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ODOR PLUMES AND HOW BLUE CRABS USE THEM IN FINDING PREY

MARC J. WEISSBURG

Department of Biology, Georgia State University, PO Box 1040, Atlanta, GA 30302-4010, USA

AND RICHARD K. ZIMMER-FAUST

Department of Biology, Marine Science Program, and Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, Columbia, SC 29208, USA

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Summary

Orientation of animals using chemical cues often takes place in flows, where the stimulus properties of odorants are affected by the characteristics of fluid motion. Kinematic analysis of movement patterns by animals responding to odor plumes has been used to provide insight into the behavioral and physiological aspects of olfactory-mediated orientation, particularly in terrestrial insects. We have used this approach in analyzing predatory searching by blue crabs in response to plumes of attractant metabolites released from the siphons of live clams in controlled hydrodynamic environments. Crabs proceed directly upstream towards clams in smooth-turbulent flows and show high locomotory velocities and few periods of motionlessness. Crabs assume more indirect trajectories and display slower locomotion and more stopping in rough-turbulent flows. This degradation of foraging performance is most pronounced as flow shifts from a smooth- to a rough-turbulent regime, where the change in hydraulic properties is associated with contraction of the viscous sublayer region of the boundary layer. Because flow in this region is quasi-laminar, the viscous sublayer may be a particularly effective vehicle for chemical stimulus transmission, such that orientation is severely compromised when it is reduced or removed. Our results also suggest that rheotactic and chemical information are both necessary for successful orientation. Perception of chemical cues acts to bias locomotion upcurrent, and feedback from odorant stimulus distributions appears directly to regulate subsequent stopping and turning *en route* to prey. Although the mechanisms of orientation to odorant plumes displayed by insects and blue crabs are largely similar, blue crabs appear to rely more heavily on spatial and/or temporal aspects of chemical stimulus distributions than has been suggested for insect systems.

Introduction

A large variety of aquatic and terrestrial animals are directed to resources by olfactory

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cues (see reviews in Bell and Cardé, 1984; Atema *et al.* 1988), often navigating through moving fluid. Consequently, distance and directional information on the location of odor sources is frequently encoded by chemical stimulus distributions in odor plumes transported by flow. In these cases, the spatial and temporal aspects of odor plume structure depend on transport dynamics occurring within the carrier fluid, and this may cause interactions between animal orientation ability and the flow environment. Surprisingly, studies explicitly examining the influence of fluid dynamics on qualities of odor plumes and orientation abilities are rare in both marine and terrestrial systems. Substantial advances in understanding the mechanics of orientation in terrestrial animals, notably insects (i.e. Baker and Haynes, 1989; Willis and Arbas, 1991), occurred only after considering the effects of flow on odor plume microstructure (e.g. Mikstad and Kittredge, 1979; Murlis and Jones, 1981). In aquatic systems, previous work has not stressed the control of the fluid environment, or the production of flows mimicking the natural habitats of the experimental organism (see review by Zimmer-Faust, 1989). Although studies have firmly established the chemosensory basis of orientation in aquatic organisms, particularly crustaceans (see review by Ache, 1988), it is currently difficult to come to firm conclusions regarding the connections between hydrodynamic forces, chemosensory abilities and locomotory performance.

Experimental verification of the interaction between hydrodynamics and olfaction is a necessary first step in determining the mechanisms of orientation in flow. Weissburg and Zimmer-Faust (1993) recently assayed the ability of blue crabs, *Callinectes sapidus* (Rathbun), to locate live prey (actively pumping clams) using chemosensation. These experiments took place under measured and controlled hydrodynamic conditions naturally experienced by crabs in estuaries along the Gulf of Mexico and south Atlantic coasts of the United States. Actively pumping bivalves liberate metabolic by-products, such as small peptides (Rittschof *et al.* 1984), which appear to act as olfactory cues guiding animals to the location of potential prey. The interaction between hydrodynamics and the success of olfactory-mediated behavior was addressed by measuring the properties of boundary layer flows, specifically boundary shear velocity and roughness Reynolds number. Shear velocity is proportional to the magnitude of turbulent mixing, while roughness Reynolds number increases as turbulent eddies penetrate closer to the substratum. This investigation revealed that the degree of boundary layer turbulence, rather than flow speed or sea bed topography *per se*, largely determines the ability of crabs to locate potential prey. Flows characterized by higher shear velocity and roughness Reynolds number diminish the capacity of crabs to track clam scents.

Documenting the effects of explicitly characterized fluid flows on orientation behavior in blue crabs raises further issues concerning the mechanisms of orientation. Theoretical treatments of odor transport indicate that changes in turbulence magnitude are principally expressed as alterations in the frequency and duration of discrete pulses of odorants within a plume, as well as by changes in odorant concentration (Murlis *et al.* 1991). Investigators have proposed several orientation mechanisms that are potentially sensitive to these changes in the distribution of odorants and which could explain the correlation between orientation success and hydrodynamic conditions observed in *Callinectes.* These mechanisms focus on the use of purely chemical cues to establish the distance and

direction to sources of odor emission. Investigators have hypothesized that some crustaceans encode information by determining differences in odorant intensity simultaneously at bilaterally paired chemosensory organs (Reeder and Ache, 1980; Devine and Atema, 1982), or possibly by successive assessment of the frequency of detectable odor pulses or the onset slope of an odor pulse (Moore and Atema, 1991). However, given the lack of detailed information on movement patterns of animals in hydrodynamically defined flows, it is difficult to substantiate whether orientation is purely chemotactic, much less to distinguish among proposed orientation strategies.

Kinematic analysis of locomotory behavior has been invaluable in understanding orientation mechanisms in other animals that function in flows, particularly insects navigating in pheromone plumes (e.g. Baker and Haynes, 1989; Willis and Arbas, 1991). Patterns of insect behavior have frequently suggested mechanisms amenable to further study in more controlled stimulus environments or suggested fruitful neurophysiological approaches. Consequently, we have attempted a detailed analysis of the search kinematics of blue crabs as they attempt to locate prey in controlled flows. As in our earlier work, explicit hydrodynamic measurements have been coupled with determinations of the locomotory performance of individuals in differing flow regimes. Not surprisingly, turbulence is found to have major effects on the kinematics of olfactory-mediated prey search. Crabs search more effectively at slower speeds and assume more indirect routes as boundary layer flows become more turbulent. Degradation of orientation performance is most pronounced as flow shifts from a smooth-turbulent to a rough-turbulent regime. This shift is associated with contraction of the viscous sublayer, a region of quasi-laminar flow adjacent to the sea bed that is apparently conducive to uninterrupted, effective transmission of chemical signals.

Materials and methods

Overview of flume design and hydrodynamic methodology

The flume

Experiments were conducted in steady flows and in fully developed boundary layers within a single-channel recirculating flume (10 m length × 0.75 m width × 0.15 m water depth) constructed from Plexiglas. The working section was a fixed drop-box (100 cm length × 45 cm width × 15 cm depth) placed 7.5 m downstream of the entry section and 1.5 m upstream of the exit weir. The drop box was filled with sand taken from local habitats and sieved to <1 mm to remove large particles (mean particle diameter 351±9.9 μm, S.D.; N=100). The entire flume bed was carefully layered to a uniform depth of 0.5 cm with this material.

Hydrodynamic methods

Benthic boundary layer flows can be defined by a variety of fluid dynamic variables. In unidirectional flows, frictional or shear velocity (u^*) and roughness Reynolds number (Re^*) are measures of boundary layer flows that are generally comprehensive enough to provide a means of establishing the dynamic similarity of various flow regimes (Nowell and Jumars, 1984). For an organism moving within the boundary layer region, such as a

macroscopic crustacean crawling along the seafloor, these measures roughly indicate the hydrodynamic forces acting upon the animal. Shear velocity and roughness Reynolds number were calculated by determining the velocity gradient through the log-layer region of boundary layer. Neutrally buoyant styrene/divinylbenene beads (350 μm diameter, specific gravity 1.03; Bangs Laboratories, Carmel, IN) were injected into the flow at known heights above the substratum. Path trajectories were recorded on video tape, and bead velocity was determined using computer-aided video motion analysis with a Motion Analysis system (model VP-110; Motion Analysis Corp., Marin, CA). Given the relationship between velocity and log height above the substratum, boundary shear velocity (u^*) was calculated using the 'law of the wall':

$$U(z) = (u^*/k)\ln(z/z_o)\,, \tag{1}$$

where $U(z)$ is the mean velocity at height z above the substratum, k is von Karman's constant and z_o is the roughness height, determined as the y-intercept of the equation regressing log$z$ against measured flow speed (Schlicting, 1979). Roughness Reynolds numbers (Re^*) were calculated as:

$$Re^* = u^*D/v\,, \tag{2}$$

where D is the bed roughness scale (the mean value of the sediment grain size) and v is the kinematic viscosity of sea water. Finally, we estimated the thickness of the viscous sublayer by reworking equation 2 and solving for D, setting Re^*=6. The onset of turbulence in outer boundary layer regions occurs at an Re^* of approximately 6 (Schlicting, 1979); thus, when Re^* is set to this value, D estimates the thickness of the region of quasi-laminar flow. Explicit details pertaining to the flume design, the calculation of hydrodynamic variables and the methods used to determine log-layer velocity gradients are found in Weissburg and Zimmer-Faust (1993).

Animal capture and holding methods

Adult blue crabs *Callinectes sapidus* (Rathbun) were captured by seine or by baited trap at local marshes and maintained in one of the holding tanks associated with the flume. Lighting was provided by standard fluorescent fixtures, with the photoperiod set at 12 h:12 h (L:D). All animals were allowed to acclimate to laboratory conditions for 4–5 days prior to experimentation and were fed an *ad libitum* diet of bivalves and fish. Crabs were starved for 48 h prior to experiments, in order to standardize their hunger level.

Water in both the holding tanks and flowing through the flume during behavioral trials passed through a 5 μm particle filter, an activated-charcoal bed and an ultraviolet sterilization unit before being returned to the system. We periodically monitored the levels of organic chemicals (ammonium and dissolved organic carbon) in the seawater system. Levels of these organic chemicals never exceeded concentrations typically found in estuarine environments.

Hard clams (*Mercenaria mercenaria*) were obtained from a commercial supplier, housed in holding tanks without blue crabs and fed every other day on a mixed diet of the flagellates *Isochrysis galbana* and *Pavlova lutheri* in logarithmic growth phase at a cell density of approximately 4×10^4 cells ml^{-1}.

Behavioral assays

Behavioral trials consisted of challenging crabs to locate actively pumping *Mercenaria merceneria* under a variety of flow regimes in the flume. Trials were performed at four different current speeds (free-stream velocity, U_∞=0, 1.0, 3.8 and 14.4 cm s^{-1}), with prey located either 1.0 or 0.5 m away from the crab's initial starting position (hereafter referred to as 'far' and 'close' starting sites, respectively). Trials were also performed at each current speed without prey (no-clam controls) at the close starting site.

All behavioral trials were started at least 1 h after sunset, since *Callinectes* generally exhibits peak foraging activity during nocturnal periods (e.g. Sponaugle and Lawton, 1990). Crabs were individually isolated, fitted with a monofilament tether and then allowed to remain undisturbed under natural illumination until used in behavioral trials. A trial commenced by placing a tethered crab into the flume containing a 0.25 m^2 patch of *Mercenaria*, at a density of 40 clams m^{-2}. This density is well within the range reported for natural populations of *Mercenaria* (Walker, 1989). Crabs often gravitated to the flume edge during the acclimation period; 30–45 min after introducing the crab (and only when the crab was at least 15 cm away from the walls), the tether was gently cut and the crab was allowed to forage for the clams for 30 min. Crab foraging behavior was filmed with a SONY CCD camera, equipped with a 1.8 mm wide-angle video lens, video tape recorder and viewing monitor. Filming was accomplished with infrared light (>820 nm), since previous work has shown that brachyuran crabs cannot detect light emissions at these longer wavelengths (Cronin, 1986). Only intermolt crabs between 75–125 mm in carapace width were used in trials, and both sexes were equally represented. Individuals were tested once, and then released several kilometers from their initial capture location.

Locomotory and orientation behavior were determined using computer-aided motion analysis of the crab movement trajectories. A path began when the crab first exhibited movement and ended when the animal either began to dig for a clam ('successful search'), or left the camera's field of view without locating the clam ('unsuccessful search'). Paths were analyzed at a rate of 2 frames s^{-1}; thus, data from each set of consecutive frames represent values averaged over a 0.5 s interval. We determined the distribution of angular bearings (0–360 °) of movement for all crabs relative to the direction of flow (0 °); that is, the angle of the vector defined by the animal's translational movement from the starting frame to the following frame. We also determined the average velocity of each path and the distribution of velocities for each path on a frame-by-frame basis. Lastly, the net-to-gross displacement ratio (NGDR) was determined for each path. The NGDR is the ratio of the shortest linear distance between the start- and endpoints of the path divided by the total travel distance. This indicator of path circuity has a maximum value of 1 (when paths are completely straight) and a minimum of zero (when paths are circular and the start- and endpoints occur at the same spatial coordinates).

Experiments separating the effects of advection (bulk transport) from eddy diffusion (turbulence)

The results of experiments with live clams indicated that increasing the free-stream

flow velocity substantially altered the search behavior of crabs (see Results). At the macroscopic scale of a blue crab, transport of odor molecules occurs principally by eddy diffusion and advection. Both of these transport mechanisms increased with free-stream velocity in our experiments with live clams; advection and diffusion thus covaried. Benthic boundary layer turbulence increases with the hydraulic roughness length of the bed (i.e. sediment grain size; equation 2); we therefore elected to manipulate the bed substratum size to increase turbulence, and hence eddy diffusion, without increasing free-stream advection.

Behavioral assays were repeated at U_∞=1.0 and 3.8 cm s^{-1}, and additional trials were performed at U_∞=1.0 cm s^{-1}, where the sand substratum was replaced with a commercially available fine gravel (particle diameter 1.93±0.65 mm S.D.; N=100). At U_∞=1.0 cm s^{-1} flowing over the gravel substratum, u^* and Re^* increased relative to their values at the same velocity flowing over sand, effectively decoupling advection from eddy diffusion (see Results).

To provide more control over the stimulus environment, these experiments utilized prey extracts emanating from model clams as the odor source. Prey extract was prepared from hard clam tissue homogenized in artificial seawater medium (ASW, Forty Fathoms Marine Mix) prepared using HPLC grade deionized water and reagent grade salts (16.5 g wet tissue mass l^{-1}). The extract was prepared as a single batch, stored at −87 °C, then diluted to a final concentration of 1:50 with ASW immediately prior to use.

To recreate the physical conditions of odor dispersal experienced by crabs foraging for live clams, the homogenate was introduced into the flow through the excurrent siphon of a model clam. Excurrent and incurrent siphons of a living clam were simulated using a pair of Tygon tubes, placed contiguously with their lips and extending 3 mm above the substratum. The excurrent flow was supplied from a small constant-head tank while the incurrent flow was taken by a gravity feed from the flume. To ensure equality of the incurrent and excurrent flow rates, we continuously monitored these flows with two separate in-line rotameters. The scale of our model bivalve (physical size and pumping rate) corresponded to a *Mercenaria mercenaria* of 15–20 mm length. On the basis of the measurements of pumping clams (Weissburg and Zimmer-Faust, 1993), behavioral trials were performed with a flow rate of 10 ml min^{-1} through a siphon pair with an excurrent diameter of 3.7 mm and an incurrent diameter of 4.7 mm.

Behavioral assays were conducted, and analyzed, using the protocol described above. Because paths were generally rather short and with high locomotory velocity, motion analysis was performed using a video sampling rate of 5 frames s^{-1}. Animals were tested only at an initial starting site of 0.5 m. A successful search was defined as one in which a crab located the siphon of the model clam and began to grasp or unbury the siphon tubes. A full treatment of the methods used in performing behavioral assays is presented in Weissburg and Zimmer-Faust (1993).

Statistical methods

For experiments involving live prey, we employed multiway analyses of covariance (ANCOVA) (Statistical Analysis Systems GLM procedure; SAS Institute, Carey, NC; SAS, 1988) to determine the effects of flow speed, starting site and search result

(successful, unsuccessful and control) on mean velocity and NGDR of crabs during searching. In this analysis, flow speed was the regression variable, and starting site and search result were classification criteria. Paths of individual crabs were replicates in the statistical analysis.

To investigate the mechanics of prey search adequately, it is also necessary to perform a more detailed analysis of crab behavior by examining the distributions of locomotor velocities and angular bearings during searching. However, statistical analysis of kinematic data is quite difficult, and there is no completely adequate method. Circular statistics (e.g. Batschelet, 1981) are generally only useful for examining a mean angle over the entire path; that is, the angle of the vector from the initial to the final position of the animal. In our experiments, the mean angle will be 0° for crabs moving directly upstream to find prey, so analysis using mean angles reveals little regarding the actual movement during searching. Calculating the mean angle for each path by averaging the bearing of the animals across all frames will also result in values near zero. Furthermore, this analysis suffers from a lack of independence, and paths with more frames are weighted unequally. The last option is to compute a time budget for each path, by expressing the proportion of travel time across various angular intervals, which become a treatment effect in an analysis of variance (ANOVA). However, because these time budgets must sum to one, the levels of the angular interval treatment of the ANOVA analysis are not independent. Given these difficulties, investigations of search kinematics typically present and analyze data using frame-wise measurements (i.e. Buskey, 1984; Butman *et al.* 1988; Willis and Arbus, 1991).

In order to examine search kinematics more explicitly, we therefore employed multiway contingency table analysis to determine the effects of flow speed and search result on the distribution of velocities and angular bearings. The raw frequency data (from the analysis of video frames) from all animals within a particular experimental treatment were pooled to give aggregate distributions of velocities and angular bearings. For multiway tables, Cohran–Mantel–Haenszel (CMH) general association statistics were used to examine the general significance of each main effect while holding the other effects constant. *G*-tests were employed in analysis of two-way tables extracted from the full data matrix.

Results

Hydrodynamic conditions

Hydrodynamic conditions during behavioral trials varied with current speed in experiments with live clams and were functions of current speed and substratum type in experiments using artificial clams (Table 1). In experiments with live clams, the measurements of Re^* indicate that boundary layer flows were smooth-turbulent at free-stream velocities of 1.0 and 3.8 cm s^{-1} and that flow was transitional between smooth- and rough-turbulence at 14.4 cm s^{-1}. In experiments using artificial clams, measurements of u^* and Re^* indicated greater turbulence, with eddies penetrating closer to the substratum, at a velocity of 1.0 cm s^{-1} over gravel than at that same velocity flowing over sand. On the basis of calculated measures of boundary-layer structure, and visual

Table 1. *Summary of hydrodynamic variables for flow treatments used in crab behavioral trials*

U_∞ (cm s^{-1})	u^* (cm s^{-1})	Re^*	Viscous sublayer thickness (mm)
0.0	–	–	–
1.0	0.07	0.4	8.0
1.0 (gravel)	0.14	2.7	1.1
3.8	0.3	1.6	1.8
14.4	1.2	6.3	0.5

U_∞, free-stream velocity; u^*, boundary shear velocity; Re^*, roughness Reynolds number. Substratum is sand except where otherwise noted.

observation of Fluorescein dye ejected through the clam mimic (Weissburg and Zimmer-Faust, 1993), plumes generated at 1.0 cm s^{-1} over gravel were more similar to those at a current speed of 3.8 cm s^{-1} over sand than to plumes at a speed of 1.0 cm s^{-1} over sand.

Experiments with live clams

Analysis of locomotory speed

Mean speeds were complicated functions of flow velocity, starting site and search result (Fig. 1A; Table 2). The large number of significant interaction effects indicates that movement patterns were often determined jointly by two or more variables, although the main effects themselves were generally significant.

Locomotory speeds of animals discovering prey (successful searchers) were significantly faster than those of animals that either failed to uncover prey (unsuccessful searchers) or were not presented with a prey item (no-clam controls; Fig. 1A; Table 2). Ryan's multiple range test (α=0.05) revealed that the speeds of successful searchers were greater than the speeds of both unsuccessful searchers and animals in no-clam controls,

Table 2. *Summary ANCOVA table for analysis of mean locomotory speed in experiments with live clams*

Effect	SS	d.f.	MS	*F*
Flow speed	1.25	1	1.25	3.11
Site	6.88	1	6.88	17.11***
Result	35.66	2	17.83	44.37***
Flow speed × site	0.16	1	0.16	0.39
Site × result	3.03	1	3.03	7.54**
Flow speed × result	2.90	2	1.45	3.61*
Flow speed × site × result	0.007	1	0.007	0.02
Error	64.69	161	0.402	

In this analysis, flow speed is the regression variable, and site (close or far) and result (successful, unsuccessful, no clam control) are class variables.

*P<0.05; **P<0.01; ***P<0.001.

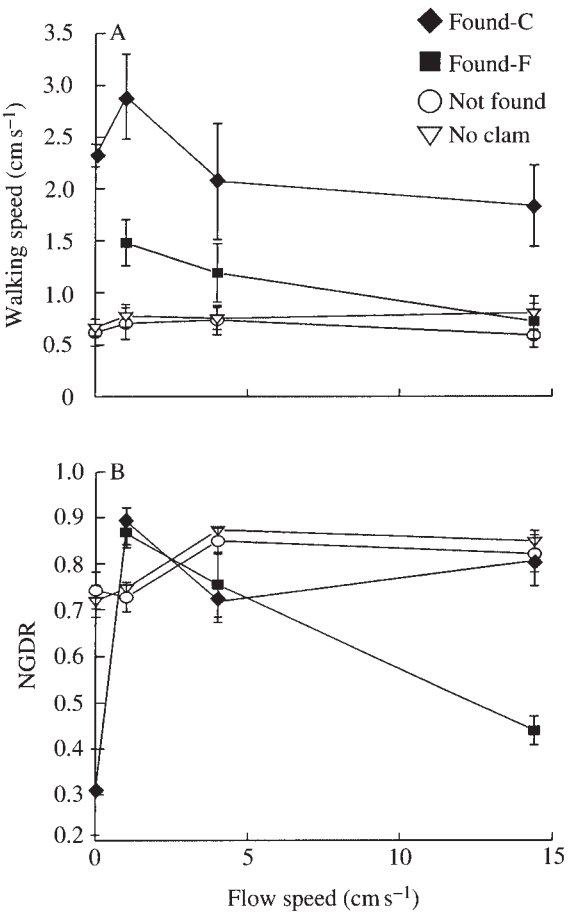

Fig. 1. Mean motion statistics for crabs in experiments with live clams. Mean ±1 S.E.M. Found-C, crabs starting from the close site and finding clams. Found-F, crabs starting from the far site and finding clams. Not found, crabs not finding clams, with data pooled across both starting sites. No clam, crabs in no-clam controls, which always started at the close site. For successful searchers starting at close positions, the numbers of crabs were 2, 9, 6, and 3 at 0, 1.0, 3.8 and 14.4 cm s^{-1} respectively. For successful searchers starting at far positions, the numbers of crabs were 6, 4 and 2 at 1.0, 3.8 and 14.4 cm s^{-1} respectively. For unsuccessful searchers starting at close positions, the numbers of crabs were 17, 9, 13 and 12 at 0, 1.0, 3.8 and 14.4 cm s^{-1} respectively. For unsuccessful searchers starting at far positions, the numbers of crabs were 17, 11, 13 and 13 at 0, 1.0, 3.8 and 14.4 cm s^{-1} respectively. For no-clam controls, the numbers of crabs were 10, 10, 9 and 9 at 0, 1.0, 3.8 and 14.4 cm s^{-1} respectively. (A) Mean locomotory speed *versus* free-stream flow velocity. No crabs found clams at 0 cm s^{-1} at the far starting site. (B) Mean net-to-gross displacement ratio (NGDR) *versus* free-stream flow velocity.

and also that the locomotory speeds of the latter two groups were statistically indistinguishable. Free-stream velocity and starting site affected locomotory speed only for crabs that found prey; Ryan's multiple range test indicated that all animals failing to

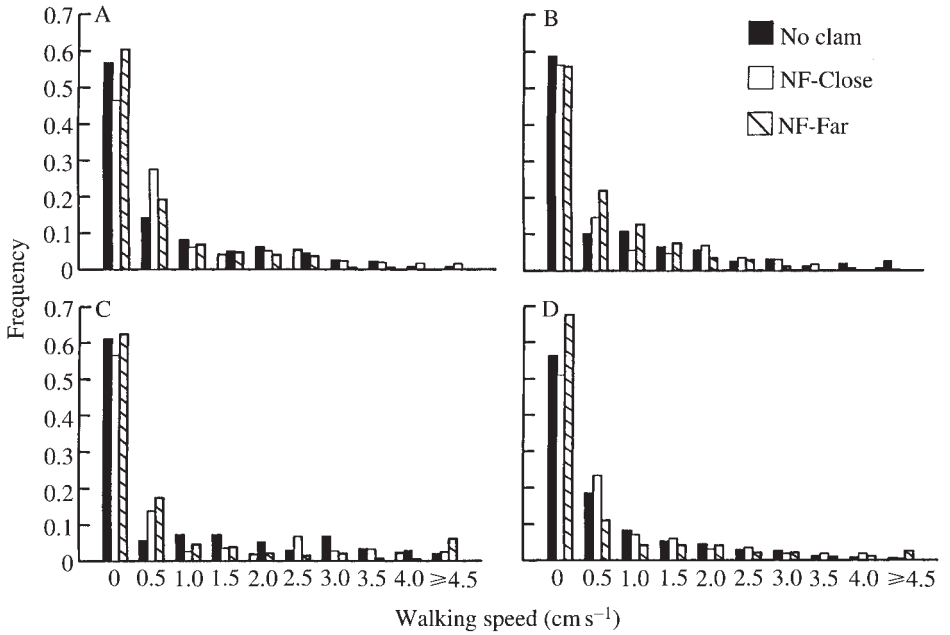

Fig. 2. Frequency distribution of locomotory speeds at each flow velocity for crabs that were not presented with clam prey or did not locate clam prey. No clam, no-clam control; NF-Close, crabs starting from the close site and not finding clams; NF-Far, crabs starting from the far site and not finding clams. (A) 0 cm s^{-1}, *N*=2801, 3042 and 10137 frames for no-clam controls, close and far starting sites, respectively. (B) 1.0 cm s^{-1}, *N*=3528, 5388 and 1496 respectively. (C) 3.8 cm s^{-1}, *N*=2273, 8175 and 1478 respectively. (D) 14.4 cm s^{-1}, *N*=2684, 1758 and 1552 respectively. Numbers of crabs in each treatment are given in the legend of Fig. 1.

locate clams or in the control group displayed similar movement speeds regardless of initial distance or free-stream velocity. This accounts for the interactions when 'result' is paired with the other main effects (Table 2). In general, successful searchers moved faster at slow current speeds and when initiating search closer to prey (Fig. 1A).

Analysis of the distributions of walking speed provides some insight into the patterns of mean velocity. Animals that did not find clams, or where no prey was present, showed a similar distribution of velocities regardless of current velocity or starting site (Fig. 2). The distributions of walking speeds are unimodal, with a large peak in the zero velocity bin (speed <0.25 cm s^{-1}) and steadily decreasing frequencies at higher locomotory speeds. By comparison, crabs finding clams had speed distributions lacking a strong peak in the zero velocity bin, but with an appreciable frequency of observations at higher locomotory velocities (Fig. 3).

Animals finding clams (at either site) exhibited a lower frequency of observations in the zero velocity class and more observations at high speeds than animals either not finding clams or in the control group. Search result significantly affected the overall distribution of speeds when controlling for current velocity (Table 3). Pairwise

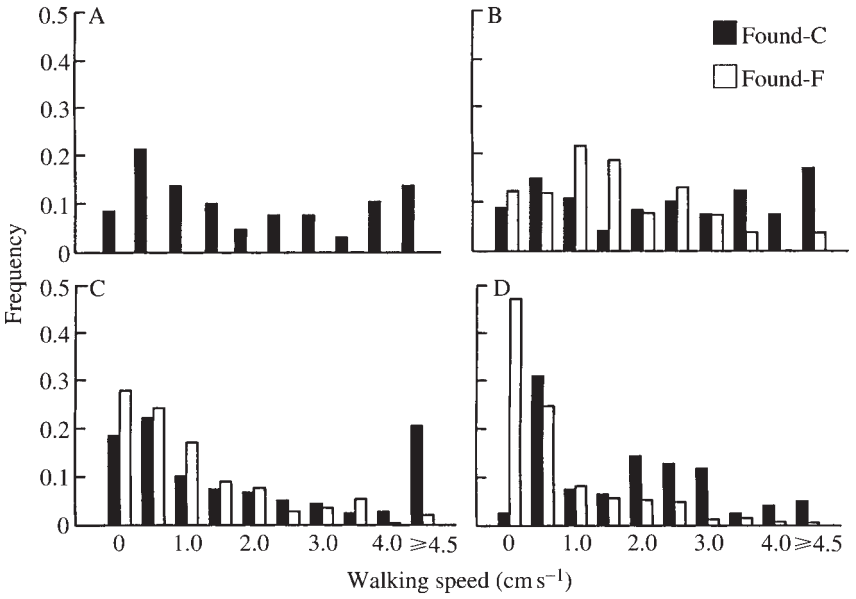

Fig. 3. Frequency distribution of locomotory speeds at each flow velocity for crabs that found clam prey at either the close or far starting site. (A) 0 cm s^{-1}, *N*=306 frames for the close site. No crabs found clams starting from the far site. (B) 1.0 cm s^{-1}, *N*=228 and 332 frames for the close and far sites, respectively. (C) 3.8 cm s^{-1}, *N*=268 and 189 respectively. (D) 14.4 cm s^{-1}, *N*=122 and 483 respectively. Numbers of crabs in each treatment are given in legend of Fig. 1.

Table 3. *Summary of analysis of distribution of locomotory speeds in experiments with live clams*

	Test statistic	Value	d.f.
Search result			
Close sites	CMH[1]	1381.2***	9
Far sites	CMH	338.01***	9
Flow speed[2]	CMH	246.40***	27
Site[2]	CMH	184.94***	9

[1]Cochran–Mantel–Haenzel general association statistic.
[2]Analysis includes only crabs successfully finding clams.
***P<0.001.

comparisons of successful *versus* unsuccessful searchers at each flow rate indicate a significant effect of search result on the overall distribution of locomotory speeds at close and far sites at U_∞=1.0 cm s^{-1} (CMH>158.18, d.f.=9, P<0.001, for all comparisons). In spite of the lack of an effect on the entire distribution of walking speeds at the remaining far sites (U_∞=14.4, 3.8 cm s^{-1}), animals that find clams show a lower frequency of observations in the zero velocity bin than do their counterparts that fail to locate clams (G>20.50, d.f.=1, P<0.001, for both comparisons).

Table 4. *Summary of characteristics of motionless periods as a function of flow speed for crabs in experiments with live clams*

	Flow speed ($cm\ s^{-1}$)			
	0	1.0	3.8	14.4
Mean ± S.E.M.	2.3±0.5	3.9±1.1	5.5±1.1	7.5±1.7
N	12	16	19	34

Mean duration of motionless period (number of video frames) is given ±1 standard error, with number of periods.

Video frame rate is 2 frames s^{-1}.

When considering only animals that search successfully, the velocity distributions of animals in low flows were right-shifted, so the modes occurred at higher velocity classes. Furthermore, crabs remained motionless less often at low flows and when initiating search closer to prey (Fig. 3). The duration and number of bouts of motionlessness are given in Table 4. Although there is considerable variability, regression analysis indicated a general association between the duration of motionless periods and flow velocity (F=4.44 d.f.=1,79, P<0.05).

The mean speed for each crab finding prey was subsequently recalculated after removing all observations in the zero velocity class, yielding average locomotory speed of crabs during the time animals were actually walking. These 'true' locomotory speeds were then analyzed with ANCOVA, again using flow speed as the regression variable and initial site as the classification variable. Crabs still moved significantly faster when initially closer to clam prey, and there was a strong, but only marginally insignificant, tendency for crabs to move faster at lower flow velocities (P<0.10; Table 5).

Analysis of NGDR and angular bearings

The analysis of NGDR reveals a more complicated interaction among the variables

Table 5. *Summary ANCOVA statistics for analysis of mean true locomotory speed of crabs successfully locating clam prey*

Effect	SS	d.f.	MS	*F*
Flow speed	1.88	1	1.88	3.37†
Site	2.05	1	2.05	4.34*
Flow speed × site	0.51	1	0.51	0.92
Error	11.76	21	0.559	

In this analysis, mean locomotory speeds have been recalculated after removing all observations in which velocity equals 0.

†P<0.1; *P<0.05.

For crabs starting close to prey, average locomotory speed was 2.8, 2.8, 1.9 and 1.5 $cm\ s^{-1}$ at flow velocities of 0, 1.0, 3.8 and 14.4 $cm\ s^{-1}$ respectively. For crabs starting far from prey, average locomotory speed was 1.8, 2.0 and 1.3 $cm\ s^{-1}$ at flow velocities of 1.0, 3.8 and 14.4 $cm\ s^{-1}$ (no crabs found clams at the far site in no flow).

Table 6. *Summary ANCOVA statistics for analysis of mean NGDR in experiments with live clams*

Effect	SS	d.f.	MS	*F*
Flow speed	0.024	1	0.024	0.59
Site	0.058	1	0.058	1.41
Result	0.052	2	0.026	0.63
Flow speed × site	0.263	1	0.263	6.36**
Flow speed × result	0.476	2	0.238	5.75**
Site × result	0.087	1	0.087	2.11
Flow speed × site × result	0.193	1	0.193	4.66*
Error	6.830	161	0.042	

In this analysis, flow speed is the regression variable, and site and result are class variables.
Net-to-gross displacement ratio, NGDR, has been arcsine-transformed to meet normality criteria.
*P<0.05; **P<0.01.

than that occurring in the analysis of locomotory speed (Fig. 1B; Table 6). There was a significant interaction among all three variables. In five of the six possible comparisons in which there is flow, animals that find prey show lower NGDRs at high current speeds and higher NGDRs at low current speeds. This trend is violated by crabs in zero flow, an observation having important implications for hypotheses on orientation mechanisms (see Discussion). Animals that do not find clams or animals in the no-clam control group show the reverse pattern, displaying lower NGDRs at low current speeds and higher NGDRs at high current speeds.

Analysis of movement direction provides explanations for the patterns of NGDR observed across the various experimental treatments (Figs 4, 5). For animals that do not find clams or are not presented with prey, the distribution of angular bearings indicates that as flow increases movement becomes increasingly aligned with the axis parallel to the current. In these treatment groups, flow velocity exerts a significant effect on the distribution of angular bearings (CMH>947.0, d.f.=21, P<0.001, for all comparisons). As flow velocity increases, animals move in a straight line parallel to the flow direction and proceed either directly up- or downstream. At low velocities, crabs take headings oblique to the flow axis and execute numerous turns while travelling either up- or downstream across the working section. There appears to be no detectable pattern with respect to frequency of movement up- *versus* downstream for crabs not finding clams. On the basis of confidence limits for percentages (data shown in Fig. 4), animals in five treatments (0 cm s^{-1} no clam; 0 cm s^{-1} close; 1 cm s^{-1} close and far; 14.4 cm s^{-1} close) moved predominantly downstream, animals in four treatments (1.0, 3.8 and 14.4 cm s^{-1} no clam; 14.4 cm s^{-1} far) moved mainly upstream, and up- *versus* downstream movement was equal in the remaining treatments (0 cm s^{-1} far; 3.8 cm s^{-1} close and far). Pooled across all trials, movement was random with respect to flow direction.

In contrast, the relationship between movement direction and flow rate in successful predators was exactly the opposite of that found in either of the other groups (Fig. 5). Typical search paths displayed by successful predators are shown in Fig. 6. As in the

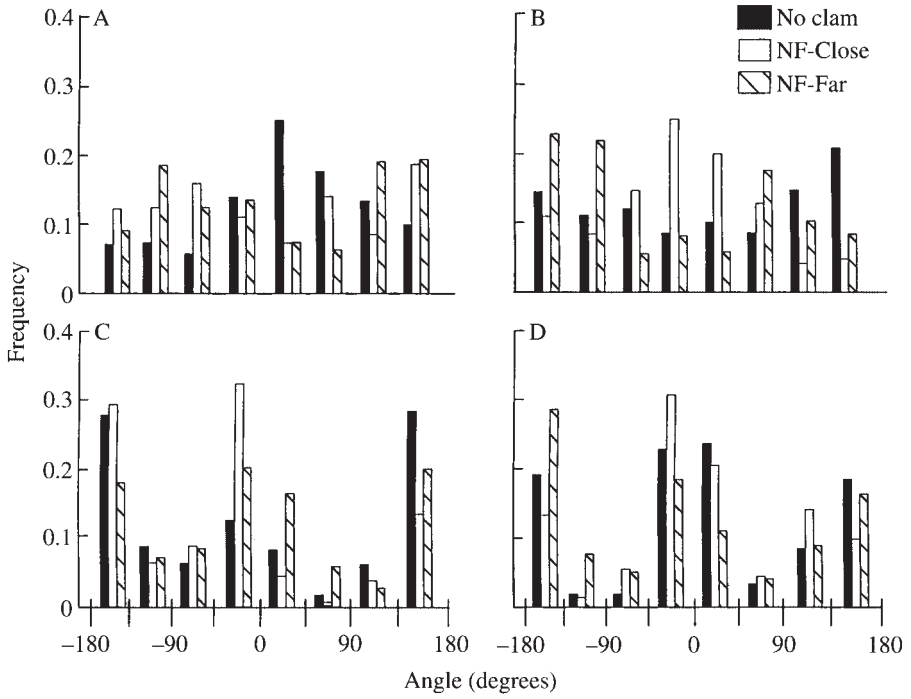

Fig. 4. Frequency distribution of angular headings at each flow velocity for crabs that were not presented with clam prey or did not locate clam prey. No clam, no-clam control; NF-Close, crabs starting from the close site and not finding clams; NF-Far, crabs starting from the far site and not finding clams. (A) 0 cm s^{-1}, N=1311, 2968 and 2372 frames for no-clam controls, close and far starting sites, respectively. (B) 1.0 cm s^{-1}, N=1655, 1635 and 343 respectively. (C) 3.8 cm s^{-1}, N=288, 465 and 840 respectively. (D) 14.4 cm s^{-1}, N=1766, 1153 and 1107 respectively. Sample sizes are different from those given in Fig. 2 because frames during which crabs remained motionless had no angular headings. Numbers of crabs in each treatment are given in the legend of Fig. 1. Clam prey is located at 0°, and ±180° points downstream directly away from prey.

previous analysis, there was a significant association between movement direction and flow velocity for crabs finding prey (CMH=302.0, d.f.=21; CMH=162.2, d.f.=14, for close and far sites, respectively, P<0.001 for both comparisons; d.f. differs because no crabs found clams at 0 cm s^{-1} at the far site). When crabs found prey, paths were most aligned to the current direction at U_∞=1.0 cm s^{-1}, whereas crabs frequently moved obliquely to the current direction at higher flow speeds. Crabs that successfully searched for prey at U_∞=1.0 cm s^{-1} appeared to move directly upstream towards the prey, while successful searchers at higher current speeds always tacked across stream at least once, and often several times, while *en route* to the prey item. Crabs in the absence of flow exhibited highly chaotic path trajectories apparently devoid of any pattern of preferential movement relative to the nominal flow axis.

Crabs that found clams also exhibited movement that was apparently polarized in the upcurrent direction. Animals moved predominantly upstream in all treatments in which

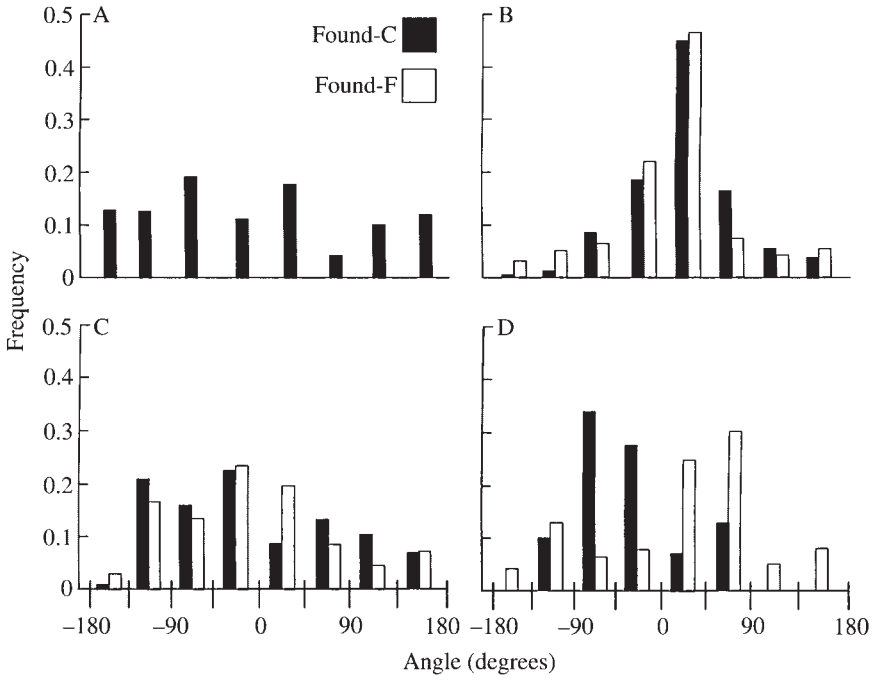

Fig. 5. Frequency distribution of angular headings for crabs that found clams at either the close or the far starting site. The coordinate system is the same as that given in Fig. 4. (A) 0 cm s^{-1}, *N*=279 frames for close site. No crabs starting from far site found clams. (B) 1.0 cm s^{-1}, *N*=206 and 303 frames for close and far sites, respectively. (C) 3.8 cm s^{-1}, *N*=226 and 158 respectively. (D) 14.4 cm s^{-1}, *N*=99 and 361 respectively. Sample sizes differ from those given in Fig. 3 because frames during which crabs remained motionless had no angular headings. Number of crabs in each treatment are given in the legend of Fig. 1.

they located prey (based on confidence limits for percentages; data from Fig. 5). Crabs in the absence of flow were a notable exception to the above pattern, showing extensive movement down-flume, i.e. away from clam prey.

The differences in behavior between crabs finding prey and the other two groups were consistent and profound. Overall, crabs finding prey showed a significantly different distribution of angular bearings compared with animals either not finding or not searching for prey, at all current speeds (CMH>184.1, d.f.=7, $P<0.001$, for all comparisons). Further, pairwise comparisons at each current speed/position combination indicate that successful predators showed significantly different distributions of angular bearings from either unsuccessful predators or crabs in the control group ($G>61.1$, d.f.=7, $P<0.001$, over all comparisons). In summary, crabs finding prey in low flows exhibited movement aligned to the direction of flow and polarized upstream, whereas crabs not finding prey or in the absence of prey, moved obliquely to the flow direction without any upstream bias. In swift flows, crabs finding clams exhibited a greater tendency to move across-stream towards prey (i.e. upstream) and crabs in the other two groups moved parallel to the direction of the flow either up- or downstream.

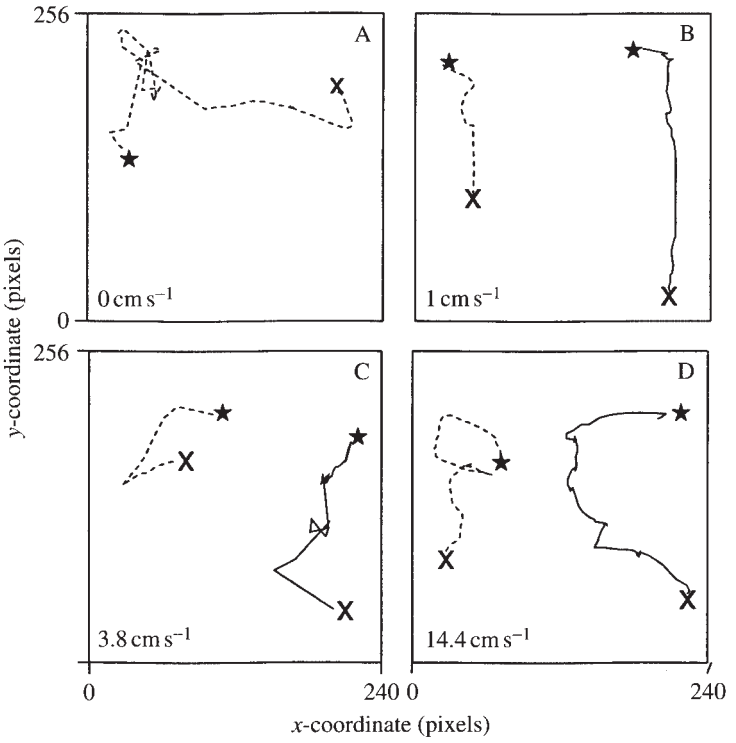

Fig. 6. Typical search paths for crabs successfully finding clams. Dashed lines are for crabs that started from the close site, solid lines are for crabs starting from the far site. Paths begin at the cross and end when the clam (denoted by a star) is located. Paths have occasionally been shifted along the *x*-axis to avoid overlap and to increase clarity. Flow proceeds from the top to the bottom of the figure.

Experiments separating the effects of advection from those of eddy-diffusion

The measurements of boundary layer structure (Table 1) indicate that the use of substrata of different hydraulic roughness alters boundary layer turbulence and the degree of eddy penetrance, irrespective of changes in flow speed. Thus, treatments at the same flow velocity, but where gravel was substituted for smaller-diameter sand, displayed increased u^* and Re^*. The effects of turbulence (eddy diffusion) were magnified without concomitant increases in flow speed (advection).

Single-classification ANOVA indicates that the boundary layer structures generated using particular substratum/flow current speed conditions appear to be responsible for effects on crab locomotory behavior during prey search (Table 7; Figs 7, 8). For both locomotory speed and NGDR, Ryan's multiple range test (α=0.05) indicates that the behavior of crabs in the low u^*/low Re^* treatment (U_∞=1.0 cm s^{-1} over sand) is significantly different from that of crabs in either of the other two high u^*/high Re^* treatments (U_∞=1.0 cm s^{-1} over gravel, and U_∞=3.8 cm s^{-1} over sand). Furthermore, the behavior patterns of animals in these latter two treatments are statistically indistinguishable from each other.

Table 7. *Summary ANOVA statistics for experiments separating advection from eddy diffusion*

Effect	SS	d.f.	MS	*F*
Mean locomotory speed				
Flow treatment	20.26	2	10.13	8.06**
Error	27.66	22	1.26	
Mean net-to-gross displacement ratio				
Flow treatment	0.338	2	0.169	10.39***
Error	0.357	22	0.016	

NGDR has been arcsine-transformed to meet normality criteria.
**P<0.01; ***P<0.001.

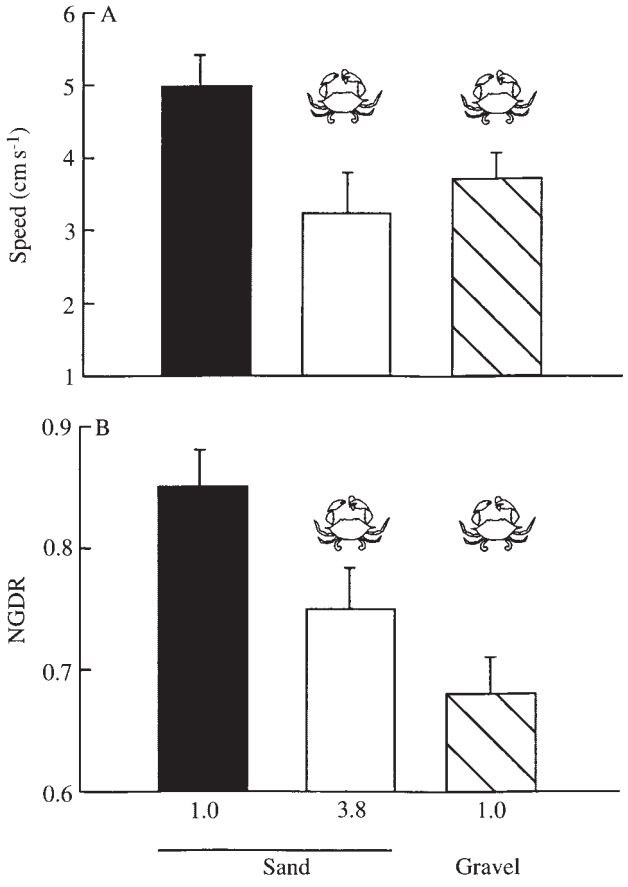

Fig. 7. Mean movement statistics for crabs successfully finding clam mimic in experiments separating advection from eddy-diffusion. Means +1 S.E.M. Crab symbols mark groups not significantly different using a Ryan's Q-test, α=0.05. (A) Mean locomotory speed *versus* flow speed/substratum combination. (B) Mean NGDR *versus* flow speed/substratum combination. Numbers of crabs successfully finding a clam mimic were six at 1.0 cm s^{-1} over gravel, nine at 1.0 cm s^{-1} over sand and nine at 3.8 cm s^{-1} over sand.

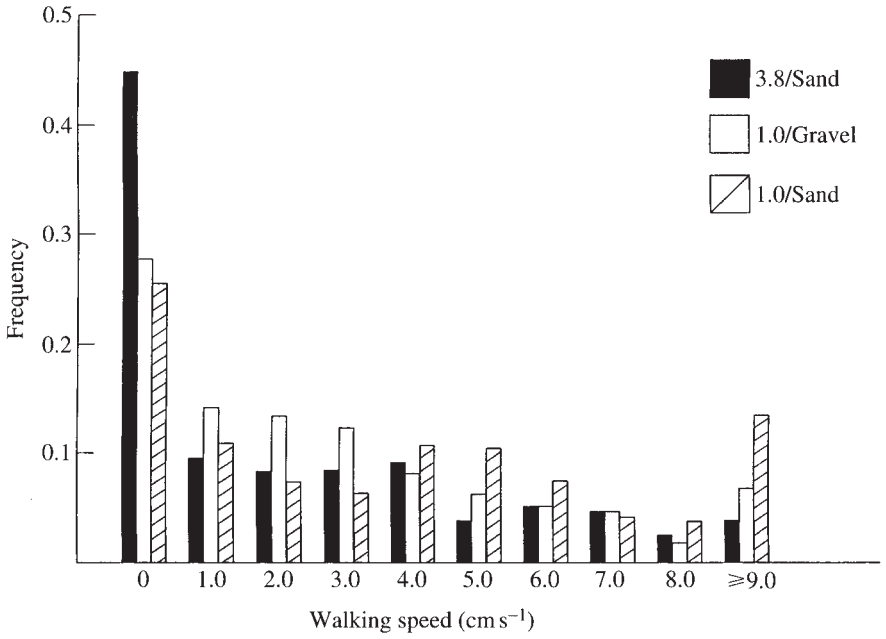

Fig. 8. Frequency distribution of locomotory speed for the three flow speed/substratum combinations in experiments separating advection from eddy diffusion. N=809 frames for 3.8 cm s^{-1} over sand, 234 frames for 1.0 cm s^{-1} over gravel and 260 frames for 1.0 cm s^{-1} over sand. Numbers of crabs in each treatment group are given in the legend of Fig. 7.

Analysis of the frequency data of crab walking speeds (Fig. 8) indicates that the boundary layer structure also has a significant effect on the distribution of movement velocities (CMH=9.4, d.f.=1, P<0.001). The frequency of observations in which crabs remained motionless follows the ranking of Re* across treatments, and pairwise comparisons indicated that all treatments are significantly different from each other (G>22.1, d.f.=9, P<0.01, over all comparisons). When locomotory speeds are recomputed after removing the zero velocity class, animals still travel more quickly in the low u*/low Re* treatment (F=9.2, d.f.=2,22, P<0.001), and a Ryan's multiple range test indicates that this behavior is different from that of a group consisting of the other two conditions.

Increased hydraulic roughness of the bed also affects angular bearings in a way similar to the effects of increased flow speed, again indicating that boundary layer structure, not flow speed *per se*, is responsible for modulating behavior. Animals in both the high u*/high Re* trials move more obliquely relative to the direction of flow (Fig. 9), with a significant overall effect of treatment on the distribution of angular bearings (CMH=65.9, d.f.=14, P<0.001). Pairwise comparisons indicate similar distributions of angular bearings in the two high u*/high Re* conditions (G=13.4, d.f.=14, P>0.05), while the behavior of the lowest u* and Re* treatment group is significantly different from that of either of the remaining two groups (G>45.9, d.f.=14, P<0.001 for both comparisons).

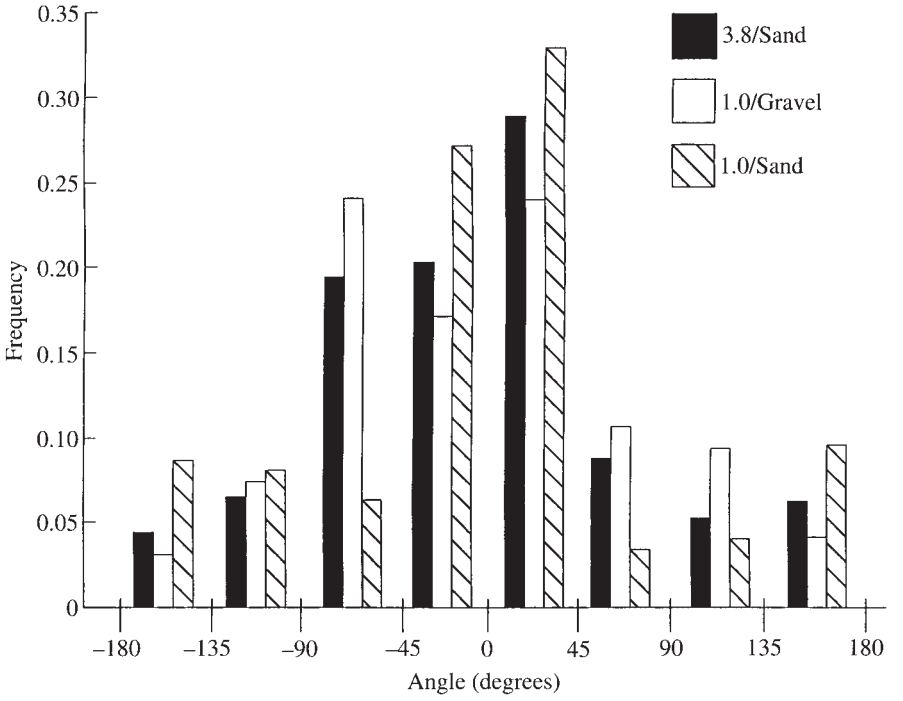

Fig. 9. Frequency distribution of angular headings for the three flow speed/substratum combinations used in experiments separating advection from eddy diffusion. N=452 frames for 3.8 cm s^{-1} over sand, 185 frames for 1.0 cm s^{-1} over gravel and 191 frames for 1.0 cm s^{-1} over sand. Sample sizes differ from those listed in Fig. 8 because frames in which animals remained motionless had no angular headings. The coordinate system is the same as that given in Fig. 4. Numbers of crabs in these treatment groups are given in the legend of Fig. 7.

Discussion

Our observations on blue crabs in controlled flow conditions show that locomotory behavior is a complex function of both the hydrodynamic properties of the flow and the presence or absence of olfactory stimulants. Locomotory speed and travel direction are affected by the flow and stimulus treatments. When crabs either fail to detect odorants (unsuccessful predators) or move in flows without odorants (no-clam controls), locomotory speeds are slow and bear no relationship to flow velocity. Movement of crabs becomes progressively more aligned to the flow axis as U_∞ (free-stream velocity) increases, although movement up- *versus* downstream occurs with equal probability. Because the behavior of unsuccessful predators is strikingly similar to the patterns displayed by crabs in the no-clam trials, it appears that unsuccessful predators have not detected odorants or, perhaps, are not responding to odorants that they do indeed detect.

Crabs responding to prey odors (successful predators) display behavior patterns very different from the movements of the two groups mentioned above. The general kinematic properties of locomotory tracks are summarized for each group in Table 8. In flowing

Table 8. *Summary of behavioral responses to increased flow speed*

	Walking speed	NGDR	Angular bearing
Successful searchers	Decreases	Decreases*	More oblique*
Unsuccessful searchers	No relationship	Increases	More aligned
No-clam controls	No relationship	Increases	More aligned

The relationship of walking speed, NGDR and angular bearings relative to the flow axis are given for crabs that are either successful or unsuccessful in locating clam prey, or are not presented with prey items (no-clam controls).

*Except crabs at $0\,cm\,s^{-1}$, which exhibit low NGDR and frequently move across the nominal flow axis.

water, locomotory speeds of successful predators decrease with elevations in U_∞. Movement paths become less parallel to the flow axis with increasing U_∞ as crabs tack across stream in search of prey, yet always move steadily upstream. The zero velocity treatment represents a special case. Animals display the high locomotory speeds characteristic of behavior in low flow, yet move along chaotic trajectories. Paths in no flow show extensive movement across the nominal flow axis and display lower NGDRs than in any other treatment.

Altering substratum roughness and flow velocity separates the effects of free-stream velocity (advection) from those of turbulence (eddy diffusion). These manipulations produced a set of treatments with similar degrees of advection, but different levels of two variables, u^* and Re^*, that describe the magnitude of turbulent flow near the sediment bed. For crabs responding to prey odors, decreased u^* and Re^* leads to greater sustained rates of locomotory speed, decreases in the extent to which animals remain at rest, and paths that are oriented more directly upstream towards potential prey. In these experiments, the changes in crab behavior mimic the effects seen in trials with live clams, suggesting that increases in current velocity and substratum hydraulic roughness both mediate behavioral changes by altering turbulence structure within the boundary layer.

Odorant transport is profoundly influenced by the physical environment of the prevailing fluid flow. It is clear that, in blue crabs, predatory search behavior is heavily influenced by the flow environment in which these olfactory activities take place. Crabs successfully locating prey behave quite differently from crabs that fail to find prey items. Crab predators successfully accomplished their task in darkness under infrared light, where chemical cues emanating from the partially exposed siphon of an otherwise buried clam were required to indicate the presence of prey. Thus, changes in the behavior of successful predators may at least partially be ascribed to alterations in the temporal and spatial properties of the chemical stimulus environment. These changes in odor plume structure are mainly a result of changes in boundary layer turbulence, as shown by similar responses of crabs in swift flows and of crabs in slower flows where u^* and Re^* are increased by manipulations of the substratum. Flow velocity itself influences some aspects of the locomotory behavior in the absence of odorants, particularly the degree of across-stream movement. This may have important consequences for determining the probability of encountering an odor plume, but has little bearing on orientation within a

plume. In any case, the net result is that crabs in more turbulent flows locate prey less often than crabs foraging in more benign hydrodynamic conditions (Weissburg and Zimmer-Faust, 1993).

Although flow-induced turbulence is detrimental to orientation ability in a plume, this argument cannot be taken to its extreme conclusion; namely, that flow itself is undesirable. When deprived of flow in an environment where molecular diffusion is the only odorant transport mechanism, crabs rarely locate prey (Weissburg and Zimmer-Faust, 1993) and display long convoluted search paths. Odorants in stagnant conditions diffuse slowly, and odorant concentrations decrease exponentially with distance from the source to establish a well-defined gradient in odorant strength (Okubo, 1980). Dye visualization studies conducted in our flume are consistent with these theoretical expectations of diffusion models. Clearly, crabs are not guided to odor sources by following this simple gradient up-flume to the odor source, ruling out an orientation mechanism based solely on changes in odorant intensity. The general orientation towards prey is mediated by the perception of flow direction, and the presence of flow acts to polarize olfactory-guided locomotion in the up-current direction. Animals as diverse as snails, flatworms, insects and sharks also appear to require flow in order to locate odor sources (Hodgson and Mathewson, 1971; Bell and Tobin, 1982; Brown and Rittschof, 1984; Baker, 1986). In other crustaceans, workers have not demonstrated convincingly that flow is either required or unnecessary for successful location of an odor source. McLeese (1973) reported that the lobster *Homarus americanus* could detect an odorant trail in the absence of flow, but not that animals could successfully use a chemical concentration gradient to find an odor source in stagnant water. In other investigations of lobster orientation, stimulus delivery involved the use of a carrier flow, and the authors concede that flow may be acting as a cue during orientation (Reeder and Ache, 1980; Devine and Atema, 1982; P. A. Moore, personal communication).

It remains to be seen whether increasing flow rate is beneficial when divorced from the effects of increasing turbulence magnitude. Swifter flows can provide greater stimulation of mechanosensory hairs, which must be deflected from a stationary position to encode a mechanical stimulus. As long as the flow remains sufficiently smooth so that chemical signal properties are not adversely affected, increasing flow velocity may allow animals to sense current more accurately and to take more direct routes to the odor source. In any case, our data demonstrate the existence of a minimum flow velocity ($<1\,cm\,s^{-1}$) below which orientation responses of blue crabs are compromised.

Crabs maneuvering within the odor plume are responding to a chemical signal with spatial/temporal properties produced, in part, by the hydraulic properties of the flow. Changes in the kinematics of search as a function of flow may therefore reveal much about the relevant chemical signal properties, since it is possible to relate fluid dynamic variables to potential changes in odor plume structure. First, increased turbulence will result in greater eddy diffusion, dispersing odorants more widely and reducing time-averaged odorant concentration in the plume (Schlicting, 1979; Okubo, 1980). Second, since turbulence is essentially the degree to which correlated fluctuations in velocity (or eddies) are present in the fluid, variance in odor concentration also increases in more

turbulent flows. A fixed location may therefore experience odorant levels considerably above or below the time-averaged concentration.

Separate experiments recently conducted in our laboratory using similar flow speeds and substratum types to those used here verify these anticipated effects of increased turbulence on dynamics of odor transport (Moore *et al.* 1992, 1994). In these two studies, tracer molecules (dopamine) were introduced *via* the same model clam and at the same flow rates as were used in the trials with blue crabs reported here. The parallel methodology ensures similarity of odorant transport dynamics in each of these investigations. At a stationary sensor (sampling at 10 Hz), odorant concentrations above background occurred in discrete bursts that last several seconds. Between these bursts, odorant concentrations fell to zero. Within a burst, odorant concentrations could be further resolved into a series of brief peaks termed odor pulses. As turbulence was increased, the time between bursts lengthened, but the total number of odor pulses within a burst increased. Concentration variance at both short (500–1000 ms) and long (>1 s) temporal scales was positively associated with turbulence intensity. Additionally, the time-averaged odorant level within these odor pulses fell with increased turbulence, reflecting greater overall dilution of odorants transported downstream within the plume. These findings are in qualitative agreement with observations of transport dynamics occurring within airborne odor plumes (Murlis and Jones, 1981; Murlis, 1986; Murlis *et al.* 1991).

The behavior of blue crabs foraging in more turbulent flows seems to reflect at least some of the predicted changes in plume structure. Average locomotory velocities, with or without the inclusion of periods of motionlessness, are lower in more turbulent flows. Similarly, modes in the frequency distributions are shifted towards lower velocities in more turbulent flows. Walking speeds presumably decrease because lower odorant concentrations in turbulent plumes induce a less vigorous locomotory response. Extensive study of crustacean olfactory receptors indicates a positive relationship between neural output and the concentration of stimulatory substances (Fuzessery *et al.* 1978; Ache, 1982; Derby and Atema, 1988). For known behavioral stimulants, observations on neural output correlate well with evidence on searching or locomotory responses to applied dose in a variety of crustacean taxa. Studies indicate that rates of searching movements (Zimmer-Faust and Case, 1982; Harpaz and Steiner, 1990) and of antennule flicking (Price and Ache, 1977; Harpaz and Steiner, 1990; Daniel and Derby, 1991) and locomotory velocity (Buskey, 1984; Weissburg and Zimmer-Faust, 1991) may all increase with stimulant concentration.

Crabs also stop more frequently in more turbulent flows. The most parsimonious explanation is that these bouts of motionlessness simply reflect long periods during which odorant concentration falls below detectable limits. As animals reach the edge of the plume, odorant concentration is low, or not above background levels. Individuals then cease walking until a burst of odorant is carried past the animal, initiating movement back towards the odor source. It is worth noting that an average of 3–4 clams, generally separated by 10–15 cm, were observed pumping during behavioral trials. We conclude from dye visualization using model clams that crabs were probably reacting to plumes from isolated clams, rather than perceiving the entire clam patch as a solid corridor of odorants.

Our data on the duration of motionless periods and on the angular bearings of the animals during searching suggest that locomotion stops when crabs lose contact with the plume. Upstream tack angles show an average difference of 76±57 °(S.D.) in the directions taken by animals before and after stationary periods. Both the number and duration of motionless periods increase as a function of increased flow speed (Table 4). Plume meandering will be more extensive in more turbulent conditions (Murlis and Jones, 1981; Elkinton and Cardé, 1984) and, hence, at faster flows. Thus, animals in a swift flow find themselves at an edge with increasing probability, so the incidence of turning and stopping will increase. This is consistent with the gross movement patterns (NGDR) and the distributions of angular headings for the animals in our high *versus* low turbulence treatments. Insects that walk to odor sources, or intersperse flying with bouts of walking, also appear to stop when they have lost contact with the plume, resuming motion when contact has been re-established (Hawkes and Croaker, 1979; Didonis and Miller, 1980; Bursell, 1984). Insects flying in wind currents cannot stop to re-establish contact, although the 'casting' behavior observed following loss of contact with a plume fulfils the same function; that is, animals can remain in a holding pattern by sweeping back and forth across the wind direction until the plume edge is relocated (David *et al.* 1983; Baker, 1986; Willis and Arbas, 1991). Although tentative, and under further study, our conclusion is that large-scale variation induced by plume meandering, not microscale structure within the plume, is affecting the stopping and subsequent turning maneuvers of blue crabs orienting to prey.

The turning behavior described here also illustrates the lack of a regular pattern of changes in course trajectory (Fig. 6), which has implications for determining the neural mechanisms underlying olfactory orientation. It is frequently observed that when insects follow pheromone plumes, they display consistent turning angles that alternate regularly between left- and right-hand turns, thus continually tacking towards the center of the plume. This phenomenon, called counter-turning, has often been interpreted as being the result of an internally guided motor program (Willis and Cardé, 1990; Willis and Arbas, 1991; but see also Preiss and Kramer, 1986). In this scenario, the alternation of turning is triggered by chemical cues, but chemical information does not provide feedback control of the actual turning angles. Rather, it is speculated that counter-turns are produced by an endogenous oscillator, possibly interneurons that act to bias motor output alternately from one side to the other (Olberg, 1983). The lack of both consistent turning angles and an alternating turning pattern argues against an internal motor program in foraging blue crabs. Furthermore, course trajectories taken by insects utilizing counter-turning behavior appear to be largely unaffected by changes in flow (wind) speed (Willis and Cardé, 1990; Willis and Arbas, 1991), whereas blue crabs clearly change their search behavior in response to flow (Figs 4, 6, 9). It appears that at least one other benthic crustacean, the American lobster *Homarus americanus*, also fails to display evidence for internally guided motor programs (Moore *et al.* 1992).

In the absence of an endogenous counter-turning motor program, crabs might remain in the center of the plume by using direct feedback from temporal and/or spatial comparisons of odorant concentration. Lobsters have been reported to use both simultaneous bilateral comparisons (tropotaxis; Frankel and Gunn, 1961) and sequential

comparisons (klinotaxis; Frankel and Gunn, 1961) during orientation to odor sources (McLeese, 1973; Reeder and Ache, 1980; Devine and Atema, 1982). These reports stress the importance of chemo-orientation but, as discussed above, cannot rule out the influence of flow in producing successful orientation. Orderly gradients of odorant concentration do not exist in naturally turbulent water flows (Zimmer-Faust *et al.* 1988; Moore *et al.* 1994), making tropo- or klinotactic methods of chemical gradient detection ineffective search strategies for most macroscopic bottom-dwelling aquatic animals. In many estuarine environments, flow is tidally driven and, hence, unidirectional over long periods. Under these conditions, flow provides a reliable cue for orientation towards the odor source. On the basis of our results, we suggest that chemotactic and rheotactic mechanisms together produce successful orientation. Upon perception of a chemical cue, crabs determine the direction of flow, producing an upstream bias in locomotion. Subsequently, features of the odorant distribution are then used by the animals to orient themselves within the plume and to mediate turning behavior if the searcher loses contact with the plume. The reliance on multi-modal cues is similar to models of insect orientation. However, in contrast to our view of orientation in *Callinectes sapidus*, insects are generally thought not to rely as heavily on direct feedback from chemical information during upwind flight (Arbas *et al.* 1993). Rather, locomotory patterns in flying insects probably result from the interaction between stimulus-induced endogenous counter-turning and optomotor guided amenotaxis (Mafra-Neto and Cardé, 1994). Continued examination of the olfactory-mediated orientation of crustaceans in realistic benthic boundary layer flows, and with carefully controlled stimulus properties, is clearly necessary to substantiate these differences between marine and terrestrial arthropods.

Our data on the kinematics of orientation implicate boundary layer structure as a crucial element in establishing patterns of locomotory performance, through effects on the spatial and temporal properties of odorant concentration. Since the hydraulic environment mediates a number of different aspects of odorant transport, the resulting aggregate properties of odorant plumes are likely to be quite complex. Determining strategies of animal navigation through turbulent odor plumes may subsequently appear daunting. However, our studies are currently aimed at examining the role of individual signal features in determining orientation patterns. These studies, in turn, may suggest the environmental features that alter the properties of odor plume structure most relevant to the ability of animals to perceive odor cues accurately. Further investigations establishing a comparative approach to olfactory-mediated orientation may help to provide linkages between animal navigational performance and the hydraulic properties of fluid environments. Most available information originates from a few well-studied insects. However, the scant information on the behavior and physiology of marine organisms suggests important differences between marine and terrestrial arthropods that are consistent with the differences in the fluid mechanical properties that characterize the environments occupied by each group. A strong comparative approach may allow predictions about the mechanisms used by various animals in searching for distant odor sources, depending on properties such as animal size and mobility, fluid viscosity and transport mechanics of odorants in fluid flows.

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