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Interaction between chemical and tactile cues in mayfly detection of stoneflies

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SUMMARY

1. Behavioural experiments were performed in artificial stream channels to determine if nymphs of the mayfly *Paraleptophlebia adoptiva* (Ephemeroptera: Leptophlebiidae) respond to (i) chemical cues from stonefly nymphs, (ii) chemical cues from disturbed conspecific mayflies, and (iii) body fluids of injured conspecifics, and (iv) whether responses to these chemical cues are different when combined with contact by foraging *Acroneuria carolinensis* stonefly nymphs (Plecoptera: Perlidae).
2. Although none of the chemical cues elicited changes in mayfly behaviour in the absence of predator contact, stonefly chemical cues significantly enhanced the avoidance response of mayflies to stonefly contact. Mayfly nymphs swam more frequently and crawled further in response to predator encounters when chemical cues were present than when chemical cues were absent. Thus, direct stonefly precontact chemical cues appear to sensitize *P. adoptiva* to the potential for an encounter with *A. carolinensis*, enabling stronger escape or avoidance responses upon contact.
3. Mayfly chemical cues that provide indirect information about the proximity of a predator (disturbed or injured conspecifics) did not stimulate increased mayfly response to stonefly contact. Indirect cues may provide less reliable information about the proximity of a stonefly than chemicals emitted directly from an adjacent predator. Mayflies might use chemical cues only in the presence of predator contact because of the high cost of premature escape in a running water system.

Introduction

Mechanisms of detection used by predators and their prey in aquatic systems have received increasing attention in the last decade. A growing body of experimental evidence indicates that chemical cues used by predators for the detection of prey (Buskey, 1984; Formanowicz, 1987; Martínez, 1987; Williams, 1987), and by prey for the detection of predators (Peckarsky, 1980, 1987; Williams & Moore, 1982, 1985; Andersson *et al.*, 1986; Sih, 1986; Martínez, 1987; Dodson, 1988; Kohler & McPeek, 1989; Crowl & Covich, 1990), are an important component of the sensory systems of many aquatic arthropods. Chemical cues have been shown to allow precise discrimination by prey (*sensu* Sih, 1986) between different signal sources (such as predators and non-predators (Peckarsky, 1980; Martínez, 1987; Williams, 1987; Dodson, 1988)). Thus, these cues might allow both direct detection of predators (allelochemicals) and indirect detection when predators disturb (pheromones) or damage (body fluids) conspecifics or other prey taxa (Snyder, 1967; Hazlett, 1985; Sih, 1986).

Peckarsky (1980) first provided evidence that mayfly larvae (Baetidae) can use chemical cues to detect and avoid their stonefly predators and, more recently, several investigators have provided additional support for the use of chemoreception by baetid mayfly larvae for predator detection (Martínez, 1987; Kohler & McPeek, 1989). While it is established that baetid mayflies can detect chemically the presence of stoneflies, it is not known how widespread this phenomenon is in other Ephemeroptera. It is also not known whether mayflies can use cues emitted by disturbed or injured conspecifics to detect foraging activity of nearby stonefly predators. Finally, it is unclear if and how non-contact chemical cues are integrated with hydrodynamic (Peckarsky & Wilcox, 1989) and tactile (Peckarsky, 1980) cues that are also used to detect and avoid stonefly predators.

This study was designed to determine whether larvae of *Paraleptophlebia adoptiva* Eaton (Leptophlebiidae) use chemical cues in pre-contact detection of predatory stonefly larvae and whether these cues are used in conjunction with tactile and hydrodynamic cues associated with predator contact. Experiments were designed to observe mayfly behaviour in artificial stream channels in order to address the following questions.

1 Do mayflies respond to direct chemical cues that indicate the presence of predatory stoneflies?

2 Do mayflies respond to chemicals emitted from disturbed conspecifics?

3 Do mayflies respond to body fluids of injured conspecifics?

4 Do mayflies alter their response to stonefly contact in the presence of these chemical cues?

Materials and Methods

Study organisms and experimental apparatus

Late instar (approx. 3 cm body length) *Acroneuria carolinensis* (Banks) (Plecoptera: Perlidae) and (approx. 1 cm body length) *Paraleptophlebia adoptiva* (Ephemeroptera: Leptophlebiidae) were collected from riffles in Bossard Run, a third order, dolomite bedrock stream in Crawford County, Pennsylvania. *Acroneuria carolinensis* is the most abundant invertebrate predator and readily consumes *P. adoptiva* (P.R. Ode, unpublished), the most abundant mayfly in this stream. Nymphs of both species were collected as needed during March 1988. We kept about fifty stoneflies at a time in a polystyrene chamber containing 3 l of oxygenated stream water and several siltstone rocks from Bossard Run. Stoneflies were fed weekly with a mixed assemblage of mayfly species (families Heptageniidae, Leptophlebiidae, Baetidae) from Bossard Run. Water from this holding cage was used for the stonefly chemical cue treatment. Mayflies were held in a 200 l circulating stream tank and were provided with periphyton-covered rocks and conditioned leaf packs as food.

All trials were conducted in three small artificial stream channels (10 cm × 10 cm × 100 cm; Fig. 1a) that were fed by an 800 l capacity reservoir (Fig. 1b). The reservoir circulated approx. 400 l of oxygenated, 12°C water. Flow was distributed into the stream channels through a gang valve and was constricted to maintain a constant flow rate of $15\,\mathrm{cm\,s^{-1}}$ in each channel. Channels (Fig. 1a) were constructed from 1 m sections of square-bottomed four-inch PVC gutter. Plexiglas caps were glued to the ends and 2 cm outlets were placed at the downstream ends. Drift nets placed in the channels above the outlets prevented study organisms from drifting out.

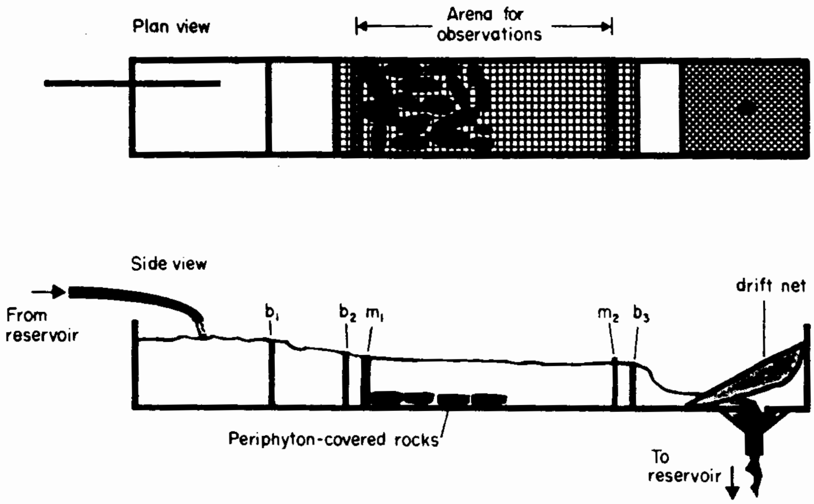

Fig. 1 Schematic of experimental stream apparatus including top and side views of a single channel showing inflow chamber, experimental chamber with rock substrate, and outflow chamber with drift retaining net (b_1, b_2, b_3 indicate wooden baffles, m_1, m_2 indicate fibreglass mesh).

Observation arenas (10 cm × 25 cm; Fig. 1a) were partitioned with fibreglass window screen mesh (0.5 mm mesh; m_1, m_2). Window screen and flat, periphyton-covered rocks from Bossard Run were glued to the bottom of the channels to provide a rough, semi-natural substrate for the study organisms. Wooden baffles (b_1, b_2, b_3) decreasing in height from upstream to downstream were added to reduce turbulence and to maintain a water depth of at least 4 cm in the observation arenas (Fig. 1a).

Experimental design

The first set of experiments was designed to determine whether four different types of chemical cues (different indicators of predator proximity) would elicit behavioural responses from the mayflies. Four mayflies were introduced together and allowed to acclimate to the observation arena until they all stopped moving (at least 5 min), and then a pulse of 100 ml of one of four treatment solutions was poured into the upstream end of the stream channel between baffle b_2 and mesh m_1. The following treatment solutions were assigned randomly to the three channels: (i) water from the stonefly holding cage (water containing dissolved organic matter); (ii) water from the experimental stream reservoir (Fig. 1b) containing the body fluids of five crushed and strained (0.5 mm brass mesh) *P. adoptiva*; (iii) stream reservoir water in which four *P. adoptiva* had been chased with forceps for 30 s and then removed (immediately prior to pouring); and (iv) unmodified stream reservoir water as a control.

Although we assumed that the 400 l of water in the reservoir provided enough dilution of chemical cues in subsequent runs, any additional chemicals introduced by the recirculation of water were present in controls as well as the chemical cue treatments. Observations were made during the 25 s following addition of treatment water (dye runs determined that the mean residence time of the plume of chemical cues in the observation arena was 25 s). Mayfly responses were recorded as: crawl (distance), swim (distance), drift (distance), posture (frequency and intensity, e.g. 'scorpion posturing' *sensu* Peckarsky & Penton, 1988), or no response. Observations were made of a total of sixty mayflies/treatment (each treatment was replicated at least fourteen times) over a 1 week period in mid-March 1988.

The second set of experiments was designed to compare mayfly responses to stonefly contact in the presence and absence of these chemical cues. These trials were similar to those in the first experiments, except that two active predators were introduced with ten mayflies to allow for more observations/trial. Flow rate was reduced (from 15 to approx 5 cm s^{-1}) to produce a longer residence time of the treatment water in the observation arena, thereby tripling observation time. The ten mayflies were placed in the observation arenas and left undisturbed until they stopped moving; then two stoneflies were simultaneously introduced to the arena with 100 ml of treatment water. The responses of each mayfly to contact by a stonefly were observed for 5 s after initial contact. The type of responses to contact (mayflies always either swam or crawled) and the intensity of the response (distance swam or crawled) were recorded for ten replicate sets of ten mayflies/treatment.

One-way ANOVAs were performed to test for differences in mayfly responses to the different chemical cues alone and in the presence of stonefly tactile cues. One-way ANOVA was also used to determine whether different chemical treatments influenced swimming and crawling distances after stonefly contact. Analyses of residuals indicated no need to transform data and Tukey's multiple comparisons tests (HSD) were used to compare individual treatment means.

Results

In the first set of experiments in which chemical cues were used alone, mayfly responses did not differ among chemical treatments. Most mayflies remained motionless during all of the trials with only mayfly ($F_{1,27} = 0.47$, $P = 0.630$) and stonefly ($F_{2,40} = 1.06$, $P = 0.357$; Fig. 2a,b) chemical cues. Mayflies drifted or swam less than 3% of the time.

In the second set of trials with stoneflies present in the arenas, mayflies responded to contact with a stonefly by either crawling or swimming in all treatments (Fig. 3). Behavioural responses such as 'scorpion posturing' (*sensu* Peckarsky & Penton, 1988) were rare. We considered crawling to be the base level response to stonefly contact and have interpreted swimming to be a stronger response because it results

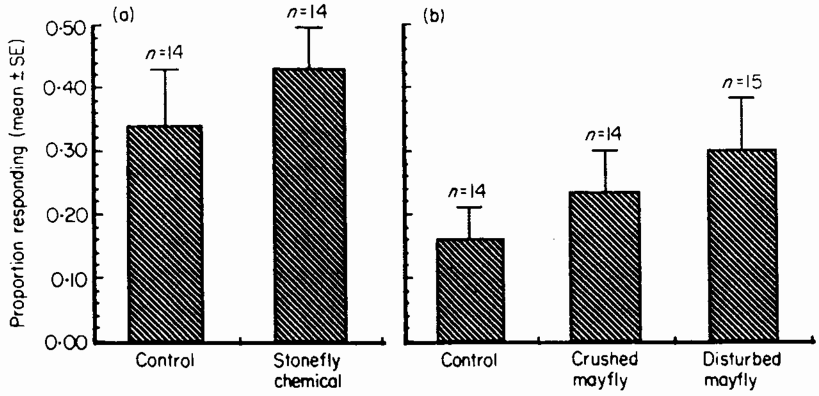

Fig. 2 Mayfly avoidance (crawling and swimming) response to chemical cues from (a) stoneflies and (b) disturbed or injured mayflies.

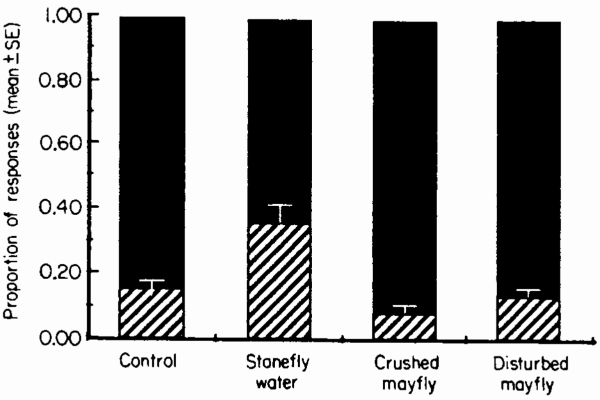

Fig. 3 Frequency of swimming and crawling avoidance behaviour of mayflies in response to stonefly contact in the presence of different chemical cues. Solid bars indicate proportion crawling and hatched bars indicate proportion swimming. Each bar represents the responses of 100 mayflies (ten sets of ten).

in greater displacement and may by energetically more expensive.

Analysis of variance indicated that mayflies swam more frequently in response to contact by stoneflies in the presence of water from the stonefly-holding chamber than to stonefly contact in the presence of control water ($F_{3,36} = 10.01$, $P < 0.0001$; Fig. 3). Although on average mayflies swam slightly farther away from the stimulus in the presence of stonefly-holding water than in the presence of control water, this difference was not statistically significant ($F_{3,30} = 2.49$, $P = 0.0795$). When mayflies crawled in response to stonefly contact, there were no differences among the treatment solutions in distance crawled away from the stonefly stimulus ($F_{3,36} = 0.81$, $P = 0.496$).

In over 400 contacts with *A. carolinensis*, *P. adoptiva* was never observed to drift. When drifting occurred in pre-trial observations, it was always associated with increases in turbulence from the introduction of treatment water. In all trials with stoneflies, many of the mayflies moved to the bottoms or sides of rocks, although they had initially remained on the tops of rocks before the introduction of stoneflies.

Discussion

Conspecific cues that indicate predator foraging activities

A number of aquatic invertebrates use chemical cues from disturbed or damaged conspecifics or allospecifics as indirect evidence of the presence of foraging predators (Sih, 1986). In addition, some stoneflies are known to be attracted to the presence of mayfly body fluids (Martínez, 1987). We found no evidence, however, that the mayfly *P. adoptiva* used the release of conspecific body fluids to detect the foraging activity of nearby predators. We also found no behavioural evidence for the release of, or response to, chemicals from stressed conspecifics, even in conjunction with contact by stonefly predators.

Two alternative hypotheses might explain the lack of response to these indirect cues: (i) concentrations of chemicals used were below a response threshold; (ii) concentrations of indirect mayfly chemicals were at such high concentrations that they reduced sensitivity to additional cues. The lack of response was probably not the result of subthreshold dosages because both mayfly chemical treatments contained higher doses of chemicals than we believe are likely to be present under natural conditions; chemical cues would be less diluted in the small volume of water in our stream channels relative to the volume present in the study stream. Since we did not repeat treatments across a range of dosages, we cannot rule out the possibility that the absence of response reflected an overdose concentration of cues (e.g. Roeloffs, 1978).

Although we cannot eliminate these alternatives, we do not think that indirect cues are as likely to predict the probability of pending attack as would a direct chemical cue from a proximate predator. We concur with Sih (1986), that effective chemical stimuli should correctly predict immediate predation risk and should depend on the potential for predator attack. Even though mayfly–stonefly encounter rates are high (Oberndorfer *et al.*, 1984; Walde & Davies, 1984; Peckarsky & Penton, 1989a), mayfly capture rates per stonefly encounter are low (Peckarsky & Penton, 1989a), thus indirect or long-distance cues are of little benefit.

Use of stonefly chemical cues in early warning

Chemical cues indicating the upstream presence of stonefly predators were not in themselves sufficient to change mayfly behaviour. Avoidance behaviours were more pronounced, however, when mayflies were contacted by a stonefly larva in the presence of a stonefly chemical cue than in the absence of the cue. There are a number of possible explanations for the combined effects of chemical and tactile stimuli including the potential that:

1 there is a simple threshold for the avoidance response that can be obtained by a single stimulus or a combination of stimuli.

2 the intensity of the response is graded (see Helfmann, 1989) and depends on the combined effects of stimuli. In this case, the response to chemical cues alone could have been so small that it was not detectable, but a response was detectable with the addition of contact stimuli. This combined effect could be either additive or synergistic (as suggested by Williams, 1987).

3 chemical stimuli themselves never elicit avoidance, but instead act as an 'early warning' signal (*sensu* Peckarsky & Penton, 1989b) that sensitizes the mayflies and enhances their ability to escape when contacted.

The simple threshold hypothesis seems improbable because we observed different intensities of response to tactile stimuli by themselves and combinations of both tactile and chemical stimuli (Fig. 2). The graded response explanation is also improbable if we assume that concentrations of chemicals from small holding tanks (25 animals/l held for several weeks) were considerably higher than concentrations that would be encountered in a natural setting; comparable natural conditions would require stonefly densities much higher than we have seen in Bossard Run. We found no evidence of a synergistic relationship between chemical and tactile cues like the one suggested by Williams (1987). Thus, we favour the sensitization mechanism, because mayfly larvae rarely exhibited any response to concentrated chemical cues alone. A similar sensitization response has been described for the mayfly *Baetis bicaudatis* Eaton (Baetidae) by Peckarsky & Penton (1989b), but in their study it was a hydrodynamic cue that alerted mayflies to the potential for stonefly attack. As in our study with *P. adoptiva*, *Baetis* rarely responded to the non-contact cue alone. We therefore argue that selection might favour chemical sensitization rather than immediate avoidance behaviour in this system.

Chemical cues in lentic and lotic systems

Most examples of the use of chemical cues by freshwater invertebrates (see reviews in Williams & Moore, 1985; Dodson, 1988; Sih, 1986) are from lentic systems, where these cues provide omni-directional information about the proximity of a predator as a function of the strength of the signal (Formanowicz, 1987; Petranka, Kats & Sih, 1987; Dodson, 1988; Sih, 1986). Chemical cues probably have long residence times in lentic systems, such that prey are exposed to predator cues long after the predation risk has subsided (Petranka *et al.*, 1987). Thus, prey should respond to a generalized cue rather than to the smell of an individual predator. In lotic ecosystems, where flow paths are chaotic (Davis & Barmuta, 1989), cues may provide very little information about the actual proximity of the predator (Dodds, 1990). Thus, such chemical cues may not be precise indicators of a threat of predation, but could be used to sensitize prey so that they can respond more effectively if they are touched.

Delayed use of chemical cues until the moment of predator contact is consistent with the hypothesis that prey should exhibit antipredator behaviours only in the presence of the predator if such behaviours are costly, and if risk of predation is low (Stein, 1979; Dixon & Baker, 1990). In this case, predation risk is low because of low capture rates by stoneflies (Peckarsky & Penton, 1989a), and the cost of a premature escape response is likely to be high because of reduced foraging efficiency (e.g. Kohler, 1984, 1985; Kohler & McPeek, 1989), and/or increased vulnerability to fish predation (Allan, 1981). This notion of delayed anti-predator response is supported by several studies that have shown that mayflies differ in their responses to predatory and non-predatory stoneflies on the basis of chemical cues (Peckarsky, 1980; Martínez, 1987; Williams, 1987).

The type of interaction between cues found in this study suggests that mayflies use precise information about the identity of a nearby individual (predator or non-predator) to decide whether to stay or flee an encounter. It seems possible that chemical cues might provide information that allows mayflies to distinguish between contacts with predators and non-predators. Different prey taxa may use different combinations of cues in this identification process depending on their repertoire of sensory capabilities and other constraints.

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