

Orientation in complex chemical landscapes: Spatial arrangement of chemical sources influences crayfish food-finding efficiency in artificial streams

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Abstract

Fluid dynamics has been shown to alter ecologically important behaviors of aquatic organisms orienting to distant chemical sources. Because the fluid dynamics and chemical plumes change across hydraulic environments, it is unclear which of these factors influence orientation behavior more. This study examined how alterations in chemical signal structure, through changes in source spatial arrangement, affect chemically mediated search behavior. Microelectrochemical measurements of tracer molecules revealed that source arrangement significantly alters the downstream fine-scale structure of chemical plumes. Flume hydrodynamic characterizations (as measured with laser Doppler velocimetry) also differed among source arrangements; however, differences were minor and existed only at select upstream regions of the flume. Crayfish (*Orconectes virilis*) found the source faster and spent less time in refuges when sources were separated, compared with sources together. Similar numbers of crayfish found the source regardless of source arrangement. Crayfish searched more efficiently with increased spatial complexity at the source. These results supported the hypothesis that spatial and temporal dynamics of chemicals within plumes contain important information that organisms use during olfactory-mediated orientation in streams.

The basis of all ecological interactions ultimately depends on an organism's ability to detect and extract relevant information about their environment. Because environmental signals have various physical properties, organisms have evolved various sensory mechanisms to extract ecological information (Dusenbery 1992). Among these systems, chemical senses are sources of ecological information for a variety of terrestrial and aquatic organisms (Bell and Cardé 1984; Atema 1988). Chemical cues can signal the presence of predators (Mathis and Smith 1993; Covich et al. 1994; De Meester and Cousyn 1997), availability of food resources (McLeese 1973), and status of mates (Atema and Engström 1971; Gleeson et al. 1984; Yen and Strickler 1996). For many organisms, chemical stimuli have particularly fundamental implications for survival, growth, and reproduction (reviewed in Dodson et al. 1994).

Transport of chemicals by the fluid environment structures the distribution of concentration spatially and temporally. At macroscopic scales (considerably larger than 1 mm), advection (i.e., bulk flow), and eddy diffusion (i.e., mixing) disperse chemical signals. The complex interaction between odor and fluid dynamics is dependent on plume width relative to the dominant mixing length scale (Davidson et al.

1995). Near the source, the plume width is smaller than dominant eddy size, which creates meander (Fig. 1). As the plume grows normal to the bulk flow axis, large eddies permeate the plume and cause rapid mixing within the plume. This size- and time-dependent process creates plumes that are spatially and temporally heterogeneous (Davidson et al. 1995). High-concentration fluctuations followed by periods of odor-free fluid often characterize plumes (Murlis et al. 1992; Moore et al. 1994; Finelli et al. 1999). Environmental transport of chemical signals generates temporal and spatial structure that has been labeled "olfactory landscapes" (Nevitt et al. 1995; Atema 1996). Chemical landscapes are analogous to a visual landscape, with peaks and valleys consisting of patches of high (i.e., peaks) and low concentrations (i.e., valleys, Fig. 1). Within these chemical landscapes, patch size, concentration gradient, and intermittence differ along the plume. The landscape structure is also shaped by how chemicals are released from the source. For example, chemicals emitted from a continuous source will have different fine-scale plume characteristics than a pulsed source.

Spatial and temporal heterogeneity of chemical plumes have potentially important implications for organisms. Since transport determines signal availability, changes in flow direction, stimulus concentration, or exposure to degradative processes can affect an organism's ability to effectively recognize and use chemical signals. Fine-scale within-plume characteristics could provide additional information about the chemical source for searching organisms (Moore and Atema 1988). For example, at distances <2 m the fine-scale filaments in plumes change predictably with distance from a source (Moore and Atema 1991; Finelli et al. 1999), and such fine-scale structure is known to play a role in the optomotor-anemotactic search in moths (Mafro-Neto and Cardé 1994). Organisms that detect temporal/spatial patterns in plume structure may, using this information, locate distant chemical sources. In addition, studies examining the chemosensory information in plumes have demonstrated the im-

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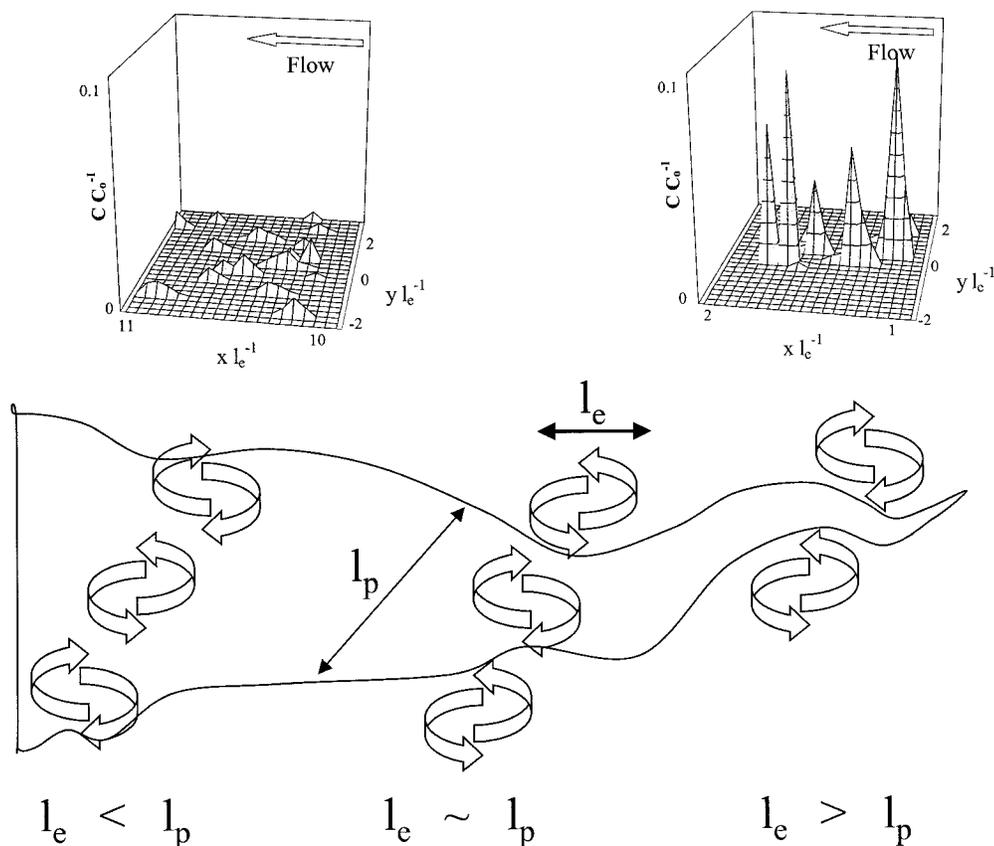


Fig. 1. Depiction of plume development (modified from Davidson et al. 1995) that generate “odor landscapes.” Flow is from right to left. Plume dimensions such as plume width (l_p) interact with dominant mixing vortices (l_e) to generate plume structure. Three-dimensional plots represent hypothetical “odor landscapes” generated within a flat horizontal sheet sliced through the plume at two distances (1 and $10 l_c$) downstream from the source. C_0 is initial concentration.

portance of hydrodynamics in altering animal behavior (Weissburg and Zimmer-Faust 1993; Zimmer-Faust et al. 1995; Tamburri et al. 1996; Moore and Grills 1999; Finelli et al. 2000). Because fluid motion propagates signals at macroscopic scales, manipulations of flow dynamics change both the fluid environment and chemical signal structure simultaneously (Weissburg 1997). It is unclear whether altered chemical signal structure or fluid dynamics is more important for explaining the effect that hydrodynamics has on chemically mediated search behavior.

To examine the role that fine-scale plume structure plays in olfactory-mediated search, we manipulated plume structure by altering the spatial arrangement of chemical sources in artificial streams. This approach was used to alter the structure of plumes while minimizing treatment effect on hydrodynamics. We characterized plume structure, fluid dynamics, and orientation behavior of the crayfish, *Orconectes virilis*, to address this important issue in sensory ecology. This study was designed to answer two fundamental questions in chemical orientation. First, how do organisms in general and crayfish in particular respond behaviorally to changes in chemosensory information? Second, how does spatial arrangement of odor sources alter hydrodynamics and fine-scale chemical signal structure? Answers to these ques-

tions will provide insight concerning how animals locate chemical sources in complex odor landscapes.

Materials and methods

Animals—*O. virilis* was chosen because it has well-developed chemosensory capacity (Tierney and Dunham 1982; Hazlett 1994) and is broadly distributed in lakes and streams throughout North America (Taylor et al. 1996). *O. virilis* is native to the northern Lower Peninsula of Michigan. Male and female *O. virilis* (mean = 29.2 mm, SE = 0.39) were collected from Maple Bay in Burt Lake. Crayfish from lakes showed effective search behavior in streams (Moore and Grills 1999). For shallow-water habitats (<0.5 m), subsurface lake currents generated by winds exceeding 1.5 m s^{-1} would be sufficient to generate conditions similar to velocities we used in our artificial streams (Wetzel 1983). Both sexes were included to characterize how crayfish in general use chemicals during orientation. Crayfish were kept in flow-through containers with access to clay pot refuges. *O. virilis* were fed an ad libitum diet of fish (*Perca flavescens*). To standardize hunger motivation, all crayfish were deprived of fish for 48 h prior to testing. All crayfish were released after testing. We collected yellow perch (*P. flavescens*), the source

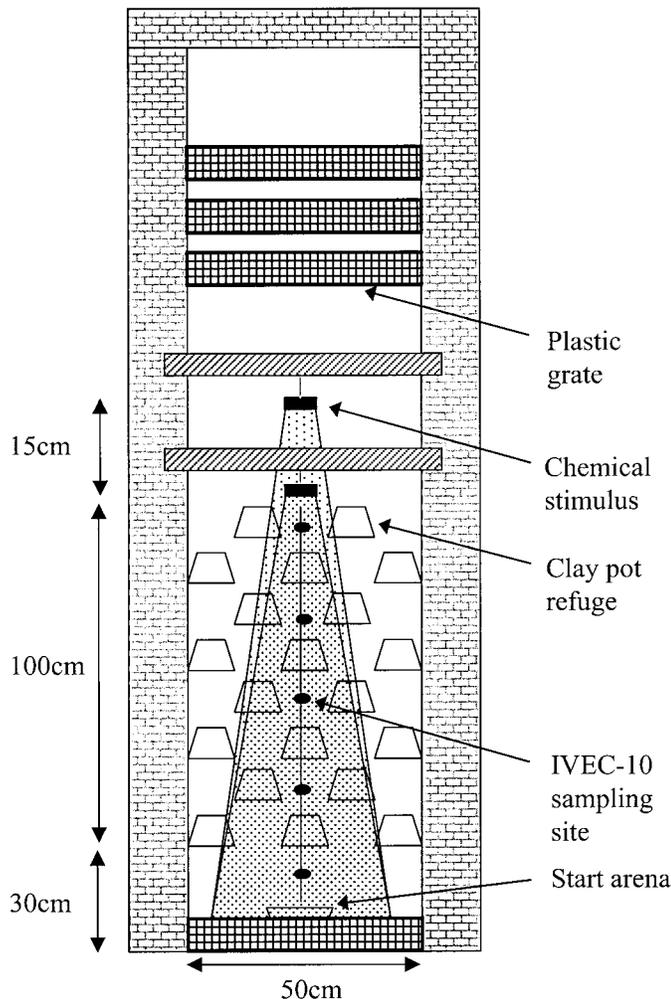


Fig. 2. Diagram of the once-through stream flumes used to test effects of source spatial arrangement on odor signals, fluid dynamics, and crayfish movement behavior.

of carrion odor, from Douglas Lake. Immediately after capture, fish were humanely killed (UM Vertebrate Permit Handling Number 7236).

Design and protocol—We examined how efficiently crayfish located a chemical source in artificial streams. To alter chemical signal fine-scale structure, we used three different source configurations. Treatments included an “odorless” control, two food sources together, and two food sources separated (one upstream of the other). Fifteen crayfish were tested in each of these manipulations ($n = 45$). Trials were randomized to prevent bias.

Three flow-through rectangular-shaped flumes (Fig. 2, 2.77 m long \times 0.5 m wide \times 0.6 m high) were constructed of concrete blocks lined with plastic. Three sheets of plastic grating (1.69 cm²) were covered in fiberglass screening (1 mm²) and were used as flow straighteners in each flume. Flow straighteners were placed perpendicular to flow, upstream of the working section of each flume (Fig. 2). To maintain the water level and ensure that the water exited the flume uniformly, plastic grates covered in plastic sheeting

permeated with 5-mm-diameter circular holes were placed at the downstream end of each flume. The Maple River was the water source for all flumes. To reduce the amount of suspended particles from the river delivered into the flumes and to maintain a constant head pressure, water was pumped into 208-liter plastic head tanks before the surface water was gravity fed into flumes. The average flow rate was measured as the time it took for a buoyant sphere (8-cm diameter) to pass through the working section of the flume. We measured flow speed before the start of each day’s experiments ($U = 3.4 \pm 0.20$ cm s⁻¹ \pm SE, $Re \sim 6,500$). Water depth was kept constant at 18 cm.

Water was pumped through flumes only during observation periods, to reduce colonization by invertebrates (i.e., potential crayfish prey). We placed sand from the Maple River throughout the flume, to provide substrate for crayfish movement. Sand was chosen because it was the substrate on which crayfish were collected and it is the most common substrate in littoral zones of Burt Lake. We leveled the sand before each day’s experiments. To provide refuge for crayfish and to add structural complexity to the search environment, 20 clay pots (8.4 cm diameter, 8.7 cm long) were placed in rows throughout the working section of flumes (Fig. 2). Clay pots were broken in half and oriented open-end downstream and were placed similarly among all treatments and flumes. We started all trials with crayfish in a clay pot (6.2-cm radius) mounted on a Plexiglas plate (110 cm from the source). This start arena had a clear, vertical sliding Plexiglas door that could be raised with minimal disturbance to the crayfish inside.

We made fish-gelatin our source stimulus, to mimic chemicals emanating from carrion and to standardize stimulus delivery. Gelatin was made by mixing 45–53 g of yellow perch, 28 g of unflavored gelatin, and 0.71 liters of boiling water in a blender, and it was solidified in flat-bottomed enamel pans (23 \times 33 cm) at 5°C. We used fish gelatin for no longer than 72 h. We made control gelatin following the same protocol, but perch was replaced by 0.24 liters of boiling water. We cut gelatin into 3.8 \times 1.9 \times 0.8 cm pieces (23.56 cm² total surface area). For the control and single odor source treatments, we placed two pieces of gelatin 2 mm apart in a single fiberglass bag (mesh 1 mm²). We hung these bags in flumes 2 cm above the bed (110 cm upstream of the start arena). This treatment is referred throughout the manuscript as the “together treatment.” The separated odor source treatment had one perch gelatin block placed in each of two bags, one positioned 15 cm upstream of the other. This design kept total flux of chemical sources constant, because surface area was constant across treatments. From this point onward, all references to chemical source or animals locating the source indicate the downstream-most source (110 cm from start arena).

To characterize how alterations in source arrangement influence the distribution of chemical signals in the flumes, we quantified the temporal distribution of dopamine (a marker chemical) at a sampling rate of 10 Hz, using an electrochemical detection technique (In Vivo Electrochemistry Computer System IVEC-10; Medical Systems). This system has been used to quantify chemical distributions in various aquatic environments (Moore et al. 1989, 1994; Zimmer-

Faust et al. 1995; Finelli et al. 1999). We released 0.5 M dopamine with 0.05 M ascorbic acid (as an antioxidant) from cylindrical bubble stones (2.54 cm long by 1.25 cm diameter; ~ 12.4 cm² total surface area) under experimental conditions to determine how source arrangement controls chemical dispersion. Delivery rate (1 ml min⁻¹) was hypokinetic (13.4 $\mu\text{m s}^{-1}$) to flume velocity. Surface area from which dopamine was released was similar to that of gelatin sources. Visual inspection of dye filaments near the source indicated that this delivery rate generated filaments matching those from gelatin sources made with fluorescein. We placed bubble stones either singly in mesh bags (separated by 15 cm as above) or paired in the downstream-most suspended bag. All measurements were conducted in one flume. We made 4-min continuous electrochemical readings five distances from the source (4.5 cm above the bottom, Fig. 2), to measure the temporal and spatial variation in dopamine concentration. We made chemical measurements using a 30- μm diameter carbon fiber electrode calibrated using four concentrations ranging from 0 to 36 μM . The electrode exhibited linearity over this range of concentrations (coefficient of determination; $r^2 > 0.99$). Further details of recording and calibration are explained elsewhere (Moore et al. 1989).

We conducted an experiment to measure how chemical source configuration affected fluid dynamics, because the plume sources are obstacles in the flow that can influence local flow dynamics. Four conditions were tested: (1) no sources, (2) two sources together, (3) two sources separated, and (4) two sources together with an additional source upstream. This design allowed us to determine the effect on velocity of adding sources (treatment 1 vs. 2, 3, and 4), separating sources (treatment 2 vs. 3), and adding an additional upstream source (treatment 2 vs. 4). Velocity measurements were made by use of laser Doppler velocimetry (LDV, Model 75 with Ion Laser, Amperometrics) in a flume constructed exactly as in the previous experiments (including clay pots and head tank). We seeded the water with kaolin to increase sampling rates and measured velocity fluctuations at the same five downstream locations and height above the bed as those taken to characterize chemical dynamics (Fig. 2).

Data collection and analysis—Time-series concentration data have several quantifiable characteristics that could potentially contain information for organisms (Fig. 3, Moore and Atema 1991). Following the terminology defined in Moore and Atema (1991), we calculated the plume qualities: peak height, rise time, and maximum peak slope for the 2 min after detection of dopamine. Only peaks that showed a final concentration $>10\%$ of peak height were included in the analysis. This 90% differentiation criterion provides detailed information on small- and large-magnitude peaks and generates a detailed characterization of the fine-scale structure of odor plumes.

We compared peak number among treatments and distances using chi-square tests of homogeneity. At each distance, we compared the number of peaks among source configurations using chi-square test of independence. We corrected for the number of comparisons using an approximation of the Bonferroni correction ($\alpha = 0.05/5 = 0.01$).

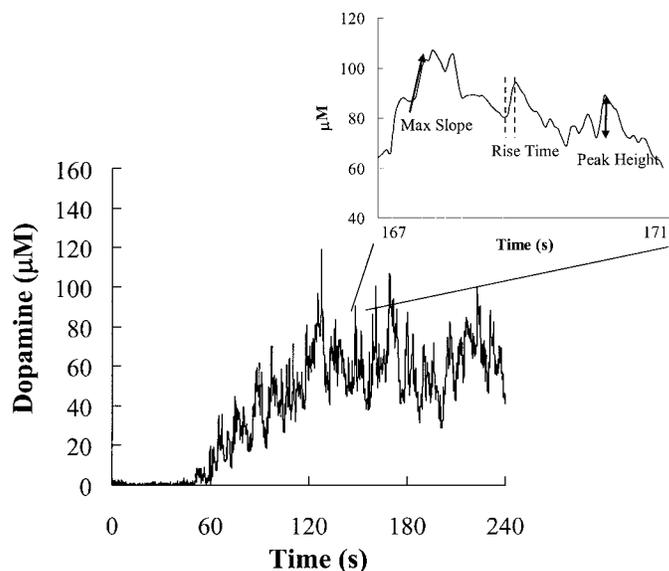


Fig. 3. Time series of the chemical fluctuations measured in flumes with sources separated (one 15 cm upstream of the other). IVEC-10 was used to sample dopamine concentrations at 10 Hz from a distance 60 cm from the downstream-most source in the center of the flume (4.5 cm off the bottom). Maximum peak height, maximum peak slope, and time to peak maximum are labeled in the inset diagram.

We analyzed peak number over a 2 min 30 s period to maximize the resolution of the analysis. Data on odor plume qualities (maximum peak height, maximum peak slope, and rise time) were analyzed by use of a two-way multivariate analysis of variance (MANOVA) with distance and source configuration as independent variables. Univariate analysis of variance (ANOVA) was used to assess treatment and distance effects on individual peak characteristics (i.e., maximum slope, maximum height, and rise time) that were statistically significant in the MANOVA analysis. We corrected for the number of tests by using an approximation of the Bonferroni method for multiple comparisons ($\alpha = 0.05/3 = 0.017$). Only peaks separated by 7 s were included in the analysis, because cross-correlation tests indicated that points were uncorrelated and thus independent beyond this interval (see Results section).

Effects of sources on fluid dynamics were evaluated using two readings from a LDV placed 10, 35, 60, 85, and 110 cm from the source. LDV sampling rate was limited in the slow flow regions (at 10 cm, sampling rate ~ 3 Hz) we could only generate a continuous velocity record using a maximum sampling rate of 2 Hz. We used 50 random values from each replicate ($n = 100$ for each treatment at each distance) from these 2-Hz data. When 100 velocity values were used for each treatment at each distance, a high level of statistical resolution even for small differences ($n = 2000$) was generated. We analyzed the effect of source configuration and distance from the source on average velocity using a two-way ANOVA. All pairwise post hoc tests were made by use of the Tukey heuristic significant difference (HSD) method.

We characterized turbulent dynamics by examining the root mean square (RMS) of the velocity fluctuations and

generating spectral plots of Fourier-transformed data. The RMS was calculated from the raw velocity data files. We compared velocity variability among together and separated source arrangements at each distance using F-tests. We corrected for the number of comparisons using an approximation of the Bonferroni method ($\alpha = 0.05/5 = 0.01$). Spectral analysis was used to characterize turbulent flow dynamics. We performed a Fast Fourier transformation of velocity time series, to determine the sine and cosine components of the time series fluctuations using Systat 7.0 for Windows (SPSS, Chicago). Frequency elements were then plotted against magnitude (Wilkinson 1997). We performed this analysis on velocity records at 35 cm for data quantified from unobstructed, separated, and together source configurations. We chose the 35 cm location for spectral analysis to maximize the distance downstream we could detect hydrodynamic differences among source configurations. Previous ANOVA analyses indicated that mean velocity differed among source configurations only at 10 cm downstream but not at 35 cm (see Results section). Because more data existed for these velocity time series, we could bin velocity values at 200 ms (effective sampling frequency 5 Hz). This sampling rate encompasses the frequencies containing the most energetic velocity fluctuations reported for similar artificial streams (Moore et al. 2000). We did not use additional smoothing, and missing data points were interpolated by use of distance-weighted least squares fitting.

Behavioral observations were conducted from 0900 to 1600 h during the period 23 June to 6 July 1998 at the University of Michigan's Streams Research Facility. Previous studies showed that crayfish orient during this period (Moore and Grills 1999). Crayfish acclimated for 30 min in start arenas. An observer sat inconspicuously beyond the outflow of the flume. Trials ended after 15 min elapsed or when the animal grabbed the source.

We characterized crayfish movement using several objective measurements. Emergence time was measured from the start of the trial until the crayfish exited the start arena. Shelter time equaled the total time crayfish spent in refuges, including time within the start arena after emergence. Time crayfish spent moving various distances from the source (>100 cm, 100–75 cm, 75–50 cm, 50–25 cm, 25–0 cm, and upstream of the source) was recorded for each animal. Search time consisted of elapsed time after emergence until trial end. A successful search was defined as animals that found the source before the end of the trial.

Behavioral data were analyzed using one-way ANOVAs with three levels (control, sources together, and sources separated). All post hoc, pairwise comparisons were made following the Tukey HSD method. A two-way repeated-measures ANOVA was used to compare time crayfish spent in different portions of the flume among odor source treatments. We analyzed the ratio of time to area ($s\ m^{-2}$), because flume sections differed in area. We included only successful animals in this analysis because we wanted to characterize the behavioral differences among orienting animals ($n = 20$). We performed a similar analysis to determine whether success differed among control versus separated and among separated versus together treatments ($\alpha = 0.05/3 = 0.017$). We used a chi-square test of independence to test whether

the sex of crayfish influenced their search success. A chi-square test of homogeneity was used to determine whether orientation success differed among odor source configurations. We compared the shelter time (independent samples t -test) and number of shelters the crayfish entered (Mann-Whitney U -test) among successful and unsuccessful crayfish (controls omitted). To examine how well the data met the assumption of normality, we examined residuals using Lilliefors' test, a test for skewness, and a calculation of kurtosis. Homogeneity of variances was tested by use of Bartlett's test.

Results

Plume structure—Plume structure was quantified at 10 Hz by use of the IVEC -10. At this sampling rate, contemporaneous data are strongly correlated. Time series separated by <3 s (lag = 30) were highly correlated (autocorrelation $r > 0.36$). Data in the time series separated by 7 s (i.e., lagged by 70 points) showed the lowest correlation (autocorrelation $r = 0.1$). To maximize the independence of odor peaks generated from these time-series data, analyses were restricted to peaks separated by this interval.

Although initial tracer concentration was equivalent among experimental treatments, temporal and spatial structure of chemical signals differed significantly between sources separated in space and those placed together. There were 45% more odor peaks in the treatments with separated odor sources (chi-square $P \ll 0.0001$). Separated sources showed more peaks at three distances from the source (Fig. 4A). The magnitude of differences depended on the distance measurements were taken from the source (chi-square $P = 0.03$).

Source spatial arrangement also affected characteristics of individual peaks in plumes (MANOVA $P \ll 0.0001$). Both maximum peak height (Fig. 4B, ANOVA $P \ll 0.0001$) and peak rise time (Fig. 4D, ANOVA $P = 0.001$) were lower when sources were separated, in comparison with those that were together. Maximum peak slope, a measure of maximum concentration gradient among pulses, was similar between treatments (Fig. 4C, ANOVA $P = 0.4$). As was seen in other studies, chemical peak characteristics changed with distance from the source (MANOVA $P \ll 0.0001$). Maximum peak height (Fig. 4B, ANOVA $P \ll 0.0001$) and maximum slope (Fig. 4C, ANOVA $P \ll 0.0001$) decreased from the odor source, whereas rise times increased significantly with increasing distance from the source (Fig. 4D, ANOVA $P = 0.017$). Structure within chemical plumes displayed a source arrangement by distance interaction (MANOVA $P \ll 0.0001$) detectable in peak rise times, maximum peak heights, and maximum peak slopes (ANOVA $P < 0.009$ for all).

Flow characterizations—We examined how chemical source arrangement altered flow characteristics. Source configuration affected velocity (ANOVA $P \ll 0.0001$). Laser Doppler velocimetry measurements taken downstream from chemical sources showed that sources reduced centerline water velocity throughout the flume (Fig. 5, $P < 0.011$ for all). At 10 cm velocity was negative, indicating that the together source configuration generates a measurable eddy. This ef-

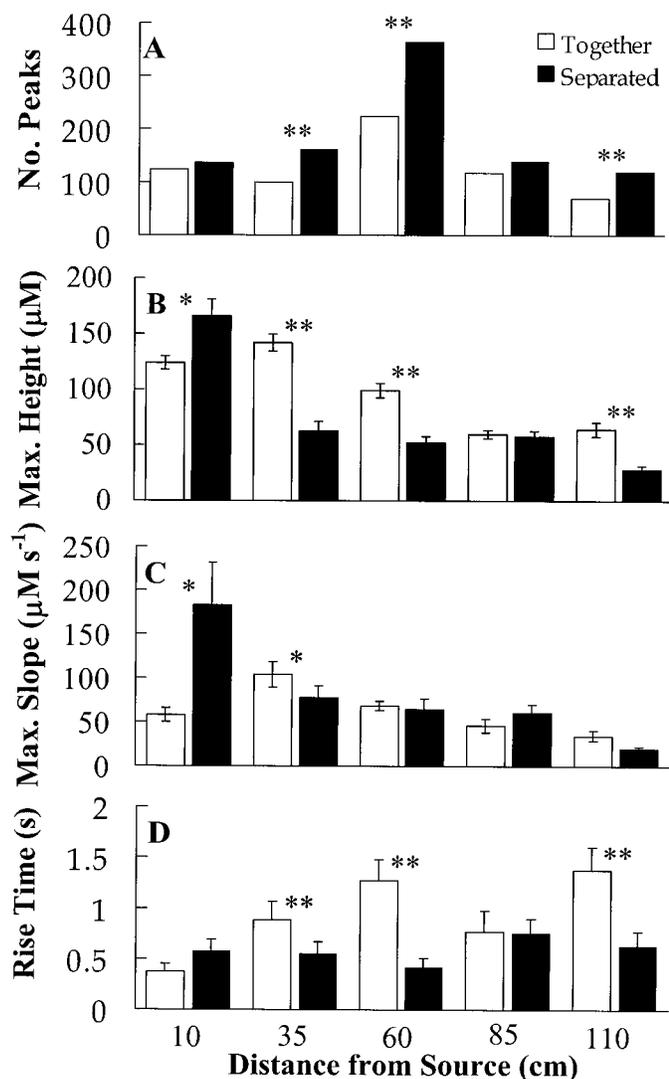


Fig. 4. Chemical peak (A) number, (B) maximum height, (C) maximum slope, and (D) rise time for plumes together and separated measured at different distances from the source (mean \pm SE). Unfilled bars represent peaks measured in a flume with sources placed together. Filled bars indicate results from measurements with odor sources separated (one 15 cm upstream of the other). Only peaks separated by 7 s were included in the analysis ($n = 191$). * $P < 0.05$. ** $P < 0.001$.

fect was detected only 10 cm ($P \ll 0.0001$) from the source (Fig. 5, $P > 0.7$ for all). The vortex was eliminated after an obstacle (i.e., mesh bag) was placed upstream of the first source, regardless of the number of odor blocks contained within bags (Fig. 5, $P > 0.9$ for all).

There was variability in the temporal sequence of velocity measurements. This fluctuating component of velocity was quantified by use of the RMS of the two velocity sequences. Values for RMS were remarkably similar among source configurations at all distances except 35 cm (Table 1). At 35 cm, turbulence intensity was greater in separated than together source configurations (F-test $P < 0.0001$). Velocity fluctuations were also characterized among source arrangements by use of spectral analysis of Fourier-transformed ve-

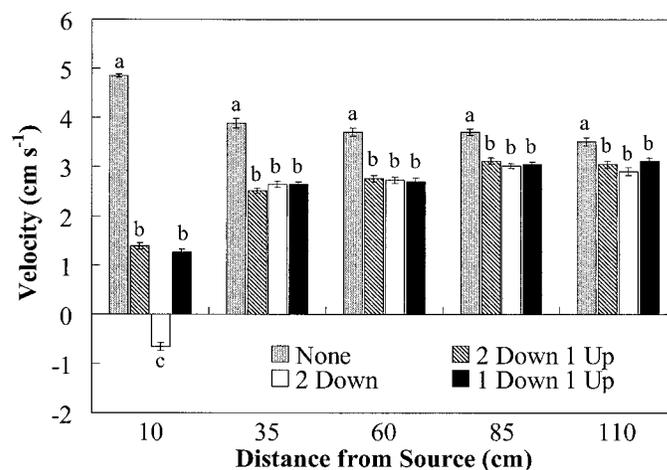


Fig. 5. Mean flow velocity (\pm SE) at 10, 35, 60, 85, and 110 cm downstream of chemical sources for four different source arrangements. Flow was measured without sources (none), with two sources in the downstream position and one in the upstream position (2 down, 1 up), with two sources downstream with none upstream (2 down), and with one source downstream with one upstream (1 down, 1 up). Data represent 50 random samples from two 60-s laser Doppler recordings 4.5 cm above the flume bed at each distance ($n = 2000$). At each distance, columns not sharing letters differ statistically ($P < 0.05$). No statistical comparisons between columns at different distances are shown.

locity recordings measured 35 cm downstream of the source. Low-frequency oscillations in velocity dominated all spectra (Fig. 6). Differences among source configurations were minor and were centered on low-frequency oscillations with a period > 5 s (Fig. 6B,C).

Search behavior—The number of crayfish that found the source differed across odor treatments (Fig 7A, chi-square $P = 0.031$), because fewer animals found the fishless source than had been expected. There were no differences in the number of successful animals between sources separated and those placed together (chi-square $P > 0.1$). Male and female crayfish located the source with similar success rates (chi-square $P = 0.87$). The total time crayfish spent searching differed among source configurations (Fig. 7B, ANOVA $P = 0.014$). The search time was greatest among animals in fishless gelatin controls and lowest in separated source trials.

Table 1. Root mean square (RMS) values calculated from velocity time series for two source configurations at 5 distances from the source.

Distance (cm)	RMS	
	Together	Separated
10	0.66–0.90	0.73–0.99
35	0.58–0.61	0.84–1.0*
60	0.66–0.74	0.63–0.78
85	0.64–0.66	0.53–0.59
110	0.77–0.83	0.73–0.99

* $P < 0.0001$.

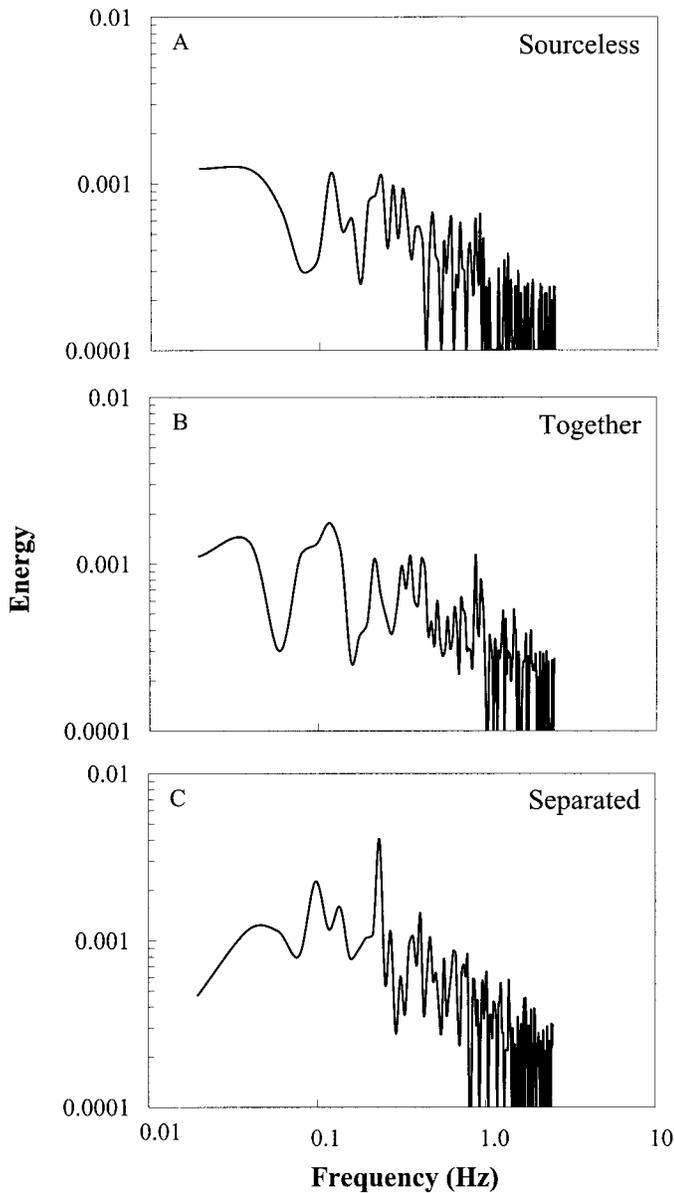


Fig. 6. Frequency spectra for (A) unobstructed, (B) together, and (C) separated source arrangements. Spectra were generated from 60 s of Fourier transformed velocity measurements taken at 5 Hz.

The time crayfish took to emerge from start arenas was similar among treatments (ANOVA $P = 0.51$).

Further analysis that used only those animals that were successful in finding the source of fish chemical confirmed that the search behavior of crayfish was influenced by source arrangement. Among these crayfish, individuals spent less time in various segments of the flumes when chemical sources were separated than when they were together (Fig. 8, repeated measures ANOVA $P = 0.049$). Crayfish spent less time in flume segments as crayfish approached the source (Fig. 8, repeated measures ANOVA $P < 0.0001$). No significant interaction between chemical arrangement and distance from the source (repeated measures ANOVA $P = 0.9$)

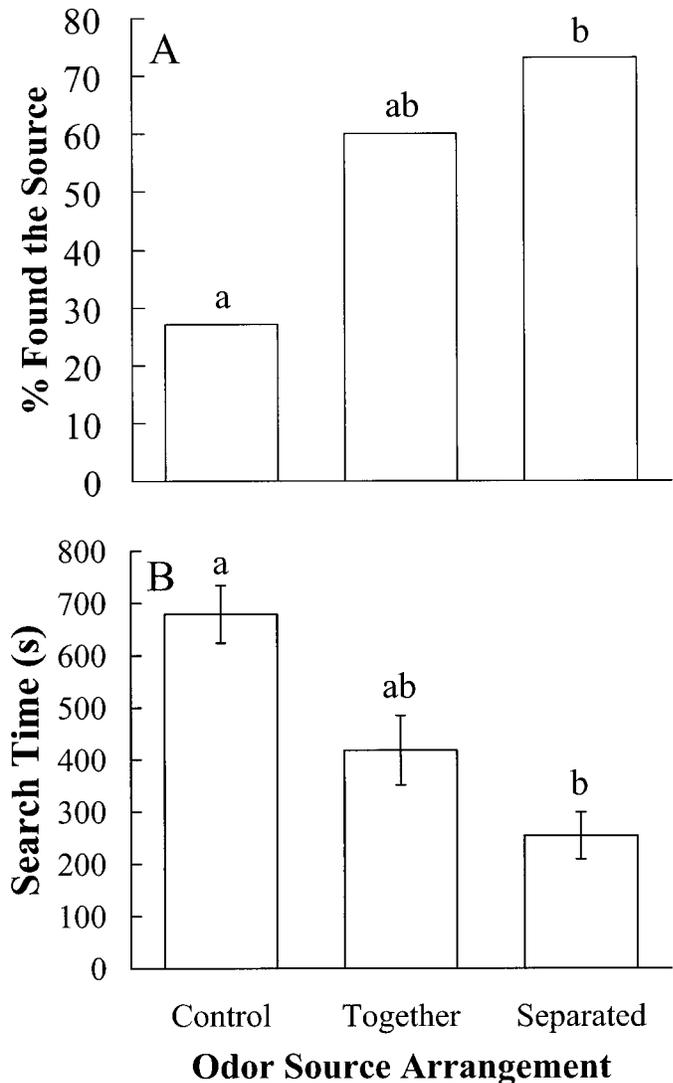


Fig. 7. Effect of source configuration on (A) percentage of success and (B) search time (mean \pm SE, $n = 45$) of crayfish during 15 min behavioral trials in flumes ($n = 45$). Search time was defined as the elapsed time from emergence to end of trial or encounter with the source. Control trials were conducted by use of only plain gelatin. For odor treatments, two fish gelatin blocks were placed either together or separated (one 15 cm upstream of the other). Columns not sharing letters differ statistically ($P < 0.05$).

indicated that crayfish behavior across distance was similar between source configurations.

Shelter use of successful crayfish differed from that of unsuccessful crayfish. Source-finding crayfish spent 33% less time in shelters than crayfish that failed to find the source (t -test $P = 0.0008$). Individuals that found the source entered shelters only half as frequently as those that did not (Mann-Whitney U -test $P = 0.004$).

Discussion

This study combined measurements of the physical/chemical world of aquatic organisms and results of behavioral

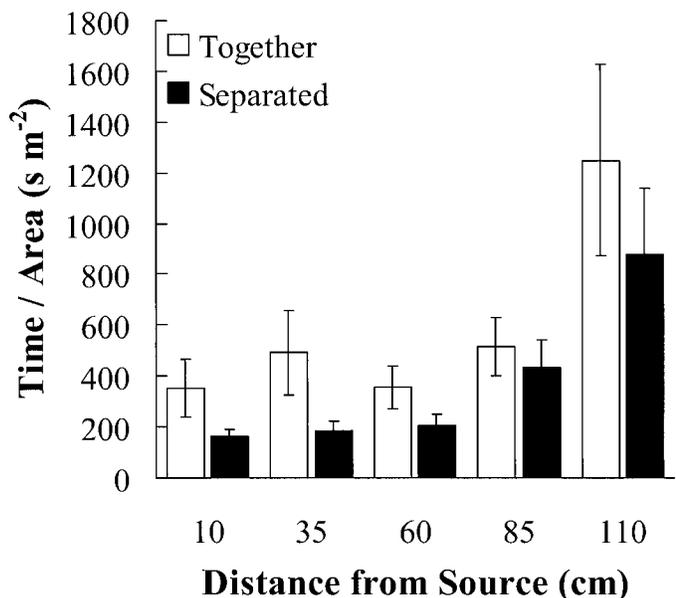


Fig. 8. Effect of chemical source arrangement on the time that crayfish spent in various segments of the flumes (mean \pm 1 SE). The fish gelatin odor sources were placed either together ($n = 9$) or separated, one 15 cm upstream of the other ($n = 11$). Only crayfish that found the source during the 15 min trials and were tested using fish gelatin were included.

experiments to examine the importance of signal structure for chemically mediated search strategies. Our experiments indicated that behaviors influencing the fitness of organisms depend on information gathered from spatial and temporal chemical dynamics within plumes. We found that source spatial arrangement influences crayfish search behavior. Among animals that successfully located the source, individuals found the source faster when chemical sources were spatially separated as opposed to together. It appears that *O. virilis* recognized within-plume differences and efficiently used chemical signals emitted from spatially distinct sources. Future research is needed to characterize what aspects of environmental stimuli organisms use during orientation (Finelli et al. 1999; Zimmer et al. 1999).

Our results are consistent with other studies examining chemically mediated search behavior. Flow rate and substrate, both of which can alter plume dynamics, effected blue crab search efficiency (Weissburg and Zimmer-Faust 1993; Weissburg 1997; Finelli et al. 2000). Crayfish chemosensory search efficiency is enhanced when orienting in habitats with increased turbulent mixing (Moore and Grills 1999). These studies indirectly support the hypothesis that fine-scale fluctuations contain information that animals use when searching for chemical sources.

Dynamics of chemicals released at the source contribute to the fine-scale structure within plumes (Murlis 1986; Monismith et al. 1990; Weissburg 1997). In this study, manipulation of the arrangement of chemical sources influenced fine-scale structure within plumes. Plumes originating from separated sources showed frequent peaks, reduced rise times, and reduced maximum peak concentration. By quantitatively characterizing stimulus profiles among treatments, we are

afforded an often overlooked glimpse into the nature of information contained in chemical plumes.

In order for complex chemical signals to provide information about the location of a stimulus source, signal characteristics must change in a predictable manner with distance downstream (Murlis et al. 1992). Rise time increased and maximum height decreased with distance downstream from the source. Our results confirm earlier studies that demonstrated that maximum peak slope decreased reliably with distance from the source (Moore and Atema 1988; Finelli et al. 1999). These fine-scale characteristics, which have been reported from field measurements (Finelli et al. 1999), may provide animals with additional information useful for effective chemically mediated orientation.

Our velocity measurements indicated that mean flow speed was unaffected by source configuration over most of the flume's working section. At 10 cm downstream from the source, mean velocity was lower in the together versus separated source configurations. This difference indicated that an eddy had developed just downstream of the single mesh bag (i.e., the "source together treatment"). This eddy was removed if another source was located upstream. The effect of this vortex on the chemical distribution would be propagated downstream and would have altered plume structure. Eddy diffusion at the source may have caused unexpected values of peak heights, peak slopes, and rise times we observed at 10 cm. Near-source fluid mixing apparently generated additional chemical information that crayfish used to search more effectively.

These experiments were designed to examine how changes in chemical dynamics influence search behavior when hydrodynamics is held constant. Estimates of the turbulence intensity showed treatment differences only at 35 cm (not at any other distance). Neither the fluctuating component nor mean velocity provided compelling evidence that differences in flow dynamics along the flume could explain the search behavior of crayfish. Crayfish can orient to potential prey using signals from fluid disturbances (Breithaupt et al. 1995). The extent to which crayfish use of fluid dynamics as a navigational cue for chemically mediated search requires additional investigation.

O. virilis used chemical stimuli to locate upstream carrion. Crayfish tested with fish gelatin were three times more likely to find the source than crayfish in gelatin controls. Crayfish success, in locating a food source >1 m away (10 body lengths), was greater than reports for other Crustacea such as blue crabs (10%–35%, Weissburg and Zimmer-Faust 1993) and lobsters (15.7%, Moore et al. 1991). However, the congener crayfish *Orconectes rusticus*, showed $>77\%$ success rates when searching for food (Moore and Grills 1999).

Using search time as a comparative measure, we found that *O. virilis* required on average <6 min to locate a chemical source >1 m upstream. Conversely, traveling farther distances, *O. rusticus* found the source in <4 min on sand and 2.5 min on gravel (Moore and Grills 1999). The efficiency (or time) with which animals locate the source of odor has ecological consequences. Longer searches increase the possibility that competitors will locate and consume a resource. Crayfish show improved growth on protein-rich diets, making fresh carrion an important source of nutrition

(Momot 1995). Carrion attractiveness to organisms changes as material ages (Zimmer-Faust 1993). Resources decompose and the state of decomposition could determine detritus availability and quality. Crayfish would be expected to have efficient search strategies for locating distant protein-rich carrion food sources before significant decomposition has occurred.

From quantitative results of both orientation behavior and signal distribution, we can begin to develop a hypothesis regarding orientation behavior in a complex "olfactory landscape" (Nevitt et al. 1995; Atema 1996). Wehner (1987) developed the idea of a "matched filter" by linking ideas concerning the nature of adaptations to the physical and physiological properties of sensory systems. This hypothesis reasons that the physical, physiological, and behavioral properties of a sensory system are adapted or "matched" to the sensory environment that carries the most relevant information. Organisms live in complex chemical landscapes—namely, multiple chemical plumes containing both attractive and unattractive chemical sources interacting in spatially and temporally dynamic ways. Applying the matched filter hypothesis, we would expect organisms to have evolved morphological, physiological, or behavioral sensory mechanisms that allow them to extract relevant information from multiple signals and respond in an appropriate behavioral fashion. Our results show that crayfish forage more efficiently when presented with more-complex signals (sources separated in space). We hypothesize that chemically mediated orientation behavior, particularly for organisms in high Reynolds-number environments, evolved to function within complex odor landscapes. Physiological, behavioral, and morphological adaptations to these complex natural stimuli enhance their ability to locate distant chemical sources.

Conclusions

This study demonstrated that the spatial arrangement of chemical sources influences search behavior of animals and modifies fine-scale chemical plume characteristics available to animals for use in orientation. These results indicated that chemical signal structure contains information used by orienting organisms. To understand the ecological significance of sensory mechanisms and the selective regime in which these systems evolved, it is necessary to characterize stimulus patterns that are present in the environment. Studies focused on mechanisms of chemoreception and chemical orientation need to recognize that signal properties provide an important source of information to aquatic animals. Experiments focused on orientation should control for signal-specific changes when manipulating the dynamics of the fluid environment. Only by characterizing chemical signal dynamics, hydrodynamics, and search behavior can we elucidate the fundamental mechanisms involved with chemical orientation.

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