The influence of evolution and plasticity on the behavior of an invasive crayfish

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Abstract

Invasion success can be enhanced by evolution and behavioral plasticity, but the importance of these processes for most invasions is not well understood. Previous research suggests there is a genetic basis for differences in growth rate between native and invaded range rusty crayfish (*Orconectes rusticus*). We hypothesized that invaded range *O. rusticus* achieve faster growth by allocating more time to foraging and less to defense. We conducted a laboratory experiment to test the effects of range (native or invaded) and plasticity (as induced by exposure to predators) on crayfish behavior. We collected *O. rusticus* adults and eggs from both ranges, hatched eggs in the lab, and then reared juveniles in common conditions either with or without predatory fish. We then quantified the amount of time that crayfish spent active in an experiment with and without predatory fish. In support of our hypothesis, invaded range adults had reduced antipredator behavior compared to native range adults. Further, invaded range juveniles were more active than native range juveniles without predators, but all juveniles were inactive with predators. Juveniles from both ranges that were raised with fish had lower activity, especially when fish were present. These results suggest the behavioral response of *O. rusticus* to predators is plastic, but activity level in the absence of predators has evolved since *O. rusticus* were introduced. Because active crayfish consume more prey, this change in behavior may be responsible for rapid growth in invasive *O. rusticus*, a trait that contributes to the strong ecological impacts of this invasive crayfish.

Keywords: invasive species, feeding, predation, foraging rate, antipredator behavior, behavioral plasticity

Introduction

Evolution can alter the success and impacts of invasive species by allowing them to adapt to novel environments (Huey et al. 2005; Colautti and Barrett 2013) and conditions that arise during the invasion process (Phillips et al. 2010). However, evolution during invasions has been understudied compared to ecological processes (Colautti and Lau 2015). Therefore to what extent invasiveness is typically caused by release of species from fitness constraints or to rapid, adaptive evolution is not well understood (Ellstrand and Schierenbeck 2000; Colautti and Lau 2015).

Evolution during invasions can occur as a result of stochastic changes, such as founder effects or genetic drift, or as a result of adaptive evolution. Species may also adapt to novel environments through phenotypic plasticity (Agrawal 2001), with greater capacity for behavioral plasticity enhancing invasiveness (Wright et al. 2010). In addition, the capacity for plasticity could also evolve during invasions (Lande 2015). Only a small fraction of species that become established in new locations become invasive (spread and cause ecological and/or economic harm), and those species that have strong impacts often have *r*-selected life history traits such as rapid growth and high reproductive rate (Sakai et al. 2001; van Kleunen et al. 2010; Lamarque et al. 2011), traits that are often subject to strong selection. In addition to traits of introduced species, characteristics of the environment and biotic community within the introduced range are often important for invasion success (Catford et al. 2009).

Some species may already possess *r*-selected traits upon introduction, but in other species, these traits may evolve during the invasion process. For example, in the early stages of an invasion, conspecific densities are low and the population is unlikely to be density regulated, and theory indicates that higher fitness is associated with high reproductive rate in non-density-regulated populations (Roughgarden 1971; Burton et al. 2010). Thus, higher reproductive rates may be selected for in invasive populations. Some previous studies have found evidence for evolution of rapid growth or increased investment in reproduction in invasive populations or during range expansions (Burton et al. 2010; Phillips et al. 2010; Flory et al. 2011); however, other studies have found no support for the evolution of these traits in other species (e.g. Bossdorf et al. 2004; Cripps et al. 2009). It has also been hypothesized that the conditions in the anthropogenic vectors that move species, e.g., the live fish bait trade in the case of crayfish, could select for aggressive individuals with associated rapid growth rates (Pintor and Sih 2009). More evidence is needed to determine in what taxa, how often, and in which conditions traits that promote invasion success evolve.

Sargent and Lodge (2014) found that growth rates of rusty crayfish (*Orconectes rusticus*) from invaded range populations were higher than those from native populations when raised in common conditions, suggesting that growth rates have evolved since *O. rusticus* were introduced to the invaded range. The mechanism by which *O. rusticus* achieves rapid growth is currently unknown, but we hypothesize that it is due to higher foraging rates. Pintor and Sih (2009) found that in some conditions *O. rusticus* from invasive populations displayed greater foraging activity and recruitment to bait than native *O. rusticus*. However, it is unclear whether these behavioral differences were genetically based or due to behavioral plasticity.

Regardless of the origins of rapid growth of *O. rusticus* in the invaded range, it may help to explain the crayfish's success following introduction. *O. rusticus* are native to the Ohio River Drainage and have been introduced widely, especially in the Midwest and northeastern United States (United States Geological Survey 2015). In northern Wisconsin, where invasive populations of *O. rusticus* have been well-studied, they have strong ecological impacts. *O.*

rusticus has greater impacts on freshwater communities than resident crayfish, *O. virilis* and *O. propinquus* (Wilson et al. 2004). When replacing these congeners, *O. rusticus* often causes dramatic declines in the abundance and richness of macrophytes and other macroinvertebrates, as well as declines in the abundance of some species of fish (Wilson et al. 2004). The ability of *O. rusticus* to replace *O. propinquus* has been attributed in part to its faster growth rate and ability to outcompete smaller individuals for shelter (Hill et al. 1993; Garvey et al. 1994; Hill and Lodge 1994). In addition, larger crayfish produce more eggs (Savolainen et al. 1997; Skurdal et al. 2011), so rapid growth among individuals may promote high *O. rusticus* population growth.

In addition to promoting growth, allocating time to foraging is likely to increase susceptibility to predation. There is a tradeoff between foraging and defense in crayfish: crayfish that spend more time moving or exposed are more vulnerable to fish predators than those that are stationary or within shelter (Garvey et al. 1994). Invasive species may leave behind their natural enemies when they are introduced, and it has been hypothesized that this process often allows invasive species to allocate less energy to defense and more energy to growth and competition (Crawley 1987; Blossey and Notzold 1995). However, for *O. rusticus*, predators and pathogens in the invaded range share a long coevolutionary history with other orconectid crayfish and readily infect or consume *O. rusticus* (Peters 2010; Sargent et al. 2014). Further, predatory fish are important in controlling *O. rusticus* abundance in the invaded range (Roth et al. 2007). Therefore, we expect predator avoidance to remain an important strategy for this species, and we were interested in how activity and foraging behavior would differ between native and invasive populations of *O. rusticus* when faced with the threat of predation.

We designed two experiments to compare behavior between native and invaded range *O. rusticus*. First, we conducted a behavioral experiment to test whether increased growth in invaded range *O. rusticus* is due to differences in activity and feeding behavior between individuals from the native and invaded range and how these behaviors are altered by the presence of a predator. For this experiment, we used adult *O. rusticus* collected from the native and invaded range. We hypothesized that *O. rusticus* from invasive populations allocate more time to foraging and less time to defense. Second, we conducted a behavioral experiment to test whether differences in activity and feeding behavior between native and invaded range crayfish are due genetic differences or to plasticity. For this experiment, we collected *O. rusticus* as eggs from the native and invaded range and raised them in common conditions either with or without exposure to predators. We then conducted a similar experiment as for adult crayfish to quantify crayfish activity and feeding behavior as influenced by the presence of a fish predator. This approach also enabled us to compare the capacity for behavioral plasticity between native and invaded range *O. rusticus*.

Methods

Adult behavioral experiment

In order to test whether behavioral differences exist between *O. rusticus* from native and invaded range populations, we collected adult crayfish from the native and invaded range in fall 2010 and examined their behavior in the presence and absence of a predatory fish. We collected crayfish from three locations within each range, and conducted a total of ten behavioral trials with crayfish from each location. Native range *O. rusticus* were hand-collected in September 2010 from the Great Miami (39°56'N, 83°44'W), Little Miami (38°54'N, 83°34'W), and Scioto (40°00'N, 83°23'W) river drainages in Ohio, USA. Invaded range *O. rusticus* were hand-collected in August 2010 from High Lake (46°08'N, 89°32'W), Big Lake (46°11'N, 89°26'W), and Lake Ottawa (46°04'N, 88°45'W) in northern Wisconsin and Michigan, USA. Until the behavioral trials began, crayfish were housed with other individuals collected from the same site in tanks with constantly flowing, aerated well-water and shelters constructed from polyvinyl chloride (PVC) pipe. Crayfish were fed shrimp pellets *ad libitum* and were exposed to a 12:12 h light dark cycle. Behavioral trials were conducted between February and April 2011. We tested the behavior of 17 males and 13 females from each range, and crayfish had a mean carapace length (± SE) of 20.0 ± 0.3 mm from the native range and 20.3 ± 0.4 mm from the invaded range.

Behavioral trials were conducted between 9:00 and 18:00 in 76 L aquaria filled with aerated well-water. Each aquarium contained a PVC pipe shelter on one end and a worm (*Eisenia foetida*) as a food source at the other end. A thin layer of sand covered the bottom of the aquaria, and a screen placed 10 cm from the base divided each aquarium into a top and bottom section (Fig. 1). In half of the trials, a smallmouth bass (*Micropterus dolomieu*) large enough to consume the crayfish was placed in the top section of the aquarium. This setup allowed the crayfish to receive visual and chemical cues from the bass, but did not allow the bass to closely approach or consume the crayfish. Smallmouth bass are voracious predators of crayfish and are common in both the native and invaded ranges of *O. rusticus* (Boschung et al. 1983; Peters 2010). We conducted a total of 60 trials (2 crayfish ranges x 2 fish treatments x 15 replicates).

At the start of the trial, we placed a crayfish in the center of the aquarium under a mesh acclimation dome and secured the screen divider in the tank. If the trial was a predator present trial, we immediately added the smallmouth bass to the top section of the aquarium. In all trials, we allowed the crayfish to acclimate under the mesh dome for 15 min. After the acclimation period, we lifted the dome using an attached string, and recorded the behavior of the crayfish on video for 15 min.

Videos were later scored and the duration of different crayfish behaviors was recorded. Behaviors included time spent active (walking or feeding) and time spent inactive (standing, crouched, or under the PVC shelter). Here, we assumed that both feeding and walking represent allocation of time towards growth and not towards predator avoidance. Previous research indicates that crayfish that are more active consume more prey than inactive crayfish (Pintor et al. 2008). In addition, crayfish that spend more time moving or exposed are more vulnerable to fish predators than those that are stationary or within shelter (Garvey et al. 1994). Therefore, combining these behaviors in the analysis allowed us to determine if crayfish were allocating time towards growth (i.e., active feeding and walking) or predator avoidance (i.e., being inactive). Only behaviors that occurred for 5 s or more were recorded, and no behaviors other than active (walking or feeding) or inactive (standing, crouched, or under the shelter) occurred for 5 s or more. Each crayfish was only used once, and aquaria were drained and refilled after each trial. All predator-absent trials were conducted first so that fish chemical cue would not be present from previous fish in the aquaria.

Juvenile behavioral experiment

In order to test whether differences in behavior between native and invaded range crayfish were genetically based or due to plasticity, we collected crayfish from both ranges as eggs and raised them in common conditions in mesocosms with predatory fish either present or absent. We then conducted a similar experiment to the one described above to quantify the foraging behavior of these crayfish, as influenced by the presence of a fish predator.

Because temperatures are warmer in the native range than in the invaded range, *O. rusticus* reproduce earlier in the native range. Therefore, juveniles from the native range were raised earlier than those from the invaded range, but important variables such as temperature, food quality and quantity, and predator presence were held constant. We hand-collected berried females (those with eggs attached to their abdomen) in early April 2012 from the Little Miami (38°54’N, 83°34’W and 39°47’N, 83°51’W) and Scioto River (40°00’N, 83°23’W) drainages in Ohio, USA. In early May 2012, we hand-collected berried females from High Lake (46°08’N, 89°32’W), Big Lake (46°11’N, 89°26’W), and Papoose Lake (46°10’N, 89°48’W) in Wisconsin, USA. We placed each female in an individual container (18 x 18 cm) in the laboratory with constantly-aerated well water, a PVC shelter, and gravel substrate. Eggs hatched, and young became independent from females 3 to 4 wks after collection. Young of year (YOY) were fed shrimp pellets *ad libitum* while in the laboratory.

YOY from the native range were placed in experimental mesocosms in late May, and YOY from the invaded range were placed in mesocosms in late June. We replaced mortalities that occurred within the first two weeks of the experiment with crayfish from the same range and, when possible, the same brood. Replacement crayfish were housed in the laboratory with the same husbandry and conditions as provided after hatching.

Mesocosms consisted of 416 L plastic tanks with flow-through, aerated well-water, which were located on the shore of Trout Lake (Wisconsin, USA). Temperature was maintained throughout the summer in each mesocosm by a 300 W heater. More details about mesocosms and rearing conditions can be found in Sargent and Lodge (2014). There were 12 mesocosms in total, with 20 YOY *O. rusticus* (10 invaded and 10 native range) in individual containers (described below) reared in each mesocosm. Half of the mesocosms contained three bluegill (9.5-13 cm total length) and three smallmouth bass (10.5-14.5 cm total length). Fish were fed *O. rusticus* once per week and earthworms (*Lumbricus terrestris*) twice per week for the duration of the experiment. Fish readily consumed both food types. Like smallmouth bass, bluegill are common in both the native and invaded range of *O. rusticus* (Boschung et al. 1983) and are major predators of juvenile crayfish (Roth et al. 2007).

Within mesocosms, crayfish were each housed in an individual clear plastic container (18 x 18 x 12.7 cm) with rectangular holes (14 x 8 cm) cut into each side and replaced with window screen. The screened containers prevented direct interaction among crayfish or with predators, but allowed crayfish to receive visual and chemical cues from predators. Two stones were glued to one side of the bottom of the container to provide shelter. On the opposite side, a small nylon nut and bolt held disks of prepared food.

Food consisted of macrophytes, earthworms, and bluegill fillets mixed with sodium alginate and water. All food was prepared at the beginning of the summer and frozen. Crayfish from native and invaded range populations were fed from the same batch of food during the same week of growth. To examine how food quality affects crayfish growth, which is described in Sargent and Lodge (2014), we made both a high quality food (40% macrophytes and 60% animal matter) and a low quality food (80% macrophytes and 20% animal matter). Half of the crayfish in each mesocosm were randomly assigned to one of these food types.

Crayfish were removed from the experiment and tested for their behavioral response to fish after they were reared in experimental mesocosms for 7 to 8 wks (or 5 to 6 wks if they were replacements). Because they grew more slowly in mesocosms, native range juveniles were smaller during behavioral experiments (16.7 ± 0.4 mm total length ± SE) than invaded range juveniles (19.0 ± 0.4 mm total length ± SE). It is possible that this size difference could contribute behavioral differences, but both size classes would be extremely vulnerable to predatory fish (Stein 1977). Between 48 and 72 h prior to the behavioral trial, crayfish were removed from outdoor tanks and placed in the laboratory. Crayfish were kept in their original individual containers, which were placed in a bucket with aerated well-water, and fed until the start of the trial.

We conducted a total of 89 trials with 2 crayfish range treatments (native or invaded), 2 fish treatments during rearing (crayfish experienced or inexperienced with predators), and 2 fish treatments during the trial (predator present and absent). We did not have an equal number of crayfish within each group because fewer native range crayfish survived in the mesocosms especially when reared with fish. Replicates of each unique treatment combination ranged from 6 to 15.

Behavioral trials were identical to those used to test adult crayfish except for the following differences. Tanks were smaller (38 L) to accommodate the smaller crayfish and time spent in the mesh acclimation dome was longer (25 to 30 min). Instead of using an entire worm as the food source during the trial, we used a 1cm section of earthworm. Tanks were used for up to two trials before well-water was changed. Predator-present trials were always conducted in the same aquaria so that fish chemical cue was not present in predator-absent trials. In addition to the behaviors recorded for adult crayfish, we recorded the number of tail flips (fast retreat backwards by contraction of the abdomen) each crayfish displayed. Tail flips are an escape behavior (Bruski and Dunham 1987). This behavior was extremely rare in the adult trials, so we only examined it in juvenile trials.

Statistical analyses

To test whether adult crayfish from the native and invaded range of *O. rusticus* responded differently to predators, we used ANOVA to examine the effects of range (native or invaded), fish (predator present or absent), and their interaction on the duration of active time (a combination of time spent walking or feeding). To test whether the observed behavioral differences were influenced by collection location, we used separate two-way ANOVAs for each range that included the effects of collection location and predator presence (and the interaction between the two) on the duration of active time. Finally, we used generalized linear models with a binomial distribution to determine whether there were differences in the occurrence of feeding or utilizing the shelter during the trial. We used likelihood ratio tests to compare models with the effects of range, predator presence, and their interaction on the occurrence feeding or utilizing shelter.

To compare adult and juvenile crayfish, we conducted a three-way ANOVA with age (juvenile or adult), range, fish, and all interactions among these variables on crayfish activity level. For this analysis, we used only the juveniles that were exposed to predators during rearing because we expected that all adult crayfish had previous exposure to smallmouth bass.

Within juvenile crayfish, we were also interested in whether previous experience with predatory fish altered crayfish activity in the experiment and whether experience had a different effect on native or invaded range crayfish. To examine the effect of experience (and interactions between experience and other variables) on the duration of active time, we conducted a four-way ANOVA with range, predator experience during rearing, food quality during rearing, predator presence during the trial, and all possible interactions. In addition, we used separate three-way ANOVAs for each range to examine the effects of collection location, predator experience, predator presence, and all interactions on duration of active time. We used the same generalized linear models as for adult crayfish to examine differences in the occurrence of feeding or utilizing the shelter during the trial but also included the effect of predator experience. To test whether there were differences in the frequency of tail flips between native range and invaded range juvenile crayfish, we used a generalized linear model with a Poisson distribution. We used likelihood ratio tests to compare Poisson regressions with the effects of range, predator experience, predator presence, and their interactions on tail flip frequency.

Results

Adult crayfish

Adult native and invaded range crayfish had similar activity levels overall ($F_{1, 56} = 0.23$, $P = 0.6362$; Fig. 2a), and crayfish activity was significantly lower in trials with smallmouth bass present ($F_{1, 56} = 20.25$, $P < 0.0001$; Fig. 2a). There was a significant interaction between range and predator presence ($F_{1, 56} = 7.15$, $P = 0.0098$; Fig. 2a), whereby native range crayfish displayed a greater difference in activity level between predator-present and predator-absent trials than invaded range crayfish. When predators were present, native range crayfish reduced their activity by 61% and invaded range crayfish reduced their activity by 20%.

Within the native range, there was a significant effect of collection location ($F_{2, 24} = 3.91$, $P = 0.0337$), a significant effect of predator presence ($F_{1, 24} = 42.16$, $P < 0.0001$), and a significant interaction between collection location and predator presence ($F_{1, 30} = 3.87$, $P = 0.0350$) on behavior, in which Great Miami crayfish were more active in the presence of fish than crayfish collected from the other two locations. Within invaded range crayfish, there was no effect of collection location, predator presence, or interaction between collection location and predator presence on duration of active time ($P > 0.2$).

Most of the adult crayfish did not consume the worm or utilize the shelter during the trial (Fig. 3). There was no significant difference in the occurrence of feeding during the trial between native and invaded range adults ($P > 0.1$), but crayfish were less likely to feed when predators were present ($\chi^2 = 4.01$, $P = 0.0454$, $N = 60$). In addition, native range adults with no predators present tended to feed more frequently than other groups, but the interaction between range and predator presence on feeding was not statistically significant ($\chi^2 = 2.71$, $P = 0.0995$, $N = 60$). There was also a non-significant tendency for invaded range crayfish to use the shelter more often than native range crayfish ($\chi^2 = 5.06$, $P = 0.0797$, $N = 60$). There was no overall effect of fish presence on shelter use ($P > 0.1$). Native range crayfish with fish present also tended to use the shelter less frequently than other groups, but the interaction between range and fish presence on shelter use was non-significant ($\chi^2 = 2.96$, $P = 0.0855$, $N = 60$). Crayfish that entered the shelter during trials with fish present tended to spend more time on average in the shelter than crayfish that entered the shelter in trials without fish (Fig. 4).

Adult crayfish vs. experienced juveniles

Adult crayfish were significantly more active than juvenile crayfish that were experienced with predators ($F_{1,94} = 9.92$, $P = 0.0022$: Fig. 2a, b), and crayfish were generally less active in the presence of smallmouth bass ($F_{1,94} = 61.15$, $P < 0.0001$; Fig. 2a, b). In addition, there was a significant 3-way interaction between age (adult or juvenile), range, and predator presence during the trial ($F_{1,94} = 13.47$, $P = 0.0004$; Fig. 2a, b). Whereas native range adults reduced their active time in the presence of predators to a greater extent than invaded range adults, we observed the opposite pattern in juvenile crayfish. Native range juveniles were generally inactive across both predator treatments, but invaded range juveniles were active when predators were absent and inactive when predators were present (Fig. 2b).

Juvenile crayfish

Within juvenile crayfish, in addition to significant effects of range ($F_{1,72} = 5.82$, $P = 0.0184$), fish presence during the trial ($F_{1,72} = 41.92$, $P < 0.0001$), and their interaction ($F_{1,72} = 22.60$, $P < 0.0001$), experience had a significant effect on activity level ($F_{1,72} = 17.84$, $P < 0.0001$; Fig. 2 b, c). Crayfish that were reared with fish were generally less active than those that were not. In addition, there was a significant interaction between fish and experience ($F_{1, 72} = 6.24$, $P = 0.0148$; Fig. 2 b, c). Crayfish that were reared with predatory fish were less active during the trials with bass than crayfish that had no prior experience with predators. The effect of food quality during rearing was not statistically significant, nor were the other interactions ($P > 0.1$).

Within juvenile crayfish from the native range, there was no effect of collection location, predator presence, or interaction between collection location and predator presence on behavior ($P > 0.2$). Within invaded range juvenile crayfish, there was a significant effect of collection location ($F_{2, 40} = 17.55$, $P < 0.0001$), predator presence ($F_{2, 40} = 75.68$, $P < 0.0001$), and an interaction between collection location and predator presence ($F_{2, 40} = 3.65$, $P = 0.0350$), in which crayfish collected as eggs from High Lake had a lesser response to predatory fish than crayfish collected from the other lakes. Invaded range juveniles from all collection locations were active when fish were absent.

Few of the juvenile crayfish consumed the worm or utilized the shelter during the trial (Fig. 3). Overall, crayfish that had experience with predatory fish tended to consume the worm less often than those that were inexperienced, but this result was not statistically significant ($\chi^2 = 3.71$, $P = 0.0542$, $N = 89$). A significant interaction existed between range and experience ($\chi^2 = 6.10$, $P = 0.0135$, $N = 89$). A similar proportion of experienced and inexperienced native range crayfish fed during the trial (Fig. 5). In contrast, invaded range crayfish were more likely to feed if they were inexperienced than if they were experienced (Fig. 5). A significant interaction also existed between predator presence and experience ($\chi^2 = 6.17$, $P = 0.0130$, $N = 89$). There was little effect of predator presence on feeding among inexperienced crayfish (15% fed in predator-present trials and 19% fed in predator-absent trials). However, for experienced crayfish, 11%

fed when predators were absent but none fed when predators were present. No other variable or interaction between variables had a significant effect on the occurrence of feeding ($P > 0.3$). In addition, there were no significant effects of any of these variables on the occurrence of shelter use during the trial ($P > 0.1$). When predators were absent, invaded range crayfish tended to spend very little time within the shelter when they entered it (Fig. 4).

Native range juveniles exhibited tail flip behavior more frequently than invaded range juveniles ($\chi^2 = 16.04$, $P < 0.0001$, $N = 89$; Fig. 6). There was also a significant three-way interaction between range, fish presence, and experience ($\chi^2 = 8.29$, $P = 0.0040$, $N = 89$). Crayfish from the native range displayed the greatest number of tail flips when predators were present and they were experienced or when predators were absent and they were inexperienced. Crayfish from the invaded range had the fewest tail flips in these treatment combinations. No other variable or interaction between variables had a significant effect on tail flip frequency ($P > 0.4$).

Discussion

Adaptive significance of behavioral differences

Our results are consistent with selection for rapid growth and high fecundity within the invaded range. Larger crayfish produce more offspring (Savolainen et al. 1997; Skurdal et al. 2011), and greater growth may be achieved by allocating more time to foraging than to predator avoidance. Juvenile *O. rusticus* from invaded range populations were more active than juveniles from the native range, suggesting that they spent more time foraging. Our results also indicate that the greater growth of invaded range juveniles observed by Sargent and Lodge (2014) is likely due, at least in part, to this behavioral difference. In addition, in the presence of fish predators, adult invaded range *O. rusticus* reduced their activity to a lesser extent than native range adults, which also suggests they could be investing more in feeding and therefore growth and less in predator avoidance than crayfish from the native range. Adults from different ranges may have had different prior experience with predators, which could be an additional influence on their behavior.

The differences in behavior observed between adult and juvenile crayfish are likely due to differences in predation risk. The threat of predation is lower for adult crayfish than juveniles (Stein and Magnuson 1976). This is because many fish are gape limited (especially *Lepomis* spp.) and fish tend to selectively consume smaller crayfish (Stein 1977). Therefore, for adult crayfish, there should be less risk than for juvenile crayfish in allocating time towards foraging when predators are present. Consistent with this, adult crayfish (especially those from the invaded range) were more active in the presence of predatory fish than were juvenile crayfish. The inactivity of both native and invaded range juveniles during trials with fish present may be an adaptation to avoid the high risk of predation. Native range juveniles were generally inactive across all conditions, though, perhaps because the benefit of avoiding predation outweighs the benefit of growing rapidly even when these crayfish do not detect predators. In contrast, invaded range juveniles were active for most of the trial when predators were absent, allowing them to spend more time foraging.

The growth and mortality of juvenile crayfish while they were raised in mesocosms suggest that native range juveniles had a greater behavioral response to predatory fish than we were able to detect in behavioral trials. During behavioral trials, native range juveniles did not have reduced activity in the presence of predatory fish; however, fish presence reduced crayfish growth rate in mesocosms (Sargent and Lodge 2014). In addition, native range juveniles had higher mortality in mesocosms with fish present than mesocosms without fish (Sargent and

Lodge 2014). Because there was high (80%) mortality of native range crayfish in mesocosms with predatory fish present, it is likely that those individuals that had the greatest response to the fish (that were most inactive and ate the least) were those that did not survive, and therefore they were not included in this behavioral study. There was 34% mortality of invaded range crayfish in mesocosms with fish present, so results from the behavioral study better represent the variation in invaded range genotypes. In combination, these data suggest that both native and invaded range crayfish respond to predatory fish presence by reducing their activity level. If there had not been mortalities, we may have found that native range crayfish had an equal or greater reduction in activity in the presence of predatory fish than invaded range crayfish, similar to what we observed in adult crayfish.

In addition to the differences we observed in activity between native and invaded range juvenile crayfish, we observed differences in escape behaviors. Crayfish collected as eggs from the native range typically exhibited tail flip behavior at least once during the trial, but invaded range crayfish often did not. This may be an adaptation to flow conditions. In the native range, *O. rusticus* inhabit lotic waters, so juveniles may be able to effectively escape predation by propelling themselves into the current. Drift is a common mechanism by which invertebrates escape predation in lotic systems (Wooster and Sih 1995). Consistent with this, native range juveniles frequently exhibited tail flip behavior when predators were present and they had prior experience with predators. However, in lentic waters, which *O. rusticus* inhabit in the invaded range, predators may be able to more easily capture juvenile crayfish in the water column, so tail flip behavior may not be advantageous. Previous research on crayfish in the invaded range of *O. rusticus* indicates that in the absence of flow swimming by crayfish increases their vulnerability to predation (Garvey et al. 1994).

Mechanisms responsible for behavioral differences

Behavior of juvenile *O. rusticus* in the absence of predators mainly differed between the native and invaded range instead of within each range, suggesting that there is a genetic basis for these behavioral differences. Our experimental design does not allow us to rule out the influence of maternal or epigenetic effects on behavior, but if these effects were important, we would expect to see differences in behavior between crayfish collected as eggs from different locations within each range. We collected crayfish from diverse habitats within each range, including a range of stream sizes in the native range and lakes with different densities of rusty crayfish (and therefore different littoral communities, resource availability, and predation regimes; Wilson et al. 2004) in the invaded range. There was no effect of collection location within the native range on the behavior of juvenile *O. rusticus*. In addition, within the invaded range, we consistently observed high crayfish activity levels when predators were absent across all collection locations, which was distinct from the behavior we observed in native range juveniles. When predators were present, juveniles from High Lake had higher activity than those from Big or Papoose Lakes; however, because our sample size of experienced crayfish from this lake was low compared to the other lakes, additional research would be needed to be confident about this difference. Interestingly, High Lake had the lowest abundance of *O. rusticus* (4 per trap) compared to other invaded range lakes (19 and 35 per trap; Sargent and Lodge 2014), and it is possible the low density within this lake could select for individuals with high activity levels. Overall, our data suggest that the differences we observed between juveniles from different ranges are most likely genetically based because we would expect maternal and epigenetic effects to differ between habitats within both ranges.

In addition to divergence in behavior between the native and invaded range, our experiment demonstrated a strong effect of experience (behavioral plasticity) on crayfish activity in the presence of predators, but not on activity level when predators were absent. Further, the magnitude of behavioral differences was similar between crayfish from different ranges and crayfish raised with and without predators. Behavioral differences between native and invaded range crayfish were most pronounced when predators were absent: in trials without fish, juveniles from the invaded range were 67% more active on average than those from the native range. Behavioral differences between crayfish that were experienced or inexperienced with predators were most pronounced when predators were present: in trials with fish, inexperienced juveniles were 87% more active on average than experienced juveniles. Overall, these data suggest that activity level in the absence of predators has diverged between the two ranges, but that the behavior of crayfish in the presence of a predator is plastic and largely depends on their prior experience. Further, the significant interaction between range and experience on the occurrence of feeding during the trial indicates that invaded range crayfish may have a greater capacity for plasticity than native range crayfish. The occurrence of feeding was similar between experienced and inexperienced crayfish from the native range, but within crayfish from the invaded range, inexperienced crayfish were more likely than experienced crayfish to feed during the trial. We did not, however, find an interaction between experience and range on overall activity. If invaded crayfish do have a greater capacity for behavior plasticity, this trait may allow invaded range crayfish to succeed across habitats with diverse predator communities.

The strong effect of experience on antipredator behavior in juveniles can inform our interpretation of the adult behavioral results because the adult crayfish had experience with predators prior to collection. We did not collect data on predator abundance, but within the native range, it is possible that the reduced behavioral response of Great Miami drainage crayfish to predatory fish could have been due to reduced prior experience of these crayfish with smallmouth bass predators. In addition, it is possible that the greater behavioral response to fish that we observed in native range adults was due to greater exposure to predatory fish. However, we think this is unlikely because we collected invaded range crayfish from lakes with substantial smallmouth bass populations. Lake Ottawa, in particular, has abundant, large smallmouth bass because regulations require anglers to release this species (Baldridge and Lodge 2013), and thus we believe crayfish from this lake would have had much experience with smallmouth bass consuming conspecifics. Therefore, native range crayfish probably had equal or lesser exposure to predators than invaded range crayfish, which suggests that the greater behavioral response of adult native range crayfish to predators was genetically based. Without predators present, mean activity was slightly higher in native range adults than invaded range adults and native range adults tended to consume the worm more frequently. These data do not support our hypothesis that invaded range crayfish have greater foraging rates; however, it is unclear whether these results are due to reduced exposure to predators in the native range or to genetic differences that manifest when crayfish are adults. There was also a tendency of invaded range adults to use the shelter more frequently during the trial, but this may be because active individuals encounter the shelter more often.

While rearing *O. rusticus* from the native and invaded range in common conditions demonstrated divergence in antipredator behavior (which is likely genetically based), it is unclear whether the same behaviors would be observed if crayfish were raised in conditions more representative of the native range. For example, mesocosm conditions such as temperature, water chemistry, and/or flow may have more closely resembled invaded range habitat than native range habitat. Therefore, if crayfish are locally adapted to their environment, native range crayfish may have been in sub-optimal condition after we raised them in mesocosms. However, we observed the most tail flips in trials with native range juveniles, suggesting they are capable of energetically costly behavior, and while they were less active than invaded range juveniles, they spent a mean of 40% the trial walking. Thus, we expect that the differences in behavior are unlikely due to negative impacts of the mesocosm conditions on the native range juveniles. Conducting a similar experiment where *O. rusticus* from both ranges are raised in conditions that reflect native range habitat would provide additional insight into how local adaptation contributes to behavioral differences between ranges.

Ecological significance

The behaviors we tested are relevant to interpreting *O. rusticus* impacts on freshwater communities. Our behavioral plasticity results highlight the capacity for early exposure to fish predators to induce enhanced anti-predator behaviors among crayfish. In addition to the suppression of activity that may result when predatory fish are in the immediate vicinity, crayfish that are raised with predatory fish exhibit reduced activity levels in general, and therefore are likely to have lesser impacts. The greater level of activity in invaded range *O. rusticus* compared to native range *O. rusticus* is likely to cause greater impacts on lower trophic levels and increased energy flow to predators in the invaded range because crayfish are investing more time in foraging and less in predator avoidance.

If the behaviors we observed in invaded range crayfish are commonly selected for when crayfish are introduced to new locations, such as when conspecific densities are low or when crayfish are in the bait trade, the introduction process may often enhance the ecological impacts of crayfish. In addition to our results for *O. rusticus*, previous studies have also found greater foraging rates among some invasive populations of signal crayfish (*Pacifastacus leniusculus*) compared to native populations (Pintor et al. 2008). To our knowledge, differences in foraging behavior and growth have not been compared between other native and invaded range populations of crayfish. Nonindigenous crayfish have caused major ecological and economic impacts globally, including extirpation of native crayfishes, harm to fisheries, and altered lake and stream ecosystems (Lodge et al. 2000; Lodge et al. 2012). If there are parallel changes in behavior across independent crayfish introductions, these changes may contribute to the strong impacts of invasive crayfish on freshwater communities. The importance of evolution during invasions is gaining attention (Phillips et al. 2010; Flory et al. 2011; Colautti and Lau 2015), and our results provide new evidence that contemporary evolution can enhance the impacts of invasive species.

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Figure Legends

Fig. 1 Setup for behavioral experiment. Experimental aquaria contained (a) a shelter constructed from PVC pipe and (b) a worm or a section of worm as a food source. The predator-present trials contained a smallmouth bass in the upper section of the tank. A screen divided the upper and lower sections of the tank so that crayfish could receive visual and chemical cues from fish but could not be closely approached or consumed by the fish. Crayfish were placed under (c) a weighted mesh acclimation dome before the start of the trial, and the acclimation dome was lifted via an attached string before crayfish behavior was recorded

Fig. 2 Interaction plots showing the effect of predatory fish presence on native and invaded range crayfish activity in (a) adult *O. rusticus*, (b) experienced juvenile *O. rusticus*, and (c) inexperienced juvenile *O. rusticus*. Experienced crayfish were reared with predatory fish that were fed live *O. rusticus* and inexperienced crayfish were reared without predatory fish present. Each trial lasted for a total of 900 s (or 15 min). Error bars represent standard error

Fig. 3 Adult and juvenile *O. rusticus* use of food and shelter resources during behavioral trials. Native range crayfish are represented by dark grey bars and invaded range crayfish are represented by light grey bars

Fig. 4 Duration of shelter use for those crayfish that used the shelter during the trial. Native range crayfish are represented by dark grey bars and invaded range crayfish are represented by light grey bars. Error bars represent standard error

Fig. 5 The effect of experience on the proportion of juvenile crayfish from the native and invaded range that fed during the trial. White bars represent inexperienced juveniles (those reared without predatory fish) and grey bars represent experienced juveniles (those reared with predatory fish). No experienced, invaded range juveniles fed during the trial

Fig. 6 Differences in the frequency of tail flip behavior between native and invaded range juveniles. Boxes represent 1st and 3rd quartiles, interior lines represent medians, and whiskers represent the range

Fig. 1

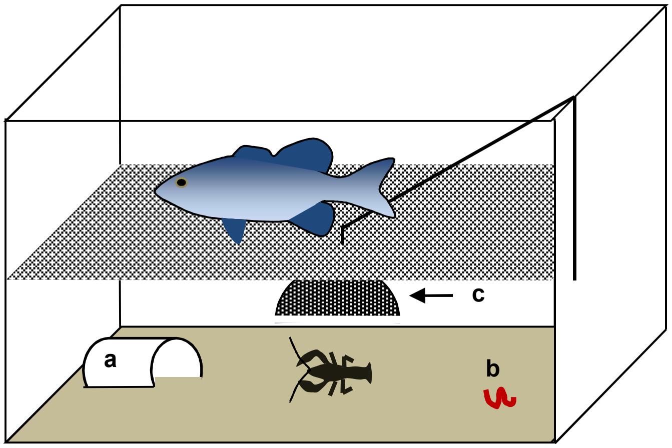


Fig. 2

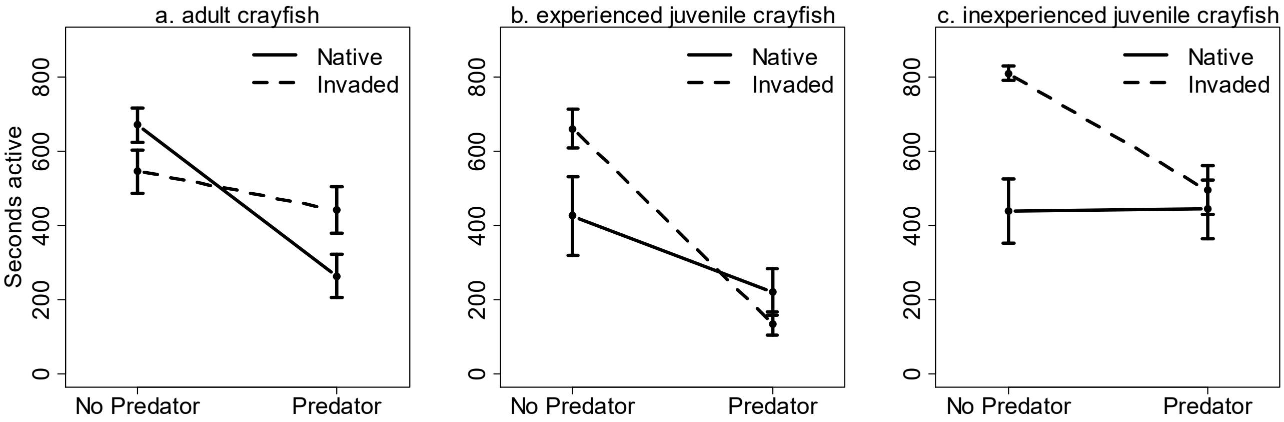
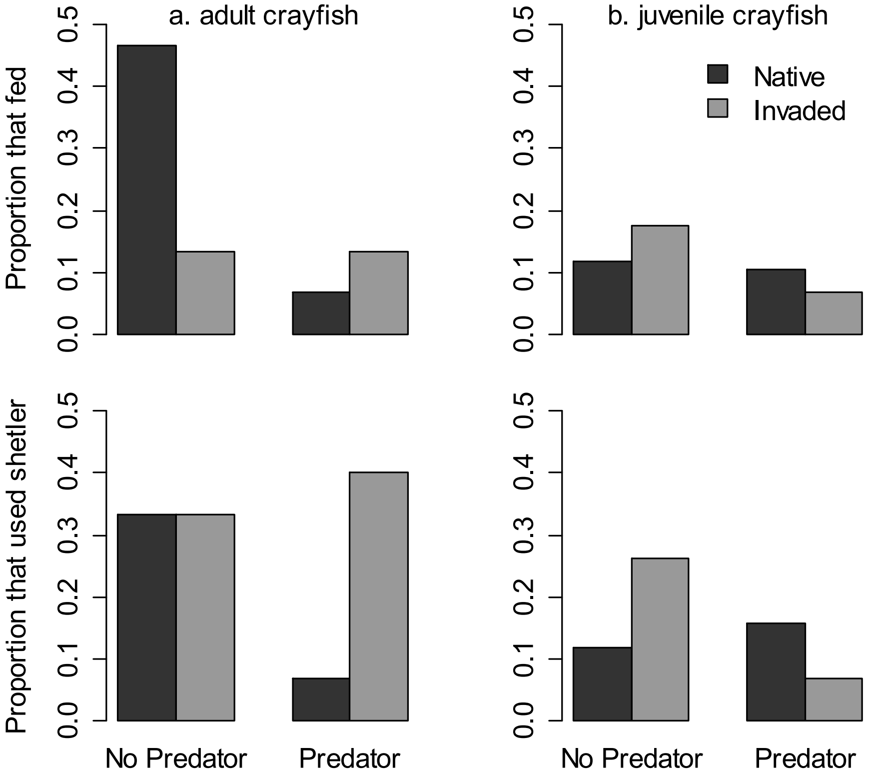

a. adult crayfish
b. experienced juvenile crayfish
c. inexperienced juvenile crayfish
Native
Invaded
Seconds active
0
200
400
600
800
No Predator
Predator

Fig. 3



a. adult crayfish
b. juvenile crayfish
Native
Invaded
Proportion that fed
Proportion that used shetler
0.0
0.1
0.2
0.3
0.4
0.5
No Predator
Predator

Fig. 4

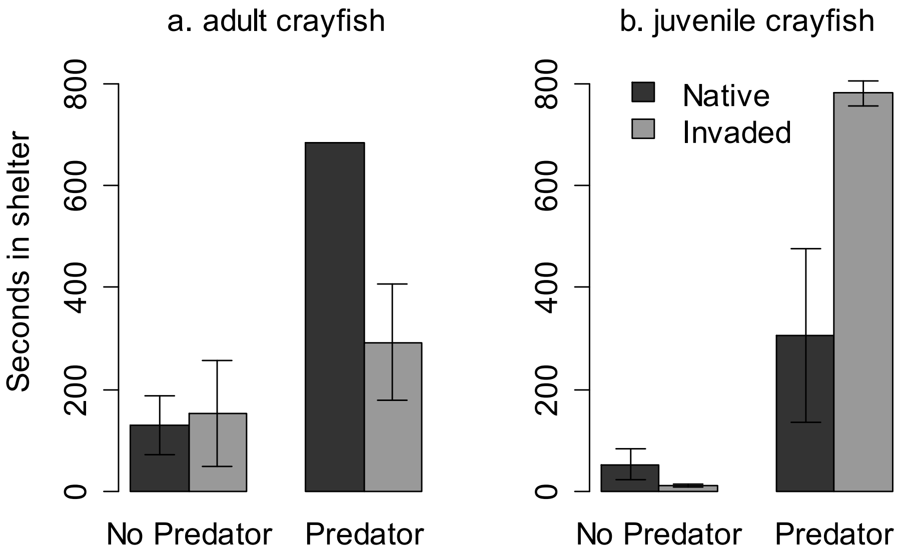
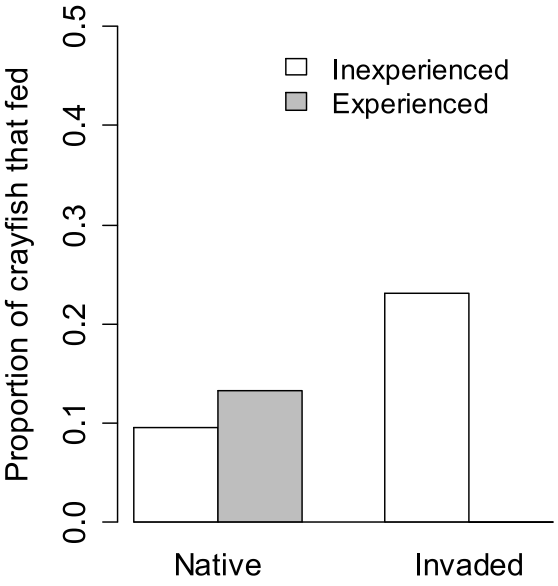

a. adult crayfish
b. juvenile crayfish
Seconds in shelter
0
200
400
600
800
Native
Invaded
No Predator
Predator

Fig. 5



Proportion of crayfish that fed
0.0
0.1
0.2
0.3
0.4
0.5
☐ Inexperienced
▨ Experienced
Native
Invaded

Fig. 6