MORPHOLOGICAL VARIABILITY IN RHIZOCLONIUM RIPARIUM (ROTH) HARV. (CHLOROPHYCEAE: CLADOPHORALES) UNDER NATURAL AND EXPERIMENTAL CONDITIONS

P. H. Nienhuis and B. H. H. de Bree

Delta Institute for Hydrobiological Research, Yerseke, The Netherlands.

ABSTRACT

Populations of the filamentous green alga *Rhizoclonium riparium* (Roth) Harv. (including *R. implexum* (Dillw.) Kütz.) growing on vertical wooden poles in the intertidal zone, show a morphocline. Filaments with wide, short cells are found high up in the intertidal zone, and filaments with narrow, long cells in the lower parts. Along the gradient these two forms gradually change into one another.

By means of culture experiments it could be demonstrated that the morphological characteristics of *Rhizoclonium* are completely changeable by the action of environmental factors. Each combination of culture conditions (temperature, culture medium, photo-period) modifies both the "wide" and the "narrow" filaments into an ecological form with uniform cell-dimensions, characteristic for those particular conditions.

INTRODUCTION

The filamentous, unbranched green alga *Rhizoclonium riparium* (Roth.) Harv. (including *R. implexum* (Dillw.) Kütz.) is very common along the estuaries in the S.-W. Netherlands. The alga grows under almost marine conditions on vertical wooden jetty-poles in the intertidal belt from mean high water-level of springtides down to mean sea-level. Quantitative population-analyses demonstrated the existence of a vertical gradient in the morphological characteristics of the alga: The populations living in the upper part of the intertidal belt are built up of woolly mats, showing long filaments with wide, short cells filled with densely packed chloroplasts, masked by starch grains. The populations living in the lower part consist of short scattered filaments embedded in the silt layer on the poles; the filaments have long, narrow cells filled with bright-green

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chloroplasts. Along the gradient these two forms gradually change into one another.

The aim of this paper is to decide by means of a series of culture experiments, whether the morphological variability observed is genetically fixed, or merely a product of interaction of environmental factors.

MATERIAL AND METHODS

Samples of *Rhizoclonium riparium* were gathered at 7 different levels from vertical wooden poles in the intertidal zone at Yerseke (Oosterschelde, S.-W. Netherlands) in January 1973 (Fig. 1).

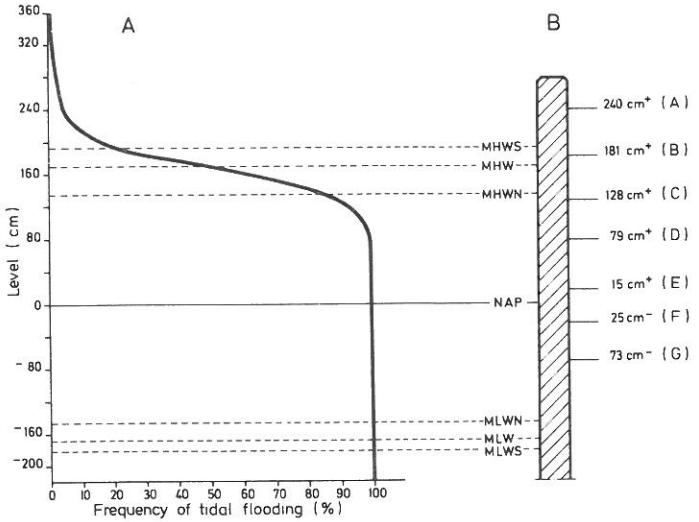

Fig. 1A. Frequency of tidal flooding at Yerseke.
1B. Wooden jetty-pole at Yerseke. The levels at which *Rhizoclonium riparium* was sampled are indicated (A-G). The hydrographical levels are in between the two figures. M = mean; HW = high water-level; LW = low water-level; S = springtides; N = neaptides; NAP = Dutch ordnance level, approximately mean sea-level.

Cell dimensions given are based on an analysis of 50 cells, chosen at random from a subsample. Uni-algal subsamples consisting of a number of filament-fragments (ca. 0.5 cm long), were cultivated in the laboratory in petri-dishes, in constant temperature rooms under 6 different conditions; temperatures: 15°C or 5°C; photoperiod: 8 hours (short day = SD) or 16 hours (long day = LD); liquid media (L) or solid agar media (S). Light intensities amounted to ca. 1100 lux at culture level, from Philips TL de luxe 20 W - 33. The culture fluid used was a modified Erdschreiber medium (0.01 g K_2HPO_4, 0.05 g KNO_3, 0.01 g GeO_2, 0.004 g Na-EDTA and soil extract in 1 l. sea-water); the salinity of the medium was 16-17°/00 Cl'. A solid medium was prepared by adding 10 g agar to 1 l. of liquid medium. The liquid medium was renewed every two weeks, and the agar plates monthly.

RESULTS

Diameter and length/width ratio of the vegetative cells are the most important characteristics in the delimitation of *Rhizoclonium* species. Fig. 2 shows the frequency distributions of the cell diameters of the field material: a series of overlapping curves with a slight discontinuity at level C. Fig. 3 shows the frequency distributions of the length/width ratio of the cells of the field material: an abrupt change at level D, owing to a small number of extremely long cells. Fig. 4 shows the ranges of cell diameter and length/width ratio, with average values and standard deviations, and, moreover, the average surface/contents ratio of the cells; the lower the levels, the narrower the filaments, the larger the length/width ratio's and the larger the surface of a cell related to its contents.

The results of the culture experiments are summarized in Figs. 5 and 6. Fig. 5 shows the modifications in cell diameter and length/width ratio obtained under 8 combinations of environmental factors. Each combination reveals that all samples from the original morphocline changed, after a growing period of some months, into one single morphological form, with an almost uniform diameter and length/width ratio of the cells, characteristic for that particular set of culture conditions. Fig. 6 combines the average diameters, length/width ratio's and surface/contents ratios's of the cells in the field samples and in the cultivated samples. Once again it can be seen that the dissimilar field samples almost uniformly adapted themselves to a particular

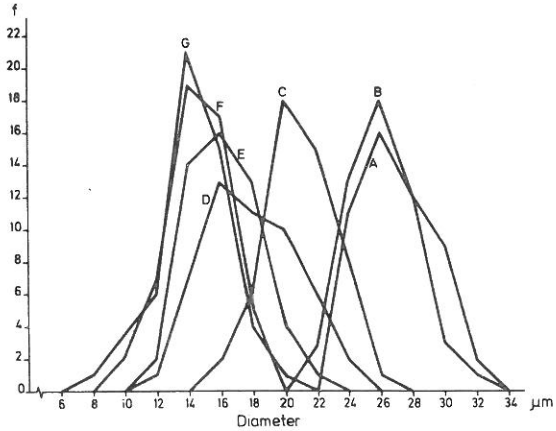

Fig. 2. Frequency distributions of the cell diameter of *Rhizoclonium riparium*. A-G correspond to the levels indicated in Fig. 1.

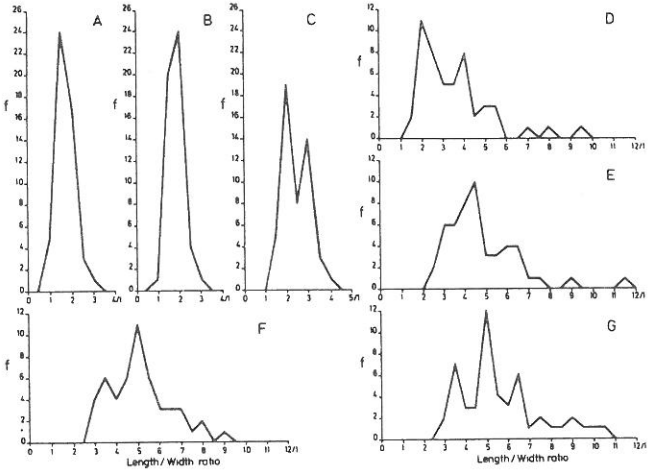

Fig. 3. Frequency distributions of the length/width ratio of the cells of *Rhizoclonium riparium*. A-G correspond to the levels indicated in Fig. 1.

combination of culture conditions. The samples kept at 15° SD-L showed the most rapid growth and appeared to be completely modified when measured after 69 days of culture. The slowest growth took place at 5° LD-S. Only after ca. 6 months of cultivation the material was adapted to its changed environment.

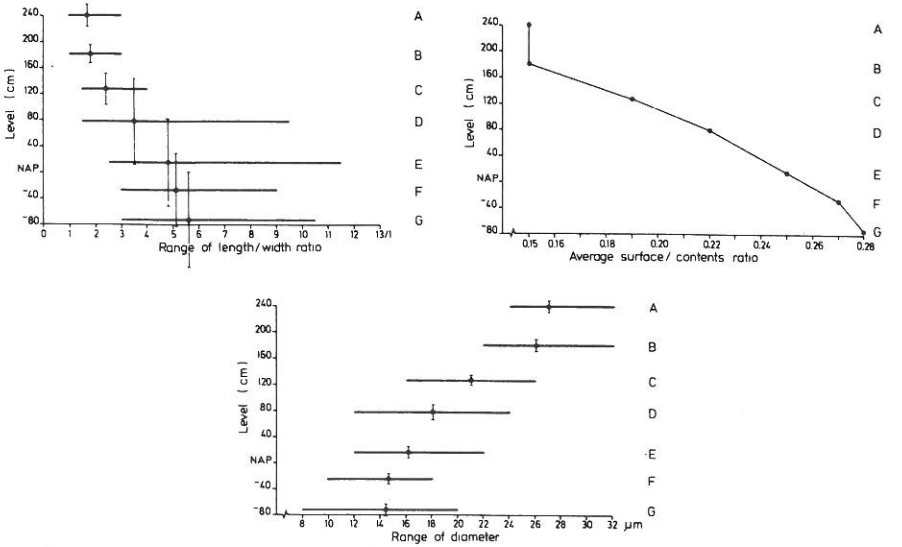

Fig. 4 Cell dimensions of *Rhizoclonium riparium*. Ranges of cell diameter and length/width ratio of the cells: thick horizontal lines; sample averages: black dots; standard deviations: thin vertical lines. The values of the average surface/contents ratio have been connected with a thick line for better visualization.

DISCUSSION AND CONCLUSIONS

It can be concluded from our results that *Rhizoclonium riparium* is highly modifiable alga; its morphological characteristics are completely changeable by the action of environmental factors. After a given growing period each combination of culture conditions produces an ecological form characteristic for that particular set of environmental factors. Temperature exercises the quickest and most significant effect on the process of modification, followed by the composition of the culture medium; daylength has only a very slight effect. The quicker the alga develops, the narrower the cells, the greater the length/width ratio of the cells and the larger the surface/contents ratio of the cells.

In nature extremely long, narrow cells are found in filaments of low-littoral populations, embedded in sediment. Short, wide cells occur in high-littoral populations, exposed to full daylight. In cultures, growth at 15°C and in a liquid medium results in relatively long and narrow cells and growth at 5°C and on a solid medium in relatively short and wide cells.

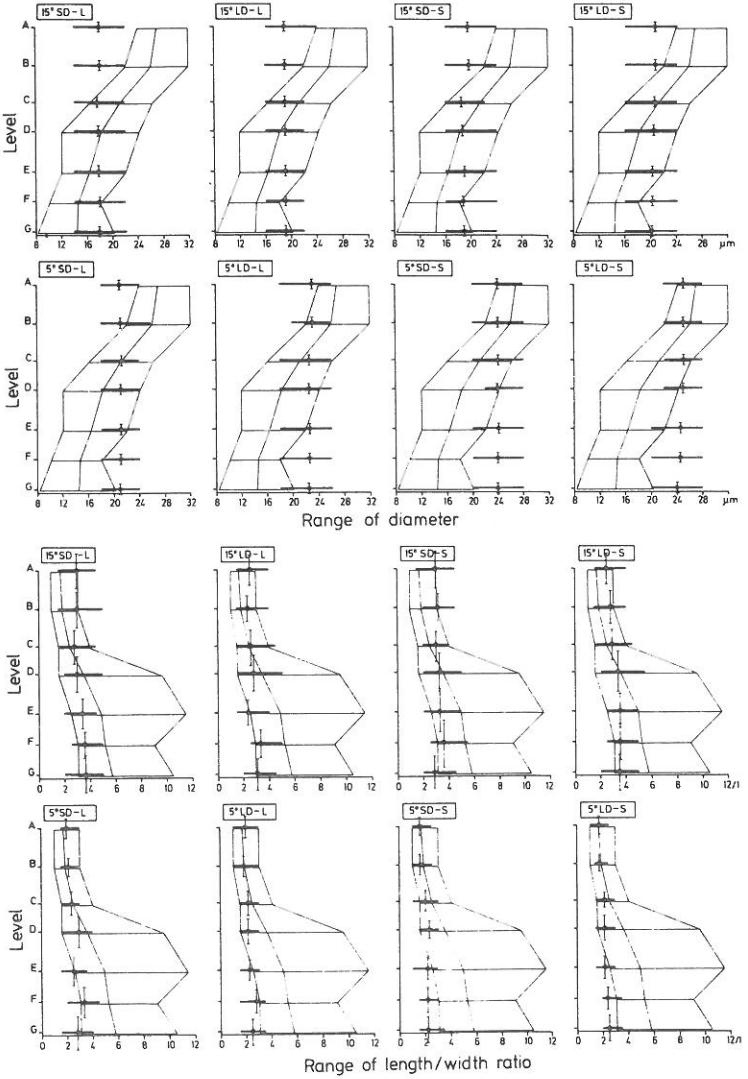

Fig. 5. Modifications of cell dimensions of *Rhizoclonium riparium* in cultures. Thick horizontal lines: ranges of cultured cell diameter and length/width ratio; black dots: sample averages; thin vertical lines: standard deviations. Thin horizontal lines: ranges of cell diameter and length/width ratio in field. A-G correspond to the levels indicated in Fig. 1. The culture conditions as shown, SD = 8 h. photoperiod; LD = 16 h. photoperiod; L = liquid medium; S = solid agar.

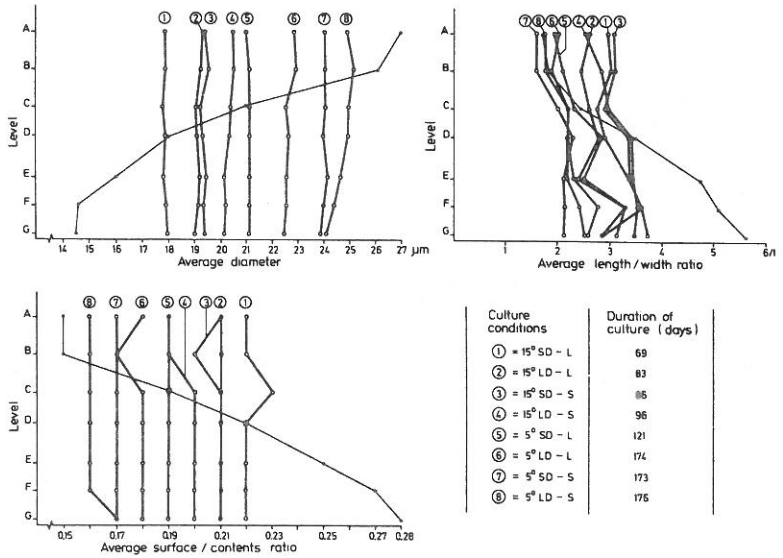

Fig. 6 Modifications of cell dimensions of *Rhizoclonium riparium* in culture (thick lines) connected by thick lines for better visualization. Thin lines = field material. A-G correspond to the levels indicated in Fig. 1.

Using the data from the culture experiments available, it is almost impossible to interpret the phenomena found in nature. In the intertidal zone a *R. riparium* morphocline could be established, coinciding with a vertical environmental gradient: from low towards high levels the flooding frequency decreases. The aquatic conditions in the eulittoral zone fade into almost terrestrial conditions in the supralittoral zone, with relatively large fluctuations in environmental factors. The morphocline is interrupted by a discontinuous change in cell dimensions just below mean high water-level of neap tides, (Figs. 2 and 3) coinciding with an abruptly decreasing flooding frequency (Fig. 1). The low littoral populations are submerged twice a day, but from mean high water-level of neap tides upwards the algal vegetation is exposed to the open air for several consecutive days every month, at neap tides. Especially during dry periods water supply may be a limiting factor for the algal growth in the higher parts of the intertidal belt. It may hypothetically be presumed that the cells of a *Rhizoclonium* filament react upon desiccation by becoming shorter and wider, thus decreasing their surface/contents ratio.

Under culture conditions moisture supply seems not to be a limiting factor, neither in liquid nor in agar cultures. Nevertheless, in agar cultures the cells become shorter and wider than in liquid cultures. The filaments growing on top of waterlogged agar in closed petri-dishes, might be thought to be exposed to "dryer" growth conditions than those in a liquid medium.

Of the three parameters tested in culture temperature showed the most significant modifying influence. It may be presumed, however, that the average temperatures under low- and high-littoral conditions differ only little, and it seems improbable that temperature is one of the major responsible factors for morphological variability of *Rhizoclonium* in the field.

In many algal groups the external form of the thallus is still accepted as the major criterion for taxonomic discrimination. However, about the morphological variability of the algae, whether or not caused by effects of environmental factors, only little is known[2]. Algae of the Cladophorales group grown in culture, may deviate morphologically from the field material, but quantitative investigations in order to study these phenomena under different culture conditions are scarce in literature (cf.1,3).

Adaptation of the morphological features of *Rhizoclonium* to the environmental conditions has taxonomical significance. According to Koster (1955)[4] two species occur in the Netherlands: *R. implexum* (Dillw.) Kütz., with cell diameter 10-21 µm and length/width ratio of cells 1-8.5 and *R. riparium* (Roth) Harv. with cell diameter 18 - 48 µm and length/width ratio of cells 0.5 - 4.5.

The morphocline in the field material shows that the "narrow" and the "wide" form spatially fade into one another. The culture experiments reveal the phenotypical plasticity, with the "narrow" and the "wide" form developing into populations with an almost identical morphology.

On account of these results, and other evidence not given here (Nienhuis, unpublished), *R. implexum* must be regarded as conspecific with *R. riparium*.

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