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Effects of Odor Flux and Pulse Rate on Chemosensory Tracking in Turbulent Odor Plumes by the Blue Crab, *Callinectes sapidus*

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Abstract. The ability of animals to track through chemical plumes is often related to properties of evanescent odor bursts and to small-scale mixing process that determine burst properties. However, odor plumes contain variation over a range of scales, and little is known about how variation in the properties of the odor signal on the scale of one to several seconds affects foraging performance. We examined how flux and pulse rate interact to modulate the search behavior of blue crabs, *Callinectes sapidus*, locating odor sources in controlled flume flows. Experimental treatments consisted of continuous plumes and plumes with discrete odor pulses at intervals of 2.5 s and 4 s at two fluxes. Crabs experienced diminished search success and reduced search efficiency as flux decreased and the inter-pulse interval lengthened. There often were significant interactions between flux and pulse length, and neither property completely determined search behavior. Thus, over the time span of several seconds, the blue crab chemosensory system is not a simple flux detector. The sensitivity of blue crabs to inter-pulse intervals in the range of several seconds indicates that larger-scale mixing processes, which create odor variation on comparable scales, may exert a significant impact on foraging success in nature.

Introduction

Many aquatic and terrestrial animals orient to fluid-borne chemical plumes to locate predators, prey, mates, or dwelling sites. A common sensory strategy of animals in these environments is to use fine-scale features of odor plumes to extract information on distance and direction (Moore and Atema, 1991; Mafra-Neto and Cardé, 1994; Vickers and Baker, 1994). These plume features, variously termed odor filaments, pulses, or bursts, are the result of turbulent mixing processes that distribute chemicals within the plume. Accordingly, odor stimulation in plumes is evanescent: although turbulent mixing may result in temporal variation in stimulus intensity over a variety of scales, odor filaments typically last less than 1 s and commonly may be less than 200–500 ms (Murlis *et al.*, 1992; Moore *et al.*, 1994; Crimaldi and Koseff, 2001; Webster and Weissburg, 2001).

The time scale of odor fluctuations may not be the same time scale that characterizes either the neural coding of chemical signals or the behavioral response, for several reasons. The physical and molecular events underlying the transduction process set limits on neuronal response times. For example, sensory neurons in crustaceans and insects cannot respond distinctly to odor pulses presented at frequencies exceeding 4 and 10 Hz, respectively (Kaissling *et al.*, 1987; Gomez *et al.*, 1994). Even when sensory systems may be able to encode information rapidly, averaging or integrating over longer periods may increase the signal-to-noise ratio and result in more accurate depiction of stimulus properties. Indeed, temporal averaging is necessary for navigational strategies that rely on mean properties of odor plumes, since stochastic variation in signal intensity in turbulent plumes reduces the accuracy of rapid assessments of local mean concentration (Webster and Weissburg, 2001).

Crustaceans such as crabs and lobsters clearly rely, at least in part, on brief odor pulses as they navigate through turbulent plumes (Moore and Atema, 1991; Weissburg and Zimmer-Faust, 1994; Mead, 2002). However, animals may alter behavior in response to larger-scale flow structures if they integrate information over time scales (*i.e.*, seconds)

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corresponding to the period over which these structures cause signal variation. Unfortunately, there is little information on how blue crabs or other crustaceans respond to odor signal variation over time scales of one to several seconds. Such information is essential for a meaningful analysis of plume tracking in nature, where large-scale flow structures (*i.e.,* eddies) may interact with small-scale turbulence to produce odor fluctuations on time scales ranging from milliseconds to minutes (Atema, 1995; Weissburg, 2000; Webster and Weissburg, 2001). Thus, this study examined the navigation of blue crabs (*Callinectes sapidus*) in turbulent flow in response to artificially pulsed odor plumes. Pulse rate and flux were varied independently to examine whether crab responses to pulsed plumes varied with the general level of stimulus intensity. The results indicate that flux and pulse rate interact to determine crab foraging behavior, suggesting that small-scale turbulent features are not the only determinants of olfactory-mediated foraging ability.

Methods

Animals

Male and female blue crabs (*Callinectes sapidus* Rathbun, 1896) were collected, using baited traps (Gulf Specimen Supplies), from habitats adjacent to Dickson Bay in Panacea, Florida (30°00′ N, 84°22′ W). Crabs were shipped to Atlanta, Georgia, kept in communal tanks filled with artificial seawater (ASW; Instant Ocean, 33 ppt, 20 °C), and tested within 20 d of collection. Animals were maintained on a cycle of 12 h light and 12 h dark, and fed freshly thawed shrimp *ad libitum* every other day. We withheld food from the crabs about 12 h before testing to ensure that they were not satiated and to standardize the time of last access to food.

The flow and stimulus environment

We characterized blue crab search behavior and hydrodynamics in an indoor, recirculating flume (12.5-m long × 0.75-m wide; total capacity about 3200 gal, or roughly 12,113 l) lined with sand to provide a natural substrate. The working section where foraging trials took place begins about 10 m downstream of the flume entrance. This facility has been described previously, and results in reproducible, realistic, and well-defined flow environments (Keller *et al.,* 2003). Average flow velocity was maintained at 4.9 cm s^{-1} ± 0.08 (mean ± SD) with a water depth of 23.0 cm ± 0.348 (mean ± SD). The vertical velocity profile of our flows showed a good fit to the Law-of-the-Wall equation (Keller *et al.,* 2003), which specifies the expected relationship between flow velocity and height above the substrate in an equilibrium boundary layer (Weissburg, 2000). At this flow speed, the boundary layer shear velocity, u^*, a measure of stress due to turbulent velocity fluctuations (Denny, 1988), was 3.1 mm s^{-1}, calculated using the Law-of-the-Wall (Weissburg and Zimmer-Faust, 1993; Keller *et al.,* 2003). This value conforms well to expectations for turbulence in open channel flows. The Roughness Reynolds number (Re*), which measures the penetration of turbulent eddies into the boundary layer, was 2.65, which suggests smooth flow (Denny, 1988). These hydrodynamic conditions during behavior trials are well within the range reported for blue crab habitats in the field (*e.g.,* Zimmer-Faust *et al.,* 1995). Trials took place between about 1400 and 1700. Light levels were lowered during trials to minimize visual cues during navigation and to reproduce the low light levels corresponding to early morning and evening periods when foraging activity peaks in the field (Clarke *et al.,* 1999).

Chemical stimuli consisted of a solution prepared by soaking whole, intact (previously frozen) shrimp in ASW drawn from the flume. Shrimp were soaked in ASW for 1 h at a concentration of either 3.5 or 7.0 gm · l^{-1}, and the solutions were prepared immediately before behavioral trials. This odorant solution was released parallel to the flow 2.5 cm above the bed from a 4.7-mm-diameter brass nozzle with a fairing to minimize the flow perturbation.

Dual inputs into the nozzle accommodated an odorless ASW source and the odorant solution. The use of two separate flows allowed us to alternate between odorless and odor-containing solutions to introduce odor pulses into the flume channel. The two flow streams converged at the horizontal arm of the L-shaped nozzle (about 2 in. long), and each was equipped with an in-line flowmeter so that the velocities could be independently controlled. Each flow stream passed through a three-way solenoid valve, and both valves were controlled by a valve driver such that one valve was on (*i.e.,* diverting the flow into the nozzle) when the other was off (diverting the flow into a waste reservoir). A pulse generator (AM Systems) provided the timing signal to the valve driver to control the on-and-off times. Air pressure imparted the necessary force to drive the fluid through the system.

Experiments were run using three patterns of stimulus release for each of the two odorant concentrations. In addition to a continuous odor plume, we also generated pulsed plumes of two types. In the first condition, 2.5 s of odorant release alternated with 2.5 s of odorless ASW. The second condition consisted of alternating 1-s odor pulses and 4-s intervals of odorless ASW. Initial flow visualization experiments suggested that it was difficult to produce coherent pulses using on-or-off times of less than 1 s. The flow rate for the odorless ASW was set to 60 ml min^{-1} to ensure cleanly separated odor pulses. The flow rate for the odorant solution was systematically adjusted to equalize the volume of odorant solution released in each of the three plume types. Release rate for the continuous case was 6 ml · min^{-1}, which corresponded to isokinetic release, as flow

velocity 2.5 cm above the bed is approximately 0.5 cm s^{-1} (Keller *et al.*, 2003). The 2.5-s and 1-s odor pulses were released at rates of 12 and 30 ml min^{-1}, respectively. Since experiments took place in the same water flow speed, a sensor of constant area would experience the same number of stimulus molecules delivered to its surface over the 5-s cycle length for plumes created with solutions of a given odorant concentration. Our manipulations using different pulse lengths and stimulus concentrations therefore decouple the temporal pattern of stimulation from rate of odorant delivery (flux) to crab chemosensors.

Flow visualization

We performed a series of qualitative flow visualizations to roughly characterize the plume conditions. Our methods were designed only to ascertain that pulsing the source results in discrete odor signals that propagate downstream, and are similar to qualitative methods employed in other studies (*e.g.*, Belanger and Willis, 1996); a full analysis of pulse properties requires methods, such as planar laser-induced fluorescence (Webster and Weissburg, 2001), that go well beyond that needed here. Initial visualizations suggested that coherent pulses generated at the nozzle orifice were disrupted as they moved downstream but maintained cohesion for at least 100 cm, setting an approximate (and probably conservative) boundary for pulse coherence. Thus, we photographed the plume as it evolved for roughly 15 cm beginning at a distance of about 85 cm downstream of the nozzle. Pictures of the plume as viewed from above were taken with a digital camera mounted 0.5 m above the middle of the working section, yielding a visualized area of approximately 15 × 10 cm (l × w). A 0.1% fluorescein solution prepared in flume water was illuminated with a 2-cm-thick horizontal light sheet produced by a slide projector and focused on the plume, and photographed against a black sand background. The three release conditions (continuous, 2.5-s pulse, and 1-s pulse) were replicated using the same delivery and flow parameters as in the behavioral trials.

The digital images were converted to high-resolution (1000 × 1500 pixel) jpeg files, then converted to grayscale and inverted using Adobe Photoshop. We selected a random subsection away from the plume (free of visible odor filaments) in each image and used this to determine the background pixel intensity, which then was subtracted from each image. Images were analyzed using the public domain NIH Image program (ver. 4.02; available at http://rsb.info.nih.gov/nih-image/) to produce a frequency histogram of pixel intensity values for each image. An average histogram for each plume condition was constructed from five images, each taken from a separate visualization.

Behavioral experiments

The ability of blue crabs to locate the odor source by navigating through the chemical plumes was tested in the flume, using methods similar to those previously described (Keller *et al.*, 2003). Briefly, blue crabs were carefully moved to the flume and placed in a flow-through acrylic plastic box (27.2-cm long, 19.5-cm wide, 16.5-cm high). Animals were acclimated in the box for 13 min prior to the release of odorant solution from a source located 1.5 m directly upstream of the center of the acrylic box. The front door was raised 2 min later. Trials lasted for a maximum of 15 min and were terminated if the animal successfully found the source and attempted to grab it, or moved either upstream of the source or downstream of the acrylic box. Trials were also terminated if the animal failed to leave the box within 5 min. Release rate and stimulus concentration were varied daily, with three or four trials on any given day. Consequently, no more than 1 l of the stimulus solution was released into the flume, which minimized accumulation of shrimp metabolites. Flume water was filtered through 5-μm particulate filters, activated carbon, nitrate- and phosphate-absorbing media, and UV sterilizers two or three times per week for a period of 12–18 h.

Crab behavior was recorded on videotape using a low-light-sensitive CCD camera mounted about 2 m above the working section of the flume. A watertight backpack containing two red-light-emitting diodes powered by a watch battery was attached to the carapace of each blue crab before behavioral experiments. We assayed the motivational state of animals that failed to find the source by placing them in a small tank (28 cm dia. containing 5 l of flume water) and offering them a single shrimp. Blue crabs that failed to respond to this food within 5 min were designated as unresponsive and were omitted from subsequent analysis. Similar to observations by Weissburg and Zimmer-Faust (1993), the percentage of unresponsive crabs ranged from about 11% to 19%, with no systematic variation across treatments. Each crab was tested only once, for a total of over 150 trials (Table 1) used for behavioral and kinematic analysis.

Table 1

Number of successful and unsuccessful searches as a function of pulse length and source concentration

		Pulse length (seconds)		
Flux*	Search outcome	1	2.5	Continuous
High	Successful	9	11	12
	Unsuccessful	8	10	6
Low	Successful	11	10	10
	Unsuccessful	26	26	13

* High and low flux rates correspond to plumes produced with odorant solutions of 7 and 3.5 g shrimp · l^{-1}, respectively.

The *x,y* coordinates of the centroid of each light-emitting diode were determined using Motion Analysis software (30 Hz; ver. 3.1; Motion Analysis Corp., Santa Rasa, CA), smoothed using a moving average algorithm (window size = 3 frames), and extracted to produce a 5-Hz time series. The tracks of successful and unsuccessful searches were used to calculate a variety of kinematic parameters of foraging crabs, including walking speed, turning angle, and net-to-gross displacement ratio (NGDR, a measure of path tortuosity; Weissburg and Zimmer-Faust, 1993).

Statistical analysis

Data on crab foraging success in various plume conditions were analyzed using log-likelihood methods for frequency analysis (Sokal and Rohlf, 1981). These methods use maximum likelihood techniques to establish the significance of treatment variables (and their interactions) for determining the frequency of observations in each table cell by attempting to express the logarithm of expected cell frequencies as a linear function of experimental parameters. To examine the influence of pulse length, flux, and their interaction on foraging success, we used LOGLIN, a log-linear analysis procedure for multiway frequency tables that is available in the SYSTAT software package, ver. 10.2 (SYSTAT Software Inc., Richmond, CA).

Analysis of variance (ANOVA) was used to examine the influence of pulse length, flux, and their interaction in determining kinematic parameters for animals that were clearly engaged in olfactory navigation (*i.e.,* successful searchers). Calculation of kinematic variables (speed, NGDR, turning angle, and time spent motionless) was based on the entire path of a given successful or unsuccessful forager. The Bonferroni method was used to establish significance values for multiple *post hoc* tests (Sokal and Rohlf, 1981) to permit comparisons across the three pulse treatments at each flux rate. We performed a similar analysis on unsuccessful searchers, but the ANOVA suggested that plume properties had no impact on behavior (see Results). Finally, we used multiple analysis of variance (MANOVA) to compare overall kinematic behavior of unsuccessful and successful foragers. In this analysis, data were pooled across pulse and flux conditions for each state of search success (see Results).

Results

Flow visualization

Qualitative flow visualization revealed that our system generated pulsed plumes that differed substantially from the continuous case, and documented small differences in spatial variability between plumes generated with 1-s *versus* 2.5-s pulses (Figs. 1, 2). Plumes created with both pulse times were composed of highly intermittent odor bursts interspersed with clean water. The continuous plume was more homogeneous than either of the two pulsed cases, although it still exhibited considerable fine-scale structure that is a general hallmark of plumes created by sources with slow release rates relative to the ambient flow (Webster and Weissburg, 2001). The concentration records along the plume centerline reinforce the perceptions gained from examining the images. The continuous plume showed some variation but had relatively long stretches with moderate dye concentration. In contrast, dye concentration in the pulsed plumes was either extremely high or undetectable. Peak-to-peak distances along the plume centerline ranged between 8 and 15 cm in pulsed plumes, which at a relative flow velocity of 5 cm s^{-1}, produced peak-to-peak intervals of approximately 1.6–3 s. The intermittency factor, defined as the total proportion of the field of view above the detection limit (*i.e.,* with a pixel intensity greater than 0; Chatwin and Sullivan, 1989), was 0.557 ± 0.23, 0.391 ± 0.19, and 0.139 ± 0.24 (mean ± std err), for continuous, 2.5-s, and 1-s pulses, respectively, indicating more signal variability in pulsed plumes as a result of highly coherent dye patches. However, pulsed plumes were more variable even when the effects of signal absence (no detectable dye) were removed. Frequency histograms of dye intensity that include only non-zero pixel values (Fig. 2) indicated that low dye concentrations occurred often in pulsed plumes. In contrast, continuous plumes had a more even distribution of intensity values, a lower frequency of dilute dye, and more frequent cases of intermediate concentration. Thus, continuous cases represented fairly constant stimulation, whereas pulsed plumes represented odor environments in which odor stimulation was highly intermittent, on the scale of one to several seconds. Plumes with the shortest pulses were the most intermittent in space, and therefore would be expected to show the greatest temporal variability as the plume is advected downstream.

Behavior

Success rate. Flux and release properties affected the ability of foraging crabs to navigate through the plume to the source (Table 1). Groups of crabs challenged with various plume types located the source at rates from 67% to 28% depending on the combination of pulse length and flux. In general, a larger flux rate (*i.e.,* plumes produced with the more concentrated stimulus solution) significantly increased the success rate (log-likelihood $\chi^2 = 21.35$, $P < 0.01$, df = 6) relative to the lower flux plumes. Pulsed plumes were less easily tracked by foraging crabs at both high and low fluxes; however, the effect of pulse on success rates was marginally insignificant (log-likelihood $\chi^2 = 13.87$, $P = 0.054$, df = 7). The decrement in foraging success when navigating through pulsed plumes was greater at the higher flux, but the interaction between pulse properties and flux was not

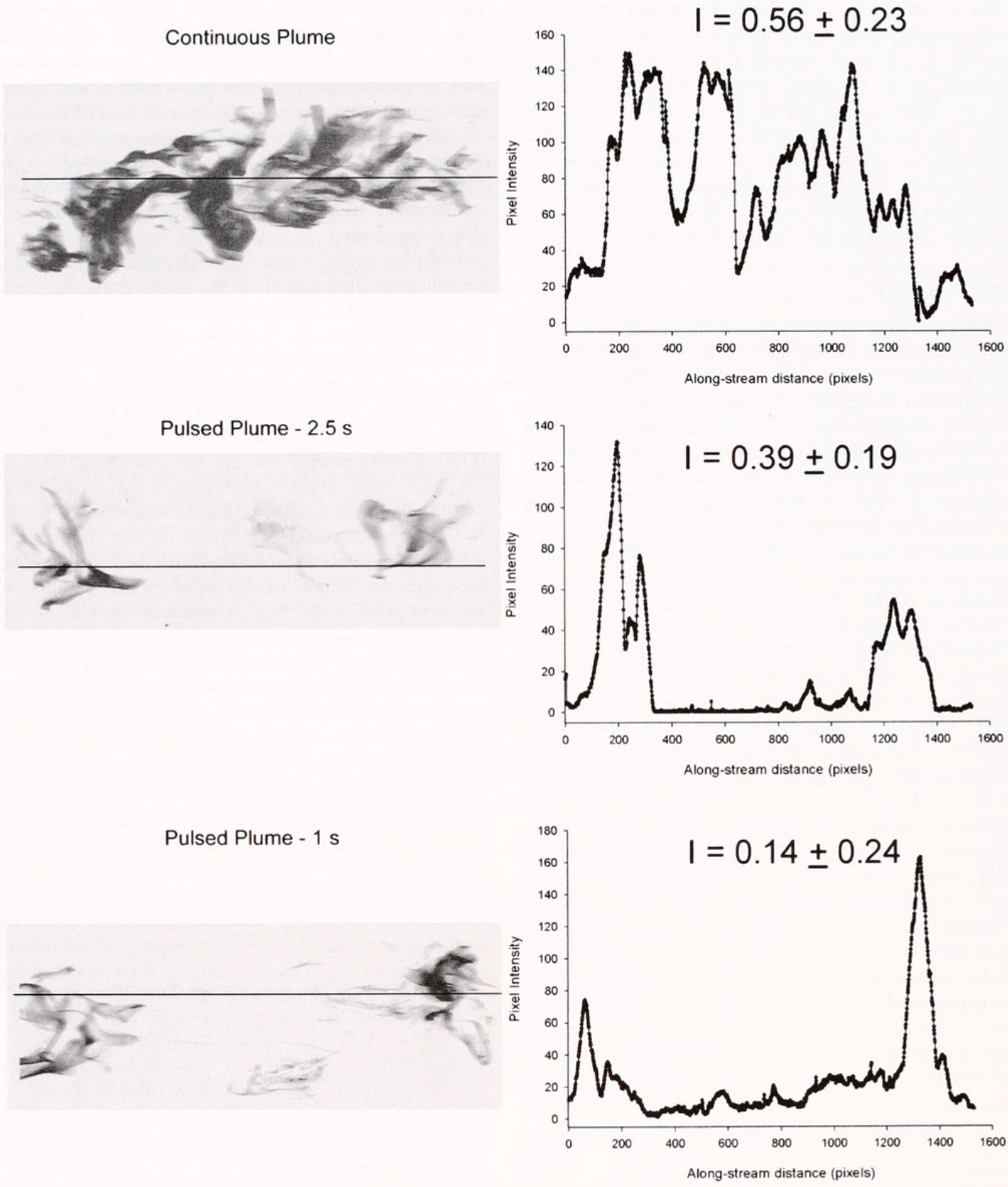

Figure 1. Characteristics for continuous and pulsed odor plumes: a side-by-side comparison of a representative plume image (left panel) and the distribution of pixel intensity values (right panel) along the plume centerline (solid line), for each of the three plume conditions. Pixel intensity values are derived from the digitally processed images, in which higher values indicate greater dye concentration. The intermittence, *I*, defined as the proportion of pixels in the image above the detection threshold (*i.e.*, with a pixel intensity greater than 0; Chatwin and Sullivan, 1989), is given above each graph of pixel intensity. The figure gives the mean *I* (± SEM) based on five images, for each plume type.

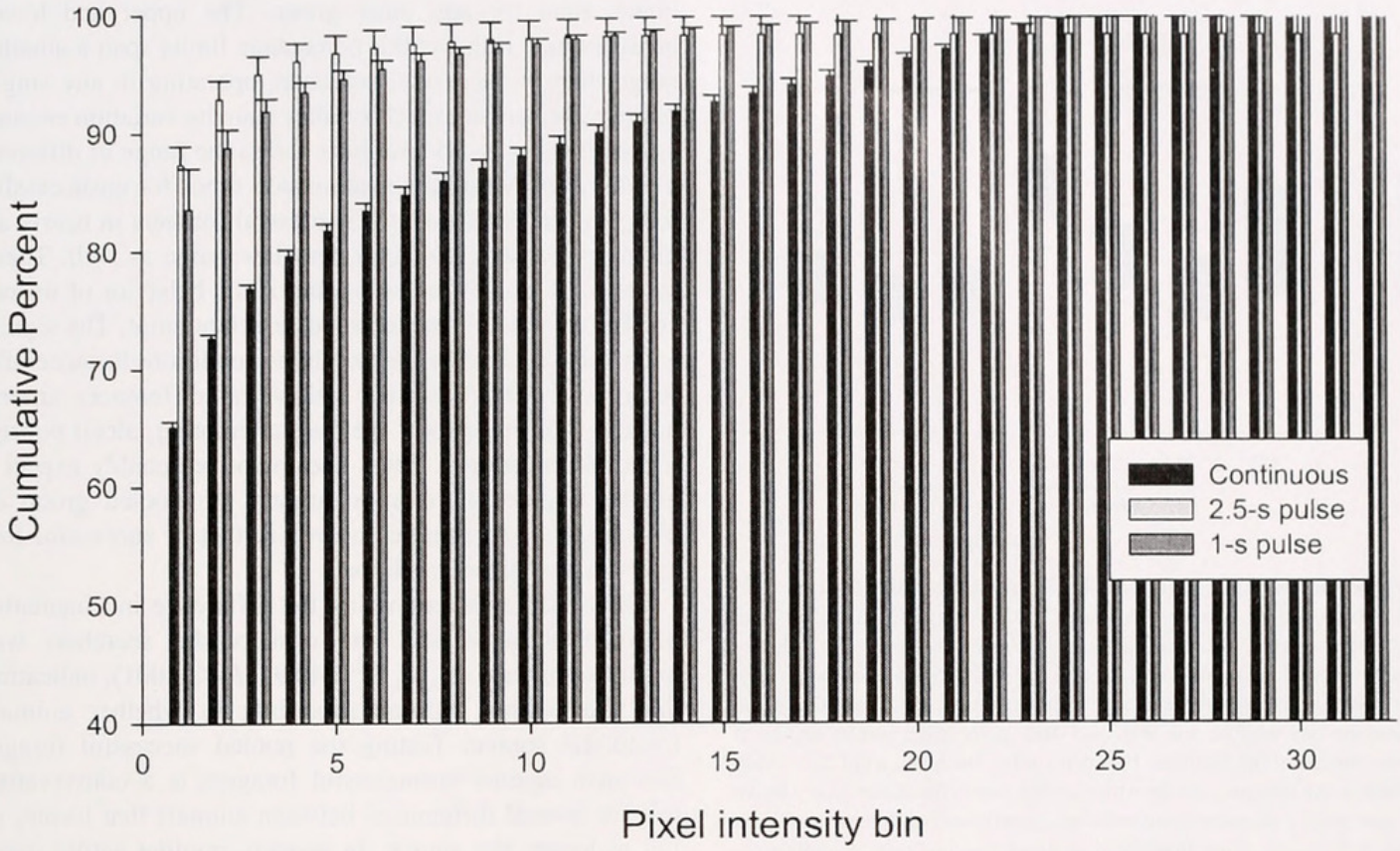

Figure 2. Distribution of pixel intensity values in continuous and pulsed plumes: the average cumulative frequency distribution (± 1 std err) as a function of pixel intensity bin for each plume type. Each bin corresponds to eight pixel intensity values, except for the first bin where the 0 pixel value (no detectable dye) has been omitted. The average frequencies are based on a sample size of five images for each plume type.

significant (pulse *concentration interaction $\chi^2 = 13.25$, $P = 0.066$, df = 7).

Search kinematics. Tracking behavior of crabs was qualitatively similar to that described in a variety of other studies on chemosensory navigation in aquatic crustaceans (Moore *et al.,* 1991; Weissburg and Zimmer-Faust, 1994; Mead, 2002). Animals moved toward the source, sometimes directly, but at other times using circuitous routes involving cross-stream tacks or meanders. Animals occasionally stopped for one to several seconds enroute to the source, but long periods of motionlessness were rare; crabs generally moved consistently upstream until they reached the nozzle and grabbed it with their chelae. Release conditions significantly affected crab foraging behavior (see below). In contrast, the behavior of unsuccessful foragers was remarkably constant and lacked detectable differences in any kinematic parameter (speed, NGDR, stop time, body angle) as a function of pulse length or flux. In fact, the majority of P values for these tests were greater than 0.5, and only one was less than 0.2. Thus, we combined the paths of all unsuccessful foragers for presentation and analysis.

Behavior of successful searchers. ANOVA revealed that flux, pulse length, and their interaction all significantly affected tracking speed for successful searchers ($F_{1,53} = 26.77$, $F_{2,53} = 17.00$, $F_{2,53} = 21.66$, respectively; $P < 0.001$ for all cases). Crabs locomoted extremely quickly under maximally stimulatory conditions (continuous plumes at high fluxes), displaying mean movement rates of approximately 10 cm s^{-1}, which exceeds the mean speed of unsuccessful foragers (Fig. 3). Crabs tracking plumes in most other conditions moved at rates of 4–6 cm s^{-1}, somewhat less than those of unsuccessful foragers. More consistent odor stimulation produced higher mean speeds only at high fluxes, whereas mean speed was similar in pulsed *versus* continuous plumes at low fluxes.

Low rates of movement in particular stimulus-release conditions were associated with increases in motionless periods (Fig. 4). Animals in high-flux plumes rarely paused during odor tracking and even at the shortest pulse length stopped for total periods of less than 5 s. Motionless periods exceeded 5 s in all low-flux plumes, and crabs remained stationary for over 20 s in plumes with short pulse lengths (long inter-pulse intervals). Flux, pulse length, and their interaction all had significant effects on the duration of motionless periods ($F_{1,53} = 12.86$, $P < 0.001$; $F_{2,53} = 23.12$, $P < 0.001$; $F_{2,53} = 4.76$, $P < 0.05$, respectively). The trend for increased motionlessness with decreasing pulse length was nonsignificant at high flux rates, although

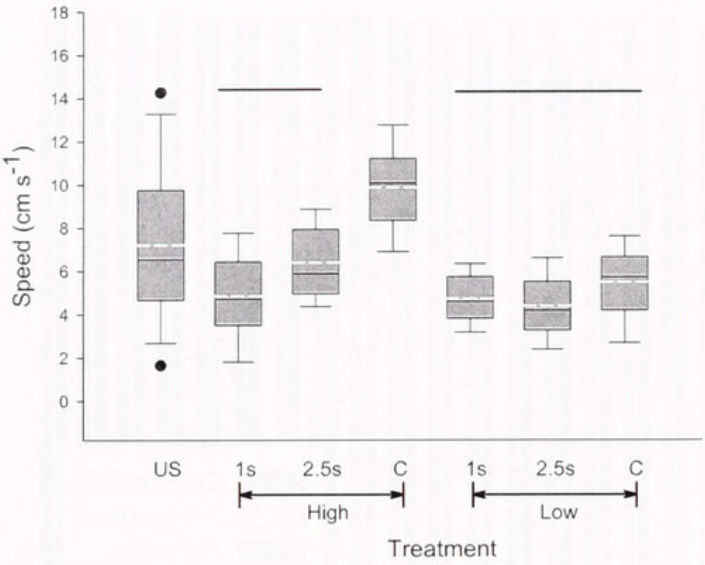

Figure 3. Walking speed statistics for all unsuccessful foragers (US) and for successful foragers in plumes with 1-s and 2.5-s pulses, and continuous plumes, for high-flux and low-flux treatments (*i.e.*, plumes created with odorant solutions of 7 and 3.5 g shrimp · l^{-1}, respectively). The gray box encloses the 25th and 75th percentiles, error bars above and below the box enclose the 90th and 10th percentiles, circles represent points outside of the 90th and 10th percentiles, the black solid line within the box is the median, and the white dashed line is the mean. Lines above the box plots join pulse treatments not significantly different from each other at $P < 0.05$ using Bonferroni adjusted *post hoc* tests. Sample sizes (numbers of paths) for these groups are given in Table 1.

post hoc tests detected significant differences among some treatment groups. The 1-s pulse length induced significantly greater motionlessness in low-flux plumes relative to the other two pulse treatments.

Path linearity varied significantly as a function of pulse length ($F_{2,53} = 5.97$, $P < 0.01$) and the flux rate–pulse length interaction ($F_{2,53} = 3.57$, $P < 0.05$), although there was no significant effect of flux itself ($F_{2,53} = 5.97$, $P < 0.01$). The overall effect of these factors was uniformly moderate path linearity at low fluxes, and moderate to high path linearity in high fluxes, with paths displaying significantly more meander at the shortest pulse length (Fig. 5).

Behavior of successful versus *unsuccessful searchers.* The behavior of unsuccessful searchers occasionally resembled that of successful crab foragers when individual kinematic parameters were examined. For instance, mean speed or stop times for unsuccessful searchers are in the middle of the range displayed by successful searchers. However, it is intriguing to note that even when mean values are similar between these groups, crabs failing to locate the source displayed more uniform behavior. For most kinematic parameters, both the median and the range (expressed as quartiles or percentage limits) indicate that crabs failing to locate the source moved more uniformly than did successful searchers. This is typified by data for path linearity and body angle (Figs. 5, 6), and to a lesser extent by mean stop time (Fig. 4). Median values for both NGDR and body angle are higher than for any other group. The upper and lower quartiles and 10th to 90th percentage limits span a smaller range than in successful searchers operating in any single plume type, and are much smaller than the variation encompassed by successful searchers across the range of different plume types. Mean and median stop times for unsuccessful foragers are lower than for successful foragers in nearly all plume types, and display a very low range as well. These patterns are somewhat surprising if the behavior of unsuccessful animals reflects a failed search attempt. The significant behavioral responses to plume conditions in successful foragers argue for similar behavioral differences among unsuccessful foragers if they are attempting, albeit poorly, to locate the source. Thus, one might reasonably expect a greater degree of variation among the pooled group of unsuccessful searchers compared to that of successful foragers in any given condition.

The MANOVA examining the difference in kinematics between all successful and unsuccessful searchers was highly significant ($F_{3,111} = 1340.97$, $P < 0.001$), indicating that movements differed according to whether animals found the source. Testing the pooled successful forager treatment against unsuccessful foragers is a conservative test for overall differences between animals that locate, or fail to locate, the source. In general, pooling across treatments that display significant differences (*i.e.*, successful foragers) is ill-advised since it compounds error variance

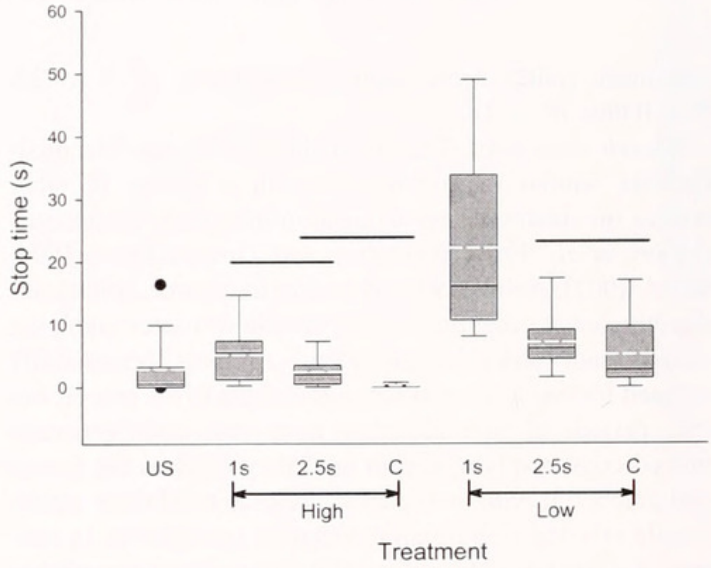

Figure 4. Stop time statistics for all unsuccessful foragers (US) and for successful foragers in plumes with 1-s and 2.5-s pulses, and continuous plumes, for high-flux and low-flux treatments (*i.e.*, plumes created with odorant solutions of 7 and 3.5 g shrimp l^{-1}, respectively). The gray box encloses the 25th and 75th percentiles, error bars above and below the box enclose the 90th and 10th percentiles, circles represent points outside of the 90th and 10th percentiles, the black solid line within the box is the median, and the white dashed line is the mean. Lines above the box plots join pulse treatments not significantly different from each other at $P < 0.05$ using Bonferroni adjusted *post hoc* tests. Sample sizes (numbers of paths) for these groups are given in Table 1.

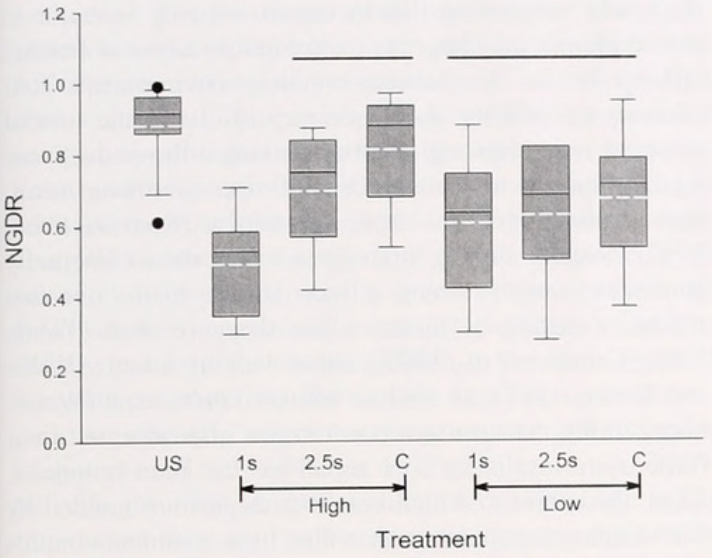

Figure 5. Net-to-gross displacement ratio (NGDR) statistics for all unsuccessful foragers (US) and for successful foragers in plumes with 1-s and 2.5-s pulses, for high-flux and low-flux treatments (*i.e.*, plumes created with odorant solutions of 7 and 3.5 g shrimp l^{-1}, respectively). The gray box encloses the 25th and 75th percentiles, error bars above and below the box enclose the 90th and 10th percentiles, circles represent points outside of the 90th and 10th percentiles, the black solid line within the box is the median, and the white dashed line is the mean. Lines above the box plots join pulse treatments not significantly different from each other at $P < 0.05$ using Bonferroni adjusted *post hoc* tests, with NGDR arcsin-transformed prior to analysis. Sample sizes (numbers of paths) for these groups are given in Table 1.

with variance due to treatment effects, and reduces the power of a test to detect a difference. Angular data require special test methods and could not be included in the MANOVA analysis. However, a Raleigh test (specific for angular data) reveals that successful foragers displayed significantly lower average body angles than crabs failing to find the source ($F_{1,113} = 13.09$, $P < 0.001$), further emphasizing the differences between the behavior of unsuccessful and successful searchers.

Discussion

Turbulent fluid motion has been shown to have a dramatic effect on the behavior of aquatic and terrestrial chemosensory foragers (Weissburg and Zimmer-Faust, 1993; Mafra-Neto and Cardé, 1994; Vickers and Baker, 1994; Moore and Grills, 1999). Investigators have focused on fine-scale mixing processes that produce variation in odor-filament structure as a key feature mediating olfactory-based navigation. The peak odor intensity of filaments and filament duration, rise time (slope), and frequency have been suggested to modulate the ability to track an odor plume to its source in a variety of creatures (Moore and Atema, 1991; Weissburg and Zimmer-Faust, 1994; Atema, 1996). The importance of small-scale mixing in modulating tracking performance is quite clear, even if the importance of particular filament features is still being debated (*e.g.*, Webster *et al.*, 2001; Webster and Weissburg, 2001).

The results presented here indicate that larger-scale odor fluctuations of one to several seconds alter the behavior of animals tracking chemical cues. Animals in plumes with short pulses (long inter-pulse intervals) showed degraded tracking performance. Similarly, field and laboratory studies of pheromone tracking in insects have indicated that larger-scale variation in plume features decreases the initiation and efficiency of search behavior (David *et al.*, 1983; Baker and Haynes, 1987; Zanen *et al.*, 1994). In aquatic creatures as well as in insects, it appears that understanding olfactory-mediated navigation will require us to investigate physical processes that create long-term fluctuations in odor concentration, in addition to examining fine-scale mixing.

Behaviorally relevant signal properties of pulsed plumes. Foraging crabs in pulsed plumes found the source less frequently, reduced their walking speed, spent less time walking, and tacked across-stream more extensively than did animals in continuously released plumes. Interestingly, decreased search success was not simply an extension of inefficient search. Unsuccessful searchers acted in a rather uniform manner as a group, showing behaviors (very straight paths, high body angles) characteristic of animals in the absence of stimulus sources (Weissburg and Zimmer-Faust, 1993, 1994; Weissburg *et al.*, 2003). This suggests that the apparent decrease in search success in pulsed con-

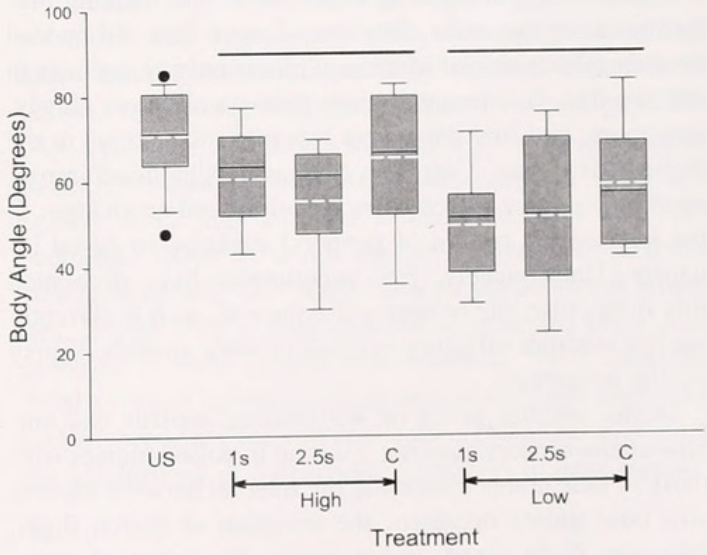

Figure 6. Body angle statistics for all unsuccessful foragers (US) and for successful foragers in plumes with 1-s and 2.5-s pulses, for high-flux and low-flux treatments (*i.e.*, plumes created with odorant solutions of 7 and 3.5 g shrimp l^{-1}, respectively). The gray box encloses the 25th and 75th percentiles, error bars above and below the box enclose the 90th and 10th percentiles, circles represent points outside of the 90th and 10th percentiles, the black solid line within the box is the median, and the white dashed line is the mean. Lines above the box plots join pulse treatments not significantly different from each other by a Raleigh test at $P < 0.05$ using a Bonferroni adjustment. Sample sizes (numbers of paths) for these groups are given in Table 1.

ditions reflects a failure to initiate search, as opposed to an inability to locate the source once search has begun. Thus, blue crabs in pulsed plumes often elect not to search, and they probably experience reduced search success when they do attempt to track to the source.

Our results suggest that the chemosensory system in blue crabs is not a simple flux detector, at least over the time scale of several seconds that is an important level of temporal variation in naturally occurring odor plumes (*e.g.*, Murlis, 1986; Finelli *et al.*, 1999). The performance of blue crabs in different release conditions ought to be similar if flux was the sole determinant of behavior, since plumes created with the same stimulus concentration had equal flux rates over the 5-s cycle length. The deleterious effects of short pulses suggest that the integration of incoming chemical signal strength requires periods substantially longer than the integration time of chemosensory neurons, which in lobsters can code stimulus intensity within several hundred milliseconds (Gomez and Atema, 1996). Integration may act to smooth out random fluctuations in signal intensity, particularly when physiological response times are shorter than the relevant scale of variation of the incoming stimulus. Indeed, it has been suggested that a major advantage of olfactory transduction is that biochemical events at the cellular level act as an integrator so that rapid binding and unbinding of stimulus molecules at the receptor does not result in spurious fluctuations in neuronal output (*i.e.*, action potentials) (Zufall *et al.*, 1994).

Flux was not completely irrelevant to the tracking performance of the crabs, however. Lower flux did indeed produce effects similar to those of plume pulsing; animals in the low-flux (low-concentration) plume walk more slowly, stop more, and find the source less often than crabs in the higher flux plume. Thus, flux can have a significant impact on chemosensory search when superimposed on changes in the longer-term pattern of temporal variation in signal intensity. Unfortunately, few experiments have decoupled flux from pulse rate or inter-pulse interval, so it is currently unclear whether olfactory systems in other animals display similar properties.

Moths, another group of well-studied animals that employ chemosensory tracking, respond to pulsed plumes similarly to blue crabs. Increasing the interval between successive odor pulses decreases the initiation of search flight, decreases flight speed, and increases the extent of cross-wind movement (*i.e.*, cross-wind casting; Vickers and Baker, 1992; Mafra-Neto and Cardé, 1995). Decreased speed and increased casting may enhance the probability that a moth will reacquire the plume if it temporarily loses the signal (Mafra-Neto and Cardé, 1995). Flying arthropods must keep moving to stay aloft, which necessitates cross-wind flight to regain the plume signal. For an animal walking on the sea floor, remaining in the same spot is analogous to casting; a motionless animal has a chance to re-contact the plume, suggesting that increased stopping behavior in pulsed plumes may improve the foraging success of benthic arthropods in intermittent-stimulus environments. At present, we lack the data necessary to clarify the role of stopping *versus* casting in other walking arthropods. Some walking insects increase the extent of cross-stream meanders as plumes become more intermittent (Kanzaki *et al.*, 1992). Such a casting strategy is based on an internally generated counter-turning scheme similar to the one that produces casting of insects when they are aloft (Tobin, 1981; Kanzaki *et al.*, 1992). Other walking insects (Willis and Baker, 1987), as well as benthic crustaceans (Weissburg, 2000), do not use a counter-turn generator, but their response to loss of an odor signal has not been examined. Thus, the extent to which search strategies are molded by evolutionary constraints rather than by a common stimulus environment remains unknown.

One difference in the responses of aquatic and terrestrial arthropods is the maximum interval between odor pulses that still allows the animal to maintain smooth and rapid upstream progress toward the source. Blue crabs responded negatively to inter-pulse intervals that exceed 2.5 s. In contrast, inter-pulse intervals in the range of 0.25 s to 1 s are sufficient to disrupt orientation in moths flying through pheromone plumes (Vickers and Baker, 1992; Mafra-Neto and Cardé, 1995, 1998). Although the difficulty of creating cleanly separated pulses at higher frequencies prevents examination of shorter inter-pulse intervals, it is unlikely that blue crabs respond significantly more quickly to signal offset than current observations suggest, given the relatively slow rate at which primary chemosensory afferents in crustaceans operate. The responses of insect olfactory receptor neurons to brief pulses remain distinct at frequencies of up to at least 10 Hz (Rumbo and Kaissling, 1989; Vickers and Baker, 1992). In contrast, lobster chemosensory neurons fuse signals when the frequencies exceed 4–6 Hz (Gomez *et al.*, 1994). The generally smaller turbulence intensity in aquatic *versus* terrestrial environments (Murlis, 1986; Weissburg, 2000) results in larger mixing lengths. In turn, this larger mixing scale (and more moderate flow speeds) translates into slower variation of odor signals in waterborne plumes, possibly resulting in longer processing times in aquatic animals than in terrestrial ones. Interestingly, the inter-pulse intervals required to disrupt plume-following in the oriental silk moth (*Bombyx mori*) are about 2–3 s (Kanzaki *et al.*, 1992). Silkmoths walk to pheromone sources and are within the low-velocity region of a boundary layer where odor signals are less disrupted by turbulent fluid motion. Thus, odor signals may be more coherent to a walking insect than to its flying brethren, resulting in a reduced sensitivity to short periods of odor absence.

The importance of longer-term signal variation. Investigators have focused on variation in the fine-scale structure of odor filaments as a key feature mediating olfactory-based

navigation. The results presented here indicate that signal variation on the order of one to several seconds also affects both the success rate and the kinematics of animals tracking chemical cues. Thus, understanding olfactory-mediated navigation will require us to investigate physical processes that create long-term fluctuations in odor concentration, in addition to examining fine-scale mixing. Unfortunately, controlled studies on the effects of such longer-term signal variation are rare compared to investigations on filament-scale variation, particularly in aquatic habitats. A few laboratory and field studies (in addition to the study reported here) suggest that odor fluctuations over one to many seconds significantly affect foraging success and behavior (Lapointe and Sainte-Marie, 1992; Keller *et al.*, 2001). Whelks, for instance, are less likely to be caught in baited traps when they are confronted with dramatic changes in current direction (Lapointe and Sainte-Marie, 1992) that likely cause long-term gaps in the odor signal or changes in its intensity.

Turbulence that generates second-scale odor fluctuations is common and may be created by a variety of mechanisms that produce eddies of an intermediate size (*e.g.*, 10–1000 cm), which are generally smaller than the largest scales and greater than the microscale flow structures (1–10 mm) that are primarily responsible for the properties of individual odor filaments. Eddies in this intermediate size range may either cause the entire plume to oscillate cross-stream or else repackage the odor into larger-scale structures, both of which will induce longer-term odor variation. The largest turbulence scales are restricted by the largest flow dimension—for instance, the water depth or channel width (Roberts, 1990). Because energy present in the largest flow features is transferred to successively smaller scales (the Kolmogorov cascade), large turbulent eddies present in open estuaries, embayments, *etc.*, will invariably create eddies of a size sufficient to induce odor variation on the scale of one to several seconds. Alternately, shallow or narrow tidal channels may initially generate eddies in the correct size range. Finally, objects in flow shed vortices at a predictable frequency. The frequency, f, produced by a circular cylinder of diameter d in a flow of velocity U is given by the Strouhal number, St, which is 0.2 over a large range of Reynolds numbers (White, 1991). Thus, $0.2 = fD/U$, so a 10-cm-diameter object will shed vortices at a frequency of 1 Hz in a flow of 50 cm s^{-1}. Given typical flow velocities in tidally dominated systems (*ca.* 1–100 cm $\cdot$ s^{-1}), biological or nonbiological objects (worm tubes, protruding shells, *etc.*) may create turbulence at scales required to disrupt olfactory foraging, at least in blue crabs.

Time-series measurements of odor plumes show fluctuations on a variety of scales that reflect the processes described above. Low-frequency fluctuations (*e.g.*, less than 1 Hz) are often a dominant component of frequency spectra for odor fluctuations in both aquatic and terrestrial environments (Hanna and Insley, 1989; Finelli *et al.*, 1999, 2000; Keller *et al.*, 2001). For instance, the slowest frequency for odor fluctuations in an estuarine tidal channel is about 0.8 Hz, which probably reflects plume cross-stream meander created by the largest eddies in the flow (Finelli *et al.*, 1999).

Many aquatic crustaceans forage most effectively in spatially and temporally coherent plumes (Weissburg, 2000; but see Keller *et al.*, 2001), which may reflect strategies that maximize the speed of navigation at the cost of decreased ability to operate in more complex stimulus environments (Weissburg *et al.*, 2002). Although turbulence may be the primary mechanism producing longer-term odor fluctuations detrimental to olfactory foraging, potential prey may exploit these signal characteristics to mask themselves from their predators. This raises the possibility that predators and prey coupled to one another *via* waterborne signals may be engaged in an arms race similar to that evolved in other systems (Vermeij, 1987); prey rendered apparent to their consumers *via* transmission of fluid-borne chemicals may modulate the release of these signals to evade predation.

Bivalves, which are commonly consumed by crabs and other olfactory predators, mimic quite well the general properties of the signal source used here. Bivalves discharge attractive metabolites from a siphon, and in principle, can modulate the flow to release odor pulses over time scales that degrade the tracking ability of their predators. In fact, any animal capable of actively controlling the release of chemical cues might profit from creating pulsed plumes rather than continuous ones. Prey species, including bivalves, use a variety of mechanisms to detect predators; once a predator is detected, the prey use a suite of strategies to decrease their apparency and vulnerability to their consumers (Cote and Jelnikar, 1999; Leonard *et al.*, 1999; Nakaoka, 2000). It is not known whether anti-predation strategies include the modification of plume signal properties, though bivalve prey exposed to predators often cease pumping entirely (Irlandi and Peterson, 1991; Nakaoka, 2000). Clearly, additional field and laboratory experiments on both predators and potential prey are required to evaluate the significance of longer-term variations in odor signals.

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