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Skeletal calcification patterns in the sea urchin *Tripneustes gratilla elatensis* (Echinoidea: Regularia)

II. Effect of various treatments

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Abstract

Morphometrically determined growth measurements and ^{45}Ca calcification assays were applied to deformed *Tripneustes gratilla elatensis* collected between 1979 and 1981 from polluted environments in the Gulf of Eilat, Israel, in order to check the effect of various pollutants and treatments on skeletal growth and structure. Deformed sea urchins showed low calcification rates and aberrant plate calcification gradients, as well as irregular sutural calcification patterns, characterized by very high vertical vs horizontal plate-edge growth ratios (v/h). This was consistent with their abnormal skeletal morphology, characterized by high height vs horizontal diameter (H/D) ratio. Similar growth and calcification patterns were shown by hard-substratum sea urchins transferred onto artificial sandy habitats. They became taller (having higher H/Ds), showing irregular sutural calcification patterns and higher v/h ratios. This phenomenon is apparently due to a biomechanical effect related to the weaker adherence of the ambulacral tubefeet suctorial discs. This experiment supports the hypothesis that the pollutants, industrial calcification inhibitors, are causing deformities in *T. g. elatensis* by affecting their calcification rate as well as their differential growth patterns. These results agree with a recently proposed biomechanical model suggesting that mechanical forces, internal liquid pressure and the activity of various contractile and elastic tissue elements control the growth and calcification of plates along their sutural edges, consequently determining the overall test morphology. It is suggested that the skeletal deformities, observed in earlier studies of this subspecies, resulted from an irreversible abnormal calcification pattern, which is often associated with changes in the proposed stress-balance.

Introduction

In a companion paper (Dafni and Erez, 1987), the basic calcification patterns were determined for the common Red Sea sea urchin *Tripneustes gratilla elatensis*, using size measurements and ^{45}Ca radioactive assays. These methods were used to examine the calcification patterns of two pollution-induced deformity types in the same sub-species: Deformity A, sea-urchins showing abnormally tall and distorted tests, from the vicinity of a power plant at Eilat, Gulf of Eilat, Red Sea. The mean height vs diameter ratio (H/D) of these urchins was 0.70, with extreme cases of $H/D > 1.3$, compared to a normal ratio of 0.53 (Dafni, 1980). Deformity B, from an artificial lagoon at Eilat, displayed flatter tests ($H/D = 0.43$), deeply depressed aborally (Dafni, 1983). The purpose of this study is to elucidate the cause of each of these deformations, and its long-term effect on the growth and morphogenesis of the sea urchins.

Following hypotheses concerning the mechanisms which determine the growth patterns and test morphology in echinoids (Thompson, 1917; Moss and Meehan, 1968; Raup, 1968; Telford, 1985), a model has been proposed, based on observations, according to which the peripheral growth and calcification of echinoid test plates are determined by a mechanical stress-balance, associated with the spatial organization of contractile and elastic tissue elements (Dafni and Erez, 1982; Dafni, 1985, 1986). More specifically, this model suggests that tensile stresses at the plate sutures facilitate sutural growth, which is alternatively inhibited by sutural compression. Possible contributors to this balance are: (1) inner hydraulic pressures produced by the expansion of the internal coelomic fluids, which are translated into sutural tension; (2) the adherence of ambulacral tubefeet to the substratum, which pulls the plates downwards, compressing them in the meridional direction; and (3) intestine supporting mesenterial threads attached to the inner side of the plates, which pull them in different directions, producing tensile stresses at certain sutures and compression in others.

The experiments described here apply the radioactive method to test Thompson's (1917) hypothesis, upon which the above model is mainly based, that tubefeet adherence to the substratum produces a mechanical effect causing flattening of regular echinoid tests and thus affecting their test morphology. Another aim was to record the long-term morphometric changes and short-term aberrant calcification patterns of the deformed sea urchins, and to compare them to the normal patterns, as well as to test a hypothesis (Dafni, 1980, 1983) that the deformities result from an abnormal array of stress-producing tissue elements. An effort to reproduce the deformities involved the application of suspected pollutants and of chemical reagents of known function, thereby simulating the effect of pollution.

Materials and methods

Growth and calcification of deformed sea-urchins

Deformed *Tripneustes gratilla elatensis* of both deformity types were collected between 1979 and 1981 from the polluted sites, and maintained for prolonged periods in flow-through seawater aquaria in the laboratory, under optimal feeding conditions. Field water temperatures were 20° to 24 °C, the ambient temperatures in the Gulf. These temperatures were maintained in captivity by seawater flow-through supply. Initial and final size and weight measurements of living sea urchins were taken, as well as their final dry skeletal weight. Individual interambulacral (IA) plates were measured after being cleaned and weighed to calculate their relative porosity, as described by Dafni and Erez (1987).

Calcification rates were determined, using seawater labelled with ^{45}Ca (RSW), in laboratory assays for: (a) whole skeleton; (b) its various skeletal components; (c) whole IA plates and (d) for calcareous samples taken from the sutural margins of IA and ambulacral (Amb) plates. The ratio between vertical and horizontal calcification rates of IA plates (v/h) was calculated by summing the uptake rates of adorad and adapicad sutural margins (the vertical component), divided by the horizontal rates – adradiad and interradiad combined (Fig. 1). Experimental procedure and analytical terminology followed Dafni and Erez (1987). RSW activity in these assays was 0.05 to 0.1 μCi/ml. All assays were preceded by a 7-d pre-incubation period in non-radioactive seawater (NRSW), with the green alga *Ulva lactuca* (L.) as food, and at least 24 h of NRSW post-incubation.

Effect of various treatments

Effect of soft substratum. Thompson's (1917) hypothesis that tubefeet adherence causes test flattening was tested by transferring *Tripneustes gratilla elatensis* from a wave-exposed rocky subtidal zone into a sand-strewn shallow aquarium with seawater supply at ambient temperatures (23° to 25 °C), resulting in significant changes in H/D (Dafni, 1986). Several of the urchins maintained on sand substrata were incubated individually, after 24 h of NRSW incubation, in RSW in a 3-liter glass container, half-filled with fine sand. The sand was pre-treated with concentrated HCl in order to prevent isotopic exchange with the tracer. A small cage, made of thin (0.5 mm) plastic covered telephone wire, prevented the urchins from contacting the solid walls. NRSW post-incubation and processing were as above.

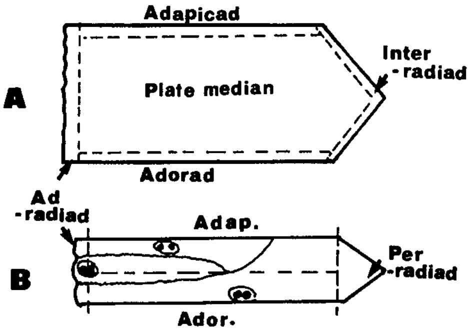

Fig. 1. *Tripneustes gratilla elatensis.* Schematic presentation of sampling method for ^{45}Ca analysis of sutural calcification. A: interambulacral; and B: ambulacral plates. Broken lines denote mode of plate-edge removal (for details see Dafni and Erez, 1987)

Effect of pollutants. The following chemical reagents were applied to normal *Tripneustes gratilla elatensis* within a two-week NRSW pre-incubation: (1) Belgard EV (Ciba-Geigy, Switzerland) and (2) CYAF 5021 (Cyanamid, USA), both carbonate precipitation inhibitors that in a previous assay reduced *T. g. elatensis* growth rates and induced test flattening (Dafni, 1983). These reagents were introduced to the NRSW medium at a concentration of 0.02% (800 μl/4l), 6 d before ^{45}Ca incubation; (3) N-200 (Zohar, Israel), a laundry detergent used extensively in a hotel whose polluted liquid outfall may have caused the flatter deformity (Dafni, 1983). It was added to the NRSW medium at 50 mg/l, 6 d prior to the radioactive incubation. The other reagents were (4) β-mercaptoethylamine (MEA) and (5) β-aminopropionitrile (APN), collagen synthesis and fiber alignment inhibitors, known to reduce the mechanical strength of connective tissue (Harkness, 1968). These were administrated by injection of 2 to 5 ml, according to size, of a 10 mg/ml solution through the peristome membrane 14 and 7 d before the RSW incubation. Radioactive incubation of 24 h was carried out in room conditions (at 25 °C ± 1 C°), in non-polluted RSW. ^{45}Ca activity and processing were as above.

Results

Growth and calcification of deformed *Tripneustes gratilla elatensis*

Growth rates and skeletal porosity. Although not directly recorded, observations showed no difference between normal and deformed urchins as to diet or feeding behaviour. The average diameter accretion of 22 deformed tall indi-

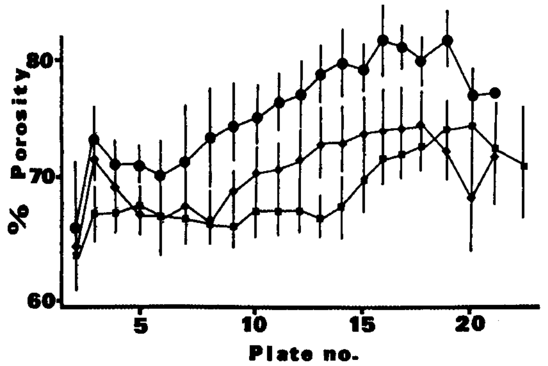

Fig. 2. *Tripneustes gratilla elatensis.* Percent plate porosity (±1 SD) of normal (●) and deformed sea urchins: (■), abnormally tall, and (◆), abnormally flat

viduals (Deformity A, $H/D = 0.90 \pm 0.18$, measured for 5 to 27 months), under optimal feeding was 8 mm per year (from an average of 41 to 49 mm). An annual growth of ca 30 mm was observed in normal sea urchins of the same size range (Dafni, 1984). Flat deformed individuals increased their diameter by only 1 to 5 mm in two years of captivity (Dafni, 1983). No significant regeneration from the abnormal H/D ratio occurred during these two years in either deformity type, although the pollutants were not present. However, these deformed sea urchins were characterized by an unusually hard and compact test. When dried tests were crushed, they often broke across the plate instead of splitting along the sutures, as normally occurs in regular echinoids tests (Strathmann, 1981). Deformed urchins' tests collected in the polluted sites shortly after the onset of the deformations were usually less compact.

The coronal plates of abnormally tall individuals were significantly less porous than those of the non-deformed ones (paired Student's *t*-test, $p < 0.05$) (Fig. 2). In deformed urchins' plates, the porosity was less than 70%, while the non-deformed plates reached > 80% porosity. The plate stereom (network of skeletal trabeculae) of abnormally flat urchins was also less porous, but since the skeletons of larger (and older) individuals are normally more compact than the younger ones (Dafni and Erez, 1987), this result may relate to the deformed larger size (> 50 mm).

It is noteworthy, however, that the porosity of the plates near the peristome was about the same (65 to 75%) in deformed and non-deformed urchins. This suggests that the lowermost plates, deposited long before the onset of abnormal growth, were least affected by the deformation.

Calcification rate of individual plates. Whole-skeleton calcification rates of the 13 deformed tests were extremely low, 0.02 to $0.05\%\ \mathrm{d}^{-1}$, compared to $1.5\%\ \mathrm{d}^{-1}$ of the non-deformed ones (Dafni and Erez, 1987). The calcification rate of the individual plates was also low, and the calcification gradient along the plate column was different from the normal pattern. Fig. 3 A to C shows representative patterns of IA plate calcification compared with the dry skeletal weight gradient of the same plate column, for both types of deformed sea urchins and for a non-deformed one. Abnormally tall urchins (Fig. 3 A) were characterized by a remarkable similarity of 8 to 9 consecutive plates, between calcification rate and dry weight. Thus, plates showing higher calcification rates weighed more than low calcifying plates and vice versa. This suggests that the abnormal calcification pattern persisted for a long time after the polluting agents had been removed.

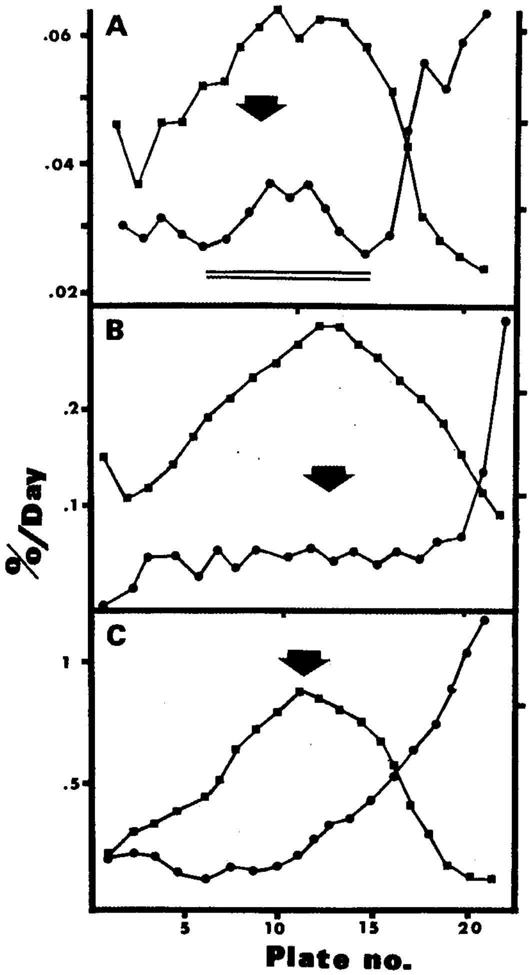

Fig. 3. *Tripneustes gratilla elatensis.* Calcification rate (circles, %/ day) and dry skeletal weight (squares, mg) of whole IA plates along a meridional column of sea urchins: (A) showing tallness (Deformity A); (B) abnormally flat (Deformity B); and (C) normal urchins. Plates are counted from the peristome. Double line in A denotes similarity of pattern between both parameters

In abnormally flat individuals (Deformity B) (Fig. 3 B), the weight pattern seemed to be normal (compare Fig. 3 C), while the calcification rates were extremely low.

Sutural calcification. The calcification patterns along the sutural margins of IA and Amb plates of deformed ssea urchins were extremely variable. One typical example of each deformity is shown in Fig. 4 A and B, while 4 C shows the normal pattern of the non-deformed sea urchin. In the IA sutures of abnormally tall individuals (Fig. 4 A), the highest calcification rates were observed along the vertical edges (adorad and adapicad), contrasting with the normal

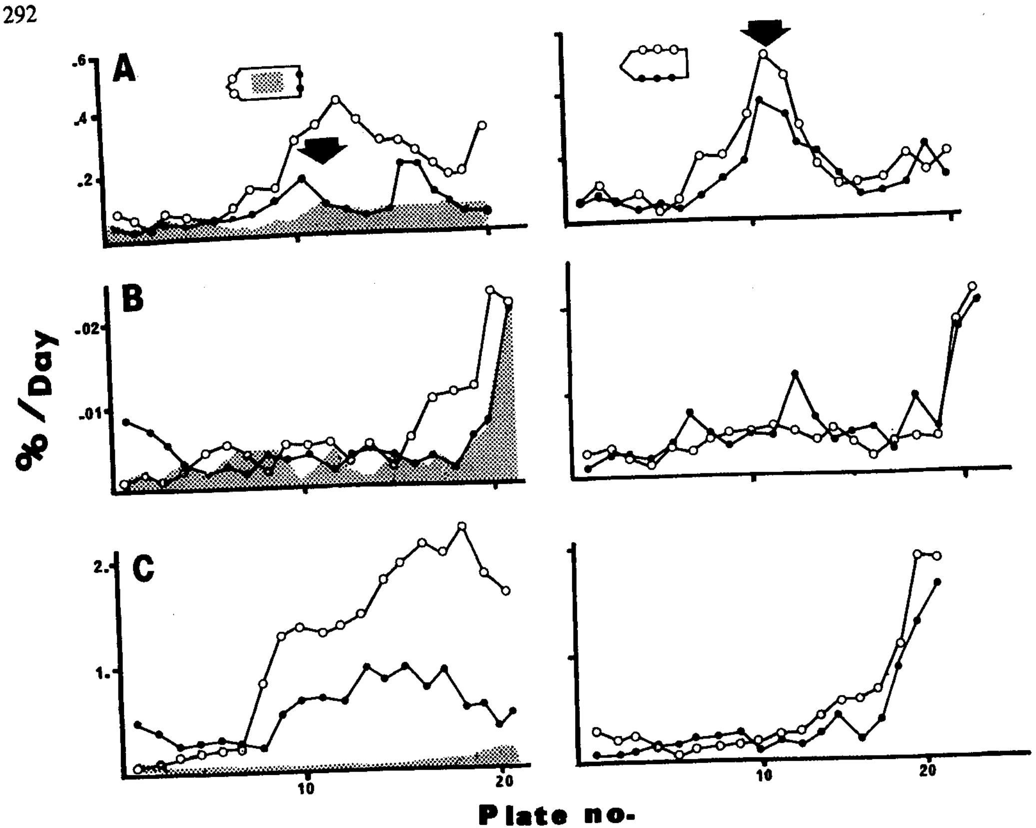

Fig. 4. *Tripneustes gratilla elatensis*. (A, B) Sutural calcification patterns of abnormally tall and flat sea urchins; and (C) normal individuals, along an IA vertical column, in the horizontal (left) and vertical (right) directions. Shaded areas represent plate median calcification. Arrows point at ambitus position

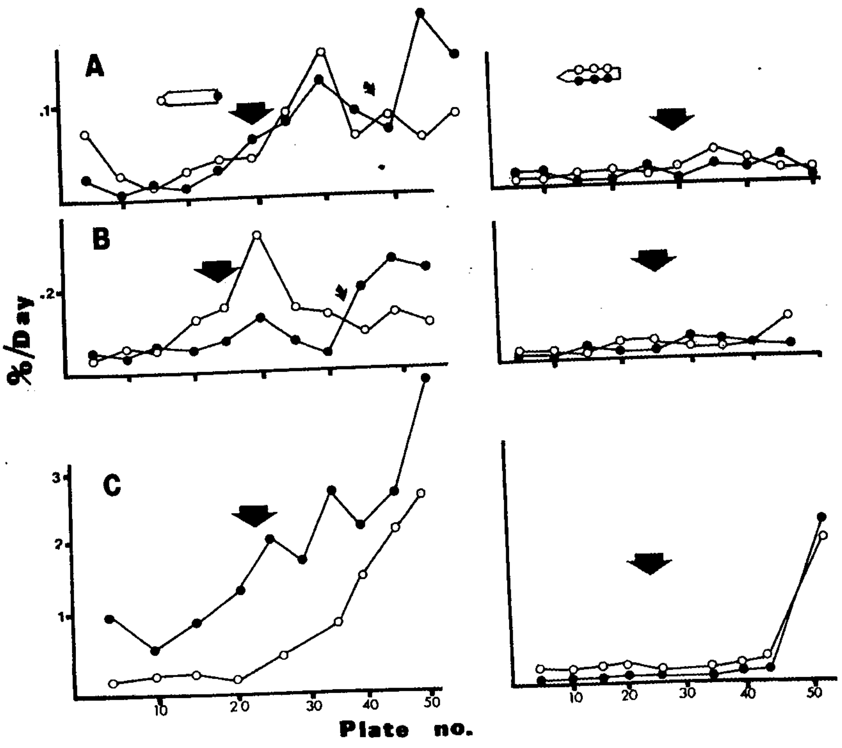

Fig. 5. *Tripneustes gratilla elatensis*. (A, B) Sutural calcification of ambulacral plates of two sea urchins showing abnormal tallness; and (C) normal urchin – horizontally (left) and vertically (right). Large arrows mark the ambital position and smaller ones point to abnormal rate decrease

pattern, in which the highest rates occur horizontally (Fig. 4C). Note that the calcification in the plate median (i.e. within the existing stereom) of the deformed urchins was appreciably higher than in the normal control, in accordance with their relatively low porosity.

In the abnormally flat individuals (Fig. 4B), low calcification rates were observed in all but the uppermost IA plates, and calcification was almost equal in all directions. It is noteworthy that in this sea urchin the calcification rate of the plate medians equalled the peripheral rates. This indicates that the net sutural calcification (peripheral minus median) in this urchin was practically zero.

The calcification patterns of the ambulacral plates of deformed tall (Fig. 5A, B) also deviated from normal (Fig. 5C). A typical trend in these urchins was a decrease in the calcification rates in the horizontal direction in superambital plates, increasing again in the plates next to the apex. This calcification pattern correlates well with the occurrence of a localized narrowing of the ambulacra in the form of pinches, which is a common occurrence in tall deformed urchins (Dafni, 1980). This may result from relatively lesser growth of the Amb plates horizontally, relative to the Amb plates above and below these points. Flatter, deformed urchins' plates were not tested for the ambulacral sutural calcification patterns.

Directional skeletal growth. In Fig. 6, the sutural calcification rates (see Figs. 4 and 5) were expressed as vectors, showing the directional accretion of the individual plates along the sutures of an entire plate column. Fig. 6A shows the non-deformed pattern, whereas Fig. 6B and C shows the patterns of abnormally tall and flat urchins (Deformities A and B), respectively. Sutural pattern of tall, deformed urchins is characterized by excessive growth in the vertical axis of circum-ambital plates, while in the latter equally low calcification rates were observed in all directions.

Although obtained in a short-term incubation, these patterns generally fit the morphogenetic trends shown by the tests, i.e. abnormally tall urchins showing excessive vertical growth and narrowing of the ambital Amb plates to form the pinches, and abnormally flat ones showing growth cessation. Another observation, obtained through the tracer method, was that in the abnormally flat urchins only the adapical plates grow and calcify at a relatively high rate (Fig. 6C).

v/h ratio. The variability of the individual patterns, shown by both normal and deformed sea urchins, is much reduced when the vertical vs horizontal (v/h) sutural calcification ratio is considered. It appears that in contrast to normal sea urchins, where all IA plates around the ambitus show a low v/h (see also Dafni and Erez, 1987), the deformed tests (regardless of deformity type) had considerably higher v/h ratios (Fig. 7). The difference between the v/h ratio of the normal urchins to those of either deformity, at the ambitus level, was significant (paired Student's *t*-test, $p < 0.05$).

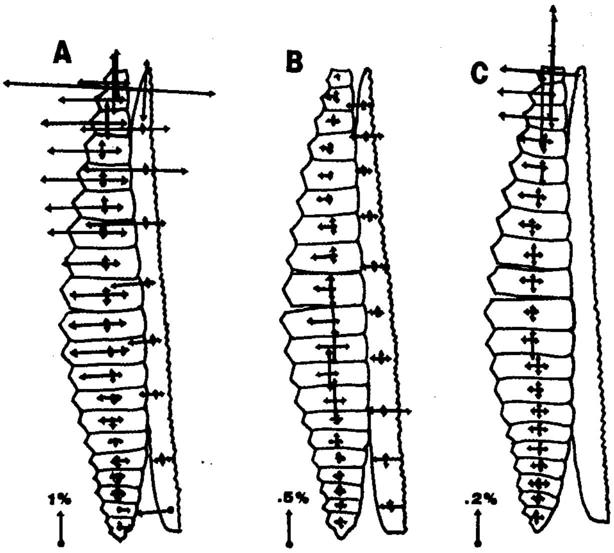

Fig. 6. *Tripneustes gratilla elatensis.* Vectoral representation of differential sutural calcification of IA and Amb plate columns of: (A) A normal sea urchin; (B) an abnormally tall one; and (C) and abnormally flat one, showing excessive vertical growth in the B ambital plates and the relative suppression of all growth directions in C. Note the different scale of the vectors, representing calcification rates (%/day)

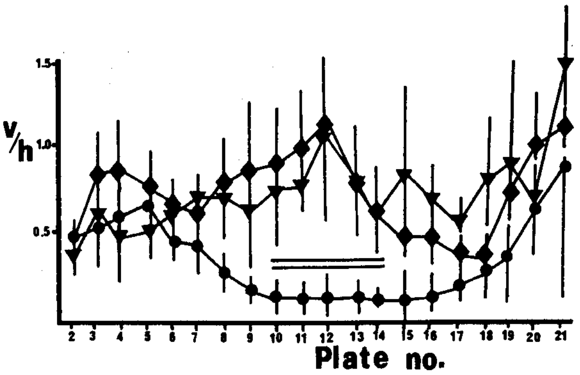

Fig. 7. *Tripneustes gratilla elatensis.* The v/h ratio (± SD) of normal (●) and deformed sea urchins' plates: (◆), abnormally tall; (▼), abnormally flat. Double line denotes significant difference between the normal and the deformed urchins (paired Student's *t*-test, $p < 0.05$). Ambitus position is at Plates 10 to 12

Effect of sand substratum

Exposure to a sandy substratum primarily affected the mobility of the urchins, due to their inability to adhere to the sand particles. Nevertheless, they grew both in diameter and height, showing a significant increase in their H/D ratios, from H/D = 0.529 to 0.539 to 0.557 to 0.572 in the various experimental groups (Table 1, Dafni, 1986). This increase (3.7 to 6.1% of the initial H/D values) was much higher than expected (2.3%) from the allometric tendency of this subspecies to grow taller with age (Table 1, Dafni and Erez, 1987).

The excessive growth vertically, observed in the ^{45}Ca assay of several sand-maintained urchins, confirmed the observed morphometric trends. One such individual, which showed exceedingly high calcification rates (> 2% d^{-1} in ambital plates), exhibited weight pattern fluctuations in the plate columns, as well as a striking similarity between calcification and skeletal weight patterns for 7 to 8 plates (Fig. 8) (compare Fig. 3 C for the normal patterns). Its IA sutural calcification pattern (Fig. 9 A) was also aberrant, closely resembling the patterns of deformed sea urchins (Fig. 4).

The v/h ratio of circum-ambital plates of two urchins maintained on soft substratum (Fig. 9 B) was > 0.6 in both analysed specimens, three times higher than the mean v/h of four normal sea urchins (0.16).

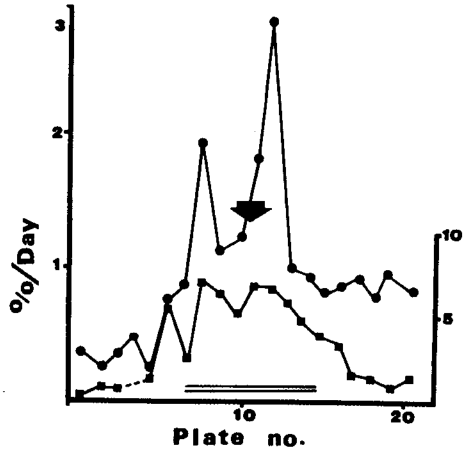

Fig. 8. *Tripneustes gratilla elatensis.* Calcification (circles) and dry skeletal weight (squares) pattern of a soft-bottom maintained sea urchin. Double line denotes similarity of pattern between both parameters

Effect of pollutants

The calcification inhibitors, CYAF, Belgard and N-200, although suppressing the overall calcification in the experimental groups, differently affected the various skeletal components (Fig. 10 A). In all cases, the coronal and apical plate calcification rates were highly reduced, whereas other skeletal components (lantern, jaws and teeth) were less affected. The calcification rate of the half-pyramids (jaws) was twice the coronal rate in sea urchins treated with CYAF and Belgard. In N-200, the calcification rates of all components, except the teeth, were roughly equal. In all samples, the calcification rates of the teeth were considerably higher than other components, quite similar to those of the control groups.

The effect of the pollutants CYAF and Belgard on the v/h ratio of the circum-ambital plates was more pronounced (Fig. 10 B). In sea urchins treated with these reagents, v/h increased more than twice the normal ratio (0.5), indicating a strong differential effect, strikingly similar to the effect of soft substratum (> 0.7 vs 0.2), or in deformed sea urchins (ca 1.0; compare Fig. 7).

In sea urchins treated with the inhibitor of collagen synthesis, MEA, a non-significant decrease in calcification was observed while the APN-treated showed the opposite trend. Furthermore, none of these reagents influenced the v/h ratio. Although affecting calcification rate, the pollutant N-200 was not tested for v/h ratio.

Discussion

Our experiments support Thompson's (1917) hypothesis concerning the influence of the physico-mechanical envi-

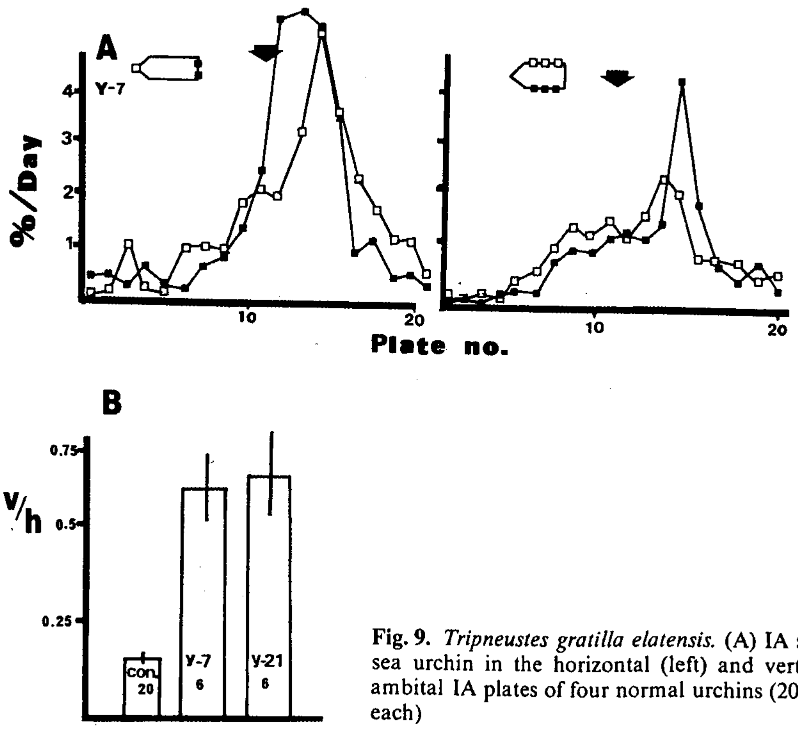

Fig. 9. *Tripneustes gratilla elatensis.* (A) IA sutural calcification pattern of a soft-bottom maintained sea urchin in the horizontal (left) and vertical (right) direction. (B) v/h ratio (± SD) of circum-ambital IA plates of four normal urchins (20 plates) and two softbottom maintained urchins (6 plates each)

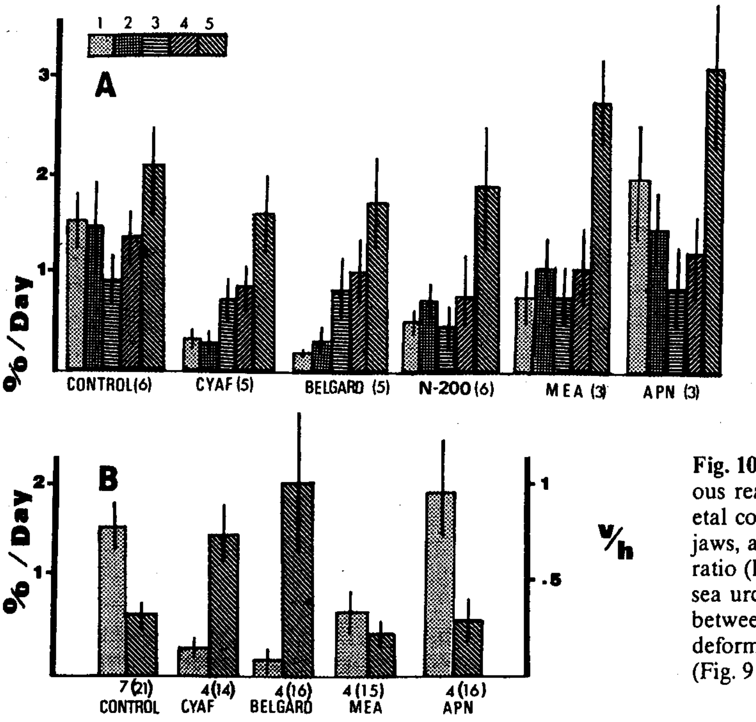

Fig. 10. *Tripneustes gratilla elatensis.* A: Effect of exposure to various reagents and pollutants on calcification rates of various skeletal components (1: coronal plates, 2: apical plates, 3: lantern, 4: jaws, and 5: teeth); B: Coronal calcification rate (dotted) and v/h ratio (hatched) of ambital plates, (Mean ± SD). Below: number of sea urchins and plates analysed (parentheses). Note the similarity between v/h of individuals treated by CYAF and Belgard to the deformed (Fig. 7) and soft-bottom maintained individuals (Fig. 9B)

ronment on the echinoid test morphology. Moreover, they show that this influence manifests itself during the ontogeny of *Tripneustes gratilla elatensis.* This principle has been incorporated in the proposed biomechanical model (Dafni, 1986).

Two main components are involved in determining the stress-tension field in which the urchins' plates grow: (1) a uniform hydrostatic pressure exerted by the coelomic fluids, whose influence on the peripheral skeleton is translated into sutural tension, spreading across the test surface; and (2) the other component consists of tethering elements, attached to the skeletal plates at definite locations, pulling inwards or outwards. The tubefeet pull the five ambulacral rays centripetally in a rather symmetrical pattern, while the inward tether of the mesenterial tissue has a more complex pattern (Dafni and Erez, 1982; Dafni, 1983, 1986).

The basic assumption in this model is that inter-plate tensional stresses induce sutural growth, while peripheral growth and calcification of compressed sutures are inhibited. Preliminary experiments showed that the application of tensional stresses to isolated pieces of test increased their calcification rates and changed their directional patterns (Dafni, 1985).

Generally, the calcification patterns of the deformed sea urchins (Figs. 3 to 5) and those resulting from the experiments (Figs. 8 to 10) are explained by this model, though the biomechanical influence may be masked by other effects. Except for a limited regeneration shown under *in-situ* conditions (Dafni, 1980), urchins removed from the polluted sites and maintained in non-polluted, large aquaria retained their distorted shapes for over two years. Partly this is due to their relatively low growth rates, insufficient to regain the previous normal test configuration. Yet, the observation that the sea urchins did not recover from their deformity suggests that the abnormal calcification patterns of these urchins became irreversibly fixed. Since non-deformed urchins grew rapidly in the same aquaria, with no apparent ill-effects, it seems that growth cessation and fixation of the deformed patterns were not caused by the artificial environment, but possibly by some regulatory mechanism. Observations have shown that irregularities and deformities in echinoids, resulting from physical damage, are frequently maintained for life (Moore, 1974; Dafni, 1986 and unpublished observations).

Fixation of calcification patterns is also indicated by the remarkable similarity between whole-plate calcification and weight patterns of the defomed urchins (Fig. 3A), suggesting that these patterns were consistent since the deformity first occurred. It was less apparent in abnormally flat urchins, which were characterized by very slow calcification rates (Dafni, 1983).

We suggest that, in addition to the two above-mentioned factors, the spatial configuration of the mineral plates controls their subsequent directional calcification. The irregular test configuration, attained during the initial stages of deformation, apparently constrains later growth and calcification by introducing a new stress-balance, different from the original one.

Assuming that the observed vectoral calcification patterns, based on the radioactive analysis (Fig. 6), conserve the sutural patterns prevailing during the deformities, the differences between both deformity types and the normal patterns become apparent: a normal sea-urchin displays a consistent anisotropic growth along their sutures. Its ambital plates grow more horizontally than vertically (low v/h ratio), whereas in the deformed ones these plates grew almost equally in all directions, as indicated by a v/h of ca 1 (Fig. 7).

Taller deformity was characterized by exaggerated vertical calcification of several plates around the ambitus and distorted ambital and superambital regions, and the flat deformed urchins' tests showed excessive calcification only in the plates next to the apex (Fig. 4). In spite of this difference and their different H/D ratio (high in taller and abnormally low in flatter), both grew isotropically. The former's tests grew taller, whereas in the latter the excessive growth of the vertical axis was turned inwards, to form aboral depressions (Dafni, 1983).

Although mainly concerned with the biomechanical influence of external forces on the echinoid test, Telford (1985) shows that the tests are normally under tension in the horizontal circumference (along the longitudinal or meridional zig-zag sutures), while the latitudinal sutures (vertically directed) are relatively compressed. The evidence we present here deals mainly with the influence of the ambulacral podia, while the possible role of mesenterial threads in such stress-balance has been pointed out in a vector analysis of the differential growth (Dafni and Erez, 1982).

Reduced activity of the contractile and elastic tissue elements attached to the test may potentially increase the v/h ratio by enabling growth in all directions, without necessarily affecting the growth rate itself, while relaxation of the hydrostatic pressure of the internal fluid is expected to decrease sutural growth throughout the test, thus increasing v/h ratio and slowing overall growth. In the latter case, the non-sutural calcification may be less affected, and will be deposited within the plate stereom, which will therefore become more compact, showing lesser porosity. This chain of events fits quite well the sea urchins showing abnormal tallness (see Figs. 2, 7).

The similarity between the results of the sand experiment and the patterns shown by the abnormally tall deformity (Figs. 8, 9), both in morphometry (Dafni, 1986) and in calcification pattern (Fig. 9), also supports the biomechanical approach. It is noteworthy that, in this case, the differential pattern was changed, but the absolute calcification rates remained high, as opposed to the results of exposure to pollutants (Fig. 10), thus suggesting that internal pressure was not affected by the soft substratum.

Chemicals used for inhibiting carbonate deposition in the polluting power plant, where taller urchins were found, induced slower growth rates, reduced H/D ratio and slight aboral depression, characteristic to the flat deformed urchins, when applied under laboratory conditions (Dafni, 1983). This firstly suggests that the same chemicals may induce both deformities under different conditions or concentrations. It is further suggested that, in these particular laboratory experiments, both components, internal pressures and forces exerted by the tethering elements, relaxed. It is noteworthy that the tallness deformity appeared mainly in urchins with ambital diameters of 20 to 40 mm (Dafni, 1980), while most urchins displaying abnormally flat tests were larger than 50 mm (Dafni, 1983). Hence, the response to the pollutant may be size-dependent. In a large sea urchin, in which sutural growth is mainly concentrated in the super-ambital region, only this region will be affected by pollutant. The apparently mechanical collapse of the apical region, which may have resulted from reduced internal pressure, caused tension in the apical part of the test, hence the relatively higher calcification rates shown there. It should be added, however, that the deeper depressions in many abnormally flat urchins were caused by a tethering effect, caused by the mesenterial threads (Dafni, 1983).

The decreased calcification rates shown in the radioactive experiment, in which sea urchins were exposed to several of these pollutants (Fig. 10), generally confirm previous observations. However, the reduced rates were mainly shown in the whole test and in the coronal plates, whereas non-coronal skeletal components (lantern, jaws and teeth) were less affected (Fig. 10A). This again suggests that the pollutants directly influence the sutural calcification, or a regulatory mechanism controlling it. The sutural patterns resulting from CYAF and Belgard EV treatment (Fig. 10B) were similar to those of the deformed urchins (low calcification and high v/h ratio of the ambital plates). This again suggests that these chemicals, which are used in the polluting power plant to inhibit carbonate precipitation in the seawater cooling system, caused abnormal tallness. There is no unequivocal conclusion as to how precisely the pollutants affected the normal urchins, but the common features shown under different treatments suggest an indirect non-specific effect. As pointed out above, reduced activity of tubefeet and lesser mesenterial pull, or relaxed inner pressures, all induce higher v/h, with or without growth retardation. The apparently conflicting effects, relaxed sutural tension causing test inflation and excessive pinches shown in certain spots, remain unexplained. We suggest, however, that in the normal test a coordination prevails between all biomechanical elements. When the stress-balance is interrupted, coordination is lost and a new stress regime may develop that ultimately becomes fixed, although the calcification rates of the deformed urchin largely drop.

The experiment that aimed to test the effect of the inhibitors of collagen synthesis and fiber cross-linking, MEA and APN (Harkness, 1968), did not show the expected effects. Considering the preliminary nature of this experiment and the arbitrary concentrations applied, we suggest that the negative results are not sufficient to reject this hypothesis. The variable tensility of the echinoid collagen (Wilkie, 1984) may play a significant part in the proposed stress-balance.

Further experiments are still needed to study the relevance of other mechanical components (lantern muscles, sutural and non-sutural collagen tissue etc.) to this model. It has been speculated that the same principles and biomechanical constraints may also influence evolutionary trends in the Echinoidea (Dafni, 1986, in press). A more detailed investigation may provide a deeper insight into the role of biomechanical elements in the ontogeny and phylogeny of both regular and irregular echinoids.

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