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Role of olfactory appendages in chemically mediated orientation of blue crabs

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ABSTRACT: Benthic crustaceans such as the blue crab *Callinectes sapidus* use various sensory appendages to navigate chemical plumes. We characterized the role of different sensory structures in blue crabs during olfactory search by deafferenting (i.e. removing or rendering inactive) particular sensor populations and by quantifying odor-plume structure and flow dynamics. Our results indicate that blue crabs use both cephalic and thoracic appendages for olfactory-mediated orientation. Cephalic chemosensor deafferentation decreased search success, reduced walking speed and increased the duration of stationary periods. All these deficiencies are manifestations of the inability of crabs to sustain upstream progress. Crabs subjected to deafferentation of thoracic sensilla failed to correctly track the narrowing plume and showed an increased frequency of large course-corrections. Whereas cephalic sensors clearly function in motivating upstream movement during the search process, thoracic receptors aid in source localization. The differing functional roles of these 2 sets of appendages may be associated with different signal characteristics impinging on their chemosensor populations. Intermittent but intense signals received by the cephalic appendages may enable the crabs to identify attractive odors and sustain searching. Chemical signals impinging on legs are more homogeneous and may allow the crabs to acquire better information on the spatial patterns of chemical signal structure that are important for navigation. The simultaneous use of chemical signals at differing heights in the plume suggest that the 3D structure of these plumes is important for foraging success, and that different populations of neural receptors may be tuned to operate optimally in particular signal environments.

KEY WORDS: Antennules · Chemical plumes · Chemoreception · Perireceptor processes · Predator–prey interactions · Sensory biology · Turbulence

INTRODUCTION

Chemical cues serve as a fundamental source of sensory information linking a variety of interacting organisms (Dusenbery 1992, Pawlik 1992, Dodson et al. 1994, Zimmer 2000). These chemical signals are often received only after having been transported by moving fluid (i.e. air or water), a process that disperses chemicals across a range of spatial and temporal scales. Thus, the physics of fluid motion influences the information available to organisms and alters chemically mediated ecological interactions such as tracking prey or mates (Weissburg & Zimmer-Faust 1993, Weissburg et al. 1998, Moore et al. 2000).

Although the effects of physics (e.g. turbulence intensity) on plume navigation by benthic organisms have been characterized, the potential role of the vertical structure of chemical plume dynamics remains unknown. Many macrobenthic marine organisms (e.g. lobsters and crabs) live at the interface between the solid bed and a moving fluid. Bed friction generates velocity gradients (i.e. a boundary layer: Nowell & Jumars 1984) which, in turn, creates vertical variation in the spatial and temporal distribution of chemicals

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(Moore & Atema 1991, Moore et al. 1994) that could have implications for sensory systems and search strategies.

Macrobenthic organisms such as decapods, gastropods, echinoderms, and fishes grow large enough to have olfactory sensilla on structures that encounter this range of hydrodynamic microhabitats. The chemosensory sensilla in these various zones may exhibit different sensory functions and neurophysiological properties when exposed to different spatial and temporal patterns of chemical signals. Receptor cells of insects (Kaissling et al. 1987) have much higher temporal resolution than those of marine crustaceans (Gomez & Atema 1996); this is possibly related to a greater variation in chemical signal intensity in terrestrial environments.

Decapod crustaceans have chemoreceptors distributed on their mouthparts (Shelton & Lavarack 1970), dactyls and propodus of claws and walking legs (Hatt 1984, Schmidt & Gnatzy 1989, Weissburg & Derby 1995), antennae (Tazaki & Shigenaga 1974, Voigt & Atema 1992, Gomez & Atema 1996), and antennules (Hazlett 1971, Reeder & Ache 1980, Devine & Atema 1982). These groups of sensilla have specialized chemosensory functions (Derby & Atema 1982). American lobsters *Homarus americanus* flick their antennules (biramus-joined appendages located above the mouthparts) to sample and identify chemical cues (Derby & Atema 1988) and to orient in chemical plumes (Hazlett 1971, Reeder & Ache 1980, Devine & Atema 1982). Chemoreceptors on mouthparts (mandibles and maxillae) and walking legs (periopods) function primarily during food manipulation (Derby & Atema 1988).

The role of chemosensory appendages during plume orientation has been best studied in various lobster species (Hazlett 1971, Reeder & Ache 1980, Derby & Atema 1982, Devine & Atema 1982). These animals have a characteristic body form that includes long bilaterally paired sensory appendages of considerable mobility (the first and second antennae). Thus, the chemosensory search strategies they employ may be, if not unique among crustaceans, contingent on the particular form of their sensory equipment.

The blue crab *Callinectes sapidus* provides an interesting comparative model system in which to examine the structure and function of appendages involved in chemically mediated search. Compared to lobsters, these decapods have small (2 cm long), antennae that are relatively less mobile, but a spatially extended and roughly circular array of thoracic walking legs that are also sensory appendages. The ecological importance of blue crabs as predators on benthic infauna and epifauna (Blundon & Atema 1982, Eggleston et al. 1984) and the general mechanisms they use during prey tracking are well established (Weissburg & Zimmer-Faust 1993, 1994). Whether blue crabs use sensory input from their different sensory appendages while tracking chemical plumes is unknown. Using blue crabs in a laboratory flume, we conducted experiments designed to examine the role played by the cephalic (i.e. antennules and antennae) and thoracic (i.e. walking legs/claws) appendages in chemical search. We addressed this issue by analyzing the behavior of individuals with experimentally induced chemosensory deficiencies and by characterizing the structure of the chemical signal at heights above the bed likely to be sampled by different sensory populations.

MATERIALS AND METHODS

Crabs. Using baited traps, male and female blue crabs *Callinectes sapidus* were collected from habitats adjacent to Dickson Bay, Panacea, Florida (Latitude 30° 00′ N, Longitude 84° 22′ W). The crabs were shipped to Atlanta, Georgia, and kept in communal tanks filled with artificial seawater (33 ppt, 20°C: ASW, Instant Ocean©), and tested within 20 d of collection. The crabs were maintained on a 12 h light:12 h dark cycle, and fed freshly thawed shrimp and squid ad libitum. We withheld food from the crabs approximately 12 h prior to testing to ensure that they were not satiated and to standardize the hunger level.

Flow environment. We characterized blue crab search behavior and hydrodynamics in an indoor recirculating flume (12.5 m long × 0.75 m wide) in which we could control fluid flow and boundary-layer conditions. The flume was lined with sand to provide a natural substrate on which the blue crabs could move (grain size = 0.894 ± 0.124 mm, mean diameter ± SD, N = 37), and was kept free of obvious ripples or other surface features. The experimental section was >7 m downstream from the flume entrance to provide ample distance for the boundary layer to become established (Weissburg & Zimmer-Faust 1993, Zimmer-Faust & Butman 2000). Water velocity was controlled with a variable-speed pump and discharge was monitored using an inline meter. Average flow velocity was maintained at 4.9 ± 0.08 cm s^{-1} (mean ± SD) with a water depth of 23.0 ± 0.348 cm (mean ± SD) controlled by a vertical tailgate. Light levels were lowered during trials to minimize visual cues during navigation and because field observations indicated peaks in foraging activity in near-dark periods of early morning and evening (Clarke et al. 1999).

Hydrodynamic measurements. We quantified the flow velocity in the center of the flume (38.5 cm from the flume walls) and 0.75 m downstream from the odor source at 23 distances above the flume bed (between 1 and 18 cm). Mean velocity values and their root mean

square (RMS, or SD) were calculated from unfiltered velocity records obtained with a laser Doppler velocimeter (60 s record length). RMS values measure the variability of the velocity at a given point to estimate turbulence intensity. We characterized boundary-layer shear velocity (u^*) and roughness by Reynolds number (Re^*) following Weissburg & Zimmer-Faust (1993). Boundary-layer shear velocities were estimated with using the well defined law-of-the-wall equation:

$$U(z) = (u^* k^{-1}) \ln (z z_0^{-1}) \quad (1)$$

where $U(z)$ is the mean velocity at a given height (z) above the bed; k is von Karman's constant (0.41). We calculated the hydraulic roughness length (z_0) as the intercept of the least-squares linear regression through mean velocity values in the log-layer (4.5 to 7.2 cm above the bed) plotted against the logarithm of height z. We determined the flow characteristics in the near-bed region (viscous sublayer layer) of the flow by calculating the roughness Reynolds number (Re^*).

$$Re^* = u^* D \nu^{-1} \quad (2)$$

Roughness height, D, was set as the mean grain size of the sand (0.894 ± 0.124 mm; mean ± SD, N = 37), as measured from digital images using NIH Image software, and ν is the kinematic viscosity.

Odor plume structure. We used electrochemistry microelectrodes (IVEC-10) to quantify the properties of spatial and temporal odorant distributions. This technique has been used successfully in field and laboratory settings to measure chemical stimulus distributions (Moore & Atema 1991, Moore et al. 1991a,b, Zimmer-Faust et al. 1995, Keller et al. 2001, Weissburg et al. 2003).

Chemical signal intensity was measured along the plume centerline after releasing the chemical marker 0.5 M dopamine at 4 ml min^{-1} through a cylindrical air-diffuser (4 cm long by 1 cm diameter) mounted inside a shrimp exoskeleton. This delivery was hypokinetic to the bulk flow so that the turbulence generated by the shrimp carapace was the primary arbiter of the resulting odor plume. A peristaltic pump introduced dopamine at a constant and controlled rate. Dopamine concentration fluctuations were measured for more than 5 min, and recordings included a 1 min period prior to odor release to determine background-noise levels.

We measured the dopamine concentrations at 1, 2, and 5 cm above the bed, the approximate height of the cephalic sense organs (antennae and antennules) and the chemosensors on the tips of the walking legs. We quantified chemical dynamics at 25, 75, and 125 cm directly downstream from the source to detect changes in odor signal properties along the plume length. Because we were interested in the information available to individual crustacean sensory neurons, we acquired data at 2 Hz, which is similar to the sampling abilities reported for *Homarus americanus* olfactory neurons (Gomez & Atema 1996).

Exceedence values represent the probability that a single measurement will meet or exceed a known concentration, and these were calculated from the concentration records. At any given concentration x, the exceedance probability is $(1–f_x)$, where f_x is the proportion of data records displaying a concentration less than x. Exceedance probabilities were calculated in 5 µM increments ranging from 0 to 120 µM. Finally, we calculated intermittence, or the proportion of the time in which no tracer was detected, at 1, 2 and 5 cm above the substrate.

Behavioral experiments. We conducted a series of experiments that selectively deafferented (i.e. removed or rendered inactive) the chemosensilla on appendages, and then challenged crabs with the task of locating a piece of fresh shrimp carrion. Selective deafferentation of chemosensilla by exposure to distilled water has been successfully used on blue crabs (Gleeson et al. 1997) and lobsters (Derby & Atema 1982). Preliminary trials suggested that distilled water was only marginally effective in blocking the olfactory function, so we added a concentrated stimulus to promote exposure of neuronal cells to this harsh osmotic stress via the opening of ion channels in the sensory neuron. A slurry was made using 14 g l^{-1} of macerated shrimp in distilled water (DI-odor treatment), which was then used to bathe particular appendages. All blue crabs (including controls) were secured with elastic bands to a plastic apparatus that positioned their walking legs and chelae under the body. We exposed the cephalic sensory appendages (antennules/antennae) and claws and walking legs to each solution for 30 min. Crab gills were moistened with sponges dipped in ASW (33 ppt) during this time. Blue crabs were randomly assigned to a variety of deafferentation and sham (control) treatments. Treatments of the antenna/antennules included (1) antennal sham: each structure was placed in a 1 ml syringe filled with ASW; (2) antennal odorant control: structures were placed in a 1 ml syringe filled with ASW-odor solution; (3) antennal deafferentation: structures were placed in a 1 ml syringe filled with the DI-odor solution; (4) a second antennal deafferentation: aesthetascs were manually removed from antennules. Walking leg/claw treatments were (1) walking leg sham: legs were bathed in ASW; (2) walking leg odor control: legs were exposed to ASW-odor solution; (3) walking leg deafferentation: legs were bathed in DI-odor solution.

We used the ASW-only treatments to control for the effects of handling and the ASW-odor treatments (shrimp slurry prepared in ASW) to determine whether

the presence of odor per se was responsible for the behavioral effects subsequently observed. We removed the chemosensory aesthetascs on the antennular filaments using methods described by Gleeson (1982) to provide independent evidence that the effects were the result of sensory deafferentation. We elected not to deafferent the antennae in this manner since the distribution and modality of sensors on this appendage remains unknown.

Blue crabs were tested for their ability to locate an upstream odor-source 17 min after treatment. Initial experiments indicated that 1 piece of freshly thawed headless shrimp (4.35 ± 0.329 g; mean ± SE) was the smallest amount that unaltered crabs could reliably locate (success rate >90%), and so this was used as a stimulus source. Blue crabs were moved carefully to the flume and placed in a flow-through Plexiglas box (27.2 cm long, 19.5 cm wide, 16.5 cm high) with a plastic-grate (1 cm^2 grid) front door and rear panel. The crabs acclimated in the box for 15 min to prior to introduction of the shrimp, which was subsequently placed on the sand 1.5 m directly upstream of the Plexiglas box. The front door was raised 2 min later. Trials lasted for a maximum of 15 min and were terminated if a crab moved either upstream of the source or downstream of the Plexiglas box. Preliminary tests indicated that blue crabs that moved upstream beyond the source or downstream of the box never found the source.

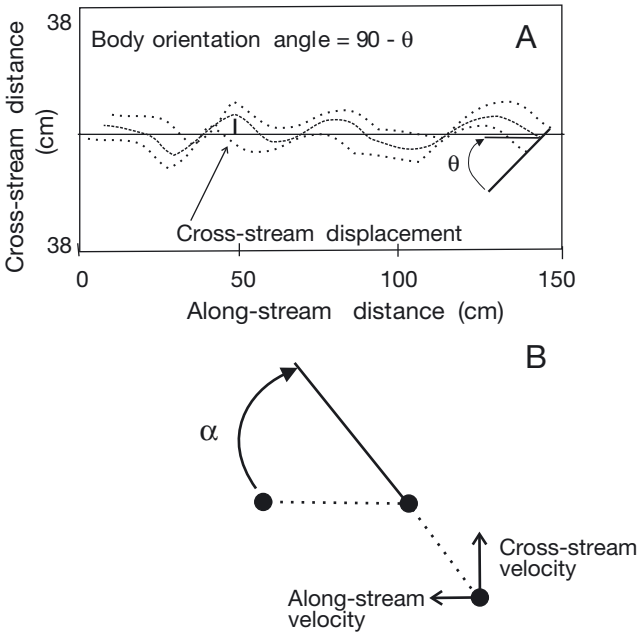

Fig. 1. *Callinectes sapidus.* Representative crab path and calculation of kinematic parameters used in analysis of crab locomotory performance. (A) Visualized area of flume showing position of left and right light-emitting diodes (LEDs) on the back of a crab at each time point, taken at 1 Hz; line drawn between the LEDs and bisected by line parallel to the flow direction gives the angle θ; body orientation angle is defined as 90–θ, ranging from 0° (crab facing directly upstream), to 90° (long axis of crab parallel to flow direction). (B) Three successive positions of a crab from time $t = -1$ to $t = 1$, showing various velocity components; turn-angle α is the angle representing change in the crab's trajectory; turn-angles ranged from 0 to ±180°, where positive angles indicate leftward and negative angles rightward turns

Crab behavior was recorded on videotape using a low-light-sensitive charge-coupled device camera mounted approximately 2 m above the working section of the flume; 2 red-light-emitting diodes (LED) powered by a watch battery and sealed in silicone were attached with elastic bands to the carapace of each blue crab before behavioral experiments. A search was deemed successful when a crab found the shrimp and attempted to consume it. We assayed the motivational state of crabs that failed to find the source by placing them in a cylindrical holding chamber (28 cm diameter) containing 5 l of ASW and offering them a single shrimp after a 15 min acclimation period. Blue crabs that failed to respond to this food within 5 min were designated as unresponsive and were omitted from the analysis.

The *x,y*-coordinates of the centroid of each LED were determined using Motion Analysis™ software (60 Hz) smoothed with a moving average algorithm (window size = 3) and extracted to produce a 4 Hz time-series. The tracks of successful searches were used to calculate a variety of kinematic parameters of foraging crabs (Fig. 1).

Neurophysiology. Extracellular physiology was used to confirm that the DI-odor treatment reduced activity of chemosensory neurons in the legs. We focused on the legs because physiological experiments are the easiest way in which to independently examine if deafferentation treatments were responsible for observed behavioral changes. Alternate deafferentation methods (e.g. scraping, coating with cyanoacrylate) may affect mechanosensors and fail to remove chemosensory pit sensilla known or suspected to be present on crustacean walking appendages (Schmidt & Gnatzy 1989, Weissburg et al. 1996).

Isolated appendages were placed into a glass and Teflon olfactometer so that the sensor-bearing distal tip was exposed to a seawater carrier while the exposed nerves lay in a saline-filled compartment. A saline-filled glass microelectrode was attached to nerves by suction, and the resulting activity was amplified using conventional AC recording techniques and digitally stored on a personal computer. The limb was perfused by delivering oxygenated saline (4 to 5 ml min^{-1}) into the main artery via a glass cannula. Identified chemosensory neurons were initially challenged with 4 to 5 presentations each of 0.5 ml of ASW or stimulus mix-

ture injected into the seawater, at 1 min interstimulus intervals. The stimulus consisted of a 1:10 mixture of ground Tetramarin fish flakes dissolved in ASW and filtered to remove large particles. The seawater carrier was then switched to the DI-odor solution. Neuronal activity in response to both ASW and the stimulus mixture was measured after cumulative exposure periods of 1, 3, 5, 10, 15, 30, 45 and 60 min. The carrier was then switched back to ASW and neuronal activity assayed at 15 and 60 min. The response was digitally stored on a personal computer using commercial software (Experimenters' Workbench, DataWave) and analyzed off-line to determine the response intensity (# of spikes) for a 4.5 s period following stimulation. Further details of the recording and analysis protocols can be found in Weissburg & Derby (1995).

Statistical analyses. The *G*-test of independence (Sokal & Rohlf 1995) was used to determine if deafferentation treatment affected source-finding success. A repeated-measures analysis of variance was used to examine effects of distance (<50, 50 to 100, >100 cm from the source) and deafferentation treatment on the following 6 kinematic parameters: total velocity, along-stream velocity (i.e. velocity component towards or away from the source), cross-stream velocity, cross-stream displacement (i.e. cross-stream distance from the source axis), time spent stationary and the net-to-gross displacement ratio (NGDR). The NGDR, which measures path linearity, was arcsine-square-root transformed before analysis (Sokal & Rohlf 1995). An NGDR of 1 indicates a perfectly linear path. Significant deafferentation effects revealed by repeated-measures ANOVA were further explored by single degree of freedom post-hoc comparisons between deafferentation treatments and their respective controls.

The effects of treatment and distance on turn angles were compared by a Kolmogorov-Smirnov (KS) test, which determined the significance probability of the maximum difference between the 2 frequency distributions. We binned all turn-angle observations according to the treatment and distance (<50, 50 to 100, >100 cm from the source) for this analysis.

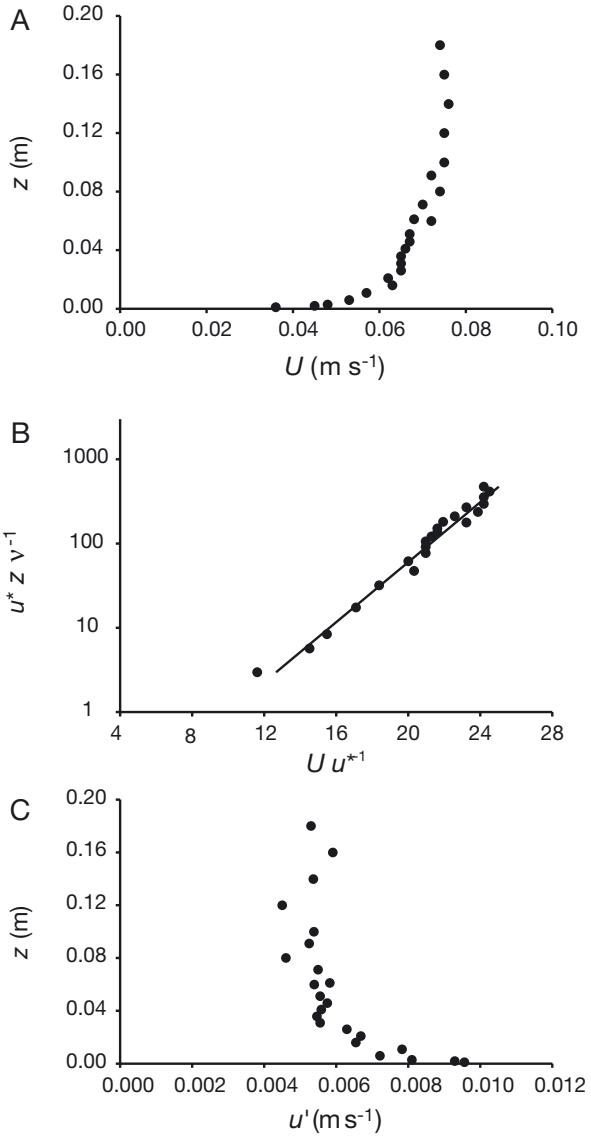

Fig. 2. Flow characteristics during the blue crab *Callinectes sapidus* behavioral trials. (A) Boundary layer profile showing velocity (U) versus height above substrate (z). (B) Plot of law-of-the-wall relationship of velocity versus height above substrate; in this plot, velocity and height are non-dimensionalized by shear velocity (u^*). In the log-layer region of boundary layer, the relationship between $U\,u^{*-1}$ and log ($u^*\,z\,\nu^{-1}$) is linear. (C) RMS of velocity fluctuations (u') versus height above substrate, showing increased turbulence intensity within maximal velocity gradient close to the bed

RESULTS

Hydrodynamic measurements

The hydrodynamic measurements confirmed that blue crabs were foraging in a reproducible, realistic and well-defined flow environment (Fig. 2). Our measurements showed a distinctive log-linear relationship characteristic of a developed boundary layer (Fig. 2A), where shear acting on the bed imposes the well-defined law-of-the-wall relationship between height above bed and flow velocity (Fig. 2B). Values of Reynolds number ($Re^* = 2.65$) indicated that the flow was hydraulically smooth near the bed, with a shear velocity, u^*, of 3.1 mm s^{-1} for these flow conditions. Blue crabs search for prey in similar estuarine hydrodynamic environments (Finelli et al. 1999). The high-velocity gradients near the bed generate considerable

Table 1. Chemical signals in plumes showing intermittence and mean (SEM) and median (SEM) periods (in seconds) when dopamine was below detection at 3 distances downstream of the source and 3 heights above the bed. N: number of intervals in which dopamine concentration was below detection; threshold for detection was set at 3 SDs above the mean baseline value

Distance (cm)	Height (cm)	Intermittence	N	Mean (s)	Median (s)
25	1	0.59	71	1.96 (0.49)	2
25	2	0.89	34	5.03 (0.94)	4
25	5	1.0	–	–	–
75	1	0.07	20	1.05 (0.45)	1
75	2	0.61	68	2.09 (0.51)	2
75	5	0.98	4	36.2 (16.2)	88.5
125	1	0.11	22	0.88 (0.12)	1
125	2	0.16	40	0.72 (0.06)	1.5
125	5	0.71	56	2.74 (0.52)	3

mixing at the height of the blue crab walking legs (ca. 1 to 2 cm), whereas velocity gradients and turbulence are less pronounced at the antennal height (ca. 5 cm; Fig. 2C). These results are consistent with those of other studies of turbulence in open channel flows (Nezu & Rodi 1986).

Structure of odor-plume signal

We characterized the spatial and temporal variability of chemicals in the fluid microhabitats where searching blue crabs maintain their sensory appendages, because the distribution of chemical signals constrains the information available to searching animals. Intermittence, the mean period without tracer, and total duration of tracer absence were all reduced in areas far from the source or close to the bed (Table 1). High intermittence close to the source reflects changes in the plume centerline (e.g. Moore et al. 1994, Webster & Weissburg 2001), which cease downstream as turbulence homogenizes and spreads the plume. Intermittence varies in the vertical dimension as a result of enhanced shear close to the bed. Sensors located 5 cm above the bed (i.e. near the cephalic appendages) experienced highly intermittent stimulation and long periods of signal absence (2.2 s to 5 min), whereas intermittence was very low close to the bed (i.e. near chemosensors on legs). Tracer was not detected 25 cm from the source at the 5 cm height because the plume had not expanded sufficiently for turbulent eddies to transport signals much above the bed.

The exceedence probabilities for tracer concentration recordings indicated clear height-dependent differences (Fig. 3). Chemical tracer concentrations recorded 5 cm above the bed rarely exceeded 10 µM, but were frequently above this level close to the substrate. Concentrations near the bed were greater than those measured higher in the water column at all distances downstream from the source. Exceedance probability-distributions shifted in a complex way depending on the measurement height as the plume evolved downstream. Signals close to the bed became somewhat homogenized, so that exceedence values decreased at the highest concentrations and increased at intermediate levels. At 5 cm above the bed, tracer was

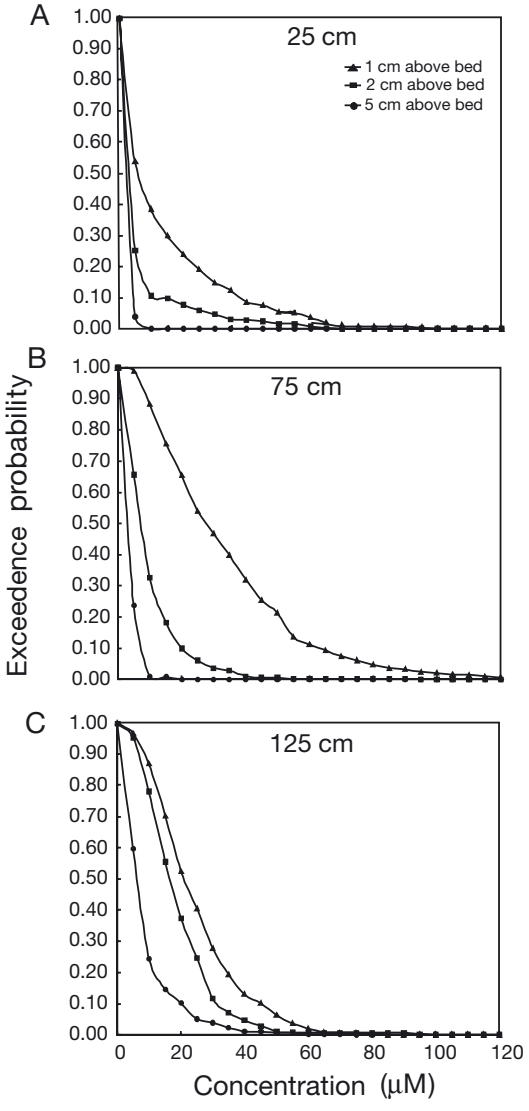

Fig. 3. Exceedance plots of chemical signals emanating from shrimp mimics. Chemical tracer concentrations and corresponding exceedance values were calculated at 1, 2 and 5 cm above the bed, which corresponded roughly to locations sampled by chemosensors on thoracic (1 to 2 cm) and cephalic (5 cm) appendages. (A) 25 cm downstream from source; (B) 75 cm downstream from source; (C) 125 cm directly downstream from source

transported vertically as the plume developed downstream so that the probability of detecting the tracer increased over much of the concentration range.

Behavioral experiments

We evaluated the impact of the various deafferentation treatments and controls first by examining patterns of search success (Table 2). Blue crabs with cephalic appendages exposed to ASW or ASW-odor solutions successfully located the source in nearly all the experiments and success rates were not significantly different from each other ($G = 0.01$, $p \gg 0.05$). The similar search success of untreated crabs (not shown) and their controls, as well as the uniformity among control groups suggests that these treatments have little effect on search performance. Search effectiveness was reduced by deafferentation of the antennae and antennules using either the DI-odor solution or by aesthetasc removal, and these 2 treatments were not significantly different from each other ($G = 1.45$, $p \gg 0.05$). We compared the success rates of pooled antennal/antennae deafferentation-treated crabs (DA) to pooled control (SA) crabs and found that deafferentation significantly reduced foraging success by roughly 50% ($G = 12.19$, $p < 0.001$). The effects of ASW and ASW-odor treatments on legs mimicked that for the cephalic appendages; crabs successfully located the source regardless of treatment ($G = 0.01$, $p \gg 0.05$) and behaved remarkably similar to untreated crabs and to each other. However, deafferentation of walking legs (DL) had no effect on search success ($G = 0.3$, $p \gg 0.05$) when tested against the pooled SL group.

An initial repeated-measures ANOVA was used to examine the effect of all 7 treatments and controls on the path kinematics of successful searchers. Fig. 4 shows representative paths of crab in these treatment groups that illustrate the general facets of the navigational paths as well as changes induced by the deafferentation treatment. ANOVA consistently failed to demonstrate differences between the ASW and ASW-odor treatments. Of the 18 individual degree of freedom post-hoc tests (3 distances × 6 kinematic variables), cephalic appendages exposed to ASW versus ASW-odor differed in only 2 cases, whereas these 2 treatments in legs only differed in 1 case. Similarly, analysis of this full model showed that the effects of aesthetasc removal and DI-odor solution were similar, with a difference detected in only 1 post-hoc comparison. Accordingly, based on the analysis of success rate and path kinematics, both the ASW and ASW-odor treatments were combined into a single control group for antennae/antennules (control or sham antennal/antennule = SA) and legs (control or sham leg = SL) for all further analysis. Likewise, we combined the aesthetasc removal and DI-odor treatments of cephalic appendages (deafferented antennal/antennule = DA).

Deafferentation treatment strongly altered crab walking speeds ($F_{3,49} = 12.98$, $p < 0.005$) and the relationship between speed and distance from the source (Fig. 5). Blue crabs in both antennal- and leg-deafferented groups (DA and DL) decreased their upstream walking speed by 15 to 50% relative to their respective controls (SA and SL), depending on treatment and distance from the source. The greatest decreases were for crabs with deafferented antennae, for which walking speeds averaged approximately 50% of the SA group. There was a general tendency for crabs to move quickly within 100 cm from the source, but then to decrease their walking speed within 50 cm ($F_{2,98} = 18.57$, $p < 0.005$). A significant distance × treatment interaction ($F_{6,98} = 2.27$, $p < 0.05$) indicated that deafferentation treatments disrupted this normal pattern of locomotion. Post-hoc comparisons indicated that these effects were more important for crabs treated with antennal deafferentation (Fig. 5); crabs in the DA group moved significantly more slowly at all distances from the source and showed little evidence of a final reduction in walking speed, whereas the DL group showed reduced walking speed only between 50 and 100 cm from the source ($F_{1,49} > 6.18$, $p < 0.016$ for all comparisons) and still displayed a velocity peak at intermediate distances from the source. Treatment effects on movement speeds in both cross- and along-stream directions were similar to patterns of total walking speed, and also were significant ($F_{3,49} = 5.41$, $p < 0.005$; $F_{3,49} = 13.59$, $p < 0.005$, respec-

Table 2. *Callinectes sapidus.* Number of successful and unsuccessful searches as a function of deafferentation treatment for the 7 separate treatments used in this study. As a result of the statistical analysis of success rate and kinematic variables, the 2 antennal defferentation treatments were pooled (DA), and the ASW and ASW-odor control groups for antennae/antennules and legs were pooled (SA and SL, respectively)

Treatment	No. successful	No. unsuccessful
Antennal deafferentation		
DI-odor	7	9
Scraping	5	6
Total	12	15
Antennal sham		
ASW	8	0
ASW-odor	7	1
Total	15	1
Leg deafferentation	8	1
Leg sham		
ASW	8	2
ASW-odor	9	2
Total	17	4

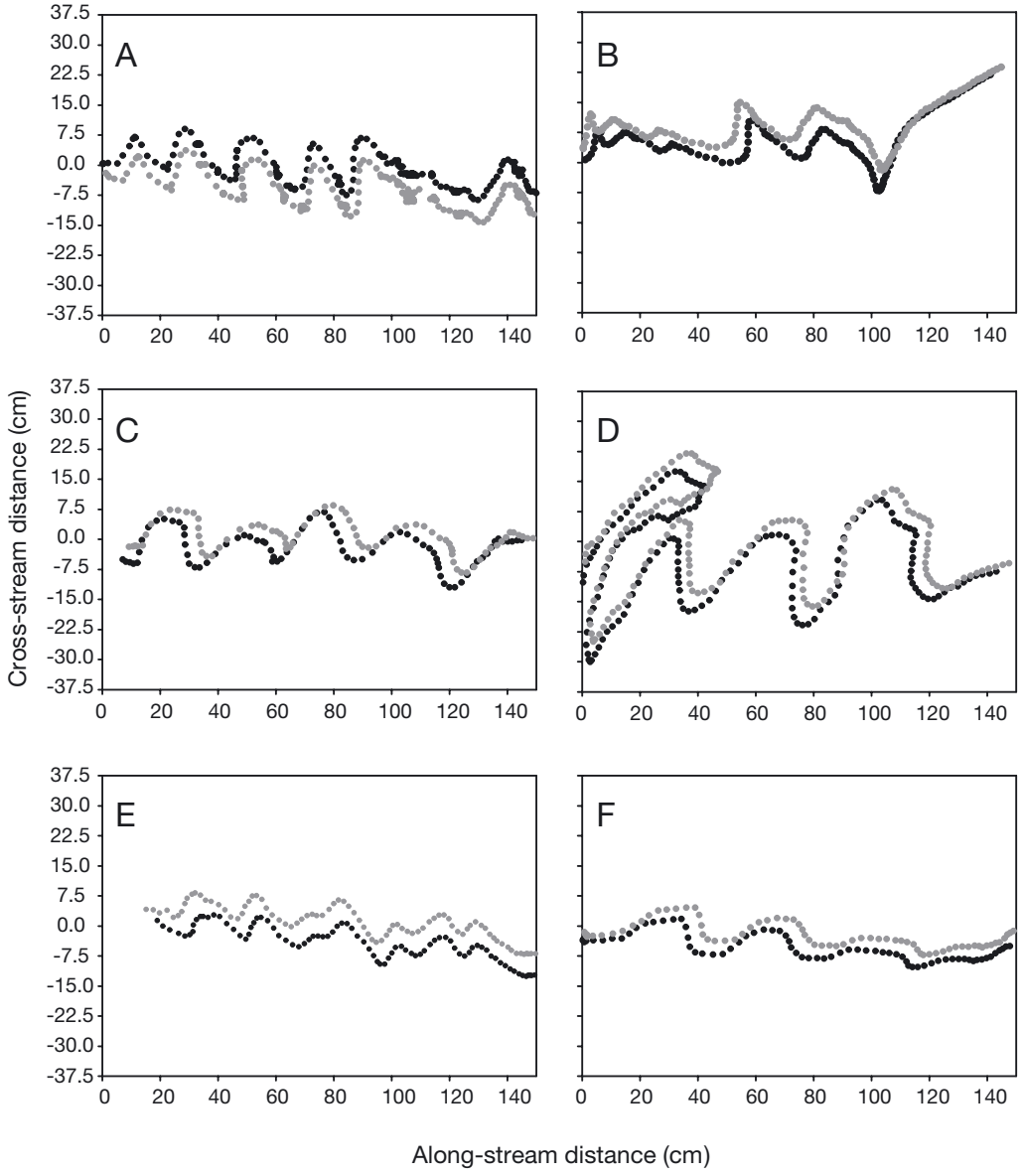

Fig. 4. *Callinectes sapidus.* Typical paths displayed by successful blue crab foragers subjected to deafferentation and control treatments. Tracks in each graph represent along- and cross-stream location of left and right LEDs as crab moves toward the source, which is located at 0.0 cm. Data points are plotted at 0.25 s intervals as described in 'Materials and methods', and flow proceeds from left to right. Treatments were as follows: (A) aesthetasc removal; (B) antennae/antennule deafferented by DI-odor solution; (C) ASW-odor treatment of antennae/antennule; (D) legs deafferented by DI-odor solution; (E) ASW-odor treatment of legs; (F) ASW treatment of the legs. DI-odor and ASW-odor treatments = exposure to slurry of macerated shrimp in distilled water or in artificial seawater, respectively, as described in 'Materials and methods')

tively). Post-hoc test comparisons showed that along-stream speeds were significantly reduced in DA group at all distances and in DL groups between 50 and 100 cm from the source, whereas the cross-stream speeds were significantly lower at all distances from the source in the DA group only ($F_{1,49} > 4.21$, $p < 0.05$ for all comparisons).

Deafferentation had major effects on the propensity of crabs to sustain upstream locomotion (Fig. 6; $F_{3,49} = 5.44$, $p < 0.005$), with a significant effect of distance ($F_{2,98} = 3.63$, $p < 0.05$). In general, crabs stopped more frequently when farther from the source. Individuals in the DA group spent more time stationary than those in the SA group ($F_{1,49} = 6.98$, $p < 0.011$ for all distances). Deafferentation of the walking legs had no effect on the time blue crabs spent stationary, regardless of their distance from the source ($F_{1,49} < 0.14$, $p > 0.71$ for all distances).

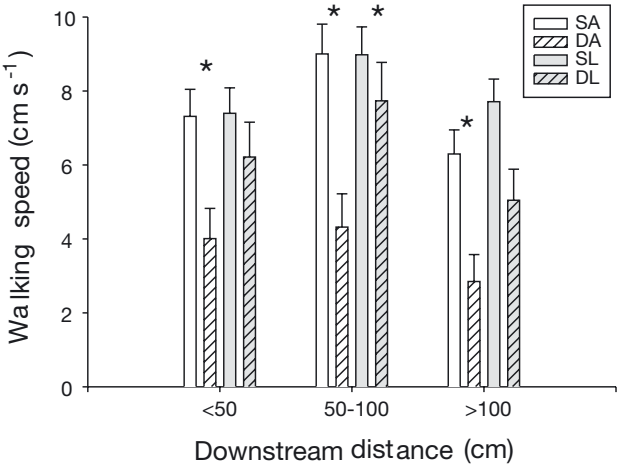

Fig. 5. *Callinectes sapidus.* Walking speed of successful crabs in deafferentation treatments and controls showing mean (+SEM) walking speed for crabs in each treatment group as a function of distance downstream from the source. Sample sizes were 15, 12, 17 and 8 for SA, DA, SL and DL treatments, respectively. Asterisks indicate treatments (DA, DL) are significantly different from their respective controls (SA, SL) at the given distance. (SA, DA = control and antennal/antennae deafferentiated crabs, respectively; SL, DL = control and walking-leg deafferentiated crabs, respectively)

Blue crabs make lateral movements relative to the axis of bulk flow during the search process (Fig. 7). The crabs generally displayed a higher NGDR as they moved upstream, indicating that they moved more directly towards the source as they approached it. The NGDR pooled across treatments equaled 0.73 ± 0.04, 0.76 ± 0.05, 0.82 ± 0.03 for downstream distances of >100 cm, 100 to 50 cm and <50 cm, respectively (Fig. 7A; $F_{2,98} = 4.10$, $p < 0.05$). Analysis also revealed a significant decrease in the lateral distance of the crabs to the plume centerline as they moved upstream (Fig. 7B; $F_{2,98} = 7.04$, $p < 0.005$). These patterns reflect the fact that under our flow conditions plumes narrow and become more coherent near their origin (see 'Discussion'). Thus, the crabs could move more directly towards their goal and orient more easily relative to the plume as they approached the source. Both path linearity and lateral displacement also changed due to deafferentation ($F_{3,49} = 3.95$, $p < 0.025$; $F_{3,49} = 2.76$, $p < 0.05$, respectively). Although crabs in the DL group had lower NGDR at all distances relative to their respective controls, none of the individual comparisons were significant. In contrast, post-hoc tests showed that crabs in the DL group were farther from the centerline at all downstream distances than those in the SL group ($F_{1,49} > 4.44$, $p < 0.05$ for all comparisons) whereas crabs in the DA group behaved similarly to their respective controls ($F_{1,49} < 2.52$, $p > 0.05$ for all comparisons).

Quantification of the turn-angles made by searching blue crabs revealed other changes in locomotory behavior that were predominantly associated with deafferentation of thoracic appendages. All crabs successfully locating the source made mostly small-angle turns. Angles less than 10°, and from 10 to 20°, accounted for roughly 40 and 30% of the observed turn-angles, respectively. Beyond 20°, the frequency of observations decreased in successive 10° bins and was generally less than 10%, although turn angles above 90° sometimes approached this value. No consistent changes were observed in the turning behavior of crabs after deafferentation of the antennae and antennules either within or across distances (Fig. 8A). Differences in the distribution of the DA group were significant only far from the source (>100 cm), reflecting a larger than expected frequency of 30 to 40° angles and a corresponding decrease in the incidence of angles between 20 and 30°. Treatment of the thoracic appendages had consistent effects on the turning behavior of crabs as they approached within 100 cm of the source (Fig. 8B). Here, blue crabs in the DL group showed a far lower proportion of turn-angles less than 40° and a concomitant increase in angles greater than 40° compared to crabs in the control group. Particularly striking was the relatively high incidence of turns greater than 90°. Far from the source, crabs in the DL group made small angular changes more frequently than those in the control treatment.

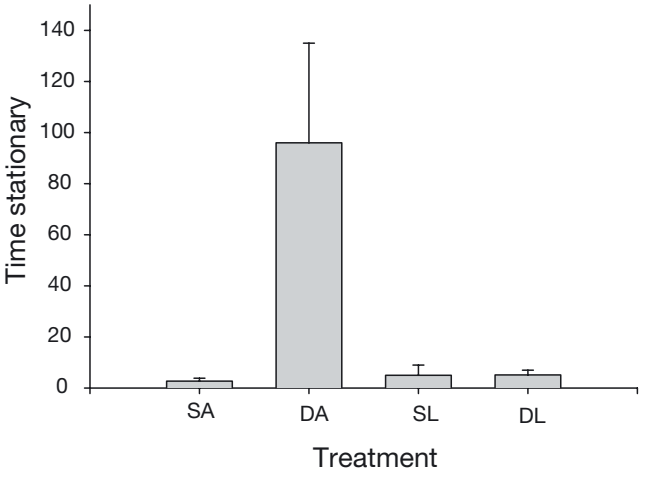

Fig. 6. *Callinectes sapidus.* Duration of motionlessness during tracking of crabs in deafferentation treatments and controls showing mean (+SEM) total period that crabs remained motionless during tracking to the source. Because ANOVA revealed a consistent effect of distance, motionless periods were pooled across all 3 distances for each treatment group. Sample sizes were 15, 12, 17 and 8 for SA, DA, SL and DL treatments, respectively

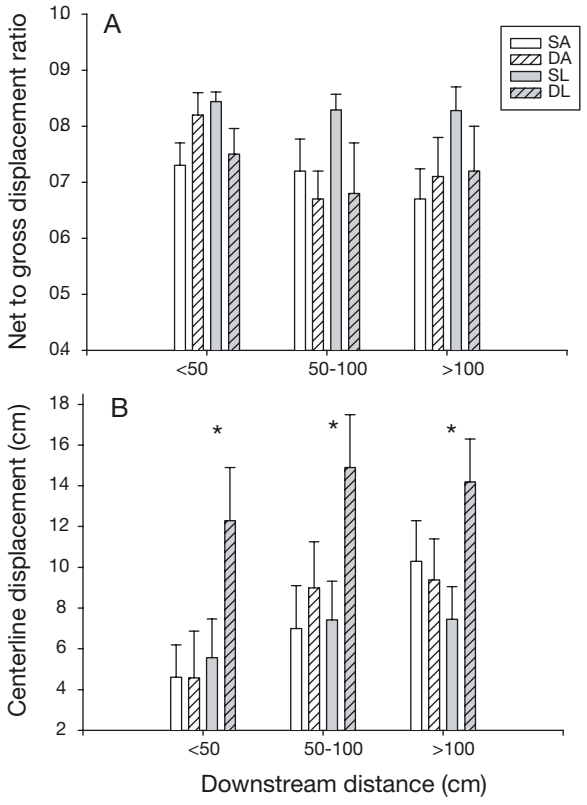

Fig. 7. *Callinectes sapidus.* Path characteristics of crabs locating odor source in deafferentation treatments and controls. (A) Average (+SEM) path NGDR displayed by crabs in each treatment group as a function of distance downstream from the source. (B) Mean (+SEM) centerline displacement in paths of crabs in each treatment group as a function of distance downstream from source. Sample sizes were 15, 12, 17 and 8 for SA, DA, SL and DL treatments, respectively. Asterisks indicate treatments (DA, DL) significantly different from their respective controls (SA, SL) at the given distance.

Neurophysiological response to deafferentation treatment

Exposure of legs to the DI-odor solution rapidly reduced the response of leg chemosensory neurons (Fig. 9). Response intensity declined by approximately 40% within the first 5 to 10 min of exposure and reached nearly undetectable levels after approximately 30 min. Although we continued to record spontaneous activity of mechanosensory neurons in response to flow, chemosensory responses were absent after 45 min of exposure, and did not reappear during the recovery period.

DISCUSSION

Many biological processes of ecological and evolutionary significance are mediated through chemicals released into fluid environments (Dodson et al. 1994, Nevitt 2000, Vickers 2000, Zimmer-Faust & Butman 2000). A clear understanding of the evolution and function of chemosensory systems is contingent on a thorough grasp of the signal dynamics created by the external environment and the ways in which animals extract information from these environmentally modified signals (Dusenbery 1992). We addressed these

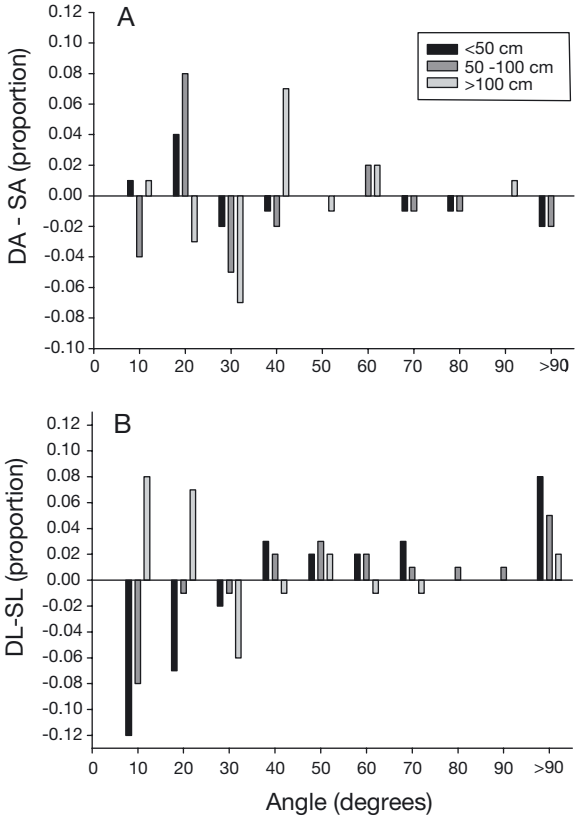

Fig. 8. *Callinectes sapidus.* Effect of deafferentation on turn-angles showing difference in turn frequency between deafferentation and sham treatments at each of 3 different downstream distances pooled across all paths in a given treatment. In this analysis, only the turn magnitude was considered, so that leftward and rightward were treated equally. Turns greater than 90° were lumped for statistical purposes. A positive value indicates that the frequency of turns in a specific bin is greater in the deafferented group (DA, DL) than in their respective controls (SA, SL). (A) Difference in turn angles displayed by antenna/antennule deafferented and sham groups; Kolmogorov-Smirnov (KS) test statistic was significant for distances of >100 cm downstream ($KS = 0.082$, $p \ll 0.01$); sample sizes consisted of 579, 710, 1215 and 660, 892, 3098 for crabs in SA and DA groups at distances of <50, 50 to 100 and >100 cm downstream, respectively. (B) Difference in turn-angles displayed by leg deafferented and sham groups; KS test was significant for all distances ($KS > 0.12$, $p \ll 0.01$); sample sizes consisted of 548, 530, 658 and 431, 573, 587 for animals in SL and DL groups at distances of <50, 50 to 100 and >100 cm downstream, respectively

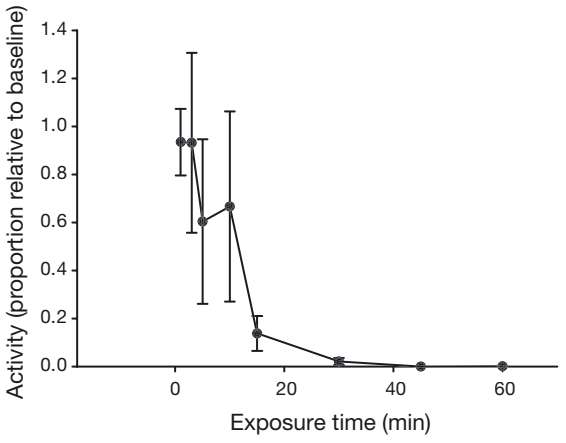

Fig. 9. *Callinectes sapidus.* Effect of DI-odor solution on chemosensory neurons in legs, showing mean (+SEM) response relative to baseline activity for 6 chemosensory neurons exposed to DI-odor solution. All responses were corrected for spontaneous activity and response to seawater

issues for aquatic chemoreception by probing the sensory function of different appendages in blue crabs during navigation using selective deafferentation of particular sensor populations. Removing either cephalic or thoracic chemosensory function alters chemosensory navigation in specific ways, and the tracking efficiency and/or success is reduced unless both sets of appendages are intact. This suggests that blue crabs in turbulent flows use sensilla on both sets of appendages during olfactory search and that they use different sensory appendages for distinct purposes during odor-guided orientation. The particular role of each appendage is associated with, and possibly caused by, the different chemical stimulus environments experienced by each sensory appendage.

Efficacy of deafferentation treatments

Taken together, the results of our various treatments and controls strongly suggest that the shrimp metabolite solution prepared in distilled water (DI-odor treatment) removed or reduced the ability of peripheral chemosensors on the cephalic and thoracic appendages to sense chemical stimuli. Given that euryhaline blue crabs are fairly resistant to osmotic shock, the DI-odor mixture was intended to increase cell permeability by forcing it into an active state. Artificial seawater-odor (ASW-odor) treatments resulted in locomotory behavior that was similar to that displayed by crabs exposed to ASW only, indicating that the presence of odor in the mixture produced no unintended effects, such as modifying of overall activity levels. The results of the physiological assay indicate that this treatment does indeed result in the eventual cessation and continued absence of odor-evoked neural activity (in legs) over the time-course of the behavioral experiments. Antennule deafferentation by immersion in distilled water reduced the length of outer dendritic segments and the impulse frequency of receptor neurons in blue crab aesthetascs (Gleeson et al. 1997). In this study we manually removed aesthetasc chemosensory structures from the antennules to generate an independent method for verifying that locomotory deficiencies were the result of elimination of sensory input. Individuals deafferented in this way responded similarly to crabs with cephalic appendages exposed to the DI–odor mixture. This suggests that the antennules are the cephalic appendages largely responsible for navigation to attractant sources. However, since our experiments were intended to clarify the function of sensors exposed to different chemical stimulus environments, further investigation of the independent role of the cephalic chemosensor-bearing appendages is required to evaluate this hypothesis. Similarly, the specific contribution of the sensors on the claws and legs requires further study.

Role of sensory appendages in blue crab chemically mediated search

Selective deafferentation of sensory appendages had perceptible effects on odor-guided navigation and implicated both cephalic (antennae and antennules) and thoracic (claws and legs) appendages in chemosensory search behavior. In our experiments, crabs with either deafferented cephalic or thoracic sensory appendages showed substantial reductions in a variety of olfactory search-performance measures such as success, upstream walking speed, time spent moving, path linearity, and the frequency of angular corrections (Table 2, Figs. 4 to 8).

Our results (Figs. 5 & 6) suggest that stimulation of the cephalic appendages is necessary for crabs to identify attractive stimulus sources and sustain up-current progress to the source; deafferented individuals with a chemosensory function remaining in their walking legs (DA) moved haltingly, but rather directly, upstream (Figs. 4A,B & 7). The hypothesized role of the antennules in initiating and maintaining upstream search is consistent with the well-established physiological function of antennae and antennules in marine crustaceans (*Homarus americanus*: Derby 1982; *Panulirus argus*: Lavarack 1964; *Callinectes sapidus*: Pearson & Olla 1977). Among the large numbers of morphologically identified chemosensilla located on the dactyls, periopods, and antennae, only the aesthetascs of the antennules have been identified as unimodal

olfactory receptors (Derby & Atema 1988). Neurons in individual sensory hairs (aesthetascs) located on the lateral flagellum of the antennule are often tuned to specific chemicals, with a weak or no response to other stimuli (Derby & Atema 1988). These cells process and extract information from complex mixtures of chemicals and provide information on the quality (identity) of specific odorant blends (Ache & Derby 1985, Derby & Atema 1988). If the aesthetascs of *C. sapidus* have a similar function, then treatments that eliminate blue crab olfactory recognition of the chemical stimulus would be expected to reduce search success and increase time spent not searching during orientation assays.

Antennule and antenna-deafferentation experiments provided only weak evidence that these appendages also play a role in orientation to the plume. Removal of antennule and antennal input had no effects on NGDR or centerline displacement (Figs. 4A,B, & 7) and significantly altered turning behavior only far from the source (Fig. 8A). The lack of a consistent response argues that these crabs may be trying to confirm stimulus identity by gathering information from the aesthetasc sensilla. They may change their position simply in an attempt to expose these sensors to the plume, as opposed to compensating for a lack of input necessary for proper orientation.

Deafferentation of the thoracic appendages also affected search behavior. Blue crabs in the DL group showed only small changes in up-current progress, and instead manifested a variety of deficiencies in their ability to orient themselves to the plume. Crabs with deafferented thoracic appendages showed significantly more large-angle course-corrections and failed to track the narrowing plume during upstream movement (e.g. Figs. 4D, 7B & 8B). These observations suggest that the extended array of thoracic appendages provides important information about the location of the crab with respect to the plume.

The importance of thoracic appendages during navigation is supported by a variety of other evidence. Previous observations of foraging blue crabs suggested that odor signals become entrained in the viscous sublayer and present an easily discernable spatial pattern in chemical signal intensity to sensors on the legs (Weissburg & Zimmer-Faust 1993). Subsequent analysis of the chemical signal structure in turbulent, aquatic plumes confirmed that sensory appendages spanning a large width relative to that of the odor plume promotes the ability of animals to determine when it is losing contact with the plume (Webster & Weissburg 2001, Webster et al. 2001). Behavioral studies indicate that animals adjust their position relative to the plume by comparisons of chemical signal intensity between broadly spaced chemical sensors (Weissburg & Zimmer-Faust 1994, Zimmer-Faust et al. 1995, Weissburg et al. 2002). For plumes created under similar flow conditions to that used herein, the integral length scale, which roughly defines the span of the odor plume, grows from the size of the source to roughly 3 times that width 1.5 m downstream (Webster et al. 2001). Blue crab antennae and antennules are rather short (2 to 4 cm), and closely spaced (<5 cm apart). Thus, these appendages can perform spatial comparisons where plumes narrow near the source, whereas farther downstream the legs (15 to 25 cm apart) are much more suited for spatial sampling. The high signal intermittence and temporal variability at the typical height of the cephalic sensory appendages the blue crab (e.g. Table 1) also makes these appendages less useful for spatial comparisons. Indeed, prior evidence of the importance of antennule stimulation for chemosensory navigation has come from animals such as lobsters, which possess large (>5 cm long) and highly mobile cephalic appendages (McLeese 1973, Devine & Atema 1982, Mead & Koehl 2000) that can sample widely in space and in different regions of the boundary layer.

The movement changes of crabs in the DL groups varied with downstream distance in a manner consistent with the local structure of the plume signal. The plume is relatively narrow close to the source and the intermittence is high. Thus, we would expect that crabs lacking leg chemosensors would have difficulty in resolving the location of the chemical signals and so display large course-corrections in an attempt to determine their position relative to the plume. Intermittence decreases away from the source as mixing process widen and homogenize the plume (e.g. Moore et al. 1994, Finelli et al. 1999, Webster & Weissburg 2001; present Fig. 3), so that adjustments necessary to maintain plume contact are small. An animal starting in the center of the plume would be less likely to experience asymmetric stimulation, and in these conditions would progress rather directly upstream.

Our working hypothesis is that chemosensors on the thoracic appendages allow blue crabs to maintain contact with the plume, but dramatic behavioral deficiencies in response to leg deafferentation occurred only close to the source due to the signal structure in our trials. Alternately, walking leg chemosensors may enhance search efficiency or help the crabs to maintain contact with the plume but their primary role may be in near-source food-finding and manipulation, as suggested by other studies on other invertebrates (Derby 1982, Derby & Atema 1982, Moore & Atema 1991). Behavioral trials in more complicated plume dynamics could resolve if signal structure, rather than distance per se, mediates the importance of leg chemosensory input during guidance. For instance, the importance of

an extended array with a large spatial span will be magnified if there is plume meander that creates significant intermittence even far from the source. In this environment, the removal of chemosensory input from walking legs would be expected to have important consequences far downstream if this is the primary sensory system used to mediate cross-stream location during tracking.

Local chemical signal structure and sensory function

Blue crab navigation in turbulent plumes is thought to comprise 2 components—upstream surges in response to odor (odor-gated rheotaxis) combined with spatial sampling (tropotaxis) to maintain contact with the plume (Weissburg & Zimmer-Faust 1993, Zimmer-Faust et al. 1995, Weissburg et al. 2002). These 2 components are sufficient to explain the behavior displayed by blue crabs navigating in turbulent plumes similar to those generated in this study (Weissburg & Dusenberry 2002). Our results suggest that information extracted from the water column using cephalic sensors primarily mediates the rheotactic component of the response and maintains upcurrent search. Without this information, blue crabs fail to find the source and stop even when located downstream from the source along the plume's center axis. Information from thoracic appendages appears to mediate the ability of crabs to orient relative to the plume. Removal of input from chemosensors on the thoracic appendages seems to interfere with spatial sampling; crabs fail to decrease their distance from the plume centerline as the plume narrows and perform an abnormally high frequency of large course-corrections.

For blue crabs tracking odor in benthic boundary layers, one potential explanation for the differing roles of these sensor populations is that the behaviors mediated by particular appendages reflects adaptation to the local signal environment. The transport and mixing processes that determine the chemical signal structure vary vertically within the boundary layer, which in turn affects the resultant flavor of the chemical signal. The proposed role of the cephalic and thoracic appendages, which are largely located in different regions of the boundary layer, is consistent with the differences in chemical signal structure impinging on their respective sensor ensembles.

Blue crab antennae and antennules are several centimeters above the substrate, in the log-layer region of the boundary layer. A number of studies have shown that chemical signals in this region display high levels of signal intermittence, with strong peak signals alternating with nearly odorless conditions (e.g. Moore & Atema 1991, Finelli et al. 1999, Webster & Weissburg 2001). Although the filamentous nature of the plume tends to be preserved farther above the bed, odor plumes become more homogenous closer to the bed, where strong velocity gradients effectively mix chemical signals (e.g. Moore & Atema 1991, Finelli et al. 1999, Webster & Weissburg 2001). Weissburg et al. (2003) measured the odor-signal structure very close to different blue crab sensory appendages: signals were dramatically affected by the local flow conditions as determined by the height above the bed, and are minimally altered by the crab's ventilatory current. Similar to the results shown here, the dactyl and propodus of the legs (and to a lesser extent, the claws) were exposed to more constant signals than the cephalic appendages.

The odor-signal structure at the height of the cephalic appendages is well suited to the proposed role of these appendages in mediating search. Aesthetasc sensilla receive periodic exposure to intense bursts of chemical cues, and foraging blue crabs appear to orient their body to maintain the coherence of these signal bursts arriving at their cephalic appendages (Weissburg et al. 2003). Periodic exposure may not present a large burden if the task of these appendages is to mediate recognition necessary to sustain upstream search as opposed to resolving the complex patterns of spatial and temporal variation in plume structure requisite for navigational decisions. In some cases, such as when odor sources are raised off the bottom, signals arriving at cephalic sensors may be more intense than those in the vicinity of thoracic chemosensors close to the bottom (Weissburg et al. 2003).

The association between signal properties and the apparent function of the thoracic appendages also suggests that adaptation to local signal structures shapes the role played by these sensory appendages. Sensors on thoracic appendages are uniquely positioned to provide information on the location of the blue crab relative to the plume. The low intermittence of signals within the plume in the region close to the substrate would create easily discernible patterns of signal asymmetry available to sensilla on legs inside versus outside the plume. The contrast between highly stimulated legs inside the plume relative to a poorly stimulated leg on the outside of the plume provides a code for the presumptive direction towards the plume center, and such asymmetry is known to be important for mediating navigation in other animals (Basil & Atema 1994). Foraging blue crabs alter their body angle in order to minimize the intermittence of signals arriving at sensors located on the tips of their walking legs (Weissburg et al. 2003).

One issue raised by the observations that multiple sensory appendages mediate tracking is whether the comparison of information from these 2 populations of re-

ceptors also aids in navigation or source-localization. Measurements of marker chemicals in the fluid microhabitats occupied by differing sensory appendages show clear differences in signal availability that may itself provide information about source proximity (Moore et al. 1994, Weissburg et al. 2002). Blue crabs could, for instance, be potentially alerted to the presence of the source when chemical information is present at the legs but missing from cephalic sensors (e.g. Table 1, Fig. 3). Blue crabs make postural adjustments during olfactory navigation by raising and lowering their body; this may be an attempt to scan the 3D structure of the odor field (Weissburg et al. 2002). A variety of aquatic animals switch behavior close to an odor source, engaging in extensive substrate-probing and other indicators of local search that suggests they recognize the nearness of their goal (e.g. Moore & Atema 1991). Future work will be required to determine if the information embedded in the 3D aspect of plume structure is important in modulating the transition to local search, or contributes to behaviors occurring during other phases of olfactory navigation.

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