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Invasive species of crayfish use a broader range of predation-risk cues than native species

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Abstract

The responses of invasive and native species of crayfish to conspecific and heterospecific alarm odors were recorded in the laboratory. Individuals of the North American invasive *Procambarus clarkii* responded just as strongly to odors from crushed *Austropotomobius pallipes* as they did to crushed conspecifics. The North American invasive *Orconectes limosus* also responded as strongly to *P. clarkii* odor as to conspecific odor. The native Italian species *A. pallipes* responded more strongly to conspecific alarm than to heterospecific alarm from *P. clarkii*. The pattern of invasive species of crayfish using a broader range of danger signals than displaced native species appears to be robust.

Introduction

It is generally assumed that species that more readily extend their ranges tend to be more flexible in many aspects of their biology than native species, which are often displaced by invasives (Schweitzer and Larson 1999). This flexibility allows the species to cope with new environmental features including new types of predators. Recognition of elevated predation risk has been shown to reduce the risk of predation in several systems (Downes 2002; Mathis and Smith 1993). In a recent paper, Hazlett (2000) suggested that one aspect of flexibility in animals could be in the breadth of information about predators that individuals of a species use. We predict that successful invasive species, those expanding their ranges with human 'help', will use a broader range of information about increased predation risk than native species that are not extending their range. In particular, invasive species will react similarly to heterospecific and conspecific alarm cues while native species will react more strongly to conspecific cues.

Hazlett (2000) presented data on one pair of crayfish species and their responses of conspecific and heterospecific alarm odors. *Orconectes rusticus* has spread throughout much of the Midwest following human introduction (Garvey et al. 1994; Hill and Lodge 1999) while *Orconectes propinquus* is not known to have expanded its range in recent times. Individuals of *O. rusticus* responded just as strongly to heterospecific alarm odor as to conspecific alarm odor. Individuals of the native species, *O. propinquus*, responded less strongly to heterospecific alarm than to conspecific alarm odor (Hazlett 2000). This difference in information use also appeared to hold for a pair of Australian species. *Cherax albidus*, which has spread in western Australia responded similarly to heterospecific and conspecific alarm cues whereas there was a reduced effect of heterospecific alarm odors in individuals of the native western-Australian species, *C. tenuimanus* (Gherardi et al. 2002). In this study we test the generality of the invasive-native dichotomy in information use by comparing the behavioral responses of three crayfish species in Italy to conspecific and heterospecific alarm odors. We tested two species which have been introduced to Europe from North America, *Procambarus clarkii* and *O. limosus* and the native species *Austropotomobius pallipes*.

Methods

The methods employed in this study are very similar to those used in Hazlett (2000). All species were tested following the same protocol. Individuals collected in the field were placed in holding containers in the laboratory and fed pieces of cod to make sure the animals treated cod odor as a food cue (Hazlett 1994). Adult animals were placed in individual, visually isolated 5 l aquaria. The aquaria contained 1.5 l of well water, were continually aerated, and contained a portion of a clay pot as a shelter. After 24 h acclimation to the observation containers, the animals were observed for 3 min following the introduction of 5 ml of different solutions. Animals were tested with one of six solutions once a day for five days. The order of test solutions was randomized among individuals each day. On day one, each animal was observed following introduction of a control of well water prior to the introduction of a test solution.

The test solutions introduced were (a) food odor, (b) conspecific alarm odor, (c) heterospecific alarm odor, (d) conspecific alarm odor plus food odor, and (e) heterospecific alarm odor plus food odor. The food odor was prepared by macerating 20 g of cod in 150 ml of well water and filtering with coarse filter paper. Alarm odors were prepared by macerating a medium-size crayfish in 150 ml of water and filtering. Crushing whole crayfish simulates the effects of predation by known predators such as snapping turtles (Hobbs 1993).

An observer with the aid of an event program and a pc recorded the behavior patterns. The data were later summarized as the total number of seconds spent executing a particular behavior or in a particular posture or condition. The behaviors recorded were cleaning movements, feeding movements of the walking legs or chelipeds, and locomotion. The postures were either raised (body elevated by extension of the walking legs, chelae raised at least to parallel to substrate, and abdomen extended) or lowered (chephalothorax in contact with substrate, chelae tips touching substrate and drawn into the body, and abdomen curled). Only the time spent in the lowered posture was analyzed since the postures are not fully independent behaviors. Animals could execute the other behaviors in combination or alone. For all except one species (*O. limosus*, $n = 8$), 20 individuals were tested.

The species tested were the invasive species *P. clarkii* (which is native to North America), the invasive *O. limosus* (which is also native to North America), and native Italian species *A. pallipes*. Individuals of *P. clarkii* were tested with heterospecific alarm odor from *A. pallipes*. Individuals of *O. limosus* and *A. pallipes* were tested with heterospecific alarm odor from *P. clarkii*. All the crayfish were collected in regions near Florence, Italy, except *O. limosus* which was collected near Parma, Italy, and all tests were conducted in the laboratory in Florence, Italy, in May 2000 and 2001.

To compare the effects of conspecific and heterospecific alarm odors, two measures of the strength of effects were calculated. For each behavior, the number of seconds spent in the control period was subtracted from the number of seconds spent following introduction of conspecific alarm and of heterospecific alarm. These differences were compared with paired t-tests because the same individuals were tested with all odors. A second measure of the strength of effects of alarm odors was generated by subtracting the number of seconds spent in behaviors following the introduction of food odor alone from the number of seconds spent following the introduction of the combination of either conspecific alarm plus food odor or heterospecific alarm plus food. These differences were also compared by paired t-tests. For all behaviors for all three species, there were no significant differences in the responses to the two sources of alarm odors when combined with food, thus those results are not reported or examined further.

Results

Procambarus clarkii. Comparison of the effects of conspecific and heterospecific alarm introduction

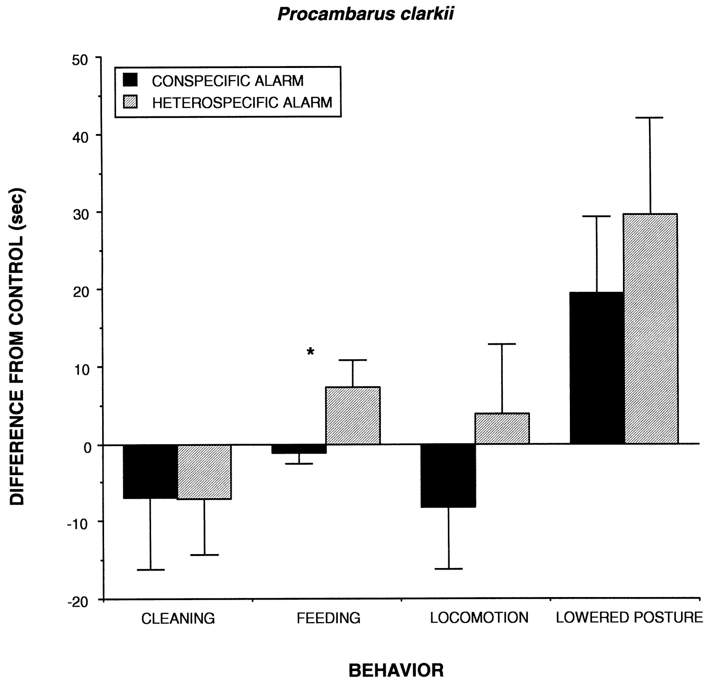

Figure 1. Differences between the number of seconds spent in control periods and following the introduction of conspecific or heterospecific alarm odor for individuals of *P. clarkii*. Means and standard errors ($n = 20$) are shown for four behaviors: cleaning, feeding, locomotion, and lowered posture. Significant differences from paired *t*-tests are indicated by *.

compared to the time spent in behaviors during the control period showed that individuals of this invasive species responded just as strongly to heterospecific alarm (from *A. pallipes*) as to conspecific alarm except in the case of feeding behaviors (Figure 1, Table 1). For time spent cleaning, in the lowered posture, or locomotion the effects of the two types of alarm odors were not different. The time spent in cleaning decreased relative to controls and the time spent in the lowered posture increased (Figure 1) but to the same degree for the two sources of alarm odor.

Orconectes limosus. Comparison of the effects of conspecific and heterospecific alarm introduction in this invasive species showed no difference in responses to conspecific and heterospecific (from *P. clarkii*) alarm odors (Figure 2, Table 1). This was true for all four behaviors tested.

Austropotomobius pallipes. Individuals of this species which is native to Europe, including Italy, responded more strongly to conspecific alarm than to heterospecific alarm (from *P. clarkii*) (Figure 3). Comparing the time spent in behaviors when alarm odors were introduced compared to control periods, there were significant differences for cleaning, lowered posture, and locomotion (Table 1). In particular, the time spent in cleaning decreased following the introduction of alarm, but more strongly for conspecific alarm and the time spent in the lowered posture increased, but more strongly for conspecific alarm compared to heterospecific

Table 1. Results of paired *t*-tests, *t*-value and associated *P*-value, comparing the effects of conspecific and heterospecific alarm odors on the behaviors of individuals of three species of crayfish.

	P. clarkii		*O. limosus*		*A. pallipes*	
Behavior	*t*	*P*	*t*	*P*	*t*	*P*
Cleaning	0.03	0.97	0.25	0.80	2.67	0.01
Feeding	2.46	0.02	1.00	0.34	2.00	0.06
Locomotion	1.80	0.07	0.21	0.81	2.17	0.04
Lowered posture	1.20	0.23	0.70	0.46	2.77	0.01

Analyses were conducted on the differences in the number of seconds spent in the control period and following the introduction of conspecific vs. heterospecific alarm odors. In the case of *P. clarkii* the source of heterospecific alarm odor was *A. pallipes*, for *O. limosus* and *A. pallipes*, the source was *P. clarkii*.

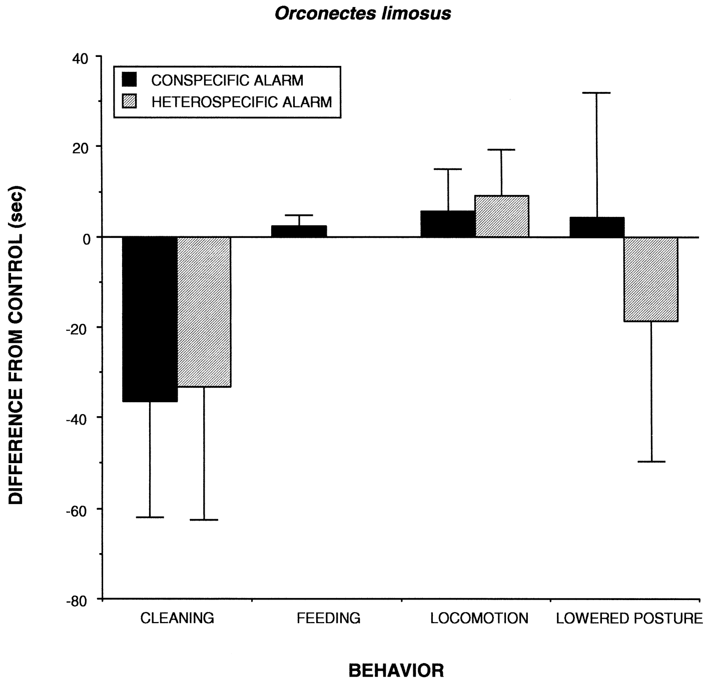

Figure 2. Differences between the number of seconds spent in control periods and following the introduction of conspecific or heterospecific alarm odor for individuals of *O. limosus*. Means and standard errors ($n = 8$) are shown for four behaviors: cleaning, feeding, locomotion, and lowered posture. Significant differences from paired *t*-tests are indicated by *.

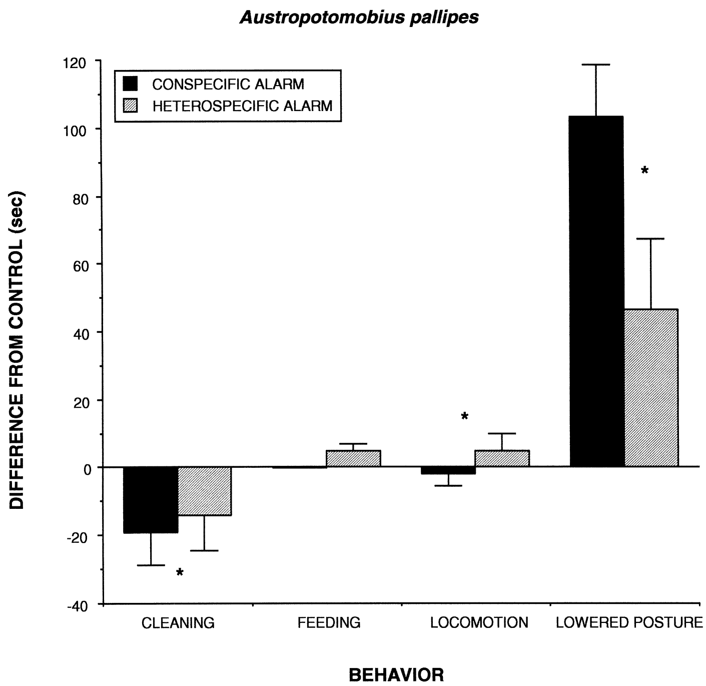

Figure 3. Differences between the number of seconds spent in control periods and following the introduction of conspecific or heterospecific alarm odor for individuals of *A. pallipes*. Means and standard errors ($n = 20$) are shown for four behaviors: cleaning, feeding, locomotion, and lowered posture. Significant differences from paired *t*-tests are indicated by *.

alarm. The difference for feeding was almost significant ($P = 0.06$).

Discussion

The results from the species tested in this study support the pattern suggested in Hazlett (2000), that individuals of successful invaders use a broader range of information concerning predation risk than do native species. Individuals of *P. clarkii* responded just as strongly to alarm odors prepared by crushing individuals of the crayfish species *A. pallipes*, as they did to conspecific alarm odor. We could predict that *P. clarkii* is using a very general cue common to the hemolymph of decapod crustaceans. *P. clarkii* has had negative impacts upon the distributional ranges of several species of crayfish following introductions related to aquaculture efforts and both the native Italian river crab (Gherardi et al. 1999) and several species of river crabs in African systems have been negatively affected (Hobbs et al. 1989).

The other North American invasive tested, *O. limosus*, has spread in several places in Europe following introduction. As was the case with the invasive *O. rusticus* (Hazlett 2000) and *P. clarkii*, individuals of *O. limosus* responded just as strongly to heterospecific alarm odor as they did to conspecific alarm odors. Similarly, the invasive *C. albidus* also responded similarly to heterospecific odors and conspecific odors (Gherardi et al. 2002). Thus, all four crayfish species tested that have undergone large range expansions following human-mediated introductions show a similar pattern of use of a broad range of chemical cues associated with increased predation risk. Assuming that such behavioral responses increase the probability of avoiding predation (Downes 2002; Mathis and Smith 1993), this ability could contribute to success in new environments that may well contain novel predators feeding on a variety of species of crayfish species.

Individuals of the native Italian species, *A. pallipes*, did not respond as strongly to heterospecific alarm odors as they did to conspecific alarm odors. This is similar to the pattern seen in the North American species *O. propinquus* (Hazlett 2000) that has not been expanding its range in recent time and has been negatively impacted by the spread of *O. rusticus* (Hill and Lodge 1994). The Australian native, *C. tenuimanus*, also responds more to conspecific than to heterospecific alarm odors and has been affected by the introduction of *C. albidus* (Gherardi et al. 2002). Thus the three native, non-expanding species studied all showed a similar pattern of reduced effects of heterospecific alarm odors relative to conspecific alarm odor.

Behavioral plasticity should be greater in species that more readily inhabit a larger range of habitats. Species that have evolved in more speciose regions may respond to a wider array of chemical cues because the evolutionary opportunities for individuals to profit from use of cues common to a wide array of species have been present. Thus *O. rusticus* originated in a more species-rich region of southeast USA than did *Orconectes virilis* or *O. propinquus* (Hazlett 2000). *Orconectes limosus* originated in the mid-Atlantic region of North America (Hobbs 1974) and overlaps in distribution with many species (Taylor et al. 1996). And *P. clarkii* originated from the center of distribution of the very speciose genus *Procambarus* (Hobbs 1989) while *A. pallipes* is the only crayfish species found in most of its range prior to various recent introductions of non-native species (Holdrich 1991).

A further aspect of behavioral plasticity which has been compared in several crayfish species is the ability to learn and remember an association between different predation-risk cues. When trained similarly to associate a novel cue (goldfish odor) with predation risk by pairing with conspecific alarm odor, individuals of the invasive species *O. rusticus* and *P. clarkii* remembered that association longer than did native species (*O. virilis* and *A. pallipes*) (Hazlett et al. 2002). It will be of interest to test invasive and native species of crayfish with additional tests of behavioral plasticity to clarify if plasticity in general is greater in those species that more readily expand their ranges.

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References

Bouwma P and Hazlett BA (2001) Integration of multiple predator cues by the crayfish *Orconectes propinquus*. Animal Behaviour 61: 771–776

Downes SJ (2002) Does responsiveness to predator scents affect lizard survivorship? Behavioral Ecology and Sociobiology 52: 38–42

Garvey JE, Stein RA and Thomas HM (1994) Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. Ecology 75: 532–547

Gherardi F, Baldaccini GN, Ercolini P, Barbaresi S, DeLuise G, Mazzoni D and Mori M (1999) The situation in Italy. In: Gherardi F and Holdich DM (eds) Crayfish in Europe as Alien Species. How to Make the Best of a Bad Situation? pp. 107–128. Balkema, Rotterdam, The Netherlands

Gherardi F, Acquistapace P, Hazlett BA and Whisson G (2002) Behavioural responses to danger odours in indigenous and non-indigenous crayfish species: a case study from Western Australia. Marine and Freshwater Research 53: 93–98

Hazlett BA (1994) Crayfish feeding responses to zebra mussels depend on microorganisms and learning. Journal of Chemical Ecology 20: 2623–2630

Hazlett BA (1999) Responses to multiple chemical cues by the crayfish *Orconectes virilis*. Behaviour 136: 161–177

Hazlett BA (2000) Information use by an invading species: do invaders respond more to alarm odors than native species? Biological Invasions 2: 289–294

Hazlett BA, Acquistapace P and Gherardi F (2002) Differences in memory capabilities in invasive and native crayfish. Journal of Crustacean Biology 22: 439–448

Hill AM and Lodge DM (1994) Diel changes in resource demand: competition and predation in species replacement among crayfishes. Ecology 75: 2118–2126

Hill AM and Lodge DM (1999) Replacement of resident crayfish by an exotic crayfish: the roles of competition and predation. Ecological Applications 9: 678–690

Hobbs HH Jr (1974) A checklist of the North and Middle American crayfishes (Decapoda: Astacidae and Cambaridae). Smithsonian Contributions to Zoology 166: 1–161

Hobbs HH Jr (1989) An Illustrated Checklist of the American Crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). Smithsonian Institution Press, Washington, DC, 236 pp

Hobbs HH III (1993) Trophic relationships of North American freshwater crayfishes and shrimps. Milwaukee Public Museum Contributions in Biology and Geology 85: 1–110

Hobbs HH III, Jass JP and Huner JV (1989) A review of global crayfish introductions with particular emphasis on two North America species (Decapoda, Cambaridae). Crustaceana 56: 299–316

Holdrich DM (1991) The native crayfish and threats to its existence. British Wildlife 2: 141–161

Mathis A and Smith RJF (1993) Chemical alarm signals increase the survival time of fathead minnows (*Pimephales promelas*) during encounters with northern pike (*Esox lucius*). Behavioral Ecology 4: 260–265

Schweitzer JA and Larson KC (1999) Greater morphological plasticity of exotic honeysuckle species may make them better invaders than native species. Journal of the Torrey Botanical Society 126: 15–23

Taylor CA, Warren ML Jr, Fitzpatrick JF Jr, Hobbs HH III, Jezerinac RF, Plieger WL and Robison HW (1996) Conservation status of crayfishes of the United States and Canada. Fisheries 21: 25–38