



# Chemical orientation to food by the crayfish *Orconectes rusticus*: influence of hydrodynamics

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(Received 28 December 1998; initial acceptance 31 March 1999;  
final acceptance 29 June 1999; MS. number: A8261R)

Many different organisms orient to chemicals in a variety of habitats. Each of these habitats has a unique hydrodynamic environment that is dependent upon the structure of that habitat. Differences in the hydrodynamics (i.e. turbulence) of an environment will be reflected in the fine-scale structure of chemical signals. To determine what role dispersion dynamics play in influencing orientation behaviour, we studied crayfish searching for food sources in different artificial streams. Streams differed only in substrate composition (sand or cobbles), and the hydrodynamics associated with different substrates were quantified. A detailed analysis of orientation paths showed that crayfish could orient to food sources in streams with either substrate. The most parsimonious explanation is that animals are using information contained in the spatial and temporal distribution of chemicals in the flow to make directional decisions. Crayfish located the source more quickly, spent more time moving, and walked faster while orienting in streams having a cobble substrate compared with those having a sand substrate. These differences between substrates were not seen in control streams. These results show that the hydrodynamics associated with chemical signal structure can greatly influence the temporal properties of orientation to food sources. For crayfish, differences in the turbulent structure of flow may actually increase orientation efficiency by decreasing search time. On a broader scale, these results show that it is important to quantify orientation behaviour in a number of hydrodynamically different environments.

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Organisms of all taxa orient to stimulus sources. Many authors have studied these behaviours and have tried to categorize either the behaviour or the underlying mechanisms (Loeb 1891, 1913, 1918; Kühn 1919; Fraenkel & Gunn 1961). These classifications in laboratory and field studies, have proven ineffective for categorizing the wide range of orientation behaviours. This has resulted in a prolonged debate in the literature and the proliferation of new terms (van der Steen & ter Maat 1979; Bell 1984; preface for Bell & Cardé 1984; Schöne 1984; Kennedy 1986; Atema 1996). With regard to orientation towards chemical signals, resolution of this debate has been prevented due to a lack of knowledge concerning the fine-scale characteristics of the underlying chemical signals.

To clarify the role that stimulus patterns play in guiding orientation and to develop a meaningful classification for chemical orientation behaviour, it is critical to quantify the stimulus patterns and types of information that are available to animals during orientation to

chemical sources. For chemoreception at macroscopic scales, we need to understand the hydrodynamics involved in fluid flow and quantify stimulus distributions at the same spatial and temporal scales at which animals perceive them.

The chemical senses are unique among all senses in that the physical process of transmission through a medium (such as fluid flow) is independent of any inherent excitatory properties of the receptor-activating signal. This is not true for light, as the quality of light (spectral frequency or wavelength) directly influences different transmission impediments such as scattering, absorption and attenuation (Jerlov 1976; Shifrin 1988). In addition, the frequency (or wavelength) of a sound is a critical factor in determining propagation through various media (Clay & Medwin 1977) and in the reflection and diffraction involved in echolocation (Griffin 1986; Nachtigall & Moore 1986). For chemoreception, there are only two physical processes of transmission: fluid flow and molecular diffusion. The relative roles that each of these two processes play in dispersing chemicals and structuring the spatial and temporal information in odour signals can be found elsewhere (Moore et al. 1994;

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P. A. Moore, R. W. S. Schneider & J. Grills, unpublished data). Consequently, changes in the hydrodynamics through changes in the physical structure of the habitat (i.e. flow velocity or habitat substrate) can have profound effects on the dispersion of chemical signals and the ability of organisms to use chemoreception. Increases in flow velocity can alter the chemical dynamics of turbulent odour plumes (Moore et al. 1994) and can alter the ability of blue crabs, *Callinectes sapidus*, to locate and prey on clams (Weissburg & Zimmer-Faust 1993).

A number of studies have demonstrated the importance that chemical signals in flowing habitats have for helping animals make ecological decisions. Male insects require turbulent pheromone plumes to locate females (Vickers & Baker 1992, 1994; Mafra-Neto & Cardé 1994, 1995). Aquatic organisms recognize and avoid predators through chemical signals (Peckarsky 1980; Sih 1986). Marine larvae use chemical signals for settlement cues (Brown & Rittschof 1984; Hadfield & Scheurer 1985; Butman 1986). Finally, many decapod crustaceans use chemical signals to orient within flowing habitats (Weissburg & Zimmer-Faust 1993; Moore et al. 1994). Unfortunately, the conclusions from these studies are somewhat limited because the spatial and temporal distribution of the chemical signal were not measured. Changes in signal structure have only been inferred from changes in the hydrodynamics, or detailed analyses of orientation behaviour are missing.

To provide some insight into how hydrodynamics and chemical signals interact to influence orientation behaviour, we studied the orientation behaviour of crayfish. Crayfish are an ideal freshwater organism for this study because they respond to a variety of chemicals within natural habitats (Hazlett 1985a, b), forage on patchily distributed food sources (fish carrion) and are known to inhabit a variety of hydrodynamically distinct environments (Lodge & Lorman 1987; Corey 1988).

We aimed to address a number of behaviourally and ecologically relevant questions in regard to orientation behaviour and hydrodynamics. Namely, whether crayfish are able to locate point odour sources in flowing habitats, whether their ability is altered by changes in the spatial information contained within turbulent odour plumes, and through what mechanisms crayfish, as model benthic aquatic organisms, are able to perceive and extract relevant information from odour plumes in different hydrodynamic environments. The results of this study will provide insight into the mechanisms of how chemical signals guide chemical orientation behaviour. This will be accomplished by altering the information in chemical signals and measuring the subsequent change in behaviour through detailed analyses of orientation behaviour. In addition, a companion paper (Moore et al., unpublished data) details the spatial and temporal distribution of chemical signals in streams with these two substrates.

## METHODS

### Animals

The crayfish used in this study were caught in the streams and lakes surrounding the University of

Michigan's Biological Station (UMBS) at Douglas Lake, Pellston, Michigan. All animals were sexed and measured, and only animals with complete sensory appendages were used (i.e. antennae, lateral and medial antennules, walking legs, and chelae). Animals were housed in a flow-through metal trough at the Stream Laboratory Facility at UMBS. In preliminary trials, animals housed in metal troughs had chemosensory behaviours that were similar to recently captured animals. Unfiltered stream water was drawn from the Maple River and gravity fed into the housing tanks. Shelters in the form of cut PVC pipes were placed within the tank as shelters. Animals were fed fish gelatin (see below) once every 2 weeks before orientation trials. All animals had a white arrow painted on their carapace using fingernail polish and had small, coloured rubber bands placed on their chelae for individual marking. Each individual was tested once and then returned to the collection sites.

### Artificial Streams

We conducted all orientation trials in a flow-through artificial stream (working section:  $170 \times 32 \times 22.5$  cm; Fig. 1). The stream was constructed using standard 20.3-cm cinder blocks as sidewalls. Plastic sheeting was doubled-over and placed inside the cinder block trough. The bottom edges of the plastic were folded under the cinder blocks to form a flat bottom on the artificial stream. Two sheets of fluorescent light grating (egg crates,  $169\text{-mm}^2$  holes) wrapped with plastic screens ( $1\text{-mm}^2$  holes) were placed upstream to serve as collimators. A small wire cage, to hold the stimulus (described below), was attached to the last collimator 4 cm above the bottom. Unfiltered stream water, taken from the Maple River, was brought into a headtank and then gravity fed into the front of the stream using 15.24-cm PVC pipes. The stream substrate consisted of either sand or cobble collected from the nearby Maple River. Sand was placed in the trough to a uniform depth of  $2.5 \pm 0.3$  cm. Sand particles had an average diameter of  $4.2 \times 10^{-2}$  cm ( $N=30$ ). Cobbles had an average diameter of  $4.7 \pm 0.2$  cm ( $N=20$ ). Average flow velocity was  $5.0 \pm 0.5$  cm/s measured in the middle of the stream with a Marsh-McBirney flow meter. Dimensions and configuration followed previous flume designs (e.g. Nowell & Jumars 1987; Weissburg & Zimmer-Faust 1993; Moore et al. 1994).

We constructed a track system for the camera from PVC pipe cut lengthwise and placed parallel to the long axis of the stream. We placed a Hi 8-mm camcorder on a wheeled cart that ran upon the track system and we controlled the movement of the cart with strings and pulleys. The camera fed a video image to a monitor on a table next to the stream. In this way the crayfish could be followed without disturbing its visual field. We covered the stream, monitor and camera set-up with a tarp to reduce, but not eliminate, ambient light conditions within the stream. There were no artificial light sources underneath or above the tarp during trials. All orientation trials were performed between 1000 and 2000 hours during the months of June and July.

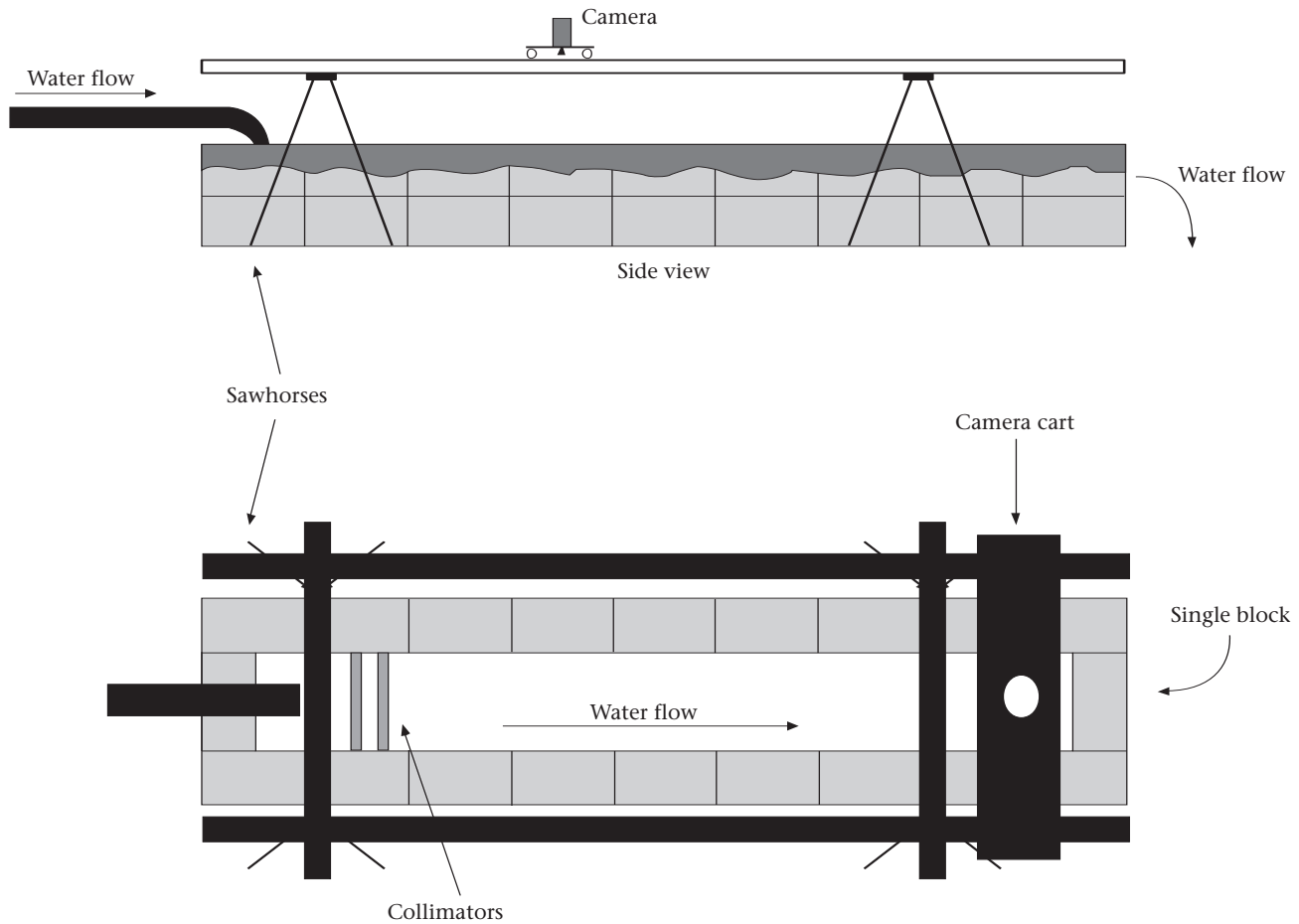


Figure 1. Schematic of the artificial stream used for orientation behaviour.

### Hydrodynamic Characterization

We determined friction velocity ( $u$ ) and roughness Reynolds number ( $Re$ ) of water flowing in the working section of the artificial stream by measuring the vertical velocity gradient. We characterized the vertical velocity gradient by timing the velocity of dye puffs at known heights above the substrate. Dye puffs were injected using a gravity-fed system into the tip of a small Pasteur pipette (1 mm inner diameter). The tip of the pipette was parallel to the flow and extended approximately 5 mm downstream. We measured velocity profiles for 10 puffs at seven different heights in the log layer section of the boundary layer following standard procedures (Nowell & Jumars 1984, 1987). Mathematical calculations followed previously published work on odour plume dynamics (Weissburg & Zimmer-Faust 1993; Moore et al. 1994).

We calculated boundary layer conditions using equations in Schlichting (1979). We calculated friction velocities ( $u$ ) using the following:

$$U_z = \frac{u}{\kappa} \ln\left(\frac{z}{z_0}\right) \quad (1)$$

where  $U_z$  is the mean velocity at height  $z$  above the bed and  $\kappa$  is von Karman's constant (0.41). We determined

the hydraulic roughness length ( $z_0$ ) as the  $Y$  intercept of the equation regressing log height above the substrate against the measured flow speed. We determined the roughness Reynolds number as follows:

$$Re = \frac{uD}{\nu} \quad (2)$$

where  $D$  is the height of the roughness elements (e.g. the diameter of the sand grains or cobble) and  $\nu$  is the kinematic viscosity of the fluid ( $0.01 \text{ cm}^2/\text{s}$ ). A summary of the hydrodynamic characterization of the benthic boundary layer for the two flow substrates is shown in Table 1.

Table 1. Hydrodynamic characterization of the benthic boundary layer for different substrates in the artificial stream

Substrate	Free stream velocity, $U_\infty$ (cm/s)	Friction velocity, $U$	Roughness Reynolds number, $Re$
Sand	5.0	0.46	1.93
Cobble	5.0	0.85	382

## Stimulus

The stimulus for all orientation trials consisted of a block of fish gelatin ( $3.5 \times 3 \times 1$  cm). Four perch, *Perca flavescens* (3–8 cm body length), were placed on ice for 20 min, quickly decapitated and homogenized in a blender. Four packets of Knox unflavoured gelatin and boiling water were mixed with the fish homogenate and poured into a rectangular pan. Control studies were performed using same-sized gelatin blocks without the addition of fish homogenate.

## Ethical Note

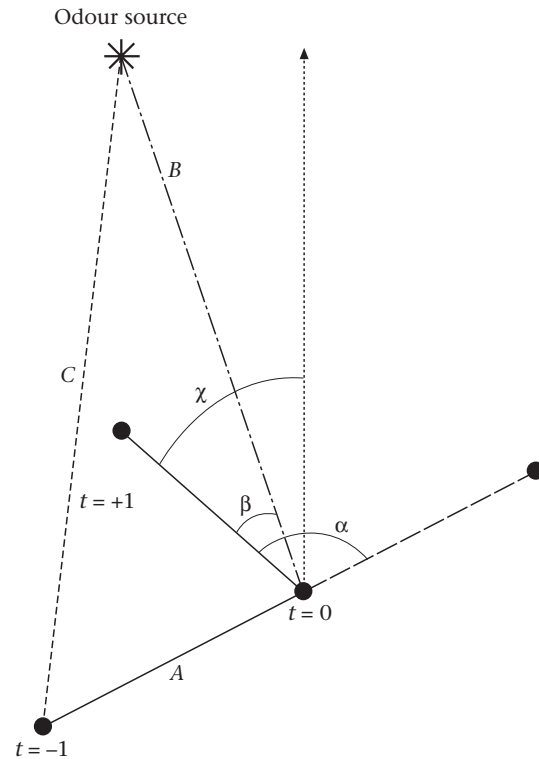
We used pilot tests on perch to determine the length of time to place the fish on ice to minimize pain during decapitation ( $N=10$ ). We forcibly pinched the tail fin of the perch and monitored the reaction from the fish. We recorded the time when the fish did not respond to this stimulus and added 5 min to this time.

## Testing Methods

Crayfish were placed at the end of the tank and allowed to explore the tank for 45 min. If the crayfish was located in the downstream end (30-cm section), we placed the stimulus source in the wire cage and started the camera. Stimuli consisted of fish gelatin, gelatin without fish homogenate (plain), and no gelatin (blank). If the crayfish did not move within 10 min from the start of the experiment for fish gelatin, the trial was ended. This occurred in only three out of 23 trials with fish gelatin. A trial was considered successful only if the crayfish approached within 5 cm of the gelatin. We recorded trials with plain gelatin and no gelatin for 30 min, but analysed only the first 4 min of each trial which was the average time for the fish gelatin trials. Ten animals were tested in each of the three odour treatments (fish gelatin, blank gelatin and plain) and for each substrate (sand and cobble). Because animals were used for only one orientation test, a total of 60 individuals are included in the statistical analysis.

## Data Analysis

We constructed paths from locations ( $X$ ,  $Y$ ) measured as a single point every second. The single point was located at the tip of the white arrow drawn on the carapace of the crayfish. Because crayfish can walk sideways and backwards without changing overall body orientation, a single reference point allowed us to follow the orientation of the animal towards or away from the odour source without regard to body orientation. From these digitized paths, several orientation parameters were analysed; these included walking speed, walking speed towards source, turn angle, heading angle, heading angle relative to directly upstream and net-to-gross ratio (NGR; Moore et al. 1991). We used linear statistics to analyse angle distributions, which ranged in values from 0 to



**Figure 2.** Three successive positions ( $t = -1$ , 0, and  $+1$ : ●) on a hypothetical orientation path used to define behavioural parameters. The dashed line represents the crayfish's projected path (if it had continued in a straight line), from which a turn angle value ( $\alpha$ ) at point  $t = 0$  is calculated. The dash-dot line represents the straight line to the odour source (asterisk) from which a heading angle relative to the odour source ( $\beta$ ) is calculated. The dotted line represents a path that is straight upstream, from which a heading angle relative to upstream ( $\chi$ ) is calculated. 'A' indicates distance moved from time point  $t = -1$  to  $t = 0$ , which is walking speed. The difference between distance to the odour source at time  $t = -1$  (dashed line, C) and distance to the odour source at time  $t = 0$  (dash-dot line, B) is the walking speed towards the source.

180°. We measured walking speed as distance travelled (cm) per unit time (s). Travel towards source was defined as changes in distance from source (cm) per unit time (s). Turn angle ( $\alpha$ ) was defined as the angle between the path connecting the previous ( $t = -1$ ) position to the present ( $t = 0$ ) position and the path connecting the present ( $t = 0$ ) and the next ( $t = +1$ ) position (Fig. 2). Heading angle ( $\beta$ ) was defined as the angle between a path connecting the source and current position of the crayfish ( $t = 0$ ) and a path connecting the current position ( $t = 0$ ) and the next position ( $t = +1$ ), with an angle of zero pointing directly at the source (Moore et al. 1991). Heading angle relative to directly upstream ( $\chi$ ) was similar to heading angle except that the source was replaced with a point directly upstream (Fig. 2). We calculated NGR by dividing the Euclidean distance from start to finish (net) by the total path length (gross). These values can range from 0 to 1, with 0 being a very circuitous path, and 1 being a linear path. NGR values and percentages were transformed using an arcsine method ( $p' = \arcsine p^{1/2}$ ; Zar 1984). We calculated maximum walking speed by taking the average

of the 10 highest walking speeds for each crayfish on each substrate. We also calculated a subsequent average for the population within treatments using the average maximum walking velocity of individual crayfish. For all of these parameters, we calculated an average value for each individual using the digitized paths. Population average values, which were used for statistical analyses, were calculated from the individual average values. Because each treatment (substrate or stimulus) had 10 crayfish, all subsequent statistical analyses used a sample size of 10 for each treatment. We compared similarities for each parameter between different trials using a  $2 \times 3$  (substrate versus stimulus) complete model two-way ANOVA. Significant ANOVAs were followed by a post hoc comparison using a Neuman–Keuls analysis. Comparison between sand and cobble orientation involving the detailed orientation parameters (percentage of time stopped, speed while moving, and time to orient) was performed using a one-way MANOVA, followed by a post hoc comparison using a Neuman–Keuls analysis. We used circular statistics to calculate average heading angle relative to the upstream. We used the Rayleigh's test to determine whether the mean angles differed significantly, and a one-sample test for mean angle to determine whether the mean angle differed significantly from a heading straight upstream (heading angle of  $0^\circ$ ; Zar 1984).

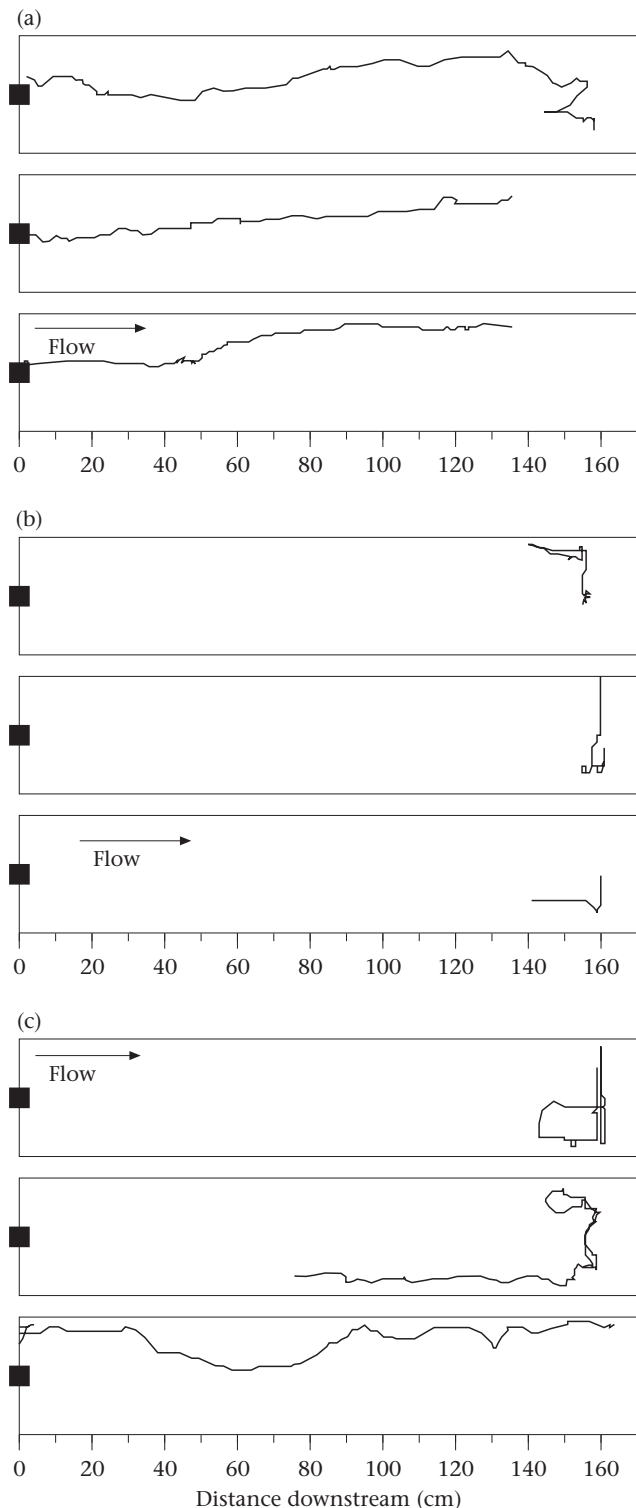
## RESULTS

### Hydrodynamics on the Difference Substrates

The hydrodynamic parameters describing the flow in the artificial stream under different substrate conditions are presented in Table 1. The cobble stream had the highest shear velocity ( $U$ ). The presence of the larger-diameter objects on the substrate clearly increased the shear velocity. This increase was also seen in the roughness Reynolds number. With these values, it appeared that the boundary layer over the sand substrate was primarily a smooth turbulent flow, and over the cobble substrate, the boundary layer was a rough turbulent flow (Schlichting 1979). The differences in shear velocity and roughness Reynolds number influenced the size distribution of eddies that distributed odour within the two streams. Further discussion and quantification of the distribution of chemical signals, including measurement of plume boundaries, on these two substrates is presented elsewhere (Moore et al., unpublished data).

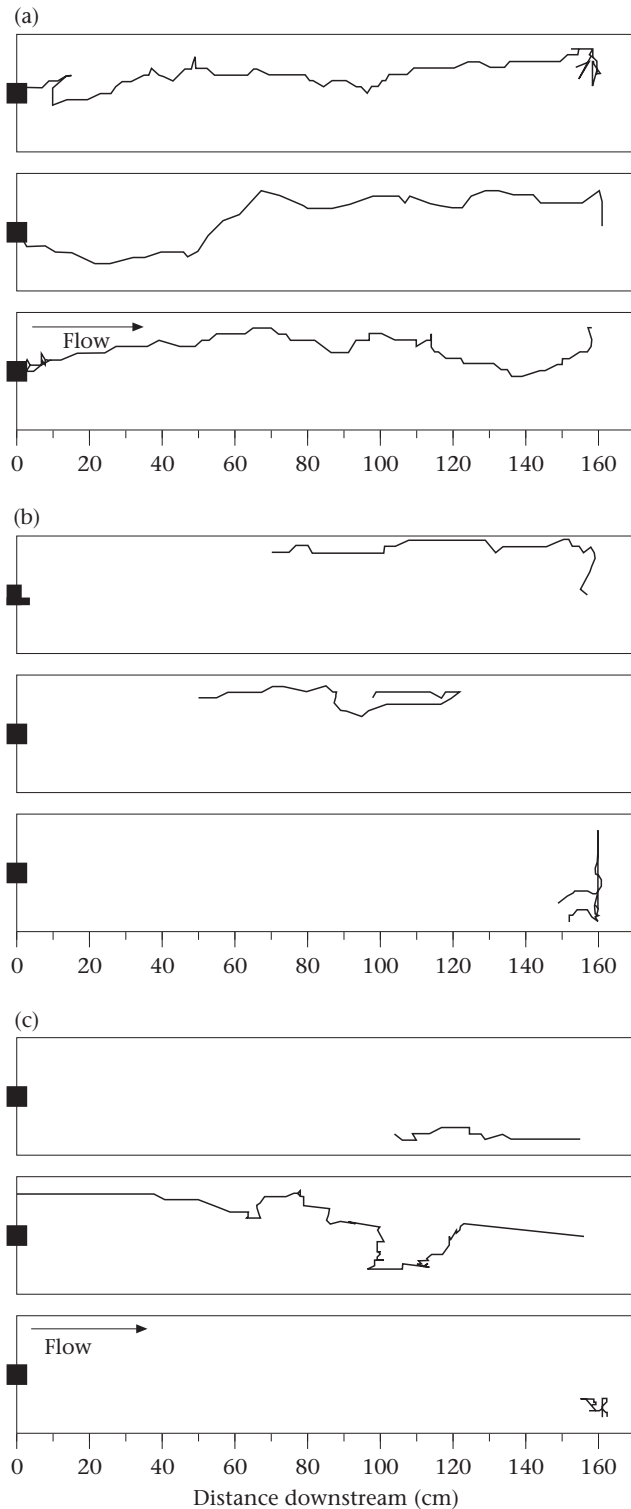
### Qualitative Description of Orientation

Qualitative analysis of the orientation paths showed that animals were positively attracted to fish gelatin and were not responding to plain gelatin or blanks (Figs 3, 4). Orientation paths looked similar (similar spatial structure) on both sand and cobble substrates. The crayfish had similar success in locating the odour source on both substrates. Ten out of 13 crayfish (77%) found the odour



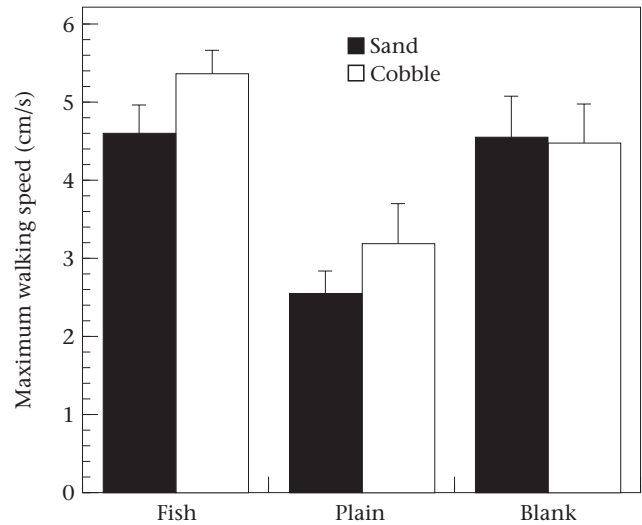
**Figure 3.** Nine representative orientation paths for crayfish orienting under different stimulus conditions on the sand substrate: (a) fish gelatin, (b) plain gelatin, (c) blank. Lines are derived from paths digitized at one point/s. Flow is in the direction indicated by the arrow. ■: Placement of the stimulus.

source on the sand substrate using our criteria. Of the three crayfish that did not find the odour source, two were not used in the analysis because they were not



**Figure 4.** Nine representative orientation paths for crayfish orienting under different stimulus conditions on the cobble substrate: (a) fish gelatin, (b) plain gelatin, (c) blank. Lines are derived from paths digitized at one point/s. Flow is in the direction indicated by the arrow. ■: Placement of the stimulus.

located within the 30-cm area at the far end of the tank after 45 min. The third crayfish did not move at all after being placed in the artificial stream. Ten out of 10



**Figure 5.** Maximum mean+SE walking velocity for crayfish orienting to different stimulus treatments on different substrates.  $N=10$  for each bar.

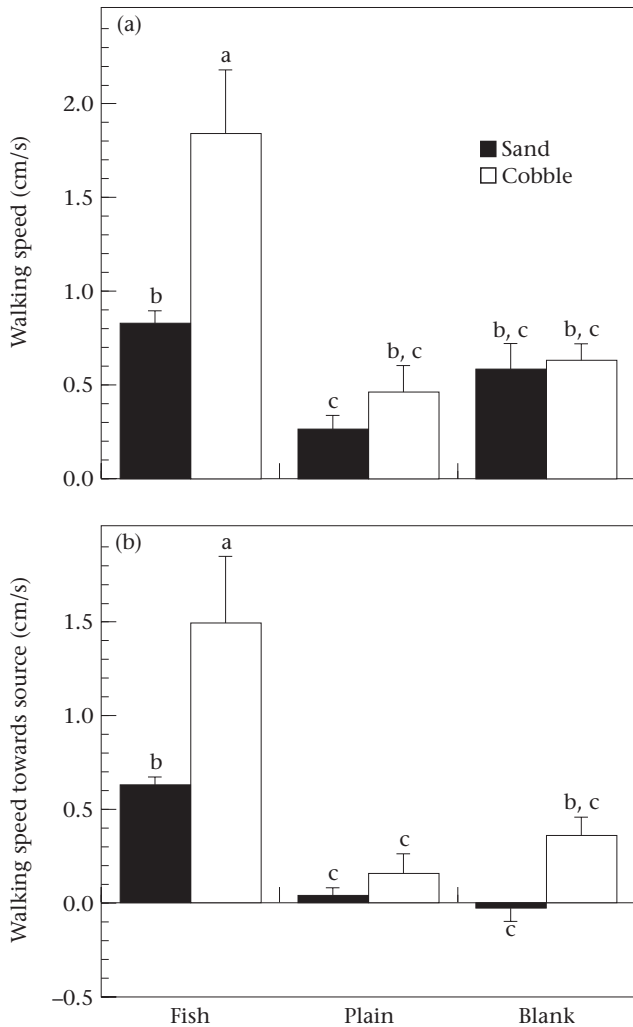
crayfish (100%) found the odour source on the cobble habitat.

### Substrate Effects on Walking

The maximum walking speeds attained by crayfish showed a significant difference for stimulus treatment (Fig. 5; two-way ANOVA:  $F_{2,54}=13.4$ ,  $P<0.0001$ ). Crayfish had significantly higher maximum walking speeds during orientation to food sources compared with the other treatments. There was no effect of substrate difference on maximum walking velocities (two-way ANOVA:  $F_{1,54}=1.72$ , NS). In these experiments, crayfish attained the same maximum velocity independent of the substrate type. There was no interaction between substrate and stimulus (two-way ANOVA:  $F_{2,54}=0.54$ , NS).

### Orientation Changes on the Different Substrates

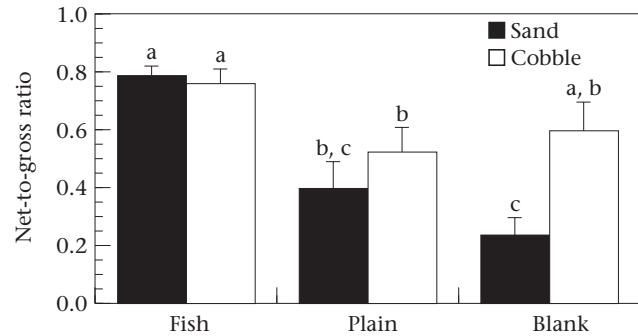
Crayfish altered their walking speeds in response to both stimulus and substrate. Crayfish had a significantly higher walking speed on the cobble substrate, across all treatments, when compared with crayfish walking on the sand substrate (Fig. 6a; two-way ANOVA:  $F_{1,54}=8.28$ ,  $P<0.006$ ). Crayfish walked significantly faster when stimulated by the presence of an odour (two-way ANOVA:  $F_{2,54}=4.38$ ,  $P<0.0001$ ). The interaction between substrate type and stimulus significantly altered the walking speed of crayfish (two-way ANOVA:  $F_{2,54}=1.72$ ,  $P<0.02$ ). Crayfish orienting in an odour plume on the cobble substrate walked more than twice as fast as crayfish without the chemical stimulus on the same substrate (Newman-Keuls post hoc:  $P<0.0002$ ; Fig. 6a, open bars). These same crayfish walked significantly faster than those crayfish orienting under chemical stimulation on a sand substrate (Newman-Keuls post hoc:  $P<0.0003$ ). Crayfish had similar walking speeds on cobble and sand substrates when not stimulated by odour (Newman-Keuls post hoc: NS).



**Figure 6.** Mean+SE walking speeds (a) and walking speed towards the source (b) for crayfish orienting to different stimulus treatments.  $N=10$  for each bar. Bars with the same letter did not differ significantly from each other (two-way ANOVA, Newman–Keuls post hoc test:  $P<0.05$ ).

Given these last statistical results, it is possible that the overall substrate effect on walking speed was solely due to the large difference in walking speeds on the cobble substrate during chemical stimulation.

In addition to changes in walking speed, crayfish altered their walking speed towards the source in response to both stimulus and substrate. Crayfish had a significantly higher walking speed towards the source on the cobble substrate compared with crayfish walking on the sand substrate (Fig. 6b; two-way ANOVA:  $F_{1,54}=11.16$ ,  $P<0.002$ ). Crayfish walked significantly faster towards the source when stimulated by the presence of an odour (two-way ANOVA:  $F_{2,54}=22.20$ ,  $P<0.00001$ ). The interaction between substrate type and stimulus did not significantly alter this parameter (two-way ANOVA:  $F_{2,54}=3.02$ , NS). As seen in walking speeds, the greatest difference in post hoc analysis occurred when comparing speeds on the cobble substrate during odour stimulation with speeds under other treatments (Newman–Keuls post



**Figure 7.** Mean+SE net-to-gross ratio for orientation paths for crayfish orienting to different stimulus treatments.  $N=10$  for all bars. Ratios were transformed prior to statistical analysis (see text). Bars with the same letter did not significantly differ from each other (two-way ANOVA, Newman–Keuls post hoc test:  $P<0.05$ ).

**Table 2.** Comparisons of heading angle relative to the odour source for crayfish orienting in streams with either a sand or cobble substrate

Stimulus	Substrate		$N$
	Sand	Cobble	
Fish	31 (3)	33 (4)	10
	80 (9)	64 (9)	
Plain	90 (6)	51 (11)	10

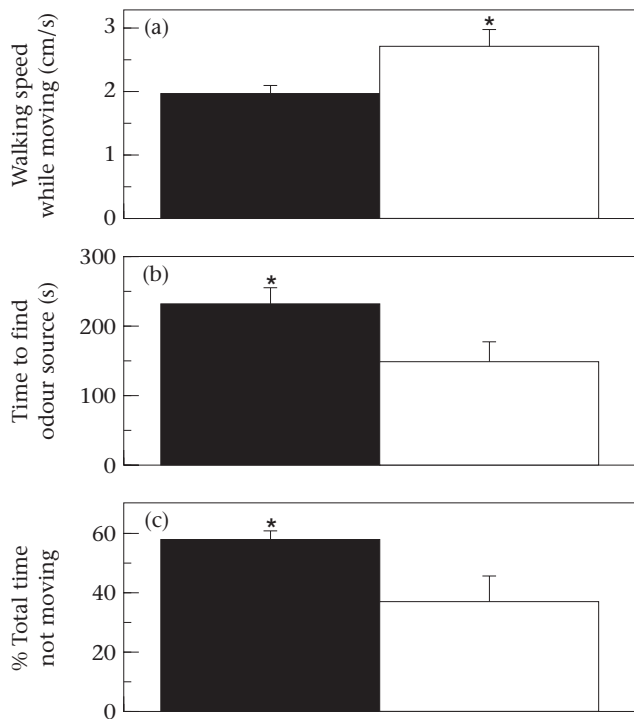
All values are in degrees and numbers in parentheses are standard errors of the mean. There was a significant stimulus effect (two-way ANOVA:  $F_{2,54}=16.49$ ,  $P<0.0001$ ). There was no significant difference in heading angles during odour stimulation between the cobble and sand substrates (Newman–Keuls post hoc: NS).

hoc:  $P<0.006$ ; Fig. 6a, open bars). Again, crayfish had similar walking speeds towards the source on cobble and sand substrates when not stimulated by odour (Newman–Keuls post hoc: NS).

### Spatial Structure of Orientation Paths

Crayfish had significantly straighter orientation paths (as indicated by higher net-to-gross ratios) during odour stimulation compared with the plain gelatin and blank control (Fig. 7; two-way ANOVA:  $F_{2,54}=11.97$ ,  $P<0.0001$ ). Crayfish also had significantly straighter orientation paths on cobble substrates (two-way ANOVA:  $F_{2,54}=5.99$ ,  $P<0.02$ ). Substrate type and stimulus had a significant interaction on NGR (two-way ANOVA:  $F_{2,54}=3.40$ ,  $P<0.05$ ). Post hoc analysis showed that net-to-gross ratios during odour stimulation did not differ significantly from each other (Newman–Keuls post hoc: NS).

Heading angle relative to the odour source is a measure of the accuracy of orientation. As expected, crayfish orienting to an odour source had a significantly lower heading angle relative to the source than other treatments (Table 2; two-way ANOVA:  $F_{2,54}=16.49$ ,  $P<0.0001$ ).



**Figure 8.** Mean  $\pm$  SE walking speed while moving (a), time to locate the odour source (b), and the percentage of time spent not moving (c) for crayfish orienting to fish gelatin on sand (■) and cobble (□) substrates.  $N=10$  for all bars. \* $P<0.05$ ,  $t$  test. Percentages were transformed prior to statistical analysis.

There was no significant difference in heading angles during odour stimulation between the cobble and sand substrates (Newman–Keuls post hoc: NS). None of these differences was reflected in the turning angles. Crayfish were not altering their turning angle, but were altering their walking directions relative to the source of the odour.

Crayfish orienting to a food source on both cobble and sand substrates had significant mean upstream heading angles (Rayleigh's test: cobble:  $Z=9.5$ ,  $P<0.001$ ; sand:  $Z=9.4$ ,  $P<0.001$ ). The mean  $\pm$  SE angles were  $60 \pm 4^\circ$  for the sand substrate and  $60 \pm 5^\circ$  for the cobble substrate. This indicates that the upstream heading angles were not uniformly distributed around a circle. Furthermore, both of the mean angles differed significantly from a straight upstream angle of  $0^\circ$  (one-sample test for mean angle:  $D=16^\circ$ , with the 95% confidence interval for both cobble and sand substrate). The mean upstream heading angles for the plain gelatin and blank controls were not significant (Rayleigh's test:  $Z<2.4$  for all tests).

From these results, it appears that the ability to find the source and the spatial structure of the orientation paths (e.g. heading angle, turn angle and NGR) was the same on the two substrates. To examine more closely how orientation changed with the two different substrates, we analysed the time to locate the odour source, the percentage of the total time spent not moving, and the average walking speed when moving (Fig. 8). Crayfish orienting to fish gelatin on the cobble substrate were able to locate the odour source faster (Newman–Keuls post hoc:  $P<0.03$

spent more time moving (Newman–Keuls post hoc:  $P<0.02$ ) and walked faster (Newman–Keuls post hoc:  $P<0.02$ ) than crayfish orienting to chemicals on sand substrates (Fig. 8).

## DISCUSSION

### Chemically Mediated Orientation

Our experiments show that crayfish can locate the source of chemical signals in turbulent flows on different substrates. All of the crayfish (100%) found the odour source when orienting in streams with a cobble substrate, while most of the animals (77%) located the source when orienting in streams with a sand substrate. None of the animals provided plain gelatin or blank controls oriented to the odour source (or the location where the source would be during chemical trials). Our results suggest that crayfish use chemical signals to locate the source of carrion in different stream habitats. Specifically, it appears that crayfish use information contained in the spatial and temporal distribution of chemical signals to make orientation decisions. In addition, it appears that crayfish stay within the plume boundary (as measured in Moore et al., unpublished data) and do not follow or react to the edge of the plume. All of the crayfish that found the odour source had straighter orientation paths than those in both control studies. This is seen in the significantly higher NGR values (Fig. 7) and significantly lower heading angles (Table 2).

Other possible orientation mechanisms, such as those similar to moths (Vickers & Baker 1992, 1994; Mafra-Neto & Cardé 1994, 1995) or blue crabs (Weissburg & Zimmer-Faust 1993; Zimmer-Faust et al. 1995) are not apparent for the crayfish under our experimental conditions. There are currently three main mechanisms of chemical orientation that have been explored in detail. Each of these mechanisms differs in the degree to which the chemical signal influences the locomotory pattern. For optomotor anemotaxis, presence of the chemical signal, sex pheromone, switches from a casting behaviour to an upwind surge (Vickers & Baker 1992, 1994; Mafra-Neto & Cardé 1994, 1995). The duration of either of these behaviours is controlled by the frequency with which odour filaments cross the antennae. Blue crabs use both the intermittency of chemical signals and the directionality of flow patterns (rheotaxis) to locate odour sources (Weissburg & Zimmer-Faust 1993). It is thought that blue crabs measure the angle relative to flow at which the animal exits the odour plume and re-enters the odour plume at this same angle. Finally, it appears in lobsters that detailed information from the chemical signal provides directional information during orientation (Moore et al. 1991; Atema 1996). Spatial or temporal comparisons of odour features, such as concentration or slope of concentration, allow the animal to locate the centre of the plume and move towards the source.

### Substrate Effects on Orientation

When we compared orientation paths between the two substrates, they were identical in the features that

describe the spatial structure of the path. Heading angles, turn angles and NGR did not differ significantly for orientation paths on sand versus cobble substrates (Fig. 7, Table 2). It appears that animals are using the same orientation manoeuvres on the different substrates. The walking speed, time spent moving and time to locate the source differed on the different substrates (Figs 6, 8). These results support two major conclusions concerning the influence of substrate differences on the orientation behaviour of crayfish. First, the search paths and the underlying behavioural manoeuvres of crayfish remained similar regardless of the substrate on which they were orienting. Crayfish had identical heading and turn angles regardless of substrate type. In addition, the orientation paths on both substrates had similar NGRs, indicating a similarity in the degree of linearity (Fig. 7). These results show that those behaviours that determine the spatial structure of orientation paths are similar although the substrate, and subsequently chemical signal structure, is changing. Second, several different measures of movement rate (walking speed, speed towards odour source, and walking speed while moving; Figs 6, 8) suggest crayfish can orient more efficiently (spend less time orienting or locating the source) when orienting in streams with a cobble substrate. This result differs from another study using decapod crustaceans on different substrates (Weissburg & Zimmer-Faust 1993). Any further discussion of these results would require a detailed analysis of the orientation paths of blue crabs and measurement of the chemical signal structure in those studies.

Crayfish inhabit a number of hydrodynamically distinct habitats, such as lakes and streams (Lodge & Lorman 1987; Corey 1988). Even within a single stream, there are spatial differences in habitat structure including riffles, pools, sandy substrates and cobble areas. Hart et al. (1996) have shown that even small changes in habitat structure can lead to large changes in the hydrodynamics of that habitat. All of these hydrodynamic changes will change the structure of information in chemical signals (Murlis 1986; Westerberg 1991; Moore et al. 1994).

Other species of crayfish use chemical signals as sources of information for a number of ecological decisions, including detection of predation events (Hazlett 1985b), dominant individuals (Zulandt-Schneider et al. 1999), and conspecifics (Ameyaw-Akumfi & Hazlett 1975; Dunham & Oh 1996). Changes in signal structure due to habitat differences can have a large impact in the ability of an organism to perceive and respond to stimuli in an appropriate fashion. Although the orientation paths are similar on different substrates, crayfish can forage more efficiently (take less time to locate the carrion food sources) on cobble substrates. If these same results pertain to other types of chemically mediated behaviours, changes in habitat, and subsequently signal structure, may be impacting habitat selection by crayfish or how crayfish behave within those habitats.

For example, several studies have shown that decisions regarding foraging effort are impacted by perceived predation risk (Milinski 1984; Lima 1986; McNamara & Houston 1986, 1987). During foraging, crayfish are

outside of their shelters and are more vulnerable to predation. For crayfish, predation risk increases as foraging time increases. If chemosensory-mediated foraging is habitat dependent, we can predict that the behavioural decisions crayfish make may differ depending on substrate type.

### Possible Mechanisms for Substrate Effects

The differences seen in movement parameters may be a result of different locomotory abilities on the different substrates. Conversely, they may be driven by differences in the chemical signal structure that arise from changes in turbulence on cobble or sand substrates. Although it is impossible to invalidate completely the former hypothesis with the present data set, several lines of evidence suggest that the basic locomotory abilities of crayfish are not influenced by substrate differences in our test arena. Comparing across substrates, there was no significant difference in either walking speed or travelling speed towards the source when the stimulus was plain gelatin or a blank control (Fig. 6). This indicates that when not stimulated by a chemical, crayfish show no differences in their ability to walk on either cobble or sand substrates. During chemical stimulation, crayfish achieved the same maximum walking speed regardless of substrate (Fig. 5). This indicates that the cobble or sand substrate does not significantly impair the ability of the crayfish to walk quickly. These two results taken together suggest that the locomotory differences seen during orientation were not the result of differences in the ability of crayfish to walk on the different substrates.

A more parsimonious explanation is that crayfish use the spatial and temporal distribution of the chemical signals to make directional, walking speed, and/or other behavioural decisions during orientation. Because the spatial and temporal distribution of chemical stimuli changes on the different substrates (Moore et al., unpublished data), crayfish would be expected to demonstrate differences in orientation on the different substrates, such as increased walking speed, more time spent moving, and less time to locate the odour source. Although decisions regarding the spatial structure of behavioural output are same on both substrates, the decisions regarding movement rates are made differently on the cobble substrate. From this result, we conclude that some aspect of the chemical signal may be providing information on when to continue movement, when to restart movement once it has stopped, or even the rate of movement towards the odour source. The most prominent change in the temporal structure of the chemical signal is the addition of higher frequencies within the signal fluctuations. This indicates shorter time intervals between odour bursts (Moore et al., unpublished data). These changes in the signal structure also seem to support the conclusion that the repetition rate of odour stimulation may be important for decisions regarding movement.

It may be possible that crayfish are using the presence and absence of a chemical signal to trigger a rheotactic search for the odour source. The size scale of turbulent

eddies differs between cobble and sand substrate (Table 1). Compared to the sand substrate, the cobble substrate generates larger eddies that will subsequently transfer their energy to smaller eddies (Tennekes & Lumley 1972). The changes in eddy structure do not translate into differences in average directional flow. If crayfish are using a chemically mediated rheotaxis based on the average directional flow, we would expect that the spatial structure of the orientations paths to remain constant. This is consistent with our findings. Orienting crayfish had significant upstream heading angles that were greater than 0° and the upstream heading angle did not change as a function of distance away from the source. We would predict that, if crayfish use a chemically mediated rheotaxis (as has been clearly demonstrated in moths), then the mean upstream heading should have been close to 0° (straight upstream) and this was not present in our results.

Habitat-specific hydrodynamics may constrain or enhance the olfactory ability of organisms, and thus, may be important for ecological decisions such as habitat selection, foraging strategies and predator avoidance. The results from the present study are beginning to shed light on how chemical signals can guide ecological and behavioural decisions in different habitats. In addition, they show the importance of measuring both the hydrodynamics and chemical signal structure when studying chemically mediated behaviours.

### Acknowledgments

We thank Brian Skrzyniecki for helping to collect the data and the University of Michigan Biological Station for supplying space at the Station's Stream Research facility. Comments from Drs Ring Cardé, Robert Huber and Troy Keller and an anonymous referee helped to improve the manuscript. This work was funded by a NSF grant IBN-9514492. The research presented here was described in Animal Research Protocol No. 98-017, approved on 28 June 1998 by the Bowling Green State University Animal Research Ethics Board.

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