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ABORAL DEPRESSIONS IN THE TESTS OF THE SEA URCHIN *TRIPNEUSTES* CF. *GRATILLA* (L.) IN THE GULF OF EILAT, RED SEA

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Abstract: Flattening and aboral depressions occurred in high proportions in *Tripneustes* cf. *gratilla* (L.) populations that inhabited an artificial lagoon in the northern Gulf of Eilat, Red Sea. The average test height to diameter (H/D) ratio of the most affected population, near a small wastewater outlet, was 0.43 ± 0.01 (SE) and 76% showed aboral depressions. The H/D ratio was 0.54 ± 0.01 in populations outside the lagoon and < 1% exhibited aboral depressions. The exposure of normal *T.* cf. *gratilla* to industrial $CaCO_3$ precipitation inhibitors affected the growth and H/D ratio in a similar manner within 30–45 days. The light-weight tests of the deformed urchins and the occurrence of skeletal resorption in the interior part of the test indicate that the unknown pollutant, possibly contained in laundry wastewater, reduced calcification by these animals and resulted in a mechanical collapse of the tests. A regular pattern of small pits at the aboral tip of the ambulacra suggests that intestinal mesenterial threads, attached to the test at the same points from within, facilitated this collapse by a mechanical pull. The normal test morphology of the regular echinoids may be regulated by the mechanical activity of various contractile and elastic tissue elements, among which mesenterial threads are probably included.

INTRODUCTION

Although cases of abnormal growth of echinoid skeletons are well documented (Chadwick, 1924; Koehler, 1924; Clark, 1925; Mortensen, 1943; Moore, 1974), high frequencies of deformities in sea urchin populations are often linked with changes in the habitat (Chesher, 1969) or with pollution (Allain, 1978; Dafni, 1980). Because mechanical stresses exerted by certain soft tissue elements on the skeletal plates may affect their rate of increase (Moss & Meehan, 1967; Dafni & Erez, in press) and determine the overall test shape (Thompson, 1917), changes in the activity or mechanical properties of these elements may induce abnormal growth (Dafni, 1980). Two-thirds of a *Tripneustes* cf. *gratilla* population, that inhabited the subtidal zone near a power plant in the northern Gulf of Eilat (Red Sea), showed abnormally tall and inflated tests and other shape irregularities (Dafni, 1980). The purpose of this paper is to describe another deformity, extreme flattening and aboral depression, in *T.* cf. *gratilla* populations from an artificial basin, which is subjected to mild pollution. Biometrical and morphological studies of living urchins, skeletons and tissues, as well as observation of the environmental conditions in the water basin support the contention that a biomechanical effect,

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imposed by soft tissues on the poorly calcified tests, may have influenced the shape of these urchins.

MATERIAL AND METHODS

Large populations of *Tripneustes* cf. *gratilla*, a Red Sea variant of the Indo-Pacific *T. gratilla* (L.) (Dafni, 1980), live in an artificial lagoon that was dug at the Eilat beach, Gulf of Eilat (Aqaba), Red Sea, (Fig. 1). The lagoon area is $\approx 1.2 \times 10^5$ m^2; it is ≈ 15 m wide at its opening, and has a maximum width of 300 m, and averages 3 m in depth. Rock jetties protect its entrance from the sea. Several hotels were built around this basin, but the northern and eastern coasts are still uninhabited.

Living *T.* cf. *gratilla* were collected at six sites inside the lagoon and on the seaward side of the breakwater. Width and height were measured with calipers and the occurrence of abnormal depression of the aboral half recorded. Relative abundance of this species in each site was determined as the number of animals collected in a 15-min search using mask and fins. Other echinoid species were noted although not counted. *Tripneustes* were also collected on unpolluted shores of the Gulf, 7 km south of the lagoon. Collection and measurements were carried out in June, 1980, and did not include newly recruited urchins. Dead urchins' tests, after cleaning in commercial bleach (NaOCl), were similarly measured and weighed in a top-loading balance with a precision of 1 mg. Water temperatures and salinity were measured in the lagoon and the open Gulf during June and January.

Small pieces of skeleton taken from a freshly killed normal urchin at the ambitus level were fixed, decalcified with Zanker's fluid (Culling, 1974) and embedded in paraffin. Sections of these pieces containing an ambulacral ray and the adjoining interambulacral plates were cut and stained with Heidenhain's Azan stain (Culling, 1974) for collagen and Milligan's Trichrome stain (Humason, 1967) for muscle. For scanning electron microscopy (SEM), skeletal parts of normal and deformed urchins were cleaned for 24 h in 5% NaOCl, washed in distilled water, air dried and sputter-coated with gold. Micrographs were made with a "JEOL-JSM 35" SEM. In some preparations, shorter bleaching time retained the soft tissues (such as intestine mesenteries).

An assay was aimed at testing the possible effects on skeletal growth in living urchins of several industrial "scale inhibiting" chemicals used by various industries in the Eilat area to reduce carbonate precipitation.

Seven small groups of ≈ 20-mm *T.* cf. *gratilla* were were kept for long periods in well-aerated closed-system 4-l aquaria. Urchins were maintained at constant temperatures (25 ± 1 °C) and artificial 12/12 h dark–light periods during October–December and were fed on epilithic algae. Diameter and test height were measured at the beginning and after 30, 43, 55, and 70 days. The tested reagents were, according to available data (reagents whose chemical composition was unavailable are marked with an asterisk):
(1) Dequest 2060 (Monsanto): diethylenetriamine-penta (methylene phosphonic acid).

(2) Dequest 2054 (Monsanto): potassium salt of hexamethylenediaminetetra (methylene phosphonic acid).
(3) P-70 (Cyanamid): sodium polyacrylate*.
(4) Belgard EV (Ciba-Geigy): based on polymeric carboxylic acid*.
(5) CYAF 5021 (Cyanamid): acrylamide-sodium acrylate resin*.
Concentrations were arbitrarily set as 0.02% (v/v), which is 1–5% of the lethal doses (Producers' manuals).

RESULTS

ENVIRONMENTAL OBSERVATIONS AND MEASUREMENTS

Temperatures in the Gulf range between 21–27 °C throughout the year (Oren, 1962; Dafni, 1974). In the lagoon they ranged between 20–26 °C in June, but in January water temperatures as low as 14 °C were recorded, at Site G (Fig. 1). Salinities were 40–42‰ in the Gulf and at Sites A–E, and 38.5 ± 1.5‰ at Sites F and G. Still lower salinities (5–15‰) found in small pools and between the boulders at Site G probably result from the mixing of fresh groundwater with the sea water. Pollution, in the form of small wastewater outlets, enters the lagoon in Sites C and E and sewage containing wastewater

the Present Site

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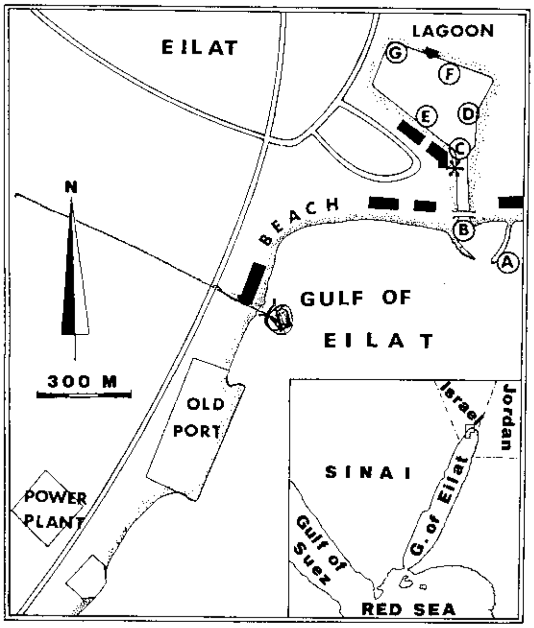


Fig. 1. Map of the northern Gulf of Eilat (Aqaba), showing the artificial lagoon and the sampling sites (A–G): *, location of the suspected source of pollution; the Marine Laboratory is on the southern beach, not shown in the map.

from a desalination plant situated 1 km southward was dumped occasionally at Site G (S. Bachar, pers. comm.).

SEA URCHIN DISTRIBUTION AND IN SITU BIOMETRICAL MEASUREMENTS

Only three of the most common Gulf echinoid species were found in the lagoon: *T.* cf. *gratilla*, *Echinometra mathaei* (Blainville) and *Diadema setosum* (Leske) (Table I). The former two abounded near Sites A–D, their abundance decreased at Sites E and F, and they were completely absent from Site G. The latter species, *D. setosum*, was equally abundant in all sites.

TABLE I

Abundance (number of urchins collected per 15-min search), horizontal diameter, relative height (H/D ratio) of *T.* cf. *gratilla* and percent occurrence of aboral depressions in sites outside (Site A) and inside (B–G) the Eilat Lagoon: the occurrence of echinoid species other than *Tripneustes* – *Diadema setosum* (*Ds*), *Echinometra mathaei* (*Em*) and *Echinothrix calamaris* (*Ec*) is given as presence (+) or absence (–).

Site	Abundance (*N*)	Diameter (mm) ± SE	H/D ± SE	Depressed (%)	Other echinoids		
					Ds	*Em*	*Ec*
A	50	67.6 ± 0.7	0.54 ± 0.01	0	+	+	+
B	76	57.0 ± 1.0	0.49 ± 0.01	14.5	+	+	–
C	89	64.6 ± 1.0	0.43 ± 0.01	76.4	+	+	–
D	38	83.3 ± 1.8	0.48 ± 0.01	31.5	+	+	–
E	8	93.7 ± 2.3	0.52 ± 0.01	12.5	+	+	–
F	7	92.2 ± 3.9	0.55 ± 0.01	14.3	+	+	–
G	–	–	–	–	+	–	–

The size of *Tripneustes* tended to increase towards the interior of the lagoon (Sites C–F). Relative height, expressed as height to diameter (H/D) ratio, was 0.52–0.55, the normal ratio of this species (Dafni, 1980), at Site A, outside the lagoon, and in Sites E and F. Abnormally low H/D ratios were shown in Sites B–D, in the central part of the lagoon. The percentage of abnormally depressed urchins (Fig. 2) was highest (76%) in Site C, but lower in B and D, and very low in Sites E and F, only 200 m from Site C. All the deformed urchins were large (> 50 mm).

The shape of the deformed urchins was variable. In most urchins flattening of the apical region was observed, but frequently the entire apical system sank deeply and in extreme cases rested almost on top of the lantern, with only a few mm left for the emergence of the oesophagus. Externally, some deformed urchins displayed dermal infection on the aboral half, associated with the sunken apex. Excreta that often accumulated in the depressions may have caused these infections. Although irregular depressions were sometimes observed in the infected areas, the depressed pattern was generally symmetrical (Figs. 2 and 5) and did not indicate overt sickness or the presence of macroscopic ectoparasites. Deformed individuals survived for 2 yr in captivity, showing reduced growth rates and did not recover from this deformity.

T. cf. *gratilla* occasionally exhibited similar deformations in non-polluted localities, outside the lagoon. In the summer of 1979, three such urchins were found 20 km south of the lagoon, and in the autumn of 1981, 15 more were collected from a nearby locality. These urchins represented <1% of otherwise healthy populations. No other echinoid species in the Gulf showed this deformity.

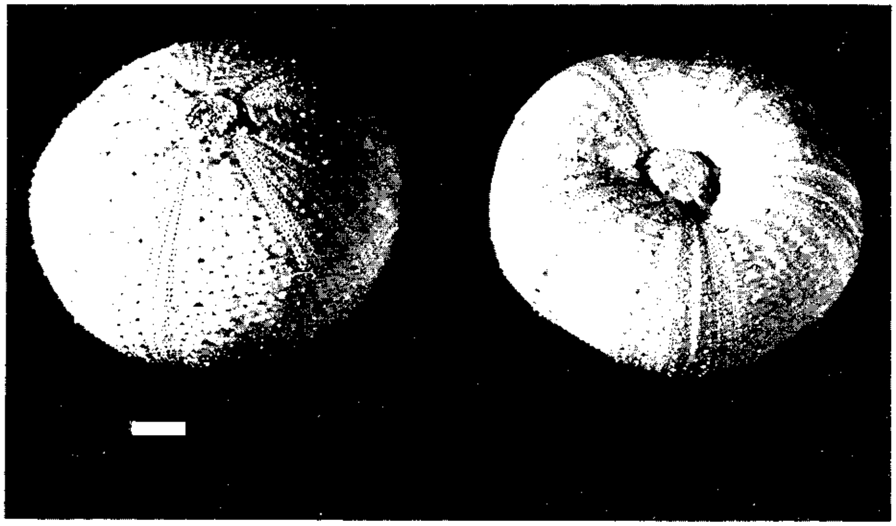


Fig. 2. Dried tests of normal (left) and abnormal *T.* cf. *gratilla*, showing the aboral depression in the latter; scale bar, 1 cm.

DRY TEST MORPHOLOGY

Abnormal tests of *T.* cf. *gratilla* from the lagoon were flatter, weighed less and had significantly fewer interambulacral (IA) plates, than unaffected urchins' tests (Table II). No significant difference was found between the relative peristome sizes of both kinds (analysis of covariance, Snedecor & Cochran, 1967). A comparison of the IA plates shapes of an abnormally flat *Tripneustes* with normal urchins of the same size shows that aboral plates of the former are smaller and their shapes deviate from the typical pattern; the zigzag line of the interradial suture is more rounded and irregular. Nevertheless, no excessive growth of the individual plates in the meridional direction, as shown in the inflated test deformations (Dafni, 1980), was observed here.

Indications of skeletal "erosion" are shown in many deformed urchin tests. Longitudinal sections in IA plates at the ambitus level show that an abnormal plate is stratified (Fig. 3A), its inner surface is extremely irregular and its median part thinner. In contrast, the sutural areas are thicker and form riblike ridges (Figs. 3A, 7B). In the eroded inner surface of the test, part of the innermost spongy layer is missing, probably due to resorption, and the underlying compact layer is exposed (Fig. 4A–C). The skeletal trabeculae at these areas are abnormally thin, and stretch to longer distance than in normal urchins, forming well oriented bars (Fig. 4D). To the naked eye, the inner surface of the plates has a wrinkled appearance. The outer surface of the deformed plates seems to be unaffected by these changes. Some deformed urchins had small gaps at the sutures of their uppermost plates due to incomplete calcification, and the linkage of these plates was weakened.

TABLE II

Allometric relationships of normal *T.* cf. *gratilla* and abnormal urchins from the Eilat lagoon, and analyses of covariance: r, coefficient of correlation; F, coefficient of covariance analysis.

A. Height (vertical diameter, H) vs. horizontal diameter (D), in mm:	
H (normal) $= 0.501 \times D^{1.021}$	($n = 101$, $r = 0.997$)
H (abnormal) $= 0.268 \times D^{1.093}$	($n = 41$, $r = 0.895$)
F_{ratio} (slopes) $= 0.425$ (N.S.)	
F_{ratio} (adjusted means) $= 428$ ($P \ll 0.001$)	
B. Peristome size (P) vs. test diameter (D), in mm:	
P (normal) $= 0.837 \times D^{0.783}$	($n = 26$, $r = 0.961$)
P (abnormal) $= 1.292 \times D^{0.677}$	($n = 38$, $r = 0.913$)
F_{ratio} (slopes) $= 2.43$ (N.S.)	
F_{ratio} (adj. means) $= 0.05$ (N.S.)	
C. Dry weight (W) in grams vs. test diameter (D) in mm:	
W (normal) $= (1.74 \times 10^{-5}) \times D^{2.979}$	($n = 31$, $r = 0.989$)
W (abnormal) $= (6.66 \times 10^{-5}) \times D^{2.834}$	($n = 30$, $r = 0.956$)
F_{ratio} (slopes) $= 2.16$ (N.S.)	
F_{ratio} (adj. means) $= 4.26$ ($P < 0.05$)	
D. Number of interambulacral plates (IA) vs. test diameter size (D) in mm:	
IA (normal) $= -4.315 + 7.435 \log_e D$	($n = 76$, $r = 0.973$)
IA (abnormal) $= -4.490 + 6.457 \log_e D$	($n = 22$, $r = 0.664$)
F_{ratio} (slopes) $= 0.316$ (N.S.)	
F_{ratio} (adj. means) $= 139$ ($P \ll 0.001$)	

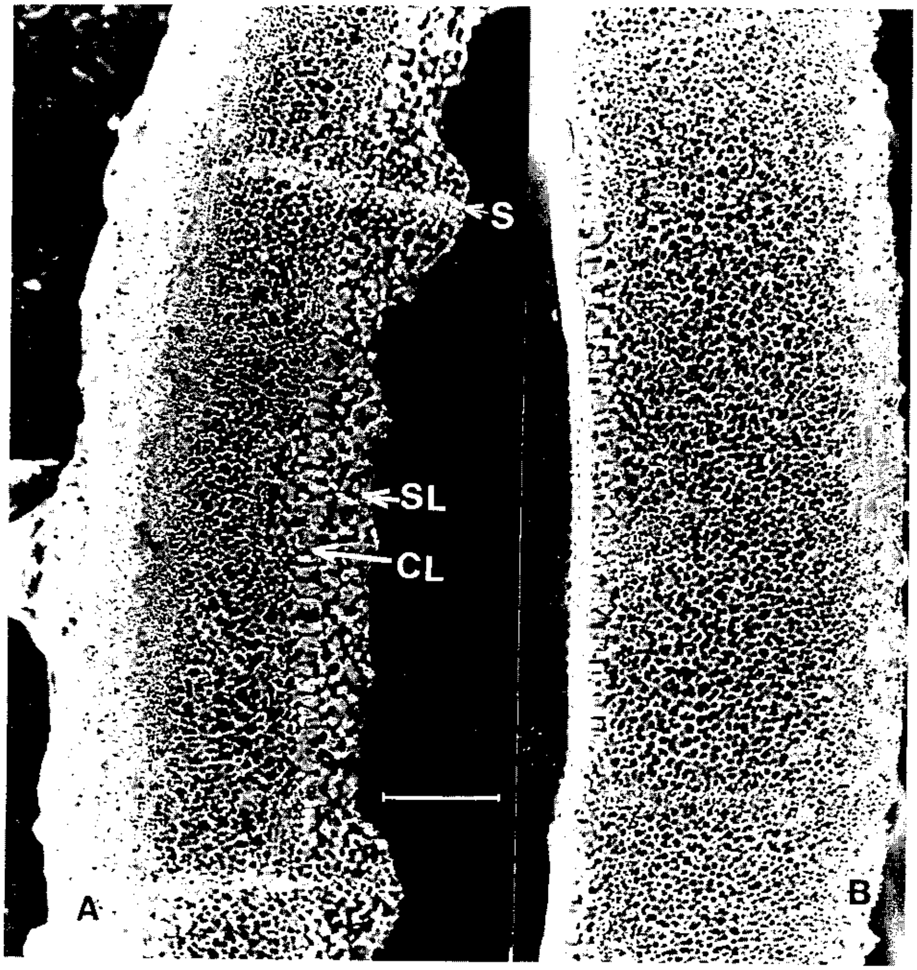


Fig. 3. SEM micrographs of meridional section in the IA plates of A, abnormal and B, normal urchins at the ambitus level: S, latitudinal sutural ridge; SL, spongy layer; CL, compact layer; in the healthy urchin's plate, layering in the inner stereom is less evident (terms according to Jensen, 1972); scale bar, 0.5 mm.

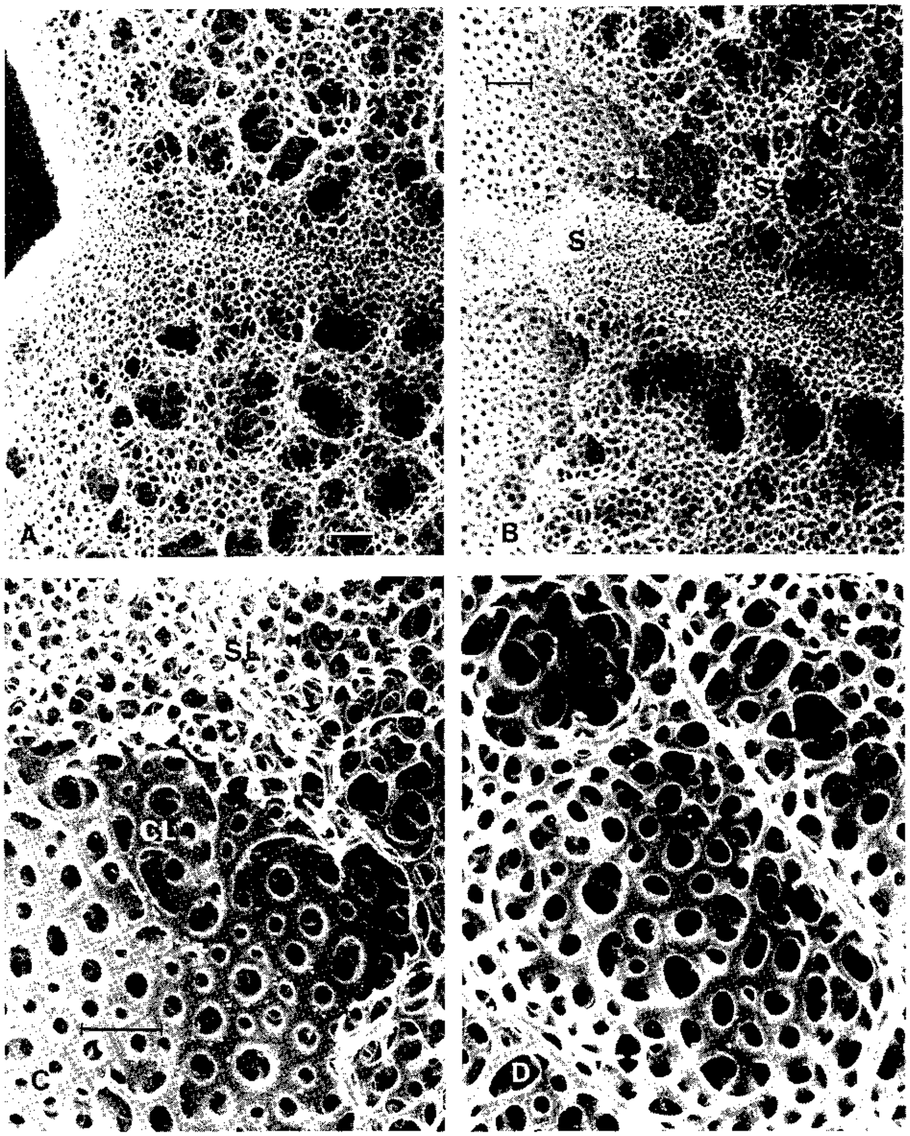


Fig. 4. SEM micrographs of the inner surface of IA plates of abnormal *Tripneustes*: A, low magnification (scale bar, 200 μm) view of the interradial edge and latitudinal suture line, showing areas with deep cavities; B, median part of two IA plates, showing sutural thickening (S), partly eroded spongy layer (SL) and the exposed compact layer (CL) (scale bar, 250 μm); C, same as B, but more enlarged (scale bar, 100 μm), note the thin trabeculae of the spongy layer and the exposed compact layer; D, abnormal spongy layer, showing strongly oriented trabeculae at the surface and deep cavity (in the upper left corner), magnification is same as C.

CONNECTION BETWEEN INNER MESENTERIES AND THE APICAL DESCENT

In many depressed tests the ambulacral plates sank deeper than the IA plates and produced a regular pattern of pits at the apex (Fig. 5). These pits coincided with the points of uppermost attachment of the mesenterial threads that fasten the large intestine to the skeleton (Fig. 6). The plates at the apical region were abnormally thin and strongly concave, instead of being convex. The inner surface of many IA plates showed conspicuous protuberances in a pattern that fitted the attachment points of the mesenterial threads (Fig. 7A). In the aboral half the protuberances were ridgelike and were accompanied by deep furrows (Fig. 7B). The alliance between the protuberances (Fig. 7C) and the threads is apparent in SEM micrographs of uncleaned tests (Fig. 7D).

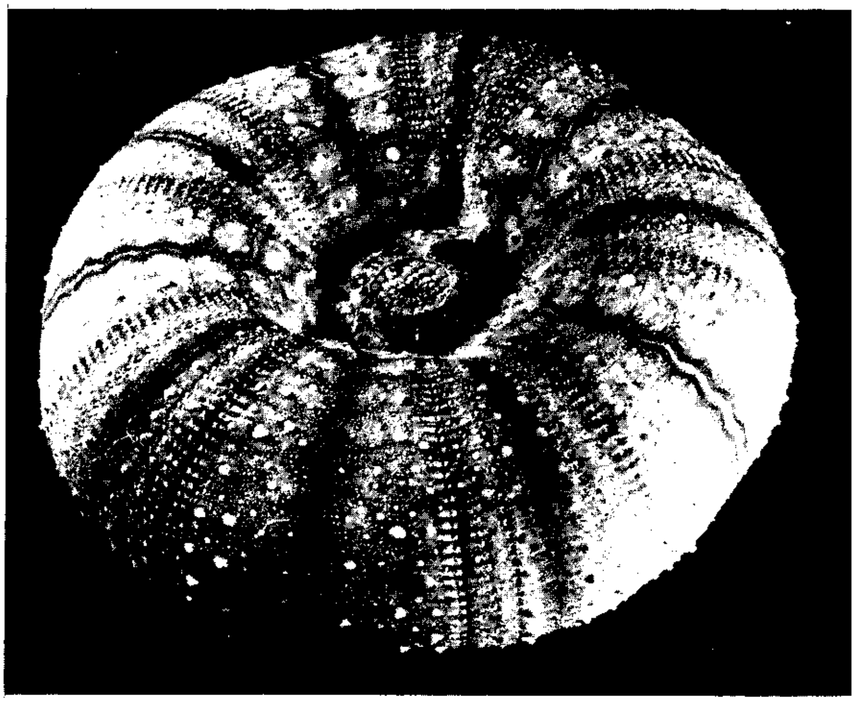


Fig. 5. Abnormal *Tripneustes*, showing aboral depression and deeper pits at the apical end of the ambulacral rays.

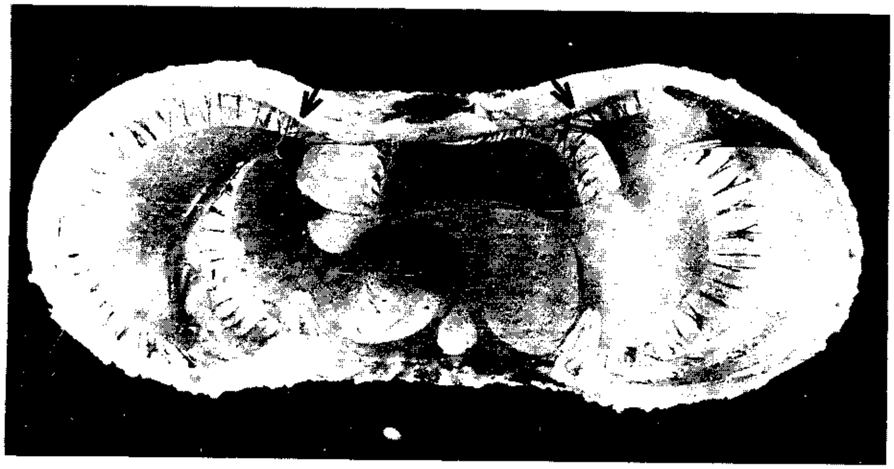
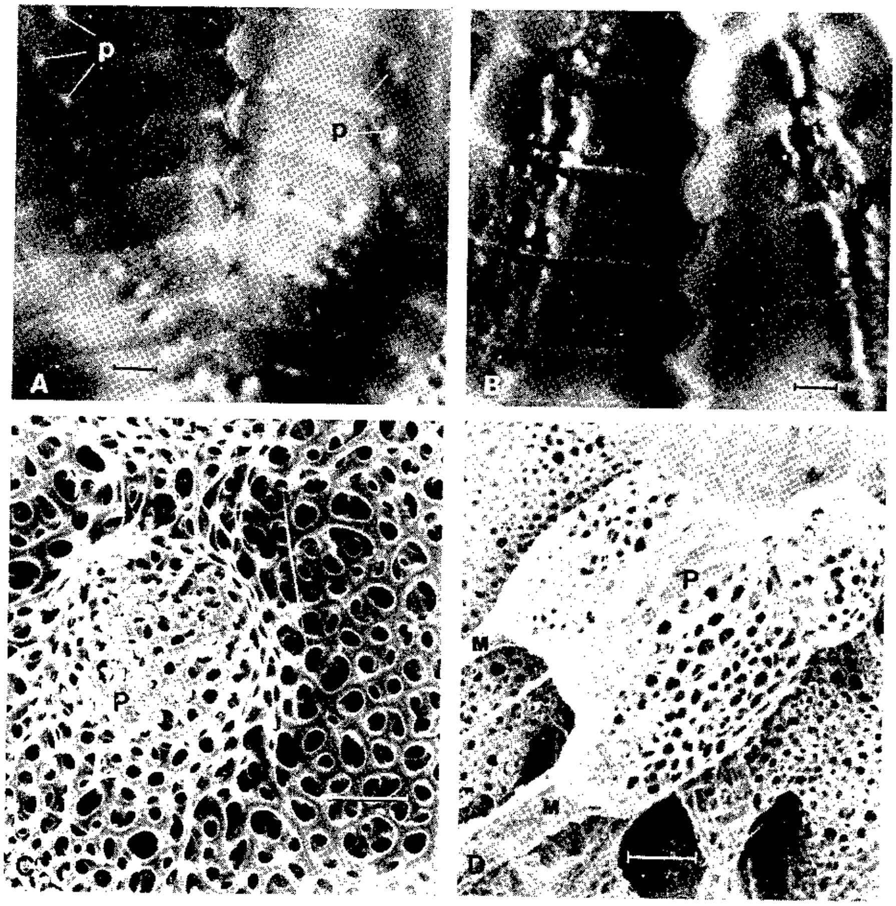


Fig. 6. Cross-section in a depressed urchin, showing the intestine, fastened to the body wall by the mesenterial threads: arrows point to the uppermost attachment points at the ambulacral rays tips (Aristotle's lantern was removed).



HISTOLOGY OF THE MESENTERIAL THREADS

Mesenterial threads, attached to the intestine and to the body wall, consist of an outer layer of epithelial cells filled with loose connective tissue (Fig. 8). A few muscle fibers appeared in the stained preparations.

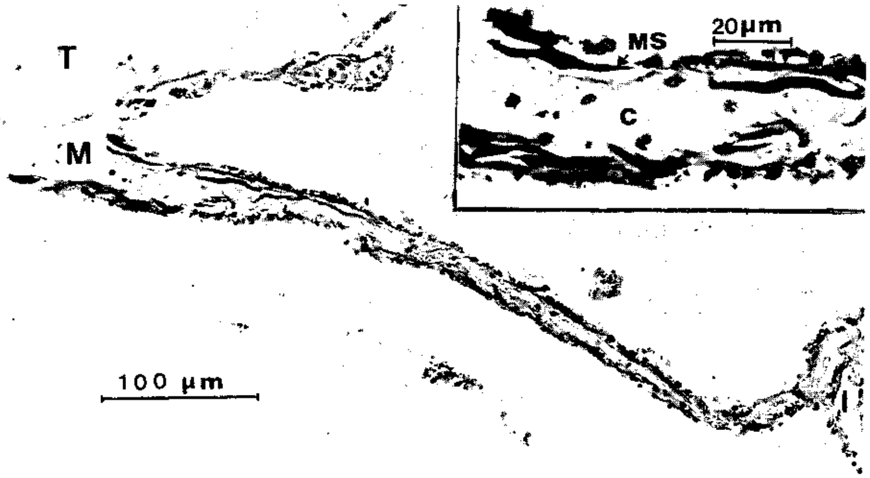


Fig. 8. A longitudinal section of a mesenterial thread: light microscope view of a 7-μm section of paraffin embedded tissue, stained with Heidenhain's Azan stain; mesenterial thread (M) emerges from the test (T) and connects with the intestine (I); C, connective tissue; MS, muscle fibers; inset, higher magnification of the left end of the thread, viewed through a 490-nm filter.

EFFECT OF CALCIUM CARBONATE PRECIPITATION INHIBITORS

The diameters of experimental groups exposed to the pollutants ceased to increase (Fig. 9B–F) and, in four out of five treatments, a significant reduction in H/D ratio followed. The two control groups that showed a continuous increase in average diameter without change in their H/D, were pooled (Fig. 9A). Dequest 2060 did not reduce the H/D ratio although it affected the growth rate. The remaining groups showed slight sinking of the apex, gaps formed between neighboring plates of the aboral region of treated urchins' tests, and the test was weakened along the meridional sutures. Scanning

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◀ Fig. 7. Light photography and SEM pictures of the inner surface of abnormal *Tripneustes*: A, oral half of the IA plates, showing the regular pattern of protuberances (p), and the thickening of the sutural areas, light photography, (scale bar, 1 mm); B, aboral half of the same, showing the developed ridge-like protuberances (scale bar, 1 mm); C, SEM micrograph, showing the protuberance (P) and a nearby cavity (scale bar, 100 μm); D, SEM micrograph of an uncleaned test, at the borderline between the A and IA plates (adradial suture), showing a protuberance (P) with mesenterial threads (M) emerging towards the lower left corner (scale bar, 200 μm).

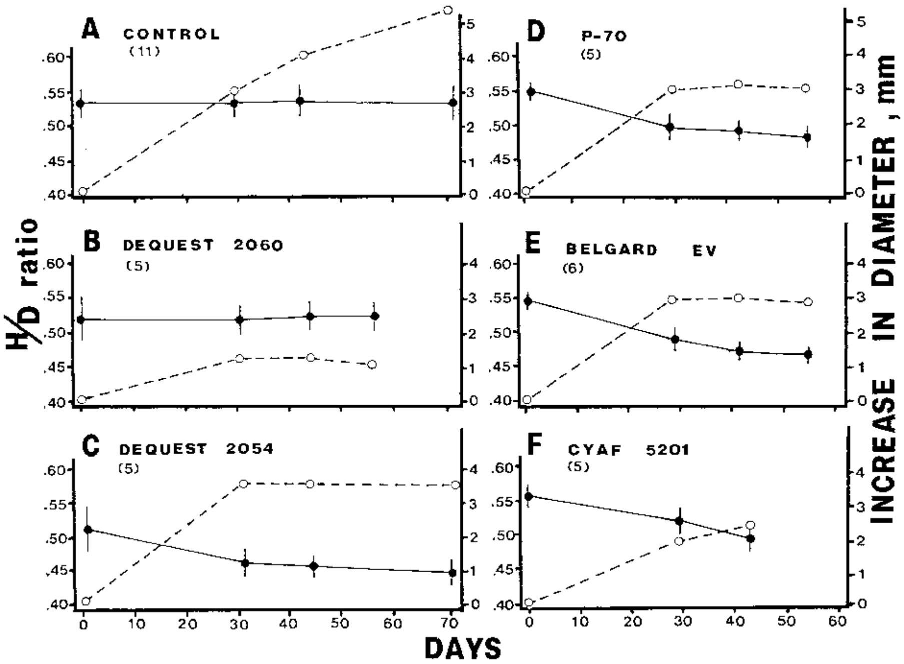

Fig. 9. Results of exposing normal *T.* cf. *gratilla* to calcification inhibiting agents: growth increment (dashed lines) and H/D ratio (mean ± SE) at various times throughout the experiment; in parentheses, number of urchins in each experimental group.

electron micrographs showed that the plates at these sutures had abnormally thin and distorted trabeculae. The CYAF 5021 experiment was terminated after only 45 days because of a complete collapse of the animal's test when the meridional sutures split open. Other injurious effects, such as complete loss of pedicellaria and dermal infections caused its termination after 55 days for some groups. After the experiment some animals were kept in non-polluted conditions, and showed only slight repair of the sunken apex deformity.

DISCUSSION

Environmental gradients that exist between the open Gulf and the lagoon seem to influence the distributions of sea urchin species in the lagoon, and to underlie the absence of most species from its farthest part. The low temperatures that were recorded in the lagoon may account for the reduction of *Tripneustes* cf. *gratilla* numbers at Sites F–G. This species is known for its sensitivity to low temperatures, whereas *Diadema setosum* is more resistant (Lawrence, 1973). The high fluctuations in salinity (15–41‰) in the innermost part of the lagoon add to the extreme conditions there. On the other hand, absence of large carnivorous fish from the lagoon (Fricke, 1971, 1974, and pers. obs.) compensates for potential environmental limitations, and may explain the larger size attained by *Tripneustes* (Table I) and other echinoids in the lagoon.

The lower H/D ratio in *T.* cf. *gratilla* in the lagoon correlated with the appearance of aboral depression. Both were at their maximum at Site C, in front of a hotel whose laundry wastewater was spilled for at least 2 yr into the lagoon. The low percentage of abnormal urchins in the nearby Sites E and F is explained by the limited movement of these urchins and by the counterclockwise direction of the prevailing currents throughout the year in the lagoon (pers. obs.). Later observations throughout 1981 confirmed that this distribution pattern (Table I) remained unchanged.

Aboral depression has been reported for *Echinus esculentus* (Chadwick, 1924), for *Lytechinus variegatus* (Moore, 1974) and for *Tripneustes gratilla* from Mauritius (l'Oriol, 1883 and Lambert & Thiery, 1914, both cited in Mortensen, 1943; Koehler, 1924; Clark, 1925). Lambert & Thiery (1914) even established a new species, *T. lorioli* for the anomalous phenotype. Although it seems that Mauritius *Tripneustes* populations were affected with this deformity for many years, Mortensen (1943) failed to find it there in the 1930's. Because of its similarity to parasite-inflicted malformations, Mortensen (1943) believed that parasites caused the Mauritius deformations, and that *T. lorioli* is only a junior synonym of *T. gratilla.*

Although our deformations conform entirely with these authors' descriptions, we were unable to establish the presence of any parasite. The "collapsed" appearance of this deformity calls, therefore, for another explanation.

The mechanical strength of the living echinoid test depends on its rigidity, attained through the close-fitted linkage of the individual plates by collagen fibers (Moss & Meehan, 1967) or nails (Märkel, 1976). Lack of this tight fit in the echinothurid echinoids renders their tests flexible and even collapsible (Hyman, 1955). The pollutant at the Eilat lagoon, whose identity is uncertain, apparently affected the urchins' rigidity. The results of the calcification inhibition assay (Fig. 9) support the view that poor calcification affected the sutures at the aboral plates and caused the physical collapse of the entire weakened aboral part. Although similar laboratory assays with the detergents that were used in the hotel laundry proved unsuccessful, it is possible that a combination of more than one chemical in the hotel's wastewater caused this effect. The light–weight tests of the deformed urchins and the erosion in the inner surfaces of their plates may also suggest defective calcification. The last phenomenon, the possible resorption of the inner parts and the significant thickening of the sutural areas were probably aimed at strengthening the sutural areas under these conditions. The abnormal orientation of the trabeculae (Fig. 4D) proved that this was not a post mortem artifact.

Flattening of the test was probably the first indication of defective skeletal growth, as shown in the assay animals. It was apparently not limited to *Tripneustes*. The average H/D of 81 *Echinometra mathaei* from Site C was 0.48 (SE ± 0.01) (D being the greatest horizontal length), significantly (*t*-test, $P < 0.01$) different from the H/D of 0.61 ± 0.01 measured for 98 urchins from outside the lagoon (S. Anzelewitz, pers. comm.).

The aboral depression resulted from the sinking of the apical system below the adjacent coronal plates. The regular pit patterns (Fig. 6), shown also in *Lytechinus variegatus* (Moore, 1974) suggest active pulling from beneath. Since the intestine mesenteries attach precisely beneath these pits, it is conceiveable that the stresses that associate with these mesenteries, force the plates to become concave and to sink. Dafni (1980) suggested that lantern retractor muscles, tubefeet and possibly also plate binding collagenous fibers produce mechanical forces that modify the growth of individual plates and consequently affect the entire test shape. The role of tubefeet was demonstrated in an experiment in which rock-dwelling *Tripneustes* cf. *gratilla* were transferred to a sandy habitat, where the tubefeet adherence to the substratum was greatly reduced, resulting in a significant increase in H/D ratio (Dafni, in prep.). Dafni & Erez (in press) pointed out the correspondence between differential calcification patterns of the plates and the spatial arrangement of tubefeet and mesenterial threads, which are reinforced with muscle fibers, and suggested that the latter may also take part in the regulation of calcification. It is noteworthy that the unique meridional muscle sheets that actively flex the soft test of the echinothurid *Asthenosoma varium* (Hyman, 1955), are in fact muscle-enriched mesenteries, probably homologous with the small intestine mesenteries of *T.* cf. *gratilla* (Dafni, unpubl. obs.).

The aboral depression deformity differs morphologically from the inflated test deformation, shown in the same species (Dafni, 1980), yet both share some characteristics. First, urchins with both types of deformity showed slower growth in captivity and their calcium incorporation rates were extremely reduced (Dafni & Erez, in prep.). Secondly, both show distorted growth patterns that could be interpreted as the results of mechanical constraints. Although it is unknown at this stage what caused the meridional directed growth by the individual plates in the inflated test deformity that yielded excessive H/D ratios, the fact that it affected mainly small urchins, whereas the aboral depression affected larger urchins (> 50 mm), may indicate different responses that relate to size.

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REFERENCES

ALLAIN, J.Y., 1978. Déformations du test chez l'oursin *Lytechinus variegatus* (Lamarck) de la baie de Carthagène. *Caldasia*, Vol. 12, pp. 363–375.

CHADWICK, H. C., 1924. On some abnormal and imperfectly developed specimens of the sea urchin *Echinus esculentus*. *Proc. Zool. Soc. London*, Vol. 94, pp. 163–172.

CHESHER, R.H., 1969. Contribution to the biology of *Meoma ventricosa* (Echinoidea : Spatangoida). *Bull. Mar. Sci.*, Vol. 19, pp. 72–110.

CLARK, H.L., 1925. *A catalogue of the recent sea-urchins (Echinoidea) in the collection of the British Museum*. British Museum (Natural History), London, 250 pp.

CULLING, C.F.A., 1974. *Handbook of histopathological and histochemical techniques*. Butterworths, London, 3rd edition, 217 pp.

DAFNI, J., 1974. Animal communities in dead corals under pollution conditions at Eilat, Red Sea (in Hebrew). M.Sc. thesis, Tel Aviv University, 76 pp.

DAFNI, J., 1980. Abnormal growth patterns in the sea urchin *Tripneustes* cf. *gratilla* (L.) under pollution (Echinodermata, Echinoidea). *J. Exp. Mar. Biol. Ecol.*, Vol. 47, pp. 259–279.

DAFNI, J. & J. EREZ, in press. Differential growth in *Tripneustes gratilla* (Echinoidea). *Proc. International Echinoderm Conference*, Tampa, Florida, 1981.

FRICKE, H.W., 1971. Fische als Feinde tropischer Seeigel. *Mar. Biol.*, Vol. 9, pp. 328–338.

FRICKE, H.W., 1974. Möglicher Einfluß von Feinden auf das Verhalten von *Diadema*-Seeigeln. *Mar. Biol.*, Vol. 27, pp. 59–62.

HUMASON, G.L., 1967. *Animal tissue techniques*. W.H. Freeman & Co., San Francisco, 2nd edition, 569 pp.

HYMAN, L.H., 1955. *The invertebrates: Echinodermata, the Coelomate Bilateria, Vol. 4*. McGraw-Hill, New York, 763 pp.

JENSEN, M., 1972. The ultrastructure of the echinoid skeleton. *Sarsia*, Vol. 48, pp. 39–48.

KOEHLER, R., 1924. Anomalies, irrégularités et déformations du test chez les echinides. *Ann. Inst. Océanogr. Monaco*, n. s. 1, pp. 159–480.

LAWRENCE, J.M., 1973. Temperature tolerance of tropical shallow water echinoids (Echinodermata) at Elat (Red Sea). *Isr. J. Zool.*, Vol. 22, pp. 143–150.

MÄRKEL, K., 1976. Struktur und Wachstum des Coronarskeletes von *Arbacia lixula* Linné (Echinodermata, Echinoidea). *Zoomorphologie*, Vol. 84, pp. 279–299.

MOORE, H.B., 1974. Irregularities in the test of regular urchins. *Bull. Mar. Sci.*, Vol. 24, pp. 545–560.

MORTENSEN, T., 1943. *Monograph of the echinoidea. Vol. III. 2.* C.A. Reitzel, Copenhagen, 553 pp.

MOSS, M.L. & M.M. MEEHAN, 1967. Sutural connective tissues in the test of an echinoid *Arbacia punctulata*. *Acta Anat.*, Vol. 66, pp. 279–304.

OREN, O.H., 1962. A note on the hydrography of the Gulf of Eylath. *Sea Fish. Res. Stat. Haifa*, Bull. 30, pp. 3–14.

SNEDECOR, G.W. & W.G. COCHRAN, 1967. *Statistical methods*, 6th edition, Iowa State University, Ames, 593 pp.

THOMPSON, D.W., 1917. *On growth and form*. Cambridge University Press, Cambridge, 793 pp.