EFFECT OF SALINITY ON THE GROWTH AND REPRODUCTION OF A BRACKISH WATER SPIROGYRA (S. SALIN A nov. sp.)

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EFFECT OF SALINITY ON THE GROWTH AND REPRODUCTION OF A BRACKISH WATER *SPIROGYRA* (*S. SALINA* nov. sp.)

by Anwar Abdel ALEEM

ABSTRACT

A new species of *Spirogyra*, inhabiting brackish waters in Etang « Canet » and Etang « Salses », Pyrénées-Orientales, France, is described as *S. salina* nov. sp.

The species thrives in salinities ranging between 5 and 17.5 °/₀₀. Culture experiments have been made to test the growth and reproduction of this species under different salt concentrations.

Profuse vegetative growth was obtained at a salinity of 13 °/₀₀, while sexual reproduction was frequent in cultures containing between 3-15 °/₀₀ salt concentration, with an optimum at 7°/₀₀.

At higher salinities, the degree of failure among cells to form gametangia or among gametes to copulate increases enormously. Sexual reproduction seems suppressed at 20°/₀₀. Parthenogenetic spores seem to develop in cultures of salinities higher than 15 ‰.

The above experiments support, to a great extent, the range of salinities tolerated by the species in nature.

INTRODUCTION

During a study of the microphytic communities in brackish water lakes along the Mediterranean coasts of France, I came across a species of *Spirogyra* in Etang « Canet » and in Etang « Salses » in the Pyrénées District in March 1952. This species flourished well in salinities ranging between 5 and 15.4 °/₀₀. A note had already been published on the ecology and distribution of this species in the brackish water environments above mentioned (ALEEM, 1952). However, its systematic position remained unclear.

The occurrence of *spirogyra* in brackish waters has only rarely been recorded. Thus KOLKWITZ and KRIEGER (1941), in Rabenhorst's Kryptogamen Flora, state that OLTMAN found a *Spirogyra* in waters of salinities ranging between 5 and 7.5 °/₀₀. TRAHMS (1939) found *S. Weberi* with conjugating filaments at 8 °/₀₀ salt concentration. As far as I am aware, no *spirogyra* seem to have been recorded in salinities higher than this latter value.

For this reason it was thought advisable to undertake a study of the influence of different salt concentrations on the growth and reproduction of the species under consideration. Laboratory cultures have been made and results are given below. At first, it may be necessary to clear the systematic position of the species and to include some more field observations on its distribution and ecology.

SYSTEMATIC ACCOUNT OF THE SPECIES.

In our previous note (*l.c.*), the affinities of this species to *Spirogyra esthonica* (Skuja) Cjurda had been suggested. However, it differs from the latter in certain morphological respects. For example, vegetative cells in our material (fig. 1, A) are somewhat larger; zygospores are also bigger, with more rounded ends. The outer and median coats of the spore (fig. 1, H) are thicker and the sculpture of the latter is different.

The essential character, which separates the two, however, lies in the fact that in *S. esthonica* the copulating cells or gametangia are differentiated from vegetative cells and are separated by intervening stretches of sterile cells. While in our species the capacity of cells to form gametangia is unlimited and there are no such stretches of vegetative cells between gametangia (fig. 1, E). One could thus meet with as many as 20 zygospores in succession on the same filament.

Another difference between the two is that the cells which fail to copulate in our species, are usually inflated, but not so in *S. esthonica.*

For these considerations and for the particular habitat in which the species has been found in nature, we tend to describe it as a new species as follows :

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(1) In a former note (PETIT et ALEEM, 1952) this species was referred to as *S. subsalina,* but this nomenclature had been used before.

Spirogyra salina nov. sp. :

Fila praelonga, ad basin contorta. Cellulae vegetativae 30-40 μ *latae,* 90-400 (450) μ *longae, chromatophoro uno anfractibus* 2-6; *conjugatio plerumque scalariformi, minus latere. Zygotae formatae aliquando parthenogenese.*

Tubum copulativum praecique emittens gametangia masculina et sic minime gametangia feminina. Gametangia non separata a non cellulis intervenientibus et sterilibus. Non copulativae cellulae tumidae ; aliquands 3 *fila copulativa.*

Gametangia masculina 20-25 μ *latae, ad* 80 μ *longae ; gametangia feminina* 25-30 μ *latae, ad* 100 μ *longae. Zygotae maturae badiae. Zygotae ellipsoideae vel globosae,* 32-62 μ *latae,* 60-85 μ *longae. Binatae zygotae non rarae. Mesosporium crassum et ornatum.*

Ad aqua salina fossa « Canet » Pyrénées et Galia.

Filaments elongated, contorted at the base (fig. 1, B); cells 30-40 μ broad, 5-10 (12) times as long, with one chromatophore of 2-6 spiral turns; nucleus median.

Copulation predominately scalariform (fig. 1, C), rarely lateral. Zygospores may also be formed by parthenogenesis. Copulation tubes are emitted by male and female gametangia, though more frequently by the former. Gametangia are not separated by intervening stretches of sterile cells; noncopulating cells inflated. Sometimes 3 filaments copulate (fig. 1, F).

Male gametes are 20-25 μ broad and up to 80 μ long; female gametes 25-30 μ broad and up to 100 μ long.

Mature zygospores are brown red in colour with more or less rounded ends; outer membrane hyaline and thick, median coat thick and sculptured.

Zygospores ellipsoid or globose, measuring ca 35-62 μ wide and 60-85 μ long. Parthenogenetic spores ellipsoid or globose, measuring ca 30 μ wide and 45-80 μ long.

Binate spores (fig. 1, G) are not infrequent.

Material has also been sent to Professor H. SKUJA, Sweden and to Dr. M. GODWARD, England and both authorities are of the opinion that this species is new. Professor SKUJA adds that « it probably represents an elementary species which comes near to *Sp. oborata.* »

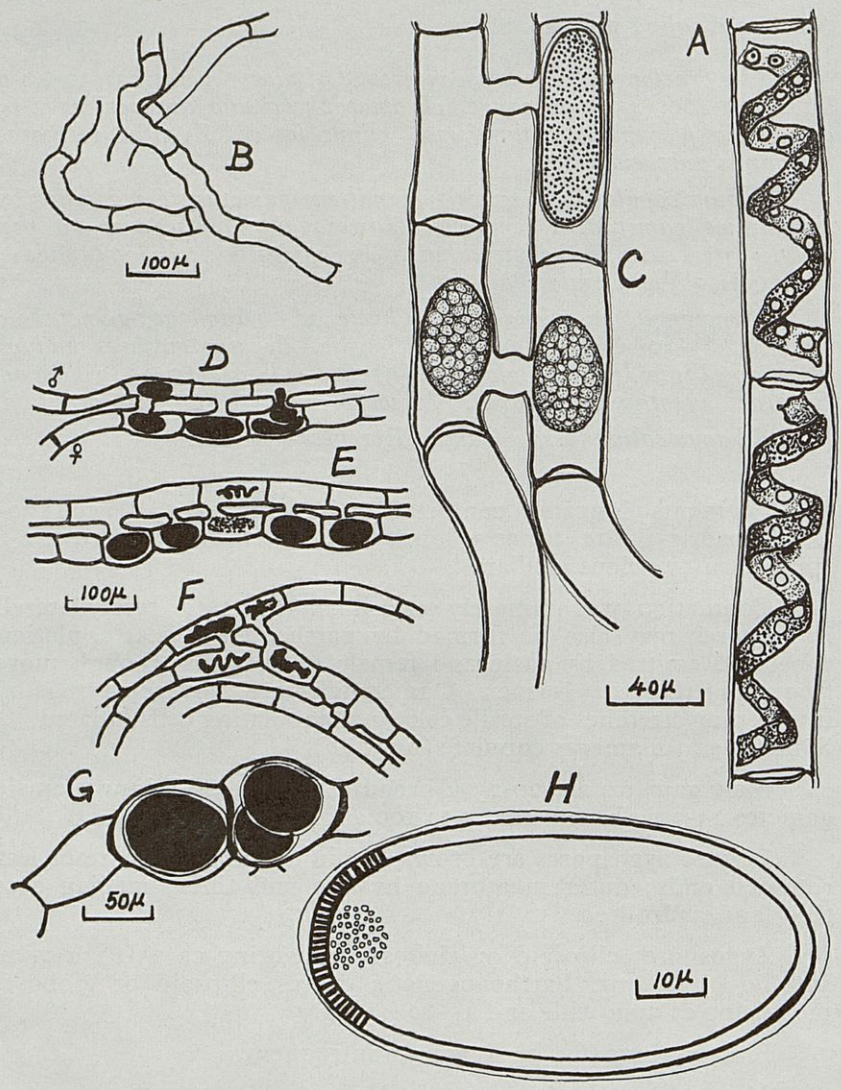

Fig. 1. — A, vegetative cells; B, contorted basal cells of filaments; C, Copulating filaments showing scalariform type, an immature zygospore and two ripe gametangia; D, Gametes in the process of copulation; E, several zygospores without intervening stretches of sterile cells, note inflated non-copulating cells on female filament; F, copulation between 3 filaments; G, globose zygospores with binate spores in a single cell; H, mature zygospore in optical section, showing outer and inner coats and the sculpture pattern of the zygospore (*highly magnified*).

Ecological observations

Apart from the observations already mentioned in our paper referred to above, the following additional notes on the ecology of the species are given.

In Etang « Canet » and Etang « Salses » further localities for the species were found, thus extending the range of salinity for the species from 5 to 17.5 °/₀₀. In Etang « Salses », however, the plant was confined only to those localities with relatively lower salinities (9-12 °/₀₀). The *p*H in these waters fluctuates between 6,6 and 8,2. The water with lower *p*H is usually confined to isolated ponds and ditches; being stagnant and mostly polluted, with brownish colour due to bacterial growth.

The plant may be found floating on the surface, attached to other algae and phanerogams or even free living at the bottom of the lake.

On one occasion, extensive growth of *S. salina* was covering the surface of a deep water pond (S = 14.2 °/₀₀) at « Canet », so that literally speaking it clogged the pond. This pond is adjoining the sea and receives influxes of sea water during rough weather.

On the lake littoral at « Canet », *Spirogyra salina* is found intermingled with other algae such as *Ulva lactuca*, *Enteromorpha intestinalis*, *Chaetomorpha linum*, and *Ceramium diaphanum*.

It also grows attached to halophytes such as *Pragmites* and *Salicornia*.

Profuse growth of *Spirogyra salina* in those lakes occurs during the spring, from March onwards.

Sexual reproduction is at its maximum from the end of april to the middle of may, after which time *Spirogyra* becomes yellowish brown in colour and is subject to severe attacks by parasitic fungi. Filaments of *Spirogyra* disintegrate and the plant disappears altogether during june.

Among the parasitic fungi encountered, mention may be made of the following species : *Myzocytium proliferum* Schenk., *Lagenidium entophytum* (Pringsh.) Zopf and *Rhizophydium Couchii* Sparrow.

The disappearance of *Spirogyra* may also be enhanced by the advent of summer and increasing water temperature in those shallow lakes. During june, the water temperature in Etang « Canet » and Etang « Salses » occasionally rises up to 30° C.

Observations in cultures

Cultures from fresh material of *S. salina* were started in the beginning of april. In these cultures equal numbers of a few, apparently similar, young vegetative filaments of equal lenghts, were thoroughly washed in sterile solution and inoculated into liquid media, kept in test tubes.

After a few preliminary experiments with enriched lake water or diluted sea water, the following medium was chosen as a basic medium for all subsequent cultures.

It has a composition of one litre of off-shore sea water, brought from 8 miles off Port-Vendres, to which are added 50 ml of soil extract and a few drops of a solution containing 50 mg. $Na_2 NO_3$ and 20 mg K_2HPO_4/l. Dilutions were made with distilled water, so as to obtain the following series of salinities :

1°/₀₀, 2°/₀₀, 3°/₀₀, 7°/₀₀, 13°/₀₀, 15 °/₀₀, 17 °/₀₀ et 20 °/₀₀. In these media the *p*H ranged between 7.2 and 8. Such a range does not seem to affect seriously the growth in cultures, since the plant in nature tolerates still a wider range.

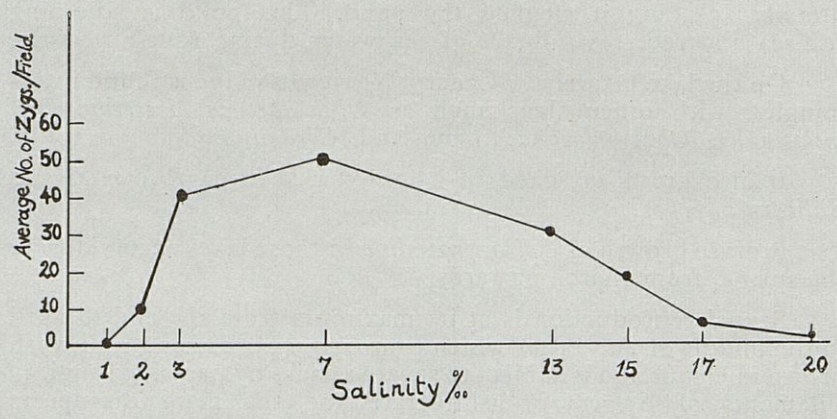

Fig. 2. — Zygospore formation at different salinities in cultures 8 weeks old.

Parallel experiments were made in similar media but without addition of nitrates, in an attempt to test whether the nitrate concentration used had any inhibiting effect on conjugation. No marked difference was observed.

Culture vessels were kept in the laboratory on a north window at room temperature and frequently examined.

The results of one such experiments, run for 8 successive weeks, are shown in the included photographs (fig. 3 and 4). The following notes are added on the growth condition and reproduction in the various salinities employed.

Cultures in 1°/₀₀ *Salinity* :

In this salinity very little growth had taken place within two months. No zygospores or copulation tubes had been observed. The cells were not healthy and filaments were often twisted and tended to break and disintegrate.

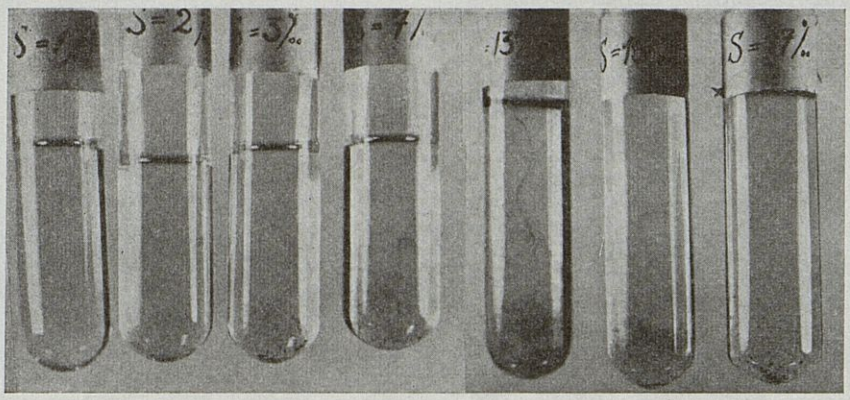

Fig. 3. — Cultures of *S. salina* nov. sp. in different salt concentrations after 4 weeks

Cultures in 2°/₀₀ *Salinity* :

Several new filaments grew, attaining a length of 3-4 cm in 2 months. Vegetative cells grew longer, reaching up to 400 μ. Gametangia and copulation tubes, at different stages of maturity were rather frequent. Several zygospores were found, but the majority were immature. The average number of zygospores, based on several random counts under low power of the microscope, was about 10 per field.

Cultures in 3°/₀₀ *Salinity* :

Still better growth took place in this salinity. Filaments grew up to 6 cm or more.

Gametangia were more frequent and more mature. In some filaments as much as 12 zygospores, in succession, on the same filament were encountered.

Average number of zygospores per field was about 40±5.

Cultures in 7°/oo *Salinity* :

There is not much difference in vegetative growth between this culture and the preceding one. The number of zygospores, however, is greater and most of these are mature ones; they amount to about 50 per field. This is the largest number of zygospores encountered among all the cultures.

Cultures in 13°/oo *Salinity* :

In this culture profuse vegetative growth took place, but gametangia and mature zygospores were less frequent than in the preceding culture.

Inflated cells are numerous. Average number of zygospores per field amounts to about 30.

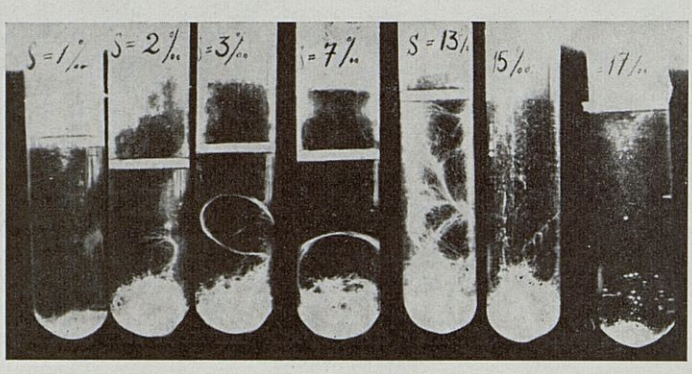

Fig. 4. — Same cultures in fig. 3 after 8 weeks, for comparison.

Cultures in 15 °/oo *Salinity* :

The behaviour of *Spirogyra* in this salinity is very much the same as in the preceding one. However, the number of zygospores is still smaller, amouting to an average of 19 per field. Of these only about one third are mature.

Inflated cells, on the other hand, are much more numerous than in all the preceding salinities. This indicates a tendancy of gametangia towards failure to copulate.

Cultures in 17 °/oo *Salinity* :

No copulating gametangia were encountered. Copulation tubes are met with rahter frequently, but they are not connected.

They are found emitted from vegetative cells that had shown no signs of gamete formation. Chromatophores are yellowish in colour, shrinking and apparently unhealthy.

The average number of zygospores encountered is very small, being 4-5 per field.

Cultures in 20 °/°° *Salinity* :

In this culture copulation tubes are occasionally present, but much underdeveloped. The majority of these are small protuberences emitted from the ends of vegetative cells containing spiral chromatophores.

Copulation, if any, must have been very rare. I have not seen any coplating gametes. The average number of zygospores per field amounts to 1.6; most of these are of small size and could have been developed parthenogenetically, since no traces of conjugation canals were seen attached to such cells.

CONCLUSIONS

From the foregoing observations on cultures of *S. salina* in different salinities, it becomes apparent that maximum vegetative growth takes place at a salinity of 13 °/°°, while sexual reproduction was at its best at a salinity of 7 °/°°, under the cultural conditions of this experiment. Both vegetative growth and sexual reproduction were suppressed at the very low salinity (1 °/°°) as well as in the highest salinity employed (20 °/°°).

If the average number of zygospores is plotted against the different salinities, we obtain the graph illustrated in figure (2). This shows that a salinity range between 3 °/°° and 13 °/°° was very favourable for conjugation. On the other hand parthenogenetic development of zygospores seems to be favoured by higher salinities. The tendency towards an increasing failure of sexual reproduction at higher salinities is shown by the emittence of copulation tubes in the form of protuberances or rather short canals which scarcely meet together. Besides, cells which fail to copulate are rather inflated.

In nature, all ranges of salinities between 5 and 30 °/°° exist in those shallow water lakes along the Mediterranean littoral of France, but the species was found only within the range recorded. Even in one and the same Etang like « Canet », where the growth of this *Spirogyra* was profuse, the species failed to grow at a station with a salinity of 21.6 °/°°.

The above experiment supports to a great extent the salinity limits observed in nature as far as the distribution of the species in concerned. However, much remains to be done on several other interesting physiological problems displayed by this. *Spirogyra.*

In concluding this paper, I whis to thank Professort G. PETIT, Director of Laboratoire Arago, Banyuls-sur-Mer, for the generous working facilities put at my disposal. Grateful thanks are due also to Professor H. SKUJA, Upsala and to Dr. M. GODWARD, Queen Mary College, London for their advise in the identification of the plant.

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