Taxis in *Aplysia dactylomela* (Rang, 1828) to water-borne stimuli from conspecifics

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Sixteen reproductively mature *Aplysia dactylomela* were observed in a unidirectional stream under each of four conditions: sea water only, one sea hare, six sea hares, and a copulating sea hare pair. Streams containing conspecific stimulation were significantly more effective in eliciting a positive taxis towards the stimulus source. A copulating pair was not different from one or six animals in producing the approach. The sea hares showed a distinct final head orientation to six sea hares when compared with sea water only; final orientation did not differ in any other comparison.

The process by which sea hares aggregate with or without copulation is not known. One theory concerning the formation of aggregations was proposed by Eales (1921). She suggested that as the animals grow they pass from the sublittoral to the littoral zone in relation to the different algae found at varying depths. Although Carefoot (1967) and Miller (1960) found that the type of migration proposed by Eales was not necessary to complete the life cycle of a population, their studies did not exclude the possibility of its occurrence. On the other hand, recent studies of feeding behavior in *Aplysia* (Audesirk, 1975; Frings & Frings, 1965; Jahan-Parwar, 1972; Kupfermann, 1974; Preston & Lee, 1973) have demonstrated that *Aplysia* respond to food stimuli at a distance, and that they will move negatively with respect to current in doing so. Thus, it is possible that in the field an aggregation can be formed in relation to food sources. Other factors such as currents (Hamilton & Ambrose, 1975; Lederhendler, Bell, & Tobach, 1975) and location (Kupfermann & Carew, 1974) may also influence the movements of *Aplysia* and therefore the aggregation process.

One of the variables which may be of considerable significance for aggregation is the role played by conspecific stimulation, which would be a critical factor in further comparative discussions of social organization in *Aplysia* (Tobach & Schneirla, 1968).

Behavioral evidence of conspecific responsiveness at a distance has been implied for opisthobranchs

in the work of Crozier (1918), Davis and Mpitsof (1971), and Zack (1975). Recently, Audesirk and Audesirk (1977) provided neurophysiological evidence for this responsiveness in *A. californica*. Aspey and Blankenship (1976) found that burrowing in *A. brasiliana* appeared to be induced by the presence of burrowed individuals, and egg-laying conspecifics seemed to affect the behavior of burrowed animals by increasing the probability of aggregation and copulation in that species. Jahan-Parwar (1976) cited data supporting an aggregation response of *A. californica* to the presence of egg-masses.

The experiment reported here was designed to determine whether *Aplysia dactylomela* responds to stimuli from conspecifics. The apparatus chosen allowed animals to respond to a source of stimulation by approach or withdrawal. By "approach" is meant any process resulting in a decrease in the distance between the head of the animal and the source of stimulation. "Withdrawal" was defined as an increase in this distance. Approach was measured in terms of total distance moved towards or away from the source of stimulation. The orientation of the animals was recorded every 15 sec in terms of the head of the animal's being either toward, away from, or neither, with respect to the source of stimulation.

Stimuli were presented in a unidirectional sea water current, as this has been used with experiments involving response to food. As many animals show rheotaxis (Fraenkel & Gunn, 1961), it was necessary to carry out preliminary studies to find a current velocity that in itself would not bias the behavior in question.

In order to ensure an adequate degree of stimulation, two "levels" were used, a single conspecific or six conspecifics in the same volume of water. In addition, a copulating pair was included as a condition to see if a qualitatively unique substance was

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given off during copulation, as evidenced by the behavior of the animals.

METHODS

Subjects

Sixteen *Aplysia dactylomela* were obtained in July and August 1975 from the shallow-water thalassia beds on the leeward side of Enrique reef on the southwest coast of Puerto Rico. Following collection, the animals were kept in outdoor 20-liter glass tanks which were continuously supplied with fresh sea water. The weights (Table 1) ranged between 195 and 510 g at the time of observation; all animals had laid at least one egg mass and had been observed copulating. (For further details regarding collection and maintenance, see Lederhendler, 1977.)

Apparatus

The influence of water-borne stimulation was tested in a narrow compartment 75 cm (length) $\times$ 15 cm (height) $\times$ 15 cm (wide) made of pine boards coated with fiberglass resin (Figure 1). Sea water entered through a 1.25-cm hole at one end, located 10 cm above the bottom. A nylon mesh screen covering the hole and a row of plastic baffles with their front line 5 cm in front of the entering water had the effect of spreading the stream evenly across the width of the container. Water flowed out at the other end through a hole of the same size set at the same height. The bottom and sides of the runway were marked by seven lines, one every 10 cm. These were used to indicate the location of an animal with respect to the current source. Preliminary experiments were undertaken to set the flow rate. In these pilot studies, plain sea water was in the source tank. Different flow rates were produced by varying the depth of water in the source tank.

Six animals that had been maintained alone for 7 to 10 days were individually placed in a perforated plastic container (the start box) with the animal's head toward or away from the source of the stream and observed in four 10-min trials at different flow rates. The startbox was located in the middle of the test chamber, 35 cm from the baffles. It enclosed the individual in a space 10 cm (length) $\times$ 15 cm (width). To start a trial, the startbox was raised clear of the animal and positioned in front of the outflow hole to prevent the animal from stopping the stream.

Table 1
Modified Latin Square Arrangement to Study Taxis
of *Aplysia dactylomela*; Final Displacement (cm)

Animal Sequence* Weight (g)	Trial†			
	1	2	3	4
1 I 190	0	70	70	70
2 II 270	30	60	25	50
3 III 230	70	30	25	70
4 IV 245	50	35	35	30
5 I 410	50	50	60	60
6 II 185	70	35	70	65
7 III 380	70	60	70	45
8 IV 400	60	0	40	40
9 I 185	5	65	50	40
10 II 500	35	30	0	35
11 III 370	60	70	60	35
12 IV 300	40	70	70	45
13 I 510	0	10	0	30
14 II 240	50	40	35	50
15 III 235	30	0	40	45
16 IV 200	40	70	0	40

*A: Sea water only; B: One *A. dactylomela*; C: Six *A. dactylomela*; D: Pair of copulating *A. dactylomela*; Sequence I = A B C D; II = B D A C; III = C A D B; IV = D C B A. †0 to 35 = 35 to 0; 35 to 70 = 0 to +35 (See text.)

Figure 1. Apparatus used to observe response of *A. dactylomela* to different stimulation.

The responses of the animals to different flow rates was observed until there was no bias in their response, according to two observers (I.L.L. and K.H.), regardless of the orientation at the start. This rate proved to be 4 liters/min. The rate was obtained by maintaining the distance between the height of the water sources and the height of the water in the compartment at 41-44 cm.

Additional evidence of the hydraulic characteristics of the situation was obtained through dye studies. Nonnutritive food coloring was placed in the "source tank"; this progressed down the runway evenly without differential rates along the sides or the middle. With 1 ml of dye in 20 liters of water, it took 2.5 min, but with the 10 ml of dye in 20 liters of water, it took only 9 sec for a concentration of dye to become noticeable at the start barrier.

Design

A modified Latin square design was used (Edwards, 1950). Every animal was observed in four 5-min trials, during which it was in water siphoned from each of the four types of source water ("stimulus conditions"). The conditions in the source tank were: (a) sea water, (b) one sea hare, (c) six sea hares, and (d) one pair of copulating sea hares.

Procedure

The animals were kept alone for 7 to 10 days in 20-liter stock tanks. Before an animal was studied, it was weighed and brought to the experimental situation in a plastic carrying bucket. To start a 5-min observation, an animal was placed in the startbox. A tap started the current, but the startbox was not removed for another 60 sec to ensure a uniform stream of stimulation. Animals were started in the center of the runway to allow the possibility for both approach and withdrawal with respect to the inlet. Between trials of a sequence all apparatus was rinsed thoroughly and wiped with a cloth.

Data Recorded

The location and behavior of the animal were recorded on a check sheet every 15 sec in a 5-min trial. The animal's displacement was converted to positive integers by adding 35 cm to every value. Thus, a displacement of 70 cm indicates that an animal reached the baffles in front of the entry point. A displacement of 0 cm indicates that the animal reached the maximum distance from the inlet; that is, the outlet point. Therefore, for purposes of analysis, the distance between the animal's location at the start of a trial and its final location was used.

Twenty locations were recorded for each animal in each trial; generally, direction of locomotion was constant and the animal's ultimate location was reached early and maintained.

RESULTS AND DISCUSSION

The experimental sequences, preobservation weights, and the individual displacement data for each of the four conditions, are shown in Table 1. There was no bias between size of the animals and sequence of presentation of the four conditions (Kruskal-Wallis one-way analysis of variance, $\chi^2 = 0.3$; Siegel, 1956). A Latin square analysis of variance (Edwards, 1950) indicated that the displacement was influenced only by the treatment conditions ($F(3,60) = 2.89$, $p < .05$). Table 2 shows the displacement means for each condition before and after conversion.

Displacement toward the source is greater when the current carries stimuli from other *Aplysia* than when the current is only plain sea water; i.e., A < B, C, D. [Cohen's (1969) power test, $p < .05$].

A McNemar test of change of the animals' final orientations indicates that only when the plain water condition was compared to water containing six *A. dactylomela* were the differences in the behavior within individual animals significantly different. This comparison was also statistically significant when other measures of head orientation during the entire trial were considered, i.e., mean and modal frequency of head orientation every 15 sec, chi-square tests, $p < .05$.

These data indicate that *A. dactylomela* will move into a current and toward a source of conspecific stimulation. When a greater number of conspecifics were in the source tank (six adults in 20 liters), a distinct movement toward the stream's origin was evident. It would appear, therefore, that the approach process may depend on the intensity of stimulation. The observational situation was designed to present a current flow that would not bias the response of the animals ("neutral rheotaxis"). Lederhendler, Bell, and Tobach (1975) reported that in the field sea hares will move negatively with respect to the currents formed by tidal changes and topographic characteristics in a grass flat, but the chemosensory context of these changes is not known.

The results of this study are consistent with the hypothesis that groupings of *A. dactylomela* can

Table 2
Displacement of *Aplysia dactylomela* in
Different Situations (cm) (N = 16)

	Conditions			
	A	B	C	D
Converted Means	31.3	45.0	48.8	46.9
Means of Actual Distance From Center	-3.7	+10.0	+13.8	+11.9

NOTE—A: Sea water only; B: One *A. dactylomela*; C: Six *A. dactylomela*; D: Pair of copulating *A. dactylomela*.

occur by an active approach response to conspecifics in the absence of food stimulation. However, since *Aplysia* approach food stimulation and possibly approach eggs as well, it is reasonable to suggest that aggregations of sea hares are formed in relation to a complex pattern of stimulation from conspecifics (including the eggs), food, and currents.

In this study, copulating individuals did not differentially influence the approach processes of a third animal. The response of the individual to the copulating pair was different from its response to plain sea water but not from its responses to other *Aplysia*, whether one or six sea hares were considered. This would suggest that during copulation, no particular substance is released that would affect the approach of another sea hare.

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