

## RESPONSES OF THE CRAB *Heterozius rotundifrons* TO HETEROSPECIFIC CHEMICAL ALARM CUES: PHYLOGENY VS. ECOLOGICAL OVERLAP

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**Abstract**—The big-handed brachyuran crab *Heterozius rotundifrons* extends the time spent in its anti-predator posture, limb extended posture, if exposed to chemical cues from crushed conspecifics. In this study, we tested whether crabs also respond to chemical cues from crushed heterospecific crabs, and if so, whether phylogenetic relations or ecological overlap is more important in influencing the duration of the anti-predator posture. Chemical cues from two other brachyuran crabs (*Cyclograpsus lavauxi* and *Hemigrapsus sexdentatus*), which do not overlap directly in ecological distribution with *H. rotundifrons*, elicited a duration of the anti-predator posture that was indistinguishable from that produced by conspecific chemical cues. In contrast, chemical cues from two anomuran crabs (*Petrolisthes elongatus* and *Pagurus novizealandiae*) that overlap in ecological distribution with *H. rotundifrons*, elicited durations of the antipredator posture that were significantly shorter than those of either conspecifics or more closely related crab species. Thus, phylogenetic relationship seems to be more important than ecological overlap in influencing anti-predator behavior in *H. rotundifrons*.

**Key Words**—*Heterozius rotundifrons*, alarm cues, heterospecific, ecological context, phylogenetic history, crabs

### INTRODUCTION

Virtually all species of animals tested show an increase in anti-predator behaviors when chemical cues from an injured or crushed conspecific are detected (Smith,

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1992; Chivers and Smith, 1998; Wisenden, 2000). Because the execution of anti-predator behavior results in a decrease in successful predation (Mathis and Smith, 1993; Mirza and Chivers, 2001), it is not surprising that selection has favored lineages that respond to cues from damaged conspecific individuals. In some species, responses to cues from damaged heterospecific individuals also occur (Wisenden et al., 1999; Chivers, 2002; Mirza and Chivers, 2003). Given the generalized diet of many predators, it is reasonable to predict that prey would be selected to utilize any information that indicates elevated risk of predation.

While it is clear that some prey learn to recognize heterospecific cues (Brown and Chivers, In Press), the literature on the use of heterospecific cues is mixed as to whether phylogenetic relationship or ecological overlap is more important in determining what cues are utilized by prey. That is, do prey treat cues from more closely related animals as good indicators of predation risk or is the extent to which animal species overlap ecologically a stronger determinant of cue utilization? Learning cues would mandate experience, and, thus, ecological overlap, while related species might release similar cues to those released by damaged conspecifics, and be innately recognized.

Individuals of the Australian hermit crab *Clibanarius infraspinus* responded to haemolymph from two congeners (*C. taeniatus* and *C. virescens*) just as strongly as to conspecific haemolymph but responded more weakly to haemolymph from the distantly related diogenid hermit crab *Diogenes avarus* (Hazlett, 1996) even though *D. avarus* overlaps in microdistribution with *C. infraspinus* and *C. virescens* does not. Rittschof et al. (1992) reported individuals of the hermit crab *C. vittatus* did not respond at all to cues from *Pagurus* species (different families) even though they overlap in microdistribution. The *Pagurus* species each responded most strongly to fluids from conspecifics, less strongly to congeneric fluids and even less to fluids from *C. vittatus* (Rittschof et al., 1992).

In contrast, Mirza and Chivers (2003) found that minnows respond to cues from injured damselfly larvae (obviously unrelated) but only when the minnows are small and in the same prey guild as damselfly larvae. In this case, ecological context appears more important than phylogenetic relatedness. Stenzler and Atema (1977) found that three species of the mud snail *Nassarius* responded to each others alarm substance, responded strongest to conspecific substance, but did not respond to cues from other gastropod genera. However, one species, *N. trivittatus*, responded to the sympatric, but taxonomically not closely related, *Urosalpinx cinerea* (Stenzler and Atema, 1977). Mirza and Chivers (2003) cite a number of cases in both aquatic and terrestrial systems where similarities in ecology appear to be more important than degree of relatedness.

Individuals of crayfish species in Australia (Gherardi et al., 2002), Italy (Hazlett et al., 2003) and North America (Hazlett, 2000), show distinct differences in whether or not they respond as strongly to heterospecific cues as to conspecific cues. However, the pattern of response does not seem to relate to phylogeny but

rather to whether species have evolved in specious regions and are successful invaders or being displaced. Those species that have recently successfully expanded their ranges respond more strongly to heterospecific danger signals, and this pattern of responses is not linked to how closely related the crayfish species are.

*Heterozius rotundifrons* is a New Zealand endemic crab that has an unusual anti-predator behavior. When stimulated by tactile input, crabs assume a limb-extended posture in which the cheilpedes are spread almost 180° apart (Field, 1990) and remain motionless in this posture for a number of minutes. This posture is an effective defense against fish predators (Hazlett and McClay, 2000), and the duration of the posture is extended upon introduction of fluids from crushed conspecifics (Hazlett and McClay, 2000).

The purpose of this study was to see if individuals of *H. rotundifrons* responded to cues generated from crushed heterospecific crabs and, if so, which was more important in determining the strength of responses, phylogenetic relationship or ecological overlap.

#### METHODS AND MATERIALS

*Heterozius rotundifrons* A. Milne Edwards, 1867 used in this study were collected from the vicinity of the Edward Percival Field Station in Kaikoura, New Zealand and transported to the salt-water facility of the School of Biological Sciences, University of Canterbury, Christchurch, New Zealand. The experiments were conducted in May 2003 at the University of Canterbury. Individuals of *Heterozius rotundifrons* were tested with alarm cues prepared from one of five crustacean species, conspecific cues, and four other New Zealand species. We tested 20 different individuals of *H. rotundifrons* per species.

In all cases, the number of seconds spent in the limb-extended posture were recorded following induction of this defensive posture by grasping a crab, turning it upside-down and then placing it right-side up on the layer of gray sand in a plastic container with 750 ml of sea water and 25 ml of alarm cue. Field (1990) has shown that this method of tactile induction is the most reliable way to induce this behavior. The end of about of the limb-extended posture was recognized by the movement of any ambulatory leg or cheliped.

The five species used to prepare the alarm cues were *Heterozius rotundifrons*, *Cyclograpsus lavauxi*, *Hemigrapsus sexdentatus*, *Petrolisthes elongatus*, and *Pagurus novizealandiae*. *C. lavauxi* and *H. sexdentatus* are brachyurans, as is *H. rotundifrons*, but do not overlap directly in distribution in the intertidal, as these grapsids occur higher in the intertidal (McLay, 1988). In contrast, individuals of *P. elongatus* and *P. novizealandiae* overlap extensively in distribution with *H. rotundifrons*, but are anomurans (McLay, 1988). The number of individuals used to prepare the alarm cue varied but was equivalent in weight to two medium-size *H. rotundifrons*. For each preparation, the crustaceans used for making the

alarm cue solutions were thoroughly crushed and mixed in 200 ml of sea water and filtered with coarse filter paper. Twenty-five ml of the crushed crustacean solution were added to the plastic container in which the test individual of *H. rotundifrons* was placed.

A one-way ANOVA was used to compare the difference between number of seconds spent in the limb-extended posture by the 20 crabs placed in the different alarm cue preparations. The five test preparation values were compared with Tukey tests.

## RESULTS

There was significant variation in the duration of the limb-extended posture among the five alarm cue preparations (ANOVA  $F = 3.37$ ,  $df = 4,95$ ,  $P = 0.013$ ). As shown in Figure 1, the time spent in the posture was similar for the three brachyuran crab preparations (*H. rotundifrons*, *C. lavauxi*, and *H. sexdentatus*), and the Tukey test values comparing those three indicated no difference between them (*H. rotundifrons*–*C. lavauxi*  $P = 0.809$ , *H. rotundifrons*–*H. sexdentatus*  $P = 0.962$ ). The number of seconds spent in the posture when the test crabs were placed in either of the anomuran crab preparations was significantly

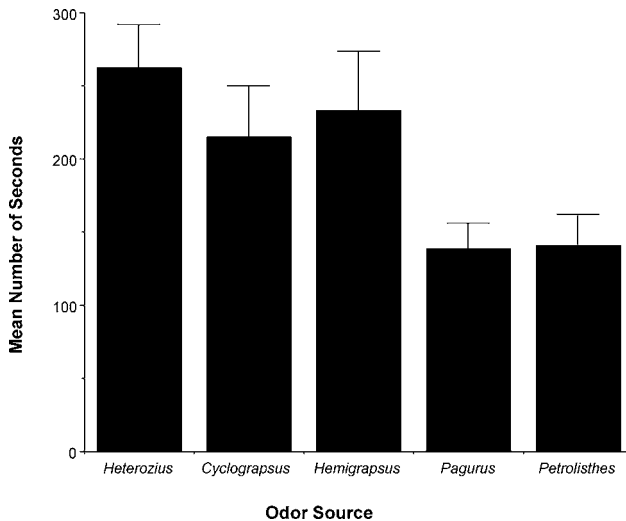


FIG. 1. Number of seconds (means + s.e.) spent in the limb-extended posture by individuals of *Heterozius rotundifrons* when placed in sea water containing chemical cues prepared with one of five crustacean species: *H. rotundifrons*, *Petrolisthes elongatus*, *Cyclograpsus lavauxi*, *Hemigrapsus sexdentatus*, and *Pagurus novizealandiae*.

lower (*H. rotundifrons*–*P. elongatus*  $P = 0.045$ , *H. rotundifrons*–*P. novaezealandiae*  $P = 0.039$ ). These values were similar to the values reported in other studies when only tactile stimulation and no chemical cues were added (Hazlett and McLay, 2000, In Press).

#### DISCUSSION

Individuals of *H. rotundifrons* responded to the cues of crushed heterospecific crabs, but those in the same broad taxonomic group produced a significantly greater response. Their response to cues from species with whom they overlap extensively in ecological micro-distribution but that are more distantly related was significantly less than to related crabs. In previous investigations, the control group of physically stimulated crabs that received no chemical cues from damaged conspecifics showed durations of response similar to those seen here to the non-related crabs (Hazlett and McLay, 2000). This suggests that the shorter responses seen for the non-related crabs could, in fact, be no response to chemicals at all, just the response to being physically stimulated. *Heterozius* is a monotypic genus placed in the Family Bellidae, closely allied to the Family Xanthidae. Thus, while it is in the same order (Brachyura) as the two grapsid species used in this study, the three brachyruans are not closely related. The crabs in the Order Anomura are only distant phylogenetically. While tests with a range of concentrations would provide additional details and an assessment of the ecological reality of responding to these cues in the much lower concentrations that might be encountered in the field, the responses of *Heterozius* to approximately equal concentration of cue from two closely related vs. two distantly related crabs is consistent.

The results of this study suggest a priority of phylogeny over ecological overlap in determining the cues to which a potential prey species will respond. This finding is consistent with the majority, but not all, of similar studies using crabs. Of course individuals of a species could respond to chemical cues more or less strongly based upon both phylogeny and ecology (Stenzler and Atema, 1977). Certainly, ecological overlap sets the stage for learning to use heterospecific cues. However, individuals of *H. rotundifrons* responded less to cues from unrelated than to cues from related crabs, and given that previous studies with this species (Hazlett and McClay, 2000) show that tactile stimulation without any chemical alarm cues can cause anti-predator posture duration that is about equal to our treatments with unrelated crab cues, it is possible that there was no response to unrelated species. It is conceivable that *H. rotundifrons* does not share predators with the porcellanid, *P. elongatus*, or hermit, *P. novizealandiae*, crabs. If that were the case there would be no advantage in utilizing cues resulting from the crushing/predation of individuals of those species. Indeed, Mirza and Chivers (2003) showed that the minnow, *Pimephales promelas*, responded to cues from

attacks on damselfly larvae, *Enallagma boreale* only when minnow size dictated the two species were in the same prey guild.

As has been pointed out elsewhere (Thacker, 1994), one might expect phylogenetic relationship to be important in determining cue use, because of the similarity of the chemicals released from the bodies of related species. Thus, individuals of *H. rotundifrons* could be responding to a cue that is common to all brachyurans but absent from anomurans. Unless a species evolved under predation pressure from a taxonomically very specialized predator, selection may favor use of a generalized cue.

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#### REFERENCES

- BROWN, G. E. and CHIVERS, D. P. In Press. Learning as an adaptive response to predation. In P. Barbosa (ed). *Ecology of Predator-Prey Interactions*, Oxford Univ. Press.
- FIELD, L. H. 1990. Aberrant defense display of the big-handed crab, *Heterozius rotundifrons* (Brachyura: Belliidae). *New Zealand J. Marine and Freshwater Res.* 24:211–220.
- GHERARDI, F., ACQUISTAPACE, P., HAZLETT B. A., 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 Res.* 53:93–98.
- HAZLETT, B. A. 2000. Information use by an invading species: do invaders respond more to alarm odors than native species? *Biol. Invasions* 2:289–294.
- HAZLETT, B. A., BURBA, A., GHERARDI, F., and ACQUISTAPACE, P. 2003. Invasive species of crayfish use a broader range of predation-risk cues than native species. *Biol. Invasions* 5:223–228.
- HAZLETT, B. A. 1996. Comparative study of hermit crab responses to shell-related chemical cues. *J. Chem. Ecol.* 22:2317–2329.
- HAZLETT, B. A. and McLAY, C. 2000. Contingencies in the behaviour of the crab *Heterozius rotundifrons*. *Anim. Behav.* 59:965–974.
- HAZLETT, B. A. and McLAY, C. In Press. Responses to predation risk: Alternative strategies in the crab *Heterozius rotundifrons*. *Anim. Behav.*
- MATHIS, A. and SMITH, R. J. F. 1993. Chemical alarm signals increase the survival time of fathead minnows (*Pimephales promelas*) during encounters with northern pike (*Esox lucius*). *Behav. Ecol.* 4:260–265.
- McLAY, C. 1988. Brachyura and crab-like Anomura of New Zealand. *Leigh Lab. Bull.* 22:1–463.
- MIRZA, R. S. and CHIVERS, D. P. 2001. Are alarm signals conserved within salmonid fishes? *J. Chem. Ecol.* 27:1641–1655.
- MIRZA, R. S. and CHIVERS, D. P. 2003. Influence of body size on the responses of fathead minnows, *Pimephales promelas*, to damselfly alarm cues. *Ethology* 109:691–699.
- RIITTSCHOF, D., TSAI, D. W., MASSEY, P. G., BLANCO, L., KUEBER, G. L., JR., and HAAS, R. J. 1992. Chemical mediation of behavior in hermit crabs: alarm and aggregation cues. *J. Chem. Ecol.* 18:959–984.
- SMITH, R. J. F. 1992. Alarm signals in fishes. *Rev. Fish Biol. Fish.* 2:33–63.
- STENZLER, D. and ATEMA, J. 1977. Alarm responses of the marine mud snail, *Nassarius obsoletus*: specificity and behavioral priority. *J. Chem. Ecol.* 3:159–171.

- THACKER, R. W. 1994. Volatile shell-investigation cues of land hermit crabs: Effect of shell fit, detection of cues from other hermit crab species, and cue isolation. *J. Chem. Ecol.* 20: 1457–1482.
- WISENDEN, B. D. 2000. Olfactory assessment of predation risk in the aquatic environment. *Phil. Trans. R. Soc. Land. B* 355:1205–1208.
- WISENDEN, B. D., CLINE, A., and SPARKES, T. C. 1999. Survival benefits to antipredator behaviour in the amphipod *Gammams minus* (Crustacea: Amphipoda) in response to injury-released chemical cues from conspecific and heterospecifics. *Ethology* 105:407–414.