

THE DISTANCE CHEMOSENSORY BEHAVIOR OF THE SEA URCHIN

LYTECHINUS VARIEGATUS

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THE DISTANCE CHEMOSENSORY BEHAVIOR OF THE SEA URCHIN
LYTECHINUS VARIEGATUS

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For Peter and Roy, two generations of Pisut men who taught me to take pride in my work, and to never give up, even in the midst of great adversity. To them, I dedicate this work.

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SUMMARY

Many organisms that lack vision rely on chemical signals to glean information from their environment. Little is known, however, about the ability of sea urchins to detect and respond to such signals. This lack of understanding is especially surprising given the ecological impact of urchins in their respective communities. Regardless of geography, urchins exert strong top down control of plants, algae, and sedentary invertebrates, and these effects are especially evident when urchins, or urchin predators, are removed from an ecosystem. Facultative omnivorous species such as *Lytechinus variegatus* may greatly alter the abundances of other invertebrates in seagrass communities by preying on juvenile and adult bivalves as well as gastropod egg masses. These potential food resources, however, are patchily distributed within seagrass beds. To find such resources before other organisms can exploit them may require acute abilities to detect signals emanating from these patches.

Experiments performed in this study demonstrated a consistent ability of *L. variegatus* to detect and orient to chemicals emanating from potential food resources over a distance of 1 m. Unlike what has been found in some other marine organisms, turbulent flow conditions did not negatively affect the ability of *L. variegatus* to find the source of this chemical cue. In fact, only the slowest flows hindered this ability; the bluff shape of the urchin formed a relatively large boundary layer at slow flows, preventing the delivery of chemical signals to the sensors. The relatively high success rates of *L. variegatus* in turbulent flows may allow it to effectively forage in areas where other organisms cannot.

Thus, turbulence may provide a selective advantage for this animal, based on its comparative ability to detect and respond to signals in its environment.

CHAPTER 1

INTRODUCTION

Chemosensation may be the most primitive of all sensory modalities. Most organisms possess some olfactory or chemosensory capacities and use them to locate food or other resources. In fact, chemosensory guidance to food or other objects is found in the most primitive viruses and bacteria to the most derived arthropods and mammals (Dusenbery 1992). The evolution of complex sensory and motility structures has made chemosensory guidance more efficient in more derived organisms. However, these behaviors also exhibit greater complexity as compared to the more ancestral organisms. For example, *Escherichia coli* detects concentration gradients of L- glutamate and undergoes different flagellated movements in the presence and absence of this stimulus (Brown and Berg 1974). In comparison, the blue crab, *Callinectes sapidus*, uses a variety of sensilla to extract sensory information from complex plume structures composed of mixtures of compounds, and exhibits 3-dimensional tracking behaviors in response to the stimuli (Weissburg et al. 2002a). Complex sensor arrangements, such as those in *C. sapidus*, allow for the potential to detect of a greater number of stimulants in a wider variety of environmental conditions.

Sea urchins are excellent examples of organisms that, like crabs, inhabit a variety of habitats, consume many different foods, and are exposed to wide-ranging hydrodynamic flow regimes (Jangoux and Lawrence 1982). Unlike crabs, however, little is known about the behavioral mechanisms underlying the foraging behavior of these organisms (Sloan and Campbell 1982, Weissburg 2000). This lack of knowledge is

surprising since urchins exert intense top-down pressure on community structure (Lawrence and Sammarco 1982, Hughes 1994, Estes et al. 1998). In response to this lack of knowledge, the goal of this study was to characterize the sensory behavior of a common species of sea urchin, *Lytechinus variegatus*. Specifically, (1) how does this species respond to chemical information in its environment, (2) how does it use this information to guide its movement, and (3) how might this behavior be altered by different hydrodynamic conditions?

Chemosensory Behavior

Behavioral responses to the presence of stimuli may be classified as either indirect or direct guidance towards the stimulus source, and further classified by whether the stimulus properties in the environment are compared in time or space. Burr (1984) refers to indirect turning responses to the presence of stimuli as a *kinesis*. In this sampling mode, behavioral responses are unrelated to the direction of the stimulus field, but the organism will indirectly guide itself to the source, albeit inefficiently by changing the frequency of some movement parameter in response to signal presence or absence. A *taxis* refers to directed changes in movement towards or away from the stimulus field. These terms are usually combined with a description of the sampling modality. The prefix *klino* refers to temporal sampling, in which consecutive sensory stimulations are compared over a series of points in space. The prefix *tropo* refers to spatial sampling, in which simultaneous sensory inputs are compared over an array of sensors (i.e., at least two sensors or sensor groups). Another common orientation behavior involves the detection of fluid flow, called *rheo* (in water) or *ameno* (in air). For example, blue crabs

(*Callinectes sapidus*) use a combination of tropotaxis and rheotaxis to locate food, where the presence of a chemical signal is compared across sensory arrays on their legs to mediate turns toward the odor plume and stimulation of antennal chemo receptors triggers movement upstream in response to flow direction (Weissburg and Dusenbery 2002, Keller et al. 2003).

However, the ability to locate resources based on chemosensory guidance is often affected by the forces of fluid motion on the chemical signal. In the absence of fluid motion, chemicals undergo diffusive spreading, decreasing in concentration in proportion to the distance from the source (Dusenbery 1992). Chemical gradients may be easily discerned in low turbulence situations because of the symmetric spreading and decreasing concentration of the signal. In these situations, a single sensor or simple sensory array may be used to detect a chemical gradient, such as in the mate tracking chemotaxis behavior of copepods (Doall et al. 1998). As the fluid velocity (U) increases, turbulent stirring distorts the predictability of the odorant concentration in a plume; the plume becomes filamentous and chemical signal structure exhibits substantial temporal and spatial variation (Webster and Weissburg 2001). Some aquatic organisms use the filamentous nature of chemical plumes to glean information and orient to the chemical source (Atema 1996). However, as turbulence continues to increase, turbulent mixing tends to homogenize the signal concentration, so that concentration bursts are not as frequent or intense as in less turbulent flow regimes (Webster and Weissburg 2001). In these latter situations where the signal plume is not uniform (as it is in diffusive spreading), a klinokinesis behavior may be ineffectual, and instead a behavior

incorporating a tropotactic orientation behavior may be needed (Dusenbery 2001, Webster and Weissburg 2001).

As a tropotaxis incorporates information from different sensors in an array, the arrangement of these arrays may impact the overall ability of the organisms to detect and orient to the stimulus. Many organisms have primary chemosensory structures localized on specific regions (e.g., nostrils, antennules, limbs), though numerous other secondary chemoreceptors may be located along the body (Derby et al. 2001). Increasing the spatial span between sensors allows for greater spatial comparison when orienting to a chemical plume, and increasing the number of sensors will increase the ability to detect the presence of a cue (Derby and Steullet 2001). Conversely, closely spaced sensors may miss a turbulent chemical plume altogether. Thus, the optimal distance between sensors should scale with the width of the plume (Weissburg et al. 2002a). This arrangement allows for the detection of the plume edges by simultaneously comparing the sensory information across the sensory array, allowing the organism to maintain an orientation course within the stimulus field.

In addition to the number and spatial arrangement of sensors, the time over which these sensors may integrate stimuli may affect tracking. Thus, the speed at which an organism moves may influence the information that can be extracted from odor plumes (Weissburg 2000). Crabs move relatively quickly. Thus, the sensory odor landscape produced by crabs is patchy, with intermittent stimulus peaks. Slower moving organisms may integrate more information at a given point in space within the chemical plume. Time-averaged concentration gradients may be discernable, if temporal sampling is rapid enough. Though sampling thresholds may be dependent on precise plume structures, at a

sampling rate of 4 Hz (equivalent to the sampling rate of some benthic crustaceans; Gomez and Atema 1996), an observer must sample a particular location for 200 sec to discern a concentration gradient as it moves along the length of a typical turbulent plume (Webster and Weissburg 2001).

The reliance on bursts of high odorant concentrations by some decapod crustaceans makes foraging in highly turbulent flows difficult, and their tracking success rates drop accordingly (Weissburg and Zimmer-Faust 1993). Increasing the sampling time of the chemical plume, along with augmenting the number of chemical sensors enhances the ability to accurately perceive the signal (Weissburg 2000). Since turbulent chemical plumes often contain patches of 'clean' and stimulant-laden water, integrating the sensory information over time or space will increase the ability of the organism to detect the patches of stimulants. Thus, slower, larger animals (or animals with numerous sensors distributed around the body) should be at an advantage for detecting waterborne chemicals, regardless of the ambient fluid motion (if sensory thresholds are equivalent). However, competition for resources in slower flow areas may be intense (Leonard et al. 1998). If faster moving organisms (e.g., crabs) can effectively forage at slow flows, slower moving organisms may be excluded from these areas, either by predation or competition. Nonetheless, the selective pressure for slow moving animals to have well developed sensory capabilities should be strong, as their deliberate pace may increase foraging times, and thus the risk of predation (Vermeij 1987).

Echinoderms and Chemoreception

Echinoderms are important consumers in a variety of habitats (Jangoux and Lawrence 1982) and represent a contrast to the typical animal models used to study chemosensory behavior. Unlike any other phyla of mobile marine invertebrates, many echinoderms are radially symmetric. Tube feet and pedicellariae are distributed across the entire exterior of urchins. Dale (2000) demonstrated that the tube feet of the sea star *Asterias forbesi* possess chemosensory function. Microscopy of tube feet and pedicellariae has elucidated structures that morphologically resemble sensilla known to have chemosensory functions (Smith 1979). Pedicellariae begin extending their length and contracting their scissor-like heads when excited—a hypothesized defensive response to grasping predators or fouling organisms. Pedicellariae from the urchin *Echinus esculentus* responded to chemical cues of predators, but not food (Campbell and Laverack 1968). However, the identity of the food chemical cue was never described in this study. If tube feet (or pedicellariae) do possess a chemosensory function, then the sensory arrangement of urchins is different than any other model organism previously studied, as hundreds of tube feet (and the associated chemoreceptors) are evenly distributed in a three dimensional arrangement over the entire exterior of the urchin test. Echinoderms may use their tube feet that are broadly separated either across the test or on separate arms for detecting plume edges. The slow speed at which echinoderms move may also be beneficial to chemosensory tracking, as the number of sensors combined with their slow speed may allow them to form crude time-averaged concentration profiles of the chemical plume (Weissburg 2000).

Echinoderms appear to perform gradient tracking during odor guided navigation. The sea star *Asterias forbesi* had a success rate of 48% when orienting to mussels or clams in nearly still water ($U = 0.05 \text{ cm s}^{-1}$; Moore and Lepper 1997). The authors predicted that the success rates were artificially low due to the lack of flow. However, success rates decreased to 20% at flows of 5 cm s^{-1} (Dale 2000). Although the stimulus differed between the two studies, the ability to track the stimulus source with out any appreciable bulk flow suggests orientation to chemical gradients. The chemoreceptors of *Asterias*, however, span a distance of 20 cm, which, when coupled with slow movement, may be adequate to detect a spatial chemical gradient. Crabs and moths, which rely on the presence of a fluid signal for orientation, do not perform gradient tracking. Extremely small organisms, such as bacteria, also cannot perform gradient tracking based on spatial sampling (Dusenbery 1996). For this behavior to be possible, sensors must be placed far enough away to detect concentration differences. The closely spaced crab sensilla on antennules work in conjunction with the leg chemoreceptors that provide spatial comparisons of the signal. This arrangement is adequate for sensing the presence of a cue and orienting in the direction of its origin or for making qualitative temporal comparisons of stimuli. The argument could be made that by moving very slowly, crabs could also discern gradients. However, the behavior of blue crabs seems to prohibit gradient tracking. Crabs may sacrifice high tracking success in all flow speeds for the ability to quickly find a stimulus source, and thus rely on the odor-guided rheotaxis that allows them to move fast while still detecting chemical cues. Echinoderms, however, are constrained by physiology to move relatively slowly, and thus must have efficient tracking mechanisms in order to compete with other predators.

Evidence for Chemoreception in Sea Urchins

Responses of several urchin species to chemical cues have been described, though detailed descriptions of urchin chemosensory behavior in controlled flow experiments have not been undertaken. Alarm responses of urchins have been known since the 1970's. A well-documented case involves the response of *Diadema antillarum* to crushed conspecifics and heterospecifics (Snyder and Snyder 1970). Though *Diadema* does not respond to the mechanical cue of hammering an urchin, the chemicals released by such an action evoke an avoidance response where urchins move away from the stimulus source in all directions. This response also occurs to the crushing of other urchin species. *Diadema* strongly responds to the crushing of *Echinometra lunctor* and *Lytechinus variegatus*, but not *Eucidaris tribuloides* and *Tripneustes ventricosus*, which suggests a chemical disparity between the former and latter groups of urchins. The authors believed this response occurred because the latter species are more ecologically dissimilar to *Diadema* than are *E. lunctor* and *L. variegatus*. Although occurring within the same geographic range, they do not commonly live in the same habitat, consume the same foods, or become victims of the same predators. Injury to *E. lunctor* or *L. variegatus*, however, may signal a potential threat for *Diadema*. Hagen et al. (2001) found that *S. droebachiensis* is sensitive to the risk of predation from both sympatric fishes and crabs. Wolf fish, *Anarhichas lupus*, and the edible crab, *Cancer pagurus*, which have recently consumed *S. droebachiensis*, release chemicals that nearby *S. droebachiensis* use to avoid the predators. This process of predator labeling has been described in other aquatic organisms (Mathis and Smith 1993, Mathis et al. 1995). Unlabeled wolf fish and crabs do not evoke avoidance by *S. droebachiensis*. The effect of predator labeling in the

lobster *Homarus americanus* has also been observed in the field. Using large inclusion cages, Vadas et al. (1986) demonstrated that *S. droebachiensis* may weigh the threat of predation against the need to feed. Crushing *S. droebachiensis* near *Homarus* caused *S. droebachiensis* to attempt to leave the cages. Otherwise, *S. droebachiensis* did not try to avoid *Homarus*, and continued feeding on algae in the cages.

Urchins also may chemically detect algae from a distance. As described by Vadas (1977), the aqueous extracts of whole *Nereocystis luetkeana* attracted *Strongylocentrotus* spp. in Y-maze experiments over a distance of 100 cm. *Strongylocentrotus* spp. significantly chose the maze arms containing *N. luetkeana* fronds or buretted aqueous extracts of *N. luetkeana* over water controls and other algae. The kelp *N. luetkeana* is not the most abundant macroalga in the Northwest Atlantic, becoming rare during the winter; however, it is one of the preferred foods of *S. droebachiensis*, *S. franciscanus*, and *S. purpuratus*. These urchins grow faster and are more fecund from consuming *N. luetkeana*, due to their high assimilation efficiencies of this alga. As a result, the ability to detect waterborne chemicals of *N. luetkeana* while foraging would have a positive effect on the fitness of this urchin genus.

In choice feeding experiments performed by Hay et al. (1986), the subtropical urchin *Arbacia punctulata* moved towards palatable species of algae in sea tables, yet either avoided or were not as attracted to algae possessing anti-herbivory chemical defenses. Interestingly, *A. punctulata* moved towards the relatively unpalatable alga *Sargassum filipendula* during daytime assays. This behavior was absent during nighttime assays, and was hypothesized to be a predator avoidance response by *A. punctulata*

(though no predators were present during the assays); escaping day-active predatory fishes by seeking shelter in the thick canopy of *S. filipendula*. The distance attraction to specific algal species implies that the orientation cue used by the urchin is probably not a common product of metabolism (e.g., amino acids, ATP, NH_4), as has been proposed by some investigators (Zimmer-Faust 1987, Carr 1988, Zimmer-Faust 1993). Like the *Strongylocentrotus-Nereocystis* example, *A. punctulata* exhibited specific food preferences, and may be cuing onto specialized chemical produced by the target food.

Like *A. punctulata*, *Diadema* seeks shelter during the day, though it prefers coral crevices to unpalatable algae (Carpenter 1984). *Diadema* forages on coral reef algae or in the seagrass beds surrounding coral patch reefs at night, and returns to the reef before daylight. The frequency of homing to the same crevice over successive nights was positively correlated to predator density and negatively correlated to urchin density. Crevices of high protective value are important predatory escapes, but are less important when high densities of conspecifics reduce the likelihood of attack to one individual, yet also make competition for reef algae high. Lacking visual navigation and a centralized nervous system (i.e., a brain) for directional memory, the ability to return to the same crevice after foraging may suggest that *Diadema* is receptive to extremely small variations in chemical signals. Also, imprinting of new chemical signals for homing behavior both on the urchin's memory and chemoreceptors must occur when a new crevice is inhabited. Castilla (1972) demonstrated that echinoderms may chemically detect new foods after a short conditioning period. However, the differences in chemicals emanating from disparate foods may be much greater than crevices that appear

similar (at least to human senses). The ability to relocate crevices suggests that echinoderms may be quite sensitive to chemical cues.

The Ecology of *Lytechinus variegatus*

The sea urchin *Lytechinus variegatus* is one of the most common large invertebrates in subtropical and tropical seagrass beds and reef habitats from Cape Hatteras, NC to Brazil (Watts et al. 2001). The distribution and abundance of *L. variegatus* may be limited more by abiotic factors such as shear, and lethal fluctuations in salinity and temperature than by predation [though crabs, gastropods, fishes, and shore birds may prey heavily on *L. variegatus* (Rivera 1977, M. Hay, *personal communication*)]. The adhesive strength of tube feet is not as great as some sympatric urchin species (Sharp and Gray 1962) and in areas of high shear stress, *L. variegatus* is easily removed from hard surfaces such as rock walls. In some seagrass beds, densities of *L. variegatus* may exceed 100 individuals m⁻² (Camp et al. 1973). Densities greater than 40 individuals m⁻² may denude seagrass beds of vegetation (Valentine and Heck 1991). Population outbreaks of this magnitude are often regulated by large freshwater inputs during rainy seasons. Low salinity inputs over short periods, such as storm runoff, may cause dramatic mortalities to populations of *L. variegatus* (Watts et al. 2001, and references therein). A cold front coupled with extreme low tides in the St. Joseph Bay, FL during the spring of 2003 also caused an extirpation of the urchin from this area, well known to support thousands of individuals (*personal observation*).

L. variegatus is traditionally associated with seagrass beds composed mostly of *Thalassia testudinum* and *Halodule wrightii*. Seagrasses communities are important in

detrital food webs and provide nursery grounds for other organisms. However, seagrasses have very little primary nutritional value (Lowe and Lawrence 1976). The lack of nutritional value in seagrasses such as *Thalassia testudinum* may be compensated by *Thalassia* specialists evolving higher assimilation efficiencies (Lowe and Lawrence 1976, Beddingfield and McClintock 1998), or by the seagrasses' high abundance allowing greater amounts of consumption. It is this high abundance that has led investigators to hypothesize that *L. variegatus* does not require any sensory input to effectively forage in seagrass beds. In fact, Klinger and Lawrence (1985) suggest that *L. variegatus* does not use distance chemosensory behavior during foraging. However, the conclusions of this study must be questioned for several reasons. A relevant test of distance chemoreception should incorporate realistic flow and stimuli, and objective determinants of behavior; none were present in this study. Agar blocks made with 5% ground *Thalassia* tissue were placed in tanks with no water circulation, and were presented to urchins that were already moving in the tanks. An observed reduced movement rate in the presence of the stimulus blocks was deemed by Klinger and Lawrence to indicate a negative chemotaxis response, though this slowing behavior actually may indicate that the urchin was attempting to detect the signal and orient to the source. Without flow, the urchin would need to rely on a pure chemical concentration gradient to determine the source location. Sensing such a slope would require a long temporal sampling effort.

It was also assumed by Klinger and Lawrence (1985) that food resources are "ubiquitous...in sea-grass meadows". However, the spatial distribution of seagrasses and its inhabitants is heterogeneous, patchy, and non-uniform (Williams and Heck 2001, and

references therein). The relatively high detritus content of the seagrass beds create ideal conditions for filter feeding molluscs, with common representatives from the genera, *Anadara*, *Chione*, *Donax*, *Geukensia*, *Modiolus*, *Musculus*, and *Semele* (Bologna and Heck 2000). The previous notion that *L. variegatus* is an indiscriminate herbivorous forager is changing due to more in-depth field observations (e.g., Beddingfield and McClintock 1999). In fact, higher densities of *L. variegatus* were found in seagrass patches containing mussels than those without mussels (Valentine and Heck 1993). Significant mortality of the mussel *Modiolus americanus* can be attributed to predation by the omnivorous urchin *L. variegatus* in St. Joseph Bay, FL (Sklenar 1994; K. Heck, *personal communication*). *L. variegatus* may, in fact, migrate to areas of higher resource value (i.e., mussel beds), though how this is accomplished is not known.

If *L. variegatus* actively migrates towards mussel beds, it might be navigating in response to chemical cues released by these potential prey. If this is the case, chemosensory behavior may be quite important to the ecology of this urchin species, especially when seagrasses are overgrazed or when the benefits of relocating to a higher resource value area outweigh the costs of the movement. Depending on the distance that must be traversed, the urchin may encounter changing hydrodynamic and benthic conditions, potentially affecting its ability to successfully orient to the chemical cue.

CHAPTER 2

METHODS

Flumes are valuable tools for investigating chemosensation; the ability to control the variables (e.g. flow speed, substrate type) found in nature allows the investigator to pinpoint certain aspects that may influence behavior, such as by decoupling flow speed from turbulence (Weissburg and Zimmer-Faust 1993). Studies using flumes to generate realistic flow environments have elucidated the basic behavioral mechanisms that are used by a variety of organisms to locate resources (Weissburg and Zimmer-Faust 1993, Moore and Grills 1999, Dale 2000). In this study, carefully controlled flows were generated in a laboratory flume to deliver chemical signals to urchins, since little is known about their chemosensory tracking. We examined foraging abilities at several flow conditions as *L. variegatus* does not live in a constant flow environment. These flow conditions were representative of speeds and turbulence levels found in urchin habitats the field.

Distance Chemosensory Behavior

Adult sea urchins, *L. variegatus* (avg. test diameter = 49 mm), were collected from *Thalassia testudinum*-dominated seagrass beds near Panacea, FL in the Gulf of Mexico and were maintained in 34 ppt seawater at 21°C, with a 12h light cycle. Urchins were fed frozen mussels, *Mytilus edulis*, once every ten days; uneaten mussels were removed after 24h. Although this species of mussel does not naturally co-occur with *L. variegatus*, its commercial availability made it a more practical food and stimulus source.

Urchins were starved at least 24 h prior to assays to increase their responsiveness to food stimulus. Urchins were tested for hunger motivation for their ability to locate foods at a distance immediately before assay. A mussel used in making the stimulus solution was presented to animals in the holding tanks. Those urchins that moved towards the food were selected haphazardly for assay. Urchins were placed in a separate holding tank so that they were tested only once.

A variable velocity recirculating laboratory flume (13.0 m x 0.75 m x 0.25 m) was used to perform assays examining chemically-mediated distance perception and tracking behaviors of *L. variegatus*. Flume specifications are presented in Weissburg et al. 2002a. Briefly, the flume bed was lined with uniform grain sand (size = 0.98 mm). Flow speed was characterized by an in-line acoustic Doppler flow meter. Water entering the flume equilibrates for 10 m before entering the test section where the boundary layer is fully developed. A tailgate at the downstream section of the flume controls the water depth.

Treatments consisted of stimulus solutions of mussels soaked in water, by placing two freshly thawed, intact mussels (mass including shell ~ 24 g) in 2 L of filtered seawater from the flume. A pressurized stimulus release system with an inline flow meter was used to generate a constant release rate that was isokinetic to the free-stream velocity of the flume. The concentration of the stimulus preparation was changed proportionally to the change in flow speed. For example, when the flow speed and stimulus release rate were increased from $U = 5 \text{ cm s}^{-1}$ to 10 cm s^{-1} , the stimulus concentration was halved. The flux of stimulants, therefore, was approximately conserved across different flow speed treatments. If the concentration was not reduced at

the higher flow speeds, the urchins in those treatments would have been exposed to greater fluxes of stimulants, potentially creating higher successful orientation rates.

The release rate and height of a chemical in flow has a significant impact on the chemical plume structure (Webster and Weissburg 2001). An L-shaped nozzle was mounted above the flume, where the vertical section was fitted with a tapered vane to reduce its fluid mechanical signal. A 90° bend in the nozzle released the stimulus parallel to the mean bulk flow and the flume bottom. The mussel conditioned stimulus was released through this nozzle positioned 2 cm above the sand bed. The nozzle was positioned 2 cm above the bed to lift the stimulus out of the viscous boundary layer, and present the stimulus at a level comparable to the height of a mussel extending out of the sand, and near the center of the urchin test. Controls consisted of filtered seawater delivered at an isokinetic rate through the nozzle.

Experiments began by positioning one *L. variegatus* 1 m downstream from the stimulus source in the center of the plume. The urchin was gently held (by hand) in this position for 30 seconds before the behavioral responses were recorded. This acclimation period allowed the urchin to acquire the signal. The behavioral responses of *L. variegatus* to the stimulus and control treatments presented at free-stream flow velocities of 1, 5, and 10 cm s⁻¹ were video recorded. These speeds were chosen since flow within a *Thalassia testudinum*- or a *Zostera marina*-dominated seagrass bed is typically around 1 cm s⁻¹, and rarely exceeds 10 cm s⁻¹ (Gambi et al. 1990, Koch and Gust 1999). Successful orientation responses to the stimulus were scored if the urchin moved the 1 m distance to contact the nozzle with its tube feet. Orientation responses were scored as unsuccessful if the urchin did not contact the stimulus source before contacting one of the

side-walls of the flume or moving upstream of the nozzle. Replicates where urchins did not move from the starting position after 10 min were omitted. The sand of flume working area was mixed and the bed smoothed between each trial to prevent urchins from following any physical or chemical signals left by urchins of previous trials. Replication consisted of approximately 15 experimentally naïve urchins for each stimulus treatment and flow speed combination. Ten urchins were tested as controls for each flow speed so that approximately 75 urchins were used in behavioral assays.

The video recordings of movement paths were digitized at a rate of 2 Hz and analyzed using Expert Vision Motion Analysis hardware and software. This system used the contrast between the body of the urchin and background to determine the outline of the urchin and calculate the resulting centroid. The movement of the centroid was converted to real distance x, y coordinates. The x, y coordinates were smoothed over 30 consecutive frames using a moving average algorithm to eliminate noise in the calculated urchin centroid, and plotted on a two dimensional grid. Differences between the movement paths were compared by computing the net to gross distance ratio (NGDR), movement speed, heading angles, and turning angles. NGDR is a measure of the efficiency (or directness) by which the urchin moves from the starting position to the source; a straight path produces a NGDR value of 1, whereas a circular path produces a NGDR value of 0. NGDR data violated parametric assumptions even after arc sine transformation, thus, differences between successfully orienting, unsuccessfully orienting, and control urchin NGDR were analyzed with a Kruskal-Wallis rank sums test for nonparametric data, followed by a Tukey-Kramer post-hoc test.

Two angular measures were used to describe how urchins moved in the flume (Figure 1). Since an urchin that moves in a straight line perpendicular to the flow (towards the flume walls) will also produce a NGDR of 1, heading angles were determined to differentiate between movements towards the walls or nozzle. Heading angles were calculated as the angle of the net urchin movement from the initial to final coordinates, relative to the bulk flow direction. Distributions of the heading angles were plotted on a 360° grid, and differences between controls, successfully orienting, and unsuccessfully orienting urchins were analyzed with Rayleigh's z test for angular dispersion and Watson's U^2 test for the mean angle for nonparametric two-sample data (Zar 1999). Since three groups (successful, unsuccessful, and control) were compared using a two-sample comparison test, the significance levels (α) for each comparison were adjusted using the Dunn-Šidák method (Sokal and Rohlf 1994). The turning angles of the urchins described the number of course corrections the urchin made while moving in the flume. The mean turning angle for each path was calculated by averaging the absolute values of the angles (relative to the direction of bulk flow) created between the urchin positions of consecutive frames collected at a rate of 0.06 Hz. This sampling rate created an average distance between points of 2 cm. Because of this relatively small distance between points, and the fact that there was no downstream movement by the urchins, none of the turning angles exceeded 70°. Differences between the mean turning angles of successfully orienting, unsuccessfully orienting, and control urchins were plotted on a 180° grid, and analyzed with Watson's U^2 test for nonparametric sample testing, with a Dunn-Šidák α adjustment.

Flow Visualization

Turbulence has a profound impact on chemical plume dynamics, and thus the sensory information available to an orienting organism. Objects impeding flow will alter the shape of odor plumes, and this interaction is affected by the fluid velocity. We performed some basic flow visualization experiments to understand how these two factors affected plume dynamics. A Kodak digital camera (DC260) mounted above the flume was used to capture overhead images of the urchin in a dyed plume at $U = 1$ and 5 cm s^{-1} . The dye (5 ml of red food color added to 2 L of seawater) was released at a height of two centimeters from the sand bed of the flume at a rate isokinetic to the free-stream flow speed. Urchins were placed in the center of the developed plume at distances of 50 and 100 cm downstream from the source. Captured images were downloaded to a computer using Adobe Photoshop 7.0 and saved as high resolution (1500 x 1000 pixels) JPEG files.

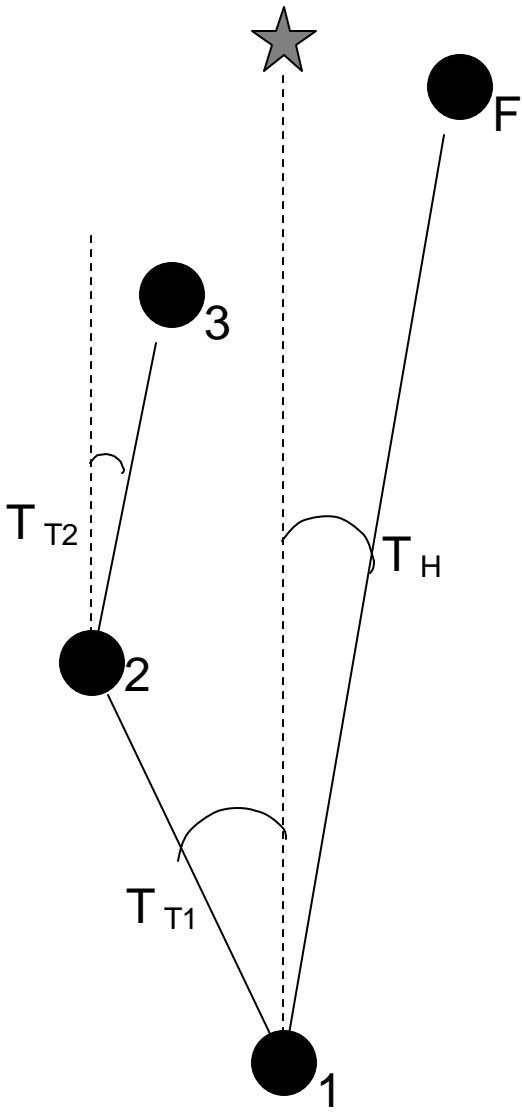


Figure 1. Angles measured from orientation paths. Heading angle (T_H) was the angle between the initial and final position of the urchin in the flume. The mean turning angle for each path was calculated by averaging the absolute values of the individual turning angles (T_T) relative to the direction of bulk flow (dashed line). Each position was calculated from x, y coordinates at a rate of 0.06 Hz. The star represents the stimulus source. Flow is from top to bottom.

CHAPTER 3

RESULTS

Distance Chemosensory Behavior

Orientation paths of urchins are presented in Figures 2-4. Overall, urchins that successfully located the odor source did so by moving in a straight line from the origin to the source. Urchins that could not locate the source typically moved up- and cross-stream, and tended to meander cross-stream more than did urchins that could successfully locate the stimulus source. The urchins from control trials displayed erratic movement and, like unsuccessfully orienting urchins, tended to move both upstream and cross-stream. Only four urchins out of the 81 trials displayed net downstream movement. Each path took approximately 15 min.

Flow speed affected the overall ability of the urchins to locate the chemical stimulus, but did not alter the kinematics of the urchins' orientation behavior. Successful location of the stimulus increased from 0% at 1 cm s^{-1} to approximately 70% at $U = 5$ and 10 cm s^{-1} . These success rates were significantly related to flow speed (Figure 5; Pearson's $\chi^2_{2,45} = 18.7$, $P < 0.001$). A two-way ANOVA was performed to test for the effects of treatment stimuli and flow on movement speed. Since there was no significant flow effect on movement speed ($F_{2,76} = 1.30$, $P = 0.28$), these data were lumped across flow treatments for further analysis. In fact, flow speed had no significant effect on any of the quantified behaviors, besides success rate. Analysis of the effect of the presence of a chemical stimulus on movement speed revealed that urchins moved at the same speed

regardless of the presence of a stimulus (avg. speed = 0.19 ± 0.02 cm s⁻¹ SD; ANOVA, $F_{2,76} = 1.50$, $P = 0.23$).

As seen in Figure 6, the heading angles defined by the initial and final positions of the urchins in the flume were not uniformly distributed (Rayleigh's z test, $z_{0.05, 77} = 294.7$, $P < 0.001$). In fact, all urchins tended to move upstream or cross-stream, but not downstream. Successful urchins moved more directly towards the stimulus source as compared to control or unsuccessful urchins. The greater amount of cross-stream movement in control and unsuccessful urchins was expected, as this behavior increases the probability of encountering an advected chemical stimulus. The consistent upstream movement of successful urchins was indicated by very uniform, low mean heading angles, and is suggestive of guided navigation. There was also very little dispersion (or deviation) around this mean heading angle. The mean heading angles of control and unsuccessful urchins were similar to successful urchins, but there was greater dispersion around the mean in these two groups.

Analysis of the movement paths showed that although the direction of movement obviously differed between successful, unsuccessful, and control urchins, subtle differences also occurred in the net-to-gross displacement ratio (NGDR) of the urchins. Similar to the analysis of movement speed, there was no flow effect on NGDR across treatments (Kruskal-Wallis rank sums, $X^2_{0.05, 2} = 2.37$, $P = 0.01$). Lumped NGDR values differed only between successful and unsuccessful urchins (Tukey-Kramer *post-hoc*, $F_{2, 45} = 0.70$, $P > 0.05$, Table 1), where unsuccessful urchins moved in the most circuitous manner. Although the NGDR of successful and control urchins was similar, the turning angles of successful urchins was lower than that of either unsuccessful

urchins or urchins in the control group urchins were significantly higher than successful urchins (Watson's test, $U^2_{29,20} = 0.74$, $P < 0.001$; Figure 7). Figures 8a-c plot the mean turning angles of each path and the amount of dispersion between the turning angles in each urchin group. Taken together, these higher NGDR and lower turn angles and dispersion of successful urchins indicates that urchins finding the source had consistently straighter paths with few course deviations than animals failing to find the source. When the frequency of the turning angles was plotted in 10° increments (Figure 9), the paths of successful urchins were characterized by a high frequency of slight course corrections, whereas control and unsuccessful urchins tended to make fewer but larger angular course corrections (Pearson's Chi, $\chi^2_{0.05,2} = 43.88$, $P < 0.001$). For example, 92% of the turns made by successful urchins were $< 20^\circ$, whereas only about 20% of the turns made by unsuccessful or control urchins were $< 20^\circ$. Referring back to Figures 2-4, the higher frequency of large course corrections is apparent by the greater number of large S-turns and circles in the control and unsuccessful urchin paths.

Chemical Plume Visualization

At the conclusion of the behavioral trials, the 0% success rate of the urchins at $U = 1 \text{ cm s}^{-1}$ was hypothesized to be explained by the lack of sensory information available to the urchin at that flow rate. The interaction of the urchin and dye plume at $U = 1$ and 5 cm s^{-1} is depicted in Figure 10. At $U = 1 \text{ cm s}^{-1}$, the plume tended to separate around the urchin, producing a distinct 1-2 cm thick boundary around the urchin where the dye did not impact the body. This boundary was not apparent in the trials at $U = 5 \text{ cm s}^{-1}$. At this faster flow speed the plume exhibited significantly more turbulent

mixing, characteristic of increasing flow speed. Flow separation around the urchin was not visible; dye readily impacted the body of the urchin. The higher flux of dye contacting the urchin may explain the significantly higher orientation success rate of the urchins at $U = 5 \text{ cm s}^{-1}$ compared to $U = 1 \text{ cm s}^{-1}$.

	Control	Unsuccessful	Successful
Control	-0.119	-0.047	-0.003
Unsuccessful	-0.047	-0.127	0.07
Successful	-0.003	0.07	-0.127

Table 1. Tukey-Kramer multiple comparison of NGDR values for nonparametric data. Numbers represented are the $q - q_{0.05, 30}$. Positive values indicate a significant difference between groups, where $P < 0.05$.

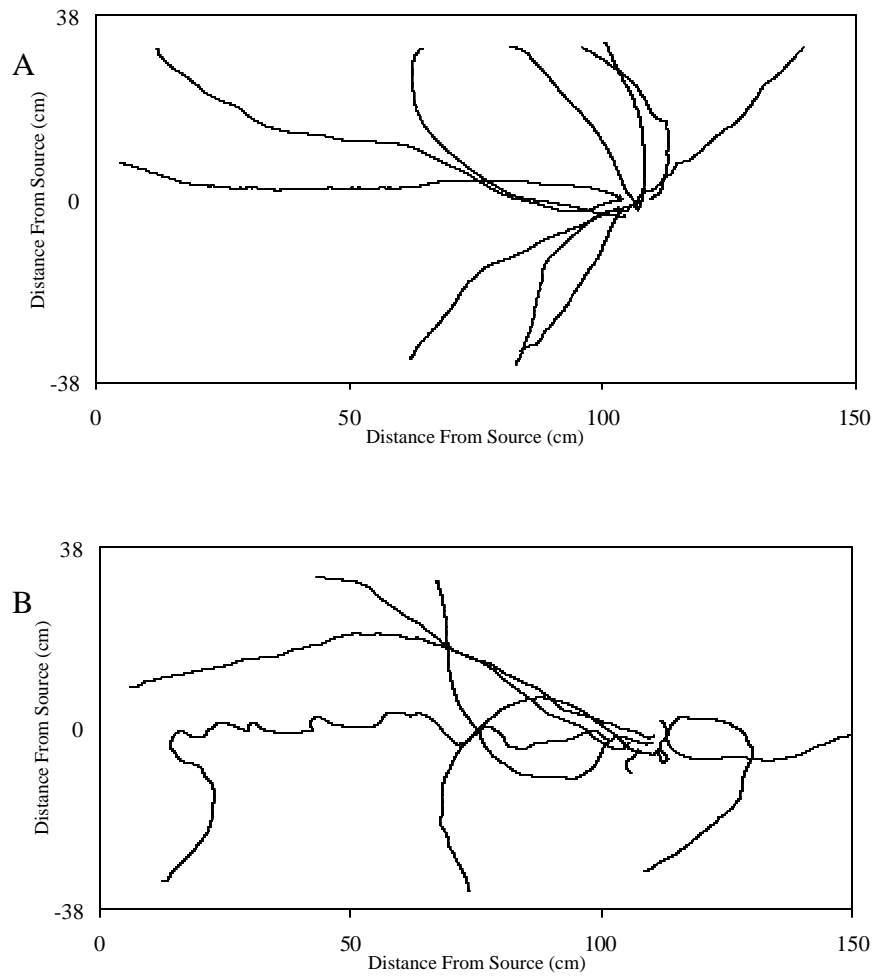


Figure 2. Superimposed path plots of urchins during (A) control and (B) unsuccessful orientation trials at $U = 1 \text{ cm s}^{-1}$. No urchins successfully oriented to the stimulus at this distance. Flow is from left to right. Stimulus is released at 0, 0. Only a representative subset of paths is depicted to reduce clutter.

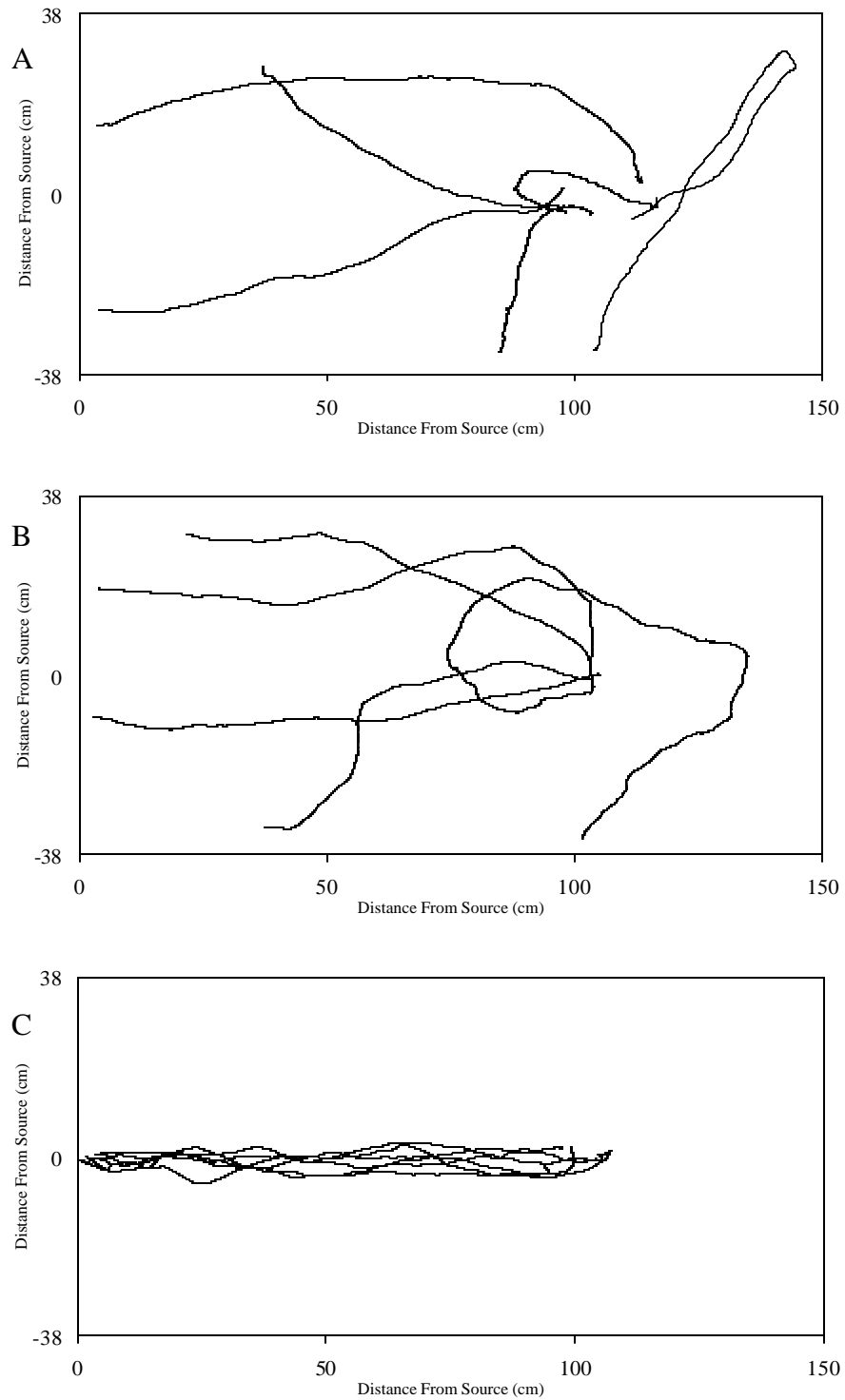


Figure 3. Superimposed path plots of urchins during (A) control and (B) unsuccessful, and (C) successful orientation trials at $U = 5 \text{ cm s}^{-1}$. Flow is from left to right. Stimulus is released at 0, 0. Only a representative subset of paths is depicted to reduce clutter.

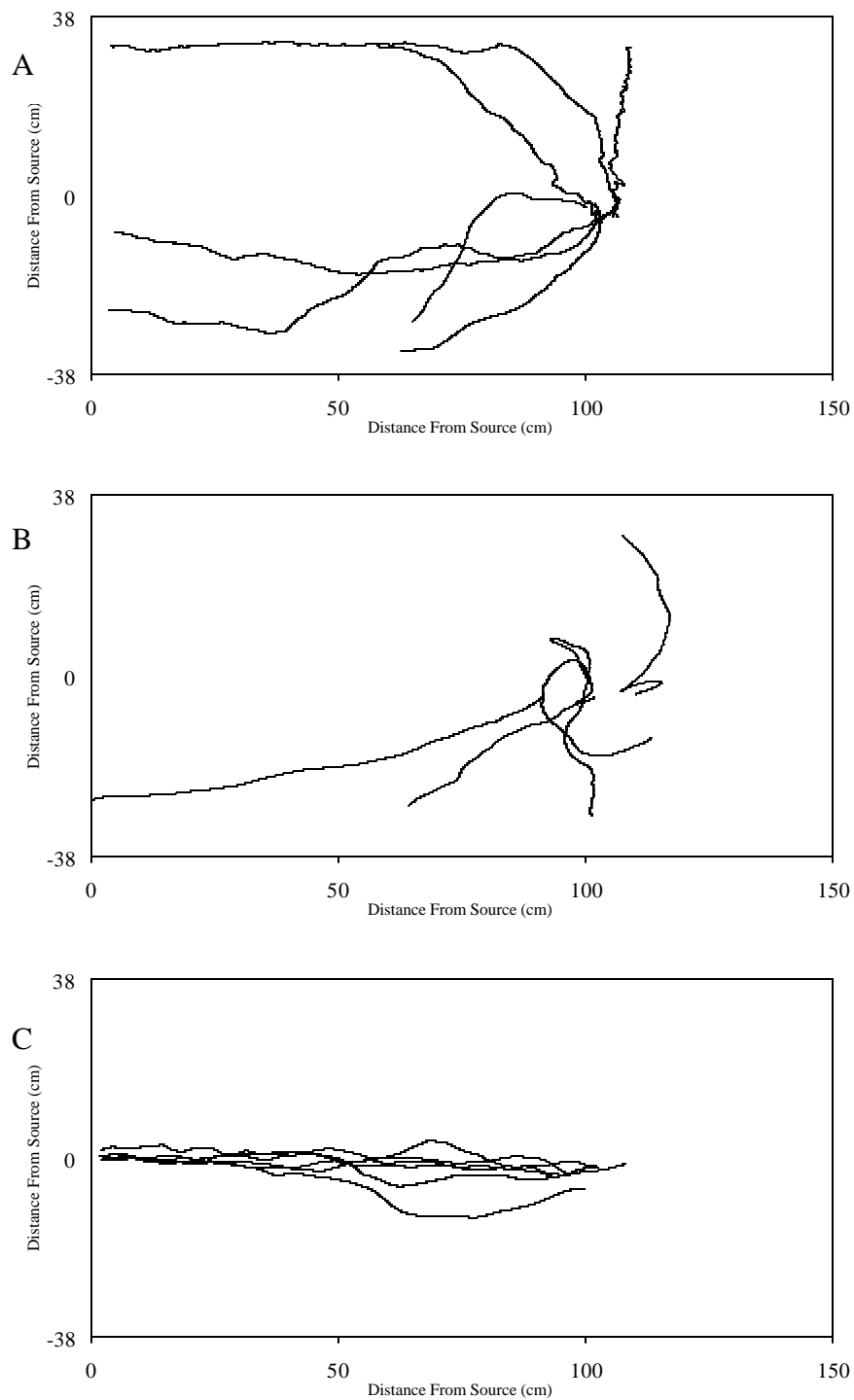


Figure 4. Superimposed path plots of urchins during (A) control and (B) unsuccessful, and (C) successful orientation trials at $U = 10 \text{ cm s}^{-1}$. Flow is from left to right. Stimulus is released at 0, 0. Only a representative subset of paths is depicted to reduce clutter.

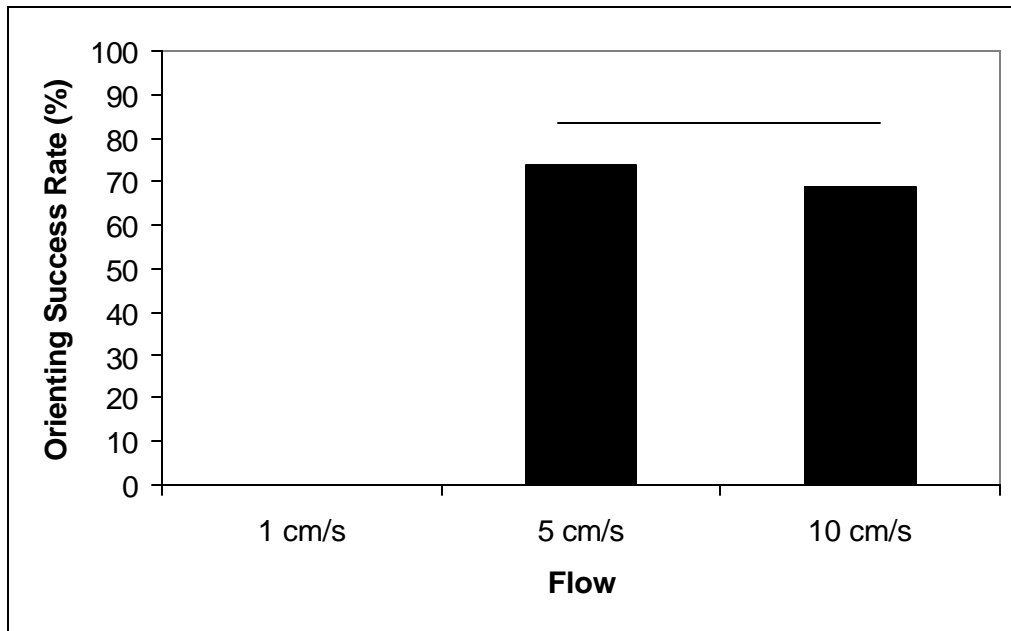


Figure 5. The percent success rate of urchins orienting to food cues at $U = 1, 5$ and 10 cm s^{-1} . $N = 15, 16, 15$, respectively. Success at $U = 1 \text{ cm s}^{-1}$ was significantly lower than at $U = 5$ or 10 cm s^{-1} (Pearson's χ^2 , $P < 0.001$).

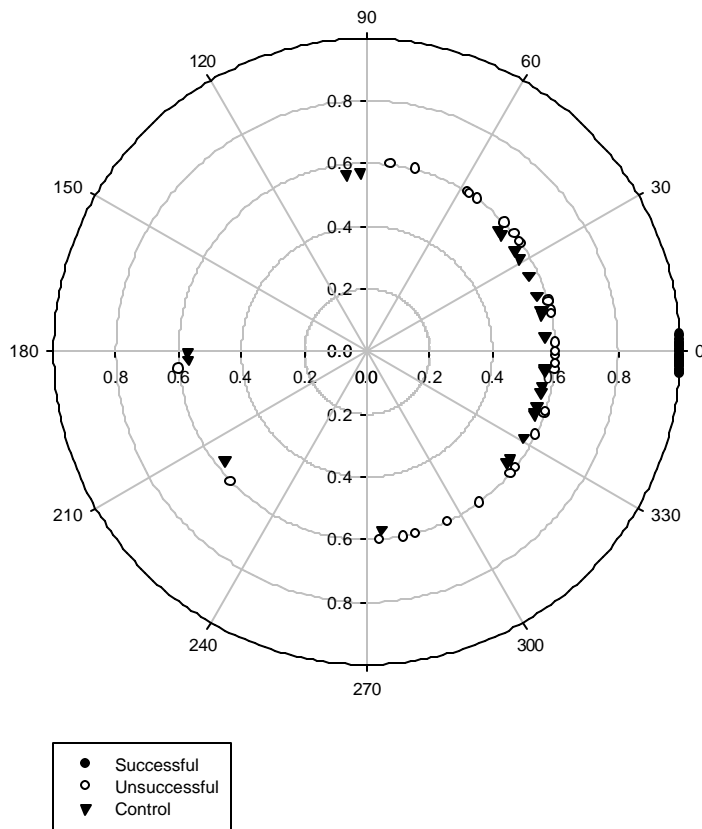


Figure 6. The heading angles of urchins in the flume for all trials. Each point is the heading angle of an urchin from the origin in the flume (radial position 0,0) to its final destination. The position of each group along the radial distance indicates the degree of dispersion for all points in the group. For example, unsuccessful urchins had the highest dispersion, and are plotted at a radial distance of 0.58. N = 25 (successful), 30 (control), and 25 (unsuccessful).

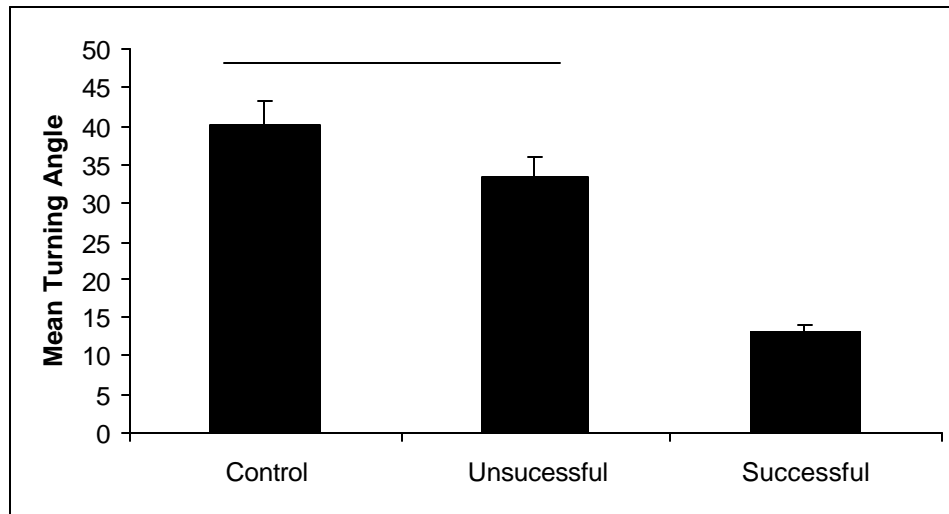


Figure 7. Mean turning angles for urchin movements in the flume. The paths created by control (N = 30) and unsuccessfully orienting (N = 25) urchins had significantly higher mean turning angles than successful urchin paths (N = 25) Horizontal bar indicates treatments are not significantly different based on Watson's test, $P < 0.001$.

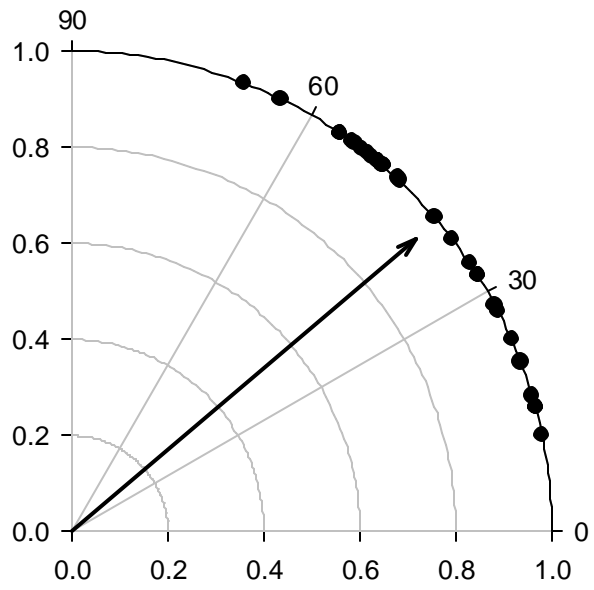


Figure 8a. Turning angles for control urchins. Each point represents the mean turning angle for each urchin path ($N = 30$). The mean turning angle for each path was calculated by averaging the absolute values of the angles (relative to the direction of bulk flow) created between the urchin positions of consecutive frames, at a rate of 0.06 Hz.

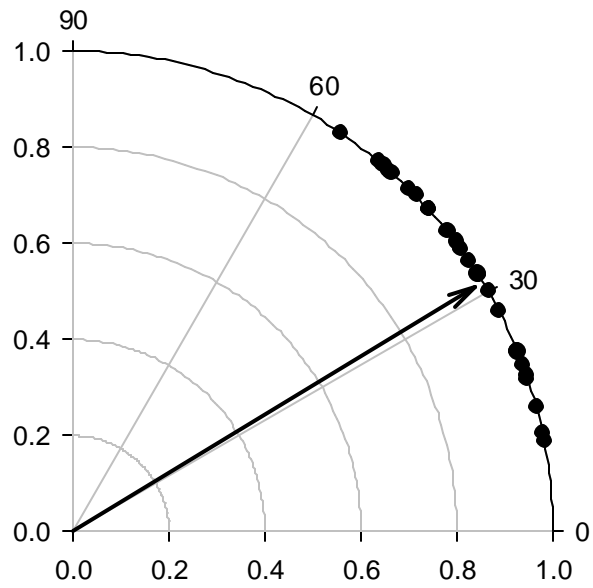


Figure 8b. Turning angles for unsuccessfully orienting urchins. Each point represents the mean turning angle for each urchin path ($N = 25$). The mean turning angle for each path was calculated by averaging the absolute values of the angles (relative to the direction of bulk flow) created between the urchin positions of consecutive frames, at a rate of 0.06 Hz.

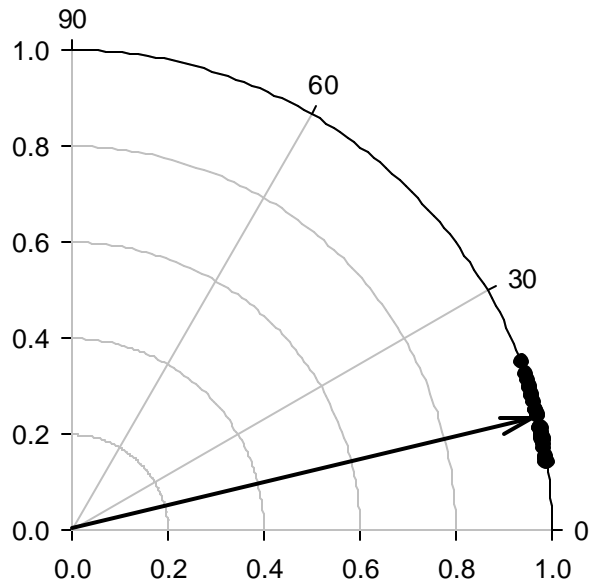


Figure 8c. Turning angles for successfully orienting urchins. Each point represents the mean turning angle for each urchin path ($N = 25$). The mean turning angle for each path was calculated by averaging the absolute values of the angles (relative to the direction of bulk flow) created between the urchin positions of consecutive frames, at a rate of 0.06 Hz.

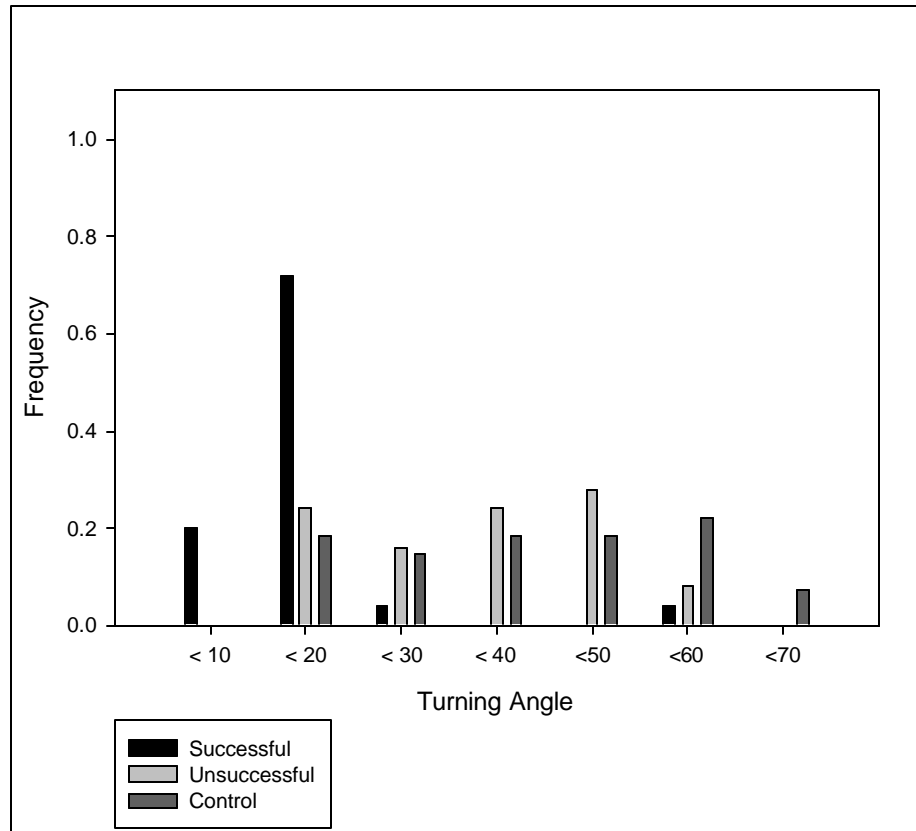


Figure 9. Histogram of turning angle frequencies. Urchins that successfully found the stimulus source had a higher frequency of small turns than those urchins that could not find the source, or were not exposed to a cue (Pearson's X^2 , $P < 0.001$).

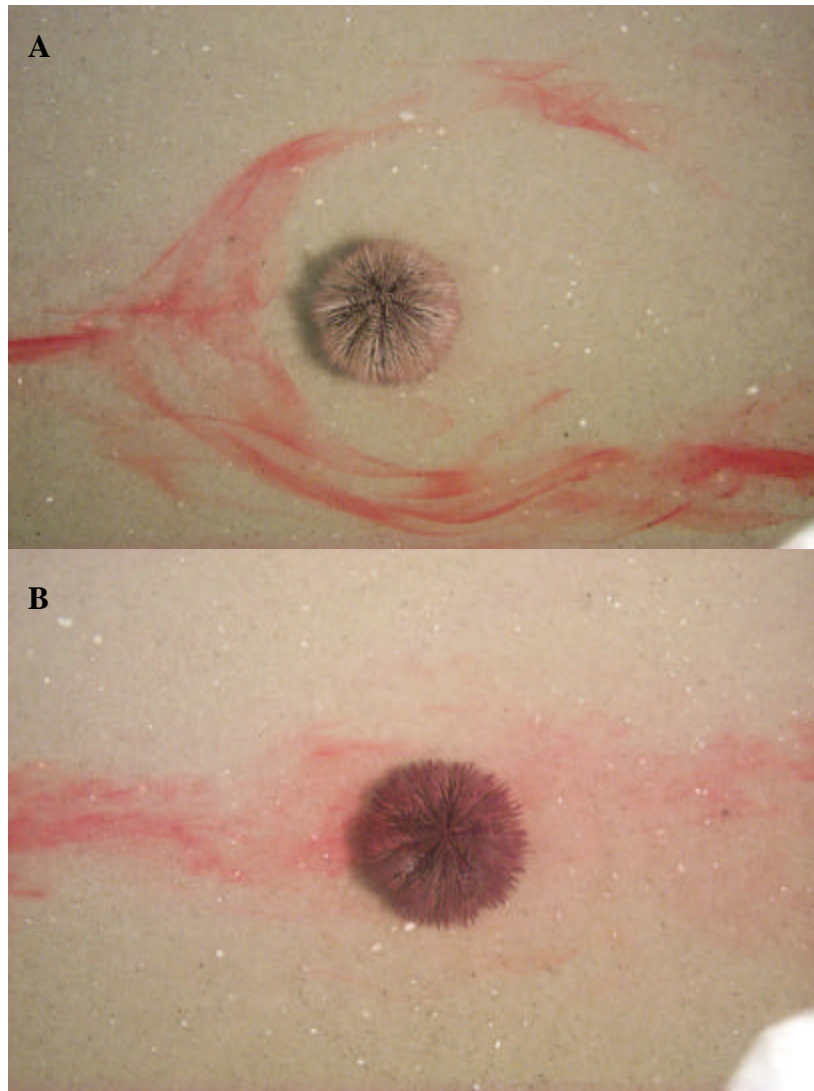


Figure 10. Chemical plume visualization. Pictures depict the interaction of the chemical plume with the urchin at different flow speeds, (A) $U = 1 \text{ cm s}^{-1}$ and (B) $U = 5 \text{ cm s}^{-1}$. The lower Re flow depicted in A results in a thicker boundary layer between the urchin and plume. This boundary breaks down at higher flow speeds and allows the urchin to contact the stimulus.

CHAPTER 4

DISCUSSION

Chemosensory Behavior

Lytechinus variegatus clearly has a well-developed chemosensory behavior. This species showed relatively high success rates in orienting to chemical cues in turbulent flows. This orientation behavior was quite efficient, as revealed by the NGDR values that approached 1.0 and low turning angles. Seventy percent of the assayed urchins (at the 5 and 10 cm s⁻¹ flow speeds) exhibited the same response—successful orientation to the stimulus source with very little deviation in the quantified paths parameters between individuals.

However, there are physical constraints placed on the ability of this urchin to effectively forage in particular flow conditions, like in other marine organisms that rely on chemical cues for ecological interactions (Weissburg et al. 2002b). The lack of success observed for urchins in slow flow (1 cm s⁻¹) may be attributable to the inability of the urchin to contact the odor plume, as a relatively large 2 cm boundary layer formed around the urchin. It must be noted that the results of the 1 cm s⁻¹ experiments should not be applied to all foraging conditions in which *L. variegatus* experiences flows around 1 cm s⁻¹. Any change in the bed roughness, release height, or release rate could drastically affect the degree of turbulence and thus the interaction of the odor plume shape with the urchin, potentially breaking down any boundary around the urchin. In fact, the shell fragments, natural bed unevenness, and seagrasses common in their natural habitat would probably increase the ability of an urchin to successfully orient at such

slow flows. Nonetheless, *L. variegatus* did exhibit considerable success at orienting to turbulent chemical plumes. (Elaborate by recapping data) These same natural features might also extend the upper limit of flows in which an urchin can effectively forage. On a uniform sand bed, a flow speed of 15 cm s^{-1} was fast enough to dislodge *L. variegatus*. However, *Tripneustes ventriocosus*, a closely related species of urchin, inhabits flow areas that exceed 1 m s^{-1} without dislodgement (*personal observation*); though these areas were characterized by a great deal of benthic topography and structure that urchins use to brace themselves. With adequate benthic structure, it might be expected that *L. variegatus* could forage in such high flow areas.

Defining the method of chemolocation has been a goal of many studies that examine the behavior of organisms such as crabs, lobsters, crayfish, asteroids, copepods, flies, etc. Many of these other organisms have well-developed sensory systems besides the ability to detect chemicals, and they use those systems in conjunction with chemosensation. From this study, it appears that *L. variegatus* uses a klino- or tropotaxis behavior. A kinesis of any kind was ruled out due to the lack of difference between the movement speed and path straightness between control and successful treatments, which ought to be apparent when comparing across these different groups. For instance, bacteria locate resources using a klinokinesis, where the presence of a signal increases the movement speed by lessening the amount of time spent 'tumbling'. This tumbling also creates undirected changes in path direction such that paths display straighter trajectories in the presence of a stimulus gradient (Dusenbery 1992).

Rheotaxis was also discounted for several reasons. Primarily, the analysis of the path data suggested that urchins may employ a searching behavior other than a

chemically guided rheotaxis. Many rheotaxis behaviors are typified by casting maneuvers incorporating large turning angles from various points within the orientation path. These angles are created by the searcher engaging in cross-stream movement to more effectively encounter a stimulus that is being advected in a unidirectional flow regime, and then moving directly upstream from the point of signal acquisition. These casting behaviors are initiated once the signal is intermittently lost. As a result, animals not exposed to a stimulus show more direct paths than those tracking a stimulus. In other words, higher turning angle and lower NGDR values are produced during orientation using rheotaxis, particularly in turbulent conditions (Weissburg et al. 2002a). In contrast, the paths of successfully tracking urchins were typically straight lines from the origin to the source, with little angular deviation. Those urchins that did make course corrections did so at angles $< 20^\circ$ (Figure 9). *L. variegatus* also had average NGDR values that approached 1 during successful searches, indicating very direct and efficient movement. Although the NGDR of control urchins also approached 1, the heading and turning angles were significantly different than those of successful urchins. This result indicates that whereas urchins not exposed to a stimulus had a constant direction of movement (albeit more cross-stream), the lack of a signal created deviations in their paths that are associated with higher turning angle values. Another reason why rheotaxis is probably not used in *L. variegatus* is that most of the areas inhabited by the urchin are not characterized by tidally-driven, unidirectional flow, but instead by more complex oscillatory flows (Koch and Gust 1999). In these latter areas, flow is not a reliable cue for directional orientation. Aquatic organisms, like blue crabs, that employ rheotaxis are typically found in areas of strong unidirectional flow, such as tidal creeks or streams. In

these latter situations, the origin of a stimulus will almost always be upstream of the individual (with the exception of turbulent back eddies).

The movement of the urchins in the plume also presents some interesting contrasts with other organisms. Weissburg et al. (2002c) hypothesized that slow moving creatures such as urchins may use time averaged concentration profiles of the odor plume during orientation. Time-averaging requires a significant number of sampling points per space and time to be effective (Webster and Weissburg 2001) in turbulent plumes similar to that used in these experiments. The slow movement speed of urchins and their numerous chemosensors may make this sampling strategy possible. It might be expected that an organism employing such a strategy would orient within the center of the plume, since time-averaged concentrations fall along a Gaussian distribution across the plume cross-section (Webster and Weissburg 2001). The higher average stimulus concentrations along the plume centerline would make orientation success more likely. Orientation within these areas of higher average stimulus concentrations in the plume was observed in *L. variegatus*. Even if the urchin moved somewhat off-center of the plume, it began to orient at an angle that would put it within the plume centerline. The lack of course deviation once the path was started suggests a tropotaxis behavior, where the average concentration of stimuli was compared across the sensory area of the urchin, creating the high frequency of small turning angles (Figure 9). A klinotaxis is also possible, where the average concentration of the stimulus was compared between consecutive points within the orientation path (i.e., temporal comparisons). Although relatively little is known about the neurobiology of urchins (Pentreath and Cobb 1972), the decentralized nervous system makes a completely spatially integrated tropotaxis

highly unlikely. The tube feet sensory neurons are connected to neuropiles, one neuropile for each of the five ambulacral grooves of the urchin. This arrangement makes comparing stimuli between tube feet on different ambulacra improbable. However, by counting the number of podia openings in an urchin test, it can be estimated that there are in excess of 200 tube feet per ambulacral groove, potentially allowing for the spatial integration of sensory information within these chemosensory regions. As neuropiles are connected via the circumoral nerve ring, the most parsimonious explanation of sensory coordination of behavior is that the levels of stimulation between neuropiles are compared, and that there is an inhibition of the less stimulated neuropile regions. Dale (2000) also proposed this mode of coordination for the sea star *Asterias forbesi*. Inhibition would promote the urchin to move in the direction of the most stimulated region. If this is the case, the urchin may engage in an intra-neuropile klinotaxis and an inter-neuropile tropotaxis.

Another alternative hypothesis is that urchins are responding to flow cues but that the lack of casting typically associated with following chemical and flow cues is eliminated due to the slow movement speed, allowing the urchin to process the chemical information more accurately. This hypothesis is possible, but would require trials of pulsed odor plumes to resolve whether an odor-guided rheotaxis orientation strategy is used. However, during several trials at $U = 1 \text{ cm s}^{-1}$, urchins appeared to orient in a cross-stream direction. At this slow flow speed, the boundary layer formed around the urchin eliminated the upstream signal, but odor filaments had a greater tendency to contact the sides of the urchin, evoking the cross-stream tracking response. This cross-stream movement during sensory stimulation would be contradictory to a behavior

incorporating rheotaxis, but further supports the idea of regional inhibition guiding movement.

The ability to time-average the information received from a chemical plume may be especially useful in turbulent conditions, and might explain the success differences between urchins and organisms that use instantaneous plume properties, which are not effective foragers in highly turbulent flow. Turbulent mixing increases the uniformity of a chemical plume, but reduces the number of high concentration stimulus peaks that are used by some animals such as moths and blue crabs (Webster and Weissburg 2001). The sensory landscape of odor plumes appears quite different to crabs foraging in low versus high turbulence flows. Urchins are not detecting individual odor peaks if they use a time averaging strategy, and thus the odor landscapes might appear quite similar between turbulent and non-turbulent plumes, assuming the flux of stimulants is conserved. As a result, there should be few behavioral differences between an urchin foraging in high and low turbulence areas if the flow speed is within a range within which the urchin's shape does not constrain its ability to move or contact the signal. Quantifications of behavior (i.e., NGDR, heading angle, and turning angle) were the same between urchins orienting at relatively low turbulence intensity (5 cm s^{-1}) and high turbulence intensity (10 cm s^{-1}), which is consistent with a strategy that incorporates time averaging.

The ability to detect chemical signals is probably under strong selection for sea urchins, as they possess no other known distance sensory modality (besides the reaction to light) that would allow them to glean information from their environment. As urchins rely predominately on chemical cues, it should be expected that they would maximize their ability to receive the chemical cues. Copepods, for example, rely predominantly on

mechanical cues for detecting food and predators (Yen 2000). The anatomical interference of self-generated mechanical signals has been minimized by copepods by placing mechanosensors outside the area impacted by their own mechanical signals. Likewise, urchins appear to employ a similar strategy. Their chemosensory tube feet extend several centimeters past their test. In fact, when urchins were orienting in the presence of a chemical cue, their tube feet were fully extended and waving in the odor plume, a behavior not typically displayed when the urchins were sedentary. It would also be expected that tube foot length should surpass spine length in chemically orienting urchins to minimize sensory interference. In fact, over evolutionary history, spine length has decreased, especially in urchins like *L. variegatus* (Paul 1977). Instead of spines, short-spined urchins commonly use camouflage to defend against predation, and many seek out covering material that chemically deters predators. The ability to find such materials may require distance chemoperception, and there is evidence that some urchins, such as *Arbacia punctulata*, have the ability to find such chemically defended algae from a distance (Hay et al. 1986). Urchins may be under stabilizing selection to maintain their slow movement speed (unlike many species that are commonly preyed upon), as increases in speed might adversely affect their ability to constantly detect chemical information. Even slight increases in speed would probably not give them any advantage over predators, but could easily decrease their ability to time-average a signal.

Ecological Significance

Previous investigators have questioned the need for *L. variegatus* to use distance chemosensory cues to find food, especially in areas where foods such as *Thalassia*

testudinum are plentiful. It is possible that *L. variegatus* is simply grazing in such instances. However, locating highly nutritive, opportunistic resources such as mollusc egg masses, would require using distance sensory information to efficiently find the resource before it is exploited by other individuals. Urchins may not be orienting to such resources from afar, but within distances of a few meters or less, where their proximity coupled with their highly effective foraging behavior might allow urchins to acquire the resource before other foraging organisms, such as crabs and molluscs. Over short distances, speed would not be as critical as success probability. There is an apparent disparity, however, between the flume success rates at $U = 1 \text{ cm s}^{-1}$ and the ability of urchins to forage within seagrass beds, where flows typically do not exceed $U = 1 \text{ cm s}^{-1}$. The turbulence levels of these areas, due to higher bed roughness, might be sufficient to allow for chemosensory success.

Two other situations may also arise when distance chemosensory cues might be needed, even in seagrass beds. During the winter, when primary production is reduced, *T. testudinum* is much more susceptible to the effects of overgrazing by urchins (Heck and Valentine 1995). In fact, areas may be denuded of seagrasses as a result of this intense herbivory. Urchins must then either move to a new patch, or forage for other foods within the depleted patch. Moreover, higher densities of urchins are found within beds of the mussel *Modiolus americanus* than in the surrounding *Thalassia* beds (Valentine and Heck 1993). The use of chemosensory cues would aid either of these habitat choices.

Not only may chemical cues be important in trophic and habitat choice, but as described earlier, the response of urchins to con- and heterospecifics is well documented.

Although the chemical cues for alarm responses and mate detection will differ, the mechanisms by which the organisms interpret and orient to (or away from) the cue source will probably be the same as in trophic interactions. Mating pheromones have been isolated from other species of urchins, and fertilization success is a function of the proximity of individuals during broadcast spawning.

The cohabitation with seagrasses is not obligatory for *L. variegatus*. The range of this species extends to coastal and offshore areas absent of seagrasses, where macroalgae and sessile invertebrates are their predominant food resource (Kaplan 1988). Offshore reefs may provide a mosaic of food choices as well as competitive interactions for these foods. In these situations, the ability of the urchin to respond to a variety of cues in dynamic flow environments will be necessary for survival.

Synthesis

The sensory behavior of *L. variegatus* may allow it to effectively respond to chemical cues in a variety of flow environments. It appears that this species uses an odor guided tropotaxis or klinotaxis, which might be quite suitable for detecting chemical cues in a variety of flow conditions. Interestingly, the major sensory constraint on this species may be imposed by its own bluff shape, which, unlike other more hydrodynamically streamlined organisms, prevents the delivery of chemical signals to its sensors in low Reynolds flows.

Heretofore, the sensory capabilities of urchins have not been fully appreciated, but this study clearly demonstrates a consistent behavioral response to food chemical cues that may give *L. variegatus* competitive advantages over other foraging animals in

some situations. This capability may be most important in finding opportunistic or ephemeral foods and in habitats that may present a complex assortment of potential food choices. Besides orientation to food cues, ecological interactions such as locating mates and shelter and avoiding predators may also make use of chemosensory behaviors. As urchins are important components of many communities, their chemosensory behavior may be an important factor in the outcome of a variety of trophic and competitive networks.

REFERENCES

- Atema, J. 1996. Eddy chemotaxis and odor landscapes: exploration of nature with animal sensors. *Biological Bulletin* **191**:129-138.
- Beddingfield, S. D., and J. B. McClintock. 1998. Differential survivorship, reproduction, growth and nutrient allocation in the regular echinoid *Lytechinus variegatus* (Lamarck) fed natural diets. *Journal of Experimental Marine Biology and Ecology* **226**:195-215.
- Beddingfield, S. D., and J. B. McClintock. 1999. Food resource utilization in the sea urchin *Lytechinus variegatus* in contrasting shallow-water microhabitats of Saint Joseph Bay, Florida. *Gulf of Mexico Science* **17**:27-34.
- Bologna, P. A. X., and K. L. Heck. 2000. Impacts of seagrass habitat architecture on bivalve settlement. *Estuaries* **23**:449-457.
- Brown, D. A., and H. C. Berg. 1974. Temporal stimulation of chemotaxis in *Escherichia coli*. *Proceedings of the National Academy of Science, U.S.A.* **71**:1388-1392.
- Burr, A. H. 1984. Photomovement behavior in simple invertebrates. Pages 179-215 in M. A. Ali editor. *Photoreception and vision in invertebrates*. Plenum, New York.
- Camp, D. K., S. P. Colin, and J. F. Van Breedveld. 1973. Overgrazing of seagrasses by a regular urchin, *Lytechinus variegatus*. *BioScience* **23**:37-38.
- Campbell, A. C., and M. S. Laverack. 1968. The responses of pericellariae from *Echinus esculentus* (L.). *Journal of Experimental Marine Biology and Ecology* **2**:191-214.
- Carpenter, R. C. 1984. Predator and population density control of homing behavior in the Caribbean echinoid *Diadema antillarum*. *Marine Biology* **82**:101-108.
- Carr, W. E. S. 1988. The molecular nature of chemical stimuli in the aquatic environment. Pages 3-27 in J. Atema, R. R. Fay, A. N. Popper, and W. N. Tavolga editors. *The sensory biology of aquatic animals*. Springer-Verlag, New York.
- Castilla, J. C. 1972. Responses of *Asterias rubens* to bivalve prey in a Y-maze. *Marine Biology* **12**:222-228.
- Dale, J. 2000. Chemosensory search behavior in the starfish *Asterias forbesi*. Ph.D. Dissertation: Boston University.
- Derby, C. D., and P. Steullet. 2001. Why do animals have so many receptors? The role of multiple chemosensors in animal perception. *Biological Bulletin* **200**:211-215.

- Derby, C. D., P. Steullet, A. J. Horner, and H. S. Cate. 2001. The sensory basis of feeding behaviour in the Caribbean spiny lobster, *Panulirus argus*. *Marine and Freshwater Research* **52**:1339-1350.
- Doall, M. H., S. P. Colin, J. R. Strickler, and J. Yen. 1998. Locating a mate in 3D: the case of *Temora longicornis*. *Philosophical Transactions of the Royal Society of London.B.* **353**:681-689.
- Dusenbery D. B. 1992. *Sensory Ecology: how organisms acquire and respond to information*. W.H. Freeman and Company, New York.
- Dusenbery, D. B. 1996. Information is where you find it. *Biological Bulletin* **191**:124-128.
- Dusenbery, D. B. 2001. Performance of basic strategies for following gradients in two dimensions. *Journal of Theoretical Biology* **208**:345-360.
- Estes, J. A., M. T. Tinker, T. M. Williams, and D. F. Doak. 1998. Killer whale predation on sea otters linking oceanic and nearshore ecosystems. *Science* **282**:473-476.
- Gambi, M. C., A. R. M. Nowell, and P. A. Jumars. 1990. Flume observations on flow dynamics in *Zostera marina* (eelgrass) beds. *Marine Ecology Progress Series* **61**:159-169.
- Gomez, G., and J. Atema. 1996. Temporal resolution in olfaction I: Stimulus integration time of lobster chemoreceptor cells. *Journal of Experimental Biology* **199**:1771-1779.
- Grant P. T., and A. M. Mackie. 1974. *Chemoreception in marine invertebrates*. Academic Press, New York.
- Hagen, N. T., A. Andersen, and O. B. Stabell. 2001. Alarm responses of the green sea urchin, *Strongylocentrotus droebachiensis*, induced by chemically labeled durophagus predators and simulated acts of predation. *Marine Biology* **140**:365-374.
- Hay, M. E., R. R. Lee, and R. A. Guieb. 1986. Food preference and chemotaxis in the sea urchin *Arbacia punctulata* (Lamarck) Philippi. *Journal of Experimental Marine Biology and Ecology* **96**:147-153.
- Heck, K. L., and J. F. Valentine. 1995. Sea urchin herbivory: evidence for long-lasting effects in subtropical seagrass meadows. *Journal of Experimental Marine Biology and Ecology* **189**:205-217.
- Hendler G., J. E. Miller, D. L. Pawson, and P. M. Kier. 1995. *Sea Stars, Sea Urchins, & Allies*. Smithsonian Institution Press, Washington D.C.

- Hughes, T. P. 1994. Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science* **265**:1547-1551.
- Jangoux M., and J. M. Lawrence. 1982. *Echinoderm Nutrition*. A.A. Balkema, Rotterdam.
- Keller, T.A., Powell, I. and M.J. Weissburg. 2003. Role of appendages in chemically mediated tracking behavior of blue crabs. *Marine Ecology Progress Series* **261**:217-231
- Klinger, T. S., and J. M. Lawrence. 1985. Distance perception of food and the effect of food quantity on feeding behavior of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea). *Marine Behavior and Physiology* **11**:327-344.
- Koch, E. W., and G. Gust. 1999. Water flow in tide- and wave-dominated beds of the seagrass *Thalassia testudinum*. *Marine Ecology Progress Series* **184**:63-72.
- Lawrence, J. M., and P. W. Sammarco. 1982. Effects of feeding on the environment: Echinoidea. Pages 499-519 in M. Jangoux, and J. M. Lawrence editors. *Echinoderm nutrition*. A.A. Balkema, Rotterdam.
- Leonard, G. H., J. M. Levine, P. R. Schmidt, and M. D. Bertness. 1998. Flow-driven variation in intertidal community structure in a Maine estuary. *Ecology* **79**:1395-1411.
- Levitan, D. R., M. A. Sewell, and F. S. Chia. 1992. How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. *Ecology* **73**:248-254.
- Lowe, E. F., and J. M. Lawrence. 1976. Absorption efficiencies of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea) for selected marine plants. *Journal of Experimental Marine Biology and Ecology* **21**:223-234.
- Mathis, A., and J. F. Smith. 1993. Chemical labeling of the northern pike (*Esox lucius*) by the alarm pheromone of fathead minnows (*Pimephales promelas*). *Journal of Chemical Ecology* **19**:1967-1979.
- Mathis, A., D. P. Chivers, and J. F. Smith. 1995. Chemical alarm signals: predator deterrents or predator attractants. *The American Naturalist* **145**:994-1005.
- Moore, P., and D. Lepper. 1997. Role of chemical signals in the orientation behavior of the sea star *Asterias forbesi*. *Biological Bulletin* **192**:410-417.
- Moore, P., and J. L. Grills. 1999. Chemical orientation to food by the crayfish *Orconectes rusticus*: influence of hydrodynamics. *Animal Behaviour* **58**:953-963.
- Paul, R. C. 1977. Evolution of primitive echinoderms. Pages 123-158 in A. Hallam editor. *Patterns of evolution as illustrated by the fossil record*. Elsevier, New York.

- Pentreath, V. W., and J. L. S. Cobb. 1972. Neurobiology of Echinodermata. *Biological Review* **47**:363-392.
- Rivera, J. A. 1979. Echinoid mortality at Jobos Bay, Puerto Rico. *Proc. Assoc. Isl. Mar. Lab. Carib.* **13**: 9.
- Sharp, D. T., and I. E. Gray. 1962. Studies on factors affecting the local distribution of two sea urchins, *Arbacia punctulata* and *Lytechinus variegatus*. *Ecology* **43**:309-313.
- Sklenar, S. A. 1994. Interactions between sea urchin grazers (*Lytechinus variegatus* and *Arbacia punctulata*) and mussels (*Modiolus americanus*): a mutualistic relationship? M.S. Thesis: Univ. S. Alabama.
- Sloan, N. A., and A. C. Campbell. 1982. Perception of Food. Pages 3-23 in M. Jangoux, and J. M. Lawrence editors. *Echinoderm nutrition*. A.A. Balkema, Rotterdam.
- Smith, A. B. 1979. Peristomal tube feet and plates of regular echinoids. *Zoomorphologie* **94**:67-80.
- Snyder, N., and H. Snyder. 1970. Alarm response of *Diadema antillarum*. *Science* **168**:276-278.
- Sokal R. F., and F. J. Rohlf. 1994. *Biometry: The Principles and Practice of Statistics in Biological Research.*, 3rd edition. W.H. Freeman & Co., New York.
- Vadas, R. L. 1977. Preferential feeding: an optimization strategy in sea urchins. *Ecological Monographs* **47**:337-371.
- Vadas, R. L., R. W. Elner, P. E. Garwood, and I. G. Babb. 1986. Experimental evaluation of aggregation behavior in the sea urchin *Strongylocentrotus droebachiensis*. *Marine Biology* **90**:433-448.
- Valentine, J. F., and K. L. Heck. 1991. The role of sea urchin grazing in regulating subtropical seagrass meadows: evidence from field manipulations in the northern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology* **154**:215-230.
- Valentine, J. F., and K. L. Heck. 1993. Mussels in seagrass meadows: their influence on macroinvertebrate abundance and secondary production in the northern Gulf of Mexico. *Marine Ecology Progress Series* **96**:63-74.
- Vermeij G. J. 1987. *Evolution and Escalation*. Princeton University Press, Princeton.
- Watts, S. A., J. B. McClintock, and J. M. Lawrence. 2001. The ecology of *Lytechinus variegatus*. Pages 375-393 in J. M. Lawrence editor. *Edible sea urchins: biology and ecology*. Elsevier Science, New York.

- Webster, D. R., and M. J. Weissburg. 2001. Chemosensory guidance cues in a turbulent chemical odor plume. *Limnology and Oceanography* **46**:1034-1947.
- Weissburg, M. J., and R. K. Zimmer-Faust. 1993. Life and death in moving fluids: Hydrodynamic effects on chemosensory-mediated predation. *Ecology* **74**:1428-1443.
- Weissburg, M. J. 2000. The fluid dynamical context of chemosensory behavior. *Biological Bulletin* **198**:188-202.
- Weissburg, M. J., D. B. Dusenbery, H. Ishida, J. Janata, T. A. Keller, P. J. W. Roberts, and D. R. Webster. 2002a. A multidisciplinary study of spatial and temporal scales containing information in turbulent chemical plume tracking. *Environmental Fluid Mechanics* **2**:65-94.
- Weissburg, M. J., C. P. James, D. L. Smee, and D. R. Webster. 2002b. Fluid mechanics produces conflicting constraints during olfactory navigation of blue crabs, *Callinectes sapidus*. *Journal of Experimental Biology*.
- Weissburg, M. J., M. C. Ferner, D. P. Pisut, and D. L. Smee. 2002c. Ecological consequences of chemically mediated prey perception. *Journal of Chemical Ecology* **28**:1933-1950.
- Weissburg, M. J., and D. B. Dusenbery. 2002. Behavioral observations and computer simulations of blue crab movement to a chemical source in a controlled turbulent flow *Journal of Experimental Biology* **205**:3387-3398.
- Williams, S. L., and K. L. Heck. 2001. Seagrass community ecology. Pages 317-338 in M. D. Bertness, S. Gaines, and M. E. Hay editors. *Marine Community Ecology*. Sinauer, Sunderland, MA.
- Yen, J. 2000. Life in transition: balancing inertial and viscous forces by planktonic copepods. *Biological Bulletin* **198**:213-224.
- Zar J. H. 1999. *Biostatistical Analysis*, 4th edition. Prentice Hall, Upper Saddle River.
- Zimmer-Faust, R. K. 1987. Crustacean chemical perception: Towards a theory on optimal chemoreception. *Biological Bulletin* **172**:10-29.
- Zimmer-Faust, R. K. 1993. ATP: A potent prey attractant evoking carnivory. *Limnology and Oceanography* **38**:1271-1275.