g\ L^{-1}$)	2,973	2,060	12,682
Total phosphorus ($\mu g\ L^{-1}$)	67	43	410
Sampling dates	June 18	July 10	June 28

Table 2. Occurrence of crayfish and snail species at each site in September 2015. The number of crayfish caught in 100 kick net samples is indicated for each site. Snails indicated as present (+) or absent (-).

	James River	Pearson Creek	Wilson's Creek
Crayfish			
Orconectes neglectus Faxon	4	19	1
Orconectes ozarkae Williams	0	1	0
Orconectes luteus Creaser	0	6	3
Orconectes longidigitus Faxon	1	0	0
Snails			
Elimia potosiensis Lea	+	+	+
Physella gyrina Say	+	-	+

Table 3. Frequency of occurrence of crayfish and snails found at near shore, vegetated, and open water sites in the James River, Pearson Creek, and Wilson's Creek. A total of 100 random quadrat locations were searched in each stream during June-July 2012. Using this collection method, I could not count crayfish to species. Raw data found in Appendix B, C, and D.

	James River			Pearson Creek		Wilson's Creek	
	Near Shore	Vegetated	Open Water	Near Shore	Open Water	Near Shore	Open Water
Number of sample locations per site	33	33	34	50	50	50	50
Crayfish	7	24	11	50	50	8	6
Snails							
Elimia potosiensis	5	11	4	50	50	0	0
Physella gyrina	5	1	0	0	0	13	3

Table 4. Co-occurrence of crayfish with *Elimia* and *Physella* in three sampling sites. A total of 100 random locations (each 1 m^2) were searched in each stream during June-July 2012. Raw data found in Appendix B, C, and D. *Cipangopaludina chinensis* was never detected during the current study. *Elimia potosiensis* was not detected in Wilson's Creek during this sampling, but was detected during a revisit in September 2015 (Table 2). *Physella gyrina* was not detected in Pearson Creek during the current study.

Location	Crayfish	No Crayfish
A. Pearson Creek		
No Snails	0	0
With *Elimia*	100	0
Total	100	0
B. Wilson's Creek		
No Snails	13	71
With *Physella*	1	15
Total	14	86
C. James River		
No Snails	33	42
With *Elimia*	9	10
With *Physella*	0	5
Elimia and *Physella*	0	1
Total	42	58

Table 5. Densities (number/m^2) of crayfish and snails at each sample site, mean (range). A total of 100 random locations were searched in each stream during June-July 2012. Using this collection method, I could not count crayfish by species. The James River was sampled on July 10, Pearson Creek on June 18, and Wilson's Creek on June 28. Raw data are in Appendix B, C, and D.

	Densities of Organisms						
Species	James River			Pearson Creek		Wilson's Creek	
	Near Shore (n=33)	Vegetated (n=33)	Open Water (n=34)	Near Shore (n=50)	Open Water (n=50)	Near Shore (n=50)	Open Water (n=50)
Crayfish	0.36 (0-3)	2.18 (0-6)	0.79 (0-4)	5.38 (1-10)	4.86 (1-10)	0.16 (0-1)	0.14 (0-2)
Snails							
Elimia potosiensis	0.45 (0-6)	0.85 (0-5)	0.18 (0-4)	33.24 (9-57)	36.68 (12-55)	0	0
Physella gyrina	0.27 (0-3)	0.03 (0-1)	0 (0-0)	0	0	2.26 (0-20)	0.06 (0-1)

Table 6. Analysis of Covariance results for predation rate in experiment 1. The square root of number of snails eaten in 12h was compared among snail species and regressed against crayfish carapace length (as covariate). Species included *Cipangopaludina chinensis*, *Elimia potosiensis*, and *Physella gyrina*. Raw data are in Appendix E.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Carapace Length	1	12.4379	10.8008	10.8008	14.90	0.001
Species	2	28.1379	2.2142	1.1071	1.53	0.235
Species*Carapace Length	2	6.2448	6.2448	3.1224	4.31	0.024
Error	27	19.5704	19.5704	0.7248		
Total	32	66.3910				

Table 7. Analysis of Covariance results for log shell thickness. Species included *Cipangopaludina chinensis*, *Elimia potosiensis*, and *Physella gyrina*. Log shell length (SL) as covariate. Raw data are in Appendix F.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Log SL	1	7.5527	4.2633	4.2633	293.76	<0.0005
Species	2	2.1592	0.0026	0.0013	0.09	0.916
Snail*Log SL	2	0.0980	0.0980	0.0490	3.38	0.037
Error	144	2.0899	2.0899	0.0145		
Total	149	11.8997				

Table 8. Results from one-sample Wilcoxon tests of Manly's Alpha (experiment 2) for significant departures from neutral selection (0.3333). Sample size = 9 ringed crayfish, each offered the 3 snail species simultaneously.

Snail Species	Manly's Alpha median (range)	Wilcoxon Signed Rank Test	
		Statistic	p-value
C. chinensis	0.7502 (0.500-0.9563)	45	0.009
P. gyrina	0.2498 (0.0438-0.500)	11	0.193
E. potosiensis	0.0 (0)	0	0.009

Table 9. Results from one-sample Wilcoxon tests of Manly's Alpha (experiment 4) for significant departures from neutral selection (0.1667). Sample size = 15 ringed crayfish, all offered the 6 species-size groups simultaneously.

Snail Species	Shell length (mm)	Manly's Alpha median (range)	Wilcoxon Signed Rank Test	
			Statistic	p-value
C. chinensis	5	0.2983 (0-1)	100	0.025
C. chinensis	10	0.4237 (0-1)	102	0.018
P. gyrina	5	0.2011 (0-0.4028)	72	0.514
P. gyrina	10	0.0470 (0-0.5690)	18	0.018
E. potosiensis	5	0.0000 (0-0.179)	1	0.001
E. potosiensis	10	0.0000 (0)	0	0.001

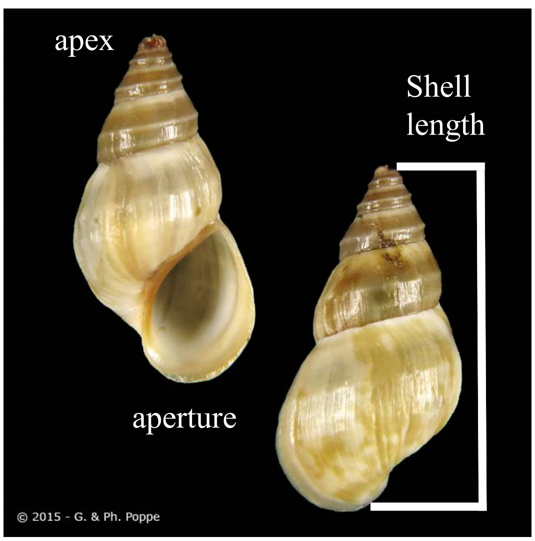
Table 10. Selected studies on size-selective predation by crayfish on snails. CL – carapace length

Study	Crayfish Species	Crayfish CL (mm)	Snail Species	Snail Size (mm)	Outcome
Olden et al. (2009)	*Pacifastacus leniusculus* (native) *Orconectes virilis* (invasive) *Procambarus clarkii* (invasive)	50.4–66.5 42.0–50.4 42.8–61.4	*Bellamya chinensis** (invasive)	10.0–14.9 15.0–22.9 23.0–29.9 30.0–39.9	The native *Pacifastacus leniusculus* was capable of eating even the largest size of snails and was a stronger competitor than the other two crayfish species.
Alexander and Covich (1991a)	*Procambarus simulans* (native)	28-40	*Physella virgata* (native) *Planorbella trivolvis* (native)	4-6 6-8 8-10 10-12 12-16	As snail size increased, handling time also increased. *P. virgata* were eaten at larger sizes compared to *P. trivolvis.*
Current Study	*Orconectes neglectus* (native)	14-31	*Physella gyrina* (native) *Elimia potosiensis* (native) *Cipangopaludina chinensis* (invasive)	4-6 9-11	*C. chinensis* was eaten at the highest rate at both the 5 mm and 10 mm sizes. 5 mm *P. gyrina* were favored over the 10 mm size. *E. potosiensis* of both sizes were not preferred.

* = *Cipangopaludina chinensis* (ITIS, 2016)

A.

B.



C.

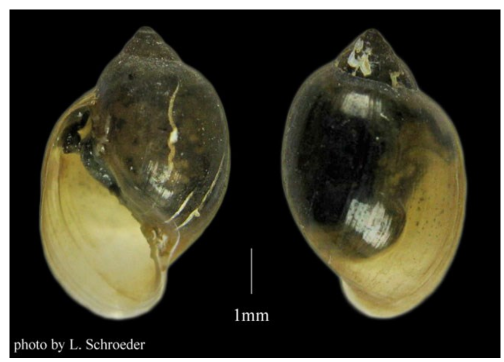


Figure 1. Snails used in experiments. A - *C. chinensis*, B – *E. potosiensis*, C – *P. gyrina*. In panel A, length of line represents 10 mm and in panel B shell length = 14.6 mm. Sources of photos: *C. chinensis*, Pacific Northwest Shell Club (2015a); *E. potosiensis*, Conchology Inc. (2015); and *P. gyrina*, Pacific Northwest Shell Club (2015b).

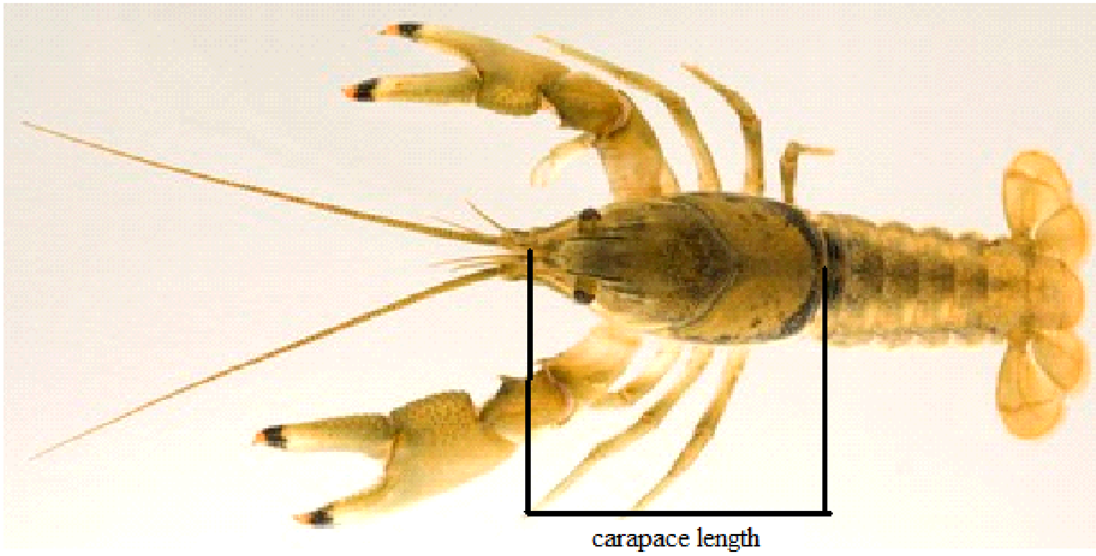


Figure 2. Crayfish carapace length. Carapace length (20 mm for crayfish pictured) was measured from the tip of the rostrum to the end of the carapace. Depicted is *Orconectes neglectus*. Picture modified from <http://www.nationalgeographicstock.com/comp/04/445/1172872.jpg

Figure 3. Crayfish housing for predation experiment. Covered individual crayfish housing (length=30cm), including a rock with an unglazed ceramic tile leaning over the rock and aeration. Water was added to a depth of 3 cm for total volume of 1.3L.

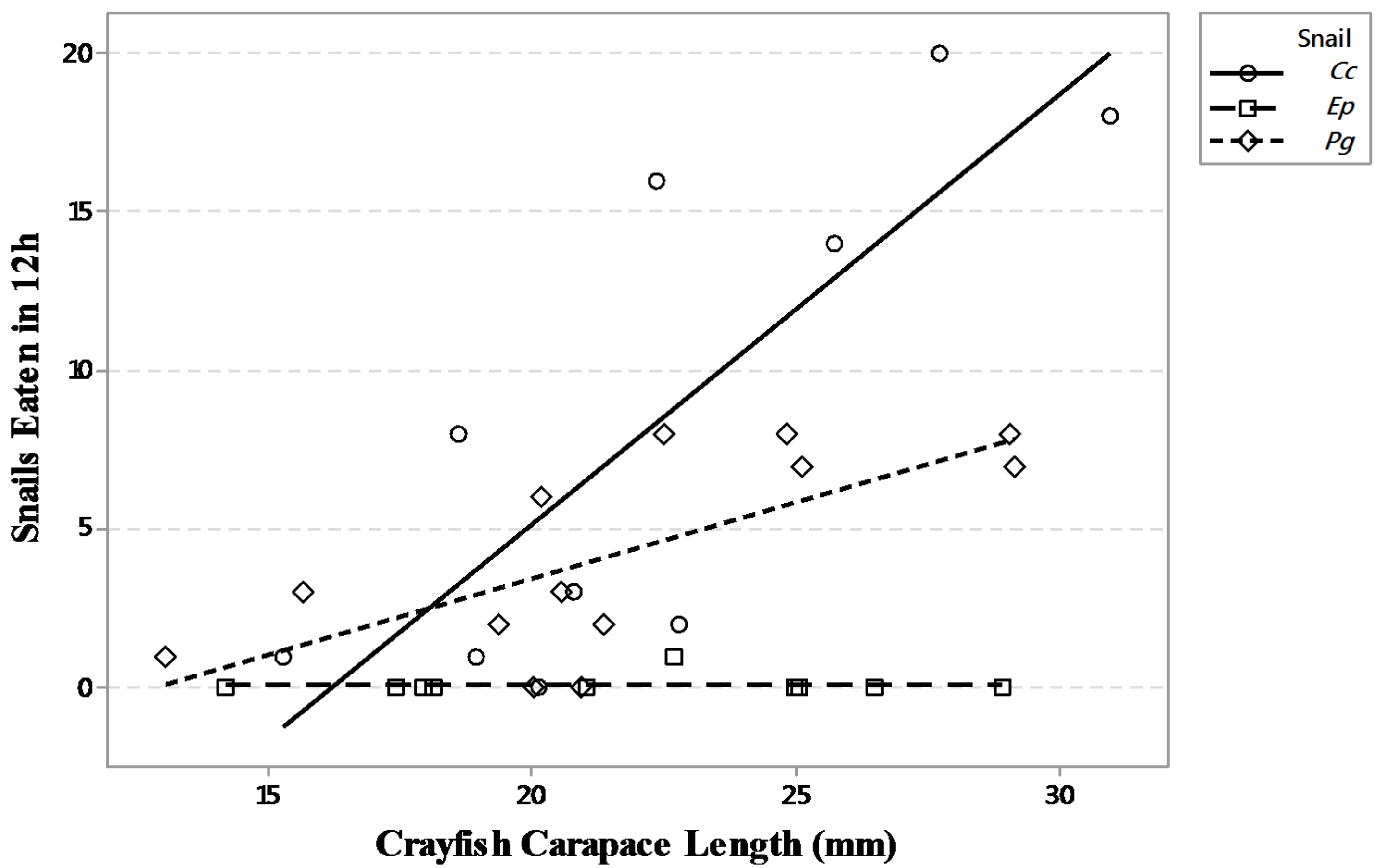


Figure 4. Results from experiment 1: predation rates on single species of snails. *E. potosiensis* (*Ep*) were not eaten, and *C. chinensis* (*Cc*) and *P. gyrina* (*Pg*) were eaten at greater rates as crayfish carapace length (CL) increased. Lines are linear regressions: *Cc* number eaten = -21.88 + 1.352 CL (R^2 = 0.638), *Pg* number eaten = -6.194 + 0.4813 CL (R^2 = 0.492), and *Ep* number eaten = -0.010 + 0.00508 CL (R^2 = 0.006). Analysis of Covariance results shown in Table 6, raw data are in Appendix E.

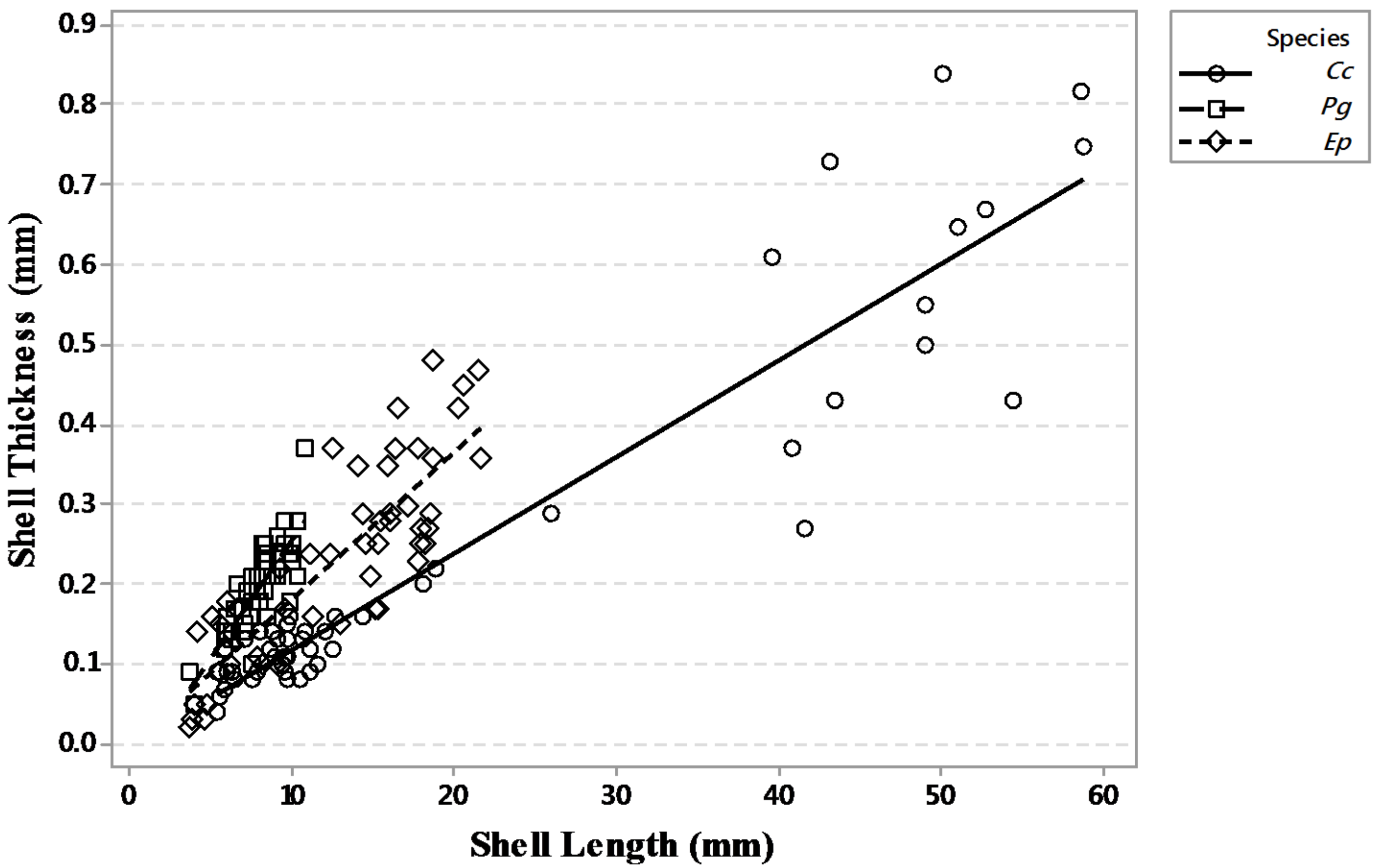


Figure 5. Shell thickness (T) versus shell length (L) for three snail species. Species are *C. chinensis* (*Cc*), *E. potosiensis* (*Ep*), and *P. gyrina* (*Pg*). Lines are linear regressions: *Cc* thickness = -0.00306 + 0.01211 L (R^2 = 0.884), *Ep* thickness = -0.00319 + 0.01851 L (R^2 = 0.724), *Pg* thickness = -0.04399 + 0.03015 L (R^2 = 0.709). Note that the size range for each snail species is different: *C. chinensis*—5-59mm, *E. potosiensis*—4-21mm, *P. gyrina*—4-11mm. ANCOVA shown in Table 7, raw data are in Appendix F.

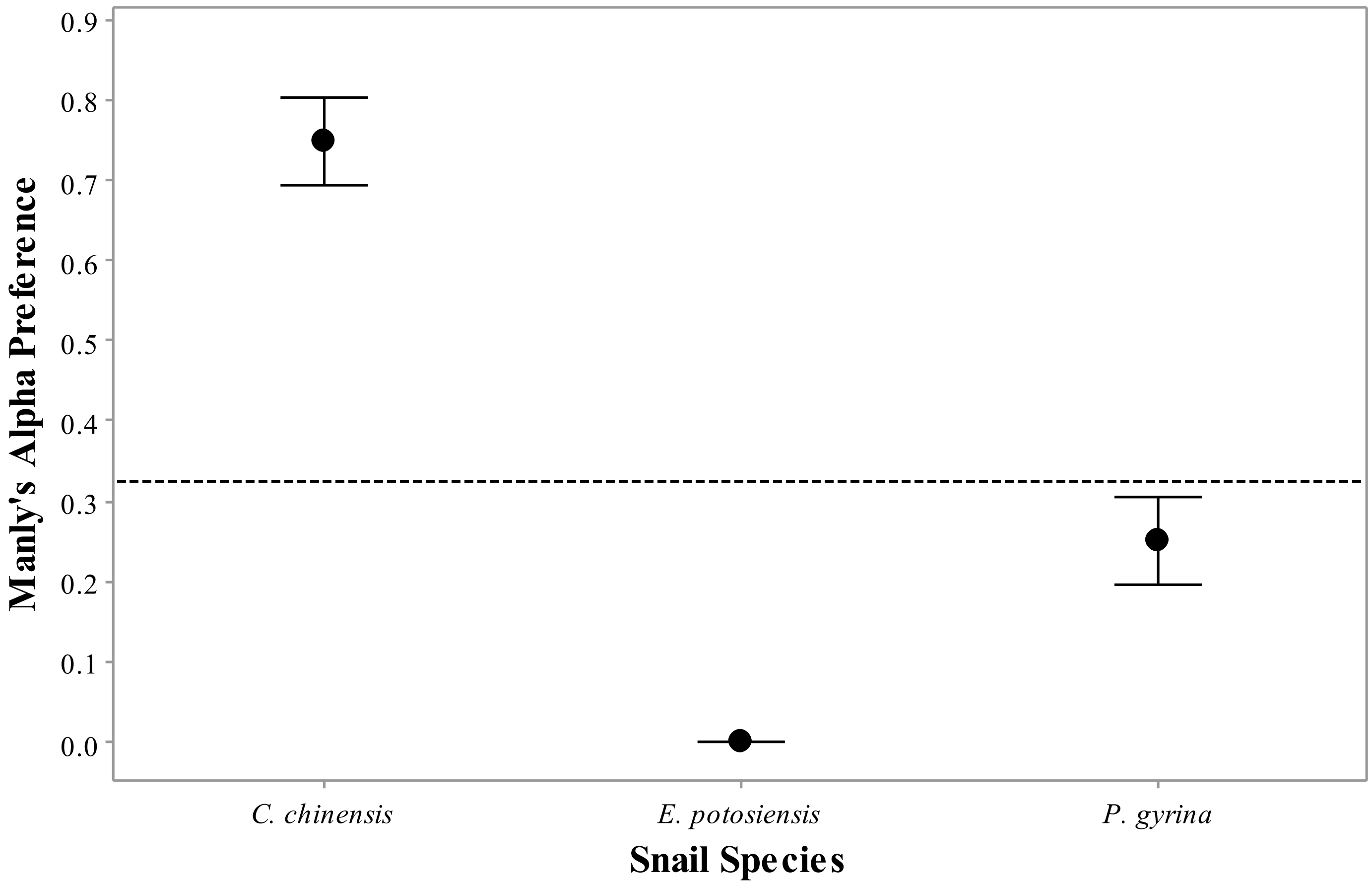


Figure 6. Results for experiment 2: feeding preference of *Orconectes neglectus* by snail species. Manly's Alpha mean ± 1 SE, based on 9 crayfish (carapace length range 19.83-28.96 mm). Dashed line at 0.33 indicates no preference. Raw data are in Appendix F.

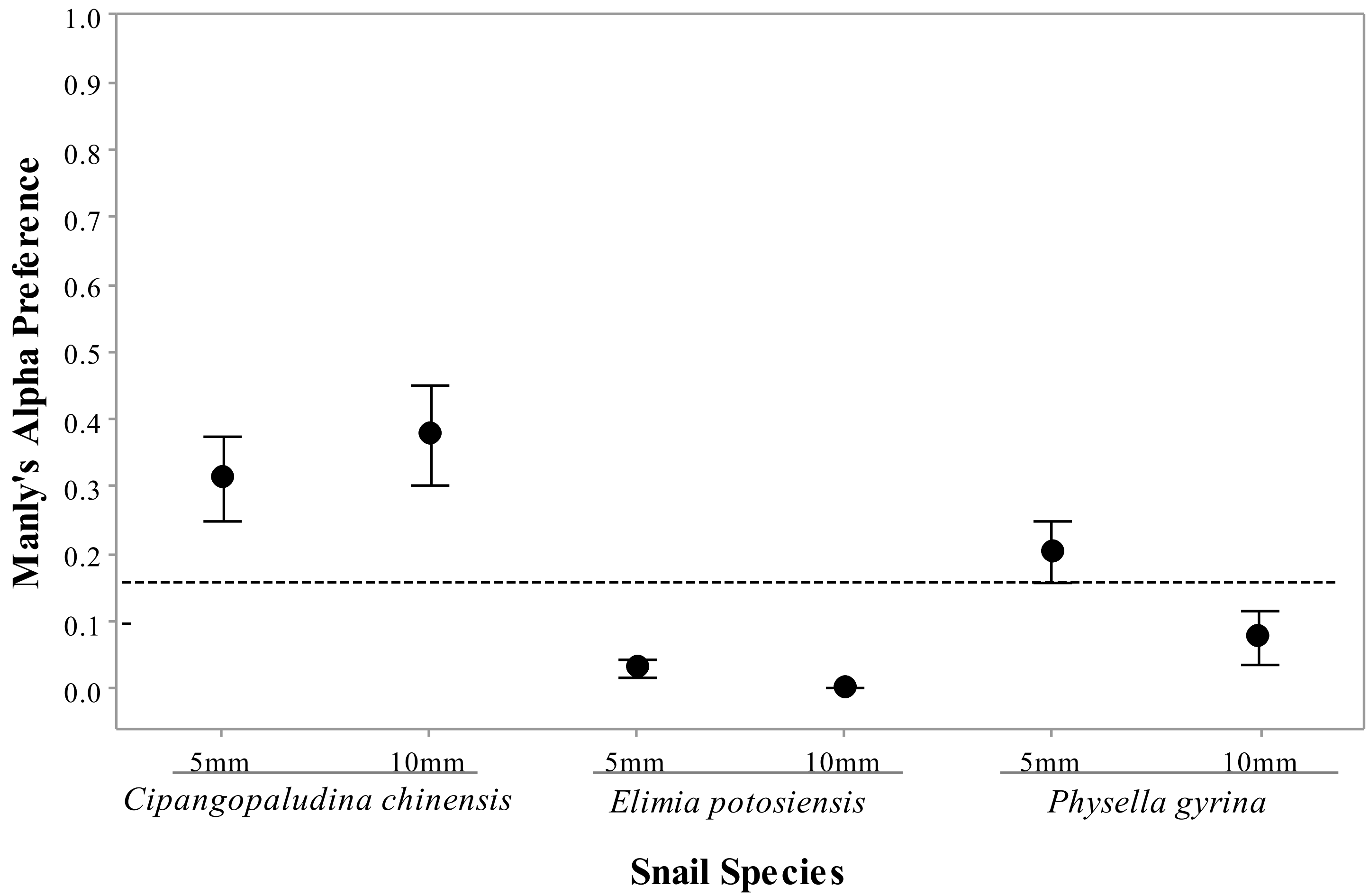


Figure 7. Results for experiment 4: feeding preference of *Orconectes neglectus* by snail species and size. Manly's Alpha mean ± 1 SE, based on 15 crayfish (carapace length 18.81-22.45 mm). Dashed line at 0.17 indicates no preference. Raw data are in Appendix G.