RESPONSES OF CAMBARID CRAYFISH TO PREDATOR ODOR

BRIAN A. HAZLETT[1] and DONALD R. SCHOOLMASTER[2]

*[1]Department of Biology, University of Michigan,
Ann Arbor, Michigan 48109*

*[2]Department of Biology, Eastern Michigan University,
Ypsilanti, Michigan 48197*

Abstract—The responses of individuals of four sympatric species of cambarid crayfish to the introduction of the odor of a common predator, the snapping turtle *Chelydra serpentina*, were recorded in the laboratory. Adult *Orconectes virilis* spent significantly more time in a lowered posture and reduced the frequency of nonlocomotory movements following introduction of snapping turtle odor but showed no change in behavior upon introduction of the odor of painted turtle (*Chrysemys picta*). Recently released young *O. virilis* did not respond to snapping turtle odor initially but did so after turtle odor and conspecific alarm odor had been paired. Individuals of *O. propinquus* did not respond to snapping turtle odor. Initial tests with *O. rusticus* did not yield any response to snapping turtle odor but after experience with paired turtle and alarm odor, individuals showed a decrease in nonlocomotory movements when just snapping turtle odor was introduced. Individuals of *Cambarus robustus* spent less time in the lowered posture, less time in their burrow, and more frequently executed nonlocomotory movements, in response to snapping turtle odor. The differences in responses to the odor of a common predator are correlated with ecological differences among the crayfish species.

Key Words—Crayfish, predator odors, chemical cues, behavior, snapping turtle, learning, *Orconectes*, *Cambarus*.

INTRODUCTION

Behavioral responses to cues from potential predators are common in a variety of animals and are to be expected if these responses decrease the probability of predation (Mathis and Smith, 1993a). In aquatic systems, one of the most commonly utilized classes of predator avoidance cues is chemical (Chivers et al., 1996; Kiesecker et al. 1996; Smith, 1992). Differences among related species

with regards to their responses to predator cues are of particular interest when such differences can alter competitive interactions (Werner and Anholt, 1993) and ultimately alter the composition of biological communities.

Past studies on the responses of crayfish to chemical cues from predators have reported that some species do not respond to predator odors, while other species do show responses. Young *Pacifastacus leniusculus* responded to chemical cues from fish predators (Blake and Hart, 1993) as did the parastracid *Paranephrops zealandicus* (Shave et al., 1994). However, Willman et al. (1994) reported no changes in the behavior of *Orconectes propinquus* or *O. virilis* when individuals were exposed to odors from largemouth bass (*Micropterus salmoides*), while individuals of *O. rusticus* increased the time spent out of shelters in the presence of the fish predator cue.

The authors of these studies used somewhat different methods and thus the differences in responsiveness could be related to either methodological differences or to ecological or phylogenetic differences among the species studied. Because a learned association between predator odor and alarm odor has been demonstrated for individuals of fish and insects (Chivers et al., 1996; Mathis and Smith, 1993b), differential experience must also be considered in explaining species differences.

The following tests were conducted to resolve the question of variation in responsiveness to predator cues among species of crayfish by using the same methodology on adult individuals of four species of crayfish occurring in the same area and all subject to predation by a common predator, the snapping turtle, *Chelydra serpentina*. Given the importance of predator–crayfish interactions in determining species replacements (Butler and Stein, 1985; DiDonato and Lodge, 1993; Garvey et al., 1994; Mather and Stein, 1993; Quinn and Janssen, 1989; Stein, 1977) and, in particular, the continuing replacement of native species by *O. rusticus* (DiDonato and Lodge, 1993; Garvey et al., 1994; Hill and Lodge, 1994; Olsen et al., 1991), it seemed particularly important to use a uniform method to examine the responses of several species of sympatric crayfish to the introduction of the odor of a predator they have in common, the snapping turtle. The role of experience in the establishment of a response by individuals of *O. rusticus* and young *O. virilis* was also examined. The specificity of the response of adult *O. virilis* was also tested by using the odor of another species of turtle.

METHODS AND MATERIALS

Experiments with Adult Crayfish. Individuals of the four most common crayfish species (Family Cambaridae) found in Michigan were tested in the laboratory. Adult *Orconectes virilis* and *O. propinquus* were collected from the

Maple River in Emmet County, Michigan, and were tested at the University of Michigan Biological Station, Pellston, Michigan. Berried female *O. virilis* collected from ponds in Saline, Michigan, were tested at the University of Michigan in Ann Arbor, Michigan. The embryos in the eggs on the pleopods of these females were well developed, and in most cases young hatched a few days after the females were tested. *O. rusticus* were obtained from a commercial supplier in Wisconsin and tested in Ann Arbor. *Cambarus robustus* were collected from Fleming Creek near Ann Arbor and tested in the laboratory at Eastern Michigan University, Ypsilanti, Michigan.

For each species, crayfish were placed in individual 10-gallon aquaria. These aquaria were visually isolated from one another, well aerated, contained 12.5 liters of continually aerated well water, and half a clay pot for a burrow. Crayfish were allowed three days to acclimate to the aquaria before testing, and each crayfish was tested just once. Behavior patterns (see below) were detected by an observer and the temporal duration of patterns recorded for 5 min on a portable computer with an event program. For every experiment, behavioral responses were recorded for the same individual during two time periods: (1) 5-min control period following the introduction of 20 ml of clean well water, and then (2) a 5-min period following the introduction of 20 ml of test water. All solutions were introduced quietly via syringe into one corner of the aquarium. The amount of stimulus water used was chosen based upon similar studies on other crustaceans (Hazlett, 1996, 1997).

The behavior patterns recorded were: (1) time spent in the burrow, (2) time spent locomoting by movement of the ambulatory legs, (3) time spent moving any appendage in activities other than locomotion (i.e., grooming, feeding movements of the ambulatory legs, movements of the chelipeds, movements of the antennae), with the exception of flicking of the antennules, and (4) time spent in each of three postures: raised, intermediate, lowered (Hazlett, 1994). The postures were characterized as follows: in the raised posture, the body was elevated off the substrate, the chelipeds held off the substrate parallel to the substrate or even higher, and the abdomen and telson were extended. In the intermediate posture, the body was held just off the substrate, the tips of the chelipeds lightly touched the substrate, and the telson was perpendicular to the substrate. In the lowered posture, the body was in contact with the substrate, the chelipeds drawn in towards the body, and the telson curled under the abdomen.

Twenty individuals were tested for each experiment. The only exception was the set of tests involving female *O. virilis* that were bearing young, only 12 of which were tested. For these animals the only behavior pattern recorded was the number of aeration movements of the egg-laden pleopods during control and test periods. All tests were run between 09:00 and 12:00 hr.

Responses to predator odor were tested for all four species of crayfish. The

source of predator odor was the snapping turtle, *Chelydra serpentina*, an important predator of all the species tested (Hobbs, 1993). For all tests, the 3.2-kg turtle was not fed for three days prior to being placed in 27 liters of clean, aerated well water and left for 24 hr. Predator odor was always freshly drawn from the snapping turtle's aquarium because the effectiveness of this odor decreases in a few hours (Hazlett, unpublished observations).

To test the specificity of the response of adult *O. virilis*, individuals were tested for responses to the odor of another turtle, the painted turtle *Chrysemys picta*. The painted turtle odor was generated by holding a 315-g turtle in 2.7 liters of water for 24 hr; this was the same weight-to-volume ratio as for the snapping turtle tests.

Because the initial tests with *O. rusticus* indicated no response to snapping turtle odor (see below) and because work with other aquatic species indicated a role of experience in recognition of predator odor (Chivers et al., 1996; Mathis and Smith, 1993b), two additional sets of tests were conducted with *O. rusticus*. To test for a response to alarm odor, individuals were exposed to 20 ml of alarm odor generated by crushing a 30-mm cephalothorax length *O. rusticus*, placing it in 400 ml of distilled water, and filtering through coarse filter paper. Individuals were then placed in a communal tank with 27 liters of water, and 400 ml of alarm odor and 400 ml of turtle odor were introduced to the tank simultaneously. This paired introduction was done three time: at 36, 24, and 6 hr prior to placing the crayfish in individual observation aquaria. Observations were made following control and snapping turtle odor introductions 18 hr later.

Pairing of odors was not done with *O. propinquus*, the other species that showed no response to predator odor, because it does not respond to alarm odor (Hazlett, 1994). Without a response to the unconditioned stimulus (alarm odor), it would make little sense to pair another stimulus with the unconditioned stimulus.

For each experiment, paired *t* tests were used to compare the number of seconds spent in the various activities and postures during test and control periods because observations were made on the same individuals under both conditions. Because the crayfish had to be in one of the three postures, the time spent in two postures dictates the time spent in the third. Therefore only two of the postures were analyzed statistically (intermediate and lowered) because less than 10% of the time overall was spent in the raised posture.

Experiments with Young O. virilis. Following testing, female *O. virilis* bearing young were placed in a 30-gallon aquarium and over the following week a number of young were released. About two weeks later young were removed, placed individually in small ceramic cups containing 10 ml of well water, and observed under 6× power with a dissecting microscope. The microscope was needed to see cleaning and feeding movements of the walking legs of the young which were 8-9 mm in total length at the time of testing. The ceramic cups had

a flat bottom and were opaque and thus visually isolated the young crayfish. After a 3-min acclimation period, individuals were observed for 3 min following the introduction of 0.5 ml of well water and then for 3 min following the introduction of 0.5 ml of snapping turtle odor. The number of seconds spend executing nonlocomotory movements (mostly cleaning and feeding movements of the ambulatory legs), locomoting, and on the sides of the observation cup were recorded on a computer with an event program. Individuals could execute two of the behaviors simultaneously or be executing none of the three behavior patterns at any point during observations. Ten individuals were observed and were then placed in a separate holding aquarium following testing. Paired t tests compared the time spent in the different behaviors during test and control periods. The young of the other crayfish species were not available for testing.

Because the young $O.$ $virilis$ did not respond in the same fashion to snapping turtle odor as adults of this species, two other experiments were conducted. First a set of 10 individuals was tested for a response to conspecific alarm odor (Hazlett, 1994). A small adult $O.$ $virilis$ (32 mm carapace length) was crushed in 150 ml of distilled water and the liquid filtered through course filter paper. Responses to the introduction of 0.5 ml control water and alarm odor were recorded, and these individuals were then placed in the holding aquarium. These young were then exposed simultaneously to snapping turtle odor and alarm odor by adding 100 ml of each to the holding aquarium 24 and 6 hr prior to testing. The young were then removed and carefully rinsed four times in clean well water by transfers to new containers (to remove odors from the holding container). Ten individuals were then tested with control and snapping turtle odor in the same manner as described above.

RESULTS

Adult individuals of $Orconectes$ $virilis$ showed two changes in behavior when the predator odor was introduced (Table 1). The crayfish spent less time in the intermediate posture and more time in the lowered posture, and the amount of time during which there was nonlocomotory movement decreased (Figure 1A). There were no other significant changes in behavior, although there was a trend towards less locomotion. In many cases, the crayfish appeared to freeze wherever it was in the tank and not move anything. Berried females significantly reduced the number of aeration movements of the pleopods when predator odor was introduced [control (mean $\pm$ SE) 212 $\pm$ 20.7; test, 147 $\pm$ 14.3; $t = 4.85$, $P = 0.001$]. Most of that reduction appeared to be in the first minute or so after odor introduction.

When the odor of painted turtle was introduced adult individuals of $O.$ $virilis$ showed no change in behavior compared to control periods (Figure

Table 1. Associated Probabilities and t Test Values from Comparisons of Number of Seconds Spent in Postures and Activities Shown by Individuals of Four Species of Crayfish ($N = 20$ for each) during 5-Minute Observation Periods Following Introduction of Control Water and Predator Water

	O. virilis		O. propinquus		O. rusticus		C. robustus	
	t	P	t	P	t	P	t	P
Posture								
Intermediate	2.1	0.048	1.2	0.22	1.4	0.16	1.7	0.097
Lowered	2.3	0.030	1.1	0.27	1.3	0.19	2.4	0.026
Movement								
Locomotory	1.7	0.096	1.0	0.31	0.1	0.91	1.2	0.23
Nonlocomotory	2.8	0.010	0.9	0.34	0.1	0.89	3.7	<0.001
In Burrow	0.3	0.74	1.6	0.12	0.9	0.34	2.2	0.037

1B). All comparisons between values for the test and control periods were associated with $P > 0.30$.

Individuals of *Orconectes propinquus* showed no change in behavior when predator odor was introduced (Table 1). There were no significant differences in either posture or activity between the test and control periods.

Similarly, individuals of *Orconectes rusticus* showed no change in behaviors compared to control periods when predator odor was introduced (Table 1). There were no significant differences in either posture or activity (Figure 2A), even though there was a tendency towards more time in the lowered posture and less in the itnermediate posture. Introduction of conspecific alarm odor resulted in a significant increase in time spent in the burrow, an increase in time in the lowered posture, and a decrease in time in the intermediate posture (Figure 2B). After experience with paired alarm and snapping turtle odor, individuals of *O. rusticus* showed a decrease in the amount of nonlocomotory movement when just snapping turtle odor was introduced (Figure 2C).

In contrast to the patterns shown by the above species, individuals of *Cambarus robustus* responded to the introduction of predator odor by spending less time in the lowered posture, less time in the burrow, and more time executing nonlocomotory movements (Table 1, Figure 3).
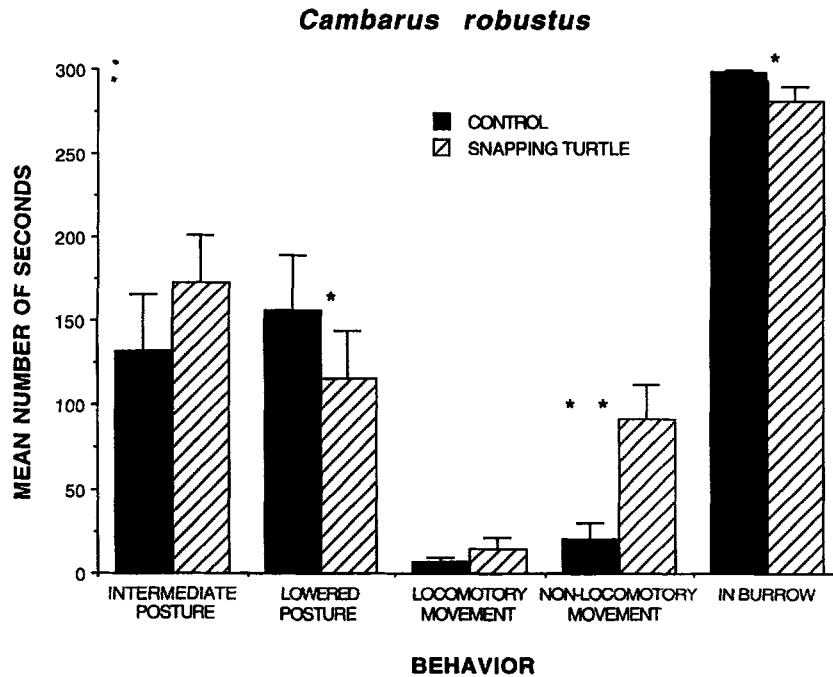
Fig. 1. Mean (+SE) number of seconds spent in postures and activities by adult individuals of *Orconectes virilis* during 5-min observation periods following introduction of: (A) snapping turtle odor and (B) painted turtle odor. Asterisks indicate a significant difference between test and control values.

Orconectes virilis

(A) SNAPPING TURTLE ODOR

(B) PAINTED TURTLE ODOR

BEHAVIOR

Orconectes rusticus

(A) NAIVE

(B) NAIVE

(C) EXPERIENCED

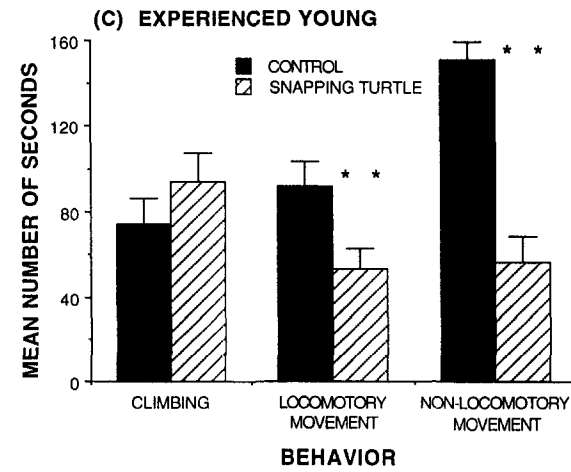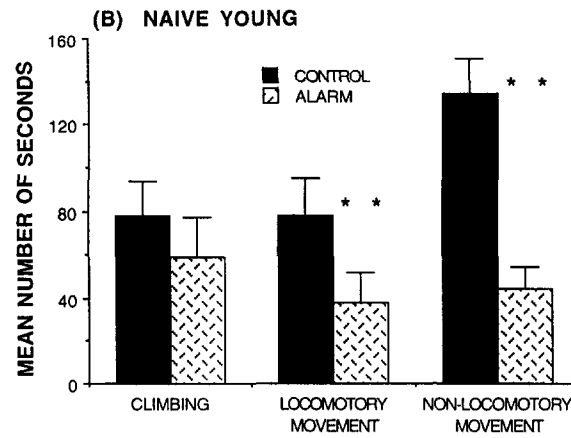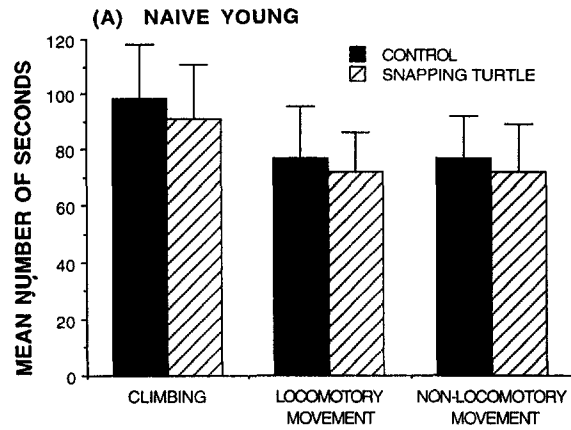BEHAVIOR
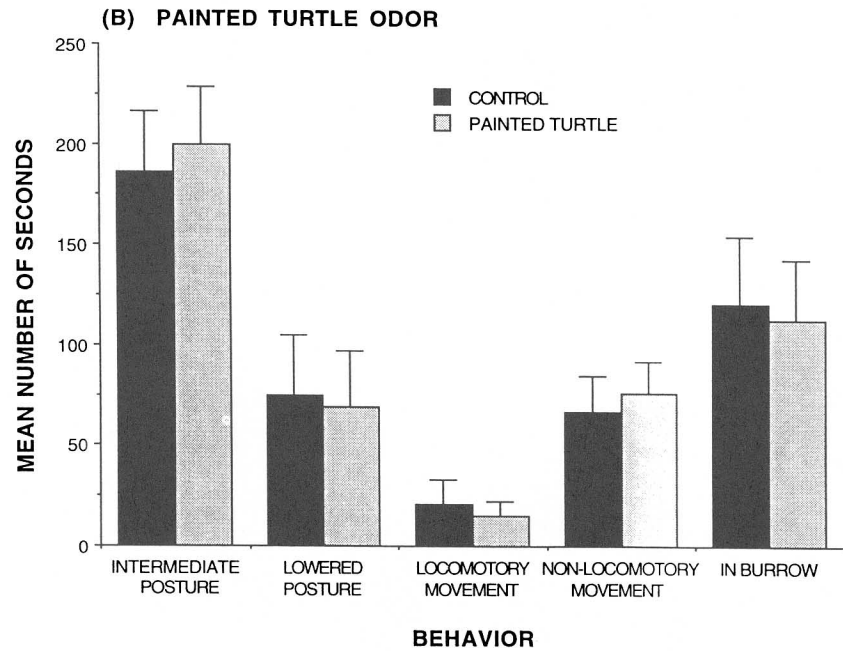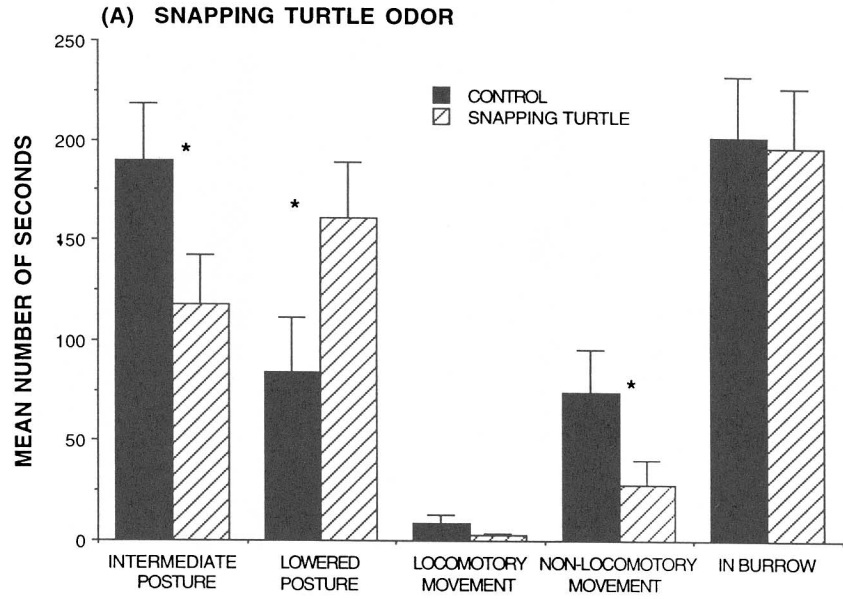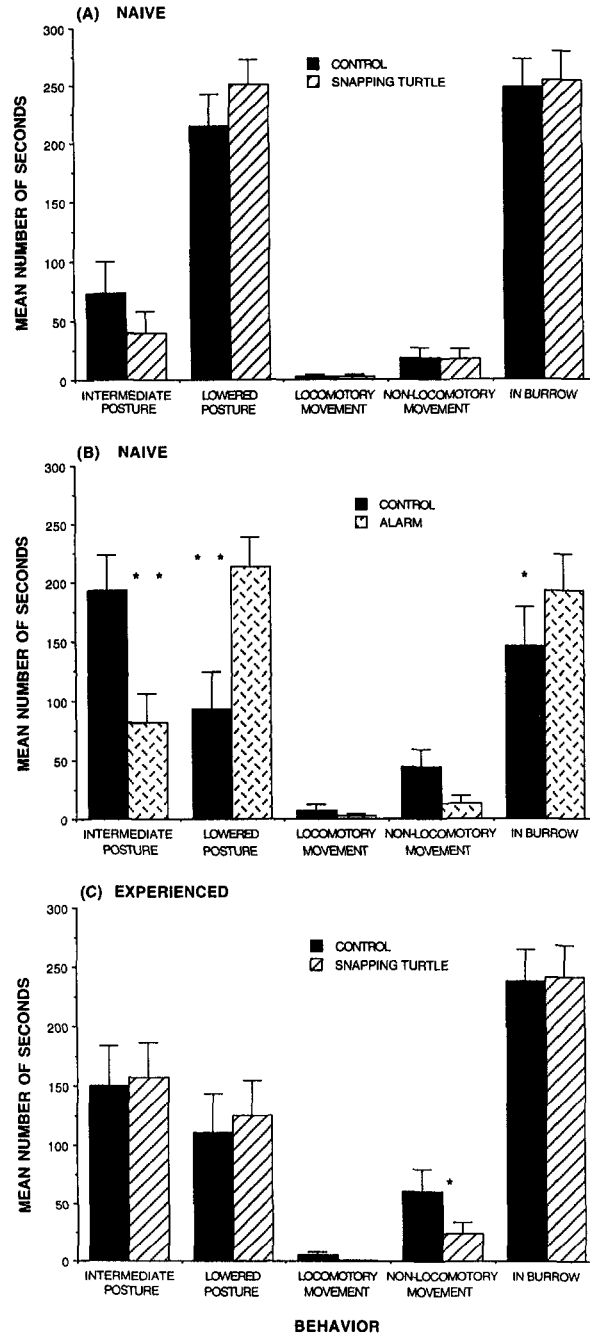
Cambarus robustus

FIG. 3. Mean (+SE) number of seconds spent in postures and activities by adult individuals of *Cambarus robustus* during 5-min observation periods following introduction of control and snapping turtle odor. Asterisks indicate a significant difference between test and control values.

When first tested, young individuals of *O. virilis* showed no changes in behaviors when snapping turtle odor was introduced (Figure 4A). Young did respond to alarm odor with a significant decrease in the time spent executing nonlocomotory movements ($t = 5.56$, $P < 0.001$) and locomotory movements ($t = 4.09$, $P = 0.003$) (Figure 4B). Following experience with alarm and predator odor simultaneously, young individuals showed a significant decrease in both locomotory movement ($t = 4.17$, $P = 0.002$) and nonlocomotory movement ($t = 8.66$, $P < 0.001$) following introduction of snapping turtle odor (Figure 4C).

FIG. 2. Mean (+SE) number of seconds in postures and activities by adult individuals of *Orconectes rusticus* during 5-min observation periods following introduction of: (A) snapping turtle odor to naive crayfish, (B) conspecific alarm odor to naive crayfish, and (C) snapping turtle odor to crayfish that had experienced simultaneous introduction of alarm and snapping turtle odors. Asterisks indicate a significant difference between test and control values.

Orconectes virilis

(A) NAIVE YOUNG

■ CONTROL
◨ SNAPPING TURTLE

(B) NAIVE YOUNG

■ CONTROL
◨ ALARM

(C) EXPERIENCED YOUNG

■ CONTROL
◨ SNAPPING TURTLE

BEHAVIOR

DISCUSSION

When tested under the same protocol, it is clear that there is variation in the responses of different species of crayfish to the odor of a common predator. Individuals of *Orconectes virilis* and experienced individuals of *O. rusticus* responded to snapping turtle odor by a marked decrease in nonlocomotory movements, primarily feeding and cleaning movements of the walking legs. A decrease in movement is a very common response to the detection of a predator (Werner and Anholt, 1993); individuals of the crayfish *Pacifastacus leniusculus* decreased movement after they detected fish predator odor (Blake and Hart, 1993). In the laboratory, snapping turtles respond strongly to movement by potential prey (Hazlett, unpublished observations). The lack of a change in locomotory movements by adult *O. virilis* may be simply a result of the low level of locomotion by these crayfish during the control periods. The lack of a response to painted turtle odor by *O. virilis* suggests that the crayfish can distinguish between the species of turtles chemically. At least for these adult crayfish, the painted turtle may not represent a serious threat of predation, even though this species of turtle does include crayfish in its diet (Hobbs, 1993).

The lack of a response by *O. virilis* to odor from a fish predator reported by Willman et al. (1994) may be because the authors did not record the time spent executing nonlocomotory movements or the time spent in different postures, the behaviors that changed the most in this study. Young *O. virilis* respond to the odor of fish predators by a decrease in nonlocomotory movements (Hazlett, unpublished observations).

Individuals of *Cambarus robustus* responded to predator odor in somewhat the opposite fashion by spending less time in the burrow, similar to the change reported for *O. rusticus* by Willman et al., (1994), and by increasing the locomotory movements shown following introduction of predator odor. The latter response would make sense for crayfish that more frequently live in a stream situation where detection of predator odor would indicate where the predator was (upstream) and directional movement away from the predator decreased predation risk. *C. robustus* is found primarily in rivers and fast-moving streams, while *O. virilis* and *O. rusticus* can be found in both streams and lakes (Crocker and Barr, 1968).

The most interesting result in this study was the lack of response by individuals of *O. propinquus*. It is always possible that these crayfish responded

FIG. 4. Mean (+SE) number of seconds spent in activities by young individuals of *Orconectes virilis* during 3-min observation periods following introduction of (A) snapping turtle odor to naive animals, (B) alarm odor from crushed conspecifics to naive animals, and (C) snapping turtle odor to animals that had simultaneously experienced alarm and predator odors. Asterisks indicate a significant difference between test and control values.

with a change in some behaviors not recorded in this study [or in that of Willman et al. (1994)] or the chemical cue primes the crayfish for detection of a second type of predator cue. The lack of a response to a chemical cue from a predator by individuals of *O. propinquus* is consistent with the lack of response to other types of chemical cues by individuals of this species, such as disturbance pheromones (Hazlett, 1990), alarm pheromones (Hazlett, 1994) and a less clear response to sex pheromones than *O. virilis* (Tierney and Dunham, 1982). I suggested elsewhere (Hazlett, 1994) that perhaps the more diurnal activity patterns of *O. propinquus* have resulted in less reliance upon chemical cues in general. Most species of crayfish are distinctly nocturnal, and this may be correlated with the use of chemical cues to various extents.

An alternative explanation for the lack of a response by *O. propinquus* is that it is considered an invasive species in the Midwest (Capelli and Munjal, 1982), and there has not been an evolutionary history of interactions with snapping turtles in that region. However, the same snapping turtle species occurs in the native ranges of this crayfish and, thus, there is an evolutionary history of interaction. A variation on this theme is that the particular individuals tested might not have had experience with the simultaneous detection of a particular predator odor and alarm substances. Experience has been shown to be necessary for predator recognition in fish (Mathis and Smith, 1993b), dameslflies (Chivers et al., 1996), and young *O. virilis* and adult *O. rusticus* (this study). In the case of the *O. propinquus* used in this study, they were obtained from the same stream as the *O. virilis* and presumably were exposed to the same experiential situations as the individuals of *O. virilis* that did respond to the predator odor. Snapping turtles readily feed upon individuals of *O. propinquus* (Keller and Hazlett, 1996). However, if in fact individuals of *O. propinquus* do not respond to alarm odors from conspecifics (Hazlett, 1994), this would mean there was no unconditioned chemical stimulus with which to associate predator odors and a learned association would not be possible for this species.

In conclusion, this study showed that there are significant differences in the responses of sympatric species of crayfish to the odor of an important predator. These results emphasize the importance of using a uniform methodology in comparative studies before any ecological explanations for differences among species can be put forth.

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