Anti-predator responses of mayfly larvae to conspecific and predator stimuli

GARRY J. SCRIMGEOUR[1]

Aquatic Ecology Group, Department of Biological Sciences, University of Calgary,
2500 University Drive, Calgary, Alberta, Canada T2N 1N4

JOSEPH M. CULP AND KEVIN J. CASH

National Hydrology Research Institute, 11 Innovation Boulevard,
Saskatoon, Saskatchewan, Canada S7N 3H5

Abstract. Hydrodynamic and water-borne chemical stimuli are considered important cues that initiate anti-predator behaviours of mayfly larvae. We tested the hypotheses that chemical stimuli from conspecifics and the predacious fish *Rhinichthys cataractae* and hydrodynamic stimuli from a *Rhinichthys* model would initiate anti-predator responses by mayfly larvae of *Ephemerella aurivillii*, *Paraleptophlebia heteronea*, and *Baetis tricaudatus*. A second set of experiments tested the hypotheses that chemical stimuli from conspecifics and the predacious stonefly *Claassenia sabulosa* and hydrodynamic stimuli from a *Claassenia* model would initiate responses by *Baetis*. These hypotheses were tested in laboratory streams where mayfly larvae received either chemical stimuli, hydrodynamic stimuli, or the combination of hydrodynamic and chemical stimuli. Responses by larvae to these stimulus types, in terms of use of substratum surfaces and drift rates, were compared with those by larvae in control streams without conspecific and predator stimulus types. Responses to chemical stimuli were variable depending upon mayfly species and the chemical stimulus type. For instance, while *Ephemerella* and *Paraleptophlebia* responded to *Rhinichthys* odours, *Baetis* did not respond to either *Rhinichthys* or *Claassenia* odours. *Paraleptophlebia* responded to conspecific odours whereas *Ephemerella* and *Baetis* did not. In contrast, all three mayfly species responded to hydrodynamic stimuli either alone or when combined with chemical stimuli. Responses by *Baetis* were more complex when chemical stimuli were combined with hydrodynamic stimuli. For instance, although *Baetis* did not respond to conspecific odours alone, drift was significantly higher in streams receiving the three stimuli of conspecific odours, predator odours from *Rhinichthys*, and hydrodynamic stimuli from a *Rhinichthys* model when compared with streams that received only the conspecific odours combined with the fish model. Similarly, an enhanced response was observed when *Baetis* odours were combined with *Claassenia* odours and the *Claassenia* model. Thus, the lack of a response to a chemical stimulus did not preclude an enhanced response when combined with other stimulus types. These observations suggest that although some mayflies are capable of detecting the presence of some chemical stimuli, a response is not elicited unless the stimulus is accompanied by additional stimuli.

Key words: behavioural responses, chemical stimuli, hydrodynamic stimuli, fish, stonefly, anti-predator responses, drift, position.

Prey display a diversity of behavioural responses to predators, including feigning death, reducing activity, assuming defensive postures, and initiating evasive flight responses, all of which act to reduce predation risk and benefit fitness (Edmunds 1974, Peckarsky 1980, Otto and Sjöström 1983, Peckarsky and Penton 1989, Holomuzki and Short 1990, Culp et al. 1991, Scrimgeour 1992, Werner and Anholt 1993, Covich et al. 1994, Scrimgeour and Culp 1994, Scrimgeour et al. 1994). In aquatic environments these anti-predator responses can be mediated through a number of stimuli including visual, chemical, and auditory types, and the perception of hydrodynamic waves produced by predator foraging motions (Peckarsky 1980, Zimmer-Faust and Case 1982, Kirk 1985, Martinez 1987, Petranka et al. 1987, Palmer 1990, Feminella and Hawkins 1994). In fact, much attention has focused on identifying the sensory stimuli that initiate these behaviours (Williams and Moore 1985, Havel 1987, Martinez 1987,

[1] Present address: Department of Zoology, CW 312 Biological Sciences Building, University of Alberta, Edmonton, Alberta, Canada T6G 2E9.

Holomuzki and Short 1988, Blake and Hart 1993). In lentic and marine systems, for instance, considerable evidence suggests that water-borne chemical stimuli induce behavioural and morphological responses of the prey species (Havel 1987, Sih 1987, Dodson 1989). In contrast, in stream environments, the relative importance of chemical and hydrodynamic stimuli as initiators of anti-predator responses to stream predators are not well understood.

In riverine systems, invertebrate prey display strong behavioural responses to stonefly and fish predators (Peckarsky 1980, Williams 1987, Soluk and Collins 1988, Kohler and McPeek 1989, Culp et al. 1991, Scrimgeour 1992, Culp and Scrimgeour 1993, Scrimgeour and Culp 1994). For instance, among mayfly larvae, *Ephemerella infrequens* exhibit the scorpion posture to deflect stoneflies and *Baetis bicaudatus* laterally flex their abdomen and turn their cerci toward stoneflies to increase detection (Peckarsky 1980, 1987, Peckarsky and Penton 1989). Mayfly prey can alter their drift rates and change their position on substratum surfaces in response to the presence of fish or stonefly predators (Malmqvist and Sjöström 1988, Soluk and Collins 1988, Kohler and McPeek 1989, Culp et al. 1991, Culp and Scrimgeour 1993, Scrimgeour and Culp 1994). While these studies and numerous others have identified behavioural responses of stream prey to predators, few studies have been designed to identify the types of stimuli initiating these responses.

In many streams across Alberta, larvae of the mayfly *Baetis tricaudatus* Dodds, *Ephemerella aurivillii* Bengtsson, and *Paraleptophlebia heteronea* (McDunnough) are numerically abundant (Culp 1989, Glozier 1989, Clifford 1991, Scrimgeour 1992). These larvae display strong anti-predator responses to nocturnal predators, including stoneflies like *Claassenia sabulosa* (Banks) and a benthic fish, *Rhinichthys cataractae* (Valenciennes) (longose dace), that consume these mayflies during the hours of darkness (Culp 1989, Glozier 1989, Scrimgeour 1992, Scrimgeour, unpublished data). Although the actual stimulus producing these responses is unknown, all of these mayfly species are nocturnal, feeding almost exclusively during darkness (Culp et al. 1991, Scrimgeour et al. 1991, Scrimgeour 1992, Culp and Scrimgeour 1993). Thus, we hypothesize that the larvae use both chemical and hydrodynamic stimuli to detect predators, because

low light levels at night should reduce their ability to perceive predators through visual stimuli.

Our study tested the hypotheses that chemical stimuli from conspecifics and predators and hydrodynamic stimuli from a predator model of *Rhinichthys cataractae* would initiate anti-predator responses by larvae of *Baetis tricaudatus*, *Ephemerella aurivillii*, and *Paraleptophlebia heteronea*. We further contend that the presence of body fluids or alarm substances from mayflies could initiate anti-predator behavioural responses of conspecifics by providing evidence of a successful predator attack and therefore an immediate risk of mortality due to predation. These hypotheses were tested in the laboratory where streams containing larvae received hydrodynamic and/or water-borne chemical stimuli. To test the generality in the responses of mayfly larvae to chemical and hydrodynamic stimuli, a second experiment determined whether *Baetis tricaudatus* responded to chemical stimuli from conspecifics and predacious stoneflies (*Claassenia sabulosa*) and to hydrodynamic stimuli from a *Claassenia* model.

Methods

Experimental animals

Laboratory experiments were performed over 4-wk periods in May and June 1989 and 1990. In each year larvae of the mayflies *Baetis*, *Ephemerella*, and *Paraleptophlebia* were collected from the Bow River (51°03′00″N, 114°03′00″W) in May, maintained in aerated stock tanks (water temperature = 10°C, light : dark cycle = 14:10 h) for 2–4 wk, and fed an ad-libitum supply of epilithon before being used in experiments. Individuals were used only once and were acclimated to individual streams for 24 h before the start of the experiments. Mean mayfly body lengths ($\bar{x} \pm 1$ SE) used in experiments were: *Baetis tricaudatus* = 5.8 ± 0.2 mm, *Ephemerella aurivillii* = 6.4 ± 0.2 mm, and *Paraleptophlebia heteronea* = 6.0 ± 0.2 mm.

Design of laboratory streams

Experiments were performed in small, circular plexiglas streams (volume = 0.25 L, planar area = 50 cm², water depth = 10 cm, water velocity = 7 cm/s; Fig. 1) to which aerated water

was supplied (Walde and Davies 1984). Water pumped from a large (600 L) reservoir entered a stream through two plastic water jets and exited through two holes in the central outflow column (Fig. 1). The bottom of each stream was covered with a 3-mm-thick layer of glass beads (diameter = 3 mm) to provide a substratum. Streams also contained two 2 × 3 × 0.5-cm plexiglas blocks which were placed on smaller 1-cm³ plexiglas stands (Fig. 1).

Experimental protocols and stimulus types

Each stimulus trial consisted of placing 12 larvae of a particular mayfly species into an individual stream (light : dark regime = 14:10 h, water temperature = 10°C). This density is within the range typically observed for all three mayfly species in Jumpingpound Creek, a tributary of the Bow River (Scrimgeour, unpublished data). During the acclimation period, water was pumped from the reservoir into streams and then recirculated back to the reservoir. To avoid the build up of chemical odours in the reservoir and contamination of control streams with chemical odours, water was not recirculated back to the reservoir after the addition of chemical odours.

Streams containing mayfly larvae received one of several chemical and/or hydrodynamic treatments which were replicated 4 or 5 times. Chemical extracts (3 mL) representing odours (described in Table 1) were introduced into streams, at 0.6 depth, over a 20-s period, using a surgical syringe. This volume of chemical extract represents only 1.2% of the total water volume in the stream, and pilot studies showed that the addition of a 3-mL volume of water placebo did not significantly affect mean drift rates or number of larvae on upper substratum surfaces (2-sample *t*-tests, $p > 0.05$). A preliminary experiment showed that 3 mL of dye added to one stream was not visible 4 min later, suggesting that residency time of chemical cues may be short. When the treatment included the addition of both hydrodynamic (i.e., the predator model) and chemical stimuli (i.e., conspecifics or predator odours), chemical extracts were added first followed by movement of the predator model through the stream.

Hydrodynamic stimuli were imposed by moving models of *Rhinichthys* and *Claassenia* through laboratory streams; these models sim-
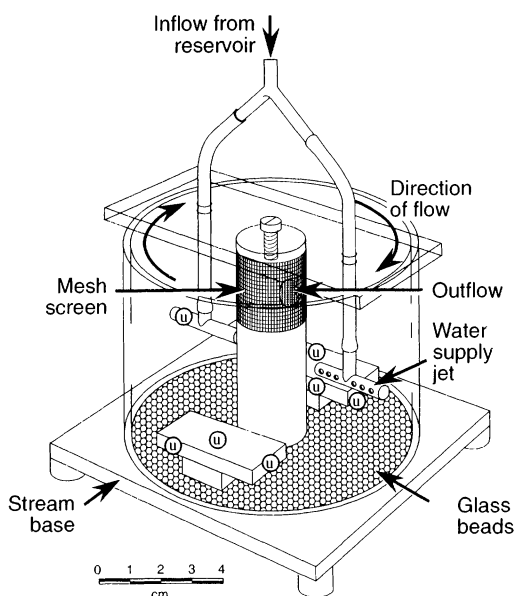

FIG. 1. Design of laboratory streams to determine whether mayfly larvae respond to conspecific and predator stimuli. Positions in the stream with the letter U represent areas classified as upper substratum surfaces.

ulate swimming and probing behaviours of *Rhinichthys* (Beers and Culp 1990) and foraging behaviours of *Claassenia* (Scrimgeour, unpublished observation). For each predator type, the model was moved into the current for two complete circuits around the stream substratum. To mimic differences in foraging speeds of the two predator species, *Rhinichthys* was moved in an upstream direction at a velocity of 1.2 cm/s whereas *Claassenia* was moved in an upstream direction at a lower velocity (0.8 cm/s). Our experiments were not specifically designed to determine whether mayflies responded to hydrodynamic or tactile stimuli produced by moving the models through the streams.

The model predators of *Rhinichthys* (total length ±1 SE = 39 ± 0.5 mm) and *Claassenia* (35 ± 0.4 mm) were developed by freeze-drying dead individuals after their dorsal surfaces had been attached to a 7-cm length of copper wire. To ensure that predator models did not give off predator odours, each model, including the wire attachment, was sprayed with urethane, a waterproofing agent and left to dry for 3 d. Models were then placed into distilled deionized water for 24 h so that any odours from the urethane

TABLE 1. Techniques used to produce predator and conspecific odour extracts. A 3-mL volume of each odour solution was introduced into streams using a syringe. Extracts were decanted to remove larger body parts including scleritized appendages and fish scales.

Chemical stimulus type	Production techniques
Mayfly odours	Six mayfly larvae of either Baetis (i.e., Baetis odours), Ephemerella, or Paraleptophlebia were macerated in 1 L of distilled water to mimic odours released from conspecifics when ingested by a predator.
Claassenia odours	One larvae of the stonefly Claassenia (total length (TL) = 38 mm) was held in 500 mL of dechlorinated tap water for 1 h and then macerated in this solution to mimic the chemical cues produced by the presence of a predacious stonefly. The 1% concentration was created by mixing 10 mL of the extract with 1 L of distilled water. The 100% concentration was not diluted.
Rhinichthys odours	Two adult Rhinichthys (TL = 56 and 62 mm) were placed in 1 L of dechlorinated tap water for 5 min. After this period, mucus scraped from a 4-cm² area from each fish was added to the 1 L solution.
Multiple odours	Three Rhinichthys (mean TL ± 1 SE = 58 ± 1.7 mm) were maintained for 4 d in a 5-L aquarium (14°C) containing 200 Baetis larvae. During this period Rhinichthys consumed 56 mayfly larvae. A 1% concentration was created by adding 10 mL of the tank solution to 1 L of distilled water. The 100% solution was not diluted. This solution was assumed to contain odours from fish, mayflies, and their faecal material.

would be released before they were used in experiments. Although individual models were used repeatedly, they were used only to impose hydrodynamic stimuli in one particular type of stimulus treatment. For example, the Rhinichthys model used in streams receiving conspecific mayfly odours was not used to impose hydrodynamic stimuli in streams which received hydrodynamic stimuli combined with Rhinichthys odours. This ensured that chemical odours that could potentially adsorb to predator models were not transmitted among streams that received different odour treatments.

Mayfly larvae display a diversity of behavioural responses to stonefly and fish predators. In the present study, we restricted our measurements to two response types commonly displayed by all three mayfly species to live Rhinichthys and Claassenia, that is, changes in drift rate and in position of larvae on substratum surfaces. Depending upon mayfly species and size, the presence of predacious fish and stoneflies typically produces an immediate drift response, or larvae move from one surface type to another (e.g., Malmqvist and Sjostrom 1988, Soluk and Collins 1988, Culp et al. 1991, Kohler and McPeek 1989, Scrimgeour et al. 1994). Because our primary interest was to determine if mayfly larvae responded to chemical and/or hydrodynamic stimuli, mayfly species were considered to respond to a stimulus type when either mean drift rate (i.e., number of larvae drifting past a predetermined vertical line from the stream substratum to the water surface) or the mean number of larvae on upper substratum surfaces were significantly different from controls that did not receive chemical or hydrodynamic stimuli. We defined upper substratum surfaces as those on the top and side surfaces of the large plexiglas blocks, as well as those on water supply jets (Fig. 1). Drift responses and changes in the number of larvae on upper substratum surfaces were measured 1 min after introduction of the stimulus and compared with larval responses in control streams (i.e., streams that did not receive chemical and/or hydrodynamic stimuli).

Statistical analyses

As the response variables in this experiment (drift rates and use of upper substratum surfaces) are interdependent, the hypotheses that

mayfly larvae responded to hydrodynamic and chemical stimuli were investigated using a multivariate analyses of variance (MANOVA) approach. Where MANOVAs were significant, subsequent separate ANOVAs were performed as per Peckarsky et al. (1993). When ANOVA tests indicated a significant treatment effect, means were compared with Least Squares Difference (LSD) criteria (Neter et al. 1990). Thus, we determined whether there were significant differences in mean drift rates or use of upper substratum in streams receiving hydrodynamic stimuli alone, chemical stimuli alone, or when hydrodynamic and chemical stimuli were combined compared with controls. Where variances were heterogeneous, data were log_{10} or $log_{10}(x + 1)$ transformed to satisfy the homogeneity of variances assumption. All statistical comparisons were conducted with SAS (SAS 1987) with an alpha value of 0.05 as argued by Carmer and Walker (1982).

Results

Responses of Ephemerella to conspecific and fish stimulus types

The overall response of Ephemerella exposed to hydrodynamic and chemical stimuli from Rhinichthys and Ephemerella was significantly affected by stimulus type (MANOVA: $F_{(10,48)} = 6.42$, $p < 0.001$). When response variables were considered separately the same pattern was observed for drift rates (ANOVA: $F_{(5,24)} = 10.30$, $p < 0.001$) and for the use of upper substratum surfaces (ANOVA: $F_{(5,24)} = 5.03$, $p < 0.005$).

Mean drift rates of Ephemerella increased significantly in response to all stimuli except for conspecific (i.e., Ephemerella) odours, compared with controls (Table 2). However, there was no significant difference in Ephemerella drift among streams receiving chemical stimuli alone, hydrodynamic stimuli alone, or when these stimuli were combined (Table 2).

Use of upper substratum surfaces by Ephemerella was significantly lower in streams receiving the Rhinichthys model combined with Rhinichthys odours and/or Ephemerella odours relative to controls (Table 2). As was the case for drift rates, there was no evidence for an enhanced response in streams receiving hydrodynamic stimuli alone, chemical stimuli alone, and when stimuli types were combined (Table 2).

TABLE 2. Responses of Ephemerella aurivillii in terms of mean ($\bar{x} \pm 1$ SE) drift rates (No. in drift/min) and number of larvae on upper substratum surfaces 1 min after addition to stream of chemical and hydrodynamic stimuli from Rhinichthys and a conspecific chemical stimulus from Baetis. Within a response, values sharing the same letter are not significantly different among treatments.

	Drift rate (no./min)	No. on upper surfaces
Control	0.6 ± 0.4^a	6.8 ± 0.7^a
Rhinichthys odours	4.2 ± 0.7^b	6.0 ± 1.6^a
Ephemerella odours	0.4 ± 0.4^a	5.6 ± 0.5^a
Rhinichthys model	3.0 ± 0.4^b	5.6 ± 1.2^a
Rhinichthys odours + Rhinichthys model	3.4 ± 0.5^b	3.2 ± 0.2^b
Rhinichthys odours + Rhinichthys model + Ephemerella odours	3.0 ± 0.4^b	2.4 ± 0.9^b

Responses of Paraleptophlebia to conspecific and fish stimulus types

The overall response of Paraleptophlebia exposed to hydrodynamic and chemical stimuli from Rhinichthys and Paraleptophlebia was significantly affected by stimulus type (MANOVA: $F_{(10,42)} = 3.39$, $p < 0.005$). When response variables were considered separately the same pattern was observed for drift rates (ANOVA: $F_{(5,21)} = 4.64$, $p < 0.01$) and for the use of upper substratum surfaces (ANOVA: $F_{(5,21)} = 9.96$, $p < 0.001$).

Mean drift rates of Paraleptophlebia were significantly higher in streams receiving all stimuli except conspecific (i.e., Paraleptophlebia) and Rhinichthys odours, compared with controls (Table 3). The absence of significant differences in drift rates among streams receiving the Rhinichthys model, and the Rhinichthys model combined with either Rhinichthys odours and/or Paraleptophlebia odours from each other, indicates that the combination of hydrodynamic and chemical stimuli did not produce an enhanced response (Table 3).

Mean numbers of Paraleptophlebia larvae on upper surfaces were significantly affected by all stimulus types with the exception of predator (i.e., Rhinichthys) odours (Table 3). Thus, in contrast to Ephemerella, Paraleptophlebia displayed a significant response to conspecific odours in

TABLE 3. Responses of *Paraleptophlebia heteronea* in terms of mean ($\bar{x} \pm 1$ SE) drift rates (No. in drift/min) and number of larvae on upper substratum surfaces 1 min after addition to stream of chemical and hydrodynamic stimuli from *Rhinichthys* and a conspecific chemical stimulus from *Baetis*. Within a response, values sharing the same letter are not significantly different among treatments.

	Drift rate (no./min)	No. on upper surfaces
Control	0.2 ± 0.2^a	5.4 ± 0.4^a
Rhinichthys odours	0.5 ± 0.5^a	3.8 ± 0.8^a
Paraleptophlebia odours	0.3 ± 0.3^a	3.0 ± 0.9^b
Rhinichthys model	2.5 ± 0.5^b	1.5 ± 0.7^b
Rhinichthys odours + Rhinichthys model	2.0 ± 0.9^b	2.0 ± 0.5^b
Rhinichthys odours + Rhinichthys model + Paraleptophlebia odours	2.6 ± 0.4^b	0.6 ± 0.2^b

terms of altering their use of upper substratum surfaces. Numbers of larvae on upper substratum surfaces in streams receiving the *Rhinichthys* model combined with *Rhinichthys* odours and *Paraleptophlebia* odours was not significantly different from that in streams that received only *Rhinichthys* odours and the *Rhinichthys* model.

Responses of Baetis to conspecific and fish stimulus types

The overall response of *Baetis* exposed to hydrodynamic and chemical stimuli from *Rhinichthys* and *Baetis* was significantly affected by stimulus type (MANOVA: $F_{(14,60)} = 7.47$, $p < 0.0001$). When response variables were considered separately the same pattern was observed for drift rates (ANOVA: $F_{(7,30)} = 23.68, p < 0.001$) and for the use of upper substratum surfaces (ANOVA: $F_{(7,30)} = 16.15$, $p < 0.001$).

Significant increases in drift rates were observed only in streams receiving the *Rhinichthys* model, either alone, or combined with chemical stimuli (Table 4). Further, while there was no significant difference in drift rates between streams receiving *Baetis* odours alone and controls, drift rates in streams receiving *Baetis* odours combined with the *Rhinichthys* odours and the *Rhinichthys* model were significantly

higher than in streams receiving only the *Rhinichthys* model and *Rhinichthys* odours. This result indicates that the combination of *Baetis* odours with the *Rhinichthys* model produced an enhanced drift response.

In contrast to the lack of response by *Baetis* to all chemical odours, all hydrodynamic stimuli, either alone or combined, significantly reduced the use of upper surfaces (LSD comparisons, Table 4). Lastly, there was no significant difference in the number of larvae on upper substratum surfaces in streams receiving the *Rhinichthys* model alone compared with streams receiving the *Rhinichthys* model combined with *Rhinichthys* odours, and the *Rhinichthys* model combined with both *Rhinichthys* and *Baetis* odours. This later result suggests that the combination of the *Rhinichthys* model with chemical stimuli did not produce an enhanced response, in terms of altering the use of upper substratum surfaces, compared with the responses solely to hydrodynamic stimuli.

Responses of Baetis to conspecific and stonefly stimulus types

The overall response of *Baetis* exposed to hydrodynamic and chemical stimuli from *Claassenia* and *Baetis* was significantly affected by

TABLE 4. Responses of *Baetis tricaudatus* in terms of mean ($\bar{x} \pm 1$ SE) drift rates (No. in drift/min) and number of larvae on upper substratum surfaces 1 min after addition to stream of chemical and hydrodynamic stimuli from *Rhinichthys* and a conspecific chemical stimulus from *Baetis*. Within a response, values sharing the same letter are not significantly different among treatments.

	Drift rate (no./min)	No. on upper surfaces
Control	3.0 ± 1.3^a	9.6 ± 1.0^a
Rhinichthys odours	3.8 ± 0.8^a	8.8 ± 0.6^a
Baetis odours	2.6 ± 0.7^a	10.0 ± 0.3^a
Multiple odours (100%)	2.5 ± 0.6^a	8.5 ± 0.5^a
Multiple odours (1%)	3.5 ± 0.5^a	8.8 ± 0.3^a
Rhinichthys model	11.4 ± 2.0^b	4.8 ± 0.7^b
Rhinichthys odours + Rhinichthys model	12.0 ± 1.3^b	4.4 ± 0.5^b
Rhinichthys odours + Rhinichthys model + Baetis odours	20.8 ± 2.2^c	5.0 ± 0.3^b

TABLE 5. Responses of *Baetis tricaudatus* in terms of mean ($\bar{x} \pm 1$ SE) drift rates (No. in drift/min) and number of larvae on upper substratum surfaces 1 min after addition to stream of chemical and hydrodynamic stimuli from *Claassenia sabulosa* and a conspecific chemical stimulus from *Baetis*. Within a response, values sharing the same letter are not significantly different among treatments.

	Drift rate (no./min)	No. on upper surfaces
Control	5.8 ± 0.6^a	$7.3 \pm 0.3^{a,b}$
Claassenia odours (100%)	6.5 ± 1.0^a	$7.0 \pm 0.7^{a,b}$
Claassenia odours (1%)	5.8 ± 1.4^a	7.8 ± 0.3^a
Baetis odours	5.3 ± 0.9^a	7.8 ± 0.3^a
Multiple odours (100%)	5.5 ± 0.6^a	$7.8 \pm 0.6^{a,b}$
Multiple odours (1%)	6.5 ± 0.6^a	$7.0 \pm 0.4^{a,b}$
Baetis odours + *Claassenia* odours (100%)	6.3 ± 0.9^a	7.5 ± 0.3^a
Claassenia model	12.8 ± 1.3^b	$5.8 \pm 0.5^{b,c,d}$
Claassenia model + *Baetis* odours	13.0 ± 1.9^b	$6.5 \pm 0.9^{a,b,c}$
Claassenia model + *Claassenia* odours (100%)	14.8 ± 2.0^b	4.5 ± 0.6^d
Claassenia model + *Claassenia* odours (100%) + *Baetis* odours	18.8 ± 2.1^c	$5.3 \pm 1.0^{c,d}$

stimulus type (MANOVA: $F_{(20,66)} = 3.13$, $p < 0.001$). When response variables were considered separately the same pattern was observed for drift rates (ANOVA: $F_{(10,33)} = 12.81$, $p < 0.001$) and for the use of upper substratum surfaces (ANOVA: $F_{(10,33)} = 3.57$, $p < 0.005$).

Mean drift rates were significantly higher in streams receiving the *Claassenia* model, and all three combinations of the model with chemical odours, than was the case among control streams (Table 5). As was found with responses of *Baetis* to *Rhinichthys*, there was no significant difference in drift rates between streams receiving *Baetis* odours alone and controls. However, drift rates in streams receiving *Baetis* odours combined with the *Claassenia* model and *Claassenia* odours were significantly higher than in streams that received only: (1) the model combined with *Claassenia* odours and (2) the model combined with *Baetis* odours. Thus, the combination of *Baetis* odours with the *Claassenia* model and *Claassenia* odours produced an enhanced drift response.

Numbers of *Baetis* on upper surfaces were significantly lowered by the introduction of the *Claassenia* model alone or combined with all chemical odours (Table 5). Unlike the enhanced drift response, number of larvae on upper substratum surfaces did not differ significantly between streams that received the *Claassenia* model alone or combined with any of the chemical odours.

Discussion

Aquatic invertebrates display a diversity of behavioural responses to their predators, including changes in drift rate and position on substratum surfaces (Peckarsky 1980, Otto and Sjostrom 1983, Malmqvist and Sjostrom 1988, Kohler and McPeek 1989, Peckarsky and Penton 1989, Culp et al. 1991). Although the antipredator behavioural repertoire of some taxa has been well documented, the stimuli initiating such responses are less well understood. Our results show that *Baetis*, *Paraleptophlebia*, and *Ephemerella* responded to several chemical stimuli and to all hydrodynamic stimuli presented either alone or combined with chemical stimuli. The observed variability in some responses depended upon mayfly species and stimulus type.

Movement of an object through the water column produces pressure waves and fluid displacement (Kirk 1985). It is widely appreciated that many stream prey respond to the movement of a predator through the water column (Peckarsky 1987, Peckarsky and Penton 1989, Culp et al. 1991). Because most studies use live predators, which produce hydrodynamic stimuli combined with other stimuli, the actual stimulus causing the prey's response can not be identified conclusively (but see Peckarsky and Penton 1989). Our results show that all three mayfly species responded to the hydrodynamic stimuli produced by the predator models, either

in terms of increased drift rates or changes in position on substratum surfaces. In general, hydrodynamic stimuli significantly increased drift rates and decreased the number of larvae on upper substratum surfaces.

Behavioural responses to predator models could have arisen as a result of contact mechanoreception if mayflies came into direct contact with the model, or non-contact mechanoreception if larvae responded to the hydrodynamic wave. Although we did not quantify the proportion of drift events attributable to contact versus non-contact drift, our impression was that most *Baetis* drift events occurred without contact with predator models. If our impression is correct, then the predominance of non-contact drift of *Baetis* to *Rhinichthys* and *Claassenia* models is similar to that reported for responses of *Baetis bicaudatus* to a stonefly model (Peckarsky and Penton 1989). In fact, our experimental design is similar to that of Peckarsky and Penton (1989) because our experiments were conducted in arenas without an algal food source for mayfly larvae. In this situation after the perception of a predator, the cost to *Baetis* of leaving a patch, in terms of lost foraging opportunities, would be assumed to be low. Further studies are clearly required to determine the economics of patch departure (Ode and Wissinger 1993, Scrimgeour and Culp 1994).

Chemical odours alone from stream predators can strongly modify prey behaviour patterns (Williams and Moore 1985, Martinez 1987, Holomuzki and Short 1988). For example, water that had previously held fish and water known to contain fish products including intestinal mucus, skin mucus, and faeces significantly reduced activity of stream-dwelling isopods (*Lirceus fontinalis*) and amphipods (*Gammarus pseudolimnaeus*) (Williams and Moore 1985, Holomuzki and Short 1988). Similarly, larvae of *Baetis bicaudatus* avoid extracts from the predacious stoneflies *Megarcys signata* and *Kogotus modestus* (Martinez 1987). Our results show that chemical extracts alone may significantly alter mayfly behaviour, by increasing drift rates and reducing the use of upper substratum surfaces. However, the presence and extent of these responses varied depending upon the type of chemical stimulus. For instance, *Ephemerella* responded to *Rhinichthys* odours but not to macerated conspecific mayfly solutions.

Responses of mayfly larvae to chemical odours were also variable depending upon the mayfly species as has been noted elsewhere (e.g., Peckarsky 1980, Martinez 1987, Williams 1987). The extent of mayfly species-specific responses to chemical extracts has not been well documented, although Martinez (1987) reported species-specific responses of mayfly larvae to extracts from predacious stoneflies. In a patch choice experiment, *Baetis bicaudatus* avoided stonefly extracts prepared from *Megarcys* and *Kogotus,* whereas the heptagenid mayfly larvae *Cinygmula* and *Epeorus deceptivus* did not avoid areas treated with *Megarcys* extract and *Kogotus* extract, respectively (Martinez 1987). Our results provide further evidence for mayfly species-specific responses to chemical extracts. For instance, whereas *Ephemerella* responded to conspecific odours, *Baetis* and *Ephemerella* did not respond to their own respective odours. Further, *Ephemerella* responded to *Rhinichthys* odours, whereas *Paraleptophlebia* and *Baetis* did not. Differences in the underlying mechanisms producing species-specific responses probably reflect species-specific differences in the relationship between the presence of a particular chemical stimulus and the impending level of mortality risk, and the evolutionary history between mayfly larvae and their predators (Peckarsky 1980, Sih 1986).

Chemical extracts used in our study were probably highly complex. For example, scrapings from *Rhinichthys* probably included proteins and amino acids derived from mucus cells, including alarm substances (Smith 1992). Chemical extracts from the macerated mayfly suspension would presumably include proteins from the cuticle, digestive enzymes, algal material from the gut, and compounds derived from the hemolymph. Multiple prey extracts would likely include all of the above. Although we have provided evidence that mayfly larvae respond to chemical stimuli alone, additional work will be required to identify the specific chemical compounds and the exact mechanisms by which they initiate these responses. This work is now under way in lentic predator–prey systems (Havel 1987, Parejko and Dodson 1990). In addition to identifying specific chemical compounds that initiate anti-predator responses, results from these studies could assist in the development of standard techniques to create predator and conspecific chemical ex-

tracts. We created chemical extracts using a diversity of techniques and assumed that these extracts would contain one or more compounds that mayfly larvae could use as a measure of mortality risk due to predation. How well these extracts mimic chemical cues released by predators and conspecifics under natural conditions remains to be determined.

The strength of a prey's response to a predator stimulus can be increased when stimulus types are combined, e.g., a chemical stimulus with other stimuli (Williams and Moore 1985, Blake and Hart 1993, Ode and Wissinger 1993). For instance, the freshwater crayfish *Pacifastacus lenusculus* and larvae of the mayfly *Baetis bicaudatus* display an enhanced response to the combined effects of chemical and visual stimuli from eels (*Anguilla anguilla*) and stoneflies (*Acroneuria carolinensis*) respectively, compared with responses to chemical or visual stimuli alone (Blake and Hart 1993, Ode and Wissinger 1993). Our analysis showed that *Baetis* larvae did not respond to *Baetis* odours alone, either in terms of drift rate or use of substratum surfaces. Despite the non-response of *Baetis* to *Baetis* odours alone, drift rate in streams that received *Baetis* odours combined with the *Rhinichthys* model and odours was significantly higher than in streams that received only the *Rhinichthys* model combined with *Rhinichthys* odours. A similarly enhanced drift response was observed when *Baetis* odours were combined with the *Claassenia* model and *Claassenia* odours compared with drift in streams that received only the *Claassenia* model and *Claassenia* odours. These results show that *Baetis* may be able to perceive conspecific chemical stimuli but do not respond unless other stimuli are also present.

Our observation that the absence of a prey's behavioural response to a stimulus did not preclude an enhanced response when the stimulus was combined with another has broad implications to the interpretation of stimulus–response experiments when the response variable is physiological (e.g., changes in neuronal action potentials) or behavioural (e.g., drift rates). The former focuses on whether the stimulus elicits a physiological response, whereas the later determines whether the stimulus alters an animal's behaviour. Behavioural response experiments do not necessarily identify whether the animal has perceived a stimulus, because the absence of a response can occur when the

perceived stimulus does not alter the animal's behaviour. The lack of a response could occur because the animal is waiting for further stimuli to obtain additional information on the level of threat or because the energetic cost of altering its behaviour outweighs the benefits. In fact, anti-predator responses by prey are highly variable depending upon, for example, an animal's level of starvation (Kohler and McPeek 1989), parasite load (Godin and Sproul 1988), size (Scrimgeour et al. 1994), and sex (Abrahams and Dill 1989). The combination of physiological and behavioural response experiments will be required to identify whether non-responses to a stimulus type is due to the lack of a physiological response or to the higher fitness cost of displaying the behaviour compared with behavioural alternatives.

In summary, our results show that anti-predator responses by *Baetis*, *Paraleptophlebia*, and *Ephemerella* were initiated by chemical and hydrodynamic stimuli both alone and in combination. Variability in responses to some stimuli was dependent upon 1) the mayfly species concerned, and 2) whether the behavioural response variable was drift rate or position of larvae on substratum surfaces. Although a diversity of research endeavours are required for a mechanistic understanding of these results, we contend that two research areas may be particularly useful. First, the specific chemical or chemicals that initiate behavioural responses need to be identified because increasing evidence has shown that lotic species respond to some chemical odours. Second, research is needed on the factors that cause a perceived stimulus to initiate a behavioural response; and such work would increase our understanding of the costs and benefits of anti-predator behaviours.

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