Fabrea salina Henneguy, 1890. A heterotrichous ciliate thriving in hypersalinity

George N. Hotos
Plankton Culture Laboratory, Dep. of Aquaculture & Fisheries Technology
Techn. Educ. Inst. (T.E.I.) of W. Greece, 30200 Messolonghi
ghotos@teiwest.gr

It was some years before when I was for the first time fascinated by the most prominent ciliate protozoan swarming the extremely saline waters sampled from the Messolonghi evaporation ponds of the local salt-works. This prominent ciliate is *Fabrea salina* and since then I kept it constantly in my laboratory exposing it to various experimental trials concerning food, light, salinity etc.

First of all I should make it clear that culturing protozoa or heterotrophic protists in general is not so easy as expected. From my samples over the years I found the hard truth that attempting to isolate each particular species and place it in small vials filled with the appropriate water (that is with salinity and temperature as close as possible to the sampled water) is not a solid road to success. Most of them will soon vanish even if you additionally supply them with various food (algae, yeast, smashed organics, let alone the pre-supposedly existence of bacterial flora). Limited success offered only some ciliates like *Condylostoma*, *Paramecium* (marine species) or *Chilomonas* and that not for long. I should nevertheless admit that the hypotrichid *Euplotes* is the exception as it multiplies so fast that not only there is no difficulty in culturing it but rather it is a real intruder (better to say a pest) in almost every other culture, demanding for frequent filtration of the water (50-80 μm nylon mesh) in order to get rid of it. Next to *Euplotes* in terms of easy culturing is *Fabrea salina* and additionally *Fabrea* is not disturbed at all in multiplication by the simultaneous presence of *Euplotes*. On the contrary it seems to do better if *Euplotes* is near-by. Sometimes for reasons not elucidated yet, the population of *Euplotes* in the *Fabrea*'s culture vessel recedes after some days and eventually totally vanishes. This phenomenon is not presented in other ciliates cultures where in mixtures of ciliate species *Euplotes* soon dominates.

Fabrea salina is cosmopolitan and (although not yet firmly established) seems to be the same species all over the world's salty lakes. My purpose here is not a scientific paper but rather a free style text to introduce *Fabrea* to scholars interested in microbiota from a naturalistic point of view.

First of all the appearance of *Fabrea* in terms of shape, color and movement cannot be mistaken and easily the microscope observer can be focused on it among various ciliates in the droplet of water. The size of *Fabrea* is highly variable and for reasons not understood there can be met populations with small individuals around 100 - 150 μm in length and populations with big ones about 300-350 μm with all in between sizes occurring at times, either mixed with medium or big individuals. I use the term population in order to point out the fact that what I mention above comes from cultured specimens kept in small vials (4-200 ml) and that occasionally from a long kept population of big *Fabrea* after some time small individuals emerge, dominate and keep so for long, the opposite never occurred (from small individuals to emerge "bigs"). This phenomenon is really puzzling and in spite of experimentation creating several microenvironments no pattern was detected. At this point to make clear, that this size variation refers to populations and not to

young daughter cells ensuing after cell division that are small and grow to maximum size after feeding. The small individuals are most of the time rather sluggish, reproduce at a lower rate or not at all and feed less (as it is seen from the negligible clearance of microalgae feed).

Description: Body length in vivo along the long axis about 180-350 µm, usually spherical, ovoid or tear-shaped with a slightly flattened and triangular-shaped anterior portion (snout) that is about 25% to 35% cell length (and tapers as proboscis). Cell size among individuals (excluding the anterior pointed portion) highly variable. Insight the cell only a slight metabolic turbulence is seen. Its pellicle is thin and flexible and with a roughened appearance of the cell's outline. Many tiny, colorless cortical granules are evident. The protoplasm is colorless or slightly yellow-grayish, often appearing opaque due to thickness of cell and numerous inclusions. A prominent band-like and irregularly shaped macronucleus is seen when not obscured by vacuolated food inclusions. Many micronuclei, although inconspicuous, are dispersed in the cytoplasm. About 150 inconspicuous somatic kineties run along the cell composed of densely packed dikinetids. Adoral zone with about 200 membranelles. It exhibits almost continuous linear locomotion in a slightly strobilized manner but sometime stops and looks suspended or drifting. It can withstand salinities from normal seawater (35 ppt) to highly hypersaline water more than 180 ppt although its upper tolerance is not exactly documented.

Classification

KINGDOM: Protista
PHYLUM: Ciliophora
SUBPHYLUM: Postciliodesmatophora
CLASS: Heterotrichea
ORDER: Heterotrichida
FAMILY: Climacostomidae
GENUS: *Fabrea*
SPECIES: *Fabrea salina* (to be named by its discoverer, Fabrea Henneguy in 1890)

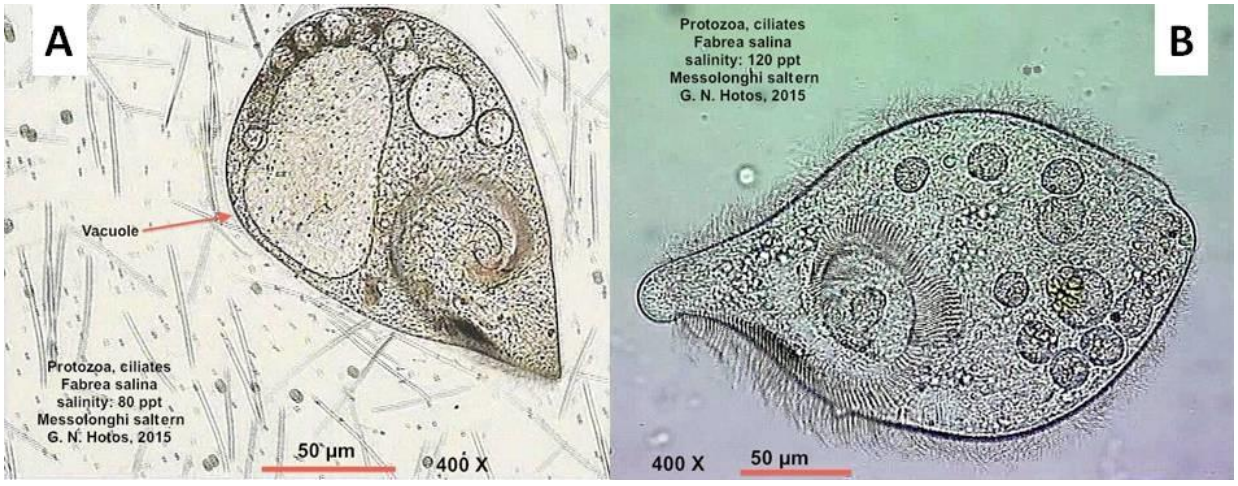

Figure 1. A typical image of *Fabrea salina*. In (**A)** the specimen has a long wide triangularly shaped snout with no proboscis. It is heavily vacuolated and in the front-most part of the adoral zone of membranelles (AZM) a dark colored area is prominent. All around the cell are numerous coccoid and filamentous cyanobacteria. In (**B)** the snout is shaped in proboscis. The cilia are evident covering the whole outline of the cell.

The salinity tolerance of this ciliate is notorious as it is able to thrive even at the extremes (more than 180 ppt) where (as it is widely and generally believed) only *Dunaliella* and *Artemia* reign the ponds. In reality at the extreme salinities a whole array of life forms exists and "prospers". There are archaea, cyanobacteria (both coccoid and filamentous), *Ateromonas gracilis* the chlorophyte "companion" of *Dunaliella*, *Fabrea salina* and the metazoans are only represented by various *Artemia* species (depending on locality).

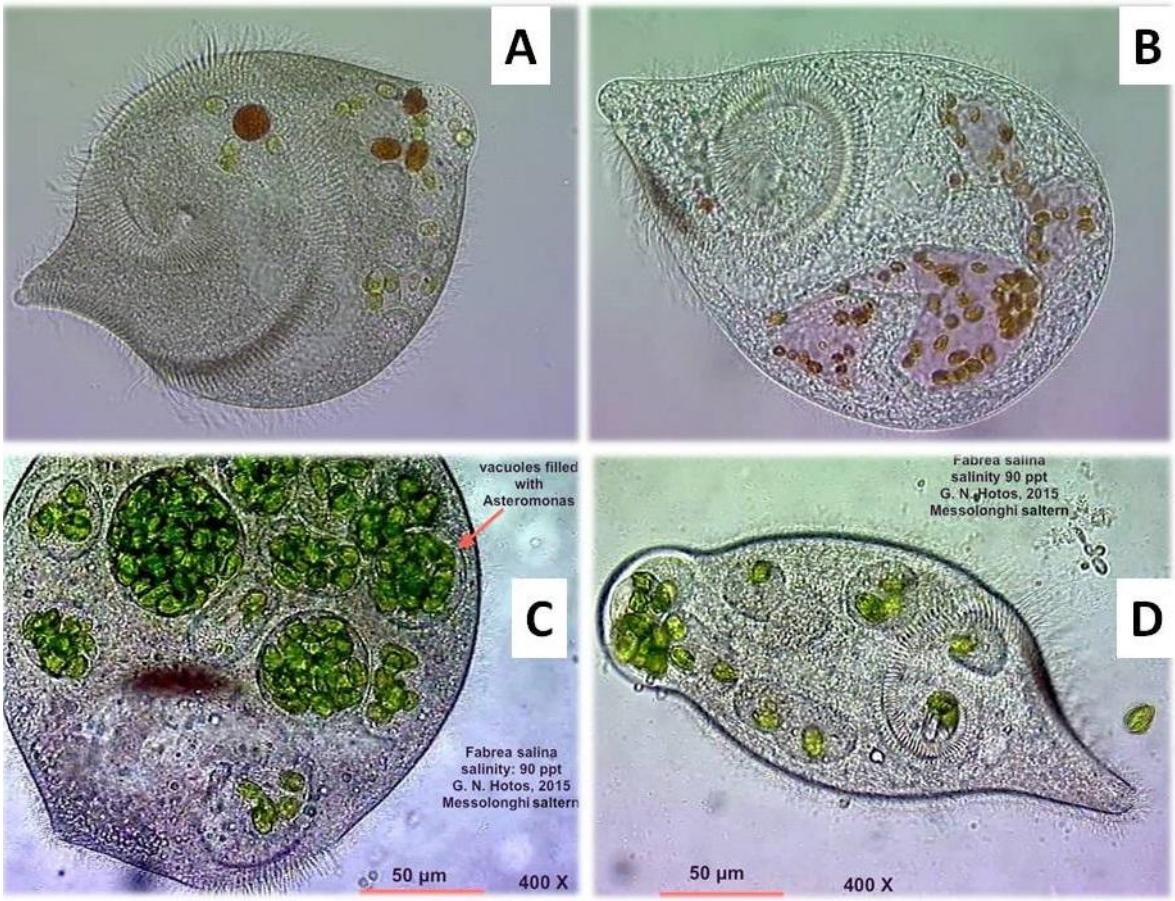

Figure 2. *Fabrea salina* fed various algal species. **(A)** fed *Dunaliella salina*, with both carotenoid full (red) and normal (green) cells, an indication of salinity (~150 ppt) where *Dunaliella* starts carotenogenesis. **(B)**, vacuoles full of the cryptophyte *Rhodomonas salina* in the process of digestion from a laboratory culture at 35 ppt salinity. **(C & D)**, with vacuoles loaded with ingested *Asteromonas gracilis* from laboratory cultures at 90 ppt. Noteworthy the snout-less form of *Fabrea* in **C** and the proboscis form in **D**.

It is really curious that this hypersalinity microbiota remains for the most part poorly recorded in the literature. The literature has not yet articulated it in a definite web-scheme that encompass abundance, succession, etc. Anyway, concerning *Fabrea* it is sure that feeds upon any kind of algae (cyanobacterial or eucaryotic) and probably bacteria. But when salinity reaches the far extremes more than 250 ppt then it succumbs leaving only *Dunaliella* and the archaean *Halobacterium salinarum* to color the water orange-red.

Talking about salinity it must be emphasized that *Fabrea* can live and multiply in normal sea-water (~35 ppt) the same as in hypersalinity and furthermore is doing better in lower salinities as it faces less osmotic pressure, higher dissolved oxygen and has more options of suspended live food. I

should also emphasize that *Fabrea* is exclusively a planktonic creature (not benthonic) never feeding on the bottom mat of the ponds and in culture conditions presents a uniform dispersal in the water volume of the culture vessel.

Conjugation in *Fabrea* is a rare event (I only noticed it 2-3 times in my multiyear cultures). It multiplies by cell fission using two ways. First the cell elongates and in an area near the middle starts to form a depression that delineates a front (snout part) and a rear bulge that after some time create two *Fabrea* resembling daughter cells united either by a common protoplasm in the "neck" front area or by a kind of linkage of the type "tail-to-snout". Eventually the protoplasmic connection thins out to nothing but a very thin highly tensible thread that eventually will break as the cells move independently. The whole process (from the beginning till separation) lasts somewhat between 4 to 5 hours and in good conditions (temperature above 22 ^{o}C, plenty of food) the generation time (doubling time) of *Fabrea* lies somewhere between 16-24 hours, not bad at all for culturing such a big sized ciliate. There is a question however if the "neck-connected" cells are the result of cell fission or a stage of the conjugation process. I am inclined to accept cell division as evidenced from the separation effort seen in several of my videos.

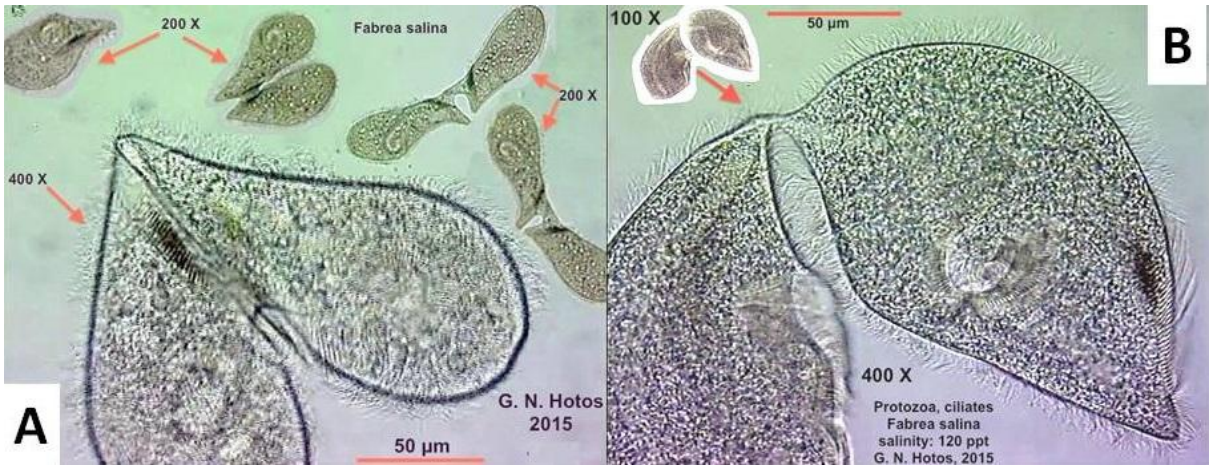

Figure 3. *Fabrea salina* separation of cells by cell fission. In **(A)** can be seen the protoplasmic connection at the "neck" area of the daughter cells (in the big depicture) and in the same photo properly arranged (from lower magnification) three different positions of stretching the connection by the movement of each daughter cell away from each other. It must be admitted however that this picture can be as well an episode of the conjugation procedure. In **(B)** the daughter cells (close to separation) are united in a "tail-to-snout" manner.

The most fascinating feature of *Fabrea* for microscopy observers is the elegant smooth way of scrolling around in the water. It swims rather slow and at times accelerates with or without strobilization. Sometimes swirls for a while and sometimes goes forwards and backwards for no obvious reason. It is very easy to take a sample of an individual *Fabrea* as it can be spotted easily and sucked properly by a microtubule even at the lower magnification of a dissecting microscope. A peculiar thing frequently encountered in culture vials is the local concentration of many individuals at certain peripheral areas leaving the center area almost devoid of cells. This happens when the population multiplies fast but has not reached yet its maximum density. When maximum density has attained (varies between 50-110 ind./ml) then all the individuals are evenly dispersed in the whole volume and no patches of crowding are created any more.

In my cultures I have observed cases of co-existence of *Fabrea* with various species of ciliates. These ciliates although at times are abundant they never dominate as populations and eventually *Fabrea* restrict them in nothing but a few cells.

The other fascinating feature of *Fabrea* is the vortex creating by its huge "mouth" region that is located inside the spiral funnel shaped by its adoral zone of membranelles (AZM). This sucking area is perhaps the most prominent region that everyone spots when examines *Fabrea*. The cilia in this region are very long and their rapid movement gives the impression of a rotary machine creating a whirl dragging in anything that falls in its action radius. Indeed we can see algal cells to reach the area, then to be forced in a cycling movement and finally by a rapid suction to be transferred inside the cytostome and then to be thrown in the cytoplasm where rapidly a vacuole engulfs them.

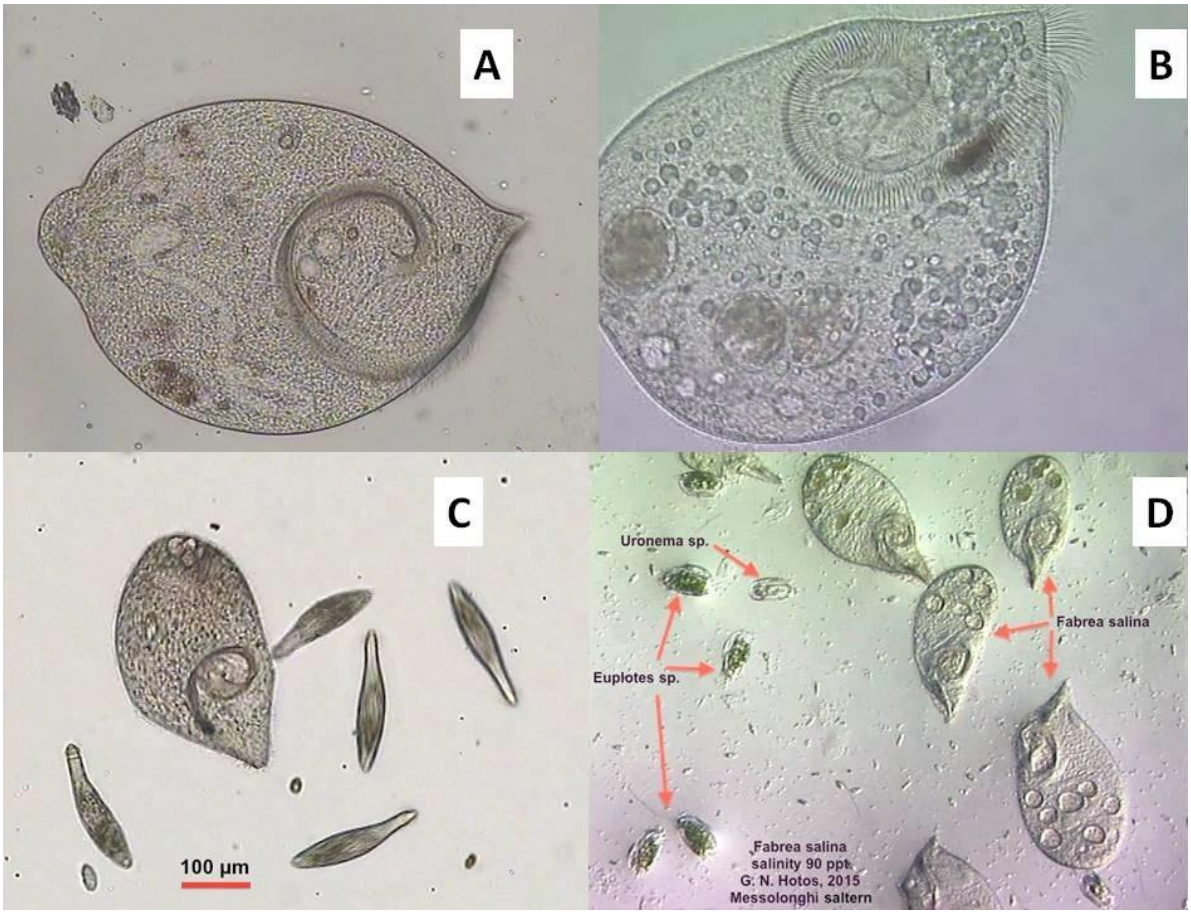

Figure 4. *Fabrea salina*. In **(A)** we can see the peculiar "S" shape of the **macronucleus** (depicted and clearly visible as lightly colored) and the circular shape of the AZM creating the big "mouth" area of the cell. In **(B)** the long cilia of the AZM in the mouth area are very prominent and also several vacuoles filled with digested material. In **(C)** *Fabrea* is surrounded by the ciliate *Hemiophrys* and in **(D)** a "bas-relief" picture of several *Fabrea* with many vacuoles accompanied by several ciliates *Euplotes*. These pictures are also useful for comparison of sizes between a very big ciliate (>200 µm) as *Fabrea* and medium to small ciliates (~50-100 µm) as *Euplotes* and *Hemiophrys*.

In cases of abundant algal availability *Fabrea* stuffs its cell with algae and its protoplasm bursts with numerous vacuoles full of green, pink or brown algal material depending on the particular

color of the algal species consumed. However not all algal cells are digested as many of them are frequently expelled through evacuation of the content of some vacuoles to the external medium and upon their freed acquire mobility like nothing had happened. It is also probable that some of the algae are not digested but instead they are used as symbionts to provide *Fabrea* with their photosynthetic products. So, based on the above, it is postulated that on abundance of food material *Fabrea* get it in excess, store it in vacuoles and then digests the proper amount at its ease.

Microalgae seems to be an excellent feed-stuff for *Fabrea* and especially the big sized and flagellated ones like *Dunaliella, Asteromonas, Tetraselmis, Isochrysis* or *Rhodomonas*. Non flagellated species like *Chlorella* or *Nannochloropsis* proved inferior to their flagellated counterparts. Coccoid cyanobacteria like *Synechococcus* sp. are also consumed but to a lesser degree. Filamentous cyanobacteria like *Oscillatoria, Lyngbya*, etc have never been observed (at least by me) to be consumed by *Fabrea*. I never met incidents of *Fabrea* consuming other ciliates nor cannibalization on its own species.

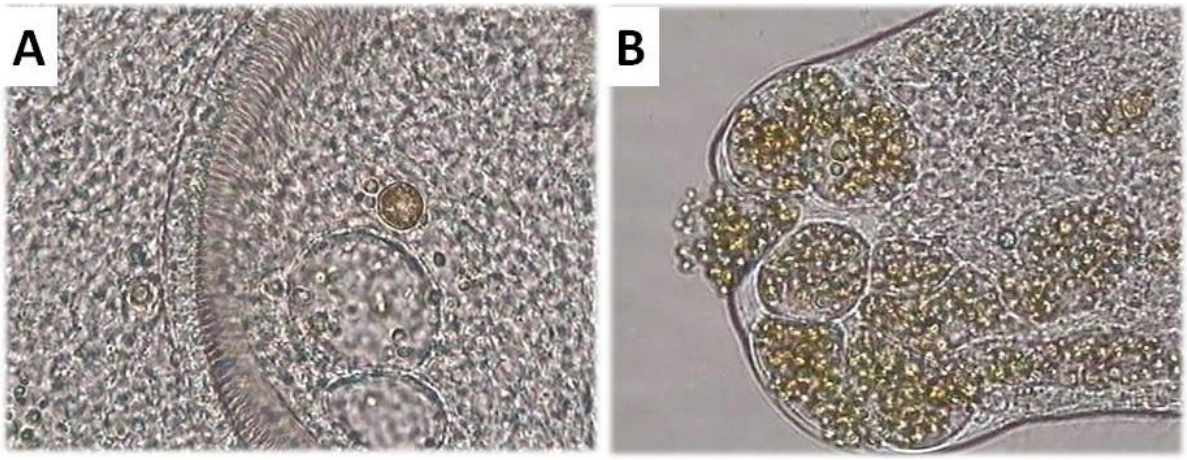

Figure 5. *Fabrea salina*. In **(A)** a close look at the "mouth" region with the big cilia on the depicted cyclical part of the AZM and an algal cell just sucked in. In **(B)** a scene with the evacuation of a vacuole while other vacuoles are stuffed with algae.

The most puzzling part in *Fabrea*'s life story is its polymorphism regarding its overall cell shape, its size, its snout length and the presence and relative size of proboscis (the variously elongated, blunt or pointed, curved or straight, part of the snout region). Probably this polymorphism is not due to genetic differentiation but rather due to an inherent ability to transform the cell into forms that suit transient needs of living. This hypothesis is strengthened by observation of a uniform *Fabrea* population that is composed of quite big individuals (~300 μm). The population kept in experimental conditions with stable temperature (~20 ^{o}C) and plenty of algal food is doing fine and multiplies intensively.

As time is elapsing the population reaches a maximum and gradually starts to create more and more smaller individuals that have all possible shapes like those depicted in Figure 6 intermingled with the initial big "normal" cells. In some cultures the small individuals stabilize in number and their proportion to the population is not changed till the collapse of the whole culture. In other cases the smaller individuals take over in proportion and in some cases the whole culture is composed exclusively of them. A prominent characteristic of these smaller cells is their lower reproductive rate and the lower feeding rate as they are most of the time quite transparent with

lower inclusions and never heavily vacuolated with full of algae vacuoles. Additionally no matter if the culture conditions are suitable (replacement of fresh water, temperature, plenty of food) the small cells never initiate again a population of big individuals.

The above phenomenon can probably be attributed to the deterioration of culture conditions but its irreversibility is highly puzzling and demands thorough investigation. Nevertheless for those intending to culture *Fabrea* continuously, the keeping of big individuals can be attained by frequent seeding new cultures with cells from a pre-existing culture that has not been left for so long in the same vessel.

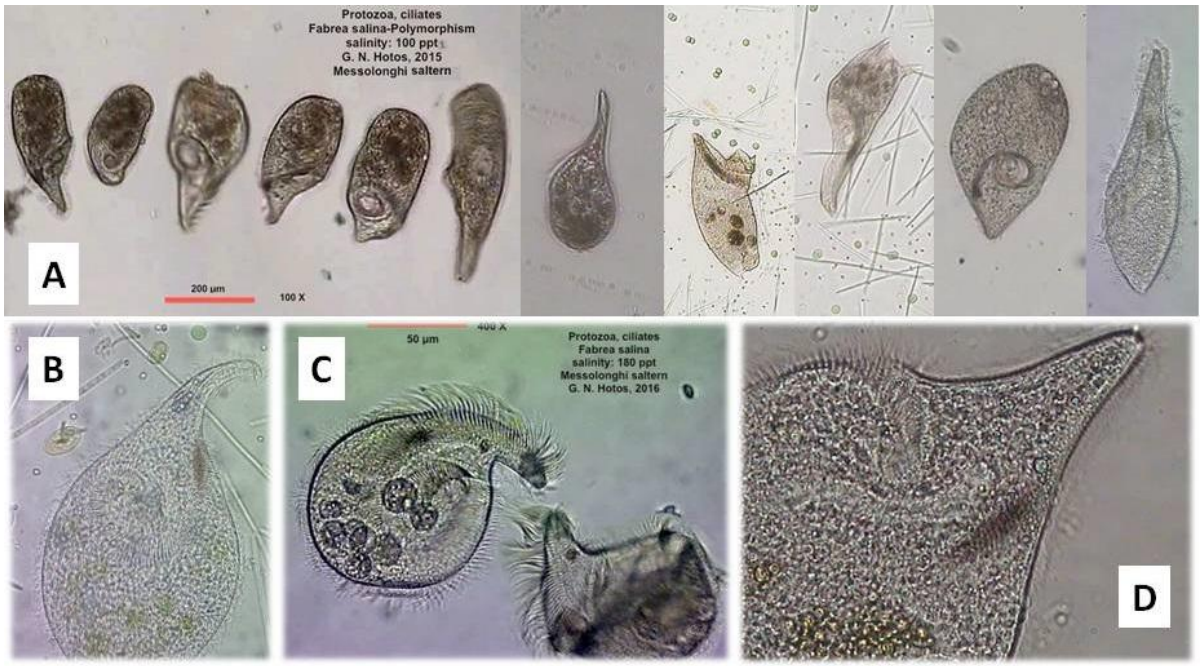

Figure 6. Polymorphism in *Fabrea salina*. In **(A)** an array of various cell forms exhibiting variations in every part of the cell (length, width, mouth region, proboscis). In **(B)** the proboscis is long and curved. In **(C)** that comes from extreme salinity (180 ppt) the whole snout area is curved, the cilia of the proboscis are very long and the AZM very small. In **(D)** is the most common snout form of *Fabrea*, to be considered (in an arbitrary way) as the ordinary and prevalent form of *Fabrea*.

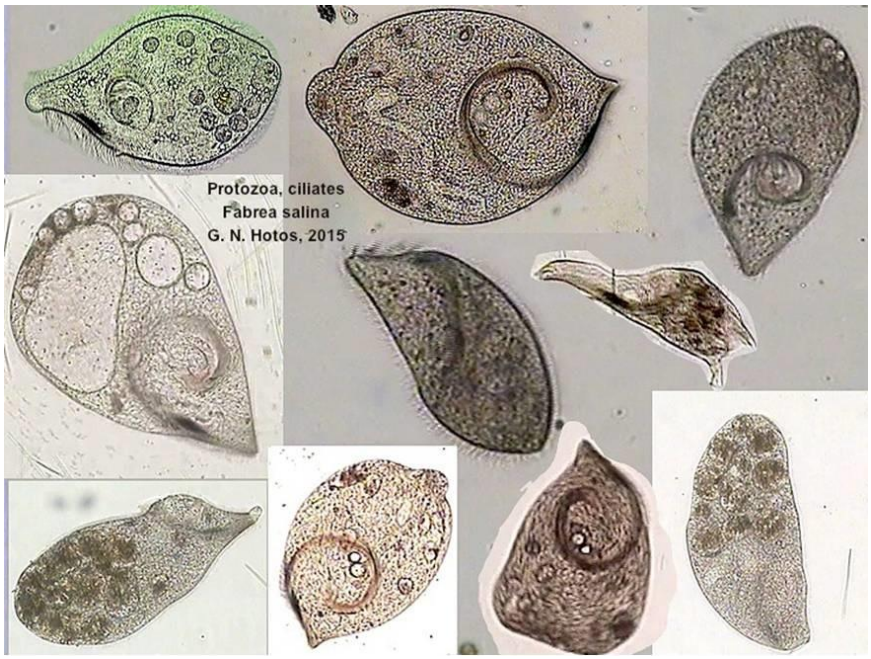

Figure 7. Polymorphism of *Fabrea salina*. This collage sums up some of the most frequently encountered cell shapes in cultures of *Fabrea* at medium to high salinities (35-110 ppt). They are from the big sized *Fabrea* (~300 µm) and most of the variation lays on the snout area.

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Another aspect of importance, puzzling as well and quite unexplained, is the encystment of *Fabrea*. It is well known based on sampling from natural sites that *Fabrea* encysts and enters cryptobiosis in order to endure the evaporation of hypersaline ponds. Its cysts are buried in the mud, withstand dryness and transform to active cells upon the re-flooding of the area. This adaptation widely spread and common among protozoa helps the colonization of new areas as the cysts are transferred there either by mud stuck on birds or by wind action. The puzzling thing is that encystment occurs also in culture vessels even when conditions are far from being adverse. This happens suddenly and not for all the individuals in captivity nor in all culturing vessels that keep the same population in the same exactly conditions. There is no trend nor a pattern and seems that the phenomenon is governed by chance. The process starts with a lowering of the speed of movement, the rounding of the body and the darkening of the cell's color. The cell moves to the bottom and the beating of cilia gradually ceases till there are no cilia observed. Around the cell a thick membranous material is created and the cell turns into a perfect dark brown sphere with amorphous inside material (no vacuoles, no AZM, no visible macronucleus). I have kept such cysts for about 1,5 years completely dry in salt crystals and I revived a c.f. 50% of them by watering their mass. The encystment of *Fabrea* can be very useful if one wishes to keep a stock of them but till the elucidation of a certain recipe for accomplishing this at will, it is nothing but experimentation.

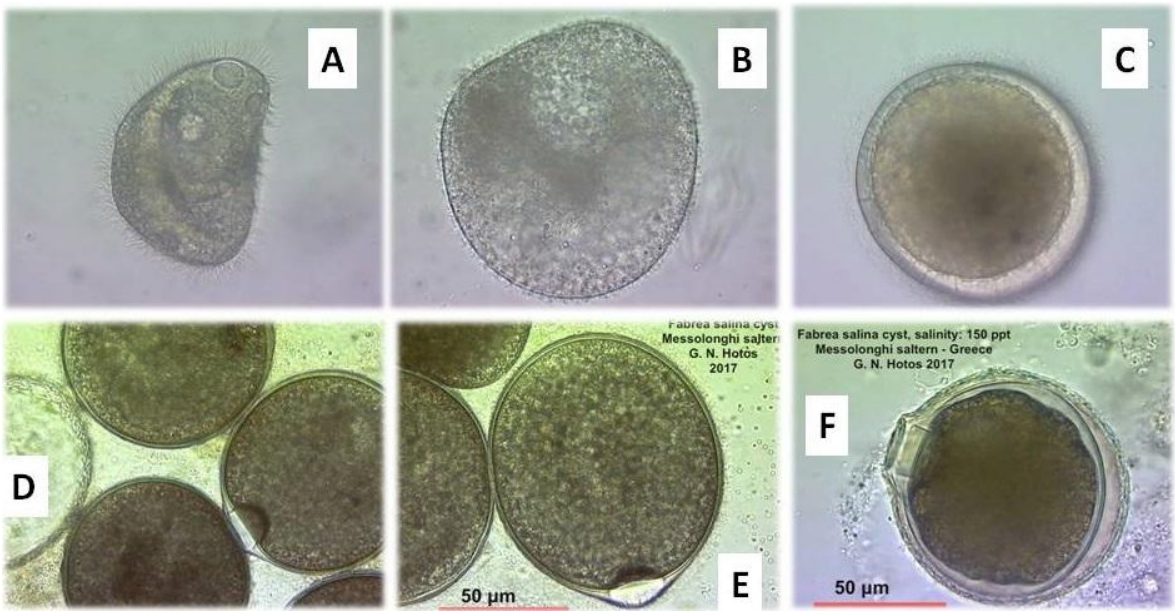

Figure 8. In **(A-B-C)** 3 steps of transformation of a *Fabrea salina* cell to a cyst. In **(D-E-F)** several views of the cysts in the process of awakening. **(C)** and **(F)** are cysts in the terminal stage but in **(F)** the cyst starts to awake creating a characteristic funnel shaped opening on the thick wall.

Fabrea salina can also reproduce by budding although this method is rarely employed by the cell and its triggering cause is unknown. It starts by an initial bulging of the cell in any location that eventually forms a small spherical outgrowth connected with the cell by a stretchable protoplasmic band. Gradually in the course of 1-2 hours the connecting band becomes thinner and thinner while the budding sphere is not changing in volume. Eventually the connection becomes nothing but a thin thread of protoplasm that eventually breaks and the sphere is freed. The sphere

starts its independent life and cilia are formed around this initial cell. Gradually it transforms to the typical *Fabrea* cell with all its organelles and grows to the normal size.

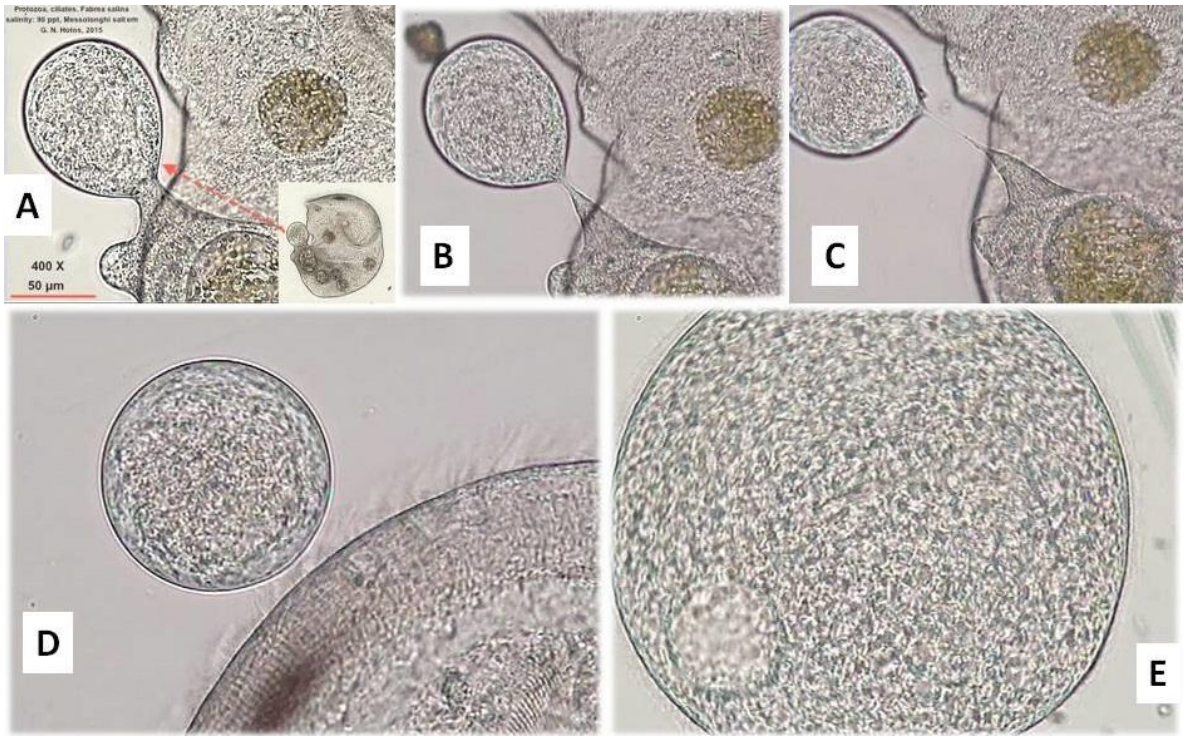

Figure 9. The process of budding in *Fabrea salina*. In **(A)**, **(B)** and **(C)** three different stages of protoplasmic connection between the spherical outgrowth and cell proper. Note the thinning of the protoplasmic connection where in **(C)** is nothing but a thin thread ready to be broken. In **(D)** the freed spherical bud and in **(E)** a closer look with the formation of its first vacuole.

In some lecture notes that I found in the web I noticed that some authors claim that budding does not occur in marine ciliates. Based on evidence from my numerous samples of marine type ciliates, this is not accurate as I have recorded budding occurring not only in *Fabrea* but also in *Euplotes*, *Holophrya*, *Condylostoma* and I deduce that is common probably in other species as well.

Fabrea salina can be useful both as an ideal tool for teaching protozoa and for experimentation in terms of osmoregulation, toxicology and as a live feed to be used in marine fish-hatcheries. In reality it is maybe the only alternative to the rotifer *Brachionus plicatilis* that is the only appropriate till today live food for the newly hatched larvae of various marine fish. Its size, color, movement, planktonic nature and constituents resembles much those of *Brachionus*. Its mono-cellular nature and its simple way of multiplication permits genetic manipulation to create appropriate strains that could serve better its role as a live feed and for other purposes.

The present work is not a scientific paper (experimentation or review) nor a monograph. It must be seen as an encyclopedian-type essay in order to support information for biology students in a simple and comprehensive way, based on the experience of a person fond of naturalistic investigations.

For the end of this essay I think it is useful to present an overall sketch of *Fabrea*'s cell to clarify the terminology used for describing its morphology. Figure 10 is the most representative and

beautiful drawing (from my personal point of view) of *Fabrea* taken from the work of Ji Hye Ki & Mann Kyoon Shin (2015) that I cite in the literature.

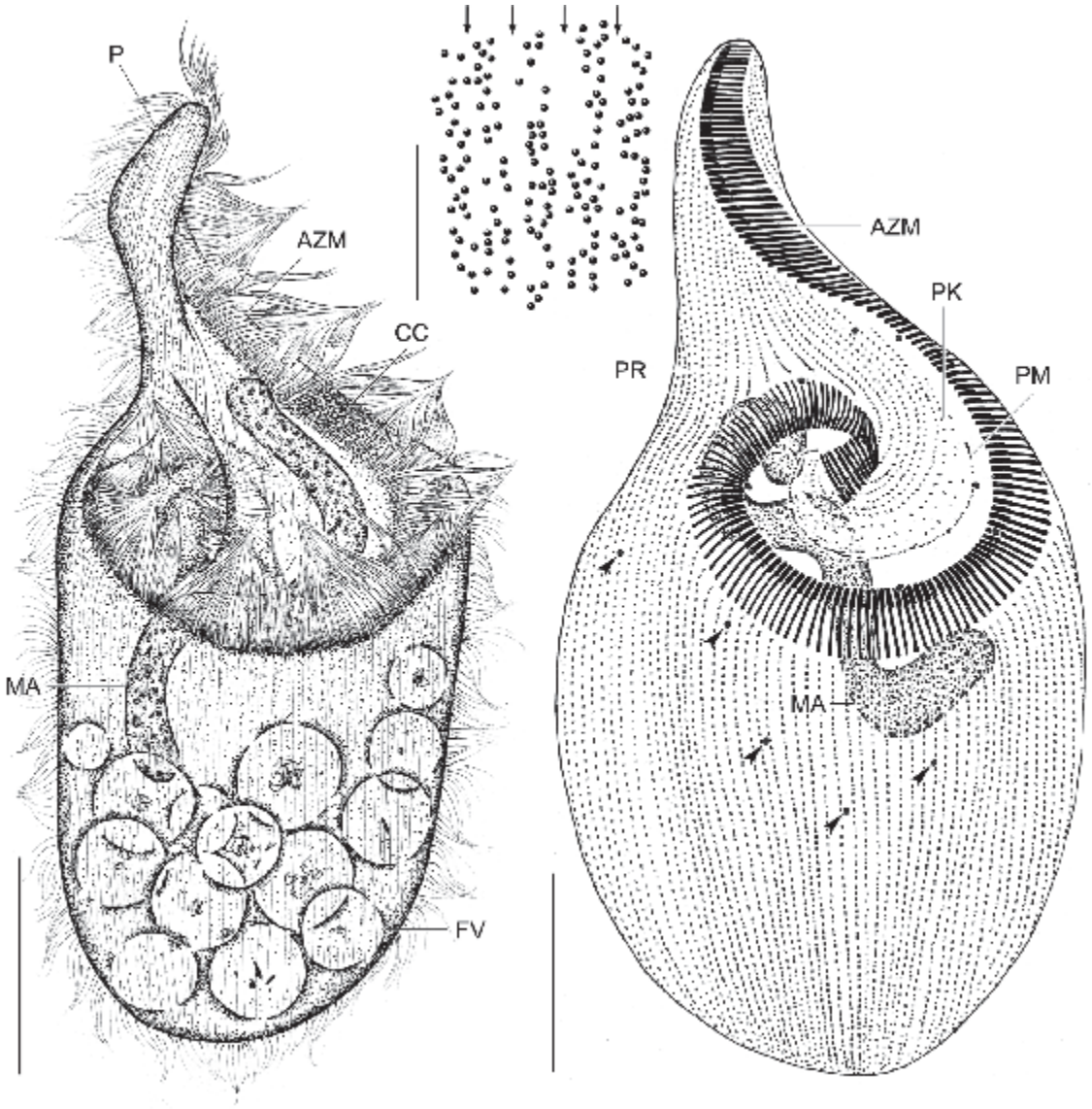

Figure 10. *Fabrea salina*. Typical body shape (left), arrangement and pattern of cortical granules, arrows indicate each somatic kineties (center) and another more detailed view of the cell's outline to show the many kineties that transverse the cell (right). Arrows indicate micronuclei.
CC, part of condensed cortical granules; **FV**, food vacuole; **AZM**, adoral zone of membranelles; **MA**, macronucleus; **P**, proboscis; **PK**, peristomial kinety; **PM**, paroral membrane; **PR**, preoral kineties. Scale bars: Left and right=50 µm, center=5 µm (after Ji Hye Kim & Mann Kyoon Shin, 2015).

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For videos about *Fabrea* those listed below created by the author can be useful.

https://www.youtube.com/watch?v=zbMe-bGWtX4 (*Fabrea salina*, evacuation of a vacuole)
https://www.youtube.com/watch?v=1BEbvQAV7Ng (*Fabrea salin*a, budding)
https://www.youtube.com/watch?v=ftmktGpurRY (*Fabrea salina*, division)
https://www.youtube.com/watch?v=QGQSKP2ZGxw (*Fabrea salina*, population)
https://www.linkedin.com/feed/update/urn:li:activity:6464178208781799424 (*Fabrea salina*)
https://www.linkedin.com/feed/update/urn:li:activity:6451864552236814336 (*Fabrea salina,* vacuoles filled with algae)