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The Influence of Invader Traits and Community Characteristics on the Invasion Success of an Exotic Crayfish

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DISSERTATION

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The Influence of Invader Traits and Community Characteristics on The Invasion Success of an Exotic Crayfish.

Abstract

Biological invasions are among the leading causes of the loss of biodiversity worldwide. Although there are many hypotheses about traits of a good invader and characteristics of native communities that increase susceptibility to invasion, the realized success of an exotic species is likely a combination of both species and community traits. This dissertation takes a multi-stage approach to examine how behavioral traits of an invader, along with characteristics of invaded communities influence the distribution, establishment and impact of the signal crayfish (*Pacifastacus leniusculus*) in freshwater stream communities. I begin in chapter one by providing an overview of each stage of the invasion process along with invader traits and community characteristics important at each stage. I then describe the patterns of crayfish invasions and provide background on the systems studied in this dissertation. In chapter two, I evaluated how native species diversity, the presence of an ecologically similar species, prey availability and stream discharge influenced the distribution and abundance of signal crayfish at fine and broad spatial scales. In chapter three, I tested whether the presence of an ecologically similar species (e.g. native crayfish) influenced the aggressiveness, activity and voracity of signal crayfish. Finally, in chapter four I evaluated the consequences of high abundances of signal crayfish on native prey and the behavior of signal crayfish. Accumulated evidence suggests that signal crayfish possess the behavioral traits (e.g. a general aggression syndrome) to overcome many challenges faced during the invasion process, but that characteristics of the invaded community (e.g. prey availability and physical attributes of streams) provide some resistance against signal crayfish reaching high abundances. When native communities do not offer much resistance (i.e. abundant prey and low flood disturbances), signal crayfish often invade at high abundances that result in large impacts on native species. The patterns and results suggest that the communities with high prey availability and infrequent disturbances are most at risk of invasion, but that the aggressive nature of this invader makes most communities vulnerable to invasion.

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Chapter 1:

Introduction: The influence of invader traits and community characteristics on the invasion success of an exotic crayfish.

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Introduction

Invasive species are one of the leading threats to native biodiversity (Vitousek et al. 1996, Mack et al. 2000). Much research in invasion biology aims to develop a predictive understanding of the invasion process by looking for generalities associated with invasion success and large invader impacts. Hypotheses have been suggested that focus on either community resistance to invasion (e.g. biotic resistance, invasional meltdown, enemy release) or traits of a species that make it a good invader (e.g. broad physiological tolerance, good dispersal ability) (Elton 1958, Rejmanek and Richardson 1996, Simberloff 1999, Kohler and Lodge 2001). However, the realized success of an invasive species likely depends on a combination of community and species traits.

When studying the invasion success of exotic species, it is important to recognize the invasion as a process with distinct stages that can impose different selection pressures on an invader and the invaded community. Invasions can be broken into three general phases: arrival/dispersal, establishment and impact/spread (Vermeij 1996, Kohler and Lodge 2001). Dispersal often requires an invader to overcome the physical and abiotic pressures of a new environment, and often invaders with broad physiological tolerances succeed (Lodge 1993, Moyle and Light 1996). Once the invader arrives, it must overcome resistance from the biotic community, including predators, competitors and parasites in order to establish a self-sustaining population (Elton 1958, Levine and D'Antonio 1999). Competitive ability has been proposed to be an important component to establishment (Bolger & Case 1992; Vermeij 1996; Holoway & Suarez 1999). High aggressiveness, foraging activity and voracity have been found to be key behavioral mechanisms behind the competitive superiority of exotic over native species (Hill and Lodge 1999, Holway 1999, Rehage and Sih 2004, Rehage et al. 2005a, b). However, the importance of these behaviors may depend on the presence of an ecologically similar species in the invaded community. In the absence of a close competitor, an invader may not need to be as aggressive and voracious since there is no competitor to displace. Additionally, if the invader is novel, then presumably the native community is naïve to the invader as either a predator or prey (Cox and Lima 2006, Sih et al. 2007, in review). It follows then that invaders may not need to be active or voracious foragers to consume poorly defended prey. Following from the enemy release hypothesis, the invader may be more bold to forage under predation risk since the potential predator is too naïve to recognize it as prey. Overall, establishment success is likely due to a combination of both invader traits and characteristics of the invaded native community.

Once established, large impacts and continual spread is often expected to occur in communities where the invader is novel and able to reach high abundances in a new environment (Ricciardi and Atkinson 2004, Hierro et al. 2005, Cox and Lima 2006). However, if aggression towards interspecific competitors (important for establishment) is associated with aggression towards conspecifics, then intraspecific aggression should keep an exotic species from becoming superabundant. Yet many invaders reach high abundances despite being aggressive competitors (Hill and Lodge 1999, Snyder 2006). Therefore, understanding the mechanisms through which exotic species are able to reach and maintain high population densities is important to predicting when and where an invader might have large impacts. This dissertation studies how behavioral traits of an invader, along with characteristics of invaded communities influence the distribution, population establishment and impact of the signal crayfish (*Pacifastacus leniusculus*) in freshwater stream communities in northern California.

Study System

Throughout much of the world, crayfish are key invaders that are having large impacts on freshwater communities (Flint and Goldman 1975, Feminella and Resh 1989, Chambers et al. 1990, Creed 1994, Lodge et al. 1994, Lodge et al. 2000, Nystrom et al. 2001). Crayfish are active, generalist omnivores (they consume plants, animals and detritus) that can thus function as keystone consumers that both directly and indirectly impact multiple trophic levels (Nystrom 2002). Crayfish possess many characteristics of successful invaders and can greatly impact native communities (Hill and Lodge 1999, Holway and Suarez 1999, Snyder 2006). They have broad physiological tolerances and can thus live in a broad range of habitats (Nystrom 2002). They are aggressive and often actively antagonize native organisms (Hill and Lodge 1999, Tierney et al. 2000). Part of the strong impact of crayfish might come from their potentially large per capita effects, but the overall effect appears to be due in part to their capacity to become very abundant.

Although North America holds approximately 75% of the world's crayfish species, only 3 of the 390 crayfish species are endemic to California (Lodge et al. 2000), all to northern California. *Pacifastacus nigrescens* was found in south San Francisco Bay area, but is now extinct. The Shasta crayfish (*Pacifastacus fortis*) is known from the midreaches of the Pit River drainage in northeastern California (Bouchard 1977). *P. fortis* is currently listed as federally endangered under the Endangered Species Act. The third species, the signal crayfish (*P. leniusculus*), is native to the lower Klamath River (northern California), however, is actively expanding its range in California and Europe, due to introduction via bait fishing or aquaculture (Lodge et al. 2000).

Within the Pit River drainage, where the Shasta crayfish is native, signal crayfish were introduced in the late 1970s through bait fishing and have been identified as the primary cause of the decline of the Shasta crayfish (Bouchard 1977, Daniels 1980, Light et al. 1995, Ellis 1999). While signal crayfish appear to be clearly having critical negative effects on Shasta crayfish, less is known about their impacts on other biota in the Pit River and adjacent watersheds. Signal crayfish are known to consume amphibian eggs and larvae and reduce growth and alter behavior of benthic fish (sculpin). Thus they appear to have the potential to have important negative effects on amphibian and fish populations (Axelsson et al. 1997, Light 2005). Interestingly, signal crayfish have successfully invaded into both regions with endemic crayfish, and regions that previously did not have crayfish, prior to invasion. Additionally, preliminary data suggest that there is variation in signal crayfish abundance among invaded streams. Together, this presents an opportunity to evaluate and compare the characteristics of invaded communities that might confer invasion resistance, and the behavioral traits of the invader that may be needed to overcome such resistance.

Dissertation Summary

In this dissertation I studied how characteristics of invaded native communities and traits of exotic species influenced the invasion success of signal crayfish (*Pacifastacus leniusculus*) in streams in northern California. I, now, summarize each chapter below:

Chapter 2

I began by evaluating the factors that influence the distribution and density of exotic signal crayfish at fine (1-m^2) and broad (> 400-m^2) spatial scales in the lower Pit and upper Sacramento River watersheds in northern California. Specifically, I looked at how native species diversity, the presence of an ecologically similar species (i.e., native crayfish), prey availability and stream discharge influenced the distribution and abundance of signal crayfish at these two scales. In doing so, I was able to make predictions about where signal crayfish are more likely to invade and where they will have the greatest impacts. Our results suggested that at a larger scale, exotic crayfish distribution and abundance exhibited a positive relationship with native species diversity and prey availability, but a negative relationship with stream discharge (e.g. scouring winter floods). Although the distribution and abundance of signal crayfish was not associated with the presence of native crayfish, native crayfish distribution was also positively associated with prey availability and negatively with stream discharge. Therefore, through prey availability and stream discharge, exotic signal crayfish were positively associated with native crayfish suggesting that streams with native crayfish are most at risk of invasion and high negative impacts of signal crayfish. In contrast, prey diversity was not related to exotic crayfish abundance at a small scale. Rather, abundance was positively related to snail abundance (preferred prey) and density, and negatively related to stream discharge. Together, the inter-relationships between prey availability, disturbance, diversity and the distributions of invasive and native crayfish put communities with native crayfish at a particular high risk of negative impacts.

Chapter 3

Here I compared the behavioral traits of signal crayfish from populations across their distribution in the watersheds surveyed in chapter two. Specifically, I tested whether the presence of an ecologically similar species (native crayfish) influenced the aggressiveness, activity and voracity of signal crayfish. I predicted that signal crayfish need to be more aggressive when invading into streams with native crayfish competitors than in streams without native crayfish. Furthermore, because the presence of native crayfish implies that the rest of the community (prey and predators) are not naïve to signal crayfish, predators and prey should both exhibit appropriate responses to signal crayfish. Therefore, I also predicted that signal crayfish need to be more active and voracious foragers on experienced prey and less bold in the presence of experienced predators in comparison to signals from communities without native crayfish. Interestingly, my results showed patterns opposite these original predictions. Signal crayfish populations from communities without native crayfish were more aggressive, more active and bolder than populations from streams with native crayfish. We suggest that the positive correlations among aggressiveness, activity and boldness are the result of an overall aggression syndrome and that the differences between populations are due to variations among streams in prey availability rather than the presence of native crayfish. These behaviors likely facilitate successful invasions of signal crayfish into streams with low prey availability.

Chapter 4

Here I evaluated the consequences of high abundances of signal crayfish on native prey and the behaviors underlying their impacts. Specifically, I compared the impacts of low and high densities of signal crayfish and a no crayfish control on a typical prey community in large mesocosm tanks. Additionally, I evaluated the effects of density on aggression, foraging activity and voracity of signal crayfish. As indicated in chapter three, signal crayfish are aggressive towards conspecific competitors. Intraspecific aggression should result in high intraspecific competition which should limit the densities reached by exotic species, yet our field surveys found that some populations become superabundant despite being highly aggressive. Our results suggest that signal crayfish do become highly aggressive towards each other at high densities. Yet, despite the increase in aggression and in the per capita number of interactions between crayfish, signals increased their overall activity which drives high feeding rates on prey. As a result, we found a strong positive correlation between crayfish behavior (aggression, activity and voracity) and impacts on prey. We suggest these behaviors are also suggestive of an overall aggression syndrome (as suggested in Chapter three) whereby some individuals are generally more aggressive/active/voracious, whereas others are less aggressive across a range of situations.

Conclusion

Accumulated evidence suggests that signal crayfish possess the behavioral traits to overcome many challenges faced during the invasion process, but that characteristics of the invaded community (prey availability and physical attributes of streams) provide some resistance against signal crayfish reaching high abundances. When native communities do not offer much resistance (i.e. abundant prey and low flood disturbances), signal crayfish often invade at high abundances that result in large impacts on native species. The patterns and results observed in this dissertation suggest that the communities most at risk of invasion are those with high prey availability and infrequent physical disturbances (e.g. winter flooding). Unfortunately, these sites are also positively associated with the endangered Shasta crayfish and suggest that this species is at high risk of extinction.

Furthermore, our results indicated that a general aggression syndrome is an important trait behind the invasion success of signal crayfish. Aggressiveness has been an important trait in the success of other exotic species, as well. Therefore, studying aggression as part of a suite of traits could identify that aggression syndromes are an important general characteristic of successful invaders. Finally, the field of invasion biology has progressed beyond case studies of specific invaders to now have a number of testable hypotheses about when and where invasions may occur. Many of these hypotheses are based on characteristics of the community, but there are also logical and testable hypotheses about the behavioral traits that correspond to these community level hypotheses. Therefore, further integration of behavior into the studies of biological invasions has the potential to illuminate invasion biology as it did community ecology in past decades.

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Chapter 2:

Scale dependent effects of native species richness, prey availability and disturbance on the invasion success of an exotic crayfish.

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Abstract

We surveyed 17 stream communities to evaluate whether the distribution and abundance of exotic signal crayfish (*Pacifastacus leniusculus*) at small (1-m^2) and large scales (~400-m^2) was correlated with the presence of native crayfish, native species richness, prey availability, and/or by other stream characteristics. Our results indicated exotic signal crayfish distribution and abundance is highest in streams with high native species richness, more prey resources, but low stream discharge at both a fine and broad scale. Additionally, although all three variables best explained signal crayfish invasion success at both scales, native species richness explained less variation at a fine scale, but the relationship still remained positive. This adds support to the positive associations between native and exotic species richness frequently observed at broad scales, but is opposite the negative relationship typically observed at finer scales. Native crayfish distribution was also highest in streams with more prey resources and low stream discharge. We suggest that the positive association between exotic signal crayfish and native species richness and prey abundance at both scales is due to biotic acceptance whereby sites good for native species are also good for exotics. However, the absence of an "equalizing process", e.g. scouring winter floods, and difference in behavior of the exotic and native crayfish suggest that eventually these communities will become dominated by the invader. Together, the inter-relationships between prey availability, disturbance, diversity and the distributions of invasive and native crayfish put communities with native crayfish at a particular high risk of negative impacts.

Introduction

Studies of biological invasions aim to understand the factors that influence the vulnerability of native communities to invasion by exotic species. An extension of the diversity begets stability hypothesis predicts that it should be more difficult for exotic species to invade more diverse native communities; i.e., there should be a negative relationship between native versus exotic species richness (Elton 1958, Pimm 1991, Lawton and Brown 1993). Ample evidence, however, suggests that the relationship between native and exotic species richness is scale dependent (Levine and D'Antonio 1999, Byers and Noonburg 2003, Davies et al. 2005, Stohlgren et al. 2006a). Exotic and native species richness are often positively associated at broad scales and negatively associated at fine scales (Levine and D'Antonio 1999, Fridley et al. 2007). Yet exotic species richness and invasion success more likely depends on multiple factors (Rejmanek 2003, Taylor and Irwin 2004). For example, various other factors including climate, resource availability, propagule pressure, competitors, and predators have been suggested to covary with native species richness while also influencing invasibility (Lodge 1993, Levine and D'Antonio 1999, Naeem et al. 2000, Byers and Noonburg 2003). Thus, understanding the susceptibility of a community to invasion is likely better understood when multiple factors are considered.

Of particular interest is the need to understand in which communities invaders may have particularly large negative impacts. Often large impacts are associated with invaders that attain high densities in invaded communities (Hierro et al. 2005). High abundances and large impacts are often expected to occur in communities where the invader is novel (e.g., mammalian predators invading an island (Elton 1958, Ricciardi and Atkinson 2004, Cox and Lima 2006)), or is a distinct ecological type or functional group not already present in the system (Ricciardi and Atkinson 2004). In communities where the invader is novel, prey are presumably naïve and lack the appropriate anti-predator responses to the invader (Diamond and Case 1986, Cox and Lima

2006, Freeman and Byers 2006). This can lead to high prey consumption facilitating the establishment of high invader abundances (Sih et al. 2007, in review). Similarly, a novel invader might be less likely to encounter enemies that can limit their abundance (Agrawal and Kotanen 2003). However, regardless of mechanism, the distribution and abundance of invasive species should also be related to the novelty of the invader to the invaded community.

The chance of an invader being functionally novel to a native community should decrease as native species richness increases. Therefore, in theory, the most diverse communities should be the least susceptible to both invasion and high abundances of an exotic species. Yet many diverse communities have been easily invaded by both novel and similar taxa that have in some cases, reached high abundances. For example, highly diverse native mussel communities in the Mississippi river that evolved in the absence of biofoulers like the zebra mussel have been severely impacted by high abundances of this invader (Ricciardi et al. 1998). Cichlid communities in Lake Victoria are among the richest native fish communities in the world, yet they have been devastated by the introduction of novel large piscivorous Nile perch (*Lates niloticus*) (Kaufman 1992). Many cyprinid minnows have become established in aquatic communities already rich in native cyprinids (Moyle 1986). One explanation is that habitats or conditions that are good for native species are also good for exotics. Therefore, although invader distinctiveness and invasion probability should be related to native species diversity, the pattern is unclear and may depend on additional community factors.

Exotic freshwater crayfish are highly successful invaders that have invaded communities both rich and depauperate in native crayfish and other invertebrate species (Hill et al. 1993, Light et al. 1995, Lodge et al. 2000, Usio et al. 2001, Olden et al. 2006). Although introduced crayfish often reach high abundances, many populations remain at low densities (reviewed in Nystrom 2002). Water temperature and chemistry, hydrologic variability, urbanization, habitat alteration and refuge availability have all been found to influence crayfish distribution and density (Light et al. 1995, Light 2003, Riley et al. 2005, Nystrom et al. 2006, Usio et al. 2006). Competition and predation have been important factors in their invasion success as well (Hill and Lodge 1994, Hill and Lodge 1999, Light 2005); however, few studies have evaluated how their distribution and abundance also relates to native species richness and whether the exotic species is novel to the invaded community.

Signal crayfish (*Pacifastacus leniusculus* Dana) have been widely introduced throughout Europe and parts of North America and have had broad negative impacts on aquatic food webs and in particular, on native crayfishes (Light et al. 1995, Lodge et al. 2000, Taylor 2002, Nystrom 2005). Native from far northern California to British Columbia, signal crayfish have been widely introduced throughout California primarily via bait-bucket introductions (Riegel 1959). Its introduction into the Pit River drainage in northern California (Shasta County) has caused the decline of the endangered Shasta crayfish (*Pacifastacus fortis*) (Light et al. 1995, Ellis 1999). Unlike the Shasta crayfish, whose distribution is limited to isolated spring-fed streams in the lower Pit River drainage, the signal crayfish has also successfully invaded run-off fed streams within both the Pit River and adjacent Sacramento River drainages. These streams have historically lacked native crayfish and offer the opportunity to test whether the invader has become more common in streams where it is novel.

In this study, we surveyed 17 streams across two watersheds to evaluate whether signal crayfish invasions are correlated with native crayfish, native species richness and other stream characteristics (algal productivity, prey biomass, evenness, discharge). Because patterns of native-exotic species richness relationships are often scale-dependent, we also compared the abundance of signal crayfish at a smaller, within-stream scale (1-m^2) versus a larger, among-stream (>400-m^2) scale. We expected the association between signal crayfish distribution and density with native species diversity to be positive across streams and either negative or unrelated at smaller spatial scales within streams. Furthermore, we expected signal crayfish distribution and density to be negatively correlated with the presence of native crayfish, i.e. lower invasion success in communities where the invader is not novel.

Study Area

The study was conducted across 17, $1^{st} – 3^{rd}$ order streams within the lower Pit River drainage (Shasta County; 9 streams) and the upper Sacramento River drainage (Siskiyou County; 8 streams) in northern California (Figure 2-1; Table 2.1). All streams in the upper Sacramento River drainage are run-off fed streams (Figure 2.2). Five of the nine streams sampled in the lower Pit River were spring-fed streams (Figure 2.3). Stream altitudes varied from 500-1400, but most were on average at about 900m elevation. Thirteen streams were sampled between July-August of 2003, and four streams were sampled in July 2004.

Methods

Within each stream, a 90-m stretch was divided into six equally spaced transects. Each transect was divided into 3 equally spaced 1-m^2 sub-sections. Transects were sampled starting downstream and working upstream. In each sub-section a standard, representative stone (~15 cm^2) was collected to measure algal biomass. Algae and organic material was scraped from the surface of each stone with a stiff brush and rinsed with 250mL of stream water into a pan, transferred into a dark bottle and frozen. In the laboratory, a 60mL subsample was filtered through a pre-ashed 45-um glass-fiber filter, dried to a constant mass at 100 °C for 24-hours, weighed and ashed at 500 °C for 1 hour, and reweighed to yield ash-free dry mass (AFDM).

Within each transect, two 30.5 cm x 30.5 cm samples of the benthos were taken from the center and left or right sub-section, alternating left or right side with each transect. Samples were taken with a D-frame net (mesh = 250μm) and preserved in 90% ethanol and returned to the laboratory for identification of macroinvertebrates to family. Samples that had a wet weight of more than 30g were subsampled to one-quarter the total weight. Macroinvertebrate diversity was calculated at each sub-section using the Shannon-Weaver Index. Evenness was calculated by taking the observed value of species diversity and the maximum diversity value that would be obtained if individuals were evenly distributed among the number of species found in the community (Morin 1999). The average mass of individuals from each taxon was calculated by weighing ten individuals from each taxon after they were dried at 100 °C for one hour. The mean mass of each taxon was then multiplied by the number of individuals per sample to get the total macroinvertebrate biomass.

Visual estimates of snails, crayfish and fish were made by snorkeling at each sub-section within transects. Snails were identified to species and counted on a 30.5 x 30.5 cm area. Crayfish and benthic fish were found by turning over cobbles and boulders within a 1- m^2 area. Crayfish were captured by hand and the following information collected: 1) species, 2) total carapace length (mm), 3) chelae length (mm) and 4) sex. Young of year crayfish (<15mm carapace length) were not captured by hand, but rather just visually counted. Fish were identified visually to genus and their total length estimated to within 1.27 cm. The dominant fish species in the streams were sculpin (rough sculpin (*Cottus asperrimus*), bigeye marbled sculpin (*Cottus klamathensis macrops*), and Pit sculpin (*Cottus pitensis*) in the Pit River watershed, riffle sculpin (*Cottus gulosus*) in the upper Sacramento watershed) and rainbow trout (*Oncorhynchus mykiss*). We were unable to easily distinguish between species of sculpin during visual surveys. Therefore, our estimates are for the genus, .

We used geographical information system software (ArcGIS Ver. 9.2) to characterize site environmental variables that are known correlates to discharge. For each site, we calculated the elevation and drainage area from United States Geological Survey 10m digital elevation models, and we calculated the annual precipitation and percent precipitation in the month of January using PRISM climatic data representing mean monthly estimations for 1971 – 2000 (Daly et al. 1994, Doggett et al. 2004). Percent precipitation was used for January to characterize annual winter flood events that occur each year in both watersheds.

Statistical analyses

Variable used in analyses included elevation, drainage area, annual precipitation, January precipitation, ash free dry mass (AFDM), evenness and diversity of macroinvertebrates, biomass and density of snails, and density of crayfish and fish. Trout were difficult to estimate visually, as they were easily disturbed by our presence in the stream, and therefore, were not included in our analyses. Data from each sub-section were pooled to derive a transect average for each variable. Transects were then pooled to come up with a site average for each variable. One-way ANOVAs using stream as the factor were run on each variable and the residuals were analyzed using a Shapiro-Wilk test to test for normality. Residuals were normally distributed for all variables. Finally, the averages of all variables at both the stream and transect scale that were not normally distributed were log transformed. All analyses were performed in SYSTAT (Wilkinson 2004).

We used multiple logistic and linear regressions with step-wise backward deletions in SYSTAT (Wilkinson 2004) to find the best model to explain the distribution (logistic) across watersheds and density (linear) of signal crayfish at fine and broad scales. All variables used in multiple regressions were examined for colinearity. When two or more variables were significantly correlated (Bonferroni $p < 0.05$), Principle Component Analysis (PCA) was used to reduce the variables into a single measure. Across streams, macroinvertebrate biomass, snail biomass and density, and evenness were all highly correlated and reduced into a single "prey availability" variable using a PCA (72.29% variance explained). At the transect scale, snail biomass and density were highly correlated and reduced into a variable as well (91.10% variance explained). Elevation, drainage area, annual precipitation and percent precipitation for January were all highly correlated and were reduced into a single "discharge" variable. The first loading score explained 45.47% of the variation.

To look how scale affected invasibility of stream communities we ran multiple regressions using either transect averages (N= 57) or stream averages (N= 17) using only sites where signal crayfish were present. To test whether the observed relationships from transect averages were consistent across streams, we used the transect averages to calculate regression coefficients for each stream, relating signal crayfish density to the independent variables in the final main model. We then used t-tests to examine whether the average correlation coefficients differed from zero.

Results

Distribution of signal and Shasta crayfish across watersheds

Prey availability was the strongest predictor of the distribution of both signal and Shasta crayfish (final model for signal crayfish, Estimate = 4.522, SE = 2.272, $p = 0.05$ and final model for Shasta crayfish, Estimate = 4.349, SE= 2.463, $p = 0.077$; Tables 2.2 and 2.3, respectively).

Density of signal crayfish at fine and broad scales

Signal crayfish were more abundant in sites with higher prey availability and macroinvertebrate diversity, however, stream discharge had a negative effect on signal crayfish density at a broad scale (>400-m^2) (final model, $r^2 = 0.952$, $p < 0.001$; 65.5%, 58.8% and 56.1% variance explained, respectively; Table 2.4).

Although the novelty of signal crayfish to the community, e.g. presence of Shasta crayfish, was not a significant predictor in the model we still explored how signal crayfish abundance was related to the presence of Shasta crayfish. Signal crayfish density was significantly higher in sites with Shasta crayfish as opposed to sites without native crayfish (ANOVA $F_{1,15} = 20.818$, $p<0.001$; Figure 2.4). Additionally, sites with Shasta crayfish had significantly higher prey availability (ANOVA, $F_{1,15} = 16.259$, $p = 0.001$; Figure 2.5). However, macroinvertebrate diversity was not significantly higher in sites with Shasta crayfish (ANOVA, $F_{1,15} = 0.512$, $p = 0.485$; Figure 2.6). Finally, stream discharge was significantly lower at sites with Shasta crayfish (ANOVA, $F_{1,15} = 28.724$, $p<0.001$; Figure 2.7).

At a fine scale, signal crayfish abundance was highest at transects with higher snail density and biomass and macroinvertebrate diversity, however, stream discharge had a negative effect on signal crayfish density (final model, $r^2 = 0.526$, $p<0.001$; 39.0%, 16.5% and 39.3% variance explained, respectively; Table 2.5). Although macroinvertebrate diversity was included in the best fit model, it did not explain a significant amount of variation in signal crayfish abundance ($p=0.109$).

Finally, snail biomass and density were, on average, consistent across streams (mean $r=0.205$) but not significantly different from zero ($SD=0.399$, $t=1.259$, $p=0.132$). Macroinvertebrate diversity and signal crayfish density was not on average consistent across streams (mean $r=0.006$) and not significantly different from zero ($SD=0.508$, $t=0.035$, $p=0.973$).

Discussion

Our results indicated that stream communities with higher native species richness were most vulnerable to invasion by signal crayfish at both a fine and broad scale, but that invasion success was best explained in combination with high prey availability and low stream discharge. This adds support to other studies that suggest that invasion success is a result of numerous interacting processes rather than native species richness alone (Davis et al. 2000, Seabloom et al. 2003, Stachowicz and J.E. 2006, Fridley et al. 2007). Additionally, although all three variables best explained signal crayfish invasion success at both scales, native macroinvertebrate richness explained less variation at a fine scale, but the relationship still remained positive. Together the patterns observed in this study add support to the positive relationships generally observed between native and exotic species richness at broad scales, but opposite the negative correlations typically observed at fine scales (Levine and D'Antonio 1999, Stohlgren et al. 2003, Fridley et al. 2007).

The positive correlation between exotic signal crayfish and native species richness may be because sites with high diversity are generally good habitats for most species and therefore accept instead of resist new species, i.e. theory of biotic acceptance (Stohlgren et al. 2003, Stohlgren et al. 2006b). Additionally, although native crayfish was not a significant predictor of signal crayfish distribution and abundance in the multivariate model, signal crayfish were also more abundant in sites with native Shasta crayfish in univariate analyses. Sites with Shasta crayfish were also higher in prey abundance, which was similarly correlated with signal crayfish abundance. The positive association of exotic signal crayfish with native crayfish was opposite our initial prediction. Yet, it adds support to the hypothesis of biotic acceptance, that sites with higher macroinvertebrate richness are generally habitats that are good for all crayfish.

However, for the theory of biotic acceptance to work, some process, such as a scouring winter flood, must prevent local competitive exclusion and facilitate co-existence of both species (Connell 1978, Chesson 2000, Fridley et al. 2007). Our results showed a significant negative association between stream discharge and signal crayfish density, e.g. more signal crayfish in sites with low discharge. Although discharge was not correlated with Shasta crayfish distribution in the multivariate model, again, discharge was significantly lower in streams with Shasta crayfish in univariate analyses and has been associated with Shasta crayfish distribution in other studies (Ellis 1999). Therefore, although high prey availability and low winter discharge may indicate habitat that is generally good for all species and hence, all crayfish, with time these sites will likely become dominated by signal crayfish in the absence of a winter flood disturbance (Fridley et al. 2007).

Although signal crayfish reached higher abundances in 'good crayfish habitat", they also successfully invaded sites with lower prey availability, higher discharge and without any native Shasta crayfish. This raises two questions: 1) what limits the distribution of Shasta crayfish to high prey availability and low winter discharge streams, and 2) how do signal crayfish invade "bad" crayfish habitat? The differences in distribution of native Shasta and exotic signal crayfish are likely due to differences in behavioral traits important to population establishment and spread. Experiments conducted on signal crayfish populations that have invaded into non-Shasta, lower prey availability communities have found that these populations are generally more aggressive, active and bold in comparison to populations that have invaded into high prey availability, Shasta communities (Pintor, chapter 3). In contrast to both signal population types, Shasta crayfish are a very inactive, non-aggressive and less bold species (Ellis 1999). This difference in behavior between populations of signal crayfish results in higher foraging activity and hence, higher prey consumption by more aggressive/active/bold populations. Such behavior is likely necessary when prey abundance is low in contrast to high prey abundance sites which should not require crayfish to exhibit as bold behavior to forage. This variation in behavior of signal crayfish across invaded communities that differ in productivity likely contributes to their success at establishing populations in less desirable habitats.

Although signal crayfish are able to invade habitats with low prey availability and high discharge, they do not reach high abundances as indicated by the significant, positive relationship with prey availability, and the significant, negative relationship with discharge at a larger scale (e.g. across streams in two watersheds). Higher signal crayfish abundance at sites with greater prey availability could suggest that these sites have a proportion of unused resources that make these communities more susceptible to invasion (Davis et al. 2000, Shea and Chesson 2002). High resource availability, and hence greater invasion opportunity, may arise not just because resources are more abundant, but also because resident species have not reduced resource densities (Davis et al. 2000) or have not interfered with access to resources too greatly (Holway and Suarez 1999). The non-aggressive, inactive (e.g. low foraging activity) behavioral traits of native Shasta crayfish suggests the later two mechanisms might further contribute to the ease at which signal crayfish are able to establish highly abundant populations in high productivity, Shasta crayfish communities. However, the same behaviors of the native crayfish that may create an abundance of resources are also likely facilitating their current displacement by signal crayfish. This further suggests that instead of more diverse/productive sites being generally "good for all", when these sites are invaded by an aggressive exotic species, they will eventually become a site that is "great for one" (Fridley et al. 2007).

In our system, inter-relationships between prey resources, disturbance, native diversity and the distributions of invasive and native crayfish at both fine and broad spatial scales appear to put the endangered native crayfish into a particularly precarious position. Streams with low prey availability without native crayfish, although still susceptible to invasion, may be the most resistant to strong negative impacts by the invader because of factors that limit the invader's population abundance, e.g. high discharge flooding and low food resources. In contrast, the sites most at risk of being overrun by signal crayfish (e.g. higher diversity and prey availability sites, but low discharge) are those with native, endangered Shasta crayfish and two threatened species of sculpin, already facing the impact of this pest invader.

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Table 2.1. Table of names of the study sites along with general descriptors of each stream.

Site Name	**Watershed**	**Spring-fed/Run-off**	**Elevation**	**Native Shasta crayfish**	**Exotic signal crayfish**
Clark Creek	Pit River	Run-off	865	No	Yes
Crystal Lake	Pit River	Spring-fed	911	Yes	Yes
Hat Creek	Pit River	Spring-fed	973	Yes	Yes
Lower McCloud River	Upper Sacramento	Run-off	1008	No	Yes
Lava Creek	Pit River	Spring-fed	1010	Yes	Yes
N. Fork Castle Creek	Upper Sacramento	Run-off	777	No	No
N. Fork Shotgun Creek	Upper Sacramento	Run-off	516	No	No
Ney Springs Creek	Upper Sacramento	Run-off	910	No	No
Rock Creek	Pit River	Run-off	923	No	Yes
Sacramento River	Upper Sacramento	Run-off	504	No	Yes
Scott Camp Creek	Upper Sacramento	Run-off	976	No	No
S. Fork Sacramento	Upper Sacramento	Run-off	1440	No	No
Squaw Creek Tributary	Upper Sacramento	Run-off	792	No	No
Sucker Springs Creek	Pit River	Spring-fed	859	Yes	Yes
Sucker Pond 5	Pit River	Spring-fed	859	Yes	Yes
Upper McCloud River	Upper Sacramento	Run-off	1154	No	No
Wagon Creek	Upper Sacramento	Run-off	1024	No	Yes

Table 2.2. Stepwise (backward deletion) logistic regression of signal crayfish presence/absence and stream variables across streams..

Variable	**Estimate**	**SE**	**t**	**p-value**
Constant	1.338	0.935	1.431	0.152
Prey Availability (PCA)	4.552	2.272	1.990	0.047

Table 2.3. Stepwise (backward deletion) logistic regression of Shasta crayfish presence/absence and stream variables across streams.

Variable	**Estimate**	**SE**	**t**	**p-value**
Constant	0.301	1.257	0.239	0.811
Prey Availability (PCA)	5.241	2.831	1.851	0.064

Table 2.4. Stepwise (backward deletion) multiple regression of signal crayfish density and stream variables across streams where signal crayfish were present. Final statistics yielded R^2=0.952 and p<0.001 (N=10, $F_{2,7}$= 39.396).

Variable	**Standardized coefficient**	**SE**	**t**	**p-value**
Constant	0.000	0.129	-0.573	0.587
Macroinvertebrate diversity	0.588	0.069	4.355	0.005
Prey availability (PCA)	0.655	0.026	4.629	0.004
Stream discharge (PCA)	-0.561	0.657	-5.069	0.002

Table 2.5. Stepwise (backward deletion) multiple regression of signal crayfish density and stream variables across transects where signal crayfish were present. Final statistics yielded $R^2=0.526$ and $p<0.001$ ($N=58$, $F_{4,53}= 19.950$).

Variable	**Standardized coefficient**	**SE**	**t**	**p-value**
Constant	0.000.	0.083	4.162	<0.001
Stream discharge (PCA)	-0.393	0.017	-2.965	0.005
Snail biomass & density (PCA)	0.390	0.023	2.897	0.005
Macroinvertebrate diversity	0.165	0.046	1.631	0.109

Figure Legends

Figure 2.1. Map of study area showing the 17 sites sampled. The inset gives the location of the study area within California. Open circles indicate streams samples without crayfish. Squares with dots indicate streams sampled, historically without native crayfish, but now invaded by signal crayfish. Squares with plus symbols indicate sites with both native Shasta crayfish and exotic signal crayfish.

Figure 2.2. Map of streams sampled within the upper Sacramento River drainage. Open circles indicate streams samples without crayfish. Squares with dots indicate streams sampled, historically without native crayfish, but now invaded by signal crayfish.

Figure 2.3. Map of streams sampled in the lower Pit River watershed. Squares with dots indicate streams sampled, historically without native crayfish, but now invaded by signal crayfish. Squares with plus symbols indicate sites with both native Shasta crayfish and exotic signal crayfish.

Figure 2.4. Density of signal crayfish in streams with and without native Shasta crayfish.

Figure 2.5. PCA score of prey productivity (snail and other macroinvertebrate biomass, density and evenness) in streams with and without native Shasta crayfish.

Figure 2.6. Shannon-Weaver Index of Diversity in streams with and without native Shasta crayfish.

Figure 2.7. PCA score of stream discharge (Elevation, drainage area, annual precipitation and percent precipitation for January) in streams with and without native Shasta crayfish.

Figure 2.1.

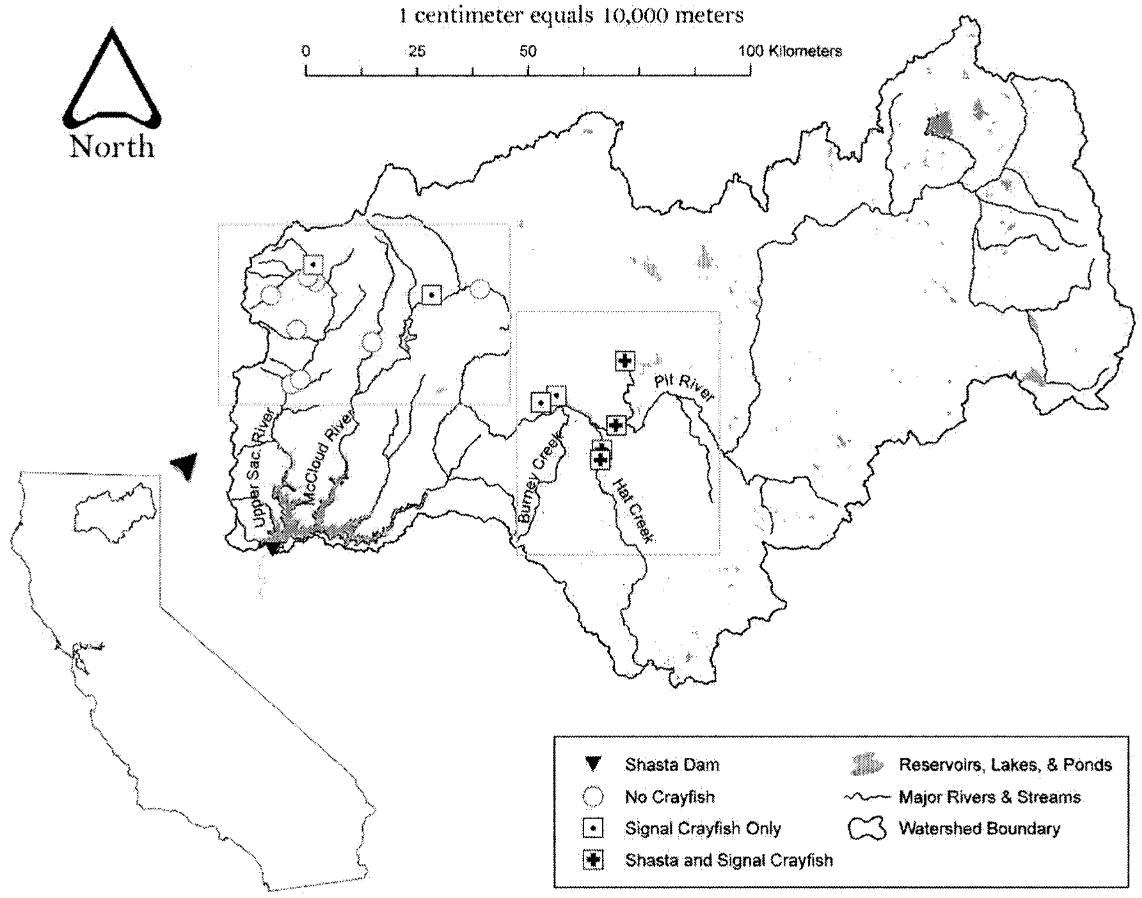

1 centimeter equals 10,000 meters
0
25
50
100 Kilometers
North
Upper Sac. River
McCloud River
Pit River
Burney Creek
Hat Creek
Shasta Dam
No Crayfish
Signal Crayfish Only
Shasta and Signal Crayfish
Reservoirs, Lakes, & Ponds
Major Rivers & Streams
Watershed Boundary

Figure 2.2.

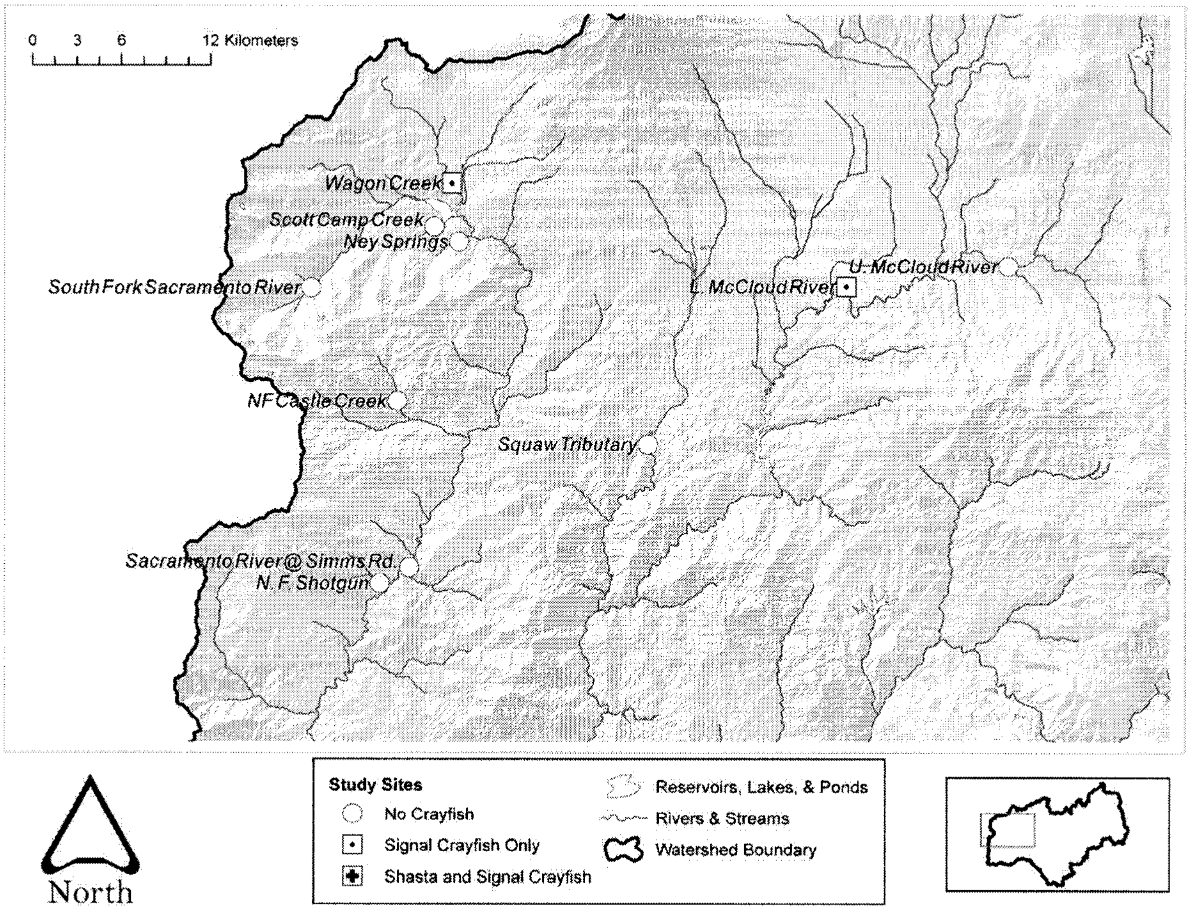

0 3 6 12 Kilometers
Wagon Creek
Scott Camp Creek
Ney Springs
South Fork Sacramento River
U. McCloud River
L. McCloud River
NF Castle Creek
Squaw Tributary
Sacramento River @ Simms Rd.
N. F. Shotgun
North
Study Sites
No Crayfish
Signal Crayfish Only
Shasta and Signal Crayfish
Reservoirs, Lakes, & Ponds
Rivers & Streams
Watershed Boundary

Figure 2.3.

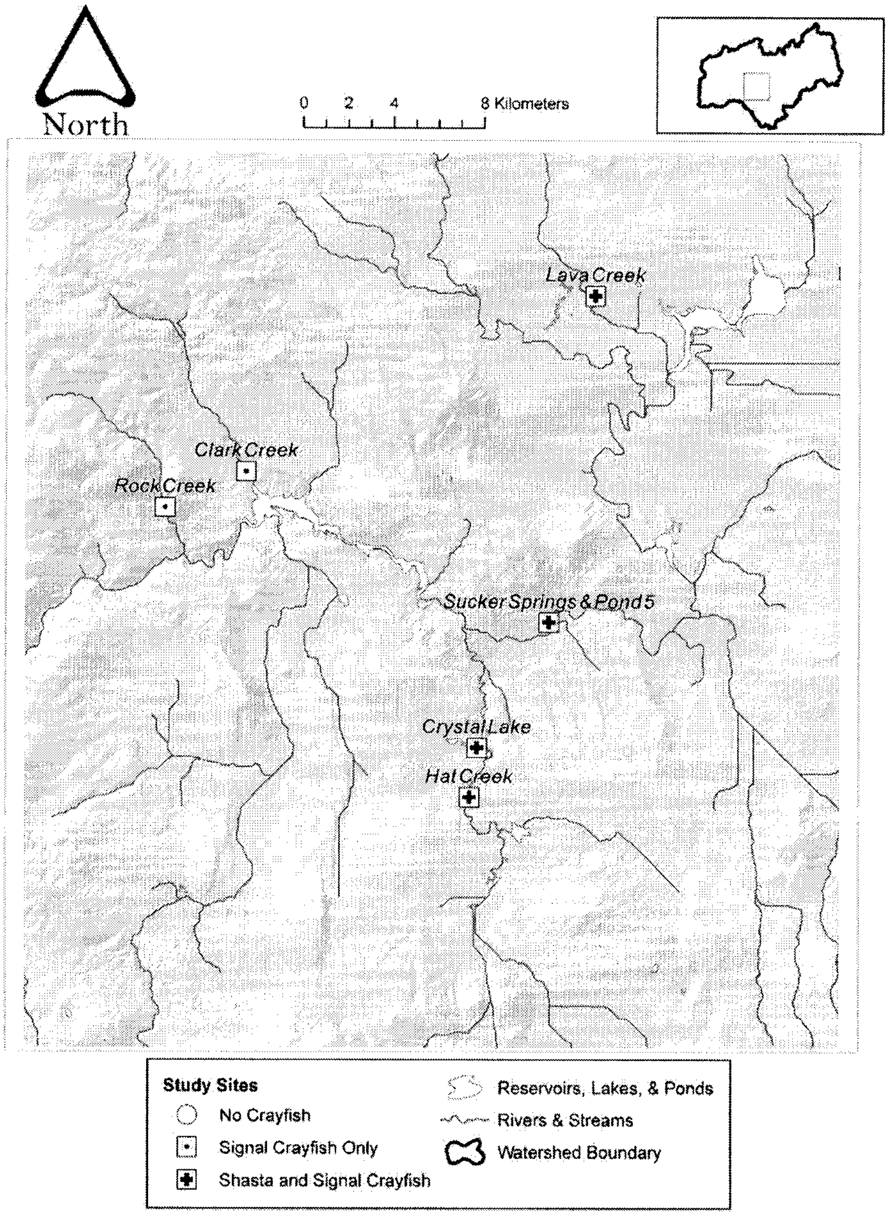

North
0 2 4 8 Kilometers
Lava Creek
Clark Creek
Rock Creek
Sucker Springs & Pond 5
Crystal Lake
Hat Creek
Study Sites
No Crayfish
Signal Crayfish Only
Shasta and Signal Crayfish
Reservoirs, Lakes, & Ponds
Rivers & Streams
Watershed Boundary

Figure 2.4.

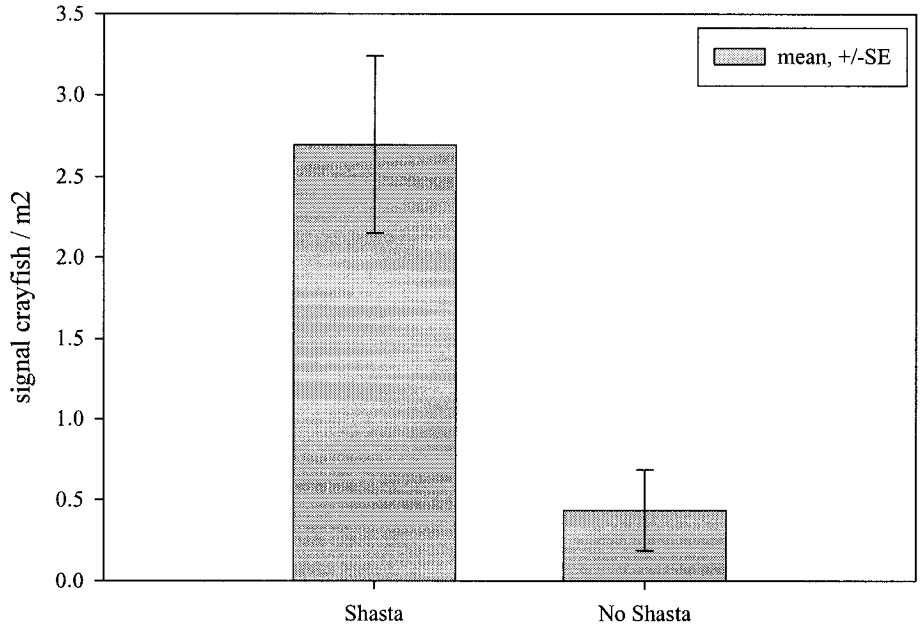

signal crayfish / m2
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
mean, +/-SE
Shasta
No Shasta

Figure 2.5.

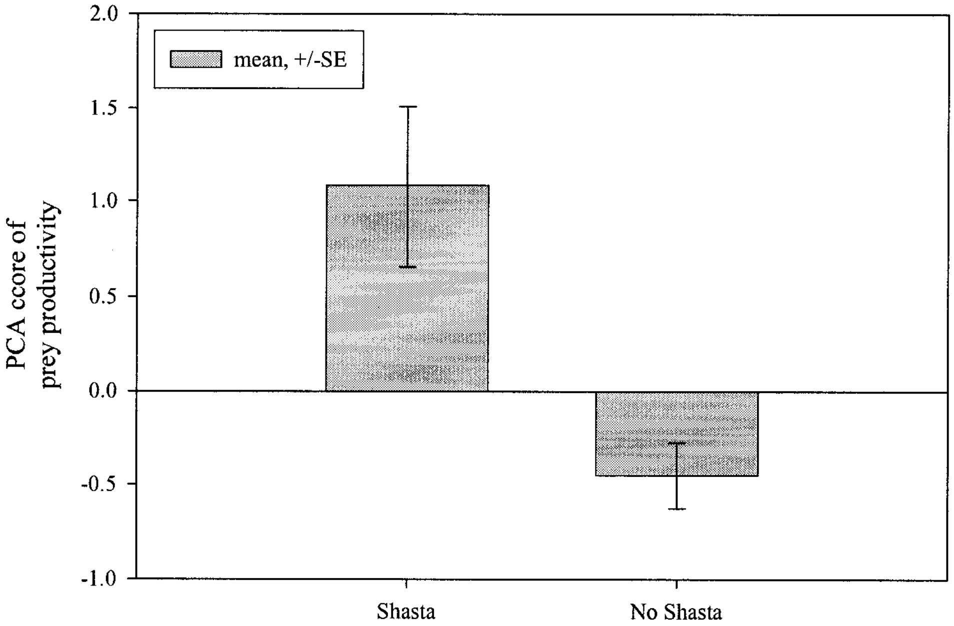

2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
mean, +/-SE
PCA ccore of
prey productivity
Shasta
No Shasta

Figure 2.6.

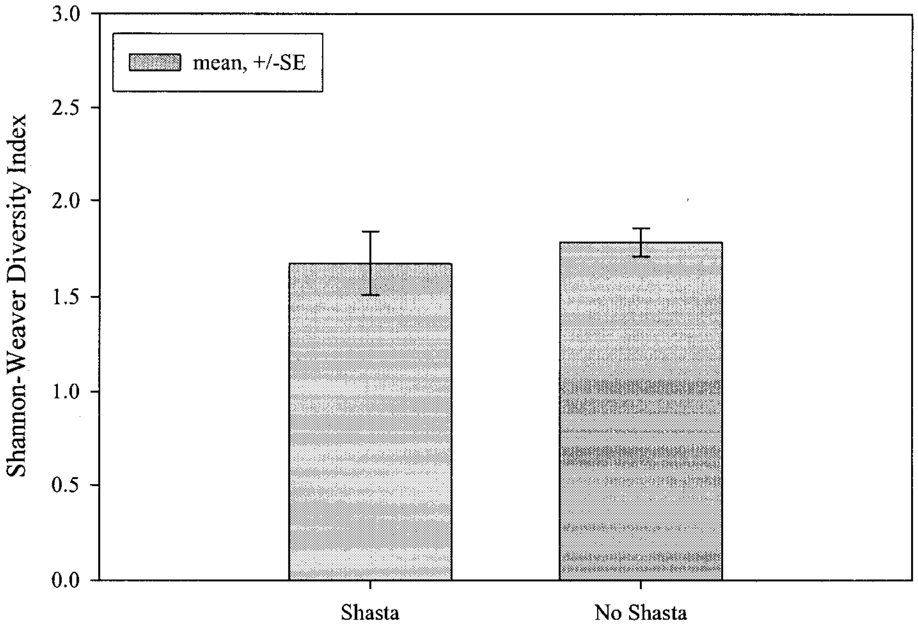
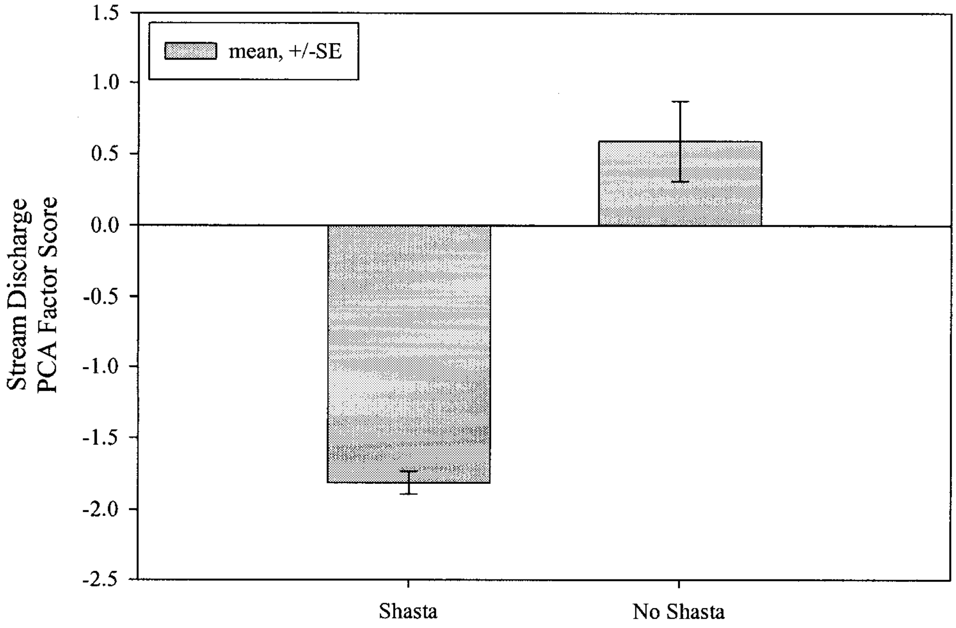

mean, +/-SE
Shannon-Weaver Diversity Index
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Shasta
No Shasta

Figure 2.7.


mean, +/-SE
Stream Discharge
PCA Factor Score
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Shasta
No Shasta

Chapter 3:

Variation in aggression, activity and boldness between native and introduced populations of an invasive crayfish.

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Abstract

We compared multiple populations of exotic signal crayfish (*Pacifastacus leniusculus*) across its native and introduce range to test whether the presence of an ecologically similar native species influences aggressiveness, boldness and activity. We predicted that signal crayfish need to be highly aggressive when introduced into communities with native crayfish competitors in comparison to communities without native crayfish. Furthermore, the presence of a similar native species also implies that the native prey and predator are not naïve and possess the appropriate behavioral responses to the invader. Therefore, we also predicted that signal crayfish should be more voracious to overcome experienced prey and less bold under the predation risk of an experienced predator when introduced into communities with native crayfish as opposed to communities without native crayfish. We conducted four behavioral assays to compare levels of intraspecific aggression, foraging activity, fight or flee threat response and boldness to forage under predation risk of signal crayfish from their native range and from their introduced range with and without native Shasta crayfish (*Pacifastacus fortis*). Exotic signal crayfish from communities without native crayfish were bolder to forage under predation risk. However, opposite to our predictions, these populations were also more aggressive towards conspecifics and more active foragers in comparison to populations introduced into communities with native crayfish and native signal populations. Higher aggression/activity/boldness was positively correlated with prey consumption rates, and hence potential impacts on prey. We suggest that the positive correlations between aggression/activity/boldness are the result of an overall aggression

syndrome and that differences in prey biomass of the invaded community may be a stronger influence on behavior than the presence of a similar native species.

Introduction

Biological invasions have expanded the geographical range of many organisms as humans take species from their native range and introduce them to areas they likely would not reach on their own. Many studies on invasive animals have focused on species traits that explain variations in the success of species outside their native range. Interestingly, in some systems, the key traits associated with invasion ability have changed following the invasion process (Suarez et al. 1999, Grosholz and Ruiz 2003, Cox 2004). For example, populations of exotic species are often larger, less aggressive towards conspecifics or more aggressive towards interspecific competitors than populations from their native range (Holway and Suarez 1999, Suarez et al. 1999, Grosholz and Ruiz 2003). These traits are important because they often contribute to the competitive displacement of native species by invaders and through biogeographical comparisons have suggested a mechanistic explanation of invasion success.

Although aggressiveness, along with foraging activity and voracity, have been found to be key behavioral mechanisms behind the competitive superiority of exotic over native species (Hill and Lodge 1999, Holway and Suarez 1999, Rehage and Sih 2004, Rehage et al. 2005a, b), their importance may also depend on the presence of an ecologically similar species in the invaded community. The presence of an similar native species may leave little resource space for an invader to successfully establish a population (Shea and Chesson 2002). If resources are not partitioned spatially or temporally, ecologically similar species are expected to compete intensely (Pianka 1981, Dudgeon et al. 1999). Therefore, if there is no similar native competitor, then the invader needs not to be as aggressive to invade since there is no competitor to displace.

Additionally, presumably if the invader is novel, both predators and prey in the invaded community will be naïve (Sih et al. 2007, in review). It follows then that invaders may not need to be active or voracious foragers to consume poorly defended prey. Furthermore, following from the enemy release hypothesis, the invader can be bold and forage even in the presence of an otherwise would be predator, since the potential predator is too naïve to recognize it as prey.

In contrast, an invader may be expected to be more aggressive when it invades communities containing similar native species and must directly compete with a close competitor. Because the invader is not as novel, it might also need to be a more active and voracious forager to overcome prey defenses that originally evolved in response to the established native competitor. In addition, the invader likely would not be as bold because predators will more likely recognize the invader as prey. Take together, variation in available resources, the competitive environment and natural enemies can lead to different niche opportunities among invaded communities (Shea and Chesson 2002). These differences may influence the behavioral traits needed by the invader to successfully invade. Therefore, we hypothesize that to successfully invade areas with similar native species (smaller niche opportunity), invaders need to be both aggressive towards congeners, exhibit high foraging activity and voracity towards defended prey, and be more cautious/less bold to forage under predation risk. On the contrary, invaders into regions without similar native species (larger niche opportunity) can successfully invade while being low in aggressiveness, foraging activity and voracity, but relatively bold in foraging.

Invasions of freshwater systems offer a great opportunity to address how differences in niche opportunities, i.e. presence/absence of a similar native species, may influence behavioral traits of an invader. In comparison to terrestrial systems, freshwater systems have been argued to function more like freshwater "islands" that are subject to a higher degree of isolation than contiguous terrestrial ecosystems (Cox and Lima 2006). Specifically, adjacent watersheds or stream stretches often differ in the presence of a particular predator or competitor; i.e., often, the same invader can be found in habitats with and without a similar native species.

Throughout much of the world, crayfish are key invaders of freshwater systems and have large impacts on native communities (Lodge et al. 2000, Nystrom et al. 2001, Wilson et al. 2004). Introduced crayfish pose the largest threat towards native crayfish diversity, often displacing natives via aggressive interactions. In particular, the signal crayfish (*Pacifastasus leniusculus*) has been shown to be competitively superior through aggressive interactions over several native crayfishes and has negative impacts on native prey. The signal crayfish is native throughout far northern California/lower Oregon and north to British Columbia, but has been introduced throughout many part of the world through bait-buckets, aquaculture and other routes. In the United States, signal crayfish have been introduced through much of northern California south of the Klamath River and has been implicated in the decline and endangerment of the native Shasta crayfish (*Pacifastacus fortis*) (Light et al. 1995). The signal crayfish is competitively dominant over the native Shasta crayfish and aggressively outcompetes it for both food and shelter resources (Light et al. 1995, Ellis 1999). In addition to its invasion into communities with the native Shasta crayfish, it has also invaded many freshwater communities in northern California that, to our knowledge, historically did not have native crayfish prior to the invasion of signal crayfish (Reigel 1959).

The presence or absence of a similar native crayfish in streams invaded by signal crayfish allows us to ask whether differences in niche opportunities among invaded communities influences behaviors of signal crayfish. Aggressiveness, foraging activity and boldness are traits known to be important to invasion success, therefore we expect these behaviors to be greater in signal crayfish from introduced populations. However, these behaviors should also depend on the presence or absence of a similar native species in invaded community. Thus, relative to signal crayfish invading into streams that historically lacked native crayfish, we expect invaders into communities that have native crayfish to be more aggressive towards competitors, and more active foragers to overcome prey defenses, but less bold under predation risk. To test this hypothesis we compared the behavior of multiple populations of signal crayfish across their native and introduced ranges in a series of behavioral assays.

Methods

We compared the behavior of signal crayfish across three geographic ranges: native range (native range), introduced range into communities without native crayfish (larger niche opportunity) and introduced range into communities currently (albeit declining densities) with native crayfish (smaller niche opportunity). Within each geographic range, we collected individuals from 2-3 populations of signal crayfish to use in a series of behavioral assays.

Adult male and female crayfish (25-45mm in carapace length) were collected from streams in Oregon and northern California. Native range signal crayfish were collected from sites the Rogue River (Jackson County), the upper Williamson (Klamath County) and the Clackamas rivers (Clackamas County) all in Oregon. Introduced populations from communities with native crayfish were collected in Shasta County from Sucker Springs Creek, Crystal Lake outflow and Lava Creek (all in Shasta County). Introduced populations from communities without native crayfish were collected from the American River (Sacramento County), Rock Creek (Shasta County) and Deep Creek (Shasta County). All crayfish were held in the lab for at least one week prior to being used in experiments. Crayfish were held individually within aerated tanks at 15-16° C under a photoperiod of 16 hours light: 8 hours dark and fed TetraMin Granules daily.

Behavioral assays were conducted on all populations within each geographic range to measure 1) levels of intraspecific aggression, 2) foraging activity on novel prey, 3) boldness to forage under predation risk and 4) initial response to predation threat. Assays were conducted during the 2004 field season (June-September). To clarify, although all populations were assayed under identical conditions, because assays were done on animals collected from the field (as opposed to laboratory-reared F1's), differences among populations could be due to either genetic or environmental causes.

Intraspecific Aggression

Because we were interested in quantifying the average level of intraspecific aggression within each population, individual crayfish were pitted against crayfish from within their own population rather than between populations or geographic range. For each population, ten pairs of crayfish were tested in 37.5 liter aquaria. Individual crayfish were sexed and measured and then sex and size-matched into pairs (within 5mm carapace and chelae length). Prior to each trial, each crayfish was allowed to acclimate to the tank separated by a divider. After 15 minutes, the divider was lifted and the interactions were recorded for 20 minutes. At each 5-second interval, each crayfish was assigned an aggression score based on the intensity of the interaction (Table 1; scoring system adapted from Karavanich & Atema 1998).

We calculated an average aggression score for each individual and used the average score for the pair to evaluate the aggression level for the entire interaction. Due to limited availability of animals, aggression assays were conducted on only one population from the introduced, no crayfish range (American River), we did not use a nested model to test for the effect of population within range, and range. Instead because there were no significant differences between populations within each range, we combined populations. Initial analyses revealed significant deviations from normality; therefore we used a one-way Kruskal-Wallis ANOVA to test for an effect of geographical range on average aggression scores

Foraging on novel prey

To examine how signal crayfish respond to and forage on novel prey, we assayed individual crayfish from each population in aquaria using New Zealand mud snails as novel prey. New Zealand mud snails are a recent invader to the western United States (Bowler 1991). All crayfish populations used were from locations that have not yet been invaded by the mud snail; therefore, all crayfish should have not previously experienced mud snails as prey. Two populations from the native (Rogue and Clackamas River) and introduced without native crayfish range (American River and Deep Creek), along with three populations from the introduced with native crayfish range (Sucker Springs Creek, Crystal Lake outflow and Lava Creek); 7 populations in total tested). Ten crayfish per population were assayed (70 crayfish total).

Individual crayfish were place in an 11.36L aquarium with 15 mud snails, along with one PVC pipe refuge. The experiment began at 1500 hr and ended at 900 the next morning. Three daytime observations were taken on the hour from 1600-1800 and three nighttime observations were taken from 1900-2100. Location (in/out refuge) and activity level (walking, climbing, and sitting) of each crayfish were recorded, along with the number of snails present and still alive in the tank. Foraging activity is the proportion of observations that crayfish were out of refuges and actively moving in the tank. Number of snails present and still alive in the tank was recorded again at the end of the experiment.

We used an unbalanced mixed model ANOVA (with a Satterthwaite approximation to estimate denominator degrees of freedom) to test for the effects of range (fixed effect) and the population nested within range (random effect). Analyses were conducted in SAS (SAS 2002-2003).

Boldness to Forage under Predation Risk

To compare levels of boldness across ranges of signal crayfish, we contrasted foraging activity of crayfish in the presence versus absence of predator cue. Trout are known predators of crayfish and are present in all populations where signal crayfish were collected (reference). Predator cues consisted of water from tanks holding eighteen, 30 cm (total length) rainbow trout.

Trials were conducted in 11.36L plastic tanks, filled with 7.57L of filtered well water at 16 °C and a 16L:8D photoperiod Crayfish were placed individually in a tank at 1200 hr without food and allowed to acclimate for 24-hours prior to the treatment being applied. After 24-hours, crayfish location (in versus out of refuge) was recorded and then 100ml of trout cue water or control water was slowly added to each tank. Immediately after that, 5 freshwater oligochaete larvae (blackworms) were added to each tank and continual observations were made for 5 minutes. Boldness was measured as the inverse of latency to begin foraging on a blackworm. We also recorded the total number of blackworms eaten within 5 minutes.

Two populations each were used in the introduced without crayfish range (American River and Deep Creek) and the native range (Rogue and Clackamas River). Three populations were used from the introduced with crayfish range. Because of the unbalanced design, the effect of treatment, range and treatment x range interaction was evaluated using a mixed model ANOVA using a Satterthwaite Approximation to estimate the denominator degrees of freedom Analyses were conducted in SAS (SAS 2002-2003).

Threat Response

Crayfish respond to the direct threat of a predator by either tail-flipping (rapid contraction of the abdomen which propels the crayfish backward, or upward into the water column) or by raising their claws. Individual crayfish were placed in a 61cm x 30.48cm sized tub filled with 16 cm of water. Crayfish were threatened by approaching the front of the crayfish by hand from a 45-degree angle and within 2.5cm of the surface of the water; their response was recorded. Assays were conducted on 2 populations each for the native (Rogue and Clackamas Rivers) and introduced no crayfish range (American

River and Deep creek), and on three populations for the introduced with crayfish range (Crystal Lake outflow, Sucker Springs & Lava Creeks). Ten individuals per population were tested. The proportion of each response observed was analyzed using a Chi-square.

Results

Intraspecific Aggression

Geographic range had a significant effect on average aggression levels for signal crayfish (ANOVA: $F_{2,65}$= 4.5, p=0.015; Figure 3.1). However, contrary to our predications, signal crayfish introduced into communities without native crayfish were more aggressive than either signals introduced into communities with native crayfish or native signal crayfish (Tukey Multiple Comparison: p=0.043 and p=0.012, respectively). Furthermore, signal crayfish introduced into communities with native crayfish were equally as aggressive towards conspecifics as were signals from their native range (Tukey Multiple Comparison: p=0.888).

Foraging Activity on Novel Prey

Geographic range tended to affect foraging activity of signal crayfish; however, the effect was not statistically significant (mixed model ANOVA: $F_{2,64}$=2.57, p=0.08; Figure 3.2a). Again, contrary to our prediction, signals from communities without native crayfish tended to be more active than signals from communities with native crayfish (Tukey multiple comparison, p=0.06). There was a significant effect of range on the number of snails consumed by signal crayfish (mixed model ANOVA: $F_{2,64}$=7.23, p=0.04; Figure 3.2b). The significant effect of range was largely due to the higher consumption of snails by signals from communities without native crayfish than signals from communities with native crayfish (Tukey multiple comparison, $p=0.04$).

Using population means, we found that foraging activity of signal crayfish was positively correlated with the average number of snails consumed during the experiment ($r=0.826$, $p= 0.022$; Figure 3.2c); i.e., populations that were more active also consumed more snails than those that were less active.

Boldness to Forage under Predation Risk

Surprisingly, predator cue treatment significantly decreased the latency of signals to forage (mixed model ANOVA: $F_{1, 135}=10.61$, $p=0.001$; Figure 3.3a); i.e., they began foraging faster in the presence of predator cue. Crayfish range had a significant effect on latency to forage ($F_{2,135} = 8.78$, $p<0.001$). However, this effect was largely driven by signals from communities without native crayfish beginning to forage significantly faster than signals from either communities with native crayfish or native populations (Tukey multiple comparison: $p<0.001$ and $p=0.002$, respectively). Although the predator treatment did not affect crayfish in all ranges, there was no significant interaction between predation treatment and range ($F_{2, 135}=2.03$, $p=0.13$).

Predation cue treatment also had a significant effect on the average number of prey consumed by crayfish (mixed model ANOVA: $F_{1, 135}=7.43$, $p=0.0073$; Figure 3.3b). Predation cue significantly increased the number of oligochaete/blackworms consumed by signals from both introduced ranges, but not by signals from their native range. Crayfish range also had a significant effect on the average number of prey consumed (mixed model ANOVA: $F_{2, 135}=5.65$, $p=0.0044$; Figure 3.3b). Signals from communities without native crayfish ate significantly more than signals from either communities with native crayfish or from their native range (Tukey multiple comparison, p=0.008 and p=0.013, respectively). There was no significant interaction between predator cue treatment and crayfish range (ANOVA: $F_{2, 135}$=0.47, p=0.62).

Using population means, we detected a positive correlation between latency to begin foraging and the average number of blackworm prey consumed for both the predator cue treatment and control (r=0.944, p=0.002 and r=0.913, p=0.004, respectively; Figure 3.3c). Populations that were more bold also consumed more prey.

Threat Response

There was a significant effect of range on the relative use of flight (tail-flip) versus fight (claw raise) in response to a simulated threat (Chi-square value =9.4409, df=2, p<0.01; Figure 3.4). Specifically, introduced signals communities without native crayfish tail-flipped 76.5% of the time, whereas introduced signals from communities with native crayfish claw raised 70% of the time. Native signal crayfish evenly displayed either a claw raise (50%) or tail flip (50%).

Behavior Correlations (Population Level)

Using population means, we looked for correlations among behaviors expressed across assays. We found that mean foraging activity (novel prey assay) was positively correlated with aggression (intraspecific aggression assay) (r=0.89, p=0.024; Figure 3.5). Additionally, foraging activity of each population to be positively correlated with boldness to forage under predation risk (r=0.702, p=0.04). Finally, boldness was positively correlated with aggression (r=0.886, p=0.025 Figure 3.5). Therefore, populations that were bolder were also more aggressive and more active.

Discussion

Although many studies have evaluated traits of successful invaders or characteristics of invaded communities, neither species nor community traits is likely to independently explain the invasion success of an introduced species. Aggressiveness is a key trait important to the competitive displacement of native species and overall invasion success (Gamradt et al. 1997, Hill and Lodge 1999, Holway and Suarez 1999, Yasuda et al. 2004). However, the importance of aggressive behavior often depends on characteristics of the invaded community (Heggenes et al. 1993, Ross and Keller 1995, Ross et al. 1996, Richards 2002). If aggression underlies competitive interactions, then the level of aggressiveness should be related to the amount of resource overlap there is between the invader and native species (Stamps 1977, Dudgeon et al. 1999, Shea and Chesson 2002). In the absence of a close competitor, an invader need not be highly aggressive nor have strong competitive abilities to successfully establish a population. Furthermore, the absence of a close competitor may also imply that the native prey and predator community lacks an evolutionary history with an ecologically similar predator/prey (Cox and Lima 2006). Native prey may lack the appropriate anti-predator responses to cope with the invader, and native predators may not recognize the invader as prey. Therefore, invaders into communities lacking a close competitor might not need to be highly voracious on naïve, native prey to be successful, but can be more bold under predation risk in the presence of naïve, native predators. Overall, we predict that invaders should be less active/aggressive, but more bold when invading into communities without an ecological similar species (as compared to when invading into communities with a similar species).

Interestingly, our results indicated the opposite pattern for aggression and activity from this prediction. Generally, across three of the four assays, signal crayfish from communities without native crayfish were more aggressive towards conspecifics, more active foragers and more bold to forage under predation risk. Higher aggression/activity/boldness was positively correlated with prey consumption rates, and hence potential impacts on prey. Although this suite of behaviors may exacerbate impacts on naïve prey and lead to higher abundances of signal crayfish, field surveys indicate that the highest densities of signal crayfish are in communities with native Shasta crayfish (Pintor, chapter 2).

A key fact that may explain the above patterns lies in systematic differences among regions in prey productivity (Pintor, chapter 2). Data from 20 streams that vary in the presence or absence of both signal and Shasta crayfish indicates that streams with native Shasta crayfish are significantly more productive than streams without native crayfish (Figure 3.6). Snail biomass (a preferred prey of crayfish) is, on average, two times higher in streams with native Shasta crayfish. Additionally, the biomass of other macroinvertebrates (e.g. Trichoptera, Ephemeroptera, Chironomidae) is also more than two times greater in streams with native Shasta crayfish. Clearly, higher productivity may explain the higher signal crayfish abundance, but it also provides insights and additional hypotheses about behavioral traits important to invasion success.

Low productivity habitats may require an invader to be more aggressive, active and bold to obtain the resources necessary to establish a population. A lack of resources, along with the absence of an interspecific competitor may lead to high intraspecific competition and aggression which may limit the densities reached by the invader, as observed with signal crayfish. In contrast, although a high productivity stream may contain an ecologically similar native species, if productivity is high enough, high aggression/activity/boldness might not be necessary to obtain resources necessary for population growth. The opportunity for invasion may arise because resources have not been fully exploited (Shea and Chesson 2002). Similarly, the theory of fluctuating resource availability argues that the susceptibility to invasion increases whenever the amount of unused resources in that community increases (Davis et al. 2000). Our results add support to this hypothesis.

The fact that native Shasta crayfish appear to be restricted to high productivity, spring fed streams may be associated with their behavior (Sih et al. 2004a, Sih et al. 2004b). Although we did not directly compare the behavior of the two crayfish species, previous work indicated that the Shasta crayfish is a very non-aggressive and generally inactive species (Ellis 1999). The non-aggressive, inactive and shy behavior of the Shasta crayfish may contribute to an inability to acquire enough resources to persist in low productivity habitats, and hence, their naturally small, restricted range (Reigel 1959, Eng and Daniels 1982). In contrast, the voracious, aggressive and bold signal crayfish can apparently successfully use these low productivity streams. Thus the signal crayfish's behavioral type contributes to their invasion success in two ways: 1) by allowing them to outcompete native crayfish in preferred, high productivity habitats; and also 2) by allowing them to establish and persist in low productivity streams.

Previous work on signal crayfish has shown that this species is generally much more aggressive than other crayfish, including other known invaders such as the rusty crayfish, *Orconectes rusticus* (Tierney et al. 2000). Although signals are generally aggressive, our comparisons of signals from different regions suggest that the invasion process might further select for aggressive/active/bold behaviors. Generally, native range populations were not significantly different from populations introduced into communities containing native crayfish. However, native populations were consistently less aggressive/active/bold than introduced signals from communities without native crayfish. Other studies that have looked at how aggressive behavior contributes to invasion success have indicated an overall increase in aggressive behavior or competitive ability following invasion. Our results support this general pattern, but the variation among introduced populations suggests that differences in this suite of behaviors (aggression/activity/boldness) may be context dependent.

The positive correlations between aggression, activity and boldness across different contexts suggest that these behaviors might be part of an overall aggression syndrome (Sih et al. 2004a, Sih et al. 2004b). Although the correlations were based on population means rather than individuals, it is still useful to consider a behavioral syndrome framework to understand the relationships between these behaviors and their implications for invasions. A behavioral syndrome, such as an aggression syndrome, has been suggested as an explanation for how a species can be successful in each stage of the invasion process (Sih et al. 2004). For example, an overall bold/aggressive/active behavioral syndrome might facilitate a species success in all three stages of the invasion process. Bold individuals may be more likely to disperse into new habitats. Aggressiveness may allow an invader to compete well when rare and active/aggressive individuals may have high foraging rates leading to large impacts on invaded communities. However, boldness might be maladaptive in some contexts, for example, in the presence of an experienced predator. Contrary to the behavioral syndrome hypothesis, but following from enemy release hypotheses, we predicted signal crayfish introduced into habitats already containing crayfish to be less bold since predators would presumably be familiar with crayfish as prey. Introduced signals from occupied communities were predicted to be more aggressive and active; therefore, boldness was expected to be negatively correlated with aggression these two behaviors. Our data fit part of the prediction that introduced signals were bolder in communities with naïve predators; however, there was a positive correlation between boldness, aggression and activity. Although this is the opposite of what we originally predicted, it supports many other studies documenting a positive correlation between these behaviors (Sih et al. 2003, Bell 2005, Johnson and Sih 2005, Bell 2007).

Although signals from communities without native crayfish were bolder, all populations surprisingly began to forage faster under predation risk in comparison to the no predator controls. Despite this being opposite of how predation risk generally affects behavior, other studies have shown that brown trout and other predatory fish prefer small, juvenile crayfish over larger adults (Englund and Krupa 2000, Nystrom et al. 2006). Furthermore, adult crayfish have been shown to increase their activity levels and aggressive displays in comparison to juveniles in the presence of predatory fish (Mather and Stein 1993, Keller and Moore 2000, Magoulick 2004). Larger adult crayfish, like those used in our experiment, typically reach a size refuge from gape-limited fish and then can afford to be bolder under the risk of predation. Organisms that experience size-dependent predation are predicted to exhibit behaviors that maximize growth rates while minimizing the risk of mortality (Werner and Gilliam 1984). Therefore, once crayfish have reached a size refuge, predation risk may promote large crayfish to become more bold/active foragers to further increase growth and body size.

Introduced signals from communities without native crayfish were more bold to forage under predation risk and generally highly aggressive, yet they responded to a simulated threat with a tail-flip (flee escape) response. Responding with a claw raise was expected to be the more aggressive response to a threat than tail-flipping. We hypothesize that the tendency to tail-flip in response to a threat might be driven by the habitat's flow characteristics. Tail-flipping is generally thought to be an inappropriate response to predation threat by fish because it raises the crayfish up into the water column where it is easier for a fish to catch (Stein and Magnuson 1976). However, a tail-flip response may be more effective in a fast-flowing stream, similar to the communities from which these populations were collected. Tail-flipping in a fast flowing stream would send the crayfish into the current possibly accelerating it and increasing its chances of escape from a predator. In contrast, populations from communities with native Shasta crayfish were from habitats with much slower flow (e.g. on average 1 foot per second in comparison to on average 4.5 feet per second). Therefore, in these habitats a claw raise would be a more appropriate response since the current is not fast enough to facilitate escape from predators.

Although aggression, activity and boldness indeed appear to be related to the invasion success and impact of signal crayfish, variation in the expression of these behaviors seems to be influenced by differences in characteristics among invaded communities. The presence of an ecologically similar native species seemed to be a plausible hypothesis for predicting when an invader should be more or less aggressive.

However, variation in resource productivity among invaded communities may be a more important driving factor behind the expression of aggression, activity and boldness. Scientists have highlighted the importance of understanding the behavioral mechanisms underlying invasion success (Holway and Suarez 1999, Sol et al. 2002, Rehage and Sih 2004). Our results suggest that future studies of the behavioral traits of invaders be considered along with variation in other characteristics of the invader and the invaded community.

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Figure Legends

Figure 3.1. Average aggression score of signal crayfish in pair-wise interactions from each of three geographic ranges. Native range ("native"), introduced range into communities with a similar species ("intro with native CF") and introduced range into communities without a similar species ("intro without native CF").

Figure 3.2a. Average foraging activity of signal crayfish on novel prey (New Zealand mudsnail) from each of three geographic ranges. Native range ("native"), introduced range into communities with a similar species ("intro with native CF") and introduced range into communities without a similar species ("intro without native CF").

Figure 3.2b. Average number of snails consumed by signal crayfish over 18 hours from each of three geographic ranges. Native range ("native"), introduced range into communities with a similar species ("intro with native CF") and introduced range into communities without a similar species ("intro without native CF").

Figure 3.2c. Average level of foraging activity verses the average number of snails consumed by each population. Foraging activity of signal crayfish was positively correlated with the average number of snails consumed during the experiment ($r=0.826$, $p= 0.022$).

Figure 3.3a. Average level of boldness (time until foraging began) by signal crayfish from each of three geographic ranges. Native range ("native"), introduced range into communities with a similar species (“intro with native CF”) and introduced range into communities without a similar species (“intro without native CF”).

Figure 3.3b. Average number of oligochaete blackworms consumed in the presence or absence of trout predator cue by signal crayfish from each of three geographic ranges. Native range (“native”), introduced range into communities with a similar species (“intro with native CF”) and introduced range into communities without a similar species (“intro without native CF”).

Figure 3.3c. Average boldness verses the average number of oligochaete blackworm prey consumed in the presence (black dots) or absence (grey dots) of trout predator cue ($r=0.944$, $p=0.002$ and $r=0.913$, $p=0.004$, respectively).

Figure 3.4. Relative use of flight (tail-flip) versus fight (claw raise) in response to a simulated threat (Chi-square value =9.4409, $df=2$, $p<0.01$).

Figure 3.5. Correlation matrix of population means of foraging activity, intraspecific aggression and boldness. Foraging activity was positively correlated with aggression ($r=0.89$, $p=0.024$) and boldness to forage under predation risk ($r=0.702$, $p=0.04$). Boldness was positively correlated with aggression ($r=0.886$, $p=0.025$).

Figure 3.6. Biomass of snails and non-snail macroinvertebrates in streams with and without native Shasta crayfish. Snail and non-snail macroinvertebrate biomass is

significantly higher in streams with native Shasta crayfish (ANOVA: $F_{1,15}=19.391$, $p<0.001$ and $F_{1,15}=8.298$, $p=0.011$, respectively)

Figure 3.1

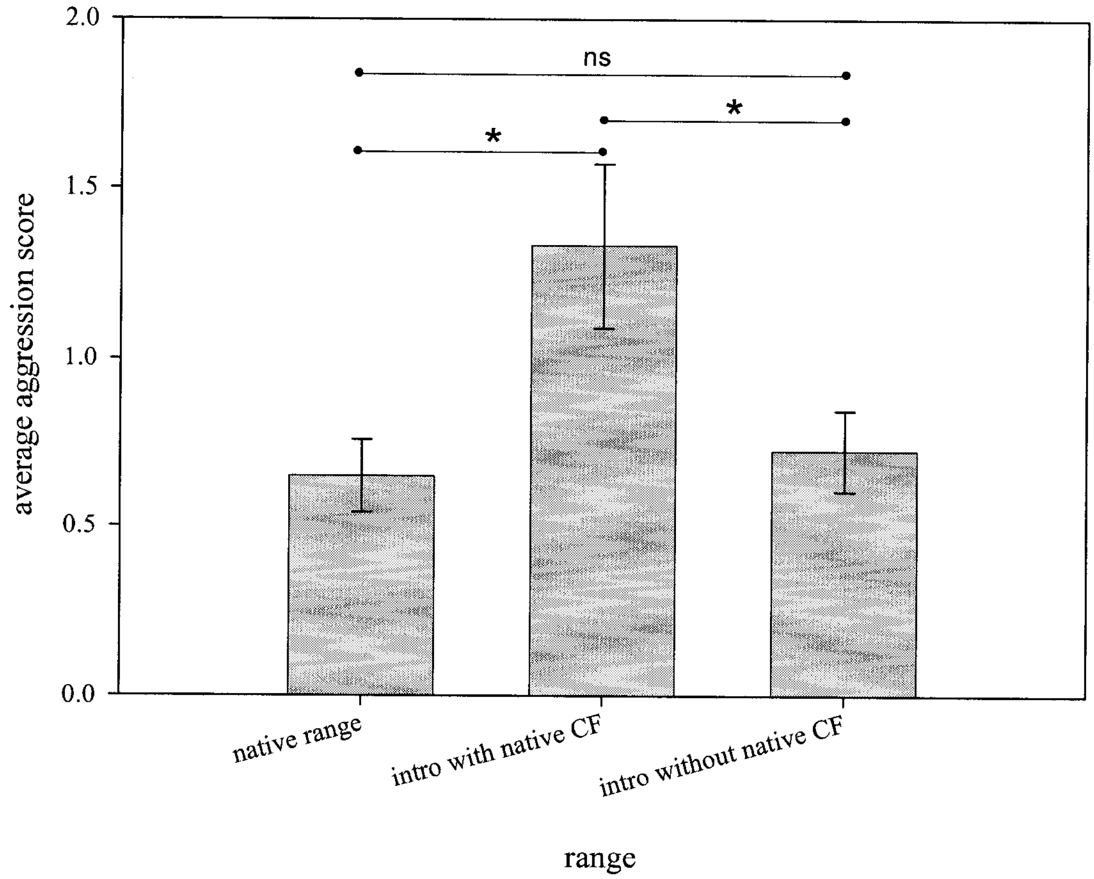

2.0
1.5
1.0
0.5
0.0
average aggression score
ns
*
*
native range
intro with native CF
intro without native CF
range

Figure 3.2a

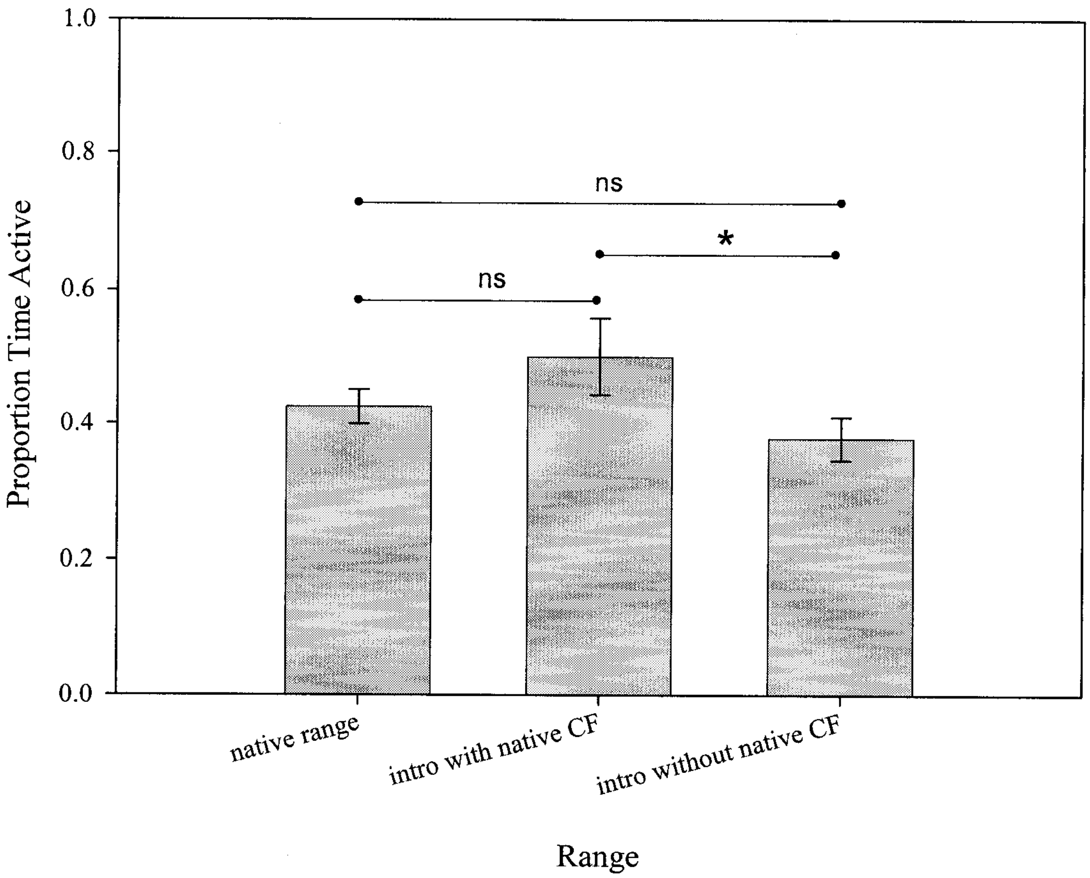

1.0
0.8
0.6
0.4
0.2
0.0
Proportion Time Active
ns
*
ns
native range
intro with native CF
intro without native CF
Range

Figure 3.2b

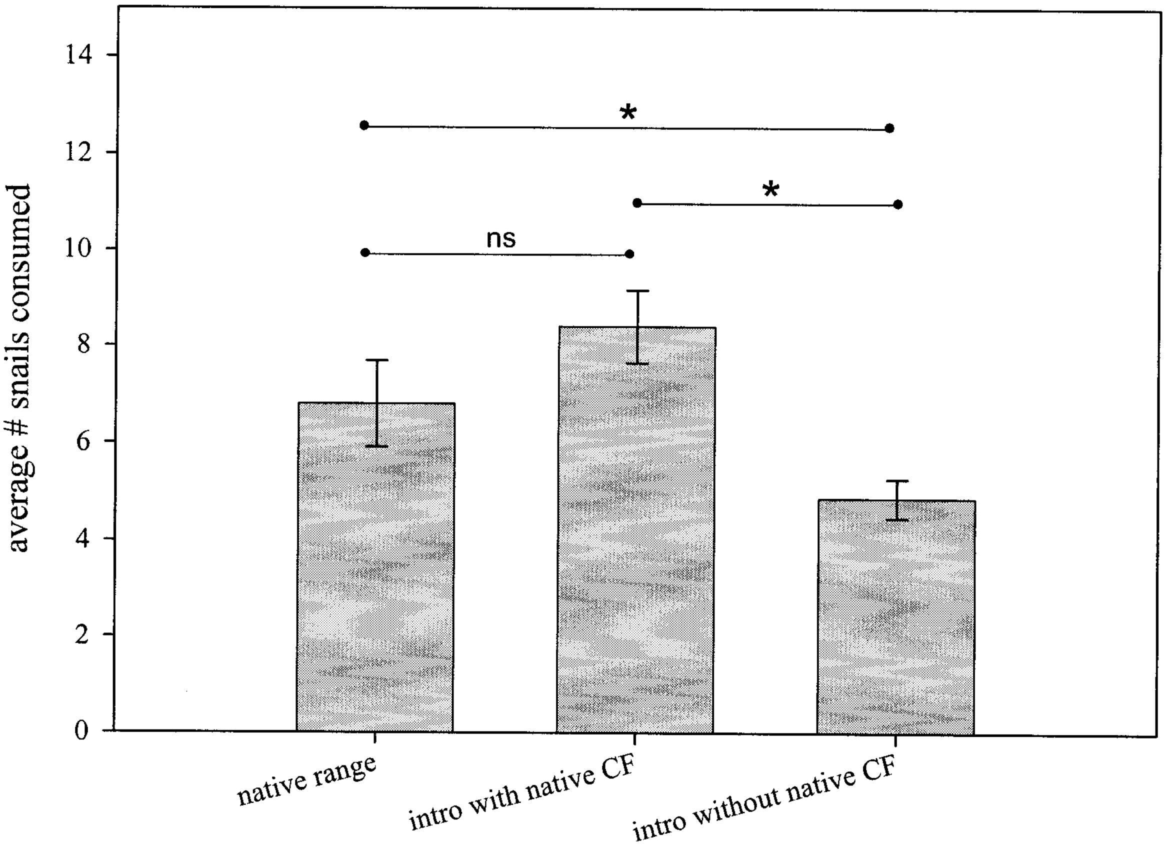

average # snails consumed
0
2
4
6
8
10
12
14
*
*
ns
native range
intro with native CF
intro without native CF

Figure 3.2c.

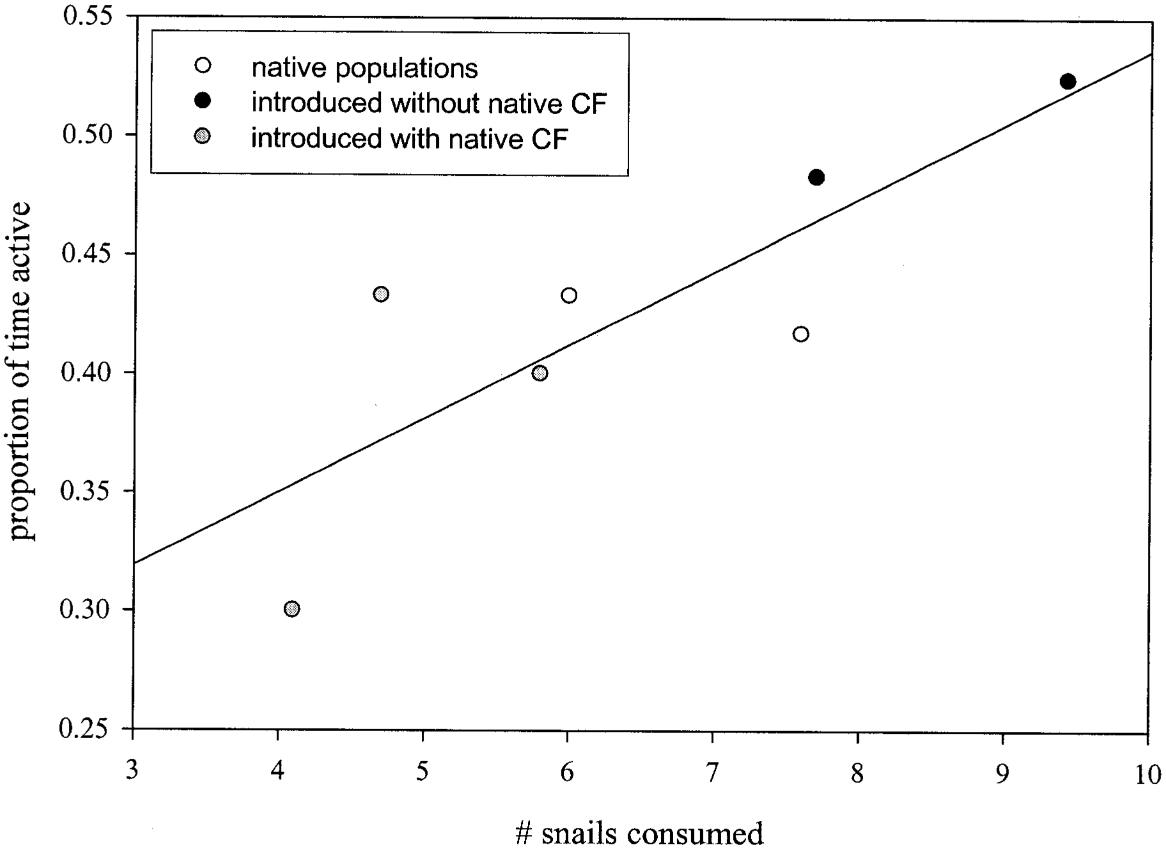

0.55
0.50
0.45
0.40
0.35
0.30
0.25
proportion of time active
3
4
5
6
7
8
9
10
snails consumed
○ native populations
● introduced without native CF
◎ introduced with native CF

Figure 3.3a.

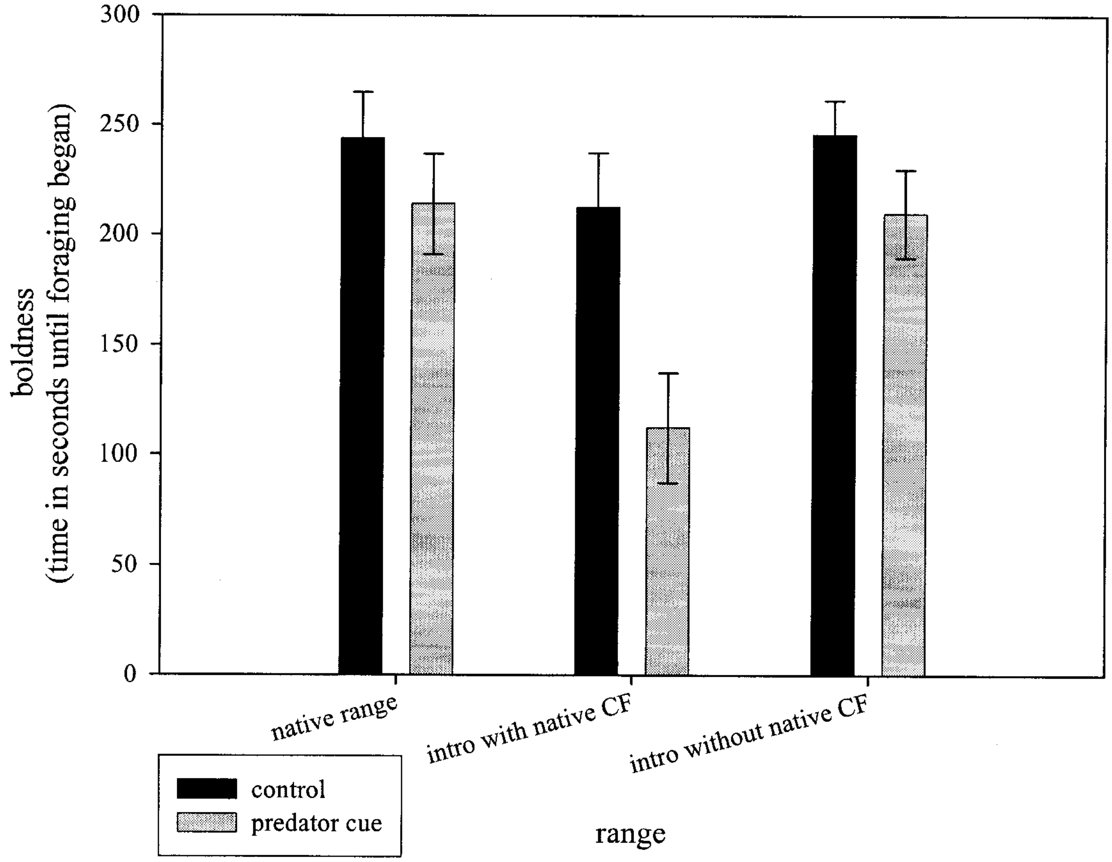

300
250
200
150
100
50
0
boldness
(time in seconds until foraging began)
native range
intro with native CF
intro without native CF
range
control
predator cue

Figure 3.3b.

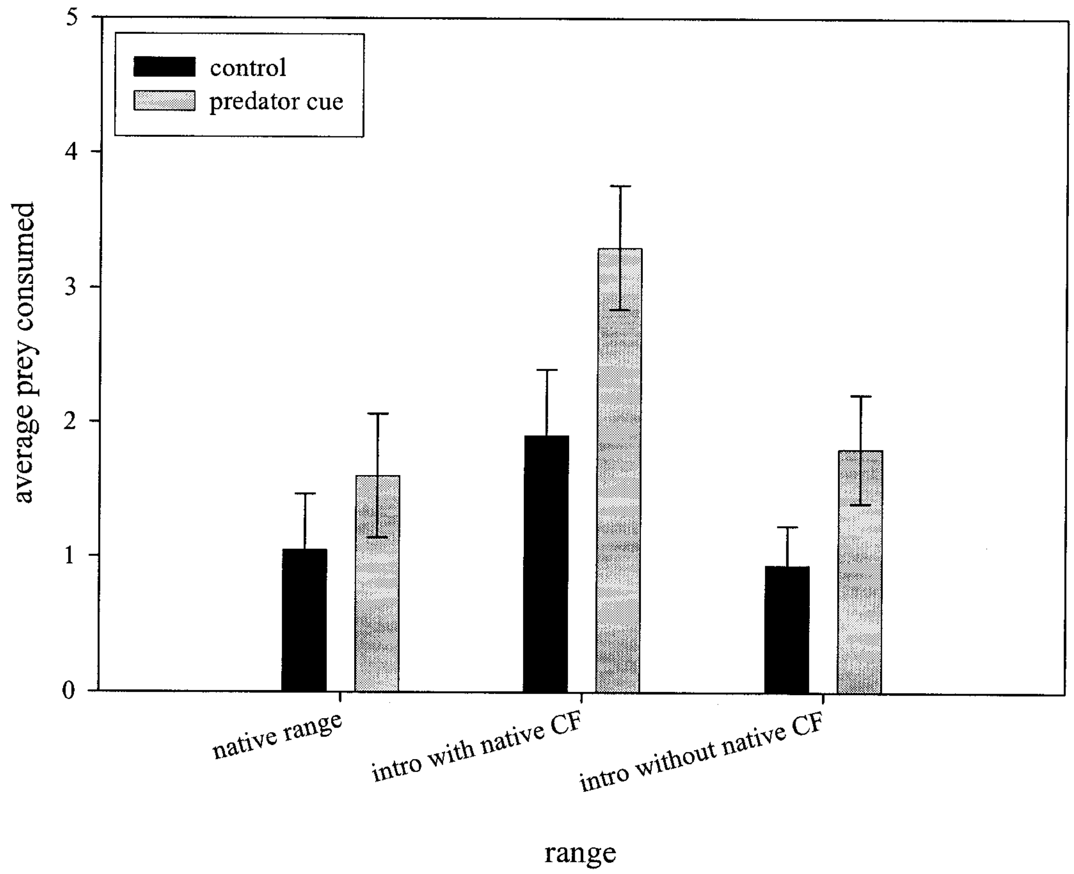

control
predator cue
average prey consumed
0
1
2
3
4
5
native range
intro with native CF
intro without native CF
range

Figure 3.3c.

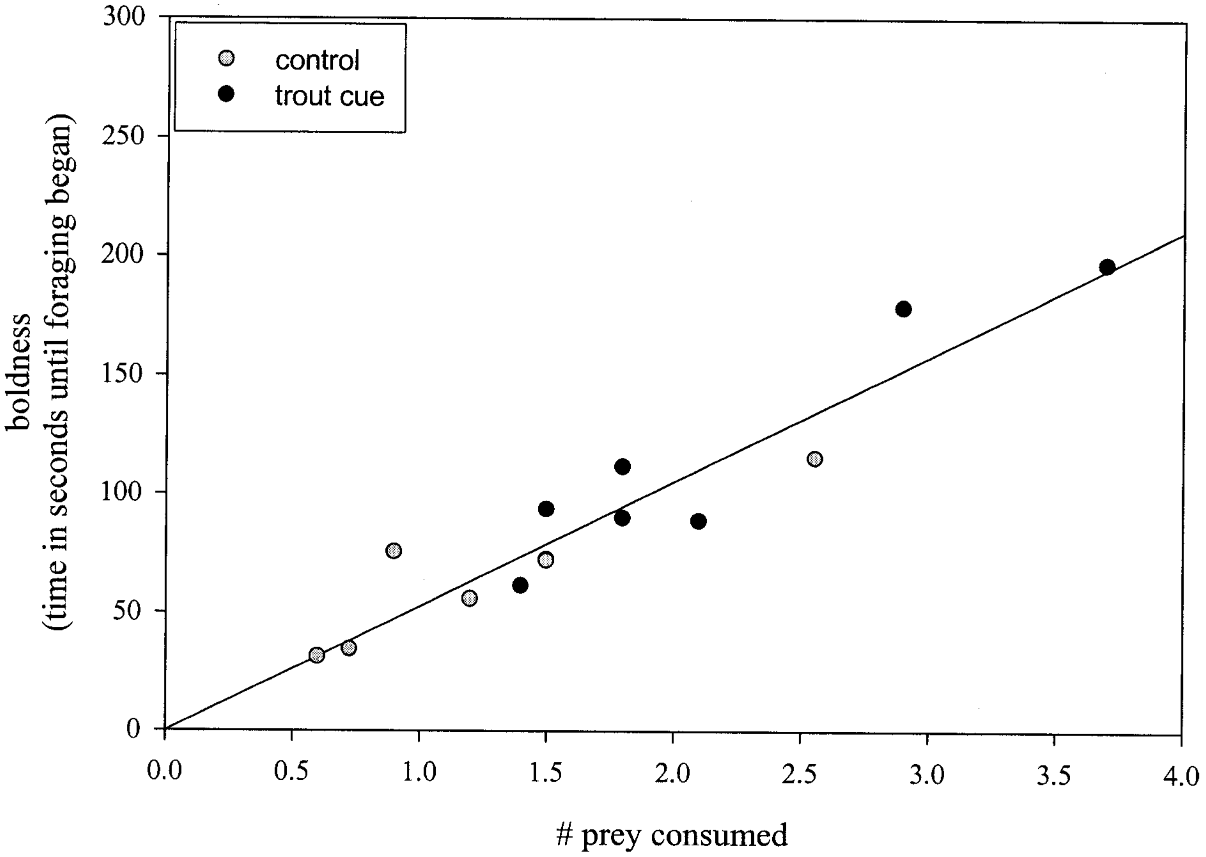

○ control
● trout cue
boldness
(time in seconds until foraging began)
0
50
100
150
200
250
300
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
prey consumed

Figure 3.4.

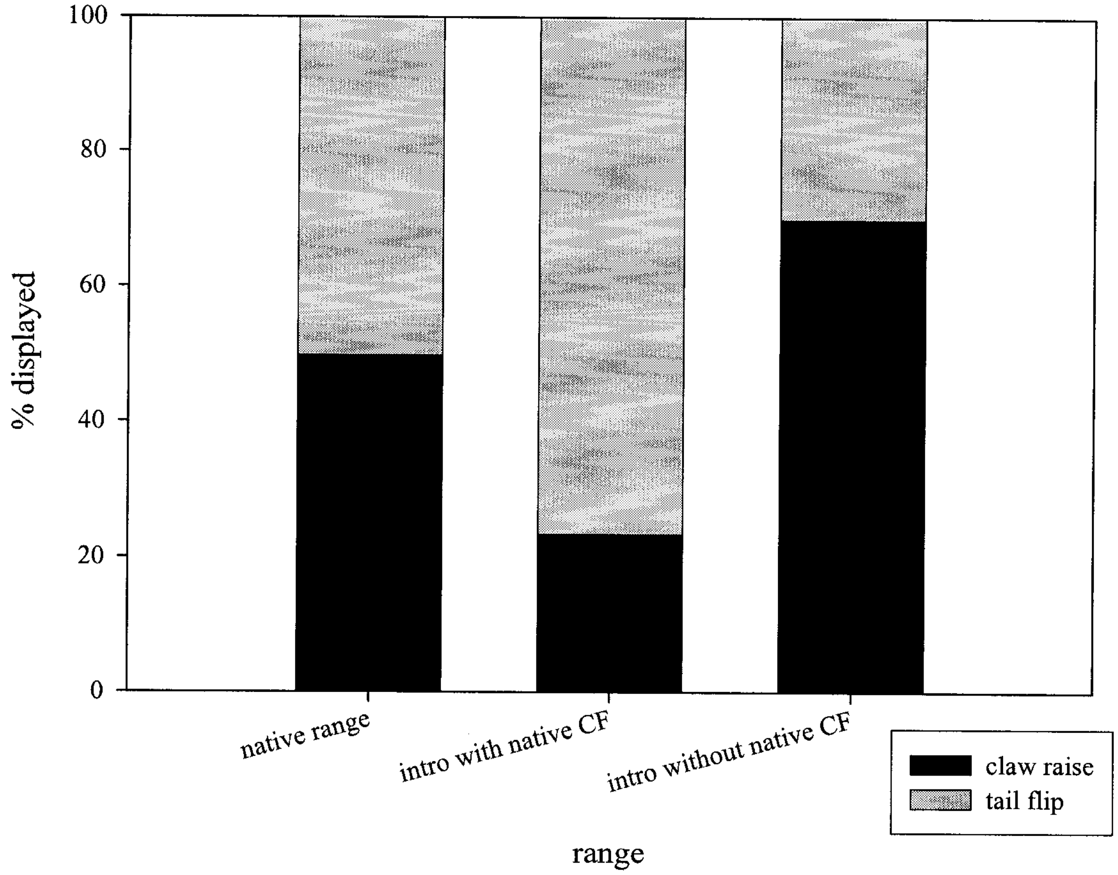

100
80
60
40
20
0
% displayed
native range
intro with native CF
intro without native CF
range
claw raise
tail flip

Figure 3.5.

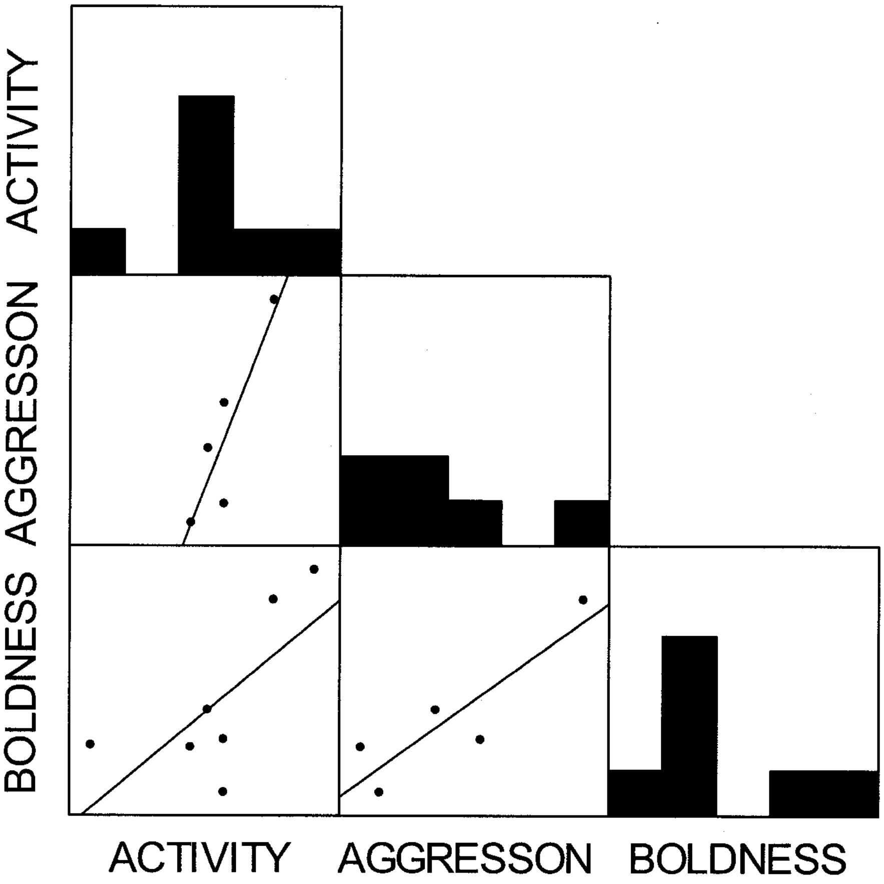
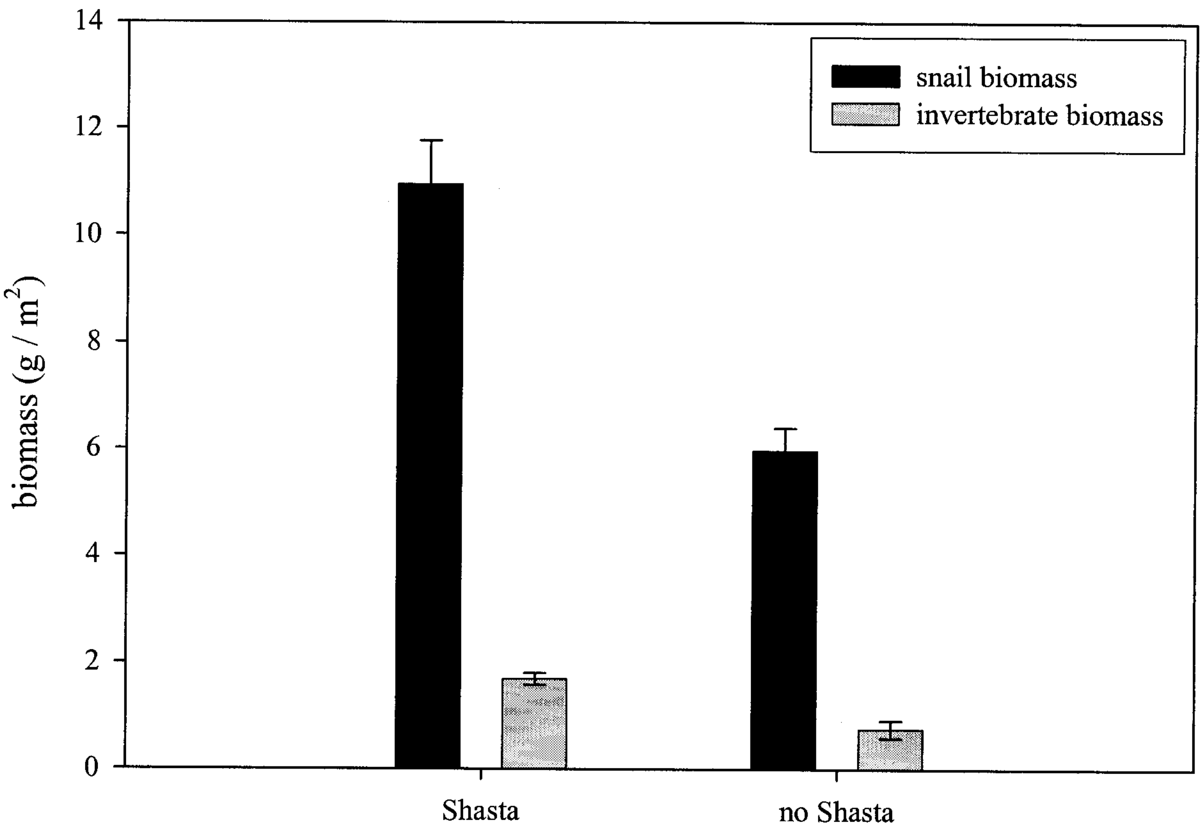

ACTIVITY
AGGRESSON
BOLDNESS
ACTIVITY
AGGRESSON
BOLDNESS

Figure 3.6.



14
12
10
8
6
4
2
0
biomass (g / m^2)
snail biomass
invertebrate biomass
Shasta
no Shasta

Chapter 4:

Synergistic effects of density and behavior on the consumptive and non-consumptive impacts of an invasive crayfish.

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Abstract

Intraspecific aggression should result in high intraspecific competition which should limit the densities reached by exotic species, yet some invaders become superabundant despite also being highly aggressive. We conducted a mesocosm experiment to address the inter-relationships between aggressive behavior, density and impacts on prey for the exotic signal crayfish (*Pacifastacus leniusculus*). Exotic signal crayfish is a highly aggressive invader that is often found at pest-like densities in invaded streams. Our results suggest that signal crayfish become highly aggressive towards each other at high densities. Yet, despite the increase in aggression and the per capita number of interactions between crayfish, signals increase their overall activity which drives high feeding rates on prey. As a result, we found a strong positive correlation between crayfish behavior (aggression, activity and voracity) and impacts on prey. We suggest these behaviors are part of an overall aggression syndrome whereby some individuals are generally more aggressive/active/voracious, whereas others are less aggressive across a range of situations. High crayfish densities had a synergistic effect on this correlated suite of behaviors and exacerbated the impacts on prey by significantly increasing the consumptive effects of crayfish on prey relative to non-consumptive effects (prey drift). Low crayfish densities still impacted prey relative to no crayfish controls; however, there was no significant difference in the consumptive verses non-consumptive effects (prey drift).

Introduction

The ability of some invaders to reach high densities or biomass often contributes to their strong impacts, yet the mechanisms underlying when and how this occurs remain unclear (Parker et al. 1999, Ricciardi 2003). This limits our ability to predict the magnitude of impact of invasive species. Studying the behavioral mechanisms underlying competitive interactions has offered insights into the strong impacts that very abundant invaders have on native species (Holway and Suarez 1999, Rehage and Sih 2004, Rehage et al. 2005). Aggressive behavior, in particular, has often strongly contributed to dominance of invaders over native species through interspecific competition (Tsutsui et al. 2000, Holway and Suarez 2004, Rehage et al. 2005). If, however, an invader's highly aggressive behavior towards other species is associated with similarly high aggressiveness towards conspecifics, then strong intraspecific competition could keep the invader from becoming superabundant. To be a superabundant, high impact pest, a species should be both highly aggressive towards other species, yet unaggressive towards conspecifics. In some well known cases, this pattern of species-specific aggression is associated with high genetic relatedness among invaders (Tsutsui et al. 2000, Ingram 2002). In other cases, however, invaders do not share high genetic relatedness, and have become superabundant despite being aggressive towards both inter- and intra-specific competitors (Mooney and Cleland 2001, Grosholz 2002, Gherardi and Daniels 2004).

An alternative behavioral mechanism for explaining the ability of an invader to attain high densities despite strong intraspecific competition involves behavioral syndromes – behavioral correlations across contexts (Sih et al. 2004a, Sih et al. 2004b). High aggressiveness can be closely associated with behaviors that are important in other ecological contexts, such as foraging, predation and dispersal (Bell 2005, Johnson and Sih 2005, Rehage et al. 2005). For example, in funnel web spiders, individuals that were more aggressive relative to other spiders showed high attack rates towards competitors, but also towards prey (Riechert and Hedrick

1993). In addition, aggressive spiders also were more voracious and showed a shorter latency to leave refuge after a simulated predator attack. For biological invasions it may be important to study aggression towards competitors together with behaviors like foraging and dispersal, because all are clearly important in predicting their impact on native prey and spread following introduction. Higher aggression may lead to higher attack rates on conspecifics, but may also be positively related to attacks on prey, i.e. increased foraging rate. If high foraging rates are maintained even at high abundances of conspecifics, an invader may be able to maintain high densities despite strong intraspecific aggression. This possible synergistic relationship between a suite of correlated behaviors and density could be a general characteristic of invaders that have a particularly strong impact following invasion. However, more studies on the proximate mechanisms underlying impacts of invasive species are needed to form such a generalization.

Crayfish are invaders where aggression has strongly contributed to invasion success; despite high intraspecific aggressiveness, they have reached overwhelming densities in some populations (Usio et al. 2001; Light 2005). Dominance in aggressive encounters has been largely responsible for displacement and local extinction of native crayfish populations throughout Europe and North America (Hill and Lodge 1999, Usio et al. 2001, Nystrom 2005). However, introduced crayfish are equally as aggressive towards conspecifics as they are towards heterospecific competitors. Yet despite the aggression towards conspecifics, many introduced crayfish reach and maintain high population densities in native communities (Lodge and Hill 1994, Lodge et al. 1994, Lodge et al. 2000). These elevated densities have also contributed to their strong negative impacts on prey; introduced crayfish impact native prey more than native crayfish when density is held constant. Aggressive behavior has been clearly shown to affect competitive interactions among crayfish, but, it has been studied in isolation from other behaviors that are likely important to predation and impacts on native prey (Usio et al. 2001, Light 2005). Studying how density affects aggression towards conspecifics and how it is correlated with foraging activity and voracity on native prey can possibly elucidate how introduced crayfish are able to reach overwhelming densities following invasion.

We addressed the inter-relationships among density, behavior and impacts on prey for the signal crayfish, *Pacifastasus leniusculus*, which is invasive in streams in northern California. This invader's highly aggressive behavior has contributed to its dominance over native crayfishes throughout Europe and North America (Gherardi and Daniels 2004). However, despite being highly aggressive, in invaded regions, signal crayfish are commonly found at densities up to twelve times higher than those reported in their native range (Bondar et al. 2005)(Pintor, unpublished data).

In northern California, the invasion of *P. leniusculus* has caused the decline and endangered status of the endemic Shasta crayfish, *Pacifastasus fortis (*Ellis 1999). When introduced, exotic signal crayfish aggressively outcompete the native species for refuge, which increases their susceptibility to predation by fish (Ellis 1999). Therefore, we know that aggressive behavior has contributed to the displacement of native crayfish. However, it is unclear how population densities affect aggression towards conspecifics and whether this is correlated to other ecologically relevant behaviors and the strong impacts they have on native prey communities. Therefore, we conducted a mesocosm experiment to look at 1) how density influences intraspecific interactions and aggression in *P. leniusculus*, 2) whether aggression during competitive interactions was correlated with foraging activity and voracity on native prey, and 3) how these behaviors and conspecific density interact to influence the impact of signal crayfish on native prey.

Methods

Study Animals

Pacifastacus leniusculus is native far northern California in the Klamath River north to Oregon through British Columbia in the Pacific Northwest United States. However, primarily through bait-bucket introductions it is now quite widespread throughout northern California. It has also been widely introduced throughout Europe and has caused the local extinction of many populations of native crayfish primarily by a fungal plague it carries (Lodge et al. 2000).

Samples from two populations of *P. leniusculus* were collected by hand from the American River (Sacramento Co., CA) and from the outflow of Crystal Lake (Shasta Co., CA) in October – November 2004. Prior to the start of the experiment, crayfish were held individually in flow-through containers within aerated aquaria and fed daily with TetraMin Granules.

P. lenuisculus is a generalist omnivore that can feed on benthic macroinvertebrates, small vertebrates (larval amphibians and fish), algae and detritus (Hanson et al. 1990, Lodge et al. 1994, Gamradt et al. 1997). In many systems, their primary prey are snails and benthic invertebrates (Nystrom and Perez 1998, Nystrom 2002, McCarthy et al. 2006). The prey community used in our experiment represented a typical prey community in northern California streams invaded by *P. leniusculus*. Snails were collected by sweep nets in Hat Creek (Shasta Co. CA). The two dominant snail species were used; *Fluminicola* sp. and *Juga* sp. The two species were added to the tanks in a 3:1 ratio, as indicated from field surveys across ~30 streams in northern California (Pintor, unpublished data). Benthic invertebrates used in the experiment included the family Chironomidae, Corixidae (waterboatman) and Coenagrionidae (damselflies) that had naturally colonized the mesocosm tanks from nearby streams in the Sacramento River drainage.

Mesocosm Experiment: Design & Set-up

We used a one-factor design, manipulating crayfish density (4 crayfish/pool & 12 crayfish/pool), along with a no crayfish control in replicate mesocosm pools. Experiments were run in four outdoor, semi-natural streams housed at the Center for Aquatic Biology and Aquaculture at the University of California at Davis. Each stream consisted of 5 pools (each 1.5 m diameter filled to a depth of 40 cm; a total of 20 pools) connected by gravel-filled riffles (1.3 m long, 30 cm wide). Pumps recirculated water through each stream. Water cascaded into each pool, flowed down the gravel riffle and and exited through a screen (mesh size: 1.5875mm) thus preventing crayfish and benthic prey from dispersing between pools. Each pool thus serves as a replicate unit. Each pool was also provided with 6 PVC pipes that served as refuge for crayfish and prey. The set of streams are housed under a screen tent (to keep out birds and reduce overheating) with natural lighting augmented if necessary by fluorescent lights. Such streams have been used for conducting numerous experiments on predator-prey interactions among aquatic organisms (e.g., (Sih and Krupa 1995, Krupa and Sih 1998).

Snails were added to each pool in equal densities across pools and allowed to acclimate and disperse for two days prior to the addition of crayfish. Crayfish were measured (carapace and chelae length, mm), weighed (grams) and individually marked prior to being added to the experiment. Crayfish were segregated by sex and size into a small (25-35mm carapace length) and large (40-50mm carapace length) group. Crayfish were added in equal proportions of sex and size group in the appropriate treatment densities, (i.e. low density treatment: one small male and female and one large male and female; high density: 3 of each size-sex combination). Experiments looking at the agonistic behavior of crayfish typically size match pairs of crayfish within 5mm of each other (Gherardi and Cioni 2004). However, we felt that size matching all crayfish within 5mm of each other would inflate aggression estimates because it would likely take more interactions and higher levels of aggression to establish a dominance hierarchy within the tank. Therefore, we chose to use a wider range of sizes to better reflect the range of sizes in natural communities. Treatments were randomly assigned to the tanks and replicated six times over two time blocks (3 replicates/block) and ran for 2-weeks per block.

Effect of Density on Aggression, Foraging Activity & Voracity

Baiting assays were conducted over the course of each two week block (four times in each replicate tank) to compare behaviors of signal crayfish at low and high densities. Prior to the start of the assay, crayfish location (e.g. in or out of refuge) and activity (e.g. walking, crawling, sitting) was recorded. The trial started after a piece of bait (a piece of a canned sardine; average mass 6-7g) was placed in the middle of the tank. Observations were made for 15 minutes.

Crayfish activity was measured as the proportion of crayfish that began actively foraging and continuously moving (more than one body length) around the tank during the 15 minute observation. Voracity was measured as the inverse of latency to recruit to the bait. Therefore, for each tank voracity was recorded as the time until the first crayfish recruited to the bait. Aggression was measured by recording all pairwise interactions that occurred over the 15 minutes. Specifically, during each interaction the behavior of each individual was recorded based on scoring adapted from (Karavanich and Atema 1998); see Appendix 4.1. In general, during each interaction one crayfish would emerge as the dominant individual and the other the subordinate. The dominant individual was assigned a scored based on the highest level of aggression it displayed during the interaction. The subordinate individual was given a score based on the behavior displayed as it retreated from the dominant individual (again based on the adapted scoring system). Because crayfish were individually marked, we were able to identify any individual that did not interact. Any crayfish that did not interact was given a zero. Therefore, aggression was calculated as the mean aggression score of all individuals in the tank during each 15 minute trial. Finally, all pairwise interactions observed over the 15 minutes were totaled and divided by the number of crayfish in each tank to compare the per capita number of interactions during each trial.

Effect of Density on Impact on a Typical Prey Community

To assess the impacts of the crayfish treatments on commonly consumed prey, snails and benthic invertebrates were sampled prior to the addition of crayfish and at the end of the experiment. Because snails can use both the bottom and sides of the tanks, we took three, 15cm x 15cm samples to estimate the density of snails; one on the upper side of the tank, one on the lower side and one on the bottom in the middle of the tank. Three, 7.6 cm x 7.6cm benthic invertebrate samples were taken using an aquarium net (mesh size: 1.5875mm) in a transect across the middle of the pool. Drift nets at the end of the riffles were sampled during the second week of the experiment. Drift nets were cleaned and then sampled 24 hours later.

The total proportion of each prey consumed was assumed to be the proportion of prey missing from each tank at the end of the experiment after accounting for the proportion of prey caught in drift nets and the average proportion of prey missing from the no crayfish control treatment. Per capita number of prey consumed was calculated by dividing the total number of prey consumed by the total number of crayfish in the tank. The same method was used to calculate the total proportion and per capita consumed for each prey type. Chironomids did not disperse from pools either via drift or emergence, thus the mean number of chironomids missing from the controls was assumed to represent chironomids lost due to natural mortality that happened across treatments.

Crayfish Growth

No crayfish molted over the course of the two week experiment. Therefore, at the end of the experiment only weight was measured and used to calculate growth over the experiment. Also, densities of crayfish were monitored each morning and any individual that was missing after two days was replaced to maintain equal densities of crayfish throughout the experiment. However, crayfish that were replaced were not included in final growth measurements.

Crayfish Dispersal

Crayfish dispersal out of the tanks was estimated by counting the number of crayfish missing from the tanks after the first three mornings of the experiment. After three days, nets were securely placed over each tank to prevent further crayfish dispersal.

Statistical Analyses

Differences between density treatments on the behavior variables (per capita interactions, aggression, activity and voracity) measured were analyzed with a MANOVA followed by repeated-measures ANOVAs. Because replicate pools were grouped in four streams, we checked for a block effect of stream channel to ensure pools did act as independent units. There was no significant block effect; therefore, we removed this factor from the analyses. When data did not meet assumptions of normality, a Kruskal-Wallis ANOVA was run instead of a parametric ANOVA.

We used a random encounter approach to calculate a null expectation for the effect of density on crayfish-crayfish interaction rates. At the low density, each of four crayfish can only interact with three others, while at the high density; each of twelve crayfish can interact with eleven others. Thus, if per capita movement rates are unaffected by density, then the expected ratio of per capita interactions in the low: high density treatment is 3:11; per capita interactions should be 3.7 times higher in the high density treatment. Given that the high density treatment also has 4 times more crayfish, mean total interactions among crayfish in the high density treatment were expected to be eleven times higher than the mean total number of interactions at the low density treatment. We used a paired t-test to compare the null expectation to the observed per capita number of interactions of averaged scores across time.

We used a series of univariate correlations to examine the relationship between crayfish aggression, foraging activity and voracity both across and within density treatments. Per capita number of interactions was excluded from the correlation analyses because it was less direct quantitative measure of behavior in comparison to aggression, activity and voracity.

Differences in density treatments on crayfish impacts on prey (proportion of each prey consumed, proportion of each prey drifted, per capita number of each prey consumed) were analyzed using MANOVAs followed by ANOVAs. The no crayfish control treatment was included in analyses of the proportion of prey consumed/missing + drifted, but not in the analyses on per capita number of prey consumed. For the proportion of prey consumed/missing + drifted, we used a Tukey's multiple comparison to compare difference between the control, low and high crayfish density treatments.

Besides the snails that we added to pools, across all pools, chironomids comprised the large majority of the benthic invertebrates that naturally colonized the pools (e.g. >95%). Therefore, we excluded other benthic invertebrates from our analyses (waterboatman and damselflies) and focused on the impact of crayfish on chironomids and snails. Because chironomids naturally colonized the pools, there was some variation among the pools in the starting densities of chironomids. To account for this variability, data were converted to rank scores to fit normality assumptions and analyzed via one-way ANCOVAs testing for effects of crayfish density on total and per capita numbers of chironomids consumed, using the initial density of chironomids as a covariate in the model.

There was no effect of snail species in any of the analyses; therefore, both species were combined into one snail measure. Because crayfish density had both consumptive and non-consumptive (prey dispersal) effects on the density of both snails species, we ran two ANOVAs to see if the proportion of the total effect on snails due to consumption and non-consumption was affected by density. Additionally, within a treatment we used a Wilcoxon signed rank test to determine whether the total effect due to consumption was different than the non-consumptive effect (snail drift).

ANOVAs were also used to look at the effect of density on crayfish growth over the two week experiment and on the per capita dispersal rate of crayfish out of the experimental tanks in the first three days of the experiment.

Finally, to determine whether variation in behavior (aggression, activity and voracity) explained a significant proportion of the variation in crayfish impact on prey and crayfish dispersal, we combined the three behaviors observed into a single behavior metric using a principal component analyses (PCA). We ran simple linear regressions to determine whether our behavior metric explained a significant amount of the variation in the total proportion and per capita number of each prey consumed, and the per capita dispersal rate of crayfish.

Results

Effect of Density on Crayfish Aggression, Foraging Activity &Voracity

Results of our MANOVA showed a significant effect of density on per capita interactions, activity, voracity and aggression (Wilk's Lambda $F_{4,19} = 6.445$, $p = 0.002$), indicating that there was variation in overall behavior between treatments.

Increasing densities of conspecifics did, indeed, significantly increase the total number and the per capita number of interactions among crayfish (repeated measures ANOVAs $F_{1,22}=16.564$, $p<0.01$ and $F_{1,22}=15.277$, $p<0.01$, respectively). There was no significant effect of time or a density by time interaction for either the total number ($F_{3,66}=1.774$, $p=0.161$ and $F_{3,66}=1.702$, $p=0.175$, respectively) and per capita number of interactions among crayfish ($F_{3,66}=1.786$, $p=0.158$ and $F_{3,66}=1.580$, $p=0.219$, respectively). The total number of interactions averaged over time was more than 60 times higher in the high density (average = 15.780) as compared to the low density tanks (average =0.233)(Figure 4.1a). The per capita number of interactions in the high density treatment was thus approximately 6x higher than expected based on the number of interactions in the low density treatment (paired t-test; t=3.004, df=11, p=0.012; Figure 4.1b).

The average level of aggression for the tank was significantly greater in the high density treatment as compared to the low density treatment (repeated-measures ANOVA $F_{1,22}=14.437$, $p<0.01$). There was no significant effect of time or a time by density interaction ($F_{3,66}=1.541$, $p=0.212$ and $F_{3,66}=1.755$, $p=0.164$, respectively). The mean level of aggression averaged over time for the tank was approximately 9x higher in the high density treatment as compared to the low density treatment (Kruskal-Wallis, $p < 0.001$; Figure 4.2a).

Foraging activity was significantly higher in the high density treatment in comparison to the low density treatment (repeated-measures ANOVA, $F_{1,22}=9.845$, $p<0.01$; Figure 4.2b). Activity initially significantly increased over time and then decreased in both treatments, but there was no time by density interactions ($F_{3,66}=4.614$, $p<0.01$ and $F_{3,66}=0.169$, $p=0.905$, respectively). Voracity (inverse of latency to recruit to the bait) was significantly higher in the high density treatment, i.e. crayfish in the high density treatment recruited to the bait significantly faster than crayfish in the low density treatment (repeated measures ANOVA, $F_{1,11}=12.293$, $p< 0.01$; Figure 4.2c). However, there was no significant effect of time or a time by density interaction ($F_{4,44}=1.639$, $p=0.182$ and $F_{4,44}=1.414$, $p=0.245$, respectively).

Correlations between Aggression, Foraging Activity & Voracity

Within and across density treatments, there were positive correlations among aggression, activity and voracity. Within the high density treatment, there was a significant positive correlation between aggression and voracity (r=0.731, N=12, p=0.01; Table 4.1) and a near significant positive correlation between aggression and activity (r=0.637, N=12, p=0.03 Table 4.1). Activity and voracity showed a positive trend, but it was not significant (r=0.494, N=12, p=0.154; Table 4.1). Within the low density treatment, there was a significant positive relationship between activity and voracity (r=0.729, N=12, p=0.01; Table 4.1). However, aggression and activity and aggression and voracity were not significantly correlated (r=0.528. N=12, p=0.233 and r=0.116, N=12, p= 0.5, respectively; Table 4.1).

Across both density treatments there were significant positive correlations between all three behaviors (Figure 4-3). Aggression was positively correlated to activity (r=0.751, N=23, p<0.001), as it was to voracity (r=0.765, N=24, p<0.001). Activity and voracity were also positively correlated (r=0.733, N=24, p<0.001). Therefore, the more aggressive crayfish were towards each other, the more likely they were to actively forage and find food. High aggressive behavior, high foraging voracity and activity were most often observed in high density treatments. Still it demonstrates the effect of density on behavior and that behaviors in a competitive context and foraging context are strongly correlated.

Effect of density on impacts on native prey (chironomids and snails)

Results of our MANOVA showed a significant effect of density on the proportion of chironomids and snails consumed (or missing in the controls) and proportion of snails that drifted (Wilk's Lambda $F_{6,46} = 7.288$, $p < 0.001$), indicating that there was variation in total proportion of prey impacted between treatments.

One-way ANOVAs indicated a significant effect of crayfish density on the proportion of chironomids eaten (Figure 4.4a). This effect primarily reflected the fact that significantly more chironomids were consumed in the presence of crayfish than in the no crayfish controls (Tukey Multiple Comparison, control vs. low: p=0.003 and control vs. high p=0.041). In contrast, although approximately three-fifths (0.612) of the chironomids were eaten in the high density treatment in comparison to approximately two-fifths (0.405) in the low density treatment, there was not a significant difference between the two treatments (low vs. high p= 0.328). For snails, there was also a significant overall effect of crayfish density on the proportion of snails consumed. On average more than one-half (0.55) of the snails were missing in the high density treatment, in comparison to approximately one-quarter (0.252) in the low and none in the no crayfish control (ANOVA, $F_{2,27}$=11.649, p<0.001) (Figure 4.4b). All treatment groups were significantly different from each other in proportion of snails consumed/missing (Tukey multiple comparison: high v. low, p=0.034, high v control, p<0.001, low v control, p=0.018).

Results of the MANOVA (per capita number snails and chironomids consumed) showed no significant effect of density on per capita number snails and chironomids consumed (Wilk's Lambda $F_{2,19}$=2.008, p=0.162). Although the multivariate analysis was not significant and therefore typically do not warrant reporting univariate results, we still found it informative to report the patterns observed. First, per capita consumption on chironomids tended to be higher in the low density treatment (4 crayfish) than in the high treatment (12 crayfish) as might be expected to occur as competition for resources increases (ANOVA $F_{1,20}$=3.809, p=0.065; Figure 4.5). In contrast, per capita consumption of snails in the high density treatment showed no tendency to be significantly different from the low density treatment (ANOVA, $F_{1,20}$=0.465, p=0.503; Figure 4.5). That is, crayfish were able to maintain the per capita amount of consumption on snails even at high competitor densities.

Crayfish density had a non-consumptive effect on snails, by causing a substantial number of snails to drift from pools with high crayfish density (ANOVA, $F_{2,27}$=9.760, p=0.001; Figure 4.6). On average one-fifth (0.232) of snails drifted in the high density treatment in comparison to approximately one-tenth (0.095) in the low density treatment (Tukey multiple comparison, p=0.008). The proportion of snails drifting from the low density tanks was not significantly higher than the proportion drifting from the no crayfish control (one-twenty-fifth or 0.04 found in drift samples) (Tukey multiple comparison, p=0.424).

Crayfish density did not have a significant effect on the proportion of the total crayfish effect on snail density that was non-consumptive (i.e., drift) or consumptive (Kruskal-Wallis, X^2= 0.013, df=1; p=0.908; Figure 4.7). In the low density treatment, consumptive versus non-consumptive effects of crayfish on snails were roughly equal in magnitude (Wilcoxon signed rank: p=0.813; Figure 4.7). In contrast, in the high density treatment consumptive effects were significantly larger than non-consumptive effects (Wilcoxon signed rank test: p=0.019; Figure 4.7).

Crayfish Growth & Dispersal

Crayfish did not molt over the course of the experiment, so growth was measured as the change in mass over the two week experiment. Both the low and high density treatments showed positive growth over the two weeks, with no significant difference in crayfish growth between the two treatments (ANOVA, $F_{1,23}$=0.049, p=0.827; Figure 4.8). Additionally, there was no observed mortality during the experiment. The per capita dispersal rates (during the first 3 days of the experiment) were significantly higher in the high density crayfish treatment in comparison to the low density treatment (Kruskal-Wallis, p=0.001), and on average were 4 times higher in the high density treatment (Figure 4.9).

Regressions of Behavior and Density on Impact & Spread

The PCA on aggression, activity and voracity compressed the data into a single component. All three variables loaded positively on the component and explained 83% of the variance. The behavior metric was positively correlated with the total proportion of snails consumed across both treatments (r=0.488, p=0.008; Table 4.2). However, there was no significant relationship between the behavior metric and the total proportion of chironomids consumed (r= -0.259, p=0.192; Table 4.2). There was a negative trend between the behavior metric and per capita consumption of both snails and chironomids, but neither was significant (snails: r= -0.194, p=0.1812, chironomids: r= -0.330, p=0.067; Table 4.2). Finally, per capita dispersal of crayfish was positively correlated with the behavior metric (r = 0.426, p = 0.019; Table 4.2). Therefore, not only are the more aggressive, active and voracious crayfish having larger impacts on snails, but they are also dispersing more frequently beyond their point of introduction in the experimental arena.

Discussion

Although invasiveness does not always translate to high invader impacts (Ricciardi and Cohen 2007), it is generally believed that high invader abundances do positively correlate with invader impact (Parker et al. 1999, Hierro et al. 2005). Competitive displacement is one mechanism by which invasive species impact native species (Bolger and Case 1992, Hill and Lodge 1999, Holway and Suarez 1999). Aggressiveness is an often cited trait of invasive species as the mechanism of competitive displacement (Gamradt et al. 1997). However, high intraspecific aggression should result in high intraspecific competition which should keep aggressive invaders from becoming highly abundant. Interestingly, some invaders, like some invasive ants, show reduced levels of intraspecific aggression which explain their ability to become highly abundant (Holway 1999). However, impacts on native ants come through their ability to dominate resources through sheer force of numbers rather than individual superiority in aggressive encounters(Human and Gordon 1996, Holway 1999). Here we know that signal crayfish outcompete native species through aggressive interactions, yet they also become superabundant. Therefore, how are signal crayfish able to be aggressive yet still attain and maintain high abundances?

Our results suggest that signal crayfish become highly aggressive towards each other at high densities. Yet, despite the increase in aggressiveness and per capita number of interactions, they also increase overall activity that drives high feeding rates. As a result, our experiment showed a strong positive correlation between crayfish behavior and impact, such that the more aggressive, active and voracious crayfish had the highest impact (both consumptive and non-consumptive) on snail prey. As a result of high prey consumption, crayfish in the high density treatment grew as much as crayfish with two-thirds fewer crayfish to compete with. Therefore, despite increased aggressiveness at high density, signal crayfish can still build up and maintain high densities in invaded communities.

We suggest that a key mechanism for this finding is the existence of behavioral syndromes. Behavioral syndromes are suites of correlated behaviors reflecting between-individual consistency in behavior across contexts (Sih et al. 2004a, Sih et al. 2004b). For example, our results suggest that crayfish that were more aggressive towards conspecifics, were also more active and voracious foragers. Although our data reflects the average behavior of a group of individuals, previous work on signal crayfish have identified suites of correlated behaviors measured on individuals across multiple situations for one of the American River populations used in this study (Pintor, unpublished data). The correlation between these behaviors may be part of an overall aggression syndrome whereby some individuals or species are generally more aggressive, whereas others are less aggressive across a range of situation and contexts. Behavioral syndromes have been suggested to have important implications for species invasions by coupling behaviors important at each stage of the invasion process (arrival, establishment and spread) (Sih et al. 2004a, Sih et al. 2004b). For example, high dispersal rates have been associated with boldness, which is often correlated with aggression and high activity. If the arrival process selects for individuals that are good dispersers, it then may also select for aggression. Boldness and aggression are both important to successful establishment and the tendency to negatively impact invaded communities. Our results not only suggest that aggressive-active crayfish had higher impacts in snail prey, but also showed that high aggression/activity/voracity was positively correlated with per capita dispersal rate of crayfish. Together this further suggests that an aggression syndrome may be a key mechanism behind the invasion success of signal crayfish. In addition, studying behavior as a syndrome allowed us to understand how high aggression, which should limit population growth and abundance, could instead facilitate the ability of crayfish to become superabundant and rapidly spread.

Many studies show that because fighting can be costly aggression among crayfish is typically reduced by the establishment of dominance hierarchies. However, in our study, levels of aggression and the per capita number of interactions did not decrease over the two week experiment. Previous studies have shown that aggression, number of interactions and the formation of linear dominance hierarchies can form within five days under experimental conditions (Goessmann et al. 2000), therefore, it is possible for them to have formed in our two week experiment . Various hypotheses may explain why these crayfish did not form the sort of dominance hierarchies that reduce aggression levels. Previous work suggests that crayfish readily form consistent hierarchies in small groups, but less often in larger groups of crayfish (Bovbjerg 1953, Capelli and Munjal 1982). Our experiments were run at the high densities typically seen in crayfish invasions. A mechanism for why high density reduces the likelihood of dominance hierarchies is the fact that stable hierarchies may require memory of the interactions with individuals in the group. High density populations would increase the number of individuals and interactions that an individual crayfish would have to remember. Therefore, perhaps the formation of dominance hierarchies is too costly to maintain beyond a threshold density of individuals. Alternatively, individual recognition and memory may become reduced following invasion, similar to the loss of nestmate recognition in introduced populations of the Argentine ant, which allow them to form super colonies (Holway et al. 1998, Suarez et al. 1999). Either way, the ecological impact of the absence of a dominance hierarchy may be that resources are not controlled by the dominant individuals, therefore, distribution and hence, consumption, of resources may be roughly equal among members of a group. This could mean higher impacts on prey because there is no dominance hierarchy to control the per capita consumption of prey by otherwise subordinate individuals.

Although there was a tendency for high crayfish density to reduce the per capita consumption of chironomids, density and increased intraspecific aggression clearly did not decrease the per capita number of snails consumed. This variation between chironomids and snails may suggest a preference for snails by crayfish. Snails are known to be a highly preferred prey item of crayfish due to their low handling times and high energy content (Nystrom and Perez 1998, Nystrom 2002) . Although crayfish do have broad diets, they are known to be highly efficient predators on snails and can have significant impacts on snail abundance and composition (Hanson et al. 1990, Alexander and Covich 1991b, a, Lodge et al. 1994). In response to crayfish predation, snails typically show strong behavioral responses by either crawling out of the water or floating to the surface and drifting (Alexander and Covich 1991b, a, Turner et al. 1999) . Although in our experiment snails did appropriately respond to crayfish by increasing drift rates in comparison to control treatments, crayfish in the high density treatment were still able to maintain high consumptive effects on snails even as competition and aggressive interactions increased.

Comparisons of the relative importance of consumptive (CE) and non-consumptive effects (NCE; snail drift) indicate that CEs and NCEs were relatively equal at low crayfish densities. This supports Preisser et al.'s (2005) meta-analysis that the NCEs of a predator can be equal or stronger than CEs. However, at high density, CEs became significantly greater than NCEs. Based in part on the Preisser et al. (2005) paper, Sih et al (in review) predicted that the relative importance of CEs and NCEs might often be roughly equal when the predator is native or when it is non-native, but similar to a native predator (Sih et al. 2007, in review). However, if the predator is truly novel to the invaded community, CEs are expected to be relatively much larger than NCEs, as prey might exhibit weak antipredator responses. In our experiment, snails exhibited the known appropriate response to crayfish predation (drift or crawl-out) and were from a population evolved with native crayfish predators, suggesting that signal crayfish are not truly novel predators. Therefore, our results suggest that the relative importance of CEs vs. NCEs may depend not just on the novelty of the predator, but also on predator density. More studies are needed to understand the effect of predator density on the relative importance of CEs and NCEs; however, our results may have important implications for the potential recovery of prey communities following crayfish invasion. For example, if prey exhibit equal or higher NCEs (e.g. drift) than CEs, they may be able to recolonize and recover in invaded sites faster than if prey show a weak behavioral response to predators. In our study, this would only be true at low crayfish densities, however, a higher probability of recolonization may give prey more opportunities to adapt to a new predator. This may suggest that total eradication of invasive crayfish may not be completely necessary for snails to persist in invaded communities.

By studying behavior as suites of correlated traits rather than individually, we were able to understand the link between two commonly observed characteristics of invasive species that appeared to be in conflict: high abundances and aggressive behavior. We identified how aggressiveness, which is important in population establishment was correlated with high foraging activity and voracity, two behaviors that can help invaders attain high densities and have large impacts on prey communities. Therefore, through a behavioral syndrome approach ecologists can look at how behaviors identified as important to invasion success are correlated across each stage of the invasion process. In doing so, we may get closer to understanding species characteristics that explain why some non-native species become invasive and others do not.

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Table 4.1. Results of correlations among aggression, activity and voracity of signal crayfish.

	Aggression	Activity	Voracity
Aggression		0.528, p=0.116	0.150, p=0.50
Activity	0.637, p=0.035		0.729, p=0.01
Voracity	0.731, p=0.010	0.494, p=0.154	

Table 4.2. Results of regression of behavior on total and per capita number of chironomid and snail prey consumed, and the per capita rate of crayfish dispersal. A PCA on aggression, activity and voracity compressed the data into a single behavior metric.

	Total proportion snails	Total proportion chironomids	Per capita snails consumed	Per capita chironomids consumed	Per capita crayfish dispersal
Behavior Metric	r=0.488; p=0.080	r=-0.259; p=0.192	r=-0.194; p=0.182	r=-0.33; p=0.060	r=0.426; p=0.019

Figure Legends

Figure 4.1a. Mean number of interactions per baiting trial averaged over time in low verses high crayfish density treatment. The total number of interactions was significantly higher than expected based on the number of inter actions in the low density treatment (open vs. closed circle in high density treatment).

Figure 4.1b. Average number of per capita interactions (i.e. interactions per crayfish) averaged over time in low verses high density treatment. The per capita number of interactions in the high density treatment was six times higher than expected based on the per capita number of interactions in the low density treatment (open vs. closed circle in high density treatment).

Figure 4.2a. Mean level of aggression averaged over time in the low verses high crayfish density treatment. Aggression level was nine times higher in the high density treatment.

Figure 4.2b. Average foraging activity over time in the low verses high crayfish density treatment. Crayfish were significantly more active in the high density treatment. Activity significantly changed over time, however, there was no significant time by density interaction.

Figure 4.2c. Average level of voracity (measured as latency to recruit to food) in the low verses high crayfish density treatment. Crayfish in the high density treatment recruited to the bait significantly faster than crayfish in the low density treatment.

Figure 4.3. Correlation matrix of average aggression, foraging activity and voracity of each tank.

Figure 4.4a. Average proportion of chironomid prey consumed across treatments. Signal crayfish in the high density treatment consumed fewer chironomids than expected based on the average chironomids consumed in the low density treatment (open vs. closed circle in high density treatment).

Figure 4.4b. Average proportion of snail prey consumed across treatments. Signal crayfish in high density treatments consumed the as many snails as expected based on the average snail prey consumed in the low density treatment (open vs. close circle in high density treatment).

Figure 4.5. Average per capita number of chironomid and snail prey consumed in the low and high density crayfish treatments. Per capita consumption of chironomid prey was higher in the low density treatment in comparison to the high density treatment. However, per capita consumption of snails was the same in the high vs. low density treatments.

Figure 4.6. Average number of crayfish dispersed per day over the first three days of the experiment. Signal crayfish dispersed more from the high density treatment in comparison to the low density treatment.

Figure 4.7. Percent of the total effect on snail prey due to consumptive (CE) vs. non-consumptive (NCE; snail drift) between treatments. CEs and NCEs were not significantly different in the low density treatment. CEs were significantly higher than NCEs in the high crayfish density treatment.

Figure 4.8. Average amount of growth (grams) of crayfish in the low vs. high density treatment. Mean mass was not significantly different between treatments.

Figure 4.9. Average per capita rate of dispersal by crayfish per day over the first three days of the experiment. Per capita dispersal was significantly higher in the high density treatment.

Figure 4.1a.

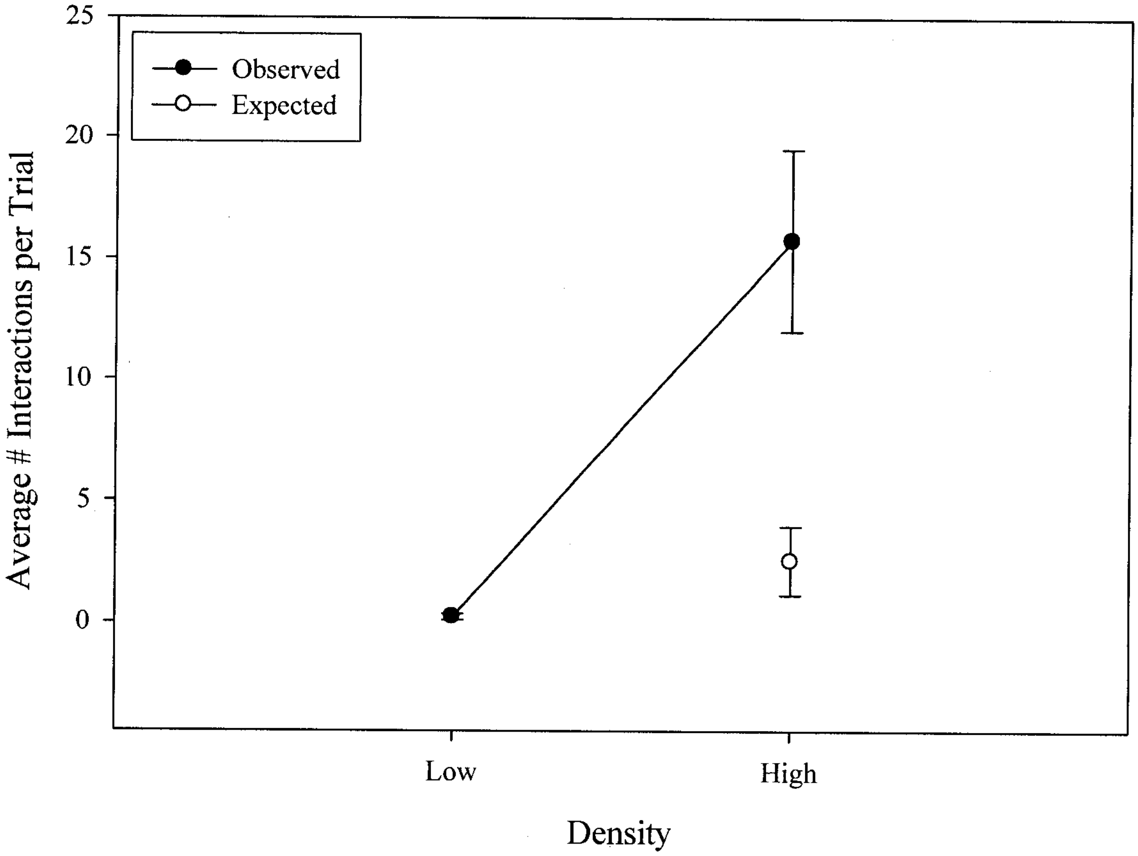

25
20
15
10
5
0
Average # Interactions per Trial
Observed
Expected
Low
High
Density

Figure 4.1b.

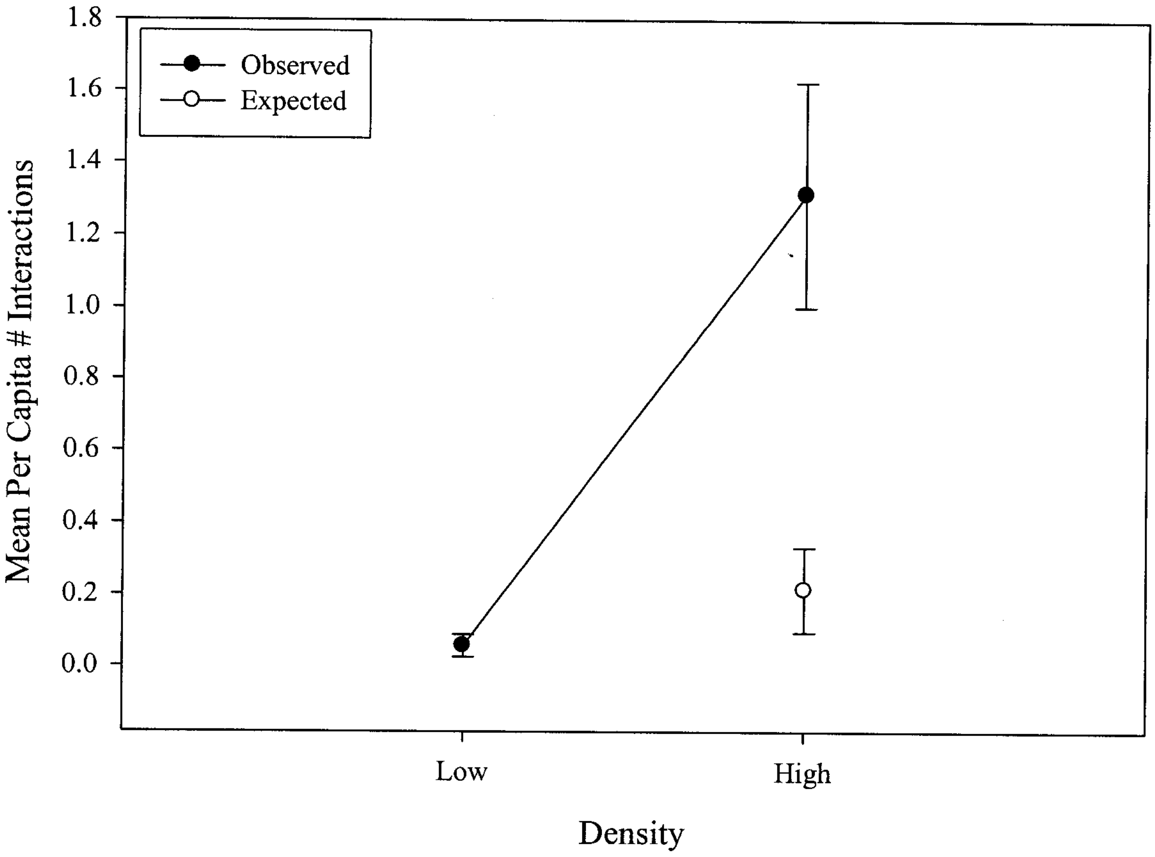

Observed
Expected
Mean Per Capita # Interactions
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Low
High
Density

Figure 4.2a.

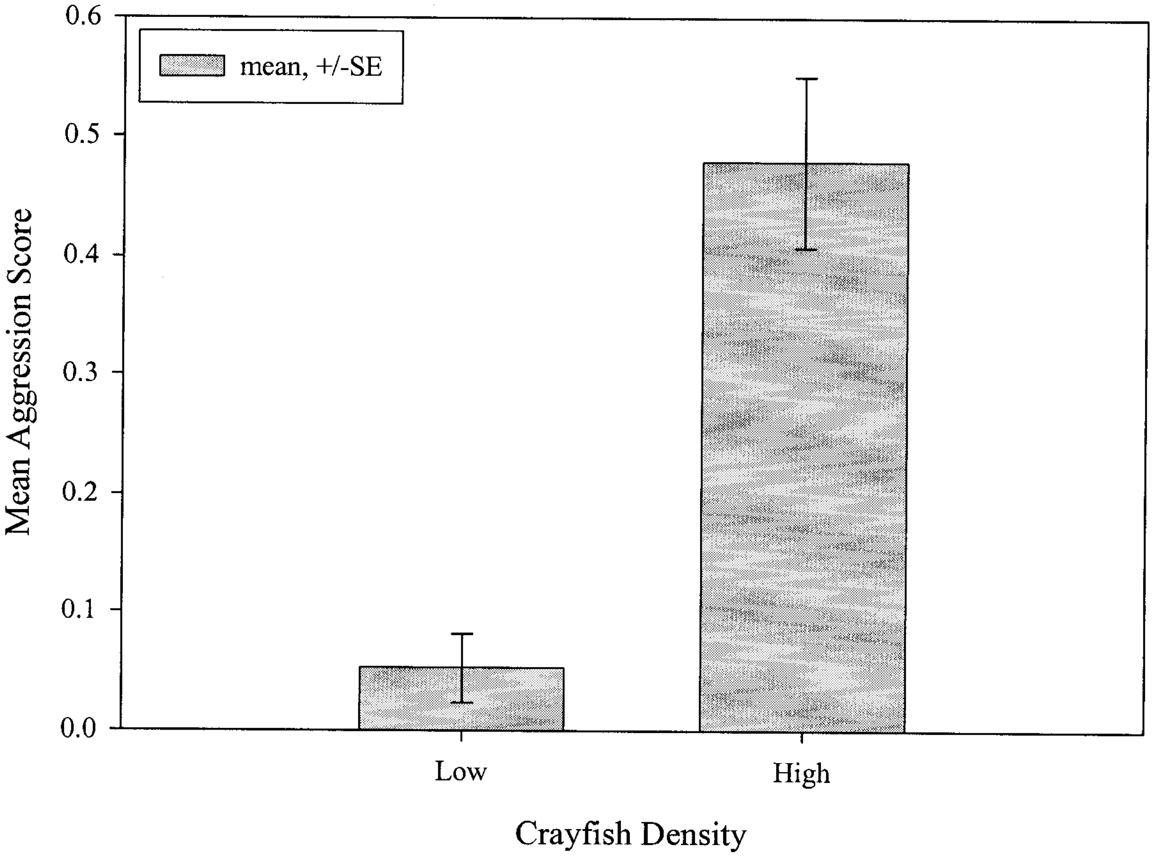

0.6
0.5
0.4
0.3
0.2
0.1
0.0
Mean Aggression Score
▨ mean, +/-SE
Low
High
Crayfish Density

Figure 4.2b

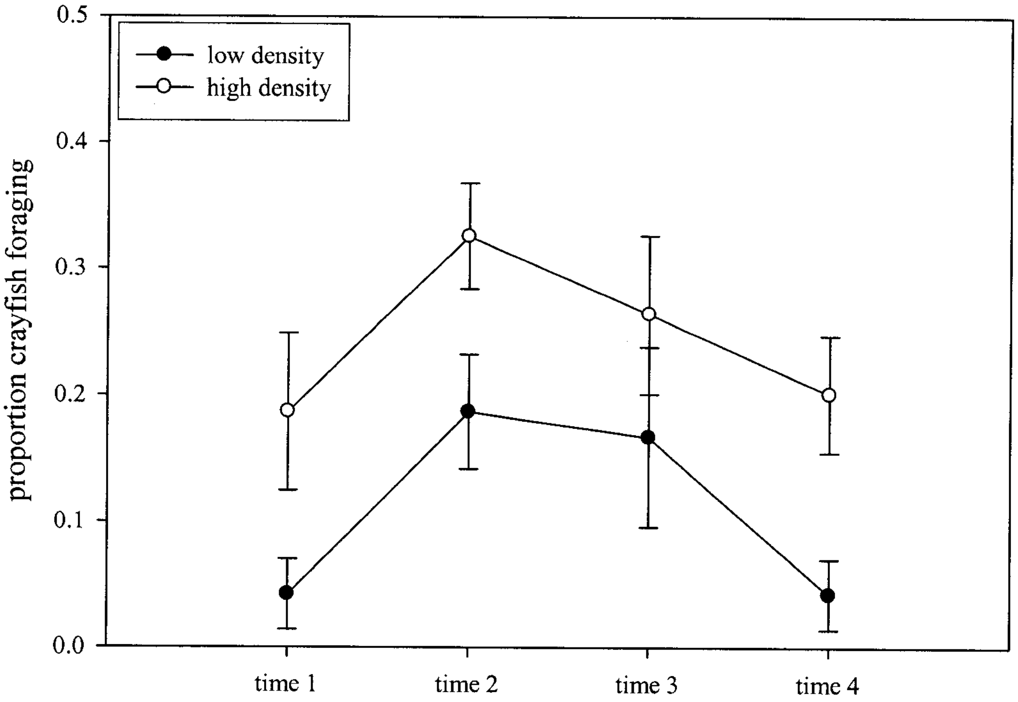

low density
high density
proportion crayfish foraging
0.0
0.1
0.2
0.3
0.4
0.5
time 1
time 2
time 3
time 4

Figure 4.2c

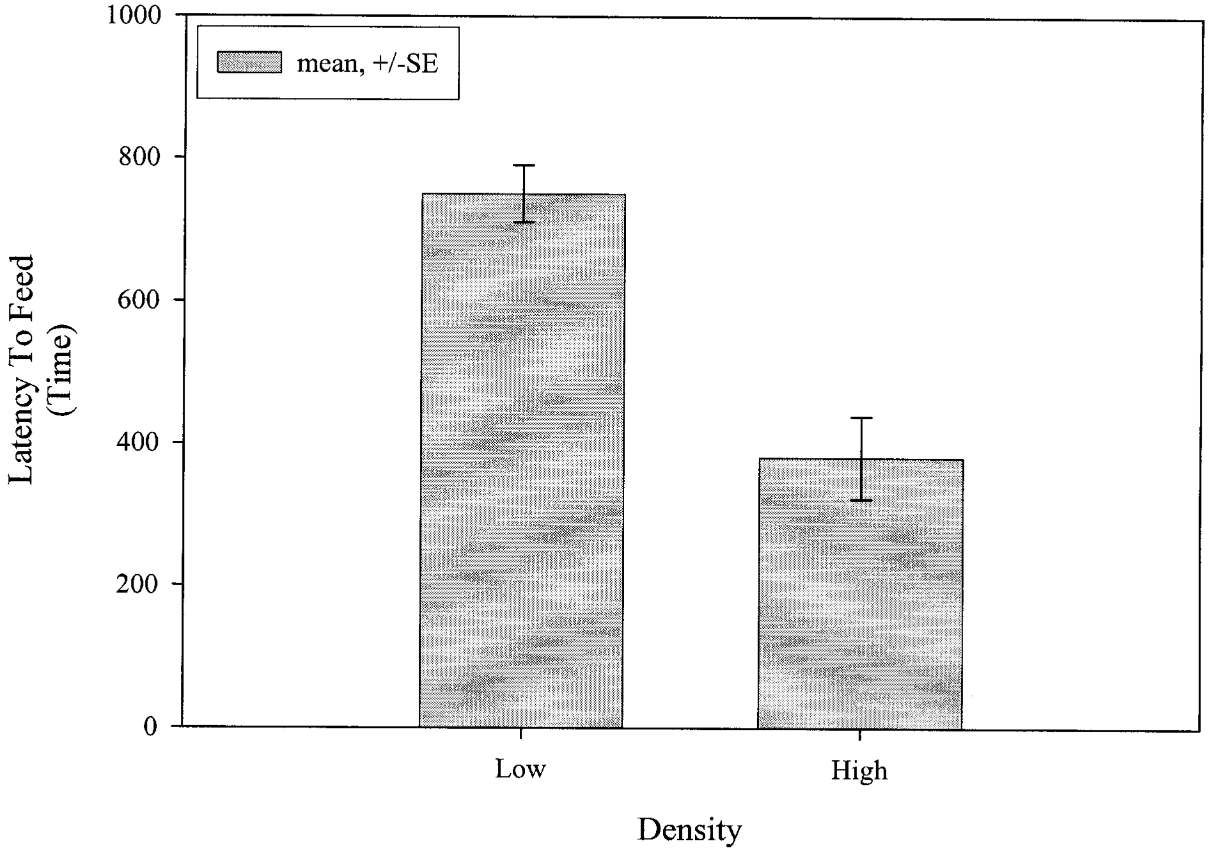

1000
800
600
400
200
0
Latency To Feed
(Time)
mean, +/-SE
Low
High
Density

Figure 4.3

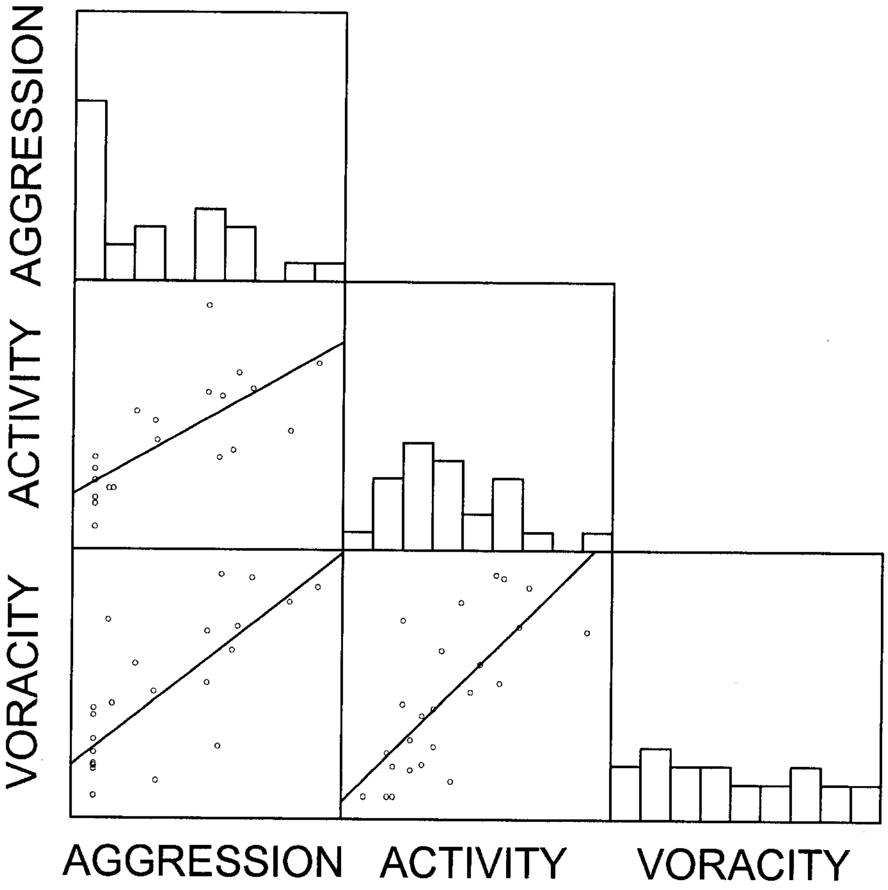

AGGRESSION
ACTIVITY
VORACITY
AGGRESSION
ACTIVITY
VORACITY

Figure 4.4a

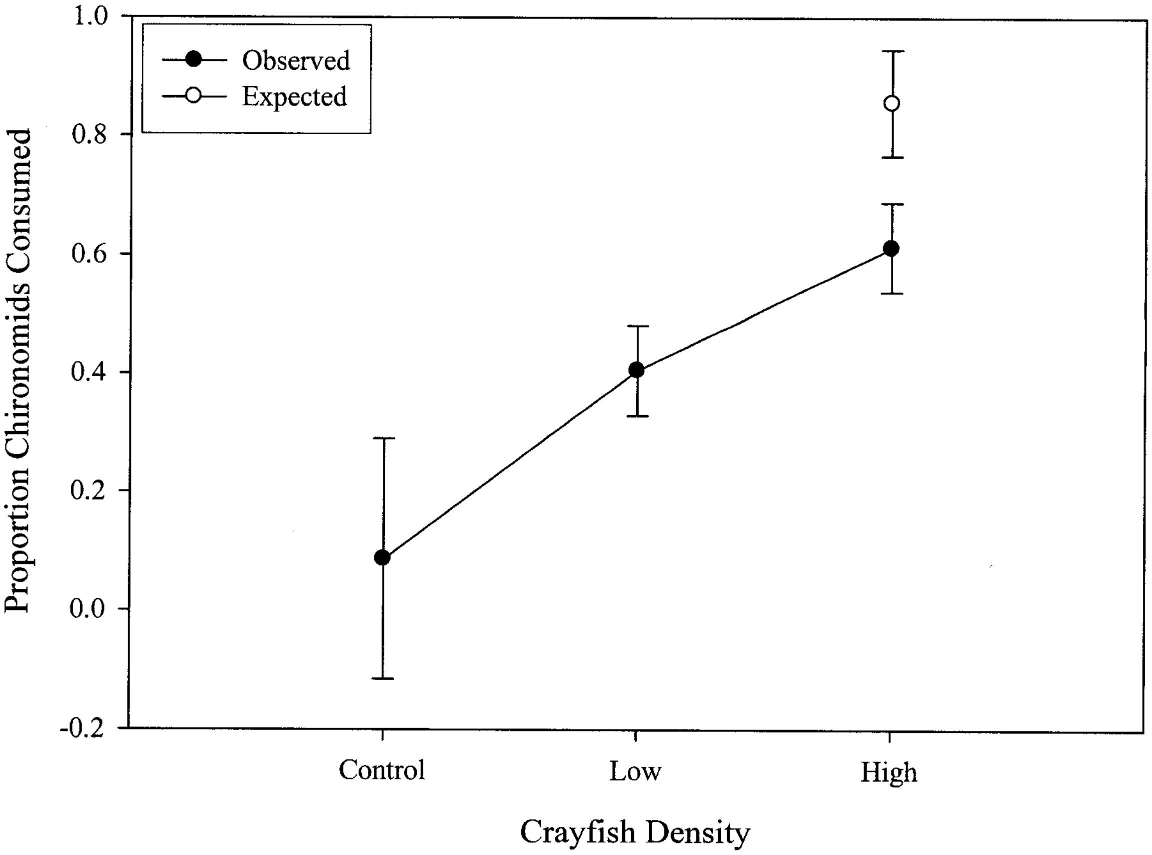

Observed
Expected
Proportion Chironomids Consumed
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
Control
Low
High
Crayfish Density

Figure 4.4b

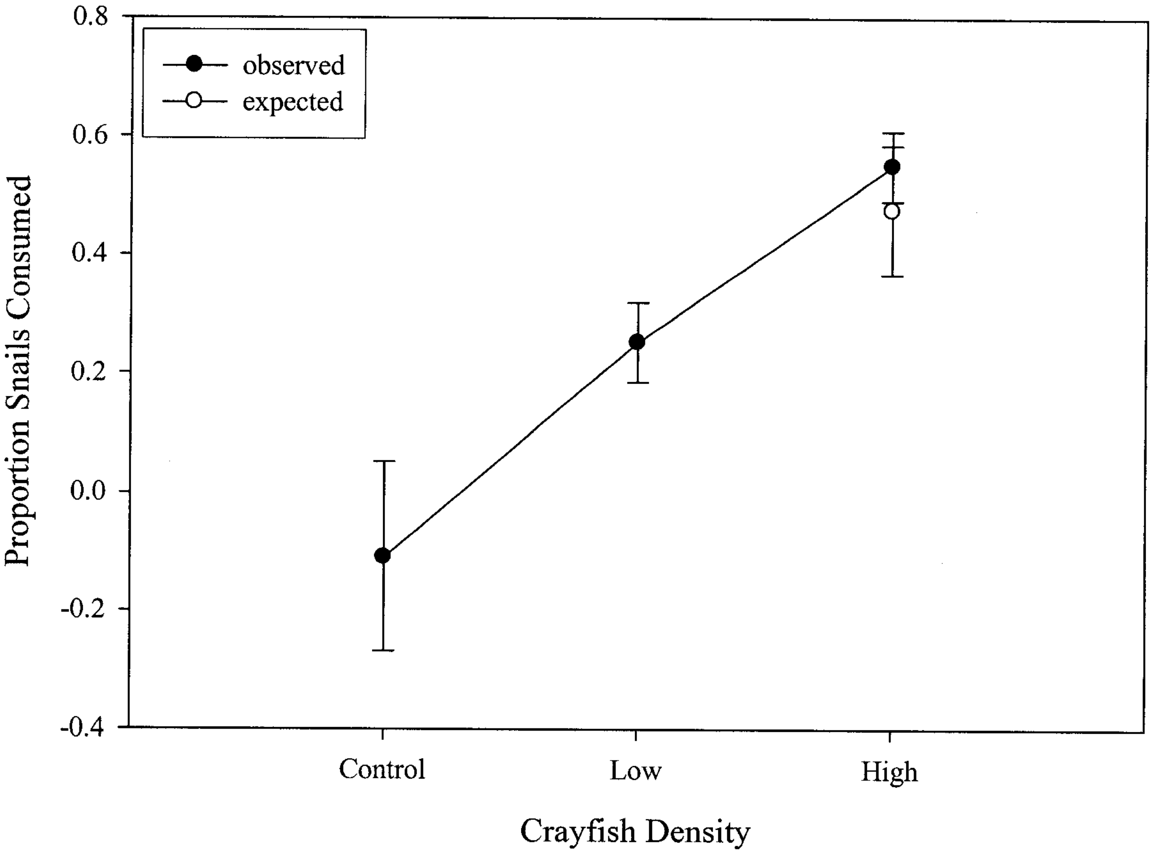

observed
expected
Proportion Snails Consumed
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
Control
Low
High
Crayfish Density

Figure 4.5

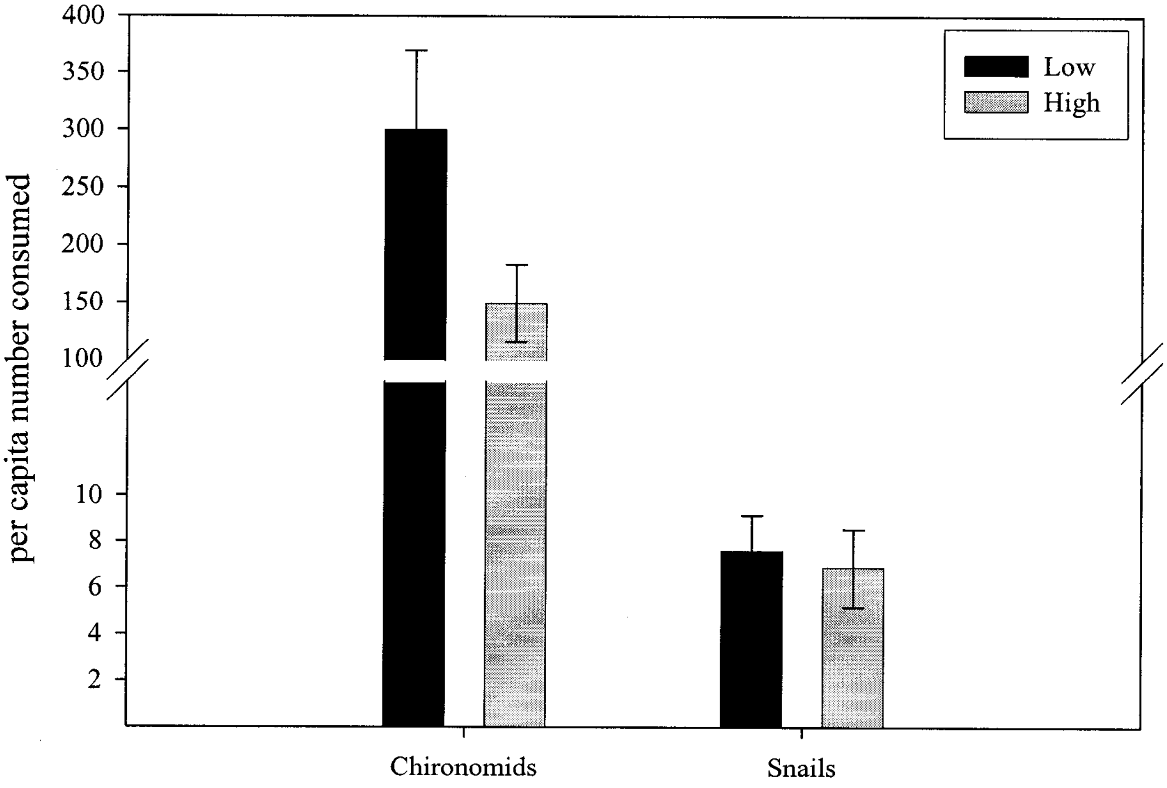

per capita number consumed
400
350
300
250
200
150
100
10
8
6
4
2
Low
High
Chironomids
Snails

Figure 4.6

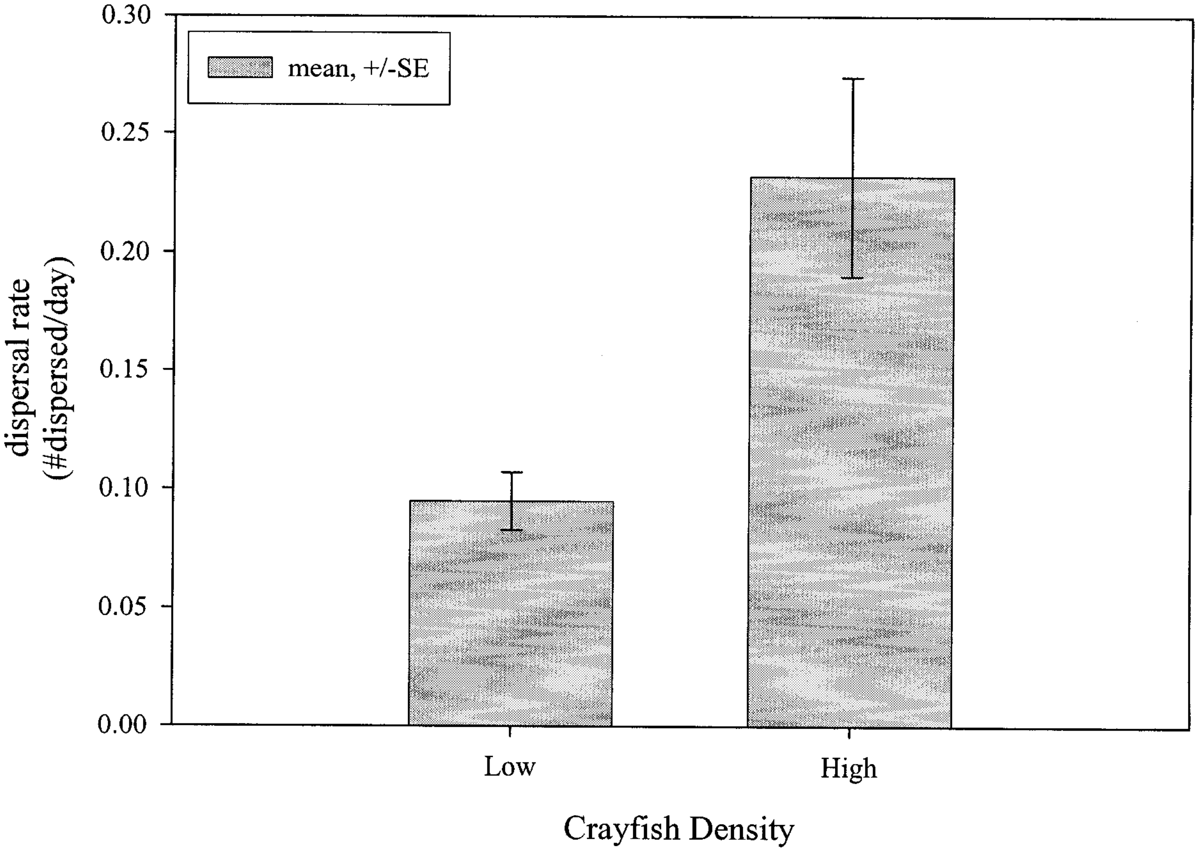

mean, +/-SE
0.30
0.25
0.20
0.15
0.10
0.05
0.00
dispersal rate (#dispersed/day)
Low
High
Crayfish Density

Figure 4.7

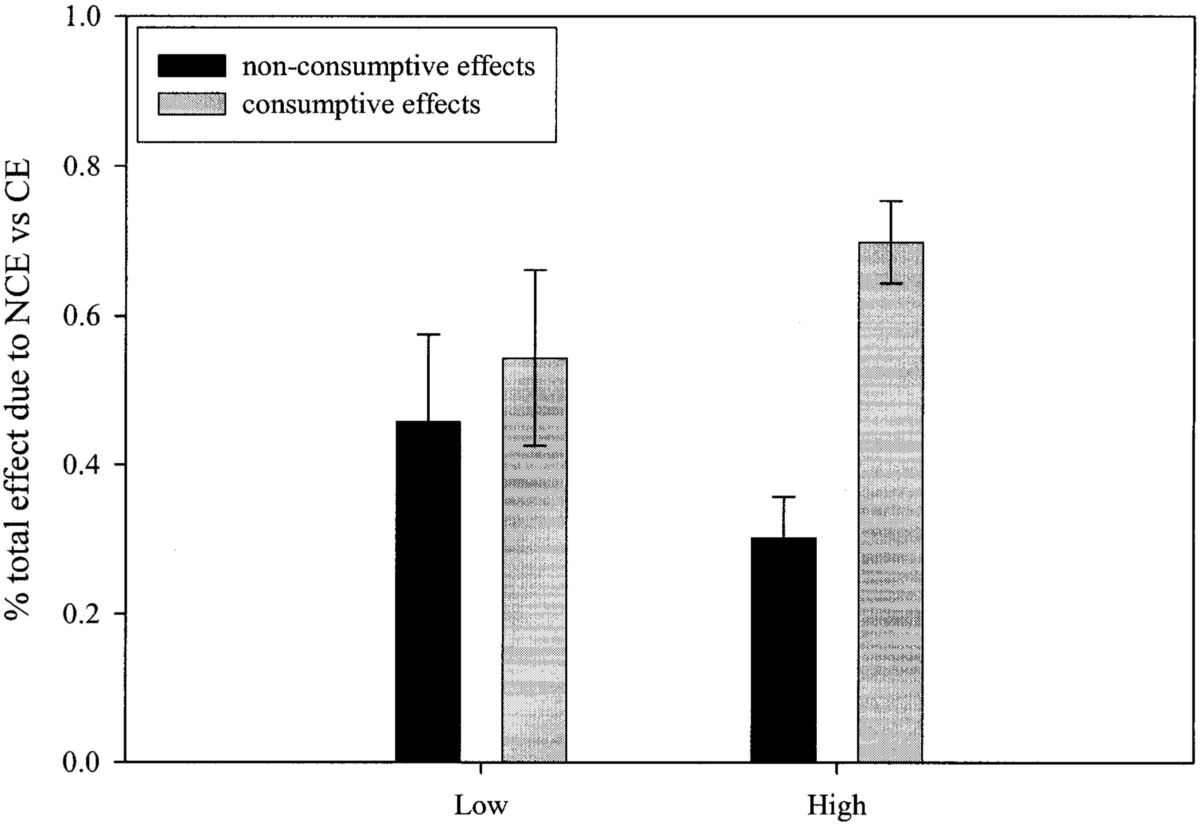

non-consumptive effects
consumptive effects
% total effect due to NCE vs CE
0.0
0.2
0.4
0.6
0.8
1.0
Low
High

Figure 4.8

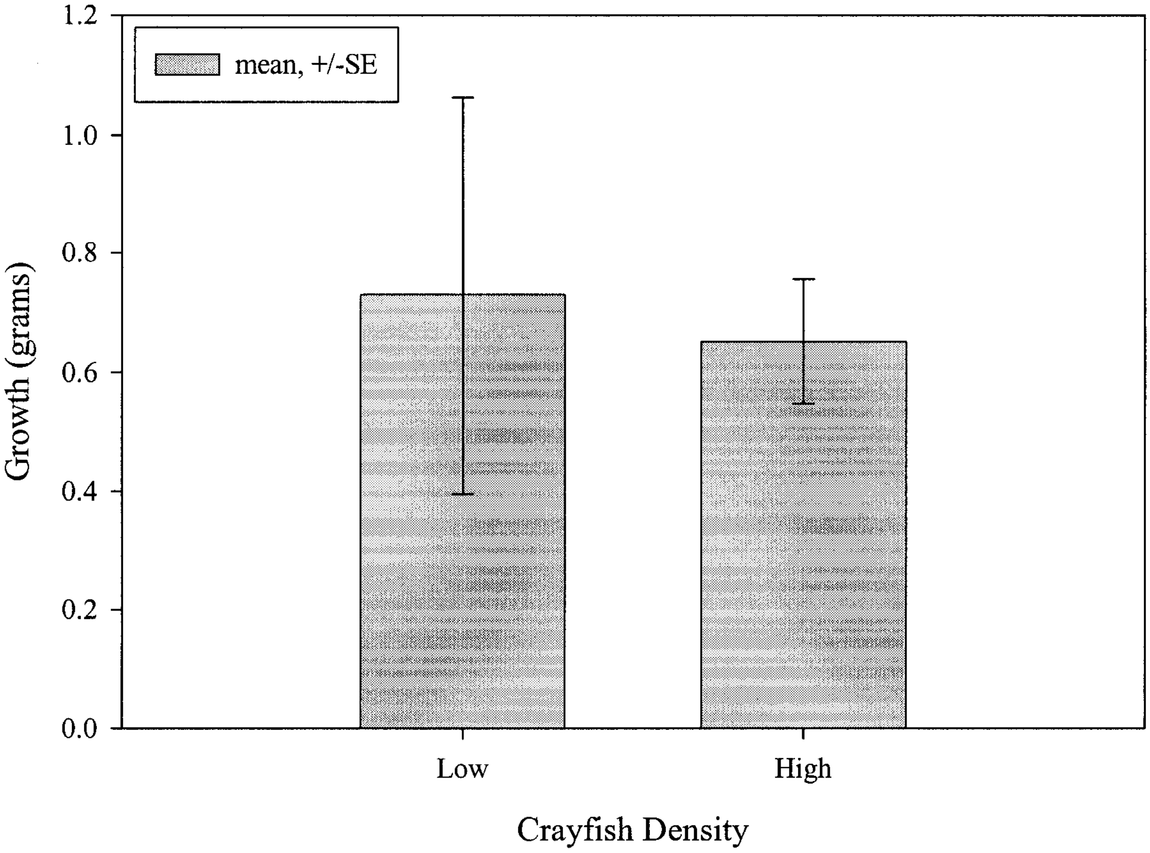
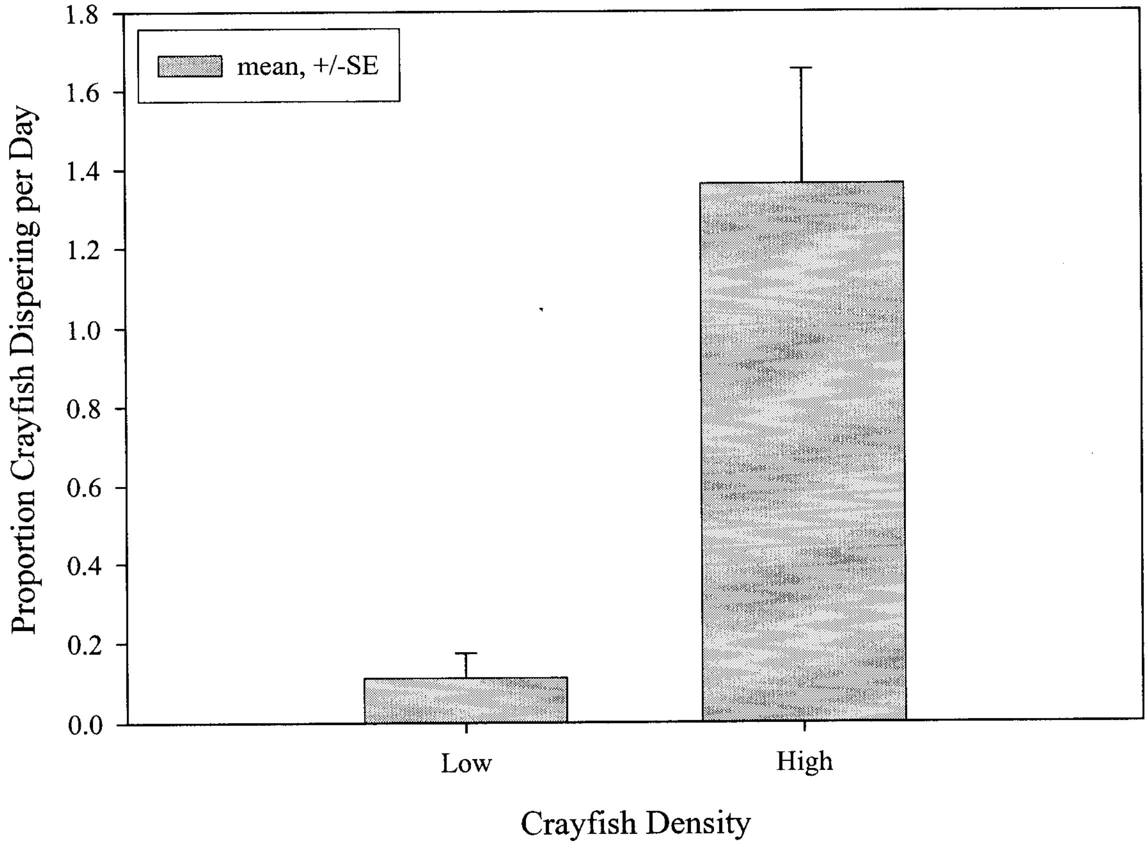

mean, +/-SE
Growth (grams)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Low
High
Crayfish Density

Figure 4.9


mean, +/-SE
Proportion Crayfish Dispering per Day
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Low
High
Crayfish Density

Appendix 4.1. Scoring system of crayfish aggression adapted from Karavanich and Atema (1998).

Score	***Behavior***	***Definition***
-2	Fleeing	Walking away (rapidly), tail flip
-1	Avoidance	Walking away (slowly), turning away
0	Separate	At least one body length apart or not facing each other
1	Initiation (no contact)	Facing, approaching, turning towards opponent
2	Threat display (no contact)	High on legs, claws open, meral spread, claws forward, antennae point
3	Physical contact (no claw grasping)	Antenna touching or whipping, claw touching, tapping or pushing
4	Physical contact (claw grasping)	Claws used to grasp opponents claws (claw lock)
5	Unrestrained use of claws	Snapping, ripping, swimming while in claw lock, grasping legs or rostrum