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*Biological Bulletin*, Vol. 183, No. 1. (Aug., 1992), pp. 138-142.

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*Biological Bulletin* is currently published by Marine Biological Laboratory.

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## Measurement of Microscale Patchiness in a Turbulent Aquatic Odor Plume Using a Semiconductor-Based Microprobe

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*The distribution of chemical signals within aquatic environments is highly patchy and heterogeneous due to dispersion by turbulent eddies. We aimed to quantify the smallest spatial scales associated with chemical patches, and therefore measured the structure of chemical signals under turbulent flow simultaneously at two chemical sensors spaced from 200 to 800  $\mu\text{m}$  apart. Measurements were done under controlled stimulus and flow conditions with a novel semiconductor-based, multisite, microelectrochemical electrode (5–2000  $\mu\text{m}^2$  surface area sensors) and a high-speed computer-based recording system. The chemical signals received at the sensor were intermittent, with wide fluctuations in concentration. Patchiness in signal structure was found at spatial scales as small as 200  $\mu\text{m}$ . Significant differences in signal height were found between recordings made at probes spaced 200, 400, 600, and 800  $\mu\text{m}$  apart. These data demonstrate that sub-millimeter patches occur in aquatic turbulent odor plumes. Such differences in chemical signal structure over small spatial scales might be important for marine animals that employ olfactory orientation. We propose alternative ways by which organisms might deal with these fine scale differences in odor concentration. Animals much larger than microscale patches may have evolved elongated olfactory organs that integrate signals, thereby smoothing variations in sensory input. Animals about the same size as micro-*

*patches may be able to capitalize on microscale variation by extracting directional information from turbulent odor plumes.*

Aquatic animals use chemical signals in identifying food and mates, selecting and colonizing substrates, and detecting and avoiding predators. One of the most demanding uses of chemical signals is in the spatial orientation to an odor source. Over the years, there has been considerable debate about the role played by chemical signals in guiding search patterns (1–4). Some of the debate has been due to misunderstanding of the structure of environmental odor signals at the spatial and temporal scales used by receptor cells and behaving animals. In previous studies, time-averaged models have been used to estimate odor concentration gradients along the plume (5, 6). These models are appropriate only for animals sampling odor signals for many minutes before making a decision (7). Because animals often operate at shorter time scales, these models are generally poor in predicting animal behavioral responses (8). Recent advances in technology and theory have shown that distributions of chemical signals are highly patchy at behaviorally relevant time and space scales (See reviews 4, 9, 10). As a result of turbulence, animals located down current of an odor source experience periods well above and below the mean odor concentration (3, 11, 12, 13). It is from these spatial and temporal fluctuations that animals extract spatial information during orientation to an odor source.

Received 2 April 1992; accepted 20 May 1992.

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Many aquatic animals extract spatial information from odor plumes using either chemosensory appendages, such as crustacean antennules and catfish barbels, or solitary chemoreceptor cells scattered along body surfaces (14). Both types of receptor populations play important roles in chemosensory orientation within turbulent odor plumes (15–17). The distance and directional information necessary for orientation toward an odor source is influenced by the spatial and temporal fluctuations within an odor plume. Therefore, knowledge of chemical signal variation within the plume is critical to determining neural and behavioral mechanisms governing chemosensory orientation.

The purpose of this study was to measure microscale chemical signal structure under turbulent conditions, to analyze the signal for potential spatial information available to orienting animals, and to determine the lower size limit of odor patches. We constructed an odor delivery system that simulates the release of chemicals from a suspension feeding clam (a common prey item for many marine predators, including crustaceans and fishes). The resulting microscale chemical signals were quantified by electrochemical recordings at 200 Hz using a novel, multisite semiconductor-based microelectrode. This sensor had five recording sites, each spaced 200  $\mu\text{m}$  apart along the electrode shank. Odor signals were measured simultaneously from two sensors spaced 200, 400, 600, or 800  $\mu\text{m}$  apart.

The chemical tracer (2 mM dopamine with 0.01 mM ascorbic acid as an anti-oxidant) was introduced into the carrier flow through the excurrent siphon of a model clam. The clam was designed on the basis of principles and procedures applied previously (18). The size and pumping rate of our model corresponded to a small hard clam, *Mercenaria mercenaria*, common in estuaries along the Atlantic and Gulf of Mexico coasts of the United States. The model clam consisted of a pair of plastic tubes that simulated the excurrent (3.1 mm I.D.) and incurrent (4.7 mm I.D.) siphons. The tubes were placed contiguously, with the tips set 3 mm above the substrate and the excurrent siphon positioned downstream. The excurrent flow was supplied from a small, constant-head tank, while the incurrent flow was taken by gravity feed from the flume. Flow rates of  $0.51 \pm 0.02$  ml/s were determined with a flowmeter. The Reynolds number of the jet flowing at the excurrent siphon was  $\approx 42$ , indicating that flow was laminar at the excurrent exit (18).

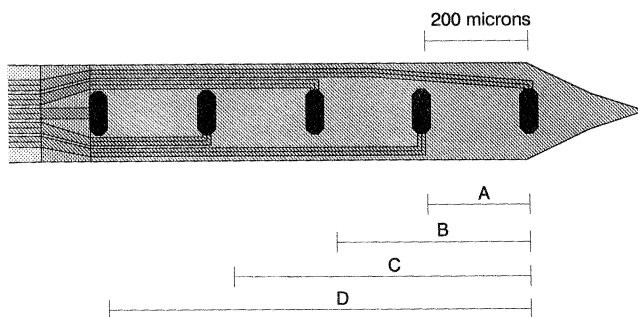
Chemical measurements were made in a fully developed boundary layer flow created in a  $10 \times 0.75 \times 0.15$  m recirculating flume (Weissburg and Zimmer-Faust, in prep.). The free-stream current speed was  $3.8 \pm 0.2$  cm/s; shear velocity was 0.33 cm/s, and roughness Reynolds number was  $\approx 1.8$ . The working section ( $1 \times 0.45$  m) was located 7.5 m downstream of the entry and 1.5 m up-

stream of the exit weir. The entire bottom of the flume was layered to a uniform depth with sand ( $351 \pm 10$   $\mu\text{m}$  diameter,  $n = 100$ ). The seawater had a salinity of 25 ppt and a temperature of  $25.0 \pm 0.5^\circ\text{C}$  during experiments.

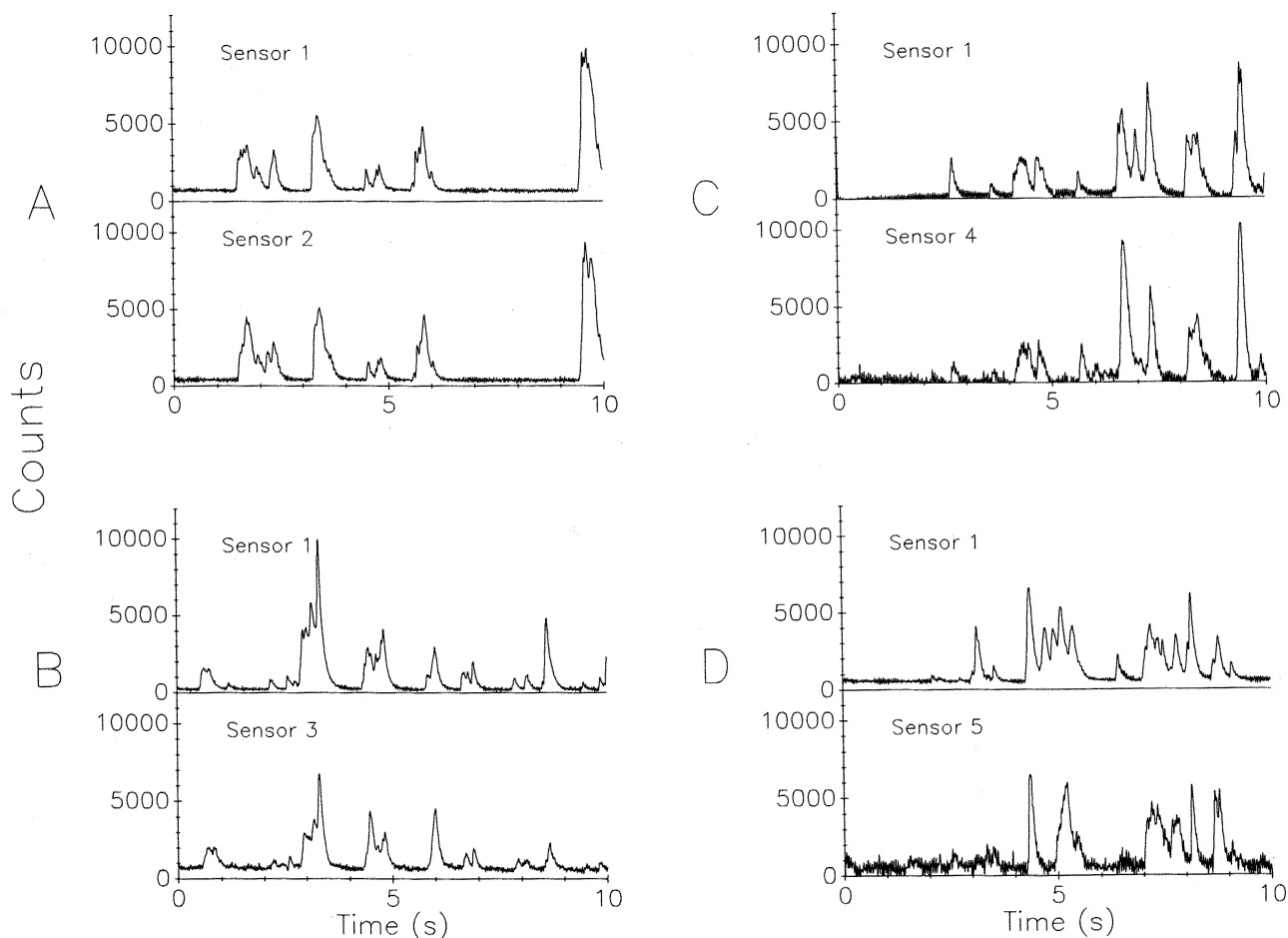
The multichannel (5 recording sites per sensor), semiconductor-based electrodes were fabricated by the Center for Integrated Sensors and Circuits, University of Michigan, Ann Arbor, using high-yield approaches (19). Each sensor site was sputter-coated with 500 nm of iridium, and then wire bonded to a circuit board carrier. The electrode recording sites were positioned 200  $\mu\text{m}$  apart (center to center of site distance; Fig. 1). The individual sites were oval in shape, each having a surface area of 2000  $\mu\text{m}^2$ . The shank was 150  $\mu\text{m}$  wide and 15  $\mu\text{m}$  thick.

Recordings were made at 200 Hz using IVEC-5 (In Vivo Electrochemistry Computer System; Medical Systems Corp.) and a customized recording program (available on request). The basic principles used in applying this technique to aquatic chemical detection can be found elsewhere (20). During each 5-ms epoch, 20 data points were collected (10 points per sensor). Each sensor was sampled alternately (*i.e.*, during any 5-ms epoch, points 1, 3, 5, 7, 9, . . . were sampled from channel 1; points 2, 4, 6, 8, 10, . . . from channel 2). The ten points were summed and stored as the final 200 Hz data point. The electrode was held at a fixed voltage of +0.55 V (*vs.* Ag/AgCl reference) during the whole recording sequence. A micromolar/count calibration factor could not be determined for the sensor prior to the experiment because of the prototypical nature of the two channel 200 Hz recording technique. Post-experiment calibration showed a typical linear response relationship between concentration and counts for all five sensors (21). Typical calibration factors for 2000  $\mu\text{m}^2$  sensors range from 100 to 300 counts/micromolar.

We placed the electrode 31 cm downstream of the excurrent siphon and 6 cm above the sand bed, with the face of the electrode directed perpendicular to the flow. The Reynolds number generated by the probe was  $\approx 3$ ,



**Figure 1.** Diagram of multichannel semiconductor-based sensor showing five, 2000  $\mu\text{m}^2$  recording sites. Recording sites were spaced 200  $\mu\text{m}$  apart (center to center distance). Sites are numbered consecutively starting from the tip (#1) to the last site up the shank (#5).



**Figure 2.** Examples of 10-s odor profiles measured at each of the four sensor pairs. Sensor #1 was used in all paired recordings as the reference point. Distances are Sensor 1:2, 200  $\mu\text{m}$  (A); Sensor 1:3, 400  $\mu\text{m}$  (B); Sensor 1:4, 600  $\mu\text{m}$  (C); Sensor 1:5, 800  $\mu\text{m}$  (D). Paired recordings were made simultaneously. X-axis tick marks represent 1-s intervals and Y-axis tick marks represent 1000 counts (See text for explanation of counts).

indicating little separation for the flow around the sensor. A total of ten 10-s periods and one 20-s period were recorded for each pair of recording sites.

We digitally filtered the recordings with a 40 Hz low-pass filter. Construction of this filter was based on C-language algorithms (22). All signal analysis procedures (except filters) were performed with a commercial signal processing program (DADiSP Worksheet). Frequency spectra showed electronic noise spikes at 60 Hz and several harmonics of this frequency. Spectra also showed that most of the chemical signal data was below 10 Hz.

Each recording was set to a baseline determined by averaging signals over a 2-s period. Profiles that were set to a baseline were analyzed for pulse height, defined previously as peak concentration level above background associated with a microscale patch (See Methods in 3). Pulse height can potentially provide directional information to animals during chemical orientation within an odor plume (3, 4, 11, 12, 23). We compared the pulse height recorded

simultaneously at two sensors by taking the absolute value of the difference. We repeated this procedure for all of the pulses within an entire data set. The differences were then averaged, the value serving as the 'mean difference' in pulse height between sensor pairs. Statistical significance was determined using ANOVAs and *post-hoc* Tukey-Kramer multiple comparisons (24).

Although dopamine was released continuously, the electrochemical signals were very heterogeneous in space and time (Fig. 2). The chemical signal measured here is similar to those previously recorded in the laboratory (3, 13) and in the field (11, 14, 25). Heterogeneity in chemical signals (Fig. 2) resulted from turbulent eddies. Mechanical forces act within a moving fluid to create large scale eddies (compared to the initial size of the odor plume) that transfer their energy to successively smaller eddies until energy is dissipated as heat. This cascade (the Kolmogorov scale) has a lower size limit below which molecular diffusion determines the distribution of odor molecules.

The smallest size at which patchiness in chemical signal structure occurs can be estimated from the differences in pulse heights at different sensor spacings (Fig. 3). The mean difference in pulse heights between sensors spaced 200  $\mu\text{m}$  apart was significantly less than sensors spaced: 400, 600, 800  $\mu\text{m}$  apart ( $P < 0.01$ ). The difference in pulse height at 200  $\mu\text{m}$  (900 counts, 10–50% of any pulse height) is less than half that at the other sensor spacings. Results indicate that the minimum size of the turbulent eddies and spatial patchiness in the chemical signals was about 200 to 400  $\mu\text{m}$ . When the distance between recording sites increases, there are periods during which only one sensor detects an odor pulse, as seen in Figure 2D (3–4 s). Therefore, chemosensory receptors located only hundreds of  $\mu\text{m}$  apart on an animal's olfactory appendage can apparently receive very different signal inputs.

The observed minimum spatial scale of concentration fluctuation can be compared to the theoretically derived values for turbulent flow  $\eta$  (26). The minimum scale of turbulent eddies is:

$$\eta = (\kappa z \nu^3 / U^*{}^3)^{1/4} \quad (1)$$

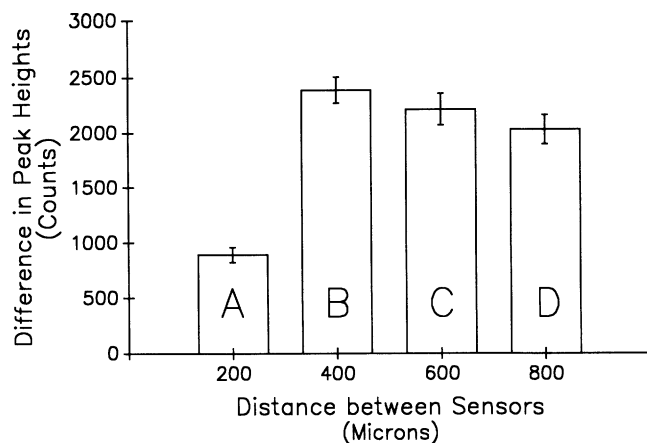
where  $\kappa$  is 0.40 (von Karman's constant),  $z = 6$  cm (distance above the sand bed),  $\nu = 0.01$   $\text{cm}^2/\text{s}$  (kinematic viscosity of water), and  $U^* = 0.33$   $\text{cm}/\text{s}$  (shear velocity). The minimum scale of concentration fluctuations ( $\eta_c$ ) is:

$$\eta_c = \eta(D/\nu)^{1/2} \quad (2)$$

where  $D = 10^{-5}$   $\text{cm}^2/\text{s}$  (molecular diffusion coefficient). Applying equations 1 and 2, we find  $\eta \approx 1000$   $\mu\text{m}$  and  $\eta_c \approx 30$   $\mu\text{m}$  for the conditions of our experiments. These order of magnitude estimates approximate the spatial fluctuations measured in our study.

The interaction between the size of the turbulent eddies and that of the odor plume determines the properties of concentration fluctuations within the plume (27, 28, 29). At the sub-millimeter scale, odor patchiness depends greatly on the lower size limit of turbulent eddies. Knowledge of fine-scale turbulence may therefore provide further insights into the form of odor signals and the evolution of the sensory systems designed to extract information from them.

In the present study, differences in odor signals occurred at spatial scales as small as 200–400  $\mu\text{m}$ . One mechanism used by aquatic animals to orient to odor sources involves the comparison of chemosensory input at paired bilateral appendages (15–17). In this case, orientation requires that microscale differences within odor patches be averaged or integrated along each receptor organ so that differences between organs may be fully resolved. The spatial integration along a chemosensory appendage would have to occur at spatial scales larger than the spatial scale of differences in odor signals to reduce the "chemical noise" caused by small scale concentration fluctuations.



**Figure 3.** Mean differences ( $\pm$ S.E.M.) between peak heights in simultaneously recorded odor profiles at distances of 200 (A:  $n = 200$ ), 400 (B:  $n = 159$ ), 600 (C:  $n = 195$ ), and 800  $\mu\text{m}$  (D:  $n = 230$ ). Mean difference for 400, 600, and 800  $\mu\text{m}$  distances were significantly different from 200  $\mu\text{m}$  ( $P < 0.01$ ), but not significantly different from each other.

Conversely, if significant spatial differences in chemical signal structure consistently occur at the scales shown here, then directional and distance information from odor plumes could potentially be derived via differential sensory input along a single chemosensory appendage for larger animals, or between paired sensors for smaller animals, e.g., copepods. In these cases, discrete spatial innervation along a chemosensory appendage would be required to preserve the integrity of spatial differences in odor signals. Spatial integrity of chemosensory innervation has been shown for the catfish taste organs (30, 31), but the innervation at the sub-millimeter spatial scales mentioned here have not been studied.

In summary, our measurements have shown that differences in chemical signal structure can exist at sub-millimeter spatial scales in benthic boundary layers. Further work is now needed to determine the relationship between hydrodynamics and the spatio-temporal structure of odor plumes, and to test whether microscale patchiness is used by animals to exact directional and distance information about odor sources. In addition to the results gathered here, the multisite electrode provided excellent sensitivity to dopamine under prolonged exposure to seawater and advective conditions. Continued studies on microscale patchiness can help to define the patterns of spatial innervation in chemosensory appendages that are necessary to preserve information enabling animals to orient in turbulent odor plumes.

#### Acknowledgments

This work was supported by USPHS grant #'s AG00441, AG06434, and NSF grant # BNS 9110308 to GAG; NSF grant #'s RII-8996152, DIR-8954231, DIR-

9013187 to RZ-F; and ADMH Drug Abuse Training Fellowship # AA07464 to PM.

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