Vol. 74: 303–306, 1991 | MARINE ECOLOGY PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published August 8

Subnose-1: electrochemical tracking of odor plumes at 900 m beneath the ocean surface

Jelle Atema[1,*], Paul A. Moore[1,3], Laurence P. Madin[2], Greg A. Gerhardt[3,]**

[1] Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts, 02543, USA
[2] Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA
[3] Departments of Psychiatry and Pharmacology, University of Colorado Health Sciences Center, Box C268-71, 4200 East Ninth Ave., Denver, Colorado 80262, USA

ABSTRACT: High-resolution (18.75 Hz) microscale (three, 200 μm average diameter sensors) measurements of the turbulent distribution of chemicals from an odor source initiated from a platform at 900 m beneath the ocean surface were studied from a deep-diving submersible, the Johnson Sea-Link. The recording sensors were attached to a movable mechanical arm on the submersible and signals from the chemical sensor were recorded using a specially designed battery-powered microcomputer-controlled instrument. The recordings showed that both rapid and slow time course chemical signals existed in the odor plume at distances of as much as 50 m from the chemical source. In addition, these studies support that this new technology can be successfully employed to study the dynamics of odor dispersal and/or nutrient chemical dynamics in a remote marine environment.

INTRODUCTION

The precise measurement of the microscale distribution of chemicals within aquatic environments is an important issue in many different fields of science. In sensory biology, a thorough description of the distribution of chemical signals will probably lead to a better understanding of chemo-orientation (Atema 1988, Moore & Atema 1988, Murlis et al. 1991) and the properties of chemoreceptors. In the field of marine ecology, there is controversy on the size and lifetime of microscale nutrient patches (for examples see Lehman & Scavia 1982a, b, Currie 1984). The direct measurement of nutrient patches will likely provide some insight into the utilization of these patches by bacteria and algal cells. In addition, understanding the types of chemicals and their distributions around different substrate types and chemoreceptor organs will also help shape theories and ideas about larval settlement. In all of these cases, it is the lack of knowledge about the fine-scale distribution of chemicals in the natural environment of chemosensory structures that has led to controversy and scientific debate.

Most current techniques for the measurement of chemical distributions, which include spectrophotometric methods (Atema 1985), conductivity probes (Moore & Atema 1988) and fast fluorometric probe designs (Zimmer-Faust et al. 1988), lack the necessary spatial and temporal resolution and/or sensitivity to measure chemical signals at biologically relevant scales. In efforts to address some of these sampling limitations, we have recently introduced electrochemical electrodes as a novel method for the high-resolution measurement of aquatic chemical signals in the laboratory (Moore et al. 1989). With this microcomputer-based recording technique, a temporal sampling rate as high as 200 Hz can be obtained with sensing electrodes that sample spatial scales as small as 10 to 30 μm. The characteristics of these electrodes have been well defined in studies of the mammalian nervous system (Rice et al. 1985, Gerhardt & Palmer 1987). In addition, electrochemical electrodes can selectively detect specific molecular species against a high salt and chemical noise background (Moore et al. 1989).

The purpose of this study was to explore the utilization of these microelectrochemical detection methods for studies of microscale chemical events in an open ocean environment. First, a portable field version of our electrochemical instrument was constructed and the electrochemical sensors were adapted to be attached to the movable arm on the deep-diving submersible, the

* Addressee for correspondence
** Addressee for electrochemical information

0171-8630/91/0074/0303/$ 03.00

Johnson Sea-Link. Secondly, preliminary studies of odor signal dynamics in a open ocean environment were conducted by releasing a chemical tracer (dopamine) from a point source (4 mm diameter opening) on a platform submerged to a depth of 900 m. These conditions were chosen to simulate a mid-water odor plume emanating from a food source.

METHODS

The basic electrochemical recording technique for measuring an odor plume is outlined elsewhere (Moore et al. 1989). These measurements were conducted using an array of sensors connected to an electrochemical instrument controlled by a portable microcomputer. The battery-powered potentiostat was a specially modified design of a similar device that has been previously reported (Gerhardt & Adams 1982). The potentiostat outputs were connected to a battery-powered analog-to-digital converter (12 bit resolution, 18.75 Hz acquisition rate) that was serially linked to a Zenith Z-183 microcomputer. The measurements were performed by applying a fixed 0.55 V potential to the electrochemical array, and the resulting oxidation current output of the sensors was digitized at the 18.75 Hz A/D sampling rate. All data acquisition and display programs were written in Microsoft Basic. Instrument schematics and software are available from the authors upon request.

The purpose of this study was to explore the possible use of these chemical detection methods for the study of microscale dynamics in an open ocean environment. To do this, the portable field version of our electrochemical instrument was constructed and the electrochemical sensors were adapted to be attached to the movable arm on the deep-diving submersible, the Johnson Sea-Link. The electrochemical sensor was composed of 3 graphite-epoxy capillary (GEC) electrochemical recording electrodes (130, 200, 270 µm diameter) (Gerhardt & Palmer, 1987) cemented in hot-melt glue. A schematic diagram of the assembly is presented in Fig. 1. A large glass fluid-filled silver/silver chloride reference electrode was included in this assembly. This electrode array 'Subnose-I' was attached to the manipulator arm of the Johnson Sea-Link, which allowed positioning of the electrode array from within the submersible. Due to the technical difficulties associated with the preliminary construction of an underwater probe assembly, the electrodes used could not be properly calibrated. However, from laboratory tests of the electronics and similar electrodes, a calibration factor for the electronics was established. These tests revealed a calibration factor of 0.29 nanoamperes per A/D count.

The preliminary study of odor signal structure in a deep ocean environment was conducted at a site 6 km south of St. Croix, U.S. Virgin Islands (17° 38' N, 64° 45' W) during the Caribbean Deep-sea Studies (CARDS) project in May 1989. An artificial odor plume was created by releasing a chemical mixture from a platform moored 5 m above the bottom at a depth of 900 m. For this test, a solution containing 50 mM dopamine (tracer), 0.5 M ascorbic acid (anti-oxidant) and fluorescein dye was prepared in raw seawater chilled to ca 6 °C. A dispensing container (diameter of opening 4 mm; total volume 8 l) filled with the test solution was taken to the bottom and placed on an underwater platform by the submersible. The chemical 'odor' signal was then released at a constant rate over a period of ca 30 min. The tidal currents measured at this site from the submersible flow velocity meter attained a max-

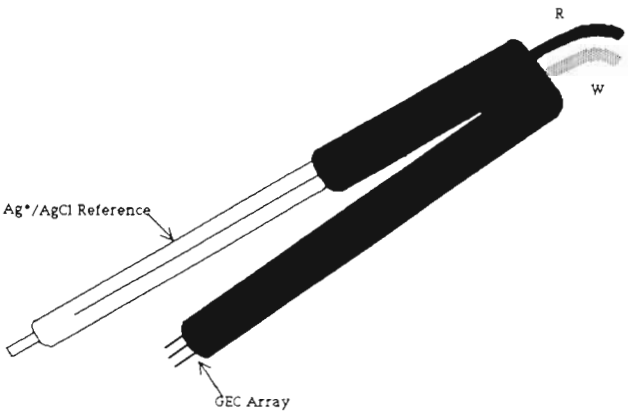

Fig. 1. Schematic diagram of the electrochemical array used for the recordings of odor signals at 900 m from the ocean surface. R: reference electrode lead wire; W: carbon array or 'working' electrode lead

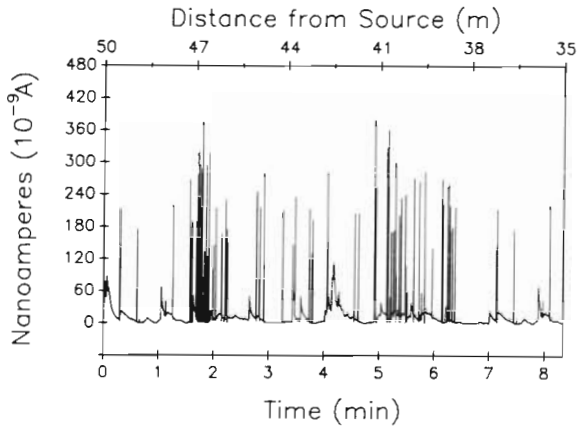

Fig. 2. First 8 min of odor profile from the complete 50 m track taken by the submersible as it moved towards the source of the odor The upper axis shows approximate distances from the odor source. Distances based on average speed of submarine during track to source

imum velocity of about 20 cm s^{-1}. The tidal current produced a turbulent odor (fluorescein and dopamine) plume visible for many meters downstream. During the trial, the submersible moved slowly towards the source from ca 50 m down-current.

RESULTS AND DISCUSSION

The turbulent dispersion of odor results in an odor signal very patchy in structure. These patches are measured as periods of large concentration fluctuations intermixed with periods of little or no concentration change. The record obtained in this study (Fig. 2) mimics those that have been measured in laboratory studies (Moore & Atema 1988, in press). Even at the high flow speeds (up to 20 cm s^{-1}) present at this test site, the patchy nature of the odor signal is seen at the greatest distances from the source (50 m; beginning of track Fig. 2). Thus, microscale (ca 0.6 to 1 mm) patches existed as much as 50 m from the source, i.e. after about 4 min of dispersion. Further work is needed to determine if this is true for other ocean conditions.

During the recording session, a total of 353 odor (dopamine) pulses were encountered during the 50 m transit to the source. Of these pulses, n = 39 (11 %) had slow rise times (100 to 1000 nA s^{-1}) and small amplitudes (Fig. 3a). These signals typically lasted from 2 to 10 s. The other 89 % (n = 314) had very steep slopes (>1900 nA s^{-1}) and large amplitudes (Fig. 3b). These very brief events were typically only 100 ms in duration but were 3 to 50 times larger in amplitude than the slower pulses. In most of these events, the rising part of the odor pulse was faster than the 18.75 Hz acquisition rate of the circuitry, resulting in signal distortion (such as occasional undershoot; Fig. 3b).

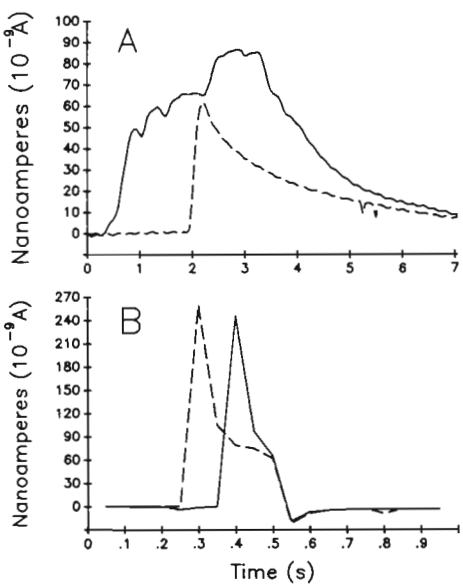

Fig. 3. Examples of electrochemical data collected from 900 m. (A) Recordings of low frequency responses. (B) Recordings of high frequency responses. Dotted lines are different examples from same record. All examples taken from the complete track, shown in Fig. 2

Laboratory calibrations of similar electrodes gave a rough idea of concentration per nanoampere of oxidation current output from the electrodes. With this calibration, we can approximate the concentration levels detected by the subnose probes. The maximum amplitude signals (Fig. 3b) are ca 250 µM signals (i.e. 200 × source dilution), while the small signals (Fig. 3a) are ca 25 to 50 µM in size (i.e. 1000 to 2000 × dilution). These numbers are rough estimates based on laboratory tests and represent only order of magnitude estimates. Future versions of this technology will have exact concentration calibration capabilities.

The high flow speed at the underwater platform (20 cm s^{-1}) resulted in signal frequencies far greater than we had anticipated. Frequency and amplitude characteristics of the micro-patches were too high to be fully accommodated by the data acquisition rate of our electronics. A redesigned version of the instrument is being developed to overcome these limitations in the recording technology for future studies in open ocean environments.

In laboratory studies (Moore & Atema 1988, in press), the slopes of odor pulses increase in value near the odor source. This has been proposed as a mechanism for orientation within a turbulent odor plume (Moore & Atema 1988). In this study we were unable to determine whether the slopes of odor pulses actually increased towards the source due to the clipping and other distortion caused by the unanticipated high slopes. In addition, the turbulence measured here may be characteristic of the wake of the odor delivering platform and not the surrounding ocean. Future studies will allow the detailed characterization of open ocean odor plumes.

In summary, this study demonstrates that the technology used allows 'real-time' measurements of microscale odor dynamics in an open ocean environment. This technique, with further refinements, will allow the direct measurement of chemical species in the natural oceanic environment at the time and space scales relevant to the organism or situation under study. We expect that this type of work will give us insights regarding questions about chemo-orientation, nutrient patch size and lifetimes, and chemical cues used in larval settlement.

Acknowledgements. This work was supported by a career development award to G.A.G. (AG00441), NSF grant (BNS 90-12952) to J.A., and a grant from NOAA's National Undersea Research Program (NA88AA-H-UR020) to L. P. M. Special

thanks to Dennis Levin for hardware and software development, Russ Peters for underwater gear and to Don Liberatore for piloting the submersible. This is contribution No. 7 from the Beebe/CARDS Project and 7598 from the Woods Hole Oceanographic Institution.

LITERATURE CITED

Atema, J. (1985). Chemoreception in the sea: adaptation of chemoreceptors and behavior to aquatic stimulus conditions. Symp. Soc. exp. Biol. 39: 387–423

Atema, J. (1988). Distribution of chemical stimuli. In: Atema, J., Popper, A. N., Fay, R. R., Tavolga, W. N. (eds.) Sensory biology of aquatic animals. Springer-Verlag, New York, p. 29–56

Currie, D. (1984). Microscale nutrient patches: do they matter to the phytoplankton? Limnol. Oceanogr. 29: 211–214

Gerhardt, G. A., Adams, R. N. (1982). Battery-powered apparatus for chronoamperometric measurements. Analyt. Chem. 54: 1888–1889

Gerhardt, G. A., Palmer, M. R. (1987). Characterization of the techniques of pressure ejection and microiontophoresis using *in vivo* electrochemistry. J. Neurosci. Meth. 22: 147–159

Lehman, J. T., Scavia, D. (1982a). Microscale patchiness of nutrients in plankton communites. Science 216: 729–730

Lehman, J. T., Scavia, D. (1982b). Microscale nutrient patches produced by zooplankton. Proc. natn. Acad. Sci. USA 79: 5001–5005

Moore, P. A., Atema, J. (1988). A model of a temporal filter in chemoreception to extract directional information from a turbulent odor plume. Biol. Bull. mar. biol. Lab., Woods Hole 174: 355–363

Moore, P. A., Atema, J. (in press). Spatial information in the three-dimensional fine structure of an aquatic odor plume. Biol. Bull. mar. biol. Lab., Woods Hole

Moore, P. A., Gerhardt, G. A., Atema, J. (1989). High resolution spatio-temporal analysis of aquatic chemical signals using microelectrochemical electrodes. Chem. Senses 14: 829–840

Murlis, J., Willis, M. A., Cardé, R. T (1991). Odour signals: patterns in time and space. ISOT Symposium (in press)

Rice, M. E., Gerhardt, G. A., Hierl, P. M., Nagy, G., Adams, R. N. (1985). Diffusion coefficients of neurotransmitters and their metabolites in brain extracellular fluid space. Neurosci. 15(3). 891–902

Zimmer-Faust, R. K., Stanfill, J. M., Collard, S. B. III (1988). A fast, multichannel fluorometer for investigating aquatic chemoreception and odor trails. Limnol. Oceanogr. 33: 1586–1595

This article was submitted to the editor

Manuscript first received: December 27, 1990
Revised version accepted: May 10, 1991