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THE INFLUENCE OF DIFFERENT SALINITIES ON GROWTH AND MORPHOLOGICAL VARIABILITY OF A NUMBER OF PHORMIDIUM STRAINS (CYANOPHYCEAE) IN CULTURE

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SUMMARY

Phormidium strains from freshwater- and marine habitats were submitted to culture experiments in media with different concentrations of seawater. The marine strains showed a greater halotolerance and less morphological variability than the freshwater ones. The results are discussed with regard to Drouets species concept within the Cyanophyceae.

1. INTRODUCTION

In his "Revision of the Oscillatoriaceae", DROUET (1968) reduces the number of species to 23, distributed over six genera. On one of these species, *Schizothrix calcicola* (Ag.) Gomont, a detailed report was published (DROUET 1963), in which 54 taxa described by GOMONT (1892) and other authors are considered as ecophenes (ecological growth forms) of this species.

According to DAVIS & HEYWOOD (1963) the ecophene – a concept of Turesson – is defined as: "The reaction-types of the ecotypes called forth by the modificatory influences of extreme habitat factors", or, in other words: "The characteristic phenotype produced in each particular locality".

The second definition fits best with Drouets conception of the ecophene or ecological growth form when we replace "characteristic phenotype" and "particular locality" by "morphological characteristics" and "natural habitat and in laboratory culture", respectively.

In conclusion it has to be pointed out that ecophenes of one species have the same genotype, and that their morphological characteristics are essentially reversible.

Apparently, according to Drouet, the considerable morphological and ecological variability of the cyanophycean species is not the expression of genetic variation within the species, but of the species' ability to produce a wide range of morphological and ecological variation on the basis of one and the same genotype, and in reaction to the environmental variation. In other words, each cyanophycean species has only one genotype, but many phenotypes. In having such a straightforward type of species, the Cyanophyceae would be an unusual class of organisms. Therefore we decided to test Drouets species concept within the Cyanophyceae with regard to the ecological and morphological variability

of his "Schizothrix calcicola", a species which encompasses saltwater and freshwater ecophenes. The present paper treats the growth, survival, adaptation and morphology in different salinities of a number of *Phormidium* strains isolated from freshwater and marine habitats.

2. MATERIALS AND METHODS

2.1 Strains

Table 1 enumerates the blue-green algal strains used, which are hereafter referred to by their strain numbers.

Table 1. Used blue-green algal strains.

strain number	species name	origin/findingplace
426	*Phormidium luridum* var. *olivacea* Boresch	Indiana culture collection (1)
427	*Phormidium foveolarum* Gomomt	Indiana culture collection (1)
482	*Plectonema notatum* Schmidle	Indiana culture collection (1)
485	*Plectonema* spec.	Indiana culture collection (1)
487	*Lyngbya* spec.	Indiana culture collection (1)
488	*Lyngbya* spec.	Indiana culture collection (1)
581	*Plectonema boryanum* Gomont	Indiana culture collection (1)
594	*Plectonema boryanum* Gomont	Indiana culture collection (1)
595	*Plectonema boryanum* Gomont	Indiana culture collection (1)
596	*Plectonema boryanum* Gomont	Indiana culture collection (1)
597	*Plectonema boryanum* Bomont	Indiana culture collection (1)
598	*Plectonema calothrichoides* Gomont	Indiana culture collection (1)
790	*Plectonema boryanum* Gomont	Indiana culture collection (1)
71/12.1	*Schizothrix calcicola* (Ag.) Gomont	Waddenzee, landreclamation sectors (2)
71/13	*Schizothrix calcicola* (Ag.) Gomont	Waddenzee, on shells (2)
71/14.1	*Schizothrix calcicola* (Ag.) Gomont	Waddenzee, on barnacles (2)
71/16.4	*Schizothrix calcicola* (Ag.) Gomont	Waddenzee, landreclamation sectors (2)

(1) According to the list of cultures of the Indiana culture collection. Freshwater strains.
(2) According to identification with DROUET (1968). Seawater strains.

2.2 Growth conditions

The algae were grown in modified Chu-10 medium (CHU 1942), consisting of: 810 mg KNO_3; 64 mg $CaCl_2$; 10 mg K_2HPO_4; 25 mg $MgSO_4.7H_2O$; 54 mg $Na_2CO_3.10H_2O$; 37.4 mg $Na_2SiO_3.9H_2O$; 5.78 mg ferricitrate.$5H_2O$; 3.82 mg citric acid.$1H_2O$; 200 mg EDTA; 10 mg $ZnSO_4.7H_2O$; 1.212 mg $MnSO_4$·

$1H_2O$; 0.05 mg $CuSO_4.5H_2O$; 10 mg H_3BO_3; 1.6 mg $Co(NO_3)_2.6H_2O$; 1 mg $Na_2MoO_4.2H_2O$; 0.18 mg vitamin B_{12}; 3.66 mg vitamin B_1; 2.44 mg biotin, added to 1000 ml demineralysed water and/or filtered seawater (whether or not concentrated by evaporation) to obtain the desired salinity. Before autoclaving the pH was adjusted to 8-9.

Mostly 0.2 ml from a three week old stock culture were inoculated into a cotton-wool plugged culture tube containing 10 ml medium. Incubation was carried out at 25 °C, 800 lux (cool white fluorescent tube, Philips TL 20W/34 de luxe) and a 12 hr. light – 12 hr. dark period.

2.3 Test procedure

Growth rates were determined qualitatively by macro- and microscopic observation and quantitatively by cell number counting in a Bürker counting chamber and by measuring the chlorophyll-a concentrations (expressed as extinctions at 665 nm and 431 nm) in cell extracts.

2.3.1 Extraction procedure

Five ml of a culture were centrifuged, and the pellet resuspended in 4 ml 90% aceton, to which 100 mg $MgCO_3$ was added. Cell walls were fractured by sonification in a ultrasonic disintegrator (MSE, 100 Watt) for two minutes at maximum output; this was followed by a 24 hrs extraction at 4 °C in the dark. After centrifugation, the extinction of the supernatant at 665 nm and 431 nm was determined in a Zeiss spectrophotometer.

2.4 Experimental procedures

2.4.1 Tolerance experiments

All strains were incubated during 42 days in medium with 0% (= freshwater), 50% and 100% seawater, and the seawater strains also in 200% seawater medium.

2.4.2 Recovery experiments

After incubation of all strains during 7 and 28 days in 0%; 100%; 200%; 400% and 1000% seawater medium (no incubation was performed with the freshwater strains in 400% and 1000% seawater medium for 28 days), the cells were centrifuged and reinoculated into 0% and 100% seawater medium for the fresh- and the seawater strains, respectively. This was followed by another ten days incubation.

2.4.3 Adaptation experiments

Freshwater strain 427 was inoculated from freshwater medium into 20% seawater medium and incubated during 7; 14; 21 and 28 days. Hereafter the cells were transferred into 40% seawater medium and, to obtain a reference for

growth, cells from one culture tube were transferred into 20% medium again. This procedure was repeated for 40% to 60%, 60% to 80% and 80% to 100% transfers, respectively.

3. DESCRIPTION OF THE STRAINS

3.1 Freshwater strains

Extensive microscopic observations of cultures in freshwater medium, led to the conclusion that the thirteen freshwater strains are morphologically identical, and can be described as follows:

Trichomes blue-green to green, not or slightly constricted at the crosswalls, 1.9-2.6 μm in diameter, straight or curved, without or with a very thin colorless sheath. Cells quadratic to rectangular, 1.9-2.9 μm in length *(table 2)*. Terminal cell rotund to hemispherical *(fig. 1)*.

3.2 Seawater strains

3.2.1 Strain 71/12.1

Trichomes green to yellow-green, forming a gelatinous green pellicle, constricted at the crosswalls, 1.3-1.9 μm in diameter, straight, with a thin colorless sheath. Cells longer than broad, 2.6-3.9 μm in length. Terminal cell rotund to hemispherical *(fig. 2)*.

3.2.2 Strain 71/13

Trichomes green to yellow-green, forming a gelatinous green pellicle, con-

Table 2. Cell dimensions of the freshwater strains. Each figure represents an average of 50 measurements. The lowest values were determined on trichomes grown on 0% seawater medium solidified with 1.5% agar, the highest values on trichomes grown in 0% seawater medium.

strain number	cell-width (in μm)	cell-length (in μm)
426	1.9–2.0	2.0–2.2
427	2.1–2.3	2.2–2.7
482	2.0–2.2	2.0–2.7
485	2.1–2.3	2.1–2.6
487	2.0–2.3	2.1–2.6
488	2.2–2.3	2.3–2.4
581	2.3–2.4	2.3–2.7
594	2.0–2.1	2.0–2.1
595	2.0–2.3	2.6–2.7
596	2.0–2.3	2.3–2.4
597	2.1–2.5	2.3–2.5
598	2.0–2.1	2.0–2.3
790	2.0–2.2	2.1–2.3

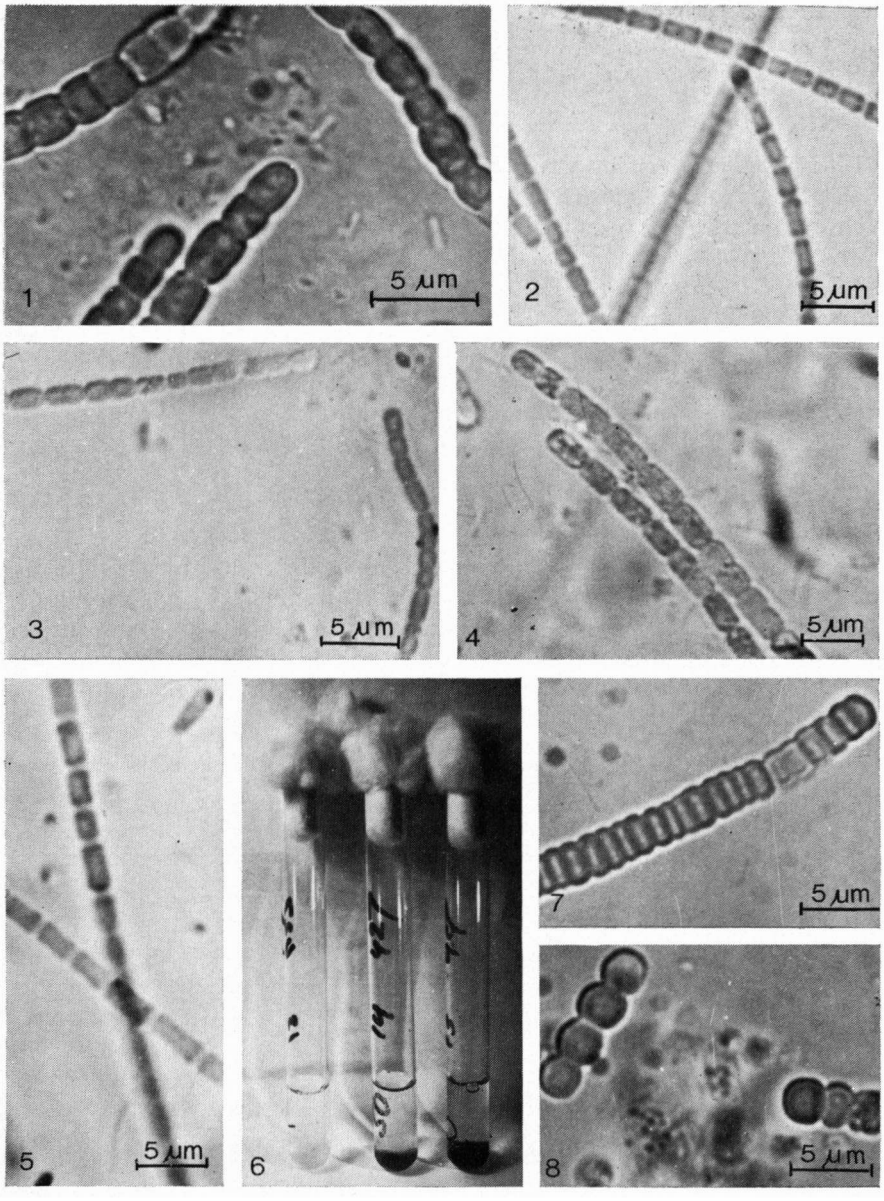

Fig. 1–5. Trichomes of a four weeks old culture of the freshwater strain 482 and the seawater strains 71/12.1; 71/13; 71/14.1 and 71/16.4 respectively. Fig. 6. Cultures of the freshwater strain 427 after six weeks of incubation in medium with, from the right to the left, 0‰, 50% and 100% seawater. Fig. 7–8. Trichomes of the freshwater strain 482, after six weeks incubation in 50% and 100% seawater medium respectively.

stricted at the crosswalls, 1.3-2.6 μm in diameter, straight or curved, having a thin colorless confluent sheath. Cells quadratic to rectangular, 2-5 μm in length. Terminal cell rotund to hemispherical *(fig. 3)*.

3.2.3 Strain 71/14.1
Trichomes green, forming a gelatinous green pellicle, constricted at the crosswalls, 1.9-2.1 μm in diameter, straight, having a thin colorless confluent sheath. Cells 1-3 μm in length. Terminal cell rotund to hemispherical, sometimes spherical *(fig. 4)*.

3.2.4 Strain 71/16.4
Trichomes green, forming a gelatinous green pellicle, constricted at the crosswalls, 1.3-2.0 μm in diameter, straight, having a thin colorless confluent sheath. Cells 1.5-3.5 μm in length. Terminal cell rotund *(fig. 5)*.

4. Results

4.1 Tolerance experiments

Table 3 shows that all freshwater strains decreased in growth by an increase in salinity, with no growth at all in 100% seawater medium *(fig. 6)*.

The four seawater strains grew well in all tested salinities, and, just like the freshwater strains, grew best in 0% seawater medium.

Table 3. Tolerance experiment.
Growth of all fresh- and seawater strains after 42 days of incubation in media with different seawater concentrations.

	concentration seawater in the medium			
	0%	50%	100%	200%
freshwater strains	+	±	−	n.t.
seawater strains	++	+	+	+

++ = good growth
\+ = normal growth
± = moderate growth
− = no growth
n.t. = not tested

As shown in *table 4* and *figs. 1, 7* and *8*, several morphological characters of the freshwater strains underwent quite dramatic changes when incubated in medium with seawater. In 50% seawater medium the cell-width increased and the cell-length decreased, and in 100% seawater medium the cells looked slightly inflated and their number per trichome was drastically reduced. The seawater strains did not show such pronounced morphological changes.

4.2 Recovery experiments

These results *(table 5)* show that the capability of the strains to survive high

Table 4. Tolerance experiment. Averages of 50 measurements of cell-width and -length of the freshwater strains after 42 days incubation in medium with 50% and 100% seawater.

strain number	50% seawater medium		100% seawater medium	
	cell-width (in μm)	cell-length (μm)	cell-width (μm)	cell-length (μm)
426	2.6	2.0	3.8	3.3
427	2.6	2.0	2.5	2.6
482	3.3	1.3	3.0	3.0
485	3.2	1.3	2.8	2.8
487	2.6	1.3	3.1	2.9
488	2.7	1.3	2.8	2.1
581	2.6	1.3	2.5	2.0
594	3.3	1.3	2.6	2.0
595	2.6	1.3	2.6	2.0
596	2.8	1.3	2.1	2.4
597	2.8	1.3	2.7	2.2
598	2.7	1.3	2.6	2.0
790	2.6	2.0	2.5	2.7

Table 5. Recovery experiment. Growth rates of all strains after 7 and 28 days of incubation in media with 0%, 100%, 200%, 400% and 100% seawater and 10 days recovery in medium with 0% seawater (for the fresh-water strains) and with 100% seawater (for the seawater strains).

seawater concentration	freshwater strains		seawater strains	
	7 days	28 days	7 days	28 days
0%	+	+	+	+
100%	+	±	+	+
200%	±	−	+	+
400%	−	n.t.	±	±
1000%	−	n.t.	−	−

+ = normal growth
± = bad growth
− = no growth
n.t. = not tested

salinities not only depended on the degree of salinity but also on the duration of the incubation.

The freshwater strains could survive salinities (100% and 200% seawater) in which they did not grow in culture. The seawater strains could endure higher salinities (up to 400% seawater). Those strains which grew after 10 days recovery, showed qualitatively and quantitatively the same morphological features as before the incubation in the media of higher salinity (cf. 3.1 and 3.2 and *figs. 1-5*).

4.3 Adaptation experiment

Increasing salinity caused a decrease in growth-rate of strain 427. This could be

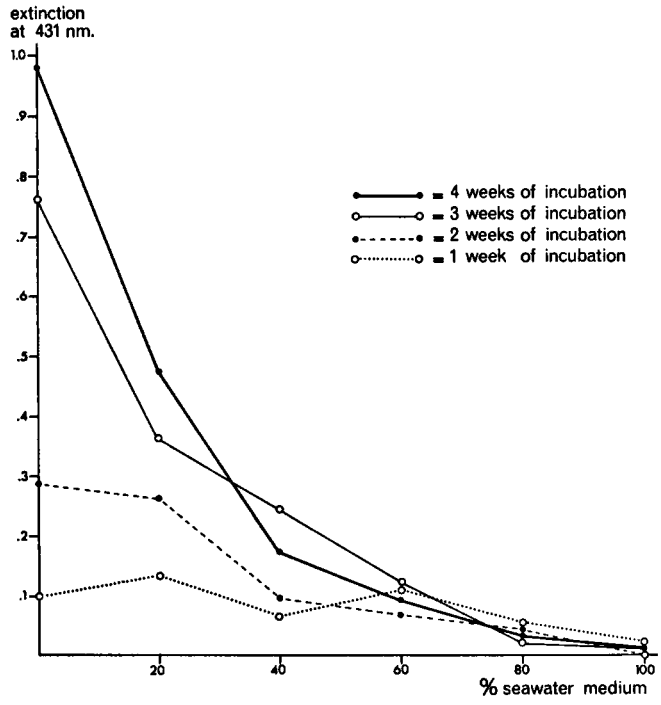

Fig. 9. Adaptation experiment. Relation between salinity and growth; expressed as the extinction at 431 nm of an acetone extract; cultures exposed to gradually increasing salinities (100% cultures derived from 80% cultures; 80% cultures from 60 % cultures etc.).

observed by comparing each adaptation culture with a reference culture (cf. 2.4.3). The extinction values at 431 nm of the acetone extracts confirmed these observations *(fig. 9)*. Growth was stopped after incubation in 100% seawater medium, and this agreed with the results of the tolerance experiments (4.1). The

Table 6. Adaptation experiment. Extinction at 431 nm of an acetone extract of cultures after *a.* Four weeks incubation in 0%, 20%, 40%, 60%, 80% and 100% seawater medium. *b.* Repeated four weeks incubation in the same medium (inoculum derived from the culture mentioned under a.).

seawater concentration	extinction a.	extinction b.
0%	—	0.98
20%	0.47	0.34
40%	0.17	0.17
60%	0.09	0.11
80%	0.03	0.02
100%	0.03	—

low extinction value of the acetone extract of the culture incubated in this salinity is due to the chlorophyll-a from the inoculated cells that survive this incubation (cf. 4.2).

The changes in morphology at each adaptation step *(figs. 10-14)* agreed with those shown in the tolerance experiments *(figs. 7* and *8)*.

Table 6 shows that repeated incubation in one and the same salinity (as performed with the reference cultures) produced no adaptation whatsoever. Therefore it is reasonable that the used incubation period was not too short for adaptation to a given salinity.

5. DISCUSSION

The freshwater strains are morphologically identical and considered to belong to the same species (cf. 3.1). The susceptibility of these strains for the cyanophage LPP-1 (SAFFERMAN & MORRIS 1963, 1964) gives also evidence for this conclusion.

Some remarks should be made about the sheath. When coloured with a 0.1-1 % toluidine-blue aquatic solution only the strains 426, 581 and 790 show a very thin sheath, just like the three strains 1462/1, 1446/3 and 1446/2 from the Culture Collection of Algae and Protozoa, Cambridge. The last three have the same origin as the Indiana strains 427, 487 and 488, respectively *(figs. 15* and *16)* These Cambridge cultures are axenic and clearly show the organisation of the trichomes in a membranous pellicle, which is due to the presence of confluent sheaths.

We suggest that the remaining freshwater strains also have sheaths, which are, however, submicroscopic or ephemeral. This reduction or short duration of the sheaths might be due to environmental influences such as the bacteria in the culture, which perhaps consume the secreted sheath material.

The existence, within one and the same strain, of axenic cultures with, and non-axenic cultures without sheaths makes this criterion on the genus level rather ambiguous. The strains can be ranged neither under *Oscillatoria* (which always lacks a sheath), nor under *Lyngbya* (which always has a sheath, apart from hormogonia) (KANN & KOMAREK 1970).

When the strains are identified with, and compared to the descriptions given by GEITLER (1932), it is evident that all strains belong to the genus *Phormidium*. The most appropriate species name, given by Geitler, for the freshwater strains is *Phormidium luridum* (Kütz.) Gomont or *Phormidium foveolarum* Gom., and for the seawater strains, which show little mutual differences, *Phormidium ectocarpi* Gom. or *Phormidium foveolarum* Gom. Hence the names of the strains 482, 487, 488, 581, 594, 595, 596, 597 and 790, as listed in *tabel 1*, are incorrect. Identification with DROUET (1968) leads to the conclusion that all strains are growth-forms of the species *Schizothrix calcicola* (Ag.) Gomont. The strains 426, 427, 482, 488, 581, 594, 597 and 598 were already identified as *Schizothrix calcicola* by DROUET himself (1963, p. 270, footnote 10).

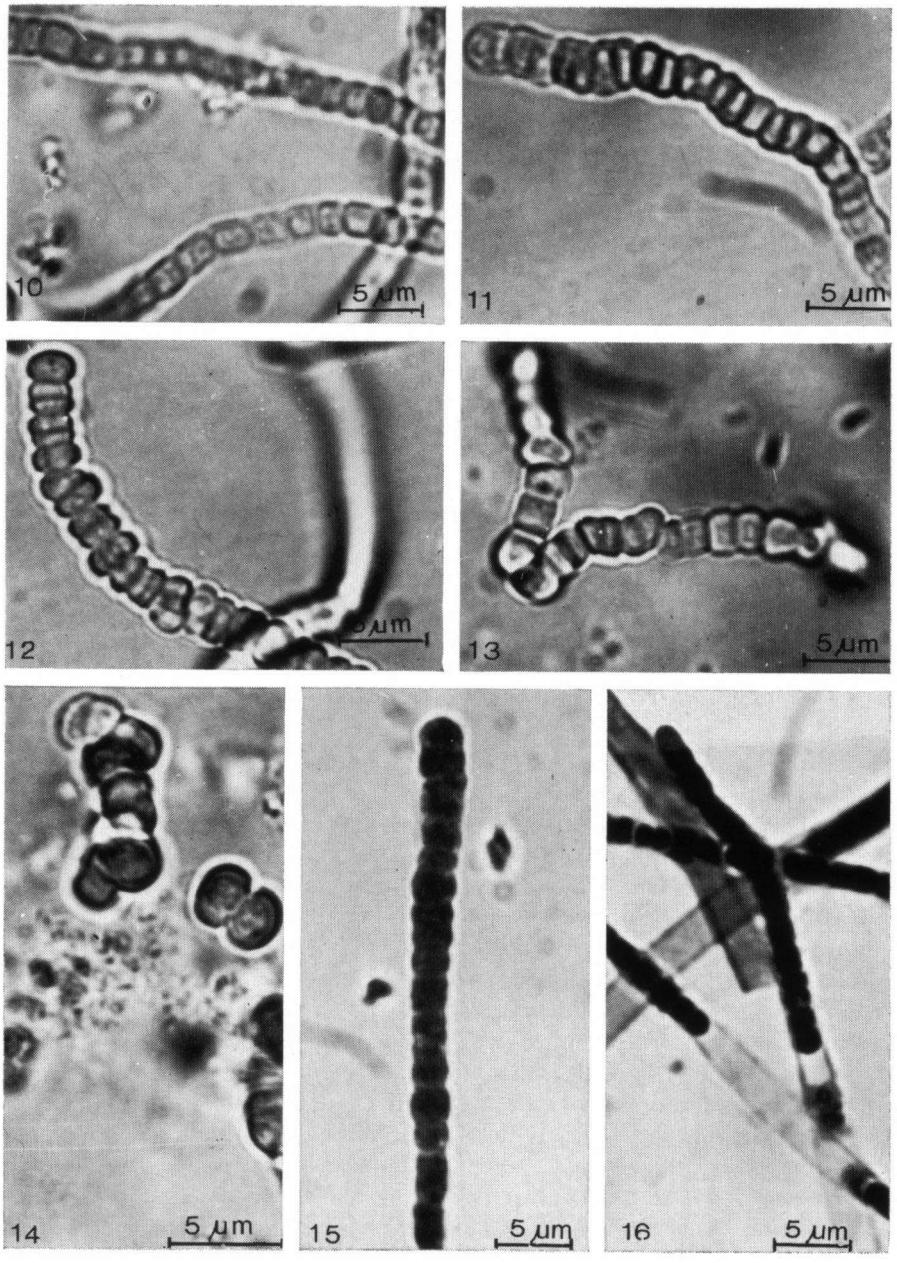

Fig. 10–14. Trichomes of freshwater strain 427 after 28 days incubation in media with 20% 40%, 60%, 80% and 100% seawater, respectively. Inocula derived from 0%, 20%, 40% 60% and 80% seawater medium cultures, respectively, Fig. 15–16. Freshwater strains 1446/2 and 488, respectively. Trichomes coloured with a 0.1% toluidine blue solution.

The strains we used can be divided into two groups: one group of strains which can only grow in freshwater to slightly brackish water media and not in seawater medium (the freshwater strains), and a second group of strains which are not inhibited by higher salinities (as in seawater) except extreme high ones (the seawater strains). Moreover, freshwater strains when cultured under brackish water conditions develop into morphologically divergent forms. These facts apparently conflict with Drouets conception of *Schizotrix calcicola* as a genetically homogeneous species with ecological and morphological variation on the basis of one genotype, for in that case both groups of strains should show – whether or not after gradual adaptation – equal growth under all conditions tested, and should show the same morphologies under identical conditions. On the contrary, the data from the adaptation experiment strongly suggest a genetical basis for the observed difference in halotolerance.

This supports the suggestion of BATTERTON and VAN BAALEN (1971) that "marine blue-green algal isolates are characteristically more halotolerant, perhaps by selection, than freshwater forms", which is, in their opinion, due to the inherent capacity of the cell to extrude Na-ions. The maximum salinity tolerated by both groups of strains used by these authors are in the same order of magnitude as that observed by us.

It should be stressed that DROUET's (1963) experiments on the saltwater ecophenes are "one-way" experiments. Drouet reports on two experiments in which he brings two "Schizothrix calcicola" samples from freshwater habitat into jars with seawater, in which these freshwater strains "remained in a healthy condition and grew well". He used these observations as evidence for his hypothesis that freshwater- and saltwaterforms of "Schizothrix calcicola" are ecophenes, i.e. genetically identical ecological variations. We think his experimental procedures are open to criticism, and that these "results" are meaningless. Our freshwater strains could survive, but not grow, in seawater medium. Surviving trichomes had an abnormal morphology – they were few celled and had an inflated appearance – and this would seem to have happened to one of Drouets seawater cultures of freshwater material, in which trichomes "broke into few celled segments". Drouet omitted to culture seawater material in freshwater. The same criticism is valid for all Drouets experiments, which only prove that samples identified by him as *Schizothrix calcicola* have a considerable potential polymorphism (as have many algae subjected to conditions that are abnormal to them), and not that samples producing comparable forms have the same, or even a similar, genotype.

DROUET's (1968) statement that variations in morphology and physiology appear when trichomes regenerate after exposure to catastrophic changes in the environment – such as sudden changes in salinity – is not supported by the results from our recovery experiments; well growing trichomes, after recovery from this "catastrophe", always have approximately the same morphology.

Still, the used blue-green algae strains have a potential morphological plasticity, which exceeds the descriptions of the *Phormidium* species mentioned above. However, the most strongly deviating growth forms are shown by ill

growing, or only surviving, cultures under adverse culture conditions, and this is a very common phenomenon in algal cultures. Well growing cultures are all rather similar.

We are still faced with the problem, whether or not the strains studied by us should be considered as belonging to one, genetically and hence morphologically and ecologically variable, species. To solve this problem, more strains and the influence of more ecological parameters on morphology and growth have to be investigated, in order to trace the mode of discontinuity of the morphological characters, and, consequently, the usefullness of these characters for the taxonomy of these strains, as has been done by KANN & KOMAREK (1970) for *Phormidium autumnale* Ag. and related species.

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