**References and Notes**

1. H. Meltzer, *Science* **159**, 1368 (1968); *Arch. Gen. Psychiat.* **21**, 102 (1969); ——, L. Elkin, R. Moline, *ibid.*, in press.
2. W. K. Engel and M. H. Brooke, in *Neurological Diagnostic Techniques*, W. S. Fields, Ed. (Thomas, Springfield, Ill., 1966), pp. 90–146.
3. W. K. Engel and G. G. Cunningham, *Neurology* **13**, 919 (1963).
4. R. Hess, D. G. Scarpelli, A. G. E. Pearse, *J. Biophys. Biochem. Cytol.* **4**, 735 (1958).
5. H. A. Padykula and E. Herman, *J. Histochem. Cytochem.* **3**, 170 (1955).
6. C. R. Ashmore, L. Doerr, R. G. Somes, Jr., *Science* **160**, 319 (1968).
7. W. K. Engel and G. G. Cunningham, *J. Histochem. Cytochem.* **18**, 55 (1970).
8. W. K. Engel, *Clin. Orthop.* **39**, 80 (1965).
9. D. Fischman, personal communication.
10. W. K. Engel, in *Exploratory Concepts in Muscular Dystrophy and Related Disorders*, A. T. Milhorat, Ed. (Excerpta Medica Foundation, New York, 1967), pp. 27–40.
11. It has been shown in human muscle biopsies (*7*) (i) that no AP-positive fibers were present in 43 biopsies from normal subjects or in 37 with various benign neuromuscular disorders (including slight and moderate type 2 muscle fiber atrophy); (ii) that many AP-positive fibers are found in active muscular dystrophies, polymyositis, and moderate and severe denervation atrophy (a small number are found in clinically normal carriers of Duchenne pseudohypertrophic X-linked muscular dystrophy, in which they are a typical lesion); (iii) that rare (two or less) AP-positive fibers occur in certain myopathies with moderate clinical neuromuscular symptoms (of 20 patients with mild denervation four had two AP-positive fibers and others none); five patients with severe type 2 muscle fiber atrophy had up to three AP-positive fibers.
12. We thank Dr. M. Matz and Miss Sarah Burnett for assistance in obtaining the muscle biopsies and G. G. Cunningham for performing the histochemical reactions. Supported in part (H.M.) by grant 17-340 from the State of Illinois and PHS grant 1-RO1-MH-16127 to H.M.

21 January 1970

## Alarm Response of Diadema antillarum

Abstract. Diadema antillarum *possesses a sensitive escape response to juices of crushed conspecific sea urchins. Stimulation usually results in urchins moving rapidly downcurrent. Strong species specificity of the response suggests that it is an adaptation to reduce predation.* Diadema antillarum *also responds with a running response to contact with* Cassis tuberosa, *a known predator.*

Swimmers who have blundered into the spines of *Diadema antillarum* know only too well how effectively this Caribbean sea urchin is shielded from harm. The slender barbed spines, sometimes over 30 cm long, are needle-sharp and brittle, readily breaking off in the flesh. As if mere possession of these formidable weapons were not enough, *Diadema* also employs a well-known shadow response when approached: the spines are waved about in a rapid manner which makes it quite difficult to grab a single spine. If one touches a spine the animal immediately brings others to bear on the point of contact. The organism is seemingly invulnerable.

We were surprised, then, to find little piles of *Diadema* spines and skeletal structures in the shallows of the Bimini lagoon in the summer of 1969. Evidently some animal was regularly feeding on *Diadema*. We soon learned that numerous species rely heavily on *Diadema* for food. Randall (*1*) lists 22 species of fishes known to eat *Diadema*. Other predators are two species of helmet shells, *Cassis tuberosa* and *C. madagascariensis*, and the spiny lobster *Panulirus argus* (*2*). Schroeder (*3*) describes the attack behavior of *Cassis tuberosa*, and Cornman (*4*) describes the paralyzing saliva of this species.

Thus, *Diadema* is far from invulnerable, and like certain other aquatic animals subject to considerable predation (*5*) the species exhibits an alarm response, or Schreckreaktion, when stimulated with juices of injured members of its own species. Alarmed urchins mount on their ventral spines and race away for a meter or two, gradually slowing down and becoming quiescent again (Fig. 1, A–D).

One questions whether an escape response of an organism to intraspecific juices is necessarily related to predation. Perhaps the urchins might respond similarly to any chemical disturbance. *Diadema* stimulated in the field with dilute (5 percent) acetic acid show essentially the same escape behavior, and there is no need to invoke predation for response to this substance. However, when we tested the response of *Diadema* to the juices of other echinoderms we found few signs of reactivity, and it is perhaps puzzling that *Diadema* might find its own juices "irritating" but not the juices of many other species.

Clumps of *Diadema* resting on a sand bottom at the mouth of Massey Creek of South Bimini were subjected to juices of other echinoderms (Table 1) Fig. 1E). Each stimulus animal was placed about 25 cm upcurrent from a *Diadema* clump and pulverized with a crowbar. Responses were noted in the *Diadema* in the next 5 minutes, then a medium-sized *Diadema* was crushed at the same spot to demonstrate that the respondent *Diadema* were at least sensitive to intraspecific juices. Control experiments in which the plain sand bottom was pulverized with the crowbar upcurrent from *Diadema* did not produce locomotory responses.

In this survey only *Lytechinus variegatus*, another sea urchin, was clearly potent in producing a response in *Diadema*. However, in one experiment with juices of *Echinometra lucunter*, two respondent *Diadema* moved rapidly away. As the *Echinometra* crushed in this experiment were quite small, it seemed possible that lack of overwhelming response might reflect insufficient concentration of stimulus juices.

In other trials with weighed stimulus urchins (Table 2), *Diadema* was most reactive to intraspecific juices, fairly reactive to *Echinometra lucunter* juices, less reactive to *Lytechinus variegatus* juices, and quite unreactive to *Eucidaris tribuloides* juices. Even massive doses of *Tripneustes ventricosus* juices produced no clear response.

Although the response of *Diadema* to echinoderm juices may be an avoidance response to irritating chemicals, it is not obvious why intraspecific juices should be so especially irritating unless some factor other than pure "chemical stress" is involved. *Diadema* juices are apparently not irritating to the myriad fishes, crabs, and lobsters of the Bimini flats (Fig. 1, A–D). We suggest that the most reasonable explanation for the observed species specificity of response is predation.

Figure 1, A–D suggests how alarm behavior might help *Diadema* avoid predators. For this sequence a single urchin in a clump of 35 urchins was crushed. Alarm reactions immediately commenced in urchins downcurrent from the crushed urchin, but not in urchins lateral to it. After a couple of minutes the responding urchins had moved varying distances downcurrent, leaving a gap in the center of the original clump. If predators of *Diadema* tend to move only short distances between meals, urchins that move downcurrent in alarm may reduce their chances of falling victim to a predator at the expense of increased danger to other urchins closer to the predator but crosscurrent or upcurrent from him and unaware of his presence. For predators feeding in schools it would not be necessary to postulate that successive victims of a single predator be close together; several predators feeding adjacent to one another could produce an equivalent advantage in response. We emphasize that response is not limited to urchins immediately downcurrent from a crushed urchin, and can be seen in urchins many meters away if the urchin crushed is large enough. In one case in which we crushed a medium-sized *Diadema*, we found urchins 8 m downcurrent moving farther downcurrent.

Under a predation hypothesis we might expect to find *Diadema* responding to juices of another species only when the juices could be a reliable signal of danger to *Diadema*. It would be relevant to know, then, to what extent predators of the species of urchins considered above also take *Diadema*. In Randall's (*1*) data almost all the fish predators of the above urchins took *Diadema* as well, but the data are much too few to indicate, for instance, that fish predators of *Echinometra* are more likely to eat *Diadema* than are fish predators of *Tripneustes*. Randall listed only one species of fish, *Diodon hystrix*, as a predator of *Tripneustes*, and this species also ate *Diadema*.

One predator of *Tripneustes* not known to take *Diadema antillarum* is *D. antillarum* itself. Quinn (*6*) reports two laboratory cases of such predation. It is most intriguing that the *Diadema* in Quinn's observations were exposed to *Lytechinus, Eucidaris, Echinometra,* and *Tripneustes*, but fed only on *Tripneustes*. One wonders if this result is directly related to the potency of the juices of the other three species in producing alarm in *Diadema*.

Could the sensitivity of *Diadema* to juices of other urchins be simply a reflection of taxonomic relationships? In the classification of echinoids given by Moore (*7*), *Diadema* is not closely related to any of the urchins considered above. It is in a different superorder from *Lytechinus, Tripneustes,* and *Echinometra* and in a different subclass from *Eucidaris*. *Lytechinus, Tripneustes,* and *Echinometra* are in the same superorder, and *Lytechinus* and *Tripneustes* are in the same family. That these three urchins, fairly close to each other taxonomically, differ strikingly in potency

Table 1. Responses of *Diadema antillarum* to echinoderm juices. Response in columns three and four is the fraction of urchins available showing locomotion. Size in column two is relative to a medium-sized *Diadema* (L, large; S, small). Medium-sized *Diadema* were crushed for column four.

| Stimulus | Size | *Diadema* response in 5 minutes | *Diadema* response to intraspecific juices |
|---|---|---|---|
| | | *Echinoidea* | |
| 1 *Lytechinus variegatus* | L | 11/15 moderate | 15/15 rapid |
| 1 *Lytechinus variegatus* | L | 9/9 moderate | 9/9 rapid |
| 1 *Lytechinus variegatus* | L | 15/15 moderate | 15/15 rapid |
| 1 *Eucidaris tribuloides* | L | 0/6 | 6/6 rapid |
| 2 *Eucidaris tribuloides* | L | 3/20 slow | 20/20 rapid |
| 1 *Tripneustes ventricosus* | L | 1/7 slow | 7/7 rapid |
| 1 *Tripneustes ventricosus* | L | 1/8 slow | 7/8 rapid |
| 1 *Tripneustes ventricosus* | L | 0/7 | 7/7 rapid |
| 1 *Echinometra lucunter* | S | 0/7 | 7/7 rapid |
| 2 *Echinometra lucunter* | S | 2/8 rapid | 8/8 rapid |
| 1 *Clypeaster rosaceus* | L | 2/16 slow | 16/16 rapid |
| 1 *Clypeaster rosaceus* | L | 0/16 | 16/16 rapid |
| | | *Asteroidea* | |
| 1 *Echinaster sentus* | S | 0/6 | 5/6 rapid |
| 1 *Oreaster reticulatus* | L | 4/11 slow | 11/11 rapid |
| 1 *Oreaster reticulatus* | L | 0/8 | 8/8 rapid |
| | | *Ophiuroidea* | |
| 1 *Ophiocoma riisei* | S | 0/8 | 8/8 rapid |
| 1 *Ophiocoma riisei* | S | 0/18 | 12/18 rapid* |
| | | *Holothuroidea* | |
| 1 *Euapta lappa* | L | 0/11 | 11/11 rapid |
| 1 *Euapta lappa* | L | 0/8 | 8/8 rapid |
| 1 *Holothuria floridana* | L | 0/8 | 7/8 rapid |
| 1 *Holothuria floridana* | L | 2/11 slow | 11/11 rapid |

* Current shifted before all 18 urchins contacted juices.

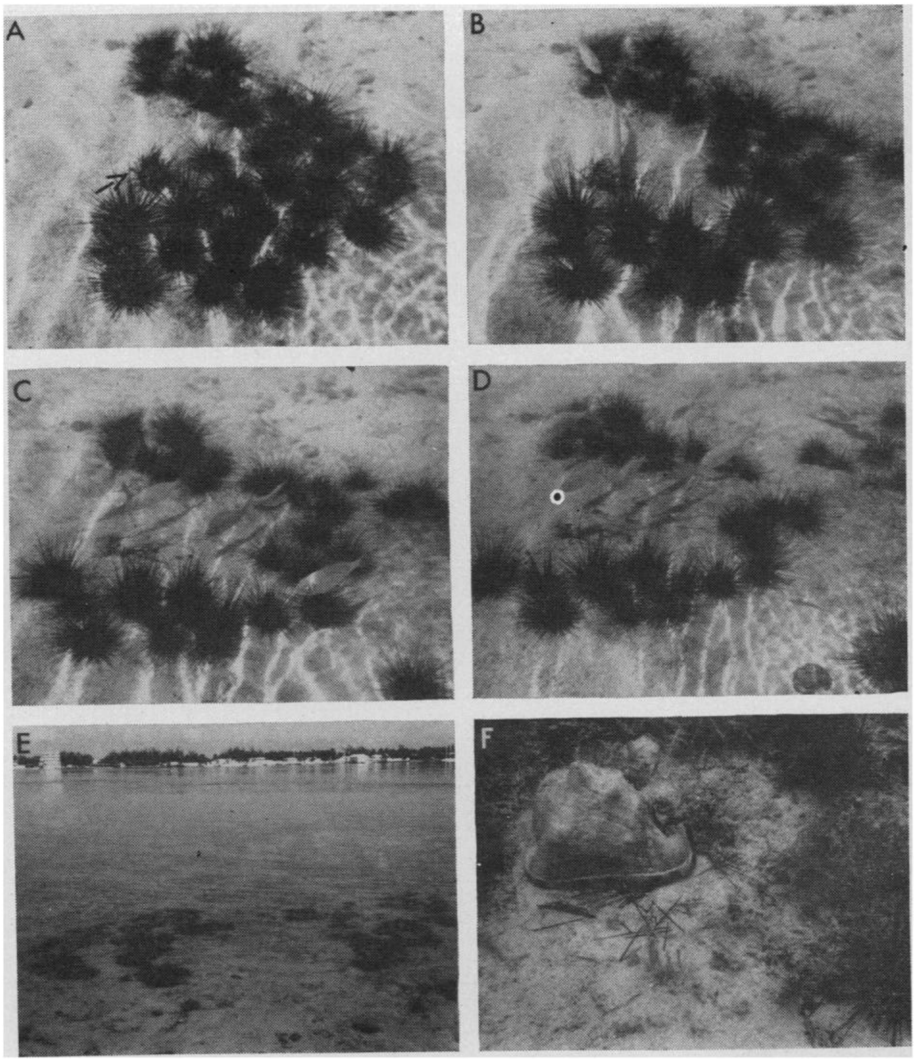

Fig. 1. (A) A *Diadema* clump immediately after an urchin (arrow) is crushed. Arrow also indicates current direction. (B) Urchins downcurrent from the crushed urchin begin to move farther downcurrent. (C and D) Fish, mostly majarras and slippery dicks, rip apart the crushed urchin as downcurrent urchins continue to race downcurrent. Time span for A–D is approximately 2 minutes. (E) *Diadema antillarum* clumps at the mouth of Massey Creek, South Bimini. (F) A king helmet, *Cassis tuberosa*, feeding on a *Diadema*.

Table 2. Responses of *Diadema antillarum* to juices of sea urchins. Response in columns three and four is the fraction of urchins available showing locomotion. Weight in column two is approximate. Medium-sized *Diadema* were crushed for column four. Diameter in column one is disk diameter.

| Stimulus diameter (cm) | Weight (g) | *Diadema* response in 2 minutes | *Diadema* response to intraspecific juices |
|---|---|---|---|
| *Diadema antillarum* | | | |
| 0.8 | .4 | 3/7 slow | |
| 1.0 | 1.0 | 7/10 moderate | |
| 1.4 | 2.2 | 7/7 rapid | |
| 1.4 | 2.2 | 8/8 rapid | |
| 1.5 | 2.4 | 6/7 rapid | |
| 1.8 | 4.7 | 8/9 rapid | |
| *Echinometra lucunter* | | | |
| 1.5 | 1.7 | 0/10 | 10/10 rapid |
| 1.8 | 3.6 | 0/9 | 9/9 rapid |
| 2.0 | 5.1 | 1/11 slow | 11/11 rapid |
| 2.5 | 8.5 | 2/10 slow | 10/10 rapid |
| 2.5 | 8.5 | 4/12 slow | 12/12 rapid |
| 2.8 | 11.2 | 5/15 slow | 15/15 rapid |
| 2.2, 2.2, 2.1 | 16.5 | 6/10 moderate | 10/10 rapid |
| 3.0, 3.0, 2.5 | 39 | 19/20 rapid | |
| *Lytechinus variegatus* | | | |
| 2.8 | 12.6 | 0/10 | 10/10 rapid |
| 3.5 | 23 | 2/8 slow | 8/8 rapid |
| 4.3 | 42 | 2/8 moderate | 8/8 rapid |
| 4.8 | 53 | 9/13 moderate | 13/13 rapid |
| 5.5 | 102 | 5/15 slow | 15/15 rapid |
| 7.0 | 144 | 3/10 moderate | 10/10 rapid |
| *Eucidaris tribuloides* | | | |
| 3.8 | 49 | 0/14 | 14/14 rapid |
| 4.0 | 70 | 1/10 slow | 10/10 rapid |
| 4.5 | 72 | 0/15 | 15/15 rapid |
| 3.0, 3.0, 2.5, 2.4, 2.4, 2.0 | 160 | 10/15 slow | 15/15 rapid |
| *Tripneustes ventricosus* | | | |
| 3.0, 4.5, 6.0 | 152 | 1/18 moderate | 18/18 rapid |
| 9.5 | 312 | 0/10 | 10/10 rapid |

in producing alarm in *Diadema* suggests that taxonomic relationships are not a reliable guide to juice potency. Since the above urchin species all frequently occur in close proximity to *Diadema* in the field at Bimini, it is also not clear that the strength of response of *Diadema* to juices of another species can be related simply to the extent of sympatry with that species.

Attempts to elicit the alarm behavior of *Diadema* in laboratory tanks gave inconsistent results, and we were unable to make an accurate determination of threshold for response. Causes for inconsistency in the laboratory are unknown. Response in the field was remarkably consistent. All sized *Diadema* tested, including the smallest individual (0.8 cm in disk diameter), were responsive to intraspecific juices and were potent in producing alarm in other urchins when crushed; and urchins in rocky crevices were as responsive as those on a sand bottom. However, urchins sometimes appeared reluctant to respond if the current was strong. Urchins that did respond in strong currents occasionally lost their holds on the substrate as they began to move downcurrent and were wafted up in the current and carried far away. Judging from observations we made of captive triggerfish and margates feeding on *Diadema* in pens, an urchin up in the current is especially vulnerable to fish predation, as under these conditions it is easy for a fish to approach an urchin from its poorly protected underside. Any advantage for an urchin showing alarm behavior in a strong current must presumably be tempered by the risk of being swept off the bottom.

Though response was usually a matter of urchins moving downcurrent or close to this direction, experiments conducted at slack tide suggest that response is a chemotaxis rather than a chemically triggered rheotaxis. In these experiments urchins in the midst of clumps were crushed, and surrounding urchins moved in all directions away from the crushed urchins. In several other experiments urchins in a current were stimulated on their downcurrent sides with intraspecific juices, and the urchins moved upcurrent to a limited extent (see urchin at far left in Fig. 1, A–D). These observations suggest that urchins orient their escape movements to concentration gradients of intraspecific juices, and not to the current per se, though the two factors may often coincide. The experimenter's position relative to the urchins was varied and appeared to have no effect on the directions the urchins took in alarm. Similarly, the presence or absence of fish feeding on a stimulus urchin appeared to make no difference in the responses of other urchins.

We performed several experiments at the Massey Creek site on the moonlit night of 21 August. The *Diadema* appeared as reactive to intraspecific juices under these dim conditions as they were under full daylight. Response at night is not unreasonable as several of the fish predators of *Diadema* are known to feed at night (*1*).

Although we never observed natural predation by fishes on *Diadema* in the field, we twice found king helmet shells, *Cassis tuberosa*, feeding on the species (Fig. 1F). We set up several interactions of king helmets with *Diadema* in the field. Though we saw no attacks we did discover that *Diadema* have a fright response to this mollusk—running away on their ventral spines. This response contrasts to the behavior of *Diadema* when a tulip snail, *Fasciolaria tulipa*, is placed in their midst. Tulip snails, which are mollusk predators not known to eat *Diadema*, must fight their way through the spines of nearly inert urchins. Apparently the running response is not simply a response to mechanical disturbance, as the *Fasciolaria* bump into the urchins at about the same rate of speed as the helmet shells. *Diadema* do not generally show response to helmet shells placed upcurrent from them, though one sometimes sees slight movement downcurrent. Perhaps with the slow movement of helmet shells, it makes little sense for *Diadema* to respond to their odor (taste) unless the odor is coupled with mechanical disturbance.

NOEL SNYDER
HELEN SNYDER

*Department of Zoology,*
*University of South Florida,*
*Tampa 33620*

## References and Notes

1. J. E. Randall, *Stud. Trop. Oceanogr.* **5**, 665 (1967).
2. ———, R. E. Schroeder, W. A. Starck, II, *Caribbean J. Sci.* **4**, 421 (1964).
3. R. E. Schroeder, *Sea Frontiers* **8**, 156 (1962).
4. I. Cornman, *Nature* **200**, 88 (1963).
5. W. Pfeiffer, *Biol. Rev.* **37**, 495 (1962); I. Hrbacek, *Experientia* **6**, 100 (1950); N. F. R. Snyder, *Cornell Univ. Agr. Exp. Sta. Mem. 403* (1967).
6. B. G. Quinn, *Bull. Mar. Sci.* **15**, 259 (1965).
7. R. C. Moore, *Treatise on Invertebrate Paleontology*, Part U, *Echinodermata 3* (Univ. of Kansas, Lawrence, 1966).
8. Our research has been supported by the Lerner Marine Laboratory, Office of Naval Research grant 552 (07), and an equipment grant from Research Corporation. We thank F. M. Bayer for identification of some of the echinoderms discussed.

5 November 1969; revised 12 January 1970