

All females seem to arrive on the spawning grounds with sperm stored in a receptacle below the mouth (2; pers. obs.); the sperm presumably come from mating in the "head-to-head" position (1, 2, 3) while offshore. Theoretically these animals need not mate again, yet all squids engage in reproductive behavior for much, if not most, of each day, both in the field and the laboratory. When eggs are placed in tank or pond systems, the males approach, investigate the eggs through sight, touch and perhaps olfaction—and then aggressive behavior begins. Large males compete vigorously in agonistic contests with other males for access to females; these contests consist primarily of bright white visual displays that can escalate to mild fin beating. Males and females exchange a variety of body pattern signals as they form temporary pairs that lasted up to two days in the laboratory trials. Large males mate the females in the "male parallel" position (1, 2, 3) and guard them as they approach the egg mass and lay each egg capsule.

"Sneaker" males, which are smaller than the typical large males discussed above, use an alternative tactic. They do not engage large males in agonistic contests; instead they jet forward suddenly onto the arms of a female as the large male and female pair approaches the egg mass. They swiftly deposit spermatophores directly on the egg capsule that is presumably amidst the female's arms (Fig. 1). Thus there are several potential sources of sperm for each egg capsule: stored sperm, the large male, and the sneaker male. To determine the winner (or

winners) of these sperm competition games, some method of paternity assessment must be used, and these are being developed.

Might the female exert some choice over sperm allocation to the more than 100 eggs in each capsule? Many such important evolutionary questions can now be addressed because the range of behaviors in the field and pond is very similar to that in the laboratory. Thus, by adding an egg mass to a group of squids that includes several male-female pairs and a small male, the mating system can be reproduced in the laboratory, and the dynamics of the interactions can be recorded and analyzed.

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Orientation Behavior of the Lobster: Responses to Directional Chemical and Hydrodynamic Stimulation of the Antennules

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Although turbulent odor plumes contain spatial chemical gradients that could be used for chemotactic orientation (1, 2), the notion that animals use purely chemotactic mechanisms to locate odor sources remains unsubstantiated. Studies of lobsters (*Homarus americanus*) with aesthetasc (*i.e.*, olfactory) sensilla of one antennule removed have shown that bilateral chemical information is important for efficient localization behavior (3). Other studies suggest that lobsters may use certain specific parameters of an odor pulse to identify a spatial gradient leading to the source of the stimulus (1, 4, 5). A direct test of this hypothesis awaits direct stimulation of the antennules with odor pulses of known shape and rate: bilateral stimulus differences leading to turning behavior would provide information on the parameters of odor pulses used for "chemotaxis" (reviews: 6, 7).

We developed a lobster-mounted, four-nozzle odor delivery

system that can jet small, measured volumes of flavored or unflavored seawater toward either antennule, or both, in freely moving, blindfolded animals. Two pulse generator-driven syringe pumps pushed brief pulses of either filtered seawater or a fish juice solution (0.015%) through two small tubing systems (134 cm of 0.76 mm inner diameter; and 200 cm of 1.19 mm diameter). Each of these systems could be switched through three-way valves to deliver pulses toward the left or right antennule, or toward both simultaneously. In these experiments we used a 1-s pulse that delivered 86 (± 38 S.D.) μ l per pulse at a rate of 1 pulse per 15 s. We used 10 food-deprived lobsters (5 male, 5 female; carapace lengths about 75 mm); each animal was used in two trials. A trial consisted of a half-hour acclimation period followed by 30 stimuli distributed in random order among the six conditions: left, right, or bilateral stimuli containing either seawater or fish juice.

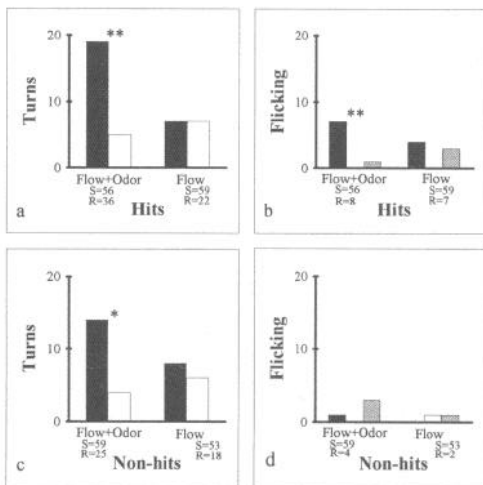


Figure 1. Directional responses of lobsters to unilateral antennule stimulation. Ordinate: number of responses: turns (a, c); number of antennule flick bursts (b, d); Direction of the response: ipsilateral (toward the stimulus, black bars); contralateral (white bars); bilateral (gray bars). Stimuli: flavored (Flow + Odor); unflavored (Flow). (S) number of stimuli; includes “no response.” (R) Number of responses; includes non-directional responses not represented in the bar graphs (i.e., stop, move forward, move backward). Hits (a, b): a dyed pulse appears to hit an antennule, determined from video analysis. Non-hits (c, d): dyed pulse appears to miss the antennule (but may still provide flow or some chemical stimulus). Significant ipsilateral responses (**P < 0.01, *P < 0.05) were turns caused by flavored stimuli (a. Chi square 8.2, c. Chi square 5.5) and flicks caused by flavored hits (b. sign test).

Lobster behavior was videotaped with two cameras: one to record locomotion was fixed above the flow tank (377 cm long, 136 cm wide, 35 cm high, 30 cm water depth, flow rate 1 cm/s), and one was hand-held to record close-up antennule position and flicking, as well as pulse delivery. Both seawater and fish juice were dyed with rhodamine to visualize pulse delivery, thus allowing us to score each pulse as either a “hit” or “non-hit.” Whether or not the animal was walking, we scored the following behaviors within 10-s post-stimulus: left or right turn

(30° or more, excluding turns near tank walls), stop, move forward, move backward.

The results show that significant ipsilateral turning occurred not only when fish juice stimuli hit the corresponding antennule (Fig. 1a), but also with fish juice stimuli judged as not hitting the ipsilateral antennule (Fig. 1c). Evidently, our ability to distinguish visually a hit from a non-hit was not shared by the lobsters. Unflavored stimuli did not cause turning toward the stimulated side (Fig. 1a, c). Turns were observed 2–8 s after stimulus release. Flicking was significantly unilateral for flavored hits and never contralateral for flavored and unflavored hits (Fig. 1b); non-hits caused very few flicks (Fig. 1d). Often there was no response, e.g., Figure 1a: 56 stimuli (S) resulted in 36 responses (R), 24 of which were either ipsi- (black bar = 19) or contra- (white bar = 5) lateral turns, and 12 were non-directional.

We conclude that unilateral stimulation of the antennule with food odor pulses can cause ipsilateral turning with 2–8-s delays even if the pulse does not hit the antennule directly, and that unilateral odor pulses hitting the antennule directly can trigger ipsi- and bilateral flicking, but no contralateral flicking. We cannot conclude that odor alone determines directional responses because all of the odor stimuli were embedded in hydrodynamic pulses—a situation common to many natural odor dispersal patterns.

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