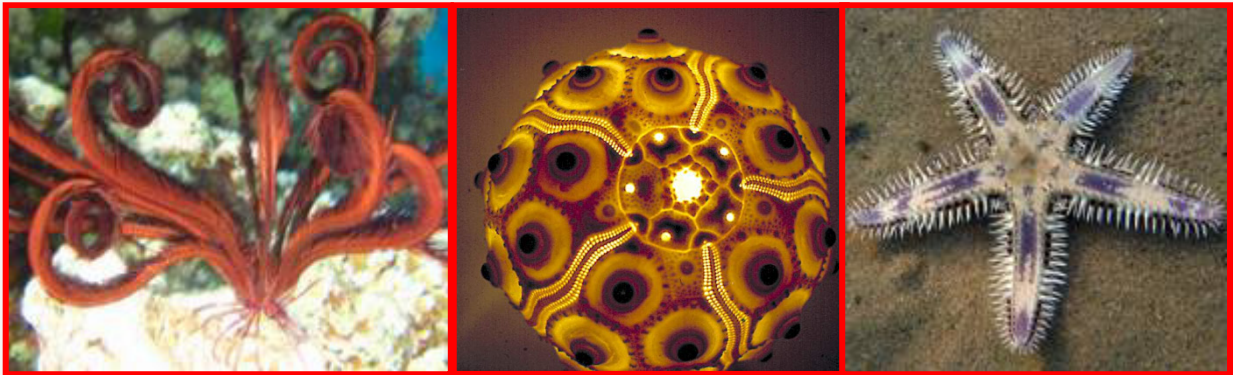


# Echinoderms

## Strange is their middle name



*Sea Stars \**

*Brittle-stars \**

*Sea Cucumbers \**

*Sea Urchins \**

*Feather-stars \**

**Jacob Dafni 2011**

This document is not a scientific publication, although it is based on a series of publications appearing in the list of sources at the end. Is distributed on an individual basis only for students and interested in the subject. For further explanations and comments please contact

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## Personal Preview

A great love wasn't there. I first met them when I was a young member of an Upper Galilee kibbutz. In the mid 1950's I went to Eilat with a friend, and endured two days of car-hiking, through the Negev Highlands and Mitzpe Ramon, who was at that time a road construction labor camp, for the newly-paved road to Eilat. The second day of our journey we drove in a huge "Titanic" truck where we were huddled in a seat next to the driver, witnesses to swearing of the driver on every bump along the dirt road to Eilat. We arrived to Eilat in a moonless night and the lights of a million stars joined faint lights from the opposite shore, from Aqaba, and the few ships moored in the bay. We fell asleep quickly, dead tired by the journey, next to the fence of the "Shekem" canteen the only building on the beach at that time. A two-story building, a hotel, was under construction then on the north coast, deserted at the weekend.

In the morning I was awakened by the blinding sunlight over the calm sea and deep blue, without a wrinkle of a wave. Before we went to look for any food or water source, I jumped into the blue water, but within two minutes I jumped outside, trying to draw black spines driven into my bare foot. This was my first encounter with a Long-spine Black Urchin– (*Diadema setosum*) (LSU). Luckily there were only a few spines, that I couldn't take off, but breaking them in the foot and bearing the pain that lasted throughout our stay in Eilat. Eilati corals, however, I met only on my second visit, six years later, made for a more practical purpose.

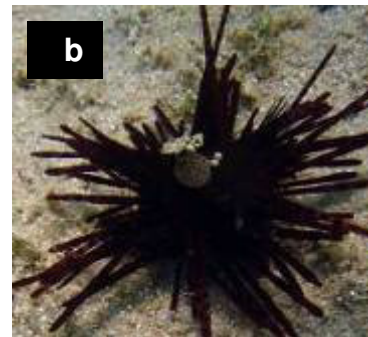
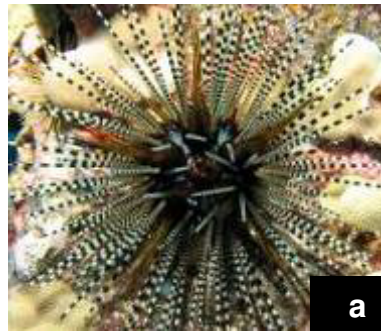
Like most teenagers at that time I also went the routine of army service and fulfilled the national pathway of the kibbutz. Like most of my generation, I finally deserted the path of collective life and found my own way in the developing Negev. Love of nature had already touched me, and the call of the *Society for the Protection of nature in Israel* (SPNI), a small non-government organization (NGO) with a long name, with an agenda to oppose the national slogan "We shall clothe the land with concrete and cement" and fight the destruction of natural landscape that accompanied the feverish development independence brought and the challenge of absorbing millions of immigrants, made me join their ranks. I was offered to go to Eilat to help stopping the looting of corals, that was then at its height. The SPNI, through an Israeli "chutzpah" decided to take charge of a small stretch of coast at Eilat, and turn it into a nature reserve. I would later learn that this nature reserve was in fact the first of its kind worldwide. The mission was a real challenge, with no existing law, or regulations at our aid, only a stubborn determination of a handful of guides-wardens, who made a living guiding tours into the desert but their "free" hours were spent on the beach, where they fought a score of "Eilati's" who made a living picking coral from the sea, drying and selling them to travelers.

Here I found the black urchin truly helpful. The few tourists who ventured into the water to pick themselves a coral were deterred by the urchins. The "experts" were not intimidated by them. They needed more elaborated methods, and it was a long struggle finally won..

As mentioned above, sea urchins are a real menace to bathers entering the sea with bare feet. Besides the Long spine sea urchin, there are more spiny devils – *Lovenia*, or “heart urchin”, an irregular sand dwelling sea urchin with long treacherous prickly spines, that before you notice them you feel the painful stab in your bare foot, and another – Black-spined urchin, who is the main “devil” of all urchins here. It is the **Double-Spined urchin** (*Echinothrix calamaris*, **DSU**). Its main danger are not the longer spines, not sharp at all, rather the extra thin spines poking through the thick forest. When it sensed danger, it lifts his thick spines and exposes the cluster of thin spines. Its normal color - dark black-brown-typical for black grown ups. The young are white and purple-striped spiked. It is probably a Red Sea variety - due to the changed conditions through the ages - of the Indo-Pacific *Double-Spined* (from Hawaii, for example) where it is named “*Banded Sea Urchin* .” Eventually I learned to deal with the *Long-spined Urchins* (**LSU**), and avoid the double-spined urchin (**DSU**) thin spines. I pick them up by grasping the spine tip, and pull the urchin up and at the same time putting my hand beneath, and let it go (so does, by the way, the Giant Wrasse, top predator of *Diadema*, thus getting access to its least protected mouth region), coming down upon my hand without harming the least, to the applause of spectators. I also manage to swim over the forest of spines on the surface of the reef, not always with complete success (no one evening I spent with my foot propped soaking in warm bath to ease the pain). I also learned that apart from two or three kinds of “bad” sea urchins - who give the whole group a bad name – most sea urchins are harmless, and are just a peaceful and interesting. The most liked is the **Velvety Sea Urchin\*** (*Tripneustes*, **VSU**). Relatively large, blunt and the surface underneath the back adorned with tiny claw-spines (Pedicellaria). It is the real “hero” of this story.

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 \*The name “**collector sea urchin**”, used in several countries is derived from their habit to collect leaves and other objects on their body (see below, p. 22 )

**Figure 1** *Double-spined Urchin DSU* (a): Indo-Pacific adult urchin) **Photo: Florent Charpin, Hawaii** (b) Red- Sea **DSU** is uniformly dark, but the juvenile (c) shows a banded temporary coloration. (d) **DSU** spread spikes in motion. In times of emergency it lifts the thick underneath and exposing the thin venomous spines



Twenty years later, in the late 1970's. By then I finished my work for the Nature Protection Society, but I was already "poisoned". Addiction to the love of the Red Sea and its wildlife led me to study marine zoology at Tel Aviv University. My field work for graduation I made in Eilat on the topic "Effect of marine pollution on the species diversity of dead coral inhabiting invertebrates". As expected, it turns out that marine pollution in general kills or suppresses many animals survival in the environment. I was then a graduate student at the H. Steinitz Marine Biology Laboratory and Hebrew University of Jerusalem, towards a Ph.D., looking for a subject for research. I tried various topics, but I was unpleased. One day a friend, Yehiam S. entered my room and said: "If you'd like to see something that will blow your mind, go to the beach at the Electrical Power Plant ."

Following my friend's advice, I went there and found that many, actually most, *Short-spines Urchins* (VSU) were highly deformed, assuming monstrous shapes. This finding encouraged me to investigate the cause of this phenomenon in a scale never reported before in scientific literature. As I was thinking of how to deal with these deformations, another aspect of mass skeletal deformities of different kind, was shown in another site, this time in the "Western Artificial Lagoon" on the north shore of Eilat, near "Ceasar Hotel". Again, the freaks were VSU, but unlike the former deformity, where they grew vertically, here I saw a different phenomenon - the descent of the skeleton dome, creating a deep depression on this top (**Fig. 39**, below). To distinguish between two types of deformities, I named the deformity that was discovered on the Power plant beach **deformity A** while the deformity of the lagoon **deformity B** (for information on those deformities and insights I got about them, see **Second Part** - p. 48 onwards)

I already knew this site. The beach in front of a power plant, used as well as desalination facility, was one of the study sites in my previous study and known to me as the most polluted beaches in Eilat. Today the beach is called "The Palm Beach". During the winter I liked to wade in the warm water - 30 centigrade and more – eluted from a water pipe spewing out into the sea the cooling water of the power plant. But despite the tendency of hot water to float up to the surface, the warm brine sank down, due to a salinity of >> 4.6% versus 4.1% of the usual Gulf of Eilat normal salinity. The brine contained unusually high concentrations of copper and iron. The hot brine was poor in oxygen, causing high mortality to the coral reef to a depth of 4 m and the location of corals, normally inhabited by crabs and molluscs, was rich with nematode worms, a known tough creature, resistant to pollution, numbering up to 90% of all individuals in the samples at that site, what explains the low species diversity there .

I was not surprised by the fact that the deformities were contaminated beaches. Relationship between mass-scale deformation (70% of individuals in the population) and the effects of pollution on the beach "Power Plant" and "West Lagoon" does not seem random at all. Relationship to the environmental cause seems adequate and reasonable. But beyond the environmental aspect, these findings encouraged me to study the mechanism of the skeletal formation of echinoids (scientific name of sea urchins), and here it was decided my PhD research topic, called "growth and skeletal calcification in the Velvety Sea Urchin *Tripneustes gratilla elatensis*."

## ***Part I – General account on Echinoderms and echinoids in particular***

### ***1. Strange is their middle name***

Even the Hebrew names of these creatures – Spiny-skinned (**echinoderms**) - suggest strangeness. Gathered here are a few thousand creatures, sometimes different from each other in many extreme traits, the common property - that is to be shared by all - and appear in their name. Later we will realize that they are indeed unique, and the closeness between them is not accidental. They include sea urchin, sea stars, Feather-stars, the Serpent stars and sea cucumbers - sea. The “sea” prefix unfolds an obvious feature shared by all echinoderms and it is life at the sea. Lobsters, crabs, snail and clams, none are restricted to sea – No spiny-skinned animal inhabits freshwater ponds, lakes and rivers, or climbed onto the land .

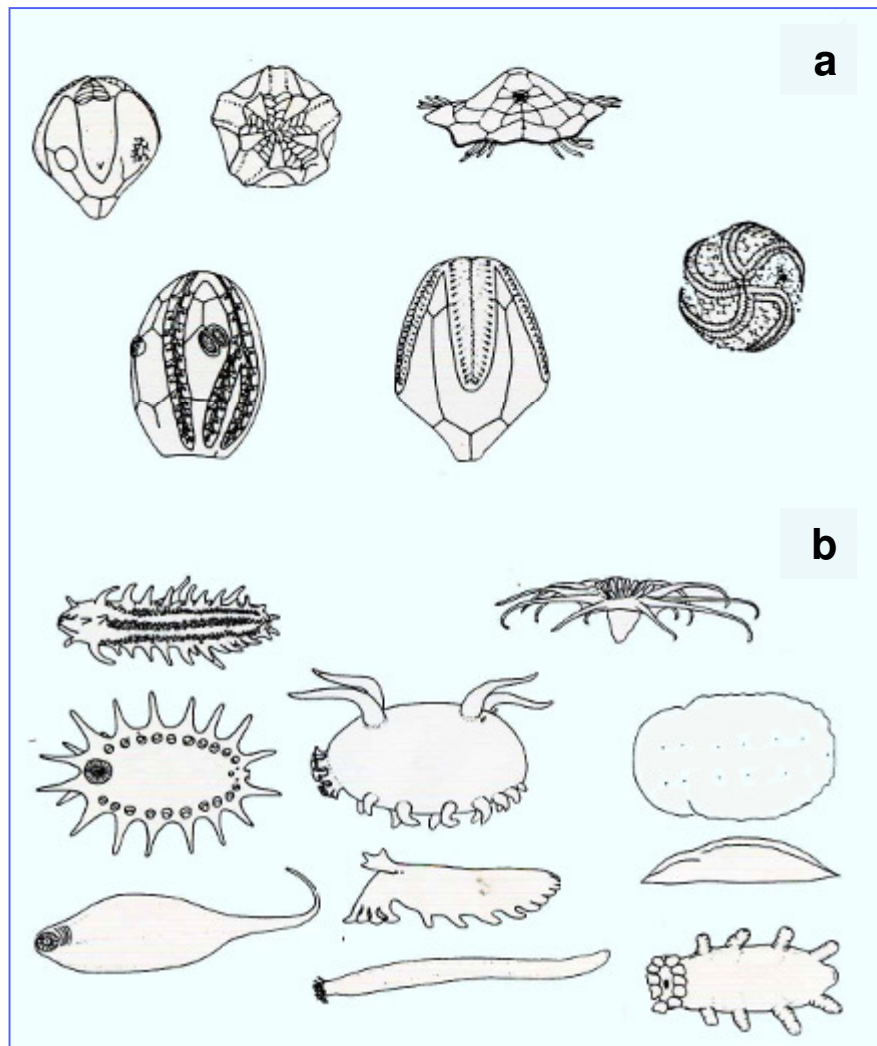
No spiny skinned animals are mentioned in our traditional literature – the Bible and later books. Otherwise we must find the following statement in Proverbs, written by the sage: "There be three things which are too bad for me among denizens of the sea, four which are most hated by man: the predatory shark; the venomous jellyfish; the eight-armed octopus, and the prickly sea urchin. But as stated, none of these sea-creatures was known to our ancestors. And if they mentioned in the scriptures, it was to warn us against eating them, or remind us of a wonderful experiences – or unpleasant experiences (please recall the frightening story of Jonah, swallowed by a giant whale, held in his bowels for three terrible days, before being dumped into the shore of Nineveh). Both testify to the primeval fears of the ancient Israeli who was probably "an earth-mouse" - from the sea and its inmates. In contrast, the peoples of the Mediterranean were familiar with the sea creatures and treated them not only their heritage, but also their art, ancient Greece and Egypt used their figures to decorate their temples, and learned the ways of life; the Philistines and Phoenicians fed on them, and learned to benefit from them raw material for linen and purple painting, and cherished the beaches inhabited by them.

Unlike sharks and octopuses, the popular and scientific literature enjoy to return to them often, and further describe the strangeness and their exploits, the popular literature does not discussed sea urchins - the sea and their relatives through the echinoderms. Despite the "conspiracy of silence" ancients, all sea-going, or rather all "going to sea," know them well enough to feel a sense of mingled fear and disgust. Sometimes it seems that simply ... People like to hate them.

The aim of our article is to discuss the echinoids and their relatives with the intention to attest for their innocence, since the damage they cause is marginal - they do not kill or harm us – and they are representatives of an ancient group that has enough positive qualities to balance their faults. As I devoted several years of my life studying these wonderful creatures, all I ask is that I will be allowed to serve as advocate to this group of strange organisms, as the Americans will say "strange is their middle name" (**Fig. 2**). Anyone reading this article to the end, maybe will not turn his heart to love them, but will at least know enough about them to balance his hatred with reason<<<< .



The present account does not pretend to be a textbook. At most, it as a personal account of an investigation I made that I find fascinating. My main intent is to share with the readers the aesthetic experience that make these creatures, despite their apparently simple shapes and lifestyles - which poses actually an intellectual challenge to understand "what makes them tick.". We have already suggested that strangeness is the common feature shared among a cucumber-like organism, a flower on a stalk, and a third which remind us a kind of a comet, significantly different from all other ocean-dwelling creatures. An American zoological, daughter of Jewish immigrants from Europe, **Libby Henrietta Hyman**, who dedicated 50 years of her life to study marine invertebrates (Hyman, 1955), summarizes her experience in the phrase *"I could only raise my hat to a group of creatures that seemed to be created especially to confuse and baffle the researchers" ...*

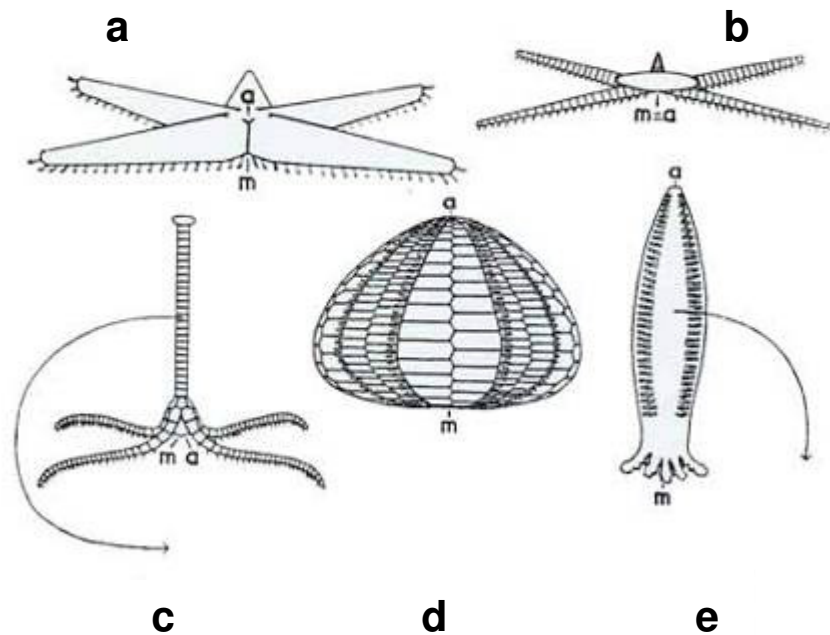


**Figure 2** (a) Echinoderms: Extinct Paleozoic Echinoderms, left to right: Blastoid, Adrioasteroid and Ophiocystoid (various sources); (b) several types of extant sea-cucumbers living in the deep sea ( Clark, 1962)

## 2. Morphological and anatomical traits of Echinoderms

Although ostensibly no members of various classes look alike, quite a few features common to all members of the Phylum Echinodermata. Some are primitive, that seems to group them with creatures at the bottom of the developmental tree, among sponges, corals, hydrozoans are:

- A. Radial Symmetry, like in primitive organisms, although it is pentamerous (five-fold) (**Fig. ,3**
- B. They have no head at all, and no eyes or other external sense organs.
- C. Inefficient organs of motion, and therefore they very slow moving and sluggish. None are swimmers.
- D. Circulatory system and their provision do not elaborate and low effectiveness.
- E. Absence of a physiology between the sea and their body fluids, a feature that restricts them in their attempt to exist in a non marine environment.
- F. Simple reproduction. Each individual is either male or female, although it is not uncommon to find bi-sexual individuals (Hermaphrodites.)
- G. Amazing capacity to rehabilitate damaged tissue and organs (regeneration), acting also in reproduction.



**Figure 3** .Radial five-fold symmetry (a) – Sea-star; (b) - brittle-star; (c) – feather-star (=)crinoid( (Upside down) (d) – Sea-urchin, (e) - Sea-cucumber, often tilted on its side (m – mouth; a - anus, sometimes a is missing, and then m = a). Arrows to illustrate the natural tilt of the body position of a feather-star and Sea-cucumber ( Clark, 1962).



In contrast, the sea urchins have an impressive collection of advanced features, placing them in line with higher organisms in the sea - chordates (**Chordata**), and we - the mammals as part of them:

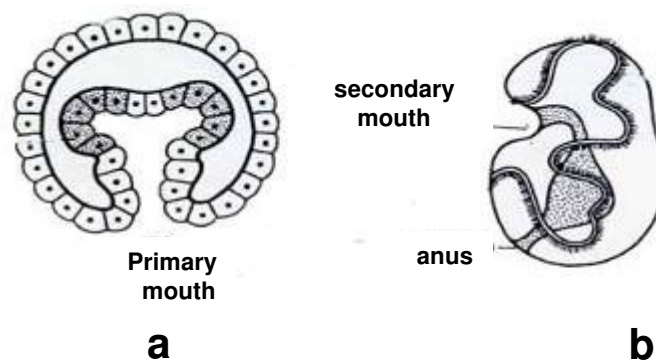
A. The symmetry of the larva is two-sided (**bilateral**). Hence the conclusion that it was probably the symmetry of their group of origin, whereas the radial symmetry is secondary and a late stage.

B. They have a secondary body cavity, called the **coelom**. This characterization is common to all higher invertebrates and vertebrates. In this cavity the internal organs - intestine and reproductive system – are nested. The coelom contains the coelomic fluid in which amoebocytes, called also coelomocytes drift, carrying oxygen and food to all corners of the body.

C. **Internal Skeleton**. Although the sea urchin's skeleton with the spines look similar to an external skeleton in fact it is an internal skeleton. **Sea urchins do not molt their shells**. Their skeleton (scientific term **test** is often used) is covered by an epithelium, a dermal tissue. Even the spines are an integral part of the inner test .

D. They belong to a group named **Deuterostomia**, a concept that expresses the shape of embryonic development as characteristic of the higher animals, especially vertebrates: At gastrulation a later hole appears in the back of the body, developing into mouth, and anterior primitive mouth becomes an anus (**Fig. .(4**

As mentioned, the common characteristic of this diverse group, sea urchins, sea stars, feather-star, sea cucumbers and brittle-star are their spines. Their collective name is merely a literal translation of scientific names – Spiny-skinned. Sometimes the name is misleading, since sea cucumbers, for example, do not seem to have spines, but all the same, they have an internal skeleton made of limestone ossicles.



**Figure 4. Deuterostomia:** two larval stages of a typical higher invertebrate and vertebrate. The appearance of a secondary mouth on the back of the larval body, that becomes its primary mouth, while the former becomes an anus: (a) Gastrula stage with a primary mouth, and (b) a more advanced stage, showing the secondary mouth and the anus (various sources.)

Sometimes the long and sharp spines turn the whole animal into spiky ball, and sometimes they are short or blunt, or they are just cogs, always part of a limestone skeleton. Formerly a few echinoderms, due to the significant roundness or branches were mistaken as plants, not animals. Actually scientists saw them first as transient stage between plants and animals. The feather-stars were termed Zoophyta (=animal-plants). Only in the 19<sup>th</sup> century science recognized them as animals.

From an evolutionary point of view the phylum **echinodermata** remains a kind of “crossroad”, neat to which marine invertebrates developed into vertebrates and went on to conquer the land. They survived in our modern world due to their adaptations to many marine environments, from the tidal zone to the ocean depths. Unlike crabs, molluscs and worms – well represented in terrestrial habitats, the echinoderms stopped at the shore, none ventured into land. The most common and typical echinoderm in shallow water is the sea urchin. We will describe it in great detail, to present and represent the entire group features

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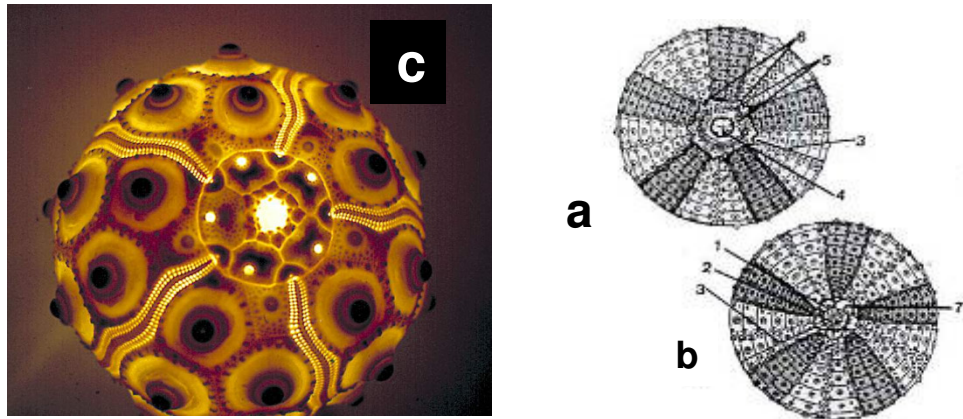
### **.3Sea-Urchins: Spine enveloped tenderness**

The Hebrew translated name, **Kipod-Yam**, does not imply imagination, and not shared by many languages. In most European languages is called the “naughty boy” (=urchin) in English and or “teddy bear” in French (Oursin). Germans are known as “Shamranim” saw the similarity to urchin, and called it “urchin of the sea (seeigel), and we, lacking a traditional name for it, followed them, so Kipod=urchin, and Yam=Sea .

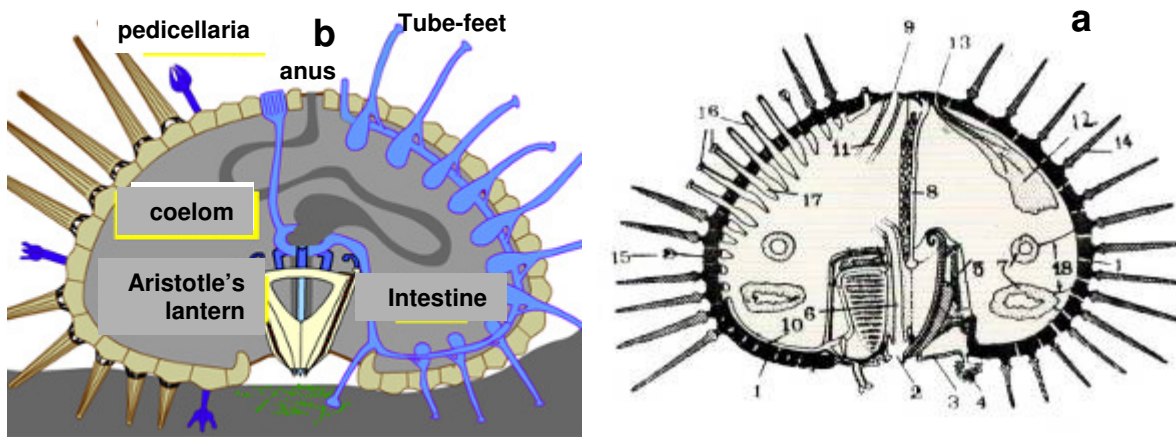
For an initial description we will mainly deal with two above mentioned Red-Sea echinoids - **Diadema** and **Tripneustes**, both included in group of the radial **Regularia**. Less common is the group **Irregularia**: bilateral sea urchin, described later on. All share the spherical shell, hard - relative, surrounding a wide inner **Coelomic** space, filled with a liquid, inside are the “floating” intestine, and other internal organs, attached to its wall with flexible wires (mesenterial threads) against the inner face of the hard skeleton. spines are an integral part of the skeleton. Later we will discuss these two characteristics - the skeletal hard structure and its fluid interior - and the biomechanical role in the body anatomy and morphology. (as well as other echinoderms). The influence of these components - skeletal and coelomic fluid - with the state and functionality of the living organism, and vice versa - the body's condition is reflected in the skeletal structure. For example, less calcium makes the sea urchin's skeleton soft and friable, whereas under starvation conditions physiological system conveys maximum resources to the digestive system resources, neglecting the body's skeletal growth. The result - a jaw, which is essential for food gathering grow at the expense of other skeletal parts

### **A. Skeletal structure**

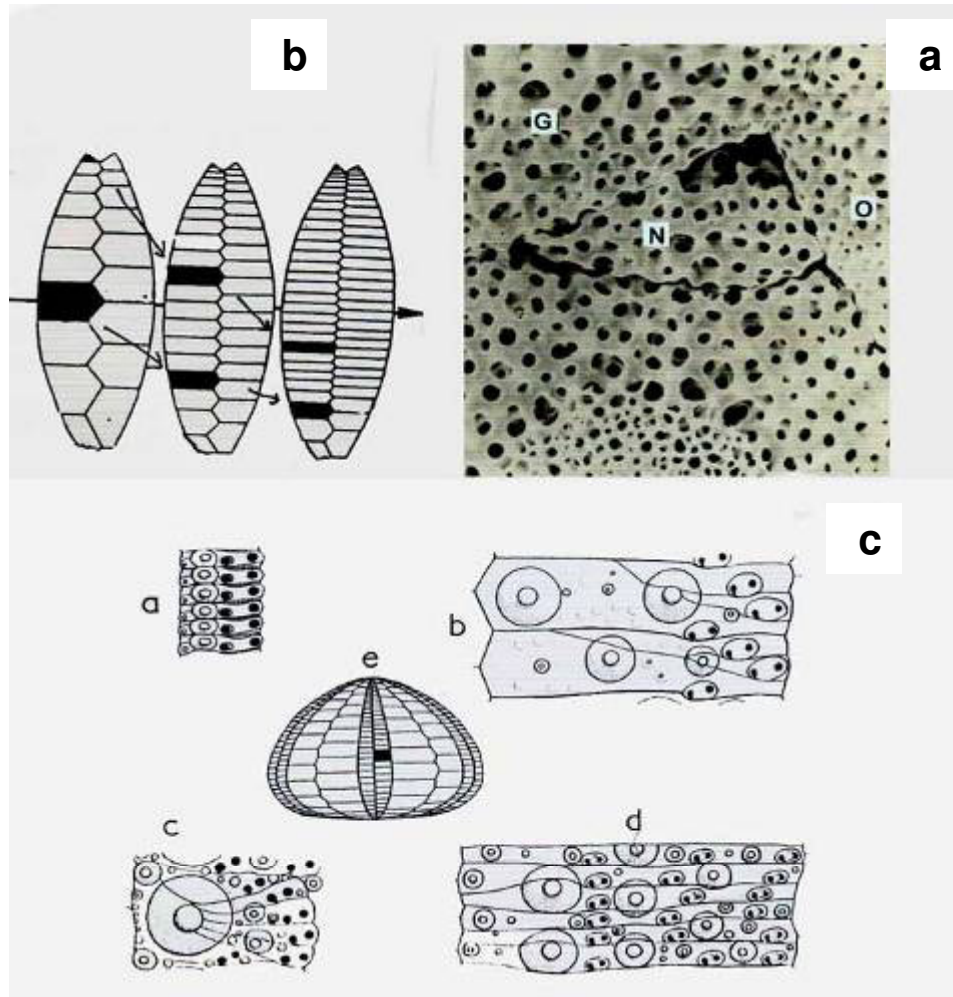
Urchin skeleton - allegedly an exoskeleton, as it surrounds an inner-filled liquid content, is in fact an **endoskeleton** (**Figs. 5,6**), externally wrapped by a dermal tissue. In contrast to skeletons of other marine invertebrates – crustaceans, molluscs etc. – of endodermic origin, echinoderm skeleton is secreted by an ectodermic tissue. The echinoid test is pentamerous, composed of twenty columns of limestone plates (five “radii”, each made of four plates columns), in a close-packing fit. New tiny plates are constantly added near the upper pole of the test (**Fig. 7a**). Each plate grows mainly at the edges and less in thickness, while they migrate down towards the **ambitus**, the body “equator”, making room for new plates to come in its wake (**Fig. 7b**). From the ambitus and down, the plates almost stop growing, become – relatively - smaller. A living urchin always adds new plates, **Amb** plates (another name: radial), perforated by tiny holes penetrating them to allow the tube-feet (see below) to go through, and partly the imperforated **inter-Amb** plates (meaning “between radial .(”



**Figure 5.** Sea-urchin tests (=skeleton) spines removed. (a) an adoral aspect (from below), (b) an aboral aspect (from above). Detail: (1) plates surrounding the anal opening (2) (apical plates), (3) The plates that form the central area of the test – ambulacral (Amb) radii (dotted) and InterAmb (light), (4) mouth and five protruding teeth of the "Aristotle's lantern" (5) flexible peristome membrane, (6) gill notches. (7) perforated sieve plate (madrepore), serves as opening of the hydraulic (=ambulacral) system of the tube-feet (Durham et al., 1966). (c) A dried test of a thick-spined cidarid urchin showing the aboral plates and anal aperture, Anus. Note the large "nipple-like" spine bases (dark) and five columns of "tube-feet perforation" (original photo.)



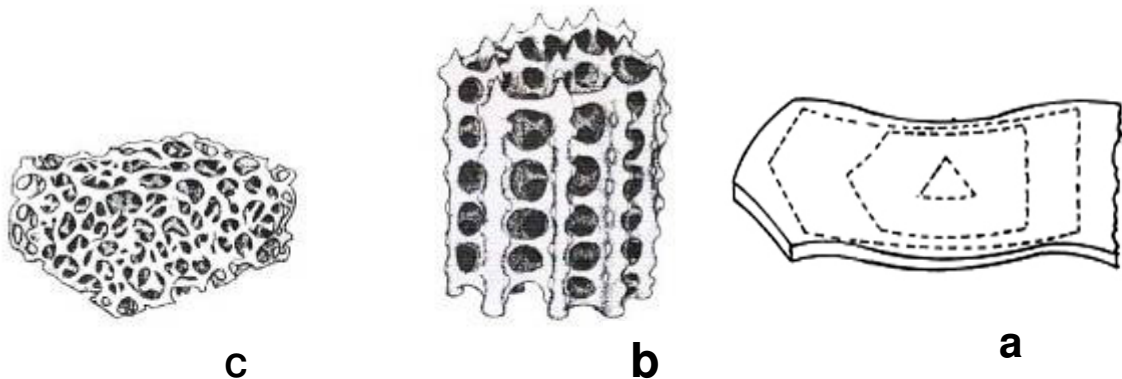
**Figure 6.** (a) A vertical section of a sea urchin showing external and internal organs: (1) test (2) mouth, surrounded by (3) peristome membrane and (4) gill ; (5), jaw, one of the five that make up the "Aristotle lantern", and (6) esophagus, (7) sections in the intestine windings in the coelom (shows only sections), (8) – stone canal axial vessel – path of the hydraulic fluid; (9) anus, and the hindgut (11) (10) radial branch - one of five - of the "ambulacral tube-feet"; (12) reproductive organ (gonad) and eggs and sperm outlet (13) (14) - large spines and - (15) – pedicellaria (16) 'tube-feet' with snapping discs at their end, and (17) – ampullae; (18) – mesenterial threads attaching the intestine to the skeleton (b) another section showing the coelom cavity (grey), intestine (dark) and Amb system (blue) (Smith, 1984 and other sources)



**Figure 7.** Skeletal plates – (a) Newly emergence and vertical shifting of **InterAmb** triangular plate (N), departing from the test plates surrounding the anus (O, G) (microscopic picture); (b) Schematic description of three InterAmb columns during the life of a urchin, from juvenile (left) to adult (right). The vertical shift is accentuated by darkening particular plates. Ambitus line shows as horizontal arrow; (based on Raup, 1968) (c) Amb plates from different urchin skeletons - a - simple plates of a primitive urchin skeleton (*Cidarid*), b-d compound plates, made of fused demiplates. b - a triangular plate urchin, c - composed of six demiplates - plate type urchins (*Echinometra*) ;d - a group of VSU (*Tripneustes*) plates in which each plate carries three pairs of holes - that is composed of three subplates each. Consequently tube-feet are arranged in three vertical rows, and e – shows the relative sizes of Amb (thin and dark) and InterAmb. Concentric circles on the plates are bases of spines ( Clark, .1962



Each of the five radii therefore has two **InterAmb** columns and two **Amb** (ambulacral) columns (**Fig. 5c**). Urchin skeleton is made of limestone including, in addition to calcium, about 10% magnesium, contributing to the relative hardness compared with other chalky skeletons. Its structure, made of a mesh of microscopic beams, contributes to its stability, (**Fig. 8**), whereas the inner space is filled with connective tissue. The surface of each plate bears round “nipple-like” spine bases. Each Amb plate is punctured by twin Amb pores, multiplied in “advanced species” of urchin species by the number of demiplates. Among the various species an evolutionary trend reduced the relative number of InterAmb plates, while Amb plates number increase, thus increase the number of tube-feet (**Fig. 7**). Ambulacral plates of the Velvety Sea Urchin for example, consists of three demiplates fused together, and arranged in three columns (hence the scientific name - *Tripneustes*) (**Fig. 7c–d**). The plates are connected to each other along the seams, called **sutures**, sewn together with sutural collagen fibers (see **fig. 58, p. (73**



**Figure 8.** The structure of the urchin's skeleton, spacious network made of limestone beams, among which organic tissue grows: (a) typical InterAmb plate of *Tripneustes* – differential growth at its margins show as concentric growth lines; (b) organized structure of spine skeleton, and (c) A less organized plate mesh of a plate. (Source: a - original; b-c: Smith, .(1984

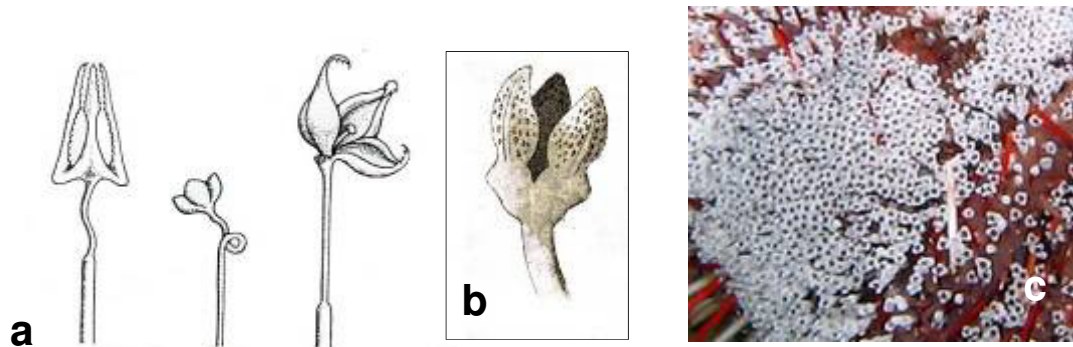
## B. Spines and pedicellaria

As mentioned, echinoderm spines (**Fig. 10**) are covered with epidermal "skin" and therefore considered part of the internal skeleton. Spine structure, especially spine of long-spined sea urchin combines firmness and flexibility. At the spine base peripheral “muscles” are shown that connect the spine to the skeleton. Muscle responds to environmental stimuli - touch, light regime or chemical and nerve stimulation, tilting the spine side-ways or in circle, responding to shadow cast on the area makes it lean side-ways or come right at the source of interference. Spines vary in size and thickness. The thin and sharp may inflict pain and deter enemies. Other spines may be used to anchor the urchin to a hole in the rock, or just prevent predation. Some spines are used to move on sand, or even move beneath the sand surface by a “rowing” movement. Another kind of spines are pedicellaria, spines equipped with plier jaws. (**Fig. (9**

Those who runs into a sea urchin spine for the first time, especially the long spiny ones are impressed by their fragility. The skeletal structure of the spine, that combines solid and durable skeleton beams with a soft tissue that fills it's inner spaces, ensures maximum flexibility. When they contract the reef rock, or even with bodies of living animals, they mostly bend, and only when pressure is applied to them, the spine punctures the living skin lodged in the soft tissue, or break .

A special type of spines, common to sea urchins and the sea stars, are the pedicellaria, *kotzvatim* in Hebrew (the name is a combined names of a spine and jawed pliers), scattered across the body surface, and between the regular spines (**Fig. 9**). These tiny pliers tipped spines have three jaws, That open and close through muscle action, thus pinching the intruder's tissue, in response to nervous stimuli. Pedicellaria jaws are an effective weapon against predators or pestering parasites. We distinguish between several pedicellaria types– Snake-head (ophiocephalid), digitate (dactylous), double tooth (bidentate), three toothed (tridentate) and clover-leaf (trifoliate) and last but not least, the venomous globe-shaped (globiferous) pedicellarium. The last one is the biggest and most developed, and equipped with blistered venom gland

Pedicellaria jaws open, cling to the tiny creature that is stubborn enough to go near them, close and snap at the opponent ,chasing him away, and sometimes even killing it. Eilat Velvety Sea Urchin (*Tripneustes*) is considered mildly venomous (as suggested by its family name - Toxopneustidae). Sea stars also have pedicellaria, smaller ones, less efficient. They usually have only two jaws. It was found that the some sea stars use them to catch their prey .



**Figure 9.** Urchins pedicellaria: (a) Snake-head (ophiocephalid), trifoliate and globiferous pedicellaria; (b) Microscopic view of the globiferous pedicellaria - three jaws (various sources). (c) External surface of a Velvety Sea Urchin covered by hundreds of globiferous pedicellaria (original (

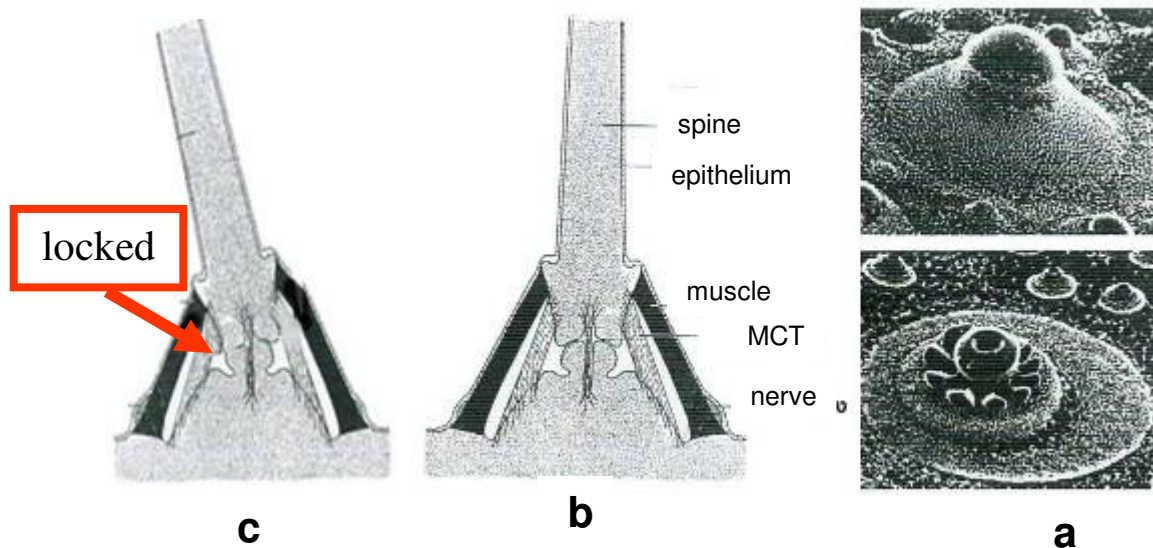


### C. A “muscle-less muscle” - unique type of connective tissue.

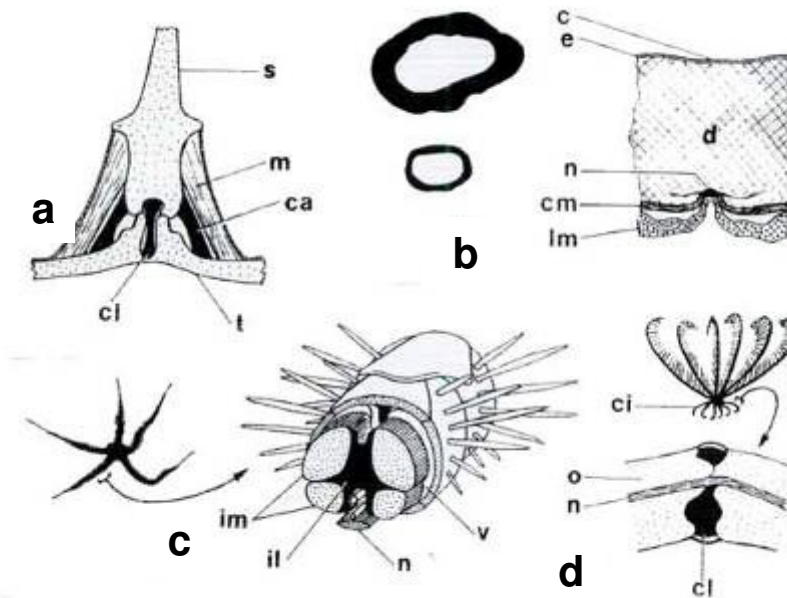
A discovery made by a Japanese scientist in the 1960s (Takahashi, 1967) stirred the biological research world: He found in the base of sea urchins spines two circles of “muscles” – an outer muscle and an inner circle of connective tissue that can undergo rapid changes in its mechanical state, relax and harden, in analogy to the muscles. This feature is attributed to a special kind of the protein collagen, found in connective tissue of most animals. The inner “muscle” was named Catch apparatus, later described and termed Mutable Collagen tissue (MCT) )Fig. .(10

MCT hardens and loosens up in seconds, without muscle any involvement. Experiments of his followers, Motokawa, (1988) and Wilkie (1984), revealed that the mutable tissue collagen plays multiple and diverse roles in physiology and anatomy of echinoids and other echinoderms )Fig. .(11 Experiments carried out by them caused hardening of collagen tissue by exposure to potassium or calcium, while fresh seawater rinsing softened them. They found also that hardening and relaxation processes controlled by the nervous system are affected by chemicals, adrenaline and acetylcholine. Sea cucumber, for example stiffens its skin under danger or emergency and soften it when danger is past. In brittle-stars, potassium caused softening of collagen, allowing the loosening and detachment of threatened arms, whereas feather-stars use MCT collagen in their lower cirri – the small branchlets that hook the brittle-star to the rock. Thus avoid to invest costly energy in gripping them to docking place. Collagen tissue is the feather-stars replaces the missing contractile system in the cirri. In this condition no predator can sever from its grasp without breaking the locks.

From personal experience I can tell that when, in the Eastern U.S., we hauled from the sea a sea star that was in the midst of oyster predation, strongly adhering to the oyster shell, through its MCT tissue, it was impossible to separate them by any conventional means. Of course we set it free to go on doing its job (p. ( 41



**Figure 10.** Sea urchins spines. (a) photomicrograph of spine bases (primary spine has larger base and secondary small ones), (b) Schematic presentation of spine attached to its base, by the two “muscle” types: outer normal muscle, activated by nerve ring, enabling the spine to bend and rotate, and an inner ring of “mutable collagen tissue (MCT) forming the **catch apparatus**”. In (c), the spine becomes locked by the MCT, unable to bend or move. The spine is covered by “skin” made of epithelium tissue (modified from Smith, .(1984



**Figure 11.** Mutable Connective tissue (MCT in black) appearance among typical echinoderms : (a) Catch apparatus at the base of echinoid spines (b) thick skin of sea cucumbers, (c) Brittle-stars arms and (d) cross section in a crinoid (feather-star) attachment cirri ( Wilkie, 1984.) Legend: s – spine; e, c - epidermis and cuticle; ci -cirri; m, cm, lm, im - muscles; n - nerve; ca - catch apparatus; cl, il - collagen ; o, v, t - skeletal components, and d- skin tissue.

### D. Tube-feet as motion organ

The echinoderms have a hydraulic moving system, unlike any other organism (**Fig. 12**). It consists of a hollow water tube, with a swollen bladder (ampulla) in the inner side and a snapping, sucker disc in the external end. The so-called tube-foot penetrate the skeleton, elongates forward or contracts, by long muscle activity, while its adhesive “suckered disc” can grasps hard objects. Echinoids rely on this so called **ambulacral system** both to anchor to the substrate and move along .

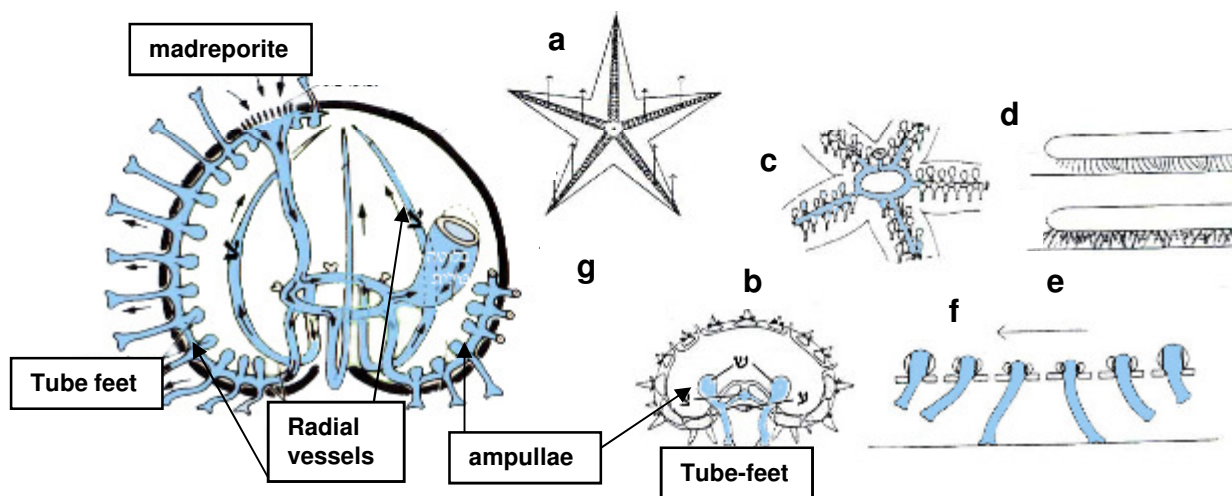
Sea urchins can extend their tube-feet at least three times the normal length (up to 5 cm in VSU, or shrink completely. Clinging to a rock is done by attaching the sucker disc to it, causing an adherence force of ca. 30 grams by vacuum suction. Multiplying it by the number of suckered tube-feet we reach the overall strength of more than a several kg (Paine, .(1926

It is not only a momentary force, they have the ability to keep anchored against displacement for long time. Experimentally held urchins for over an hour could apply a constant force (or weight) of 250 g, and it is possible that they could resist the sudden wave in a force several times stronger (Sharp & Grey, 1962.). Each urchin has five double sets of tube-feet, to match the number of Amb plates pores. *Velvety Sea Urchin* , largest urchin in the Gulf of Eilat (diameter of up to 13 cm, without spines), has more than 1,500 tube-feet, and as many pairs of tiny holes in the skeleton.

Tube feet attach the urchin to the substrate similar to tent strings, and stabilizing it, but at the same time serving as a primary means of movement. Let us describe the mechanism of action of the Amb system and the tube-feet; Water is driven forward by the contraction of the ampullae, within the skeleton. Since the interior of the tube-foot is sealed, the adhesive sucker is pushed forward, and the foot extends, by hydraulic pressure, aided by the peripheral muscle contraction of the tube-foot itself.

Muscles that operate the adhesive discs allow them to hold on to solid objects along their motion. Upon adherence of the tube-feet sucker, the length of the tube muscles shrinks, and the urchin is connected. There is a remarkable similarity between this system the hydraulic system, which transfers printing paper in contemporary printing machines. It is unlikely that those echinoderms stole this hydraulic principle from mankind ...

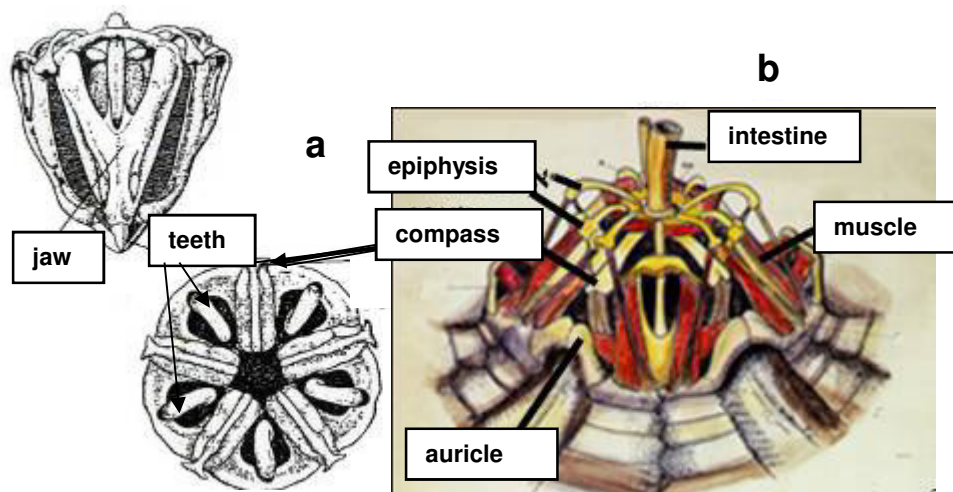
Strange: this hydraulic system is connected to the sea with no separation of any organic membrane. Water enters through the tiny pores in the sieve plate called **madreporite** ("mother of pores"), which is one of the plates surrounding the apical (anal) ring, from which the water goes down to a peripheral ring pipe that splits into five branching columns (radial vessels), along which the **ampullae** are arranged (**Fig. 12 c,g**). A complex valve system divides the system into separate units, allowing the creation of hydrostatic pressure in each of the hundreds of tube-feet simultaneously. Tube feet system of sea stars is substantially similar to that of the urchin, but the number of plates pores, scattered across the "back" of the star may reach up to .11



**Figure 12.** Amb system of sea stars and sea urchins: (a) lower aspect of a sea star, showing the motion directions (arrows) each leg has to move to lead the star move in one direction, (b) section of one arm, demonstrating the appearance of the column's double ampullae in the leg's inner coelom ( Clark, 1962), (c) General view of the entire sea star's Amb system and comparison between "legs" of a millipede worm (d) and the rhythmic regularity of a sea star's arm tube-feet (e), moving and attaching. (f) Description of the moving cycle of a sea star's leg along the path of movement (arrow), and (g) the interior of a sea urchin, showing the Amb system components (modified from Fell.)

The directions of pull and attachment of the tube-feet is coordinated by the nervous system. To move, a sea urchin must disengage, bolt out and stretch in the desired direction, attach the adhesive suckered disc to a hard object and pull the body towards it. As dozens of suckered tube-feet do it simultaneously, at different directions, the nervous system has to dictate each tube-foot when to relieve its hold, when and at what direction to stretch, and when and where to use its muscles to attach and shorten, to move at that direction. Such coordination - perfect for a creature whose "brain" is only a bundle of nerves surrounding the mouth, and branching off to the Amb system, is undoubtedly one of the wonders of nature. Part of the tube-feet are used for other purposes: to carry and maintain various objects – leaves or pebbles - on the body surface (“**covering reflex**”, **p. 22**), gathering food and carrying it to the mouth, and in several types of sea urchins – also used as gills to breathe (**p. 30**). In the predatory sea stars, tube-feet serve primary means to hold, and pry open the oysters’ twin valves, to reach their soft flesh. Here also, the tube-feet must have the sensing ability to distinguish between objects it touches – hard, to hold on to, and soft, appropriate to feed upon .

It is believed that the original role of the Amb (tube-feet) system, in the ancient sea, was to gather food and move it to the mouth, and as such they are used in several less advanced echinoderm groups – feather-stars (Crinoidea) and brittle-stars (Ophiuroidea) In echinoids it is used as gills (sea urchin gills are apparently tube-feet adapted for this task). Food gathering by tube-feet is shown by sand dwelling urchins, where tube-feet are useless as moving device, or in those living inside rock crevices, gathering algae or micro-organisms from the water environment.



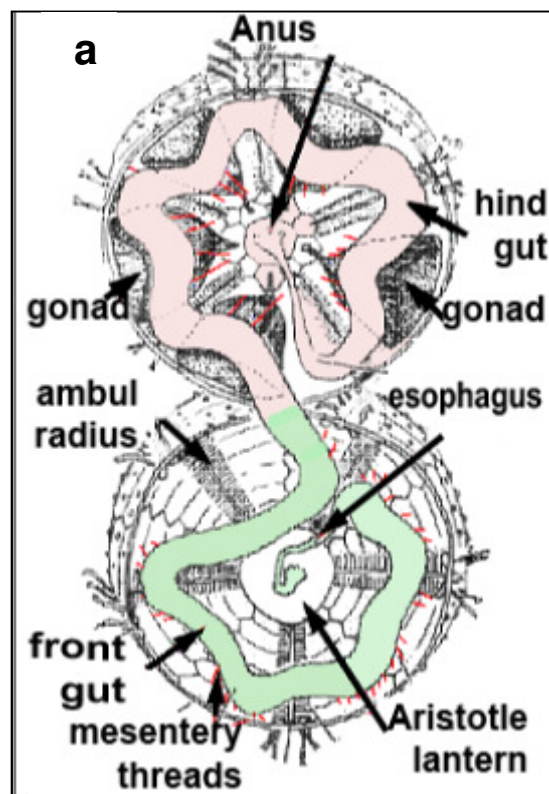
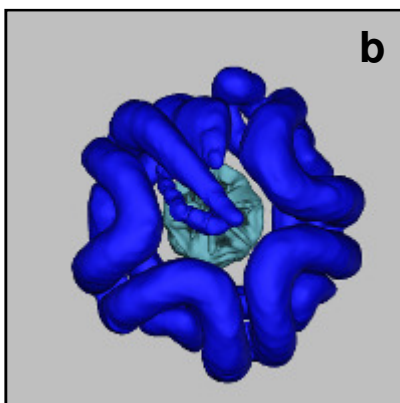
**Figure 13.** “Aristotle's lantern”: (a) Side view of the jaws and the five teeth sheeted inside them, meeting at a point, and a view from above, showing other parts - compass and epiphyses, as well as the soft upper origin of the five teeth. (b) Aristotle's lantern, seen from within the lower part of the test (skeleton) showing also the muscles attaching it to the test and manipulating its jaws (modified from various sources).



## E. Digestive system of a Sea urchin

Digestive system includes the mouth and jaws, the esophagus and intestine, with its two parts - the fore gut, and the hind gut, and the anus (**Fig. 6**). Echinoid's mouth is always in the lower pole, called Oral, a wide opening at the center of the test, partly covered by an elastic tissue – the peristome. It is equipped with one of the most bizarre feeding organ, a symmetrical arrangement of five jaws, each used as "sheath" to a long tooth. All five teeth tips meet at one point, slightly protruding at its lower end. The entire organ is consisted of 25 hard parts (ossicles), manipulated by no less than 45 (!) muscles that open and close the jaws, protruding forwards each tooth separately, to pinch and cut algae, or other organic matter and bring it into the esophagus. This special jaw system has aroused great interest on the part of the Greek naturalist Aristotle, in the 4th century BC, and is named after him "Aristotle's lantern" (because of its similarity to the lanterns used at his day) (**Fig. 13**). Normally the "lantern" is used to cut and collect algae or other sedentary organisms, but some echinoids use their hard teeth (enriched in magnesium carbonate) to erode the rock, cutting holes in it (**Fig. 16**). The intestine is long, as predicted for vegetarian food, and begins at the upper side of the lantern, and draws a typical route through the fluid filled skeletal cavity, and ends at the anus, which ends at the anus, at the upper pole (**Fig. 14**). The Intestine is held to the inner wall of the skeleton by wires **mesenterial threads** (similar to vertebrate mesentery) and their grip at the inner wall is sometimes showing clearly (**figures 52, .55**

**Figure 14.** (a) Sea urchin cut in half its middle, exposing the intestine and other interior parts - foregut (green, lower half), leaves Aristotle's lantern, winding around the inner wall of the body in an counterclockwise direction. After drawing almost a full circle, it turns to the opposite direction, and continues as hindgut (red), attached to the wall side through mesenterial threads, in between the Amb radii (**see also fig. 51**), and ends at the anus. (b) A MRI image of a sea urchin's interior, shows the "Aristotle's lantern" (green), and the intestine in blue ( Ziegler et al.).



Most echinoids are vegetarians, scraping the short algae covering intertidal and sub-tidal rocks. Only few are predatory, feeding on sedentary animals – sponges, hydroids, worms etc. Recently it was discovered that some sea urchins absorb dissolved nutrients from the water, through pores in the spines - amino acids and sugars (Pequignat, .(1970

## **F. Body cavity and its functions**

Sea urchin did not develop internal fluid transport systems (heart or arteries) like in most animal species. The liquid aqueous fluid filling the central coelom volume (**Figs. 14, 6**), is in constant motion, and inhabited by floating free cells, called Coelomocytes, that pass through walls of the various tissues, using the pseudopods, like those of amoebas (hence their name: amebocytes). These cells ingest food particles and move them to the various body tissues. Ion concentration in these body fluids is almost identical to that of seawater, which indicates their external origin, and the full dependence of these fluids on the watery medium surrounding them. Another fluid mass, separated from the main body mass is the “blood fluid system”, whose function is not fully known. The celumocytes, which are the echinoderm equivalent of blood corpuscles, transport food and oxygen as well as removing waste and acting as an immune mechanism.

## **G. Respiratory System**

Respiration in sea urchins is carried out by tiny gills, which are small projections of the flexible body wall, next to the mouth, on the border between the solid skeleton and the elastic perisome membrane. In some species there are no gills, but they can breathe through adapted tube-feet, or through the skin's epidermis tissue itself. *Diadema* and *Echinothrix* have a kind of ball-shaped appendix, which crops up from the anal opening, whose role is still on dabate. The currently explanation is that it might help in breathing .

## **H. Sensory organs and vision**

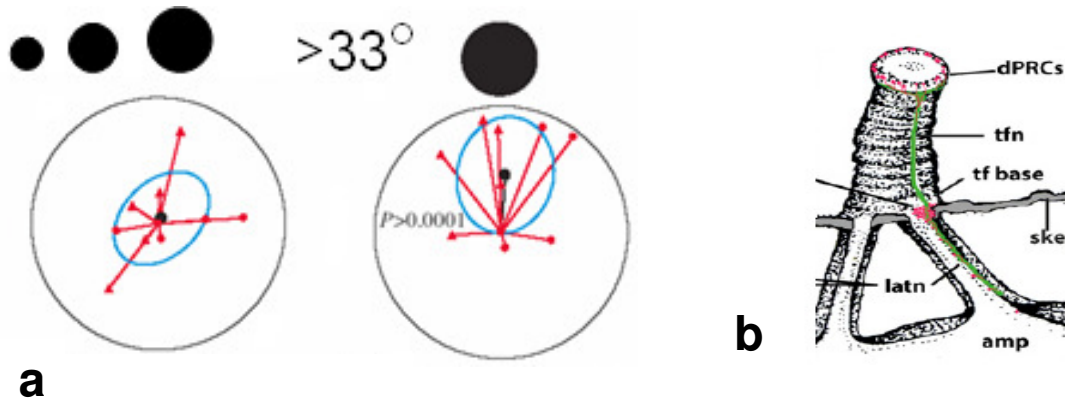
A diver who sees the *Diadema* sea urchin for the first time in its natural habitat, and distinguishes five small white “eyes”, sparkling among the blue stripes adorning the dark test's surface among the longitudinal plates. Research shows that these found that these so-called “iridiophores” that adorn the bodies of urchins of the order Aulodonta, are definitely no eyes (Millott & Manly, 1961). They might, however, be used as tiny mirrors that reflect light falling on them. It was found that the vision pigment – kind of Rhodopsin – is scattered all over the skin, translating incident light perception into a nervous visionary reflex to the central nervous ring that surrounds the mouth and jaws .

Various echinoid species differ in their tendency to light, some are attracted, while others avoid being exposed to light. It was found that urchins living exposed to light will prefer to move toward the light, while individuals who normally live in low light will prefer dark corners. A study made by Belvins & Johnsen (2004) found that the urchins can see a dark stain with a diameter exceeding 33<sup>0</sup> circumference of the arena around them (**Fig. 15a**).

There is no doubt that tube-feet are sensitive to touch, and distinguish between the different substrate types - soft, solid, crumbled etc. Sensory cells, sensitive to small concentrations of chemicals, are spread all over the body, communicating with the nervous system. There is no evidence that echinoids respond to the sound or different water pressures.



In a recent study (Ullrich-Lüter et al., 2011) (**Fig. 15b**), they found that the visual sensitivity of a sea urchin is concentrated on the periphery of the tube-feet, and the nervous link to the central system. They assume that all light sensitive points on the entire body of a sea urchin function act as parts of a compound eye, like those in insects or crabs, and used to navigate to or from light, as needed.



**Figure 15:** (a) Belvins & Johnsen (2004) results showing that urchins placed in the center of an arena are attracted to a dark spot presented to them, only if its size exceeds the peripheral width of 33°. (right circle). Smaller sized spots (left) caused no significant response ; (b) A tube-foot attachment to the body wall, and activated by the central nervous system. Photo receptor cells (in red) are shown on the tube-foot disc, and their link (green) with the nervous system (Ullrich-Lüter et al., 2011).

## I. Ecology and behavior of sea urchins

As in other groups of animals, among echinoids (sea urchins) you may find a large variety of ways of life and preferred habitats: Several species live in the sub-tidal region, where unstable conditions may change rapidly. This forces them to be adaptive, using every available opportunity, and fulfill their living tasks rapidly. On the other hand, species who inhabit the more stable coral reef (although wave energy may change suddenly), tend to live longer and show typical ways of life. The long-spined sea urchin (LSU) is usually short-lived and highly adaptive, whereas Slate-pencil urchin (*Heterocentrotus*) (SPU) whose spines are thick and blunt, is considered to be perennial. Against waves it anchors itself in small reef crevices, using its spines to hold it to the walls. Both species use their spines for locomotion and protection. *Diadema* use their lower spines as “stilts”, to move upon less stable sand, at night when they move around to feed. During daylight they aggregate in large and densely-packed group, where the multiple spines protect them against predatory fish or mammals. In both species the mutable collagen help them to maintain erectile spines with minimal energy invested. The SPU earned their name “slate-penciled” from the habit of some tropical natives to scorch the spines in fire, and use them as simple dark coal-like pencils, to draw and write on slate.

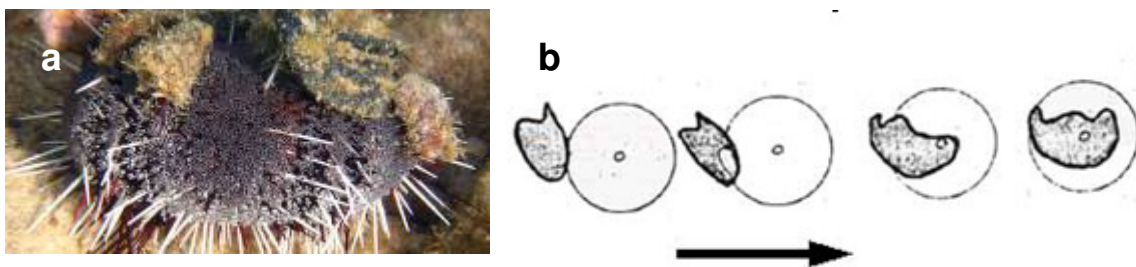
Velvety Sea Urchin (*Tripneustes*), to which we will devote a considerable part of this article, is “super-adaptable”. It is common in tropical oceans, to a latitude of ca. 30° in both the northern and southern sides of the equator. The Red Sea variant of *Tripneustes* (as discovered in this study) shows properties of an annual type in beaches where strong water energy and winter waves - rapid growth and reproduction in the first year. Under more predictable conditions - protected from winter sea storms, but under predation, it acquires a perennial way of life, reflected in high survival and long-life (see p. 83). This feature is called adaptive opportunism. The smallest species among regular echinoids is the “nude” sea urchin (*Nudechinus*), rarely reaching the size for more than one inch across, but the skeleton and hard underneath and let him dig in between grains of gravel sub-tidal zone.

Sea urchins have many enemies. Their slowness of movement made them target for predatory fish, snails and crabs. But various sea urchins species learned to avoid them. Some nocturnal active species hide in crevices of the rocks or reef during the day, or they cover their bodies with pebbles or plant leaves which obscure them (avoidance of harmful light is also a possibility), and leave for feeding at night. A recent study on a long-spined urchin showed that if an echinoid was attacked at their vicinity, its conspecific individual quickly seek for shelter. It was found that they react to the smell of internal fluid of the victim they disperse at different directions, which make it harder for the predator to get them .

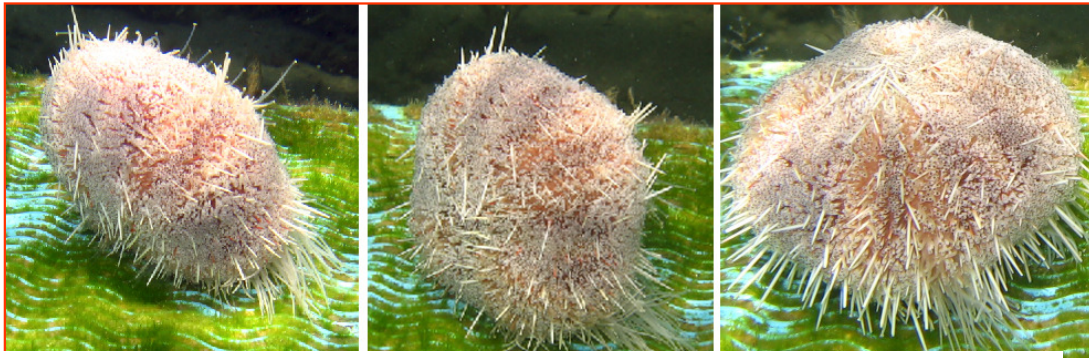
Predators can influence the way of life of an urchin. LSU live in closed bays, less likely to predators to move in. There they are active around the clock, while their con-specific urchins, living in fear of their lives, are mostly nocturnal, spending their daylight hours in congregations or in caves in the reef (Fricke, 1974). It seems that their sensitivity to light is involved. It appears also that predation has a more elaborate ecological role of Red Sea *Tripneustes* (VSU), crucial in determining their behavior and survival .

### Covering reflex

Sea-urchins, sensitive to light, as the VSU, pile upon their “backs” leaves, shell fragments or pebbles - a feature known as covering reflex - to protect them from radiation or as camouflage against predation )Fig. 16A). A similar reflex is shown also by sea-cucumbers, that load on their “backs” herbal leaves, protecting themselves from light and predators alike.



**Figure 16 A.** Ecology and behavior of echinoids: (a) Leaves and other objects accumulated on *Tripneustes* (VSU) (original). (b) Recorded sequence of uptake and covering a strongly illuminated sea-urchin: Drawings show a foreign object collected from the environment and its uploading on the body surface following light beam, focused on the back (small circle), (Lawrence, .1976



**Figure 16 B.** Left to right :Three stages in straightening of a VSU. From an undesirable upside down position they manage to align within minutes (original.)

### **Straightening**

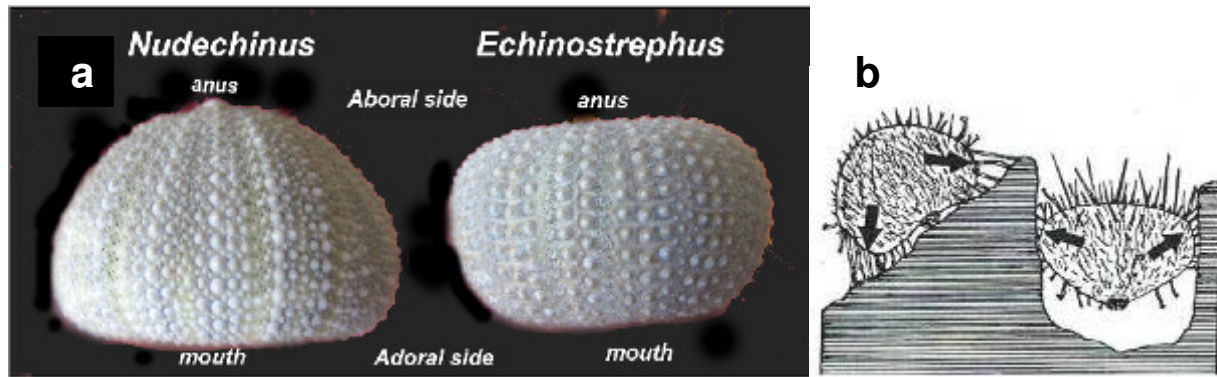
Fish or other larger animals often topple a sea-urchin into an upside down position, vulnerable to predation. Most urchins manage to right themselves using the tube-feet. In long-spine species, the spines must turn at one side, to enable them to do so. In sandy unstable substrate, where tube-feet cannot attach, this may be an impossible mission. In such cases they use any hard object to tilt their weight and roll back to normal position )**Fig. 16B.**(

### **Sea-urchins as a ecological key group**

Being one of the most salient groups of marine vegetarians, ecologists see in them as **key organisms**, capable of profoundly affecting plant cover and its exploitation by other herbivores. Gnawing off the algae, they clean the substrate exposing it to recruitment of corals and other sedentary organisms. Long-spined black sea-urchin come out at night to graze sea-grass not too far from the reef. They form “halos” around coral solitary reef knolls. Those who do not manage to return to their daylight shelter aggregate into large and dense groups ,protected by their long spines. If an urchin is broken, pried open by a predator, it emits coelomic fluids, which is used as a chemical emergency message, to which all its neighbors react by running away in panicked flight (Snyder & Snyder, 1970). Echinoids are considered a major rock eroding agent in tropical beaches. They drill holes and crevices in the reef frame, producing limestone rich sand )**Fig. 17).** The following video clip illustrates the ecological impact of various echinoderms – sea-urchins, sea-stars and brittle-stars. <http://www.youtube.com/watch?v=D3W4OCnHyCs&feature=related>

### **J. Virulence and toxicity**

Poisonous spines of echinoids are not made for aggression. They protect these vulnerable slow moving creatures, going around their innocent feeding habits. However, the spines of several sea-urchins are mildly poisonous. Upon their entering the human body they usually break, excreted a non-toxic fluid which stimulate the nerves system, causing severe pain. The pedicellaria of certain urchins, mainly VSU and others of the family Toxopneustidae, contain highly toxic proteins, whose effectiveness was proven by injection into mice (Alender et al., 1965). This protein is immune against anti-histamines, often used to counteract many medical toxins )Mebs, .(1984



**Figure 17** (a) A comparison between profiles of a typical Regular echinoid *Nudechinus* and the burrowing urchin *Echinostrephus*; (b) Drawing of two attitudes of the same urchins: left a regular echinoid grazes upon the rock surface, having the normal profile of a substrate-living urchin. Arrows indicate the average pull of its suckered tube-feet (which will be later mentioned as influencing its profile shape). Next to it, an unusual profile of the unusual stone burrower, drilling a round hole in the rock, settling inside it with its mouth downward and all its spines turn upwards. Tube feet pull it in an upward direction (arrows), causing the extraordinary upside-down profile of this urchin. It feeds on floating material caught by the tube feet. (Dafni, 1988)

### K. Variety of Regular Sea-urchins in Eilat reefs

The following page (fig. 18) shows a gallery of Regular echinoids, found in the northern Red Sea and the Gulf of Aqaba-Eilat. Please Note the different spine sizes – thickness and length, as well as color variation, influenced by color pigments of the spines, and in some cases the test (skeleton). The primitive Cidarids *Prionocidaris baculosa* and *Eucidaris metularia* are thick-spined, but the spines are rougher than the **Slate-Pencil Urchin** (*Heterocentrotus*) (SPU), whose spines are smooth and hard. Regular sea-urchins vary in size, from the smallest *Nudechinus*, to the large Velvety Sea-Urchin (*Tripneustes*, VSU) the color variation has three components – spines, pedicellaria and tube feet. Each of these components may range from pure white, through brown or red to pitch black. The afore-discussed pedicellaria, normally used to deter parasites has developed into an efficient weapon in *Toxopneustes* – a highly venomous urchin, con-familial with VSU (absent from the Red Sea). Another unique toxic urchin is the Red Sea endemic Fire Urchin (*Asthenosoma marisrubri*), carrying toxic swollen blisters (ampullae) containing acute venom. Luckily none of these sea-urchins is lethal, although the spines of earlier discussed **Long-spined Sea-urchin** (LSU), the former with breakable fragile long spines, and the thin sharp spines of the **double spined Echinothrix calamaris** (DSU) have the ability to inflict severe pain and in some cases cause allergenic responses, or just long-lasting inconvenience. Innocent, non-toxic sea-urchins are *Echinometra matheii*, a common Indo-Pacific species, which is responsible to most rock or reef erosion, while eating the shorter rock-covering algae, turning reefs into chalk sand, typical to that region. A rare sea-urchin, predator of sedentary invertebrates, is *Microcyphus rousseaui*, decorated with a beautiful zigzag pattern of naked zones.





*Microcyphus rousseaui*



*Nudechinus scotiopremnus*



*Heterocentrotus (SPU)*



*Prionocidaris baculosa*



*Asthenosoma marisrubri*



*Eucidaris metularia*



*Echinothrix calamaris (DSU)*



*Diadema setosum (LSU)*



*Echinometra mathaei*



*Tripneustes g. elatensis (juv.)*



*Echinostrephus molaris*



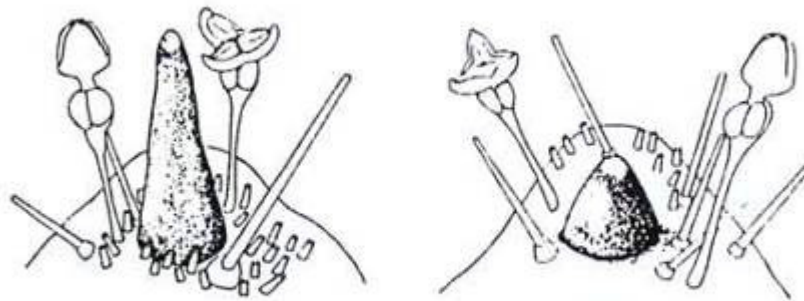
*Tripneustes g. elatensis (VSU)*

**Fig. 18.** Variety of regular echinoids from the Red Sea. Photos: O. Lederman, Y. Shlesinger, A. Colorni, M. Levin, K. Stander, J. Dafni

If you were unlucky and stabbed by sea-urchins spines, don't panic. Most sea-urchins spines are just prickly, but do not really harm. Try to extract them out of the skin using pincers or a sharp needle. Extracting long-spined urchin and the smaller double-spined urchin spines, however, is almost impossible, since the spines have numerous side-spurs, which keep them rooted deep under the skin. The black pigment that spreads rapidly inside the skin is scary. Most sea-urchins are non-venomous. Venomous to a certain extent are Fire Urchin (*Asthenosoma*) and also Double-spined Urchin, (*Echinothrix*) (Fig. 18) but none are lethal. If you are sensitive (some people are sensitive to the **pedicellaria** venom of VSU – Fig. 9) seek medical aid, otherwise try to crush them in the skin using a mild massage, rinse with hot water or alcohol to disinfect the wound, and leave the spines inside, until the pain subsides. The spine's mineral is normally absorbed into the blood system, which may take a few days. Use of a non-sterile sharp object may aggravate the situation. Longer-lasting pain is normally the result of spine touching a nerve fiber, and it may endure some time, a souvenir of the unhappy encounter .

Spines of Slate-pencil-urchin (*Heterocentrotus*, **SPU**) and urchins and urchins of the family Cidaridae, *Eucidaris* and *Prionocidaris* are harmless, used for motion on the ground, like crutches, or strengthen their attachment to reef crevices. **SPU** spines show when broken dark-light concentric rings - reminiscent of the annual rings in trees, but since in the tropics there are less pronounced seasons, no correlation was demonstrated with annual rings .

Type oceanic bizarre **Shingle urchin** (*Colobocentrotus*) - not found in the Red Sea – which lives in highly exposed wave stricken shores, is extremely flat and has very thick and short spines, with truncated ends, that end in the same length, forming a “flattened shingle” as shield against the pounding waves. Only spines around and below the ambitus line are somewhat longer and flattened (Fig. 65b)



**Figure 19.** Male-female sexual difference in Velvet Sea-Urchin (VSU - *Tripneustes* ):. Significant difference in length of papilla from which eggs and sperm are emitted - a long papilla of the male (left) and a shorter one in the female. Shown also are several pedicellaria (Tahara and colleagues, .1958



## L. Reproduction and larval development

The five reproductive glands (Gonads) of echinoids open directly to the sea by five small pores (gonopores) that surround the anus. Injection of potassium chloride (KCl) at a 0.5 Mol/liter concentration stimulates the ripe urchins, males or females, to spawn their eggs and sperm. Minutes after injection the rosy eggs or yellowish sperm appear at the genital pores. Often, a synchronizing “social” hormone is involved, stimulating neighbors - both male and female (fig. 19) - to join the spawning, thereby increasing the chances of fertilization. Eggs are tiny and perfectly round, with a diameter not exceeding 0.1 mm. After external fertilization they start cleavage - dividing into two, four, eight etc. Since they have almost no yolk or other material to support the developing egg, the larval shape and development are morphologically “classic” and therefore often used to illustrate in schools and universities the juvenile development in animals in general.

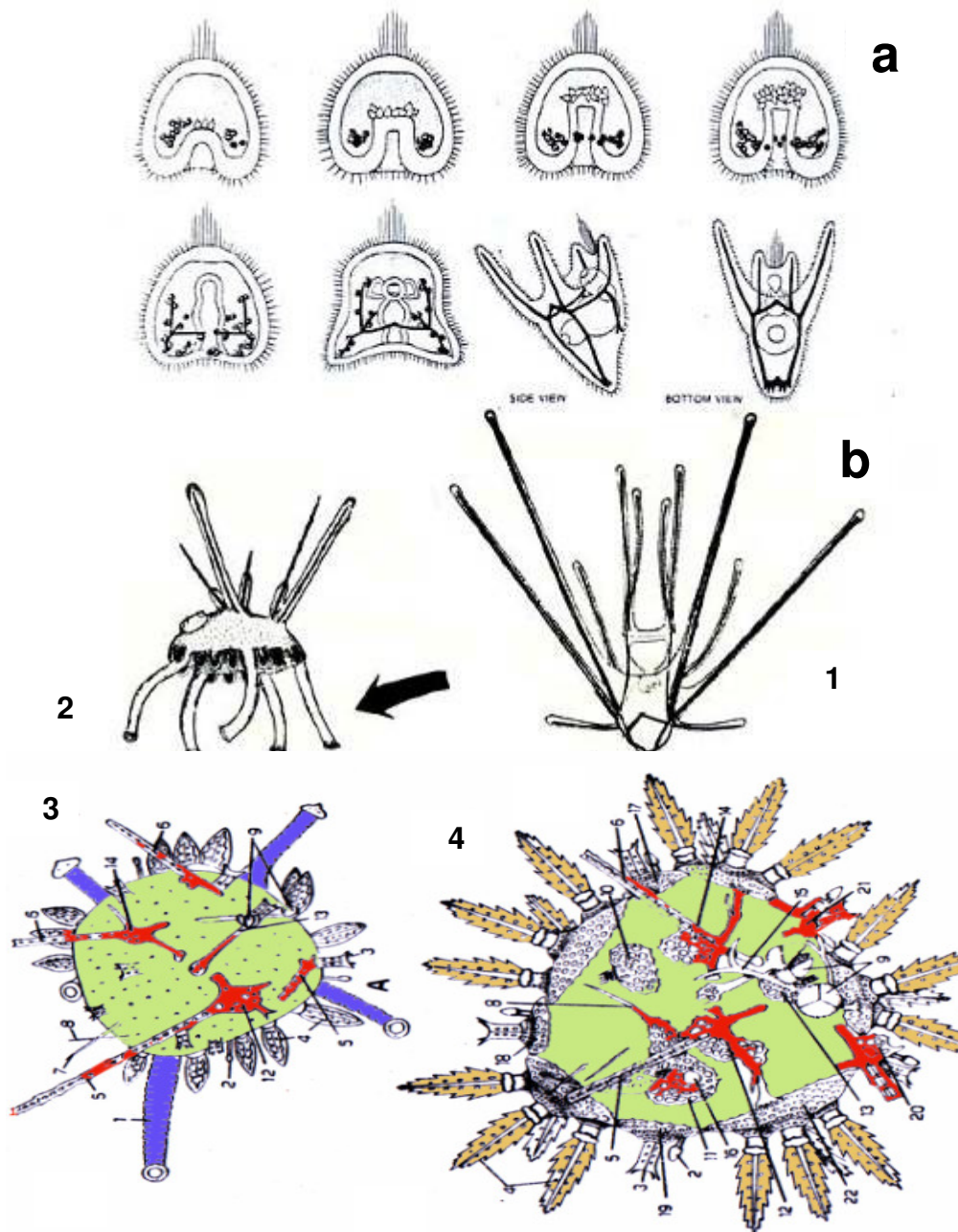
At the end of the first phase is formed **Blastula**, which is a strawberry like ball-shaped. Next it invaginates - a depression appears in the lower side, where the fetal mouth appears, at a second stage – the **Gastrula**, at this stage the coelom starts as two small cell-enveloped cavities, and the larval form, **Pluteus** (Fig. 20a) emerges. It moves about by numerous cilia, long or short flagella that flip in unison to help in swimming in the water and catching micro organisms. At the next stage – the In the central space of the pluteus the embryonic skeleton starts to form, in shape of small needles developing into a typical “chair-like” structure the at the first needle of the fetal skeleton.

Breeding of urchin species in the Red Sea has been studied in the 1960's by the American scientist **John Pearse** (1969-1970), who compared their seasonal reproductive patterns to those in other parts of the Indo-Pacific Ocean.

Each class of Echinoderms has a unique larval form. In Echinoids (sea-urchins) it is called **echinopluteus**, in brittle-stars (Ophiroids) it is a superficially similar **Ophio-pluteus**, while other groups has different types of larvae - **Bipinnaria** in Sea-stars, and **Auricularia** in Sea- cucumbers. All larval stages show a bilateral symmetry, and it seems that radial symmetry in echinoderms seems to be secondary development .

The echinopluteus drifts for several weeks or months, as planktonic stage. It can exist this form for several months, until right conditions - water temperature, substrate type, food variety etc – are achieved. Then the metamorphosis from larva to adult will begin, also induced by hormones. The adult stage is a soft liquid globe - the **echinus** (Fig. 20b): The larval spines are absorbed into the body, and new spines develop on the spherical test (skeleton). Echinus becomes gradually the adult sea-urchin as we know it.

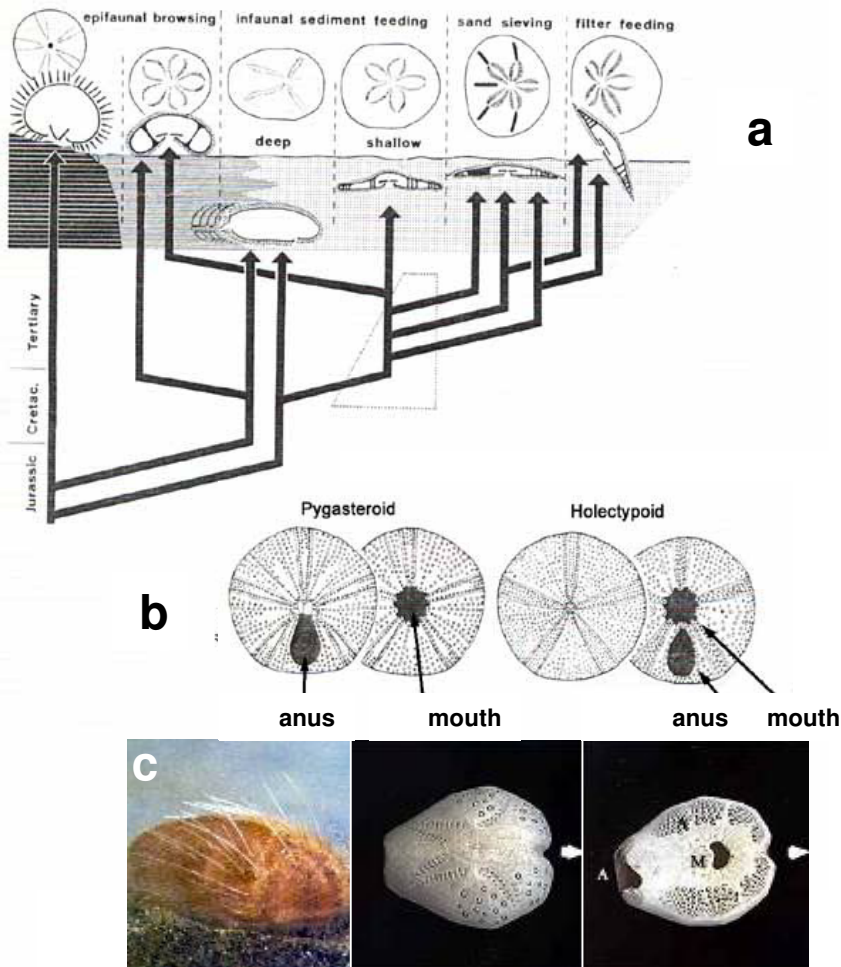
In some urchins, like the toxic **Fire Urchin** in Japan, a direct development from the large egg (1.2 mm) to the echinus stage is shown, skipping the pluteus stage (Amemiya & Tsuchia, 1979). In all sea-urchins reproduction is a social event dictated by maturity of the reproductive glands in individuals taking part in the process, apparently triggered by water temperature, lighting regime (night, moonlight, etc.) or other celestial factor .



**Figure 20.** Skeletal parts of echinopluteus larvae of regular echinoids and their metamorphosis into the adult. **(a)** Development of the early larval from gastrula stage to the echinopluteus. (Black – skeletal calcite spiculae of embryo, to the “chair-like” skeleton) **(b 1-2)** The process of metamorphosis – from long-spine pluteus to first echinus, showing the first five tube feet. Larval spines are already partly absorbed. **(b 3-4)** First adult spines are broad and appear among the tube feet and partly absorbed larval spines (red). Gradually skeletal plates cover the soft bubble-like urchin, to be arranged in the typical five-radial system. In stage 4 pedicellariae also show (right). (based on Gordon, 1926 and other sources).

## M. The transition toward irregular sea-urchins

Irregular echinoids (Echinoidea, sub-class **Irregularia**) are different from the Regular urchins in several characteristics (Fig. 21a-b). They represent the tendency to be either (a) flat and having bilateral symmetry, and live buried inside the sandy sea-bed, known as “**sand dollars**”, or swollen “**heart urchins**” (c), but showing bilateral symmetry, they move through the sand by “rowing” using their somewhat flattened spines. Sand dollars are named so, because of their similarity to coins, accentuated by a five-ray “**petaloid**” pattern. Four evolutionary processes were involved in the development of this advanced group (Gordon, :1926)



**Figure ) .21a** The evolution of various irregular sea-urchins (from the Jurassic to present day), related to their tendency to inhabit soft bottom (infaunal) habitats, compared with the epifaunal hard bottom Regularia (left). Their morphology is determined by their habits, from grazing of algae to feed on organic debris in the upper sand layer, to “swim” and in the deeper layers. **(b)** Two early Mesozoic Irregular Pygasteroid and Holoctypoid urchins, illustrate stage of anus withdrawal from the apical plate ring, to its final position, next to the mouth; **(c)** Living urchin and dried test of a *Lovenia* “**Heart urchin**”, a common infaunal urchin, well adapted to move in the sand using its “oar-like” shorter spines. (Source: (a) A. Seilacher, 1979; (b) A. Smith, 1984, (c) A. Shub & originals.)

### I. Migration of the anus

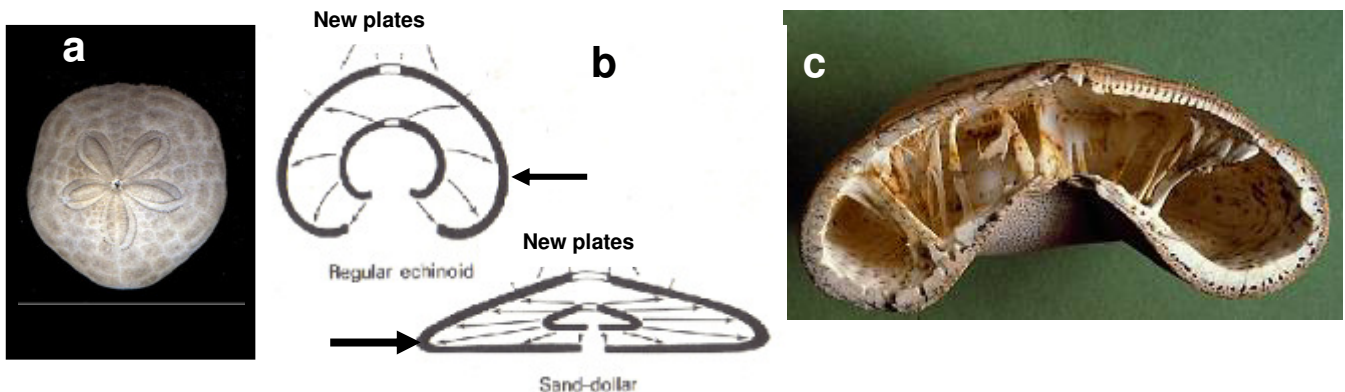
In Regular echinoids, the anus is situated at the test apex (apical pole), in close association with the ambulacral and genital systems openings. The evolution of the Irregularia started with the anus shift to a lower–posterior position **Fig. 21b** shows this process in two Mesozoic extinct orders, **Pygasterioids** and **Holoctypoids**. In the former, the anus opening is oval, elongated, and sideways to the apex, whereas in the latter it reached already the lower half of the test, and established itself next to the mouth, resulting with bilateral symmetry. By the way, in Eilat mountains, in Cretaceous limestone rocks, the species *Coenholectypus larteti* is a common fossil.

## II. Shift to live on sand and moving within

In their new habitat - diving and moving beneath the sand surface both the spines and tube feet have lost their former roles and assumed an adverse position: Tube feet, which require a solid substrate to stick to in order to hold or move the body, are of no use under these circumstances, assumes a respiratory role – gaseous exchange - oxygen with carbon dioxide. On the upper part of the irregular urchins the Amb system forms “petaloids” (**Fig. 22a**). of adapted tube feet to breathing .

Spines are not used as protection in most irregular urchins. In the sand they can either move on the surface by walking, as "crutches" or “swimming” in the sand, using them as “oars” as main means of mobility. In the most advanced **Spatangoida** order, such as **Lovenia**, the spines rotate in a half circular, coordinated motion, back and forth, pushing the sand backwards moving the body forward, just like oars in a boat are manipulated. This development was made in several stages: the spine base, that in regular urchin is a rounded protrusion (boss), sinks into the frame, creating a semi-circular furrow (**Fig. 21c**), which enables the spines to move in one direction. Next, the spines assume an “oar-like” flat surface, and become crescent-shape, to optimize their rowing movement .

Muscles also adapt to the semi-circular orientation of sand-swimming movement. This adaptation to move in soft sand demanded changes in the overall shape of the test, from circular and domed regular shape of the test into a hydrodynamic shape, with minimal drag or friction while moving through the sand. Scientists believe that a curved shape creates also a lift force, resulting from a negative pressure at the top, similar to the aeroplane wing (Telford, 1981) that prevents the test from sinking during movement. Various species of **Sand-dollars** have “key-holes” and recesses at the skeleton edge, which also facilitates their movement in the sand. An interesting fact is that most irregular urchins radically change their plate growth patterns – new plates added at the apex, do not cross over the ambitus line, and the number of plates in the lower part, beneath the “equator” (ambitus) remain fixed (**Fig. 22b**). In result, the lowermost plates grow immensely. This is in part due to the supportive “pillars” (**Fig. 22c**). that fix the test shape, and stops the plate downward migration.

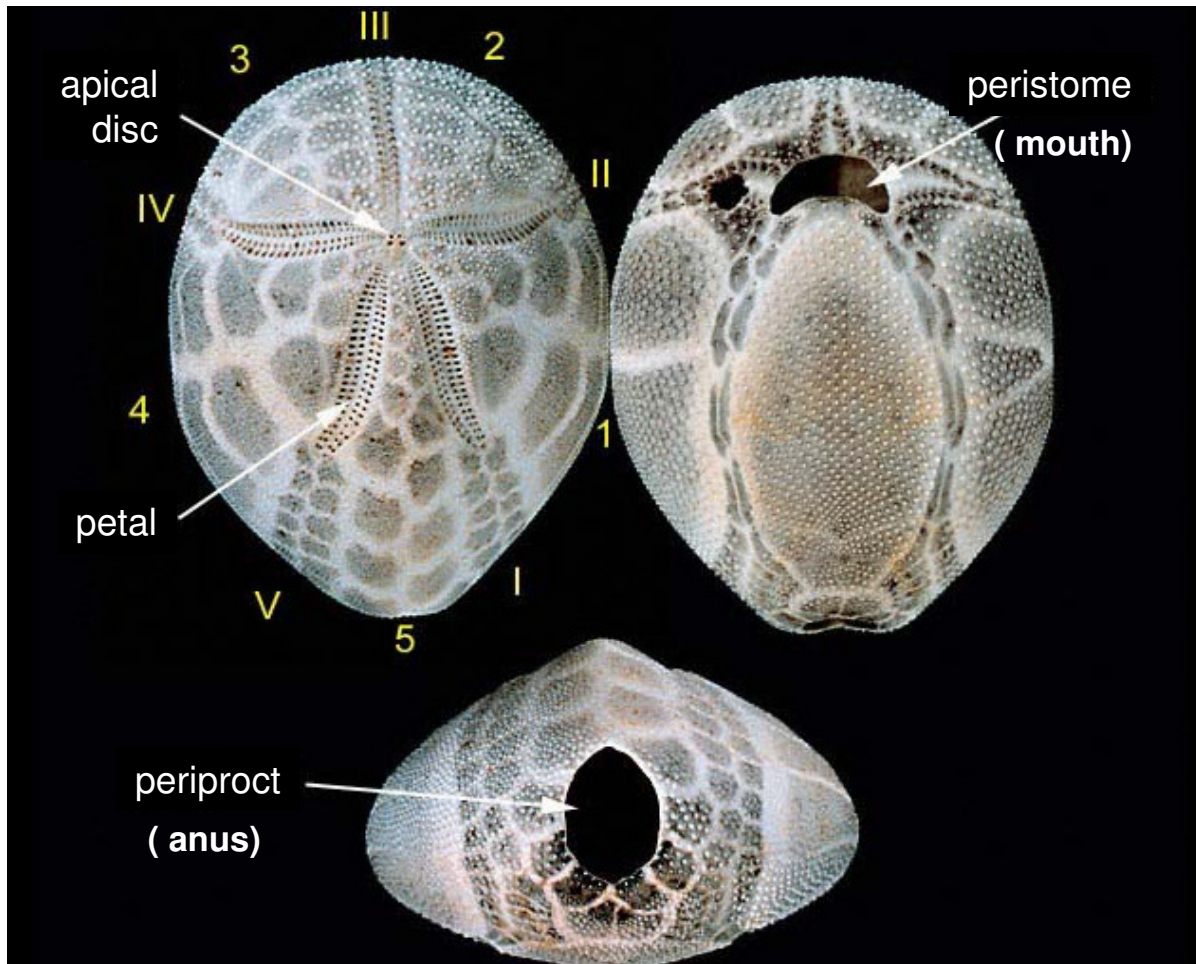


**Figure ) .22a)** An irregular echinoid, a sand dollar )**Clypeaster**) **(b)** The difference between a regular echinoid, whose added new plates are continuously moving downwards, and a flat irregular echinoid, a sand dollar, where the new plates are limited to the area above ambitus (arrows). The number of subambital plates is fixed, growing continuously. This process is mainly caused by appearance of **(c)** Supporting pillars between the aboral and adoral halves - ( A. Smith, 1984, and original.)



### III. Tube feet as breathing organ

Tube feet of the upper, aboral part of irregular sea-urchins are mostly adorned by a five-branched petaloid (flower petal-like ) shape (**Fig. 22a, 23**), in which the tube feet pores are aligned in parallel line, meeting at the distal end. At the lower, adoral side, the tube feet are mainly used for foraging, gather food particles and deliver them to the mouth.



**Figure 23.** An irregular echinoid (spatangoid) test, showing three aspects: **aboral** (left), **adoral** (right) and **posterior** view. Note the relative larger size of the adoral (lower) plates whose largest one is called **Plastron**. Note also the plates' growth zones – older are dark, darker) and widening sutures are whitish ( NHM Echinoid Directory.)

### IV. Changes in the digestive tract

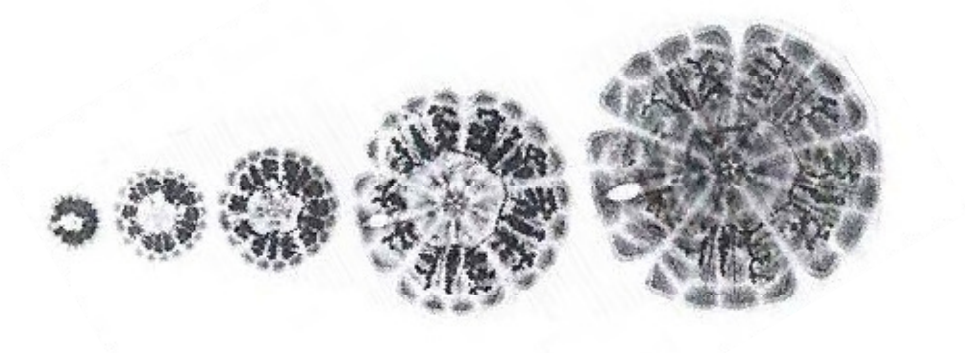
Digestive system turned from grazing algae to digest organic matter particles found in sand. In san dollars the "Aristotle's lantern" is extremely flat, used to as his role to crush shells. In the "heart urchins" it entirely disappeared.

## N. Unique phenomena in Irregularia

A unique phenomenon was observed in some Irregular urchins (genera *Echinocardium* and *Brissopsis* from the Mediterranean) – Ferric iron ion storage in their guts and connective tissue (Buchanan et al., 1980). More strange seems their ability to accumulate **magnetite**, another mineral of iron found in the guts of sand dollars (Fig. 24). As Implied by its name, this last-mentioned ferrous mineral shows explicit magnetic properties, being attracted by iron metal and magnetometers. At first it was thought magnetism is used to navigate in the sand (following a discovery that certain bacteria do so, thus exploiting the earth's magnetic field to coordinate), but a prevailing theory is that the heavy metal is used in the sand dollar, especially juvenile ones, as “weight belt” to “dive” into the sand, stabilize them against the sweeping movement of the waves and moving sands .

In the South and Central America sand dollars are collected as decoration, and their shapes produced from silver or even gold, that are many dollars worth.

The most common “heart urchin” in the Gulf of Eilat is *Lovenia* (*Lovenia elongata*) (Fig. 21c). This small and fragile species, not exceeding 5 cm, is agile: it digs in the sand in moments, (Ferber & Lawrence, 1976). Its path in the sand is easily shown as furrows plown in the sand. *Lovenia*’s many long spines look like fur around its test, but they are sharp to the touch and painful if they penetrate the skin. The greatest of their kind is the short-spined *Metalia* (*Metalia sternalis*) reaching 18 cm, its bare skeleton (test) often shows beautiful whitish growth zones around the formerly darker plates (Fig. 22c) – very useful for growth pattern analyses. It inhabits sand at a depth of 5-10 m. More rare is *Paraster gibberulus*, the shape of which shows deep furrows along the dorsal side. The smallest of this group is *Echinocyamus crispus* with an average size of about 0.5 cm. It is often brought up by deep sea-bottom dredging.

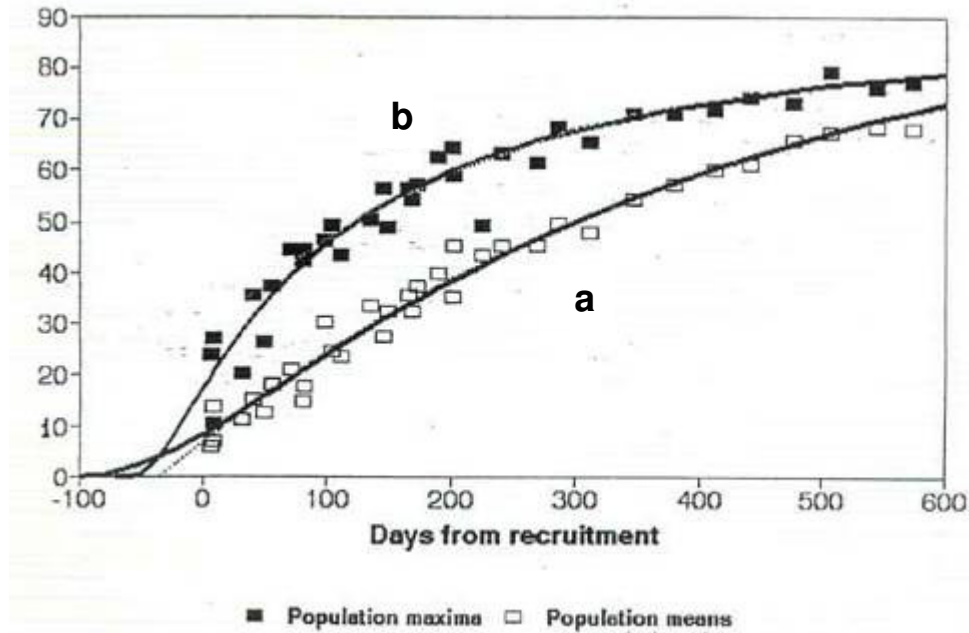


**Figure 24.** Storage of the mineral magnetite in different sizes of san dollars, non-permeable to light rays, shown in X-ray pictures of sand dollar. Adults, whose digging into the sand is easy, no longer need the “weight belts” ( Seilacher, .(1979



### O. Growth rates

Regular sea-urchins settle on the ground when the size of 1-3 mm, after undergoing transformation (metamorphosis). The rate of growth depends on food availability, and if they could use them. Under optimal conditions initially the skeleton increases steadily and the growth rate gradually diminished, creating a "sigmoid" (S-shaped, Figure 23) growth curve. Older urchins may maintain their minimal growth rate for many months or even years. Restrictive conditions - lack of food, high population density and other stresses – strongly affect their growth rate, and sometimes the individuals remain stunted, resulting in dwarf skeletons. The echinoid expert Ebert suggested that under extreme food shortage, the food resources are channeled to essential body parts, like "lantern of Aristotle" which is relatively large compared with other body and skeletal parts, which are neglected. He even suggested that under such conditions "negative growth" may result (Ebert, 1967-1980), but later contradicted this idea. Growth cessation does not usually involve dissolution of skeletal part, however, except when the increasing Aristotle's lantern grows and causes the increase of the peristome opening by dissolution of oldest plates .



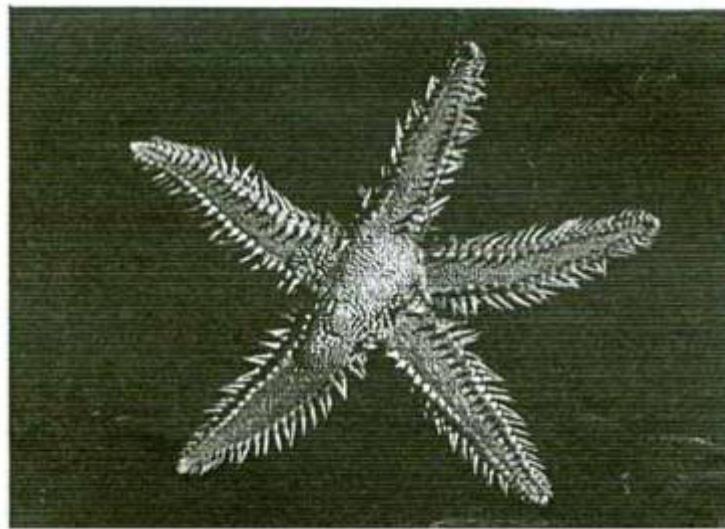
**Figure 25.** Sigmoid growth-curve of Velvet Sea-Urchin (*Tripneustes*) diameter, in mm, from Eilat, as measured in urchins from day of recruitment in the sub-tidal zone. Two methods are shown: (a) The mean size for the entire population, and (b) the largest individuals in each sampling event, aims to show the maximal size they reach potentially under optimal conditions. Raw data, the distribution of sizes at different times, appear in **Figure 67a**. Johnston's growth curve is adapted to the data (Dafni, .1992)

#### .4Other echinoderms :

##### I. sea-stars and Brittle-stars: similarities and differences

Ostensibly, there is almost no difference between a five-armed sea-star (some call them erroneously starfish), and a brittle-star with a similar number of arms, and distinction between them is not always obvious. However, anatomic differences and the various ways of life are larger than a superficial resemblance. First, brittle-star arms are made of highly flexible units - "vertebrae", and are distinct from the central disc, covered by thick dermal plates, whereas the arms of sea-stars are an organic extension of the central part of the body, and the intestine branches into those five or more arms..

A second difference is very significant and it is their diet: Sea-stars are carnivores of molluscs, which can easily overcome creatures many times larger than themselves. As such they are equipped with sense organs, to smell, heat and touch and, on top of it – "eye spots" consisting of many ocelli, epithelial cells that respond to light. Each ocellus is covered by a transparent cuticle that acts as a lens. Brittle-stars are not predators. They feed mainly on organic matter - crumbs, dead invertebrates etc. Some species roam the seafloor collecting debris and excrement. One notable species, Shore Brittle-star )*Ophiocoma scolopendrina*), spread their arms against rising water when tide comes in, catching drifting and floating food particles )**Fig. 36**). Other brittle-stars )*Ophiothrix spp.*) inhabit stony and soft corals, adhering to their surface. Special feature, shown by them is a voluntary shedding of arms, possibly by softening the collagen tissue against opposing muscle contraction.



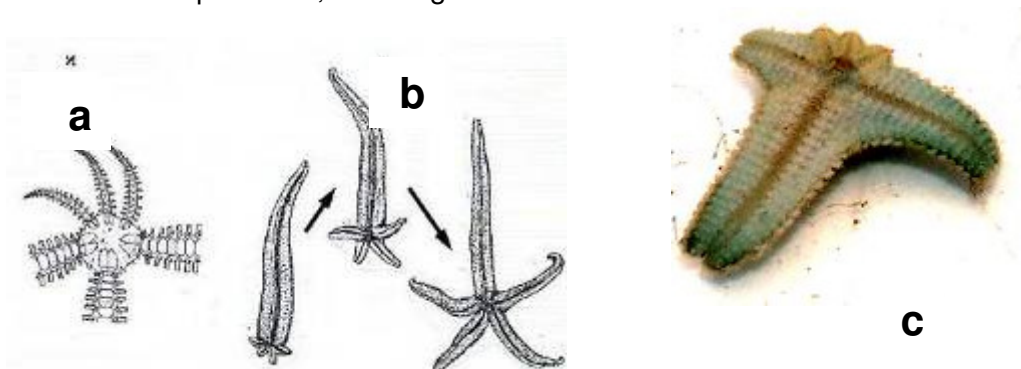
**Figure 26.** Comb Sea-star (*Astropecten*) swallowing its mollusc prey intact, later ejecting the empty shell. Here, an individual blown up by its food, a snail shell before being ejected (source - Clark, .(1962

## II Sea-stars: External predation

Despite their almost phlegmatic and sluggish appearance - creatures devoid of head or real arms, “surfing” the soft substrate or crawling on rock and reef formations - sea-stars are flexible, agile and firm in their own way. Like sea-urchins, their skeleton is made of calcium carbonate ossicles, but in them the limestone is submerged in thick connective tissue, which provides maximum flexibility to their bodies. Their need for long hours bending over their prey - an oyster locked itself in its shell or coral which shrank into its stone basement - requires considerable energy investment for a long period of time, well beyond what it could raise from its own sources (Eylers, 1976). So evolution has equipped him with the unique kind of connective mutable collagen tissue (MCT). This type of connective tissue can harden instantly, on command from the nervous system, or in response to external stimuli. Softening of the collagen at will enables it to disengage, move from its position, and shift to muscle action. Some sea-stars ingest snails or clams alive, and after digestion of the edible tissue, release the prey's shells through the mouth (they lack an anal opening) to the sea-bed (**Fig. 26**). As later discussed, some sea-stars can digest prey larger than themselves, by pulling out their frontal part of the stomach out of the body and spreading it on the coral tissue, secreting digestive juices, and ingesting the prey.

## III Regeneration as life style

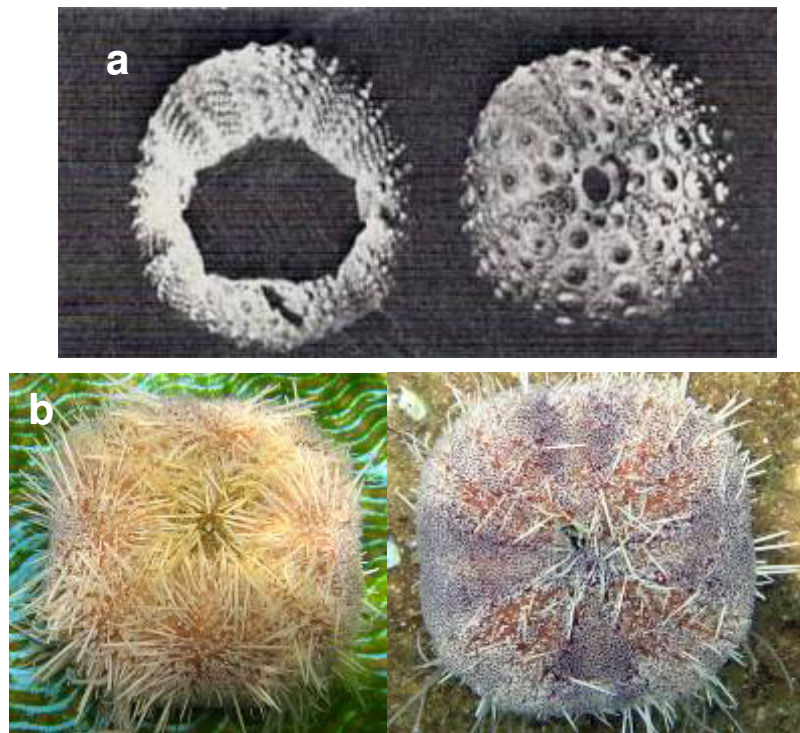
sea-stars possess an amazing ability to recover up to 80% of their body mass after fragmentation (rehabilitate 4 out of 5 arms). Brittle-stars are also able to restore damaged or missing inner and outer organs. If we add this feature to their ability to shed one or more arms voluntarily, we may consider it as another way of reproduction. In fact, several, mostly smaller kinds of sea-stars, as well as brittle-stars, utilize this ability, primarily aimed to rehabilitate after an accident, as legitimate way of asexual reproduction. Some of them reproduce in the proper season through sexual reproduction, whereas in other seasons, or on different ecological conditions, they reproduce only asexually, in fragmentation. Noticeable among the sea-stars, such as Pigmy Sea-Star (*Asterina*), that among them we often find more split or broken individuals at regeneration than intact ones. One brittle-star (*Ophiactis savignyi*), is known as having six arms, half of them original and the other half are shorter and smaller arms – apparently regenerated from amputation. (**Fig. 27a**). Sexual reproduction is seasonal. Some sea-stars are hermaphrodites, or change sex from male to female .



**Figure 27.** Regeneration in sea-stars and brittle-stars. (a) brittle-star (*Ophiocoma savignyi*) half intact half regenerating (Source - Clark, ), (1962b) stages in rehabilitation of Comet sea-star (*Linckia*), from one remained arm with 4 more stubs (called “comet”) to almost equal arms; and (c) Pigmy Sea-Star (*Asterina*) after fragmentation, regenerating new arms (various sources.)

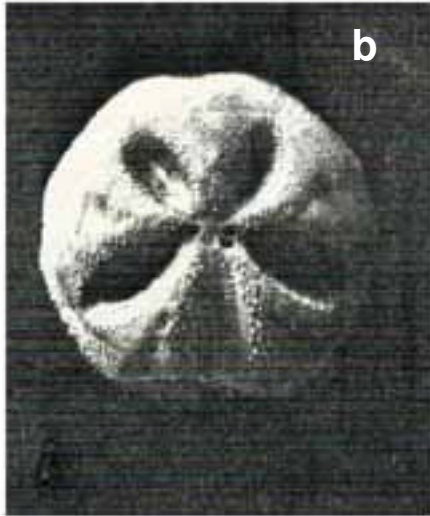
Asexual reproduction through fragmentation is usually limited to small sea-stars and brittle-stars. Larger species of the same can at best restore severed or amputated arms. For the process of regeneration – rehabilitation - at least the central part that contains the nerve center and a “tube feet” system, must remain. In smaller species like *Linckia* – a stub of an arm is enough to induce restoration of the whole body. In fact, in some circumstances voluntary breakage of the entire star into five equal arms, each one replaces missing organs - the mouth, ambulacral, digestive and nervous systems. After such division, we usually find a single arm, which the fractured side is strongly constricted to prevent inner parts from being exposed. Within weeks the central part is restored, and arm buds appear. At this stage, one intact long arm is shown, and four (or more) very short arms, shows a typical “comet”. )**Fig. 27b,c** .(

Regenerative power is not limited only to sea-stars and brittle-stars. Feather-stars also regenerate broken “feathered” arms, and sea-cucumbers are capable of blurting out parts of the intestine guts, or be cut into two voluntarily and quickly restore the damaged parts. sea-urchins recovery of missing body parts is limited by the necessity to maintain the peripheral skeletal structure. However, limited recovery of one or two skeletal sectors can be restored. The phenomenon of four-fold symmetry in sea-urchins (as well as in sea-stars etc.) is not uncommon. They show only four tube feet columns, compared to the normal pentamerous symmetry - usually associated with regeneration after injury. In cases where one can identify the damaged part , a good sign is that in these urchins the “Aristotle’s lantern” has five jaws (**Fig. 28a**).. In more extreme cases also the lantern is four fold, which indicates an early age damage (**Fig. 28b**.)



**Figure 28.** “Square” sea-urchins: (a) Cause of the deformity - an early injury to one Amb column. Such urchins are characterized by a five-jawed Aristotle’s lantern. (b): A four-jawed lantern Velvety Sea-Urchin (*Tripneustes g. elatensis*), seen from adoral side (left) and from above (right) (original photos.)





**Figure 29.** Brooding pouches in sea-urchins: (a) An extinct “marsupiate” regular urchin *Paradoxechinus* showing dorsal sinking to form space for egg brooding. (Philip & Foster, 1971) (b) An extant (living today) irregular sea-urchin, *Abatus*, with deep depressions resulting from deeper than usual “petaloid” to accommodate larval incubation (original.)



Although eggs shed to the outer medium, and inseminated in open water is the rule among echinoderms, in many sea-urchins we find adaptation to environmental stress conditions, exhibited among other adaptations to keep the first reproduction stages of the developing larvae in a protected pouch or enclosure, among the spines next to the mouth or in skeleton depressions in the female (**Fig. 29a**). Care of the offspring appears especially in harsh environmental habitats - in the arctic regions. In These cases the larvae skip the earliest embryonic stage and develop directly to a pre-adult stage (see the Fire Urchin case, **page 27**). In an irregular sea-urchins living in South American shores and the coast of Antarctica, we find special pockets modulated from the petaloid – a five-fold depression for incubation (**Fig. 29b**).

The dense forest of spines is a privileged shelter from predation for nocturnal fishes during daytime, as well as a “kindergarten” for coral reef juvenile fishes, and night shelter for day-active fish and invertebrates .



#### IV The case of Crown-of-Thorns Sea-star

Until the year 1966 nobody paid attention to the important role of sea-stars in nature, although there was ample evidence on their predation in oyster ponds. Then, the Natural Museum in Australia published an alarming report on unusual increase in the Crown-of-Thorn Sea-star (**COTS**) as the main predator capable of killing entire coral reef colonies in the worldwide Australia's Great Coral Reefs, affecting the entire reef. Following this article, similar reports began to arriving from all tropical sea - the islands of Guam, Malaysia, Borneo, Fiji, Palau and Australia, heralding a new era in marine research – an attempt to deal with a global event - the destruction of tropical coral reefs. Recognizing the importance of protecting the reefs that prevent the destructive action of waves on the tropical coasts, the pessimists predicted that "Judgment Day" of the reefs is imminent. They imagined a scenario that the death of the coral will expose the shore to oceanic waves, with significant damage to human settlements. Even the optimists had difficulty to digest the threatening news. The question raised was what causes sea-star population to grow so immensely, and how can we stop it. Marine research laboratories launched intensive and comprehensive research on the biology of these stars - their relationship to coral reef, and at the same time, attempting to discover who are their predators. The researchers also conducted hunting operations, to kill as many they can, thus preventing the the crown-of-thorns march. At the same time, they launched basic research aimed to learn who is this creature, how does it hover on top of a coral colony devouring it without even putting it into its mouth, and threatens the entire world of coral reefs?



**Figure 30. Crown-of-Thorn Sea-star:** (a) Perching on top of a stony coral, relishing on spreading its tissue; (b) Juvenile of 5 cm across (one arm missing); (c) Giant Triton snail (*Charonia tritonis*), the most effective predator of sea-stars, (d) and a Harlequin Shrimp (*Hymenocera elegans*), a small shrimp predator, capable to match the giant sea-star (Photo, in the same order: A. Lederman; J. Dafni; D. Vered and A. Diamant)

COTS is common throughout the Indo-Pacific Ocean. Its diameter reaches 60 cm, has multiple, 12 to 18 arms. Its entire body is covered by long spines-thorns (up to 3 cm long) thick and sharp (**Fig. 30a**). In the Red Sea it is not uncommon, but rarely appears in daytime, and most of its activity is nocturnal. At night it moved onto the reefs using its tube-feet, while during the day it hides in the shade of coral or dark crevices. Like most sea-stars it lacks real eyes – although sensitive organs sense the light while chemical sensory organs detect their preferred prey, stony corals, and the smell spread out of body fluids of the corals attract them. For some reason it prefers the stony branched corals to the soft or massive ones. It is explained by their inability to hold them against erosion and displacement by waves. Since it cannot not swallow the entire coral heads, protected by massive limestone skeleton, and because of lack of teeth that can tear the corals flesh and separate it from the skeletons, COTS "invented" a unique form of predation, unparalleled in the animal world: it pulls out its fore part of the stomach through the mouth and stretches it over coral head or branches tissue, and secrete gut digestive juices. It was found that adding sea-water which passed through a container which held live corals, to cause the COTS to draw out the stomach through its mouth. After several hours of preliminary digestion by enzymes the COTS begins to absorb the macerated coral tissue, leaving behind bare coral skeleton. A normal sized COTS may digest in one night a coral colony 30 cm in diameter, the result of many years of slow growth. The bare limestone attracts within days settlement of green and brown algae. Usually the coral dies - rehabilitation was occasional .

Under normal conditions there is a balance between predator and prey, and COTS will not "sawing the branch on which it seats", the rate of predation does not exceed the rate of coral growth, recruitment and renewal. Sometimes, for some reason not yet fully explained a "population explosion" of COTS causes disruption of the balance (**Fig. 30b**). Some ecologists blamed the disruption of the reef food web, to commercial removal of COTS main predator, the Giant Triton (*Charonia tritonis*) (**Fig. 30c**), for sale to tourists, thus causing the COTS to thrive beyond the normal rate. Guilt also slammed into the U.S. nuclear policy, which turned some Pacific islands to experimental sites for nuclear bombs. Explosions caused, according to these researchers, the destruction of the reefs, which has, after a decade, caused uncontrolled proliferation of the coral predator.

It is interesting, that five years prior to the alarming population explosion of COTS, the known American marine scientist **Thomas F. Goreau**, who joined the Israeli expedition to the South Red Sea noticed their high numbers, and predicted that under certain ecological circumstances the balance between predator and prey may get out of control, causing extensive coral reef destruction. And indeed, at the height of the outbreak, between the years 1968 to 1975, it seemed that nothing would save the corals, and most of them will find their way into its protruding stomach. In affected reefs scientists counted more than 600 COTS per diving hour (an arbitrary measure developed to quantify their frequency), or ca. 1 COTS for each square meter (a thousand times more than the in normal reef). Reports on progress of a "moving front" were that they move at a rate of 1 km per month, leaving behind a wake of dead corals, partly or fully covered by algae. Attempts to fight back by capturing them, and injecting a poison - concentrated formaldehyde – into their bodies, were expensive - a modest estimate held that the killing of all of each dead COTS came to a cost of \$35 and their effectiveness was little.

.In the meantime, a comprehensive study found the unusual reproductive potential rate of COTS. During breeding flocks of 50 to 100 individuals having an "orgy " through which each female lays several hundred thousand eggs. The total number of eggs laid reach year by such flock were estimated as 60 million. Their growth rate is rapid and an average individual comes within three years to a diameter of an adult, 40-20 cm. This study identified several COTS predators unknown before: one is the tiny Harlequin shrimp )*Hymenocera elegans*) )Fig. 30d), living on the reef, feeding with small sea-stars. A pair of these shrimps is capable, a way that is still poorly understood, handle the larger COTS – paralyze them and eat their flesh. Polychaete worms and small crabs living among the coral branches are able to harass and chase away the predator, thus repairing their host.

All the same, the COTS frenzy faded within a decade or so. In the damaged reefs COTS started to starve, and the reefs began a long rehabilitation process. In retrospect, it turned out that it was another of the "population explosion" cases that accompany the biological history of Earth. From a "core", area of intensive breeding from which the massive reproduction began, the larvae spread out by the currents. Some open questions remain, among which was "how much was involved in these breakouts the human factor". These studies were worthwhile from several aspects: one, it was the first time biologists had deal with natural catastrophes in a large scale: It was necessary to locate the factors involved and recommend chemical and physical short and longer-term solutions, as well as to study the impact of these phenomena on the global ecological balance. Two, the "take-away lesson" was that the sea, despite being vast and having a unimaginable volume of water is still limited and destructive processes may spread out like wild fire .

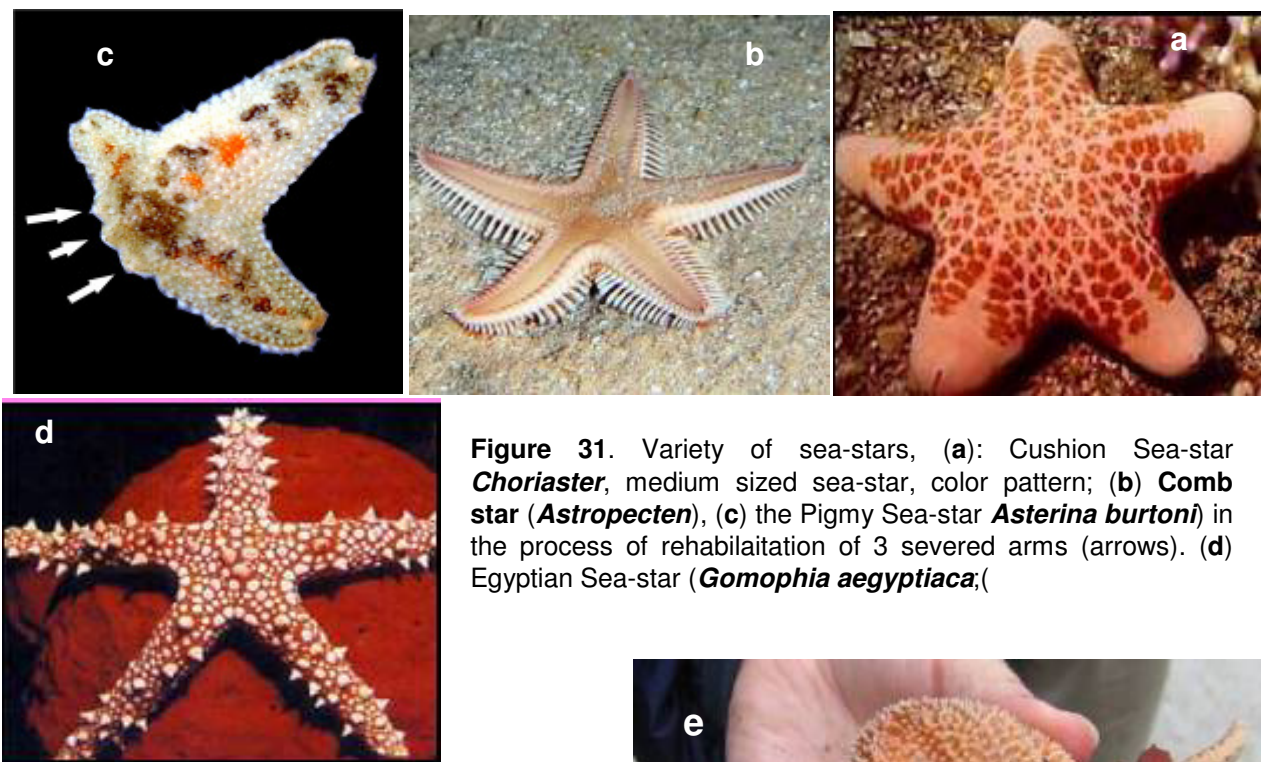
The decade after these events was characterized by several phenomena of over proliferation, as well as mass mortalities in echinoderms of tropical and temperate sea. Over-increase of sea-urchins in the coasts of North America was followed by over grazing of algae, the "feeding ground" of many commercially valued fish and crustaceans with commercial value.

On the other hand, disease outbreaks caused total disappearance of sea-urchins from other areas, which had also an impact on natural marine habitats. It is possible that the marine biology science is now more equipped to deal with these phenomena .

Other large sea-stars were also suspected to be coral predators, but so far it seems that they have only a marginal impact. Short-armed sea-star (*Culcita*), best known as "needle cushion" because of its similarity to that accessory, used by tailors long ago. This is a sea-star with extremely short arms, and shows a perfect pentagonal shape, covered with few short spikes. Another species – Cushion Sea-star )*Choriaster granulatus*) )Fig. 31a) has longer arms, but it is smooth to the touch and adorned with red spots on a off-white background. It is uncommon on the seabed at various depths, feeding apparently living foraminifers and other organic food, but is not a coral predator. In the Gulf of Eilat about 15 species are commonly seen, the most colorful among them are Egyptian Sea-star )*Gomophia aegyptiaca*) and Pearl Sea-star )*Fromia ghardaqana*), both endemic to the Red sea. Red controls the coloring, and white and blue spots decorate them. Comb star (*Astropecten*) has comb-like rows of spines, and *Choriaster*) )Fig. 31), not to forget the Crown-of-Thorns sea-star (*Acanthaster*), which has many arms and prickly thorns )Fig. .(30

Most sea-stars inhabit rocky habitats, The Comb star, however, lives in sand. Unlike other sea-stars its tube-feet lack sticking pads, They burrow into the sand, and their tips expand, thereby gaining hold in it, to pull the star inward. It is a predator, swallowing entire snails or clam, and after digesting it they throw the shells )Fig. 26).The Mutable collagen tissue and its role we have already mentioned (p..(15





**Figure 31.** Variety of sea-stars, (a): Cushion Sea-star *Choriaster*, medium sized sea-star, color pattern; (b) **Comb star** (*Astropecten*), (c) the Pigmy Sea-star *Asterina burtoni* in the process of rehabilitation of 3 severed arms (arrows). (d) Egyptian Sea-star (*Gomophia aegyptiaca*);



(e): A sea-star caught in the midst of oyster-hunting, locking its arms tightly around the prey through the mutable collagen tissue. It was impossible to pry it open by conventional means - proof of the effectiveness of this rigid-tensile MCT unique to Echinodermata (**see p. 15**) (photos: L. Dafni, M. Levin, J.. Dafni, A.. Colorni.)

## V Sea-cucumbers: "upon your belly you shall go"...

It is customary to divide animals into vegetarians, carnivores and omnivores - all. In this division cucumbers are missing - that they eat... sand. Quite a few animals (e.g., irregular urchins) are feeding on tiny creatures or organic debris above and within the sand - But sea-cucumbers have some more strange features that eating the sand is not the most peculiar of them all...

One common sea-cucumber in Eilat shore is Black cucumber (*Holothuria atra*), an elongated, cucumber-shaped creature. It tends to dig in the sand and only a small ripple indicates its presence there. Patience is required to notice its slow movement upon or under the sand. With little effort you can distinguish between the front side – in which the mouth, wherefrom five short tentacles protrude to feel and collect sand, swallow them - and the rear, where the anus opens. No head, or eyes, antennae or any other notable sense organ.

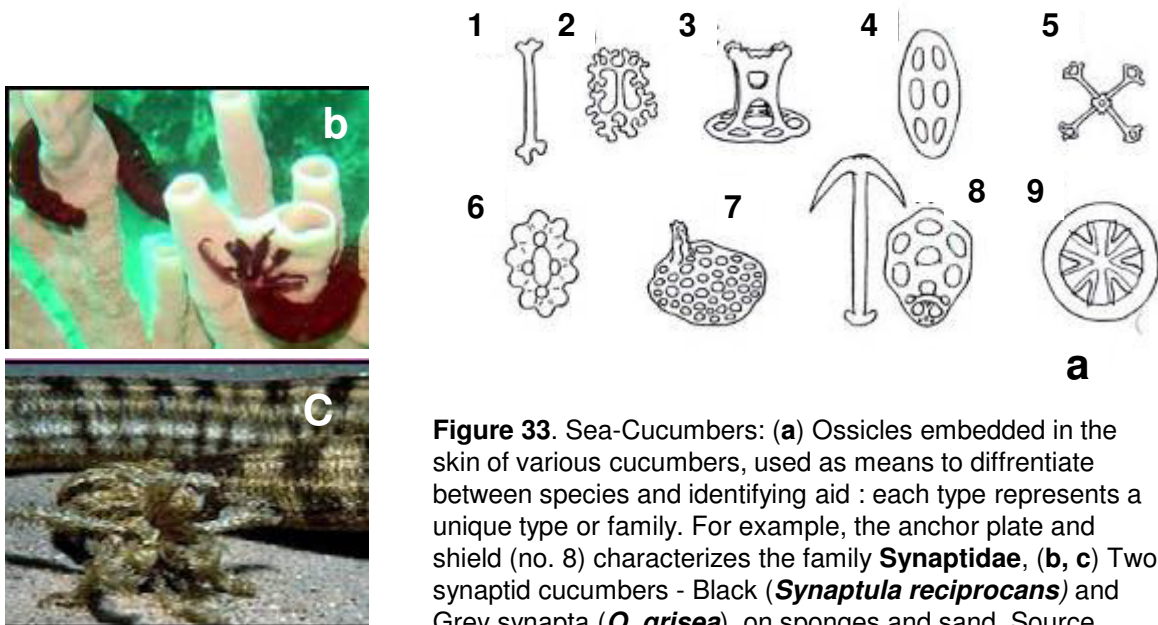
Unlike other echinoderms, a sea-cucumber has bilateral symmetry. This unusual feature drew the attention of scientists, who showed that the symmetry is actually secondary. In fact, many cucumbers especially those who belonging to another order **Dendrochirota**, of which ***Cucumaria*** is a common species in the Mediterranean sea – it is perfectly circular, sedentary in a vertical posture, collecting plankton from the water. Cucumbers of the common order **Aspidochirota** -lie in fact on their side (**Fig. 3e**) and crawl over a bed of sand or inside, gradually adopted an elongated posture, shown by the common Pale cucumber (***Actinopyga***) - they move around sluggishly. Secondly, they have created a distinction between "ventral-lower side", on which they crawl, aided by three rows of tube-feet, whereas on the dorsal side are scattered the tube-feet of the other two rows, used to carry debris or other foreign material. The cucumber body structure seems a regression from an active life style to a more primitive way of life, reminiscent of certain worms. But many aspidochirote cucumbers show more advanced behavior: Their body is soft, but a large number of calcite plates (ossicles) are embedded in the skin. Species are normally classified by the ossicle shape to families and species (**Fig. 33a**), whereas coloration is seldom reliable for identification (**Fig. 33a**). A third group of common cucumber in Eilat are of the order **Synaptida**, "snake-like" flexible and elongated (**Fig. 33 b,c**). The intestine of all cucumbers is long, and ends in the anus. The hind part of the intestine is called "respiratory tree", in the form of double tubes, unique for cucumbers, possibly a respiration aid. The Black (***Holothuria atra***) and the Edible cucumber (***Holothuria edulis***), who dive into the sand, are equipped with unique white and sticky tubuli, called "Cuvierian tubuli" (named after the French scientist Cuvier). These tubuli are ejected from the anus when the cucumber is disturbed by a predator, to hinder and annoy the intruder. Fish or crab limbs who got near them get entangled, and must retreat. When endangered, the cucumber is able to emit voluntarily all their internal organs - the respiratory tree, intestines and gonads. As mentioned above, cucumbers are also able to change the rigidity of their skin, from very soft to almost stone-hard, by using their MCT collagen. The Pale cucumber (***Actinopyga***) is notable for having five ossicles-teeth in the anus (**Fig. 31a**), possibly a means of preventing parasite or predatory fish and crabs, from entering the intestine from behind. A special menace are pearl-fish, **Carapidae**, known to enter the anal opening of cucumbers, feed on the intestine (**Fig. 34b**). The cucumbers which are not equipped in any protection are vulnerable to these parasites. However, they are able to regenerate these organs within a few weeks. For feeding they spread forward small mouth tentacles, collecting sand grains and carrying them to the mouth, to being swallowed. The digestive tube is long (at least twice the length of the body) absorb the organic nutrients. The "cleaned" sand, of organic matter, is ejected in the form of a typical long "sand sausage" (**Fig. 34f**). Common along the Sinai coast is the colorful elegant Bread cucumber- (***Pearsonothuria graeffei***) (**Fig. 32a**), the only sea-cucumber that practically walks upon the coral reef and living corals scanning the surface to gather food. Its colors are white and cream-colored, striped pattern and dark and brown spots all over his body. The juvenile is colorful (**Fig. 32b,c**), often mistaken as a colorful sea-slug, which may be explained as mimicry



**Fig. 32 (a)** Adult ***Pearsonothuria graeffei*** and **(b)** juvenile, resembling **(c)** a mimic sea-slug (***Fryeria rueppelii***). Photos: A. Harpaz, B.& S. Koretz.

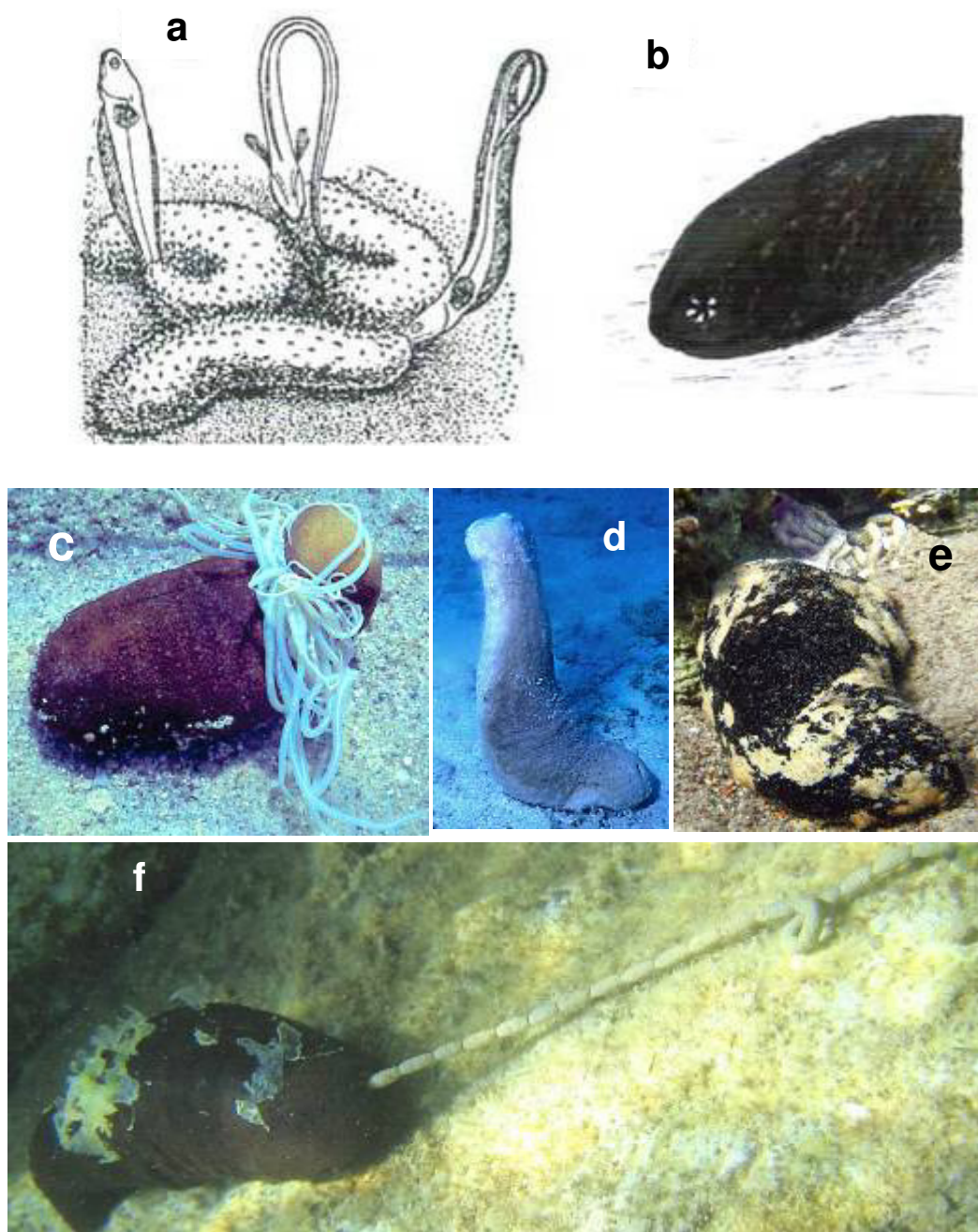
Special and peculiar are the sea-cucumbers of the family Synaptidae, whose common representatives are grey and black “rope cucumbers” (**Fig. 33b-c**). The unique characteristics of these flexible and agile “worm-like” cucumbers are the extremely thin body wall and lack tube-feet, all along the body, except for the oral tentacles (around the mouth). They move by peristaltic stretching or contracting of their body length, and contraction of the body width, by “ring muscles” - a form of movement typical of worms. They use their oral tentacles to gather food particles from the substrate. These animals are usually nocturnal, and the light sensitive organs at the base of their tentacles sense the approach of potential predators, contracting quickly when disturbed tentacles. They live above ground, and move while holding to it by using the “anchor-shaped” plates which is a kind of unique skeletal plates protruding from the skin. These plates form an anchor, that connects to plate “shield” is characteristic (**Fig. 33a sub-fig,8**). The size of the anchor not exceeding half an inch. This movement is a form of specialization for walking the few sand grains in solid bodies. Very common is the Grey synapta (*Opheodesoma grisea*) (**Fig. 33c**) which lives among sea-grasses.

In terms of breeding sea-cucumbers are not different than other echinoderms. During the breeding season, day or night, under the influence of external cues (like a full moon), cucumbers rise to erect position and emit tens of thousands of eggs or sperm cells into the water, from genital pores near the mouth (**Fig. 34d**). A strong stimulus is probably a “social hormone” (pheromone) emitted into the water and makes all of the individuals of the species to take part. Within hours all eggs are fertilized and begin development of the larva in the water. After several stages of development they settle on the ground before adopting the lifestyle of adults



**Figure 33.** Sea-Cucumbers: (a) Ossicles embedded in the skin of various cucumbers, used as means to differentiate between species and identifying aid : each type represents a unique type or family. For example, the anchor plate and shield (no. 8) characterizes the family **Synaptidae**, (b, c) Two synaptid cucumbers - Black (*Synaptula reciprocans*) and Grey synapta (*O. grisea*), on sponges and sand. Source paintings (a) Clark, 1962. Photos: (b) M. Levin, (c) Z. Livnat





**Figure 34.** Sea-Cucumbers (a) Pearlfish (*Carapus*) internal parasite of cucumbers (source – Y. Margolin: **Zoology**, published by the Kibbutz Hameuchad, Tel Aviv. Volume II). (b) “anal teeth” surrounding the anus, in *Actinopyga* (original), (c) *Holothuria edulis* showing Cuvier’s tubuli, and (d) Sea-cucumber spawning posture, (e) Black and white Cucumber *Holothuria nobilis* and (f) A sea-cucumber emitting faeces of sand “sausages” (photos: Y.. Schlesinger, Unknown, M.. Levin, J.. Dafni)



## VI. Crinoids and Brittle-stars - “living plants”

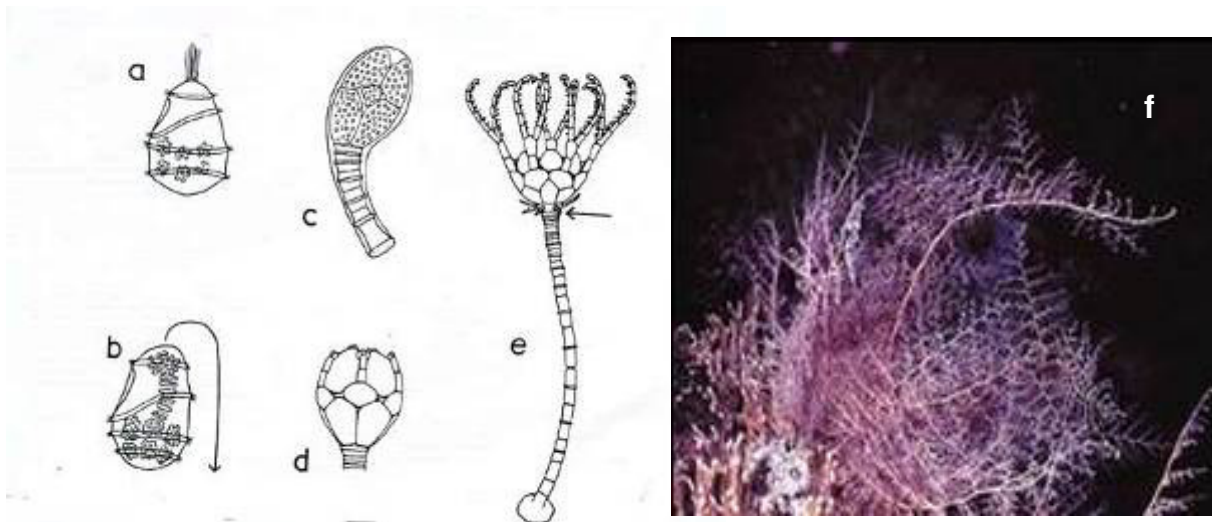
At night predatory fish activity in the reef slackens, and the tiny plankton creatures rise close to the surface. Then, the plankton feeders wake up for action. Corals polyps spread their tentacles. Soft corals also catch the passing plankton. Many echinoderms, especially feather-stars (=Crinoids), and the magnificent specialized form of brittle-stars, called “Basket-star” (*Astroboa*) (**Fig. 35 f**), are important plankton feeders. Their shape is plant-like, therefore in the Middle Ages they were erroneously considered plants, only in the 1700 recognized as animals. From the main stem, many feature-like branches spread to form a living sieve (**Fig. 36**). The caught plankton is driven to the mouth, and ingested into the internal organs, all concentrated in a small disc .

As mentioned above, both kinds – crinoids and basket stars are nocturnal. In daylight they hide coiled or clumped in crevices and among coral branches. At sunset, the eye-less creature sense the light waning, they spread their arms, climbing to reef top, to feed .

In pre-historic ages most common crinoids were **sea-lilies**, long-stemmed, and permanently attached, and their Arm-crown spread out in the water. Some lilies were floating, attached to a gas-filled bladder, and carried far and to wide distances. Recently the stemmed crinoids are found only in deep-sea, lost in the competition for life in the shallow water .

These ancient forms survived as developmental sub-adult stage in extant crinoids (**Fig. 35 e**), while the adults are not stemmed (**Fig. 36 b,c**).

In Eilat reefs there about 20 crinoid species were found, the most common is the multi-colored *Lamprometra klunzingeri* which used to be widely spread in the reef of Eilat. In the past, a night diver could record dozens of individuals per square meter along the reef edge (Fishelson, 1974). The Lifestyle of this species has been studied in Eilat (Rutman & Fishelson, 1984). In the 1990's, apparently due to chronic pollution their numbers fell dramatically. Only lately their number increased again, yet they did not reach the past plentitude .



**Figure 35.** (a-e): Larval stages in a crinoid (feather-star). **e** is a “stemmed” subadult attached to the substrate, not unlike the deep-sea **sea-lillies**. Arrow points to the separation zone, where the adult breaks from the stem, during transition. (**f**) Basket star (*Astroboa nuda*), a plankton filter feeder related to the **brittle-stars**, attached to the reef at night (see **Fig. 11**). (source - Clark, 1962; M. Levin(

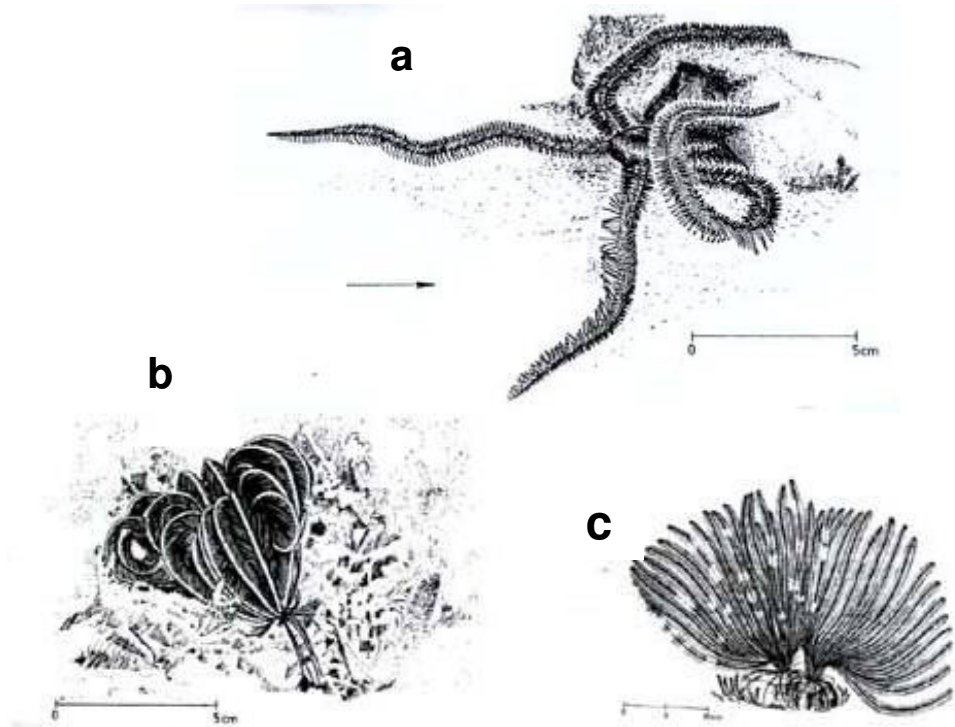
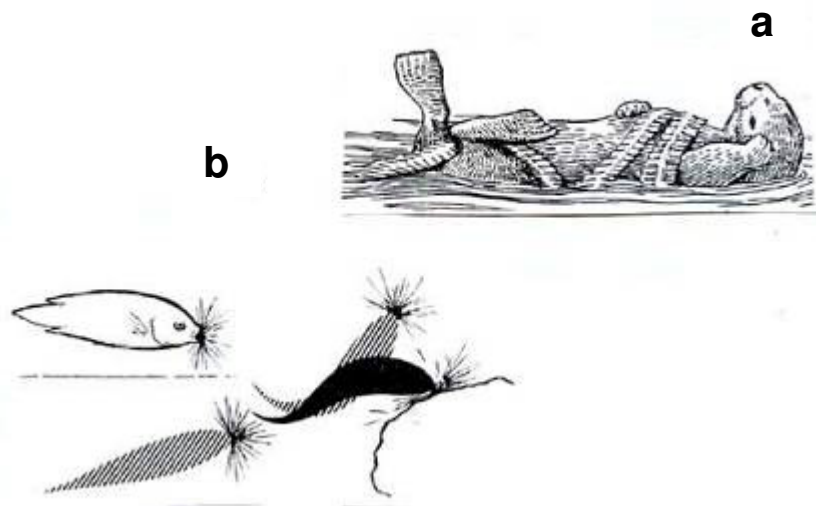


Fig. 36. (a) *Ophiocoma scolopendrina*, a common intertidal brittle-star in feeding posture, when its body hides in a crevice, and two feeding arms scan the water surface from beneath (b) *Capillaster*, a black Feather-star (crinoids) hiding among algae, arms coiled; and (c) two crinoids with spread arms, holding fast by means of small cirri, while feeding ( Magnus, 1964)

**Figure 37** Sea-urchin predators: (a) Sea-otter (*Enhydra lutris*) dives to the bottom, bringing in a sea-urchin or abalone – a snail, and crushing with a flat stone on the belly (source unknown), (b) A Broomtail wrasse (*Cheilinus lunulatus*), grabs the urchin by its spines and crushes it on the reef rocks ( Fricke, .1974



## **.5 Who eats them and what is edible?**

Echinoderms are not a favorite food to most creatures. Their meager nutrient content on the one hand, and the inconvenience of access to their edible internal body parts due to spines or spiculae on the other hand, do not encourage their consumption. This situation changes dramatically in the breeding season, when the coelom of these creatures is overflowing with nutritious proteins, that build and constitute the gonads, reproductive glands, and fat store to sustain the developing larvae. Many organisms make a living by feeding on echinoderms. Each group of echinoderms has its predators, that covet the inside fleshy gonads.

Sea-urchins, which are common in shallow water habitats, have an important ecological impact on their environment. Ecologists view them as "key species", organisms whose population size - increase or decrease - affects the delicate balance between the various components of their habitat. For example, unrestrained proliferation of sea-urchins in the coast of the United States caused several years ago the total denudation of large marine areas, previously covered by giant algae (kelps), which caused immense reduction in invertebrate and fish population, even affecting commercial fisheries .

In coral reefs sea-urchin grazing had a crucial effect on the balance between coral and algae. Protection of reefs from sea-urchins ensures greater cover of hard coral, calcareous and coralline algae, and greater substrate diversity and topographic complexity than unprotected reefs which had greater algal turf and sponge cover. Coral cover and topographic complexity were negatively correlated with total sea-urchin density .

Sea-urchins are an important food source for animals and Man. Predation of urchins became expertise for many creatures. The Rock lobsters (*Panulirus spp.*) are able, despite lacking pincer claws, to devour hard-shelled and heavily spined urchins. Some octopuses also do it successfully. A unique expertise is showing in Sea-otters living along the coast of North-West America and Eastern Asia. This mammal, an experienced diver, dives to a depth of 5-15 m and takes out from the seabed sea-urchins (or abalone – a large fleshy snail). To crack the shells he gets a flat stone, and in a typical back swim he slams the stone at the urchin which lies on its belly until it breaks (**Fig. 37a.**)

Some reef fish have also adapted to predate on urchins: the Giant Triggerfish (*Hemibalistes*), for example, has very thick and hard scaled skin, whose mouth is thrust out and away from eyes, vulnerable to being stung by the spines. He grabs the tips of the spines, and releases the urchin in mid-water. While it falls, the fish turns around and attacks it in the urchin's lower parts, next to the mouth, where a flexible tissue, the peristome, is vulnerable, and pries the shell open. The hard teeth of Stellate puffer (*Arothron stellatus*) allow it to crash and devour spiny urchins. Giant Wrasses (*Cheilinus*), also fond of sea-urchins catch them with their thick lips, carry it to the next reef or rock, and beat it until the shell is broken. Then it crushes the shell with his pharyngeal teeth (hard plates in the throat) (**Fig. 37b**). Short spined urchins like Velvety Urchin are more vulnerable to medium size wrasses, like clown wrasse (*Coris aygula*), who prefer brittle-stars to urchins. They wander along the shore of Gulf of Eilat at mid tide, and can be seen with their dark body half submerged, half out of water, when digging into the beach rock crevices where brittle-stars hide.

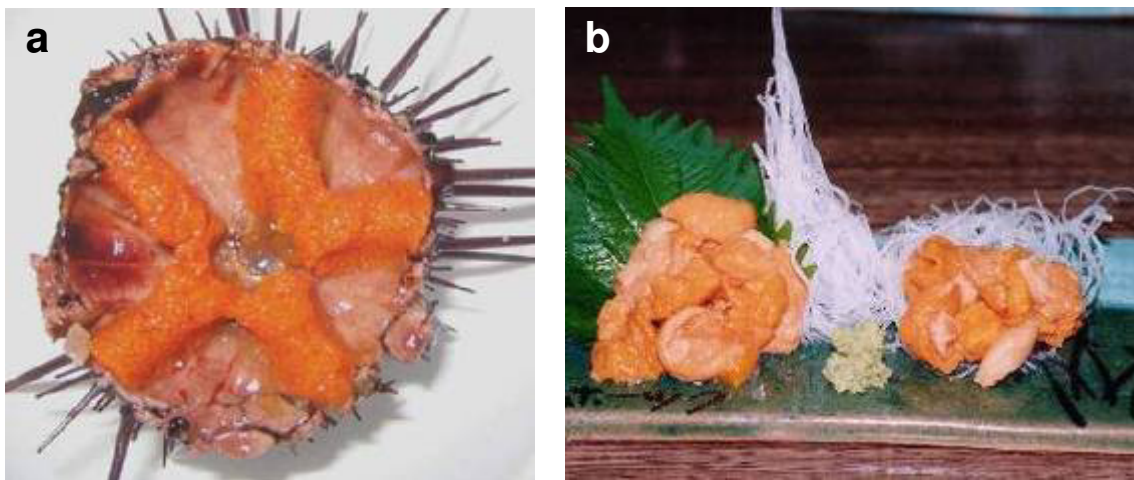
Predators are known to influence urchins ways of life and behavior: LSU (*Diadema*) living in closed bays, into which fish predators seldom enter, are active around the clock, while in open sea, in danger of their lives, these urchins are mostly nocturnal, hiding in daylight in reef crevices, or creating dense groups, mutually protected by the spine thickets, on the sandy sea-floor. They adapted an "alarm response". When one sea-urchin is "punctured" by a predator, and its inner fluid spilled into the sea, all its neighbors scatter, running for shelter (Snyder & Snyder, .(1970

As always, the ultimate predator is Man. In many countries different echinoderms are food in high demand, some considered delicacies. The most wanted are sea-urchins (known as “sea eggs”), the edible part of which are the roe, actually the gonads - reproductive glands (ovaries and testes filled with eggs and sperm cells - Raw in English). The breeding season is thus the season when the fishermen fish many tons of sea-urchin roe, prepared in a variety of recipes for sea-food lovers. Most of their exploitation as food is in the Far East (in Japan they are known as “**uni**”). They are eaten greedily also along the beaches of Europe - Ireland, Portugal, France, Italy and North Africa (in Italian they are called “**Ricci di mare**”). Usually their gathering is forbidden while reproducing, because of environmental protection.

Sea-cucumbers are edible and highly prized in the Far East. In Malaysia is “**trepang**” eaten dried and salted, sun-dried or smoked, and it is widespread throughout the Indo-Pacific coasts. In China's coastal cities sea-cucumbers - named “**Hong hor**” – are an essential component of the diet of fishermen and the poor. Japanese also eat their eggs and sun dried skin, and their growth culture is centuries old. In the Philippines sea-cucumbers are called “**Oh-Leo**”, and eaten raw, smoked, or pickled with salt. In Europe they are known in their French name “**Beche-de-mer**”. They cook them and serve with salad or soup.

Sea-stars - a natural predator in oyster pools - are fished in large quantities and used primarily for drying and producing “fishmeal” a poultry food supplement.

World Fishing yield of echinoderms amounts to 70 000 tons per year, 50,000 in sea-urchins, and the rest in sea-cucumbers. Sea-stars yield comes to a hundred tons per year (Sloan, 1984). In some countries, like Japan and the Soviet Union, gathering was much reduced to a daily allowance, which offenders are fined (**Fig. 38**). In certain countries – Italy for example - the gathering of sea-urchins is restricted by ministerial decree: No tool other than rakes can be used for the commercial fishing of sea-urchins. Sea-urchins can also be gathered manually by professional divers using underwater breathing apparatus and by non-professional divers. Daily bag limits are 1,000 individuals for professional fishers and 50 individuals for non-professional fishers. It is prohibited to gather sea-urchins less than 7 cm in diameter. Sea-urchin collection is suspended during the months of May and June every year.



**Figure 38.** (a) Edible roe, gonad contents of a sea-urchin. Egg or sperm mass occupies much of the inner coelom space and their volume reaches 30% of overall weight (b) Urchin roe as main component of the traditional **sashimi** in Japan ( Wikipedia(

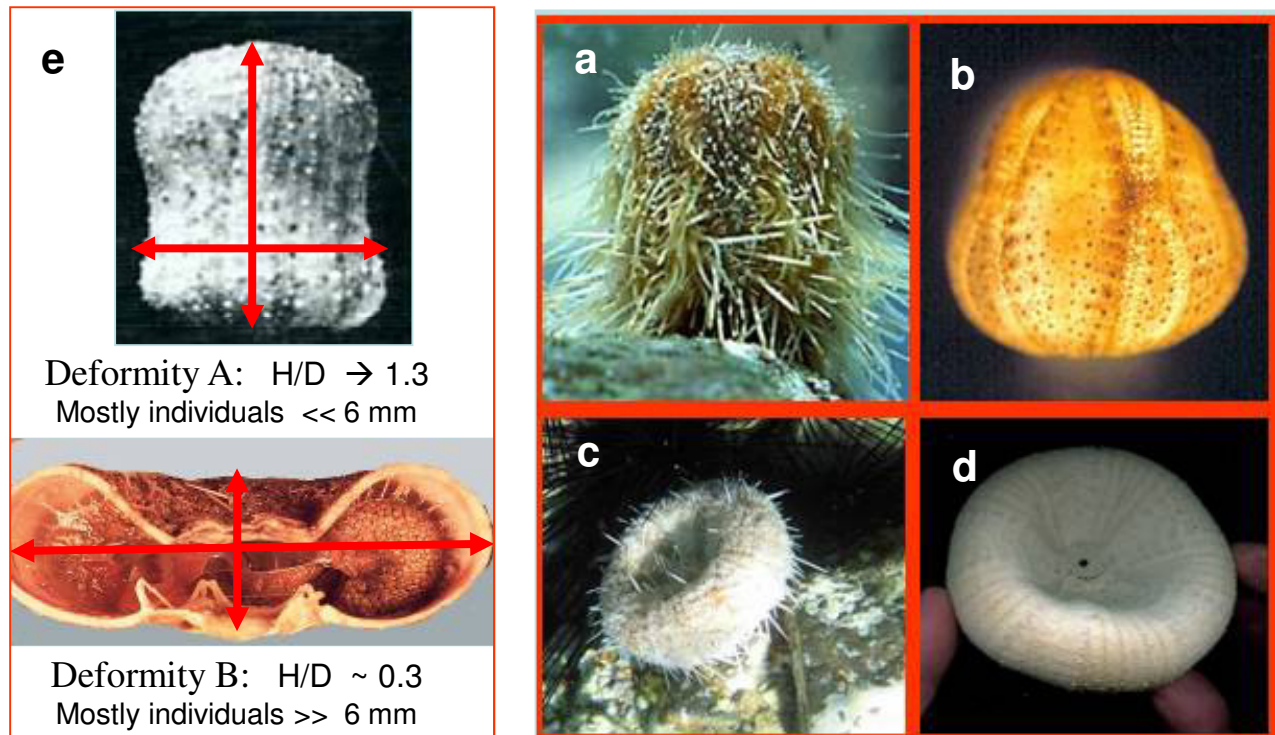


## Part II - The author's studies in skeletal growth & calcification in sea-urchins

### 1. Deformations in polluted sites

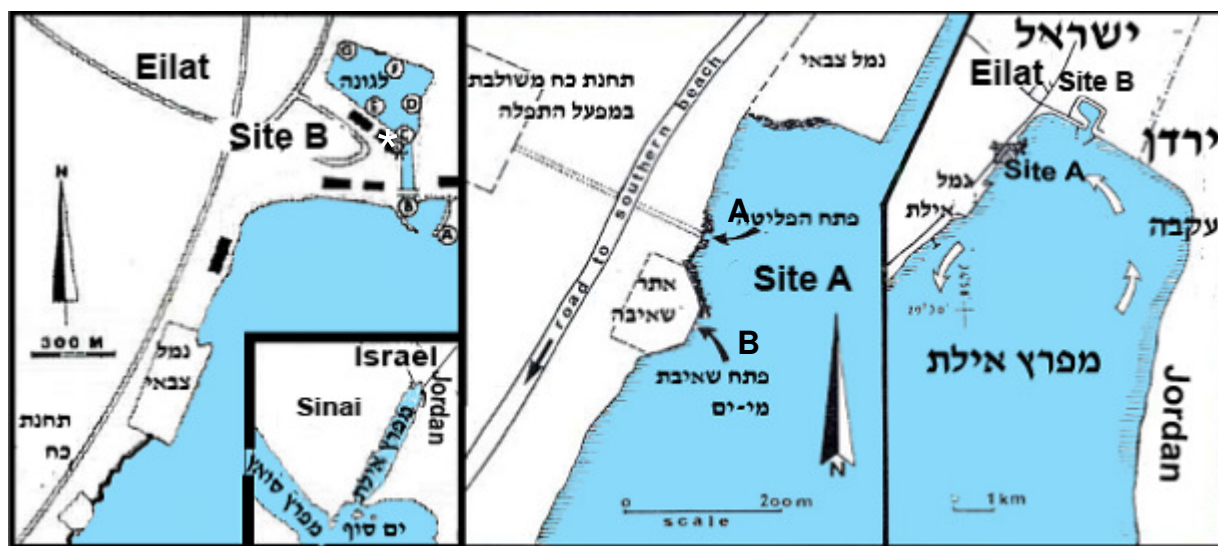
In 1979 acute skeletal deformations in the Velvety Sea-Urchin (*Tripneustes g. elatensis*, VSU) were discovered by the writer in polluted sites at Eilat. Deformations were of two types (**Fig. 39**):

**Deformity A** – Sea-urchins grew excessively in the vertical axis (**Fig. 39 a,b,e**). Compared with the normal ratio between test (skeleton) height and diameter (H/D), which is approximately 0.5 the deformed showed a relative height that was equal or more than twice their normal ratio (i.e.  $H/D \gg 1$ ). Their overall shape of the deformed also deviated from the normal flattish-circular shape - and their shapes were reminiscent of pears or peanuts, and the skeletons had on their side conspicuous "pinches" of the Amb column - by all accounts "monstrous". This deformation was found in large numbers (frequency of 66%) of one population, who inhabited the shore opposite a power plant in Eilat (**Fig. 40 - Site A**).



**Figure 39:** two deformity types in *T. g. elatensis* : **a,b**: Living urchins and skeletons of Deformity A; **c,d**: Deformity B. (**e**): H/D proportion shown by both deformities Sources: Dafni, 1980, 1973, 2010).

**Deformity B:** Sea-urchins became extremely flattened (the ratio of body height and diameter did not exceed 0.4). The aboral (dorsal) side, which is normally dome-like, most often showed deep depressions, collapse of the "architectural" dome (**Fig. 39 c,d,e**). This deformity was found in high frequency in a VSU population in an artificial lagoon in the northern shore of Eilat (**Fig. 40 - Site B**). Later this deformity spread to other beaches along the Israeli shore, to apparently non-polluted sites.



**Figure 40.** Deformations Sites Map – Right: Overview of Northern tip of Eilat Gulf; middle: **Site A** location, where deformity A occurred. Left: **Site B**, white asterisk marks location C in the artificial boats lagoon in the North shore. A-G – investigated locations from the sea inlet to the furthest location - **Table 2** (Dafni, 1980, 1983b.)

**Table 1. Distribution of deformity A in site A – power plant water spill**

Site test location in the shore opposite the Power plant	No. of tested urchins	Average D/H ratio	%deformed
A – North of polluted warm water spill (arrow in map)	95	0.59 > normal	Not counted
B – South of polluted warm water spill (arrow in map)	117	0.70 >> abnormally high	66%
C – Away from polluted warm water spill (not in map) -	139	0.53 = normal	None

**Table 2. Distribution of deformity B in the artificial lagoon site B**

Site test location in Site B	No. of urchins	Average D/H ratio	% deformed
A Open Sea	50	0.54 normal	0
B – Lagoon opening	76	0.49 < abnormal	14.5%
<b>C – Hotel laundry discharge*</b>	<b>89</b>	<b>0.43 &lt;&lt; normal</b>	<b>76.4%</b>
D- inner lagoon east	38	0.48 < normal	31.5%
E - inner lagoon west	8	0.52 ~ normal	12.5%
F – Lagoon far up	7	0.55 normal	14.5%
G – Furthest location North-West	0	-	**

\* asterisk in map ; \*\* low salinity here. No living VSU there .

## what caused the deformities ?

An initial hypothesis raised by some observers was that a genetic error caused by mutation resulted by skeletal deformities in these sites. This hypothesis was rejected for the following reasons:

.1An Individual born as a result of mutation is generally a rarity, and differs from in normal (usually, not always) by a particularity rather than an extreme complex of characteristics.

.2Sea-urchins development goes through a large period of larval life, a **pluteus (Fig. 20b)**, drifting among the plankton. Thus, descendants of one mutant are expected to disperse over a range of dozens, perhaps hundreds of kilometers of coastline, rather than concentrate on such a small location.

The hypothesis that the deformation caused apparently by a parasite, whose sting may cause skeletal deformities, or are the result of recovery after an attempt to predation on the part of any predator was rejected, mainly because the integrity of the urchin was not impaired, only their growth was distorted.

.3After rejection of 1 & 2 hypotheses, we further hypothesized that an environmental factor, in these sites, may has caused the deformations. We decided to study further this hypothesis for the following reasons:

**A.** Concentration of hundreds of deformed urchins in one locality supports the hypothesis and suggests a deleterious impact on the growth of sea-urchins, after undergoing metamorphosis from larva into the adult, and recruiting on the particular site.

**B.** On **Site A** more sea-urchins were deformed down the steady north-south stream from the power plant spill, and the deformations were more severe than those upstream direction (**Fig. 39 and table .(1**

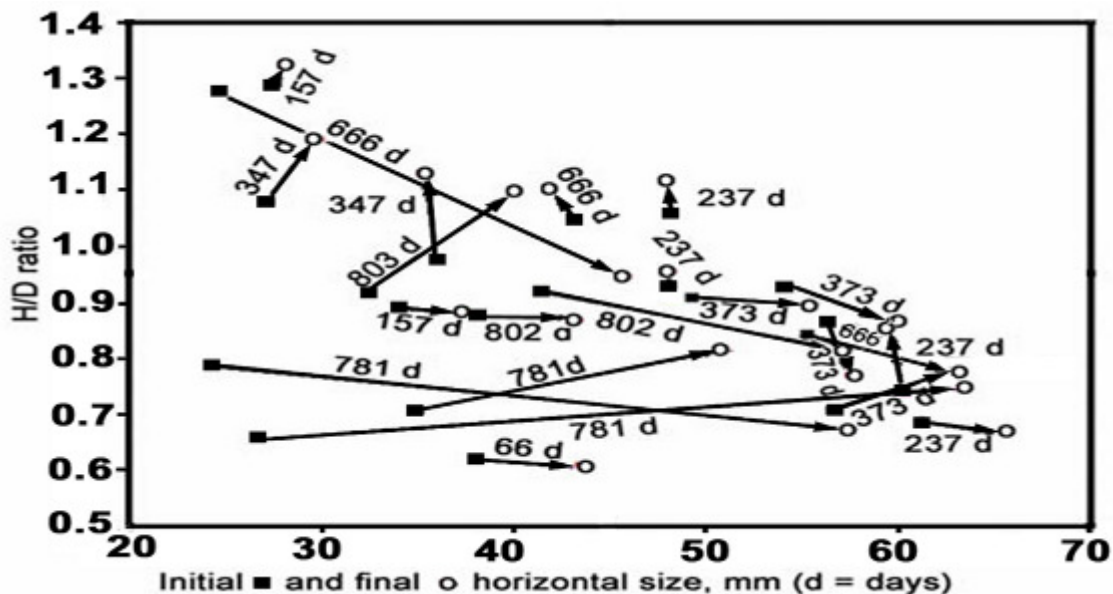
.4Hypothesis 3 was greatly supported by the correlation between pollution degree, from the less polluted open sea conditions to the various locations inside the lagoon (site B), and the number of deformed urchins and degree of deformations. One location stood apart (**location C**), next to a small waste water discharge from the laundry of a hotel, as it showed a maximum number of deformed individuals and high rate of deformity, showing both flattening and collapse of the aboral part of the skeleton (**Table .(2**

## Deformities in the scientific literature

Skeletal deformities urchins are rarely mentioned in scientific literature, but there were some interesting clues that related to the possibility of deformation of infection: A French explorer, J.Y. Allain (1978), found a certain number of deformed urchins in Cartagena Bay, Colombia. He attributed the phenomenon to pollution. Another scientist, H. B. Moore (1974), attributed some of the deformed skeletons of urchins, collected over the years, to physical damage, and also to environmental or climatic factors. One case of mass deformities in regular echinoids has been described by Th. Mortensen In his book *Monograph of Echinoidea* (1944). He mentioned that the British Museum in London was given at the end of the 19th century some tens of *Tripneustes g. gratilla* from Mauritius Island in the Indian Ocean, showing **deformity B**, extreme flattening and aboral depressions. I wrote to the local museum in Mauritius, exploring the circumstances of this deformity, and find if there were any "potentially polluting" factories during this period –tanneries, fish or copra production (raw materials for oils), in these shores, that may have caused it - but got no answer.

### Experiment 1. Long-term growth and rehabilitation of deformed urchins

Deformed sea-urchins, collected at the shallow water next to the power plant spill were kept in captivity, fed with algae for up to 800 days (2.5 years), and measured several times during the period. **Figure 41 a** shows the initial (dark squares) and final size (hollow circles) in mm attained during these periods, as well as changes in height to diameter ratio (H/D). It proves that (a) Highly deformed urchins (top left group) almost did not add height, and most of them remained deformed. (b) Urchins who were least deformed ( $H/D < 0.8$ ) grew larger and in some cases reduced the H/D ratio during the experiment.



**Figure 41 a:** Changes in the initial (squares), final (hollow circles) size (mm) and H/D ratio of deformed *T. g. elatensis* collected in site A and held in captivity for a duration of up to 2.5 years. An arrow is drawn to connect the initial and final measurements of each individual urchin .d- days elapsed from beginning to the end of the experiment (Dafni, 1983, 2010).



## Experiment 2. Who caused are the deformities?

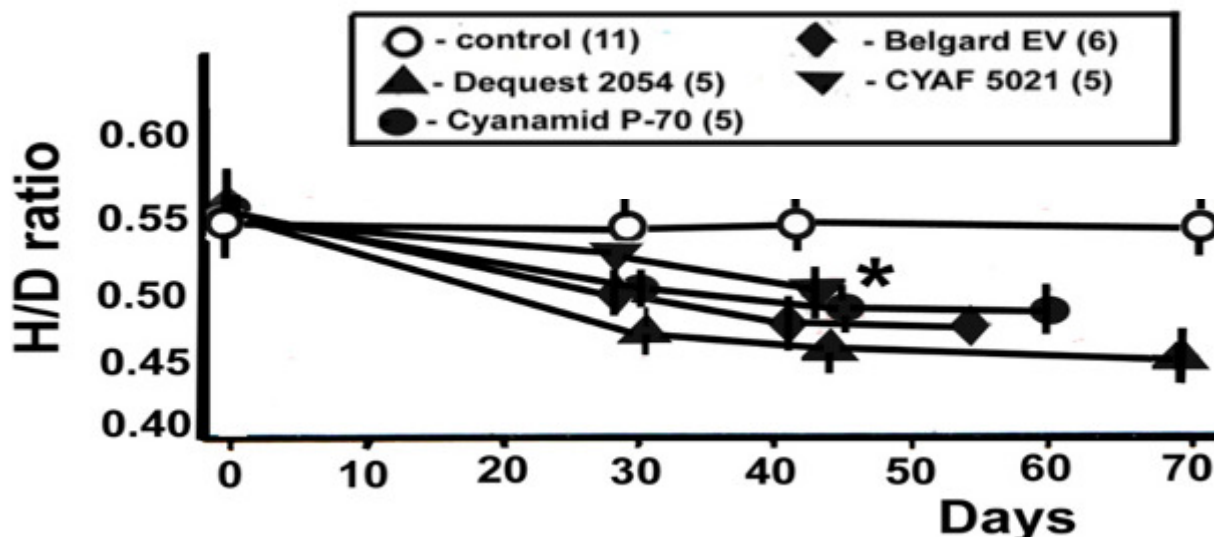
Juvenile VSU's groups (ca. 20 mm in diameter, in parentheses – number of individuals) were held in captivity, exposed to four chemicals (Table 3), added to the plumbing system of the power plant to prevent calcification (next to site A). Concentration were about 200 parts per million (0.02%), which were 1% - 5% of lethal dose, as determined by the manufacturers. Measurements were made after 30, 45 and up to 70 days.

The results (Fig. 41b) show that all the experimental groups – except the control – showed decreased growth rate, and that ceased altogether ca. 30 days after the onset of the experiment, compared with an average growth of 5 mm diameter of the control group. But the more interesting findings were (1) Significant change in body proportions, especially the ratio between body heights to diameter (H/D) - all experimental groups became flatter and the appearance of minor depressions in the urchins' dome, similar to those found in deformed urchins and (2) Appearance of small gaps at the sutures (seams) of a width of < 1 mm between the plates of the skeleton, especially the longitudinal sutures, occasionally causing the entire skeleton to mechanically weaken and even in some cases to collapse, while the urchin itself was still alive (Dafni, 1983b.)

**Table 3. Chemical used for experiment 2:**

Calcium carbonate inhibitors (used occasionally in power plant)

- Belgard EV (Ciba-Geigy). Based on polymeric carboxylic acid.
- CYAF 5021 (Cyanamid). Acrylamide-sodium acrylate resin.
- Monsanto Dequest 2054. Potassium salt of hexamethylene-diamine-tetra (methylene phosphonic acid).
- P-70 (Cyanamid). Sodium polyacrylate



**Fig. 41 b :** Vertical to horizontal (H/D) ratio  $\pm$  SD change in a long-term exposure of non-deformed urchins to 4 industrial calcification reagents. In parentheses: number of tested urchins (Dafni, 1983, 2010).

## Working hypotheses

If pollution caused deformation, we ask: how does it work? The above experiments and observations indicate a clear connection with a source of pollution for each deformity. But what causes skeletal deformities in such “monstrous” intensity? For this study we raised a few hypotheses:

- A. Growth of a sea-urchin can be described as two distinct processes: (1) growth of the organic tissue - muscles, organs, skin, etc. and (2) Skeletogenesis formation of a skeleton by chalk ( $\text{CaCO}_3$ ) deposition in an organic matrix prepared in the previous step. Calcification apparently follows organic growth, and thus ensures skeletal stability. In some cases, delay in calcification damages the integrity of the entire structure, characterized by feeble attachment of the test plates, and the entire skeleton softens.
- B.  $\text{CaCO}_3$  deposition may be impaired by polluting agents, such as the chemicals tested in experiments 1 and 2. The results of the experiments answer some of the questions asked, but still there remains a puzzle: Surely these polluting agents were used only in the power plant, where deformity A appeared, showing excessive vertical growth. In the experiment, only one individual showed such deformity (**Fig. 63 a**), not enough to draw any conclusion.
- C. Calcification, which follows organic growth, can be affected by mechanical properties of muscles and other tissues – pressure or tension. Pressure at the plate margins will slow down the calcification rate and tension will increase it.
- D. There must be some balance between mechanical stresses and tensions. Damage to this balance may cause imperfect skeletogenesis and deformation.

But before we go forward to discuss deformities and their causes, we present another approach to the control of skeletal growth and morphogenesis – the biomechanical approach.

## .2 Biomechanical approach.

One of the most intriguing questions in modern biology is how and who determines the exact body shape of animals and plants?

Everyone knows that the genetic code controls - and directs - all metabolic processes in the body, including growth processes, morphogenesis and shape stability. On the other hand, there is growing evidence that plants and animals, affected by mechanical injuries, showed accelerated growth in the damaged body area or parts, in a process aimed to restore normal body shape. Trees of which fronds were cut off grow new branches, firstly to fill the void space caused by the damage, until the former shape is re-established. Similar phenomena were shown in branching corals. There is no doubt that the last process is not necessarily dictated by the genetic code, but it is a response to environmental cues, mechanical or chemical, that exhibit themselves following injury.

In plants it was found that growth hormones from the treetop encourage or inhibit the growth of lower branches, in a so called “apical dominance” over the lower branches. If the treetop is cut off, rapid growth of lateral branches ensues, until the former “ideal” shape is achieved. There are good reasons for arguing that the mechanical stress is one of the factors that influence or regulate the speed of growth of many tissues in animals and plants, and their morphogenesis.

## D'Arcy–Thompson's hypotheses

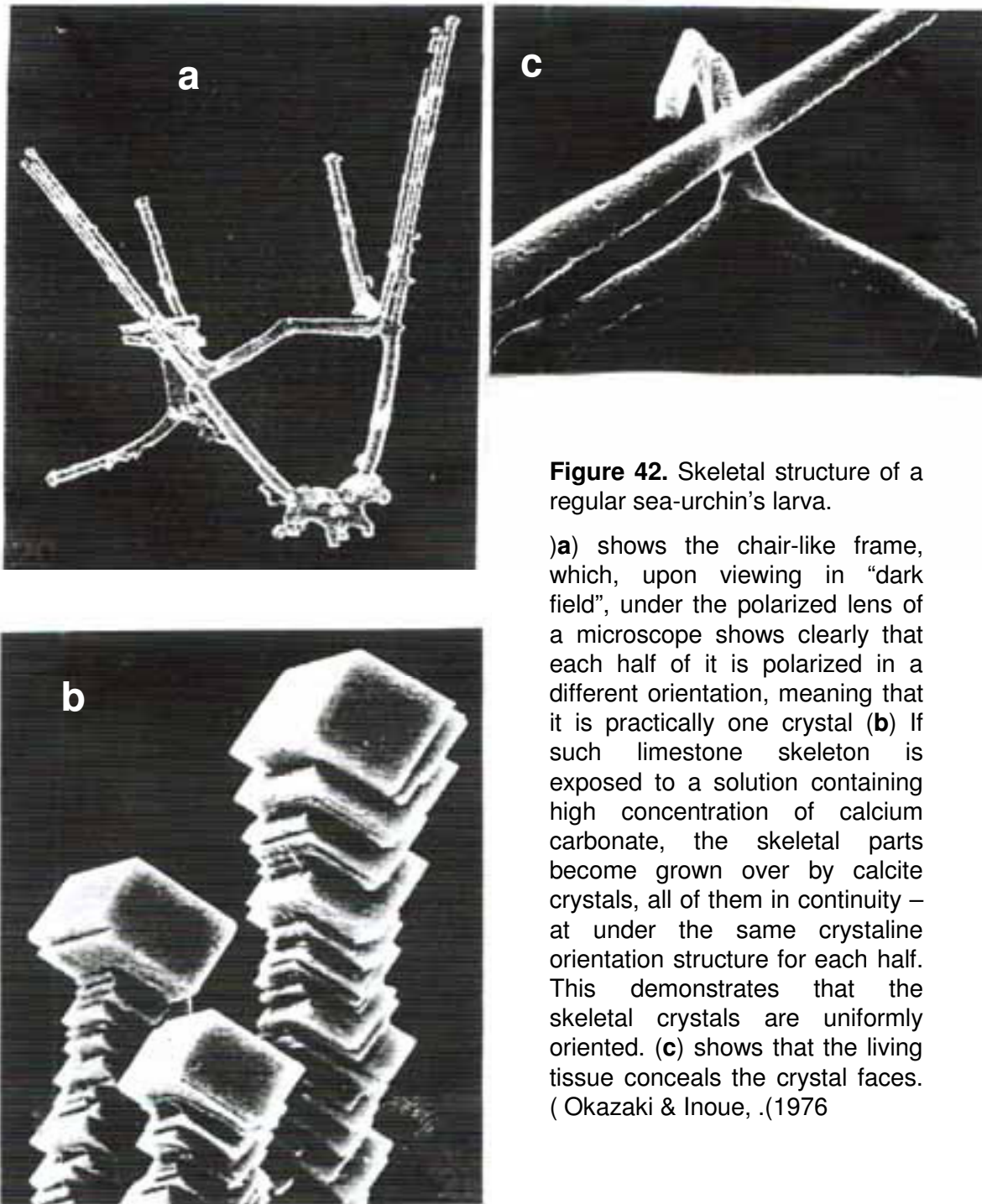
The book "***On Growth and Form***", written an English scholar, **D'arcy Thompson**, published in 1917, revolutionized the minds of many morphology researchers. Thompson drew their attention to the similarity that exists between the shape of organisms, or parts of their bodies, to inanimate objects, shaped by mechanical forces in nature. In it he held that ) –**(A)** Mechanical principles, controlling the shape of inanimate structures, are also valid in controlling the growth of living organisms, and; **(B)** Adherence to these principles characterize the development process of both the individual (ontogeny) and the group level – genus, family, order etc. (Phylogeny). He also argued that the growth of an organism, and morphogenetic changes occurring within, involve a deviation from an ideal "forces balance", generally by applied mechanically active tissues - muscles or connective tissue – that regulate its shape from inside. According to Thompson forces and pressures from the external environment have also influence on the growth, and eventually on the shape of the individual. Among the examples he brought to illustrate his ideas, he pointed to the body and skeleton of sea-urchins. Shaped like a balloon, anchored to the substrate by "wires" (tube-feet), and their morphology is dictated by the strength of this anchoring stretch. He believed that like a balloon, a sea-urchin if not strongly attached to the ground, tends to be spherical, whereas an urchin strongly wired to the ground, will adopt a more flattened body shape. The ideas of Thompson, who is considered Father of modern Biomechanics, have revolutionized the world of many biologists, but still many ignore or dismiss them as simplistic.

These hypotheses remained dormant for decades, mainly because it was difficult to reconcile them with the ruling Darwinian concept, that evolution is entirely an interaction of random new combinations - by way of mutations and permutations - and natural selection to choose the fittest, preferably if they match the mechanical design principles. Thompson's ideas were conceived as an illustration of the degree of efficiency, where natural selection "uproots" the offspring not complying with these principles.

Although since the book was published ample information was gathered on the mechanical design of biological organisms, few experiments have been made to show the effect of mechanical forces on changes in their morphology.

Recent development in genetics during the last half-century revived the hope that by decoding the genetic code the genes that determine each detail and individual variation in living creatures will be found. However, the question still remains: "How can a single genetic code in DNA – two-dimensional – present itself such variety of three dimensional forms?". Some scientists believe that the formal design of the body and limbs is strongly influenced by interaction between the physical forces - Mechanical, acting on the inside and outside the body (the epigenetic concept). Animal development researcher **J.T. Bonner**, an avid admirer of the biomechanical approach (also the author of an abbreviated edition of Thompson's book) tried to bridge the gap between opposing genetic and epigenetic approaches stating that ... "response of the developed organism to the physical environment body - is in itself adaptive, and so will naturally be favored by natural selection". Influence of genes on the morphogenesis, according to this approach, is limited mainly to materials and the forces that regulate its growth, when the forms themselves are largely determined by the interaction between body and environmental forces and constraints.

Recently new data support mechanical effect on a Human skeletal structure. With departure of astronauts from the influence of gravity their normal growth and metabolic processes were affected, and their bone became fragile. This and the importance of "body building" practices through exercise, led to the revival of developmental biomechanics.



**Figure 42.** Skeletal structure of a regular sea-urchin's larva.

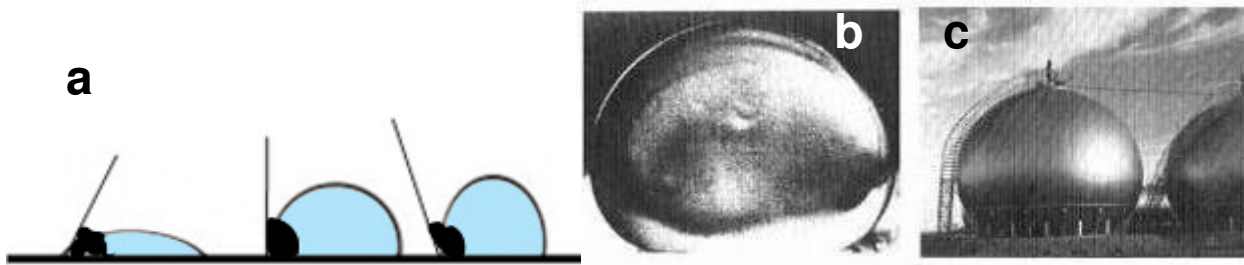
(a) shows the chair-like frame, which, upon viewing in "dark field", under the polarized lens of a microscope shows clearly that each half of it is polarized in a different orientation, meaning that it is practically one crystal (b) If such limestone skeleton is exposed to a solution containing high concentration of calcium carbonate, the skeletal parts become grown over by calcite crystals, all of them in continuity – at under the same crystalline orientation structure for each half. This demonstrates that the skeletal crystals are uniformly oriented. (c) shows that the living tissue conceals the crystal faces. (Okazaki & Inoue, .1976



### ***.3Echinoid skeleton as a pneumatic structure***

A picturesque example of the principle of epigenetic morphogenesis are the pneumatic structures. Body organs of most living organisms tend to be round and swollen, when they shapes are controlled by internal pressure (turgor) of the fluids or internal tissues, against which the surface tension of the outer envelope. Such are most of the juicy fruits, plant and animal organs, and sometimes the entire plants or animals. The same principle also prevails among architectural structures – containers and round tankers )**Fig. 43c**). Many invertebrates lack a solid skeleton, maintaining their stability through a “**water skeleton**” - (hydroskeleton), which is in fact a pneumatic system. Human body organs also agree with the pneumatic principle (inner pressure vs. outer envelope tension: skin, stomach, breasts, intestines, etc.). Wherever the apparent pneumatic principle does not explain the shape of the organism - plant or animal, we usually find a solid wall, reinforced the supporting structures, which are usually created in response to mechanical constraints. Thompson suggested that sea-urchins skeleton is a structure illustrating well the advantages of a design following the physical-mechanical principles. Beyond the “forest” of spines, that protect the urchin from outside a solid spherical structure, made of limestone plates that surround a fluid-filled cavity, in perfect analogy of a pneumatic structure. It resembles a air or gas filled balloon, or alternatively – a thin shell egg (sea-urchins are often called “sea-eggs”). In addition to being a well designed architectural masterpiece - a symmetrical dome-shaped sphere, capable to resist considerable external loads (Telford, 1985), it constantly increases its size: Every skeletal plates the test is made of grows at different rates in each direction, and yet the overall shape proportions of the sphere does not change. D'Arcy Thompson compared the regular (subclass Regularia) sea-urchin's shape to a drop of water lying on a dry surface, tending to flatten because of its weight )**Fig. 43a**), but will assume a perfect sphere if suspended in another liquid, such as oil. He claimed that it is not a mere analogy, but a demonstration of the effect of real physical forces determining is morphology.

The paradigm of the pneumatic flexible structure, the **pneu**, was adopted by several biologists, providing them a new means to examine the physico-mechanical structure and function. In living organisms, internal pressure system interacts with shell flexibility and durability. For example, the curved shape of a crab's body shell, is also caused by the same principle: we find that after moulting their exoskeleton, to be replaced by another, still softer shell from underneath. At that point, the crab pumps water into its tissues, to inflate its body cavity, before the outer shell is hardened, to stabilize the new shape, a demonstration of the pneumatic principle.



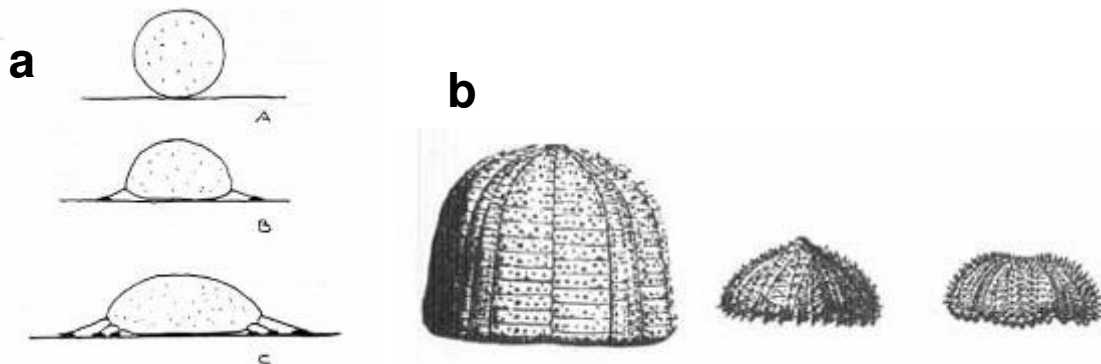
**Figure 43.** (a) Liquid drops on different surfaces, differences lie on the contact angle between the liquid interface meeting the solid surface. The smaller the contact angle the flatter drop will become. The angle depends on surface tension of the various liquids. Left - a small angle - the drop is flat, like the contact between water and glass  $< 90^\circ$ ; Center: water on a fat covered surface, and right: high-angle as in mercury on glass ( $140^\circ$ ). (b) blocks of plaster cast from a pneumatic bag and (c) architectural structures based on this principle ( Stevens, .(1974

Back to our discussion - if we suspect that a violation of any mechanical balance as the factor responsible to sea-urchin deformities - we have to study the biomechanical balance. In both types of deformation there were features suggesting that mechanical forces were involved – the "pinches" shown along the Amb columns of deformity type A (**Fig. 39b**) and the collapse of the domed structure in deformity B (**Fig. 39c-d**). These two could easily occur if mechanical forces were applied to an urchin's flexible structure at any degree of softening. Similar pattern were found in sea-urchins suffering from deformity caused by mechanical damage (Chadwick, 1924). Since evidence pointed at an environmental effect, we offered a hypothesis that in these urchins the natural "forces balance" which according to D'Arcy Thompson regulates the process of growth and  $\text{CaCO}_3$  deposition, resulting in uncontrolled vertical growth, as in deformity A, and the mechanical collapse of the "dome", as in deformity B. To confirm or reject this hypothesis we started to gather facts about growth and development of regular sea-urchins, and look for a controlling mechanism, testing it through experiments.

Following Thompson's hypotheses we wanted to find out whether attachment to the substrate is such a controlling factor. With no such attachment, the echinoid test will tend to be spherical, while those urchins who have to hold tightly, will be more squashed flat (in analogy to a tent with or without strings attached to the substrate - **Fig 44a** ).(These ideas inspired later researchers, although most of them have not been tested in reality. We found, however, evidence that in accordance to his suggestion that sea-urchins living on very soft sandy or muddy substrate are indeed flatter below, apparently to prevent their sinking in the mud (**Fig. 44b**).

#### 4. *Skeletogenesis in echinoids*

Before studying echinoid skeletal growth, we inquired how calcification in organism takes place. It is well known that animal skeletal calcification occurs within specialized cells – osteocytes or osteoblasts – where calcium carbonate saturation, or in ectodermal or mesodermal tissues. In calcareous algae the body cells produce organic material, that stimulates the non-organic environment to deposit calcium carbonate. In animals the skeleton is usually produced in contact with organic matrix, that acts as platform for the mineral deposition. The matrix controls the amount and direction of crystal growth. In echinodermata the skeleton is mainly in the form of calcite crystals, with some magnesium. Teeth, however, are rich in magnesium, which makes them harder.



**Figure 44.** Biomchanics of sea-urchins tests. (a) Shapes of balloons attached to the substrate by strings of varying force - as analogs to the skeletal shape of sea-urchins (Otto, F. in: Dafni, 1986b). (b) Deep-sea echinoids, flattened below, adapting to muddy substrate )Mortensen, 1935-;(1944

Like in vertebrates, even in echinodermata, the skeleton is of mesoderm origin. It is made of ossicles (pieces of bone), that are at first elongated needle-like spikes, which branch and divide until they change into different spatial forms. Most of the information on calcium carbonate deposition in echinoderms has been obtained from observations in the skeletogenesis of sea-urchins larvae. After several cell divisions the earliest larval stage, the **blastula** is formed, as tiny ball of cells surrounding an internal cavity. A short while later the outer wall sinks-in, forming a deep depression in the lower pole which becomes the primitive mouth. This is the **gastrula** (**Fig. 20a**). At that stage several separate inner cells are divided from the inner tissue, and settle at two points in the gastrular space. These cells send out pseudopods ("false legs") and are grouped, tied into dense knots, and around them the first mineral crystals are formed. The first, larval skeleton is spiny tristar, that further branches to form the typical "chair-like" larval structure, carrying in some species longer spines (**Fig. 20b-1**). After **metamorphosis**, i.e. turn into the adult form, the embryonic spines and skeleton disappear, their minerals dissolved and absorbed into the body, to form the adult skeleton (**Fig. 20b-4**).

From the above the reader might understand that the sea-urchins skeletal plates are not something opaque of solid mineral as some believe. In the skeletal elements two components are seen: (1) **stereom**, the mineral component, made of a tri-dimensional mesh of porous magnesium-rich calcium microscopic bars, the trabeculae (**Fig. 8**). And (2) **stroma**, a soft tissue made of organic tissue and active living cells, which fill interstices among the skeletal trabeculae, that branch continuously and reunite, forming a solid structure which is partly inorganic mineral and partly organic, live material. The specific weight of the skeleton is obtained in precise measurement and weighing of plates of a sea-urchin. It is about 1.2 g/cm<sup>3</sup> - not far heavier than water (1.0 g/cm<sup>3</sup>), while the density of the magnesium rich limestone - building the skeleton - is 2.73g/cm<sup>3</sup>. From these data I calculated the **average porosity** of a plate (% volume occupied by soft tissue) as  $100 \times (1.00 - (1.20/2.73)) = .56\%$ . In fact, I found that recently added aboral skeletal plates are more porous (80-85%) than plates that ceased to grow (porosity of .50-60%).

Another important unique peculiarity of echinoderm skeletal plates – the organic stroma is a **syncytium**, tissue made of many cells, each having a nucleus, but no borders between them. From a hydrodynamic point of view it is a multinuclear continuous fluid. A thin layer lines the stroma with the porous skeletal stereom. The result is that each ossicle – or plate – is a volume unity, in which the mineral component is one crystal and the soft tissue is a continuous fluid, in contrary to vertebrate bones or mollusc shells or which are a mixture of randomly oriented crystals, and a many cell matrix. It was also established that the proteins in the stroma can induce the calcification under the condition of over-saturation, or inhibit calcification under different conditions (Politi et al., 2004). This fact has interesting implications: For decades it was known that the sea-urchin's skeletal plate is optical one continuous crystal (**Fig, 42**), where all mineral molecules are arranged at the same crystalline orientation. There is, however, two exceptions: the spines and teeth of sea-urchins stereoms are multi-crystalline, which contributes to their strength and flexibility (Uppsala University :Echinodermata) Later I intend to show that a sea-urchin plate is actually a unity also in terms of biomechanics. It consists of a continuous fluid volume (stroma), contained in an uninterrupted tri-dimensional mineral mesh (stereom) and enveloped by an outer covering, the epidermis. Thus it should be considered a **pneumatic** entity, with hydromechanical qualities discussed below .

## **5 Differential growth of sea-urchin skeletal plates .**

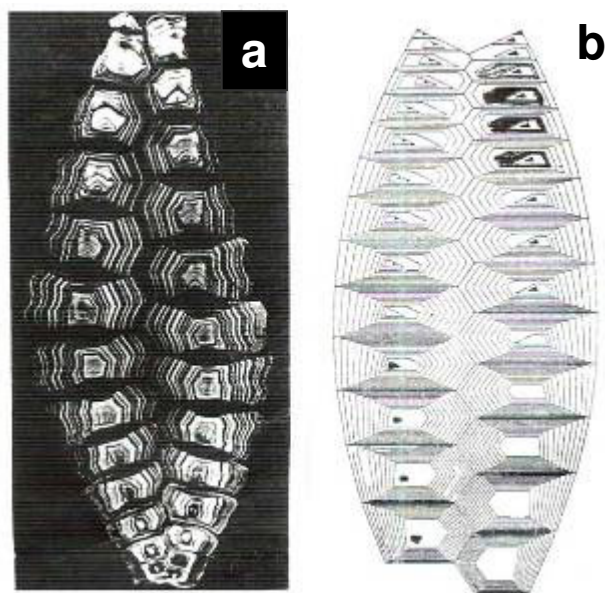
At the metamorphosis stage, on the flank of the pluteus larva an **echinus** stage appears. It assumes the shape of a "liquid ball", with 5 primary tube-feet. Gradually its outer surface is covered with newly formed skeletal plates and spines, while the vestiges of the larval skeleton dissolve (**Fig. 20b**). The new plates are of two types: (1) plates perforated by minute pore pairs, from which the tube-feet emerge from the coelom to the outer environment – called **Amb** plates (sometimes called radial plates); and (2) imperforate (intact) plates called **interAmb** plates (interradial) (**Fig. 5**). At the end of the metamorphosis the number of plates, arranged in 20 vertical columns - the lower pole (adoral, mouth) to the upper pole (aboral - where the anus), is ~ 4-5 per column, their number steadily increase with age and size. The anal opening is surrounded by 10 **apical** plates, whose number remains constant throughout the entire life, while around the mouth the plates end, replaced by an elastic tissue with minute embedded plates, the **peristome**. In the midst of which five teeth protrude, parts of a complex chewing organ, "Aristotle's lantern", made of five jaws, each composed of several limestone ossicles (**Fig. 13**). Spines are also part of the internal skeleton, although in many cases they do not seem to be covered with an epidermis. Each spine is attached by a collagen tissue to a rounded base and is powered by a muscular ring at the base. Unlike a turtle carapace, composed of a fixed number of dermal plates, a sea-urchin's test adds new plates throughout its entire life, on to of the existing ones, which grow and move downwards from their origin - the aboral circle (**Fig. 7**). But, like the dermal plates of the turtle, or tortoise, all echinoid plates show circular growth lines at their margins. These lines are seen on the urchin's plate after applying specific treatments that emphasize them (**Fig. 45a**). The growth increments shown in the growth lines patterns, indicate a typical differential growth, different rates in various directions, common to most "regular" sea-urchins. In my study I invested much thought to the significance of these patterns and the factors that may determine the differential growth – or calcification – patterns, as I will show later, may have caused the deformed sea-urchins patterns .

An American scientist (Raup, 1968) made an attempt to examine the differential growth in a theoretical model by producing a computer program, in which initial size, growth gradient, number of plates and distance from the apical origin interacted. His computerized pattern, quite similar as those found by using the **charring** technique (see next page) (**Fig. 45b**). Raup did not assume that this differential pattern is of any genetic origin, but related to the mechanical or biological environment of the living creature, without elaborating his view.

In this work we assume that a **sea-urchin's skeletal plate has no "inherent information", dictating how far and at what rate and direction to grow and add their skeletal limestone deposits**. If so, we must look for the factors that determine the rate and intensity of this peripheral plate growth.

Before investigating our mechanical hypothesis that differential growth is related to the effect of mechanical forces ,we must first find a way to measure and quantitatively describe the various peripheral directions of growth in an immediate and efficient mode .In this study we examined the differential growth patterns of each plate in two different methods simultaneously to compare and correlate them:



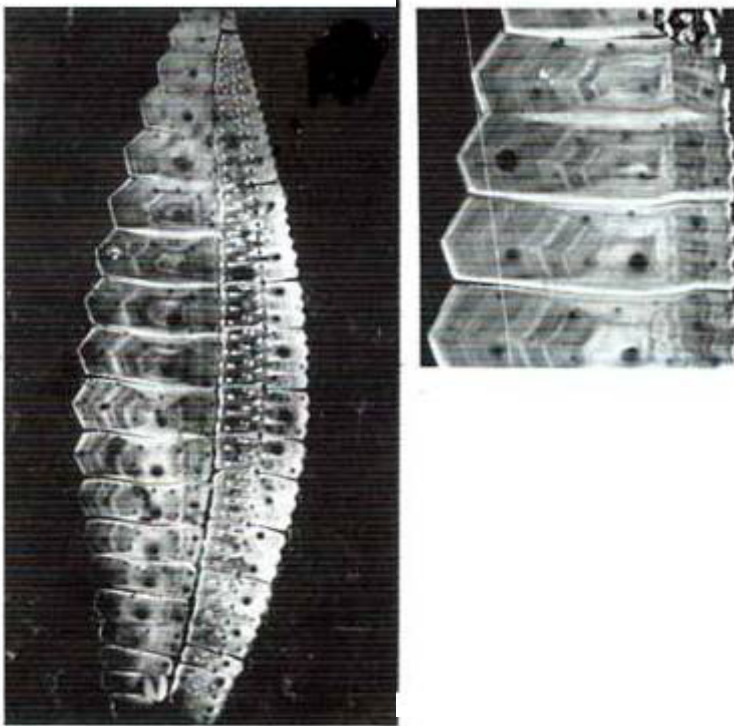


**Figure 45** (a) skeletal plates of the sea-urchin *Strongylocentrotus* showing growth patterns with the charring technique; (b) Theoretical growth model, from data obtained from real urchins (left) drawn by computer, according to his mathematical model (both Raup, 1968)

- A. The first method involved charring – incomplete combustion of organic matter – of cleaned plates in a furnace at a temperature of 300°. In this temperature a pattern of growth lines appears at all plate edges, which are later immersed in the solvent xylene, and enhanced by fixing in **Canada balsam** resin. The data were obtained for an entire InterAmb column, by selecting the an arbitrary identical (and therefore synchronous) growth line, shown in all plates. Measurement of the distance in mm, to the nearest margin determines the total growth in the same direction at the time elapsed since this line had been formed and the plate death demise. The same procedure was made in all four directions – aborally (upwards), (adorally) downwards, (Interradially) towards central suture, and (adradially) at the suture between Amb and InterAmb plates. The time line is equal for all the plates in the entire column (**Fig. 46**).
- B. A second method - uses a radioactive calcium isotope ( $^{45}\text{CaCl}_2$ ). A living urchin of an similar size as in A, to transfer into an aquarium with radioactive sea-water for several hours, after which it was sacrificed. The skeleton was bleach-cleaned and the individual plates separated. A sharp scalpel removed minute  $\text{CaCO}_3$  samples of the extreme edge of each plate, in the above mentioned directions, each put into a vial, where the calcium carbonate dissolved in acid, and radioactivity measured in a Liquid Scintillation Counter for each sample, of an entire Iamb column.
- C. A third method, similar to A, but the artificial growth pattern induced by exposure to tetracycline, which deposits in the living skeleton a bright light pattern, visible in UV light.. This method was not implemented here

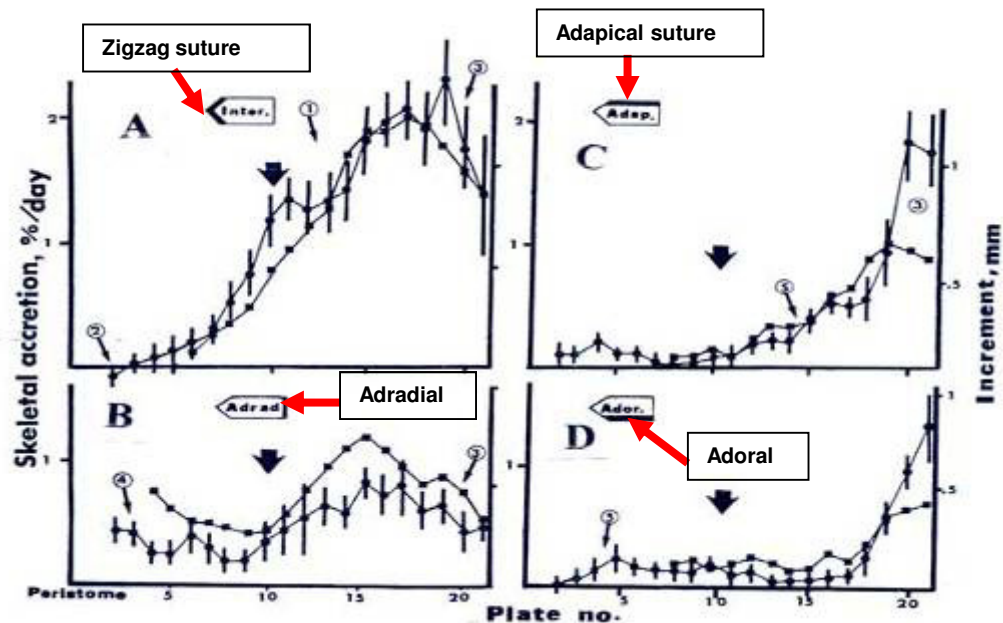
**Figure 47** shows the results and correlation between growth, in mms, for a given duration, obtained in method A, and calcification, in % increment per day, measured by method B. The patterns shown here are remarkably similar to each other and to a pattern shown in a Mediterranean Sea-urchin (*Paracentrotus lividus*), using method C. (Märkel, 1975).

From these results we conclude that each test plate undergoes a regular growth pattern, which depends on its age and its position along the plate column. As a rule, it grew faster in the horizontal direction and much slower in the vertical direction (had a low **v/h ratio** - see below). Also, there is a significant difference between growth rate of the interr radial - zigzag suture (between two neighboring Iamb columns) than the adradial suture.



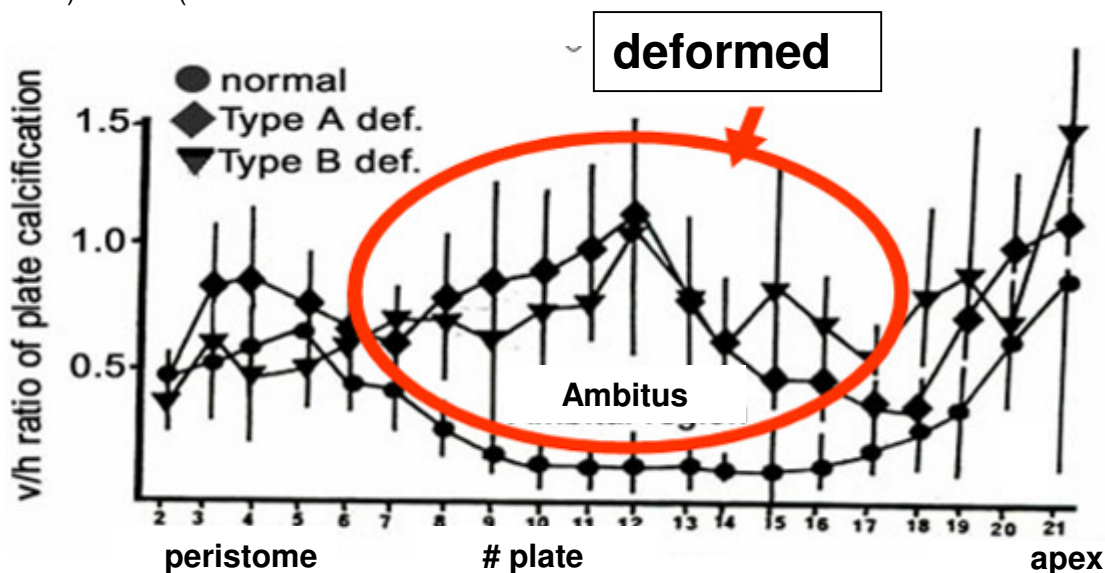
**Figure 46.** Charring patterns of growth lines in Amb and InterAmb plates of a non-deformed Velvety Sea-Urchin, that its increment measurements are depicted in **Fig. 47**

It is important to note that a similar, almost identical pattern appears in most regular sea-urchins, therefore it is considered the normal pattern (Dafni, 1980).



**Figure 47.** the correlation between **method A** measurements (mm, **square symbols**) and skeletal radioactive Ca accretion – **Method B** (% per day, **dots**) in the skeleton (in mm,) and measured increments in a non-deformed Velvety Sea-Urchin, like the one depicted in **Figure 46**, in the different sutures (red arrows): **A** - zigzag suture **B** – suture in opposite direction (Adradial); **C** - the upper (Adapical) and **D**-- the lower (Adoral) of the plate. Plate are displayed from the bottom (peristome, left) upwards, to the apex (apical, right - the youngest plates). (Dafni & Erez, 1987a)

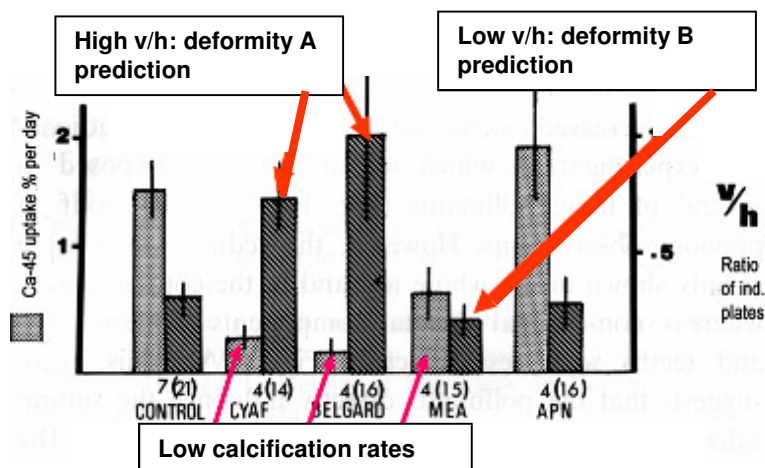
A much different pattern was shown in deformed VSU's in both deformities. An analytical feature that defines both deformities is a abnormal ratio between growth and calcification in the vertical direction (**v**) vs. horizontal growth (**h**), i.e. **v/h ratio**. Urchins showing these two deformity type showed an atypical pattern of v/h: The lowermost plates, as well as the uppermost ones showed a normal ratio, whereas in plates at the **ambitus**, v/h ratio is ~0.2 in normal, non-deformed VSU's, while the deformed of both deformities showed a v/h ratio of ) 0.5-1.0**Fig. 48**). This seems to be a meaningful analytical feature. If our hypothesis is correct, the factors that constrain or regulate the growth pattern affect the v/h ratio. This ratio, obtained in the radioactive <sup>45</sup>Ca method B, has been used in the ensuing experiments (3 -5) testing the influence of pollutant agents )e.g. **Fig. 49**) or under abnormal mechanical constraints )**Table .(5**



**Figure 48.** The v/h ratio between calcification rates in the vertical direction (**A+B** in the previous figure) vs. in the horizontal direction (**C+D**) along an **InterAmb** column in normal sea-urchin (**circles**), and in both deformities (**triangle & diamond**). Number of plates: as before (Fig. 47). The difference between the deformed and normal urchins in this aspect was highly significant. Hereafter we will discuss the "prophetic" value of this measured property in early detection of skeletal deformities (Dafni & Erez, 1987b (

### **Experiment 3 – influence of pollutants on v/h ratio in VSU**

**Figure 49.** A radioactive Ca experiment in which plates from the ambitus region of a healthy VSU's, who were exposed to pollutants, two of which are chemicals used for preventing buildup of Ca deposits in the power plant (**see Table 3**), and two chemicals, **APN**,  $\beta$ -amino-propionitrile, and **MEA**,  $\beta$ -mercapto-ethylamine, both cause relaxation of collagen systems, using v/h as parameter. **APN** was the only chemical that did not affect these parameters. Below: number of samples. (Dafni & Erez, 1987b).



## 6. Tube feet, attachment strings – regulation and deformation

The Amb system is a highly hydromechanical system. It consists of many fluid filled hydraulic “feet”, each carrying a sucker disc, that snaps and adheres to a solid object by vacuum. Their “roots” are inside the body cavity, in a muscle activated ampulla (**Fig. 12**), which contracts or relaxes, and the internal pressure pushes the tube, extending it forwards. The tube-feet shorten when the longitudinal muscles contract. Thus, the tube-feet penetrate the body wall through the perforated Amb plates, where they are attached to the solid wall plates (**Fig. 15b**). As mentioned above, the tube-feet are arranged in five double-rows in perfect pentamerous symmetry around the test perimeter. So, regular sea-urchins are attached, or linked to a rock or any other hard substrate by hundreds of “strings” at once (**Fig. 44**). The movement of an urchin is done in steps: (1) the sucker disc releases its previous grip, (2) and is pushed forward by hydro-pressure to find a firm hold in the desired direction; (3) where the its sucker attaches by vacuum (4). Then, the longitudinal muscles contract and (5) pull the entire urchin body towards it. At any moment tens of tube-feet pull at all directions, and even at rapid movement, a healthy urchin never releases all its tube-feet at once. Each skeletal plate (even InterAmb plates, not directly connected to the tube-feet), is under intermittent pull outwards, and down, towards the hard bottom it attaches to. A deviation from the normal pull pattern may cause deformation.

## 7. Impact of mechanical stresses on the sea-urchin’s test morphology

The hypothesis that the tube-feet attachment has influence on the sea-urchin test shape gained some circumstantial evidence: Moore (1935) and Nichols (1982) noted that regular urchins living on solids substrates tend to have a flattened body profile than those living in soft bottom habitats, where weaker forces are applied by the tube-feet. This hypothesis, however, was never tested in a controlled experiment. Experiment 4 intended to test it.

### Experiment 4. Transfer of sea-urchins from hard to soft substrate

In this experiment we moved urchins from solid substrate (reef) to a soft substrate (sand) and measured over time the change in their external profile (**H/D ratio**). And indeed, during 17-42 days significant changes occurred (**Table 4**). Reversible changes, H/D decrease when these sea-urchins were carried back to a solid substrate were less significant (Dafni, 1986).

**Table 4. transfer sea-urchin juveniles from hard to soft bottom and vice versa**

group	Sample size	initial measurements		Dura- tion	final measurements		Statistics
	number	Avg. diameter, mm	H/D $\pm$ SD	days	Avg. diameter, mm	H/D $\pm$ SD	T-test, probability
<b>A. Transfer to sand substrate (increase in H/D ratio)</b>							
control	11	21.05	0.542 $\pm$ 0.023	70	26.36	0.539 $\pm$ 0.020	0.33 ( - )
Exp. A	10	28.71	0.529 $\pm$ 0.028	38	30.27	0.559 $\pm$ 0.035	2.12 **
Exp. B	18	10.59	0.539 $\pm$ 0.034	42	26.44	0.572 $\pm$ 0.026	3.27* **
Exp. C	19 (21)	36.35	0.537 $\pm$ 0.032	17	36.81	0.557 $\pm$ 0.031	2.00 **
<b>B. Transfer back to hard substrate (decrease in H/D ratio)</b>							
Exp. C	16 (19)	36.81	0.557 $\pm$ 0.028	30	37.75	0.541 $\pm$ 0.028	1.68*

Statistic probability two-tail: ( - ) N.S. ; (\*)  $p < 0.1$  ; (\*\*)  $p < 0.05$  ; (\*\*\*) highly significant-  $p < 0.01$



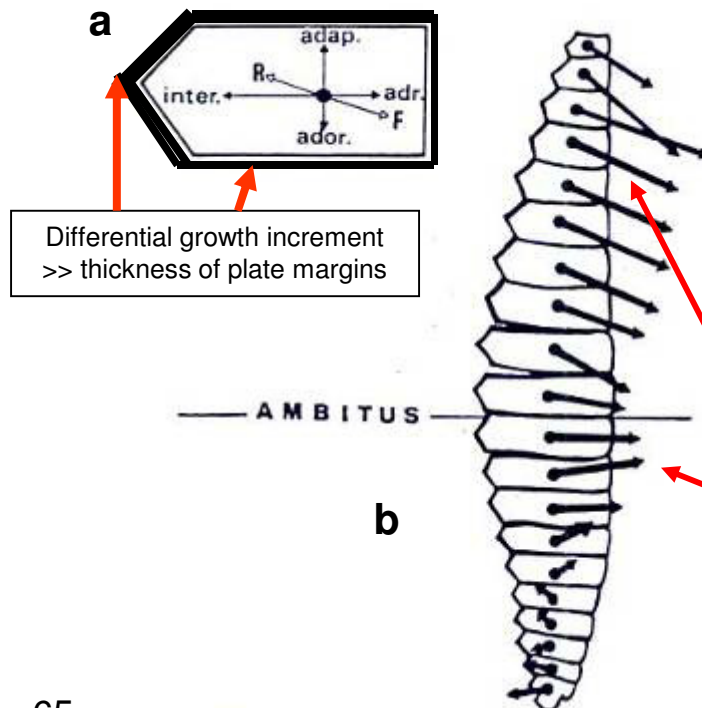
## .8The biomechanical role of the mesenterial threads

As our working hypothesis suggests, the differential sutural growth and Ca deposition in the plates margins are affected by mechanical forces – pressure or tension between them. We already showed that tube-feet are able to exert such forces. The plates are bound together by flexible collagen sutural fibers, allowing their growth, but prevent their falling apart. We assume that when plates are drawn forcibly to each other, their sutural growth is inhibited, because the  $\text{CaCO}_3$  crystals need space to grow. Tensional forces that threaten to pry the suture open encourage calcification. Furthermore, we assume that when a plate grows equally at all directions, it means that the forces around it are equal. Therefore, the growth lines pattern we observe reflects the forces that may have caused them. Analyzing the growth and calcification patterns may reveal the biomechanical constraints around and within the sea-urchin's skeleton. Such analysis (Fig. 48) shows that both deformity types show at the ambitus level greater growth and calcification in the vertical direction (increased v/h ratio) than the non-deformed urchins .

In the next analysis (Fig. 50a) we try to "translate" the growth increment (=calcification rates) in each marginal direction of one putative plate into a **vector**, which depicts the dominant direction and intensity of growth (**R**). For this vector we hypothesized that a **force vector F** exists, whose pulling at the opposite direction within the close-packing plates array may have produced the tension that caused the directional growth, denoted by **R**. Repeating this process for the entire **InterAmb** column (Fig. 50b), we have put together the putative **F** tensional vectors .

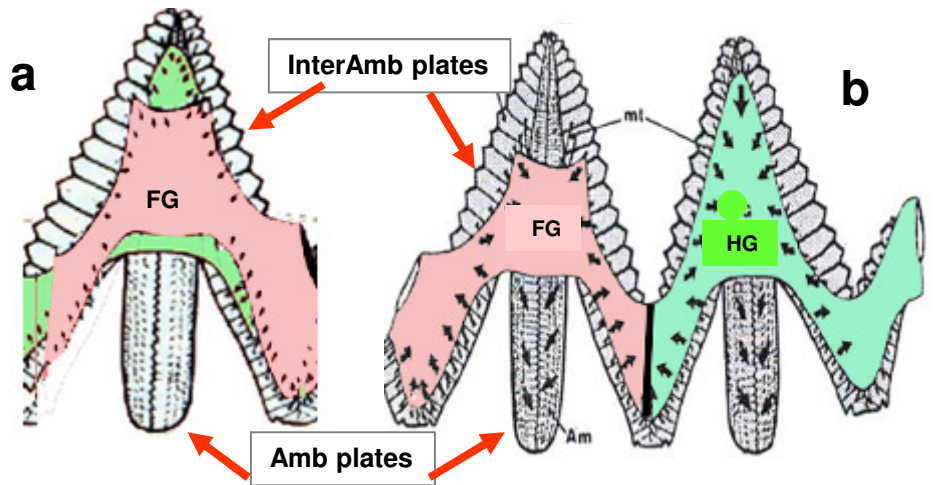
The **F** vectors of plates above the ambitus were longer and pointed clearly downwards, whereas below the ambitus the picture was different – vectors were shorter and their direction turned upwards .

In the following we will show that this pattern conforms well with our observation that the mesenterial threads, that bind the intestine to the skeleton, are arranged in a similar pattern.



**Figure 50.** A typical column of InterAmb plates and tensional vectors, derived from growth/calcification measurements (Fig. 47) based on the above hypothesis. **R** in (a) is a **weighted vector**, derived from an arithmetic sum of growth/calcification in four directions (compare thickness of sutural margins): vertical and horizontal. **F** is a putative tensional force, pointing to the opposite direction as **R** and comparable size. (b) The entire column, with **F** vectors. Horizontal line indicates the midline (**ambitus**) (Dafni & Erez, .(1982

**Tensional force F vectors**



**Figure 51** .Sea-urchin intestine attachment to inner wall by mesenterial thread (**Fig. 14**). (**a**) One sector of Amb and InterAmb columns is shown. The intestine sections are hung on the inner wall of the test by mesenterial threads (thin arrows). The **foregut (FG)** “covers” the **hindgut (HG)**, that hangs higher than **FG**. (**b**) shows them separately, marking the putative tensional forces direction that applies to the plates by the intestine (thicker arrows), in a pattern which fully conforms with the vectors pattern (**Fig. 50b**) )Dafni & Erez, .(1982

**Fig. 51** shows that there was a clear fit between the spatial vectors pattern of these and the distribution pattern of the mesenterial threads, that hang the urchin’s intestine on the inner wall surface. The sea-urchin’s intestine, after emerging from Aristotle’s lantern upper part and a short esophagus, draws two complete loops around the coelom cavity, before ending in the anus, in the apical region )**Figs. 14**). The first loop (foregut) runs anti-clockwise and the second loop (hindgut), after a recurving, runs in a clockwise direction. The entire intestine is tied to the wall by the above mentioned threads. The mesenterial threads are usually overlooked, since they are thin, transparent and barely noticed to the casual observer’s eye. In microscopic survey )**Fig. 23**) we found that they made of collagen, apparently MCT )**p. 15**), able to solidify and relax, they show a small number of muscle fibers, which may provide them contractile power, and the ability to exert real pulling forces. In the following pages we will bring circumstantial evidence that forces, applied by these threads, are the best candidate to control the differential growth of the sea-urchin’s plates .

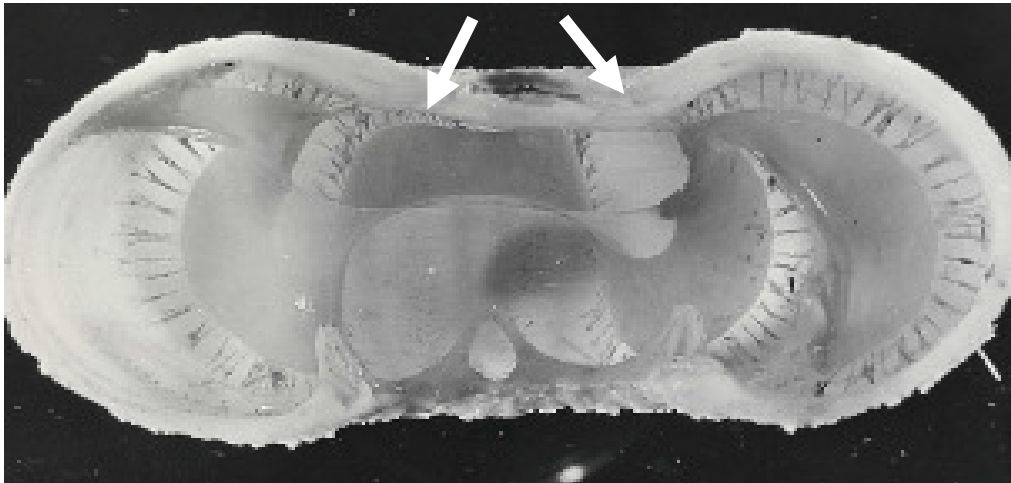
1. A longitudinal section in a slightly deformed VSU after its intestine was emptied from food shows the intestine loop, and its suspension from the inner wall by the mesenterial threads (**Fig. 52**).
2. Fire Urchin )***Asthenosoma*** )**p. 25**) is an aberrant genus of sea-urchins, whose unique property is its ability to change its shape immediately, from flattish to high, round to triangle, as shown in **Fig. 54a**. Its anatomy reveals **articulated plates** rather than fused ones, and a muscle system unique among Regular sea-urchins made of **radial muscles**, often named “**mesenterial muscles**” )Jensen, 1985)) **Fig. 54b**), which are responsible for its flexible morphological - clearly homologous with our mesenterial threads system.

Careful observation of the radial (mesenterial) muscles of Fire Urchins shows that they are homologous with the mesenterial threads of other regular urchins. These muscles differ from other echinoderm muscles in that they do not respond to stimulants like adrenalin, dopamine etc., with pronounced response to other muscles (Amemiya & Tchuchia, 1979), this proves that it is unique muscle tissue, of a different origin.

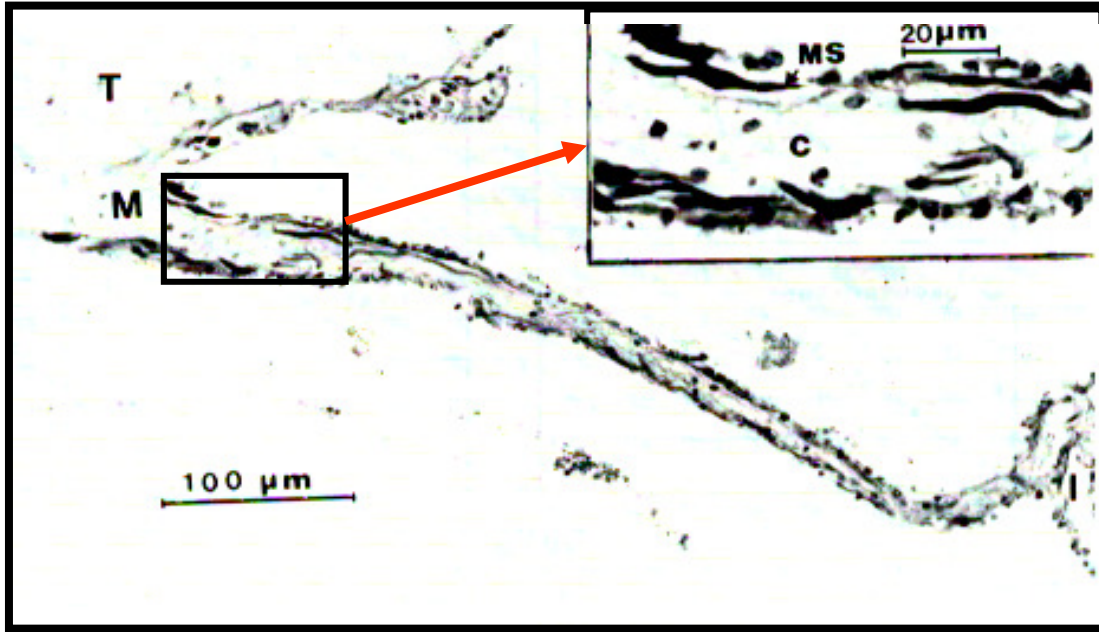
3. In the inner wall of sea-urchin tests various protuberances are shown on the inner area of the plate, in the exact points where the mesenterial threads hang (**Fig. 55**). In some cases they are elongated, forming a pattern like the one suggested by our analysis (**Fig. 51**), indicating that they were controlled by tensile forces that follow this attachment.

From all the above, it becomes clear that if there is a pull of the body downwards, as proposed by Thompson, the Amb plates, through which the tube-feet protrude, must calcify more in the upper margin, opposite to the direction of the pull. And, indeed, when we measured the calcification pattern of the Amb plates in both methods – growth increment and redioactive Ca – it was clearly shown that they also do. This pattern was consistent to other sea-urchins (Märkel, .(1975

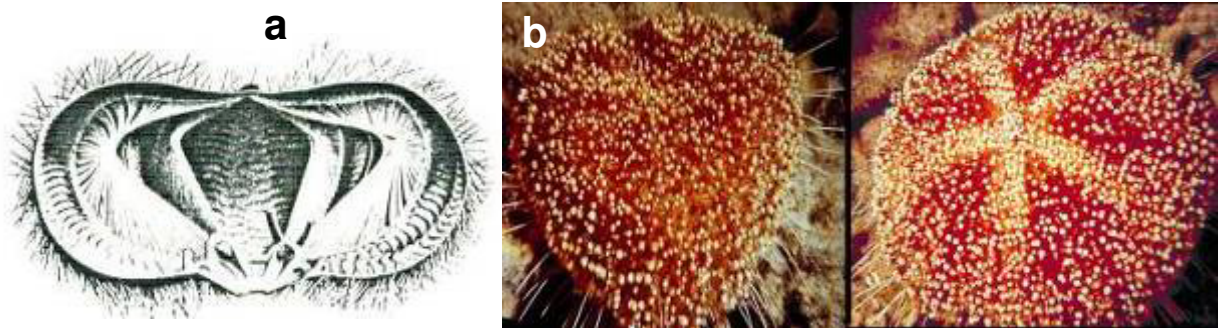
4. Later, I wanted to demonstrate that calcification along the sutures between neighboring plates, bound by sutural collagen fibers, is also influenced by tensional or compression forces. For this purpose experiment 5 was carried out.



**Fig. 52.** Longitudinal section in VSU, showing the intestine loop, suspending from the inner wall of the test by mesenterial threads. Arrows show the uppermost hanging points of the threads. (original).

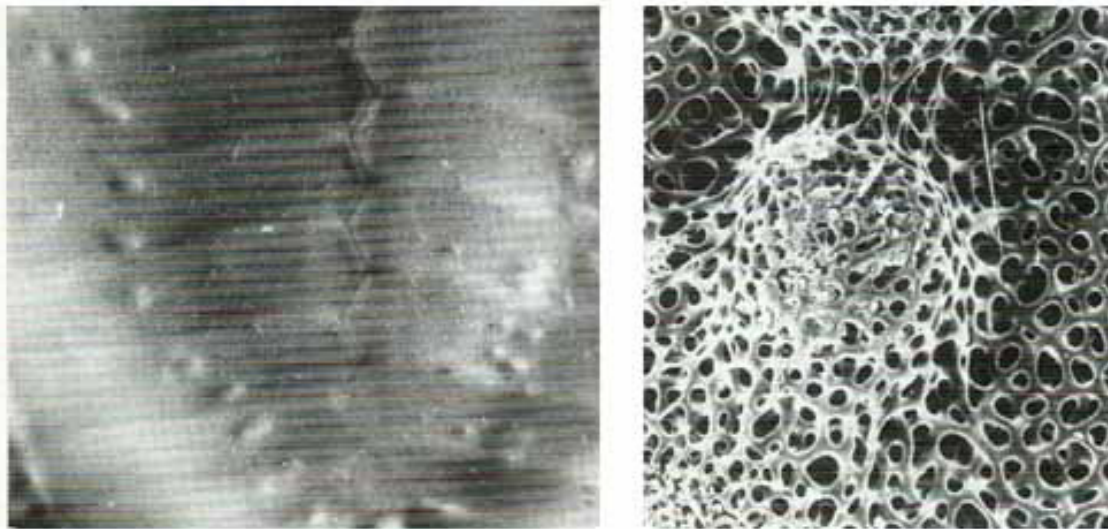


**Fig. 53.** Microscopic enlargement of **M**, a mesenterial thread, connecting the test **T** and the intestine **I** : **MS**, muscle fibers, **C**, collagen mass (Dafni, 1983b).



**Figure 54.** (a) The unique radial-mesenterial muscle system of *Asthenosoma*, as described by Sarasin & Sarasin (1888). (b) Two pictures showing the remarkable plasticity of the Red Sea Fire Urchin (*A. marisrubri*), obtained through the function of the mesenterial muscles (Photo: A. Colorni)





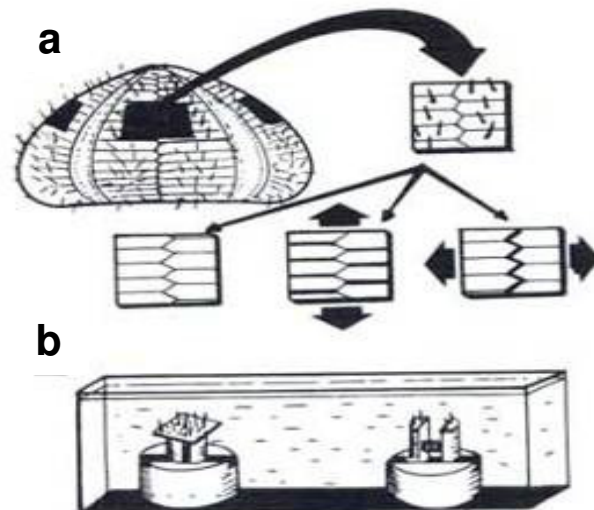
**Figure 55.** Scanning electron microscopy (SEM) in low (x5), and high (x200) magnification of skeletal protuberances associated with the attachment of mesenterial threads (compare **Fig. 51**). Note that most of the protuberances are elongated, apparently by pull in a direction pattern like the one suggested by our analysis (**Fig. 50** arrows) (Dafni, 1988).

### **Experiment 5 – Effect of tensional stresses on v/h of VSU plates**

In previous observations we noticed that skeletal plates, severed from a living sea-urchin, and maintained in sea-water, continue to move their spines and pedicellaria continued to open and close their "Jaws" for at least two days, and calcify for several hours, although at lesser rate than on an intact living skeleton.

Five InterAmb plate squares were cut from the test using a cutting disc from a medium sized Tripneustes from the super-ambital region, with an area of 2.5 cm<sup>2</sup> each (**Fig. 56a**), and placed in a aerated and well lit aquarium, in <sup>45</sup>Ca radioactive sea-water, at room temperature. Manipulation included impaling the explants on two moving spring tensed spikes, with an intensity of 50-100 g - an experimental device shown in **Figure 56b**. These spikes applied tensional forces to the skeletal explants, in two directions – vertically and horizontally (lower row in **Fig. 56a** for 12 hours of incubation. Control explants were fixed on the spikes but with no mechanical forces applied. Procedure and results followed the described below (**Table .5**)

**Figure 56. Experimental setup for Experiment 5 :** (a) Square explants cut from a live Velvety Sea-Urchin (VSU) test and impaled on an experimental device shown in (b), The experimental device which applied tensional forces (spings) in the directions shown by thick arrows. Left – control. (Dafni, 1984



**Table 5**

Results of mechanical stretching of skeleton explants of VSU in (A) calcification rates on whole plates or marginal sutures - horizontal, vertical, and control (no stretching). (B) their effect on the ratio between vertical - horizontal )**v/h ratio**) (Fig.56a) In parentheses, number of plates samples examined. T-test results were applied and their significance shows at the bottom of the table ( Dafni, .(1984

<b>A. Calcification rates</b>				<b>(% day<sup>-1</sup> ±SD x 10<sup>3</sup>)</b>			
Individual no.	Parts analysed	Direction of stretching			Comparison between		
		Horizontal	vertical	control	Hor-vert	vert-cntrl	hor-cntrl
Y-23A	Whole plates	----	30±10 (17)	22±5 (18)	----	1.39*	----
Y-9	Sutural edges	104±55 (10)	----	64±24 (13)	----	----	1.63*
Y-12	"	108±26 (20)	82±21 (22)	74±11 (9)	1.32***	1.1 (-)	1.46***
Y-17	"	64±14 (18)	68±13 (19)	----	0.94(-)	----	----
Y-20	"	87±47 (15)	49±26 (20)	42±20 (9)	1.77***	1.17 (-)	2.07*
<b>B. Effect on v/h ratio</b>							
Y-9	Sutural edges	0.47±0.19 (10)	----	1.11±0.64 (13)	----	----	0.42***
Y-12	"	0.71±0.22 (20)	1.09±0.26 (21)	1.20±0.34 (9)	0.65***	0.91(-)	0.59***
Y-17	"	0.73±0.25 (18)	1.17±0.48 (19)	----	0.63***	----	----
Y-20	"	0.12±0.11 (15)	0.26±0.28 (20)	----	0.46 (-)	----	----
Y-23	"	0.47±0.16 (19)	0.73±0.42 (30)	----	0.65*	----	----
Level of significance: (-) N.S.; *, p<0.05; **, p<0.01; ***, p<0.005							

The observations showed that the explants maintained their vitality throughout the experiment. Calcification rates measured in the radioactive method were 5-10% of the in vivo ratios. However, the hypothesized ratios and patterns were stable and fixed, and similar to that obtained in whole, normal urchins: The results, shown here are that vertically stretched whole explant calcified at a rate 1.39 times higher than in the non-treated control explant, cut from the same urchins as the treated explants. In sutural calcification, horizontal margins showed 1.46-2.07 higher rates than the non-stretched (control) or the vertically stretched. Vertical vs. control did not show significant differences. Both, horizontal and vertical stretching changed the v/h ratio in the sutural edges significantly. v/h ratio of horizontal stretching were lower than the vertically stretched. In the former a ratio of 0.47 – 0.73 was different than 1.11-1.20 of the control. Vertically stretched did not show significant change from control. These results are explained by the assumption that in vivo the plates are under mechanical compression, and their mere extraction from the natural close packing cause them to change their calcification rates .

Under stressed conditions, like the conditions in which this experiment was carried out, sea-urchins calcify less than in the wild, yet their differential ratio may support the hypothesis that the deposition of limestone in the skeleton of sea-urchins is related to tension, caused either by an internal pressure, or due to pulling by contractile or elastic elements, creating tiny gaps, in which the mineral deposition occurs (see below, the biomechanical model .(

### ***.9Soap bubbles model of echinoid skeletal plates.***

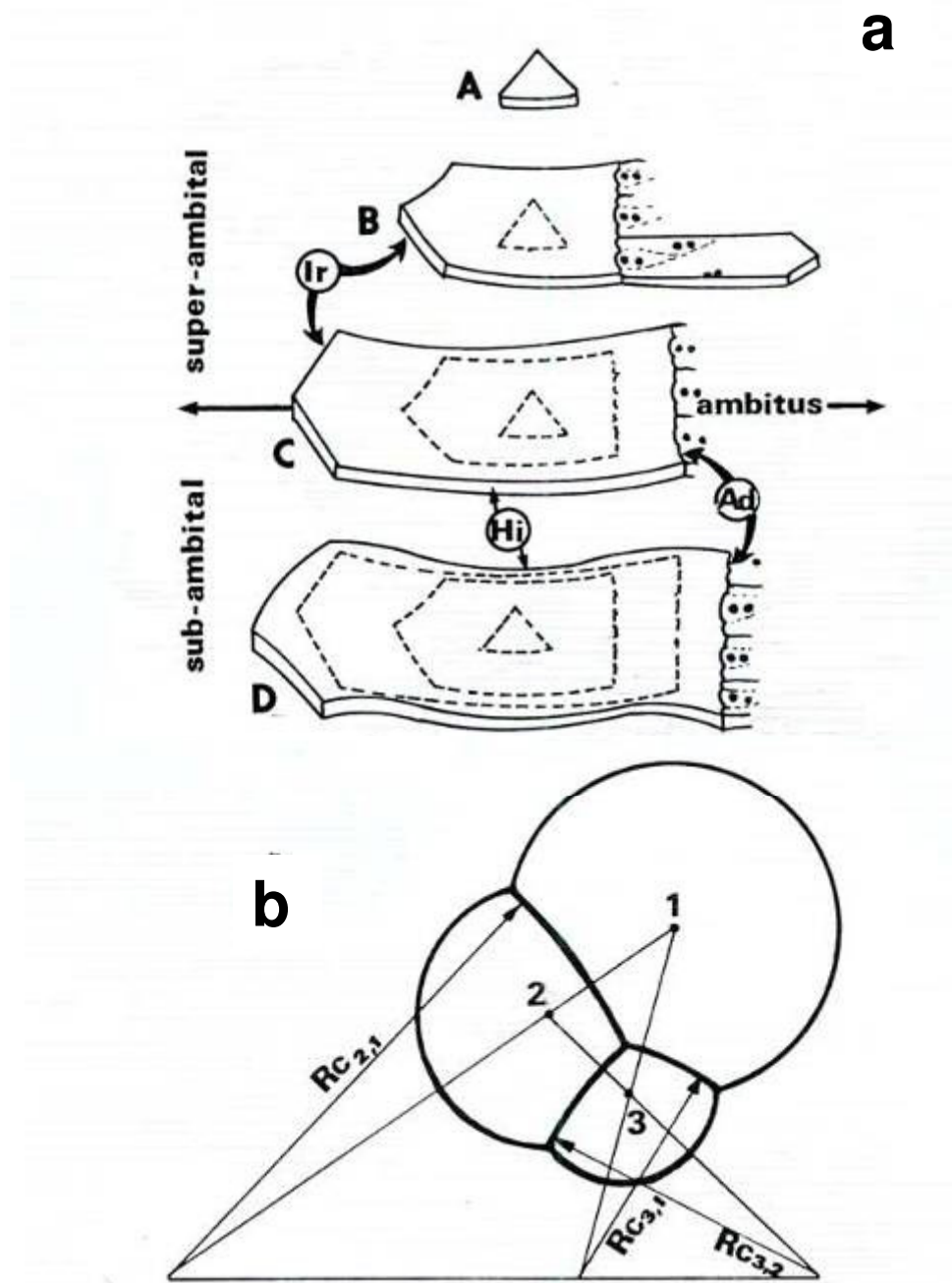
An observation I made in an early stage in my investigations, was that the suture lines between neighboring plates are seldom straight. It is evident from my own observations (**Fig. (57,46** and was also documented by Raup (1968, his text-fig. 2, ) (**6Fig, 45**). Contrary to patterns of close-packing, shown by honeycombs or hexagonal basalt columns, here the boundary between the units is curved – smaller plates convex at the boundary of larger plates and vice versa, larger plates concave at the boundary of a smaller plate. The boundary between two equal-sized plates is straight. Actually it was D'arcy-Thompson's who first wrote about the analogy between the plate margins of sea-urchins and the array of soap bubbles. Raup wrote that soap bubbles boundary area tends to be minimized with respect to volume, meaning that like soap bubbles, the echinoid plate's **volume** is a factor in determining the nature of the curvature of the boundary between neighboring plates .

**Figure 57** shows five representative interambulacral (IAmb) plates out of the 15-21 plates, along the interr radial - zigzag suture, showing their shape changing with growth. An “elderly” plate, charred and embedded in Canada balsam, shows growth lines patterns (see **Fig. 46**). The uppermost, just added plate is triangular (**Fig 57aA** – see also **Fig. 7A,N**), but convex along the suture attached to the plates beneath. Another, somewhat lower, IAmb plate shows concavity toward an upper plate across the zigzag (**Ir**) suture, and convexity toward a larger plates beneath and across the suture )**Fig. 57aB**). The third plate (**C**) At the midline (ambitus) where the plates across the suture are approximately equal - the zigzag margins is straight )**Fig. 57C**). Plates from beneath the ambitus grow almost exclusively in the horizontal direction, and the concavity-convexity pattern in **Ir** suture reverses – convex upwards and concave downwards )**Fig. 57D**.(

Back to the soap bubble, whose shape is balanced between internal air or fluid pressure and the surface tension around the outer membrane. Internal pressure causes the bubble to grow, until stopped by outer membrane surface tension. Assuming that the Concavity-convexity denotes a balance between inner volume pressure and surface tension of an outer membrane, Raup asked, like other students of the echinoid morphology: how can we explain solid plates behavior like soap bubbles ?

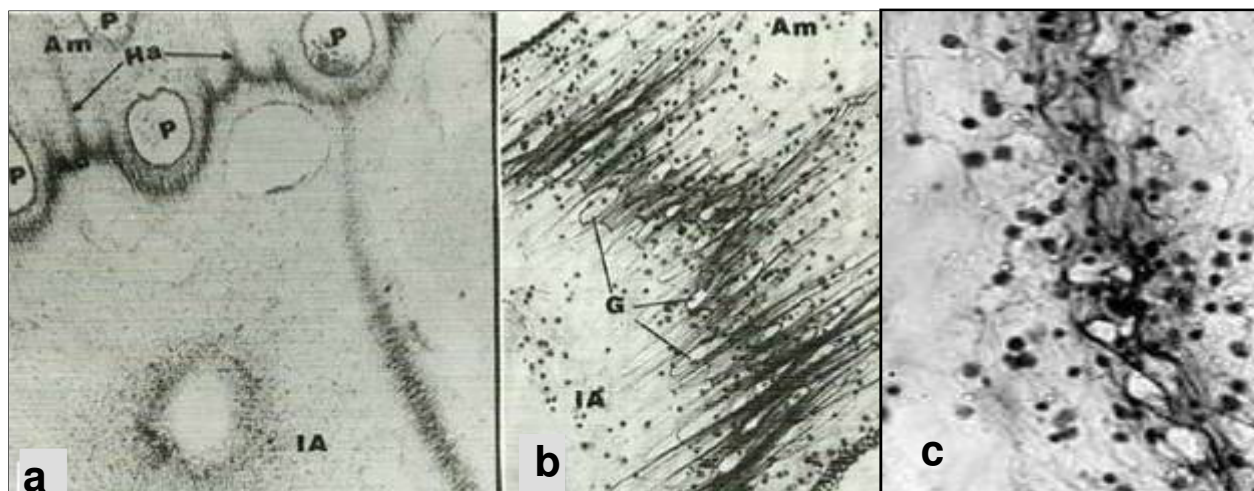
I propose that this apparent contradiction is easily solved by proving that the internal structure of the plate has volumetric properties. Moss & Meehan (1967) showed that a echinoid plate is actually an inner space covered by an outer membrane, It is logical that this volume consisting of a highly porous mesh of mineral trabeculae, the **stereom** )**Fig. 8**), intertwined with **syncytic stroma** )**p. 59**) tissue may be under hydraulic internal pressure, and tension in its surface – a pneumatic “bubble”. Indeed, if we ignore the mineral fill, we may assume that the calcium carbonate deposition is secondary to its primary shape designing (morphogenesis) determined by the soft tissues, as ice freezing in a plastic container gets the shape of the container, the plate shape is dictated by its pneumatic properties.

The “Soap bubbles model” well describes the mutual relations between the plates, and allows us to predict that if a plate breaks in the middle, regeneration includes creating a new dividing collagen fibers barrier, similar to sutures that surround the plates, the two halves will start to behave as independent bubbles. Indeed, when examining regenerated plates, they always behaved as expected from this model, i.e. if the halves were equal the new suture was straight, or convex or concave if the halves were not equal sized. Indeed, many observations of plates' arrays in Regular and Irregular sea-urchins proves the validity of this “pneumatic” approach.



**Figure 57.** (a) Changes in the concavity-convexity pattern of the echinoid plate edge throughout its lifetime. **A** – shortly after appearance; **B** plate in super-ambital region, concave upwards, and convex downward; **C**- plate at ambus: The zigzag (**Ir**) margins are straight, and **D**, plate in the sub-ambital region – the zigzag margin shows reversed concavity-convexity patterns. In the upper and lower horizontal sutures (**Hi**) which grow vertically, the reversal is shown only in those margins that continue to grow (**Fig. 46-47**). Note the concavity-convexity of the adradial vertical suture (**Ad**). (b) Size-geometry of soap bubbles, and the relative size of adjacent bubbles (**Rc2,1** - bubble radius of concavity-convexity between 2 and 1, for example) (Dafni, .(1986





**Figure 58** (a) A microscopic section of a decalcified VSU plates (at the adradial suture - IAmb-Amb boundary) showing collagen fibers of different length along the edge of the sutures between them (x100). (b) x400 enlarged fast-growing suture, with very long fibers, oriented in a parallel array, showing elongated "ghosts" (G), remains of the trabecular beams that have been dissolved. (c) an almost non-growing suture, showing few less-organized fibers and rounded "ghosts". Abbreviations: IA – IAmb plate, Am – Amb plate, P – tube-feet pores in the Amb plates. Ha – inactive boundary between partly fused together "demiplates" of a compound plate, characterized by a few fibers (Dafni, 1986)

### **.10Collagen fibers in the inter-plate sutures**

A question often asked: if the echinoid test grows as result of inner pressure, and other elements exert mechanical forces at opposite directions, that aim to explode or deform the test shape, how the plates, and consequently the test as a whole do not explode, and the sutures do not rip open? The answer is simple: the sutures join together adjacent plates, act as boundaries with a common wall, made of a dense network of collagen fibers, who "stitch" the plates together, like the seams and patches of a football. The fibers are wrapped around the limestone beams (trabeculae) that form the stereom, across the suture gap (**Fig. 8, 59**), preventing the plates ripping off. When an urchin is injured by mechanical damage, it seldom breaks along the sutures. Even after its death, the test will not collapse, unless the suture fibers are consumed (prolonged immersion of the skeleton in bleach solution digest the fibers). Microscopic sections, specially dyed, after decalcifying the plates, removal of  $\text{CaCO}_3$  shows the sutural fibers (**Fig. 58**), which delineate the sutures around the plates array (**Fig. 58 a**). Decalcification of the plate Ca leaves behind "ghosts", empty voids that mark the shape and density of the trabeculae "beams" (**Fig. 58 b**). Assuming that the mechanical role of the collagen fibers is to resist sutural tensional stresses, it is easy to imagine that sutures exposed to stronger tension, we will find multiple, well-oriented collagen fibers, and much elongated "ghosts". Beams with an ellipsoid cross-section are mechanically stronger than with a round cross-section. Where compression was predicted, less fibers were seen, their orientation was more chaotic, and the "ghosts" were roundish (**Fig. 58 c**).

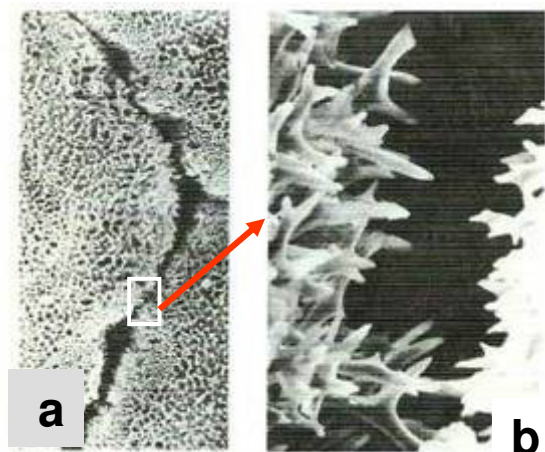
From analyzing the vector pattern (**Fig. 50**) we knew already that along the vertical zigzag suture the tensional forces are strongest, especially in the super-ambital region, and beneath the ambitus we expect some compression. Compression was evident also in horizontal sutures. In stretched sutures we found the longest and well-organized fibers, showing higher **second moment of inertia**, a property of that can be used to predict the resistance of a beam to bending and deflecting )**Table 6** .(6

**Table 6**

Length measurements of collagen fibers and “ghosts” of fiber-associated trabeculae in a decalcified section of a 10 mm VSU plate. > 30 ghosts were measured (in micrometer= $\mu\text{m}$ ). Differences in **ovality**, length/width of the ghosts were highly significant ( $p < 0.05$ ). Stiffness, or **2<sup>nd</sup> moment of inertia** ( $I = a^3b\pi/64$ ), a measure of strength and stiffness was highest for interradially oriented beams (Dafni, 1986).

suture	Fiber band width ( $\mu\text{m}$ )	Trabecular ghosts measurement ( $\mu\text{m}$ )			ovality	2 <sup>nd</sup> moment $\mu\text{m}^4$
		Length (a)	width (b)	area $\mu\text{m}^2$		
<u>Longitudinal</u> Adradial	200	13.6 $\pm$ 3.5	7.6 $\pm$ 1.8	81.0 $\pm$ 28.6	1.8 $\pm$ 0.5	<b>941</b>
Interradial	250	15.6 $\pm$ 8.5	6.5 $\pm$ 1.1	79.7 $\pm$ 45.3	2.4 $\pm$ 1.0	<b>1213</b>
<u>Latitudinal</u> Central part	120	13.0 $\pm$ 3.0	9.5 $\pm$ 3.3	96.9 $\pm$ 32.7	1.4 $\pm$ 0.4	<b>1027</b>
Terminal part	50	12.2 $\pm$ 2.8	10.1 $\pm$ 2.3	96.6 $\pm$ 31.6	1.2 $\pm$ 0.2	<b>893</b>

Another function of the array of collagen fibers along the sutures is to direct the growth of the trabeculae. Normally the trabecular fibers grow across the sutural gap, to attach to an adjacent plate. The trabeculae grow in between them, apparently using the fibers as indicators. As mentioned above, recent studies have shown that echinoderms have a unique collagen, which undergoes immediate changes in hardness, controlled by the nervous system. We speculate that when it grows the fiber collagen softens, and hardens when growth stops, or if the urchin is in stress. The inner pressure apparently force the sutural gaps open, and rapid growth of fibers and calcification follow. Under stress, mechanical or environmental, trabeculae growth stops and the urchin is defending itself by hardening the fibers and compacting the plate array across the sutures. In these conditions calcification is much reduced



Sometimes for some unknown reason, abnormally wide gaps appear in sutures of an otherwise healthy living sea-urchins, along existing sutures. These gaps may compromise the stability of the test, and must close as fast as possible. We observed one such a gap in a healthy VSU individual. We found that collagen fibers were intact and elongated, bridging across the gap. For about 3 weeks we observed rapid growth of the trabeculae on either side of the gap. Just before the gap closed we sacrificed the urchin, removed the soft tissue and took a SEM picture. Indeed, long trabeculae, poorly branched, were shown in a definite directional arrangement )Fig. .(59

**Fig. 59.** Scanning Electron microscope (SEM) of the zigzag (inter-radial) wide suture of a healthy urchin, fibers removed, showing the closure of an inter-plate gap by rapid growth of  $\text{CaCO}_3$  trabeculae. (a) low magnification x40; (b), x400 (Dafni, 1986).

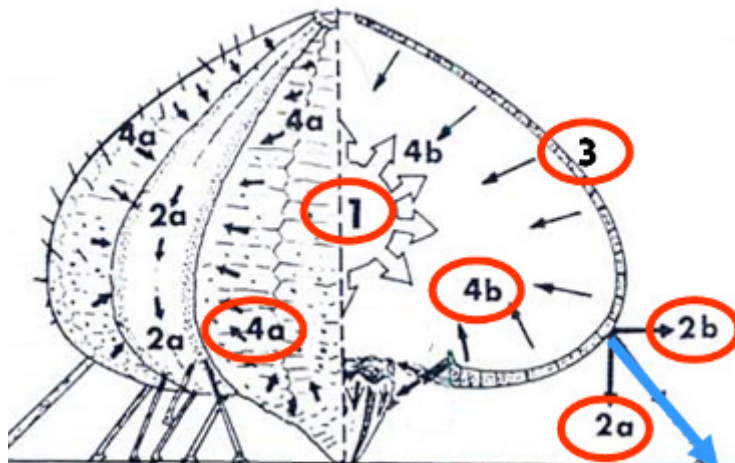
## **.11A biomechanical model for the growth of sea-urchins**

At this stage of the work we proposed a "Biomechanical Model" to describe the growth and calcification of sea-urchins, both the normal growth and as deformations. Assumptions underlying the model are:

- .1 The plates that make up the echinoid test do not have "knowledge" in which direction to grow, or calcify, responding to "messages" from their immediate environment.
- .2 Contrary to common belief, that the fluid content of the coelomic cavity of the echinoid skeleton has no "constructive" role, our model assumes that in analogy to Thompson's "water drop" model, the echinoid test is under contradictory forces - inner fluid pressure and outer surface tension. The model assumes that in state of non-growth a balance exists between these forces, and growth is initiated by internal pressure from the inner bulge of space, and the tension builds up from collagen fibers, which bind the plates to each other. The solid test and flexibility of the fibers resists explosion. Hence, growth is an unsteady state, in which inner pressure forces the sutures to open, and calcification is the reaction, stabilizing the new shape .

Four main components were identified as taking part in this model (**Figure :60**

- **1** Expansion of the coelomic fluid exerts an inner pressure while it grows. **2b, 2a** – Amb tube-feet, that pull the test toward the substrate (**2a**) and the horizontally to the periphery (**2b**); **3** – Sutural collagen fibers' mechanical properties - mainly tensile stresses, and. **4b, 4a** – the "centripetal" pull of the mesenterial threads (**Figs. .50,52**

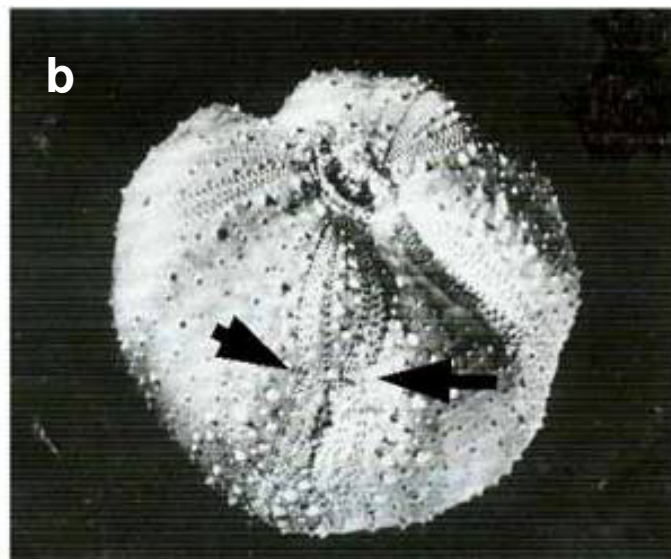


**Figure 60** Components of the biomechanical model for the growth of a regular sea-urchin (the left half shows an external view, with thicker arrows showing the tensional force vectors (**Fig. 50**) affecting plates growth, whereas the right half shows the inner distribution of these components: **(1)** Expansion of the coelomic fluid contained in the inner volume; **(2a, 2b)** Forces exerted by tube-feet in the vertical and horizontal directions, **(3)**, Tension of inter-plate collagen fibers; and **(4b, 4a)** – Inside pulling forces (thin arrows) associated with the mesenterial threads, that affect the directional growth patterns of the test plates. (Dafni, 1986) .

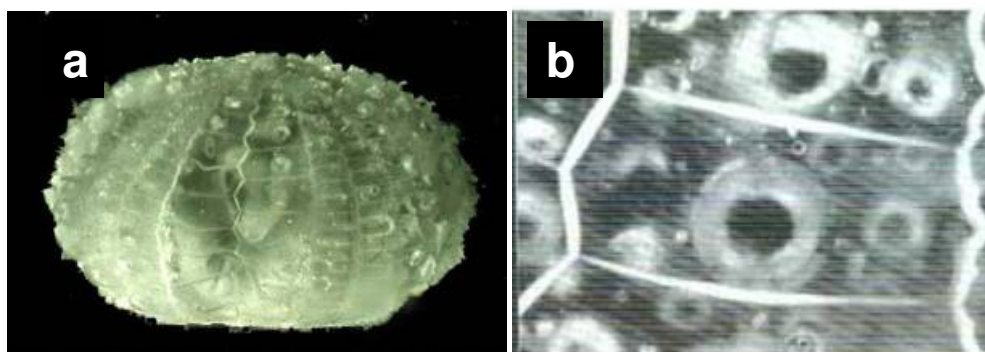
Growth, according to the current model, is a temporary violation of an everlasting force balance, when transient internal pressure increases the sutural tension, forcing sutural gaps to open between the plates. These forces are balanced by the mechanical strength and resistance to tensile stresses of the collagen fibers. Calcification, according to this model, is a secondary process, aimed to close the gaps resulting from these tensile forces, and fasten the test with mineral deposits. When an urchin does not grow, the tension on the fibers overpowers the inner pressure, the sutural gaps close and calcification stops. Several researchers tried, by devising various experiments, to confirm or reject components of this model. **Ellers & Telford** (1992) failed to detect constant positive pressures in the coelom. **Baron** (1991), on the other hand, recorded coelomic pressures in living urchins, and developed an alternative a “**tensile stress growth model**” which basically supports the “**pneu**” paradigm ).<sup>57</sup> The problem rests, in my opinion, in the methodology applied by the investigators. Urchins respond to any human manipulation in a apprent defence reaction, which causes growth cessation. Such a manipulation, manual size measurement with calipers, common practice in echinoid rsearch, often results in the so-called “negative growth”, apparent shrinkage of a few mms in their diameter (Ebert, .(1967

Our model explains well deformations under pollution conditions: (1) A reduced rate of calcification, caused by pollution, softens the test rigidity, and pre-existing gaps do not close. (2) Damage to the soft tissue – collagen strength and/or adverse muscles activity – creates stresses and tension in unpredicted directions. A good example is the excessive swellings of the entire test on the vertical axis )**Fig. 61a**), and the “pinches” )**Fig. 61b**), narrowing of the Amb plate columns at the exact hanging points where the foregut has its hanging to the ambulacral column, typical to deformity A )compare **Fig. 61 ,51b**). A similar effect – decrease of the plates stability, accompanied by inner pull of the mesenterial threads, results with the collapse of the apical “dome” into deep aboral depressions, typical to deformity B )**Fig. 63**). In the next pages I will show that deformations may result also from artificial, or other abnormal physical manipulations, unrelated to pollution. Furthermore, some deformations follow evolutionary trends shown by extant and fossil echinoid species.

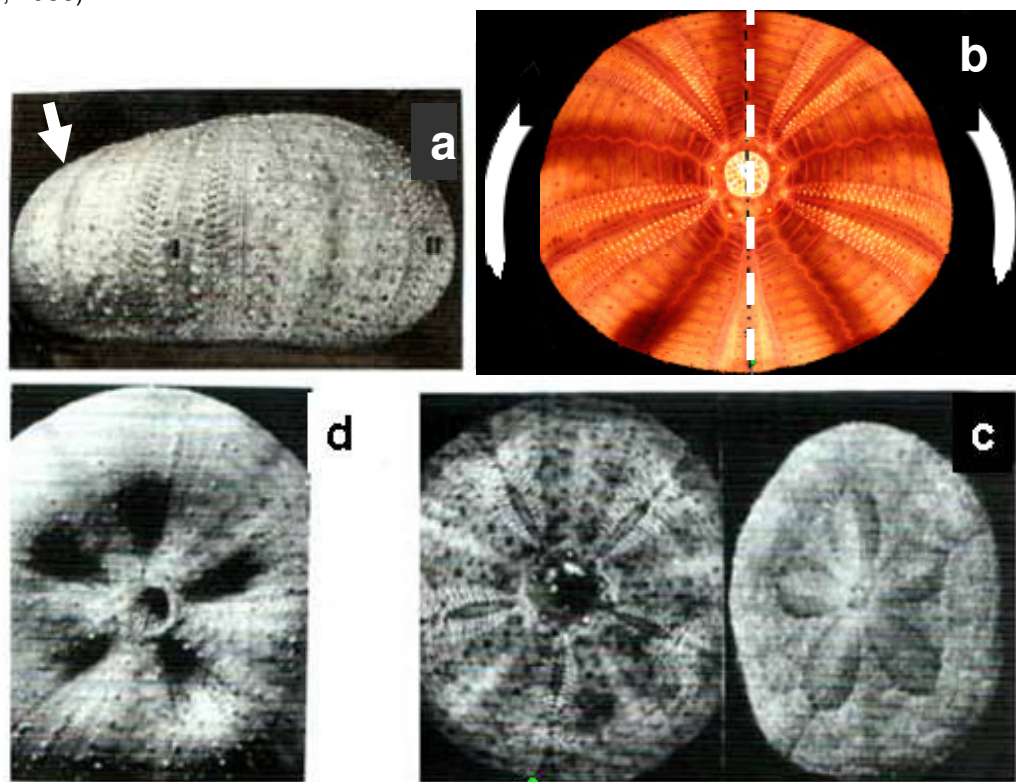
**Figure 61** (a) A Velvety Sea-Urchin that shows deformity A (abnormal swelling) following treatment with a calcification inhibitor (Belgard EV), (b) “Pinching” (between arrows) and the depressions in a deformed urchin's test (Dafni, .(1980





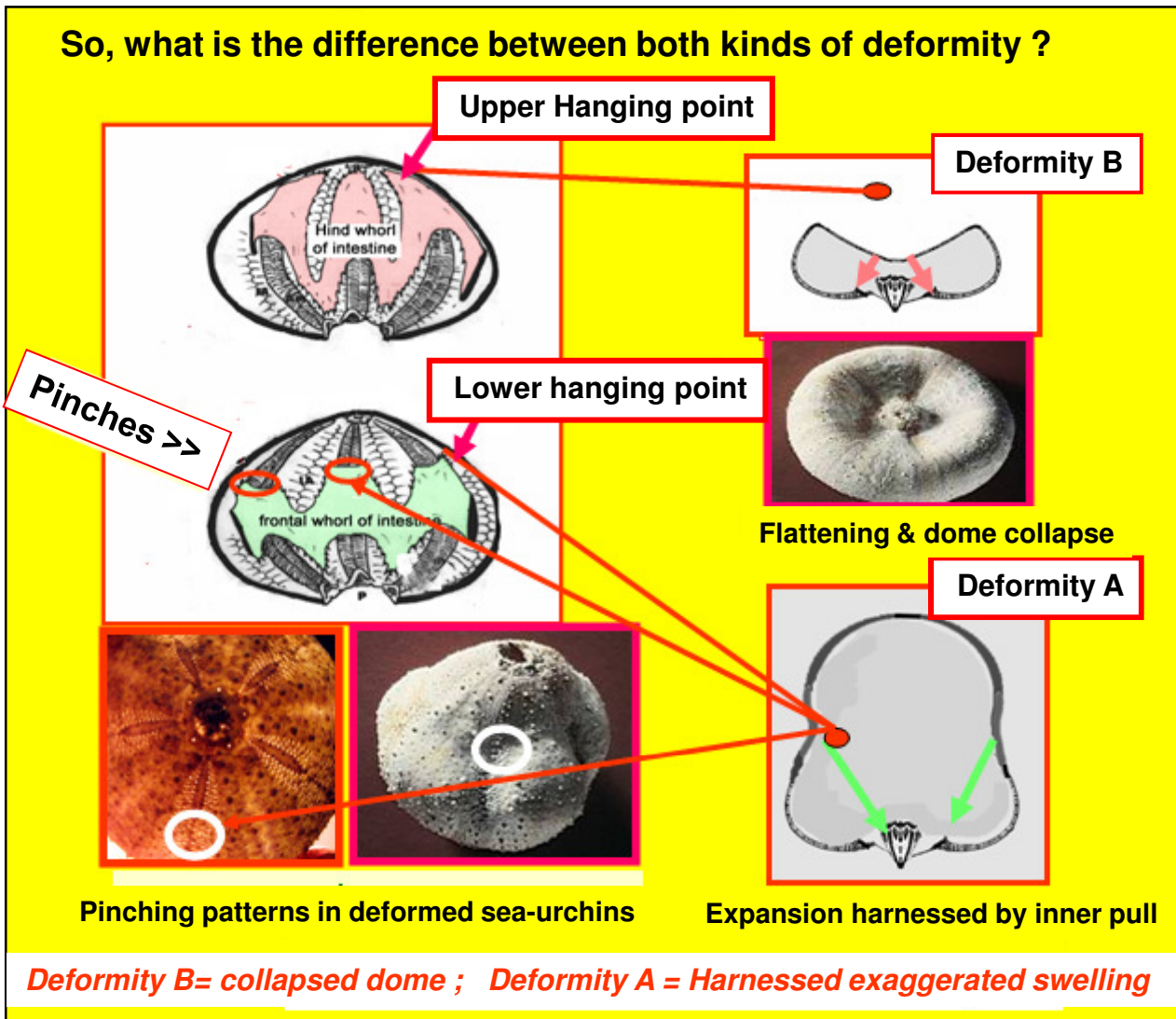


**Figure 62.** (a) A decalcified regular sea-urchin without consuming the soft tissues. Through the transparent plate enveloping membranes the intestine and other internal organs are seen. Note that although not mechanically supported by mineral, the skeleton keeps its shape, relying on the hydrostatic pressure of the included fluid, balanced by the tensional properties of the envelope. (b) An enlarged picture of the same, taken in “**Dark-field microscopy**” shows the spine bases (large circles), and a white boundary made of collagen fibers (glaring due to a **double refraction** property of collagen in this technique) along the plate’s periphery. Differential width of the glaring collagen boundary reflects the actual width of the sutural collagen band there (compare **Fig.58a**). (Dafni, 1988).



**Figure 63.** (a) Results of experimental damaging of one Amb radius of a normal *Tripneustes* next to the mouth: Atrophy of the damaged radius (white arrow), and the appearance of (b) an apparent “bilateral symmetry” with the damaged Amb column as axis (white broken line), and other Amb columns skewed towards the defected ambulacrum (curved arrows). (c), “pinched” Ambs in a deformed *Tripneustes* (Left) show incidental similarity to a natural “petal” of a irregular sand dollar (Right). (d) Five-fold Amb depressions in deformity B *Tripneustes* (compare with brooding pouches of *Abatus* (**Fig. 28b**)).(Dafni, (1988

# Bottom line



## What did we want to show, and what did we demonstrate?

- A.** The deformities were caused by polluted chemicals, that reduced the calcification of the mineral skeleton and affected the mechanical properties of collagen and other soft tissues.
- B.** A sea-urchin skeleton is a geodesic ball, stabilized by mechanical forces that encourage growth or if fail, causes its apex to collapse.
- C.** In the last process are involving contractile and elastic elements (such as sutural collagen fibers, mesenterial threads, tube-feet, etc.), playing important roles both in normal function and in the deformed growth processes.

The attached scheme shows how the two hanging points of the intestine and the way they pull at the skeletal walls from within may cause the both the collapse (**deformity B**) and the harnessed swelling and pinching (**deformity A**)

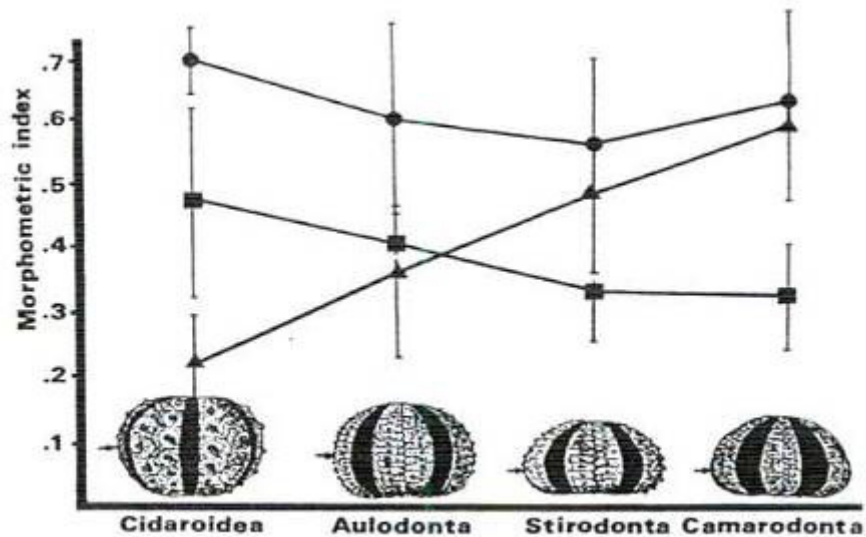
## 12. Proof of connection between biomechanical forces and deformity

Most observations, presented above, support the biomechanical model. It is common knowledge that real forces creating pressures and tensions are usually associated with the growth process. There is also a reason to speculate that an internal pressure exists, although possibly weak and ephemeral, in the coelomic cavity of the echinoid test. When the skeleton of a dead and conserved sea-urchin was decalcified with EDTA, a mild acid chemical, that did not harm the soft tissue and retained the fluid included in the plates inner spaces, the urchin's shape was retained whole, even if put on flat dry surface, the typical globular shape did not collapse (**Fig. 62a**). However, when living sea-urchins were exposed to chemicals known to affect collagen systems, **APN** and **MEA** (see **Fig. 49**), caused the entire collapse of the skeleton, and significant gaps were formed at sutures of the upper part of the living test. In one extreme case the whole skeleton collapsed and disintegrated to individual plates, long before the urchin died. The "poster" shown in the previous page (**p. 78**) clearly shows that real contractile and tensile forces were involved in those deformities.

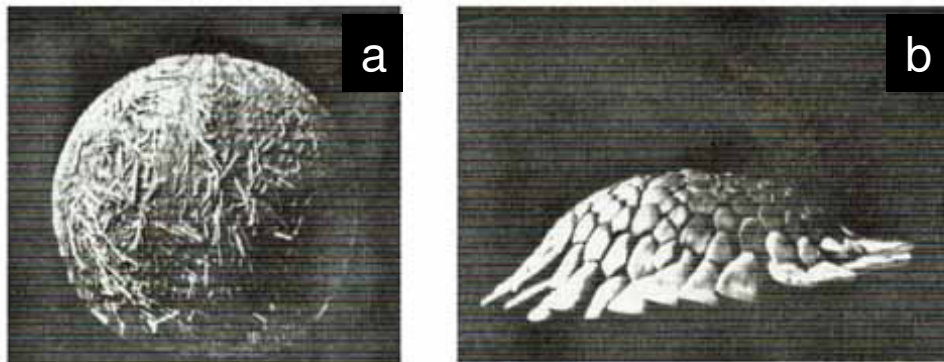
## 13. why does the evolution of sea-urchins look as a natural deformity ?

Several bizarre shapes appeared in the deformed *Tripneustes* : Bilateral symmetry, "pinched" ambulacra, symmetrical depression patterns etc.). These shapes remind us of genetic traits in the evolutionary scale. Aboral depressions, for example, are shown in sea-urchins as brooding pouches in several fossil species (**Fig. 28b**). Deliberate neutralizing one Amb column (**Fig. 63a,b**) resulted in bilateral symmetry, similar to irregular urchins. The same analogy is shown by the "pinches" – in several cases they mimic the irregular echinoids Amb "petals" (**Fig. 63c,d**). Small changes in the ontogeny of deformed urchins exhibit patterns that are part of a "repertoire" that appears here and there along the echinoid evolution. It is tempting to define these evolutionary changes (petals, brooding pouches etc.) as "natural deformities" that were selected in more advanced groups.

The possible connection between the evolution of the Amb system in regular echinoids in context to their overall test morphology was studied in a small survey, in which I examined photographs of 147 species of regular echinoids, from four orders, namely the primitive **Cidaroidea**, which did not change much from the Triassic Era, the **Stirodonta** and **Aulodonta** to the most advanced order, the **Camarodonta** (to which the Velvety and the Slate Pencil are included). I examined three parameters – (1) relative height (H/D), (2) relative height of the ambitus, and (3) the relative width of the Amb system, as part of the entire perimeter (**Fig. 64**), in order to find if an evolutionary trends exists in these regular urchin orders. The results show that **Sea-urchins became more flattened** (from H/D = 0.7 on average to H/D = 0.6, **Ambitus line tends to descend** from 0.5 of the total height to an overall height of 0.3, in modern sea-urchins. The last trend - **The relative width of the Amb system increased** considerably, from 0.2 in the primitive Cidarids to 0.6 in the modern Camarodonts. The results further emphasize the relative importance this system to the motion efficiency of these urchins, and supports our speculations about the role of this system in morphogenesis. The most significant correlation was between ambitus position and the relative size of the Amb system. ( $p = 0.01$  in Spearman test). It is also related to biomechanics. The greater efficiency of the ambulacral system, that in primitive echinoids had fewer tube-feet, caused the ambitus line to drop closer to the substrate, and their downward pull affected both the ambitus line and the entire profile of the sea-urchin.



**Figure 64.** Developmental trends in different series of urchins - regular sea, as reflected in relation to **H/D** (circles), relative width of the Amb system (painted black) (triangles) and the vertical position of the midline (ambitus, squares). Means and s.d. (original). The importance of the tube-feet system is expressed in the relative % of the periphery, from primitive series (left) to advanced (right). (Dafni, .1986)



**Figure 65.** regular sea-urchins extremes: (a) a totally spherical (*Holopneustes*), a sea-urchin that lives in algal thickets; (b) An extremely flat sea-urchin whose spined are peg-like, short, and stubby shingle urchin (*Colobocentrotus*) that lives in the wave beaten beaches of the Indo-Pacific Ocean (original).



Another "evolutionary" evidence that supports our hypothesis that tube-feet exerts a significant mechanical constraint, is the observation that sea-urchins living among algae or seagrass which do not allow tight attachment - like the Australian canopy dwelling **Holopneustes** (Fig. 65a) – tendency to be perfectly spherical. In contrast, the pillow-like Indo-Pacific Shingle Urchin (**Colobocentrotus**), living on rocky tidal zone in the Pacific Ocean, where maximum adherence is needed to withstand the destructive waves, are extremely flattened (Fig. 65b).

One exception, and as such it apparently proves the rule, is the burrowing urchin (**Echinostrephus**), that drills a round hole in the beach-rock or reefs, and dwells within, "hanging" at the upper rim of the burrow. The mean vector of the tube-feet pull is upwards, as opposed to all other regular echinoids, whose vectors are directed downward, as shown in Fig. 17a,b. This explains the inverted shape of its test profile, which is flattened at the top, while below, next to its mouth, it is pointed.

From these and other examples emerges an undeniable connection between the profile or other morphological features of the urchins animals and their normal function. There is ample evidence that the rich morphological "repertoire" of creatures in the wild have a more immediate significance, expressed in their response to biomechanical stimuli in terms of evolutionary adaptation.

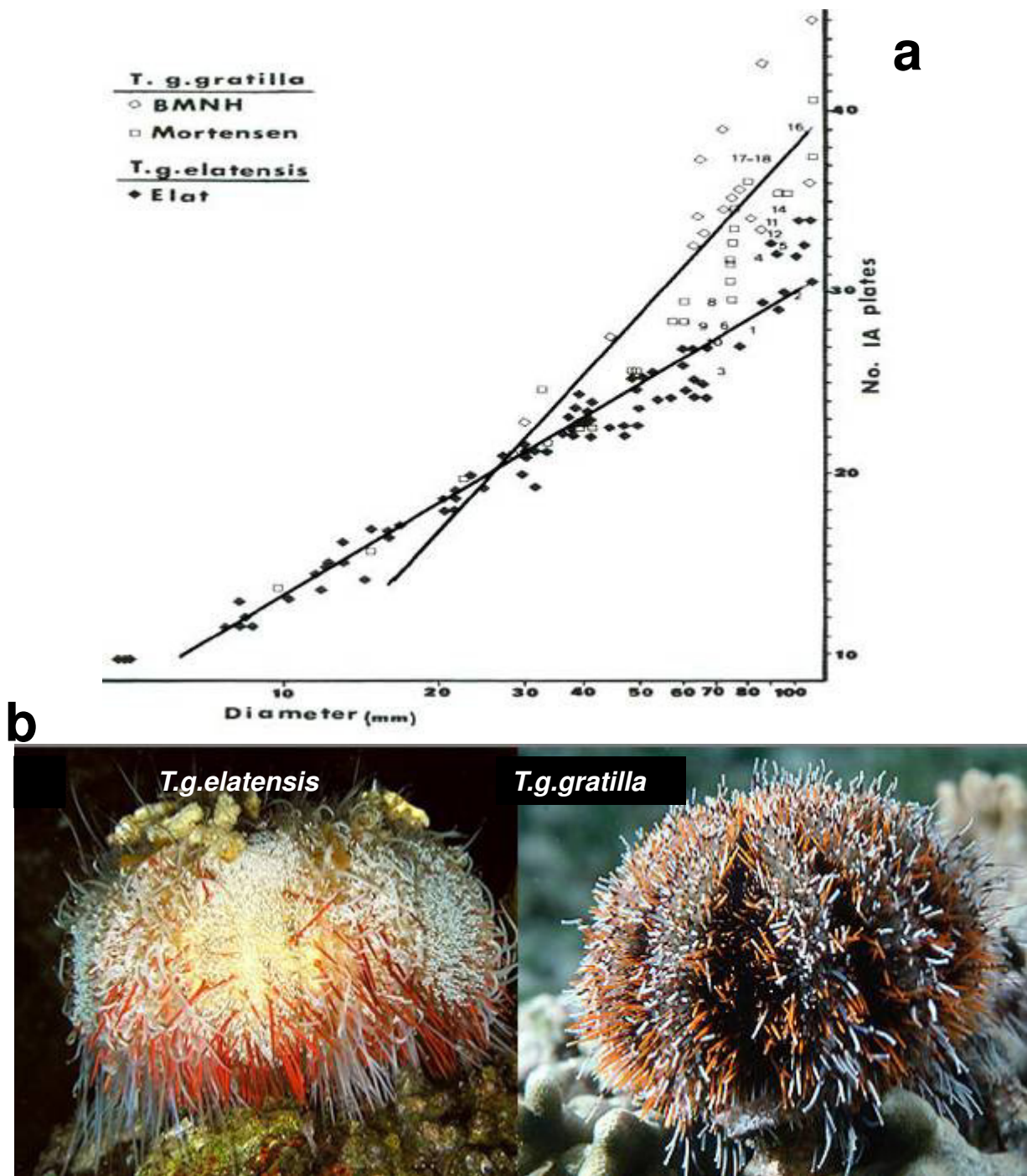
#### 14. **Velvety Sea-Urchin – is it a Red Sea-endemic ?**

One of our study results was the discovery that the Red Sea *Tripneustes* is quite different from its Indo-Pacific conspecific. In a series of measurements and correlations we found that despite Mortensen's (1944) assertion that ***Tripneustes gratilla*** is the same species throughout the Indo-Pacific Region (extending across the entire Indian Ocean and in the Pacific). It turned out that the differences between the properties of the oceanic *T. gratilla*, and those of the Red Sea variety, are enough to justify a description of the latter as an endemic subspecies (***Tripneustes gratilla elatensis***, Dafni 1983). The differences:

##### I. Morphology

A common way to quantify morphological differences between species or populations is by allometry. Fig. 66 is an allometric graph in which two parameters – number of plates in an IAMB plate column for different size urchins in relation to size (test diameter) of both, Red Sea ***T.g. elatensis*** and Indo-Pacific ***T. g. gratilla***. The following summarized the results :

1. The number of IAMB plates per column in *T.g.elatensis* is considerably lower than *T.g.gratilla*. (Fig. 66a)
2. The oceanic *T.g.gratilla* have more spines – at least one for each IAMB plate, whereas *T.g.elatensis*. had fewer spines, one spine to 2-3 plates in several regions of the column, but much more pedicellaria (Fig. 66b, compare also Fig, 9). It seems that the latter replaced its spines with pedicellaria as main means of protection.
3. Peristome in *T.g.elatensis*. Is 25% larger than *T.g.gratilla* suggesting that also the "lantern of Aristotle" is larger (Dafni, 1983a).
4. *Tripneustes.g.elatensis* colors are more vivid and variable than those of the oceanic conspecific. It appears in shades of white, pink, gray and black, compared with the more uniform colors of the latter. Three different components: tube-feet, pedicellaria and spines (Fig.18, .82



**Figure 66. (a):** Comparison of allometric Indices of two subspecies of *Tripneustes gratilla*, Red Sea variant (*T. g. elatensis*), from Eilat (closed diamonds) and Indo-Pacific variant (*T. g. gratilla*) (open diamonds, in loan from the British Museum collection, and from literature (Mortensen, 1943); **(b):** Two typical *Tripneustes gratilla*, *T.g. elatensis* (left), showing the fewer spines and considerably larger pedicellaria “fields” than Oceanic *T.g.gratilla* with more spines, and fewer pedicallaria. ( Dafni , 1983a, 2010) (Photos:: J.Dafni, M. Boyer.)

## II . Ecology and demography

**1The Red Sea subspecies is almost limited to reefs** and sub-littoral hard bottom habitats, while Indo-Pacific subspecies prefers sea-grasses habitats .

### 2Demographic and ecological characteristics

A demographic–ecological research, that joined the main study (Dafni & Tobol, 1987, Dafni, 1992), revealed some surprising facts. It seems that the life history of sea-urchins dramatically changes due to environmental factors. we found that–

**Growth and reproduction cycles.** In the Marine Laboratory shore at Eilat (Site A) *Tripneustes g. elatensis* show a fast and concised life history: They reproduced at the age of 4-6 months and the post-larval individuals, appearing in the sub-tidal zone at the end of winter (May - June), (**Fig. 67a-b**). I found them hiding beneath flat rocks. Within two months they reached a diameter 20-25 mm. In this wave stricken shore the juveniles were ready to settle by the middle of winter, but winter storms probably prevented them from doing so . .

**3Influence of winter storms.** In wave-exposed shores (**site A**) the winter storms posed a major environmental stress. In two consecutive years 1979-80 and 1980-81 waves wiped out the entire population, which had to renew in the next spring (i.e. **an annual life history**)(**Fig. 67a**). The rapid growth and early reproduction enables them to survive there .

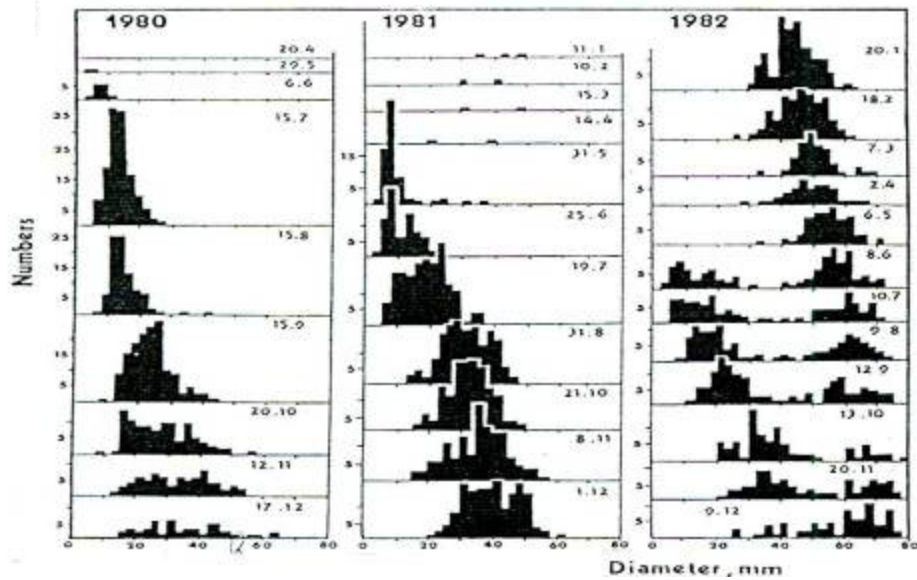
**4Migration offshore into deeper water.** The growing urchins have to move into deeper water - 1-5 m - to find shelter (**Fig. 67c**). This transition is fraught with danger, and predatory fish (wrasses, groupers etc.) catch many of them. In the more wave protected **Coral Reserve (Site B in Figure 67c)**, many urchins are killed during the “rush” to the reef. Those who were unable to leave the sanctuary near the shore, and stayed behind where they settled, became stunted (dwarfs), possibly due to starvation. Those who migrated reached the reef, sheltered there, grew to a considerably size (>> 50 mm). At that size they are relatively protected from predation, and live longer (**a perennial life history**). Consequently, I found in site B two sub-populations – juveniles and “dwarfed” population in the subtidal, and only adults in the reef crevices, with total absence of intermediate size (**Fig. 67b**). (

These observations confirm the ecological principle that in a harsh habitat, in which abiotic factors are dominant, an annual life history is the rule, whereas in more physically predictable environments, where the biotic factors – predation, competition etc. – are predominant, a perennial life history is more common

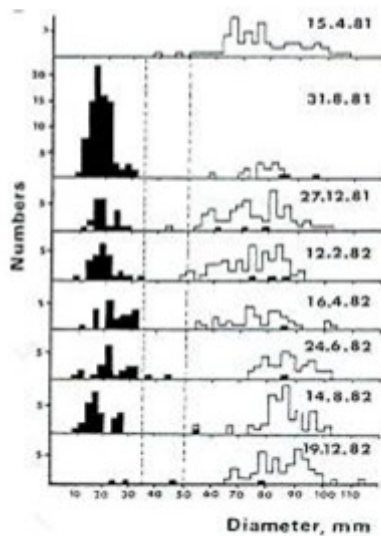
### 15Natural History of *Tripneustes gratilla elatensis*

It is reasonable to assume that the Red Sea subspecies (that will probably gain in the future the merit of full species, endemic to the Red Sea), underwent speciation in the Red Sea, as result from unique global events taking place during the ice ages, when sea levels lowered in 100-130 m, and a shallow sill existed at the southern opening of the Red Sea. Some scientists believe that the Red Sea may have dried out partially or totally, and its populations perished or found shelter in the north-western corner of the Indian Ocean, to re-establish in the Red Sea after the crisis. Another option is that the local variation is the result of the extreme environmental conditions in modern Red Sea (low water temperatures, high salinity, etc. - Dafni, .(2010

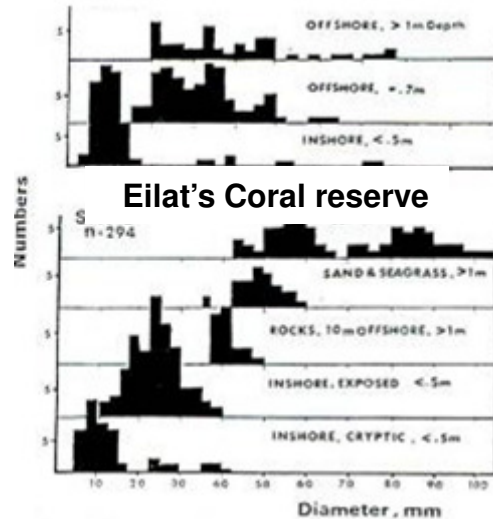
a site A – 3 years of settlement and growth patterns



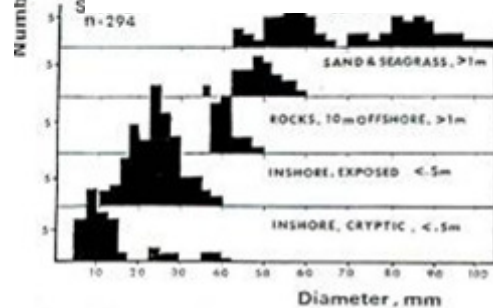
b. site B coral Reserve



c. Sites A & B movement to deeper waters



Eilat's Coral reserve



**Figure 67.** Size distribution of *T.g.elatensis* average sizes (diameter in mm) in two sites, **Site A**, Marine Biological Lab. **Site B**, Coral Nature Reserve. **(a)** Three years of recruitment and survival in Site A.. In the winters of 1979-80 and 1980-81 the entire population was wiped out by wave storms. In 1981-2 it survived **(b)** Site B survival pattern, showing two population size modals "dwarfed" in the sub-littoral (in black), and reef (white), and adults at the reef **(c)** showing the migration into deeper waters in several measurements in one week: in site A inshore and offshore >> 1 m deep. In **site B**: measurements taken in 5 locations, from the shore to the reef. (Dafni & Tobol, (1987



## **.16 Epilogue and “take away lessons”**

The study does not provides answers to the all questions we asked. Answer to one question usually opens new more questions that deserve to be studied. But as many other basic and applicable studies, this study raised several points that their study may be useful:

- Pollution in its various forms enters the developmental history of organisms and its effects may take us back many years, during which these evolutionary developments took place.
- We studied the life style of an interesting creature, which some parts of its body are not only edible, but are also favorite food in the several coastal regions. It is quite possible the growing them will become an important item in aquaculture in our country too.
- The similarity between the skeleton of sea-urchin and Humans is surprisingly high, and I have no doubt that certain principles are shared between them. Understanding life processes of sea-urchins can give us more insight into the processes in our own life.

All the studies listed and described in this booklet were published in articles between the years 1980-2010 (see list of sources.)

### **And one more thing about the model:**

This research has made progress in several areas of research relating to sea-urchins calcification and morphogenesis.

My biomechanical model, published in 1986-1988 was regarded with mistrust by various colleagues, that thought that it is rather a simplistic model and in contrast to the common belief that morphology, like all other developmental characteristics, is exclusively regulated by genes. The first to accept the model was an interdisciplinary group in Germany, who dedicated its researches to the “pneu” paradigm, according to which the epigenetic processes that determine the final morphological shaping of living creatures are basically biomechanical processes, in which mechanical properties of contractile and elastic elements in the organism are the dominant factors. In the auspices of this group I published in 1986 the booklet “**Echinoid Skeletons as Pneu Structures**” Konzepte SFB 230, Universität Tübingen und Stuttgart. Stuttgart 13:9-96.

Several young students, who attended my conference lectures got interested and designed various experiments to investigate certain aspects of these ideas. They published their results in the 1990's (Baron, 1990; Ellers, 1993; Ellers et al., 1998; Johnson et al. 2002). In a personal letter Olaf Ellers told me that a conference lecture of mine pushed him towards his research, that was later continued with a colleague, Amy Johnson, now a professor at Bowdoin, Maine, USA. In a paper (1993) he wrote: “*No conclusive evidence exists that tests this hypothesis, but circumstantial evidence supports it in regular urchins... the pneu model predicted the correct shapes given the observed pressures*”. In 1998, Ellers and co-workers wrote that “*the possible structural role of the sutural ligaments has not been a focus [in earlier works]*”. Nevertheless, in an official note to the press (2004), they claimed that they were the first to understand the exact way in which sea-urchins grow (see overleaf .)

I do not envy their scientific achievements. In the Hebrew language we have a saying: “**one should not be jealous in his son and his pupil**”, and yet, the present booklet proves that they weren't the first...

## Bowdoin Researchers Seek Methods to Spur Sea-Urchin Growth

Posted July 26, 2004

When Bowdoin Biology Department Chair Amy Johnson and Biology Research Associate Olaf Ellers published their discovery of the mechanism by which sea-urchins grow in 2002, marine biologists took note. *No one had yet understood how echinoderms could increase the size of their shells, or skeletal plates, without shedding them. They discovered that as urchins grow, the collagen tissue inside, outside, and between their skeletal plates softens. The shell inflates like a balloon. The collagen stretches and expands gaps between the plates from the inside, while containing them from the outside. Eventually, the tissue between the plates is reabsorbed and is replaced by hard shell. This mechanism is similar to the growth of a vertebrate skull\*.*

While this discovery stands on its own as a bit of interesting basic biology, the bust of Maine's sea-urchin industry now makes this information invaluable to researchers, regulators and harvesters struggling to find ways to replenish Maine's once-bountiful sea-urchin population along the southern coast - while protecting remaining stocks in the north...

<http://www.bowdoin.edu/news/archives/biology/000222.shtml>

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\*In my humble opinion, my explanations are more comprehensible and preceding theirs by ~15 years .

### Acknowledgments

Although this is a personal summary based on a study done three decades ago, It is a pleasure to mention those who stood beside me for help, guidance and advice during the study and over the years that followed.

I am greatly indebted to **Dr. Ailsa M. Clark**, whose book "*Starfishes and their relations*" was my "bible" for many years, and to **Dr. Andrew Smith's** important book "*Echinoid Paleontology*", to **Dr. Yehiam Schlesinger**, who brought to my attention the deformation phenomenon, Ms. **Ilana Farber** who encouraged me and helped in so many ways; professors **F.D. Por** and **Jonathan Erez**, friend and teacher, who guided me in his radioactive laboratory and spent with me many long hours brainstorming and looking for solutions; Profs. **John M. Lawrence** and **Thomas Ebert**, senior colleagues who helped me to uproot many mistakes I have made, **Prof. A. Seilacher**, from the **Geological Research Institute in Universität Tübingen**, Germany, in which some of the studies were made, to Dr. Andrew Smith and Ailsa M. Clark from the British Museum, for letting me copy figures from their books. and last but not least, the staff of the **H. Steinitz institute for marine studies** at Eilat.

I dedicate this booklet to the memory of two persons who influenced my career most: **Rachel Carson**, the author of the book "*The sea around us*" and **D'Arcy Thompson**, whose book "*On growth and form*" was an important milestone in the acceptance of the epigenetic biomechanics. Each, in his own way, showed me my way to the sea and its inhabitants.

## Appendix 1

### Sea-urchins and the Human genome\*

Most of us know the sea-urchin - a pin cushion shaped creature living on the seabed and often near beaches - mainly due to its long, sharp spines. Not many people know that this little priky creature has the same qualities as humans and the higher animals - the vertebrae, a fact known to researchers for years .

Now, a group of international scientists, that were able to map the genetic material of the sea-urchin, was surprised to find that sea-urchins are more similar to us, much more than estimated so far. Furthermore, It was found that humans and sea-urchins share more than 7,000 out of 23,300 genes (30%). These are the most important genes responsible for some of the important mechanisms in the body

\*From an article on YNET (following completion of the sea-urchin genome research – in U.S).

#### Surprise! Your Cousin's a Sea Urchin

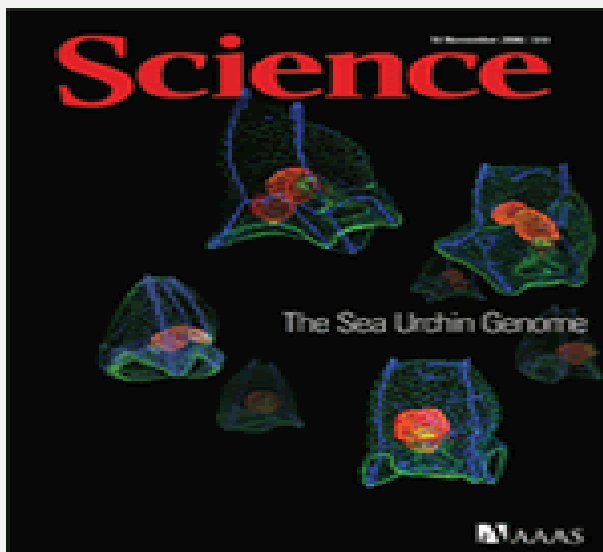
By **Jeanna Bryner**, LiveScience Staff Writer  
posted: 09 November 2006 02:02 pm ET

Share this story

The group estimates the sea urchin has 23,300 genes. For comparison, the fruit fly has about 13,600 and humans 20,000 to 25,000.

“ It turns out that the sea urchin is very much like us ”  
Dr George Weinstock, HGSC

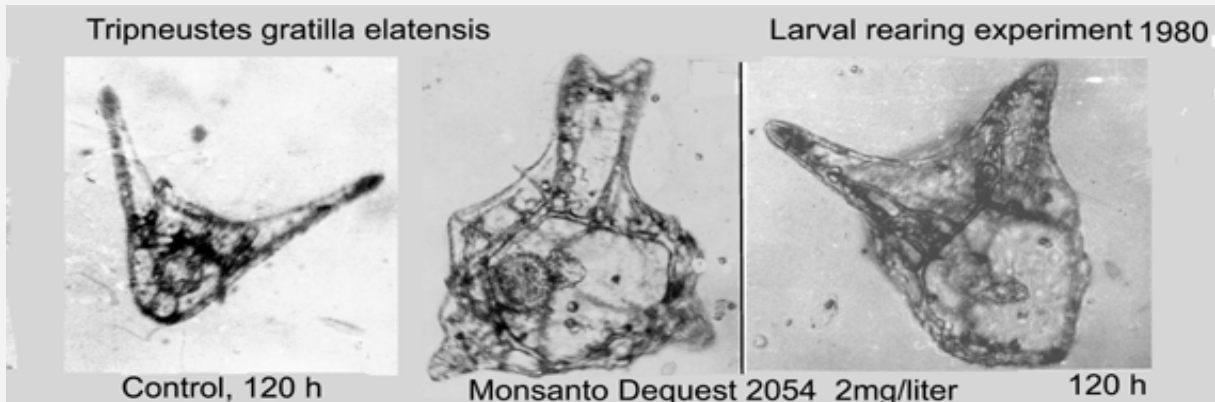
30% identity was found between sea-urchin genes to those of Man - and in particular sensory system and the immune system. The researchers hope that you can use the sea-urchins to investigate human diseases and disorders



The purple sea urchin is a closer genetic cousin to humans than suspected. Credit: Charles Hollahan/Santa Barbara Marine

## Appendix 2

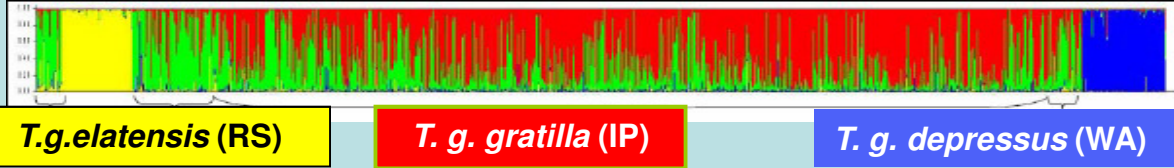
### Experiment on influence of pollutants on Tripneustes larvae



- In an experiment I also checked the effect of pollutants on the growth of larvae (= embryonic sea-urchins). Deforming sea-urchin larvae under the influence of pollution is known and recognized worldwide and used for testing sea-water quality.
- The process to induce eggs shedding is known: an adult sea-urchin is injected with 0.5 Molar potassium chloride solution, and ejected eggs and sperm from which the cells that develop into larvae. The larvae are exposed to various concentrations of polluting chemicals, discover their potential effect.
- In this case the pollutant **Monsanto Dequest 2054** of a concentration of 2 ml/liter of water in which the larvae were immersed. After 120 hours 5% of them deformed, in the form, shown in the photos, listed above .

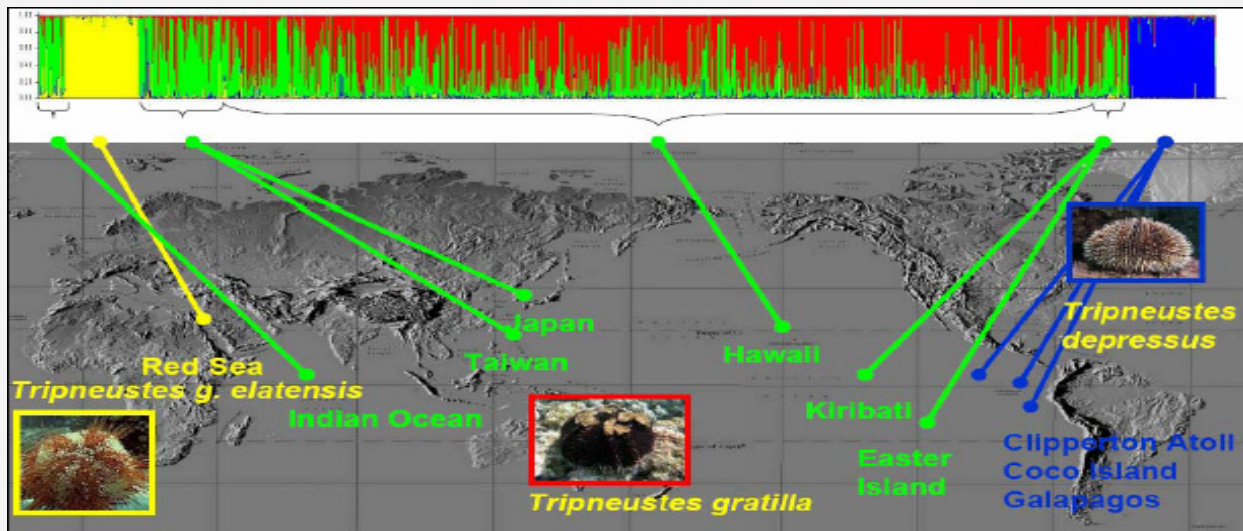


## Taxonomic status of *Tripneustes gratilla elatensis*



Since I started the study, I became aware to the unique properties of the Red Sea variety of the Indo-Pacific *Tripneustes gratilla*, which I later described as subspecies *elatensis* (Dafni, 1983a, p. 81), I waited for a thorough systematic review of the genus *Tripneustes*, that will confirm my findings and gain more insight in this subspecies natural history among the many populations of this genus all around the tropical coasts, especially those in the Indo-Pacific Ocean. Dozens of species and subspecies of *Tripneustes* have been described over the 250 years the since this sea-urchin was first describes by **Linnaeus**. The last taxonomist – Mortensen, 1943 - narrowed the list and determined that *T. gratilla* is the only valid species from the Red Sea to the remotest islands of the central Pacific Ocean. Two more extant species, *T. ventricosus* from the Carribeans, and *T. depressus* from the Eastern shore of The Pacific Ocean, and several fossil species from the Miocene and Pliocene - 20-3 millions B.P. Since then, the Red Sea underwent several crises during the ice ages, and it is logical that a certain isolation took place, that may account for some of the unique phenomena (p.81-82). The application of new DNA techniques, that may define the differences between populations, held hope that these questions will be answered soon. To my disappointment, a study of **Lessios et al. (2003)**, which aimed to base the taxonomy of *Tripneustes* on molecular evidence, did not find such differences, and stated emphatically that *T. gratilla* is the only valid species throughout the entire indo-pacific, from the West Coast of South and Central America, to the West Indian Ocean and the shores of Africa.

When I read this paper, I immediately wrote him. He apologized for not including *Tripneustes* from the Red Sea I this study, being unaware of my findings. To clarify my case I was asked to transfer biological material, preserved in absolute alcohol to him. Even so, no significant difference in the DNA and no significant differences were found. Later on, correspondence was addressed to me by a Hawaii researcher, Dave Carlon, who developed a special technique of higher resolution – the microsatellites markers technique. I sent him material, and some preliminary, non-final findings are included in a summary on the next page. He concludes that in addition to the almost homogenous *Tripneustes gratilla gratilla*, widely distributed throughout the entire Indo-Pacific span, two subspecies are to be found in both margins of the ancestral species range, driven by either isolation, natural selection, or both: *T. g. elatensis* in the Red Sea and *T. g. depressus* in West America.



## Phylogeography and population structure of tropical reef animals

By David Carlon\*

The spatial distribution of genetic and phenotypic variation within species plays a key role in the potential for evolutionary change. A parallel question is now being asked by resource managers, who need to know the number of independent populations (and potential management units) that are contained in species with large geographic ranges. Understanding spatial patterns of genetic and phenotypic variation is crucial for developing fisheries management tools such as MPAs to protect the large fishing economies and biodiversity benefits provided by coral reefs. My labs work in this area has been funded by the Hawaii Seagrant College and the Hawaii Coral Reef Initiative. For a recent summary of this research, see my HCRI presentation on YouTube\*. We are analyzing patterns of genetic structure in heavily fished reef organisms that have biogeographic distributions across the Pacific and Indian Oceans. One species is the “collector urchin” *Tripneustes gratilla*. Adults are found on a variety of benthic habitats including coral reefs, rocky substrata, and seagrass beds, while planktonic larvae require 1-2 months to complete development. A previous study by Harilaos “Haris” Lessios and colleagues found that common mtDNA haplotypes are distributed from the eastern shores of Panama to the Indian Ocean, and no unique mtDNA variation that separated *T. gratilla* in the Central Pacific from the subspecies *T. gratilla depressus* in the Eastern Pacific. **There is one more subspecies relevant to our questions: a unique phenotype called *T. gratilla elatensis* lives in the Red Sea and was described by Jacob Dafni.** I have been collaborating with Haris and Jacob by applying new microsatellite markers developed in my lab [Carlon and Lippe 2007] to larger samples sizes that include both subspecies, as well as more intensive sampling in the Central Pacific. We find strong population subdivision associated with subspecies. The STRUCTURE plot above provides a visual representation of the strong linkage disequilibrium between the two subspecies and a broadly distributed population of *T. gratilla*. This result is particularly exciting in light of a peripatric model of speciation as advocated by Ernst Mayr, where population differentiation occurs at the margin of an ancestral species range, driven by either isolation, natural selection, or both.

\* Included in a Youtube video →: <http://www.youtube.com/watch?v=XWAZAq3JUcg>

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