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HABITAT-SPECIFIC SIGNAL STRUCTURE FOR OLFACTION: AN EXAMPLE FROM ARTIFICIAL STREAMS

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Abstract—Many animals use chemical signals to acquire information about their habitats. The structure of this information is dependent upon specific features within a habitat, and the information in signals can be habitat-specific. We quantified the spatial and temporal information in an aquatic odor plume in three different artificial stream habitats with different substrate types by measuring turbulent odor plumes with an electrochemical detection system. Streams had one of three substrate types that correlated with typical aquatic habitats: sand ($\approx 4.2 \times 10^{-2}$ cm diameter), gravel (≈ 2.5 cm), and cobble (≈ 4.5 cm). As predicted from the hydrodynamics, the spatial and temporal structures of the signals were different on different substrates. Spectral analysis showed that the sand and cobble substrates had signals that were dominated by lower frequency fluctuations, whereas gravel had the highest and broadest range of signal fluctuations. Cross- and autocorrelations showed that signals on the gravel substrate had the largest spatial and shortest temporal components. Our results imply that the information obtained from chemical signals may be limited in some habitats. These constraints on information may affect how organisms perform chemically mediated behaviors.

Key Words—Chemoreception, fluid dynamics, benthic crustaceans, stream ecology, chemical orientation, odor plume, chemical signals.

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INTRODUCTION

A common behavior seen across a number of different taxa and in a number of different habitats is orientation to a chemical source. In terrestrial habitats, many insects move upwind to locate pheromone sources (Kennedy, 1982; Bell and Tobin, 1982). Lobsters and blue crabs locate food sources by using odor plumes in near-shore marine environments (Moore et al., 1991b; Weissburg and Zimmer-Faust, 1993). Nurse and lemon sharks respond to food odors within flowing water in open ocean habitats (Hodgson and Matthewson, 1971). In freshwater habitats, crayfish can locate carrion sources in artificial streams (Moore and Grills, 1999), and predator–prey interactions can be influenced by chemical signals (Peckarsky, 1980; Petranka et al., 1987). These investigators have shown quite convincingly that fine-scale chemical signals played an important role in the behavior of animals, but virtually nothing is known of the spatial and temporal characteristics of chemical signals across different habitats.

Many researchers have tried to elucidate the underlying mechanisms in initiating and controlling orientation of an organism to a chemical source in both laboratory and field studies. Recent work has outlined three possible behavioral mechanisms for chemical orientation: optomotor anemotaxis in many insects, chemically mediated rheotaxis in blue crabs, and chemotaxis in lobsters.

The temporal information contained within odor signals is particularly important for controlling upwind flight behavior in several species of moths (Vickers and Baker, 1992, 1994; Mafra-Neto and Cardé, 1994, 1995). The intermittent nature of the chemical signal, in particular the timing associated with arrival of odor filaments, is important in mediating either upwind flight or casting behavior of moths. The key feature within the odor signal is the frequency information associated with the turbulent nature of odor plumes. While this work, in particular, has shown conclusively that odor filaments are important for modulating upwind flight or casting behavior, a broader conclusion cannot be drawn because this work has been done for only one hydrodynamic condition and been analyzed in only two dimensions. These moths fly, however, in a number of hydrodynamically different habitats. Other authors have studied the influence of vegetation on the ability of organisms to locate the odor source but have not directly measured odor plume structure (Suckling et al., 1996; Suckling and Karg, 1997).

A mechanism shown in blue crabs is a combination of both chemo- and rheotaxis (Weissburg and Zimmer-Faust, 1993). Blue crabs follow the edge of odor plumes to the source. When they exit an odor plume, they measure their angle with respect to the main flow direction and reenter the plume by reversing that angle. The ability of blue crabs to orient to chemical sources is hindered by increases in fluid velocity (Weissburg and Zimmer-Faust, 1993). While the researchers studied the effect of different flow velocities, the studies were done in only one type of habitat and the fine-scale structure of chemical signals with the different flow velocities was not measured.

Lobsters use true chemotaxis in that the structure of the chemical signal guides orientation to the odor source (Moore et al., 1991b), and results suggest that lobsters follow a spatial gradient in peak slopes or height to locate the odor source. Although the distribution of chemical signals in the orientation tank was measured (Moore and Atema, 1991), any broader conclusion from the lobster study is limited due to the lack of variety in the types of habitats and signal structures tested. Although all of these studies have been critical in drawing a detailed picture of the orientation capabilities of organisms, a broader and more comprehensive ecological theory of chemical orientation is missing because of the limited knowledge of the signal structure during orientation in all of these trials and the lack of any variety in the physical environment for chemical dispersion.

Classical concepts of odor plumes and the mathematical equations describing them are based on spatial and temporal averages of odor concentrations in a terrestrial rather than aquatic environment (e.g., Sutton, 1953; Bossert and Wilson, 1963). These equations characterize odor concentration gradients as smooth functions. The odor diffuses homogeneously from a source to fill a predictable space with gradually decaying concentrations down current. This diffusion concept is generally correct for small organisms that live in environments dominated by molecular diffusion. The random movement of individual odor molecules is still orders of magnitude smaller than the millimeters to meters scale at which small organisms measure the diffusion gradient. Within this scale the diffusion model provides a biologically useful description of odor distribution.

For larger organisms operating in a turbulent fluid environment, the diffusion model loses its power. Although some animals may indeed use average odor concentration to determine spatial information, as in the Sutton model (1953), in most cases it takes an animal far too much time to find the spatial or temporal average of a patchy distribution. A reasonable approximation of the Sutton model for an insect pheromone plume (in air) requires 10 min of averaging (Pasquill, 1961; Gifford, 1968). In many normal behavioral situations this is intolerably long; more efficient competitors will arrive at, and exploit, potential mates or food sources first. Moreover, we know from many orientation studies that animals make correct directional decisions in seconds or less (see Payne et al., 1986, for reviews). To clarify the role that stimulus patterns play in ecologically relevant behaviors, the quantification of stimulus patterns and types of information that are available to animals in a variety of different habitats becomes essential.

This study was designed to quantitatively describe aquatic odor plumes emanating from a biologically relevant source in artificial streams with different substrates. The streams were designed to mimic natural habitats for benthic invertebrates. These same streams were used for a companion study examining the orientation behavior of crayfish to carrion odor sources in streams with different substrates. Specifically, the present study was designed to answer several questions regarding chemical signals: How do habitat structures influence the spatial and temporal information in odor plumes? What hydrodynamic features of stream substrates are correlated with differences in signal structure? Can habitat differences constrain or alter potential information contained in the temporal fluctuations of aquatic odor signals? The results from this study will help build a broader picture of chemosensory information in different habitats and provide some understanding of the environmental processes that structure that information.

METHODS AND MATERIALS

Artificial Stream. All odor plume measurements were performed in a flow-through artificial stream (working section: 170 × 32 × 22.5 cm; Figure 1) constructed in the Stream Research Facility at the University of Michigan's Biological Station. The stream was constructed with standard 20-cm cinder blocks as the sidewalls with two layers of plastic sheeting (6 mil) laid inside the cinder block trough. The bottom edges of the plastic were folded under the cinder blocks to form a flat bottom on the artificial stream. Two sheets of fluorescent light grating (egg crates, 1.69-cm^2 holes) wrapped with nylon screens (0.01-cm^2 holes) were placed upstream to serve as collimators. Unfiltered streamwater, taken from the Maple River, was mixed with groundwater and gravity fed into the front of the stream by 6-in. PVC pipes from a headtank. The stream substrate consisted of sand, gravel, or cobble collected from the Maple River. Sand was placed in the trough to a uniform depth of 2.5 ± 0.3 cm. Sand particles had an average diameter of $4.2 \times 10^{-2} \pm 0.3 \times 10^{-2}$ cm ($N = 30$). Gravel had an average diameter of 2.5 ± 0.1 cm ($N = 25$). Cobbles had an average diameter of 4.5 ± 0.2 cm ($N = 24$). The average flow velocity in the middle of the stream was maintained at 13 ± 0.5 cm/sec as measured with a Marsh-McBirney flowmeter. The dimensions and configuration of the artificial stream were based on accepted principles of flume design and followed previous flume designs (e.g., Nowell and Jumars, 1987; Weissburg and Zimmer-Faust, 1993; Moore et al., 1994).

Odor Source. We chose to mimic an odor source that is potentially important to a number of aquatic organisms, that of a female crayfish releasing urine. This one source could convey a potential mate to male crayfish, a potential predator to small benthic invertebrates, and a potential prey item to stream fish. In addition, the body of the animal influences how chemicals are dispersed downstream. To construct a mimic, the carapace of a female crayfish, *Orconectes rusticus*, was shellacked, fixed with the chelae in front of the animal (total body length = 12 cm), attached to a thin piece of Plexiglas (0.31 cm thickness) and

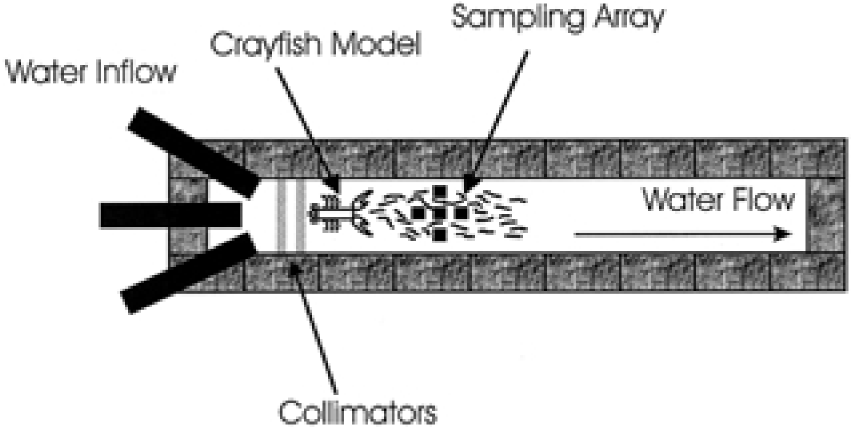

FIG. 1. Schematic (not to scale) of the artificial stream showing the relative positions of the odor source (crayfish) and sampling array. Five points at three different heights above the substrate were used to reconstruct a three-dimensional analysis of the odor plume.

weighted with metal washers. A Pasteur pipet (tip diameter = 1.5 mm) was fed through the tail of the crayfish such that the tip of the pipet exited the crayfish at the front right nephropore. This placed the tip of the pipet 2 cm above the substrate. The large end of the pipet was connected via Tygon tubing to a flowmeter (Manostat No. 2) and then to a reservoir that held the tracer chemical. The model crayfish was placed at the head of the working section and in the middle of the stream. The legs of the model were placed at the level of the surrounding substrate.

The tracer chemical (1 M dopamine) was delivered by gravity feed at a constant rate of 10 ml/min. This resulted in a flow velocity of 2.4 cm/sec (U_∞, 13 cm/sec). These values were chosen to mimic the release of urine from a female crayfish and matched values for urine release as measured in our lab (R. Zulandt and P. A. Moore, personal observations).

Electrochemical Microelectrodes. We used standard IVEC recording procedures for quantifying the fine-scale distribution of aquatic chemical signals (Moore et al., 1989, 1994; Moore and Atema, 1991). We used a graphite–epoxy capillary electrode (Gerhardt et al., 1984) with a tip diameter of 150 μm at a sampling rate of 10-Hz with the IVEC-10 (In Vivo Electrochemistry Computer System; Medical Systems Corp., Greenvale, New York). Each 100-msec epoch for the 10-Hz sampling rate was composed of a 50-msec epoch at +0.55 V (oxidation) followed by a 50-msec epoch at 0.0 V (reduction). The recording electrodes were sampled every 50 msec. Analog-to-digital conversions of the samples occurred at 4 kHz, and data were averaged for the 50-msec time epoch.

Further details of recording and digitizing are explained elsewhere (Moore et al., 1989). Electrodes were calibrated in solutions of dopamine prepared in natural stream water and exhibited excellent linearity over a concentration range of 0.1–1 M ($r^2 > 0.95$). We used a source concentration of 1 M dopamine (and 0.1 M ascorbic acid as an antioxidant).

Recordings were performed at 15 points for each of the three substrates (Figure 1). For clarity, the x dimension was parallel to the flow and the y dimension was the cross-stream dimension. The z dimension was height above the substrate. The middle point in the diamond-shaped array was 60 cm downstream from the odor source. The four additional points in this x–y plane were 10 cm away from this central point ($x = \pm 10$ cm and $y = \pm 10$ cm). Each of these five points was sampled at three different heights above the substrate ($z = 2$, 4, and 6 cm). These heights were chosen to match the heights of various chemoreceptor locations on a crayfish, that is, the midpoint of the major chelae approximately 2 cm above the substrate, the midpoint of the lateral antennules 4 cm above the substrate, and the midpoint of the antennae 6 cm above the substrate. Chemical flux was measured over a period of 4 min in an established odor plume.

Data and Statistical Analysis. All measurements were converted from computer counts to micromolar concentrations of dopamine by using the calibration factor determined for the electrode in static solutions. Odor profile parameters (Figure 2) were extracted from the calibrated odor plume signals based on the definitions set forth in Moore and Atema (1991) and redefined elsewhere (Moore et al., 1994). While many statistical measures and signal analysis procedures could be used to analyze the signals presented here, we chose those parameters and analysis procedures that are most relevant to neuronal detection and behavioral responses to chemical signals. Computer simulations (Moore, 1994) and electrophysiological studies (Gomez et al., 1994; Borroni and Atema, 1988, 1989) have demonstrated that stimulus parameters such as pulse slope, height, length, and off time are important for determining the temporal response characteristics of primary chemoreceptor cells. A general MANOVA (height vs. substrate) was used to analyze for differences in mean values of odor plume parameters. If this was significant, a Newman-Keuls post-hoc test was used to determine individual differences. In addition, standard time series analyses were used to analyze the temporal nature of the chemical signals. Each time series was composed of 2400 data points that were truncated to 2048 data points for Fourier analysis. The data were further divided into five subsamples. This subdivision results in the best estimate of the frequency spectrum, but also includes some loss in frequency resolution at the low frequency end (Moore, 1994; D. Mountain, personal communication). Each of these subsamples was detrended and had the mean subtracted from each data point before the spectral analysis was performed. The final spectral analysis was an average of the spectra from five separate subsamples. Conventional methods for spectral density estimates

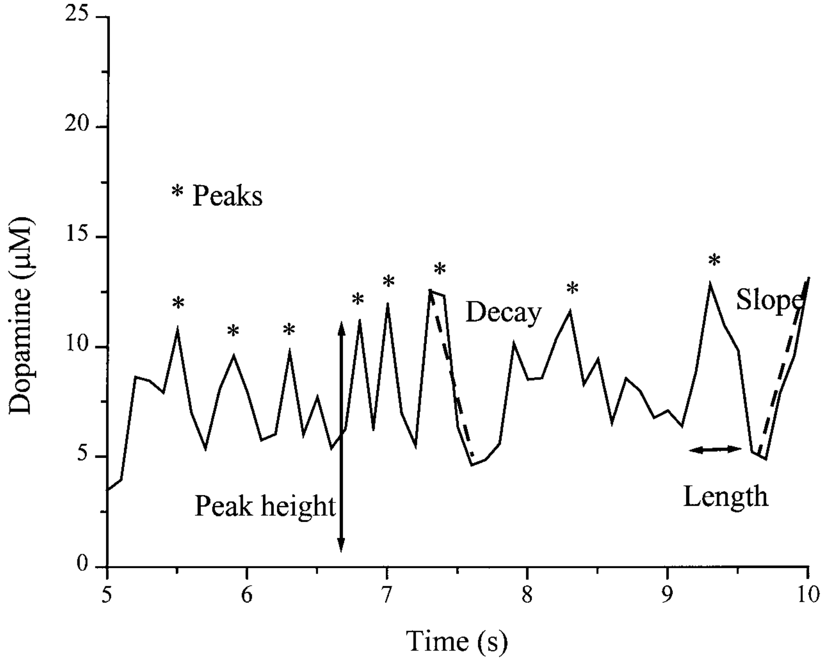

FIG. 2. A representative odor trace showing the parameters analyzed for behavioral information. Peaks (asterisk) are defined elsewhere (Moore et al., 1994). Peak height (in μM) is concentration over baseline level and peak slope (in μM/sec) is the maximum slope during the rising phase of any peak. Complete definitions are found elsewhere (Moore et al., 1994).

were performed with a commercial statistical package (Statistica). Cross-correlation and autocorrelation analysis were used to compare signals at different spatial points (cross-correlation) and signals at different temporal points (autocorrelation).

Hydrodynamic Characterization. The friction velocity ($u*$) and roughness Reynolds number ($Re*$) of water flowing in the working section of the artificial stream were determined by measuring the vertical velocity gradient. The vertical velocity gradient was characterized by timing the velocity of dye puffs at known heights above the substrate. Dye puffs were injected by a gravity-fed system into the tip of a small Pasteur pipet (ID 1 mm). The tip of the pipet was parallel to the flow and extended approximately 5 mm downstream. Velocity profiles were measured for 10 puffs at seven different heights in the log layer following standard procedures (Nowell and Jumars, 1984, 1987). Mathematical calculations follow from previously published work on odor plume dynamics

(Weissburg and Zimmer-Faust, 1993; Moore et al., 1994).

Boundary layer conditions were determined from equations in Schlichting (1979). Shear velocities ($u*$) were calculated by using vertical velocity profiles in equation 1, which was rearranged to solve for $u*$ as follows:

$$U_z = u*/\kappa \ln(z/z_0) \tag{1}$$

where U_z is the mean velocity at height z above the bed and κ is von Karman's constant (0.41). The hydraulic roughness length (z_0) was determined as the y intercept of the statistically significant regression between height above the substrate and the measured flow speed at that height. Roughness Reynolds number ($Re*$) was determined as follows:

$$Re* = (u * D)/\nu \tag{2}$$

where D is the height of the roughness elements (e.g., the diameter of the sand grains, gravel, or cobble), and ν is the kinematic viscosity of the fluid (0.01 $\mathrm{cm}^2/\mathrm{sec}$).

Other hydrodynamic parameters were estimated from the time series of chemical recording data following standard practices (Sanford, 1997; Hart et al., 1996; Kundu, 1990). The root mean square of velocity fluctuations was estimated from the root mean square of chemical fluctuations and averaged across all spatial points for a single substrate. The Peclet number of our flow conditions was approximately 1.3×10^8, indicating that our chemical fluctuations were due to bulk flow and can be used to estimate U_{rms} (Sanford, 1997). To estimate boundary layer characteristics, we used an integral time scale. This was an estimate of the time over which current velocity was highly correlated to itself. This was calculated by performing an autocorrelation on the chemical recordings and determining the area under the curve for each time series (Hart et al., 1996; Kundu, 1990). Integration of the area was stopped at the 95% confidence interval for a random time series. The integral length scale (l) is an estimate of the largest eddy size in the flow and was calculated by multiplying the average integral time scale for a substrate by the average U_{rms} for that same substrate. The turbulent energy dissipation rate (ϵ) was estimated from equation 3 (Sanford, 1997) below.

$$\epsilon = (A_1)(U_{\mathrm{rms}}^3/l) \tag{3}$$

This estimate assumed a constant of 1 for A_1 (Kundu, 1990). From these values, it is possible to calculate both the Kolmogorov microscale (η, equation

TABLE 1. HYDRODYNAMIC CHARACTERIZATION OF BENTHIC BOUNDARY LAYER IN AN ARTIFICIAL STREAM[a]

Substrate	U_∞ (cm/sec)	U* (cm/sec)	Re*	ϵ (cm^2/sec^3)	η	η_s
Sand	13.0	1.12	4.7	26	0.09	0.004
Gravel	13.0	1.87	470	500	0.04	0.002
Cobble	13.0	2.10	950	21	0.09	0.004

[a] Abbreviations: U_∞ = free stream velocity, U*= shear velocity, Re*= Roughness Reynolds number, ϵ = turbulent energy dissipation rate, η = Kolmogorov scale, and, η_s = Batchelor scale.

4) and Batchelor microscale (η_s, equation 5) (Mann and Lazier, 1996). These scales are measures of the smallest eddy sizes (Kolmogorov) and the smallest distance for differences in chemical concentrations (Batchelor).

$$\eta = 2\pi(\nu^3/\epsilon)^{1/4} \tag{4}$$

$$\eta_s = 2\pi(\nu D_s^2/\epsilon)^{1/4} \tag{5}$$

where D_s is the molecular diffusion coefficient of dopamine (2×10^{-5} cm^2/sec). A summary of the hydrodynamic characterization of the benthic boundary layer for the three flow substrates is shown in Table 1.

RESULTS

Qualitative Description of Odor Plumes. The shape of dye filaments as an indicator of the odor plumes was clearly different over the three substrates. With the sand substrate, the dye filaments were very coherent and stayed visible for a long distance downstream (greater than 1 m). The plume on the cobble substrate looked very similar to the plume on the gravel substrates, and over these two substrates the dye filaments were quickly dispersed and moved much more laterally than on sand. The plume on the cobble and gravel substrates exhibited greater effects of turbulence. The filament trajectory was very unsteady and fluctuated both horizontally and vertically in response to turbulent fluctuations in the flow.

As in previous odor measurements, odor profiles were heterogeneous in time, although the odor source was emitting dopamine at a constant rate (Figure 3). All odor time records displayed a number of peaks. The records on the sand substrate typically had the fewest number of peaks (approximately 160 per 4 min), while those from gravel and cobble substrates (at the same locations) had the greatest number of peaks (approximately 270 per 4 min).

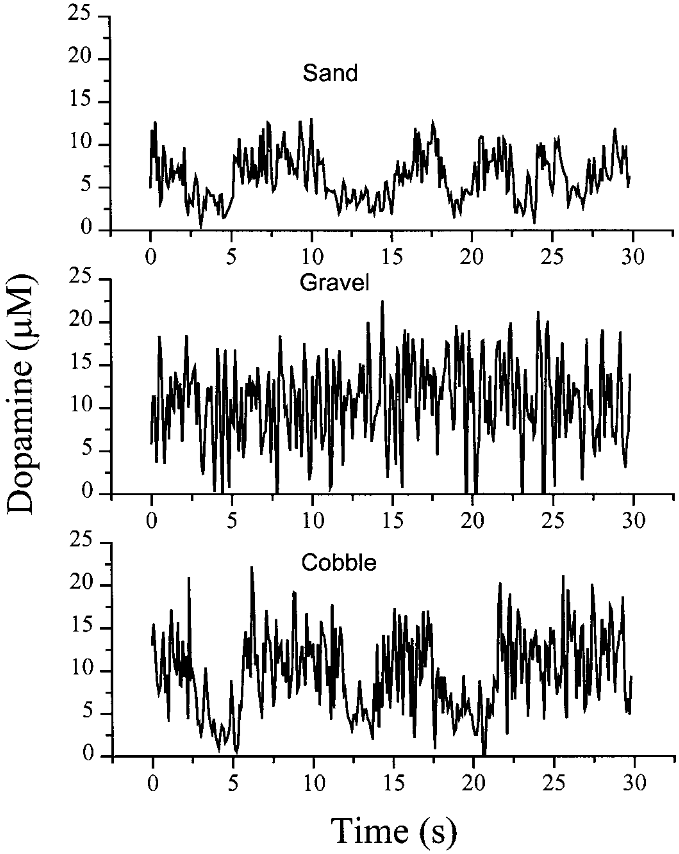

FIG. 3. Typical concentration fluctuations of the dopamine tracer during 30 sec taken from a 4-min record, measured with the IVEC 10 system. Each graph shows odor signals over a different substrate type: sand (top graph), gravel (middle graph), and cobble (bottom graph). Sections were chosen at random from the total 4-min records.

Hydrodynamics. The hydrodynamic parameters of the artificial streams are represented in Table 1. The cobble stream had the highest shear velocity, ($u*$), ~2.10. The gravel and sand streams had lower shear velocities at 1.87 and 1.12, respectively. The presence of the larger diameter objects on the substrate clearly increased the friction velocity. By definition, this increase was also seen in the roughness Reynolds number ($Re*$).

The hydrodynamic variables calculated from the odor signals showed a somewhat different picture of the temporal dynamics. The turbulent energy dissipation rate (ϵ), Kolmogorov microscale (η), and Batchelor microscale (η_s)

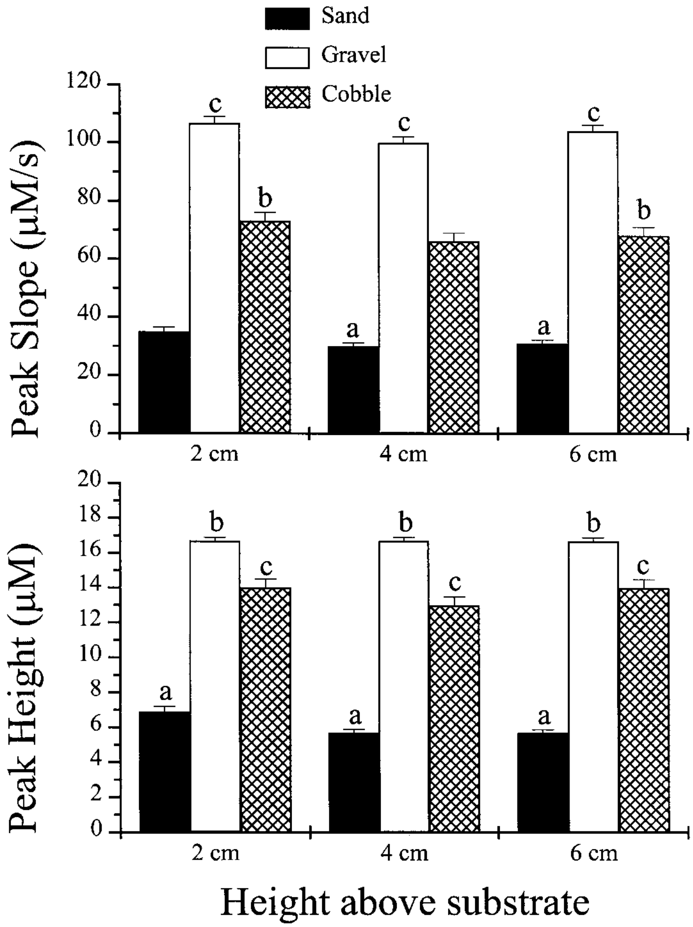

FIG. 4. Mean (+ SEM) values for peak slope (top graph) and peak height (bottom graph) for the different substrates and at different heights above the substrate. Number of valid cases range from 148 (sand, 2 cm height) to 296 (gravel, 2 cm height). Columns with the same letter are not significantly different from each other. Significant differences were determined by a two-way MANOVA followed by a Newman-Keuls post-hoc test. All significant levels were set at $P < 0.001$.

were similar for both the cobble and sand substrate (Table 1). The gravel stream showed a much smaller microscale and a larger turbulent energy dissipation rate.

Pulse Height and Slope. The odor plume on the gravel substrate had significantly higher peak slope and heights than the odor plumes on cobble or sand substrate (two-way MANOVA; Newman-Keuls post-hoc, $P < 0.001$; Figure 4).

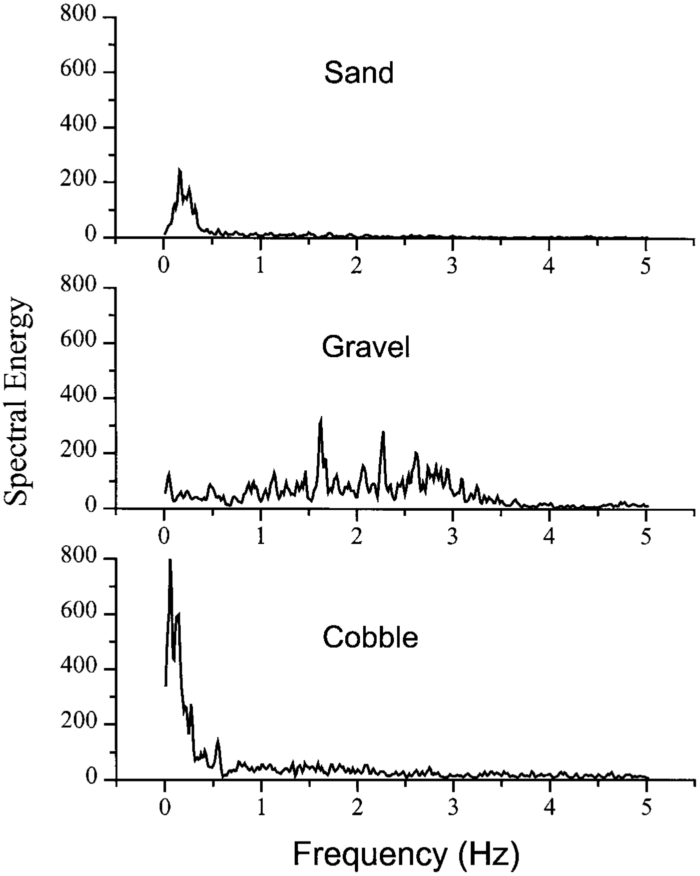

FIG. 5. Spectral analysis of odor signals on three different substrates: sand (top graph), gravel (middle graph), and cobble (bottom graph). The lines consist of points at 0.0125-Hz intervals. Spectral analysis is taken from the odor signal measured at the first point of the diamond array (see Figure 1) at a height of 4 cm above the substrate.

This difference was seen at every point in the spatial array and was consistent across the different heights above the substrate. As seen with previous measurements, both the mean peak slope and height increased closer to the odor source.

Spectral Analysis. Spectral analysis of the odor profiles from the three different substrates showed the change in the temporal characteristics of odor signals. The signals on the cobble and sand substrate were dominated by low frequency fluctuations (Figure 5), with most being well below the 1-Hz range. The signal on the cobble substrate had a much higher density of signal fluctuations

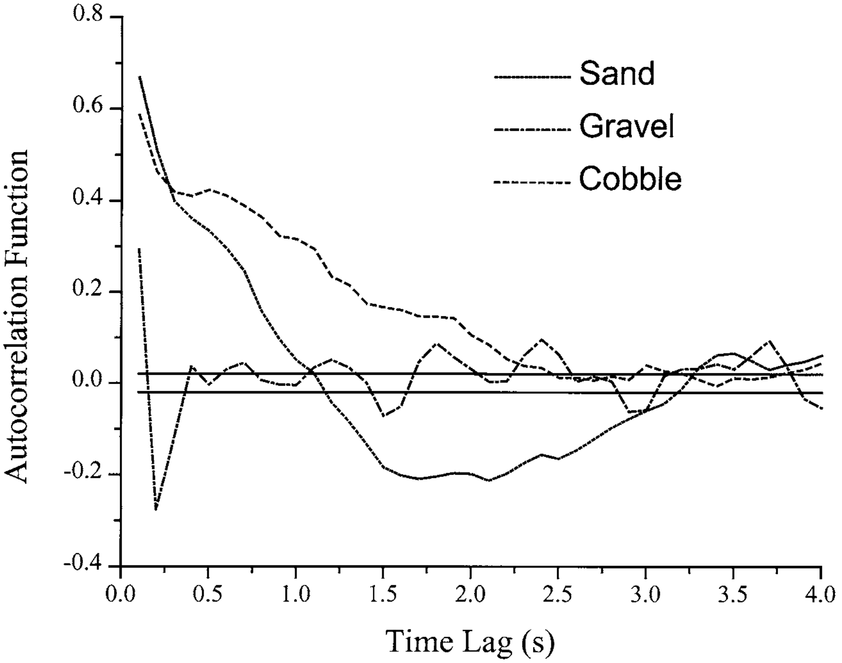

FIG. 6. Autocorrelation function for the odor signals shown in Figure 2 on different substrates: sand (dashed line), gravel (dotted line), and cobble (dash-dot line). The autocorrelation indicates the degree of similarity between the signal and the signal offset time lag (*x* axis). The function ranges in values from -1 (a perfect negative correlation) to 1 (a perfect correlation). The solid black lines are the 95% confidence levels generated from autocorrelation of a white noise signal. Any point beyond the 95% level is statistically significant.

within this band than the signal on the sand substrate. In contrast, the gravel substrate had very little energy in the lower frequency bands (Figure 5, middle graph) with most signal fluctuations occurring in a frequency band from 1 to 4 Hz. There was also a reduction in the spectral density of the signal on the gravel substrate as compared to the signal on the cobble substrate.

To examine any possible repetition in the odor signals and to estimate the temporal scale of correlation, an autocorrelation was performed. As in the spectral analysis, the autocorrelation for odor signals on the sand and cobble substrates were more similar to each other than to those from the gravel substrate (Figure 6). The autocorrelation function for the signal from the sand stream showed a significant positive correlation to a time lag of 1 sec, and then a significant negative correlation from a time lag of 1.25–3 sec. The autocorrelation for the cobble stream showed a significant positive correlation to a time lag of 2.25 sec. Finally, there was no significant correlation with the signal from the gravel streams. These results show that patches on the cobble substrate were twice as long as patches on the sand substrate (2.25 vs. 1.25 sec) and that the temporal fluctuations were much slower on the cobble substrate as compared to the other two substrates.

Cross-correlation analysis can give some estimate as to the spatial variation of odor signals by analyzing similarity in signals from two different locations. A cross-correlation was performed on signals (from the same substrate) that varied by 10 cm along the long axis of the stream (Figure 7). There was no significant correlation associated with different spatial signals on the gravel substrate. The two signals from the sand substrate appeared to have a repeated pattern of chemical fluctuations. The peaks of the correlation occurred at 0.6, 4.2, and 7.7 sec. On the cobble substrate, there was a single positive correlation that had a peak at 5.4 sec. The single peak for the cobble substrate, taken together with the longer positive time lag from the autocorrelation and larger spectral density function, indicated that the chemical fluctuations on this substrate had a much longer and possibly larger temporal and spatial scales than on the other substrates.

DISCUSSION

This study demonstrates three major consequences of different stream substrates for structuring chemosensory information in natural habitats. First, habitats with different substrates can have dramatically different spatial and temporal profiles of chemical signal distribution. Substrates with smaller roughness elements (such as sand) will have chemical signals that are dominated by lower frequency signals, and these signals will have lower peak heights and onset slopes. Temporal features of the odor signal will change independent of the source of the signal. If organisms are relying on the temporal features of odor plumes to make decisions about their environment, these decisions may be habitat specific or the organisms may have a sampling strategy that depends upon the hydrodynamic conditions.

Second, the plume structure depends upon the interaction between plume size and eddy size. Turbulence creates a cascade of eddies that transfer their energy to successively smaller eddies (Kolmogorov scale). This cascade has a lower size limit, below which molecular diffusion is the predominant transport mechanism. The spatial range of eddies is an important phenomenon that structures the information within an odor plume. The interaction between eddy size and plume size is partially responsible for the size and length of concentration fluctuations within the odor plume. Previous authors have described this interaction as three phases of plume growth (Aylor, 1976; Aylor et al., 1976). When the

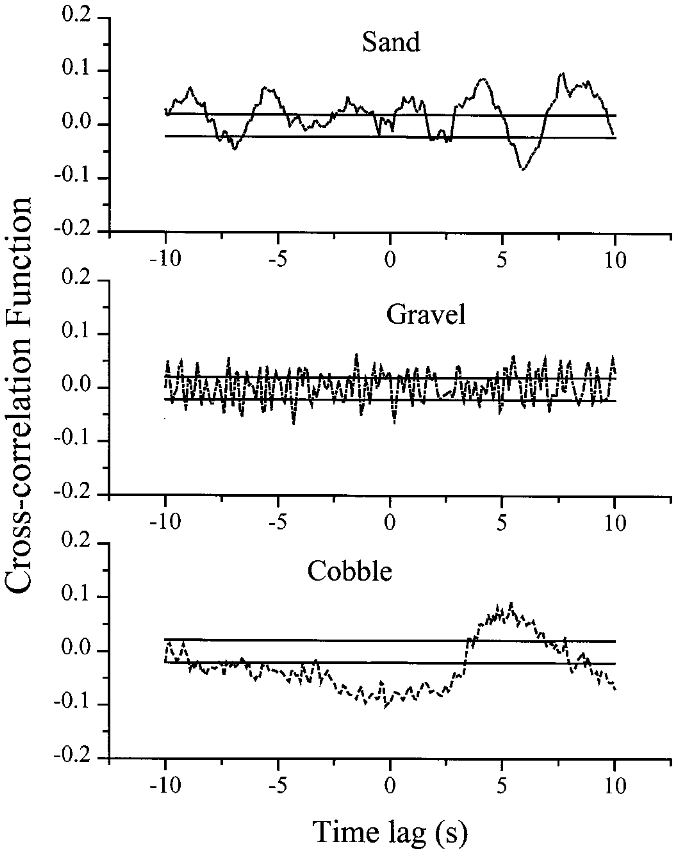

FIG. 7. Cross-correlations of the signals shown in Figure 2 correlated with the signal 10 cm downstream on the same substrate and at same height above the substrate. The cross-correlation indicates the degree of similarity between two signals by using an offset for each signal, hence, the positive and negative time lags. The function ranges in values from −1 (a perfect negative correlation) to 1 (a perfect correlation). The solid black lines are the 95% confidence levels generated from autocorrelation of a white noise signal. Any point beyond the 95% level is statistically significant.

plume is smaller than the smallest eddies, the eddies move the plume as a whole. As the plume begins to expand, it approaches the size of the eddies present in the flow. At this point, the plume is broken into smaller more discrete patches of odor. When the plume is larger than the largest eddies, they redistribute the odor within single patches and begin to homogenize the odor between patches.

Other factors, such as shear, may also play a role in smoothing patch–

nonpatch boundaries (Tennekes and Lumley, 1972). Higher levels of turbulence will have smaller minimum eddy sizes and, thus, smaller and more diffuse odor patches. Lower levels of turbulence will not have as many small eddies, and odor patches will tend to be larger and of higher concentration. Although the cobble substrate had the highest level of turbulence as measured by hydrodynamic methods, the chemical signal generated on this substrate was similar to the sand substrate in fine-scale structure, as found in the values for minimum eddy size (η and η_s; Table 1) and in dye trials. On the cobble substrate, we sampled the odor plume before it could reach phase two of plume growth, so we mainly saw lower frequency fluctuations of the signal.

The third consequence of different stream substrates is the demonstration that increased turbulence generally resulted in lower spatial correlations of the signal. On the sand substrate, signals were correlated in time over short distances (10 cm), whereas the signals on the gravel substrate showed almost no spatial correlation. On the cobble substrate, there appeared to be a rather significant correlation downstream at a time lag of 5 sec. This longer time lag is present because the plume appears to be in the early stages of plume growth. Previous studies have begun to explore microscale properties of chemical distributions within odor plumes and how these properties may contain sensory information valuable to animals (Murlis and Jones, 1981; Murlis, 1986; Moore and Atema, 1991; Zimmer-Faust et al., 1988; Moore et al., 1994). These studies have concentrated on spatial information within a single plume as possible sources of directional information and have shown clear correlations between distance from a source and changes in plume parameters. In this current study, our goal was to describe the sensory environment for chemically mediated behaviors in different habitats (i.e., different substrates). Our study has found that the spatial and temporal relationship of odor plume parameters is highly dependent upon the hydrodynamic forces of that particular flow. Information contained within the temporal fluctuations will vary on different substrates even when the production, release dynamics, and background velocities are the same. Animals using this information for ecological decisions are faced with an interesting problem: How do organisms make the same decisions about an odor source if the information contained in the signal is perceived differently?

The most challenging problem faced by aquatic organisms is using chemical signals as a source of environmental information for orientation to food, mates, or escape from predators. The characteristics (height, slope, frequency) of odor patches constitute information about source distance, direction, size, and age (such as age of carrion) that is potentially useful to organisms. A biological receptor or high-resolution microelectrode moving through these odor patches converts the spatial pattern into a temporal sequence of odor pulses. The odor dispersion described above predicts that the shape of an individual odor pulse will change depending upon its distance from the source and the type of substrate for the stream. Patch gradients and concentration slopes should be steep close to the source and shallow at greater distances from it (Murlis, 1986; Murlis and Jones, 1981; Moore and Atema, 1991; Moore et al., 1994). A comparison by the organism of these odor parameters for several sequential patches encountered while moving could indicate the direction to the source. Given some independent knowledge of the local turbulence (from various mechanoreceptors) and of the nature and probable strength of the source (from the qualitative identification of the odor source, i.e., predator vs. food vs. mate), organisms also might be able to derive an estimate of the distance to the source.

Bilateral sampling of odor allows an animal to measure pulse parameters on its left and right side to detect concentration gradients. The degree of concentration difference between left vs. right stimulation that can enable an animal to determine signal differences is not yet known. This directional decision making (right vs. left side of stimulation) has been shown for lobsters (Devine and Atema, 1982), catfish (Johnsen and Teeter, 1980), and blue crabs (Weissburg and Zimmer-Faust, 1993). If these bilateral concentration differences change on various substrates, these search strategies may become inefficient.

Our results have shown that temporal signal characteristics are altered with changes in turbulence. Morphology and sampling behavior of sensory appendages influences the hydrodynamics of flow around and through them (Rubenstein and Koehl, 1977; Cheer and Koehl, 1987), and this in turn will affect the structure of chemical signals near receptor cells (Moore et al., 1991a; Gleeson et al., 1993; Schneider et al., 1998a,b). Schneider et al. (1998b) hypothesized that the structure of the boundary layer around sensory appendages may be "temporally tuned" for specific odor plume dynamics. For example, some appendages have boundary layers that allow higher frequency signals to arrive at the receptor cells, whereas other boundary layers allow only lower frequency signals through (Moore et al., 1991; Schneider et al., 1998b). Our present study demonstrates that odor plumes and chemical fluctuations may be habitat specific. Thus, it is possible that the morphology and sampling behavior of a chemosensory appendage will be correlated with the hydrodynamics of the flow regime in which they are normally located.

In summary, the information in chemical signals is due to three important factors: hydrodynamics of the environment, hydrodynamics of the source (Moore et al., 1994), and position of the source within the boundary layer (Westerberg, 1991). Even if the latter two factors are kept constant, small changes in the habitat (size of particles in the substrate) can result in large changes in chemical signal structure. Aquatic animals living in different benthic environments may have specific orientation strategies, chemosensory sampling strategies, or locomotory patterns maximizing their ability to extract information from a fluctuating signal. Such strategies would need to be sensitive to hydrodynamic conditions between habitats and even microscale changes within a single habitat.

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