

RESEARCH ARTICLE

Staying the course: chemical signal spatial properties and concentration mediate cross-stream motion in turbulent plumes

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SUMMARY

This study examined the role of broadly distributed sensor populations in chemosensory searching, especially cross-stream heading adjustment. We used three-dimensional laser-induced fluorescence to collect chemical concentration data simultaneously with behavior observations of actively tracking blue crabs (*Callinectes sapidus*). Our analysis indicates that the spatial distribution of the odorant concentration field is necessary and sufficient to mediate correct cross-stream motion, although concentration provides information that supplements that obtained from the spatial distribution. Crab movement is continually adjusted to maintain an upstream heading, with corrections toward the source modulated only in the presence of chemical cues. Crabs detect and respond to shifts in the position of the center-of-mass (COM) of the odorant concentration distribution as small as 5% of the leg span, which corresponds to ~0.8–0.9 cm. The reaction time after a 5% threshold shift in the position of the COM is in the range of 2–4 s. Data also indicate that these steering responses are dependent on stimulus history or other characteristics of the plume, with crabs taking longer to respond in conditions with large-scale spatial meanders. Although cross-stream motion is determined by chemical signal inputs to receptors on the walking legs, crabs do make rotational movements in response to chemical signals impinging on the antennules. These rotational movements do not affect the direction of travel, but rather, determine the crab's body angle with respect to the flow. Interestingly, these body angles seem to represent a compromise between reducing drag and obtaining better chemical signal information, and this trade-off is resolved differently under different plume conditions.

Key words: chemical plume, chemical sensing, odor-guided navigation, spatial processing.

INTRODUCTION

Chemical signals can convey information on the location of distant sources by both spatial and temporal variation (e.g. Webster and Weissburg, 2009). The role of temporal information is particularly well understood in a variety of animals. Bacteria for instance, continue moving forward as long as they perceive increases in chemical signal concentration through time, and randomly change direction when challenged with decreasing concentrations (Block et al., 1982; Segall et al., 1986). Trail-following ants determine trail direction by temporal comparisons (Calenbuhr and Deneubourg, 1992). Perhaps the best known example is provided by odor-gated responses to flow seen in pheromone-tracking moths (Mafra-Neto and Cardé, 1994; Vickers and Baker, 1994), as well as a variety of other organisms including fish and crustaceans that navigate through turbulent chemical plumes (e.g. Hodgson and Mathewson, 1971; McLeese, 1973; Weissburg and Zimmer-Faust, 1993; Baker et al., 2002). In this case, the perception of discrete odorant bursts causes upstream surges, and the rate of progress towards the source is determined largely by the odorant burst arrival frequency.

Whereas the ubiquity and importance of temporal (i.e. sequential) sampling is established, the importance of spatial sampling (i.e. simultaneous collection of spatially distributed signals) is less well understood. Large rod-shaped unicells, for instance, may benefit from spatial more than temporal sampling and have spatially separated receptor distributions that can supply the requisite

information (Dusenbery, 1998). Ants use their bilateral antennae to provide information on spatial patterns of intensity that allow them to stay on the trail (Calenbuhr and Deneubourg, 1992), as do other walking insects (Martin, 1965; Willis and Avondet, 2005).

Turbulent airborne or aquatic plumes are highly variable, particularly in time (Webster and Weissburg, 2001; Webster, 2007), and thus comprise signal environments where spatial sampling may be advantageous. Spatial sampling requires that animals have sensor spans large enough to detect spatial variation, and this seems a reasonable assumption for many animals (Weissburg, 2000; Webster et al., 2001). Behavioral observations suggest that aquatic animals may, in fact, utilize spatial sampling (Atema, 1995; Zimmer-Faust et al., 1995; Jackson et al., 2007). Definitive proof is difficult to establish, particularly as there are substantial limitations on our ability to present defined chemical signals to naturally foraging aquatic creatures.

We developed a system for the simultaneous visualization and quantification of odorant signal structure and animal movements during navigation (Dickman et al., 2009) to examine how animals use chemical signal structure to navigate through turbulent odorant plumes. This allows us to define relationships between chemical signal input and the subsequent behavioral output both over an entire tracking event and in different chemical signal environments. Here, we concentrate on the role of spatial information during chemosensory navigation by blue crabs, *Callinectes sapidus* Rathbun

1896, and show that the local spatial distribution of odorant concentration mediates directional changes. Further, we show that multiple aspects of these signals provide information and that the behavioral response changes in different plume types. Thus, stimulus history or state-dependent responses to spatial patterns are an important, but previously unrecognized, aspect of navigational strategies in turbulent plumes. A companion paper examines the role of temporal sampling in mediating upstream progress (Page et al., 2011).

MATERIALS AND METHODS

We present an abbreviated description of our experimental method. A full description is found in the companion paper (Page et al., 2011).

Hydrodynamic environment

Simultaneous odorant signal quantification and tracking experiments were conducted in a recirculating saltwater flume housed in the Environmental Fluid Mechanics Laboratory at Georgia Tech. The clear acrylic channel of the flume measured 0.76×13.5 m with a ~ 2 m working section at the downstream end where the boundary layer was fully developed. Water was maintained at a depth of 21.2 cm by a weir and recirculated at a mean flow speed of 5 cm s^{-1} . The bed of the flume was covered in sand with a mean diameter (d_{50}) of 1 mm. These conditions approximate environments that blue crabs would realistically encounter in the field while foraging (Finelli et al., 1999; Smee and Weissburg, 2006) and resulted in a boundary layer with a roughness Reynolds number of ~ 3 and a bed shear velocity (u^*) of 3.01 mm s^{-1} (Jackson et al., 2007).

Chemical signal preparation

The attractive odorant stimulus was created by soaking 2.21 g of shrimp in 1 l of seawater for 1 h, with fresh solutions made each day and tested within 2–3 h of preparation. This general preparation has been used in many behavioral assays involving aquatic organisms and, in particular, blue crabs have effectively tracked sources of chemical exudates of shrimp in previous behavior trails (Jackson et al., 2007; Keller et al., 2003). The stimulus solution was mixed with a fluorescent dye, Rhodamine 6G, which served as an optically measurable proxy for the relative concentration of the attractive odorant. A stimulus delivery system was established in the working section of the flume that utilized a 4.2 mm diameter nozzle suspended 25 mm above the substrate.

Animal behavior was examined in three plume types (continuous, meandering and pulsed release), which were chosen to alter signal structure in distinct ways to evoke particular aspects of tracking behavior [see Page et al. (Page et al., 2011) for flow visualization images]. Specifically, the continuous plume provided a straight, relatively simple plume shape for basic comparisons of plume properties with resulting behavior. The meandering plume was used to test the effects of large-scale spatial variation, and the pulsed plume was used to test the effects of large-scale temporal intermittency on crab tracking behavior. We expected large-scale spatial structure to evoke behaviors concerned with spatial localization and temporal intermittence to elicit responses directing upstream motion. The release conditions for the continuous and meandering plumes were created by the uninterrupted isokinetic release of stimulus from the nozzle. Meander was induced by a 10.1 cm cylinder placed 50.8 cm upstream of the nozzle, thereby creating a von Karman vortex street in the cylinder wake. The pulsed plume was released at a frequency of 0.1 Hz and was created by a 5 s odorant pulse followed by a 5 s release period without odorant.

Odorant signal visualization

We designed a three-dimensional laser-induced fluorescence (3DLIF) system to measure the chemical signal structure impinging on the chemosensory organs of animals used during orientation, which consist of both cephalic (antennules and antennae) and thoracic (walking legs) appendages (Keller et al., 2003). The system consisted of an Argon-ion laser (Coherent Inc., Santa Clara, CA, USA), a pair of orthogonally mounted mirrors (Cambridge Technology, Lexington, MA, USA) used to scan the laser beam across the test section in both the horizontal and vertical directions, and an overhead CMOS camera (Mikrotron GmbH, Unterschleißheim, Germany), all controlled by a computer system running Video Savant image capture software (IO Industries Inc., London, ON, Canada).

The 3DLIF system illuminated a three-dimensional volume created by 20 sequential horizontal scans, each separated vertically by 8.4 mm. The lowest and highest scan positions were located at 0.5 and 16.5 cm above the substrate, respectively, and were chosen to encompass the extent of the chemical plume within the sampling volume with the lower limit being set by the approximate level of *C. sapidus* leg chemosensors. The camera lens allowed 1 mm pixel resolution at an elevation of 50 mm above the channel substrate and created a depth of field that allowed an adequate level of focus for each of the 20 scan elevations. The resulting 3DLIF image data were collected with Video Savant real-time-to-disk software at a collection rate slightly less than 5 Hz (i.e. the 20 planes required 0.21 s to collect).

Behavior measurements

Blue crabs were collected and maintained as outlined in Jackson et al. (Jackson et al., 2007), and mean crab size did not statistically differ over the three treatments (continuous= 13.85 ± 0.4231 cm, meandering= 14.76 ± 0.3263 cm, pulsed= 14.35 ± 0.2827 cm; $F_{2,36} = 1.6319$, $P = 0.2097$). Intense laser light is an aversive stimulant to foraging blue crabs, so they were reversibly blinded with electrical heat shrink-wrap, which created no adverse effects (Dickman et al., 2009). Crabs were outfitted with a light-emitting diode (LED) backpack to indicate their position, which was recorded by the CMOS camera simultaneously with the Rhodamine 6G fluorescence data.

Data collection and analysis

Crab position

The bright spots on the recorded 3DLIF images created by the individual marker lights were easily distinguished from the fluorescence. The crab velocity, acceleration, angle with respect to flume centerline, and angular velocity were calculated based on the mean of the LED locations for each 3DLIF set (i.e. mean of the 20 layer images) every ~ 0.2 s. The angle between the line drawn connecting the two LEDs (across the widest axis of the crab carapace) and the x -axis (i.e. the axis parallel to the flow) yielded the crab's angle of orientation with respect to the flow (Ψ). The mid-point between the LEDs along this line was considered to be the center of the crab (x_{crab} , y_{crab}).

To analyze crab position and motion relative to the source location, we defined the source radius vector (\mathbf{R}) as the direction and distance from the crab to the source. We also defined a crab displacement vector (\mathbf{r}) that indicates the direction of the crab's movement between two time steps. The source-heading angle, α , is calculated as the normalized dot product of the source radius vector and the crab displacement vector at each time step:

$$\frac{\mathbf{r} \cdot \mathbf{R}}{|\mathbf{r}| |\mathbf{R}|} = \cos \alpha \quad (1)$$

The dot product provides a measure of how directly a crab is moving towards the source.

Movement towards the mean plume centerline (from either side of the centerline) is considered an adjustment towards the source and is accordingly labeled as a positive angle (Fig. 1). Movement away from the mean plume centerline by tracking crabs results in a negative angle.

Odorant concentration at the legs

Shadowing of the concentration data by the appendages influenced how we extracted the odorant signal structure impinging on leg chemosensors. First, only crabs that tracked the plume leading with their left claw (i.e. crab mouth facing the laser) were used for analysis. We were able to encourage the correct orientation by how we initially placed the crab in the flume, and our extensive prior work with these animals indicates tracking behavior does not differ in leftward- *versus* rightward-facing animals (Keller et al., 2003; Weissburg et al., 2003; Jackson et al., 2007). Second, signals very close to the body often were shaded by appendages regardless of the crab orientation. For this reason, the concentration arriving at the outer chemosensors was estimated by evaluating the structure of the approaching plume [see Dickman et al. (Dickman et al., 2009) for more details]. Concentration data were extracted in a rectangular region sufficiently upstream to avoid shadow or reflection interference from the leading claw (Fig. 2). As described in Fig. 2, the advection velocity, time delay and translation distance were employed to map the sampled concentration distribution from the upstream location (Fig. 2B) to the near-body location (Fig. 2A). A correlation analysis revealed that the odorant signal structure in the leg sampling box is largely unchanged over this short advection distance (correlation coefficient of 0.8 or greater) (Dickman, 2008). The cross-stream dimension (H_b) matched the projected cross-stream width of the crab and was calculated for each frame based on the crab's orientation angle and size. The concentration measurements for these chemosensors were taken from the laser plane located at $z=0.5$ cm, which was the closest to the chemosensors on the walking legs.

We characterized the transverse (cross-stream) distribution of the signal structure impinging on leg chemosensors by calculating the location (h) of the cross-stream concentration center-of-mass (COM), based on the instantaneous distribution of odorant concentration within the box:

$$h(x_0, y_0, t) = \frac{\sum_{x_0-w/2}^{x_0+w/2} \sum_{y_0-H_b/2}^{y_0+H_b/2} yc(x, y, t)}{\sum_{x_0-w/2}^{x_0+w/2} \sum_{y_0-H_b/2}^{y_0+H_b/2} c(x, y, t)}, \quad (2)$$

where x_0 and y_0 are the midpoint coordinates of the leg sampling box, t is time, c is concentration, x is the streamwise coordinate, y is the cross-stream coordinate and w is the along-stream dimension.

The hypothesis is that the location of the COM relative to the crab's current position encodes information about the local spatial bias; e.g. a COM located to the left of the crab's midline indicates a leftward bias in chemical signal intensity. We examined several ways in which the location of the COM may be used and examined whether any of these measures were associated with cross-stream motion.

Relating odorant signal properties to directional changes requires us to define cross-stream motion. We identified a cross-stream motion event as a sustained translational movement in the y -direction, which corresponded to the following two conditions: (1)

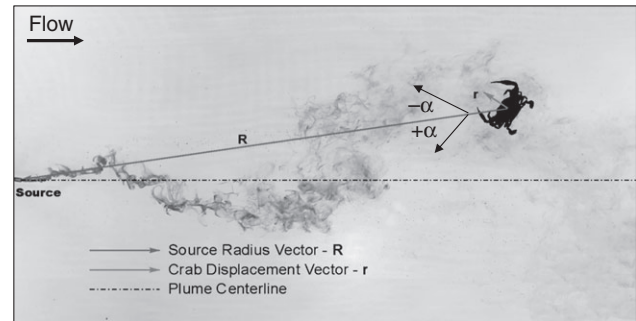


Fig. 1. Definition of vectors used for analysis of blue crab movement. The source radius vector (\mathbf{R}) is drawn from the center of the crab directly to the source. The crab displacement vector (\mathbf{r}) represents the magnitude and direction of movement of the crab between two time steps. The source-heading angle (α) is positive when the crab is moving towards the centerline and negative when the crab heads away from the centerline, with direct movement towards the source defined as $\alpha=0$ deg.

a cross-stream velocity of greater than 0.75 cm s^{-1} maintained for at least 1 s, and (2) a forward along-stream velocity of greater than 2 cm s^{-1} during the same interval. These criteria systematically excluded events where crabs repositioned their body without translation and apparent small translations associated with shifts in the location of the marker lights due to a crab tilting its body.

We also examined the influence of odorant concentration spikes arriving at the antennules as potential signals influencing cross-stream motion. As described in Page et al. (Page et al., 2011), we determined the instantaneous filament concentration arriving at the antennules for each searching crab, and identified a relative stimulus threshold (i.e. the concentration of an individual odorant spike relative to the mean) that evokes behavior. The use of a relative threshold rule allowed us to designate a set criterion that takes into account inter-individual variation in absolute stimulus concentration.

Statistical analyses of kinematic data

The analyses in the present study cover data obtained from crabs tracking in continuous ($N=15$ records), meandering ($N=13$ records) and pulsed ($N=12$ records) plumes. Most of the statistical analyses presented here were performed in SYSTAT 12 (version 12.00.08, SYSTAT Software Inc., Chicago, IL, USA). General linear model analyses were used for both univariate and multivariate tests of variance or covariance. We employed a repeated-measures design when appropriate, such as for cases where we examined the relationship between odorant properties and different behavioral outcomes or physical measurements on the same crab (e.g. analysis for data in Figs 9 and 11). Data were transformed where necessary to meet ANOVA assumptions of normality and homogeneity of variance. Statistical analyses on angles and related angular measures were performed in MATLAB R2008b (version 7.7.0.471, MathWorks, Inc., Natick, MA, USA) and utilized ANOVAs developed specifically for circular data as outlined in Zar (Zar, 1999) and Harrison et al. (Harrison et al., 1986), with tables provided by Mardia (Mardia, 1972).

RESULTS

Source-heading angle

The frequency distribution of the source-heading angle (i.e. α defined in Eqn 1) over the entire path was examined for each plume type to determine whether a crab's steering was affected by

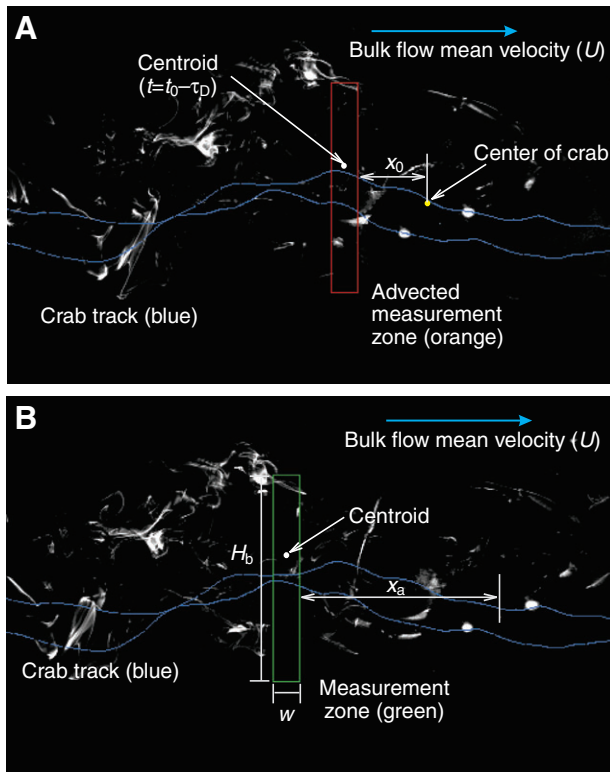


Fig. 2. The location of the leg sampling box, used to evaluating the signal at the walking appendage chemosensors. The panels depict an overhead view of a tracking crab at two points in time: (A) time $t=t_0$ and (B) time $t=t_0-\tau_D$, where τ_D is the time delay between images. Due to shadowing concerns, concentration data are extracted at an earlier upstream location (green box in panel B) and are assumed to be advected to the chemosensory location (orange box in A). The desired sampling box position is initially located a distance upstream of the crab center position (x_0 , shown in A). To evaluate the sampling box location at an earlier time ($t_0-\tau_D$), the position is shifted upstream by the advection distance ($U\tau_D$). In addition, the crab moves forward during this time period (distance of Δx_{crab}), which also shifts the relative position of the sampling box to the crab. Hence, the distance from the crab center to the measurement zone (green box) equals $U\tau_D+x_0+\Delta x_{\text{crab}}$. The τ_D shown corresponds to ~ 1 s. Figure from Dickman et al. (Dickman et al., 2009).

differences in patterns of chemical signal structure (Fig. 3). Crabs in all plume types displayed a strong peak in source-heading angles of ~ 0 deg (crab moving directly towards the source; Fig. 1), with the majority of observations occurring at small source-heading angles (between -45 and 45 deg). There was a bias towards positive source-heading angles, which indicates that the crabs preferentially make minor course corrections towards the plume centerline. Despite the positive bias, the angular distribution for crabs in the meandering and pulsed plumes showed minor peaks at small negative angles, whereas there was no indication of a corresponding peak at negative angles for crabs in continuous plumes. These minor negative peaks indicate that crabs in the meandering and pulsed plumes have a tendency to make small path corrections away from the plume source, which suggests that crabs in these plume types are less able to discern the direction of the plume source. The peak in the source-heading angle distribution for crabs in the meandering plume was lower compared with crabs in the pulsed plume (i.e. approximately -24 versus -9 deg, respectively). This indicates that crabs in the meandering plume make more extreme course

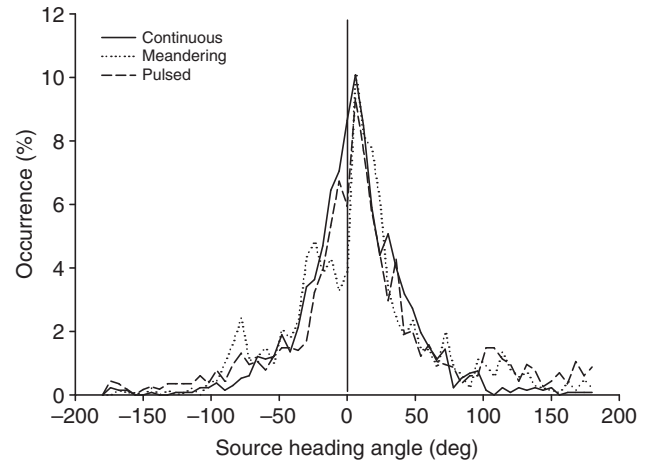


Fig. 3. Discrete probability distribution of source-heading angle (α) for crabs in various plume types. The vertical line highlights headings of 0 deg, i.e. pointing directly towards the source. Source-heading angles to the left of this line (i.e. negative) indicate that the crab is headed away from the plume centerline, and source-heading angles to the right of this line (i.e. positive) indicate that the crab is headed towards the plume centerline. Angles less than -90 deg or greater than 90 deg indicate movement away from the odorant source.

corrections away from the source (negative α) compared with the crabs making small course corrections in the continuous and pulsed plumes.

The distributions of the source-heading angle show that crabs do not universally move toward the source. Both the meandering and pulsed plume source-heading angle distributions showed local peaks at approximately -90 and 90 deg (crab is moving sideways with respect to the source radius vector and making no net movement towards the source), whereas the distribution for crabs in the continuous plume showed only a small local peak at ~ 90 deg. Movements away from the source (i.e. angles less than -90 deg or greater than 90 deg) were rare in all plume types and essentially nonexistent in the continuous plume. Crabs in the meandering and pulsed plumes showed a tendency for course corrections towards the center of the plume whenever they moved away from the source (i.e. more samples with angles greater than 90 deg than less than -90 deg).

Body angle during orientation

An animal's angle relative to the flow direction indicates how easily the animal may extract information from the chemical plume as well as the drag force of the flowing fluid. In general, crab orientation facing the flow (0 deg; Fig. 4) corresponds to the greatest drag force, but also to the position in which the crab can extract the most chemosensory information from the turbulent plume (Weissburg et al., 2003). Plume type significantly affected the body angle of the crab over the total path ($F_{2,37}=222.8$, $P<0.001$; Fig. 4). Crabs in the meandering plume consistently maintained body angles smaller (i.e. rostral-caudal axis more parallel to the flow) than those of crabs in the continuous and pulsed plumes, which tracked at similar body angles.

Angular velocity

We examined angular velocity to discern changes in body angle in response to odorant signal properties. Analysis revealed that statistical significance cannot be determined for the mean path

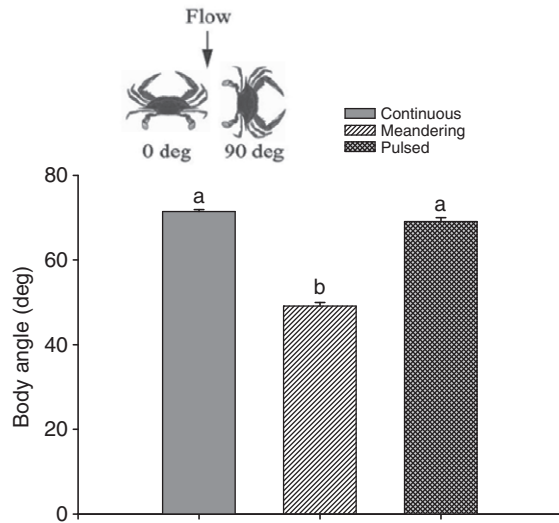


Fig. 4. Mean (\pm s.e.m.) crab body angle in relation to flow direction for crabs in various plume types. As shown in the sketch, body angle is defined such that 0 deg corresponds to the rostral-caudal axis aligned parallel to the flow direction and 90 deg corresponds to the rostral-caudal axis aligned perpendicular to the flow direction. Letters denote groups that are significantly different based on pairwise tests with Bonferonni-adjusted probability values.

angular acceleration of tracking crabs because the standardized magnitude of a crab's angular acceleration was too small for a valid statistical test (Zar, 1999). Hence, we present the analysis of angular velocity only. This relies on a conventional ANOVA approach (for circular statistics) as no accepted repeated-measures method for circular data exists. Substantial analysis indicated that body angle did not change during cross-stream motion (i.e. cross-stream motion was translational) and was generally unaffected by the direction in which crabs were moving (Page, 2009). Because body rotation was therefore not involved in determining direction of travel, we sought to determine whether crabs rotated their body axis relative to the flow as a result of acquiring chemical signals. Note that for this analysis, we defined angular velocity such that it is positive when crabs rotate upstream (i.e. a 0 deg orientation, see Fig. 4 inset) and negative when crabs rotate cross-stream (i.e. a 90 or -90 deg orientation).

The mean angular velocity of tracking crabs was not significantly affected by plume type over the total path ($F_{2,37}=1.31$, $P>0.90$; Fig. 5). However, the data suggest that chemical signal conditions modify the rotational behavior in response to odorant signal reception. Angular velocity for crabs in the continuous plume was contingent on whether they received an odorant spike at the antennules in the previous 1 s ($F_{1,28}=5.06$, $P<0.05$): crabs that received an odorant spike rotated so that their long axis was parallel to the flow (i.e. negative angular velocity) and those that did not receive a spike rotated their body to be more perpendicular to the flow (i.e. positive angular velocity). Note that the method for defining and extracting an odorant spike at the antennules is discussed in Page et al. (Page et al., 2011). Interestingly, the converse occurred with crabs in the meandering plumes, although the effect was not statistically significant; crabs that received an odorant spike rotated their body so that the long axis was more perpendicular to the mean flow direction, whereas those crabs that did not receive a spike rotated so their long axis was more parallel to the flow. The

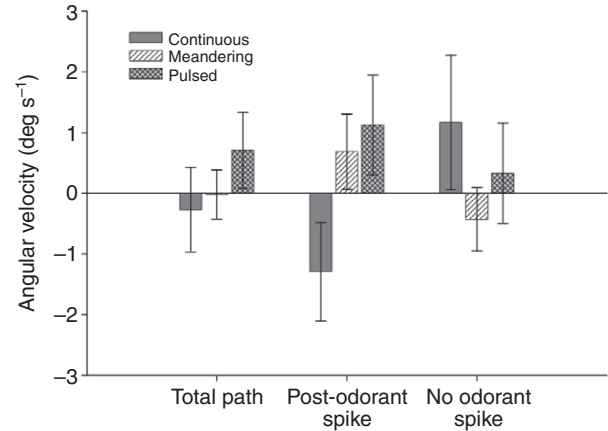


Fig. 5. Mean (\pm s.e.m.) angular velocity of crabs in various plume types. Mean angular velocity is displayed for the total path, for crabs 1 s after an odorant spike at the antennules (post-odorant spike) and for crabs that have not received an odorant spike (no odorant spike) within the previous 1 s. Positive angular velocity corresponds to rotation towards 0 deg and negative angular velocity corresponds to rotation towards 90 deg.

angular velocity of crabs in the pulsed plume was always positive, and appears unaffected by reception of an odorant spike. These differences potentially relate to both the varying structure of the odorant plume and the difficulty of extracting spatial information from these differing plume types, as we discuss below.

Cross-stream motion in response to chemical signal properties

The goal of this analysis was to test the hypothesis that crabs encode and use concentration variation across the areas sampled by chemosensors on legs to determine their course corrections while tracking. As outlined previously, we determined a concentration COM of odorant signal impinging on the legs as a measure of the distribution of the stimulus field relative to the crab's position. We initially analyzed cross-stream motion by asking whether the most frequent location of the COM prior to a cross-stream motion predicts the motion direction. We calculated the COM at each 0.2-s time step for 1 s prior to a cross-stream motion event (see definition in Materials and methods), and defined the 'mean' bias as that corresponding to the most frequently observed position (left or right) of the COM relative to the crab's midline. We then calculated the percent of the time that cross-stream motion events were as predicted by the COM bias for each path. The analysis revealed a high degree of concordance between the bias of the transverse odorant distribution and subsequent cross-stream movement. The sign of the mean bias of the transverse distribution of odorant corresponded to the cross-stream motion direction at least ~70% of the time across the various plume types (Fig. 6), with little evidence of differences across plume types. Similar relationships hold for the time period of 1–2 s prior to the cross-stream motion event (data not shown). We elected not to examine longer prior intervals, as crabs sometimes moved cross-stream several times during periods exceeding 2 s, particularly in continuous plumes.

Pooling data yields a mean percent correct cross-stream movements of ~74%, which is significantly different from the random expectation (50%) using a one-sample *t*-test ($t=5.33$, $N=39$, $P<0.001$). This suggests that stimulus bias in the transverse direction is a code for direction to tracking animals, and that it is measured

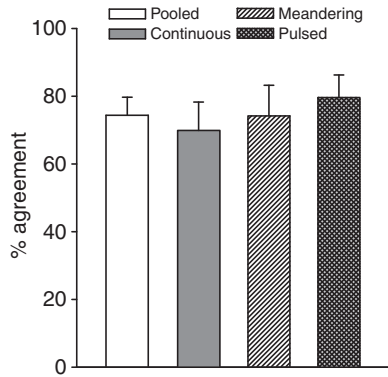


Fig. 6. Mean (\pm s.e.m.) percent agreement of the sign of the mean bias of the location of the center-of-mass (COM) of the concentration distribution in the leg sampling box 1 s prior to the cross-stream motion event and the direction of the cross-stream motion for crabs in various plume types.

by the leg chemosensors and used by the animal to locate itself relative to the plume structure.

Initial analysis suggested that the distance of the mean location of the COM from the crab body midline often was relatively small in instances where the mean bias did not correlate with subsequent cross-stream movements. Recall that the parameterization of the bias is binary (i.e. to the left or right of the crab's midline) in the above analysis, and that a change in the COM location as small as 1 mm (our pixel resolution) could produce a change in the sign of the bias. Thus, we elected to use the degree of the COM shift as a variable rather than simply examining the direction of the COM relative to the crab's midline.

We examined shift events of the COM that occurred in the opposite direction from the direction of the crab's concurrent cross-stream velocity (V_y) to observe instances when it would be advantageous for the crab to change direction. Starting from a shift event, we determined the time period that it took the crab to react to the stimulus, indicated by when the crab stopped accelerating in its current direction ($A_y=0 \text{ cm s}^{-2}$). We also determined the time period before the crab initiates cross-stream motion in the direction of that shift. We defined a 'turn' as starting when $V_y=0 \text{ cm s}^{-1}$ (Fig. 7) because cross-stream motion in the direction opposite to the previous direction requires that the y -component velocity changes sign.

We evaluated the probability that crabs move in the correct direction as a function of the magnitude of the shift of the COM (Fig. 8). Even small shifts ($<2 \text{ cm}$) in the location of the COM produced cross-stream motion in the corresponding direction, and the frequency of correct cross-stream movement increased with increasing shift distance such that crabs nearly always moved in the correct direction when the COM shift exceeded 6 cm. These general patterns remained consistent across all of plume types.

Because the size of the leg sampling box (H_b) changes as a function of crab size and body angle relative to the flow, we sought to clarify the relationship between COM shift and cross-stream motion by expressing the shift relative to the size of the crab's sampling array (i.e. the legs). We defined COM shifts relative to H_b and compared two potential thresholds for defining the smallest COM shifts that could be discerned by the animal: 2.5 and 5% of H_b . These thresholds correspond to distances of approximately 0.4–0.5 cm and 0.8–0.9 cm, respectively (Fig. 9). The differences in shift size across plume types were consistent with the effect of

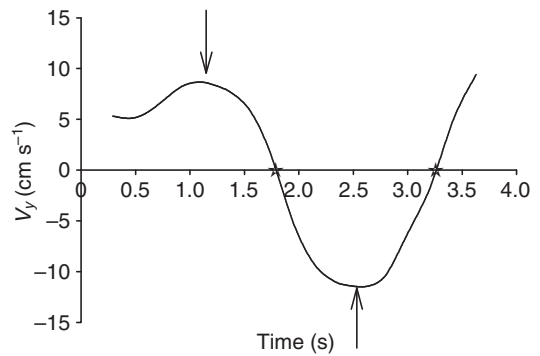


Fig. 7. Illustration of reactions and turns. The crab begins to change the direction of its prior motion by accelerating in a new direction at the moments indicated by arrows ($A_y=0$ at 1.1 and 2.5 s), which we call a reaction. The change in acceleration may eventually result in a directional change as the cross-stream velocity changes sign at the moments indicated by stars ($V_y=0$ at 1.8 and 3.25 s), which we call a turn. The time interval between a shift of the COM of the odorant concentration distribution in the leg sampling box and the events marked by arrows and stars defines the reaction and turn time periods, respectively.

plume type on mean body angle (Fig. 4), as there was no statistically significant difference in crab size across each plume type ($F_{2,37}=1.63$, $P=0.21$). The need for a relative measure of COM shifts is clearly shown by the analysis of the size of the leg sampling box, which is significantly affected by plume type (repeated-measures analysis, $F_{2,37}=6.40$, $P=0.004$). Body orientation angle was smaller, and consequently the leg sampling boxes were larger, for crabs in the meandering plume compared with those for the continuous and pulsed plumes (Fig. 4). There was also a significant effect of threshold applied on the size of the leg sampling box ($F_{1,37}=5557.2$, $P<0.001$), which was expected because the absolute and threshold sizes are directly related (i.e. 5% of H_b is exactly two times greater than 2.5% of H_b). There was no significant interactive effect of plume type and threshold ($F_{2,37}=1.28$, $P=0.28$).

A three-way repeated-measures ANOVA was used to examine the effect on response time period as a result of an antennule spike as a factor of threshold and plume type, with behavior (i.e. reaction or turn) constituting the repeated-measures variable (Fig. 10). The analysis revealed a significant effect of plume type on response time period ($F_{2,322}=8.24$, $P<0.001$), with crabs in the continuous plume showing the most rapid reactions and turns. This pattern was retained across plume types and thresholds as there was no interaction effect between plume type and reaction/turn time periods ($F_{2,322}=2.19$, $P=0.11$), plume type or threshold ($F_{2,322}=2.20$, $P=0.11$), nor any three-way interaction between plume type, threshold and reaction/turn time period ($F_{2,322}=1.04$, $P=0.36$). The time period required for crabs to react is, by definition, always shorter than the time period necessary for a crab to make the actual turn, accounting for the significant, but trivial, effect of behavior ($F_{1,322}=1346.05$, $P<0.001$).

A threshold of 5% of the width of the leg sampling box yielded response time periods that were significantly shorter than those observed with a threshold of 2.5% ($F_{1,322}=13.32$, $P<0.001$). The response time periods for 5% thresholds were less than the mean time period observed between turns, suggesting that observed responses are coincident with COM shifts exceeding 5% of the cross-stream dimension of the leg sampling box (and leg chemosensor array). In contrast, a threshold of 2.5% yielded data with

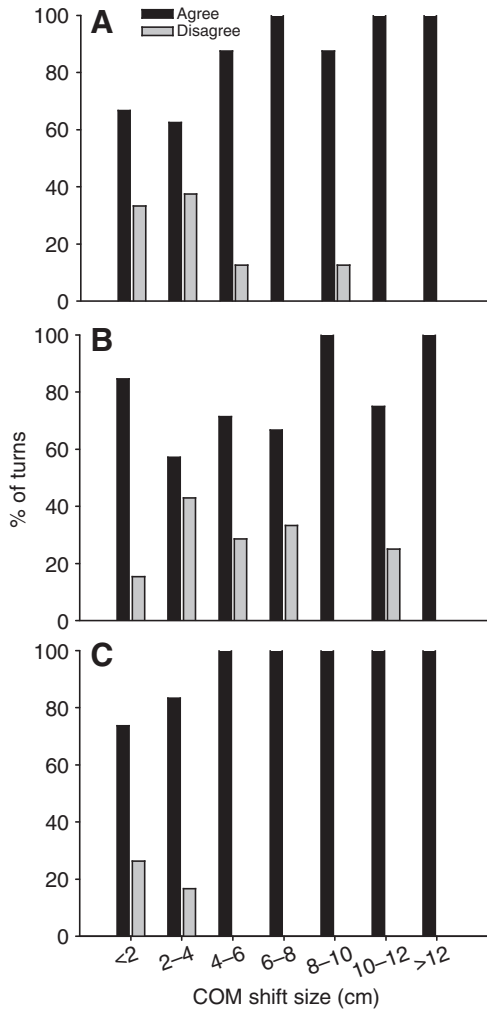


Fig. 8. Percentage distribution for cross-stream motions of crabs that agreed or disagreed with the COM shift direction as a function of the size of the COM shift. $N=53$, 19 and 16 shift events for the (A) continuous, (B) meandering and (C) pulsed plumes, respectively.

reaction/turn time periods that were shorter than the mean time period between turns only in the pulsed plume. In summary, this analysis suggests that a COM shift threshold of 5% of the leg sampling box size is consistently associated with turning, and that a threshold of 2.5% does not adequately predict turning across all plume types. Therefore, the threshold distance over which blue crabs behaviorally respond to spatial asymmetry in signal distribution is approximately 0.8–0.9 cm.

The association of directional changes and COM shifts indicates that spatial sensing of chemical distributions provides information about the plume location. However, this does not indicate that other stimulus parameters are unimportant. Therefore, we examined whether stimulus concentration played a role in directional changes by examining the concentration of chemical signals in the leg sampling box for events where cross-stream motion agreed *versus* disagreed with the COM shifts, using our previously determined 5% threshold. Interestingly, chemical signal concentration interacted with spatial distribution: the chemical signal intensity in the leg sampling box was greater in crabs that moved correctly in response to an above-threshold COM shift compared with animals that made incorrect cross-stream motions [repeated-measures analysis, $F_{1,26}=10.52$, $P=0.003$

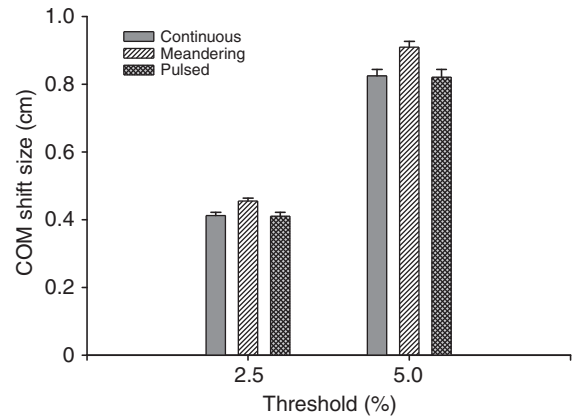


Fig. 9. Mean (\pm s.e.m.) size of the shift of the COM of the odorant concentration distribution in the leg sampling box relative to the threshold definition for crabs in various plume types.

(some paths had no incorrect cross-stream motions, so sample sizes differ from previous analysis); Fig. 11]. This effect was consistent across plume types (i.e. there is no plume type \times cross-stream motion interaction; $F_{2,26}=1.571$, $P>0.05$), even though there was a significant effect of plume type on concentration ($F_{2,26}=4.30$, $P=0.025$).

DISCUSSION

Behavioral analysis of animals navigating in turbulent plumes suggests that spatial information from the chemical signal distribution or information derived from the flow, or a combination of both, allows animals to extract directional information (Vickers, 2000; Weissburg, 2000; Grasso and Basil, 2002). A crucial factor is the sensory ambit relative to the scale of the plume (Weissburg, 2000). Animals with small sensory spans may be unable to achieve sufficient contrast across their sensory array to allow them to orient relative to the plume (Webster et al., 2001), which may explain why insects steer relative to the flow direction by using an

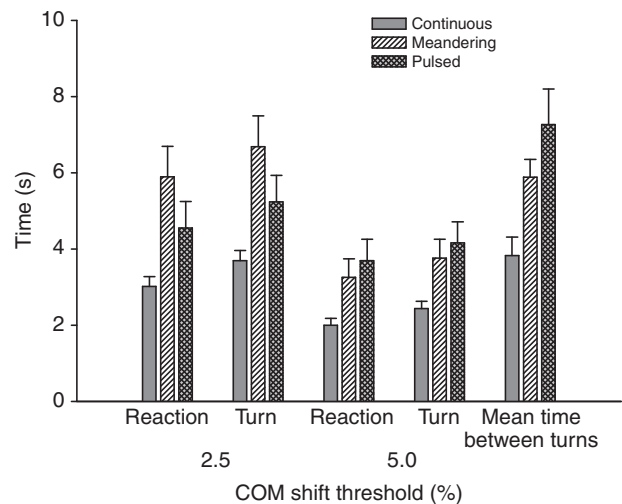


Fig. 10. Time period between a shift in the COM of the concentration distribution and a reaction ($A_y=0$) or a turn ($V_y=0$) in the corresponding direction. Data are means \pm s.e.m. for COM shift thresholds of 2.5 and 5% of the width of the leg sampling box. Mean \pm s.e.m. time periods between turns are shown for comparison.

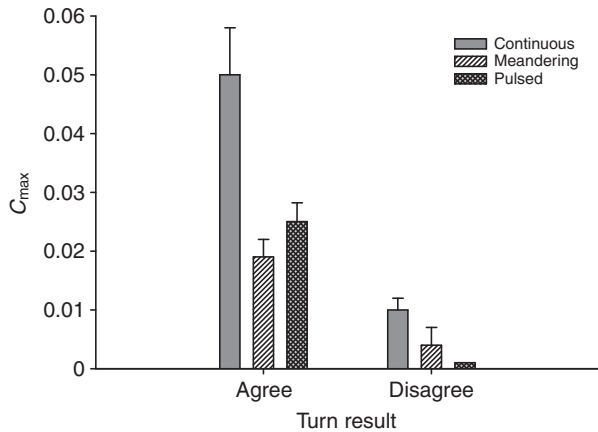


Fig. 11. Mean (\pm s.e.m.) magnitude of C_{max} in the leg sampling box prior to a cross-stream motion event for crabs in various plume types. C_{max} is the highest measured odorant concentration normalized to the source concentration, C_0 . Data are separated by whether the shift direction of the concentration COM of the concentration distribution in the leg sampling box disagreed or agreed with the subsequent cross-stream motion direction. Data are for the 5% COM shift threshold.

endogenous counter-turning mechanism that produces alternating left/right movements when they receive an odorant signal (Arbas et al., 1993). Such a mechanism does not rely on quantifying the spatial distribution of the odorant signal.

Larger animals, such as crustaceans in turbulent aquatic plumes, may have sensor spans that sample widely enough to determine the general direction towards the plume midline by encoding the spatial contrast (Webster et al., 2001). Evidence suggests that animals often move towards the time-averaged borders of the plume where there is a strong contrast between sensors located towards the interior of the plume and those on the outside (Finelli et al., 1999; Jackson et al., 2007). Additional evidence using point sensors indicates that foraging lobsters react to intensity differences of signals arriving at paired cephalic appendages by moving towards the more strongly stimulated side (Atema, 1996), although these observations are qualitative. Signal contrast has also been proposed as a mechanism of chemosensory navigation for a variety of walking insects (Borst and Heisenberg, 1982; Martin, 1965) and at least one flying insect (Duistermars et al., 2009), although these studies used narrow chemical plumes that may or may not be characteristic of natural sources.

Using a combination of odorant signal and animal visualization, we have established that spatial properties of odorant signals are necessary and sufficient to evoke correct cross-stream motion responses in blue crabs. Chemosensors on the walking legs seem to encode information on local spatial variation that is used to determine the direction towards the source. Interestingly, spatial properties of the odorant field may be combined with other aspects of incoming signals to shape the response (discussed in more detail below). Further, foraging crabs use odor stimulation to rotate towards a body orientation that improves their ability to resolve plume spatial properties if this does not conflict with increasing drag-induced locomotory costs (Weissburg et al., 2003). Finally, animals in different signal environments seem to react differently to similar instantaneous bias in odorant distribution, suggesting context-dependent responses to spatial properties contingent on stimulus history or other aspects of plume structure.

The role of flow information

Quantifying the gross aspects of crab cross-stream movements in varying plume conditions shows patterns consistent with previous investigations and also novel features. As in numerous studies (Moore et al., 1991; Baker et al., 2002; Wyeth et al., 2006), movements of blue crabs in the present study produced largely symmetrical distributions with predominantly small source-heading angles. Our data also provide strong evidence that odorant signal properties, rather than flow direction, are the primary drivers of steering.

Crabs in pulsed and meandering plumes sometimes moved away from the source. This is particularly evident from our analysis of crab responses to the meandering plume, where the distributions of source-heading angle showed a pronounced local maximum at approximately -90 deg, indicating that these crabs were moving perpendicular to the direction to the source. Crabs in the meandering plume followed the instantaneous plume structure as it meandered to the side, attempting to find the local, instantaneous plume centerline even if this resulted in movement perpendicular to the main flow direction. Further, we observed that crabs in the meandering and pulsed plumes moved downstream away from the source and towards the centerline of the plume for brief periods. Downstream movement in the meandering and pulsed plumes occurred when isolated odorant patches detached from the main plume structure and contacted predominantly the rear thoracic appendages, inducing the crab to chase discrete odorant patches downstream (personal observation).

The tendency to move down- or cross-stream is difficult to explain unless the navigational strategy uses directional information from chemical signal distributions as the primary source of information (Weissburg and Dusenbery, 2002). Unlike in moths, which turn with respect to the wind direction (Arbas et al., 1993), the role of flow here is much more limited. Although crabs move against the flow upon reception of an odorant spike at their cephalic appendages (Dickman et al., 2009; Page et al., 2011), the spatial distribution of odorant mediates cross-stream motion directly.

We see little indication that crabs consistently react to coherent flow structures comprised of odor-laden fluid. In other words, it does not appear that local flow dynamics interact with odorant properties to determine movement. Orienting to such structures in meandering plumes should result in cross-stream motions directed away from the source (e.g. Fig. 1), and we would further expect relatively symmetrical distributions of source-heading angle because meandering plumes are characterized by an alternating pattern of vortex shedding. Although source-heading angles for crabs in the meandering plume showed a greater incidence of cross-stream movement directed away from the source than in the other two plume types, these still comprise a small fraction of the overall directional changes and there is little evidence of the expected symmetry.

The role of odorant spatial properties

The detailed analysis of cross-stream motion in response to odorant signal structure suggests that information on the spatial variation of odorant filaments is provided by the array of chemosensors on the walking legs, and that this information mediates steering. The odorant concentration distribution, quantified by the location of the COM relative to the crab's midline, clearly predicts the crab's cross-stream motion (Fig. 6). This indicates that crabs position themselves relative to the plume by relying on the local spatial distribution. Further, our data provide evidence that dynamic changes in the location of the COM are associated with cross-stream motion. Examining crab movements in response to COM shifts demonstrates

that crabs move cross-stream in response to changes in the COM of less than 1 cm. In particular, crabs are able to detect and respond to a shift in the COM that is only 5% of the span of their legs (represented by the size of the leg sampling box), corresponding to approximately 0.8–0.9 cm (Fig. 9), and this change in the local spatial distribution is sufficient to induce movement in the appropriate direction (Fig. 10). Larger shifts resulted in more accurate responses (Fig. 8). We were unable to discern correlations between COM shift magnitude and other odorant properties such as mean filament intensity (data not shown), suggesting that the magnitude of the shift itself improved the accuracy of crab responses. This indicates that the ability to encode bias in the spatial distribution increases with the degree of bias, possibly as more sensory appendages become involved in coding. Conversely, crabs do not respond precisely to COM shifts on the order of 0.5 cm, which could be because these shifts are too hard to resolve based on the physical separation of the sensory appendages (i.e. legs) – such shifts probably would not result in sensory contrast across multiple legs so may not provide sufficient information on the local spatial distribution. Alternatively, there may be a threshold such that small changes in spatial properties are ignored as they are not reliably indicative of the plume source location.

We note that crabs can extract spatial information using a set of walking legs on one side of the body, as they often walk at an angle relative to the flow where a single set of walking legs faces upstream (Fig. 4). Thus, although signal contrast is often thought of as occurring across the midline in bilaterally symmetric animals (e.g. Martin, 1965; Borst and Heisenberg, 1982; Duistermars et al., 2009), this is not essential for blue crabs that function as radially symmetric organisms during navigation. The association between correct cross-stream motion and the degree of COM shift suggests that incoming chemical signal information is compared across multiple appendages even if they are one side of the body.

Although odorant spatial distribution is clearly necessary and sufficient for mediating cross-stream motion, our observations indicate that other odorant signal properties also contribute to steering responses. When the COM shift was above threshold, the mean instantaneous filament concentration was greater for correct than incorrect cross-stream movements (Fig. 11). Interestingly, there was no evidence of association between correct cross-stream movement and concentration when the COM shift was below threshold ($F_{1,14}=1.52$, $P>0.05$; data not shown). As noted, there was no evidence of correlation between COM shift and mean instantaneous concentration. Thus, information on filament intensity only has an effect on cross-stream motion once the COM shift is above a certain magnitude, acting to supplement information on the spatial distribution. Previous studies have relied on point sampling of spatial information (Atema, 1996) or unilateral sensory removal (e.g. Duistermars et al., 2009) to discern the role of spatial information. These techniques make it difficult to examine how various stimulus properties interact to modulate navigational decisions.

The time period required for blue crabs to detect and respond to odorant signals is consistent with other reports, although comparative data are sparse. The 2–4 s latencies we observed in blue crabs (at the 5% threshold) are the same as the 2–4 s lag between the arrival of bilaterally asymmetric stimuli and subsequent steering movements previously documented in lobsters (Atema, 1996).

Significantly, the reaction/turn time periods in response to changes in spatial information differed according to plume type. Crabs in the continuous plume reacted to a concentration COM shift quickly (~2 s), whereas crabs in the meandering and pulsed plumes

took longer (~3.5–4 s; Fig. 10). The direction of a cross-stream COM shift is a more reliable indicator of the direction to the plume centerline in the continuous plume compared with the meandering plume, where large-scale flow patterns produce instantaneous plume structures that are not aligned with the mean flow direction or the direction to the source (Dickman, 2008). Further, the pulsed plume provides intermittent information relative to the continuous plume. Our data, then, suggest that the difference in reaction time periods between the plume types may be due to the crab's perceived directional quality of the information present in a COM directional shift, where different conditions alter the weighting of information on the spatial distribution. We are continuing to examine the cues that mediate such context dependency, and hypothesize that the variation in COM shifts may be important. Regardless, these context-dependent responses are presently unknown in other systems and clearly are advantageous for tracking *via* fluid-borne chemical signals, which display random fluctuations in both space and time.

Recent reports suggest that differences in arrival time of stimuli at bilaterally paired olfactory sensors can mediate steering (Gardiner and Atema, 2010). We are unable to assess the extent to which this aspect of signal structure may be used by blue crabs. However, any difference in arrival time between sensors would be affected by the animal's body orientation, the location of a filament and its orientation in the flow. It's not clear how these parameters might be disambiguated so that arrival time differences provide a reliable directional signal, nor is the advantage of this seemingly more complicated scheme relative to spatial sampling. Further, the arrival time differences are likely to be extremely small, particularly under natural conditions. The time lag between adjacent legs, roughly 1.5 cm apart for a large crab, traveling at 10 cm s^{-1} in our 5 cm^{-1} flows can be no greater than 100 ms with a body angle of 0 deg (when the downstream distance between legs is greatest). This is the minimal time period producing oriented responses reported by Gardiner and Atema (Gardiner and Atema, 2010). In most conditions, the time lag is expected to be shorter because crabs assume a body angle of 45–75 deg (Fig. 4) and flows commonly approach 25 cm s^{-1} in the field (M.J.W. and D.R.W., personal observation).

Behavioral responses to increase information acquisition

Acquiring information about the environment may conflict with other important activities that animals must perform. Blue crabs rotate their body angle in relation to the flow while tracking, causing a trade-off between drag force (and cost of locomotion) and signal acquisition (Weissburg et al., 2003). A crab facing more directly into the flow (0 deg) experiences increased drag on its body relative to when its longest body axis is parallel to the flow (90 deg) (Weissburg et al., 2003). Concurrently, rotating to face the flow increases the transverse span of the animal (size of the leg sampling box; compare Figs 4 and 9) and thereby permits crabs to detect odorant spatial distribution information over a larger cross-stream area. This indicates that we should expect crabs to sacrifice drag in order to take advantage of stimulus asymmetry across their body only in environments that require them to make comparisons across a wide spatial expanse. The body angles of the crabs seem to support this prediction: crabs that were tracking plumes with large spatial spreads (meandering) faced more directly into the flow than crabs that were not faced with great transverse spatial variation (continuous and pulsed) and had corresponding larger transverse sampling spans.

Information acquisition has both costs and benefits, particularly because appropriate sensor deployment may compromise locomotion. For instance, effective scanning in the weakly electric fish occurs at

the cost of propulsive efficiency, and so these creatures have distinctly different body orientations for prey targeting *versus* normal swimming (MacIver et al., 2010). Changing body orientation in response to signal acquisition also allows foraging crabs to manage trade-offs between obtaining chemical signals and minimizing locomotory costs. Crabs in the continuous plume rotated their body more parallel with flow when they received an antennule spike whereas the crabs in the meandering plume rotated their body perpendicular to the flow in response to an antennule spike (Fig. 4). This difference in behavioral response seems to be associated with the signal characteristics of the plume that the crabs are tracking. Crabs in the continuous plume receive spikes more frequently than crabs in the meandering plume (Page, 2009). In addition, the smaller spatial spread of the continuous plume relative to the meandering plume permits accurate spatial localization over smaller distances. Crabs that receive frequent and easily perceivable spatial information may be able to afford to reduce drag force and therefore move upstream more quickly or with less energy. Conversely, crabs that receive less frequent and more diffuse signals may react to receiving an antennule spike by increasing their signal perception to take advantage of the 'rare', information even if this imposes greater drag costs.

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