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Odor Plumes and Animal Navigation in Turbulent Water Flow: A Field Study

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Turbulence causes chemical stimuli to be highly variable in time and space; hence the study of animal orientation in odor plumes presents a formidable challenge. Through combined chemical and physical measurements, we characterized the transport of attractant released by clam prey in a turbulent aquatic environment. Concurrently, we quantified the locomotory responses of predatory crabs successfully searching for sources of clam attractant. Our results demonstrate that both rheotaxis and chemotaxis are necessary for successful orientation. Perception of chemical cues causes crabs to move in the upstream direction, but feedback from attractant distributions directly regulates movement across-stream in the plume. Orientation mechanisms used by crabs differ from those employed by flying insects, the only other system in which navigation relative to odor plumes has been coupled with fluid dynamics. Insects respond to odors by moving upstream, but they do not use chemical distributions to determine across-stream direction, whereas crabs do. Turbulent eddy diffusivities in crab habitats are 100 to 1000 times lower than those of terrestrial grasslands and forests occupied by insects. Insects must respond to plumes consisting of highly dispersed, tiny filaments or parcels of odor. Crabs rely more heavily on spatial aspects of chemical stimulus distributions because their fluid dynamic environment creates a more stable plume structure, thus permitting chemotaxis.

The ability to detect chemical stimuli is nearly universal among animals. Chemical signals are generated by liberating stimulus molecules into a fluid, where they are transported by advection and diffusion (eddy and molecular) until detected and acted upon by a biological sensor. Thus the physical process governing chemical transport has a profound impact on the nature and success of chemosensory-mediated behavior (1, 2, 3, 4, 5, 6). Except at microscopic scales, turbulence causes a stimulus pattern whose concentration is highly variable in time and space (3, 4, 5, 6, 7). As a result, the study of chemoreceptive behavior presents a formidable challenge. It demands simultaneous measurements of stimulus release, fluid dynamics, and animal responses to perceived chemical cues. Until now, however, these determinations have not been combined in a single field study.

We experimentally establish a direct link between the transport of chemical stimuli and animal navigation in a natural, turbulent flow environment. Attraction of predatory blue crabs (*Callinectes sapidus*) to odor released by clam prey (*Mercenaria mercenaria*) was investigated. The mechanisms directing predator to prey were identified for crabs foraging naturally in estuarine tidal creeks, where water flows unidirectionally for hours at a time. These creeks were shallow enough to permit direct, noninvasive observations of crab locomotory behavior.

Experiments were conducted in the North Inlet Estuary, near Georgetown, South Carolina, USA (32° 20′ N, 79° 15′ W). Flow records were obtained with an electromagnetic flow meter (Marsh-McBirney 523) equipped with a two-dimensional sensor (1 cm diam), mounted on a flat base, and submerged in the tidal creek. The base was positioned flush with the sandy bottom, and the sensor was placed 5 cm above the substrate. Sensor height was chosen to match the elevation of a typical, adult crab body. A data logger (Campbell CR10) was used to record both horizontal and vertical flow velocities continuously measured by the sensor at 1 Hz, between 26 June and 8

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August 1993. Horizontal flow (downstream advection) typically ranged from 0 cm/s at slack tides, to 30 cm/s during peak ebb and flood tides. We applied the eddy correlation method to calculate shear velocities, at 1-min intervals, using the correlation between the horizontal and vertical flow velocities (8, 9). Shear velocity (u_*) is a measure of the strength and correlation of turbulent fluctuations in flow speed near the substratum. Finally, we determined the coefficients of turbulent mixing as the products of shear velocity, sensor height above the substratum, and Von Karman's constant (9). These mixing coefficients were remarkably low, ranging from 0.5 to 1.5 cm^2/s. They indicated that across-stream mixing occurred very slowly in the tidal creek even though water flow was turbulent.

The dynamics of odor plumes were characterized by measuring the transport of fluorescent dye (sodium fluorescein) and an electrochemical (dopamine) following their combined release from a point source. Input concentrations of fluorescein and dopamine were 1.0 g/liter and 2 mmol/l, respectively, with ascorbic acid added to the mixture at 20 mmol/l as an antioxidant. The mixture was introduced through polyethylene tubing (0.5 mm ID) at 6 ml/min.

Fluorescein provided a visual marker, and fluorometric determinations were used to establish the time-averaged distributions of dye at downstream and at across-stream sites, relative to the release point. Water samples were collected (at 1 ml/s) simultaneously over 1-min intervals by syringes placed at each of 18 to 30 sites per trial. These sites were distributed in a grid, with six sites placed across-stream (0, 2, 4, 6, 8, 10 cm distant from the plume midline) at three to five locations downstream of the release point (5, 25, 50, and in some trials, 100 and 275 cm distant from the source). The bell-shaped distribution of fluorescein concentration with gradual decay downstream (Fig. 1) is what a Gaussian plume model would predict (Pearson's product-moment correlation: $r^2 \geq 0.95$; $P < 0.001$; all replicate plume measurements). Concentration dropped sharply at the plume's visible lateral edges (Fig. 1).

Fluorometric measurements also provided an alternative method of calculating the mixing coefficient for comparison with determinations made using the electromagnetic flow meter. Temporal changes in the across-stream variance in fluorescein concentration were used to estimate the mixing coefficient (9). Results from the two methods matched well. For example, estimates based on fluorometric determinations ranged from 0.5 to 1.2 cm^2/s during a time when estimates of 0.5 to 0.8 cm^2/s were made from electromagnetic flow meter records.

When measured at fast temporal scales, chemical stimuli in odor plumes are patchily distributed due to turbulence. Mean concentrations and time-averaged distributions of fluorescein dye, therefore, may not be indicative of the information available for crabs attempting to orient towards an odor source (3, 4, 5, 6, 7). Because arthropod chemoreceptors detect intermittent (or pulsed) chemical stimuli applied at a maximum frequency of 4–10 Hz (10, 11), we employed carbon fiber microelectrodes (150 μm diam) and a computer recording system (MedSystems Corp. IVEC-10) to sample dopamine at 10 Hz (12). Electrode recordings were made at the fluorescein sampling sites (see above). Turbulent mixing caused the concentration of dopamine sampled downstream of the source to fluctuate strongly in time and space. Bursts of highly concentrated chemical passed over the sensor, alternating with periods of low or zero concentration (Fig. 1). The plume's lateral edge, as defined by our high-speed dopamine measurements, was positioned identically relative to the edge detected both by our time-averaged fluorescein measurements and by our visual observations. This lateral edge, separating clean from chemical-laden water, was very narrow (2–4 cm wide) compared to the body size of an adult crab (10–15 cm carapace width). Thus a steep concentration gradient was found across-stream, but not downstream of chemical release.

We previously demonstrated that some crabs search for and find intact live clams, and that these crabs are responding to odor plumes created by the excurrent release of attractant metabolites at low concentration (13). Once a clam is found, however, it is chipped open by a crab and attractants are released to form a plume of high concentration. High-concentration plumes then immediately attract other crabs to the predation site. Hence, depending on the situation, crabs may be exposed either to low or high attractant concentrations, and crabs respond effectively in each case.

Concurrently with hydrodynamic and chemical measurements, we assessed crab orientation in odor plumes. Our field studies focused on plumes characteristic of chipped clams. We chose to work with chipped clams because high attractant concentrations would better ensure an effective stimulus through the broad range of hydrodynamic conditions encountered by crabs in the field. Stimulus plumes (dyed with fluorescein for visibility) were created, either presenting a chipped clam or introducing clam mantle fluid (membrane filtered to 0.22 μm) at a rate mimicking its release from a chipped clam. Each stimulus plume was always paired with a control plume that delivered fluorescein in filtered seawater. Both stimulus and control solutions were introduced at 6 ml/min, with inputs separated by 60 cm across-stream.

Free amino acid compositions of effluent leaking from chipped clams (n = 8 clams assayed), mantle fluid of intact live clams (n = 8 clams), and homogenized clam flesh (n = 7 clams) were all determined using a Beckman 6300 System Gold high-performance liquid chromato-

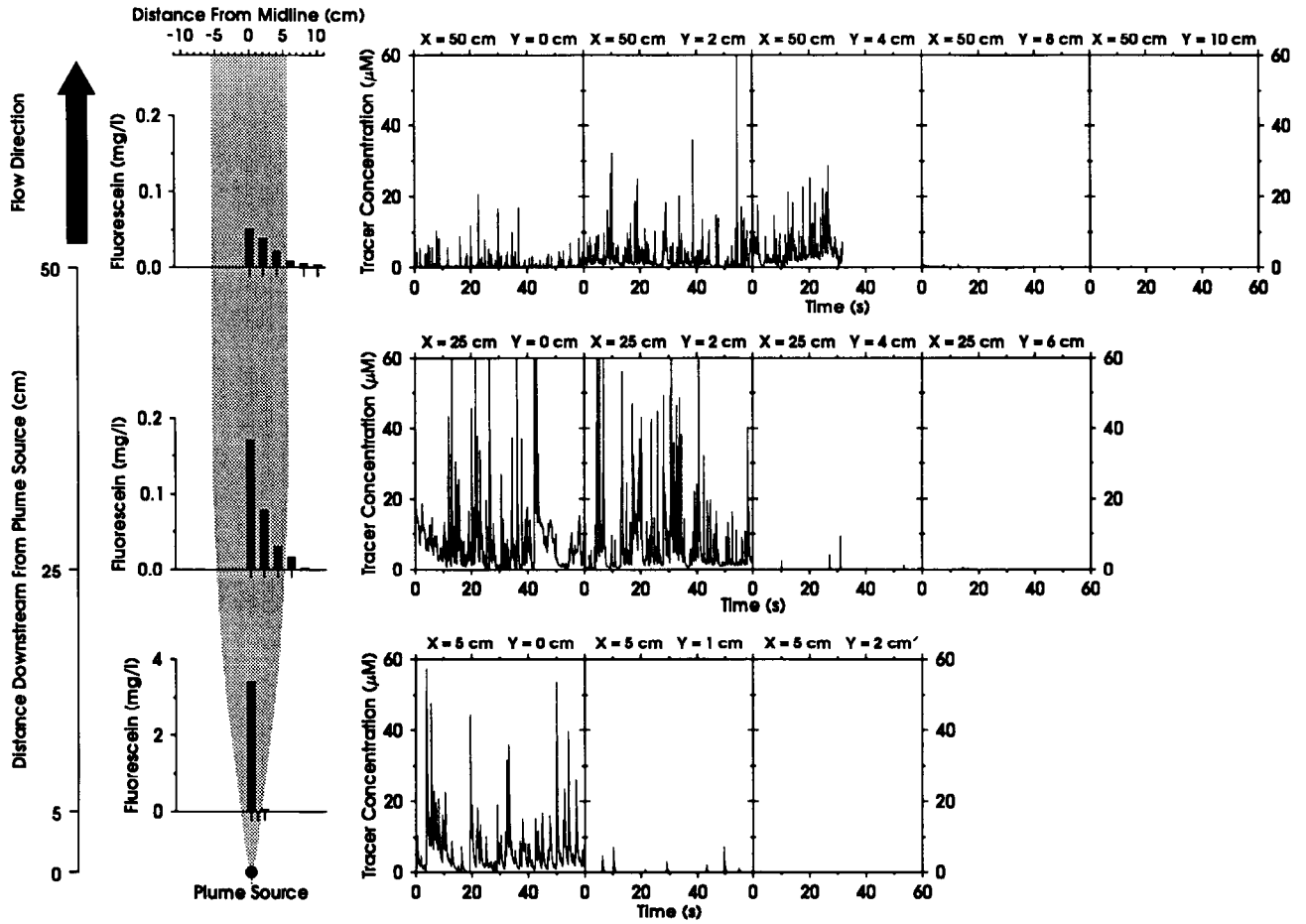

Figure 1. Representative concentration distributions downstream from a point source in a tidal creek. Histograms represent fluorescein concentrations (mg/l) in samples collected at 5 cm (bottom row), 25 cm (middle row), and 50 cm (top row) downstream from the source and at 0, 2, 4, 6, 8, and 10 cm from the midline of the plume. Note the scale difference between fluorescein concentrations at downstream locations. The visible region of the fluorescein plume at each position is denoted by the shading. Panels to the right of the histograms represent 60-s records of instantaneous fluctuations in dopamine (tracer) concentration, measured at 10 Hz with a carbon fiber electrode, at locations where the fluorescein was sampled. The leftmost panel in each row is the sample from the midline of the plume, and successive panels are samples from 2, 4, 8, and 10 cm from the midline (see tick marks on histogram axes for sampling sites). Highly concentrated bursts of dopamine were common in all samples taken within the visible portion of the plume.

graph with a sodium ion-exchange column (4-mm ID × 120 mm; Beckman) for separation. In this system, amino acids were monitored spectrophotometrically after post-column reaction with ninhydrin. Compositions of clam effluent and mantle fluid were almost identical (Pearson's product-moment correlation: $r^2 = 0.998$; $P < 0.001$; $n = 18$ amino acids chromatographed), indicating that mantle fluid was the source of effluent material. Because taurine was by far the most abundant amino acid in both clam effluent and mantle fluid (accounting for >50% of the total amino acid composition), we used it as a marker to measure the rates of fluid release from chipped clams. In the laboratory, clams ($n = 12$) of various sizes were chipped by using a metal rod to deliver a single firm blow to the lateral shell margin. The resulting chip was similar in size and shape to one produced by a blue crab as it begins to feed. Each chipped clam was then placed individually into a separate beaker of artificial seawater. The beakers were stirred, and they were maintained at the same temperature and salinity as seawater in the tidal creek from which clams were collected. Artificial seawater was sampled from each beaker before placement of the clam, and again at 30- to 60-s intervals for 15 min after placement; HPLC analysis of this seawater indicated that taurine (and mantle fluid) release was constant throughout the trial period. The relation between taurine release rate and clam size was then used to scale our delivery of mantle fluid in field experiments, simulating the

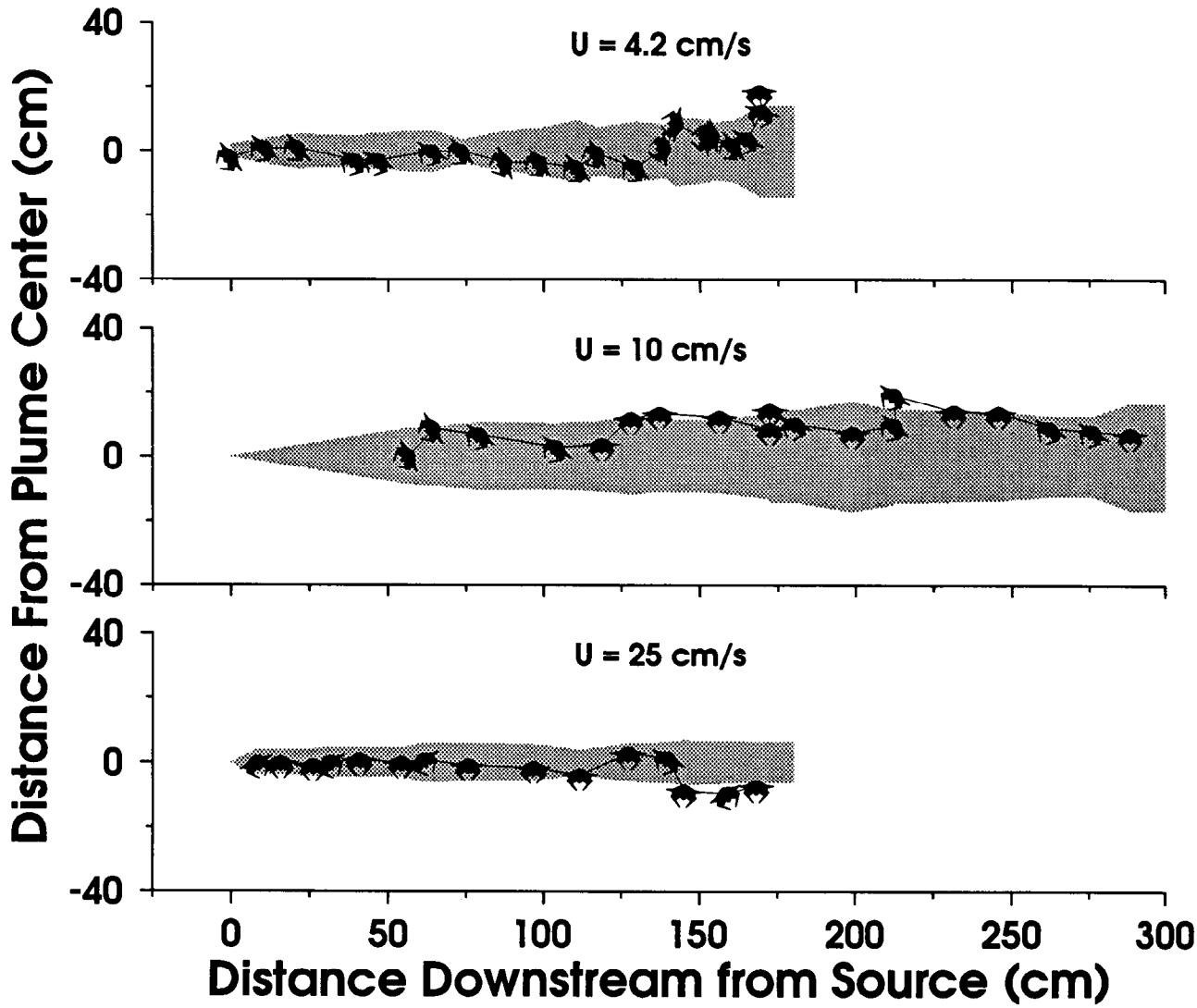

Figure 2. Representative tracks of crabs following odor plumes. The crab symbols represent the positions and orientations of individuals at 1-s intervals as they moved upstream toward the odor source. Naturally foraging crabs normally walk sideways as well as forward. The visible region of the fluorescein/odor plume is noted by the shading. The water velocity (U) at 5 cm above the bottom was 4.2 cm/s (top panel), 10 cm/s (center panel), and 25 cm/s (bottom panel). Distances downstream and across stream are in centimeters.

fluid input from an intermediate-sized chipped clam (6 cm total shell length).

Quantitative observations of foraging crabs were made noninvasively with a video camera (Sony TR81) mounted 4 m above the tidal creek. Video records of crabs responding to plumes were made during ebbing tides. A 3-m field of view was dictated by the resolution of the video camera and the size of the crabs (10–15 cm carapace width), whose positions could not be reliably quantified in wider field images. A scale bar in the field of view was employed to measure distance and to correct for distortion due to perspective. In the laboratory, plume edges and crab positions at 1-s intervals were traced onto acetate sheets from video playbacks to a monitor. Both crab location, in relation to the plume edge, and crab locomotory kinematics were measured (Fig. 2).

Crab responses to the plumes were dramatic and unambiguous: 29 crabs contacted the control plumes, but only 4 of these crabs walked upstream towards the input source. The near absence of positive responses to control plumes demonstrated a lack of attractant effect by fluorescein dye. In comparison, after contacting stimulus plumes, 68 of 80 crabs walked upstream to the input source. Crabs turned upstream within 1.5 s (±0.3 SD) of contacting an odor plume. Percentages of crabs responding positively to plumes from either chipped clams or mantle fluid were nearly identical, being 86% (n = 52 crabs) or 82% (n = 28 crabs), respectively (G-test for ho-

mogeneity: $G^2 = 0.270$; df = 1; $P > 0.50$). Our results demonstrate an odor-conditioned rheotaxis that orients crabs upstream. Previously, we reached an identical conclusion for blue crabs foraging in a laboratory flume. Crabs walked upstream to find intact live clams in flowing water, but they oriented indiscriminately and searched unsuccessfully for clams in still water (13).

Oriented movements by crabs lateral to water flow are controlled by chemotaxis. As crabs walked upstream towards an attractant source, they frequently approached the lateral edges of the plume. When crabs did reach the edge, they nearly always turned directly back to the plume (50 of 61 turns; *G*-test for goodness-of-fit, 1:1 hypothesized ratio, $G^2 = 14.97$; df = 1; $P < 0.001$), without exhibiting either casting or zigzagging (Fig. 2). Lateral movements were initiated as crabs began to exit a plume and partially contacted clean water. Fluorescein did not act as a visual cue, because crabs displayed identical oriented responses when tests were conducted in the dark (under infrared illumination) and without fluorescein (13, and in prep.). It took, on average, less than 1 s (0.8 ± 0.2 s SD) for crabs to renew upstream walking after they had begun moving laterally towards the plume midline. Remarkably, we did not observe walking speeds to change significantly as crabs moved closer to attractant sources (analysis of covariance: $F = 0.60$; df = 4,237; $P = 0.66$; walking speed: 12.8 ± 0.4 cm/s SD), and we found no significant correlation between walking speed and water flow velocity (Pearson's product-moment correlation: $r^2 = 0.037$; df = 1,64; $P = 0.12$). We hypothesize that crabs perceive clam attractant as a binary cue (present/absent), both in their upstream movement and in their across-stream walking. Because the plume edges were very sharp, when crabs partially exited the plume, some pereiopods (legs or claws) were outside the plume while others remained inside. A comparison of simultaneous chemosensory inputs from the appendages inside and outside the plume would presumably allow the crabs to determine the correct direction and return to the plume. This binary response would lead crabs to locations of higher concentration of clam attractant.

Orientation mechanisms used by crabs in upstream movement are similar to those of flying insects. However, crabs differ from insects in their across-stream response. Insects provide the only other system in which navigation relative to odor plumes has been coupled with fluid dynamics. Flying insects locate a source of chemical attractant by moving upwind upon contacting a filamentous trace of attractant odor (14, 15). After several seconds of flying in clean air, insects shift to casting (regular reversals of flight directed across-stream) until contact with another odor trace causes a return to upwind flight (16). Flying insects, therefore, do not use chemical concentration gradients to determine either their upwind or across-wind directions (16, 17, 18). The use of chemotaxis may be impractical in their environment, where complex fluid dynamics do not permit stable zones of high attractant concentrations to exist. Crabs in contrast, consistently turn back into the attractant plume rather than zigzagging after losing the plume signal.

The difference between estuarine tidal creek flow and atmospheric winds may explain why blue crabs and insects use contrasting mechanisms for successful navigation towards an odor source. The crop fields and forests used as experimental models for insect flight are hydraulically rough, with high advection. Eddy diffusivities in insect habitats are 100 to 1000 times greater than those we recorded in estuarine tidal creeks (19, 20). Higher diffusivities yield plumes consisting of tiny, highly dispersed filaments or parcels of odor. Wind direction changes frequently, causing plumes to meander (3, 6). The dispersal pattern of odor, coupled with the relatively fast flight speed of insects, means that a flying insect has little chance to detect more than the occasional pulse of passing odor. Casting, zigzagging, and rapid behavioral modulation in response to fine-scale changes in odor concentration may be strategies appropriate for situations in which the entire plume meanders away from the animal.

In contrast, the flow environment of estuarine tidal creeks is markedly less turbulent, yielding relatively stable, straight, and sharply delineated odor plumes. Plumes cannot meander substantially, because flow is constrained by water depth and by the sides of the creeks. A stable plume structure permits direct binary comparisons of chemical concentration inside and outside the plume, to guide movement lateral to flow. The more direct plume-following behavior and across-stream chemotactic responses shown by crabs reflect a strategy appropriate to the plume structure characteristic of their environment. Mechanisms of plume-following behavior, therefore, arise in response to chemical stimulus distributions, as determined by the specific fluid dynamic environments in which animals must naturally navigate.

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