

Behavioral observations and computer simulations of blue crab movement to a chemical source in a controlled turbulent flow

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Summary

The behavior of crabs tracking odor in turbulent chemical plumes was compared to the performance of computer simulations of search behavior operating in similar chemical signal environments. The movement of blue crabs (*Callinectes sapidus*) towards a source of food odor was studied in controlled flow conditions in a flume. The evolving chemical stimulus field of a similar chemical source in an equivalent flow environment was captured by recording concentration patterns of a fluorescent tracer. Hypotheses about the sensory mechanisms employed by the crabs were tested by computer simulation using the recorded fluorescence as the stimulus. The results demonstrate that a simple model combining chemotaxis (simultaneous, spatial comparisons of chemical signals) and odor-stimulated upstream movement

(rheotaxis) is sufficient to explain the efficient movements towards the source displayed by foraging crabs. Spatial integration around each sensor improves performance significantly, but the number of sensors does not have a large impact on performance. The weighting of information from chemical versus flow signals can substantially change simulation performance, resulting in more or less congruence between the behavior of simulations and that of crabs, which suggests the general importance of both sources of information for successful odor-guided navigation.

Key words: blue crab, *Callinectes sapidus*, chemo-tropotaxis, rheotaxis, navigation, odor, turbulence.

Introduction

Chemical stimuli are the most ancient and universal sources of information for organisms. They are often used to locate food and mates, presenting animals with three problems: a search problem, of making contact with appropriate chemicals that have dispersed away from the source (which usually provide a much larger target than the source itself); an approach problem, of using information from the chemical stimuli to move to the source (Dusenbery, 1992); and a termination problem, of determining that the source has been reached. The first problem has been addressed by a number of theoretical and empirical studies (Bell, 1991; Dusenbery, 1992), and many studies have examined how animals behave when solving the second problem (Arbas et al., 1993; Atema, 1995; Vickers, 2000; Weissburg, 2000), although the exact mechanisms are sometimes unclear. The third problem remains for future study.

In this paper, we address mechanisms employed by animals when challenged by the second problem. Understanding this process is complicated by the fact that, at scales above a few centimeters, chemicals are dispersed by turbulent flows of wind or water and the resulting distribution patterns are complex and unpredictable on the relevant temporal and spatial scales. It is now widely appreciated that turbulent mixing is

such that animals cannot simply follow the average gradient because large fluctuations in turbulent plumes force animals to sample for long time periods in order to estimate average concentrations accurately (Jones, 1983; Murlis, 1986; Murlis and Jones, 1981; Webster and Weissburg, 2001; Wright, 1958). Other hypotheses have been proposed, and among the most prominent is that animals make use of flow direction information in conjunction with information derived from odor properties. One whiff of the appropriate chemical indicates that there is a source upwind or upstream.

Extensive research using male moths locating females has provided evidence to support this general idea, and suggested more specific models (Arbas et al., 1993; Belanger and Arbas, 1998; Mafra-Neto and Cardé, 1998; Murlis et al., 1992; Vickers, 2000). However, these studies are difficult because it is impractical to visualize chemical stimuli in the air and correlate behavioral with stimulus patterns. By contrast, visualization of chemical tracers in water can now be carried out with high resolution using fluorescent dyes, lasers and video cameras. Furthermore, flying insects face very different problems from walking animals, and aquatic environments may impose different constraints than air.

These considerations led us to study the behavioral

mechanisms by which blue crabs move to the source of food odors. In particular, we suspected that these relatively large and slow-moving crustaceans might employ spatial comparisons between receptors on different appendages to gain information that is not available to a flying insect. Such information, possibly in conjunction with odor-triggered movement upstream, is thought to underlie strategies that blue crabs and other crustaceans use to navigate in turbulent odor plumes to find a source (Atema, 1996; Grasso et al., 2000; Weissburg and Zimmer-Faust, 1993).

Our specific objectives were to observe the behavior of crabs in a well-defined hydrodynamic environment, observe the pattern of chemical distribution under the same conditions, and test computer models simulating hypotheses about how the chemosensory behavior of aquatic crustaceans is controlled.

Materials and methods

Flow environment

We characterized blue crab search behavior in a recirculating flume (10 m long \times 0.75 m wide) with controlled fluid flow and boundary layer conditions. The details of this facility have been previously described (Weissburg et al., 2002), but briefly, this facility is capable of generating equilibrium boundary layers in the test section (located 7.5–9.5 m downstream from the head of the flume) at flow velocities as low as 1 cm s^{-1} . Water velocity was controlled using a variable speed pump and discharge was monitored using an inline meter. Average flow velocity was maintained at $4.9 \pm 0.08 \text{ cm s}^{-1}$ (mean \pm S.D.) with a water depth of $23.0 \pm 0.348 \text{ cm}$ (mean \pm S.D.) controlled by a vertical tailgate. At this velocity, the boundary layer shear velocity u^* , calculated using the Law-of-the-Wall equation (Weissburg and Zimmer-Faust, 1993) was 3.1 mm s^{-1} , and boundary layer structure conformed well to expectations for turbulence in open channel flows (Nezu and Rodi, 1986). These hydrodynamic conditions during behavior trials are well within the range reported for blue crab habitats in the field (Finelli et al., 1999; Weissburg and Zimmer-Faust, 1993). Flume water salinity was kept between 28–32 p.p.t. and the temperature at 24–27°C, which matches the conditions in the animal holding tanks.

Fluid dynamics measurements took place in a fully developed, uniform open channel flow of freshwater established in a 1.07 m wide, 24.4 m long tilting flume with a rectangular cross-section and smooth bed. This flume provided more precise control over the flow conditions, but was not designed for use with seawater, preventing its use in behavioral trials. Average velocity in the flume was 50 mm s^{-1} and the flow depth, H , was 200 mm. Flow was uniform in depth to within 0.3 mm for a distance of at least 12 m upstream of the measurement location. The turbulent boundary layer over the bed had a friction velocity, U^* , equal to 3.55 mm s^{-1} , indicating that the conditions were very similar to those under which the crab behavior was observed.

The plume source for both fluid physical and behavioral studies consisted of a brass 4.7 mm diameter nozzle with a

brass fairing attached to minimize the flow perturbation. The effluent velocity matched the channel flow velocity, thus creating an iso-kinetic source. For the data presented here, the nozzle was located 25 mm above the floor of the flume.

Chemical concentration records

Long time-histories of instantaneous concentration fields were obtained by planar laser-induced fluorescence (PLIF). The source effluent contained $519 \mu\text{g l}^{-1}$ of Rhodamine 6G that was made to fluoresce using a horizontal laser sheet. The laser sheet was located $25 \pm 1 \text{ mm}$ above the bed of the flume, which approximates the position of the antennules of the crabs and intersects their legs. Video records of 1024×1024 pixels were acquired at a frame rate of 10 Hz and processed to obtain accurate representations of concentration in the range $0\text{--}104 \mu\text{g l}^{-1}$ at each position of a 1008×1018 array.

In order to obtain sufficient spatial resolution (1 mm), the camera was focused on a 1 m square field of view, and three different camera positions were combined to obtain a record of 6000 images with 1024×1860 pixels covering a field of view of $1.0 \times 1.9 \text{ m}$. This procedure produces artifacts at the two boundaries between the three camera fields of view (because the three records were made at different times), but this does not appear to affect the performance of these simulations. Further details of the acquisition and processing of concentration measurements may be found in Webster and Weissburg (2001) and Webster et al. (2002).

Animals

Male and female blue crabs *Callinectes sapidus* L. were collected using baited traps from habitats adjacent to Dickson Bay, Panama, FL, USA (latitude $30^{\circ}00'N$, longitude $84^{\circ}22'W$; Gulf Specimen Supplies). Crabs were captured from February 2000 through September 2001, shipped to Atlanta, kept in communal tanks (artificial seawater 28–32 p.p.t., 24–27°C), and tested within 20 days of collection. In the laboratory, animals were maintained on a 12 h:12 h light:dark cycle, and fed freshly thawed shrimp and squid *ad libitum*. We withheld food from blue crabs approximately 12 h prior to testing to ensure that the animals were not satiated and to standardize hunger level.

Behavioral testing

Blue crabs were moved carefully to the flume and placed in a Plexiglas box ($27.2 \text{ cm} \times 19.5 \text{ cm} \times 16.5 \text{ cm}$, length \times width \times height) with a plastic grate (1 cm^2 grid) forming the front door and rear panel. This design enabled the flow to penetrate the box freely while keeping the crab in a known starting position. Animals were placed in the box for a 15 min acclimation period prior to the introduction of the stimulus. The stimulus consisted of a solution made by soaking 7 g l^{-1} of intact shrimp in flume water for 30 min and was introduced as previously described. Trials lasted for a maximum 15 min but were terminated if the crab failed to exit the start box within 5 min after the door was raised, if the crab found and grabbed at the source, or if the crab walked upstream of the odor source (Weissburg et al., 2002).

The behavior of crabs in the odor plume was recorded on videotape using a low-light-sensitive CCD camera mounted approximately 2 m above the working section of the flume, which corresponded to a resolution of approximately 5 mm per pixel. Trials were performed in near-darkness (light intensity $\ll 1$ lux) because field observations indicate peaks in foraging activity occur in the early morning and evening periods (Clarke et al., 1999). Animals were unresponsive to visual stimuli during the trials. Each animal was tracked using two red-light-emitting diodes in a self-contained unit affixed to the dorsal carapace. The diode backpack was approximately 6 cm \times 2 cm \times 1 cm (length \times width \times height) and weighed 25 g, which is less than 10% of the weight of crabs typically used in our study (300–350 g). The backpack had no detectable effect on crab movements (M. Weissburg and T. Keller, unpublished observations) and kinematic parameters of tracking crabs reported here are similar to that of previous studies using animals without backpacks (Weissburg and Zimmer-Faust, 1994). The centroid of each light was calculated using Motion AnalysisTM software in each frame, generating a 60 Hz time series of x - y pixel values for each light. These x and y pixel locations were smoothed using a moving average algorithm (window size=3) and every 12th frame was extracted to produce a 5 Hz time series. Time series-pixel data were then converted to real world distances using a calibration function.

Computer simulations

The general simulation software has been described (Dusenbery, 2001). A major element of this software is reasonable representation of noise in both sensory inputs and motor outputs. Care was also taken that information about its position was not used by the searcher. The only directional reference was to flow direction.

Simulating turbulent flows at the appropriate scales is impractical, so the video records of odor plumes entrained in turbulent flow (described above) were used for the stimulus field. With the frame rate of 10 Hz and flow of 50 mm s⁻¹, parcels of fluid travel 5 pixels, on average, between frames. This would produce an unrealistic jumping of stimulus parcels between frames. Fortunately, the flow is nearly uniform at this scale and a linear interpolation between frames smoothed the flow without distorting the odor properties. This is consistent with Taylor's hypothesis, which states that over small spatial and temporal scales, bulk flow simply advects scalar distributions without changing their structure (Tennekes and Lumley, 1972). The simulation was run with a time step representing 0.02 s (5 steps per frame), so that on average the flow only moved one pixel between steps. Each run (with 1–1000 searchers) was started at a randomly chosen frame. The record of 6,000 frames was treated as an endless loop, which produced a rare temporal discontinuity when going from frame 6000 to frame 1.

The basic model (which was thought to most closely represent a crab) had ten chemical sensors distributed evenly around a circle of 50 mm radius (representing sensors on the

walking legs and antennules) and a sensor indicating flow direction. Each chemical sensor reported the value of 1 pixel (small integration radius) or the average value of all pixels within a radius of 15.5 mm (large integration radius). We also used a variety of simpler sensor configurations to explore the effects of sensor number, size and position on navigational performance. Integration over time was not extended beyond the step time of 0.02 s.

To emulate the behavioral experiments, all searchers started 1.5 m downstream of the source. The searcher did not move unless chemical stimulation reached a threshold level, which corresponded to a 0.003 dilution of the source solution. The searcher's speed of locomotion was limited to 150 mm s⁻¹, the maximum speed attained by crabs in behavioral trials of odor tracking. Reaching the source was defined as 'contacting' it by coming within a 55 mm center-to-center distance.

Individual searchers were simulated for up to 20,000 steps (400 s of simulated time) for generating tracks to compare with individual crabs. The statistical success of large numbers of searchers was calculated using simulations that were usually run for 1500 steps (30 s), which is three times longer than necessary for success.

We simulated crab search in this study by adding flow direction sensory capabilities to a previous tropotaxis model (Dusenbery, 2001). The searcher moved in a given time step only if stimulated and then at maximum speed. Thus the direction of locomotion was the primary behavior controlled by stimulation. The commanded direction (the direction of vector **D**) was based on two components: chemical gradient and flow direction. A vector **C**, pointing in the direction of higher chemical stimulation, was determined by one of two alternative models. In the best-receptor model, the vector direction was that of the most highly stimulated sensor, and had a magnitude proportional to the strength of stimulation of this receptor. In the center-of-gravity model, the vector was identical to a vector from the geometric center to the center of mass, where mass is proportional to the stimulation of each of the sensors. If only one sensor is stimulated, both models produce the same result; otherwise they usually generate somewhat different vectors. A vector **F** of identical magnitude pointing in the upstream direction (except for noise in flow direction; see below) is also generated. The desired direction of movement at each time step is therefore determined by a weighted sum of these two vectors: $\mathbf{D} = w\mathbf{F} + (1-w)\mathbf{C}$, where the weight w varies from 0 to 1 in different simulations but is fixed for a particular individual.

Noise was incorporated in a manner parallel to what was done in previous simulations of tropotaxis (Dusenbery, 2001). Adding an individual bias and a temporal component modified each determination of flow and chemical gradient directions. The bias (constant for each individual but different for different individuals) and the temporal component (selected anew at each time step) were both random samples from a Gaussian distribution of mean zero and standard deviation (S.D.) equal to 0.01 of a revolution (3.6°). With the geometry assumed and this degree of bias, a searcher always commanded to move

upstream had a 0.40 chance of contacting the source. Noise levels for all parameters were set to 0.01 (1% S.D.), which was found to give good fit to experimental data for cells moving in smooth chemical gradients (Dusenbery, 2001).

Results

Behavior of crabs

The search behavior of blue crabs to chemical cues in this study was similar to that described in a variety of other investigations (Finelli et al., 1999; Weissburg and Zimmer-Faust, 1993). Animals moved upstream immediately on leaving the starting position and traveled consistently towards the nozzle. Animals occasionally paused during their search for 1–2 s, during which time they often waved their claws or bobbed up and down. Animals rarely moved directly upstream, but instead often tacked across the flume. During these maneuvers they generally stayed within half a body diameter of the mid-line, although extensive cross-stream movement was not uncommon. Crabs found the source of the shrimp-conditioned water approximately 75% of the time, averaging 30.0 ± 1.6 s to complete the task.

Performance of simulation models

An example of a simulated searcher in the chemical plume is shown in Fig. 1. This illustrates the variation of plume signal structure and the relative spatial sampling ability of the simulated 10-sensor crab-like searcher.

The performance of simulations varied with different weightings of rheotaxis (using flow direction information) and tropotaxis (using chemical gradient information) (Fig. 2). Performance was excellent with intermediate weightings, and all searchers reached the source in close to the minimum possible time. However, there was a very sharp decline in performance with weightings below 0.5, and no searcher reached the source. Observation of the simulations in progress revealed that, with low weightings, searchers follow blobs of chemical stimuli downstream (see Fig. 3A). Weightings above 0.8 produced large variations in success, with some searchers reaching the source in minimal time while others never reached it. Observation of the simulations in progress revealed that, with these high weightings, searchers move upstream but some contact the source and some are off to the side (see Fig. 3B). This result is not surprising given that searchers with a high bias in flow direction sensing will veer laterally as they move upstream and miss the source.

Comparison of the two plots in Fig. 2 shows very little difference between the performance of models using the best-receptor and center-of-gravity methods of determining gradient direction.

We compared the results of the basic searcher model discussed above with simulations employing other sensor configurations to examine hypotheses about the importance of sensor number, location and area of integration. A two-sensor model was tested as the simplest that can provide any spatial comparison. (This might correspond to a comparison between



Fig. 1. Illustration of a simulated searcher in the chemical plume. The searcher's ten sensors are located at the ends of the 'spokes'. The dark patches are areas where the chemical stimulus is above threshold. The image is to scale, with the plume extending approximately 150 cm downstream of the source and the diameter of the searcher equal to 10 cm.

two opposite legs). A three-sensor model was chosen as the simplest that can resolve all directional ambiguities. All models were tested with two integration areas corresponding to a single pixel (approximately 1 mm diameter) and the largest radius that avoided overlap of integration regions in the 10-sensor array (31 mm diameter). As before, the previous models all used the same radius for the array (100 mm diameter) and thus had the same spacing between sensors. In addition, a two-sensor model was tested with the array reduced to 31 mm

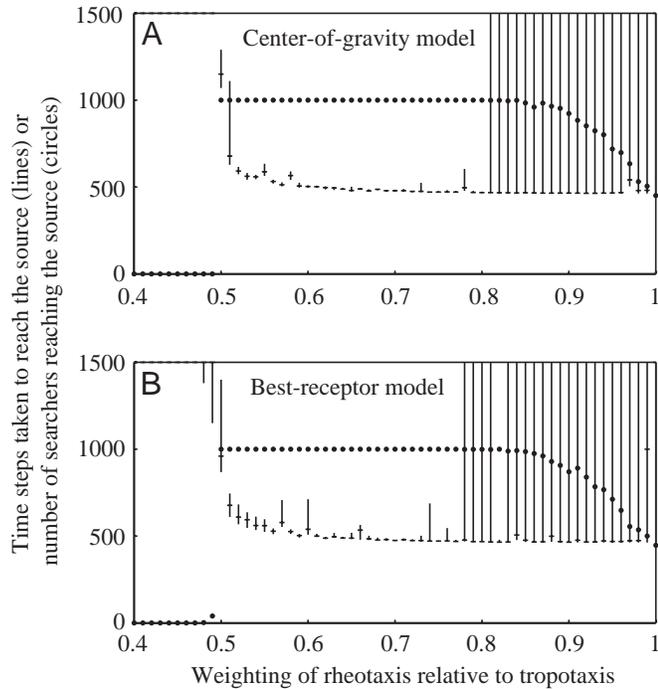


Fig. 2. Performance of center-of-gravity (A) and best-receptor (B) models with different weightings of rheotaxis and tropotaxis. For each weighting, 1000 searchers were simulated for up to 1500 time steps (30 s), as described in Materials and methods. The vertical lines span the range of time steps taken to reach the source, while the horizontal lines indicate the median time taken. The closed circles indicate the number of searchers that reached the source. Few searchers reached the source with a weighting of less than 0.5, whereas weightings greater than 0.8, resulted in some searchers reaching the source while others never did. All searchers reached the source in close to the minimum time with intermediate weightings. For comparison, the average crab found the source in approximately 1500 time steps (30 s).

diameter, the minimum that avoided overlap of the integration areas of the two sensors when the larger integration area was used. (This might correspond to a comparison between two adjacent appendages, such as the pair of antennules.)

Surprisingly, different sensor configurations did not alter the basic results concerning the effects of rheotaxis weightings (Fig. 4). Performance fell off rapidly outside the 0.5–0.8 range of rheotaxis weights for all searcher models. With any weighting within this range, at least 70% of searchers reached the source within 10,000 time steps (Fig. 4A,B). However, the median time taken to reach the source varied 20-fold (Fig. 4C,D). All models with the large integration areas outperformed all those with the small integration areas. In addition, models with more sensors were better than those with fewer, especially for those with the small integration areas. For the two-sensor models, there was little difference between the two array sizes. We again see that the center-of-gravity models were not superior to the best-sensor models (Fig. 4).

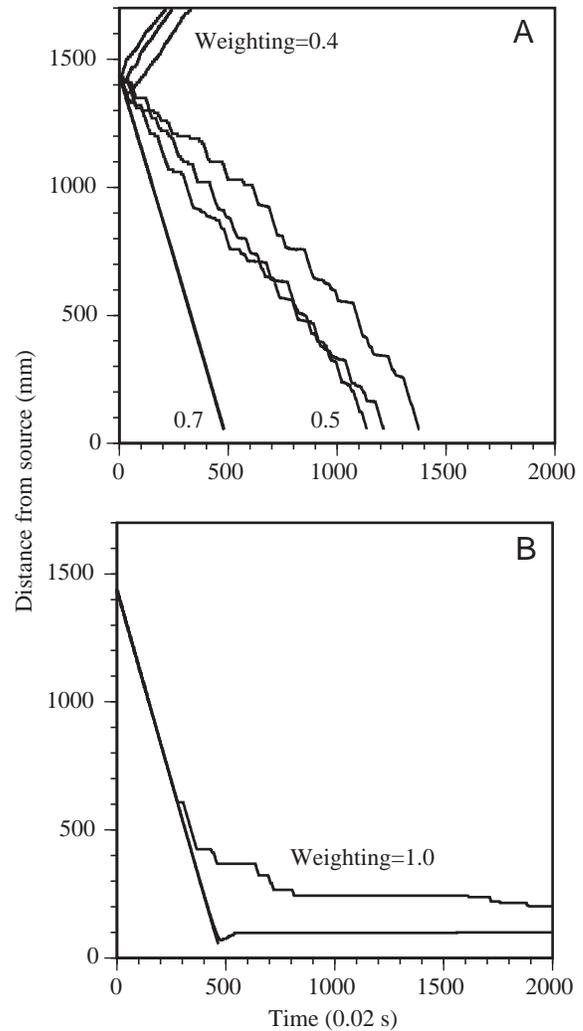


Fig. 3. Distance from the source as a function of time is shown for an arbitrary sample of three simulations at four different rheotaxis weightings. (A) Searchers with a weighting of 0.4 eventually move downstream, away from the source, following blobs of chemical stimulus. All searchers with a weighting of 0.5 move upstream to the source, displaying varying periods of little movement, as stimulus blobs move downstream of the searcher. Searchers move upstream to the source at close to maximum speed, when rheotaxis weightings are 0.7 (the 3 tracks overlap) (B). With a weighting of 1.0, searchers blessed with a low bias in determining flow direction move directly upstream to the source, while others veer cross-stream of the source and move upstream of it, or move out of the plume and rarely progress.

Comparison of real and simulated crabs

We analyzed the path kinematics of 14 tracks each from real crabs that successfully located the odor source, and from simulated crabs using the center-of-gravity model (10 sensors, large integration radius) with rheotaxis weights of 0.7 and 0.5. The success rate of the artificial searchers was 100%, somewhat higher than that of real crabs (see above). In simulations with high rheotaxis weightings, searchers found the source in 10.1 ± 0.1 s (mean \pm S.E.M.), which is about the

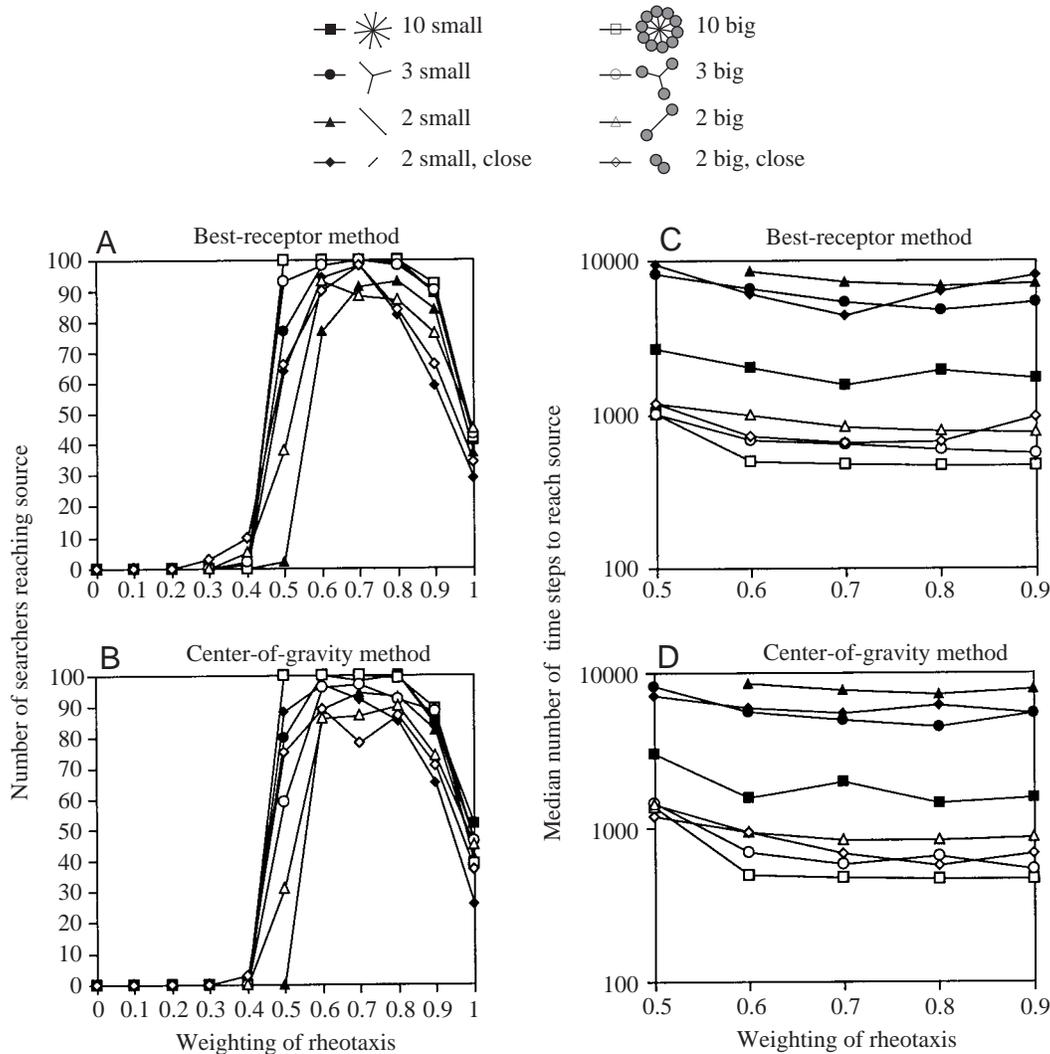


Fig. 4. Comparative performance of searchers with different numbers of sensors and different integration areas (big or small). Sensor arrays are illustrated by 'spokes' from the array center to the center of each sensor and by a filled area of integration (top). With all models, performance falls off rapidly outside the 0.5–0.9 range of weighting rheotaxis. With any weighting within this range, at least 70% of searchers reached the source within the 10,000 time steps simulated (A,B). However, the median time taken to reach the source varied 20-fold (C,D). All models with the large integration area outperformed all those with the small integration area. In addition, models with more sensors were better than those with fewer. With the two-sensor models, the larger array was marginally superior. The more complicated center-of-gravity models (B,D) are not superior to the simple best-sensor models (A,C).

minimum possible time (given the assumed speed limit). Simulations with weights of 0.5 had search times averaging 35.6 ± 7.6 s, suggesting their performance levels were comparable to that of real crabs (see above).

In addition to the similarity in overall behavior, the fine-scale details of locomotory kinematics in simulated crabs strongly resembled the movements of real animals. Typical trajectories of both real and simulated crabs were characterized by relatively straight paths from the starting point to the source (Fig. 5). In general, none of groups produced tracks with any dramatic excursions from the plume centerline, although simulations with lower rheotaxis weightings exhibited more abrupt directional changes. Searchers maintained fairly steady progress towards the source and large velocity fluctuations

were common in both along-stream and cross-stream components, particularly for crabs and simulations with low rheotaxis weightings (Fig. 6). The latter showed more saltational movement than either crabs or simulations with high rheotaxis weightings. Bouts of motionlessness or sluggish movement in simulations with low rheotaxis weightings were associated with relatively large directional changes (Fig. 5, correlation coefficient 0.4). Real crabs occasionally moved downstream in pursuit of the odor plume. In simulations with weightings greater than 0.5, the computer algorithm resulted exclusively in upstream movement, although with lower weightings, downstream movement was common (Fig. 3).

We used a variety of statistical measurements to quantify further the differences in movement between real and virtual

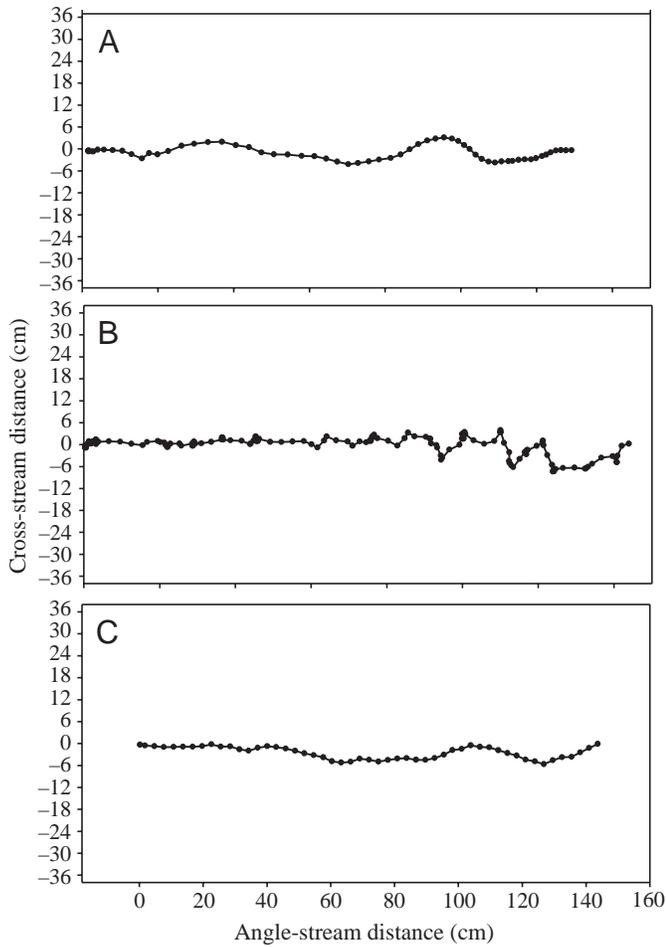


Fig. 5. Typical search paths of real and simulated searchers. (A) Real crab, (B) simulated crab with a rheotaxis weighting of 0.5, (C) simulated crab with a rheotaxis weighting of 0.7. Values represent the coordinates of the animal rendered at 5 Hz.

searchers. Simulated searchers relying more on rheotaxis moved significantly faster and took a more direct path to the odor source than did either simulations with low rheotaxis weightings or real creatures tracking shrimp metabolites (Fig. 7). Simulated searchers with high rheotaxis weightings never paused during locomotion, which distinguished them from the other two groups. However, high variance in motionless periods displayed by crabs obscured any clearly defined trend and the resulting difference among groups is marginally significant.

It may seem surprising that some virtual crabs show few stopping bouts when one rule of the algorithm is for them to stop in the absence of odor stimulation. Since these animals maintained close proximity to the plume centerline, they experienced odor loss only very rarely, so that there were few intervals where movement velocity was zero. By contrast, virtual crabs with high rheotaxis weightings and high bias in flow direction sometimes deviated far from the centerline, where they experienced longer periods in the absence of odor, causing them to cease locomotion. Simulations with rheotaxis

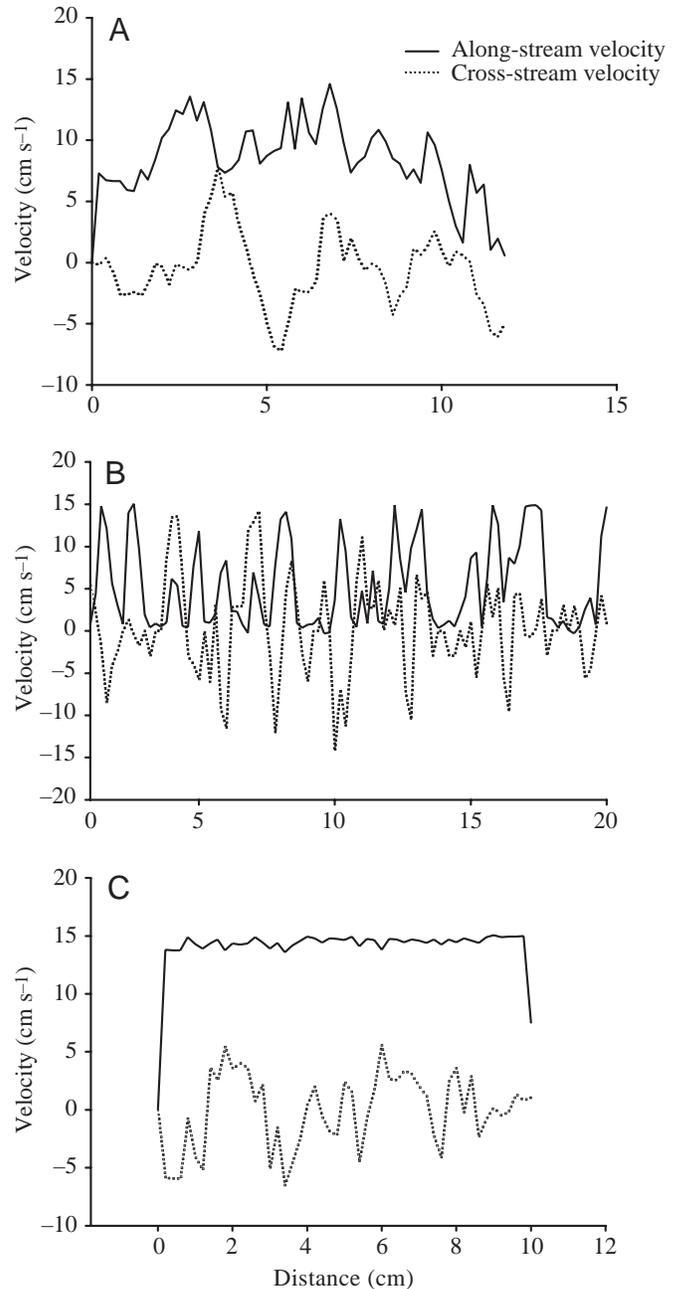


Fig. 6. Typical velocity records in real and simulated searchers. (A) Real crab, (B) simulated crab with a rheotaxis weighting of 0.5, (C) simulated crab with a rheotaxis weighting of 0.7.

weightings of 0.5 apparently followed individual odor features off of the plume centerline, where they ceased movement for short periods of time in which odor was absent (Fig. 3).

Interestingly, real *versus* simulated crabs modulated their behavior differently as they traversed up the plume (Table 1). When we divided the tracking area into three equal regions, based on distance downstream from the source, the analysis indicated that the walking velocity of live crabs decreased as they progressed towards the source, but there was no consistent change of speed in the simulations. Both real and simulated

Table 1. Path kinematics in real and simulated crabs in three different downstream plume regions

	Velocity (cm s ⁻¹) in plume region			Centerline displacement (cm) in plume region		
	100–66 cm	66–33 cm	33–0 cm	100–66 cm	66–33 cm	33–0 cm
Simulations (0.7)	14.3±0.05	14.4±0.05	14.5±0.06	2.55±0.32	2.31±0.21	0.64±0.08
Simulations (0.5)	6.79±0.30	6.73±0.34	6.08±0.23	2.94±0.25	1.83±0.19	0.72±0.54
Real crabs	7.21±0.82	7.26±0.91	5.52±0.73	6.29±1.33	4.85±0.79	3.34±0.32

Weightings in the simulations are given in parentheses.

Mean movement velocity and distance from centerline are shown in three plume regions characterized by their downstream distance from the source.

Values are means ± s.e.m. ($N=14$ paths for each treatment).

Repeated-measures design analysis indicates that movement rate is significantly different in the three regions for real but not for simulated searchers ($F_{2,26}=4.70$, $P<0.05$; $F_{2,26}<2.07$, $P>0.05$, for real and both simulation groups, respectively).

Movement velocity in real crabs declined linearly as a function of distance category ($F_{1,13}=6.07$, $P<0.05$).

Centerline displacement (absolute distance) was significantly different in all groups ($F_{2,26}>5.17$, $P<0.05$ for all groups) and was a linear function of distance category ($F_{1,13}=5.74$, $P<0.05$ for all groups).

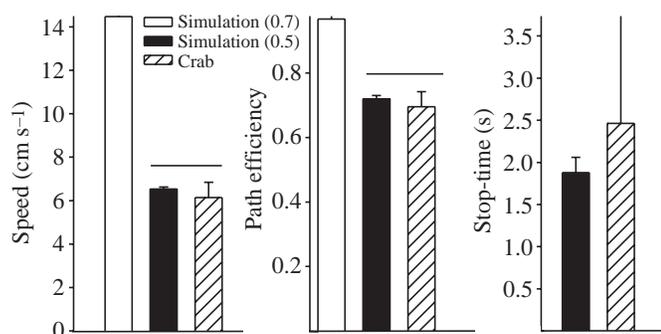


Fig. 7. Path kinematic parameters of real and simulated searchers. Values are means per path ($N=14$ paths for each treatment). Analysis of variance (ANOVA) indicates that speed and path efficiency are significantly different across these groups ($F_{2,39}>39.07$, $P<<0.001$ for both comparisons), whereas the ANOVA analysis of stop time indicates a marginally significant effect ($F_{2,39}=3.02$, $P=0.06$). Horizontal lines join pairs that are statistically indistinguishable based on Scheffe's test, for speed and path efficiency. Path efficiency is the total distance traveled relative to the shortest distance from the origin to the destination, and is unity when the searcher takes the shortest, most direct route to the source. Data are from real (hatched bars) and simulated crabs (open and filled bars; rheotaxis weighting in parentheses).

searchers showed a decrease in their average distance from the plume centerline as they approached the source. Real crabs were generally farther away from the centerline at any given distance downstream than were simulations with high rheotaxis weightings.

The most dramatic differences between the performance of real and simulated crabs are seen in the distributions of speed and directional changes (Fig. 8). The velocity distribution for real animals is relatively uniform, with a modest peak at an intermediate value. The velocity distribution of simulations with high rheotaxis weightings shows a single mode at nearly the maximal velocity, which is consistent with their extremely rapid, sustained movement towards the source. The velocity

distribution of the simulations with low rheotaxis weighting was strongly bimodal, with peaks at both intermediate and high velocities. Intermediate velocities are the result of averaging motionless periods and intervals of movement at maximal speed. The low frequency of remaining stationary reveals that real and simulated crabs rarely went for long without stimulation.

Distributions of turning angles reveal that, compared to real crabs, simulated animals with high rheotaxis weightings generally did not change course drastically, which is a function of their reliance on flow direction cues that are essentially invariant. In contrast, animals with a low rheotaxis weighting made more large-angle course corrections compared to the other two groups. The consequences of these different patterns are easily seen when examining the paths themselves (Fig. 5). As noted, the paths of the low-weighting simulations show dramatic directional changes and sudden bursts of locomotion interspersed with periods of near-stillness, whereas real crabs show more consistent movement and more gradual turning.

Discussion

A particular strength of this study is the ability to examine the kinematics of locomotion by searching animals in both real and virtual environments with similar stimulus properties. It often has been difficult to study the chemosensory abilities of animals in turbulent flows because it is not possible to reproduce the stimulus environment accurately or to know the pattern of stimulation. Our approach relied on empirical data on odorant distribution collected at biologically relevant time and space scales to provide the stimulus environment in the simulations, and on our ability to generate similar flow, and hence odor, conditions to challenge real creatures. Several investigators have proposed that up-stream locomotion in response to odor, combined with spatial sampling, is an efficient way of moving in the direction of a source while maintaining contact with the plume to ultimately result in source localization (Finelli et al., 1999; Weissburg, 2000;

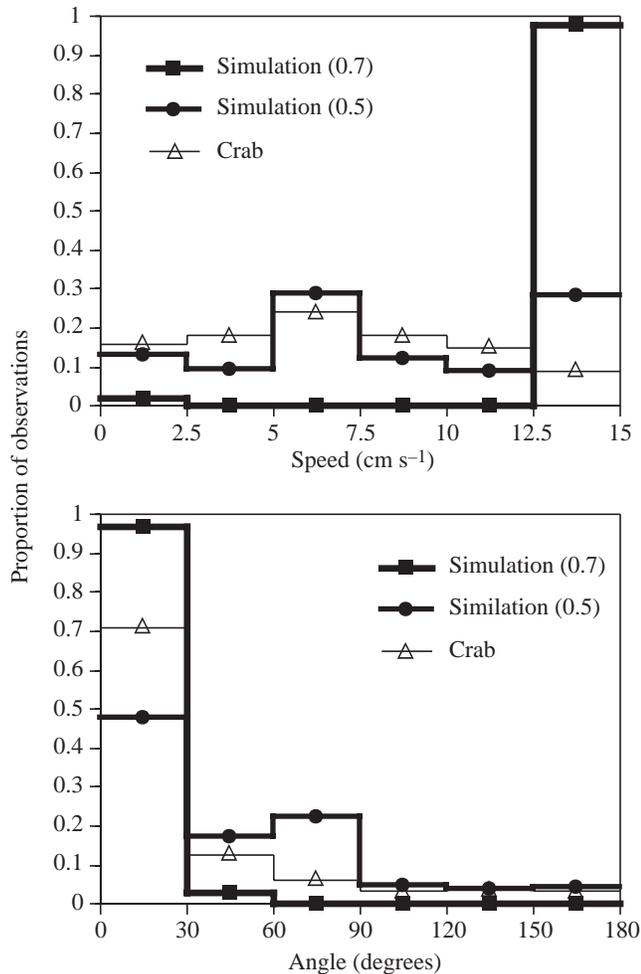


Fig. 8. Distributions of movement speed and turning angles of real and simulated searchers. Data from all paths in a particular group were pooled. Turn angle is the absolute difference between the heading measured over successive frames (crabs) or simulation steps. Values are from real (open triangles) or simulated crabs (filled squares and circles; rheotaxis weighting in parentheses). Sample sizes, representing the number of video frames in all 14 paths in each group, were 758, 2100 and 2504 for simulated searchers with a rheotaxis weighting of 0.7, simulated searchers with a rheotaxis weighting of 0.5 and real crabs, respectively.

Weissburg and Zimmer-Faust, 1993). Similar to real animals, simulated crabs with this combination of sensory mechanisms traveled consistently upstream while narrowing their distance from the cross-stream location of the plume to find the source easily. The correspondence of movement patterns seen in real and virtual crabs indicates that the hypothesized mechanisms are indeed adequate for tracking in turbulent flows, and suggests that aquatic crustaceans may use some variant of these relatively simple strategies *in vivo*.

The present study appears to be the first report of performance comparable to that of real animals when using a good representation of realistic stimulus patterns in a turbulent flow. A few studies have tested specific algorithms for solving

the task of following a chemical stimulus dispersed in a turbulent flow (Belanger and Arbas, 1998; Grasso et al., 2000; Ishida et al., 1996a,b; Kuwana and Shimoyama, 1998; Russell et al., 1995). These studies employed either mechanical robots to implement the algorithm, or computer simulations, and present valuable demonstrations that algorithms produce successful tracking that can be implemented in hardware. Explicit comparisons with the behavior of real organisms are rare, but generally the performance of these artificial agents lags behind that of the creatures used as their inspiration, which are primarily aquatic and terrestrial arthropods. Unfortunately, few previous investigations contained realistic, naturally relevant, quantified odor plumes, which complicates efforts to test the adequacy of proposed mechanisms for solving problems that animals actually face. Using an elegant simulation approach explicitly based on known behavioral and physiological properties, Belanger and Arbas (1998) examined a variety of models for pheromonal tracking in moths. Although simulations took place in a simplified odor environment, their approach could, in principle, be used on plume data sets similar to ours. Belanger and Willis (1996) address the use of video-images of smoke plumes as a dynamic stimulus for simulating moth orientation. In addition to the technical challenges discussed by these authors, visualizing smoke plumes with conventional optics and illumination results in an estimate of concentration that is integrated over the plume volume at any given x,y location. This is a less accurate representation of the stimulus pattern than is obtained using PLIF.

Grasso et al. (2000) examined the performance of a robotic lobster mimic with simple odor sensing mechanisms in a semi-natural odor environment. The robot mimic performed poorly relative to lobsters, particularly in the farther reaches of the plume, where a lack of upstream movement caused the robot to move outside of the plume or simply hold station.

Weissburg et al. (2002) simulated the performance of animals in turbulent odor plumes with the same PLIF data as used here. The virtual searcher had three sensors and both the sensor area and array size were varied systematically. Explicit comparisons of kinematic parameters were not performed, but simulation search time and success rate were similar to those of crabs in the same environment. The major finding was that optimal performance depends on matching the array size and integration area to the scale of the plume. Small array sizes and integration areas result in reduced contact with the plume and an inefficient search, whereas large integration areas erode the spatial contrast and also reduce search success.

Simulation assumptions

The simulations demonstrate that a particular behavioral hypothesis can perform as well as the animals. This, of course, does not prove that the animals employ the same mechanism. In the present case, we have tested what we believe is the simplest hypothesis consistent with known features of the anatomy and behavior of blue crabs.

The assumed sensory capabilities are at least plausible. Several crustaceans are known to have chemosensory hairs on their appendages (Derby and Atema, 1982; Reeder and Ache, 1980; Schmidt and Gnatzy, 1989; Weissburg, 2000) and there is evidence that chemosensors on the walking legs help blue crabs (T. Keller and M. Weissburg, manuscript in preparation), and perhaps other crustaceans (Devine and Atema, 1982; Moore and Atema, 1991), move to a source of food. The source of flow-direction information is less clear, although crustaceans possess an abundant supply of mechanosensors (Ebina and Wiese, 1984; Laverack, 1962; Schmitz, 1992). Information regarding flow probably originates from mechanosensory hairs on various body surfaces that are stimulated by water movement, but might come from joint sensors that respond to deflection caused by the flow acting on the body.

The simulation algorithm assumes that all sensors on the animal are equivalent. This is the simplest model and is a logical starting point, but may not be biologically realistic. Individual chemosensory neurons in crustaceans vary in the suite of compounds that will elicit a response (Derby and Atema, 1988). Whether variation in neuronal sensitivity produces differences in sensitivity or other response properties across appendages remains unknown for most animals. Studies in the lobster *H. americanus* suggest that different sensory appendages may have populations of neurons tuned to different chemicals but similar levels of sensitivity (Voigt and Atema, 1992). Future studies may be required as more data becomes available on physiological properties of particular sensor populations or the relative contribution of different sensory appendages to distance orientation.

Chemotaxis is valuable

The results demonstrate that a simple model combining rheotaxis (using flow direction information) and tropotaxis (using chemical gradient direction information) is sufficient to explain the observed crab behavior. Although extensive studies of chemically mediated guidance in the American lobster *Homarus americanus* have focused on extracting information from the kinetics of the chemical signal (Atema, 1995), it has recently been argued that the nature of chemical plumes requires more time than these animals take to acquire useful samples of directional information (Grasso et al., 2000; Webster and Weissburg, 2001). The efficacy of the simple mechanisms employed by our simulations suggests there is no need to invoke sophisticated temporal processing of signals in order to explain the navigational ability of crabs, and perhaps of other aquatic animals.

The more sophisticated calculation of mean gradient direction across the array of sensors did not yield a superior performance to that obtained by simply choosing the direction of the most highly stimulated sensor (Figs 2, 4). This is particularly surprising for arrays with only two or three receptors. The lack of sensitivity to this difference probably results from the high levels of noise (intermittancy) in the signals, and is consistent with the notion that chemotaxis serves mainly to keep the animal near the plume centerline.

Perhaps the most valuable result of the simulations is the demonstration of the importance of appropriate balance between moving upstream and moving toward higher concentrations. This is consistent with other simulation studies, which suggest that the absence of either chemical gradient or flow information severely compromises performance (Belanger and Arbas, 1998; Grasso et al., 2000). Although our models performed well with a fairly broad range of weightings (0.5–0.8), there were sharp declines in performance outside this range. The successful model can be understood as using chemical information to stay directly downstream of the source (correcting for fluctuations and errors in flow direction) while using flow direction information to move towards the source. This strategy keeps animals close to the centerline of the plume and allows them to track the narrowing width of plume as they travel towards the source. The sharp decline in performance at low weightings can be understood as the result of the net effect of two opposite tendencies (to move downstream following blobs of chemical stimuli and to move upstream as commanded by rheotaxis). This sharp cutoff has been seen in numerous other simulations (not shown), indicating that this result is surprisingly independent of where in the video loop the simulation started.

One of the more interesting findings is that, although success is robust over a range of weightings of chemical gradient *versus* rheotactic information, there is considerable difference in the fine-scale kinematics of virtual animals that vary in their reliance on flow information. In particular, simulated crabs with lower rheotaxis weightings display behavior that more strongly resembles the real organism. This similarity suggests that, like the simulations in virtual plumes, animals in turbulent aquatic plumes extract important information from the chemical signal, as opposed to simply using odor to evoke movement that is guided by the perception of flow direction. The importance of directional cues from odor signals has been postulated previously on the basis of the different behaviors of aquatic *versus* terrestrial arthropods. Flying moths, the archetypal example of odor-tracking proficiency, are thought to be too small and fast to employ spatial comparisons and generally display stereotyped directional changes as a result of an endogenous motor program triggered by odor detection, but steered using perception of flow (Vickers, 2000). This contrasts greatly with the behavior of animals such as blue crabs, which display highly variable course trajectories that are assumed to result from unpredictability in the structure of turbulent chemical plumes (Moore and Atema, 1991; Weissburg and Zimmer-Faust, 1993, 1994).

Although the behavioral concordance of simulated searchers with intermediate rheotaxis weightings ($w \approx 0.7$) and real animals is encouraging, the differences may indicate important disparities between the hypothesized and actual mechanisms. In particular, virtual crabs exhibit bimodal movement velocities and more frequent course corrections than real creatures. Our simulations included neither physical constraints on their movement, nor latency in responding to odor features. Either factor may alter some of the properties

displayed by virtual searchers or robots (Belanger and Arbas, 1998; Grasso et al., 2000).

Spatial integration is valuable

The simulations indicate that the area over which each sensor integrates the signal is quite important. Sensors integrating over a large area are more likely to detect a signal and provide a more accurate indication of the direction of the plume axis. In principle, integration could be performed by summing the outputs of many sensors or by moving one sensor around rapidly. Since the crabs do not move their appendages rapidly and they are covered with many sensory hairs, the summation method is most likely. Although it is not clear what value of integration radius would best emulate the crabs, we expect that it would fall between the two values we used in the simulations. Unfortunately, basic anatomical information that could be used to assess the degree of integration of crustacean chemical sensors is lacking. Most studies examining projections of olfactory neurons focus on how neural substrates convey information about odor quality, not mechanisms relating to preservation or encoding of spatial information.

The results displayed in Fig. 4 demonstrate that more sensors are better, as expected. However the effect is most pronounced for sensors with small integration areas, where ten sensors reached the source in one-third less time than three sensors. With large integration areas, the effect was much smaller. This suggests that the main benefit of having more sensors is simply to increase the probability that one of the sensors detects a signal, rather than to increase the precision of determining the chemical gradient direction.

Other constraints on strategies for navigation

The observation that simulated animals with high rheotaxis weightings perform more efficiently than real creatures does not lead us to believe that we have, in fact, built a better crab. Rather, it is probably an indication that this model works very well in our relatively simple odor environment, which is not the only situation in which real creatures must be competent trackers. Variation in the fluid environment and bottom topography in natural flows will result in more complex plume features, such as large-scale meanders, which are not present in our odor landscape. A heavy reliance on flow direction weakens an animal's ability to use odor cues to move to the plume centerline where it is most likely to continue receiving chemical signals. This is a particularly severe problem where flow direction fluctuates or the source is moving. We expect that adding such features to our stimulus environment would increase the performance of strategies with greater reliance on chemical information, such as we hypothesize that crabs employ.

Secondly, our simulated crabs have no internal mechanism for determining that they have reached their goal, in contrast to real crabs that sense their close proximity to the source. In fact, in our trials we have never observed even the most excited blue crab to overshoot the source and have to backtrack. Since the odor source is released iso-kinetically into the flow,

distance information must come from some odor signal characteristic, and a variety of odor signal parameters may contain the necessary cues (Atema, 1996; Moore and Atema, 1991; Webster and Weissburg, 2001). Blue crabs may slow down as they approach their goal in order to acquire better information on proximity to the source. Other animals (Moore and Atema, 1991), but not simulations, display similar patterns. It appears from our observations that moving to the location of a prey or mate may be easier than knowing the goal has been reached, and that dividing, at least conceptually, the navigation process into these distinct phases may be operationally convenient. Incorporating mechanisms into our simulations that can account for the ability of animals to stop once they have reached their goal is required to evaluate potential constraints between processes that mediate movements towards, *versus* stopping at, the source.

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