The Journal of Experimental Biology 214, 1498-1512

doi:10.1242/jeb.049312

RESEARCH ARTICLE

Getting ahead: context-dependent responses to odorant filaments drive along-stream progress during odor tracking in blue crabs

Jennifer L. Page[1], Brian D. Dickman[2,*], Donald R. Webster[2] and Marc J. Weissburg[1,†]

[1]School of Biology, Georgia Institute of Technology, Atlanta, GA 30332-0230, USA and [2]School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA 30332-0355, USA

*Present address: Department of Mechanical Engineering, Colorado State University, Fort Collins, CO 80523, USA

†Author for correspondence (marc.weissburg@biology.gatech.edu)

Accepted 24 January 2011

SUMMARY

The chemosensory signal structure governing the upstream progress of blue crabs to an odorant source was examined. We used a three-dimensional laser-induced fluorescence system to collect chemical concentration data simultaneously with behavior observations of actively tracking blue crabs (*Callinectes sapidus*) in a variety of plume types. This allowed us to directly link chemical signal properties at the antennules and legs to subsequent upstream motion while altering the spatial and temporal intermittency characteristics of the sensory field. Our results suggest that odorant stimuli elicit responses in a binary fashion by causing upstream motion, provided the concentration at the antennules exceeds a specific threshold. In particular, we observed a significant association between crab velocity changes and odorant spike encounters defined using a threshold that is scaled to the mean of the instantaneous maximum concentration. Thresholds were different for each crab, indicating a context-sensitive response to signal dynamics. Our data also indicate that high frequency of odorant spike encounters terminate upstream movement. Further, the data provide evidence that the previous state of the crab and prior stimulus history influence the behavioral response (i.e. the response is context dependent). Two examples are: (1) crabs receiving prior odorant spikes attained elevated velocity more quickly in response to subsequent spikes; and (2) prior acceleration or deceleration of the crab influenced the response time period to a particular odorant spike. Finally, information from both leg and antennule chemosensors interact, suggesting parallel processing of odorant spike properties during navigation.

Key words: chemical plume, chemical sensing, odor-guided navigation, rheotaxis, turbulent plume.

INTRODUCTION

In turbulent flow conditions (i.e. chaotic and unpredictable flow), organisms such as decapod crustaceans and flying insects rely on the filamentous nature of chemical plumes to find odorant sources (Vickers, 2000; Weissburg, 2000). Turbulent stirring has dramatic effects on the structure of chemical plumes, creating filaments of highly concentrated odorant interspersed with regions of low or zero concentration that vary greatly in space and time (Murlis and Jones, 1981; Webster and Weissburg, 2001; Crimaldi et al., 2002). The spatial and temporal characteristics of the fine-scale structure of chemical plumes provide information for olfactory-mediated behaviors (Moore et al., 1991; Mafra-Neto and Cardé, 1994; Finelli et al., 1999), and variation in the plume structure influences how organisms perceive and respond to chemical signals in their environment (Willis et al., 1994; Jackson et al., 2007).

The importance of instantaneous plume properties to successful chemosensory search of rapidly tracking organisms has been widely reported (e.g. Vickers and Baker, 1994; Webster and Weissburg, 2009). Reiterative contact with discrete odorant filaments is necessary to sustain up-current progress. Both aquatic (Mead et al., 2003; Keller and Weissburg, 2004) and terrestrial arthropods (Mafra-Neto and Cardé, 1995a; Willis and Avondet, 2005) display straighter search trajectories and improved source localization with increasing filament contact frequency. In some moths, in-flight arrestment occurs in homogenous pheromone plumes (e.g. Willis and Baker, 1984; Justus and Cardé, 2002). This leads to the speculation that the frequency resolution of the pheromone-sensing elements sets an upper limit; frequencies exceeding a critical value cause fusion of incoming pulses such that animals perceive a single continuous pulse that stops upwind progress. However, not all moths seem to show in-flight arrestment in putatively continuous pheromone sources (Justus and Cardé, 2002), making the role of high contact frequencies unclear. An alternative explanation is that organisms stop once they experience high odorant concentrations. Indeed, evidence from moths (Baker et al., 1988; Baker and Haynes, 1989) suggests that adaptation of antennal neurons associated with in-flight arrestment is frequently related to differences in the concentration of incoming pheromone pulses, and that adaptation as a result of high pulse rates is weaker than that due to concentration differences.

Our understanding of odor-guided navigation is based largely on experiments where behavioral strategies are examined in specific odor environments, allowing us to interpret behavioral responses in light of the general properties of the odorant signal (e.g. Moore et al., 1991; Keller et al., 2003). However, the chaotic and unpredictable nature of turbulent plumes makes it impossible to precisely define the temporally varying signals experienced by a given searcher. Only a single study has directly resolved the relationship between odorant signal structure and behavior (Vickers and Baker, 1994) by simultaneously recording physiological responses of isolated insect antennae mounted to moths flying through a plume. In another study, Mead et al. examined the effect of waves on odorant filament

structure and intermittence using laser-induced fluorescence (Mead et al., 2003). Mantis shrimp in waves experienced more odorant pulses (and higher concentrations), but there was no difference in tracking success or kinematics for shrimp in wave-driven *versus* unidirectional flow.

The lack of direct observations on the relationship between odorant signal structure and behavior means we are left with few concretely verified hypotheses, particularly for aquatic invertebrates that have been less well studied than their terrestrial cousins. As noted, the role of intermittence in terrestrial insects is ambiguous. Conclusions on the role of intermittence in aquatic invertebrates are largely inferential, except for the study by Mead et al. (Mead et al., 2003). We have no direct evidence regarding the role of odorant concentration or other odor-burst properties such as duration, and investigators have advanced widely varying views on their importance. Additionally, because few previous experiments directly examine the signals that are encountered by a given searcher, we are unable to evaluate whether animals use a predetermined set of rules to govern movement as opposed to flexible strategies that consider the particular odorant environment. Similarly, we have not examined the role of stimulus history in modulating behavioral strategies. The substantial variability of odorant plumes suggests benefits for the ability of animals to have rules that adapt to the particular odorant environment or that depend on stimulus history. Finally, nearly all studies of insects and crustaceans have focused on a single olfactory appendage, normally the antennules. We have yet to determine the behavioral consequences of interactions between stimuli arriving at different chemosensory appendages in spite of their potential importance. Studies in at least two decapods have showed that, although the antennules are sufficient to produce navigation to food cues, navigational efficiency is increased in animals that use their full complement of functioning chemosensory organs (Keller et al., 2003; Horner et al., 2004). This suggests that parallel processing of odorant signals may be important during normal navigation.

In order to make a direct link between chemosensory signals and behavior, we used a three-dimensional laser-induced fluorescence (3DLIF) system to quantify the time-varying three-dimensional plume structure and the concentration of odorant filaments that reach blue crab sensory structures. The corresponding tracking behavior was simultaneously recorded. This approach provides a comprehensive examination of odorant signal input-behavioral output functions for animals in turbulent plumes.

Our focal animal is the blue crab, which provides excellent subjects for studying the role of instantaneous properties of odorant plumes in chemosensory search behavior. These animals display most of the well-documented characteristics of chemosensory guidance in large mobile animals, including odor-triggered movement in response to flow and spatial orientation to odorant signal asymmetry (Weissburg and Zimmer-Faust, 1994; Zimmer-Faust et al., 1995; Jackson et al., 2007). Crabs (as well as related large crustaceans) move quickly through plumes and the time period needed for adequate time-averaged sampling is too long to be practical for their use (Webster and Weissburg, 2001; Weissburg et al., 2002); hence, these crustaceans must extract odorant plume information from the filaments themselves. Substantial research has suggested that upstream surges and spatial orientation within a plume are governed by antennule and leg chemosensors, respectively (Keller et al., 2003). These two populations of sensors are located at different heights in the water column and encounter different spatial aspects of the plume (Rahman and Webster, 2005; Jackson et al., 2007). Separation of behavioral control into two sets of sensors that encounter unique plume environments provides an opportunity to dissect the control of tracking behavior into its components and to examine interactions between information arriving at these two sensor populations.

In the present study, we analyzed the signal structures governing the successful along-stream progress of blue crabs, *Callinectes sapidus* Rathbun 1896, to an odorant source. Our goals were to: (1) examine whether odorant signal arrival rate is sufficient to mediate upstream motion in the absence of information on other properties (e.g. filament concentration); (2) distinguish the role of high pulse arrival rates *versus* concentration in mediating near-source stopping; (3) evaluate whether navigational algorithms are fixed or context-dependent; (4) determine the role of stimulus history; and (5) identify whether interactions between antennular and dactyl (leg) inputs affect upstream motion.

MATERIALS AND METHODS

Flume system

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 an average diameter (d_{50}) of ~1 mm. These conditions were chosen to 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).

Odorant plume

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 daily within 2–3 h of testing. This general preparation has been used in many behavioral assays involving aquatic organisms, and blue crabs in particular have effectively tracked sources of shrimp chemical exudates in previous behavior trials (Keller et al., 2003; Jackson et al., 2007). The specific concentration of stimulus was chosen as a value in the middle of the dose response range of blue crabs. This created an odorant plume that crabs could easily follow under continuous isokinetic release conditions, yet allowed us to see marked changes in behavior due to introduced plume structure complexity. 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. The combined solution was neutrally buoyant, non-reactive and transported passively by the flow. The diffusivities of Rhodamine 6G (Schmidt number of ~1250) and the chemical exudates were approximately equal in seawater and did not chemically interact. Hence, the scalar filaments of dye and odorant were assumed to be identical. 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. The elevated release location leads to stirring by eddies in the turbulent boundary layer and a concentration field with great spatial and temporal variation (e.g. Webster and Weissburg, 2001). The specifics of the flume and delivery system, like all other aspects of the following methodology, have been fully described in a number of publications (Jackson et al., 2007; Dickman et al., 2009).

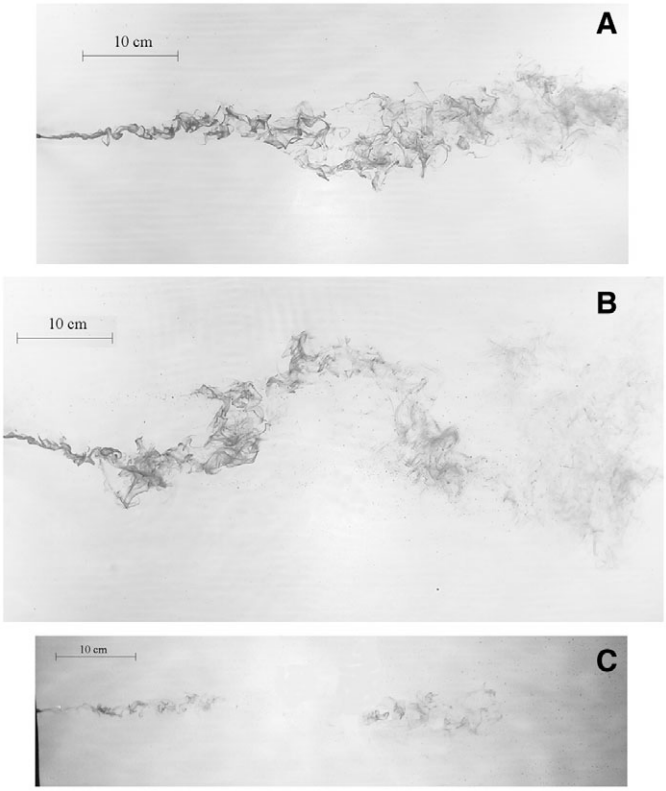

Fig. 1. Flow visualization images taken from above the (A) continuous, (B) meandering and (C) pulsed plumes. Flow direction is from left to right for each image.

Animal behavior was examined in three plume types (continuous, meandering and pulsed release; see Fig. 1), which were chosen to alter signal structure in distinct ways to evoke particular aspects of tracking behavior. Specifically, the continuous plume provided a straight, relatively simple plume shape that could be compared with the other plumes. The meandering plume was used to test the effects of large-scale spatial intermittency, and the pulsed plume was used to test the effects of large-scale temporal intermittency. We expected that large-scale variation in spatial structure would evoke behaviors concerned with spatial localization whereas changes in temporal intermittence would elicit responses directing upstream motion. The release conditions for both 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 shedding frequency was ~0.1 Hz, which corresponds to a Strouhal number of 0.2 (note that the Reynolds number for the cylinder flow is ~5000). The pulsed plume was released at a frequency of 0.1 Hz created by a 5-s odorant pulse followed by a 5-s period of no odorant. The odorant pulse was released with a slight momentum relative to the surrounding flow in order to match the scalar mass release rate of the other two plumes [see Dickman (Dickman, 2008) for fluid dynamical characterization of the three plume environments].

3DLIF system

Laser-induced fluorescence (LIF) permits non-intrusive quantitative measurement of instantaneous concentration fields by utilizing low concentrations of a tracer dye (i.e. a light-reactive fluorophore) to illuminate the plume structure. We designed a 3DLIF system to measure the chemical signal structure impinging on various chemosensory organs of animals during orientation (Dickman et al., 2009). 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 horizontal and vertical directions, and an overhead CMOS camera (Mikrotron GmbH, Unterschleißhiem, 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 0.5 and 16.5 cm above the substrate at the flume centerline, respectively, and were chosen to encompass the extent of the chemical plume with the lower limit being set by the approximate height of *C. sapidus* leg chemosensors. The system imaged the region 150 to 40 cm downstream of the source location. References to total path plume data (see Results) refer to data from 150–40 cm downstream of the source whereas data analyzed by section of the plume refer to regions covering 150–100 cm (downstream), 100–50 cm (middle) and 50–40 cm (upstream) from the plume source. Although this results in unequal segment lengths, we chose these divisions to remain consistent with other experiments in this same flow facility under similar conditions (e.g. Jackson et al., 2007). 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). As previously reported (Dickman et al., 2009), crabs were reversibly blindfolded with heat-shrink tubing to avoid the deleterious behavioral effects of intense laser light. This manipulation does not harm crabs, and does not alter kinematics or tracking success relative to untreated controls (Dickman et al., 2009). Mean crab size (width at the lateral spines) 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).

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. Trials were conducted in a dark room, and crabs were acclimated in a cage at the downstream end of the test arena for 10 min. During this time period, odorant plume release was initiated to ensure that crabs had contact with the stimulus plume prior to entering the test section and that a developed plume was present for the entirety of the experiment.

Data collection and analysis

Crab position

The recorded light intensity of the LEDs on the crab backpack was generally an order of magnitude greater than that of fluorescence from the Rhodamine 6G. The LEDs' location was followed on a frame-by-frame basis using an area search algorithm and converted to Cartesian coordinates. The LED coordinates were validated during post-processing by overlaying the calculated frame-by-frame

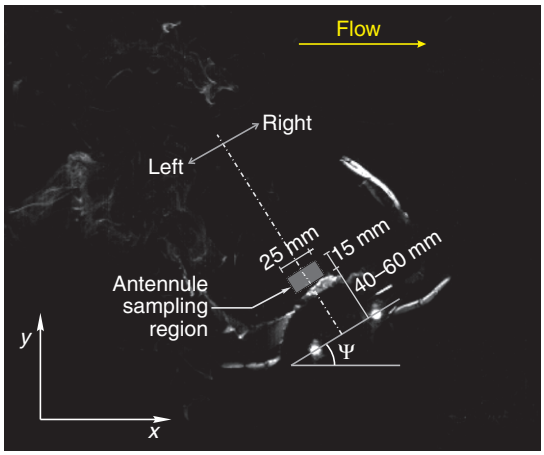

Fig. 2. Location of the sampling zone for the blue crab antennule region. The two bright dots in the lower right region of the image are the light-emitting diodes used to quantify the crab position. Light reflecting from the carapace and mouthparts is observed in the space between the bright dots and antennule sampling region. Ψ, crab orientation angle with respect to the *x*-axis (and mean flow direction). The sampling box is a standard 25×15 mm rectangle oriented at angle Ψ. Figure from Dickman et al. (Dickman et al., 2009).

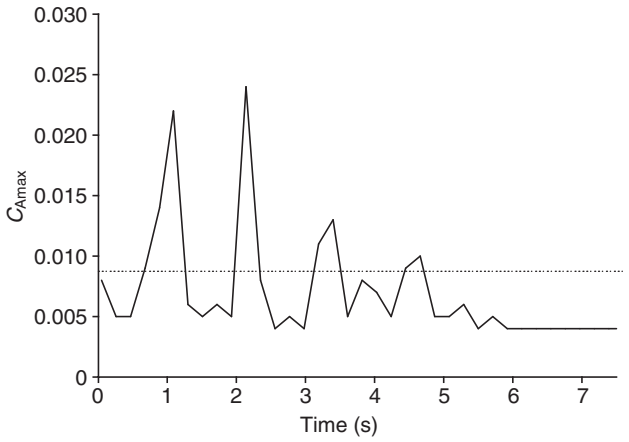

Fig. 3. Representative C_{Amax} record for a blue crab tracking in the meandering plume, showing the definition of the odorant spike. C_{Amax} is the observed maximal concentration in the antennule sampling region at a given time point normalized by the source concentration C_0. Dotted line represents an example of the spike threshold determined by calculating the mean C_{Amax} as described in the text. Odorant peaks below this line would be excluded. See Results for details.

trajectory of the marker lights with their actual trajectory on the raw image data. The crab's velocity, acceleration and body angle with respect to flume centerline were calculated based on the mean of the LED locations for each 3DLIF set (i.e. mean of the 20 layer images) at every ~0.21 s. The angle between the line connecting the two LED locations (across the widest axis of the crab carapace) and the line of the *x*-axis (i.e. the axis parallel to the flow) yielded the crab's angle of orientation with respect to the mean flow direction (Ψ). The mid-point between the LED locations along this line was considered to be the center of the crab (x_{crab}, y_{crab}), which was used to determine the distance of the crab from the mean centerline of the plume (*d*). The crab's velocity was calculated in both the *x* and *y* directions (V_x and V_y, respectively) using central differencing, and the combined change of position in time defined the total velocity (V_T). The crab's acceleration was derived from the velocity values (i.e. A_x, A_y and A_T). Positive velocity and acceleration values in the *x*-direction correspond to the upstream direction (i.e. opposite to the *x*-coordinate direction shown in Fig. 2). Positive velocity and acceleration in the *y*-direction indicate cross-stream motion to the right of the plume centerline when facing upstream (i.e. consistent with the *y*-coordinate direction shown in Fig. 2).

Concentration measurements

Only crabs that tracked the plume leading with their left claw (i.e. crab mouth facing the laser) were used for analysis because crabs facing the opposite direction shaded the signal impinging upon the antennule chemosensors. We were able to encourage the desired orientation by how we initially placed the animal in the flume, and our extensive prior work with these animals indicates tracking behavior does not differ in leftward versus rightward facing animals (M.J.W., unpublished observations). The signals impinging on crab antennules were quantified by analyzing chemical concentration within a sampling region in front of the antennules. The size of this region (shown in Fig. 2; 15×25 mm in the three LIF data planes nearest the crab mouth, thus spanning ~16 mm in the vertical direction) was chosen to represent the volume that the antennules potentially access within one sampling time step, taking into account animal velocity and odorant advection. We determined the elevation of the antennules by manual examination of each 3D image set (i.e. the 20 images constituting a volume scan).

The objective was to relate signal properties to animal movements, hence we extracted the maximum concentration within the antennule sampling region to facilitate the analysis. The substantial variation in stimulus concentration that crabs experience (across individuals, across plume types, and through time) complicates the task of determining what constitutes a relevant signal. In addition, sensory systems adapt to constant stimuli and often respond to change as opposed to absolute intensity (e.g. Gomez and Atema, 1996a). Thus, a search for a reasonable characterization of odorant signals focused on the concentration of individual odorant peaks relative to the background concentration (Fig. 3). The greatest concentration within the two-dimensional antennule sampling box at each time point was normalized to the source concentration (C_0), resulting in a non-dimensionalized maximum concentration value (C_{Amax}=$C_{greatest}$/C_0).

In this analysis, a concentration peak was defined initially as any point that was greater than the values immediately previous and subsequent in time. It is unlikely that tracking crabs process all peaks as viable signals; therefore, further analysis was necessary to determine what constitutes a signal to a tracking crab. We determined for each crab the mean C_{Amax} over the duration of the track, and signals were defined relative to this value by using a threshold criterion. Expressing the threshold as a function of the per crab mean C_{Amax} accounts for the variation experienced by individual crabs while still constituting a consistent criterion. We evaluated various thresholds in order to identify the minimum level of above-threshold signal intensity – termed an odorant spike – that evoked behavior (see Results).

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. We used repeated-measures analyses to examine behavior of individuals as functions of their downstream position (i.e. plume section), although for some records we did not have enough data in the farthest upstream region for

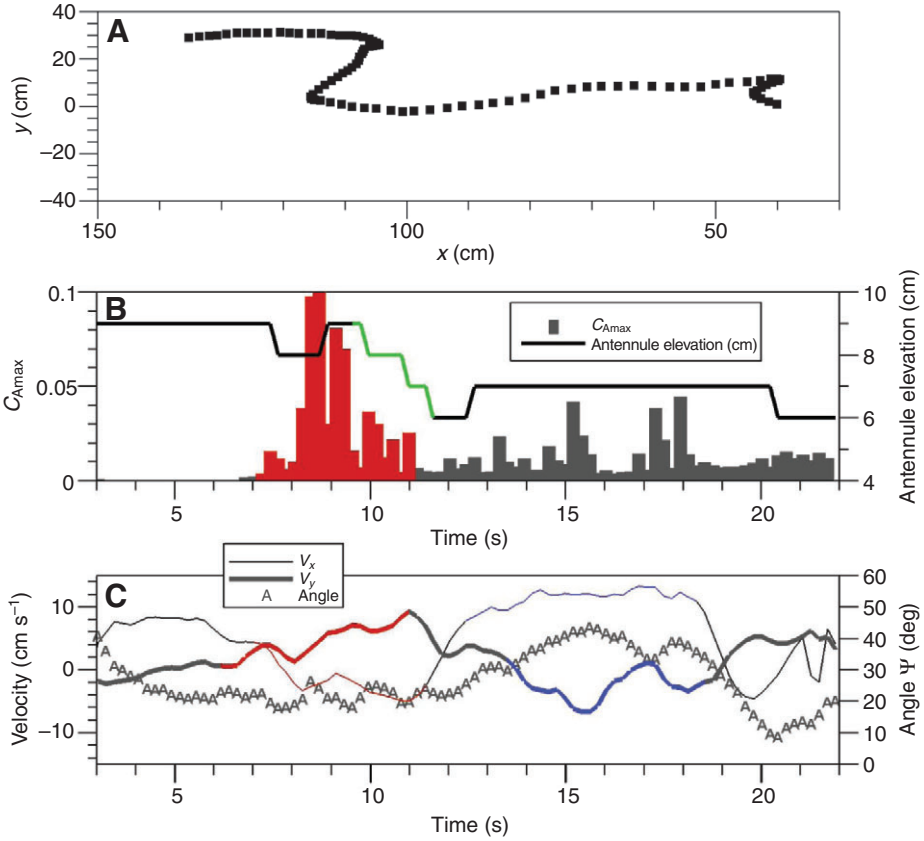

Fig. 4. Extracted time traces for blue crab 1286Q in the meandering plume. (A) Crab trajectory with data points separated by 0.21 s, (B) concentration and antennule elevation data and (C) streamwise and transverse walking velocities and crab orientation angle data. High concentration bursts, shown in red, occurred after the crab moved transversely (also shown in red in B). The interception of the high concentration bursts resulted in a lowering of the crab antennules by ~2.5 cm (shown in green). The crab moved rapidly upstream after the transverse and vertical adjustments (shown in blue) while making transverse adjustments in the opposite direction of those shown in red. The crab's body orientation angle remained between 10 and 40 deg throughout most of the track.

analysis. Frequency analyses were based on records at each time step rather than means, and therefore sample size corresponded to ~900–1200 discrete measurements across the various conditions. Most of the statistical analyses 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, with variables transformed to meet normality assumptions as necessary. Analysis of categorical frequency data was performed by log-linear analysis using an online interactive statistics tool provided by Vassar College and authored by Richard Lowry (http://faculty.vassar.edu/lowry/VassarStats.html).

RESULTS

Fig. 4 shows an example time record for a crab in the meandering plume. Up to 7 s, the crab received no odorant signal at the antennules and maintained a modest upstream velocity. High concentration bursts were intercepted after 7 s as the crab moved transversely. This crab chased an odorant packet in the downstream direction for several seconds, as shown in red (shown *via* negative *x*-velocity). During this time period, the crab also lowered its antennules in the boundary layer, as shown in green. At 12 s the crab accelerated upstream and maintained a relatively high *x*-velocity while moving in the negative *y*-direction. No general trend is apparent in the record of the orientation angle Ψ.

Fig. 5 shows an example time record for a crab in the pulsed plume. The trajectory is considerably straighter compared with the example in the meandering plume (compare Fig. 5A with Fig. 4A). After the first stopped period (noted by red arrow), the crab received a burst of odorant at ~11 s, which was followed by upstream movement (shown in red). After passing through the odorant cloud, the crab decelerated and actually moved downstream for ~1 s (shown in green). During deceleration, the crab altered its body orientation. A train of low-intensity odorant bursts, shown in blue, corresponded to rapid upstream movement and rotation of the crab orientation. After the low-intensity odorant cloud passed, the crab decelerated and remained stopped for several seconds, as noted by the second red arrow. The high concentration burst at ~23 s did not elicit an obvious response.

Dickman et al. presents and discusses two additional example time records for crabs in the continuous plume (Dickman et al., 2009). Further, all individual records used for the results, analysis and discussion below are presented in Dickman (Dickman, 2008).

Time periods during tracking

Mean search period

We broadly describe the effects of spatial (meandering plume) and temporal (pulsed plume) intermittency on the successful tracking behavior of blue crabs before addressing the role of specific instantaneous plume properties. We evaluated the time period for successful searchers to traverse the test section as a measure of the ability of crabs to obtain signals leading to upstream progress. The time period crabs spent searching for the source was significantly affected by plume type ($F_{2,37}$=5.50, P=0.008), with crabs in the meandering and pulsed plumes taking ~30–60% longer to traverse the entire test section compared with crabs in the continuous plume (Fig. 6).

We hypothesized that the tendency to make upstream progress would change with distance from the source; therefore, search time period was analyzed using a repeated-measures two-way ANOVA, with plume type and plume section as main effects. We omitted the 50–40 cm region given the differences in length between this section and the more downstream sections. The analysis again reveals a significant effect of plume type on the time period it

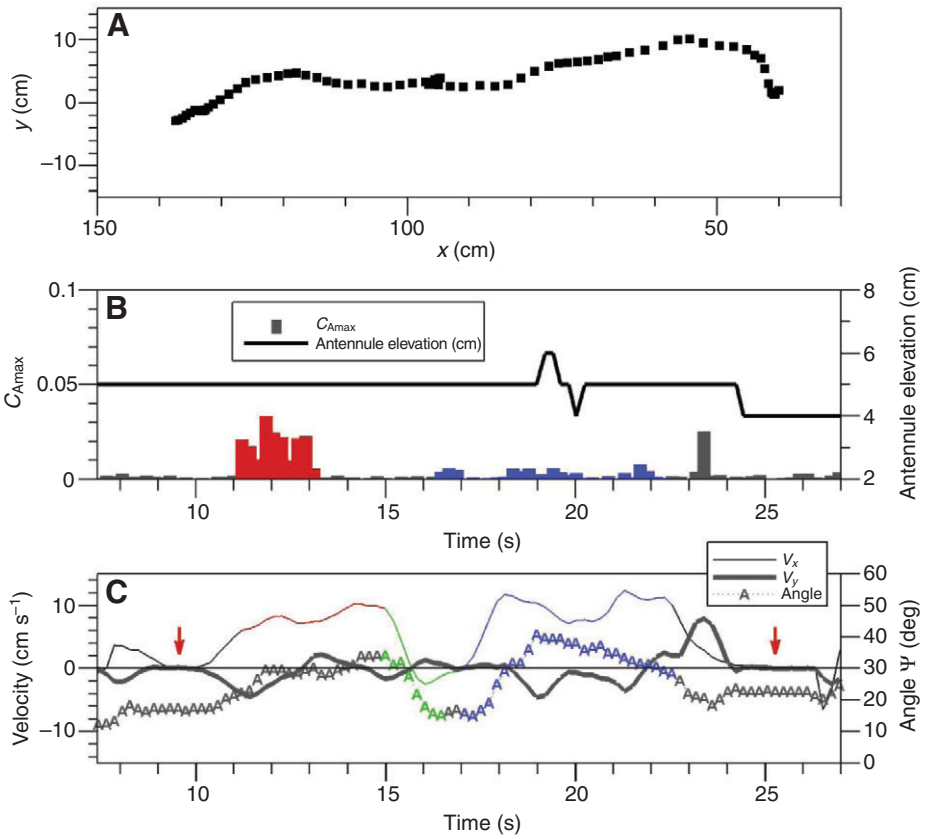

Fig. 5. Extracted time traces for blue crab 1201M in the pulsed plume. (A) Crab trajectory with data points separated by 0.21 s, (B) concentration and antennule elevation data and (C) streamwise and transverse walking velocities and crab orientation angle data. Periods of stopping are noted with red arrows. The crab accelerated upstream after receiving a burst of odorant (shown in red) following a stopping period. After passing through the odorant cloud, the crab decelerated and rotated and even headed downstream for ~1 s (shown in green). The moderate concentration bursts shown in blue correspond to a second period of acceleration and body rotation.

takes for crabs to navigate the downstream and middle sections of the plume ($F_{2,37}$=5.48, P=0.018). Crabs in the continuous plume took less time to traverse a particular section than crabs in either the meandering or pulsed plumes, which is consistent with the lowest total search time periods displayed by crabs in the continuous plume (Fig. 6). The effect of plume section was not significant ($F_{1,37}$=1.68, P=0.20), but there was a marginally significant interactive effect of plume type and plume section ($F_{2,37}$=3.04, P=0.06). Data indicate that crabs in the continuous and pulsed plumes took longer to traverse the middle section of the plume (100–50 cm) than the downstream section (150–100 cm), whereas crabs in the meandering plume spent statistically equivalent amounts of their search time period in the downstream and middle sections of the plume.

Mean percent of time period stopped

The period of time that a crab spends stopped directly affects the time required to locate the source and indicates a searcher's ability to make upstream progress. We considered a crab stopped when its total velocity was sustained between –0.5 and 0.5 cm s^{-1} for greater than 0.5 s. This criterion is consistent with our previous definitions (Jackson et al., 2007) and was chosen to avoid apparent non-zero velocities due to error in encoding the coordinates of the marker lights. Shifts in the crab's body posture and other effects can change the apparent location of the crab without corresponding translational movements.

The percent of time period that crabs spent stopped differed over plume type and plume section (Fig. 7). Because the percent of time period stopped is not a function of the absolute time period spent in a section, we can justifiably incorporate data from all three sections in this particular analysis. Crabs in the meandering and pulsed plumes spent a significantly greater percentage of their track time period stopped than crabs in the continuous plume (two- to three-fold increase; $F_{2,37}$=5.94, P=0.006; Fig. 7). A repeated-measures ANOVA on percent of time period stopped as a function of section indicates there was a significant effect of plume section on the percent of search time period stopped ($F_{2,74}$=3.35, P=0.04), with the greatest percent of search time period stopped closest to the source. There was also a marginally significant interactive effect of plume type and plume section on the percent of time period stopped ($F_{4,74}$=2.349, P=0.06). Crabs in the continuous plume spent a progressively greater percent of search time period stopped as they moved upstream towards the source, whereas the percent of track time period stopped did not appear to change in relation to plume section for crabs in the pulsed plume.

Along-stream movement

Analyzing the frequency distributions of along-stream velocity across plume types reveals plume-specific differences in upstream and downstream movement that can be easily masked by examining the mean values alone. Plume type had a significant effect on the distribution of a crab's along-stream velocity while tracking (χ^2=313.56, d.f.=18, P<0.001; Fig. 8). The along-stream velocity distributions depict a bimodal distribution in the pulsed plume and, to some extent, in the meandering plume, whereas the velocity distribution in the continuous plume is unimodal with a peak at higher velocities (~8 cm s^{-1}). The bimodal distribution in both the pulsed and meandering plumes has a local minimum at 6 cm s^{-1}, which also roughly equals the mean along-stream velocity for crabs in both plumes. Crabs in the meandering plume had a strong tendency to move along-stream at low velocities (<4 cm s^{-1}) and to stop along-stream motion. The along-stream velocity distribution of crabs in the pulsed plume appears to be intermediate between crabs in the meandering and continuous plumes, with the bimodal

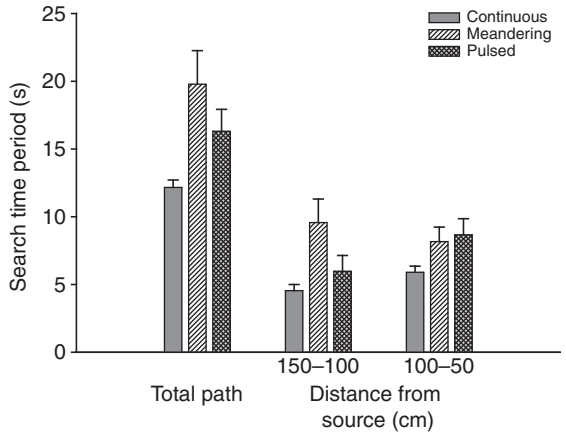

Fig. 6. Mean (±s.e.m.) search time period (s) for blue crabs in various plume types. Data are shown for crabs over the entire length of the track (total path) and for each segment of the track, determined by distance downstream from the source (150–100 or 100–50 cm).

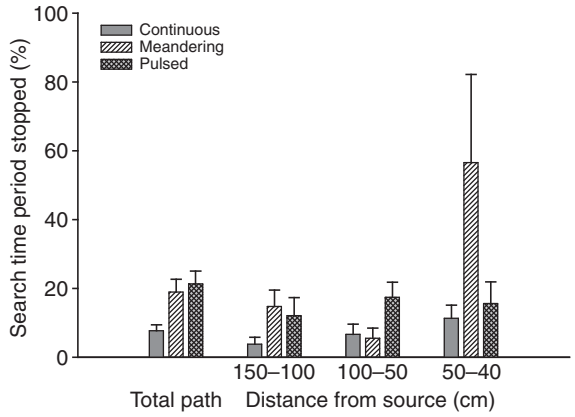

Fig. 7. Mean (±s.e.m.) percent of search time period that blue crabs in each flume section spend stopped, for crabs in various plume types. Data are shown for crabs over the entire length of the track (total path) and for each segment of the track, determined by the distance downstream from the source (150–100, 100–50 or 50–40 cm).

peaks of the pulsed plume aligning with the major peak in the data of each of the other plumes.

In summary, our analysis shows that crabs in the continuous plume moved faster, moved upstream more consistently and stopped less than crabs in the other two plume types. The pulsed plume elicited the slowest speeds, the greatest degree of stopping and the least consistent upstream movements, with the meandering plume showing similar but less dramatic effects on these parameters.

Concentration and spike record

Mean maximum concentration

Analysis of the mean maximum filament concentration experienced by a given crab ($\overline{C}_{Amax}$) provides insights into the signals experienced by each crab and reveals signal differences across the plume types. Examining the mean values in each plume demonstrates that C_{Amax} was significantly affected by plume type ($F_{2,37}$=3.45, P=0.04; Fig. 9). Specifically, crabs in pulsed plumes encountered filaments of lower mean concentration than crabs in the continuous and meandering plumes, which experienced similar concentrations.

The analysis of how filament concentration changes as crabs approach the source re-sampled individual crabs in different parts of the plume. A repeated-measures two-way ANOVA of plume type and section indicates there was a marginally significant effect of plume type on $\overline{C}_{Amax}$ ($F_{2,37}$=2.87, P=0.07). There was a significant effect of plume section on $\overline{C}_{Amax}$ encountered ($F_{2,74}$=8.5, P<0.001) and a significant interaction between plume type and plume section ($F_{4,74}$=2.62, P=0.04). Crabs in the pulsed plume encountered the lowest concentration filaments over the entirety of the track and experienced concentrations that were 80% less, and much less variable, than the other plumes in the section closest to the source (50–40 cm downstream from the source). Crabs in the continuous and meandering plumes encountered the highest concentration filaments closest to the source and also experienced the greatest spike variation in this section.

Defining the spike threshold

As discussed above, the substantial variation in stimulus concentration that crabs experience (across individuals, across plume types and through time) complicates the task of determining a reasonable concentration threshold. We examined how three threshold rules performed in defining above-background stimuli: $\overline{C}_{Amax}/4$, $\overline{C}_{Amax}/2$ and $\overline{C}_{Amax}$ (shown in Fig. 10). We examined the relationship between threshold and the mean concentration of odorant spikes above and below the threshold and analyzed the number of potential peaks excluded by each threshold.

We report detailed data only for the continuous plume (Fig. 10), but our results are consistent across plume types in suggesting that a threshold of $\overline{C}_{Amax}/2$ defines odorant bursts without excluding potentially meaningful smaller odorant peaks. As shown in Fig. 10, the $\overline{C}_{Amax}$ values calculated by including only peak values that exceed the threshold value of $\overline{C}_{Amax}/2$ (triangles) are greater than the $\overline{C}_{Amax}$ values for the entire track (open circles) by a factor of 1.5 to 3.2. Further, the $\overline{C}_{Amax}$ values calculated by including only suprathreshold peak values (triangles) are greater than the $\overline{C}_{Amax}$ values calculated for sub-threshold non-spike peaks (filled circles) by a factor of five to 14. Hence, the threshold choice of $\overline{C}_{Amax}/2$ provides a clear separation of spikes in the sequence of signals received by the antennules. In contrast, a threshold choice of $\overline{C}_{Amax}/4$ fails to provide separation of the spikes; spikes extracted using this threshold rule yield a $\overline{C}_{Amax}$ value that does not substantially exceed the value for the entire track (data not shown). In addition, the time period between crab acceleration events matches the inter-spike time periods observed in each of the plume types for the $\overline{C}_{Amax}/2$ threshold, but the time periods match considerably less well for the $\overline{C}_{Amax}/4$ threshold (see Acceleration in response to odorant spikes, below; Table 1).

Although a threshold of $\overline{C}_{Amax}$ is clearly above background odorant levels, our analysis suggests this choice excludes quite a few peaks that are potentially meaningful. Our analysis defined a total of 200 peaks across all 15 paths for animals in continuous plumes. The number of odorant spikes detected by the $\overline{C}_{Amax}$, $\overline{C}_{Amax}/2$ and $\overline{C}_{Amax}/4$ threshold definitions equaled 95, 160 and 185, respectively. Hence, the spike threshold of $\overline{C}_{Amax}$ was by far the most exclusive. For an individual path, this rule eliminated ~37–53% of all peaks that arrived at a crab's antennules, whereas thresholds of $\overline{C}_{Amax}/2$ and $\overline{C}_{Amax}/4$ excluded ~18–28 and ~5–15% of all peaks, respectively.

Further, our analysis indicates that the threshold definition rules perform similarly across all plume types. There was a significant effect of threshold rule on the percentage of peaks excluded over

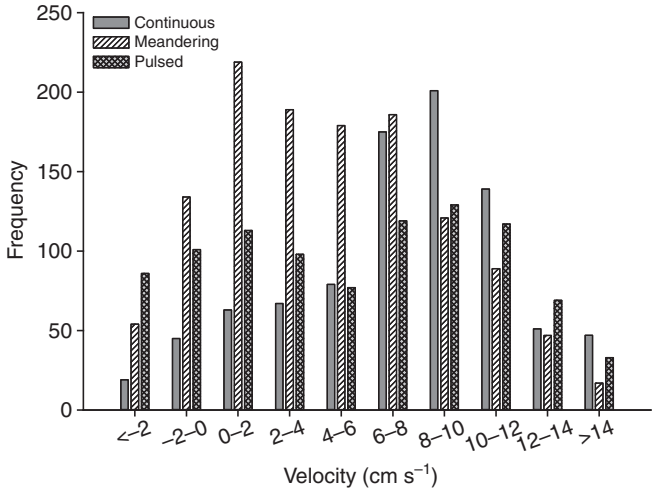

Fig. 8. Frequency distribution of blue crab along-stream velocity (V_x) in various plume types. Note that positive values of V_x are defined in the upstream direction, which is opposite to the axis shown in Fig. 2. Negative velocity values indicate downstream movement.

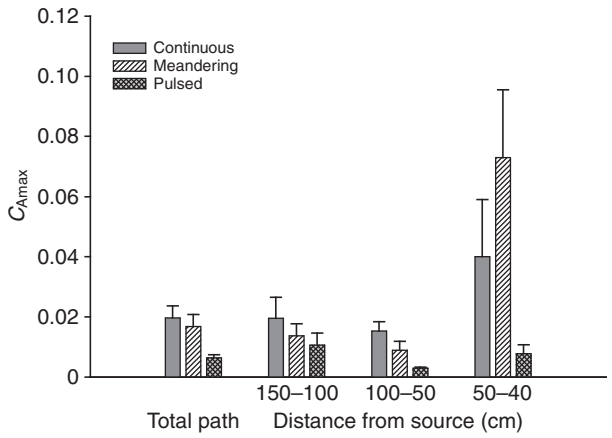

Fig. 9. Mean (±s.e.m.) C_{Amax} values for individual blue crab tracks in various plume types. Data are shown for crabs over the entire length of the track (total path) and for each segment of the track, determined by the distance downstream from the source (150–100, 100–50 or 50–40 cm).

the entire path length ($F_{2,111}$=34.25, P<0.001), but no significant effect on plume type ($F_{2,111}$=1.87, P=0.16) or interactive effect of plume type and threshold ($F_{4,111}$=0.61, P=0.66). Additionally, a repeated-measures analysis on the plume broken into downstream sections indicates that there was no significant effect of the interactions between plume type and plume section ($F_{4,156}$=1.78, P=0.14), threshold and plume section ($F_{4,156}$=0.70, P=0.59) or plume type, threshold and plume section ($F_{8,156}$=0.55, P=0.81) on the percentage of peaks excluded by our analyses. Thus, a given threshold rule performs similarly for individual crabs regardless of the plume type. This serves to further justify the use of a standardized threshold. Based on these considerations, we used a threshold of $\bar{C}_{Amax}/2$ to identify odorant peaks in our subsequent analyses.

Mean inter-spike interval

Because of the high variability of spike concentrations between and within paths (Fig. 10), it is unlikely that absolute concentration governs the behavior of blue crabs as they move towards the source. In fact, we were unable to detect any robust relationship between upstream movement and odorant spike concentration using a variety of analyses (Page, 2009). For instance, there was no significant difference between the spike concentrations when crabs accelerate after an odorant spike *versus* when they decelerate. In addition, we examined whether the changes in odorant concentration in the 2 s period preceding a given spike could explain subsequent velocity changes. There was no difference in the change in concentration or the first or second derivatives with respect to time of the change in concentration for crabs that either accelerate or decelerate in response to an odorant spike. As a result, our analysis focused on the frequency of odorant spike encounters as a determinant of upstream progress towards the source.

The time period between odorant spikes arriving at the antennules (inter-spike interval) differed as a function of plume type ($F_{2,37}$=4.06, P=0.04; Fig. 11) and section ($F_{2,74}$=8.86, P=0.001). The length of the inter-spike interval was also significantly affected by the interaction between plume type and plume section ($F_{4,74}$=2.83, P=0.04).

In general, the inter-spike interval for crabs in the continuous plume was shorter than those experienced by crabs in the meandering or pulsed plumes. The magnitude of this effect was contingent on distance downstream from the source as inter-spike interval generally increased with decreasing distance from the source. Farthest downstream, crabs in the meandering plume experienced roughly 25–50% longer inter-spike interval than crabs in the continuous and pulsed plumes, which had roughly similar inter-spike intervals. Crabs in the meandering and pulsed plumes experienced an intermediate, yet similar, inter-spike interval in the middle section of the plume, whereas crabs in the continuous plume experienced their longest inter-spike interval in this section. The time period between spikes increased dramatically (50–100%) as crabs traversed the upstream section of the meandering and pulsed plumes relative to inter-spike interval farther from the source. These inter-spike intervals were

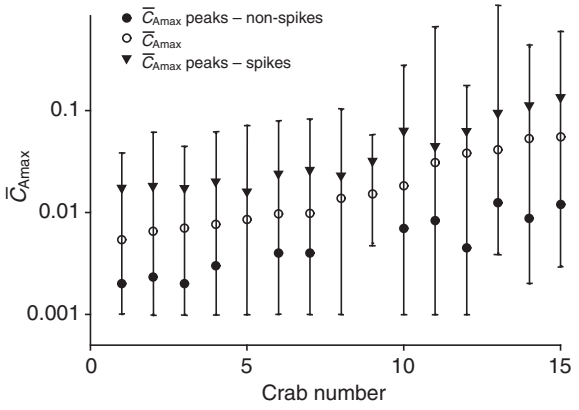

Fig. 10. Odorant peak $\bar{C}_{Amax}$ values for individual blue crabs in the continuous plume. $\bar{C}_{Amax}$ values are indicated by open circles, and the error bars report the maximum and minimum values during the track. Crab number (*x*-axis) was arbitrarily assigned to crabs in order of increasing $\bar{C}_{Amax}$ value. The $\bar{C}_{Amax}$ values are also reported in relation to a spike threshold of $\bar{C}_{Amax}/2$: the mean spike values (filled triangles) include only peaks that are greater than the threshold, and the mean non-spike values (filled circles) include only peaks that are less than the threshold. Note that crabs 5, 8 and 9 only experienced concentration peaks that exceeded the threshold, hence those cases lack the non-spike symbol.

Table 1. Mean time period (interval) between odorant spikes for the three plume types and four threshold levels, and mean time period (interval) between blue crab acceleration events for the three plume types

Threshold	Mean interval between odorant spikes (s)		
	Continuous	Meandering	Pulsed
$\bar{C}_{Amax}$	1.444±0.144 *N*=95	2.092±0.309 *N*=94	1.933±0.209 *N*=77
$\bar{C}_{Amax}/2$	1.066±0.057 *N*=160	1.452±0.155 *N*=148	1.384±0.131 *N*=115
$\bar{C}_{Amax}/4$	0.865±0.039 *N*=185	1.130±0.085 *N*=198	1.296±0.188 *N*=129
No threshold	0.803±0.030 *N*=200	1.063±0.060 *N*=214	1.188±0.102 *N*=144
	Mean interval between acceleration events (s)		
	Continuous	Meandering	Pulsed
	1.2±0.08 *N*=115	1.41±0.15 *N*=123	1.37±0.13 *N*=117

$\bar{C}_{Amax}$, mean maximum filament concentration experienced by a given crab.
Data are means ± s.d.

two to three times greater than the interval experienced by crabs in the continuous plume, where the interval exhibited relative constancy with distance downstream from the source.

Mean time period to above average velocity after odorant spike

There may be a link between the frequency of spike reception and a crab's velocity, as crabs in the continuous plume experience shorter intervals between spikes (Fig. 11) and take the least amount of time to traverse the test section compared with crabs in the meandering and pulsed plumes (Fig. 6). Such a link suggests that crabs surge upstream in response to odorant spikes; therefore, we examined lag time periods between stimulus arrival and the subsequent crab response. Specifically, we examined the time period that it took for a crab to reach above-average velocity after receiving a concentration spike at the antennules (Fig. 12).

The analysis reveals a strong link between stimulus arrival and velocity; crabs overall took less than 1 s (0.4–0.7 s) to reach above-average velocity following a spike at the antennules. The interval between periods of above-average velocities vastly exceeded the time lag between odorant-spike encounter and attainment of above-average velocity in all cases (interval=1.46±0.37, 2.57±0.97 and 2.92±1.16 s for crabs in the continuous, meandering and pulsed plumes, respectively; mean ± s.e.m.; *N*=45, 39 and 49, respectively). The difference in these two lag times suggests that the association between odorant spike encounter and above-average velocity is not simply fortuitous.

Crabs in the continuous plume took the shortest amount of time to respond to concentration spikes over the entire path, and reached above-average velocity in under 0.5 s. Crabs in the meandering and pulsed plumes appeared to take longer to reach above-average velocity on average (~0.6 s), but this difference across plume types was not statistically significant ($F_{2,37}$=1.63, P=0.21). Reaction time was significantly affected by plume section itself ($F_{2,74}$=11.35, P<0.001). The interactive effect of plume type and plume section on reaction time also was significant ($F_{4,74}$=3.33, P=0.02). Crabs in the continuous and meandering plumes respond two to three times faster in the middle section of the plume (~0.3 s) than they did in the downstream section of the plume (~0.5 and 0.7 s, respectively). Crabs in the pulsed plume did not change reaction time as a function of plume section. Note that all of these intervals are shorter than the mean inter-spike arrival time period, suggesting that perception of a single odorant spike is sufficient to elicit upstream motion.

Prior research has indicated that blue crabs utilize information from their antennule chemosensors to mediate upstream motion and

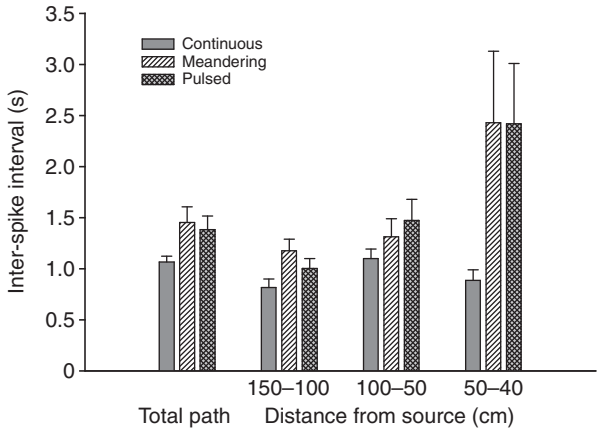

Fig. 11. Mean (±s.e.m.) time period between encountering single spikes (inter-spike interval) for blue crabs in various plume types (spike threshold defined as $\bar{C}_{Amax}/2$). Data are shown for crabs over the entire length of the track (total path) and for each segment of the track, determined by the distance downstream from the source (150–100, 100–50 or 50–40 cm).

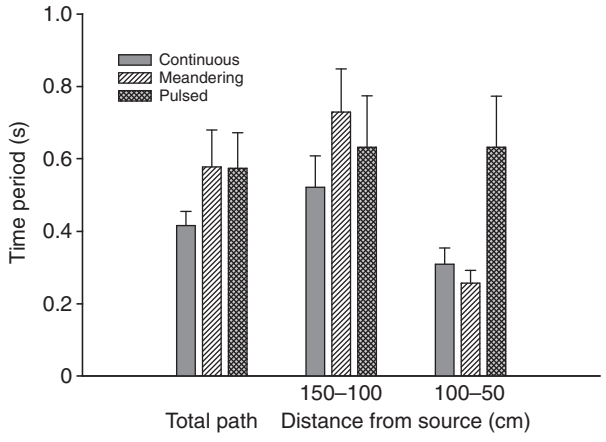

Fig. 12. Mean (±s.e.m.) time period that it takes blue crabs in various plume types to reach above-average velocity following an antennule spike (spike threshold defined as $\bar{C}_{Amax}/2$). There were not enough data points in the 50–40 cm section of the plume to include those spikes in the graph or analysis. Crabs that reach above-average velocity within 0.2 s (the shortest sampling interval) may already have been at above-average velocity prior to spike arrival.

signals from their leg chemosensors to modulate cross-stream motion (Keller et al., 2003). The simultaneous collection of concentration records at both sets of chemosensors, coupled with behavioral measurements, allows us to investigate the relative contribution of antennule *versus* leg chemosensors to each aspect of movement.

The role of both the antennule and leg chemosensor information in the motion towards the source was investigated by analyzing the time period it takes a crab to reach above-average velocity following a spike at the antennules as a function of spikes at its antennules or legs during the previous 1 s period (Fig. 13). This analysis employed a three-way ANOVA with main effects consisting of plume type, the presence/absence of a prior leg spike and the presence/absence of a prior antennule spike.

Interestingly, receiving a prior odorant spike at the legs within 1 s had a significant effect on the time period it takes for a crab to reach above-average velocity following an antennule odorant spike ($F_{1,99}$=5.63, P=0.02), although there was no significant interactive effect between plume type and previous leg spike ($F_{2,99}$=0.01, P=0.99). Receiving a previous spike at the antennules within 1 s had a marginally significant effect on time period to above-average velocity ($F_{1,99}$=3.26, P=0.07). The response to an antennule spike for any given combination of prior leg or antennule spikes was the same regardless of plume type ($F_{2,99}$=0.43, P=0.66), and there was no significant effect of the interaction between plume type and previous antennule spike ($F_{2,99}$=1.90, P=0.16). There was no significant two-way interactive effect between receiving a prior antennule spike and receiving a prior leg spike ($F_{1,99}$=0.57, P=0.45) and no significant three-way interactive effect of plume type with prior reception of an antennule or leg spike ($F_{2,99}$=0.83, P=0.44). In summary, receiving a prior spike at either the legs or (likely) the antennules reduced the response time period of crabs receiving a subsequent antennule spike regardless of plume type, and the response time period was not further reduced if crabs received prior spikes at both legs and antennules.

Acceleration in response to odorant spikes

Analyzing crab behavior in relation to the time period to above-average velocity indicates strong reactions to stimulus spikes, and intuitively relates the upstream progress of animals to the reception of odorant spikes. Another measure of the importance of odorant spikes is the time lag between contact with an odorant spike and a subsequent response. Thus, we analyzed the timing of acceleration/deceleration after receiving a spike at the antennules.

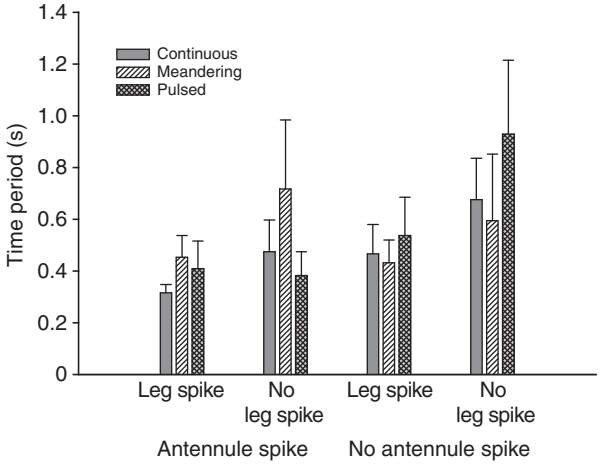

Fig. 13. Mean (±s.e.m.) time period that it takes blue crabs in various plume types to reach above-average velocity following an antennule spike (as a function of prior spike reception). Data are analyzed by whether the crab received an odorant spike at its antennules and/or legs within 1 s preceding the antennule spike.

We initially examined the relationship between the interval between odorant spike arrival and the interval between the onset of acceleration, reasoning that these two intervals should be similar if odorant spikes produce velocity increases. This analysis also served as an independent check of our choice of the odorant spike threshold of $\overline{C}_{Amax}/2$. There was a strong correspondence between inter-spike and inter-acceleration intervals (Table 1), with the threshold $\overline{C}_{Amax}/2$ providing the best match. Overall, the continuous plume showed the lowest inter-spike and inter-acceleration intervals, whereas crabs in pulsed and meandering plumes experienced longer periods between odorant spikes and showed correspondingly longer periods between events of acceleration. The interval between spikes defined by the most stringent threshold criterion ($\overline{C}_{Amax}$) was longer (particularly for pulsed and meandering plumes) than the inter-acceleration intervals, whereas thresholds lower than $\overline{C}_{Amax}/2$ (i.e. $\overline{C}_{Amax}/4$ or no threshold) produced the opposite trend: inter-spike intervals were shorter than inter-acceleration intervals.

The analysis presented in Table 1 suggests that velocity increases are produced by suprathreshold odorant spikes. The higher movement speeds, reduced motionless periods and faster search time periods for crabs in continuous plumes are likely a product of the greater frequency of odorant spike arrivals. These results also suggest that we have defined an appropriate value for the threshold. Applying the $\overline{C}_{Amax}$ threshold excludes some spikes that are stimulatory so that the interval between behavioral responses is less than the inter-spike interval. Thresholds less than $\overline{C}_{Amax}/2$ include many non-stimulatory odorant-spikes and hence produce the opposite result.

Frequency distributions of the post-spike acceleration patterns indicate, as expected, that crabs accelerate within a short period following a suprathreshold odorant spike, and further that the prior state of the crab affects the time lag between odorant spikes and subsequent acceleration (Fig. 14). For crabs accelerating prior to spike arrival (Fig. 14A), nearly 70% continued to accelerate in the interval 0–0.25 s, whereas the remaining 30% decelerated. Crabs that were decelerating prior to a spike (Fig. 14B) were also capable of rapid acceleration; ~30% accelerated within 0–0.25 s after receiving a spike, which is consistent with the rapid response time period of crabs seen in earlier analysis. However, despite their capacity for rapid acceleration, a substantial fraction of these crabs took considerably longer to accelerate, sometimes up to 2 s.

A log-linear analysis (VassarStats) indicates that the full model incorporating plume type and pre-spike state (accelerating *versus* decelerating) is significant (G^2=104.14, d.f.=17, P<0.0001). In addition, plume type significantly affects the frequency distribution of acceleration time periods following an antennule spike (G^2=18.9, d.f.=6, P=0.004), which is seemingly due to the behavior of crabs that are decelerating when they receive an odorant spike. Further, the distribution time period to acceleration following a spike also is significantly affected by pre-spike acceleration or deceleration state (G^2=77.06, d.f.=3, P<0.0001); crabs that were decelerating prior to a spike take longer to accelerate than crabs that were previously accelerating, as evinced by the smaller proportion of rapid acceleration (0–0.25 s) and larger proportion of acceleration over long time periods (0.25–2 s). There was no interactive effect between pre-spike acceleration or deceleration and plume type (G^2=0.16, d.f.=2, P=0.92).

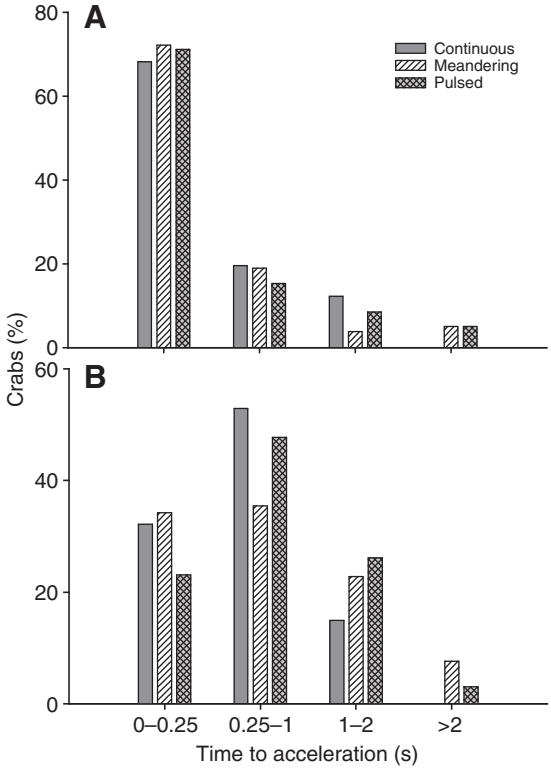

Fig. 14. Percent distribution of post-odorant spike acceleration patterns of blue crabs in various plume types. The *y*-axis gives the percentage of crabs that accelerate within a given time interval for crabs that were (A) accelerating or (B) decelerating prior to receiving an odorant spike at the antennules.

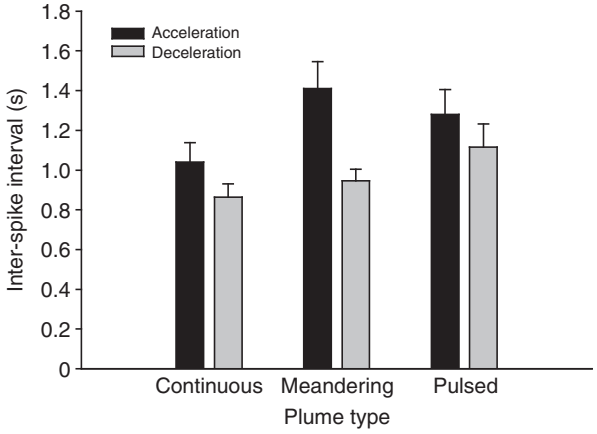

Fig. 15. Mean (±s.e.m.) inter-spike interval prior to receiving an antennule spike for blue crabs accelerating and decelerating in various plume types. Smaller inter-spike intervals correspond to more frequent odorant spike encounters.

In contrast to data on acceleration, the time period over which crabs decelerated was not substantially affected by plume type or prior state (e.g. accelerating or decelerating when they received a spike) (data not shown). Crabs decelerated ~0.56 s after receiving a spike across plume types (mean ± s.e.m.=0.53±0.06, 0.61±0.07 and 0.52±0.06 s for crabs in continuous, meandering and pulsed plumes, respectively; N=51, 68 and 56, respectively). We saw no correlation between the time period to deceleration and the preceding inter-spike interval (Pearson correlation coefficient=–0.050, P=0.528, N=175).

Mean inter-spike interval associated with post-spike behaviors

The preceding analysis indicates that crabs respond to odorant spikes by increasing their movement speed (Figs 12 and 14), but does not provide a fine-grained analysis of the effect of inter-spike interval on subsequent responses. Consequently, we determined whether the inter-spike interval was related to subsequent post-odorant spike movement (acceleration *versus* deceleration) (Fig. 15). For clarity, data were analyzed only for crabs already moving forward when they received an antennule spike.

The analysis showed a significant relationship, indicating that smaller inter-spike intervals are associated with deceleration ($F_{1,281}$=9.10, P<0.01; data square-root-transformed to meet normality criteria; Fig. 15), a significant effect of plume type ($F_{2,281}$=4.49, P<0.05), but no significant plume type×movement interaction ($F_{2,281}$=0.80, P⩾0.05). Thus, inter-spike intervals preceding deceleration are smaller in all plume types, but the inter-spike intervals corresponding to acceleration or deceleration are larger in meandering and pulsed plumes relative to the continuous plume. The data show a striking pattern: the inter-spike intervals that produce accelerations are all close to the mean inter-spike interval for the respective plume type given in Table 1. We saw no influence of concentration on deceleration or stopping. Specifically, we calculated the change in normalized concentration for accelerating *versus* decelerating/stopping crabs (where a negative concentration indicates a decrease in concentration from initial to final odorant spikes). These values were –0.003±0.005 *versus* 0.0015±0.005, –0.0075±0.023 *versus* –0.015±0.035 and –0.0045±0.01 *versus* –0.002±0.075 (mean ± s.e.m.) for crabs in continuous, meandering and pulsed plumes, respectively (N=57, 57; 64, 43; and 34, 32 for accelerating, decelerating/stopping in continuous, meandering and pulsed plumes, respectively).

DISCUSSION

Small-scale structure of a turbulent odorant plume has been implicated as a key determinant of the success of many rapid searchers in both terrestrial and aquatic habitats (Moore et al., 1991; Mafra-Neto and Cardé, 1994; Vickers and Baker, 1994; Weissburg and Zimmer-Faust, 1994; Webster and Weissburg, 2001; Jackson et al., 2007). This conclusion comes from studies in which the general properties of the stimulus environment have been obtained separately from the behavioral investigations. These studies have been key to developing the important idea that small-scale filament structures and instantaneous signals, rather than time-averaged gradients, are what mediate tracking success in turbulent environments. However, the presumed importance of instantaneous properties has been tested only rarely using techniques that directly examine relationships between odorant stimulus input and behavioral output. This means we have yet to establish the relevance of particular stimulus properties, such as filament concentration. Additionally, the lack of quantified stimulus input–behavioral output relationships in different odorant environments over an entire crab track prevents us from examining the context-dependency of sensory processing schemes, the role of stimulus history, and the interaction of inputs from multiple chemosensory organs.

Our study addresses unresolved issues by simultaneously examining the signal structure impinging on multiple olfactory organs in relation to animal movements to clarify the relationships

between sensory input and behavioral output that govern chemosensory tracking. Our data and analysis support the following conclusions: (1) tracking crabs respond to odorant filaments in an all-or-nothing fashion, and seem not to display graded responses to odorant concentration; (2) although crustaceans require lower pulse arrival rates than terrestrial insects to cue locomotion, consistent arrival of odorant spikes are required to sustain locomotion, whereas higher odorant pulse arrival rates cause deceleration and stopping; (3) context and state dependency are common and important aspects of odor-guided navigation, and are expressed as variable stimulus thresholds, variable responses to odorant stimulus frequency and differing behavioral lag times depending on stimulus history; and (4) input from multiple, different sensor populations is used during search.

Olfactory perception and attraction

Our results suggest that graded responses to odorant spike concentration are unimportant. Rather, odorant stimuli seem to elicit responses in a binary fashion by causing upstream motion as long as the concentration exceeds a specific threshold. We base this hypothesis on several lines of evidence. First, the absolute concentration range experienced by tracking crabs is rather large; even within an individual track instantaneous concentrations can span four or more orders of magnitude. Second, large changes in odorant concentration may occur even at the same position in a plume, which limits the utility of finer responses to concentration. More importantly, our data strongly relate velocity increases in tracking crabs to the presence of an odorant spike occurring above a certain threshold (Figs 12 and 14, Table 1) and indicate that, in general, more consistent upstream progress is associated with smaller inter-spike arrival time periods during animal tracking (compare Figs 6–8 with Fig. 11). Finally, whereas this simple threshold rule proved explanatory, we never found clear relationships between odorant spike concentration and crab movements. As previously noted, movement velocities following an odorant spike were unrelated to the concentration of the odorant spike or the first or second time derivatives of spike concentration during the prior period (Page, 2009). Similarly, changes in plume temporal structure have more drastic effects on search kinematics in moths than concentration changes over three orders of magnitude (Mafra-Neto and Cardé, 1995b).

Physiological evidence from crustacean olfactory systems is also consistent with strategies that do not depend heavily on graded responses. Integration time periods for sensory cells seem too long to provide meaningful estimates of concentration, particularly for repetitively applied stimuli. Gomez and Atema previously demonstrated that stimulus concentration discrimination generally takes 100–200 ms (Gomez and Atema, 1996b), a period greatly exceeding the duration of most pulses experienced by crabs in our trials. Note that acceleration in response to individual odorant spikes of crabs in the present study was on approximately the same time scale (i.e. often within 250 ms; Fig. 14), suggesting the importance of odorant spike detection rather than concentration encoding. Additionally, adaptation to repetitively applied stimuli further erodes the capacity of chemosensory neurons to encode concentration. For instance, adaptation to 2 Hz stimuli renders lobster hydroxyproline cells incapable of discriminating concentrations varying over three orders of magnitude (Gomez et al., 1999). The large variation in odorant burst intensity suggests that intense spikes may arrive at sensors, to be followed later by less concentrated odorant stimulation (even if the animal is moving upstream). Adaptation would clearly diminish the capacity of neurons to accurately code stimulus concentration under these conditions, rendering simple threshold responses more effective.

We obtained clear relationships between crab velocity changes and odorant spike encounters by using thresholds that were scaled to the mean of the instantaneous maximum concentration (near the antennules) that a given crab experiences (i.e. $\bar{C}_{\mathrm{Amax}}$). The use of a relative concentration threshold rule is certainly intuitively appealing given the large concentration range experienced by crabs within and across a given tracking event (Fig. 10). Additionally, the universality of adaptation as an important determinate of sensory neuron responses [e.g. spider mechanoreceptors (Juusola and French, 1998), mice olfactory receptors (Boccaccio et al., 2006), vertebrate auditory hair cells (Hudspeth et al., 2000) and vertebrate photoreceptors (Fain et al., 1996)] has been taken as evidence that sensory systems encode relative rather than absolute changes. Nevertheless, behavioral evidence for this phenomenon is rare when considering the task of chemosensory tracking, where it has been difficult to either precisely manipulate the signals presented to tracking animals or quantitatively relate them to animal movements.

Our use of a relative threshold is consistent with previous attempts to determine proportional differences [e.g. just noticeable differences (JNDs) or Weber fractions] that elicit behavior, although we know of no evidence that defines this for any olfactory tracking event. The employed threshold yields a $\bar{C}_{\mathrm{Amax}}$ value, calculated by including only suprathreshold peak values, that is greater than the value for the entire track by a factor of 1.5 to 3.2 (i.e. $10^{0.2}$–$10^{0.5}$). This provides a rough estimate of JNDs during tracking and yields a two- to three-fold difference. This estimate is similar to the JND that evokes feeding behavior of lobsters, but is considerably larger than the JND that evokes flicking (2–8%) (Zimmer-Faust, 1991). It may be that the large variability of turbulent plumes renders small JNDs strategically unsound and physiologically difficult to encode. Such small differences are well within the variation experienced by foragers in our plumes (e.g. Figs 3 and 10). Intermittent, large-concentration odorant spikes may result in neural adaptation such that lower concentrations cannot be detected, or pulses may be so brief as to disallow such small differences above background to be discerned.

Behavioral relevance of signal intermittency

Signal intermittence appears to be a key feature that explains the success of plume navigation. Previous studies in both aquatic and terrestrial arthropods have shown decreased tracking success in more homogeneous turbulent plumes (Weissburg and Zimmer-Faust, 1993; Willis and Avondet, 2005; Jackson et al., 2007), or those with experimentally reduced pulsed frequency (e.g. Mafra-Neto and Cardé, 1995a; Keller and Weissburg, 2004). However, concentration and flux tend to vary with intermittence in these situations, so the causal relationship is unclear.

Elegant experiments by Vickers and Baker (Vickers and Baker, 1994) recorded electroantennograms of moths in free flight, and showed that contact with a single strand of pheromone results in an upwind surge, thus providing a plausible mechanism that relates the response to individual odorant stands to the behavior observed in plumes with differing frequency of filament arrival (see also Mafra-Neto and Cardé, 1994). Using a direct behavioral-odorant visualization system similar to ours, Mead et al. showed that stomatopods encounter more odorant filaments (and more concentrated filaments) in wavy flows compared with unidirectional flows (Mead et al., 2003). However, they were unable to strongly relate odorant properties to search kinematics or success, neither of which differed in their two flow environments.

Our data indicate that upstream movement and odorant spike frequency are strongly related in aquatic arthropods. Odorant environments in which searchers encounter odorant spikes more consistently result in the most efficient and rapid progress towards the source (compare Figs 6–8 with Fig. 11). Crabs in the continuous plume had the fastest movement velocities and the shortest stop times, whereas crabs in plumes with increased inter-spike intervals showed longer search time periods (Fig. 6), in part due to increased stopping while tracking (Fig. 7). The stimulation frequencies required for blue crabs to sustain upstream progress are clearly less than their terrestrial counterparts, which move upwind only when odorant spike encounter frequencies are greater than 3–5 Hz (Vickers and Baker, 1994; Mafra-Neto and Cardé, 1998). This follows physiological evidence (Gomez et al., 1994) suggesting that differences in the fluid dynamic environment of odorant signal transport have resulted in slower processing of chemical signal information in aquatic *versus* terrestrial arthropods.

The relationship between the frequency of suprathreshold odorant spikes and overall progress to the source is again more explanatory than concentration differences. For instance, crabs in the continuous *versus* meandering plumes experienced largely the same $\overline{C}_{\mathrm{Amax}}$ (for total path; Fig. 9), but crabs in the continuous plume moved more quickly and stopped less, which matches the difference in odorant spike arrival periods. Similarly for crabs in pulsed *versus* meandering plumes, there was a large difference between odorant spike concentration (for total path; Fig. 9), but a minimal difference for odorant spike arrival periods (for total path; Fig. 11). These crabs had much more similar behavior (at least overall and farther than 50 cm from the source) than would be expected if spike concentration was a crucial governing property.

Crabs can accelerate within 0.25 s of encountering individual odorant spikes. As in insects, the response to a single odorant spike constitutes a template for upstream surges, and consistent progress requires repeated contact. The response time periods displayed by blue crabs are similar to those of other organisms. For instance, *Drosophila melanogaster* tracking in odorant plumes reorient their flight trajectory within 250 ms of plume contact (Budick and Dickinson, 2006). Further, moths turn upwind and increase velocity within 250 ms of encountering pheromone filaments in plumes (Vickers and Baker, 1994; Mafra-Neto and Cardé, 1996). Interestingly, similar to moths, we found no evidence that the reception of individual odorant spikes causes turning as well as increasing velocity. Heading angles and angular velocity immediately before and after odorant spikes were statistically indistinguishable [data not shown, but see Page et al. (Page et al., 2011)]. This adds further evidence that turning and upstream movement are largely decoupled in blue crabs, possibly because their large size allows them to use spatially distributed sensing to orient to the plume (Page et al., 2011). Such a system is probably not possible in moths, given their small size relative to the odorant plume width (Weissburg, 2000).

The latency between odorant spike contact and accelerations is less than the time period required to turn in response to perceived odorant asymmetry. Blue crabs appear to make turning decisions with latencies of ~2 s (Dickman et al., 2009; Page, 2009), which is similar to the 2–4 s lag time between the arrival of bilaterally asymmetric stimuli and subsequent turning in lobsters (Atema, 1996). The difference between lag times of tenths of a second for stimuli producing upstream surges and lag times of seconds for turning may reflect a number of neurological processes. Information mediating upstream motion simply may be processed faster because, in contrast to signals mediating turning, stimuli mediating upstream movement do not require spatial integration of multiple signals. Additionally, crabs may react to antennule stimulation more quickly because along-stream movement is coupled with information from the mechanosensors, providing clear information about source directionality.

Our data clearly demonstrated that signal intermittence is a key feature mediating both upstream progress and cessation of movement; crabs are more likely to decelerate when they encounter more frequent odorant spikes (i.e. smaller inter-spike intervals; Fig. 15). We suggest this apparent contradiction reflects two distinct aspects of the navigational strategy: the requirement for a minimum odorant spike encounter rate and the use of particularly large odorant spike arrival frequency as an indication of closeness to the source. A minimal contact rate would conceivably reflect the time period of the decay of upstream surges in response to stimulation. As the inter-spike interval increases, the time period that crabs spend accelerating in response to odorant spikes would diminish, resulting in decreased velocity. In contrast, it has been suggested that sensory adaptation or increased inhibitory inputs in homogeneous plumes (or plume regions) suppress movement (Baker et al., 1988; Baker and Haynes, 1989). However, evidence for this conclusion is based on experiments where sensory neurons were stimulated with pulse properties whose relationship to actual pheromone clouds is unclear, and neuronal adaptation (and in-flight arrestment) under the experimental conditions was more strongly related to source concentration than pulse parameters. Our data show that reduction and cessation of movement of navigating crabs is clearly related to odorant pulse arrival rate rather than concentration. The increasing likelihood of large odorant spike arrival rates close to the source suggests the suitability of this mechanism as an indicator of near-source proximity.

The role of stimulus history and state-dependent responses

The response to a given stimulus may be affected by the previous stimuli encountered because of a variety of neural responses such as adaptation, inhibition and habituation. This is a particularly vexing aspect to examine in odorant-mediated navigation given the difficulty in either manipulating stimuli or observing organismal responses to known stimulus patterns. With the exception of endogenous counter-turning, most accounts of odor-mediated navigation have not incorporated the role of history or state dependency despite its potential importance. The need for, and success in, applying the per crab mean maximal concentration signal ($\overline{C}_{\mathrm{Amax}}$) to construct the threshold rule is strongly indicative of the need to incorporate state-dependent responses; an absolute threshold would be difficult to apply given the large differences between crabs. As discussed below, there are other behavioral observations that are difficult to explain without hypothesizing state or history dependency.

Our data suggest several incidences where responses to specific stimuli are affected by previous stimulus patterns or prior state. First, crabs that recieved prior odorant spikes attained elevated velocity more quickly in response to subsequent spikes (Fig. 13). Second, the prior state of the crab (acceleration *versus* deceleration) also had a bearing on how quickly the crabs reacted to a particular odorant spike. Crabs that were accelerating immediately prior to receiving an odorant spike accelerated on average more quickly than crabs that were previously decelerating (Fig. 14). Given that even crabs decelerating prior to receiving an odorant spike were capable of accelerating within 0.25 s of receiving stimuli, these data suggest that the post-spike acceleration patterns of blue crabs are an effect of signal processing strategies and not simply a time lag due to a motor effect.

Our interpretation of these two observations is that they are representative of a neural processing scheme that weights subsequent stimuli more heavily when coupled to previous stimuli. If crabs receiving previous odorant spikes accelerate more rapidly upon encountering a subsequent spike, then they will reach high velocity more quickly and sustain it for a longer period, decreasing the time period spent searching. As noted, crabs in plumes with higher rates of spike encounter progressed more rapidly to the source and spent the least amount of time stopped, suggesting the importance of these state- and history-dependent responses. Further, the hypothesized mechanisms have obvious utility given the physical aspects of turbulent odorant plumes. Odorant spike encounters are more frequent close to the plume centerline (Jackson et al., 2007; Moore and Atema, 1991); thus, more frequent stimuli indicate a higher probability of being within the plume and justify giving more weight to odorant spikes that follow a series of previous ones. An isolated spike may be indicative of being off-axis, which suggests that rapid upstream movements would reduce the likelihood of subsequent contact as plume width narrows towards the source. Strategically, it makes sense to acquire more information when presented with such isolated stimuli, and the neural processing underlying olfactory navigation seems constructed to produce such behaviors.

Similar state dependencies may have been observed in moths where animal responses in pheromone plumes were recorded simultaneously with recordings of pheromone-induced electrical activity of the antennae (Vickers and Baker, 1994). A moth early in the flight track received a series of pheromone odorant spikes (detected by the electrical activity of the antennae), but these bursts failed to elicit sustained upwind motion and the moth began to cast. After a period in which the moth was flying consistently upstream, another series of bursts with roughly the same temporal profile elicited a sustained upwind surge [see fig. 4A in Vickers and Baker (Vickers and Baker, 1994)]. Although anecdotal, these observations suggest the need to incorporate state dependency into our models governing the relationship of odorant stimulation to upstream movement and reinforce the value of quantitative, simultaneous records of stimulus patterns and responses.

A third example of state or history dependency in our data is the relationship between upstream progression and odorant spike encounter rate. As noted, despite a general relationship between upstream progress and small inter-spike arrival time periods, crabs are more likely to decelerate when odorant spikes occur particularly close together (Fig. 15). Interestingly, the inter-spike intervals eliciting accelerations *versus* decelerations/stopping are scaled to the prevailing plume dynamics. The inter-spike intervals at which crabs accelerate are rather close to the mean interval for the respective plume type, increasing in the plume types where odorant spikes arrive less frequently (compare Figs 11 and 15). The inter-spike interval characteristic of deceleration/stopping increases (odorant arrival rates decrease) in plumes with lower arrival rates; that is, the definition of what constitutes a sufficiently high rate of odorant simulation is context dependent.

One implication of the changes in these behaviorally relevant arrival rates across plume types is that crabs must have some mechanism to encode odorant-spike intervals or encounter rates and use this information as a way to achieve a reference point. Crabs in our experiments encountered the odorant plume for several minutes before they were released, and naturally foraging crabs can pause for short periods, at least, before they begin what seems like tracking (M.J.W., personal observation). Thus, it may be that crabs acquire such information through time sampling. In addition (or alternatively), there may be multiple independent pathways, each of which constitutes a discrete signal providing independent estimates of odorant-spike arrival. A potential complicating factor is that estimates of closely spaced sensors will not be truly independent (i.e. they may be sampling the same odorant filament), and there may be a limit to how much information is obtainable in this way.

Complementarity and redundancy in sensory systems

Sensory systems are remarkable in that they can serve multiple functions and interact in a complementary manner, thereby providing a mechanism of redundancy that is useful if one or more sensory system is impaired. Removing input from particular chemosensor populations can suggest which sensors are sufficient, or necessary, to evoke a particular task (Derby and Atema, 1982; Keller et al., 2003; Horner et al., 2004), but sheds little light on how different chemosensory populations interact during the performance of a specific task. The results of these deafferentation experiments have often been interpreted to be the result of providing for equivalent functions in case a particular population is damaged (e.g. Horner et al., 2004), but our data suggest that redundancy may not be the only, or even the major, contributing factor.

Previous studies suggest that olfactory search for food in blue crabs and lobsters is not contingent on input from aesthetastc sensors on the antennules. Animals in which these inputs have been removed can still find distant food sources by navigating through turbulent plumes, although their locomotory efficiency is reduced relative to the intact animal (Horner et al., 2004). Blue crabs lacking antennular input show deficits in upstream movement, which suggests that antennular input mediates upstream locomotion (Keller et al., 2003). Although the present data confirm the hypothesis that antenullar input mediates upstream motion, blue crabs also appear to employ input from receptors on the walking legs to move upstream. Interestingly, Aggio and de Freitas demonstrated that chemical stimuli (taurine) delivered to the dactyls, walking legs or swimmerettes of *Callinectes danae* induced these crabs to move as if to grab something in front of them or begin walking, whereas the same stimuli delivered to the antennules did not evoke any movement (Aggio and de Freitas, 2007). This supports the idea that, at least under certain circumstances, stimuli arriving at the leg chemosensors can help to initiate and perhaps sustain motion. Despite the wide variety of systems in which chemically guided search is known, we are unable to identify any other systems in which the participation of multiple sensory populations is known to occur during normal behaviors in intact animals.

ACKNOWLEDGEMENTS

The authors thank Kimberlee Berkenkamp for assistance with the crab trails. Thanks also to the National Science Foundation for financial support under grants IBN-0321444 and OCE-0424673 to M.J.W. and D.R.W. and for an NSF IGERT fellowship awarded to J.L.P.

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