1 HISTORY

The laboratory was established in 1992 with the mission of culturing microalgae, rotifers and *Artemia* for students to learn the techniques of producing live food for the larvae of marine fish produced in
fish hatcheries.

2 FACILITIES

It covers an area of 80 m^2 divided into two rooms. One room of 30 m^2 with the facilities and devices for cultures and one of 50 m^2 with microscopes, instruments and teaching aids for research and teaching.

3 ACTIVITIES

The laboratory is active in finding, cultivating and preserving local strains of microalgae species from the lagoons of Western Greece and from the hypersaline waters of the Messolonghi salworks. At the same time, it searches for, isolates and preserves zooplankton organisms from these areas. Only those species that can be maintained in thriving cultures under the rationale of being easily cultured under normal environmental conditions when mass production is attempted, are grown on trials.

In order to achieve the above, continuous field trips and water sampling are carried out. Then the water samples undergo the appropriate procedures in the laboratory to end up after a long time, continuous renewals, and various tests, in monospecific cultures which prove to be vigorous and sustainable.

PLANKTON CULTURE LABORATORY

Department of Fisheries and Aquaculture
University of Patras
Messolonghi, Greece

To date, 10 species of microalgae (6 eukaryotic, 4 cyanobacterial), 3 species of protozoa, 2 species of copepods and 1 species of rotifers have been isolated.

The creation of monospecific cultures is not guaranteed for every species, either phytoplankton or zooplankton, of those present in the water samples that undergo the isolation and recultivation procedures in the laboratory. The majority of species after the initial phase of isolation do not persist for long nor do algae persist in monoculture. Therefore, the effort made in the laboratory has value for this very reason, that is, of maintaining only dynamic and vigorous species that are easily cultivated.

Species maintained continuously in the laboratory are available to universities, research institutes and companies interested in their exploitation.

Laboratory head

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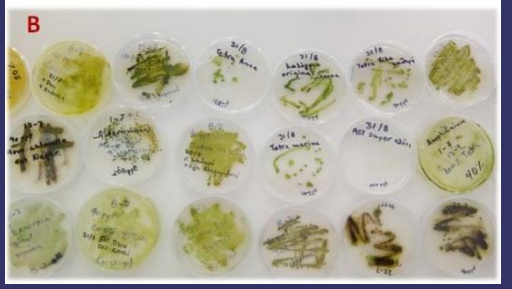
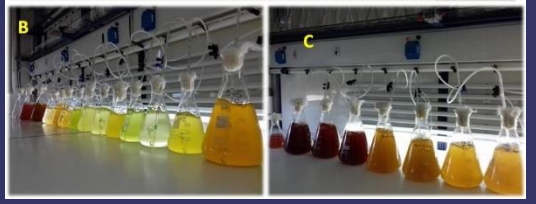
SURVEYANCE

1 LAGOONS

2 SALTWORKS

ISOLATION





CULTURES

ORGANISMS MAINTAINED AND AVAILABLE TO INTERESTED PARTIES

A. EUKARYOTIC MICROALGAE

Amphidinium carterae

Taxonomy

Supra-kingdom: Eukaryota
Kingdom: Protista
Division: Dinoflagellata
Class: Dinophyceae
Order: Gymnodiniales
Family: Gymnodiniaceae
Genus: *Amphidinium*
Species: *Amphidinium carterae*

Unicellular planktonic species with a range of sizes from 11.93 ± 1.3 (sd) µm along the long axis and 8.64 ± 0.92 (sd) µm along the width. Mobile with characteristic rectilinear movement and frequent sudden change of direction. They don't swirl. No "armor"-cellulose shell.

Sometimes the cells appear completely stationary within a vitreous cyst. Cell shape (Figures 1 & 2) varies between spheroidal and fusiform and compressed along the long axis. Their color varies between shades of olive yellow and yellow-orange with a slight greenish tint sometimes. The epicone is much smaller than the hypocone and unique in its shape among dinoflagellates as it resembles a curved "proboscis". Among the cells there is variation in the size of the envelope, in some it is voluminous and in others it is smaller. The transverse groove (cingulum) is not as obvious as in other classes of dinoflagellates because the epicone, small as it is in relation to the hypocone (which occupies most of the cell), is not separated from it by a large diameter groove. The vertical groove (sulcus) is evident running perpendicular to the transverse groove along the entire length of the hypocone. Both transverse and vertical flagellum are prominent with the transverse lying partly within the transverse groove and part of it projecting in a characteristic undulating motion and the longitudinal projecting well beyond the vertical groove and moving less undulating than the transverse. The nucleus is at the bottom of the hypocone. The olive-green colored chloroplast occupies most of the protoplasm and is lobed in shape. The cytoplasm is vitreous with a variety of inclusions and varying shades depending on the growth phase of the cultured cells.

Propagation is by simple cell division and although amphigenic reproduction and cyst production are mentioned in the literature, this was not observed in this strain. It grows well in salinities of 35-55 ppt and the typical color of its cultures is orange-yellow at first to brown-orange at maturity (Figures 3 & 4).

Its culture emits a characteristic pungent smell which resembles rotting organic matter of marine origin. A characteristic of its mature culture is its yellowish color and its intense foaming with the foam accumulating on the surface of the water. It is thought to produce a lot of organic matter which it excretes into the water. Another remarkable feature of its cultures is that there is no presence of diatoms, which very easily invade even the most carefully preserved cultures of other types of microalgae. It is possible that the phenomenon of allelopathy exists and that *Amphidinium* produces substances toxic to diatoms.

A remarkable characteristic of *Amphidinium* is the compactness of the precipitate which results when a quantity of its culture is centrifuged. The sediment is so thick and firm at the bottom of the tube after centrifugation, that it will not re-suspend no matter how hard you stir. Only by mixing with a rod does it dissolve. Apparently, extracellular substances resulting from cell leakage due to centrifugation act adhesively on the cell mass.

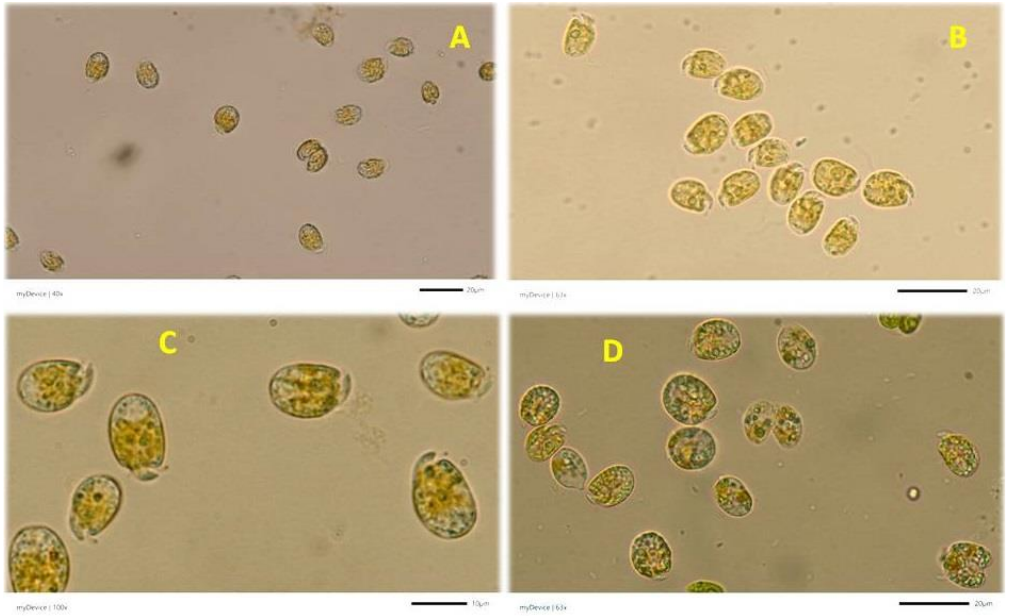


Figure 1. Photomicrographs of *Amphidinium* cells from cultures in the exponential growth phase. The proboscis-like form of the epicone is characteristic. The scale is shown by the line at the bottom right of each photo. A: 20 µm-400X, B & D: 20 µm-630x, C: 10 µm-1000X.

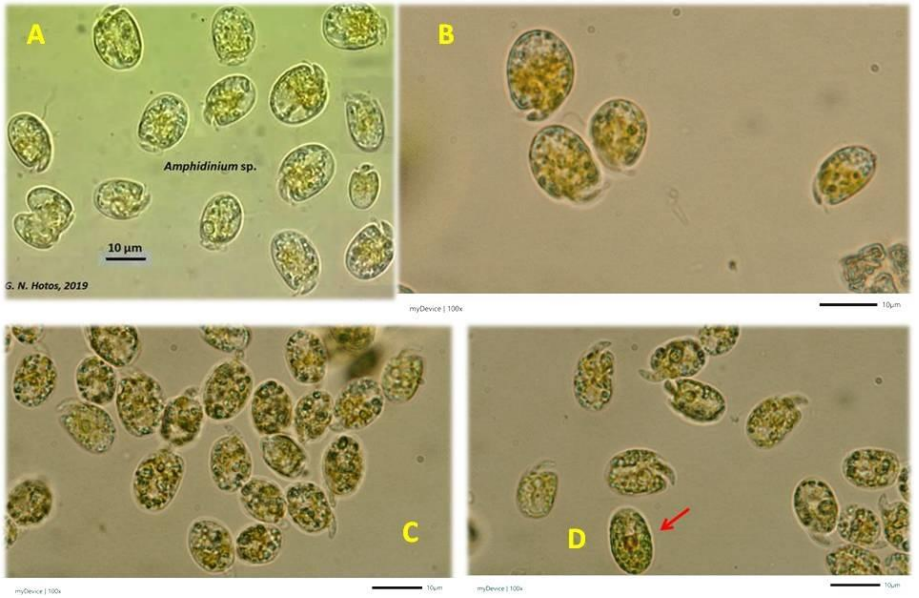


Figure 2. Photomicrographs of *Amphidinium* cells from cultures in the exponential growth phase. Characteristic is the proboscis-like shape of the epicone and the olive-yellow color of the cells. A *Tetraselmis* cell is shown in arrowed D for comparison. The scale is shown by the line at the bottom right of each photo. A, B, C & D: 10 µm-1000X.

Figure 3. Culture of *Amphidinium carterae* in the initial phase.

Figure 4. Culture of *Amphidinium carterae* in the mature phase.

Nephroselmis sp.

Taxonomy

Kingdom: Protista
Division: Chlorophyta
Class: Nephrophyceae
Order: Nephroselmidales
Family: Nephroselmidaceae
Genus: *Nephroselmis*
Species: *Nephroselmis* sp.

Unicellular planktonic chlorophyte (Figures 5 & 6) with single longitudinally flattened bean-shaped cells each bearing two unequal flagella which arise from the middle part of the hollow part of the cell. Cells lack a cell wall and measure 5.28 ± 0.35 (sd) µm along the long axis and 3.81 ± 0.5 (sd) µm along the width. The movement of the flagella provides intense motility to the cell with a characteristic rectilinear movement that is slightly swirling and slightly jerky. The shorter flagellum moves in the forward direction and the longer flagellum pulses at the rear. Each cell contains a large lateral chloroplast within which is a pyrenoid. The chloroplast occupies most of the volume of the cell and also has a photosensitive spot in a position corresponding to the root of the short flagellum. The color of the cells under the microscope is dull green while in the culture vessel light - dull green (Figures 7-9). Reproduction takes place either by cell division (usually), or amphigenically, in contrast to the oligogamous way, i.e. by the fusion of two identical germ cells to create a zygote, but without the formation of cysts. It grows well in salinities of 35-65 ppt.

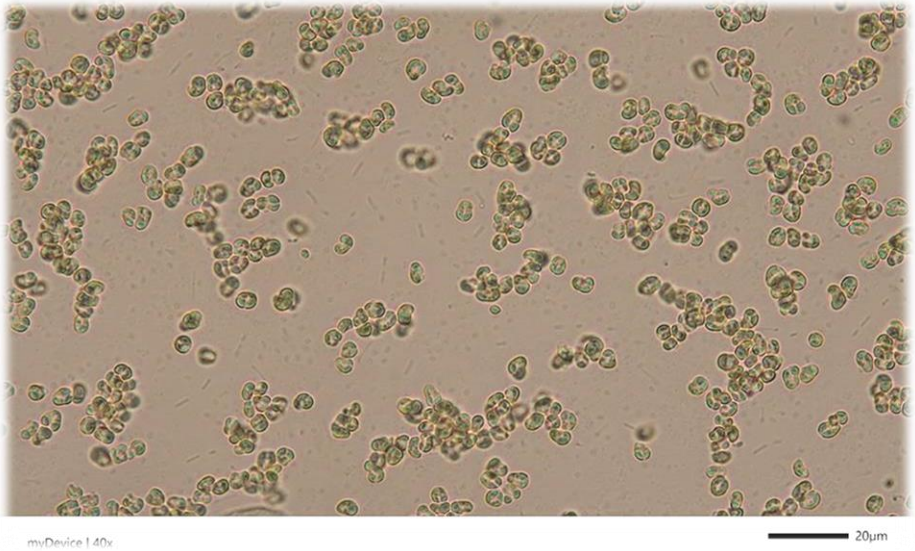


Figure 5. Microscopic photograph of *Nephroselmis* cells. The cells have a green tint and tend to clump together. The scale is shown by the line at the bottom right of the photo, 20 µm-400X.

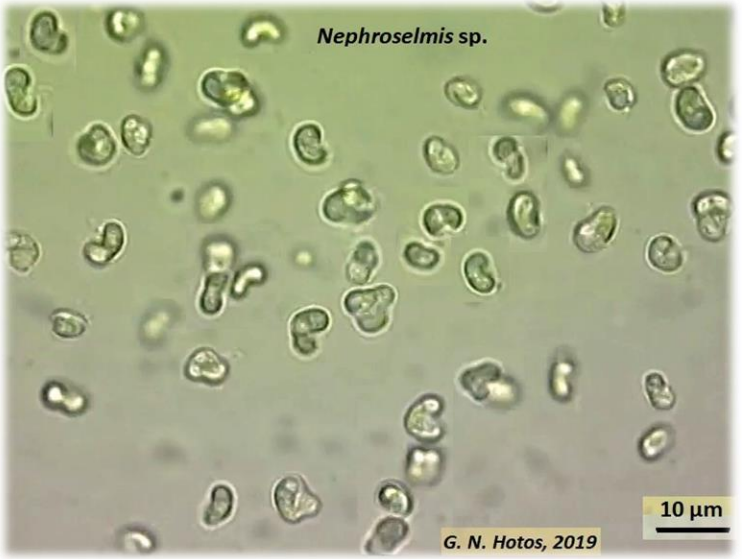


Figure 6. Microscopic photograph of *Nephroselmis* cells. Scale 10 µm-1000X.

Figure 7. *Nephroselmis* cultures in initial phase (day 2).

Figure 8. *Nephroselmis* cultures in exponential phase (6th day).

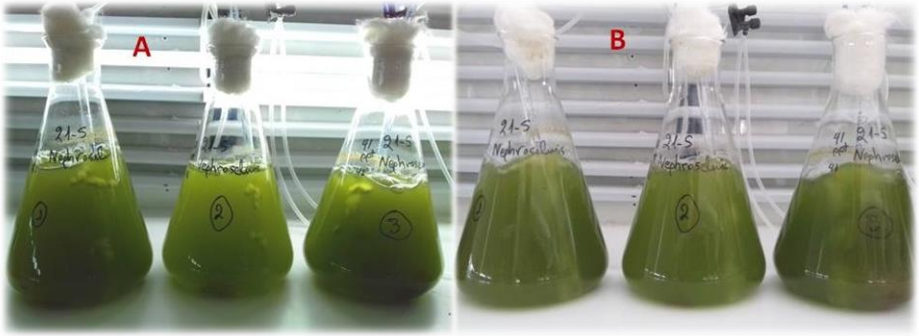
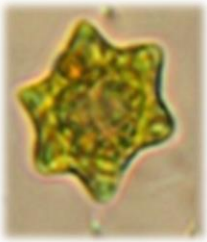


Figure 9. Color of *Nephroselmis* cultures, bright green in exponential phase (A) and dull green in mature stationary phase (B).

Asteromonas gracilis



Taxonomy

Supra-kingdom: Eukaryota
Kingdom: Protista
Division: Chlorophyta
Class: Chlorophyceae
Order: Chlamydomonadales
Family: Asteromonadaceae
Genus: *Asteromonas*
Species: *Asteromonas gracilis*

Unicellular planktonic chlorophyte (Figures 10-14) without a cell wall (naked). The most important morphological characteristics of normal, mature cell (vegetative) of *Asteromonas gracilis*, microscopically are:

- fusiform shape, which is sometimes narrow and sometimes not (Figures 10, 11 & 13),
- sometimes due to the characteristic jerky movement of the cell it is placed in the space that looks like a star (Figures 10, 11 & 13),
- 2 flagella attached to their upper part,
- absence of cell wall,
- 3 to 6 (most typical 6) "notches" (like a keel), running lengthwise laterally throughout the cell,
- average length 18.84 ± 2.88 (sd) µm along the long axis and 13.09 ± 2.4 (sd) µm along the width and with extreme sizes of 12-22 µm along the long axis and 8.7 - 16.36 µm across,
- length of flagella equal to 1½ to 2 times the length of the thallus,
- external surface without scales, nevertheless, fine fibrous deposits (characteristically visible with an electron microscope) can often be seen on the surface of the cell and flagella.

In a living cell of *Asteromonas gracilis*, a set of cell organelles and formations can be seen inside the cytoplasm. One of the most basic is the chloroplast with an almost basin-like shape. It is thin and pale (no bright green color) on its larger surface. It extends to the upper part of the cell, almost to the base of the flagella. It has an asymmetric pyrenoid (a proteinaceous “body” inside the chloroplast), where starch is formed, which is adjacent to the posterior part of the large nucleus. In the upper part of the cell and inside the chloroplast, an orange spot can be seen, the eye spot (stigma). The nucleus is large and contains a distinct nucleolus. It is placed almost in the center of the cell and its anterior part extends almost to the base of the flagella. In addition, in the inner side of the cell, on the periphery of the cytoplasm, many small mitochondria can be seen. In the front part of the cell there are distributed more or less symmetrically around the nucleus several reticulosomes 4-6 in number, which together form the Golgi system. The large number of micro-vesicles, which can be seen in the front part of the cell, between the reticulosomes, probably ensued from them.

Many thin-walled cysts (Figure 12) often accumulate at the bottom of an old culture. No evidence has been found to link cyst formation to the reproductive process. The cells that are to form cysts are greatly enlarged, keeping the pyrenoid relatively small. The cyst wall initially appears to be formed as a discontinuous layer peripherally to cytoplasm, which then thickens, starting from the interior. Fully mature cysts have a diameter ranging from 13 to 18 µm and a wall thickness of 2 to 3 µm.

It reproduces by cell division (Figure 14) with separation of the daughter cells along the longitudinal axis, i.e. the imaginary line connecting the extremity of the flagella with the posterior protuberance of the cell. No sexual reproduction was observed, therefore, no zygote formation of some form (swollen cell, cyst or palmella) and of course no spores (aplanospores or multiple zygote divisions).

Asteromonas gracilis is one of the most halotolerant eukaryotic algae and possibly together with *Dunaliella* among the chlorophytes, the most resistant to very high salinities. After all, both were collected and isolated from the saltworks of Messolonghi. Its culture progresses well at salinities above 90 ppt and "suffers" at typical seawater salinities (30-40 ppt) while exhibiting unstable growth in the 40-60 ppt range. The color of the cultures ranges from light

green (initial phase) to dark green in the mature phase. If the cells are abruptly transferred to very high salinity then their shape is distorted without any pattern (Figures 11C & 13D) but they remain alive with low motility. Over time, however, they adapt to the new salinity and regain their usual spindle-star shape and their intense straight tremorous motion. At high salinities, *Asteromonas* (as well as *Dunaliella*) accumulates intracellular glycerol to cope with osmotic stress. Unlike *Dunaliella*, however, it does not seem to accumulate β-carotene. If, on the contrary, they are transferred from a high salinity to a much lower one (e.g. from 90 ppt to 35 ppt) then the cells swell due to water absorption and become spherical and take a long time to regain their normal shape.

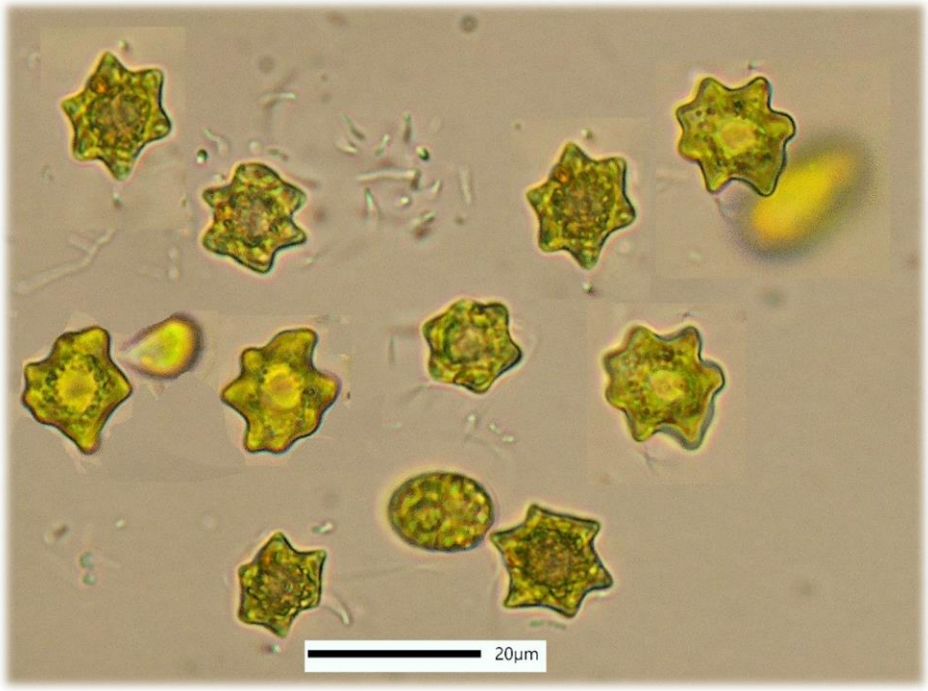


Figure 10. The star-like appearance of *Asteromonas gracilis* cells.

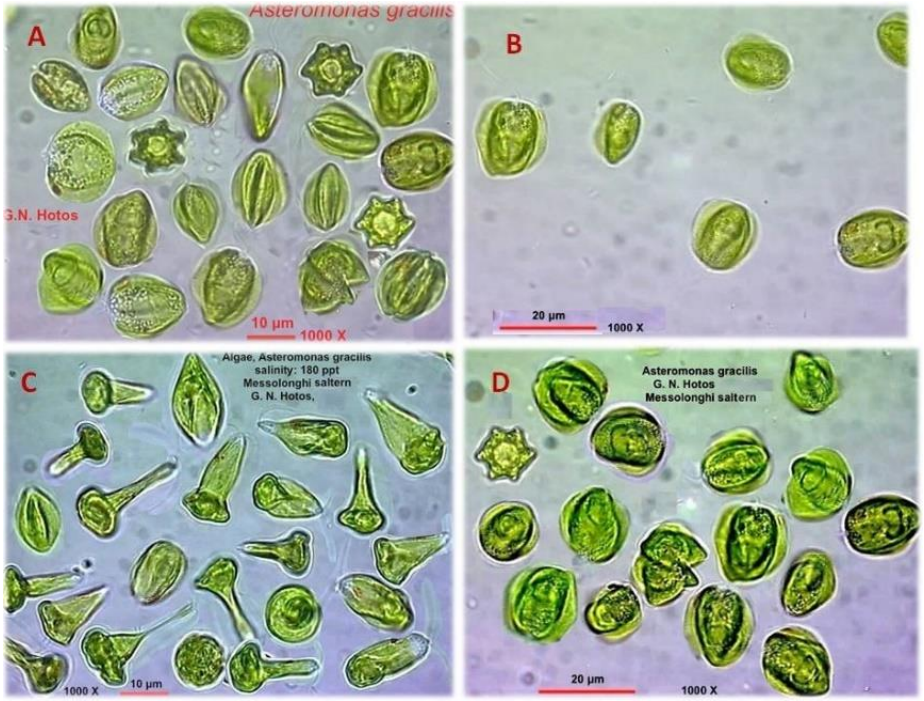
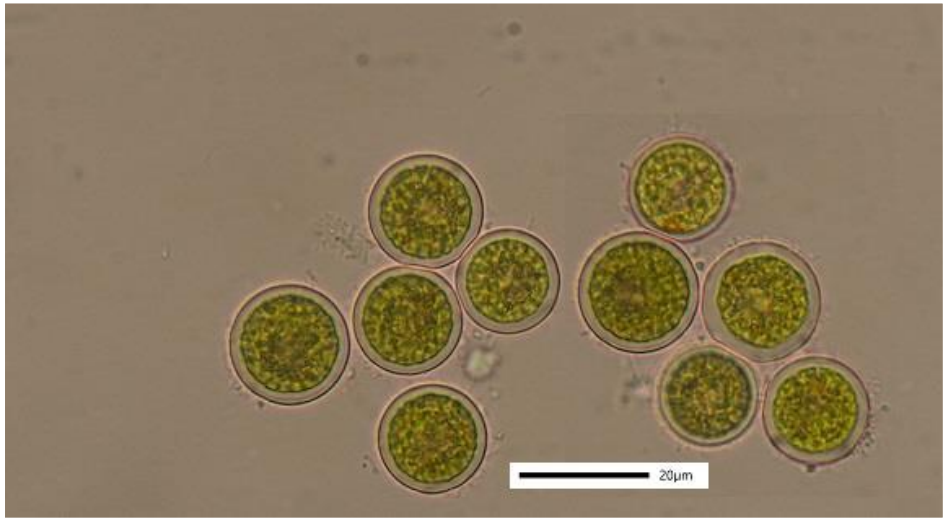


Figure 11. Polymorphism of cells of *Asteromonas gracilis.*



Εικόνα 12. Cysts of *Asteromonas gracilis* .

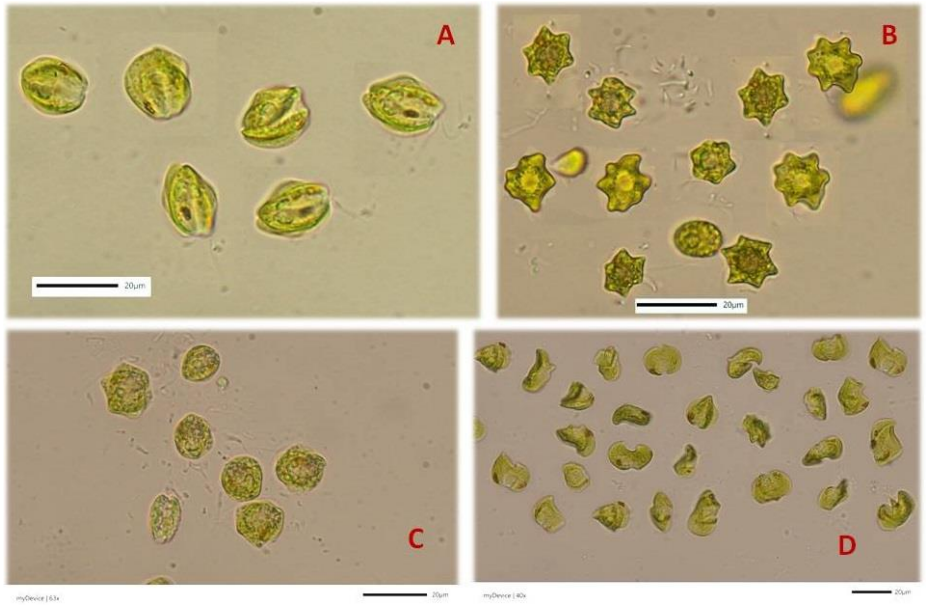


Figure 13. At A, B & C vegetative cells of *Asteromonas gracilis,* many stellate at B and strongly deformed (but alive) due to osmotic stress at C & D, 20 µm, 630X.

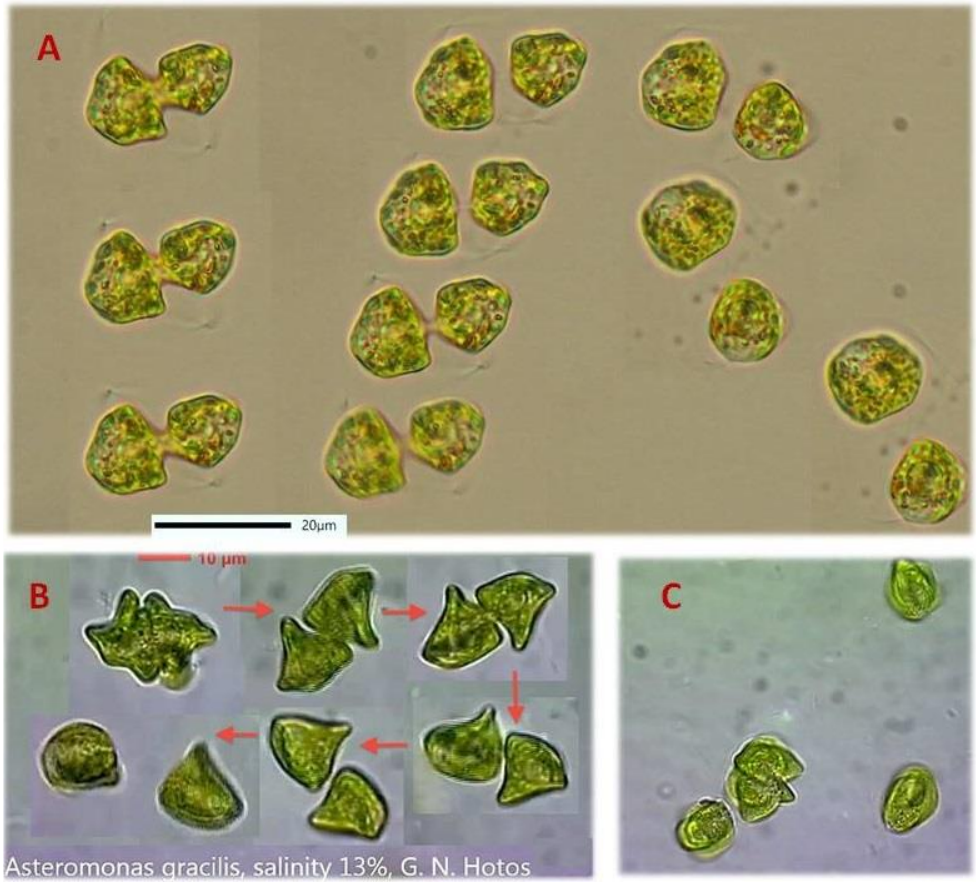


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Figure 14. Time-lapse photos of cell division in *Asteromonas gracilis.*

Dunaliella salina



Taxonomy

Supra-kingdom: Eukaryota
Kingdom: Protista
Division: Chlorophyta
Class: Chlorophyceae
Order: Chlamydomonadales
Family: Dunaliellaceae
Genus: *Dunaliella*
Species: *Dunaliella salina*

Unicellular planktonic species without cell wall (naked) and bright green in color (Figure 15 A, C & D) in the vegetative phase of cells (no cysts, no extreme salinities). The most characteristic feature of all species of the genus *Dunaliella* and especially of *Dunaliella salina* that we isolated from the salt marshes of Messolonghi is its exceptional resistance to hypersaline waters.

All species of the genus *Dunaliella* are unicellular, without a cell wall (naked) and have 2 equal-sized flagella longer (1.5-2 times) than that of their thallus. The nucleus is large and centrally located in the anterior part (the part where the flagella emerge) of the pear-shaped typical cell shape and as anterior we call the narrow part while as posterior the rounded part at the opposite end. The nucleus is surrounded for its most part by the large chloroplast which almost fills the protoplasm and by various small vacuoles. The absence of a cell wall does not allow the rigidity (stability) of the cell shape which, due to being very malleable, is easily affected by the osmotic state of the surrounding water and swells or shrinks accordingly (up to where it is allowed to, because the cell has an osmoregulation mechanism) depending on whether the water is much dilute or denser than the cytoplasm. The size of the cell varies greatly both among different species and within the species itself according to prevailing conditions. A typical size is that of 7-12 μm along its long axis but very often it is also found in sizes of 16-20 μm. It is a very mobile species and the very rapid strokes of its flagella propel it forward in a characteristic course of sharp and twisting movements along the horizontal axis of the cell. The average (typical) cell size of *Dunaliella* is however much smaller compared to other species of flagellated chlorophytes found in hypersalinity such as that of *Asteromonas gracilis* (18-25 μm) or *Tetraselmis marina* (20 - 25 μm) or in normal salinity that of the cryptophyte *Rhodomonas salina*, (10-15 μm).

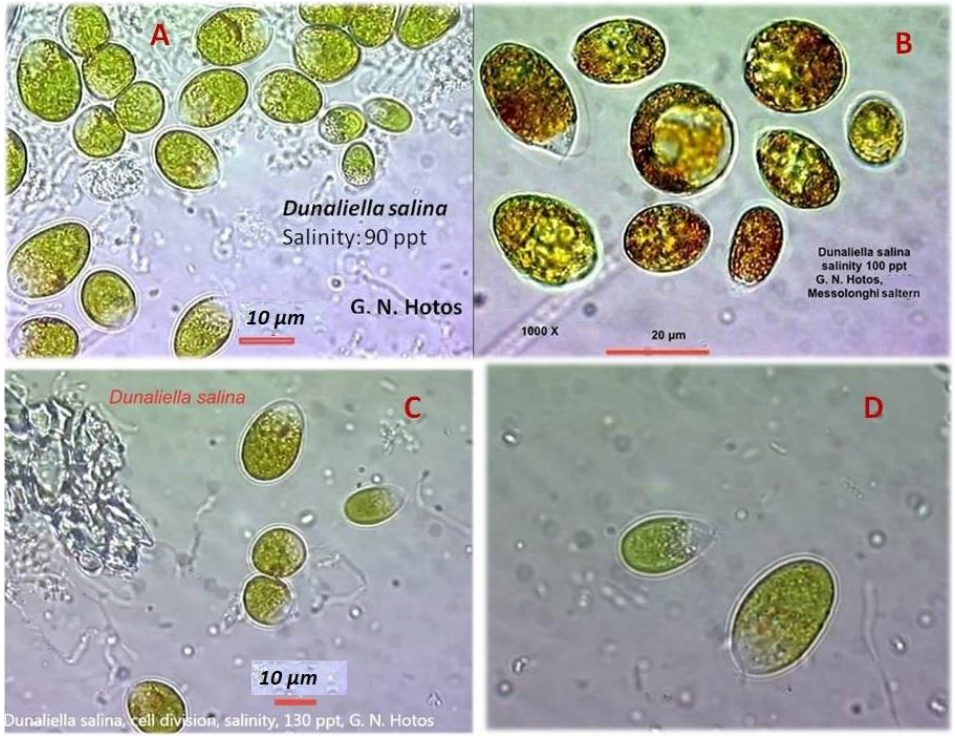


Figure 15. *Dunaliella salina* with variation in cell sizes and bright green color at salinities below 100 ppt (A, C & D) and swelling with redness due to β-carotene accumulation at salinities above 100 ppt in B.

The shape of the cell of *Dunaliella* shows great diversity and while its typical form is pear-shaped it can become cylindrical, oval, ellipsoidal, fusiform or spherical (Figure 16) according to changes in conditions, i.e. temperature, light, nutrients and above all salinity. As a general rule, however, we can say that in unfavorable conditions (mainly depletion of nutrients) it switches to the spherical form.

Cell size also varies greatly and in a thriving culture with cells in the exponential phase of population growth, a variety of sizes are observed (Figure 15 A, C & D) with the majority at a typical size of 8–10 μm (along the long axis) and 4–5 μm (in width), and a smaller proportion of smaller cells of the order of 5–8 μm apparently derived from recent propagation by zygotes or aplanospores. In very high salinity, condition where cell proliferation is dramatically reduced, the cells grow in size and can reach up to 15 μm while at the same time they begin to acquire an orange hue due to the accumulation of carotenoids (Figure 15B).

There are many reports of *Dunaliella* forming cysts (Figure 16) either in palmelloid form (enclosure in a mucous capsule, although we did not observe this) or by transformation of the germ cell, or by zygote formation. Apart from the case of the zygote, the other two cases of encystment seem to be caused by strong changes in the characteristics of the water, and indeed in terms of the two extremes of salinity, i.e. either a sharp drop in salinity or a dramatic increase in it, which sometimes results in the complete evaporation of the water mass. However, frost can also cause encystment, as can the lack of nutrients. These cysts sink and settle to the bottom where they can remain dormant for a long time (unknown for how long) and transform into motile (flagellated) vegetative cells when conditions improve. In movement and cellular appearance, *Dunaliella* is often mentioned in scientific texts as resembling the much-researched chloro-microalga *Chlamydomonas*, but in addition to the fact *Chlamydomonas* has a cell wall, the shape of their cells differs since *Chlamydomonas* is almost spherical in shape while *Dunaliella* pear-like. Instead of a rigid cell wall, *Dunaliella* possesses a remarkable mucilaginous covering composed mainly of glycoprotein.

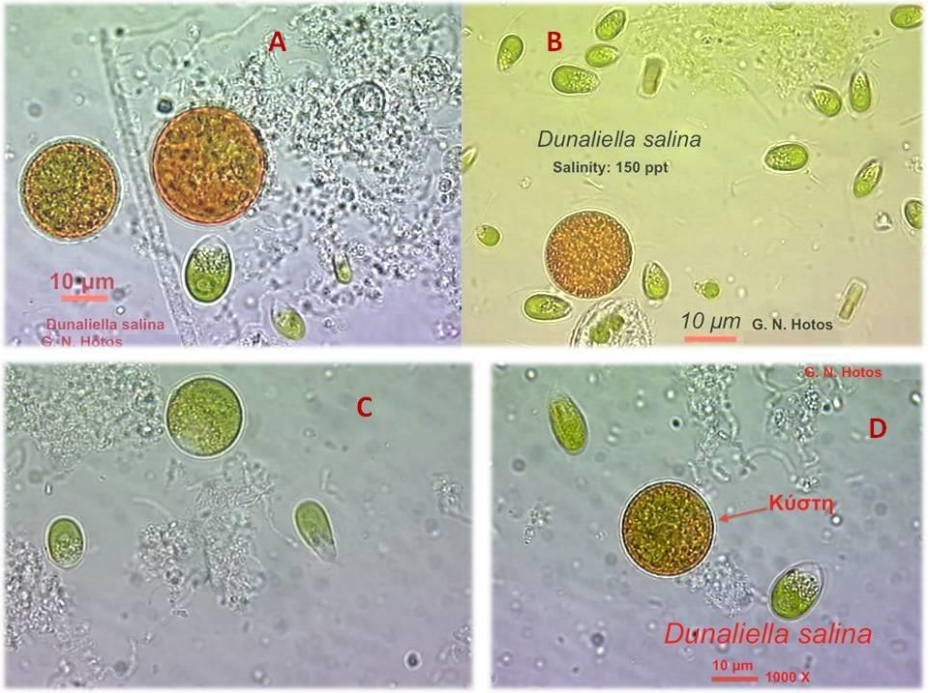


Figure 16. *Dunaliella salina*. Spherical cysts between green vegetative cells and with intense accumulation of β-carotene (A, B & D). In C the cyst is green and is presumed to be a zygotic cell undergoing meiotic and mitotic divisions.

It lacks a pulsatile vacuole (usually found at the front of the cell) that helps other algae in osmoregulation because *Dunaliella* has another "strategy" to deal with osmotic pressures by producing and accumulating glycerol intracellularly. However, it is similar to *Chlamydomonas* in that they both have a large cup-shaped chloroplast, and they differ in that *Dunaliella*, which is found in saline and hypersaline waters, has in the center of its chloroplast a pyrenoid surrounded by starch aggregates (products of photosynthesis). At the front end of the cell and depending on the physiological state, a varying number of oil (lipid) granules (droplets) can be distinguished. The glycerol (glycerin) which, as mentioned above, *Dunaliella* produces at high salinities, is sometimes so great in quantity that its excess is secreted from the cell and the culture water accumulates on the surface a dense white creamy foam (Figure 18D). The other notable feature of the very halotolerant species *Dunaliella salina* and *D. parva* is the accumulation in their chloroplast of large amounts of the pigment β-carotene which, in the form of droplets, occupies the periphery of the chloroplast and colors the cell orange-red at high salinities (Figure 17 C & D). The change in the color of the cell from green (when it has little β-carotene) to orange-red (high β-carotene) is due to the covering by the carotenes of the green chlorophyll and because the chloroplast comprises the largest mass of the cell the color of the cell is determined from the carotenes that flood the chloroplast.

The phenomenon of orange-red coloration finds its classic and most intense expression in the species *D. salina* which thrives in basins with very high salinity (withstands up to 300 ppt), usually in saltworks ponds (Figure 18 A, B & C) where it prevails gradually (with increasing salinity) as the only microalgae there. The role of β-carotene, in addition to acting as an auxiliary pigment for chlorophyll (it transfers photons to chlorophyll-a of the photosynthetic center), is also to protect chlorophyll as well as cellular DNA from excessive exposure to solar radiation (and from part of the UV spectrum) in the conditions prevailing in sun-drenched shallow hypersaline basins. In other words, it acts as a sun protection shield. In addition, the many β-carotenes also act as a "repository" of the excess carbon that is bound by the intensively photosynthesizing cells which, however, in these conditions do not multiply intensively. In other words, the existing population of *Dunaliella salina* in hypersalinity and bright light does not grow but its cells photosynthesize and "get fat"".

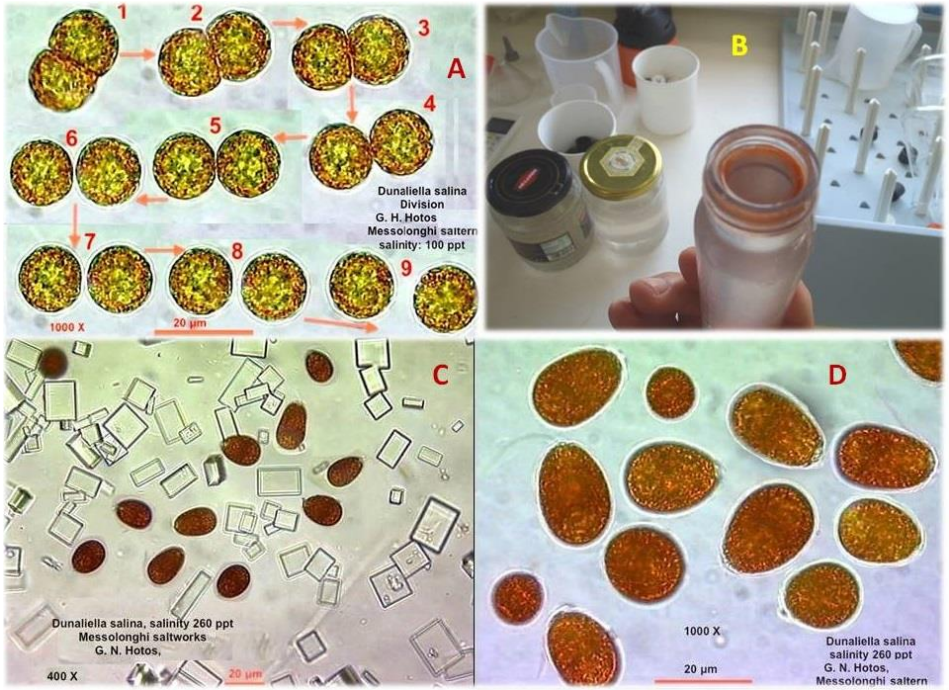


Figure 17. *Dunaliella salina*. In A, collage of photographs of successive phases during mitotic division of a spherical cell with a large amount of β-carotene. In C, pear-shaped strongly stained cells with a lot of β-carotene at very high salinity >250 ppt among already formed salt crystals. In D large pear-shaped cells from high salinity, pulsing with β-carotene flooding the cell. In B a typical photograph of *Dunaliella* culture centrifugation in water salinity > 200 ppt, where the very high viscosity of the water does not allow the cells to settle so they condense on the surface of the container as a red layer.

Figure 18. *Dunaliella salina*. A: Collection of a sample from a high salinity basin of the Messolonghi saltworks with a reddish color due to the growth of the archaeum bacterium *Halobacterium salinarum* and the chlorophyte *Dunaliella salina*. In B & C *Dunaliella salina* culture in very high salinity with red color due to large amounts of β-carotene intracellularly. In D, *Dunaliella salina* culture at lower salinity with the typical green color but they have started to undergo artificial elevation of salinity (salt addition) and produce a lot of glycerol which accumulates on the surface as a white foam.

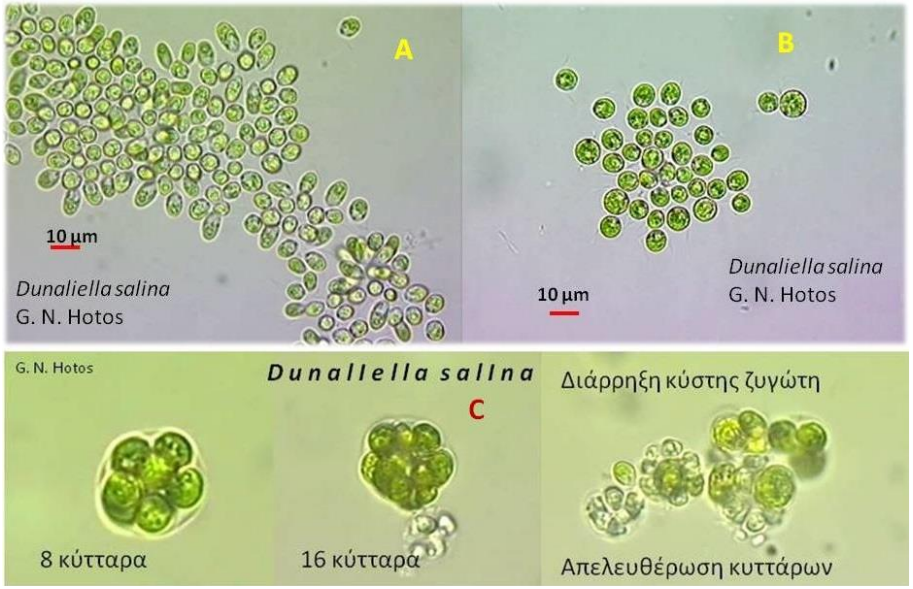
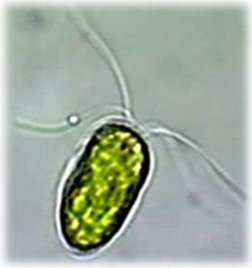


Figure 19 *Dunaliella salina*. In A & B, cell aggregates at normal salinities (~40 ppt) with the characteristic green color and pear-shaped (A) and spherical (B) forms. C shows 3 phases of daughter cell generation from a green zygote cyst (like the one in Figure 16C).

Dunaliella multiplies by cell division along the long axis especially when it is in its motile phase (there is also a phase that rounds and does not move). When the culture is observed microscopically and is in the exponential growth phase, the cells are very motile, the flagella pulsate and the movement is rapid with rather irregular movements and intervals of "rest" with flickering. Sometimes the cells aggregate in large numbers into clusters resembling grape "bunches" that are irregular in shape and size (Figure 19 A & B). Some consist of a few cells and others of hundreds. In 'clusters' these cells are arranged in some way that seems to serve a purpose. The sharp parts of the cells where the flagella emerge are towards the inside of the bunch, while their opposite side (the swollen one, don't forget that the cell is pear-shaped) "faces" outwards. The flagella in these aggregates, although densely packed, appear to retain some mobility. With careful observation we see that periodically new cells come to be added to each cluster, while at other times some cells detach from it, move away and acquire their usual high mobility. Sometimes again, the removal of many cells in a short period of time dissolves

the aggregate. The significance of this phenomenon for the survival of the species remains unexplained in the literature (it has not even been reported as far as we have studied it), but what has been observed is that it occurs in vigorous cultures. The phenomenon of aggregation (Figure 19 A & B) was mentioned to describe cases of cell unions and more specifically the one observed during the pairing of gametes (isogamy), i.e. the union of two cells with their anterior sharp part for the purpose of fusing their haploid nuclei and the creation of the diploid zygote. This is the other mode of reproduction, amphigenic, where each mated cell plays the role of a "female" or "male" gamete. During mating, in addition to the nuclei, a common larger cytoplasm is created and the zygote that is formed is round, has lost the flagella, is green or red and is surrounded by a smooth and thick cell wall with the dominant component being sporopollenin. The zygote first undergoes a dormant phase (its exact duration is unknown) and then, initially with a meiotic division and then with mitotic ones, it forms up to 32 haploid microscopic cells (Figure 19C) which are released after rupture of the maternal wall and then move freely with their flagella and grow to the normal size of the species.

***Tetraselmis* sp.** (marina var. messolonghi)



Taxonomy

Supra-kingdom: Eukaryota
Kingdom: Protista
Division: Chlorophyta
Class: Chlorophyceae
Order: Volvocales
Family: Chlamydomonadaceae
Genus: *Tetraselmis*
Species: *Tetraselmis* sp. (marina var. messolonghi)

Unicellular planktonic algae with a bright green color (in cells and culture medium-Figure 20). Its cell is elongated, cylindrical in shape but slightly compressed and slightly ellipsoid (Figures 21-24). Anterior part of cell with characteristic indentation from which emerge 4 flagella of equal length distinct in 2 oppositely placed pairs (Figure 22C). The flagella impart intense motility to the cell which follows a straight course with occasional swirling and frequent changes of direction.

Sometimes the cells acquire a spherical shape, shed the flagella and become immobilized. Very often also the cells are enclosed in a membranous, transparent bubble-like structure with watery and sometimes slightly granular contents. In this form they are called palmelloid cells (Figures 23B & 24B) and while they retain their bright green color, they are stationary without flagella and most often filled with distinct spherical inclusions apparently containing storage material (starch or oils). Their metabolism during the palmelloid stage seems to remain active as cell divisions are observed in several of them.

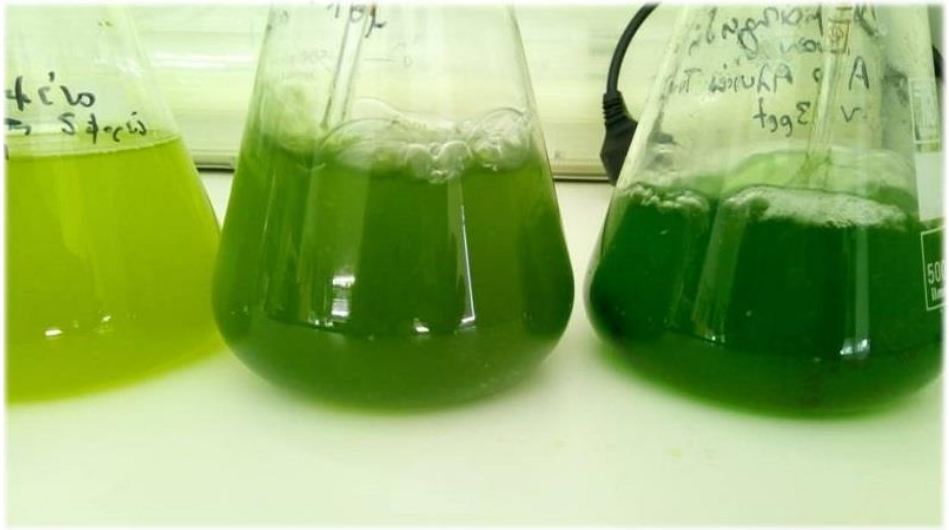


Figure 20. 500 mL Erlenmeyer flasks with *Tetraselmis* sp. (marina var. messolonghi) in various growth phases to show the bright green color of the culture.

Figure 21. Cells of *Tetraselmis* sp. (marina var. messolonghi) from a young culture (A) with a bright green color and from a mature culture (B) with pale green cells and 2 reddish cysts due to the accumulation of carotenoids. 20 µm line, 630X.

In "aged" cultures palmelloid cells partially or completely lose their green color and appear filled with spheroidal colorless inclusions (Figure 23A). In the cell there is a large cup-shaped chloroplast with a pyrenoid in a central position. A large orange “eyespot” is evident in a position toward the upper middle of the cell (along the long axis) and in a lateral position. In the center of the cell is the nucleus adjacent to the “eyespot”.

Reproduction is by simple cell division in the vegetative motile stage as well as in the palmelloid stage. Sexual reproduction consisting of fusion of quasi-gametes has not been observed. However, thick-walled spheroid cysts are often formed, the cell divides into 4 daughter cells (Figure 24C) which are then released and increase in size as motile vegetative cells.

The specific strain which we call *Tetraselmis* sp. (marina var. Messolonghi) is of very large dimensions compared to the known species of the genus *Tetraselmis* (*T. suecica, T. chui, T. tetrathele* etc.) and shows great variety in shape, size, the presence of the pyrenoid, in the shape of the chloroplast and in the various inclusions of the cell, traits that more or less characterize this genus and make the taxonomic arrangement of its species difficult. However, the usual size of *Tetraselmis* sp. (marina var. Messolonghi) ranging at 17.62 ± 1.79 (sd) µm along the longitudinal axis and 10.02 ± 0.62 (sd) µm along the width is much larger (perhaps the largest of all species of the genus) as well as its great resistance to very high salinities of the order of 150 ppt clearly differentiate it from the rest.

Figure 22. Cells of *Tetraselmis* sp. (marina var. messolonghi). A: Large bright green ones. B: in phase palmellae globose. C: the 4 flagella. D: arrows show cell division in the palmelloid stage, cells also transitioning to palmella, smaller mitotically derived cells can also be seen.

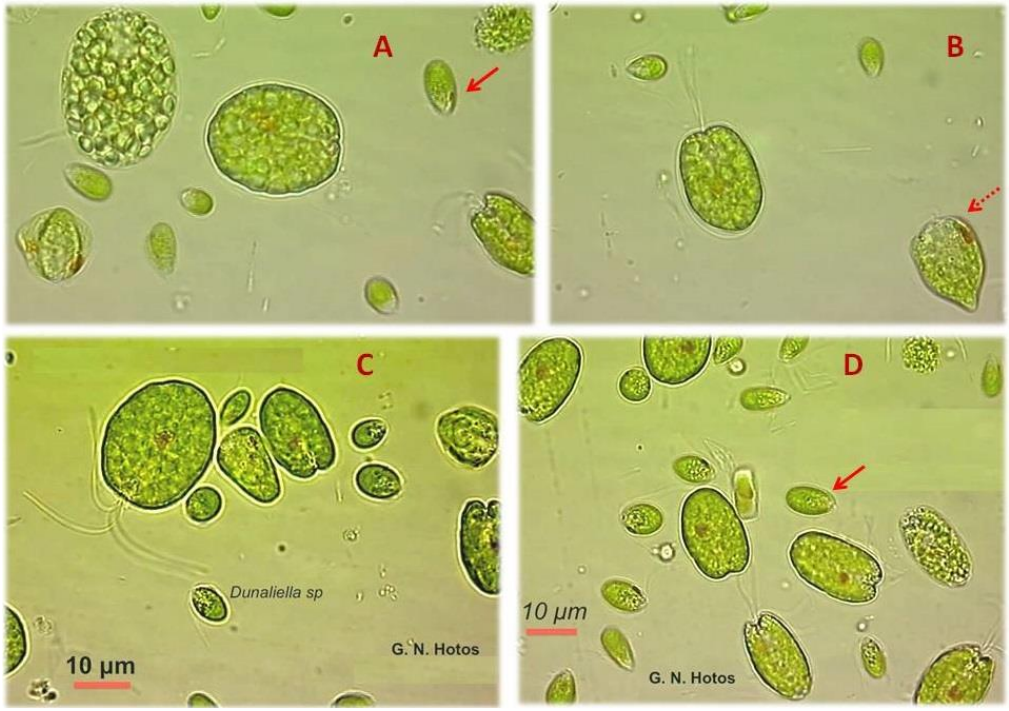
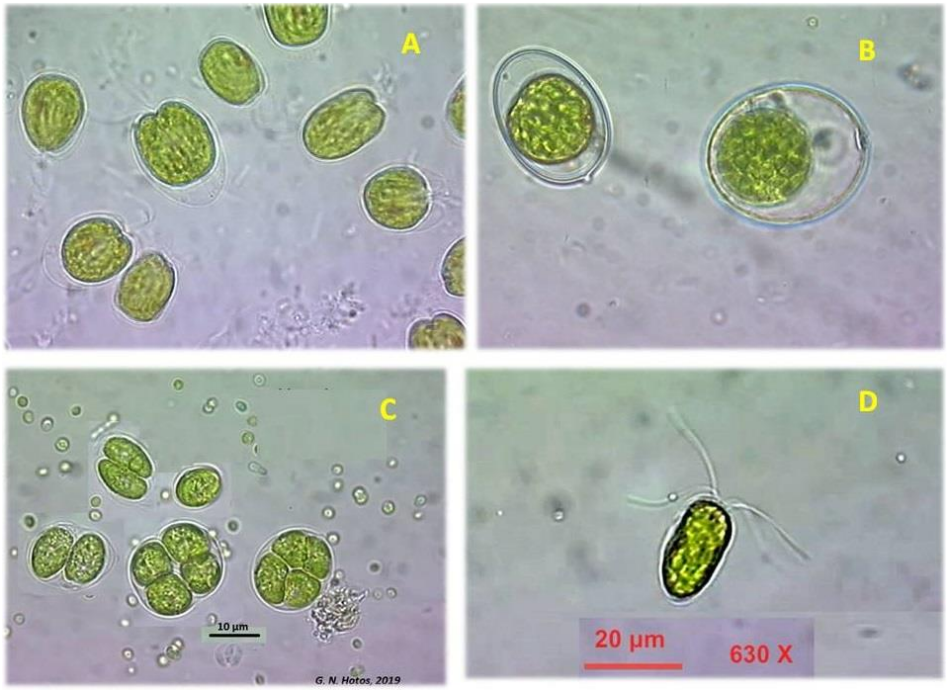


Figure 23. Cells of *Tetraselmis* sp. (marina var. messolonghi). In A & D a *Dunaliella* cell is shown with an arrow for comparison, in B an *Asteromonas* cell is shown with an arrow.



Εικόνα 24. Cells of *Tetraselmis* sp. (marina var. messolonghi). A: vegetative, B: palmelloid, C: mitotic division inside a palmelloid cell. D: vegetative with 4 flagella.

***Tetraselmis* sp.** (red var. pappas)

Taxonomy

Supra-kingdom: Eukaryota
Kingdom: Protista
Division: Chlorophyta
Class: Chlorophyceae
Order: Volvocales
Family: Chlamydomonadaceae
Genus: *Tetraselmis*
Species: *Tetraselmis* sp. (red var. red pappas)

Unicellular planktonic chlorophyte similar in morphology to *Tetraselmis* sp. (marina var. messolonghi) described above but with significantly smaller cell dimensions of 10.52 ± 1.2 (sd) µm along the long axis and 8.11 ± 1.08 (sd) µm along the width for the species isolated from the Kotychi lagoon and 11.32 ± 0.78 (sd) µm along the longitudinal axis and 7.95 ± 1.06 (sd) µm along the width for the species isolated from the Pappas Achaia lagoon. The extremely interesting feature of this microalgae is the dark-red coloration that the water in its culture container acquires when the culture matures. Thus, while in its initial phase (low concentration of cells) the culture is dark-green in color (Figure 25), with time advancing acquires a brown-red color (Figure 27A, Figure 28 A, B, C & F). The reddish color of the culture is naturally due to the corresponding reddish color of its cells (Figure 26, Figures 29-32) which is observed under the microscope as granules of various diameters enclosed throughout the mass of the cell mixed with corresponding green ones.

Apparently, these are various carotenoids which are not found in such intensity in any other known *Tetraselmis* species. In fact, two varieties of this microalgae were isolated, one from the lagoon of Kotychi which shows the usual green coloration of the cells when they are in the initial exponential phase of growth and then when the culture is very mature (static phase) they turn slightly red and then acquire a dark burgundy color and another from the lagoon of Pappas Achaia in which the cells are initially reddish. We call the first variety *Tetraselmis* sp. (red var. kotychi) and the second *Tetraselmis* sp. (red var. pappas).

Both varieties when centrifuging a sample taken from their cultures, exhibit the unique phenomenon of the supernatant in the centrifuge vessel being red and the sediment (cells) being green in *Tetraselmis* red var. kotychi and dark-red in *Tetraselmis* red var. pappas (Figure 27B). We did not find a similar case in the literature. Apparently, the red color of the water in the supernatant is due to unspecified substances produced by the cells and excreted into the water with varying intensity.

Figure 25. Cultures of *Tetraselmis* sp. (red) in 500 ml Erlenmeyer flasks where the green color of the culture can be seen with the bottle on the left having already acquired a brown-red color

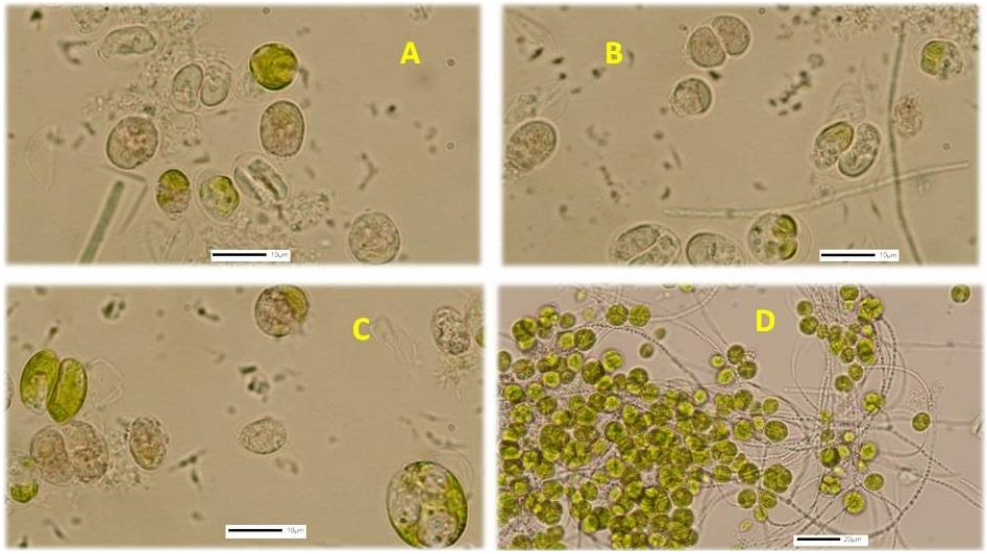


Figure 26. Photomicrographs of *Tetraselmis* sp. (red) from the Pappas lagoon with green and brown-red cells. In A, B & C, scale bar 10 µm, 1000X, in D, 20 µm, 400X.

Figure 27. In A, supernatant red water from a centrifuged quantity with the cuvette next to it that will be photometered. In B, the tubes with the centrifuged sample where the supernatant is reddish and the pellet containing the cells is greenish.

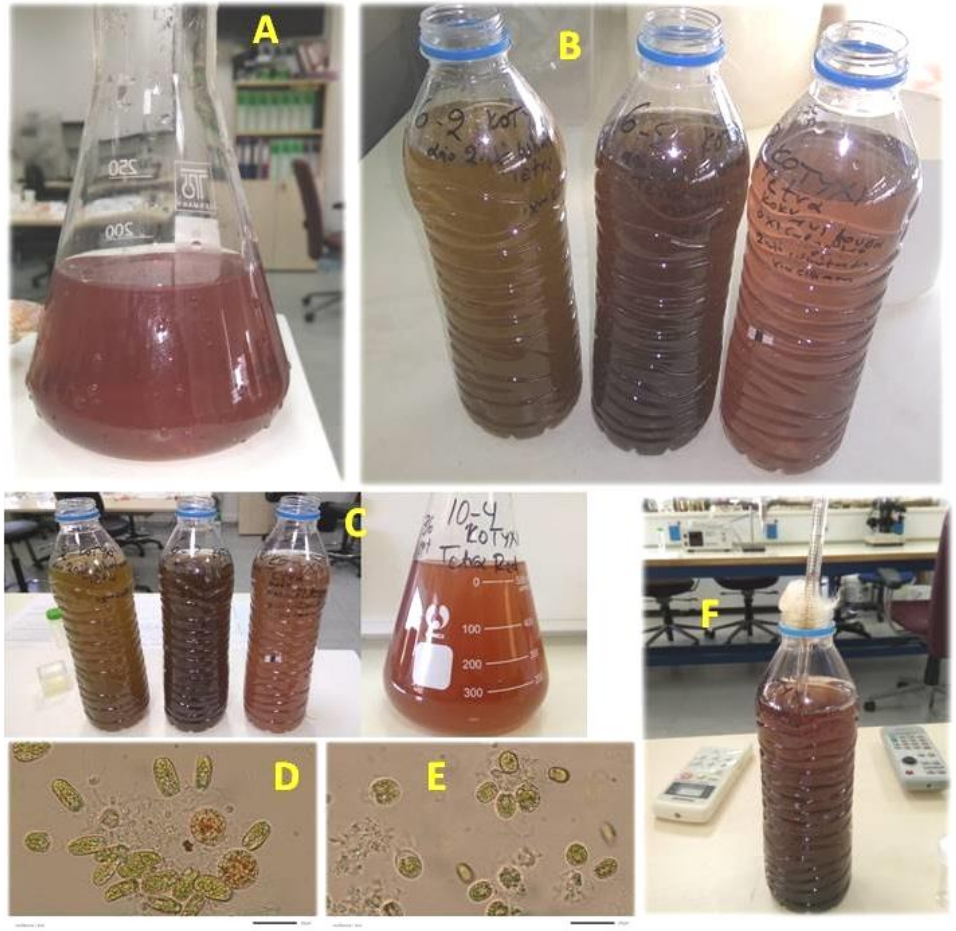


Figure 28. Cultures of *Tetraselmis* sp. (red) that have acquired an intense reddish color. In A, F and B (bottle on the right), the liquid contains no cells but only the supernatant of the centrifuged amount. In C, cultures at different stages of maturation to show the color transition from brown on the left to reddish brown and red (right). In D, *Tetraselmis* (marina var messolonghi) cells for comparison with *Tetraselmis* (red) in E, scale bar 20 µm, 630X.

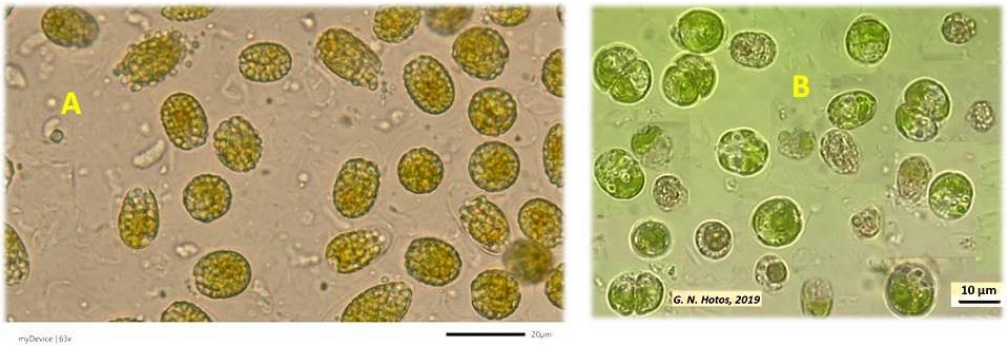


Figure 29. Photomicrographs of *Tetraselmis* sp. (red) from Pappas lagoon (A), scale bar 20 µm, 630X and from Kotychi lagoon (B). In A, all cells slightly reddened in B, green.

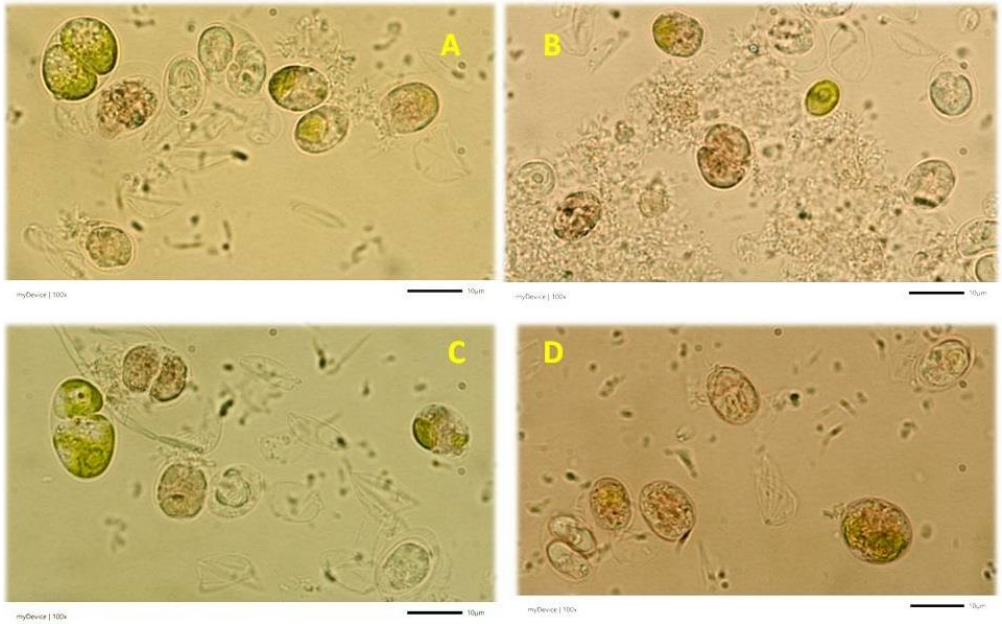


Figure 30. Photomicrographs of *Tetraselmis* sp. (red) from Pappas lagoon, scale bar 10 µm, 1000X. The variety in sizes is obvious. In A and C, green and red cells in division.

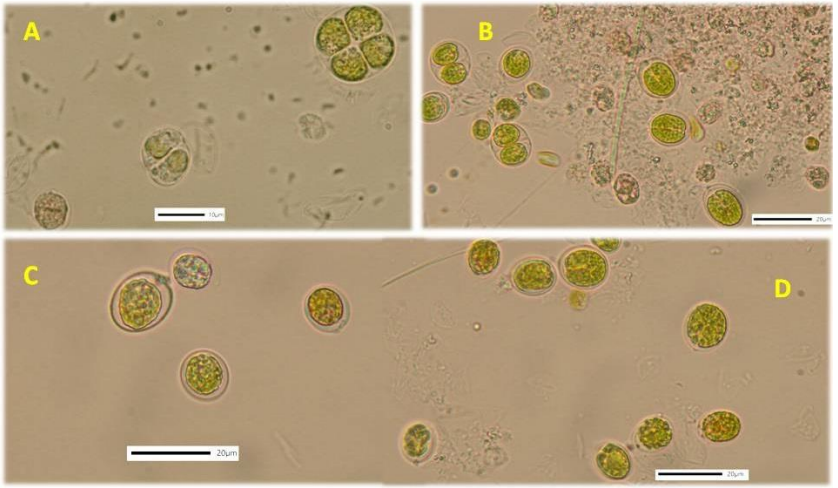


Figure 31. Photomicrographs of *Tetraselmis* sp. (red) from the lagoon of Kotychi. In A, cell division with 4 daughter cells, scale bar 10 µm, 1000X, in B, wide range of sizes and cell division into palmellae, 20 µm, 630X, in C, various palmelloid cell sizes, 20 µm, 630X, in D, various sizes, 20 µm, 630X.

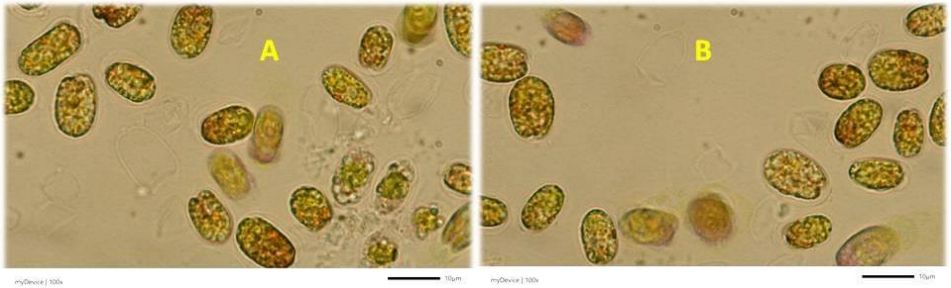
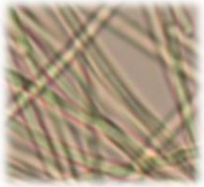


Figure 32. Photomicrographs of *Tetraselmis* sp. (red) from Pappas lagoon with prominently reddened cells, 10 µm, 1000X.

B. CYANOBACTERIA

Phormidium sp.



Taxonomy

Supra-kingdom: Prokaryota
Kingdom: Eubacteria
Division: Cyanobacteria
Class: Cyanophyceae
Order: Oscillatoriales
Family: Oscillatoriaceae
Genus: *Phormidium*
Species: *Phormidium* sp.

Filamentous type cyanobacterium (Figure 33). Filaments (trichomes) unbranched, generally straight, very long, sometimes twisted, bending slightly when in large masses. Sometimes some filaments are wound in a spiral. The filaments have a smooth appearance and have a slow gliding ability. Their color varies according to the stage of culture from light green, to green-olive to olive-yellow (Figures 37, 38C & 42B). The terminal parts of the filaments (tips) not pointed but slightly curved. The mucus sheath covering the filaments is compact, very thin and absent from the tip cells (end cells).

The species is not nitrogen-fixing as it does not possess heterocytes (special cells for binding atmospheric nitrogen -N_2). It does not form akinetes. Filaments consist of cylindrical cells with a length slightly longer than their thickness [2.37 ± 0.2 (sd) µm]. Adjacent cell walls at the cell contact sites do not form obvious divisions in their microscopic observation in the sample isolated from the Tourlida lagoon of Messolonghi, while they are faintly visible in the sample isolated from the Kotychi lagoon.

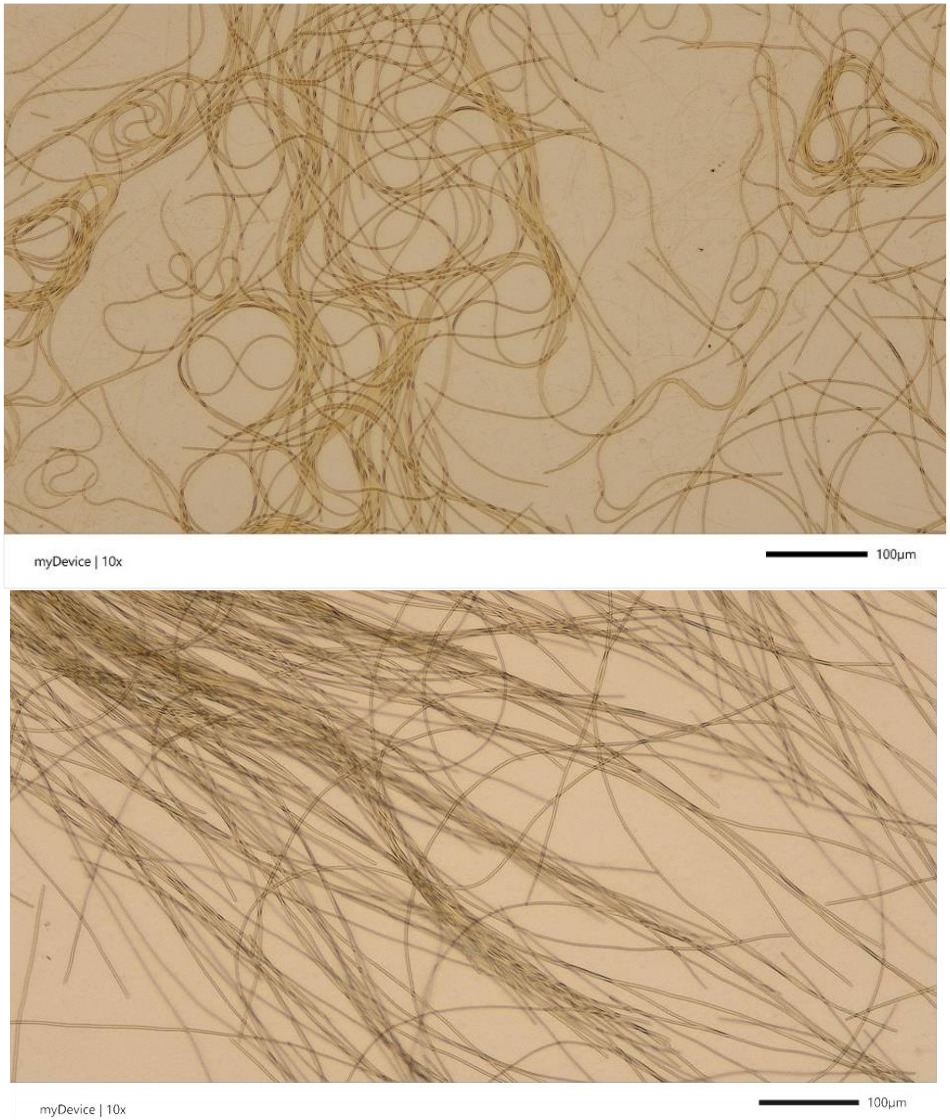


Figure 33. Dense masses of long filaments of the cyanobacterium *Phormidium* sp. isolated by the Tourlida lagoon of Messolonghi. In the filaments there are no heterocytes and no akinetes. Scale bar: 100 µm, magnification 100X.

Aerotopes are not usually observed in the cells, but sometimes a small number of small aerotopes appear in some cells. The contents of the cytoplasm under high magnification appear granular with very small granules of a green-cyan hue.

Propagation takes place by fragmentation of the filaments (Figure 34) into pieces of varying length which are then elongated by cell divisions. The cell divisions (fissions) of the cells take place transversely i.e. perpendicular to the axis of the filament. The cell first grows to the appropriate double size and then divides. Only the terminal cells of the filament do not have such an ability. The points of the thread where the break is to take place develop necrodia, i.e. cells that will dissolve to cause the break and separation of the thread.

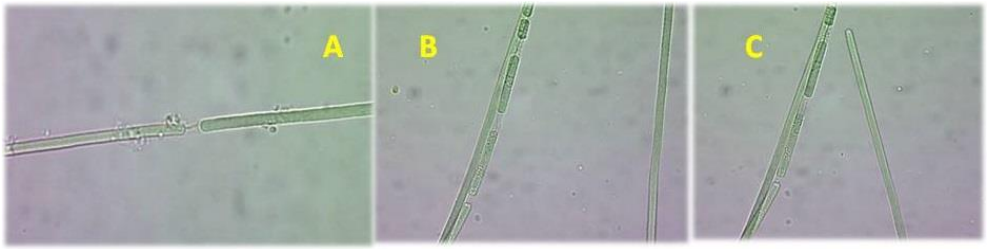


Figure 34. Breakpoints of the cyanobacterium *Phormidium* filament from which new long filaments will arise with cell proliferation.

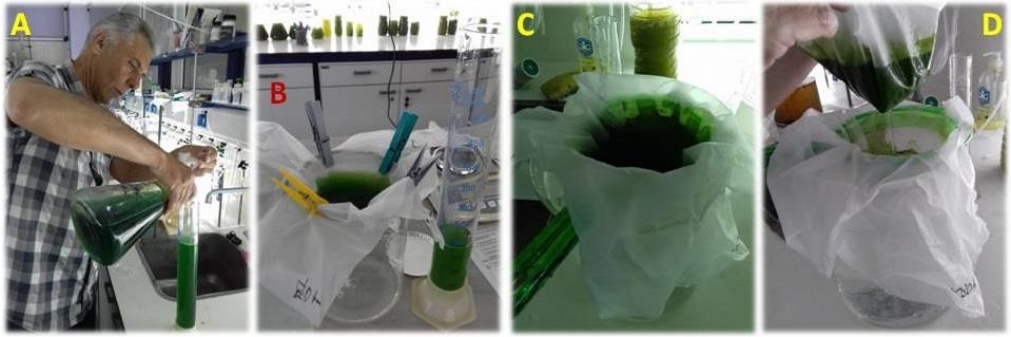


Figure 35. Process of concentrating the *Phormidium* culture (A) by pouring it into a 100 µm planktonic mesh net (B) and draining the culture (C) so that only a dense mass of *Phormidium* filaments remains in the net (D).

Phormidium grows rapidly under normal conditions (20-22 °C, ~4000 lux) and is very halotolerant, tolerating a wide salinity range of 15-60 ppt with best growth around 40 ppt. Very quickly its initial culture which is green in color (Figures 39-41) creates a mass of filaments that are visible to the naked eye as they swirl with aeration in the culture container. After a few days the culture acquires an olive green color and finally olive yellow when the growth reaches a big biomass. Water with *Phormidium* left undisturbed for long enough will show intense and quick sedimentation of the filaments creating a compact sediment with almost clear supernatant (Figures 36, 37 & 38). Sedimentation is completed in about 3 hours and occurs at the same rate regardless of the color phase of the culture.

If the precipitate from the green phase is left for a long time and especially in a freezer (-19 °C) a strong blue color will appear in the supernatant after thawing (Figure 36D & E), due to the release from the cells of the water-soluble pigment phycocyanin which is used by industry as powerful antioxidant. Therefore, *Phormidium* offers many advantages for its mass cultivation as:

1. It grows rapidly and reaches dense concentrations.
2. It is easily collected as it soon forms a dense sediment with undisturbed sedimentation.
3. It produces a lot of phycocyanin.
4. Its culture is greatly facilitated in achieving monospecificity as its filaments are retained by passage of the culture medium through a ~50-100 µm planktonic pore net (Figure 35), thus all other single celled microalgae are washed away.

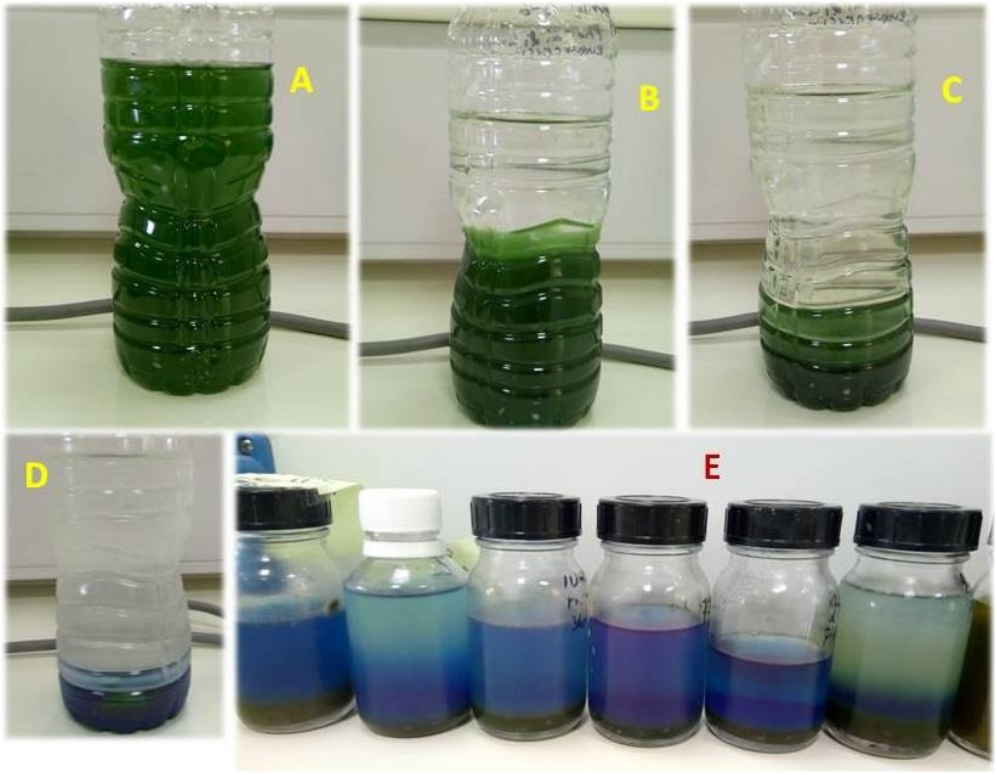


Figure 36. In A, B, C & D, *Phormidium* culture in the green phase and in the time advancing sedimentation of the mass of its filaments forming a thick green precipitate (in about 3 hours). In D, after a 24-hour stay in a freezer (-19 °C) the precipitate released the water-soluble phycocyanin. In E, sediment samples with impressive release of phycocyanin quantities that color the water blue. Only the green phase readily releases phycocyanin when refrigerated.

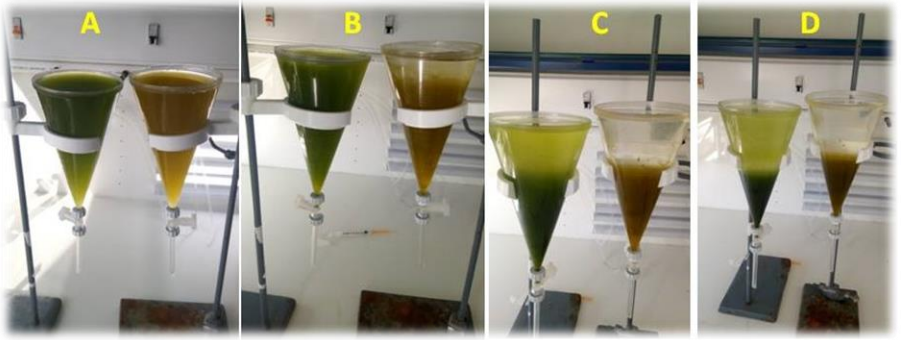


Figure 37. *Phormidium* cultures in the green and olive phases placed in conical flasks to show the developing sedimentation and in D, the sediment finally formed, green for the green phase, olive for the olive.

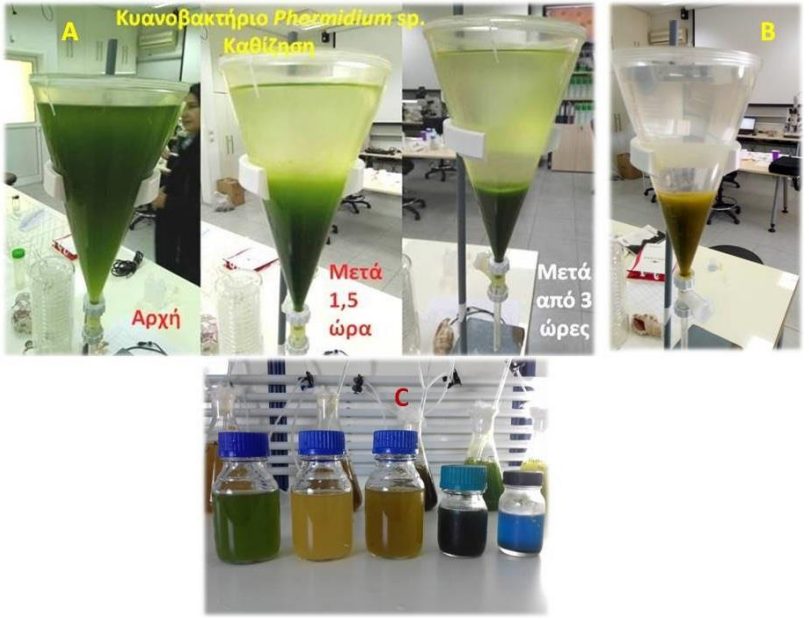


Figure 38. Close-up of a *Phormidium* culture in the green phase placed in a conical separation flask (A) showing the settling of the green sediment in about 3 hours while the overlying water is almost clear (without cyanobacterial filaments) and in B, the corresponding olive sediment by similar precipitation of *Phormidium* culture in the olive phase. In C, samples of *Phormidium* cultures in green, yellow-olive, olive and olive-green phase with the container at the far right having released phycocyanin after cooling for 24 hours.

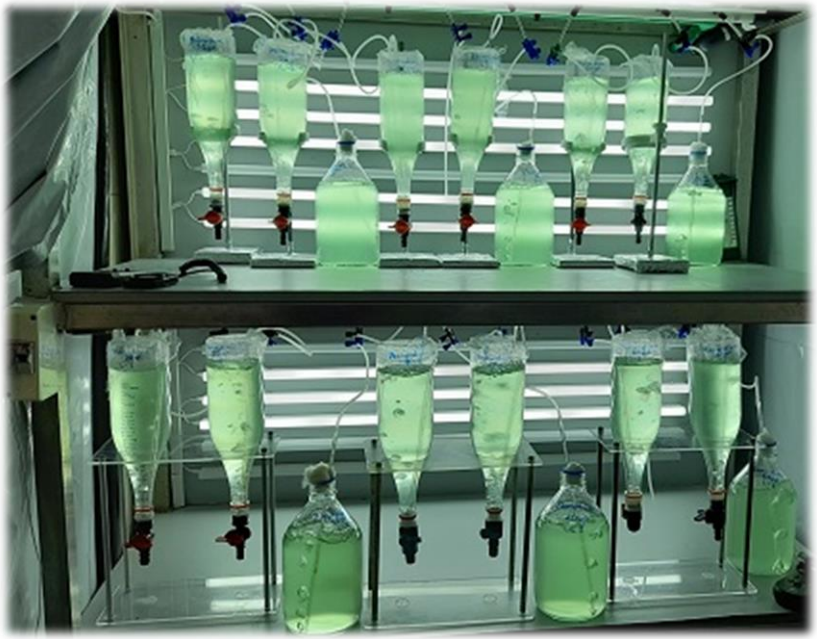


Figure 39. *Phormidium* culture 1st day.

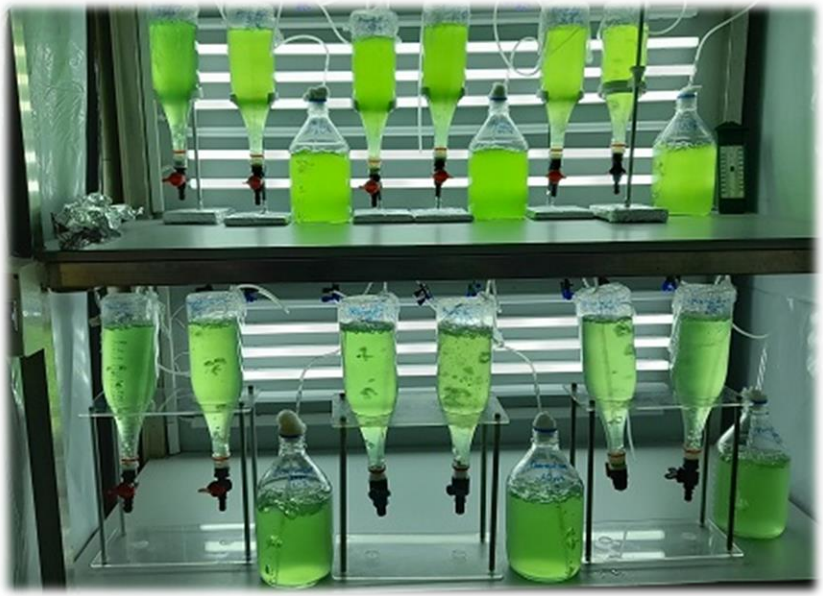


Figure 40. *Phormidium* culture 4th day.

Figure 41. *Phormidium* culture 8th day.

Figure 42. In A, *Phormidium* cultures at different stages of (start-middle-mature). In B, culture samples with dark green color and one olive yellow.

Cyanothece sp.

Taxonomy

Supra-kingdom:	Prokaryota
Kingdom:	Eubacteria
Division:	Cyanobacteria
Class:	Cyanophyceae
Order:	Oscillatorialles
Family:	Cyanothecaceae
Genus:	*Cyanothece*
Species:	*Cyanothece* sp.

Planktonic nitrogen-fixing cyanobacterium with single cells (Figures 43, 44, 45, 47 & 48) and never colonial. Sometimes, however, the cells united to form chains-rows of short length and sometimes curved (Figure 44B) but in no way similar to those characterizing *Anabaena* or *Nostoc*. Neither heterocytes nor akinetes cells are also observed in these apparently occasional chains of cells, even though this species is characterized as nitrogen-fixing. Movement of the cells was not observed and movement is probably not a characteristic of them.

However, once we observed and recorded in a video (https://www.youtube.com/watch?v=gR8yxSM9Yt4) their apparent slow movement, something that leads us not to completely exclude such a possibility of having the ability for movement which, for unknown reasons, they rarely express occasionally.

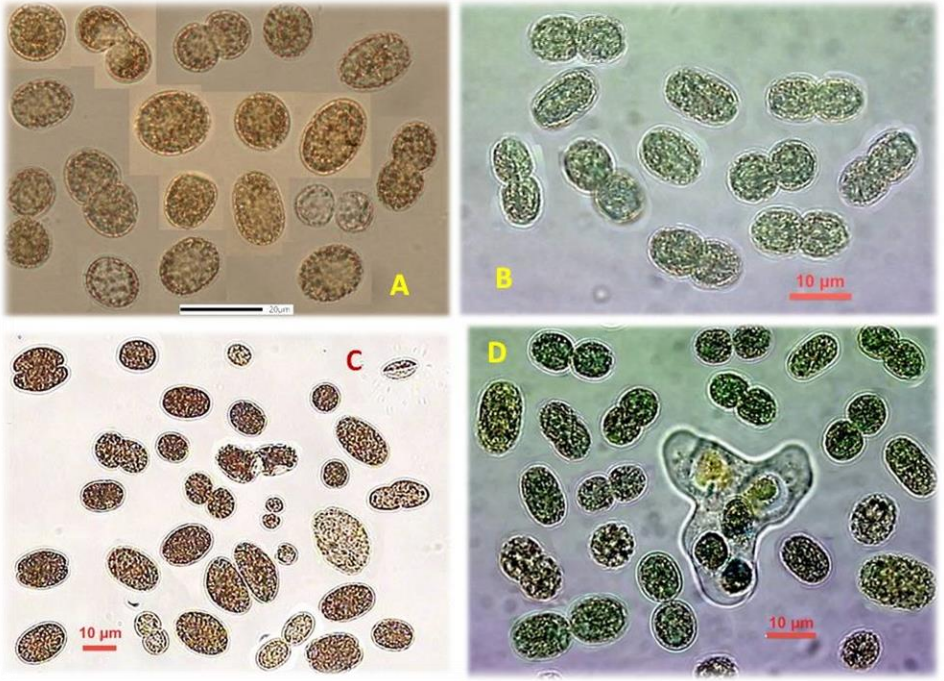


Figure 43. Intense cell diversity of the coccoid cyanobacterium *Cyanothece* sp. isolated from the Messolonghi salt marshes. In A, collage of cells with brown color, symmetrically oval and visible cell wall, scale bar 20 µm, 630X. In C, brown color with a wide variety of sizes and shapes. In B and D, cells blue-green apparently due to a large amount of the pigment phycocyanin. In D, an amoeba has engulfed some cells and digested some of them. In all the photos, the granularity of the cytoplasm, the cell wall and various stages of cell divisions are evident

The shape of the cells is sometimes almost spherical, sometimes oval, sometimes rod-shaped and sometimes slightly curved or slightly sigmoid, but always rounded at the ends. The size varies greatly for each sample we examined and according to the ripening phase of the culture. In general, the cells when single and spherical have a diameter of 8-12 µm and when they are found as subsidiaries of the division united ~15 µm. The cytoplasmic content is homogeneous or with numerous uniformly distributed granular inclusions. The coloration of the cell varies according

to the physicochemical conditions of the water, the age of the culture and the color of the aforementioned inclusions. This is how chromatic adaptation occurs, with light blue-green (cyano-green), bright cyan-green, olive-green, brown-red to light pink cells. At a macroscopic level, i.e. in terms of the color presented by the cultures of this cyanobacterium, we can observe cultures that are sometimes blue-green, sometimes olive-green, and sometimes orange. It is not clear whether this variation in its coloration is due to the known chromatic adaptation that occurs in cyanobacteria or is another manifestation of metabolic process. No obvious aerotopes were observed.

The cell is covered by a distinct layer of cell wall. Internally, the faintly distinguishable thylacoids are arranged concentrically following the contour of the cell. The cells have the ability to produce mucus (Figure 47C & D) which they sometimes (especially in mature cultures) produce and secrete in large quantities resulting in excessive foam production on the surface (Figure 46) and creaminess of the water containing the cells.

It reproduces by cell fission (always 2 daughter cells) in a direction perpendicular to the longer axis of the cell (Figures 43, 44, 45 & 47). Perfectly symmetrical daughter cells grow to the original parent size to divide again. Upon completion of cell division, the resulting cells either separate completely, or remain united for some time in short filament-like chains (pseudofilaments). When the culture conditions worsen (e.g., depletion of nutrients) the cells are slightly deformed (Figure 48) losing their symmetry (asymmetrical) and dividing asymmetrically.

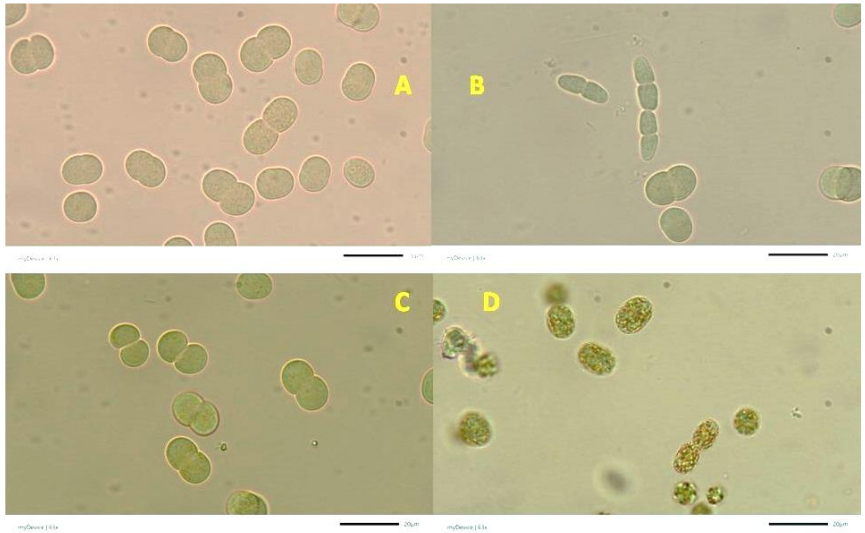


Figure 44. Coccoid cyanobacterium *Cyanothece* sp. with a variety of cell sizes and many cell divisions. In A, B & C, the granular cytoplasm has been replaced by uniform content after centrifugation for an unknown reason. In D, the cells have not been centrifuged and are normally granular. In B, a chain of cylindrical cells. Scale bar 20 µm, 630X.

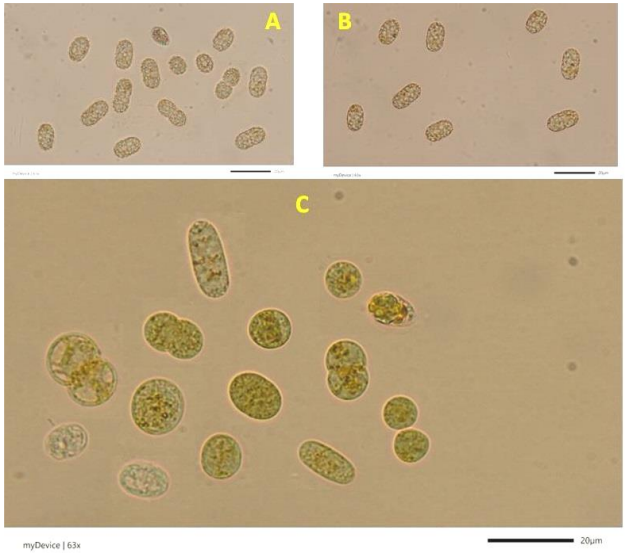
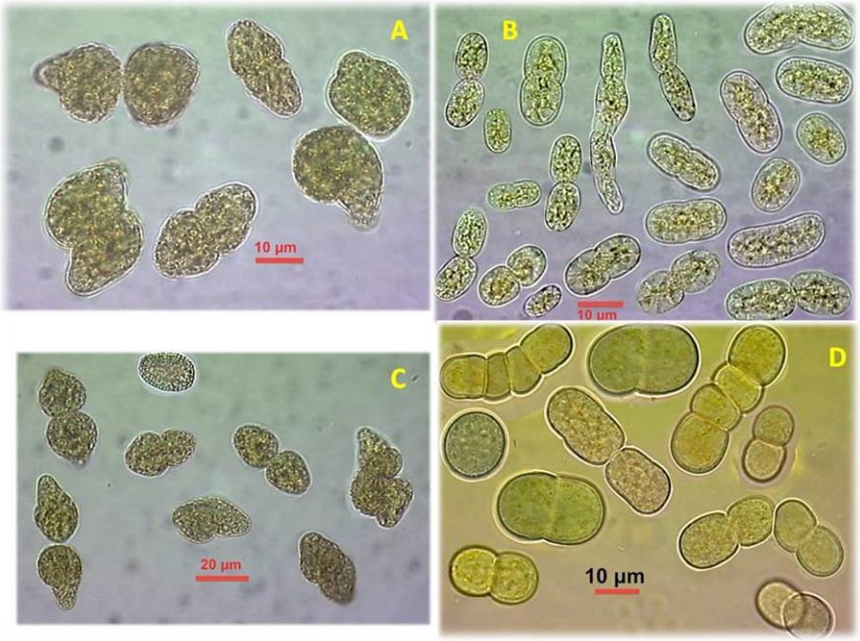


Figure 45. Cells of the coccoid cyanobacterium *Cyanothece* sp. in intense cell division. Before dividing, the cell elongates and from spherical it becomes cylindrical followed by constriction around the middle and then separation of the daughter cells. Daughter cells may remain attached for a long time at the point of constriction.

Figure 46. Intense foam formation in cultures of *Cyanothece* sp.



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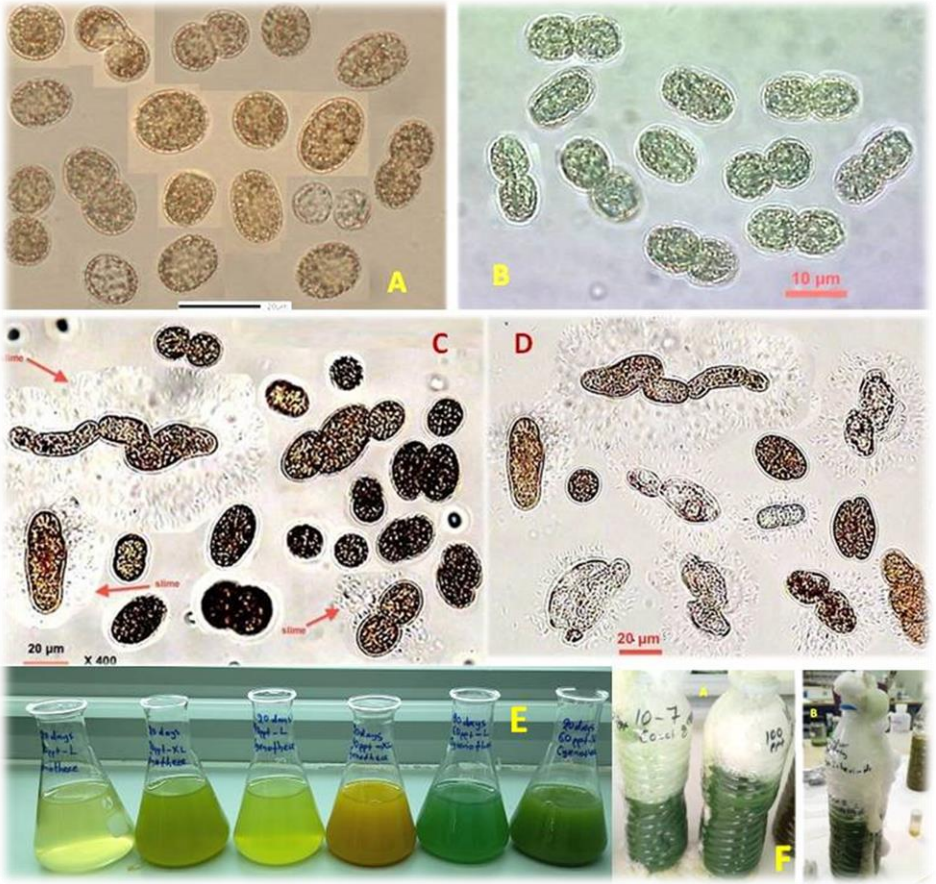
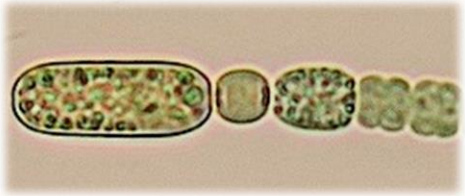


Figure 47. Intense cell diversity of the coccoid cyanobacterium *Cyanothece* sp. with mucus masses covering several of them (arrows in C, all cells in D). Asymmetric cells and chains of cells in B, C & D. In E, samples of intense color adaptation depending on light intensity and stage of culture. In F, intense foam production.

Figure 48. Impressive variety of asymmetric cell forms of the coccoid cyanobacterium *Cyanothece* sp.

Anabaena sp.



Taxonomy

Supra-kingdom: Prokaryota
Kingdom: Eubacteria
Division: Cyanobacteria
Class: Cyanophyceae
Order: Nostocales
Family: Nostocaceae
Genus: *Anabaena*
Species: *Anabaena* sp.

Filamentous cyanobacterium, nitrogen-fixing, with distinct cells in the filaments. Filaments are unbranched mostly straight, varying in length from very short on the order of a few cells (5-10, ~20-30 µm long), to very long (> 250 µm) with hundreds of cells (Figure 49). The long filaments are folded to form large curves (Figure 53B). There are no twists or tangles of threads. The filaments in dense cultures are somewhat arranged in parallel lines. The filaments do not present gliding and only occasionally very slow movement occurs. It shows no obvious sheath-coelom covering the filament, but sometimes there is a thin glassy and colorless covering of mucus. The cells that make up the filament are clearly distinct from each other with obvious tightening of the adjacent cell walls. There are 3 types of cells. The vegetative ones which are the most and make up the "thallus" of the algae, the heterocytes which are the nitrogen-fixing ones found sporadically along the filament and the akinete cells which are obviously the largest cells and are found in various places of the filament. The vegetative cells are uniform in size along the filament and barrel-cylindrical to spheroid in shape slightly longer than wide (~4.2 x 3.8 - 4.7 x 4.5 µm). In other filaments the cells are uniform and in others due to cell division they are distinguished into normal size and into cells which are half the normal size due to division evident from the constriction in the middle. The proliferating daughter cells will grow to the normal size of the typical vegetative cell. Their color varies depending on the physiological condition of the filament from light green, to blue-green or olive green and olive (Figures 49 & 50). However, whatever color they have, it characterizes the entire thread, that is, heterogeneous color areas cannot be distinguished in the same filament. In the cytoplasm of the vegetative cells there are granular aggregates and sometimes aerotopes. The terminal cells of the filaments are conical (Figures 49, 50 & 52C), lighter in color and slightly larger than the others and show no granular content or aerotopes.

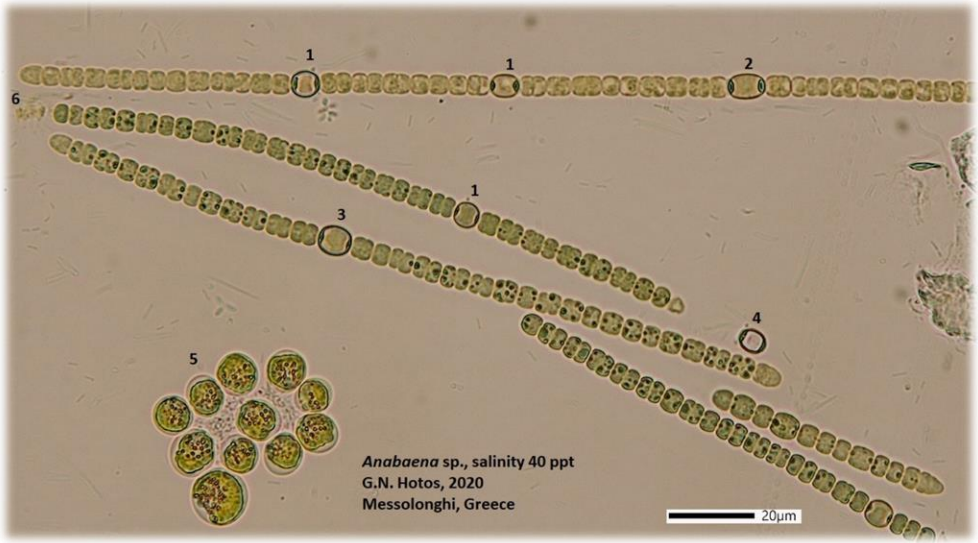


Figure 49. Characteristic filaments of the cyanobacterium *Anabaena* sp. The variety of cell color from olive to olive-green is remarkable. 1= small spherical heterocytes, 2= oval heterocyte, 3= large spherical heterocyte, 4= detached heterocyte, 5= *Tetraselmis* cyst cells, 6= disintegration of extreme cell (not known why). On careful observation along the cells a thin transparent sheath is faintly visible.

Heterocytes are clearly distinct from vegetative cells, spherical to oval in shape and sometimes slightly cylindrical, rarely prominently cylindrical (Figures 49, 50, 53 & 54). However, in any case, they are larger than the vegetative ones (~5.6 µm the spherical ones, ~6.3 x 5.7 µm the cylindrical ones). They are characterized by an increased thickness of that part of the cell wall that is in contact with the neighboring vegetative cells. Heterocytes appear in varying numbers from 1, 3 to 9 in each filament and never two heterocytes border each other, several vegetative cells are always interspersed. Sometimes neighboring cells of the heterocytes are akinetes (Figures 52A & 54) apparently because some adjacent vegetative cell had become akinetes. Sometimes a neighboring akinete can also acquire a neighboring akinete.

The akinetes (Figures 52, 53 & 54) are large cylindrical cells much larger than vegetative or heterocytes (~14.4 x 6.4 or ~19.7 x 7 µm, two typical sizes). They have a thick cell wall and highly granular cytoplasm. They are observed as single cells interspersed in the vegetative row or 2 (rarely 3) together in a row. Several filaments have heterocytes that are adjacent on both sides with akinetes (Figure 52C). Very often the akinetes are released from the filaments (which is their destination after all) and exist as single cells in the water (Figures 52C, 53A&B & 54).

Anabaena filaments elongate by many divisions of their vegetative cells. In addition, the filaments in some places are broken as some vegetative cells turn into necridia and disintegrate. Thus, the filament breaks into fragments and these fragments are elongated by proliferations of their vegetative cells. In laboratory cultures (Figure 51), *Anabaena* showed high sensitivity to the antibiotic erythromycin and less to oxytetracycline. After a few days of introducing the antibiotic into the culture vessel (~100 mg/L), both the filaments and any solitary akinetes began to disintegrate (Figure 53D) and after about 10 days they disappeared. Its sensitivity to urea is also noteworthy as it showed clear signs of decomposition at doses of ~0.5 g/L.

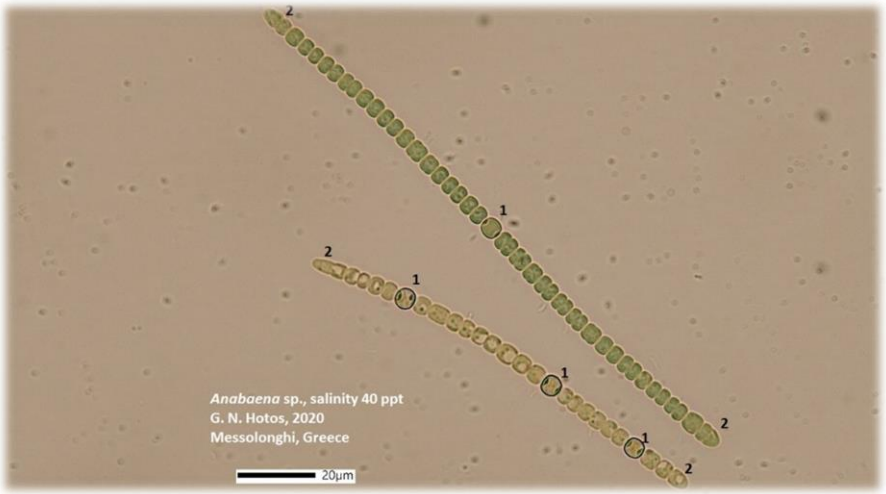


Figure 50. Two relatively short filaments of the cyanobacterium *Anabaena* with a strongly different color. One is yellow and the other is green. 1=spherical heterocytes, 2= cone-shaped extreme cells. There are no akinetes. The distance between heterocytes is remarkable.

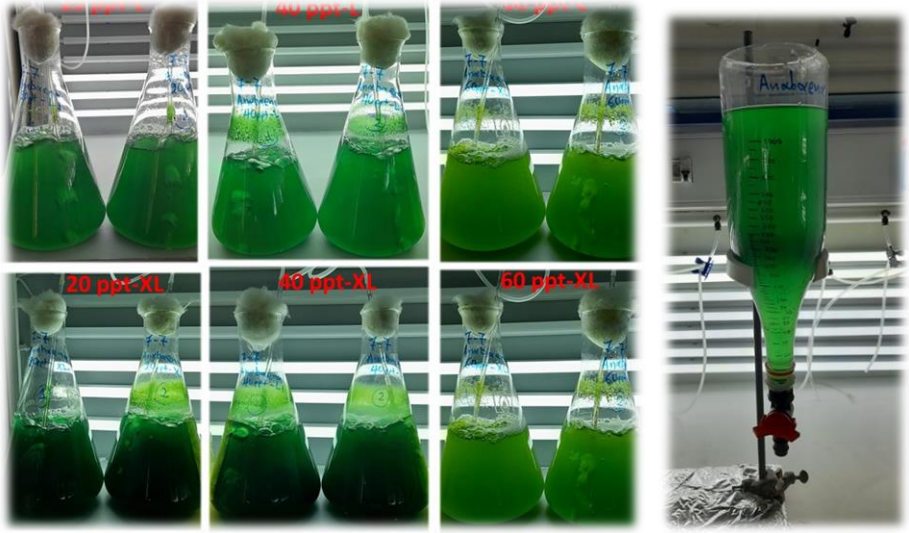


Figure 51. Cultures of *Anabaena* at various phases.

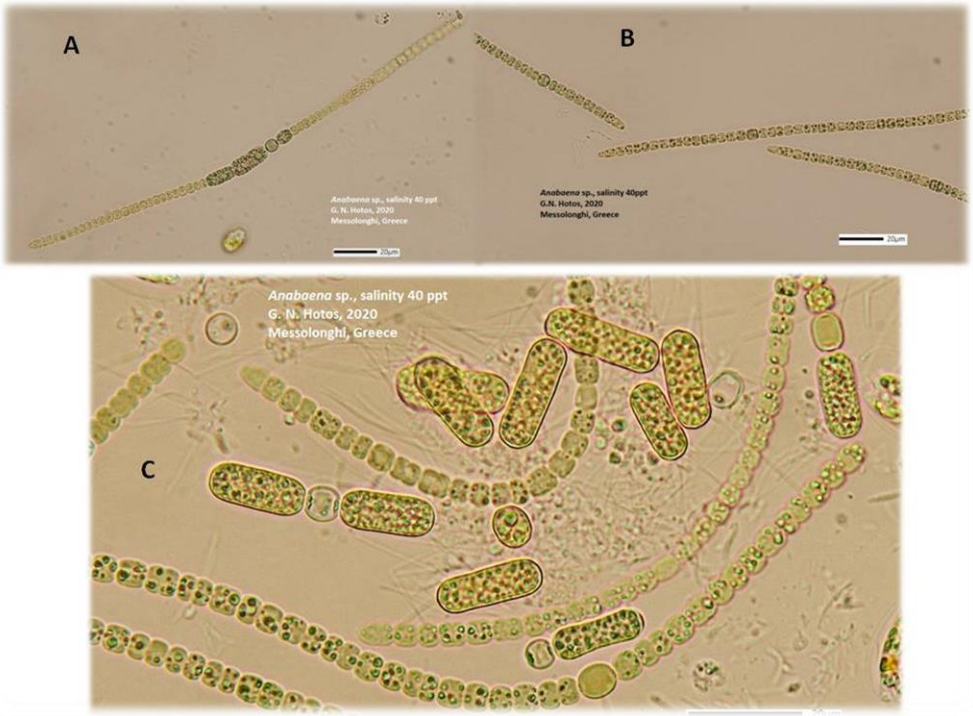


Figure 52. A: A centrally located *Anabaena* filament has a spherical heterocyte adjacent to its left end by two akinete cells (large and small) and to its right by a vegetative cell that transforms to an akinete cell. B: Filaments with conical end cells evident. C: *Anabaena* filaments with granulomatous vegetative cells among liberated strongly granular akinetes some of which are attached to heterocytes as fragments detached from the filament.

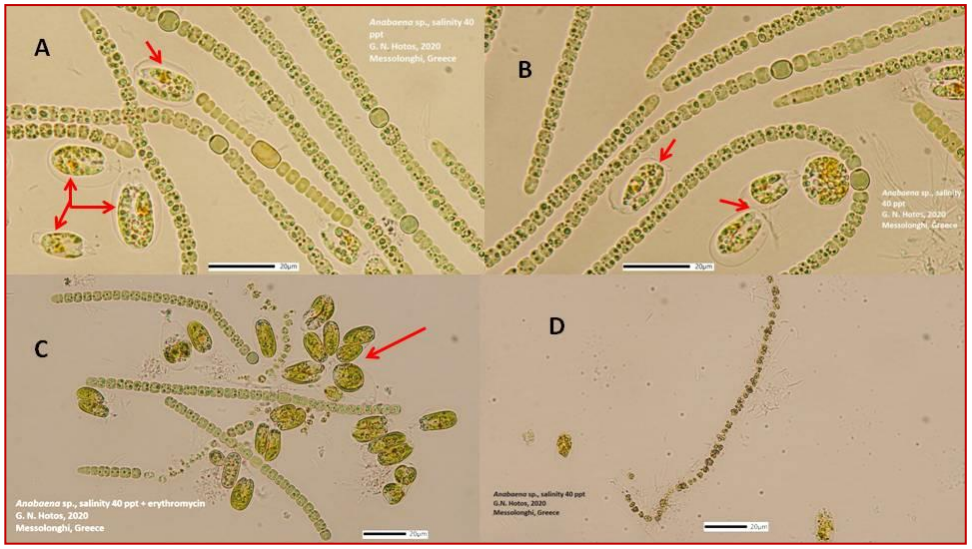


Figure 53. A, B & C: *Anabaena* filaments between cells of the microalga *Tetraselmis* indicated by arrows. D: *Anabaena* filament in an advanced stage of decomposition after a few days' exposure to antibiotics.

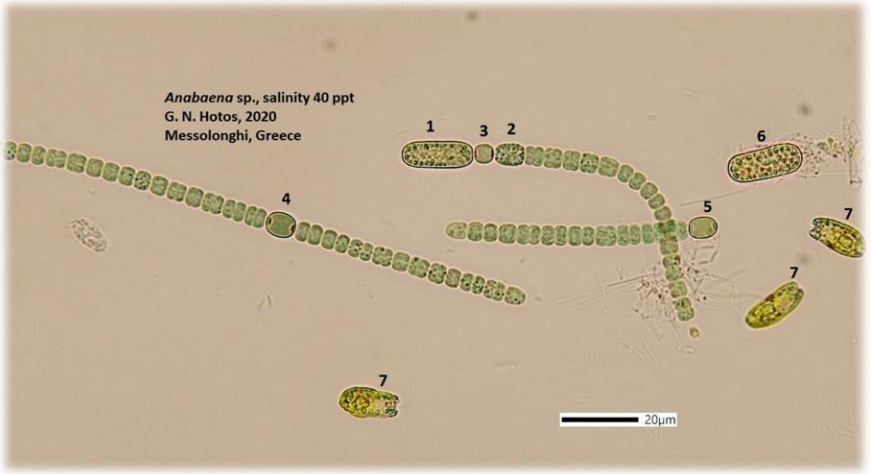
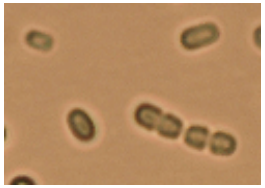


Figure 54. One long and two short filaments of the cyanobacterium *Anabaena*. 1= apical large elongated akinete. 2=vegetative cell converting to an akinete, 3=spherical heterocyte between two akinetes, 4=large spherical heterocyte, 5=apical spherical heterocyte, 6=detached from the filament akinete, 7=*Tetraselmis* cells. The strongly granular cytoplasm of the akinetes is very characteristic.

Synechococcus sp.



Taxonomy

Supra-kingdom:	Prokaryota
Kingdom:	Eubacteria
Division:	Cyanobacteria
Class:	Cyanophyceae
Order:	Synechococcales
Family:	Synechococcaceae
Genus:	*Synechococcus*
Species:	*Synechococcus* sp.

Coccoid planktonic non-nitrogen-fixing cyanobacterium with cells spherical when solitary or slightly cylindrical when joined in short chains (3-15 cells). Cell size varies from 1.9 µm to 2.8 µm. The population of this cyanobacterium shows the characteristic of being mostly composed of cell chains with many cells at the initial densities of the cultures and over time short chains (2-4 cells) predominate first and finally in the very mature cultures almost only single spherical cells (Figures 55 & 56). Obviously, this phenomenon is due to the intensity of the multiplication rate, which is more intense at the beginning of the culture. This strain of *Synechococcus* that we isolated, according to our first experiments, exhibits a very rapid growth rate and grows equally well in a wide range of salinities of 10-40 ppt. It is also characterized by the strong chromatic adaptation depending on the light intensity, yellowish at more than 20,000 lux, green at 2000 – 8000 lux (Figures 57, 58 & 59).

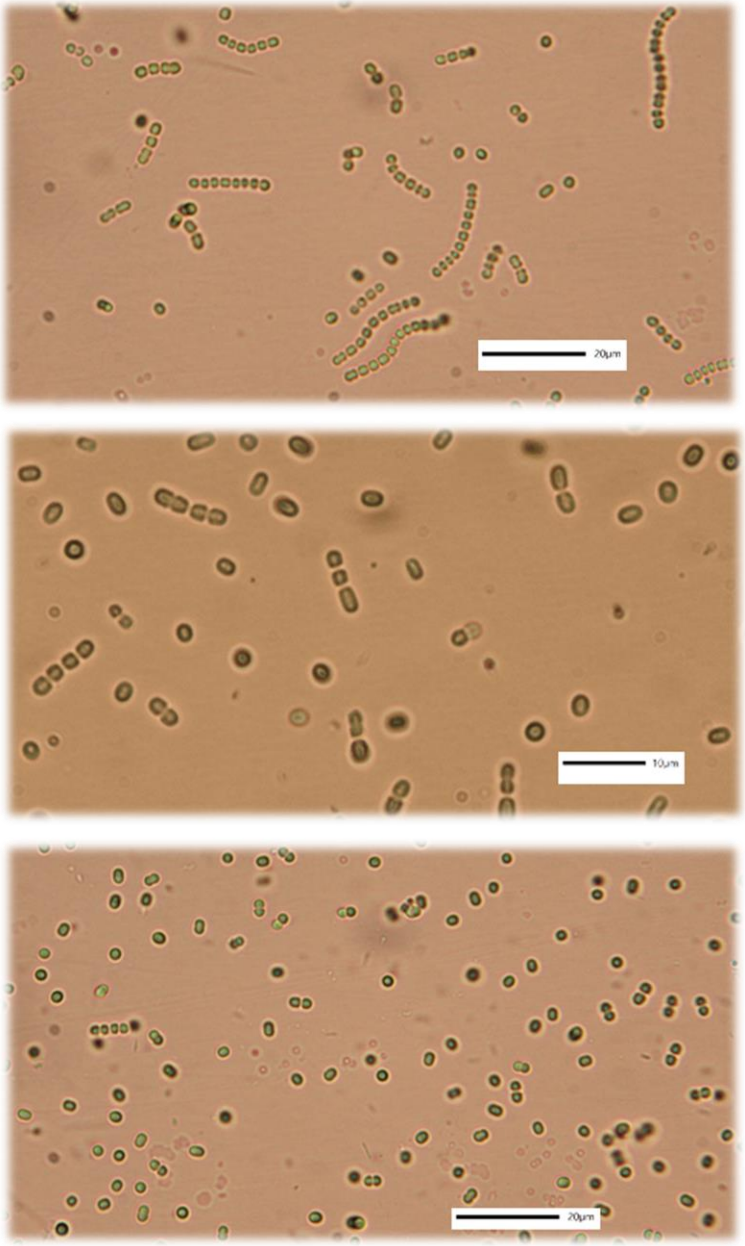


Figure 55. Photomicrographs of cells of *Synechococcus* sp. from initial culture (top), advanced (middle), and mature (bottom).

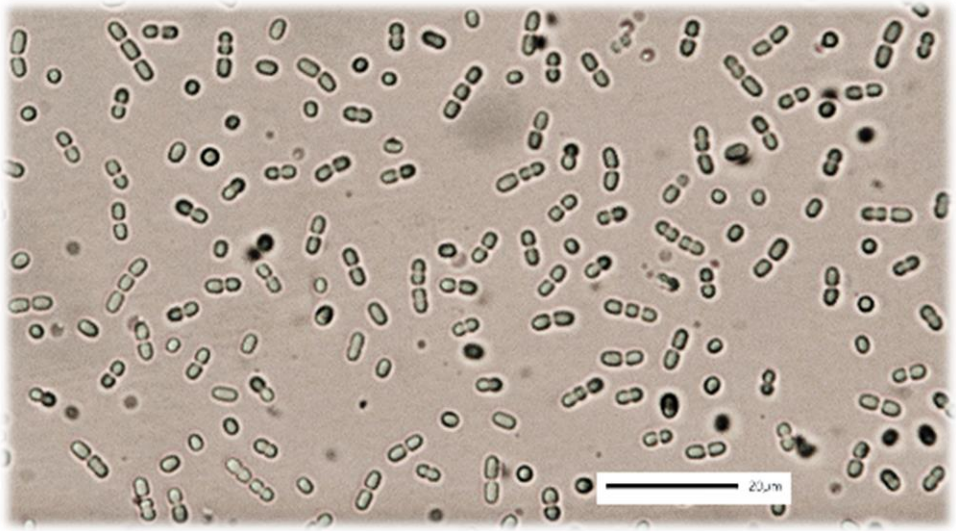


Figure 56. Photomicrograph of cells of *Synechococcus* sp. from initial culture with the presence of chains and single cells.

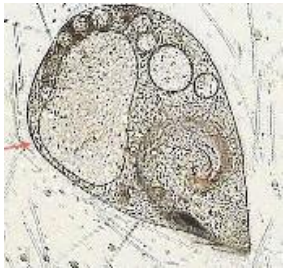
Figure 57. Characteristic bright green culture color of *Synechococcus* sp. in the middle of the growing period (4th – 5th day), lighting 6000 lux.

Figure 58. Characteristic chromatic adaptation of *Synechococcus* sp. in the mature phase of the culture period (8th – 10th day), illumination 22,000 lux in the back yellow bottle, light green in the middle (8500 lux), dark green in the front (4000 lux).

Figure 59. Characteristic chromatic adaptation of *Synechococcus* sp. in the samples from various cultures of different light intensities.

C. PROTOZOA

Fabrea salina



Taxonomy

Kingdom: Protista
Phylum: Ciliophora
Sub-phylum: Postciliodesmatophora
Class: Heterotrichea
Order: Heterotrichida
Family: Climacostomidae
Genus: *Fabrea*
Species: *Fabrea salina* Henneguy 1890

The cell of *Fabrea salina* is quite large showing a wide range of sizes (150-350 μm along the long axis) and generally larger than other ciliated protozoa. The shape of the cell is varied (Figure 60) as it consists of a massive globular somewhat main "body" and a prominent snout-like structure which contributes much to the polymorphism at various lengths. The snout makes up 25 to 35% of the cell length. The muzzle gradually tapers and resembles a proboscis. The coloration of the cell is brownish but can become very dark sometimes approaching black especially when the protozoan has fed to saturation, a condition which appears as cytoplasm filled with vacuoles full of food particles (Figures 70-73). Sometimes if the vacuoles are filled with green microalgae the appearance of the cell becomes greenish. Sometimes also the appearance (apparently due to feeding) becomes yellow-grey. If the cell has been deprived of food for a long time the appearance of the protoplasm is semi-transparent and then various granules of varying size can be distinguished which, under high magnification of the microscope, exhibit a "shuffling" movement within the cell.

The macronucleus with careful observation is distinguished as a zonal region twisted in an "S" shape (Figure 63). But often the macronucleus is difficult to be distinguished as the cytoplasm is full of vacuoles. The micronuclei are numerous and scattered in the cytoplasm and are very difficult to be distinguished. Along the cell there are inconspicuous or obscured about 150 congested lines of microkinetids. A large funnel-like mouth-like cavity is formed at the base of the proboscis "outgrowth" leading into the cell via the cytopharynx. The funnel-like area has long cilia, the membranelles (about 200) around its circumference, which pulsate continuously creating a vortex that drags microparticles of food into the funnel (Images 61, 62 & 63). All over the surface of the cell there are cilia that pulsate. The movement of the cilia gives the cell motility which is usually linear smooth, rapid or occasionally a little fast but without jerks or lightning shifts. Sometimes the cell swirls loosely while moving and occasionally stops and gives the impression of floating in the water. According to studies, cilia are also used to crawl along surfaces, as well as for attachment and sensation. Therefore, in addition to helping the organism move from one area to another, they allow *F. salina* to sense any changes in its environment and therefore be able to respond effectively. Compared to flagella present in other unicellular organisms, cilia are more numerous and small and may cover the entire surface of the cell. Through their coordinated movement it is able to move faster.

Figure 60. *F. salina* cells characterized by great flexibility in form.

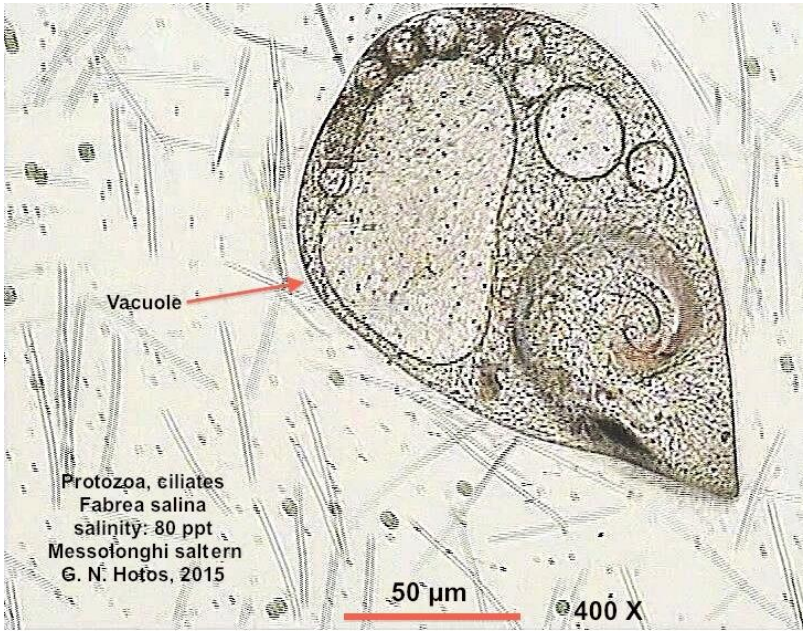


Figure 61. Typical cell of *F. salina* with one large and several small vacuoles.

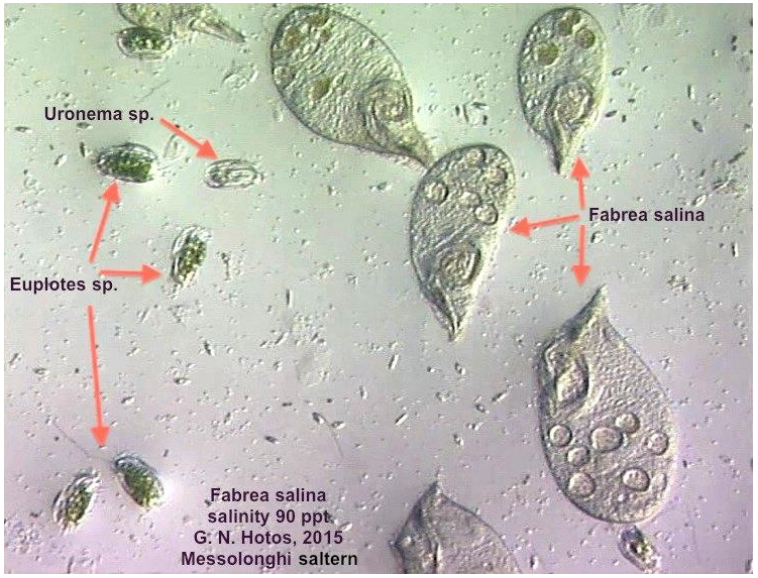


Figure 62. Cells of *F. salina* compared to other smaller ciliated protozoa (*Euplotes, Uronema*).

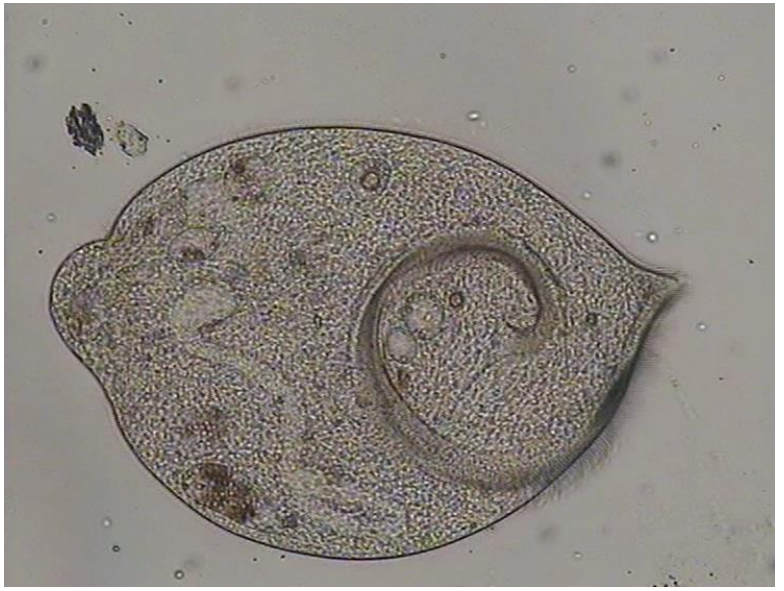


Figure 63. The sigmoid-shaped macronucleus of *F. salina.*

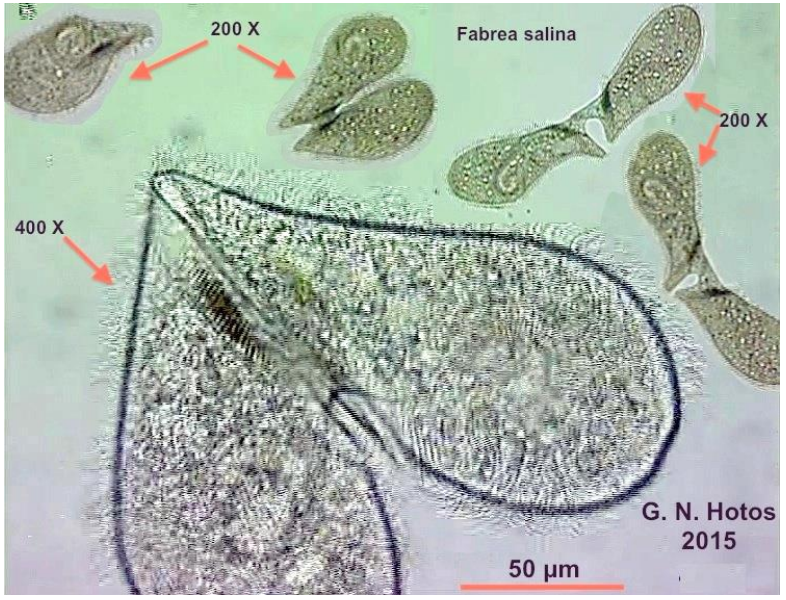


Figure 64. Collage of various phases during the conjugation of *F. salina*.

F. salina reproduces asexually by simple cell division (Figure 65) and by the peculiar mode of conjugation of two individuals exchanging genetic material (Figure 64). During mating, two *F. salina* individuals come into contact with each other forming a cytoplasmic bridge. This is followed by a process known as cell micronucleus meiosis to produce haploid micronuclei. Some of the haploid nuclei undergo dissolution while the rest divide into two by mitosis in both cells. One of the two nuclei then moves to the other cell through the cytoplasmic bridge where it contacts the other cell's micronuclei to form a diploid nucleus that eventually forms a macronucleus once the cells divide. This is followed by the division of the protoplasm (while the macronucleus divides in two) to form two daughter cells. Each of the daughter cells will have a macronucleus and a micronucleus. During the contact phase of reproduction, the micronucleus of the cell undergoes mitosis (two diploid micronuclei) while the macronucleus divides into two. The cell then divides in two with one of each macronucleus and micronucleus in each of the new cells. Sometimes (although rarely for reasons that are not understood) and although not mentioned in the literature, we observed reproduction by budding (Figure 66).

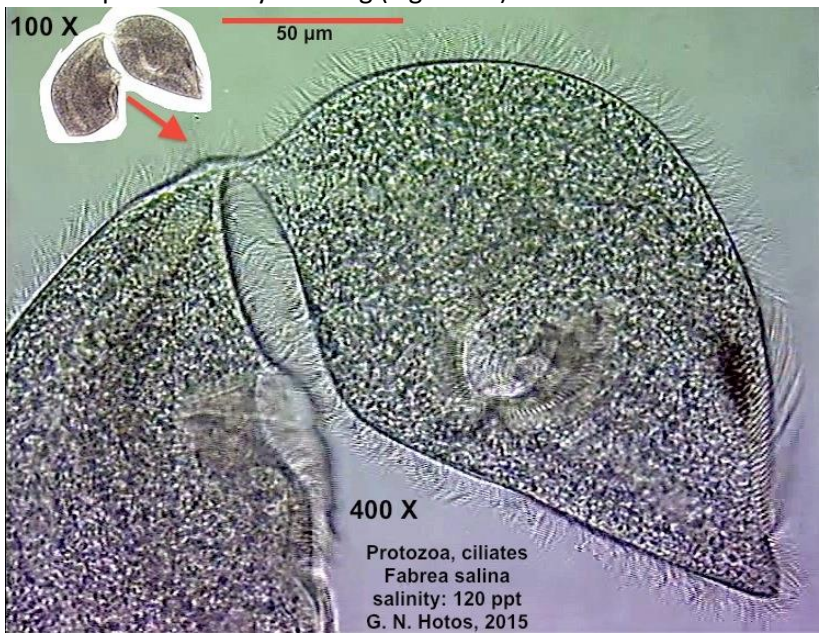


Figure 65. Final stage of separation of daughter cells during asexual reproduction of *F. salina.*

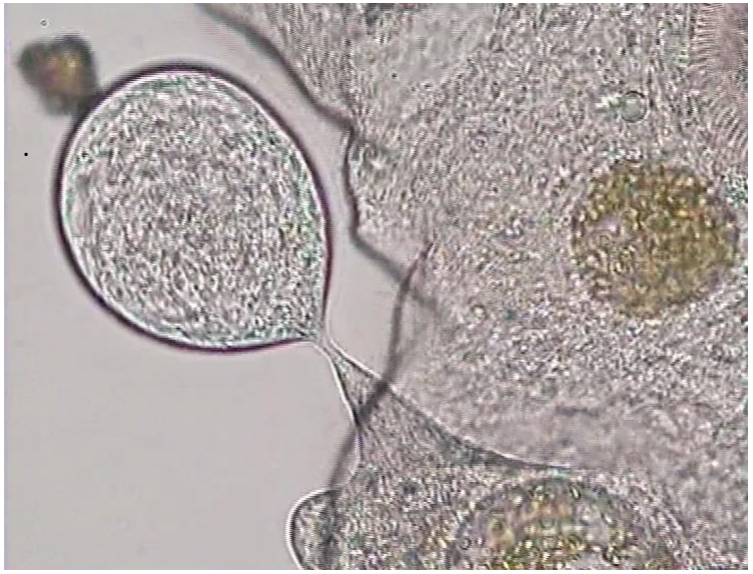


Figure 66. Detail of budding in a cell of *F. salina.*

Fabrea salina is an exclusively marine protozoan, solitary, pelagic-planktonic, does not form colonies and thrives in high salinity basins. Its salinity tolerance is remarkable apparently due to a special osmoregulating ability and although its upper limit of tolerance has not been sufficiently studied, there are findings both in nature and in the laboratory which show that it can withstand up to 200 ppt. On the contrary, its resistance to low salinity water is not as great and although the minimum salinity limit has not been sufficiently studied either, it seems to be somewhere between 25 and 30 ppt. Other remarkable properties of it are (according to observations in our laboratory) the gradual reduction in the size of the cells in old cultures and the final predominance exclusively of very small-sized individuals (shrinkage) with a characteristic shape (Figure 67). If the cultures are left any longer, eventually the individuals dissolve or become encysted (Figure 69). But encystment can also be observed in large individuals for reasons that are not completely understood. It may be caused (the encystment) by a drop in temperature or salinity, but surely an unknown combination of factors causes it. From these cysts (Figure 68) which resist dehydration for a long time, healthy normal cells can then arise.

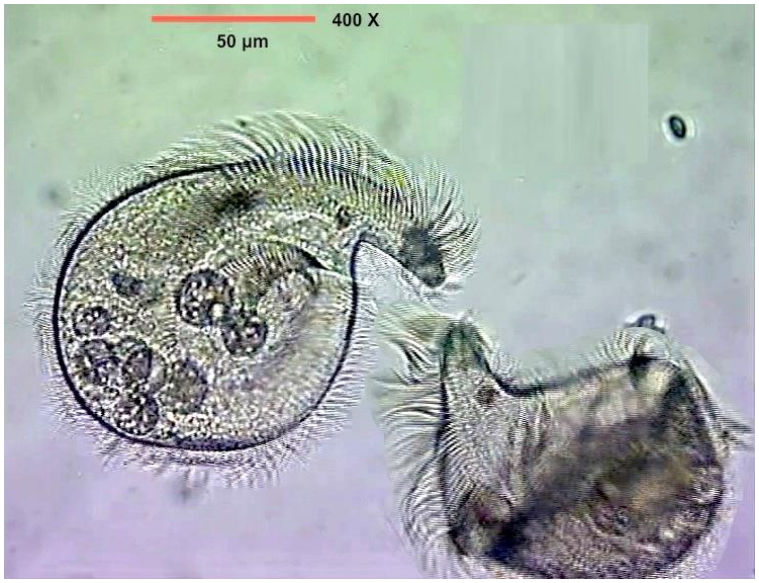


Figure 67. Characteristic cells of "dwarfed" *F. salina*.

Figure 68. Cyst of *F. salina*.

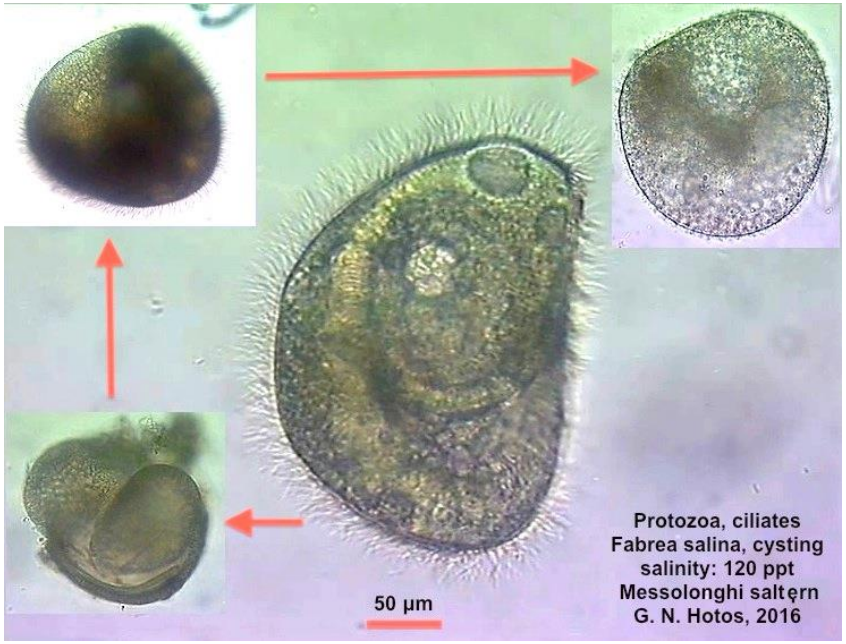


Figure 69. Collage of phases of encystment of *F. salina*.

In hypersaline saltworks basins, *Fabrea* is sometimes the dominant protozoan apparently because of its superiority in resistance to hypersalinity over other species. It feeds by filtering the water and retains large quantities of the dominant microalga *Dunaliella salina* which also grows massively in hypersalinity. But it has been shown experimentally that *Fabrea* can also feed on a variety of other planktonic microalgae such as the halotolerant chlorophyte *Asteromonas gracilis* as well as a variety of cyanobacteria or other bacteria. In well-nourished individuals there are many vacuoles filled with the ingested algae and while in some vacuoles digestion appears to have progressed, in others the microalgal cells appear intact (Figure 71). It may be a form of symbiosis in which *Fabrea* benefits from the excess photosynthetic production of the microalgae while the alga is unknown as to what kind of benefit derives. Sometimes these vacuoles empty their content into the water and then it is observed that the freed microalgae regain their mobility. The significance of this phenomenon remains unknown. However, *Fabrea* has not been observed consuming other smaller protozoa.

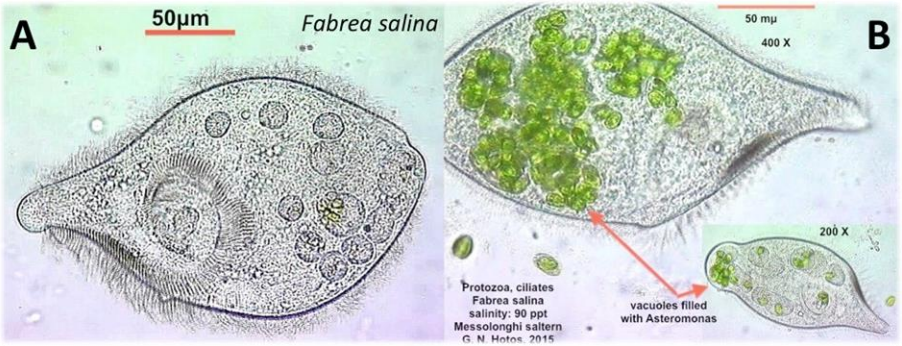


Figure 70. A: Cell of *F. salina* non-fed. B: Vacuoles full of chlorophyte cells.

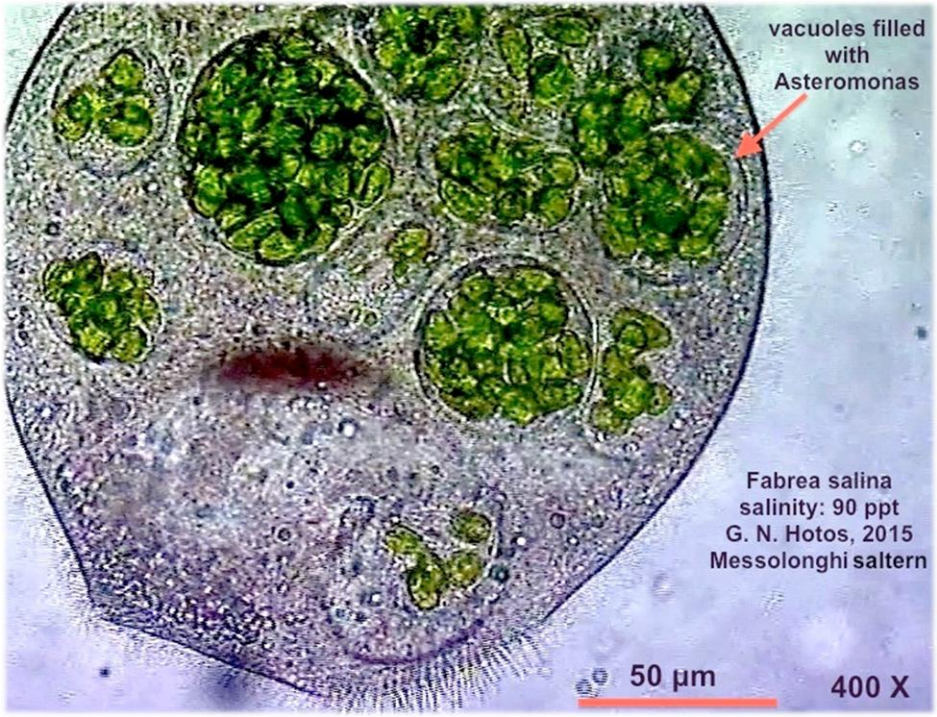


Figure 71. *F. salina* cell with vacuoles filled with *Asteromonas gracilis* cells. They may not undergo digestion but coexist beneficially for the protozoan.

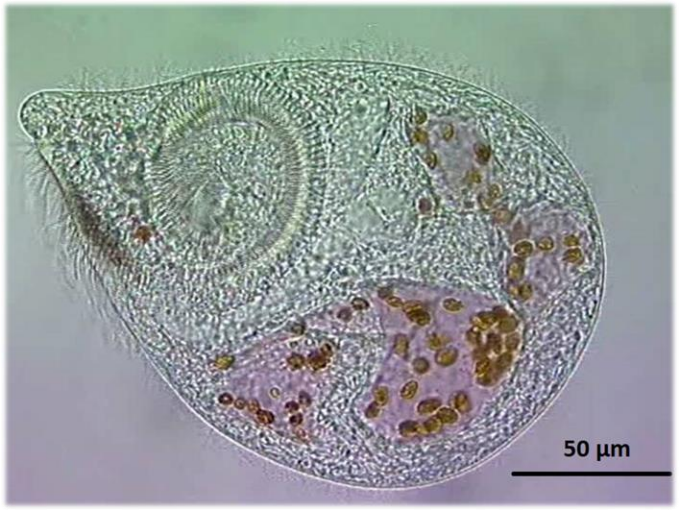
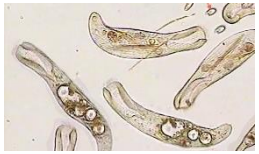


Figure 72. *F. salina* cell fed with the cryptophyte *Rhodomonas salina* whose cells are digested staining the vacuoles pink with their contents.

Figure 73. *F. salina* fed with cells of the chlorophyte *Dunaliella salina* with their characteristic red color (due to carotenoid pigments) in the high salinity basins of Messolonghi saltworks.

Condylostoma sp.



Taxonomy

Kingdom:	Protista
Phylum:	Ciliophora
Sub-phylum:	Postciliodesmatophora
Class:	Heterotrichea
Order:	Heterotrichida
Family:	Condylostomatidae
Genus	*Condylostoma*
Species:	*Condylostoma* sp.

The isolated species *Condylostoma* sp. proved to be very hardy in laboratory conditions, reproducing rapidly, feeding on almost any species of microalgae, reaching high densities. Contrary to the usual appearance of the cells of the species of this genus (Figure 74), this specific strain that we are cultivating is dark-red to black in color so that nothing can be distinguished from its internal structure (Figures 75-77). Its size ranges from 260-330 µm along the longitudinal axis and 66-72 µm along the transverse axis. The surface of the cell covers about 13,000 μm^2. It is a benthic species, but often also floats in the water column. Its movement is very slow with torsional contractions in an eel-like manner. The cell structure is very delicate and almost most cells are dissolved when collected with a planktonic net. It reproduces by simple cell division (Figures 76 & 77). It accepts as food almost all microalgae that we grow in the laboratory. Tolerates a wide range of salinities but suffers at less than 15 ppt. When its culture reaches a high density (Figure 78) and remains for a long time, the water acquires a brownish-red hue and massive cell death follows. This can be an extremely useful protozoan in bioassays.

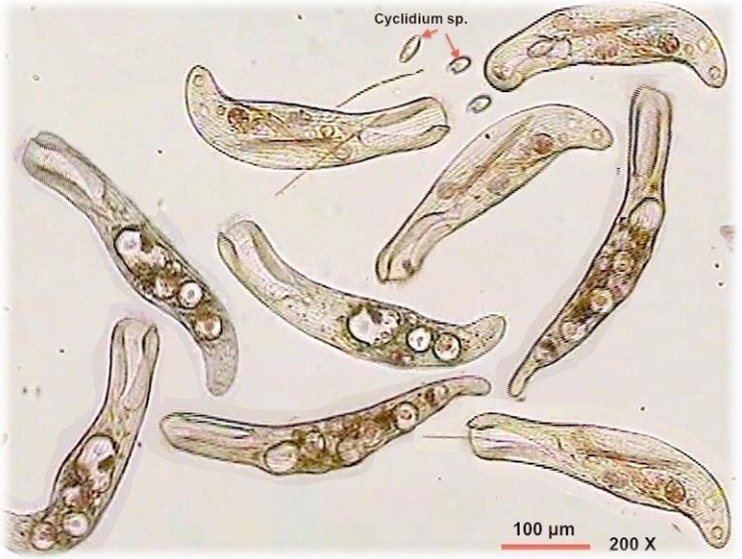


Figure 74. Typical appearance of *Condylostoma* cells from our other samples but not kept long in culture.

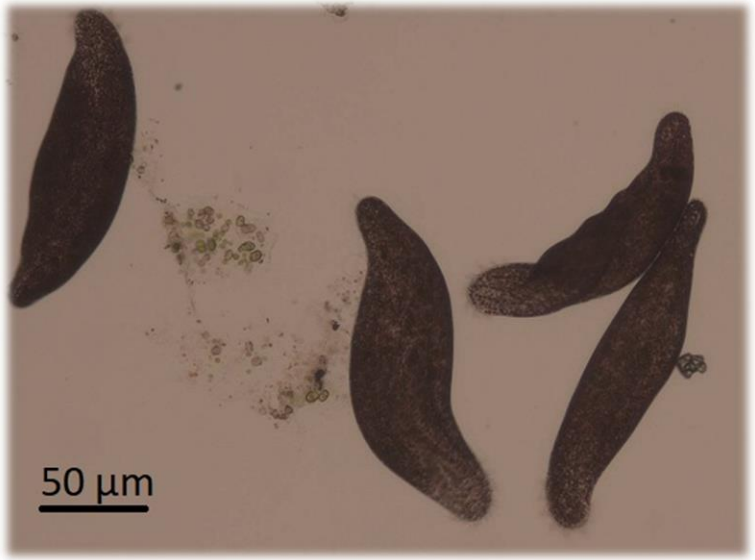


Figure 75. Typical appearance of cells of the specific strain *Condylostoma* sp. from laboratory cultures.

Figure 76. Division of the cell of *Condylostoma* sp. in the middle of the process.

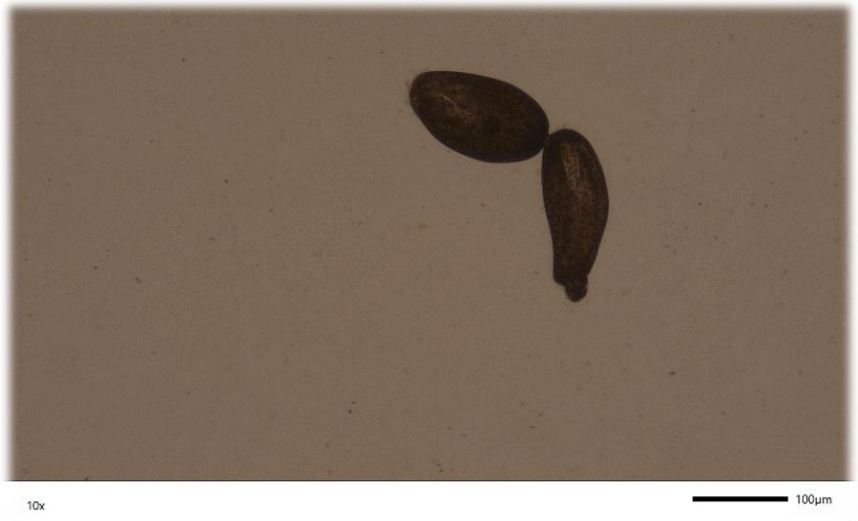


Figure 77. Division of the cell of *Condylostoma* sp. at the end of the process just before the separation of the daughter cells.

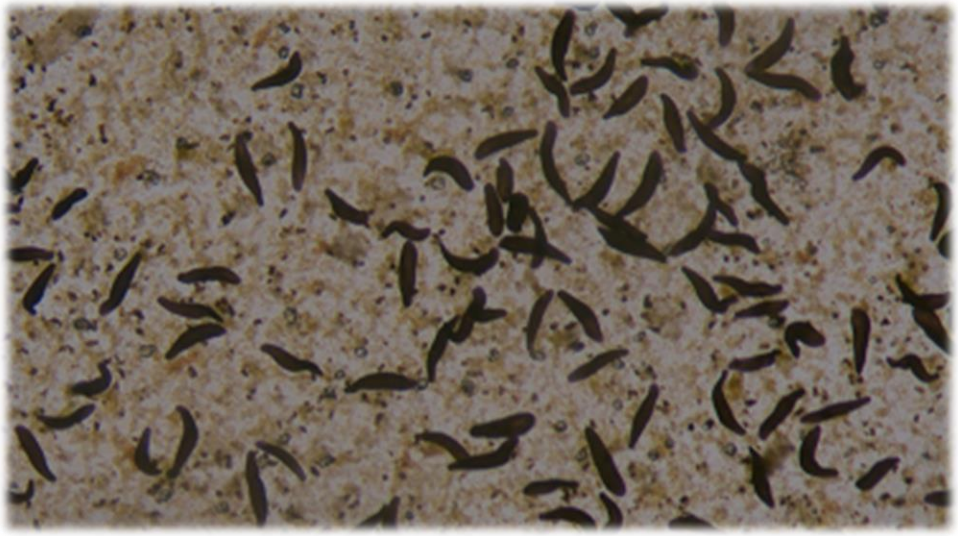
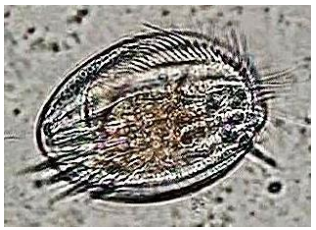


Figure 78. Dense population of *Condylostoma* sp. in 2 magnifications at the bottom of the culture vessel.

Euplotes sp.



Taxonomy

Kingdom:	Protista
Phylum:	Ciliophora
Class:	Spirotrichea
Sub-class:	Euplotia
Order:	Euplotida
Family:	Euplotidae
Genus:	*Euplotes*
Species:	*Euplotes* sp.

The cells of the ciliate *Euplotes* are rigid, dorsally flattened and generally oval in shape (Figures 79-81 & 84), with a very large stomatal region (peristome) bounded by a long "pale membranous zone" (AZM). *Euplotes* moves and feeds with the help of complex ciliated organelles called "cirri", which consist of thick tufts of cilia that are sparsely distributed throughout the cell. The robust "cirri" on the ventral surface of the cell allow *Euplotes* to “walk” or crawl on solid surfaces. All species of *Euplotes* have a group of stiff hairs (caudal cirripodia), protruding from the back of the cell. The number of caudal cirri varies even within a species, but the usual number is 4 or 5. The macronucleus is typically long and narrow and roughly horseshoe-shaped or shaped like the number “3”.

Euplotes in addition to the fact that they are found as a variety of species, often in any algal culture without strict cleanliness, are considered as a "pest" of algal cultures. Growing rapidly in almost any range of environmental conditions, they can cause culture collapse or dominate crops of other protozoa or rotifers. Their size (~50-110 µm along the longitudinal axis and ~30-70 µm along the transverse axis) nevertheless allows their capture with a 20 µm planktonic net as the rigidity of the cell does not allow their passage (at least the large ones) through its pores. *Euplotes* despite their relatively small size can be used as food for heterotrophs or can be a host symbiotic organism for large algal cells (Figures 80 & 81) such as *Asteromonas* (~> 20 µm). They reproduce by simple cell division (Figures 80 & 81), sometimes even (though rarely) by budding (Figure 83). They can withstand very brackish water (~3 ppt) to over 100 ppt. Very often they become encysted (Figure 82). Because of the above it is ideal for bioassays.

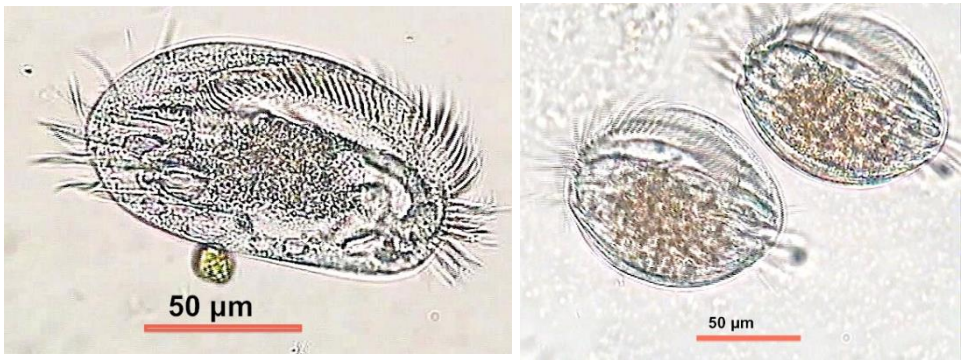


Figure 79. Typical image of 2 species *Euplotes* sp.

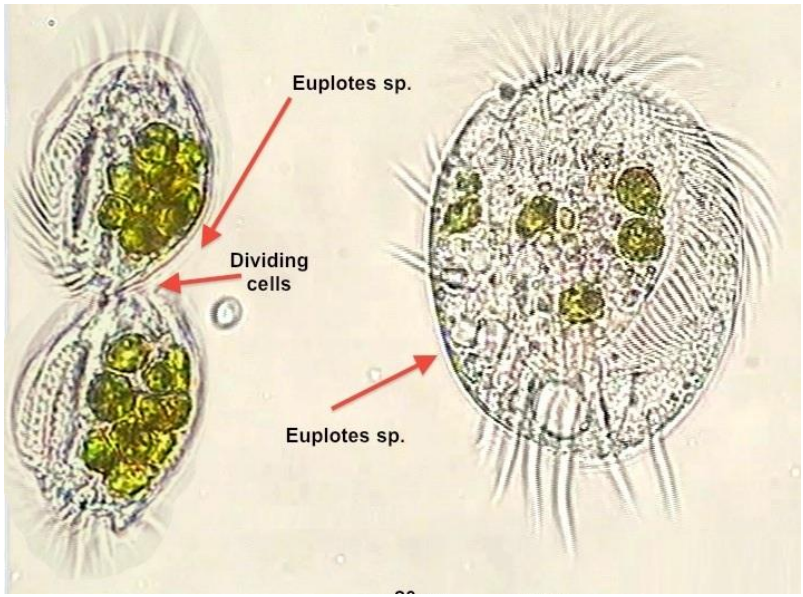


Figure 80. *Euplotes* sp. in division with a remarkable ability to feed on microalgae while the daughter cells have not yet fully separated.

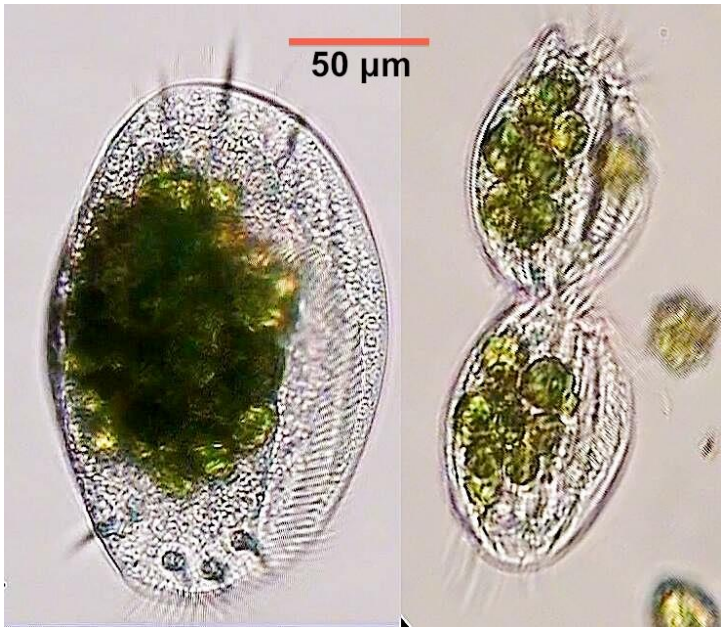


Figure 81. Two species of *Euplotes* sp. Left filled with *Asteromonas gracilis* cells. Right in division with the daughter cells already feeding on this chlorophyte as well.

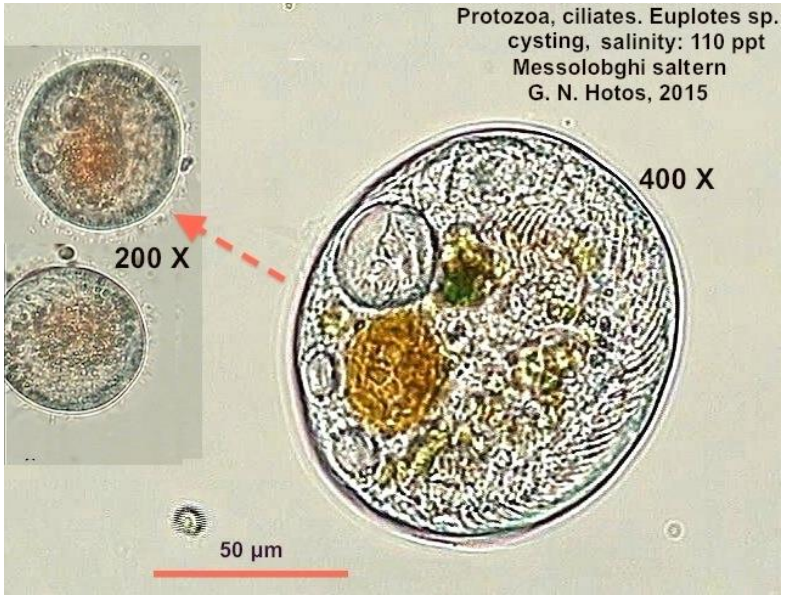


Figure 82. Phases of encystment in *Euplotes* sp.

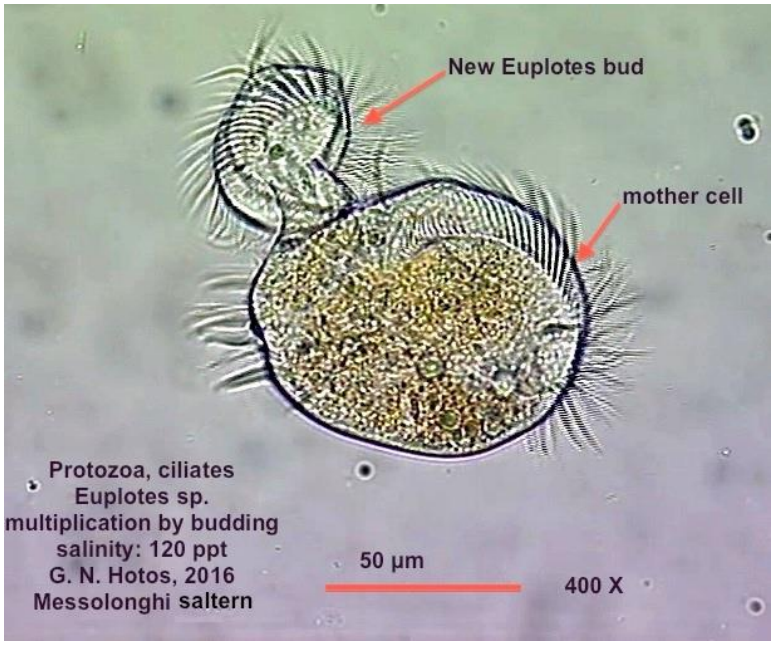


Figure 83. A rare image of budding in *Euplotes* sp.

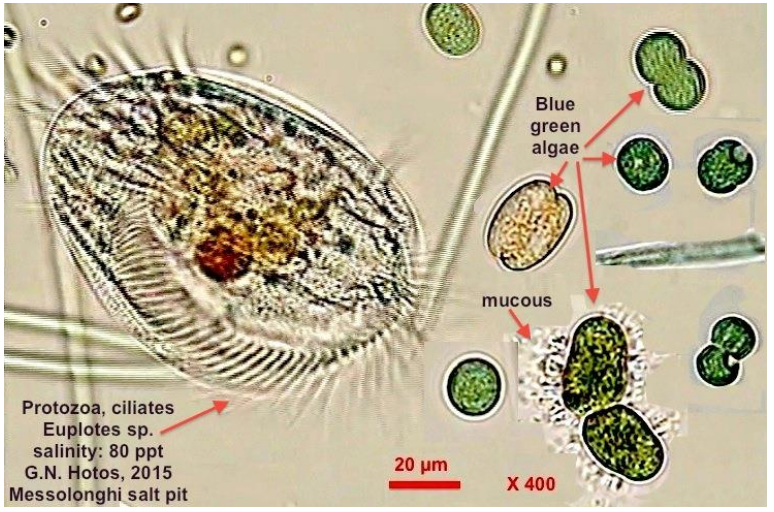


Figure 84. *Euplotes* sp. among various cyanobacterial species.

D. COPEPODS

From the lagoon of Messolonghi since 2017 we have isolated, acclimatized and continuously maintained in the plankton culture laboratory 2 species of harpacticoid copepods. One is a species of the genus *Tigriopus* (probably *T. fulvus*) and the other a strain of *Tisbe holothuriae*. We recently isolated another species of the genus *Tisbe*, probably *T. furcata*. Harpacticoid copepods are much easier to culture than other orders of copepods such as calanoids or cyclopoids, because they exhibit calmer swimming activity, without sudden jerks, which makes their capture attempts by larval fish easier (with *Tisbe* slower than *Tigriopus*). In the laboratory, these species grow rapidly and unimpeded fed on the microalgae we culture. Although harpacticoid copepods are characterized as benthic in the literature, in practice they occupy the entire water column in the culture vessel, thus making them ideal for mass production.

Tigriopus sp.

Taxonomy

Super-kingdom:	Eucaryota
Kingdom:	Animalia
Sub-kingdom:	Bilateria
Sub-sub-kingdom:	Protostomia
Supra-phylum:	Ecdysozoa
Phylum:	Arthropoda
Sub-phylum:	Crustacea
Supra-class:	Multicrustacea
Class:	Hexanauplia
Supra-sub-class:	Neocopepoda
Sub-class:	Copepoda
Supra-order:	Podoplea
Order:	Harpacticoida
Family:	Harpacticidae
Genus:	*Tigriopus*
Species*:*	*Tigriopus* sp*.*

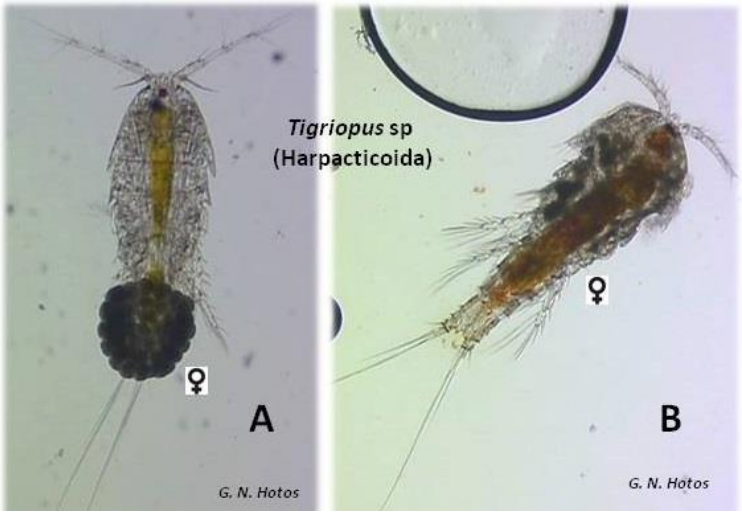


Figure 85. A: Female with egg-sac and B: male adult *Tigriopus* sp.

All species of the genus *Tigriopus* are dioecious (Figures 85-87). Before fertilization, the female individual is captured by the male with the help of its 1st pair of antennae (Figure 86). After a single mating the female produces successive egg-sacs (Figures 88 & 90) with fertilized eggs which hatch into nauplii (Figures 89 & 91) while the sac is still attached to the female's body.

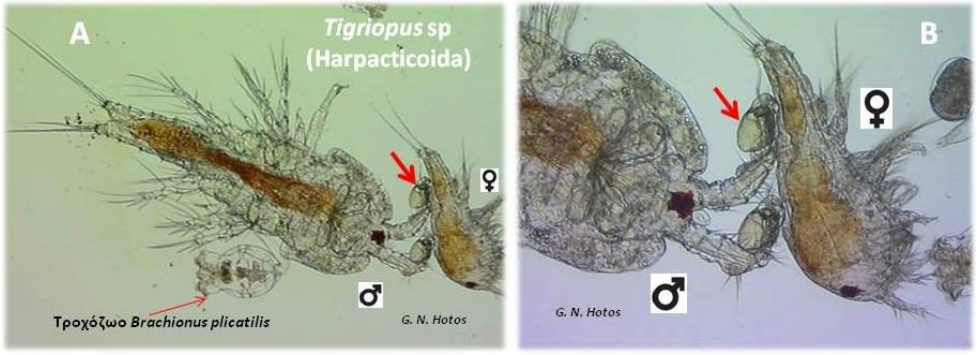


Figure 86. A & B: Capture of a small female in advanced copepodite stage by an adult male *Tigriopus* sp. for the purpose of fertilization which will take place after they swim as a pair for hours or even days. In B: the characteristic swellings on the male's 1st pair of antennae can be seen.

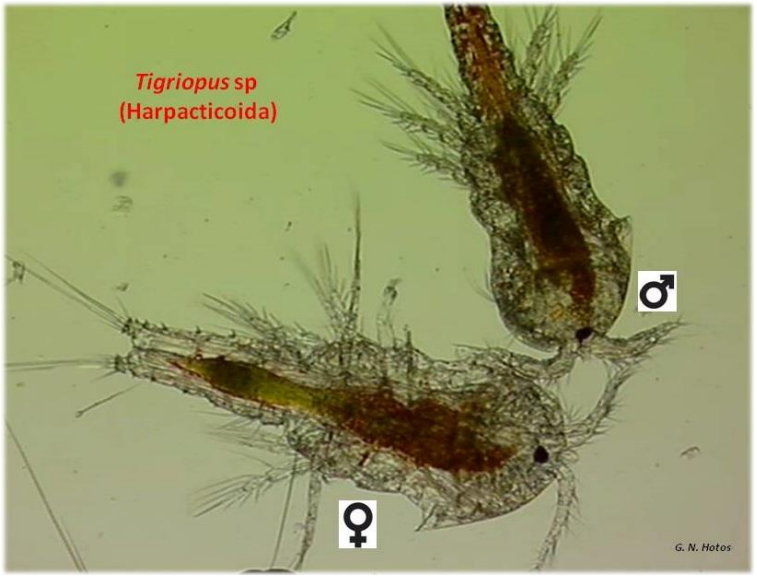


Figure 87. Male and female adults of *Tigriopus* sp.

Tigriopus in the adult stage is over 1000 µm long (including caudal spines) with the two sexes almost equal in size. Newly hatched nauplii (Figure 91) are ~120 µm long and develop (in size and ontogenetically) through 6 naupliar stages and 5 copepodite stages before developing into the adult stage, a process that takes a total of 10-20 days depending on temperature.

Figure 88. Close-up of a female *Tigriopus* egg-sac with the embryos inside the many eggs (~30) at an advanced stage of development.

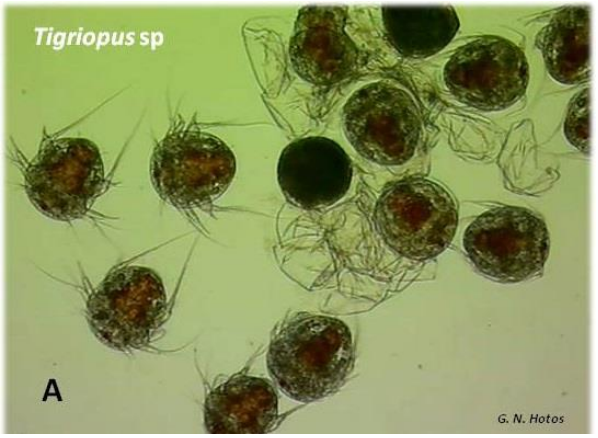


Figure 89. Hatching of *Tigriopus* eggs with nauplii released.

Figure 90. Female *Tigriopus* with the egg-sac attached to the body.

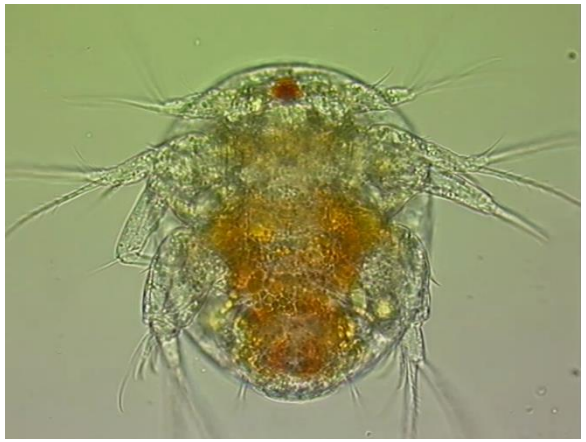


Figure 91. Nauplius of *Tigriopus* just hatched.

Tisbe holothuriae

Taxonomy

Supra-kingdom:	Eucaryota
Kingdom:	Animalia
Sub-kingdom:	Bilateria
Sub-sub-kingdom:	Protostomia
Supra-phylum:	Ecdysozoa
Phylum:	Arthropoda
Sub-phylum:	Crustacea
Supra-class:	Multicrustacea
Class:	Hexanauplia
Supra-sub-class:	Neocopepoda
Sub-class:	Copepoda
Supra-order:	Podoplea
Order:	Harpacticoida
Family:	Tisbidae
Genus:	*Tisbe*
Species:	*Tisbe holothuriae*

The harpacticoid copepod *Tisbe holothuriae* (Figures 92, 93 & 94) is noticeably smaller (~700 µm) in size than *Tigriopus* and much slower in its swimming movement, tending more to concentrate on the bottom of the container but not avoiding swimming in the water column as well. Despite their name "harpacticoids" they are not predators of other zooplanktonic organisms but rather filter-feeders of phytoplankton cells which they lavishly consume growing unimpeded. Both species tolerate an extremely wide range of salinities from very brackish (~2 ppt *Tigriopus*, ~10 ppt *Tisbe*) to very hypersaline (~100 ppt *Tisbe*, ~120 ppt *Tigriopus*). Also noteworthy is their ability to be unaffected by degraded water quality and low dissolved oxygen, as our observations of containers with thriving populations of water with no renewal or aeration showed neither suffering nor collapsing of their population. Therefore, they are considered ideal organisms for bioassays and of course as cultured live food species for fish larvae. Similar to *Tigriopus, Tisbe* also is dioecious with conception of the female by the male (Figure 96), long swimming period of the pair, single fertilization, multiple egg-sacs, 6 naupliar and 5 copepodite stages with nauplii but slightly smaller than those of *Tigriopus* (Figures 97 & 98). Another characteristic of *Tisbe* is their distinctive folding of the body and the sometimes inexplicable attachment of a second male to the swimming breeding pair (Figures 95 and 96 respectively).

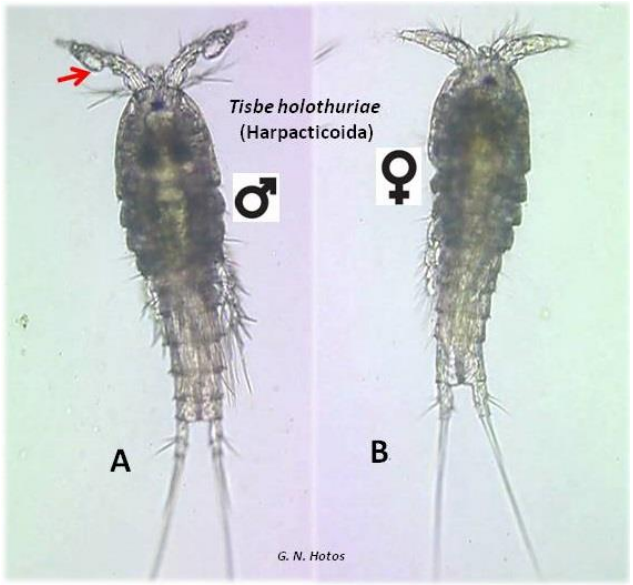


Figure 92. Typical appearance of male and female *Tisbe holothuriae*. The arrow shows the male's characteristic bumps on the first pair of antennae.

Figure 93. Another typical view of male and female *Tisbe holothuriae*. The female carries a large egg-sac. Characteristic of both species of *Tisbe* and *Tigriopus* is the single egg-sac, in contrast to calanoids and cyclopoids that carry 2 egg-sacs.

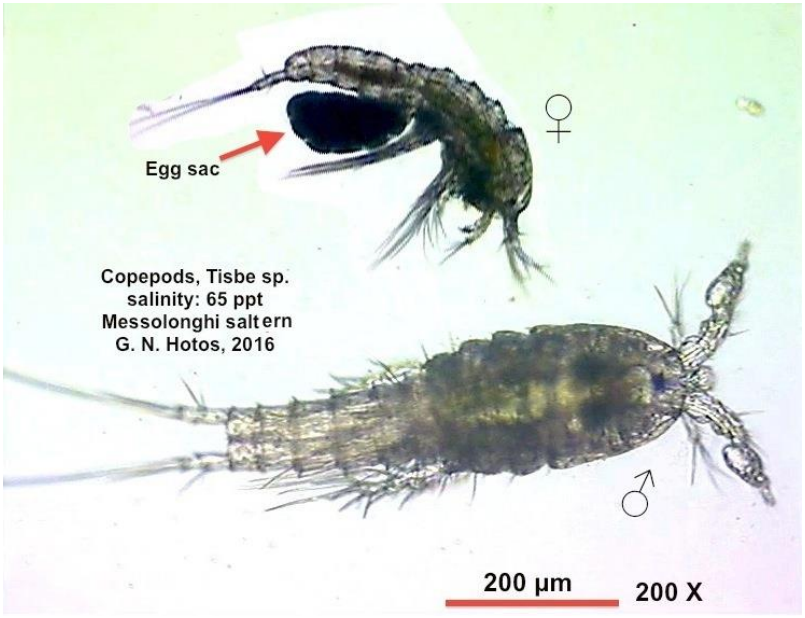


Figure 94. Another typical view of male and female *Tisbe holothuriae*. The female carrying a large-sized egg-sac which in this case and contrary to usual, is visibly smaller. Very evident in this photo are the characteristic bumps (bubbles) on the 1st pair of antennae of the male.

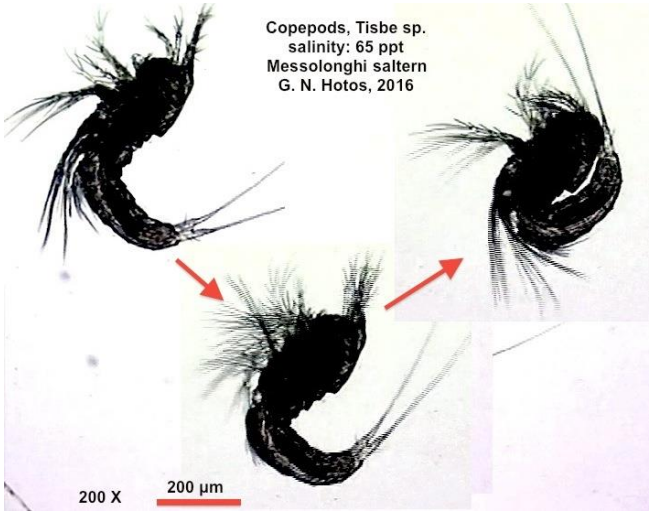


Figure 95. The great plasticity of the body of *Tisbe holothuriae*.

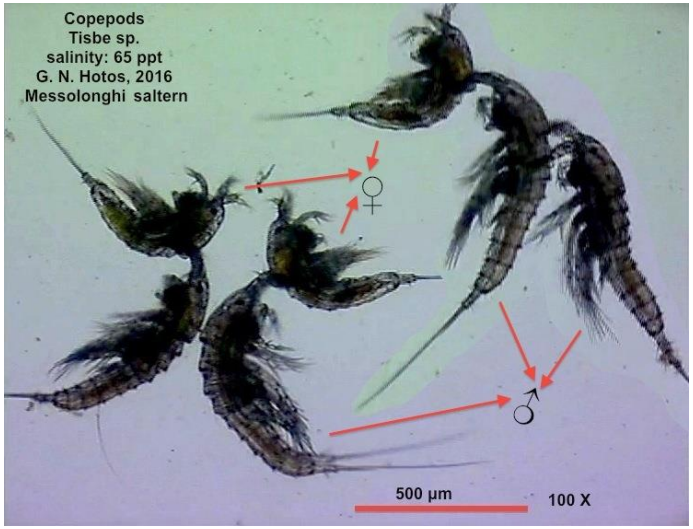


Figure 96. Typical breeding pairs of *Tisbe holothuriae* simultaneously with the inexplicable addition of one more male to a typical pair.

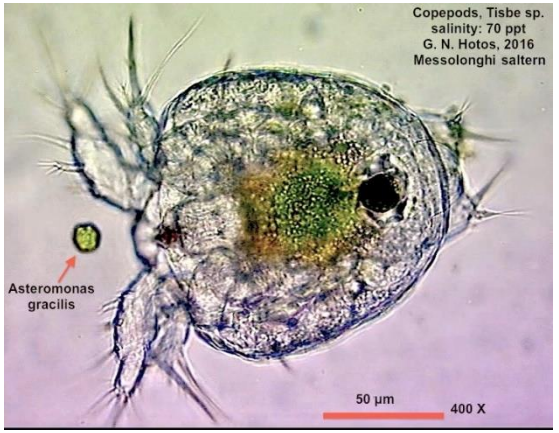


Figure 97. Typical appearance of a *Tisbe holothuriae* nauplius that has begun to feed on phytoplankton.

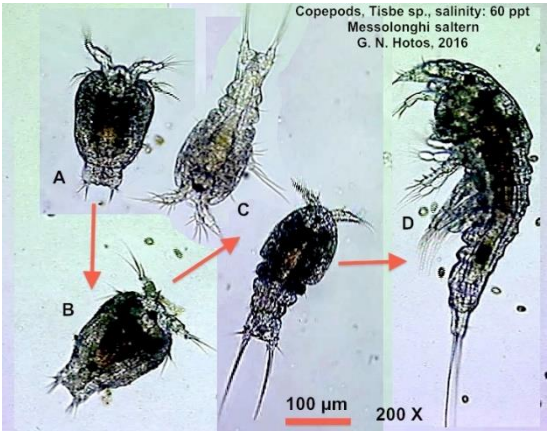


Figure 98. Photo collage of *Tisbe holothuriae* nauplius development in a copepodite and then in an adult.

Apart from the above copepods *Tigriopus* sp. and *Tisbe holothuriae* which are particularly easy to cultivate in the laboratory, recently 2 other harpacticoids were isolated which, however, do not give completely satisfactory results in terms of the ease of maintaining them in thriving cultures. Despite this, the efforts continue and are maintained even in a limited number with constant renewals. One species is likely to be *Tisbe furcata* (Figure 99) and the other (Figure 100) not yet identified (probably of the genus *Tisbe*).

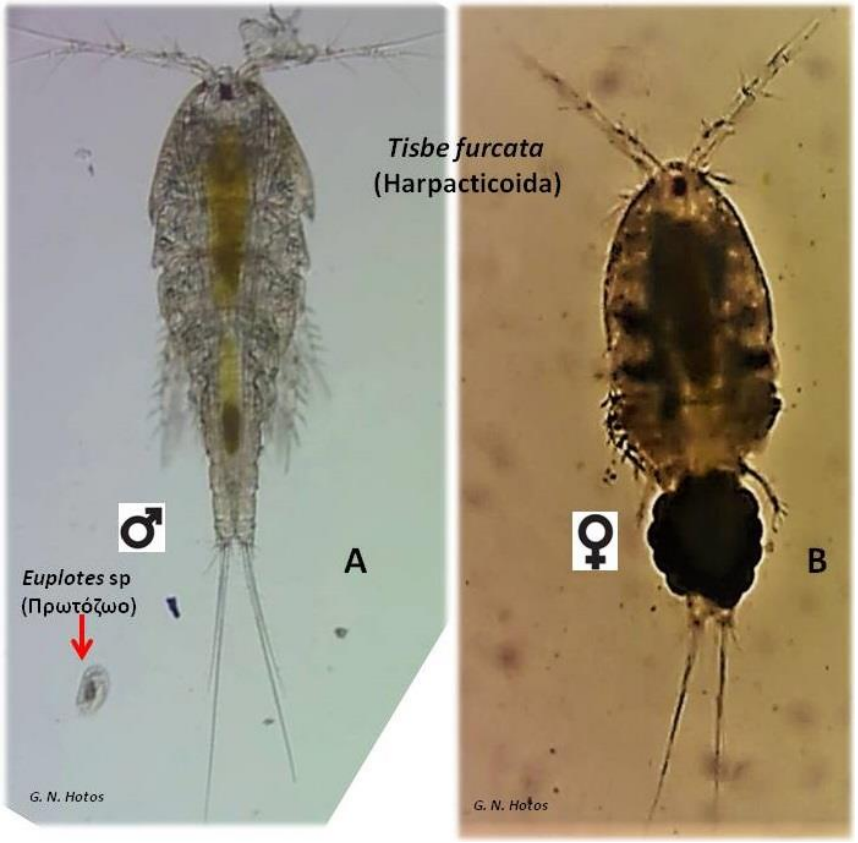


Figure 99. Typical appearance of male and female *Tisbe furcata.*

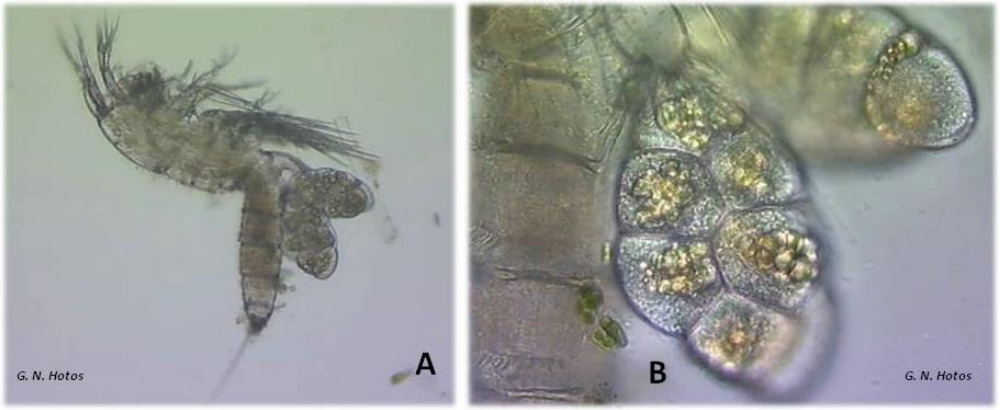


Figure 100. In A, an unidentified species of harpacticoid copepod from the Klisova lagoon carrying 2 egg-sacs. Characteristic is the wide urosoma and long thoracic legs. In B, close-up view of an egg-sac containing relatively few eggs (~10).

E. ROTIFERS

Brachionus plicatilis

Taxonomy

Kingdom: Animalia
Phylum: Rotifera
Class: Monogononta
Order: Ploimida
Family: Brachionidae
Genus: *Brachionus*
Species: *Brachionus plicatilis*

From the lagoon of Messolonghi we isolated a local strain of the well-known trochozoan species *Brachionus plicatilis* (Figure 101) with growth characteristics in culture equivalent to those known from the "enormous" literature that exists for this animal, which is the only and irreplaceable first live food for fish larvae in marine fish hatcheries. An extensive description of the morphology and biology of this species will not be presented here as they are anecdotal. Only key evidence of its successful culture will be given with specimens produced in the laboratory. *Brachionus plicatilis* reproduces mainly parthenogenetically (amictic reproduction) or after the influence of certain factors sexually (mictic reproduction). During parthenogenesis the female produces exact genetic copies of itself, i.e. clones. However, if there are strong dramatic changes in the living environment, e.g. sudden change in salinity or temperature, then mictic reproduction is induced resulting in the appearance of tiny (compared to females) males and, after fertilization, production of special ``dormant diapause eggs or resting eggs'' (Figure 102), biological entities analogous to *Artemia* cysts. A female *B. plicatilis*, depending on the conditions of its culture, can produce up to 20 eggs (offspring) during its approximately 10 days of life. The eggs it produces at a time (1-8) are attached to the back of its body until they hatch (Figures 103-106). The reproductive capacity of this animal has occupied several researchers and several data have been collected for application to its culture. For example, depending on the type of algal diet the highest fecundity was observed when animals were fed *Isochrysis galbana* at 20-22°C. Production of 21 offspring/female, length of reproductive period of 6.7 days, life span of 10.5 days and mean adult length (*B. plicatilis*) of 234 μm were observed. Speaking of rotifer sizes even for one and the same species (we are most interested in *B. plicatilis*), some key issues will be mentioned below.

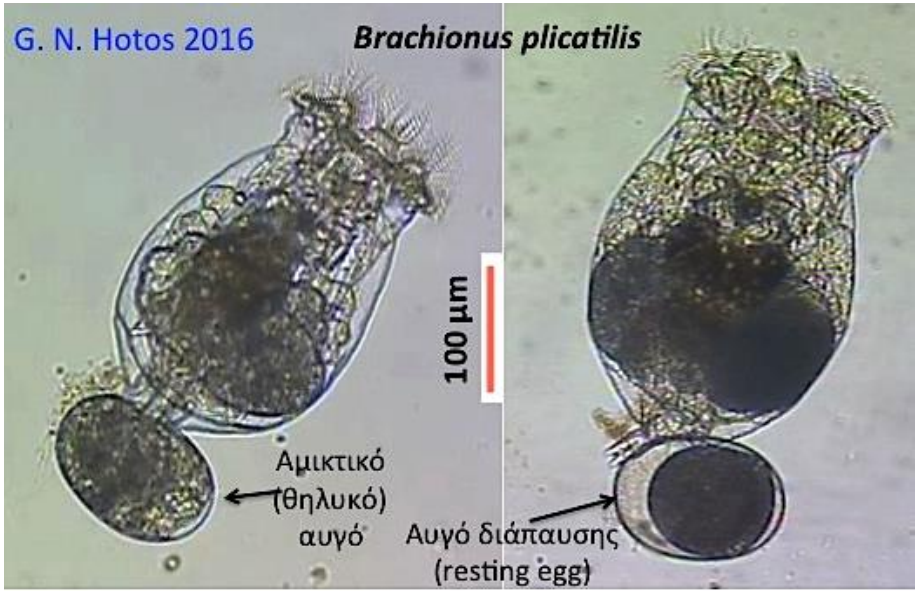


Figure 101. Left: female with an amictic egg. Right: with a resting egg.

In rotifers, the phenomenon of polymorphism is observed in the same species depending on the conditions of the environment where they live. Polymorphism reflects differences in the various types of a species both in the general morphology of the individual and mainly in size in general. Each type has its own particular requirements and abilities to adapt to different conditions of the culture environment (temperature, salinity, type of food, etc.) and characteristics of its biological entity (rate of reproduction, growth, etc.). According to various researchers, variations in size and shape of rotifers are characteristics that correspond to genetically isolated populations and as such are followed by ecological physiological adaptations, i.e. different types of animals.

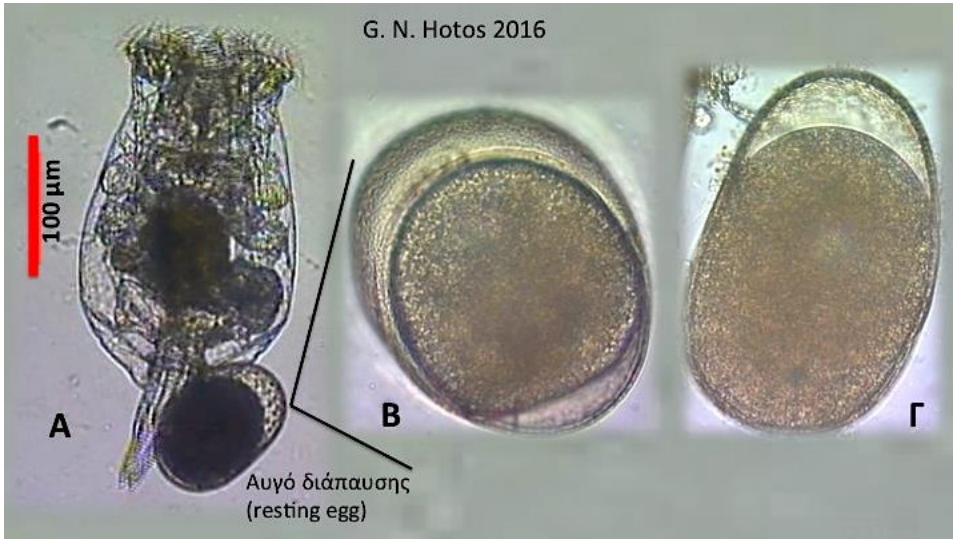


Figure 102. Mictic female *B. plicatilis* with a diapausing (resting) egg (left) and two enlarged views (right) of the resting egg.

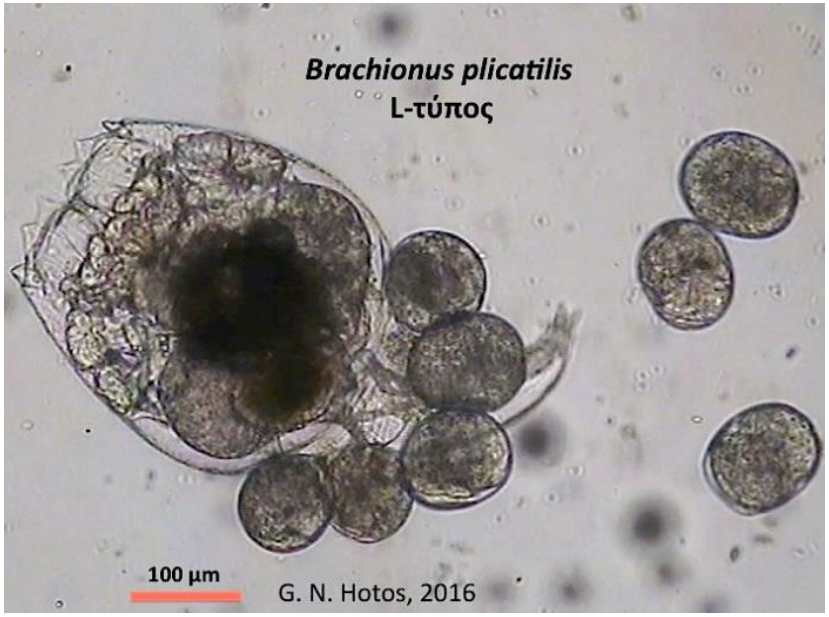


Figure 103. Female *B. plicatilis* with many amictic eggs, some still attached to its body and some detached. Upon their hatching, female individuals will emerge.

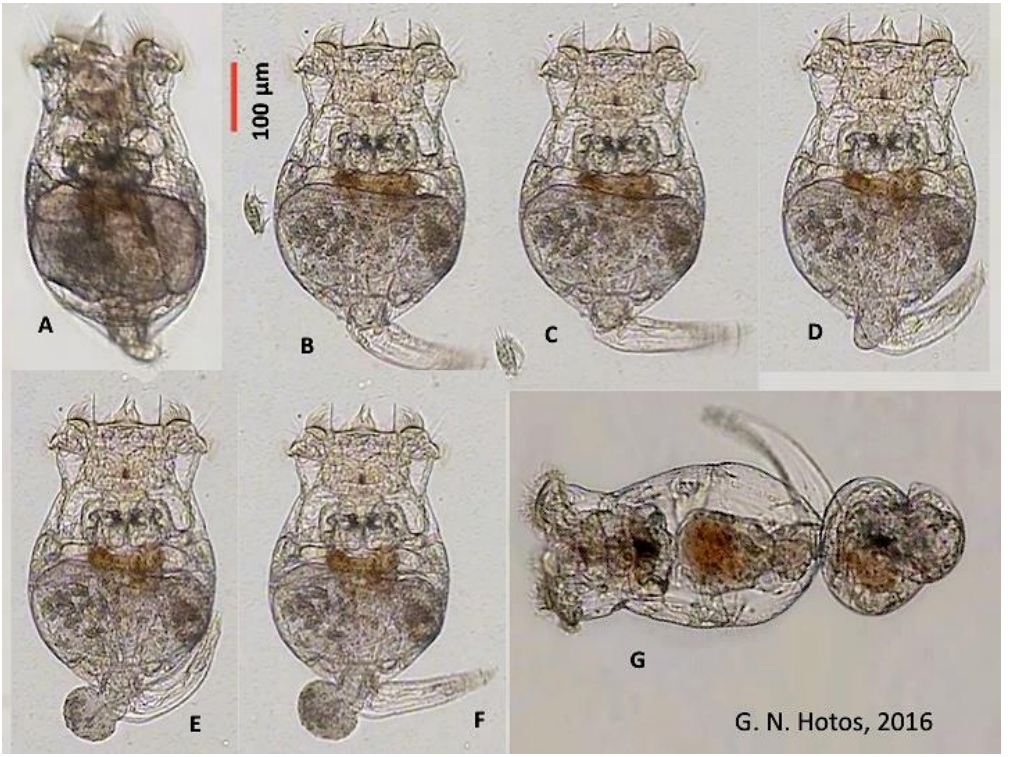


Figure 104. Capture of the exit phases of an egg from inside the body of a female *B. plicatilis.*

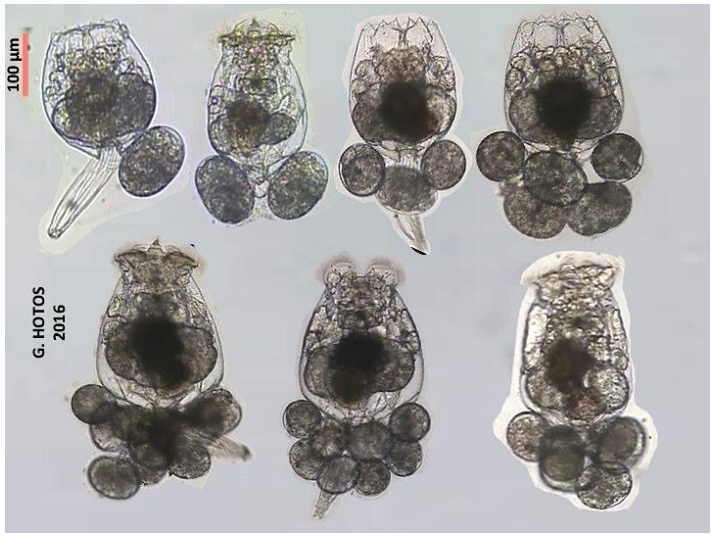


Figure 105. Amictic rotifers of *B. plicatilis* with varying numbers of amictic eggs attached to the mother individual. 1, 2, 3, 5, 6, 8 and 10 eggs can be seen held on the back of the body.

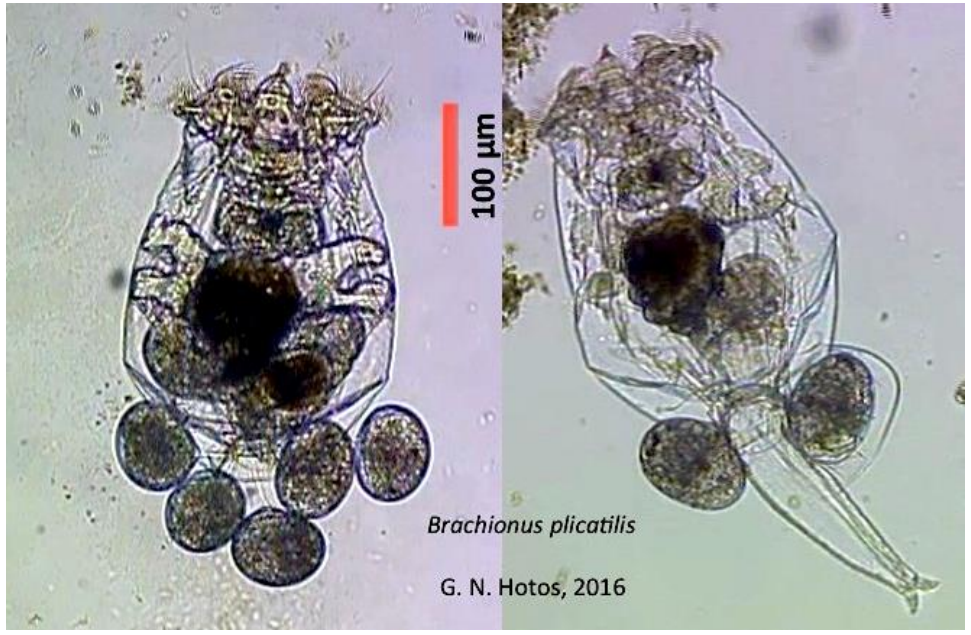


Figure 106. Mictic female individuals of *B. plicatilis* with 5 and 2 eggs that will produce male individuals.

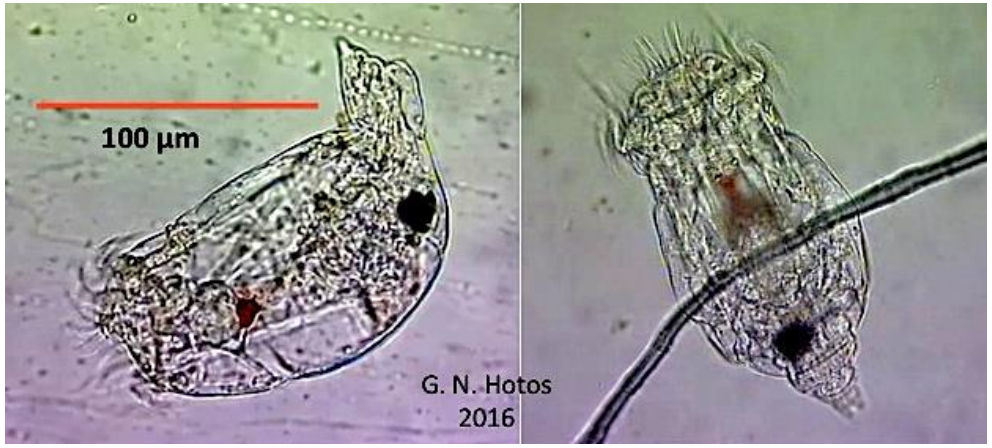


Figure 107. Male *B. plicatilis* in two views.

Figure 108. Photo collage of the fertilization process in *B. plicatilis*. A: approach, B: receptivity detection, C: preparation, D: fertilization. Remarkable fact that fertilization is also done in mixed females already carrying male eggs.

Summary of reproduction in *B. plicatilis*:

• Reproduction takes place in two ways: A. Amictic mode or parthenogenesis, B. Mictic mode or amphigenic

• In the amictic way there is no fertilization. Eggs are produced by parthenogenesis, which gives amictic females.

• Some females that become "mictic" produce special small eggs (Figure 106) which will produce males (Figure 107).

• Mictic females are fertilized by the resulting males (Figure 108) and produce dormant eggs (or resting eggs). After hatching all give amictic female individuals.

• Amictic reproduction is faster and therefore the population increases faster, which is necessary for the intensive culture of rotifers.

• When the conditions in which rotifers live are unsuitable, then, they use mictic reproduction. This is how dormant eggs manage to cope with these adverse conditions.

• The production of mictic females depends mainly on three factors that interact with each other: a) salinity, b) population density, c) quality and quantity of their food.

• Amictic reproduction can be interrupted and replaced by phases of amphigenic reproduction (mictic reproduction), which is caused either by exogenous factors – signals (low temperature and salinity, decrease in quality and quantity of food, increase in population density, etc) that characterize a deterioration of the environment, or by endogenous factors (age).

• In the mictic way, the mictic females are fertilized by the males and produce dormant or resting eggs with a mandatory latent period of at least one month.

• The preferred method of reproduction is the amictic for the following reasons:

- ✓ It is faster
- ✓ The mictic way causes culture collapse
- ✓ Males produced by the mictic method have a lower nutritional value for fish larvae due to the lack of a digestive system.

HOME BIBLIOGRAPHY

- **ΧΩΤΟΣ, Γ**. & Δ., ΑΒΡΑΜΙΔΟΥ, 1995. Μελέτη της αύξησης του μονοκύτταρου αλόφιλου φύκους *Asteromonas gracilis* (Chlorophyta) σε συνθήκες μαζικής καλλιέργειας με τη χρήση διαφορετικών αλατοτήτων, φωτοπεριόδου και έλλειψης πρόσθετων βιταμινών. *Γεωτεχνικά Επιστημονικά Θέματα (Geotechnical Scientific Issues)*. 6(2): 37-45.
- **ΧΩΤΟΣ, Γ**., ΑΒΡΑΜΙΔΟΥ, Δ., ΑΘΑΝΑΣΟΠΟΥΛΟΣ, Η. & ΑΡΒΑΝΙΤΑΚΗΣ, Γ., 2000. Ρυθμός αύξησης, διήθησης και πέψης του τροχόζωου *Brachionus plicatilis* σε πειραματικές συνθήκες με χρήση του αλοανθεκτικού χλωρόφυτου μικροφύκους *Asteromonas gracilis* Artari. *6ο Πανελλήνιο Συμπόσιο Ωκεανογραφίας & Αλιείας.* Χίος, 23-26 Μαϊου 2000, τ. ΙΙ: 268 - 273.
- **ΧΩΤΟΣ, Γ**., ΑΒΡΑΜΙΔΟΥ, Δ., ΑΘΑΝΑΣΟΠΟΥΛΟΣ, Η. και ΑΡΒΑΝΙΤΑΚΗΣ, Γ., 2000. Επιλεκτικότητα για κατανάλωση από το τροχόζωο *Brachionus plicatilis* μεταξύ δύο μικροφυκών, μεγάλου (*Asteromonas gracilis*) και μικρού (*Chlorella* sp.). *9ο Πανελλήνιο Συνέδριο Ιχθυολόγων Υπ. Γεωργίας*. Μεσολόγγι, 20-23 Ιανουαρίου 2000.
- **ΧΩΤΟΣ, Γ**., ΑΒΡΑΜΙΔΟΥ, Δ., ΑΘΑΝΑΣΟΠΟΥΛΟΣ, Η. και ΑΡΒΑΝΙΤΑΚΗΣ, Γ., 2000. Αποτελεσματικότητα της διατροφής του τροχόζωου *Brachionus plicatilis* με συνδυασμούς 4 ειδών μικροφυκών το καθένα με *Chlorella*. *9ο Πανελλήνιο Συνέδριο Ιχθυολόγων*. Μεσολόγγι, 20-23 Ιανουαρίου 2000.
- **HOTOS, G.N**., 2002. Selectivity of the rotifer *Brachionus plicatilis* fed mixtures of algal species with various cell volumes and cell densities. *Aquaculture Research*, 33 (12), 949-957. DOI: 10.1046/j.1365-2109.2002.00746.x
- **ΧΩΤΟΣ, Γ**., ΑΝΑΣΤΟΠΟΥΛΟΣ, Π. & ΠΕΤΖΑ, Δ., 2003. Επίδραση της θερμοκρασίας στο ρυθμό αύξησης δύο ειδών μικροφυκών *Asteromonas gracilis* Artari και *Tetraselmis* sp. *11ο Πανελλήνιο Συνέδριο Ιχθυολόγων*, Πρέβεζα 10-14 Απριλίου 2003.
- **ΧΩΤΟΣ, Γ**., ΑΝΑΣΤΟΠΟΥΛΟΣ, Π. & ΠΕΤΖΑ, Δ., 2003. Επίδραση του περιβαλλοντικού φωτισμού και της φυγοκέντρησης των φυτοπλαγκτονικών κυττάρων των ειδών *Asteromonas gracilis, Tetraselmis* sp. και *Dunaliella* sp., στη διατροφή του τροχόζωου *Brachionus plicatilis*. *11ο Πανελλήνιο Συνέδριο Ιχθυολόγων, Πρέβεζα 10-14 Απριλίου 2003.*
- **HOTOS, G. N**., 2003. Growth, filtration and ingestion rate of the rotifer *Brachionus plicatilis* fed with large (*Asteromonas gracilis*) and small (*Chlorella* sp.) celled algal species. *Aquaculture Research*, 34 (10), 793-802. DOI: 10.1046/j.1365-2109.2003.00868.x
- **HOTOS G**., 2016. The halotolerant green alga *Asteromonas gracilis*, an ideal tool for research, education and mass culture. A preliminary report. *2nd Intern. Congress on Appl. Ichthyology & Aquatic Environment. 10-12 Nov. 2016*. Messolonghi Greece.
- **HOTOS, G.**, 2018. Protists, Cyanobacteria, Rotifers and Crustacea from the hypersaline lakes of Messolonghi saltworks (W. Greece). *10th World Salt Symposium, 2018, Park city, Utah, U.S.A.*.
- KOROVESSIS, A. K., **HOTOS, G**. & G. ZALIDIS, 2018. The role of the ciliate protozoan *Fabrea salina* in solar salt production. *10th World Salt Symposium, 2018, Park city, Utah, U.S.A.*
- **HOTOS, G**., 2019. Feeding with various microalgae the salt "loving" ciliate *Fabrea salina* in normal salinity 35 ppt. *Journal of the Science of Food and Agriculture, 3(3):150-152.* DOI: 10.26855/ijfsa.2019.07.001.
- **HOTOS, G**., 2019. A short review on the halotolerant green microalga *Asteromonas gracilis* Artari with emphasis on its uses. *Asian Journal of Fisheries and Aquatic Research*, 4(3): 1-8. DOI: 10.9734/ajfar/2019/v4i330054..
- **HOTOS, G.,** AVRAMIDOU, D. & V., BEKIARI, 2020. Calibration curves of culture density assessed by spectrophotometer for three microalgae (*Nephroselmis* sp., *Amphidinium carterae* and *Phormidium* sp.). *European Journal of Biology and Biotechnology*, 1(6): 1-7. https://doi.org/10.24018/ejbio.2020.1.6.132

- **HOTOS, G**., 2021. A preliminary survey on the planktonic biota of a hypersaline pond of Messolonghi saltworks (W. Greece). *Diversity*, 13, 270: 1-12. https://doi.org/10.3390/d13060270
- **HOTOS**, **G**., 2021. "Culture Growth of the Cyanobacterium *Phormidium* sp. in Various Salinity and Light Regimes and Their Influence on Its Phycocyanin and Other Pigments Content" *Journal of Marine Science and Engineering* 9, no. 8: 798. https://doi.org/10.3390/jmse9080798
- **HOTOS**, **G**. and Despoina Avramidou, 2021. The Effect of Various Salinities and Light Intensities on the Growth Performance of Five Locally Isolated Microalgae [*Amphidinium carterae*, *Nephroselmis* sp., *Tetraselmis* sp. (var. red pappas), *Asteromonas gracilis* and *Dunaliella* sp.] in Laboratory Batch Cultures. *Journal of Marine Science and Engineering* 9, no. 11: 1275. https://doi.org/10.3390/jmse9111275
- **HOTOS**, George N., and Theodoros I. Antoniadis. 2022. The Effect of Colored and White Light on Growth and Phycobiliproteins, Chlorophyll and Carotenoids Content of the Marine Cyanobacteria *Phormidium* sp. and *Cyanothece* sp. in Batch Cultures. *Life* 12, no. 6: 837. https://doi.org/10.3390/life12060837
- **HOTOS**, George N. and Ioanna Touloupi. 2022. Response of the Ciliates *Fabrea salina* and *Condylostoma* sp. to Different Salinities and Microalgal Feeds. *Ecologies* 3, 2, 225-234. https://doi.org/10.3390/ecologies3020017
- **HOTOS**, G.N., Avramidou, D., & Samara, A., 2022. The Effect of Salinity and Light Intensity on the Batch Cultured Cyanobacteria *Anabaena* sp. and *Cyanothece* sp. *Hydrobiology*, 1, 278–287. https://doi.org/10.3390/hydrobiology1030020
- **HOTOS**, G.N., Avramidou, D., & Samara, A., 2022. The effect of salinity and light intensity on the batch cultured cyanobacteria *Anabaena* sp. and *Cyanothece* sp. *Marine and Inland Waters Research Symposium*, Porto-Heli, Greece, 16-19 Sept. 2022.
- **Χώτος**, Γ. & Ι. Φωτοδήμας, 2022. Η επίδραση της αλατότητας και διαφόρων μικροφυκών ως τροφή, στην επιβίωση και γονιμότητα των αρπακτικοειδών κωπηπόδων *Tisbe* sp. και *Tigriopus* sp. *18ο Πανελλήνιο Συνέδριο Ιχθυολόγων*, Μεσολόγγι, 3-6 Νοεμ. 2022.
- **Χώτος**, Γ. & Σ. Κολυροπούλου, 2022. Η επίδραση της έντασης φωτισμού και της αλατότητας στην καλλιέργεια δύο μικροφυκών *Dunaliella* sp. και *Tetraselmis* sp. από τις αλυκές Μεσολογγίου. *18ο Πανελλήνιο Συνέδριο Ιχθυολόγων*, Μεσολόγγι, 3-6 Νοεμ. 2022.
- **Χώτος**, Γ., Αβραμίδου, Δ. & Βλ. Μπεκιάρη, 2022. Χρήση της Φασματοφωτομετρίας και της φθορισομετρίας για τον υπολογισμό της πυκνότητας φυτοπλαγκτονικών καλλιεργειών. *18ο Πανελλήνιο Συνέδριο Ιχθυολόγων*, Μεσολόγγι, 3-6 Νοεμ. 2022.
- **HOTOS**, George N., Evi Kourelea, and Ioannis Fotodimas,. 2022. "Salinity Tolerance and the Effect of Salinity and Algal Feed on the Demographics of Cultured Harpacticoid Copepods *Tisbe holothuriae* and *Tigriopus* sp. from the Messolonghi Lagoon (W. Greece)" *Journal of Marine Science and Engineering* 10, no. 11: 1663. https://doi.org/10.3390/jmse10111663

SPECIAL HOME BIBLIOGRAPHY

- Hotos, G. & Th. Antoniadis, 2022. An illustrated procedure of phycocyanin, allophycocyanin and phycoerythrin measurement in cyanobacterial cultures. ResearchGate, DOI: 10.13140/RG.2.2.29018.62404
- Hotos, G., 2020. Βιολογικές χρωστικές και φωτοσύνθεση. ResearchGate, DOI: 10.13140/RG.2.2.13148.54406/1
- Hotos, G., 2019. Κωπήποδα (Βασικά βιολογικά στοιχεία). ResearchGate, DOI: 10.13140/RG.2.2.25424.46081
- Hotos, G., 2019. COCCOID CYANOBACTERIA IN THE HYPERSALINE PONDS OF MESSOLONGHI SALTERNS). ResearchGate, DOI:10.13140/RG.2.2.36501.27363

- Hotos, G., 2019. *Fabrea salina* Henneguy, 1890. A heterotrichous ciliate thriving in hypersalinity ResearchGate, DOI: 10.13140/RG.2.2.33424.87041
- Hotos, G., 2019. *Dunaliella*, το θαυμαστό φύκος των αλυκών ResearchGate. DOI: 10.13140/RG.2.2.36763.41763
- Hotos, G., 2018. Τοξικά Μικροφύκη. ResearchGate, DOI: 10.13140/RG.2.2.21109.32486
- Hotos, G., 1995. Περιβαλλοντική μελέτη λιμνοθάλασσας Κλείσοβας Μεσολογγίου με μέτρηση επιλεγμένων φυσικοχημικών παραμέτρων κατά τα έτη 1993-94 ResearchGate, DOI: 10.13140/RG.2.2.20322.89280
- Hotos, G., 2016. Η μικροσκοπική υδρόβια ζωή στις υπεράλμυρες λεκάνες των αλυκών Μεσολογγίου. Εκδοση Φορέα Διαχείρισης Λιμνοθάλασσας Μεσολογγίου-Αιτωλικού. Στο ResearchGate: https://www.researchgate.net/publication/308326410_E_MIKROSKOPIKE_YDROBIA_ZOE_STIS_YPERALMYRES_LEKANES_TON_ALYKON_TOU_MESOLONGIOU_The_microscopic_water_life_in_the_hypersaline_saltern_ponds_of_Messolonghi-W_Greece

RELATED VIDEOS ON YOUTUBE FROM THE LABORATORY

For the chlorophyte *Dunaliella*:

- https://youtu.be/a7X_0walwRQ Characteristic colony-like concentration of *Dunaliella* cells.
- https://youtu.be/HqWxnhv-Ka0 *Dunaliella* among other microalgae used in fish hatcheries.
- https://youtu.be/kQJOdTnFLcc One-of-a-kind time-lapse video of *Dunaliella* cell division.
- https://youtu.be/ky85Piwy6EQ Another one-of-a-kind time-lapse video of *Dunaliella*'s cell division, with cells that are spherical and red due to the accumulation of β-carotene.
- https://youtu.be/acGUObR2ibQ A very enlightening video about chlorophytes found in hypersalinity including *Dunaliella*.

For the chlorophyte *Asteromonas*:

- https://youtu.be/x-hjmBv8Ql8 My first *Asteromonas* video with the "journey" of a single cell.
- https://youtu.be/h2MxCBngUN4 *Asteromonas'* signature twitchy movement in all its "glory".
- https://youtu.be/n2KbrHprsKc A unique and rarely found elsewhere time-lapse video of *Asteromonas* cell division.
- https://youtu.be/W393jYY1MJM The characteristic appearance and movement of *Asteromonas* in very high salinity water (>150 ppt).
- https://youtu.be/Uk8uudd6mP8 Another unique time-lapse video of *Asteromonas* cell division.
- https://youtu.be/TYgiSGC9rYU Another video with *Asteromonas* in hypersaline water where salt crystals have actually formed.
- https://youtu.be/_t8HNZ457XQ Another video with *Asteromonas* in hypersaline water where the cells show a special shape and coloration.

For the chlorophyte *Tetraselmis*:

- https://youtu.be/3Vew3G9IRUE A video clearly showing the cell movement and reddish color of this aforementioned strain of *Tetraselmis* (var. red pappas) that we isolated from the neighboring lagoon of Pappas of Achaia.

- https://youtu.be/HqWxnhv-Ka0 In this video showing microalgae used in fish hatcheries, all three microalgae are presented which we culture continuously but did not isolate from the surrounding lagoons. These are *Tetraselmis suecica*, *Isochrysis galbana* and *Rhodomonas salina*.
- https://youtu.be/acGUObR2ibQ In this video about the chlorophytes found in hypersalinity, a dominant position is occupied by the large *Tetraselmis* sp. (*marina* var. messolonghi).

For the dinoflagellate *Amphidinium carterae*:

- https://youtu.be/R8ue4H6zuYQ Characteristic shape, color and movement of this dinoflagellate.

For the chlorophyte *Nephroselmis* sp.:

- https://youtu.be/giZ430t15Sc The lively and swirling unique movement of this dynamic chlorophyte.

For the cyanobacterium *Phormidium* sp.:

- https://youtu.be/QNFw4LQb9Tc One of the few videos on the internet showing the slow gliding motion of this filamentous cyanobacterium.

For the cyanobacterium *Cyanothece* sp.:

- https://youtu.be/gR8yxSM9Yt4 Online unique video showing that even single-celled cyanobacteria can exhibit some degree of motility.

For the ciliate protozoan *Condylostoma* sp.:

- https://youtu.be/qqI9goQGmb8 The high density, black coloring and slow movement of the protozoan *Condylostoma* sp.

For the ciliate protozoan *Fabrea salina*:

- https://youtu.be/nUykguv8eMg The slow graceful movement of *Fabrea salina* in a snapshot of high population density and size diversity.
- https://youtu.be/RfvxkqCMhmw A close-up of a *F. salina* cell which after persistent observation I managed to get stationary, filtering and "ingesting" cells of the cryptophyte *Rhodomonas salina*.
- https://youtu.be/zbMe-bGWtX4 A swollen *F. salina* cell with its vacuoles filled with the chlorophyte *Asteromonas gracilis* which at some stage empties some of them, releasing the microalgal cells intact. One of a kind online video.
- https://youtu.be/1BEbvQAV7Ng Online unique video of the budding process in *F. salina*.
- https://youtu.be/ftmktGpurRY Laborious to capture video of cell division phases of daughter cells during division of *F. salina*.
- https://youtu.be/QGQSKP2ZGxw An artistically textured video showing the slow and majestic movement of the cells of a dense culture of *F. Salina* amidst the simultaneous presence of many smaller cells of the ciliate *Euplotes* sp.

For the copepods:

- https://youtu.be/mKWNr5dhJGY Clear difference in locomotion between harpacticoid and calanoid copepods.

- https://youtu.be/zmwXeoN1wTM Snapshots of egg hatching and nauplius form of *Tigriopus* sp.
- https://www.youtube.com/watch?v=evBBMgLzpwM The amazing male-female pair of *Tigriopus* sp. swimming around in a precopulation stage.
- https://www.youtube.com/watch?v=w8CSu1zTg8I Nauplius, copepodites, adults, ovigerous females of *Tisbe holothuriae* in a fast-track presentation.

For the rotifer *Brachionus plicatilis*:

- https://youtu.be/F61cHnGih54 A male B. plicatilis immobilized at high magnification with its anatomy fully displayed.
- https://youtu.be/NFVNFpkxa2w A satisfying video of the hatching of an amictic egg still attached to the mother's body and the release of a small female.
- https://youtu.be/6bgyUuwHjDk Video with many separate snapshots of the process of defecation in rotifers.
- https://youtu.be/qPPvSRzoXrQ One of the strangest videos I've ever filmed showing inexplicable brief contacts between *B. plicatilis* individuals as if they were exchanging some kind of information.
- https://youtu.be/nr9-gCuZ_tM Another video of an egg hatching and releasing a young individual only this time it's a male.
- https://youtu.be/X0zPJT9CiGM The frantic and orgiastic process of courting and impregnating mictic females by the tiny males of *B. plicatilis*.
- https://youtu.be/q_tfEO6Caqk Another video with snapshots of the long process of courting a female by a *B. plicatilis* male.
- https://youtu.be/fe7aGS67XuE Another video with snapshots of the long process of courting and copulation of a female with a *B. plicatilis* male.
- https://youtu.be/xqjIPIMx72Q Unique video of egg hatching and release of newborn *B. plicatilis*.
- https://youtu.be/ibtQeq_U_6U A unique close-up video of the microalgae filtering, trapping and chewing process in *B. plicatilis*.
- https://youtu.be/ajz5JgvlYCA Even more impressive video of *B. plicatilis* filtering, trapping and chewing large-sized microalgae.
- https://youtu.be/ciK91WIuxm8 The only (so far) worldwide videorecorded lightning process of the exit of an egg from the mother's body. Endless hours and efforts were needed to make this recording a success.
- https://youtu.be/co8z9KWKaoA Another amazing recording of the exit of the egg from the mother's body.
- https://youtu.be/j0W_xpupOyc A video "landmark" in the biology of *B. plicatilis* as it captures the immediate fulfillment of the purpose of life of a male, which, having just emerged from its egg, immediately seeks to mate.
- https://youtu.be/kQC3PVDhcVg Another video with several separate snapshots of the defecation process in *B. plicatilis*.
- https://youtu.be/3MnlEgw8Zbs Amazing and unique video with very close-up and clear presentation of *B. plicatilis* filtering, swallowing and chewing the large cells of *Asteromonas gracilis*.
- https://youtu.be/GgPwwJar8iI A hard-to-take video showing a close-up of a female being fertilized by a male.
- https://youtu.be/63kS5lSMiCk In this video, females are shown carrying small eggs that will be hatched giving males as well as the resting eggs that result from the fertilization of mictic females by males.

- https://youtu.be/LSJAXMcqtvs A potpourri of highly fertile amictic females with varying numbers of eggs as the case may be.

Similar videos:

In my channel's site:
https://www.youtube.com/channel/UCoams0_M5zKLtcv0LsRClyQ
there are many other related videos with ciliate protozoa, diatoms, polychaetes, *Artemia*, etc.
Some of them have a globally original theme such as e.g. the capture of an elongated pennate diatom by a marine amoeba:
https://www.youtube.com/watch?v=isN_L58hhbc&t=4s

The budding process in the ciliate *Holophrya* sp.
https://www.youtube.com/watch?v=GkAJ2X6q33c

The great plasticity of the cell of the ciliate *Phialina* sp.
https://www.youtube.com/watch?v=23pEzj7KQJ4

The amazing tremors of the ciliate *Uronychia* sp.
https://www.youtube.com/watch?v=2gfE1m1fASM
And many more.

INDICATIVE USEFUL NOTES

• All items mentioned herein may be given to interested parties for their use after prior consultation with the writer.
• Of the multitude of protozoa that can be found in a sample of natural waters, only a few species can be subsequently preserved in viable cultures. Such are the species in this brochure. The situation in salty waters differs from the corresponding one in fresh waters, where e.g. *Paramecium* is referred to as an easy-to-culture organism.
• The situation of microalgae is somewhat similar. Not all species of a sample respond equally well to culture efforts. Only the "robust" species will make it.
• Microalgae cultures result in monocultures of species that can prevail over others present at the same time. This situation is probably due not only to the competition for available nutrients but also to the very little studied phenomenon of allelopathy.
• All algae maintained in the laboratory have been shown over the years able to be maintained in almost absolute monoculture.
• Only cyanobacteria can catalytically "contaminate" a culture of eukaryotic algae and eventually prevail. Getting rid of the cyanobacteria requires repeated doses of a cocktail of antibiotics.
• If, on the contrary, other eukaryotic algae appear (even if they do not prevail) in a cyanobacterial monoculture, then their elimination can be done easily with the appropriate doses of cyclohexamide. Although cyclohexamide acts as an inhibitor of mitosis, its effect on possibly present protozoa is weaker and requires continuous inoculation with it and a long time to eliminate them.
• Diatoms that can easily overwhelm an algal monoculture are completely eliminated with appropriate doses of germanium dioxide.
• The filamentous cyanobacterium *Phormidium* sp. requires the least care to be cultured continuously monospecifically.

• The coccoid cyanobacterium *Synechococcus* sp. proved to be an excellent food for protozoa, rotifers, copepods and *Artemia*.
• Only basic information about the organisms kept in the laboratory is mentioned here. In particular, data from research on their culture can be found in the relevant literature.
• In addition to the described species, the well-known microalgae, *Tetraselmis suecica*, *Rhodomonas salina* and *Isochrysis galbana* have been preserved in the laboratory for 25 years.
• Long-term maintenance of these organisms in the plankton culture laboratory requires constant vigilance and attention to the required renewals. In other words, "Did you leave them?", “They left you."
• A number of worthwhile graduate theses and doctoral theses can be produced in the plankton culture laboratory if candidates decide that they are willing to devote themselves completely to the chosen organisms.

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