Chapter 12

Orientation of Marine Invertebrates to Odor Sources

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Contents

General Introduction

One of the most fascinating questions in Animal Behavior is how animals use series of immediate local stimuli to be successful at long-range "goals". These goals may be food acquisition or reproductive success, among others. A clear example is found in orientation behavior in odor plumes. Since animals can sense odor only in their immediate environment they must engage in some form of sampling and searching behavior to derive directional information to locate a far-away odor source. We may assume that competition for food resources acts as significant selective force on efficiency of odor source localization, particularly for predators and scavengers hunting primarily by scent. Many aquatic animals use underwater scent to determine the identity, distance, and direction of an odor source. Furthermore, to effectively use and detect chemical signals, animals must either control the fluid motion around themselves or "design devices" to interface with the flow. The first set of experiments demonstrates how odor dispersal is dependent on flow pattern and release rates and how animals actively alter their environment by producing currents to enhance odor reception. A second set of experiments demonstrates the orientation behavior of marine invertebrates in a straight flume or a Y-maze.

Notes for the Instructor

The experiments described in this chapter allow for a lot of flexibility. Depending on the available equipment students could conduct the experiments by just using a note pad or by using sophisticated video and computer analysis. Therefore, the analysis methods need to be adapted. The experiments outlined suggest how to explore the chemical world of aquatic animals.

Flume Design

In our courses students successfully build their own flumes of various sizes made out of Plexiglas. (Plexiglas can easily be joined together by using solvents like methylenechloride. *Caution*: use only in well ventilated areas.) The size of the flume needs to be scaled to the size of the animal and especially the width should allow the animal to freely move sideways. Collimators at the input end made out of straws (5 cm long) or fluorescent lighting grate help to generate an unidirectional flow.

Recirculating flumes are also easily made out of Plexiglas. A regular aquarium pump with an airstone is sufficient to move water around. A propeller and a motor combined with a rheostat allows for better control of flow speed.

Choice of Experimental Animals

The following experiments can be carried out with a variety of different marine invertebrates such as lobster, hermit crab, green crab, horseshoe crab, starfish, and mud snail. These animals are readily available through the MBL or other suppliers. Marine invertebrates can be replaced by freshwater species such as crayfish.

Some of the animals have an advantage in the laboratory over others. We successfully used the following marine invertebrates in our courses:

1. The American Lobster, *Homarus americanus*. The advantage of studying this animal would be the extensive literature that exists on lobster chemoreception and orientation. Lobster produce powerful information currents which they use in intraspecific communication. However, lobsters are large and can be difficult to work with for certain experiments. Small juveniles may be available if ordered sufficiently in advance.
2. Hermit Crabs, *Pagurus* sp. These animals are very easy to work with. The presence of their shell (which can alter the fluid dynamics of their long-distance chemoreception) poses an interesting point of investigation.
3. Green crabs, *Carcinus maenas*. These animals are also very easy to work with. They are relatively easy to handle (e.g., their claws don't do too much damage to a human finger, unlike lobsters) and are very responsive to food odors.
4. Starfish, *Asterias forbesi*. Starfish use chemoreception extensively and chemosensory input seems to play a very direct role in their coordination. Observing starfish behavior requires some patience, but they do move surprisingly well.
5. Mudsnails, *Illyanassa obsolete*, are common gastropods living on the salt marsh mud flats of the eastern United States and Canada, from New Brunswick to Cape Canaveral, Florida. *Illyanassa* is easily attracted to dead animal material which is a secondary food source for these snails.

Flow Visualization Techniques

Structure of Odor Plumes

Background Information

Animals live within a fluid medium whether it is a terrestrial or aquatic environment. This medium not only exerts a physical force upon the animal but also serves to deliver oxygen, nutrients, and chemical signals. It is the physical motion of fluid movement that ultimately determines the characteristics of environmental odor signals. Classical concepts of odor plumes and mathematical equations describing them are based on spatial and temporal averages of concentration (Sutton, 1953). This averaging treatment describes odor concentration gradients as smooth functions as if odor diffuses homogeneously from a source to fill a predictable space down-current with gradually decaying concentrations away from the source. This concept is generally correct for those microscopic organisms that live in environments dominated by molecular diffusion. In their case the random walk of individual odor molecules (Brownian motion) is still orders of magnitude smaller than the spatio-temporal scale at which the organism measures the diffusion gradient (which is based on averages) and thus the diffusion model gives a biologically useful description of odor distribution.

However, animals larger than about 1-10 mm live in environments dominated by turbulent fluid flow and not molecular diffusion. At these size and time scales, molecular diffusion plays a minor role in the dispersion of odor signals. Turbulent flow breaks up an initially homogeneous odor distribution into a dynamic pattern of odor patches of various and constantly changing sizes and concentrations. This results in a patchy distribution of odor down current from the source. A patch is an area in which odor concentration is higher than the surrounding area, in some cases there will be no odor around a patch as is seen commonly at plume edges. A stationary sensor or receptor organ will experience a fluctuating odor signal with chaotic periods of high and little to no concentration (Figure 1). An arbitrary spatial or temporal average of odor distribution will not tell us what an animal will actually perceive within a odor plume. Thus, we need an instantaneous description of the patchy nature of odor plumes rather than spatially or temporally averaged descriptions.

Odor plumes are dispersed by differential fluid velocities of the surrounding medium producing complex patterns of patches with a wide range of size and time scales. We will use two common models of an odor plume: a plume emitted from a stationary, constantly flowing or from a “leaking” point source. Realistic examples of a flowing (“jet”) source are the filter currents of bivalve molluscs or odor from a signaling mate; an example of a leaky source is a wounded or dead animal. Large-scale turbulence, of a size the length of the plume or larger, causes shifts of the entire plume. This results in large scale meandering of the plume as a whole. Medium-scale turbulence (*i.e.*, at the scale of the plume diameter) breaks up the plume into large patches. Small-scale turbulence causes the larger patches to break up into smaller ones and to “fray” patch edges (Atema,1988; Moore and Atema,1991). Finally, molecular diffusion will become more and more efficient at further “softening” the edges of the smallest patches. In the end, the odor will tend to become uniformly dispersed, but long before that the concentration gradients of its spatial distribution will become undetectable. Thus, with increasing time and distance from the source we will see an increase in the number of patches spread over a larger area, but a decrease in patch size, edge gradients, and internal patch concentration (Moore and Atema,1988; Atema, 1996; Murlis et al. 1992). Such patch features constitute a number of odor dispersal parameters that contain potential information about source distance and direction. A biological receptor moving through this odor space converts this spatial pattern into a temporal

sequence of patch encounters. A "patch" in the space domain is converted into a "pulse" in the time domain (Figure 1). From this temporal pattern we can now begin to define and describe odor pulse parameters such as pulse slope, height, width, area under the curve, frequency spectrum, intermittency, off time, etc. (Figure 2). Some of these pulse parameters may be used by the animal's nervous system to derive directional and distance information faster than would be possible by concentration averaging.

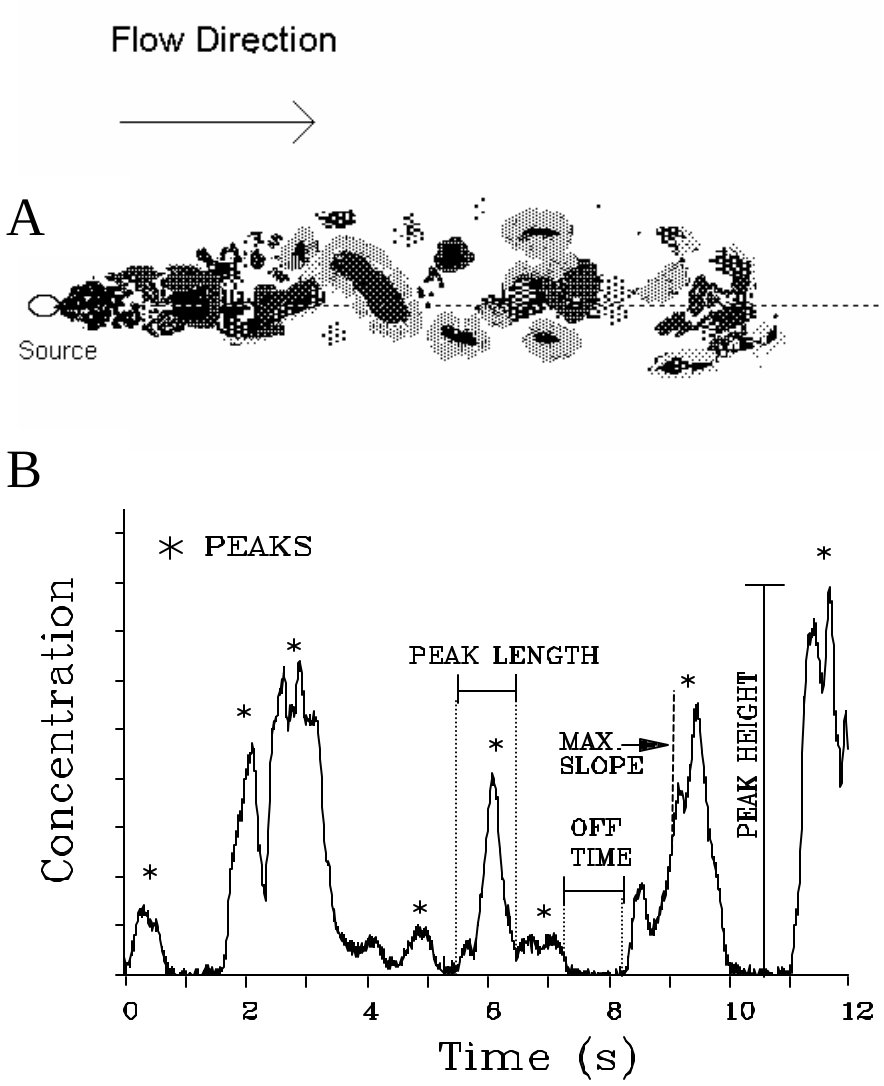

Figure 12.1. (A) A chemical signal emanating from its source (e.g., prey) into a unidirectional flow stream. The chemically affected area is called a "plume". The turbulent nature of the flow environment breaks the odor plume into a series of various size patches. The three-dimensional plume is shown here as a two-dimensional projection. (B) Definition of commonly measured odor patch (= pulse) parameters. (from Moore and Atema,1991)

Materials

Flume (flow through or recirculating)
Collimators (made out of straws or fluorescent lighting grate)

Stimulus delivery system:
Dyes: fluoresceine, rhodamine, crystal violet , food color, or milk
Aspirator bottle 250-ml to 2 liter (or plastic bottle with outlet near bottom)
Plastic tubing, 1/8" to 1/2"
Pasteur pipette with bent tip (or syringe needles of different sizes)
Regulator (Hoffman) clamp or valve
Ring stand with clamps to hold pipette in place
(Flowmeter, optional)
Cotton balls
Glass aquarium
Acrylnitrile-Butadien-Styrole particles
Slide projector
Slides with different size of light slits (glass slide covered with black electric tape)

Optional:
35-mm camera
Strobe light
8-mm Video camera (or camcorder and 8-mm VCR)
MacIntosh computer and NIH-Image software (free common domain program)
Conductivity-meter

Student Outline

This exercise introduces the equipment and techniques used to create, control and visualize fluid flow. It demonstrates two kinds of flumes: a recirculating (Figure 2) and a straight flow-through flume (Figure 3). Both dye and particle visualization techniques are easy methods to measure the size and direction of a flow field and flow speed.

Odor Dispersal Under Various Flow and Release Regimens

Odor dispersal is best studied in a flow-through flume (Figure 3). Jet plumes of odor are released by gravity flow through a pipette tip. Leaky sources release odor more slowly. They can be realistically mimicked by using a cottonball-(sponge) in a perforated container as the source of dye. Dyes such as fluoresceine, rhodamine, crystal violet, food colors, or milk are classical tools for tracing currents and odor dispersal. This method is excellent to obtain a qualitative impression of dispersal but it is difficult to quantify flow velocities and odor concentrations at proper biological size scale. Quantification of odor concentrations can be achieved in a freshwater flume by mimicking the odor plume with a salt water plume; conductivity measurements give an excellent description of the local odor concentration in time and space.

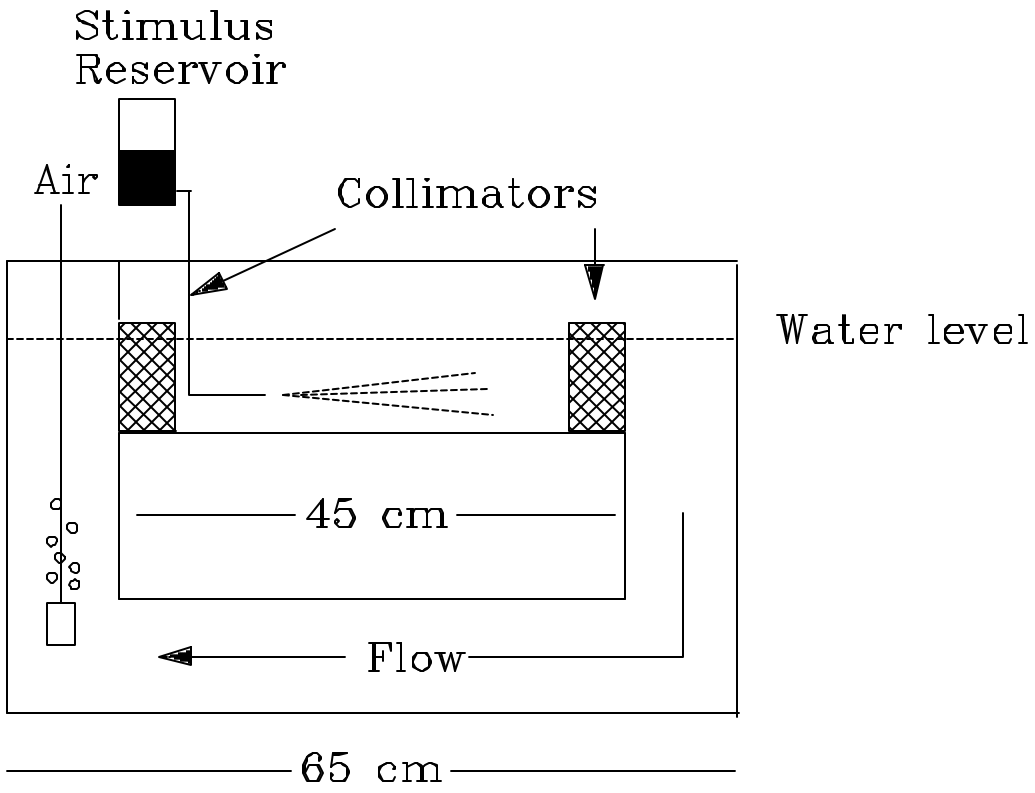

Figure 12.2. Diagram of a recirculating flume. The flume can be driven by air or a small propeller. This set-up is preferable for experimentation with marine invertebrates when running seawater is not available. However, eventually, built-up of stimuli will adapt the animal's chemoreceptors and prevent the animal from exhibiting orientation behavior.

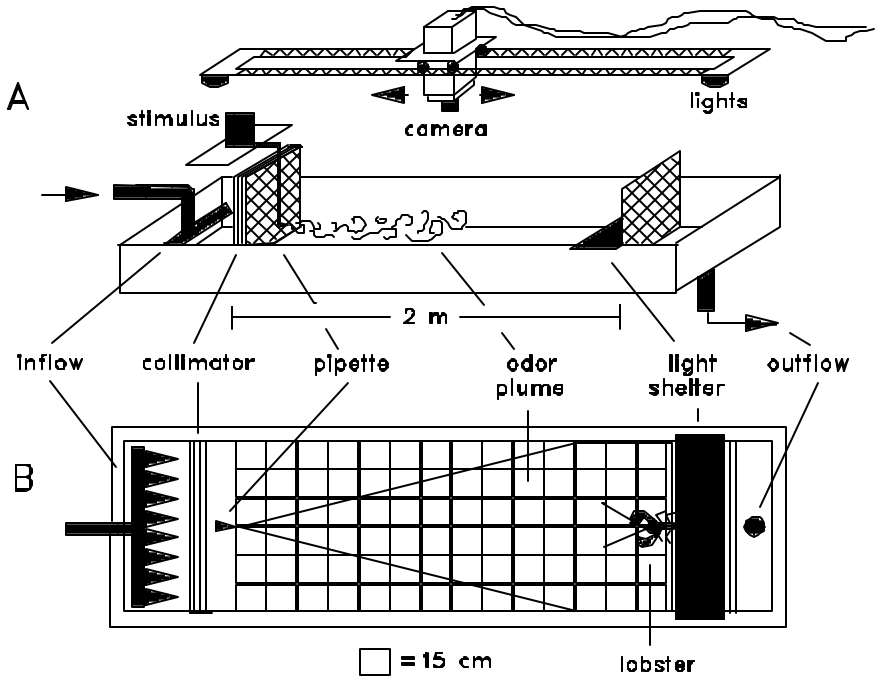

Figure 12.3. Side and top view of a flow-through tank (or "flume") used to study chemically-mediated behavior. The flow-through system is needed to insure that the animal does not habituate to the stimulus. Collimators help produce even flow across the tank, and can be easily constructed from straws or fluorescent light grating. The size of the tank is adapted to the size of the animal, at least four body lengths, but preferably more. (from Moore et al., 1991)

Quantification of velocities in the flow field can be achieved by particle tracking techniques. A suspension of fine, neutrally buoyant particles is lit by a sheet of light (Figure 4). Observation and filming perpendicularly to the plane of the light sheet allows the tracking of single particles in the "slice" area. With or without stroboscopic illumination one can follow particles and thus measure flow velocities at all points in the slice volume. Slices in three perpendicular planes can give three dimensional flow quantification. The entire flow field with outgoing currents and return/replacement flows can be measured simultaneously. However, very low flow rates cannot be measured easily and dilution of odor cannot be measured at all with this technique.

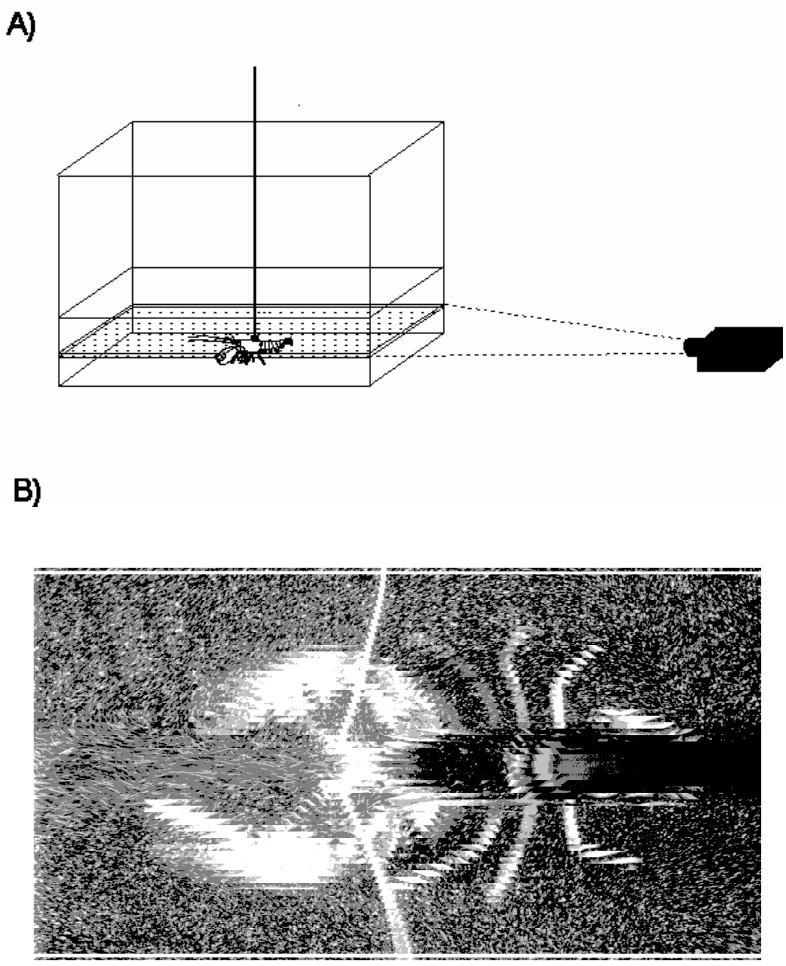

Figure 12.4. (A) Diagram of a "no flow" tank used for visualization of animal-generated currents. The size of the tank needs to be large enough to prevent "wall effects", i.e., distortion of the currents by the aquarium walls. The animal is held in place by a plastic bolt and a rod with a plastic screw. **(**B**)** Long exposure photograph of lobster gill current visualized with neutrally-buoyant particles. (Photograph by Thomas Breithaupt and George Gomez)

Video Analysis and Flow Visualization by Single Particle Tracking

Flow fields are visualized by submerging ABS (Acrylnitrile-Butadien-Styrole = Plastic) particles of 0.065 - 0.5 mm diameter in the water. Since the particles have the same density as water (1.03 kg/liter) they follow the movements of the surrounding water molecules much as balloons trace wind patterns. Tracking speed and direction of single particles will it make possible to quantify flow fields.

After introduction the particles will float in the water for more than 15 minutes until they start to settle. A horizontal or vertical layer of water is illuminated, using a slide projector and slides with slits of different widths. It is recommended to use more than one slide projector (positioned on different sides) to evenly illuminate the whole field of interest. Movements of the particles in the illuminated layer can either be

filmed by a video-camera (or 35-mm camera) and analyzed on the MacIntosh Computer or photographed for documentation. Single particle tracking can be achieved by grabbing single frames from a 8-mm video recorder and storing and analyzing them on a MacIntosh-Computer with NIH "Color Image" (Version 1.32 is a free public domain program). The path of individual particles is followed by "clicking" its position with the mouse on subsequent pictures. Alternatively, long-exposure photographs record direction and velocity of the particles if they remain in the illuminated plane.

Preparation of particles: Since the particles are originally too big to provide a good resolution for flow visualization they have to be ground down to a fine powder. Before grinding, particles are submerged in liquid nitrogen. This makes the material brittle, which enables cutting it down with a commercial coffee-grinder. Preferred particle size can be selected by the use of sieves of different sizes (0.065 mm and 0.2 mm are recommended).

Suggested experiments:

Describe the effect on the stimulus dispersal by changing:

1) the bottom from smooth to sandy to rocky at the same carrier and stimulus flow rates.
2) carrier and/or stimulus flow rates.
3) the tip diameter of the stimulus delivery system.
4) the height above bottom of the stimulus delivery system.
5) Put differently shaped objects in the path of the stimulus plume.
6) Put animals (restraint, see below) in the path of the stimulus plume.

Notes for the Instructor

Flow visualization techniques can be used to demonstrate the principles of fluid dynamics. Vogel's book (second edition, 1994; with Instruction Manual available from the author) gives an excellent introduction to these topics. These experiments can also be carried out in freshwater. Especially recirculating flumes are easily built and can be used to demonstrate flow fields around objects. The experiments could be combined with a behavioral study observing orientation or other behavior in a flume.

A problem frequently occurring is that the densities of the stimulus (dye and food odor) and carrier flow are different. This results in rising or dropping of the stimulus plume. Therefore it is important to keep both solutions at the same temperature. The dye concentration should be kept at a minimum, e.g., at a concentration that allows good visibility. Overdyed stimulus plumes may not only sink, but also may repel animals.

Similar precautions should be taken with food odors. Too high a concentration of food odor often repel animals. On the other hand too low concentrations might be below detection threshold. We suggest pilot studies to determine an efficient concentration.

Animal-Generated Currents

Background Information

To understand chemical communication we must consider (advective) flow and turbulent dispersal of odor as it is carried away from the odor source (Atema,1985, 1988). Many animals that depend on chemical signals utilize water flow, e.g., they generate their own currents and inject them into environmental currents. Many decapod crustaceans give good examples. Here we describe the American lobster,

Homarus americanus, as an example (Figure 5). *H. americanus* utilizes three current-generating mechanisms that can operate separately or in combination; all three are involved in chemical communication: gill current, exopodite current and pleopod current.

The scaphognatites inside the gill chambers generate a powerful gill current which jets forward from bilateral "nozzles". This current reaches distances of up to seven body-lengths in adults (Atema, 1985), and velocities of 3 cm/second near the nozzle. It is usually a bilateral current and it carries the animal's gill metabolites. Mature-sized lobsters under summer temperatures almost continually produce this breathing current; in winter, the current stops for episodes of several seconds, presumably reflecting the animal's lower metabolic demands. In addition, urine can be released into this current from bilateral bladders through small ventrally directed excretory pores (nephropores) at the base of the antennae.

Further control of signaling is possible through a redirection of the gill current by the exopodites of the three maxillipeds. It appears that the exopodite of the first maxilliped can be positioned to - partially - cover the gill chamber outflow nozzle, thus deflecting and redirecting forward water flow. The large feathery exopodites of the second and third maxillipeds then fan the deflected water backwards, while drawing in a slow flow of water from around the animal's head (Atema, 1985). The lateral filament of the antennules flick and thus sample odor within this area, the radius of which is about the length of the antennule. The exopodite fan current represents the second lobster-generated current. It can be bilateral or unilateral on either side.

Together, the two lobster-generated currents that can be measured around the animal's anterior end are complex and carefully controlled. They are ideally suited to carry urine and gill metabolites away from the lobster to specified directions. Simultaneously, the water displaced by these outgoing currents results in incoming currents with chemical signals from the environment that can be sampled by the antennular chemoreceptors.

The third and most powerful lobster-generated current is the pleopod current, which draws water from below the lobster and blows it posteriorly (Atema, 1985). Typically, the lobster raises its tail and beats its pleopods to generate this current which is sufficiently powerful in adult animals to help the animal in forward motion and in climbing onto rocks.

Materials

Aquarium
Acrylnitrile-Butadien-Styrole particles
Slide projector
Black slides with different size of slits (glass slide with black electric tape)

Optional:
35-mm camera
Strobe light
8 mm Video camera (or camcorder and 8-mm VCR)
MacIntosh computer and NIH-Image software (free common domain program)

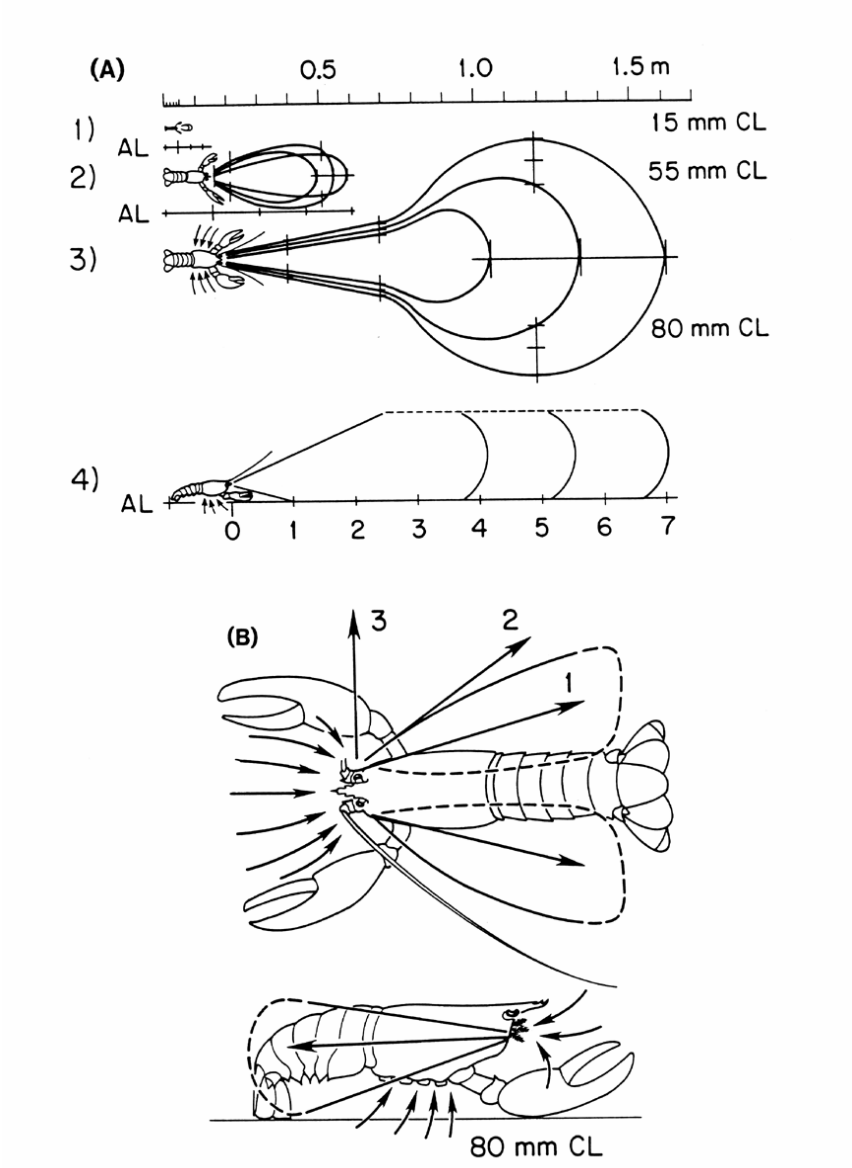

Figure 12.5. Information currents of the American lobster, *Homarus americanus.* **(A)** Forward gill currents with mean and standard deviations; top view of three different-sized animals (1-3). Side view of mature animal (4) - broken line indicates that vertical expansion of plume is limited here by horizontal stratification of water. Arrows indicate water uptake into gill chamber. **(B)** Exopodite 'fan' current. Direction 1 is commonly observed; directions 2 and 3 occur occasionally. Small arrows show water flow drawn toward the lobster. (Pleopod current is not shown. These are created by the fan-shaped appendages under the tail of the lobster.) (from Atema and Voigt, 1995).

Student Outline

Both dye and particle visualization techniques are easy methods to measure flow fields and flow speeds generated by aquatic invertebrates, especially crustaceans (Figure 5). The shape of an animal affects the flow patterns around it, e.g., shell shapes affect the flow dynamics around an animal and thus what it can sense with its chemosensory appendages. Questions as to how these currents are generated or when they are used can be combined with morphological studies. These experiments can be modified by presenting different odors. In recirculating flumes stimuli may accumulate and the animals may adapt after several cycles around the flume. Therefore, a flow-through flume is of advantage. Particle visualization is better done in a recirculating flume so that particles are not lost; also accumulation and adaptation are not a problem when using particles. Experiment with different particle sizes and densities for best results.

Orientation of Marine Invertebrates to Odor Sources

Background Information

Different odor plume structures result in different orientation behavior. We expect to learn about decision-making in animals which when faced with different plume conditions may adopt different search strategies: some animals may change their sensory integration scale with the fine structure of the plume, or with the biological meaning of the odor (food, mate, predator). Given some independent knowledge of the local turbulence (from various mechanoreceptors) and of the size of the source (from odor quality identification) an animal could derive an estimated distance from the source. Bilateral sampling of odor (spatially) or comparison of sequential samples (temporally) allows an animal to measure patch parameters to establish a likely gradient, and to make a behavioral choice (Atema, 1996). Lobsters take bilateral olfactory samples ("flicking" antennules) and make remarkably efficient directional decisions. Lobsters with unilateral olfactory lesions loose orientation efficiency (Devine and Atema, 1982).

Materials

Straight flume (see above)
Y-maze (=straight flume with divider in the middle)
Stimulus delivery system (see above)

Food extracts are excellent odors to attract crustaceans or gastropods. Stimuli are prepared by blending mussel, fish or squid meat (10 - 100 g/liter make a good stock solution) and centrifuging (or filtering) the extract. Commercial clam juice also works well. The extract is diluted 10-1000-fold and dyed with rhodamine or food color to visualize the flow field. The stimulus is released upstream through a gravity fed pipette. Dyed sea water serves as control.

Notes for the Instructor

Motivational State of Animals

It may be important to point out that much of behavioral ecology and optimal foraging theory is based on probabilities of encounter and on risk assessment. Similarly, we consider that extraction of information from the environment is based on probabilities of encountering certain sensory signals. For instance, a decision to turn right may be based on the just-measured probability distributions of odor pulses on left and right sides of the animal. The animal's motivational state (hunger, danger) may modify criteria for decision; i.e., a hungry animal may leave shelter safety based on lower probability of success than a well-fed animal. The latter example indicates also the importance of distance estimates: if a (food) source is judged to be far away, a well-fed animal may not want to risk leaving shelter to find out that a competitor closer to the source had beaten him to it. A hungry animal given the same odor information may assume that risk. Distance-to-source estimates are also important throughout searching behavior. Many animals change their search behavior patterns as they come near the source.

Student Outline

In this section we will demonstrate the orientation behavior of different marine invertebrates to an odor source in a straight flume or Y-maze.

Orientation Behavior in a Straight Flume

Orientation behavior is best studied in a flow-through tank to prevent stimulus build-up. Crustaceans, starfishes, and gastropods rely on a given odor release pattern in their environment to successfully find the odor source. By modifying flow rate and/or odor release rate we can determine how an animal uses the available information by observing its orientation path. Figure 3 gives a general overview of our set-up. The size of the tank is adapted to the size of the animal. Collimators at both ends help to produce an even flow across the tank, and can be easily constructed from straws or fluorescent light grating.

Prior to each run the animal is placed in a holding cage for acclimation. The cage is placed downstream from the pipette in the middle of the odor plume. Ample time is given to establish the plume structure. The plume structure can be easily changed by varying stimulus release rate or pipette diameter. Each trial run is videotaped. In play back, the animal's track is digitized by taping an overhead acetate sheet on a TV screen and marking the position of the animal every second. Walking speed and other orientation parameters (heading and turning angle; Figure 6) are commonly derived from the orientation paths taken by the animal (Figure 7).

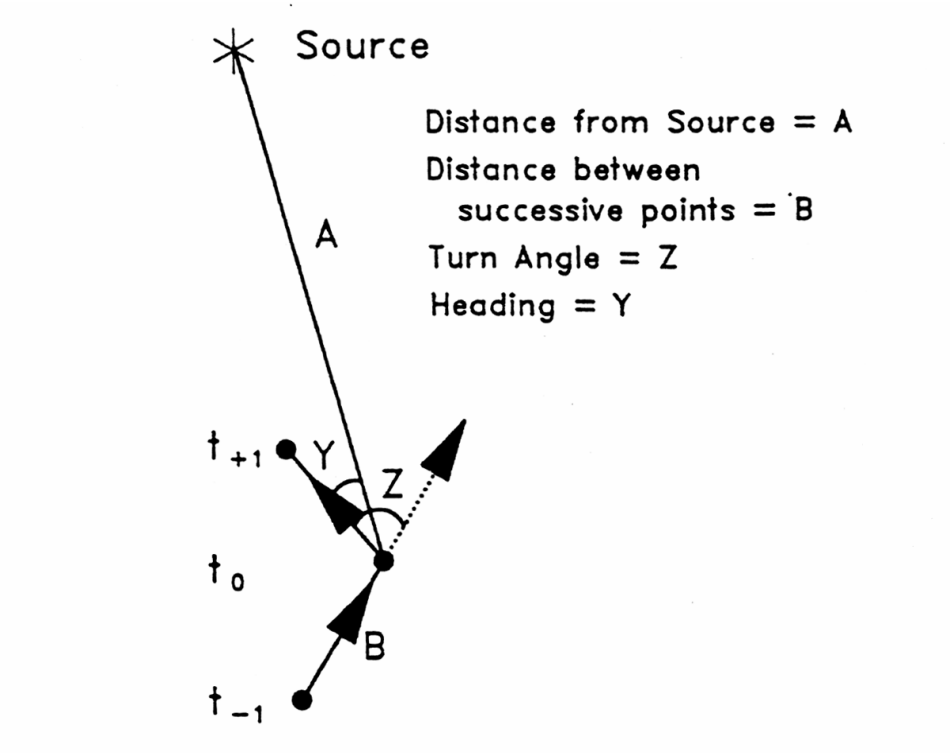

Figure 12.6. Commonly measured parameters of animals orienting to an odor source. Three successive positions (solid circles) are shown on a hypothetical orientation path (t= -1, 0, 1 are time relative to middle position). The dotted line represents the animal's projected path had it continued in a straight line, from which a turn angle value (Z) at point t = 0 is calculated. A (*) indicates distance to the source, from which a heading value (Y) is calculated at point t = 0. Walking speed at t= 0 is B cm/second, since time between successive digitizing points on the path was set at one second. By measuring these behavioral parameters and either measuring or controlling the chemical stimulus parameters simultaneously, we can begin to correlate environmental signals to behavioral decisions (from Moore et al., 1991).

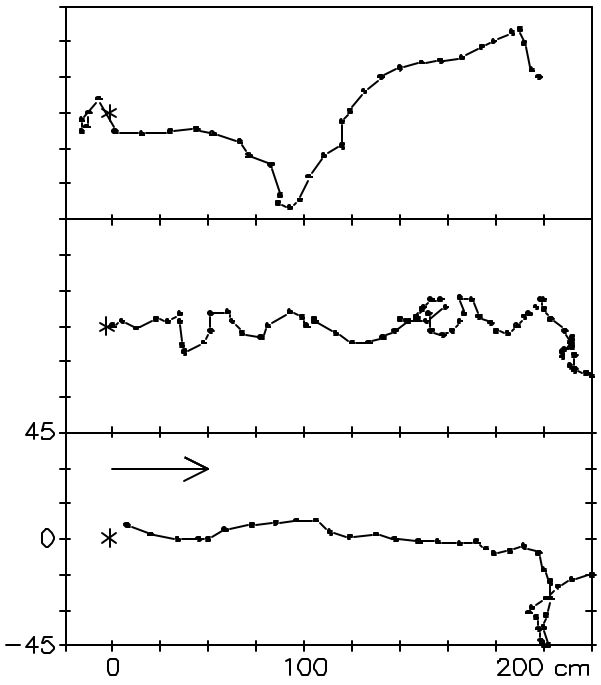

Figure 12.7. The digitized orientation paths of three lobsters through a flow-through tank toward an odor source (*). Animals presented with a jet of mussel extract walked slower and made more turning decisions on their way to the source than control animals, presumably being careful not to lose the odor trail. (after Moore et al., 1991).

Choice Tests (Orientation Behavior in a Y-Maze)

Our straight flume can be easily converted into a simple Y-maze by adding a separation into the middle of the tank. We can test the effect of stimulus composition and concentration (single compounds versus mixtures), the relevance of food odor freshness (decomposition of food), food odor preferences (extracts of different natural food items) or the effect of aversive stimuli or alarm substances on the animal's choice.

Again, each run begins with an acclimation period. We deliver different stimuli at identical release rates into each arm of the Y-maze and allow the animal to smell both. Then we remove the cage and record the animal's choice. First choice, time spent in each arm, or final preference can be measured. Stimuli are presented at random in each arm of the maze.

Suggested experiments:

Observe:

1. the effect of odor dispersal on the behavioral decision the animal makes.
2. the orientation behavior as it changes with distance from the odor source.
3. the effect of the animal's motivational state on its behavioral decisions.
4. the effect of stimulus quantity and quality on the behavioral decision.

Chemosensory Sampling Behavior

Background Information

In our model animal, the American lobster, all cephalic, thoracic, and abdominal appendages (Figure 8) appear specialized for a great variety of behavioral functions, from locomotion, to grooming, to sensing (Atema and Voigt, 1995). Peripheral specialization of appendages seems to be a crustacean characteristic, not limited to lobsters. The appendages gain their special functions in part through appendage-specific arrays of different setal types. Setae are cuticular "hairs"; sensilla are setae with sensory function and thus innervated.

Typically, chemoreception has been divided into at least two different categories, smell and taste, and the criteria for this decision in crustaceans have been debated (Atema,1985). Behavioral studies have identified stimulatory amino acids and other components of food extracts in crustacean feeding behavior (reviewed by Carr, 1988) as well as tank water from aquaria containing prey or conspecifics. Natural extracts of cod, shrimp, and lobster muscle are more stimulatory than any of the single compounds or simple artificial mixtures. Subtle differences in mixtures can have important behavioral consequences; for example, lobsters can discriminate between the body odors of two mussel species (*Mytilus edulis* and *Geukensia demissus*) and show significantly improved detection and localization behavior for the species odor they have been exposed to for a few weeks (Derby and Atema,1981).

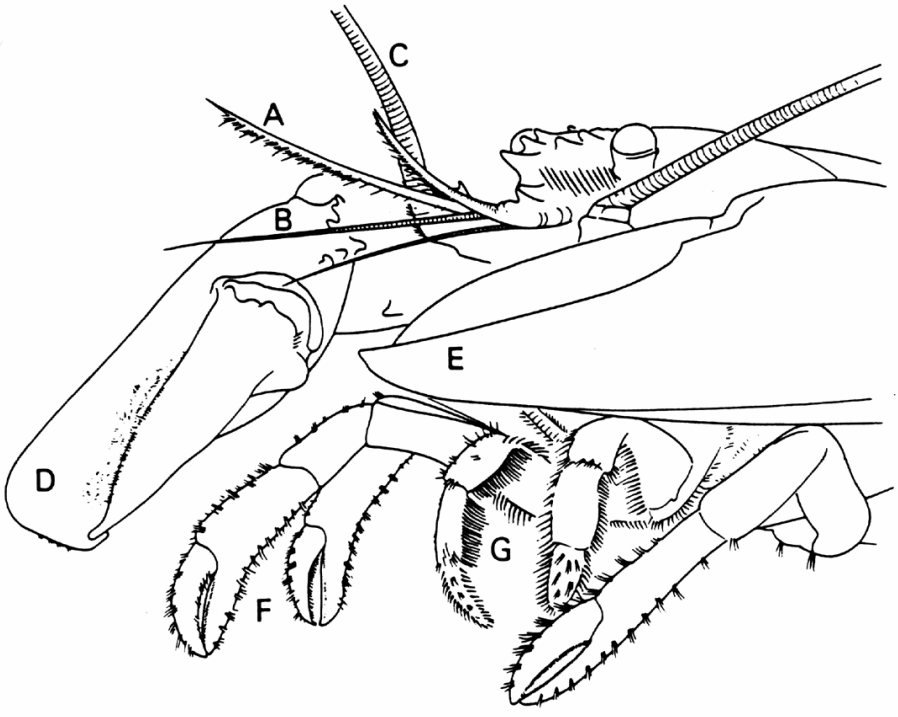

Figure 12.8. Schematic representation of the cephalothorax of *Homarus americanus* and its chemosensory appendages. (A) Lateral flagellum of the antennule (first antenna); (B) medial flagellum of the antennule; (C) second antennae; (D) crusher claw; (E) seizer claw; (F) chelate walking legs; and (G) third maxillipeds. Note the hair-like structures on each appendage through which chemical information is received. (from Atema and Voigt, 1995).

Lesion Experiments

Initial evidence for causal relationships between stimulus and response comes traditionally from lesion experiments. Typically, a suspected sensory organ is removed or its innervation severed and behavioral responses to the stimulus disappear or diminish. Experiments with lobsters, lesioning either antennules and their parts (Devine and Atema,1982) or chemoreceptors in the walking legs (Derby and Atema,1982) have provided important results for our understanding of aquatic chemoreception. In lobsters or other marine crustaceans, we can eliminate chemoreception by dipping the appendages in deionized water for 5 minutes; these lesions are reversible and the animal will regain its chemosensory abilities within several days. From these experiments we learned that the main input for odor orientation behavior comes from the lateral antennules and specifically from their aesthetasc receptor sensilla. The walking leg chemoreceptors provide only a minor contribution to tracking behavior; their main behavioral task appears to be food recognition and local (about 10 cm) search by probing around. Lesion experiments always carry the danger that non-specific damage occurs, or that lack of sensory input decreases motivation; the results also are more difficult to interpret in terms of specific stimulus parameters.

Materials

Straight flume (see above)
Stimulus delivery system (see above)
Nylon bolts and nuts, 1/4"
Wooden rod
Cyano acrylate glue, (“Krazy glue”)
Ring stand with clamps to immobilize animal

Notes for the Instructor

Lobster as other crustaceans are more relaxed if their abdomen touches an obstacle and the bottom of the tank is rough.

Student Outline

As mentioned above, crustaceans have different chemosensory organs. The lateral antennules are thought to function as olfactory organs and flick to decrease the boundary layer. This increases stimulus access to the chemoreceptor cells in the antennule. Antennular flicking can be easily counted and has been used to determine the biological relevance of stimuli.

Experiments are best carried out in a flow-through flume. The crustacean is restricted in its movements by gluing a plastic nut on its carapace; a rod with a plastic screw on one end is mounted on a ring-stand. Stimuli are released by a gravity feed in the vicinity of the antennules. Antennular flicks before and after stimulus introduction are counted.

The walking legs of crustaceans function behaviorally as taste organs. We can test their function by placing odor laced agar cubes (2 % in sea water) close to the legs and measure the handling time. Different stimuli and blank cubes are presented at random. The results allow us to determine threshold, preference and biological relevance of different stimuli.

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Appendix A

Supplies

General supplies can be obtained from laboratory supply companies like Fisher Scientific, Cole-Parmer, or others.

Chemicals can be obtained from Sigma Chemical Company.

Acrylnitrile-Butadien-Styrole particles:
Polymerland
13801 Reese Blvd. West
Suite 150
Huntersville, NC 28078
Tel: 1-800-752-7842

Marine specimens can be obtined from:
Marine Biological Laboratories
Aquatic Resources Division
7 MBL Street
Woods Hole, MA 02543–1015
Tel: (508) 289-7375 ordering
(508) 289-7455 information
(508) 289-7900 fax
email: specimens@mbl.edu