Table 1. Numbers of banded and unbanded snails infected with larval trematodes.

Condition	Banded	Unbanded
Infected	46	140
Uninfected	769	1415
Total	815	1555

1555 unbanded snails of various ages were dissected and examined for larval trematodes. Representatives of the families Heterophyidae, Echinostomatidae, Philophthalmidae, and Schistosomatidae were present. Heterophyids accounted for 80 to 81 percent of the infections in both banded and unbanded snails. Small juveniles are not infected. The results of the dissections are given in Table 1. They show that the incidence of larval trematodes in banded snails (5.64 percent) is significantly less than that in unbanded snails (9.00 percent). ($\chi^2_1 = 8.34$; $p = .01$ when $\chi^2_1 = 6.64$).

Banding is probably genetically controlled, and it would appear that a lowered susceptibility to larval trematode infection is associated with it. Parasitized snails are usually sterile and may have a shortened length of life (*2*). Other evidence suggests that banding may be a balanced polymorphism (*2*); if this is so, parasitism is partly responsible for its maintenance.

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Gymnodinium breve: Induction of Shellfish Poisoning in Chicks

Abstract. *Oysters exposed to laboratory cultures of the fish-killing dinoflagellate,* Gymnodinium breve, *are toxic when fed to chicks. The ecological significance of this result is interpreted in relation to the scarcity of well-documented reports of shellfish poisoning in higher animals from those areas of the Gulf of Mexico in which* G. breve *"blooms" occur.*

Ecological and epidemiological studies have strongly implicated two dinoflagellates, *Gonyaulax catenella* (*1*) and *G. tamarensis* (*2*) as causes of shellfish poisoning along the Pacific and Atlantic coasts of North America. Studies with cultures have clearly established *G. catenella* as a specific agent for shellfish poisoning along the Pacific coast (*3*). Despite the periodic occurrences of catastrophic mortalities of various marine animals associated with "blooms" of dinoflagellates (red tides) in the Gulf of Mexico (*4*), the incidence of shellfish poisoning in humans definitely traceable to eating molluscs from this region is strikingly low. At least two fish-killing dinoflagellates, *Gymnodinium breve* (*5*) and *Gonyaulax monilata* (*6, 7*) occur in this area.

Our interest in reasons for the infrequency of reports of shellfish poisoning from the Gulf area has led us to consider several possibilities: (i) failure of molluscs to feed on these dinoflagellates; (ii) failure of toxin (or toxins) to accumulate in molluscs; (iii) lack of susceptibility of higher vertebrates to the particular toxin (or toxins); and (iv) infrequent occurrence of toxic dinoflagellate "blooms" in areas of shellfish (mollusc) production.

Recently, in perhaps the only well-established case on record, *G. breve* has been thought to cause human illness. In December 1962 several persons suffered a mild illness, suggestive of a paralytic poisoning, after eating oysters taken from Sarasota Bay, Florida, during the occurrence of a red tide (*8, 9*). Extracts of oysters from the suspected batch were toxic to kittens and mice (*9*). Moreover, McFarren and co-workers extracted a ciguatera-like poison from the oysters, clams, and *G. breve* cultures. Eldred and co-workers observed a rough correlation between the toxicity of oyster and clam extracts to mice and concentrations of *G. breve* at several locations along the west coast of Florida during 1963 (*8*).

However, it has been noted that "blooms" of dinoflagellates, including species of *Gonyaulax*, may coincide with *G. breve* "blooms" in the implicated area (*10*). Thus the evidence is primarily circumstantial and has only associated *G. breve* with an unusual occurrence of shellfish poisoning. The need for controlled studies to determine the toxicity of molluscs exposed to a single species of dinoflagellate suggested this work. The results of three such experiments are presented in this report.

In our studies assays were conducted by controlled feeding of oyster (*Crassostrea virginica*) tissues to test animals rather than injecting or feeding extracts, since the use of extracts of such tissues might circumvent the influence of some natural process on a potential toxin. The molluscs were held in the laboratory in noncirculating, aerated aquariums containing sea water for 1 to 4½ days before use. We used mass unialgal cultures of *G. breve* grown in artificial sea water media (*7, 11*) at 25° ± 1°C and approximately 8100 lu/m² (750 ft-ca) illumination.

Experimental oysters in 2 to 4 liters of culture were exposed to from 1.2 ×

Table 1. Toxicity to chicks of oysters exposed to *Gymnodinium breve.*

No. chicks	Mean weight (g)	Type of oysters fed to chicks	No. *G. breve* per gram of chick*	Time (hours) to	
				Equilibrium loss†	Death
		Experiment No. 1			
2	100	Control	0		
2	100	Exposed	1×10^4	6, 17	
		Experiment No. 2			
2	60	Control	0		
2	60	Exposed	3×10^5	3, 10	10, 22
		Experiment No. 3			
4	33	None	0		
4	33	Control	0		
4	33	Exposed	9×10^4	1½, 2, 2½, 5	6, 6½, 8, 17

* Calculated as follows:

$$\left(\text{No. } G.\ breve \text{ filtered} \times \frac{\text{wt. exposed oysters fed per chick}}{\text{total wt. exposed oysters}}\right) / \text{ chick wt.}$$

† Birds in this condition were lying on side or back.

10^6 to 20×10^6 dinoflagellates. The quantity and typical pale green color of pseudofeces and feces indicated that the oysters filtered the culture. Microscopic counts showed that the oysters had reduced the *G. breve* population by 90 percent after 7½ to 56 hours, at which time the molluscs were removed from the medium and homogenized. Control oysters, each exposed to 2 to 4 liters of uninoculated culture medium, opened occasionally during the experiments, but no pseudofeces and only a small amount of dark feces were observed. The assay animals, male white Leghorn chicks, did not receive food or water for 8 to 11 hours prior to the experimental period. Each of the experimental chicks was force-fed portions of a tissue homogenate of the oysters exposed to the dinoflagellates. Control chicks were each force-fed a tissue homogenate of the oysters from the uninoculated culture medium. In one experiment four additional chicks were maintained as unfed controls to determine the effect of lack of food. After the force-feeding of the chicks, they were provided water but no food for the duration of the experiments.

All eight of the experimental chicks showed a marked loss of equilibrium, and six of them died in 6 to 22 hours (Table 1). None of the control chicks showed gross toxic symptoms, such as loss of equilibrium, at the end of 24 hours, at which time they were returned to a normal diet.

Although the degree of toxicity to chicks varied from batch to batch of culture, in no instance did oysters exposed to *G. breve* fail to produce toxic symptoms in experimental animals. The age of the culture, composition of medium, and stability of toxin in the molluscs may contribute to such variability.

Another indication of the toxicity of *G. breve* was provided by the behavior of the polychaete "mudworms" (*Polydora* sp.), which inhabit the oyster's shell. In *G. breve* cultures the response of the "mudworms" varied from greatly reduced antennal movement to complete emergence from the shell, whereas in uninoculated medium these worms displayed normal antennal activity and remained in the shell.

Having established that oysters can ingest *G. breve* and in the process become toxic to higher animals, we suspect that the scarcity of such occurrence in nature is based on ecological barriers to contact between oysters and *G. breve*. Commercially exploitable quantities of oysters usually occur in Gulf areas having average salinity levels of 25 parts per thousand or less (*12*). Such estuarine salinities inhibit the development of *G. breve* (*11, 13*). Thus salinity is probably a most important factor in preventing frequent contact between these two organisms. The scarcity of shellfish poisoning of humans in areas indigenous to *G. breve* may be attributed to this ecological pattern.

Gonyaulax monilata, the other known toxin-producing dinoflagellate from the Gulf of Mexico, remains to be investigated. Mass cultures of this organism have been established to permit controlled study of its toxic potential.

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Two-Stage Paired-Associate Learning and Eye Movements

Abstract. *Eye movements of 20 male students were photographed continuously throughout the course of their learning verbal paired-associates. As learning progressed, proportionately less and less time was spent scanning the response when the stimulus and response were presented together. These findings are interpreted as supporting a two-stage theory of verbal learning.*

In experiments on verbal paired-associate learning, subjects are required to associate pairs of items, usually nonsense syllables, such that the presence of the first or stimulus member comes to elicit the second or response member. A number of investigators (*1*) regard the learning of such lists as a two-stage process, subjects consolidating the responses during the first or "response-learning" phase and then connecting them to the appropriate stimuli during the second or "hook-up" stage. We evaluated this notion by examining the eye movements of subjects throughout the course of their learning paired-associates, concentrating on the proportion of time each subject spent fixating the stimulus and the response when the two were presented together. If subjects scanned the response out of proportion to the stimulus during the early learning trials, the two-stage concept would be supported.

Twenty male students of introductory psychology were used as subjects. As each entered the laboratory he was placed in a dental chair, the various attachments of which facilitated the recording of eye movements, and was given standard instructions for paired-associate learning. We told him that he would be required to learn a list consisting of seven pairs of nonsense syllables. Each syllable had a Nobel (*2*) m' value within the range 1.80 to 1.93. For each syllable-pair, we exposed the stimulus alone and then with the response by projecting slides onto a green-surfaced chalkboard 1.37 m in front of the subject. We told him that when the stimulus was presented by itself he had 2 seconds in which to anticipate the response and when the two appeared together, the stimulus on the left and the response on the right, he would discover whether he was correct or incorrect. The list was given in each of four different random orders