LIFE-HISTORY STUDIES IN THE GENUS BRYOPSIS (CHLOROPHYCEAE) IV. LIFE-HISTORIES IN BRYOPSIS HYPNOIDES LAMX. FROM DIFFERENT POINTS ALONG THE EUROPEAN COASTS

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SUMMARY

Zygotes of *Bryopsis hypnoides* from Banyuls and the Rade de Brest grew into filamentous germlings which divided into stephanokontic zoids. Some of the germlings from Banyuls directly developed new *Bryopsis* plants. In culture the germlings of *Bryopsis hypnoides* collected in Banyuls have a longer "dormancy" than those of the *Bryopsis hypnoides* collected in the Rade de Brest. Zinc-chlor-iodine and Congo red positively stain the wall of the *Bryopsis* phase and negatively the walls of the germling phase. The germling phases of *Bryopsis hypnoides* from the Rade de Brest and of several other *Bryopsis* species are uninucleate, each containing one giant nucleus.

1. INTRODUCTION

Recently NEUMANN (1969a) investigated the life-history of *Bryopsis hypnoides* from Helgoland, by cultural as well as caryological methods. *Bryopsis hypnoides* appeared to be monoecious, one half of a gametangium giving rise to microgametes, the other half to macrogametes (see also FELDMANN 1957). Zygotes grew into diminutive creeping germlings which each contained one giant zygote nucleus. New *Bryopsis* thalli sprouted from the creeping germling phase after (presumably mitotic) division of the giant nucleus into a large number of smaller nuclei. NEUMANN (1969a) assumed meiosis to take place preceding gamete formation. Apparently he based this assumption on the work of SCHUSSNIG (1932) and ZINNECKER (1935), for he did not produce convincing caryological evidence that meiosis actually takes place preceding gamete formation.

In the present paper the life-histories of *Bryopsis hypnoides* collected form some other places along the European coasts are described.

2. MATERIAL AND METHODS

Bryopsis hypnoides collected from the following localities was investigated:

a. Rade de Brest; July 1968; Pointe de Rostoviec; epiphytic on *Laurencia pinnatifida, Chondrus crispus, Ceramium rubrum, Hypoglossum woodwardii, Gelidium crinale*, and also on rocks; upper sublittoral zone.

b. Banyuls; September 1967; Grotte du Troc; epiphytic on *Pterocladia capillacea*; depth about 0.5 m.

The life-histories of two plants from the first-mentioned locality and of one plant from the second locality were studied. *Bryopsis hypnoides* plants from the Rade de Brest differed slightly from those collected at Banyuls (cf. *fig. 1* and *fig. 16*).

For methods of isolation of unialgal cultures and for culture methods see RIETEMA 1969 and 1970. If not stated otherwise, cultures were kept in a 16 ± 1 °C culture room and under a long day regime (16 hours light – 8 hours darkness).

Germlings and thalli of *Bryopsis hypnoides* derived from material collected in the Rade de Brest were caryologically stained with acetocarmine and embedded in phenol balsam, according to the method of VON STOSCH (1952).

The reactions of cell-walls of germlings and mature thalli to staining with Congo red (a vital stain) and zinc-chlor-iodine were investigated, both for Banyuls and Rade de Brest material.

3. RESULTS

3.1. Bryopsis hypnoides from the Rade de Brest

3.1.1. The life-history

Subcultures derived from determinate laterals had grown into plants about 1.7–2 cm high after about two weeks (*fig. 1*). These plants were morphologically similar to the original plants from nature. Male (*fig. 2*) and female (*fig. 3*) gametes were produced in determinate laterals transformed into gametangia: male gametes were produced in the upper half of each gametangium, female gametes in the lower half (*fig. 4*). The production of male and female gametes in the upper and lower half of each gametangium, respectively, was constant in one plant and also remained constant in vegetative subcultures. However, there were also plants in which the upper half of each gametangium produced female and the lower half male gametes (*fig. 5*) and this also remained a constant feature in vegetative subcultures.

Mixtures of swarms of male and female gametes formed in one and the same or in different gametangia produced large numbers of creeping filamentous germlings. After about two to three months these germlings were about 6 mm long (*fig. 6*). After transfer into fresh medium and short day conditions (8 hours light – 16 hours darkness) three months old germlings (30 observed) started to divide their contents into numerous stephanokontic zoids (*fig. 7*) within four weeks of the beginning of the observation. After release these zoids (*fig. 8*) grew directly into new *Bryopsis hypnoides* plants (*fig. 9*, *10* and *11*).

3.1.2. Caryological investigations

The filamentous germling phase of *Bryopsis hypnoides* was uninucleate (*fig. 12* and *13*). The diameter of such a nucleus was about 30 to 45 μ, eight to ten times the diameter of nuclei in a multinucleate thallus. In such a thallus the diameter of the nuclei amounted to 3–4.8μ. Cleavage of the contents of the germling phase into stephanokontic zoids was achieved by divisions of the giant nucleus in such a way that each stephanokontic zoid received one nucleus. The diameter

of these nuclei amounted to about 10 μ. The details of this division are obscure (*fig. 14* and *15*).

3.1.3. Staining reactions of the cell-walls of the *Bryopsis* phase and the germling phase to zinc-chlor-iodide and Congo red

The cell-walls of the thalli of *Bryopsis hypnoides* stained red and violet by Congo red and zinc-chlor-iodine, respectively. The cell-walls of the germlings, however, remained uncoloured in both staining solutions.

3.2. Bryopsis hypnoides from Banyuls

Subcultures derived from determinate laterals had grown into plants about 1.4–2.1 cm high after about two weeks (*fig. 16*). These plants were morphologically similar to the original plants from nature. Of about 55 six months old germlings two directly developed new *Bryopsis* thalli whereas only one germling produced stephanokontic zoids after several months of observation. These zoids grew into *Bryopsis* thalli morphologically similar to the original plants from nature.

3.2.1. Staining reaction of the cell-walls of the *Bryopsis* phase and the germling phase to zinc-chlor-iodine and Congo red

The cell-walls of the gametophytic phase stained red and violet in a Congo red and zinc-chlor-iodine solution, respectively, the cell walls of the germlings were not stained at all.

Figs. 1–15. Structure and reproduction of *Bryopsis hypnoides* from the Rade de Brest:
Fig. 1. Habit of *Bryopsis hypnoides* from the Rade de Brest about two weeks old (grown, in culture, from a determinate lateral).
Fig. 2. Male gametes.
Fig. 3. Female gametes.
Fig. 4. Part of a plant with gametangia; male gametes are produced in the upper half, female gametes in the lower half of each gametangium.
Fig. 5. Part of a plant with gametangia; female gametes are produced in the upper half, male gametes in the lower half of each gametangium.
Fig. 6. Habit of a ca. three months old germling phase.
Fig. 7. Stephanokontic spores shortly before release
Fig. 8. Zoospore viewed from above.
Fig. 9 and 10. A germinating stephanokontic zoid (three days old).
Fig. 11. A juvenile *Bryopsis hypnoides* plant 7 days after germination of a stephanokontic zoid the form of which is still discernable.
Fig. 12. Germling with its single giant nucleus.
Fig. 13. Detail of the giant nucleus (Acetocarmine- phenolbalsam).
Fig. 14. Stephanokontic zoospores before release. Each spore contains one nucleus (Acetocarmine).
Fig. 15. Spore-formation, showing the centrally placed nucleus.

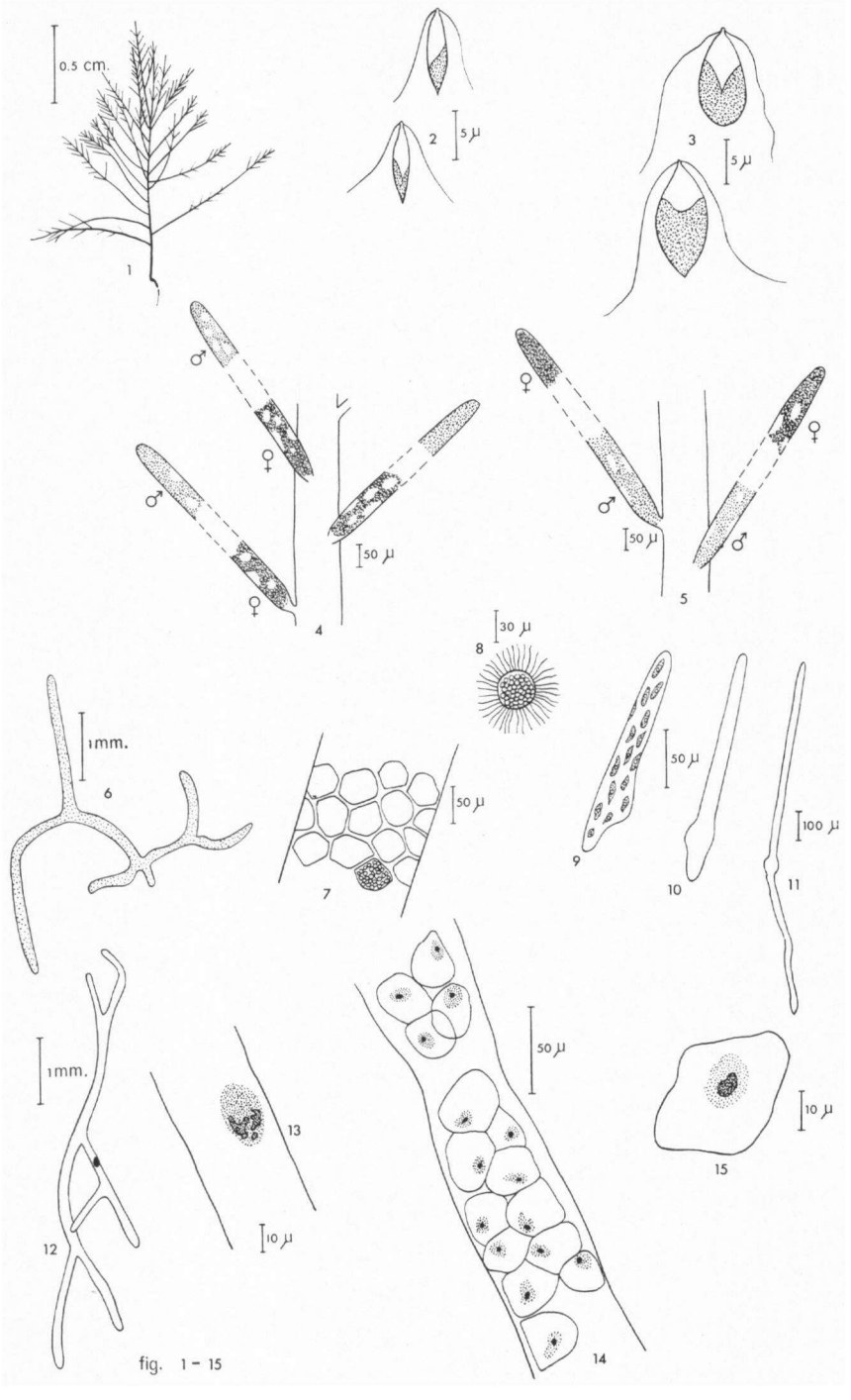
0.5 cm.
1
2
5 µ
3
5 µ
♂
♀
50 µ
4
♀
♂
50 µ
5
30 µ
8
1mm.
6
50 µ
7
50 µ
9
50 µ
10
100 µ
11
1mm.
12
13
10 µ
50 µ
14
10 µ
15

fig. 1 – 15

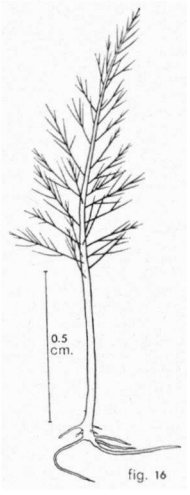

Fig. 16. Habit of *Bryopsis hypnoides* (Banyuls material) about two weeks old (grown in culture from a determinate lateral).

4. CONCLUSIONS AND DISCUSSION

Both *Bryopsis hypnoides* from Banyuls and from the Rade de Brest produce zygotes that grow into diminutive creeping filamentous germlings: the sporophytic phase. These germlings are capable of dividing their contents into numerous stephanokontic zoids, each of which grows into a new *Bryopsis* plant. A diagram of the life-history of *Bryopsis hypnoides* collected in the Rade de Brest is reproduced in *fig. 17*. It is much easier to "break" the "dormancy" of the gametophytic germling phase of the material from Brittany (Rade de Brest) than that of the Banyuls material. Furthermore, the germling phase of the Banyuls material is also capable of directly developing into new *Bryopsis* thalli.

The cell-walls of the gametophytic phase of Bryopsis hypnoides show a positive reaction to zinc-chlor-iodine and Congo red, whereas the cell-walls of the sporophytic phase show a negative reaction to both stains. In all above-mentioned respects the germlings of *Bryopsis hypnoides* behave like the germlings of *Bryopsis plumosa* (RIETEMA 1969, 1970) from Brittany (Roscoff) and Banyuls, respectively.

The investigations carried out so far by the present author strongly suggest that the life-histories of *Bryopsis hypnoides* and *Bryopsis plumosa* vary in an identical way at different latitudes along the European coasts, roughly as follows:

Mediterranean populations of both species have germling phases that can both form stephanokontic zoids and develop new *Bryopsis* plants directly, and in addition the "dormancy" of the germling phase is difficult to break; Breton populations of both species have germling phases that are, under the present culture conditions, only capable of forming stephanokontic zoids and in ad-

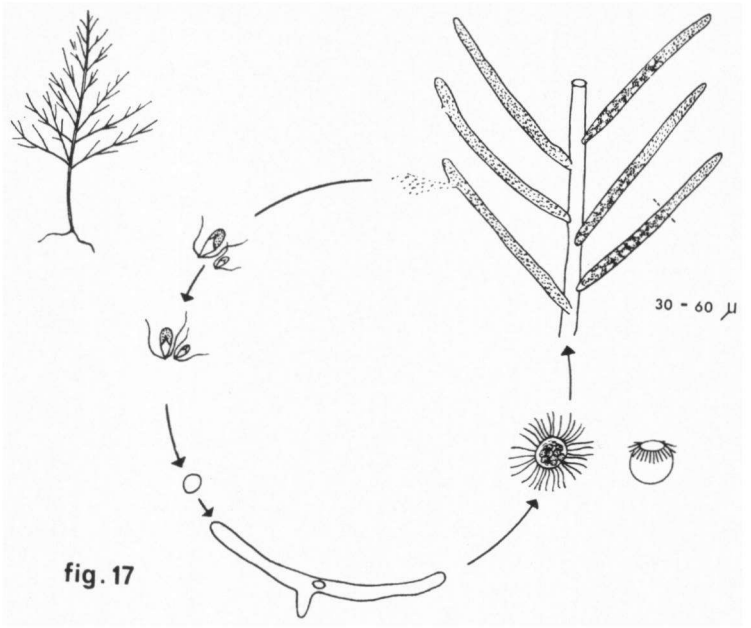

Fig. 17. Diagram of the life-history of *Bryopsis hypnoides* from the Rade de Brest. Within 14 days determinate laterals grow out into plants of 1.7–2 cm.

dition the "dormancy" of the germling phase is easy to break. Zeeland populations of *Bryopsis plumosa* (RIETEMA 1969) and *Bryopsis hypnoides* (unpublished) also have germling phases that are only capable of directly developing into new *Bryopsis* plants, and in addition the "dormancy" is easy to break after transfer into a fresh medium.

Observations by NEUMANN (1969a) on *Bryopsis hypnoides* from Helgoland and by the present author on *Bryopsis hypnoides* from Kiel (Baltic sea), Fiskebäckskil (Swedish West Coast), and Drøbak (Oslofjord) (RIETEMA unpublished) fit into this overall picture quite well. All these northern populations of *Bryopsis hypnoides* have approximately the same type of life-history as that of *Bryopsis plumosa* and *Bryopsis hypnoides* from Zeeland (Netherlands); the "dormancy" of the filamentous germling phase is easy to break and the germling phase is only capable of directly developing new *Bryopsis* plants and never forms stephanokontic zoids. A diagram of the life-history of *Bryopsis hypnoides* collected in northern latitudes is reproduced in *fig. 18*. In all cases the cell-walls of *Bryopsis* plants stain positively with zinc-chlor-iodide and Congo red, whereas the germling phases are always negative to the stains, even when they directly develop new *Bryopsis* plants.

It remains a matter of definition or interpretation whether to call the life-histories of *Bryopsis hypnoides* from northern latitudes and that of *Bryopsis plumosa* from Zeeland (Netherlands) monophasic or biphasic heteromorphic.

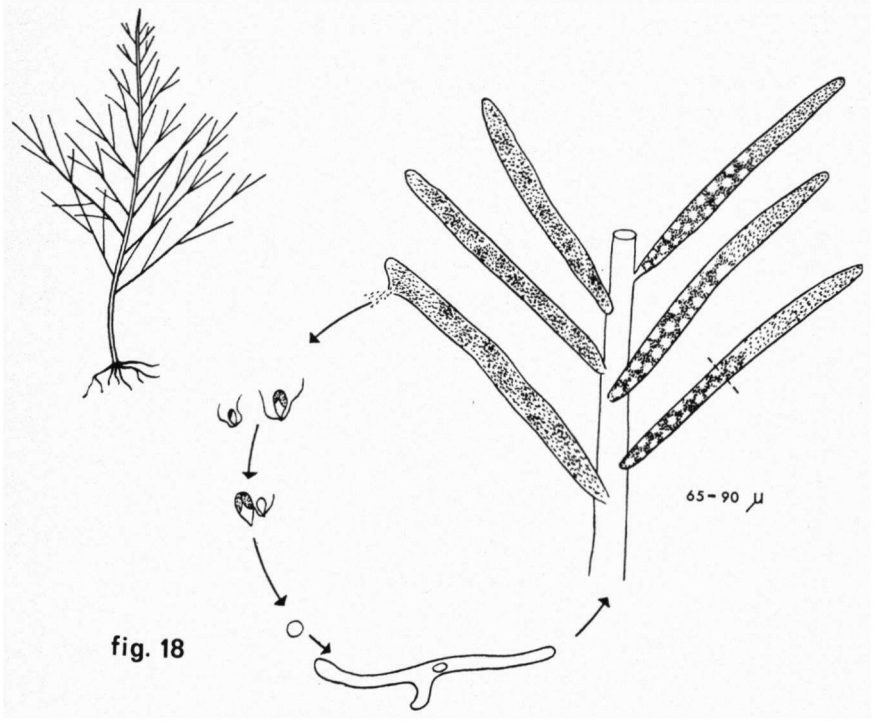

Fig. 18. Diagram of the life-history of *Bryopsis hypnoides* from northern latitudes. Within 14 days determinate laterals grow out into plants of 2.5–3.0 cm.

On the basis of comparative studies in the life-histories of both species at northern and southern latitudes along the European coasts the present author tends to interpret the diminutive creeping germlings of the northern populations as separate sporophytic phases that have lost the capacity to form stephanokontic zoids.

Caryological observations on the germling phase derived from *Bryopsis hypnoides* collected in the Rade de Brest showed that each germling has only one single giant nucleus. This observation is in accordance with that of NEUMANN (1969a) on the giant nucleus in the germling phase of *Bryopsis hypnoides* from Helgoland. Equally *Bryopsis plumosa* from Zeeland and Roscoff and *Bryopsis monoica* from Banyuls alwasy have one such single giant nucleus in each germling (RIETEMA unpublished). These observations also support the opinion that the germling phases developing directly into *Bryopsis* plants and those dividing into stephanokontic zoids are fundamentally similar. According to NEUMANN (1969a) meiosis takes place preceding the formation of gametes. He apparently based this opinion on the unconfirmed work of ZINNECKER (1935) and SCHUSSNIG (1932) for he did not produce any caryological evidence to support his opinion. There are some indications (e.g., the segregation in the offspring of one

germling phase plant into male and female plants in *Bryopsis plumosa*) that meiosis takes place in the germling phase. In this respect it is interesting that *Bryopsis halymeniae* has in its life-history a *Derbesia*-like sporophytic phase with sporangia producing stephanokontic zoids (HUSTEDE 1964; RIETEMA, unpublished). The site of meiosis has not been investigated for this species. However, in *Derbesia marina* it was shown caryologically that meiosis takes place preceding the formation of stephanokontic zoospores (NEUMANN 1969b).

Dr. D. R. Kreger, working in the department of plant physiology (State University, Groningen), has recently made x- ray diffraction patterns of physically cleaned cell walls of the gametophytic phase of *Bryopsis hypnoides* (collected in the Rade de Brest) and of the germling phase derived from it. According to his interpretations of these patterns the cell walls of the gametophytic phase contain xylan as the skeletal polysaccharide. However, the cell walls of the germling phase after mild treatment with boiling dilute hydrochlorid acid yielded x- ray diffraction patterns corresponding to those of ivory-nut mannan and not to xylan. By thin-layer chromatography only mannose was detected in the acid extract.

The chemical composition of the cell walls of the gametophytic (probably haploid) phase apparently differs also considerably from that of the uninuclear (probably diploid) germling phase as suggested by the staining reactions. The significance of these observations in connection with the taxonomy of siphoneous green algae will be discussed later.

ACKNOWLEDGEMENTS

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