THE ROLE OF THE CILIATE PROTOZOAN FABREA SALINA IN SOLAR SALT PRODUCTION

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ABSTRACT

Production of salt from seawater involves the selective recovery of pure NaCl, free of other soluble or non-soluble salts and other substances. To this end, condensation of seawater through solar evaporation results in the fractional crystallization of all contained salts; a process based on their varying solubility in seawater.

Current, industrial Solar Saltworks consist of a series of successive lakes where a salinity vector is being developed by solar and wind energy. It is well known that throughout the lakes system, along with the salinity vector, an extremely important biological process is also developed consisting of planktonic and benthic communities of microorganisms in large populations [2, 4]. As each lake functions at **steady state**, a unique ecosystem is created where regular and hyper saline environments coexist.

The physical and the biological process of Solar Saltworks interact strongly and affect both the quantity and quality of their final product. Every microorganism present in the Saltworks pond system has an impact (major or minor) to the solar sea salt production process.

In this paper the role of the protozoan ***Fabrea salina*** is examined in the rare and unusual case that **dominates in part of the Saltworks pond system**. This fact became reality after an unfortunate conjuncture in Messologhi Saltworks and gave us the opportunity to study on a large scale this rare occurrence and report on its impact on the biological process in the pond system and crystallizers and the final product quality.

INTRODUCTION

Current industrial Solar Saltworks recover salt (NaCl) from seawater using a system of interconnecting successive lakes [7]. The available area for salt production is divided into a number of connected in series mainly, **lakes** (ponds) which are distinguished in two main groups. The first group usually called *Evaporation Ponds **(EPs)***, is used to condense seawater up to saturated (with respect to NaCl) density and the second group usually called *Crystallization Ponds* ***(CPs)*** or *Crystallizers* or *Pans* is used to produce salt.

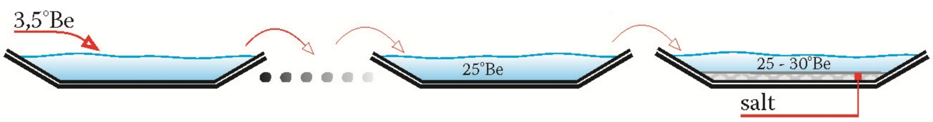


Figure 1. Interconnecting successive lakes system used for sea salt production.

The first EP of the system is fed by seawater into the by pumping and/or tidal action. The density of the seawater gradually increases by solar evaporation as it flows into the system of EPs. Finally the last Evaporation Pond, usually called *Feeding Pond **(FP)***, feeds with saturated brine the CPs where solar evaporation causes salt crystallization. The driving force that causes brine evaporation is the incident solar radiation and its rate depends on the prevailing microclimate of the region, particularly wind action, air temperature and air moisture.

Hence, a salinity (brine density) vector is developed throughout the Saltworks production area (EPs & CPs), with a simultaneous brine volume reduction, which eventually leads to NaCl crystallization/production. This constitutes the ***physicochemical (or physical) process*** of Solar Saltworks.

In parallel with the physical process, an extremely important microbial community develops in the EPs and CPs of Solar Saltworks, composed in planktonic and benthic communities and covering all three domains of life, Eukaryotes, Bacteria and Archaea [7].This chain of organisms constitutes the ***biological process*** of Solar Saltworks.

MESSOLOGHI SALTWORKS

Messologhi Saltworks is located in Western Greece next to the important lagoon of the historic town of Messologhi. It is the largest saltworks in the country and occupies an area of 11.000 acres.

It is worth mentioning a particular feature of the production area of Messologhi Saltworks that enabled us to do some extra testing to achieve more comprehensive results. In particular its crystallization area consists of two separate groups of crystallizers. As a consequence a part of the EP's area is also divided are two groups according to the following scheme:

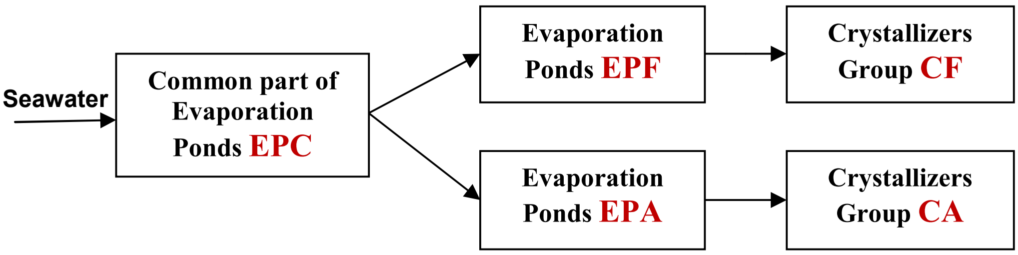


Figure 2. Production scheme of Messologhi Saltworks.

All Saltworks in Greece are seasonal. That is they operate/produce salt only during the summer season, from April to September each year when the produced salt is harvested and stockpiled. From November to March each year they lie fallow as the prevailing weather conditions do not favor evaporation. The unused amounts of concentrated brines are stored in specially constructed deep lakes (**BD**, Brine Deposit Ponds) where dilution from winter rainfall will be low, in order to be reused at the beginning of the next year's production.

UNFORTUNATE CONJUNCTURE

The stored brines in Messologhi Saltworks Brine Deposit (BD) ponds, was found with excessive organic load which resulted in poor salt crystallization and product quality. It was decided then to reduce the amount of stored brines by emptying one BD pond. This decision gave also the opportunity to optimize the relevant pond's design by constructing new dikes using a suitable earthmoving machine (Crawler Drag Line).

Unfortunately, on the completion of the project, the machine was trapped in the middle of the pond and by the operator's attempt to drive the machine out, the surface of the pond was disturbed and agitated to a large extend. The problem actually arose when the brine (~13 ^{o}Be) used to fill the pond into consideration, at the start of the production, was not thrown away but was led, with the entire suspended biological load, to the crystallizers.

This happened because when the pond was filled with brine, the unfortunate conjuncture of the disturbance and agitation of its surface, a vast population of *Fabrea salina* cysts released from the surface of the dried pond and also nutrients released from its benthic community to the planktonic community of the circulating brine. Under these conditions the population of *Fabrea salina* exploded and dominated in the planktonic communities of both groups of crystallizers (CF & CA) and evaporation ponds EPF & EPA.

Therefore, at the early beginning, the production process in Messologhi Saltworks was locked and forced to operate with an extensive disturbance in the developing biological process in almost all high salinity ponds and the crystallizers. According to samplings carried out by the Inter Balkan Environmental Center, the evaporation ponds EPA & EPF and the crystallizers CA & CF, were filled with brines dominated by *Fabrea salina*.

FABREA SALINA

There are vague notations in the literature concerning the role of *Fabrea salina* on the production of good quality salt. In bibliography and published papers the protozoan *Fabrea salina* is not reported as a main organism in the biological process of Solar Saltworks. Its occurrence has reported occasionally [1, 3, 5, 6] and its presence is connected with a severe decline of *Artemia* population, excessive reproduction of *Aphanothece halophytica* and increased export of organic substances to the downstream ponds [3].

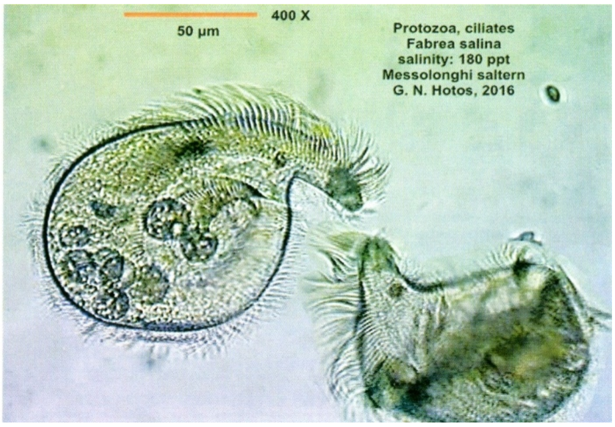


Figure 3. The ciliate protozoan *Fabrea salina*. (G. N. Hotos, Messologhi Saltworks 2016)

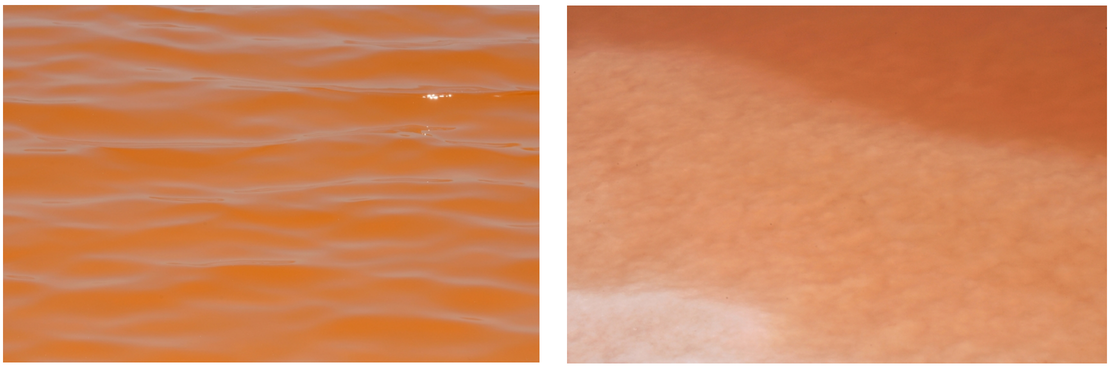
In fact its presence is neglected in most studies concerning saltworks. Davis (2000) is more specific as he considers *F. salina* rather a nuisance than a benefactor. In several relevant studies (Coleman & White, 1993; Davis, 1993, 2000, 2006), the importance of the biota on the production of good salt is emphasized and general outlines of its proper manipulation are presented. However it is very difficult to find clear-cut instructions as to what should be specifically done concerning *Fabrea salina* and its role in solar salt production.

IMPACTS ON BIOLOGICAL PROCESS

The first observation with the naked eye was the complete disappearance of *Artemia* from all ponds where *Fabrea* was dominating. The brine became turbid and cloudy, its color turn to dark brown and it was hard to see the pond bottom. According therefore to our knowledge and experience it was reasonable to expect a catastrophic year with minor production of poor quality.

Dark brown turbid and cloudy brines dominated by Fabrea salina.

However, with great surprise we found that the system evolved in an entirely different way! As the brine in crystallizers group CA reached concentration density first (it was fed with higher density brine than the group CF) and by the time that salt starts to precipitate the color of the brine gradually changed from brown to red! It was like a miracle! *Fabrea salina* disappeared and in its place, the *Halobacterium salinarum* developed into large populations and occupied its dominant position in the CA crystallizer's brine! The brine also became crystal clear and transparent!



Clear red brines in crystallizers dominated by Halobacterium.

It looks like the F. salina has successfully replaced *Artemia* in the pond's biological chain. Its predominance in the brines resulted in the consumption of *Dunaliella*, *Synechococcus and Asteromonas* whereas the *Halobacterium* was kept in a prolonged state of inertia and dormancy. When the brine density in crystallizers rose above 26 ^{o}Be, the population of *F. salina* collapsed and since almost all other organisms have disappeared, the room was free for *Halobacterium* to proliferate.

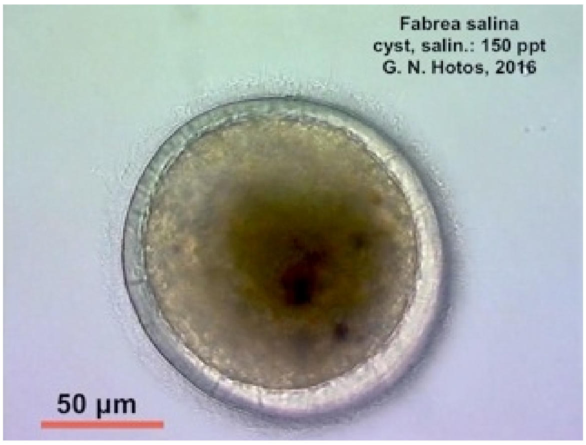


Figure 4. *Fabrea salina* cyst. (G. N. Hotos, Messologhi Saltworks 2016)

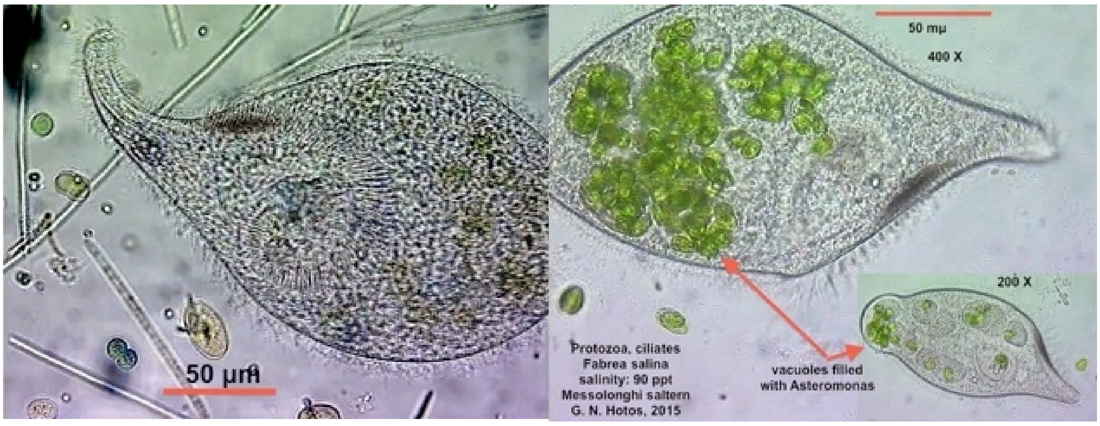


Figure 5. *Fabrea salina* digesting *Synechococcus* cells (left) and with ingested *Asteromonas* cells (right). (G. N. Hotos, Messologhi Saltworks 2016)

Capitalizing on this trend, we made the appropriate arrangements in brine movement, in order to accelerate the relevant process in the CF group of crystallizers. Finally we end up with a biological process where the brines in all high salinity evaporation ponds EPA & EPF including the feeding ponds were dominated by *F. salina* whereas the crystallizers were dominated by *Hallobacterium* and the salt produced was of excellent quality.

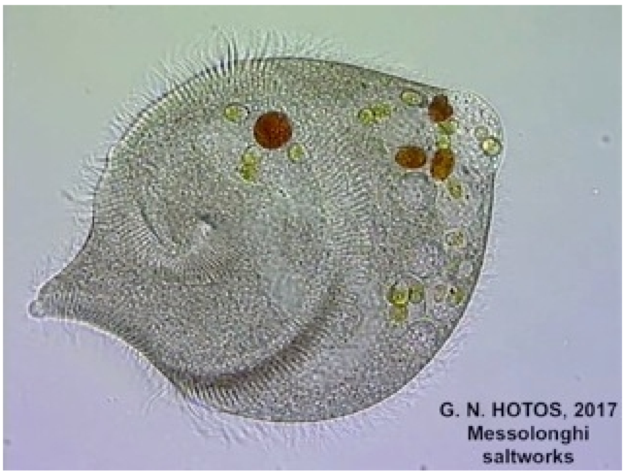


Figure 6. *Fabrea salina* with ingested *Dunaliella* cells.
(G. N. Hotos, Messologhi Saltworks 2017)

DISCUSSION AND CONCLUSIONS

According to a logical scenario supported from key facts on the life cycle of *F. salina*, it is probable that a lot of dormant cysts (Fig. 4) of this ciliate had been previously accumulated in the mud of the dried pond. From unpublished data (Hotos, 2016, personal observations) it was found that after its dormancy, *Fabrea* that emerged from cyst is very active in feeding. Actually this ciliate is a voracious consumer of phytoplankton (Fig. 5, 6) and presumably bacteria. It can consume large-celled chlorophytes (*Dunaliella*, *Asteromonas*) and cocciform cyanobacteria (*Synechococcus*) that can be seen en mass into its digestive vacuoles. It is logical to assume that it consumes bacteria as well. By *Synechococcus* we mean the mucilage producing cyanobacteria in general as the frequently used in many papers synonyms *Aphanothece* sp. and *Coccochloris* sp. are probably based on their rough morphology than to molecular indentification.

In our case *Fabrea* that "awoke" from its cyst dormancy found favorable conditions and its population exploded. It rapidly consumed the bulk of microalgae and at the same time kept grazing on bacteria. The bacteria including *Halobacterium* was thus prevented from reaching self-limiting numbers and were kept in a prolonged state of "biological youth" [6] with a high rate of assimilation of organic material. Soon after the brine density rose above 26 ^{o}Be, the population of *Fabrea* collapsed. *Halobacterium* took over and excellent salt crystallization occurred. We finally note that the finding that the Fabrea salina releases mucilaginous substances [3] was not verified by the results of this study.

It is obvious that the role of *Fabrea salina* should be examined more thoroughly throughout the whole biological process of Solar Saltworks. In order to exploit the results presented here, we believe that an elaborate study is needed since the case we are examined arose from an unusual disturbance rather than from the normal operation of Solar Saltworks.

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