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RESPONSES OF THE CRAYFISH *ORCONECTES VIRILIS* TO CHEMICAL CUES DEPEND UPON FLOW CONDITIONS

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ABSTRACT

The responses of individuals of the crayfish *Orconectes virilis* to the same set of chemical cues were tested under flow (lotic) and non-flow (lentic) conditions. The cues presented to the crayfish were food cues, alarm (= crushed conspecifics) cues, and the combination of food + alarm cues. Crayfish behavior (time in burrows) and posture (lowered posture) were significantly affected by the odor treatment, the flow environment, and an interaction of treatment and environment. While the general patterns of responses to odors (more time spent feeding when food cues were introduced, and more time spent in lowered posture and in burrow when alarm cues were introduced) were qualitatively similar in the two environments, responses were stronger (especially to food odors) in the environment with flow. However, the different strength of these responses seemed not to be related to the directional information provided by the flow but rather was probably the effect of a difference in crayfish behavior between lentic and lotic environments in the control (no added chemical cues). In flow, crayfish spent less time executing feeding movements and more time in the lowered posture and in the burrow than in no-flow conditions.

INTRODUCTION

Animals must always respond to particular pieces of information (stimuli) they receive in the context of other information available to them. That context would include motivational variables, past experience with the stimuli, and other information about the current environment in which the stimulus is detected (Acquistapace et al., 2003). For example, how prey respond to cues indicating the presence of a predator depends upon hunger level (Hazlett, 2003a), motivational level related to other activities such as mating (Hazlett and Rittschof, 2000; Rohr and Madison, 2001), the temporal pattern of recent experience with the cue (Pecor and Hazlett, 2002), the availability of other sensory categories of information (Mitchell and Hazlett, 1996), and the structural complexity of the environment (Sih et al., 1988; Hartman and Abrahams, 2000).

The responses of many species to chemical cues depend upon flow of the medium in which the cue is carried. Many terrestrial species move upwind upon detection of a cue such as food odor or sex pheromone (Able, 1991). Flow direction, detected by sense organs other than the primary chemical sense organs, is part of the environmental context in which the chemical is detected and that information alters the responses shown to the chemical cue (Vickers, 2000). The effects of flow can be either subtle, as in the case of hermit crabs selecting heavier shells in flowing conditions (Hahn, 1998), or critical, as with the gastropod *Urosalpinx cinerea*, which does not respond to prey cues unless there is fluid flow (Brown and Rittschof, 1984). In aquatic environments, there is a general dichotomy of environments into those with little or no flow (lentic), such as ponds and lakes, and those with substantial unidirectional flow (lotic), such as streams or rivers. In both environments, complexities of local fluid movement arise (Moore and Atema, 1991; Moore et al., 1994), but, as a general rule, directional information about the source of chemical cues is more available in lotic environments, although cues are on average more patchily distributed (Weissburg, 2000).

Relatively few studies have compared the responses of the same species under different flow conditions. Sherman and Moore (2001) found that brown bullhead catfish (*Ameiurus negulosus*) swam faster under flowing conditions but in a more circuitous pattern. The bullheads also seemed less successful in finding an odor course under the flowing conditions, perhaps because of eddies and other complexities in the odor plume set up by flow. The blue crab *Callinectes sapidus* oriented to a food cue better in low-flow (less turbulence) conditions than in either high flow, or no-flow conditions (Weissburg and Zimmer-Faust, 1993).

The crayfish *Orconectes virilis* (Hagen, 1870) occurs in both lotic and lentic environments (Pearse, 1910; Crocker and Barr, 1968). Individuals living near the mouths of streams and rivers as they empty into lakes can experience both conditions over a short period of time. While young may be moved downstream in fast flowing water, upstream movement by adults can maintain populations in fast-flowing lotic environments (Hazlett et al., 1979). A considerable number of studies have been conducted on the responses of individuals of *O. virilis* to chemical cues both in lentic environments (aquaria without directional flow) (Hazlett, 1985a,b, 1989, 1994a, b, 1999, 2000, 2003a,b; Hazlett and Schoolmaster, 1998; Pecor and Hazlett, 2002; Acquistapace et al., 2003) and in lotic environments (artificial streams) (Keller et al., 2001; Tomba et al., 2001). However, no study has examined the responses to the same array of chemical cues in the two environments recording the same behavioral variables.

We sought to compare the responses of individuals of *O. virilis* to food cues, alarm odor, and the combination of food and alarm cues in lotic and lentic conditions. Responses to

these cues have been reported earlier but only under no-flow conditions using one set of behavioral measures (Hazlett, 1999) or under flowing conditions using a different set of behavioral measures (Tomba et al., 2001). In particular, we asked if (a) in the absence of added cues, do crayfish behave differently depending on flow conditions?, (b) do crayfish respond to odors differently depending on flow conditions?, and (c) do crayfish respond to different types of odors differently depending on flow conditions?

Materials and Methods

Individuals of *Orconectes virilis* were collected from ponds in Saline, Michigan, for the no-flow condition tests and from Burt Lake, Michigan, for the flow condition tests. In both field environments, crayfish experience basically no-flow conditions.

The general methods followed are very similar to those used in Hazlett (1999). After 24 h of starvation, crayfish were observed and behavior patterns recorded on a laptop computer with an event program during two consecutive time periods: (1) a 5-minute control period following the injection of 10 ml of distilled water, and, immediately following, (2) a 5-minute period following the injection of a test solution. Each replicate lasted 10 minutes, the control water always preceding the test solutions.

Test solutions consisted of (1) food odor (FOOD), (2) alarm odor (ALARM), or (3) food plus conspecific alarm odor (FOOD + ALARM). Twenty crayfish were observed under all three test solutions, a single treatment each day. The food solution was prepared by macerating 2 g of cod fish in 200 ml of distilled water and filtering with coarse filter paper, removing all particulates. The alarm solution was prepared by macerating a 45–50 g male *O. virilis*, mixing the pieces thoroughly in 200 ml of tap water and filtering with coarse filter paper. Test solutions were prepared just prior to use because freezing or even refrigeration may alter the alarm odor (Hazlett, 1994a).

The behaviors and postures recorded were: (a) in burrow, (b) feeding, the crayfish moved their maxillipeds and /or scraped the substratum with the chelipeds and pereiopods, and (c) raised, intermediate, or lowered posture (Acquistapace et al., 2002). Differences in these behavior patterns and postures have previously been shown to be related to food and predator detection in crayfish (Hazlett, 1994b; 1999). When only food cues are present, crayfish increase the time spent in all the feeding-related activities (feeding, and the raised posture) and decrease the time spent in shelters and in the lowered posture. In contrast, the two feeding-related activities are depressed (Blake and Hart, 1993; Hazlett and Schoolmaster, 1998; Hazlett, 1999), and both burrow occupancy and time in the lowered posture increase (Hazlett, 1994b), when crayfish detect predator-related odors as alarm odors alone or in association with food cues. We recorded the time spent in both the raised and lowered posture but because the crayfish had to be in one of the two postures, the values of time spent under different conditions and treatments are not independent, thus only the time in the lowered posture was analyzed statistically.

The no-flow condition experiment was conducted at the University of Michigan, Ann Arbor, during January 2001. Twenty crayfish were placed in individual aquaria (25 × 40 cm bottom area) filled with 15 l of water and supplied with aeration and half of a clay pot for shelter. All individuals used were adults (cephalothorax length between 36.3 and 40.7 mm) with approximately equal numbers of males and females. Each aquarium was visually isolated from other aquaria and from possible sources of disturbance. All tests were conducted between 1100 and 1500 hours. Crayfish were fed cod for several days to familiarize them with that odor as a food stimulus (Hazlett, 1994a). Ten ml of solution were added in the case of FOOD and ALARM, and 20 ml of solution were injected in the case of FOOD + ALARM. Solutions were injected via a syringe into the corner of the aquarium nearest the observer. The order of test solutions introduced to individuals over the two days of testing was determined by using a random number table.

The flow condition experiment was conducted at the Stream Laboratory Facility at the University of Michigan Biological Station, Pellston, during July and August, 2001. Sixty adult crayfish (cephalothorax length between 37.9 and 40.8 mm) with approximately equal numbers of males and females were placed in a communal tank for several days and fed pieces of cod fish to familiarize them with the odor as a food stimulus. Crayfish were then isolated in individual chambers of the test apparatus. This apparatus consisted of four sections of plastic gutters that were 300 cm long and 20 cm wide. Each gutter was divided into five chambers that were 50 cm long and 20 cm wide. The chambers were separated by plastic walls with small holes for the flow of water that was delivered to each gutter directly from the Maple River. Chambers were supplied with a clay pot for shelter and contained one crayfish in each chamber. All tests were conducted between 100 and 1500 hours. Water flow in the gutters was adjusted to 0.12 liters/s and 25 ml of each solution (CTRL, FOOD, ALARM, and FOOD + ALARM) were slowly injected through a syringe pump during the entire 5-min period. Thus cues were available to the test animals throughout the observation periods. To avoid exposure of test crayfish to chemicals before the control and test phases, we started experiments with the animals that occupied the chambers farthest from the introduction of the water flow and ended with the chambers closest to the start of the water flow. To control for the effect of position of the animals in the gutter sections, the position and order of solutions introduced to individuals over the two days of testing were determined using a random number table.

In both the no-flow and flow experiments, crayfish were exposed to the same total intensity of the stimuli, thus eliminating the possibility of differences in responses due to diverse amounts of chemical detected. For the no-flow condition, dye tests conducted by Gherardi et al. (2000) in a similar experimental protocol showed that the injected water was mixed throughout the aquarium in less than 30 s. For the flow condition experiments, problems could have arisen if the intensity of odors changed across the flow chambers (Vickers, 2000). The position of animals relative to the stimulus source should be the same at least at the beginning of each experiment and this was the case in our experiments, since crayfish were always under the shelter in the center of gutters and facing the point source of stimuli at the beginning of each trial.

We used *t* tests to compare crayfish behaviors between flow and no-flow conditions during the control periods. The difference between test and control solutions in the time spent in each behavior was calculated to examine responses of animals to the different odors under flow and no-flow conditions. Analyses were done with two-way repeated-measures ANOVAs because each individual crayfish was tested under each of the three test solutions. The two main effects were flow condition (flow or no-flow) and odor treatment (food, alarm, or food + alarm). The interaction term between odor and flow condition indicates if crayfish responded to odors in general in a similar fashion under the two flow conditions.

Results

The time spent feeding during the control periods varied significantly between flow and no-flow conditions ($t = -3.08$, $d.f. = 118$, $P < 0.003$). Crayfish spent greater amounts of time by almost an order of magnitude feeding in no-flow than in flow conditions (Fig. 1). Time spent feeding in response to odors was not significantly different in the two flow conditions, but feeding responses varied for the different odor treatments (Table 1). Food odor stimulated crayfish to spend 20–30 times longer executing feeding movements, whereas alarm odor (either by itself or in combination with food odor) caused much smaller increases in the time spent feeding (Table 1, Fig. 1). In addition, patterns of feeding responses to odors were extremely similar between flow and no-flow conditions, as evidenced by the lack of a significant interaction between odor treatment and flow condition (Table 1).

In contrast, the time spent in the lowered posture was significantly longer under flow than under no-flow conditions ($t = 12.3$, $d.f. = 118$, $P < 0.001$). Crayfish responded more strongly to odors in flow than in no-flow conditions (Table 1, Fig. 2) and crayfish responded differently to the three odor treatments in the two flow conditions (Table 1, Fig. 2). This interaction probably results from a greater decrease in the time spent in this posture in response to food odor in the flow than in the no-flow conditions, as well as from an increase in the time spent in this posture in response to alarm odor in no-flow condition only (Fig. 2).

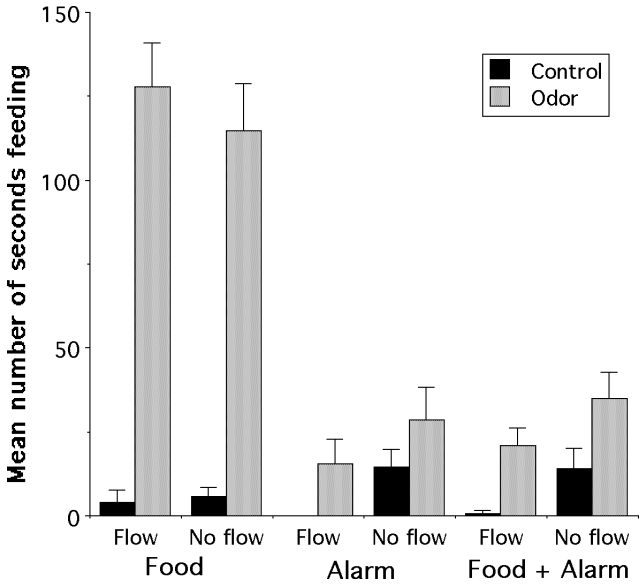

Fig. 1. Number of seconds (means ± SE) spent executing feeding movements by individuals of *Orconectes virilis* when exposed to different odor treatments (controls, food odor, alarm odor, or food + alarm odors) under flow and no-flow conditions.

Table 1. Comparison between odor treatments and flow conditions in the number of seconds spent by crayfish in Feeding, in the Lowered Posture, and in Burrow. Two-way repeated-measures ANOVAs were applied to differences in the time spent by crayfish in the three behaviors between test and control solutions.

	d.f.	*MS*	*F*	*P*
Feeding				
Between subjects				
Flow	1	5754	1.8	0.18
Residual	38	508		
Within subjects				
Treatment	2	24791	57.9	< 0.001
Flow × Treatment	2	4544	0.25	0.78
Residual	76	621		
Lowered Posture				
Between subjects				
Flow	1	230388	25.6	< 0.001
Residual	38	240844		
Within subjects				
Treatment	2	473206	63.7	< 0.001
Flow × Treatment	2	49045	6.6	< 0.01
Residual	76	7425		
Burrow				
Between subjects				
Flow	1	47720	13.9	0.001
Residual	38	3441		
Within subjects				
Treatment	2	65653	23.3	< 0.001
Flow × Treatment	2	42116	15.0	< 0.001
Residual	76	2812		

Animals spent significantly more time in their burrow under control conditions in the flow than in the no-flow conditions ($t = 7.6$, $d.f. = 118$, $P < 0.001$). As with time spent in the lowered posture, animals also responded more strongly under flow conditions than no-flow conditions (Table 1, Fig. 3). Again, patterns of response to the odor treatments varied under the flow and no-flow conditions (Table 1), with animals leaving the burrow in response to food odor only in flow conditions.

Discussion

In this study, individuals of *O. virilis* responded to chemical cues signaling increased feeding opportunities or associated with increased risk of predation in a similar fashion as reported earlier for the same species in lentic (no directional flow) conditions (Hazlett, 1999). Detection of food odor results in increased feeding movements and decreased time spent in the lowered posture and in the shelter. Detection of cues associated with predation risk results in more time in the lowered posture and in the shelter, and lower amount of feeding movements compared to the responses to food cues. This was the case when both food and alarm cues were presented simultaneously, that is alarm cues inhibited the responses to food cues as reported earlier (Hazlett, 1999). These patterns of responses were qualitatively similar in the flow and no-flow testing conditions and this is to be expected given that increased feeding when food cues are detected and lower activity when predation risk is higher is adaptive whether the fluid medium is moving or not moving.

While qualitatively similar, the responses shown to the same cues under different flow conditions were quantitatively different in some cases. The additional contextual information of directional flow resulted in a stronger response to test chemicals; however, these stronger responses seemed to be the effect of the different behavior shown by crayfish in the two conditions in absence of added cues. Because the crayfish were less active and spent more time in the lowered posture and in the burrow during control period in flowing water, their responses to food cues in particular appeared stronger.

The time spent in all four behaviors was very different during control periods for the two flow conditions. In the flow conditions, crayfish spent less time engaged in feeding behaviors and more time in the lowered posture and in the burrow. The clear difference in behaviors during the control periods in the two conditions may well point towards the more "natural" levels of behavior being shown in the flowing environment. In the field, individuals of *O. virilis* are rarely observed moving during the hours when these laboratory tests were run and appear to spend the vast majority of their time resting under a rock or in a burrow (Hazlett et al., 1979). The low level of activity observed in the flowing condition appears closer to the patterns observed in nature and the moderate level of activity seen in aquaria during the day may be slightly elevated over that seen in at least some natural situations. Quantitative field studies of activity in the two environments would be needed to see if the differences in control period behaviors observed in this study reflect behavior in the two conditions in the field.

In a similar study on *O. virilis*, Pecor and Hazlett (in press) reported no significant differences in responses to chemical cues under different flow conditions. However, they did not include control periods in their methods and the

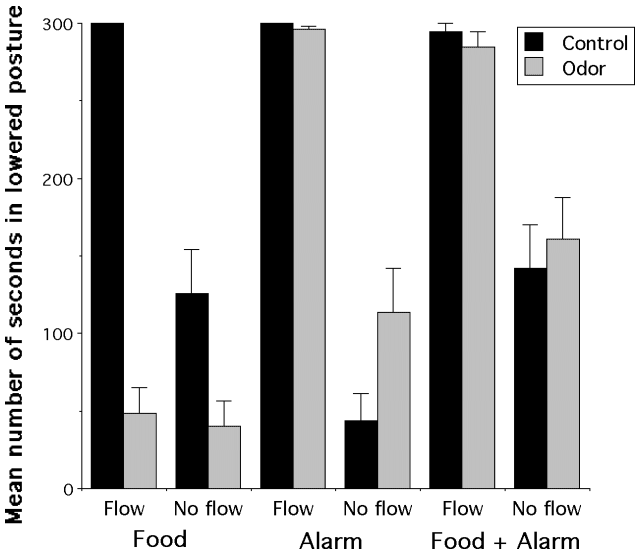

Fig. 2. Number of seconds (means ± SE) spent in the lowered posture by individuals of *Orconectes virilis* when exposed to different odor treatments (controls, food odor, alarm odor, or food + alarm odors) under flow and no-flow conditions.

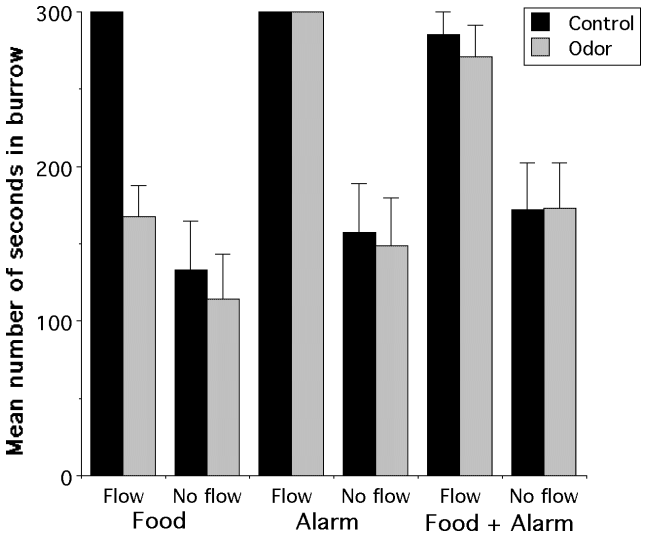

Fig. 3. Number of seconds (means ± SE) spent in the burrow by individuals of *Orconectes virilis* when exposed to different odor treatments (controls, food odor, alarm odor, or food + alarm odors) under flow and no-flow conditions.

responses to added chemical cues were very similar to those reported in this study. Without the control periods for contrast, no flow effects were significant. In addition, the flow rate used in the Pecor and Hazlett study was 0.4 L/min (= 0.007 L/s) compared to 0.12 L/s used in the current study. Brown and Rittschof (1984) showed that there can be a threshold effect of flow rate determining whether flow affects responses to chemical cues.

There are several confounding factors in our experimental design that both require attention and point to some interesting questions for future work. The quantitative differences in behavioral responses in the two environmental conditions could be ascribed to one of three differences in our methods in addition to the physical difference in directional flow we have concentrated upon. First, while the crayfish used in both studies came from lentic environments (ponds in the Ann Arbor study, Burt Lake in the Pellston study), genetic differences in the population could contribute to the behavioral differences observed. However, earlier studies of *O. virilis* from streams (Hazlett, 1994a) and lakes (Hazlett, 2003a) and from populations in two different habitats reported very similar responses to chemical cues presented in a lentic situation—Hazlett (2003a) utilized Douglas Lake individuals and Hazlett (1999) used animals from the ponds in Saline, Michigan. A second confounding factor is the time of year that the two experiments were done. The lentic studies were done in January, while the lotic studies were done in the summer. While a fully crossed design looking for possible effects of season would be interesting, the fact that very similar responses to chemical cues of crayfish tested in the fall (Hazlett, 1999) and in summer (Hazlett, 2003a) would seem to make season a less important factor than flow condition. Third, individuals used in both studies were from lentic habitats. While this means all crayfish had experienced the same type of flow environment prior to testing, it also means the individuals placed in a lotic environment had not recently experienced that situation. Thus, part of the low level of activity shown in the lotic environment, in theory, could be ascribed to being placed in an unusual environment. It would be very interesting to compare individuals from lotic and lentic environments in both flowing and no-flowing conditions to see if experience with a given environmental context might influence responses to chemical information.

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