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# Growth Zones in the Echinoid Skeleton

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SYNOPSIS. Growth zones in echinoid skeletal ossicles are mainly the result of differences in structural characteristics. In the test plates, opaque zones, which appear light in reflected light, dark in transmitted light, and are X-ray dense, have relatively larger trabecules and smaller intertrabecular channels. Translucent zones, which appear dark in reflected light, light in transmitted light, and are less X-ray dense, have relatively smaller trabecules and larger intertrabecular channels. Organic material in the plates, especially when pigmented or charred, enhances the appearance of the growth zones. Opaque zones result from relatively fast plate growth while translucent zones result from relatively slow plate growth; food deprivation leads to the formation of translucent zones. The growth zones appear to be formed seasonally, at least in some cases, probably in relation to seasonal changes in growth rates, and perhaps in relation to seasonal reproductive activity.

## INTRODUCTION

The echinoid skeleton is made up of an orderly arrangement of calcium carbonate ossicles which can be divided into three main categories: (i) the external spines and pedicellariae; (ii) test plates, including the corona with five double columns of ambulacral plates alternating with five terminal (ocular) plates and five genital plates, one of which is the madreporite; and (iii) the jaw apparatus, or Aristotle's lantern, with some 40 ossicles, including demipyramids, epiphyses, compasses, rotules, and teeth (Hyman, 1955; Melville and Durham, 1966). Except for the polycrystalline laminate teeth, all echinoid ossicles are quite similar (Raup, 1965). Each ossicle is a highly fenestrated meshwork of trabecules (Nissen, 1969; Weber et al., 1969; Heatfield, 1971). Living tissue occurs throughout the spaces in the meshwork of each ossicle. Growth and fusion of the individual trabecules on the surfaces of each ossicle enlarges the ossicle, and different surfaces grow at different rates to give each ossicle its characteristic shape (Moss and Meehan, 1968; Raup, 1968; Kobayashi and Taki, 1969).

Although each ossicle behaves optically as a single crystal (Raup, 1965, 1968; Donnay and Pawson, 1969; Nissen, 1969), the arrangement of the meshwork and trabecules varies within each ossicle. The spines of many echinoids, especially the camarodonts, have layers of small and large trabecules, giving the appearance of concentric rings in cross-section (Mortensen, 1928-1951; Heatfield, 1971) (see Fig. 4*A*). The test plates also have a well-organized variation in their internal meshwork (Becher, 1914; Deutler, 1926) (see Fig. 1). The outer surface has a fine meshwork of small trabecules generally oriented perpendicular to the plate, while the inner surface has a coarse irregular meshwork of large channels and large trabecules, the "callus" (see Fig. 4*B*). In between, the meshwork of trabecules is generally oriented parallel to the plane of the plate. Variation in the meshwork also occurs in the lantern ossicles, and these have a complex, yet orderly, pattern (Becher, 1914; and unpublished observations).

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Portions of most of the ossicles have alternating light and dark zones. These zones have been seen and interpreted in various ways by different workers. The concentric rings in camarodont spines reflect the growth pattern of these ossicles (Heatfield, 1971). Growth zones in the coronal plates were reported by M'Clelland (1840) as evidence that test growth took place by plate growth as well as by plate addition. Agassiz (1904) figured the lines in the plates of *Salenia miliaris* as evidence of the mode of growth. Deutler (1926) showed that growth zones occurred in the spines, spine bosses, apical plates, coronal plates, and lantern ossicles in a widespread array of echinoid species. In a careful and detailed analysis of the growth zones in the coronal plates of one individual of *Echinus esculentus*, Deutler (1926) showed that echinoid growth and shape results from individual plate addition and growth, with little plate resorption or remodeling. More recently, Raup (1968) used the growth zones as an aid for computer simulation of echinoid plate shape and growth.

Kume (1929) and Moore (1935) used the growth zones in the genital plates of the apical system to age individuals of *Pseudocentrotus depressus* and *E. esculentus*, respectively. Similarly, Dix (1972) showed that the genital plates of *Evechinus chloroticus* might be used for aging individuals. The genital plates in many species are small, however, and in any case the lines are difficult to see clearly. Consequently, suggestions also have been made to age individual sea urchins using the concentric rings in the spines (Moore, 1966; Weber, 1969*a*) or the growth zones in the coronal (especially interambulacral) plates (Kume, 1929; Zoeke, 1952; Durham, 1955; Gamble, 1967; Jensen, 1969*a,b;* Pearse et al., 1970; Birkeland and Chia, 1971; Miller and Mann, 1973; Sumich and McCauley, 1973). Evidence that these growth zones can be used as "sclerochronometers" remains unconvincing, however. New cycles of rings in the spines can be generated merely by breaking the spine tips (Ebert, 1967; Heatfield, 1971). Coronal plates are added as the animal grows, and many are much younger than the animal (Gordon, 1921, 1929; Durham, 1955; Moss and Meehan, 1968). Neither the structural, chemical, nor growth basis of the zones in the test plates, including the apical plates, or the lantern ossicles has been described, nor have they been well correlated with time or environmental changes. Without such information, it seems premature to use these growth zones as sclerochronometers. In this paper we examine the structural, chemical, and growth basis of the growth zones, particularly in the interambulacral plates of *Strongylocentrotus purpuratus* and *S. franciscanus,* and then try to relate these to a long-term maintenance experiment.

## TECHNIQUES

When present, the concentric rings in the spines are relatively easily seen under low magnification after the spine is sectioned transversely or longitudinally (e.g., Ebert, 1967; Weber, 1969*a*; Heatfield, 1971) (Fig. 4*A*). The growth zones in the test plates and lantern are more difficult to examine. The growth zones in the test plates occur largely between the fine-mesh outer plate surface and associated tubercles, and the coarse-mesh inner "callus" (Becher, 1914; Deutler, 1926; Moore, 1935) (see Fig. 1). Usually the inner or outer surface needs to be removed by grinding before the growth zones can be seen clearly. M'Clelland (1840, p. 168) observed the zones " . . . by reducing the several parts of the test of a large *Echinus* to a thin transparent film, when the successive layers of deposit were everywhere observable around the margins of each plate . . . ." Deutler's (1926) technique included cleaning the plates of organic material by boiling in sodium hypochlorite solution (Eau de Javelle), washing in detergent, dehydrating in ethanol, embedding in balsam, grinding and polishing, and finally clearing for examination in a terpineol-methylbenzoate mixture. Deutler's preparations are exquisitely clear and beautiful, but his techniques are tedious and laborious. Durham (1955) attempted differentiation of the growth zones by several techniques, including grinding, staining, acid etching, and photographing in transmitted and reflected light.

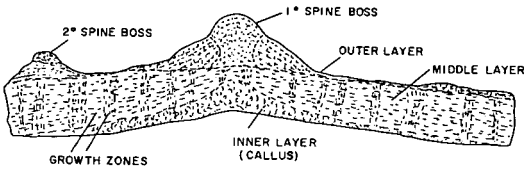


FIG. 1. Diagram of a latitudinal cross-section through an interambulacral test plate of a sea urchin. Different sizes and orientations of trabecular meshwork distinguish the different regions labeled. The growth zones are in the middle layer; the spine bosses also show concentric banding.

Jensen (1969*a*) described a technique to show the zones in coronal plates which is both easy and rapid. After fixing the plates in ethanol and removing extraneous tissue with forceps and brush, she charred the plates over an ethanol flame. The zones could be seen when the plate was immersed in xylene or methylbenzoate. Jensen's technique has been used by Pearse et al. (1970) and Miller and Mann (1973). Charring is not always necessary; for example, Birkeland and Chia (1971) and Sumich and McCauley (1973) simply dried the plates at 75 to 85°C (the former after sanding the surface, the latter after cleaning the plates with sodium hypochlorite, commercial bleach) and cleared them in xylene.

We found charring most helpful with the test and lantern ossicles of *Strongylocentrotus* spp. However, since charring in an alcohol flame is difficult to control and reproduce, we charred the hypochlorite-cleaned plates in a muffle furnace at 300°C for 10 min. Both sides of the plates were ground, either before or after charring, on 600 grinding paper, to remove the spine bosses and the inner layer of callus that usually obscured the zones. For ease of handling and viewing, the plates were permanently mounted in a xylene-based mounting medium.

The plates of individuals of some species contain pigments which enhance the appearance of the growth zones. Both Moore (1935) and Dix (1972) made use of these "pigment" bands in the apical plates. Both of these workers ground off the outer surface of the plates; Moore then cleared and mounted them in balsam while Dix further "developed" the zones by immersing the plates in lactophenol-glycerine with 0.5% ferrous sulphate.

We also thin-ground skeletal ossicles by methods similar to those used by Moss and Meehan (1967) and Heatfield (1971). Plates were mounted on petrographic glass slides with thermoplastic (quartz) cement (No. 70C Lakeside Brand, Hugh Courtright, Chicago) and ground on a lapidary wheel with 600 grit grinding paper. After the desired level was reached, the cement was remelted, the plate turned over, and the grinding completed to 30 to 50 $\mu$m thickness. The thin-ground sections were mounted in Permamount, covered with a coverslip, and examined with polarizing, phase, and ordinary light microscopy.

Scanning electron micrographs were made of plate surfaces cleaned in 3% sodium hypochlorite (commercial bleach diluted 1:1 with water), vacuum-coated with gold, and examined in a JEOL JSM-2 scanning electron microscope operated at 25 kv and equipped with a goniometer.

X-radiographs of whole or thick-ground (about 1000 $\mu$m) plates were taken by placing the plates on Kodak AA or R film and using a Faxitron 805 X-ray unit. Best results were obtained at 25 kv with 2 to 5 min exposure times (see Knutson et al., 1972).

Ossicles were labeled in living sea urchins with tetracycline hydrochloride, following the technique of Kobayashi and Taki (1969). A solution of 1 mg tetracycline per 0.1 ml sea water was injected through the peristome; animals received 1 mg tetracycline per 10 g urchin live weight. Whole and thick- and thin-ground tetracycline-labeled plates were examined under reflected UV light (300 to 350 nm) using a Zeiss Fluorescence Microscope with Exciter Filter II (BG3) and Barrier Filters 44 and 65.

## CHARACTERIZATION OF THE GROWTH ZONES

### *Structural characters*

Under conditions of reflected light, with which most workers have viewed the growth zones in cleared test plates, alternating light and dark zones can be seen. Viewed with transmitted light, passing through a ground and cleared preparation, plates also show a pattern of alternating light and dark zones. The pattern observed with

transmitted light, however, is the "negative" of the pattern seen in reflected light (Fig. 2). This contrast indicates that visualization of the growth zones depends on differences in their qualities of light transmission and reflection. Optically *opaque* areas reflect relatively more light, permitting little transmission; these areas appear white in reflected light and dark (often brownish after charring) in transmitted light. Conversely, optically *translucent* areas reflect less light, permitting more light to pass into and through the plate; these areas appear dark in reflected light and bright in transmitted light. References in the literature to "dark" and "light" or "pigmented" and "unpigmented" zones are thus often ambiguous, and indeed if the light conditions are not specified, these terms have little meaning. We will refer to the growth zones simply as opaque or translucent.

X-radiographs of interambulacral plates of *Strongylocentrotus franciscanus* show striking X-ray opaque and X-ray translucent zones (Fig. 3). X-ray opaque zones (dark lines in Fig. 3*C,D*) appear to correspond to the light opaque zones (white in Fig. 3*B*), while X-ray translucent zones correspond to light translucent zones.

The appearance of the interambulacral plates in reflected and transmitted light, and in X-radiographs, indicates that the alternating visible zones result from structurally different zones within the plate. In the spines, differences in trabecular arrangement clearly account for the visible concentric zones, as has been well described by Heatfield (1971) (Fig. 4*A*). A similar structural difference which could account for the visible zones is observable in the character of the trabecular meshwork within the plates (Fig. 4*C,D*). When the plates are thoroughly cleaned in hypochlorite solution and fine-ground to 30 to 50 $\mu$m thickness, the zonal pattern becomes obscure; possibly, the pattern depends partly on the three-dimensional organization of the meshwork and how different layers of trabecules are oriented with respect to each other. However, as shown in Figure 4, the size of the intertrabecular channels and the degree of orientation of the channels and trabecules vary, with tracts of "isometric" meshwork occurring in patterns parallel with those of the gross opaque and translucent growth zones. Because the grossly visible zonal pattern is indistinct in thin-ground plates, it is difficult to correlate mesh-sized differences and gross growth zones with certainty. How-

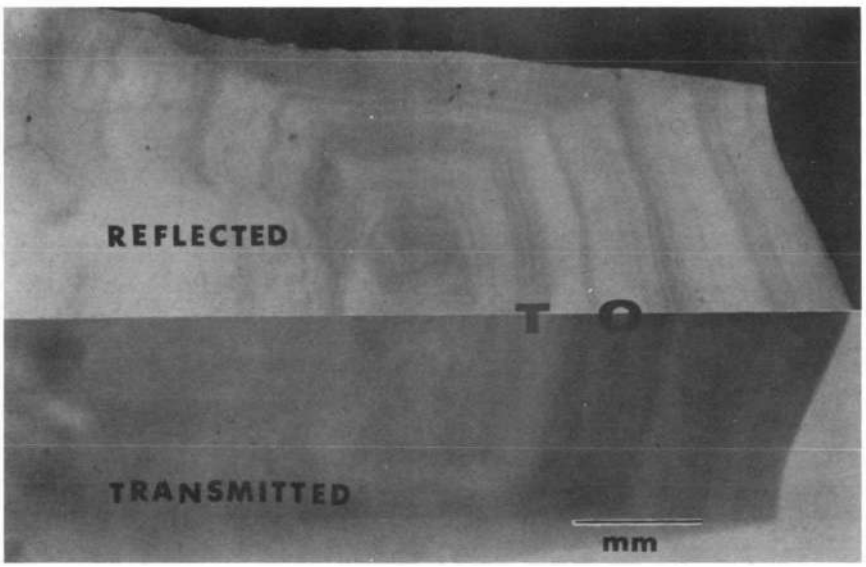


FIG. 2. A photo-collage of an aboral interambulacral test plate of *Strongylocentrotus purpuratus*, ground to 1 mm thickness and cleared to show the growth zones. The translucent (T) and opaque (O) zones are dark and light, respectively, in reflected light and appear reversed in transmitted light.

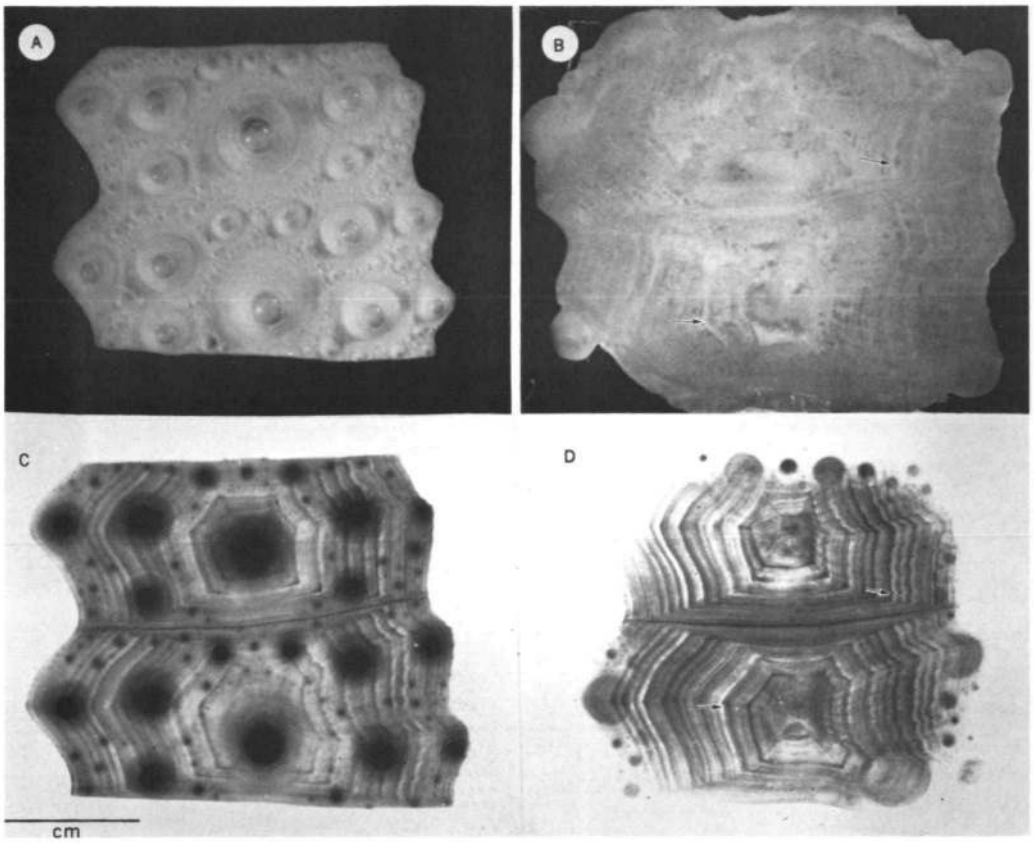


FIG. 3. Aboral coronal plates from a large specimen of *Strongylocentrotus franciscanus*, all from corresponding positions in different interambulacral columns. *A*, Outer surface of two intact cleaned plates with spines removed (reflected light). *B*, Plates thin-ground to ~ 50 $\mu$m thickness (reflected light). *C*, X-radiograph positive print of plates shown in *A*, with spine bosses obscuring portions of the growth zones. *D*, X-radiograph positive print of plates ground to ~ 1000 $\mu$m thickness. ARROWS in *B* and *D* indicate corresponding growth zones. (X-radiographs by R. W. Buddemeier.)

ever, it appears that opaque growth zones have relatively smaller channels (approximately 15 $\mu$m diameter), larger trabecules, and perhaps a greater degree of orientation, while translucent growth zones have relatively larger channels (approximately 20 $\mu$m diameter), smaller trabecules, and a lesser degree of orientation. These differences seem consistent with what we would expect from the transmission properties of the zones, that is, the more dense trabecular meshwork would absorb or reflect relatively more light and X-radiation and thus would appear opaque, while the more open meshwork would transmit more visible- or X-radiation and thus would appear translucent. (Further correlates from growth experiments are presented below; see Figs. 8, 9.)

Several other kinds of observations underline the structural character of the zonal patterns in the test plates. Durham (1955) used weak acid to etch and differentiate the growth zones. Decalcification with disodium ethylenediamine tetracetate (Moss and Meehan, 1967) or with Bouin's solution (unpublished observations) often follows the pattern of the growth zones. It appears that the translucent (more open mesh) areas decalcify more rapidly than the opaque areas. Grinding of plates, especially on coarser grits, often produces low relief along zonal lines, the dense mesh left as ridges with the open mesh hollowed out between. When a dry test plate is placed in xylene for clearing, the liquid often is observed to clear the plate by zones, penetrating rapidly to a certain point all around the

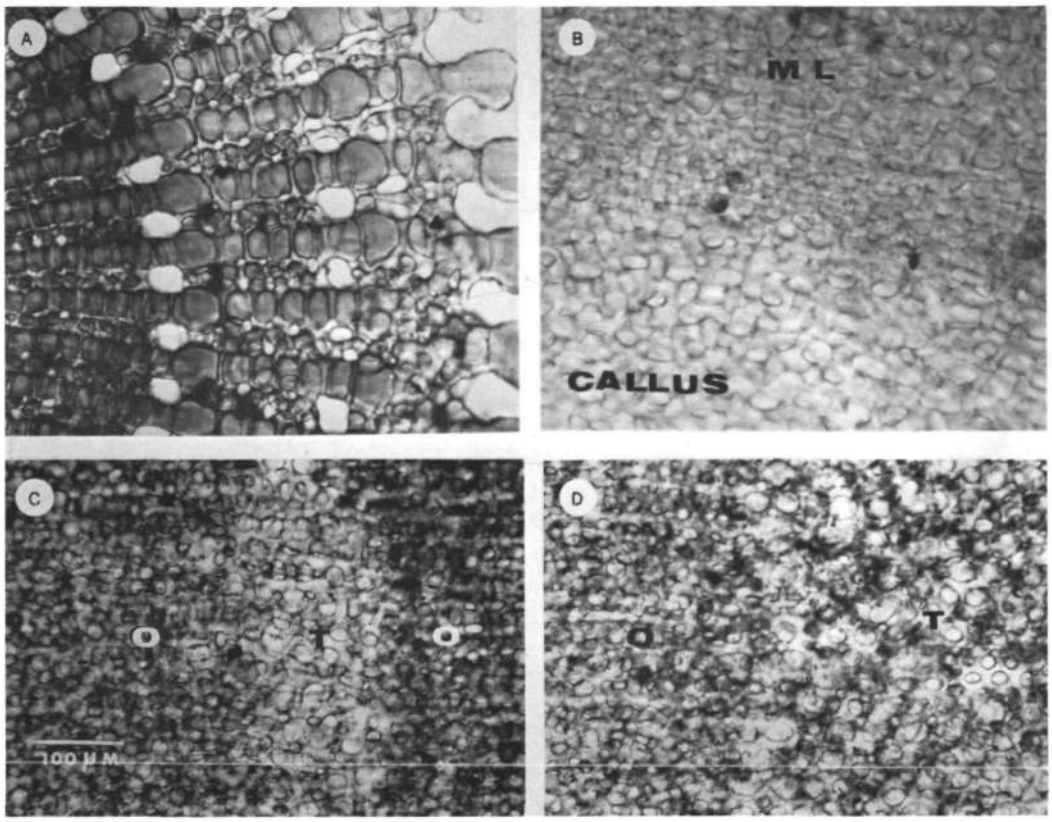


FIG. 4. Skeletal sections thin-ground to ~ 50 μm thickness; transmitted light. *A*, Cross-section of a spine. *B*, Cross-section of a plate showing junction of middle layer (ML) above and larger-mesh callus layer below. *C*, *D*, Tangential sections of a plate showing smaller channels in trabecular meshwork of opaque (O) and larger channels in translucent (T) growth zones. *A*, *Strongylocentrotus purpuratus; B-D*, *S. franciscanus*.

plate, then proceeding towards the interior only after a short time lapse; this could result from differences in capillary forces in the different zones of trabecular mesh. Finally, what appear to be growth zones are sometimes evident in the coronal plates of fossil echinoids (Zoeke, 1952; Durham, 1955; and unpublished observations). Fossils of other echinoderms also sometimes show distinct growth zones (see Macurda, 1967, for particularly beautiful figures of growth zones in blastoids). Such fossils suggest differential mineral replacement or erosion which could result from variations in the plates' mesh structure. In addition to simple mesh size differences, differences in the physical or chemical properties of the surfaces or internal structure of the trabecules may also be reflected in the phenomena described above.

### *Chemical characters*

Various workers have attributed the growth zones to patterns of deposition of organic material, especially pigments. In many species, the spines and test plates contain pigments which at least enhance the appearance of the zones (Fig. 5). The pigment in the test plates is usually confined to the outer layer of the plate, or occurs particularly along the boundary of the outer and middle layers of the plate (Merker, 1916) (see Fig. 1). Deutler (1926) observed that a deep violet coloring made the growth zones especially visible in some species. Moore (1935, 1937) believed that the growth lines which he saw in apical plates were periodic extensions of the outer pigment layer deeper into the plate, and that the pigment was a napthoquinone

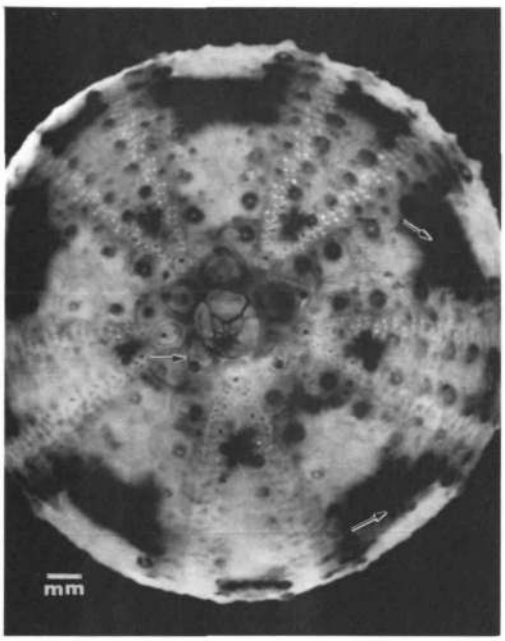


FIG. 5. Sea urchin test cleared in xylene (clean of spines but not bleached; reflected light). Arrows indicate growth zones in apical and interambulacral plates accentuated by the presence of pigment. *Lytechinus pictus*, 16 mm diameter, 18 months old, laboratory-reared in recirculated seawater aquaria by R. T. Hinegardner.

("echinorubin") which came from the animals' food. Because the apical plate zones seemed to be enhanced by ferrous ions, Dix (1972) suggested that they contain melanin. Ebert (1966) extracted pigments from coronal plates, after decalcification, which he tentatively identified as carotenoids and thought might be responsible for the zonal patterns. It is unlikely, however, that alternating deposits of pigment or other organic materials are ever the only basis for zonal patterns. Birkeland and Chia (1971), for example, demonstrated the striking banding patterns in the interambulacral plates of the sand dollar *Dendraster excentricus*. These are shown in sanded, oven-dried specimens, cleared in xylene (Fig. 6*A*). The dried specimens appear greyish purple and the banding pattern is indistinct before clearing in xylene. After being thoroughly cleaned in hypochlorite solution (commercial bleach), the dried plates are uniformly snow-white with no trace of any pigmentation; the bands are distinct, however, when such cleaned specimens are cleared in xylene (Fig. 6*B*). Other workers have also shown that hypochlorite cleaning does little to the banding patterns in cleared plates (e.g., Deutler, 1926; Durham, 1955; Sumich and McCauley, 1973), indicating that at least intertrabecular pigments or organic materials are not involved. Moreover, growth zones are observed in colorless ossicles, such as those of the oral half of the corona, unpigmented in some species, and of the lantern (Merker, 1916; Deutler, 1926; unpublished observations). (The "pigmented" zones of Taki [1973*a*] are opaque zones viewed in transmitted light.) Merker (1916) and Deutler (1926) believed that the opaque white zones contained organic material incorporated within the trabecules which resulted in optical heterogeneity. By this reasoning, the translucent zones, containing relatively less organic material, would reflect less light and would appear dark in reflected light.

Jensen (1969*a*) showed that charring enhances the banding pattern in coronal plates (we found this to be true also for the lantern ossicles). Cleaning the plates with hypochlorite solution to remove intertrabecular material has little or no effect on the enhancement of the pattern by charring. Charring appears to increase the contrast between translucent and opaque zones in both reflected and transmitted light. The appearance of the plates after cleaning and charring suggests the presence of organic material deposited within the crystalline trabecules. Although Klein and Currey (1970) showed that echinoid ossicles are remarkably free of intracrystalline organic material compared with vertebrate bone, some organic material is present and incorporated within the calcite crystals. The appearance of broken trabecular surfaces suggests that trabecules grow by laminar accretion of calcite, and organic material present may also occur as thin concentric layers (Fig. 7; see also Nissen, 1969). The hypochlorite-resistant purple color in the spines and plates of many echinoids is due to echinochrome deposits within the calcite (Fox and Hopkins, 1965).

Whether the intracrystalline organic material is an essential characteristic of the

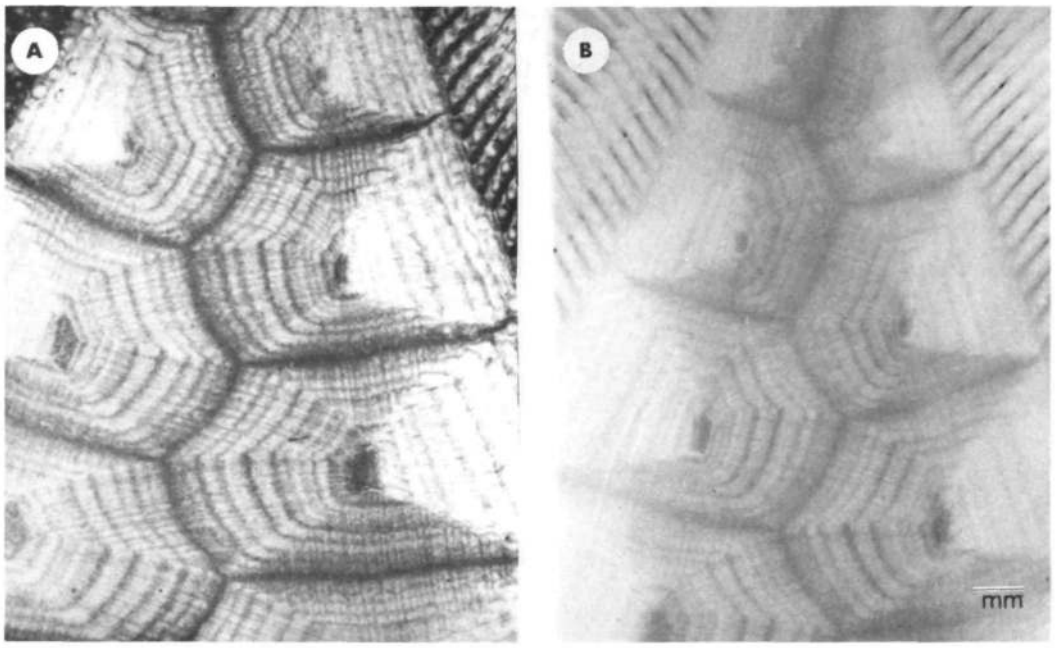


FIG. 6. Aboral plates of a sand dollar; outer surface, reflected light. *A*, Test dried, sanded, cleared in xylene. *B*, Test bleached in 3% sodium hypochlorite, then prepared as in *A*. *Dendraster excentricus*, 8.5 cm max. diameter; Sand Hill Bluff, Santa Cruz, California, 10-m depth.

growth zones, or whether it is incidentally incorporated under particular conditions, is unresolved. The material (pigmented or colorless) could be evenly distributed but differentially visible according to the transmission properties of the growth zones. Alternatively, the material could vary quantitatively in the zones and perhaps even be directly involved in regulation of calcification and mesh size. Growth rings in fish otoliths, which appear grossly similar to the growth zones in echinoid plates, seem to result from alternating amounts of organic material (Degans et al., 1969; Pannella, 1971).

The more open mesh translucent zones may serve as "tissue tracts" in the plates to provide more effective communication between the outside and the inside of the animal. Tritium-labeled material, for example, moves rapidly through the body wall (Pearse and Pearse, 1973). However, there is little evidence for any organization of the cellular tissue in the intertrabecular channels of the plate meshwork which would correspond to the skeletal growth zones; cells appear scattered without pattern in histological preparations of decalcified plates (e.g., Moss and Meehan, 1967; Pearse, 1970, and unpublished). Nevertheless, decalcification is an extremely disruptive process and may well obliterate loose or subtle patterns of cellular organization.

In addition to possible variation in the organic content of the trabecules in different growth zones, it is not unlikely that some inorganic constituents may vary. The proportions of Mg and Sr present appear to be related to ossicle growth rate and can be correlated with various environmental factors (Weber, 1969*b*; Davies et al., 1972). Calcium concentrations seem to vary in relation to growth rings in clam shells (Rosenberg, 1973). It would therefore be interesting to investigate whether the alternating growth zones in echinoid ossicles vary in their Ca:Mg:Sr ratios.

### *Growth characters*

Moore (1935) and Dix (1972) both correlated the "pigmented" (translucent?) zones in apical plates with periods of slower growth in the summer in *Echinus esculentus*

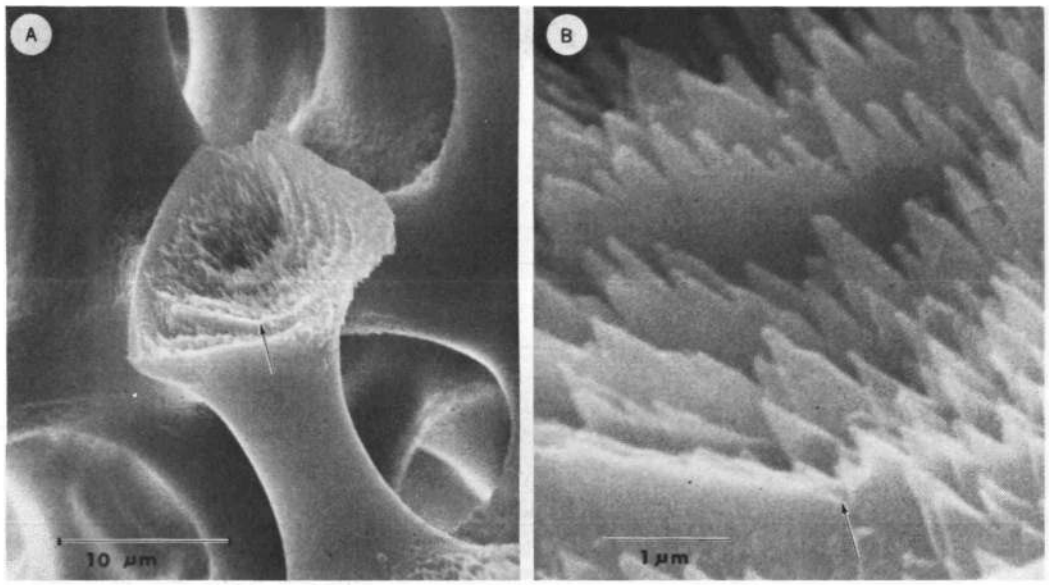


FIG. 7. Scanning electron micrographs of a broken trabecule in a longitudinal suture of a young plate of a rapidly growing sea urchin (*Strongylocentrotus purpuratus*), showing concentric laminated structure. Arrows indicate same feature for comparison.

and *Evechinus chloroticus,* respectively. Taki (1972*a,b*) correlated the "pigmented" (opaque) zones in the coronal plates and lantern ossicles of *Strongylocentrotus intermedius* with periods of faster growth in the winter. To examine the relationship of growth rate to the mesh structure of test plates in *Strongylocentrotus purpuratus,* we maintained two groups of eight specimens each in running sea water aquaria from 27 July to 27 October, 1973. One group was given a constant supply of the brown alga *Macrocystis* for food, while the other group was given no food. The mean wet weight of the fed animals changed from 16.3 g to 21.9 g (35%) while that of the unfed urchins changed from 19.2 g to 20.3 g (6%); the latter group probably derived some nourishment by ingesting wood from the aquarium floor and walls, pieces of which were found in their guts.

Two weeks after the maintenance period began (13 August 1973), all the animals in both groups were injected with tetracycline. Using the tetracycline label as a reference point, we found that plate growth over the 3-month period was much greater in the animals given food than in those without food. In the fed animals, the longitudinal margins of aboral interambulacral plates grew outward about 200 μm over the 3 months, while comparable growth in the unfed animals was only about 80 μm (Fig. 8).

The trabecular meshwork in the plates of fed animals (Fig. 8*C*) is regular and well oriented perpendicular to the edge of the plate and has relatively small channels, as is characteristic of opaque growth zones. In contrast, the meshwork near the edge of the plates of unfed animals shows an abrupt discontinuity (Fig. 8*D*). The location of the tetracycline label indicates that this discontinuity corresponds to the beginning of the maintenance period without food and, presumably, to a slowing in the growth rate of the plate. This edge area shows the more open meshwork with larger channels and less regularly oriented trabecules characteristic of translucent growth zones. From this experiment we conclude tentatively that opaque growth zones in the coronal plates correlate with periods of fast plate growth while translucent zones correlate with periods of slow plate growth (see also further experiments described below).

Scanning electron micrographs of the longitudinal suture edge of comparable

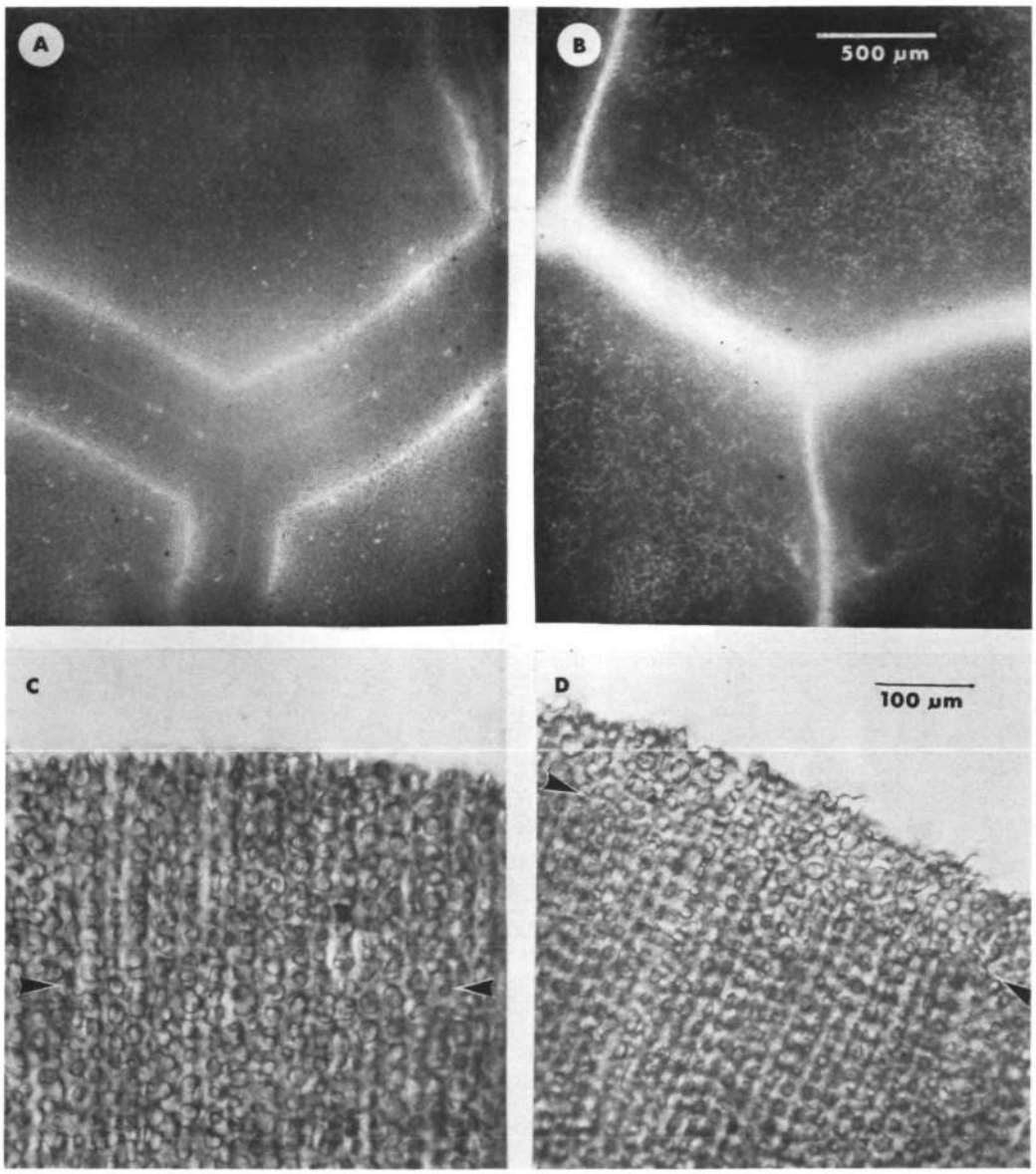


FIG. 8. Interambulacral plates of two sea urchins (*S. purpuratus*) injected with tetracycline and kept for 3 months with or without food. Top, fluorescence photos of inside surface of young plates (4th-6th aborally) of (*A*) fast-growing fed and (*B*) slow-growing unfed sea urchins. Bottom, thin-ground sections (50 μm) of longitudinal edges of the same plates (5th aborally), transmitted light; *C*, fed sea urchin, showing no visible change in trabecular mesh; *D*, unfed sea urchin, showing abrupt transition to mesh with larger channels. Arrows mark inner limit of tetracycline label.

aboral interambulacral plates (same as Fig. 8) also indicate differences between faster-growing opaque zones and slower-growing translucent zones. The edge of a faster-growing plate shows a very regular organization and has prominent, well-spaced, growing trabecules (Fig. 9*A,B*). In contrast, comparable regions of slower-growing plates show a less regular spatial organization, and the ends of the growing trabecules are low and uneven (Fig. 9*C,D*).

Some of the characteristics of opaque and translucent growth zones which we have assembled, and others gathered from

the literature, are summarized in Table 1.

## GROWTH ZONES IN LABORATORY-REARED SEA URCHINS

### *Experimentally induced growth zones*

Juvenile individuals of *Strongylocentrotus purpuratus,* all 4 to 5 mm diameter and 35 to 90 mg wet weight were collected from sand and shell at 3 to 5 m depth in Papalote Bay, Baja California, Mexico, on 11 October 1970. Spawning occurs in *S. purpuratus* at Papalote Bay in the spring (Pearse et al., 1970), and these juveniles, about 3 months post-metamorphosis, were the result of the 1970 spring spawning. The animals were reared in plastic containers in running seawater aquaria at the Kerckhoff Marine Laboratory (Corona del Mar, California) with a continuous supply of the brown alga *Macrocystis* for food. Some were sacrificed on 23 December 1970; they were 6 to 13 mm diameter, 130 to 930 mg wet weight, and about 6 months old.

Seven of the remaining animals were transferred to the Hopkins Marine Station (Pacific Grove, California) on 15 January 1971 and maintained as before in running seawater aquaria with a continuous supply of *Macrocystis.* On 23 August 1971, when they were about 1 year old, the largest (13.6 g), smallest (5.6 g), and one medium-sized individual (8.3 g) were killed. The four remaining animals were divided into two lots of two animals each and maintained in one aquarium divided by a plastic screen until 1 July 1972, when they had reached about 2 years of age and were all killed. Two of the four animals were fed continuously while no food was given to the other two during four 3- to 4-week periods of the second year.

The calculated daily growth rates of the seven animals maintained for 1 to 2 years are shown in Figure 10. All seven animals grew rapidly, and at increasing rates (mg/day), during their first winter and spring, in 1971. The growth rate decreased abruptly in each animal in May, June, or July. The cause of this abrupt change in growth rate is unclear. Since the three animals killed in August, 1971 contained large gonads full of gametes, the abrupt change in growth rate may correspond to attainment of sexual maturity and a reallocation of materials from general body growth to gonad growth. It is important to note that the growth rate slowed at different times and to different degrees in different animals, even though all were held together in one aquarium. The change in growth rate therefore cannot be related with confidence to any specific environmental change or event.

During the second year, the growth rates in the two animals continuously provided with food varied considerably. Both showed a general increase in growth rate in the fall and a general decrease in the spring, but, again, these changes were not synchronous.

The pattern of changing growth rates was strikingly modified by discontinuous feeding. When no food was provided, growth decreased promptly, and in the last

TABLE 1. *Characterization of echinoid test growth zones.*

| | Opaque | Translucent |
|---|---|---|
| Reflected light | Light | Dark |
| Transmitted light | Dark | Light |
| X-ray | More dense | Less dense |
| Intertrabecular channels | Smaller | Larger |
| Trabecular diameter | Larger | Smaller |
| Trabecular orientation | Greater | Lesser |
| Acid etching | Slower | Faster |
| Coarse grinding or weathering | Positive relief | Negative relief |
| Organic material | Present; Responsible for opacity (Merker, 1916; Deutler, 1926) | Present; Responsible for charred dark appearance (Jensen, 1969*a*) |
| Pigment (Moore, 1935; Dix, 1972) | Absent? | Present? |
| Growth rate | Faster | Slower |

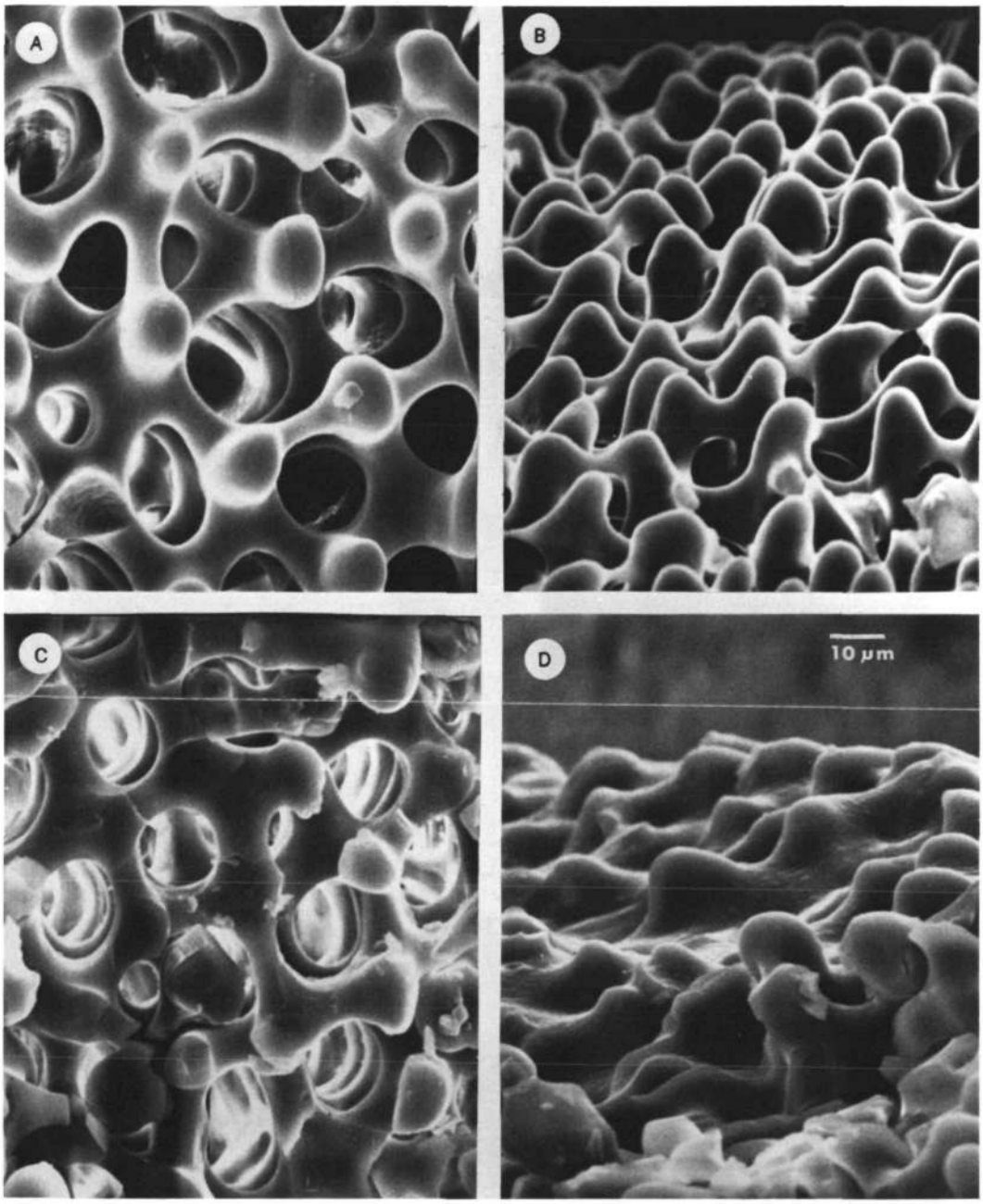


FIG. 9. Scanning electron micrograph of longitudinal sutures of plates corresponding to those shown in Figure 8. *A, B,* Fast-growing fed sea urchin; *C, D,* slow-growing unfed sea urchin. *A, C,* Suture viewed vertically; *B, D,* suture viewed obliquely. All at same magnification.

two deprivation periods, weight decreased. When food was provided after deprivation, growth rates usually increased sharply, but, conveniently, not always to the same extent (see below).

Comparable interambulacral plates from the discontinuously fed animals clearly show four narrow translucent zones which correspond to the four periods of food deprivation (Fig. 11, arrows). The interven-

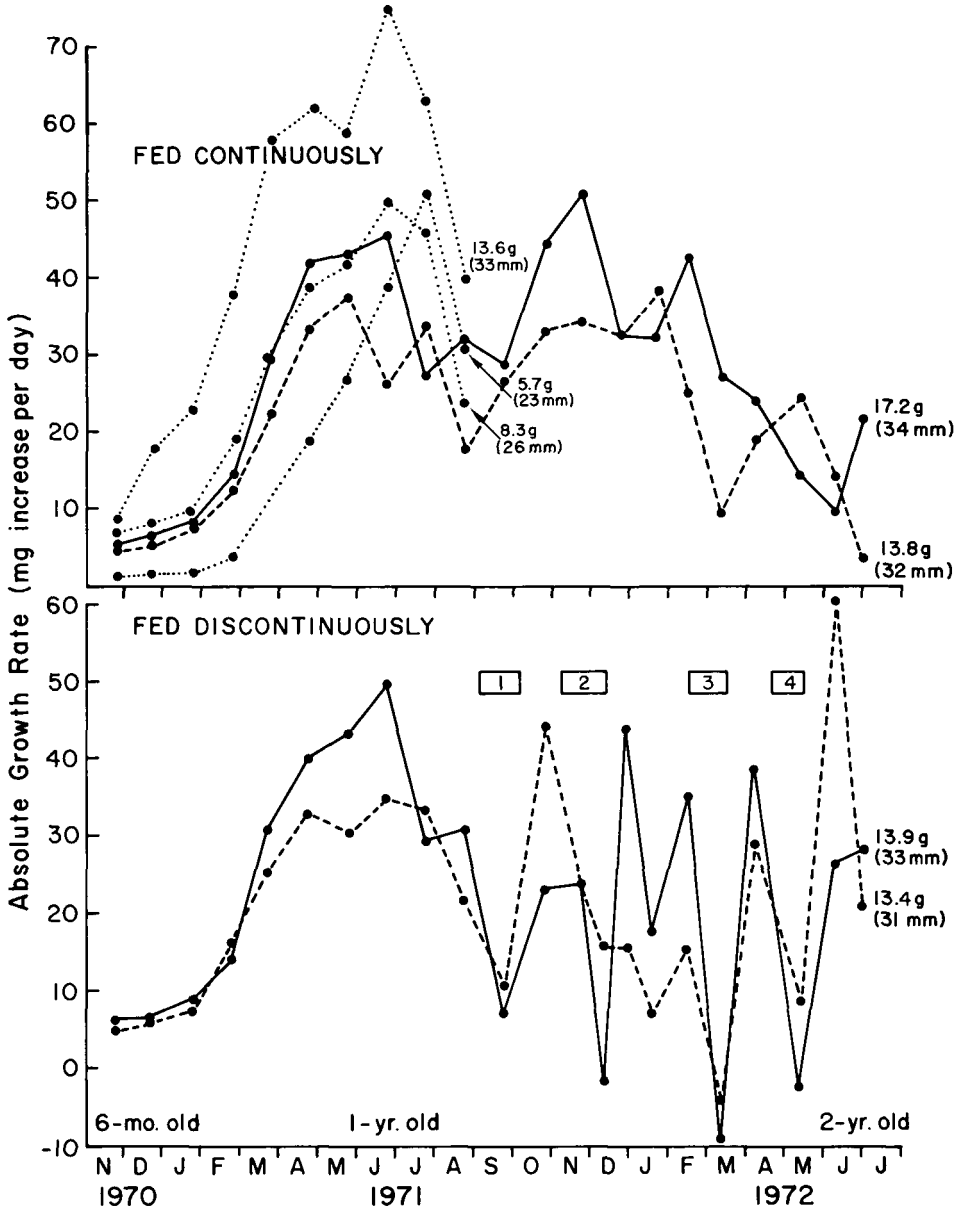


FIG. 10. Growth rates of sea urchins (*S. purpuratus*) collected when 4 to 5 mm diameter and laboratory-reared for nearly 1 and 2 years. The numbered bars (1-4) in the lower graph indicate the periods during which the discontinuously fed sea urchins were given no food.

ing opaque zones correspond to the periods of increased growth rates when food was again provided and reflect the growth curves (Fig. 10) in remarkably faithful detail. The marked growth rate increases of the smaller animal (31 mm diameter) after the first and fourth deprivation periods are represented by relatively wider opaque zones following the first and fourth translucent zones; while the most pronounced growth rate increase of the larger animal (33 mm diameter) occurred after the second deprivation period and is represented by its widest opaque zone, following the second translucent zone.

Patterns of opaque and translucent

growth zones also occur in the plates of the continuously fed animals, and although individual zones were altered by the periods of food deprivation in the second year, all four animals have similar clusters of growth zones (Fig. 11). In all four, a large central opaque area is surrounded by a prominent translucent zone, which probably corresponds to the sharply decreased growth rates shown by all of the animals in the spring and early summer, 1971 (Fig. 10). The following patterns of thin alternating translucent and opaque zones in the continuously fed animals are difficult to relate to the growth curves; perhaps the distinct wide opaque zone corresponds to the higher growth rates in fall and early winter, 1971-72 (Fig. 10). Nevertheless, the growth zone clusters seen in all four plates in Figure 11 suggest fairly regular changes in growth rate with time. The two continuously fed animals, at least, have two broad clusters of translucent zones which may correspond to two spring-summer periods.

*Test plate addition, position, and growth zones*

Both plate addition and plate growth contribute to the growth of echinoids (Deutler, 1926; Moss and Meehan, 1968; Raup, 1968; Kobayashi and Taki, 1969). At metamorphosis, the newly formed echinoid has 10 apical plates and, depending on the species, between about 3 and 10 plates in each ambulacral and interambulacral area (Gordon, 1926, 1929). New

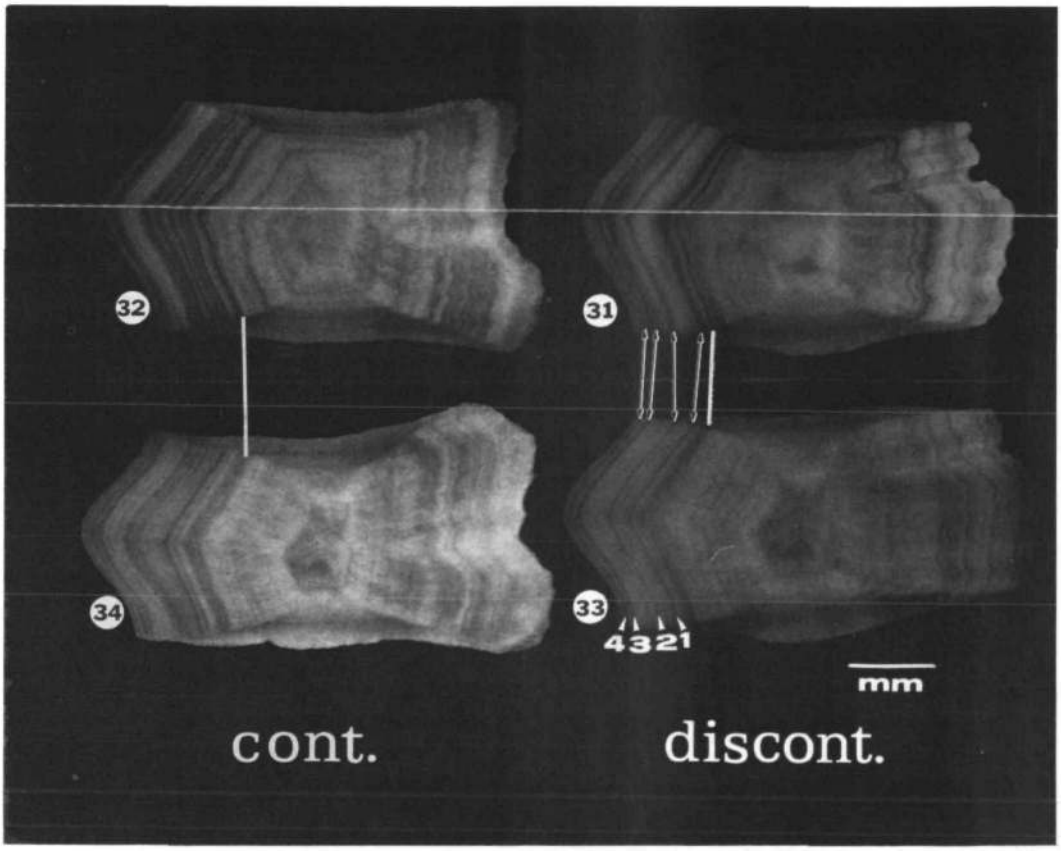


FIG. 11. No. 20 plates (see Fig. 13) of four laboratory-reared 2-year-old *S. purpuratus*, whose growth rates are recorded in Figure 10. Left, continuously fed sea urchins; right, discontinuously fed sea urchins. Circled numbers give diameter of test in millimeters when killed at 2 years. White bars mark edge of plate at the end of the first year, and the beginning of the feeding experiment (Sept. 1971). Arrows indicate four narrow translucent growth zones which correspond to the four periods when these sea urchins were without food (see Fig. 10). Reflected light.

plates are added at the apical (aboral) end of each coronal column. The number of coronal plates added varies among different species; sand dollars add only about 4 to 6 per interambulacrum while some sea urchins add well over 40 (Durham, 1955; Swan, 1965; Moss and Meehan, 1968). The number of plates generally increases nearly linearly with size. In *S. purpuratus* the number of plates per interambulacrum seems more closely related to size than age; our largest laboratory-maintained 1-year-old animal had more test plates than the smallest of the 2-year-old animals (Fig. 12).

Because coronal plates are added throughout the life of a sea urchin, each plate is of a different age, and only the original plates on the oral side of the test are as old as the animal. Consequently, different coronal plates have different patterns of growth zones, depending on their position in the test. The situation is complicated further because the plates have different growth rates depending on their position in

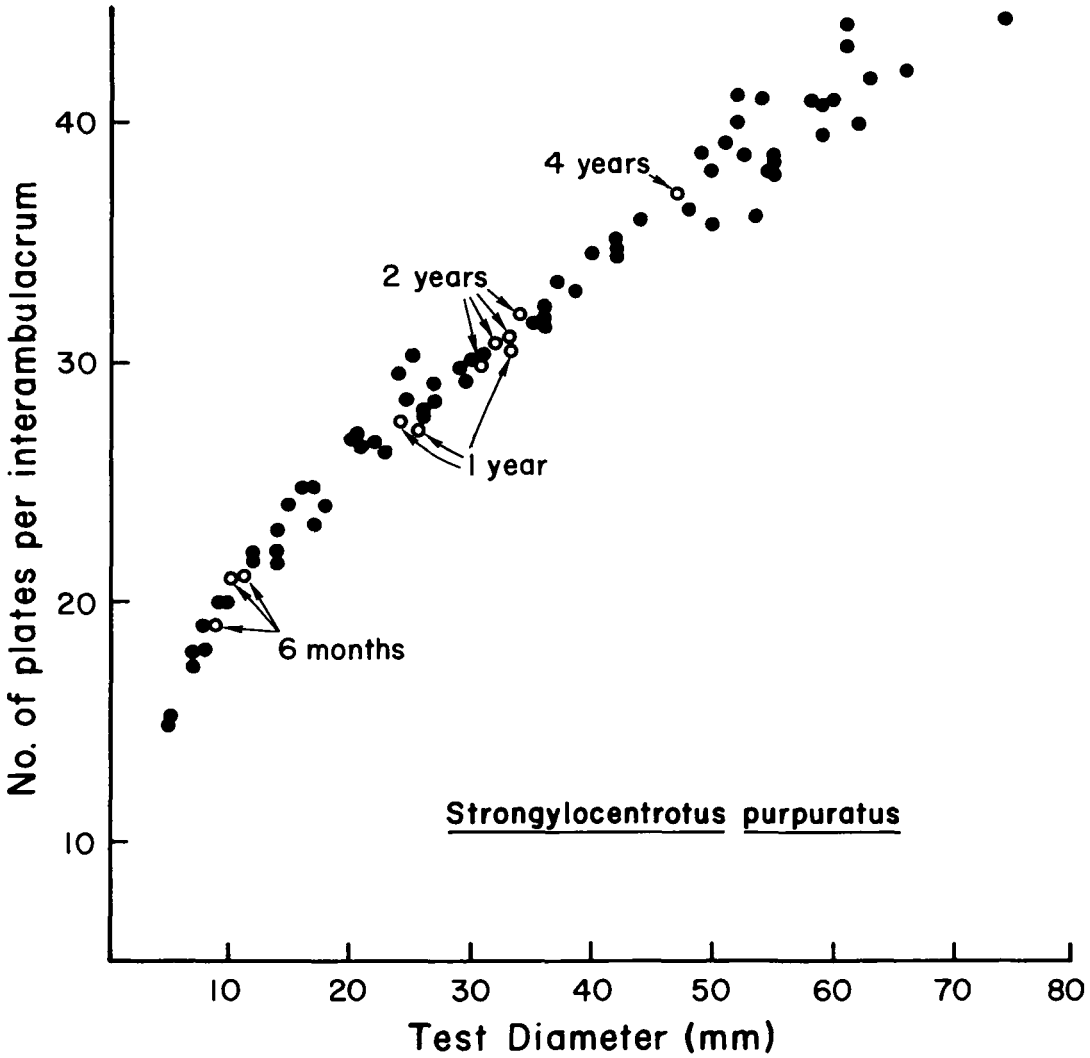


FIG. 12. Number of plates per interambulacrum vs. test diameter. Open circles, laboratory-reared sea urchins of known age. Six-month, 1- and 2-year-old animals from experiments described in this paper (see Fig. 10 for growth record of 1- and 2-year-olds). Four-year-old animal collected from Half Moon Bay, California, when about 3 mm diameter and laboratory-reared in recirculated seawater aquaria by R. T. Hinegardner. Solid circles, field specimens from several sites in California and Baja California.

the test (Deutler, 1926; Raup, 1968; Kobayashi and Taki, 1969). Young coronal plates on the aboral surface increase rapidly both in length and width as animal growth proceeds; while the older oral plates grow very slowly (Deutler, 1926; Raup, 1968; Kobayashi and Taki, 1969; and our unpublished observations from plate measurements and tetracycline labeling), and a few plates may be resorbed at the peristome. Moreover, as elegantly shown by Deutler (1926), the number and outline shapes of the growth zones within each plate tend to correspond to the number and outlines of the plates above (aborally). Such correspondence suggests that growth zones may be added with each plate addition. This situation could result coincidentally if plate addition and growth zone addition were both seasonal events. Or, each plate addition and attendant stresses could lead to relative re-alignment and re-positioning through growth of already formed plates, resulting in a new growth zone (Raup, 1968).

At metamorphosis, individuals of *S. purpuratus* have approximately 10 plates per interambulacrum (5 in each column), while at 6 months the number has increased to about 20 plates (Fig. 12). This difference of 6 months appears to result in considerable difference in the growth zone patterns in the center of each plate (Fig. 13, 14). The No. 10 plates have a translucent center which corresponds to the summer growth period. The No. 20 plates do not have the large translucent center; rather they show a large opaque central zone which corresponds to growth in the winter and spring after the plate was formed. When the animals reach 2 years of age and have about 30 plates per interambulacral area (Fig. 12), the original No. 10 plate is well below the ambitus (Fig. 13) and further plate growth is very slow. This slow growth produces a broad translucent area which borders older plates and in which well-defined growth zones are no longer discernible. Similar observations led some workers (e.g., Deutler, 1926; Sumich and McCauley, 1973) to conclude that growth ceases in these older plates. However, tetracycline labeling and measurements of corresponding plates in tests of different sizes indicate that slow growth, at least in plate width if not height, continues indefinitely (Kobayashi and Taki, 1969; and unpublished observations). Growth in plate height slows very early even in plates which are still rapidly growing in width, and this is reflected in the narrow indistinct growth zones and greater overall translucence of the oral and aboral sides of each plate (see Figs. 2, 11, 14). By 4 years of age, the No. 20 plate is also below the ambitus, its growth is slow, and new growth zones are obscure on all sides (Fig. 14). However, the indistinct zones in these slow-growing translucent areas show up much more clearly in X-radiographs (see Fig. 3), which may provide better resolution to approach this problem.

Figure 15 diagrammatically summarizes changes in plate number, position, and growth zones in an interambulacral area of *S. purpuratus* for a period of 3 years. Within 1 to 2 years, all the original plates are well below the ambitus, and plate growth is too slow for well-defined growth zones to form. In the 3-year-old urchin, a number (about seven) of the most aboral plates are less

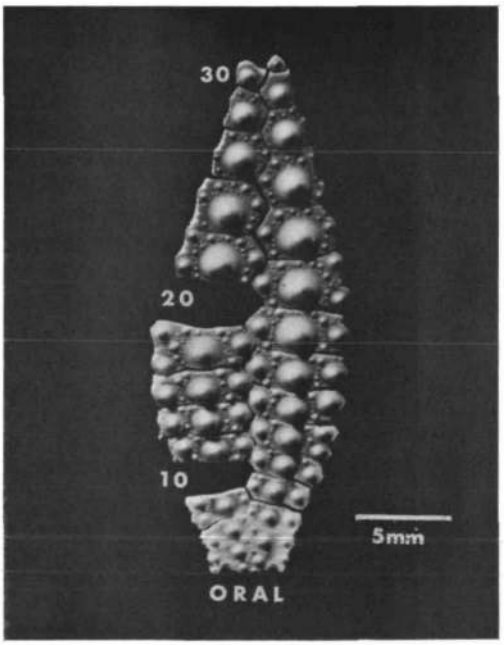


FIG. 13. Interambulacrum of a laboratory-reared 2-year-old sea urchin (*S. purpuratus*) containing 31 plates. The no. 10 and no. 20 plates have been removed for examination of growth zones (see Fig. 14).

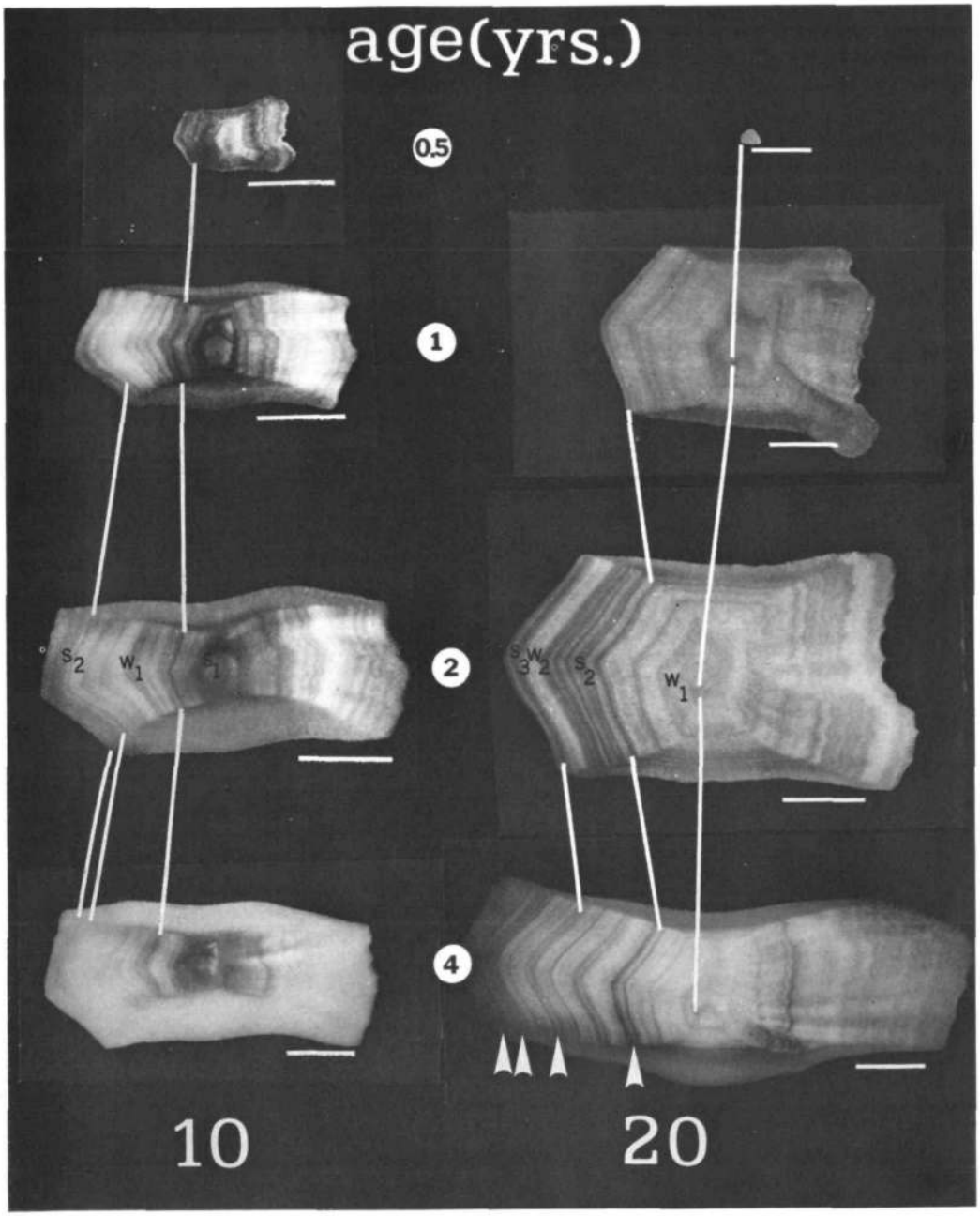


FIG. 14. No. 10 (left) and no. 20 (right) interambulacral plates of laboratory-reared *S. purpuratus* of known ages: 0.5, 1, 2, and 4 years (top to bottom) (see Figs. 12, 13). White connecting lines suggest growth zones of corresponding age. On the 2-year-old plates are tentative identifications of zones which may represent the first summer ($S_1$), first winter ($W_1$), etc. The second winter ($W_2$) and third summer ($S_3$) are not represented by distinct growth zones in the no. 10 plate, which instead shows the peripheral translucent area characteristic of older plates. The arrows on the no. 20 plate of the 4-year-old suggest limits of yearly growth, based both on appearance of zones and on measurements of plates of laboratory-reared urchins. Size scales = 1 mm. Reflected light.

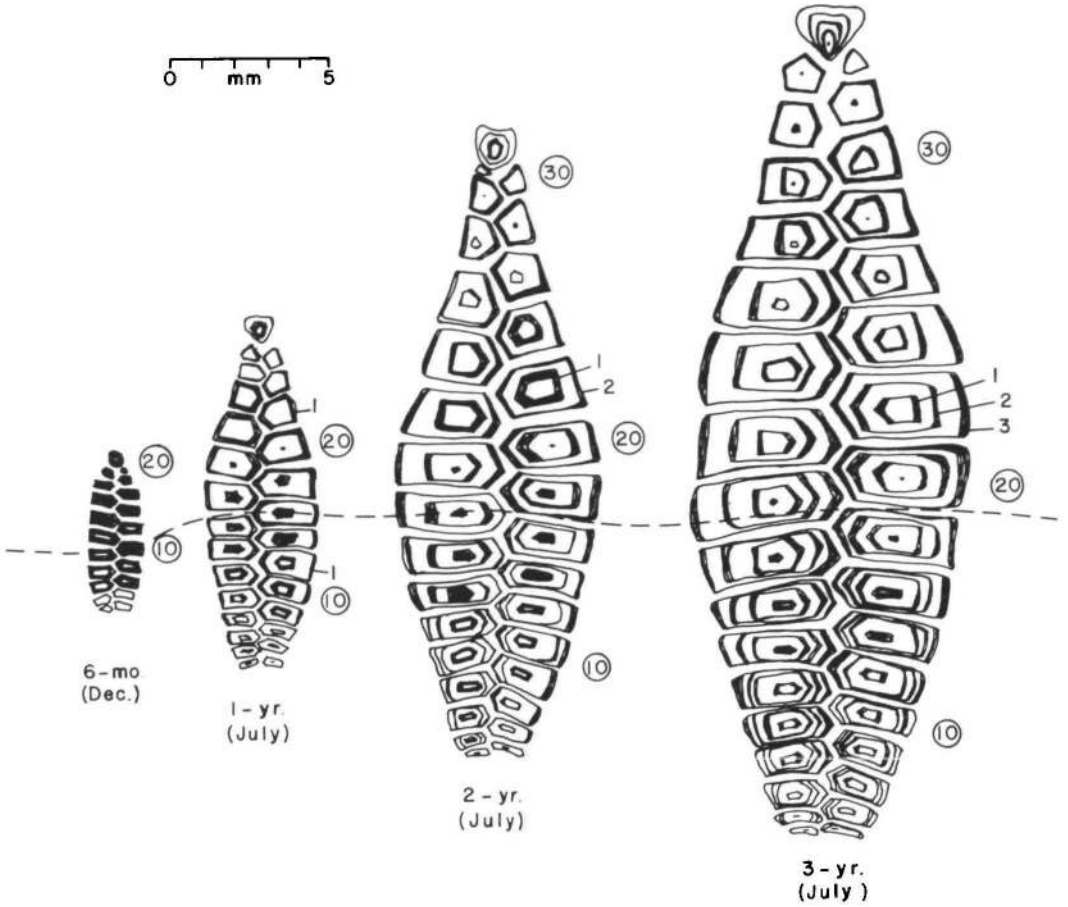


FIG. 15. Diagrammatic representation of interambulacral growth zones of sea urchins (*S. purpuratus*) at 6 months, 1 year, 2 years, and 3 years of age. Dark areas represent translucent zones, and the end of the 1st, 2nd, and 3rd years are marked. The plate position numbers are indicated in circles. The dotted line shows the position of the ambitus (greatest test diameter).

than 2 years old and lack the first year's growth zones. Thus, because of the regular sequence of changes in plate growth as an animal grows larger and because the relative positions of plates are shifted orally (the plates do not "migrate"), a record of all the growth zones could be difficult or impossible to obtain from any single plate, especially in older animals. Suitable and comparable plates must be selected in comparing growth zones of different individuals. Sumich and McCauley (1973) proposed that all the growth zones in an entire interambulacrum be counted, growth zones in the older oral plates being added to counts from the younger aboral plates to obtain an accurate total.

It should be emphasized that the growth zones shown in Figure 15 are diagrammatic; actually, the growth zones are often (if not usually) indistinct and ambiguous, and generally cannot be counted with much confidence (see Figs. 2, 3, 11, 14, 16 for examples). The same difficulty is seen in actually counting the growth zones in coronal plates of other sea urchins (e.g., see photographs of Deutler, 1926; Ebert, 1966; Raup, 1968; Jensen, 1969*a*; Sumich and McCauley, 1973). The corresponding positions of growth zones in the plates shown in Figure 14 could not have been determined if the animals had not been laboratory-reared, regularly measured, and of known age. We therefore suggest that caution and skepticism be applied to counting such zones and assigning them ages without

good reference data based on (i) animals of known ages; (ii) extensive size frequency data showing clearly separable year classes; and/or (iii) established seasonality of growth zones. Unfortunately, data are not yet available for the coronal plates of any echinoid which would permit us to determine the age of field animals with confidence.

*Growth zones as chronometers*

If the growth zones can be counted, they at least need to be shown to correlate with seasons before they can be used with any confidence as chronometers. Such correlations are more convincing if the change in growth zones can be related to specific environmental and/or physiological changes.

Growth zones in the apical plates, which have the advantage of being original plates in all species, have been convincingly correlated with seasons by Moore (1935, 1937) and Dix (1972) for *Echinus esculentus* and *Evechinus chloroticus,* respectively. In both cases "pigmented" zones (probably translucent zones) occurred on the outer edges of the plates in most animals during the summer, when growth was slowest. Ebert (1972) found a close agreement between Moore's (1935) age estimates based on growth zone counts and computer-programmed age estimates based on changes in animal size distribution. Using tetracycline label, Taki (1972*b*) found the opaque zone ("pigmented" under his transmitted light observations) "to be the winter ring in each test plate during the active growth period in the winter." Jensen (1969*b*) also reported seasonal deposition of "pigmented" zones in coronal plates apparently related to seasonally differing growth rates. Formation of translucent zones in the interambulacral plates of our laboratory-maintained specimens of *S. purpuratus* appeared to be mainly in the spring and summer (Figs. 11, 14, 16), which correlates with changes in our growth data (Fig. 10). All these observations suggest that seasonal changes in growth rates may result in seasonal patterns of growth zones in the plates. This gives hope that chronometry may indeed be possible, but more convincing evidence for more species is much needed.

Another requirement of a chronometer consisting of a series of lines or bands is that it must be size independent. It is well known that different specimens of echinoids grow at different rates, depending partly on food quality and supply, and individuals of identical age can be of quite different sizes (e.g., Swan, 1961, 1965). Moreover, the mean size of individuals in different populations is often very different (e.g., Fuji, 1967; Ebert, 1968; Pearse et al., 1970). In such contrasting populations, the smaller mean size may be the result of slow-growing "stunted" animals which are quite old, or fast-growing animals which experience high mortality and do not live long enough to reach large sizes. If the growth zones are size dependent, so that larger test plates in larger animals simply have more lines, then they cannot be any more useful as chronometers than animal size or plate number. Jensen (1969*b*) presents a diagram of the coronal plates of an individual of *Psammechinus miliaris* with many more growth zones than shown in a larger individual from another locality. This comparison shows that the number of growth zones can be indeed size independent, although her conclusion that each zone represents a year is not convincing; she found, as did we (see Fig. 11), that growth zones can be altered by altering food conditions.

The pattern of clusters of growth zones also appeared to be size independent in our laboratory-maintained animals. The largest 1-year-old animal was larger than the smaller continuously fed 2-year-old (see Fig. 12). However, among comparable plates, the pattern of growth zones in the largest 1-year-old was nearly identical to the pattern in a smaller 1-year-old, but identifiable only in the central (1-year-old) portion of the plate of a similar-sized 2-year-old urchin (Fig. 16). These observations strongly suggest that when animals are kept under similar conditions, the formation of growth zones in their plates is size independent and that differences in growth zone patterns in different animals may be at least partly referable to differences in animal age.

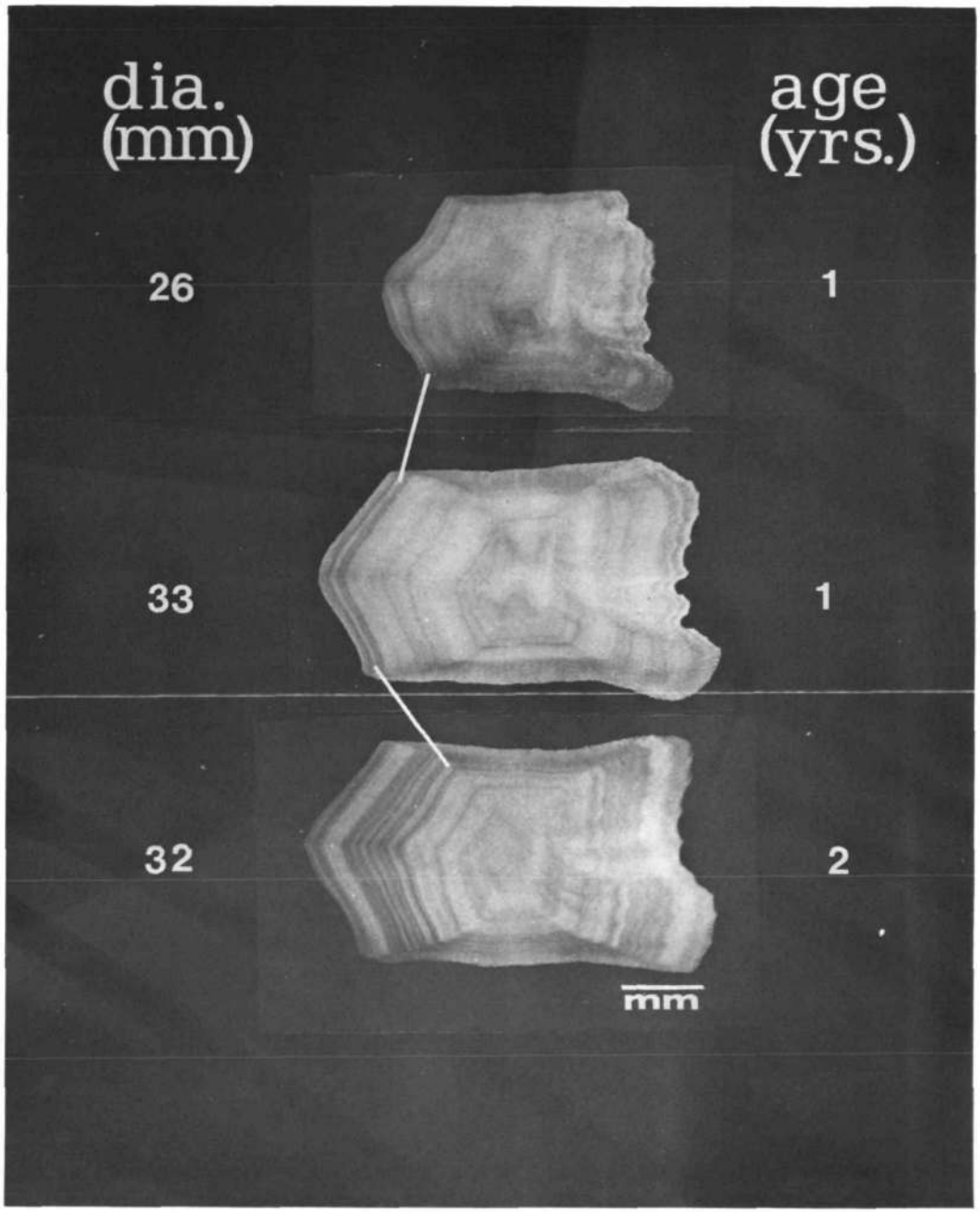


FIG. 16. No. 20 interambulacral plates of two 1-year-old sea urchins (*S. purpuratus*) of different sizes and one 2-year-old urchin approximately the same size as the larger 1-year-old. The white connecting lines mark the first prominent translucent zone formed at the end of each urchin's first year. The zonal patterns appear to be size-independent and closely correlated with age. Reflected light.

The physiological and environmental events leading to the formation of annual growth zones in echinoid plates are still unclear. We have shown that the different growth zones probably reflect changes in growth rates, and food deprivation results in the formation of a translucent zone. Feeding has been shown to be seasonal in

some species of sea urchins (e.g., Moore, 1935, 1937; Fuji, 1967) and perhaps these seasonal feeding rhythms lead to seasonal growth zones.

Other seasonal changes also occur in echinoids which may or may not be related to skeletal growth zones. Holland et al. (1967) found low protein levels, similar to those found in starved animals, in the coelomic fluid of *S. purpuratus* collected in the spring. Shimizu (1970) found low coelomic fluid protein levels in *S. intermedius* in the summer coinciding with the reported period of slow test growth. Striking seasonal changes in the amount of organic material in the body wall of *S. purpuratus* were reported by Ulbricht (1974); the mean percentage of whole-urchin ash-free dry weight fluctuated from about 75% in the spring to 50% in the fall. Moreover, catabolism ($CO_2$ production) in the body wall was maximal in the fall while anabolism (lipid synthesis) was maximal in the spring. These changes in weight and metabolism suggest that the test is a dynamic body component with dynamic seasonal changes, and the skeletal growth zones may reflect these changes.

Perhaps the most dramatic seasonal event occurring in echinoids, as well as other marine animals, is reproduction (see Giese and Pearse, 1974), and as suggested by Zoeke (1952), Sumich and McCauley (1973), and others, the skeletal growth zones may be related to reproduction. Gonad growth in *S. purpuratus* occurs mainly in the summer and fall, during which time the gonads grow from 2 to 4% to about 12% of the body weight (dry weight) (Gonor, 1973), and the body wall loses a similar amount of organic material (Ulbricht, 1974). Gonad growth therefore may proceed at the expense of body growth, resulting in translucent growth zones in the skeleton. Moore (1935, 1937), Fuji (1967), and Dix (1972) also found an inverse relation between gonad and body growth, and Moore and Dix related these changes to the apical plate growth zones.

The environmental changes which lead to seasonal reproduction in marine animals are still unresolved. Because reproduction and other events, including perhaps formation of skeletal growth zones, are seasonal, and different individuals in a population are more or less synchronized, regulation of these events is not likely to be wholly endogenous. Understanding both endogenous and exogenous regulation of these seasonal events remains a challenge.

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