

# LIFE-HISTORIES OF *BRYOPSIS PLUMOSA* (CHLOROPHYCEAE, CAULERPALES) FROM EUROPEAN COASTS

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## SUMMARY

Zygotes of *Bryopsis plumosa* from Naples, Banyuls, and Biarritz grew into filamentous germlings which divided into stephanokontic zoids. Some of the germlings from Naples and Banyuls developed directly into new *Bryopsis* plants. In culture these germlings have a longer "dormancy" period than those of *Bryopsis plumosa* collected in more northern regions (Zeeland, Roscoff). *Bryopsis plumosa* from Naples could interbreed with Roscoff material to produce germlings that only developed through stephanokontic zoids. *Bryopsis plumosa* from Naples could also interbreed with Zeeland material and produced germlings that only developed directly into new *Bryopsis* plants (which is characteristic for *Bryopsis plumosa* from Zeeland). Zinc-chlor-iodide and Congo red positively stain the walls of the *Bryopsis* phase, and negatively the walls of the germling phase. On the basis of these results the life-histories and the site where meiosis takes place in the life-history of *Bryopsis plumosa* are discussed.

## 1. INTRODUCTION

Until recently it was generally accepted that all *Bryopsis* species are characterised by a monophasic diplontic life-history and by pronounced anisogamy. Biflagellate male and female gametes (in *Bryopsis plumosa* produced by male and female plants, respectively) fuse to produce zygotes that grow immediately into new gametophytes. Meiosis was considered to take place preceding formation of gametes in the determinate laterals of the gametophytes. In fact the only evidence, caryological as well as cultural (though incomplete), for the existence of the above type of life-history in *Bryopsis plumosa* was that of ZINNECKER (1935) on material from Naples.

In comparing the life-history of *Bryopsis plumosa* from Roscoff with that of *Bryopsis plumosa* from Zeeland (south-western part of the Netherlands), however, the present author observed that the life-history of *Bryopsis* from Roscoff was heteromorphic biphasic (RIETEMA 1969) whereas the life-history of the material from Zeeland corresponded with the monophasic life-history observed by Zinnecker. In the heteromorphic life-history of *Bryopsis plumosa* a zygote grew into a small, creeping, filamentous germling phase that produced stephanokontic zoids which developed into new erect *Bryopsis* thalli.

In the present paper the life-histories of *Bryopsis plumosa* plants collected in some other places along the European coasts are described. Crosses were made to investigate whether there are interbreeding barriers between *Bryopsis plumosa* plants collected in different places along the European coasts.

## 2. MATERIAL AND METHODS

Material investigated was collected from the following localities on European coasts.

- a. Naples (Italy); May 1968; Porto Santa Lucia; north side of a perpendicular quay; depth about 0,5 m.
- b. Banyuls (Pyrénées Orientales, France); September 1967; small pool containing seawater discharged from the aquaria of the Laboratoire Arago.
- c. Biarritz (Basses-Pyrénées, France); August 1969; Rocher de la Vierge, in rock pools, upper sublittoral zone.

The plants all differ from one another in morphological details (which will be treated in forthcoming papers), but would be identified as *Bryopsis plumosa* according to the current taxonomic concepts of the genus (HAMEL 1930; NEWTON 1931; TAYLOR 1957).

Unialgal cultures of this material were obtained as described by RIETEMA (1969). A slightly modified Erdschreiber solution was used as a culture fluid. To one litre of filtered North Sea water were added: 25 cc soil extract; 3.72 mg EDTA; 0.278 mg FeSO<sub>4</sub>; 0.269 mg Na<sub>2</sub>HPO<sub>4</sub> · 12H<sub>2</sub>O; 42.5 mg NaNO<sub>3</sub>; 0.02 mg MnCl<sub>2</sub>; and 5 mg GeO<sub>2</sub> to prevent growth of diatoms.

The present experiments were started from cut-off determinate laterals or tips of main axes of unialgal cultures which developed into new *Bryopsis* thalli.

Motile male and female gametes from mature *Bryopsis* thalli were brought together by rupturing the cell walls of the gametangia in a watch glass. In this way it was often possible to obtain large numbers of germlings.

Thalli and germlings were kept in a 16 ± 1 °C temperature room and exposed to long day conditions. Germlings which were 5 mm or more long were transferred into culture tubes (one in every tube) filled with 10 cc culture fluid and were exposed to 16 ± 1 °C short day conditions. Under short day conditions only a small number of the germlings died after transfer into fresh medium. Daily photoperiods of 16 hours are termed "long day conditions"; photoperiods of 8 hours "short day conditions".

The light was emitted by white fluorescent tubes (Philips TL 34). The intensity of the light falling on the culture tubes amounted to about 1600 lux (as measured with an AEG lux meter).

The reactions of cell walls of germlings and mature thalli to staining with Congo red and zinc-chlor-iodide was investigated. A few drops of a saturated Congo red solution were added to the culture tubes. This is a vital stain: *Bryopsis* easily survives addition of Congo red. The reactions of the cell walls of thalli and germlings to zinc-chlor-iodide were investigated by putting some material into a few drops of this stain on a microscopic slide.

## 3. RESULTS

### 3.1. *Bryopsis plumosa* from Naples

Determinate laterals from a male and female plant had, after two weeks, grown into plants about 2 to 3.5 cm high (figs. 1 and 2) and produced male and female

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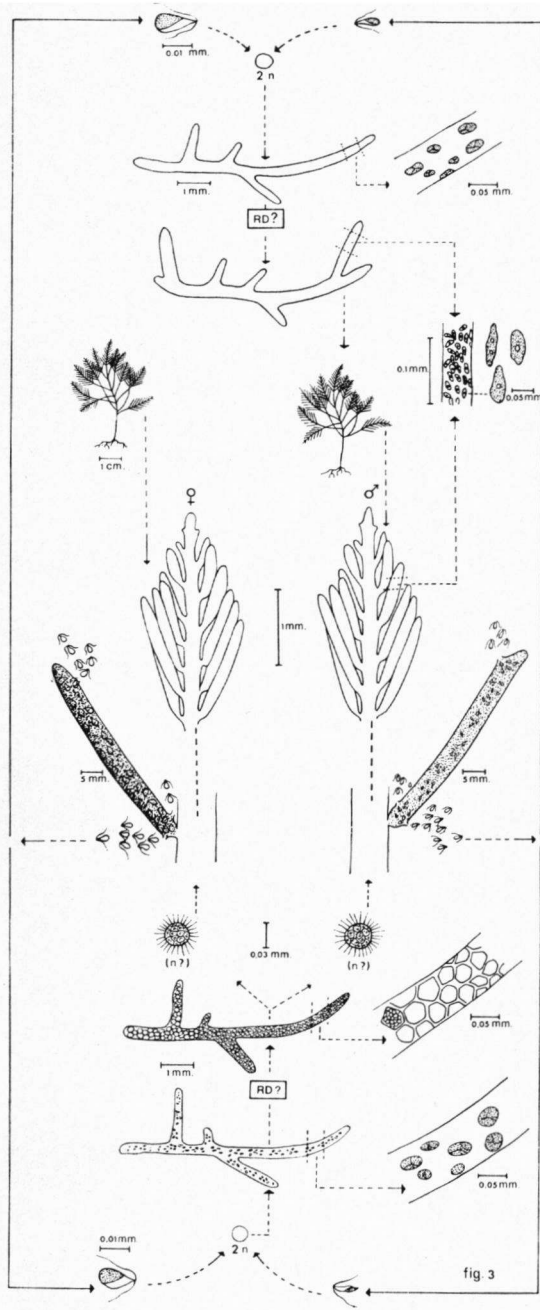


Fig. 3. Diagram of the life-history of *Bryopsis plumosa* from Naples.

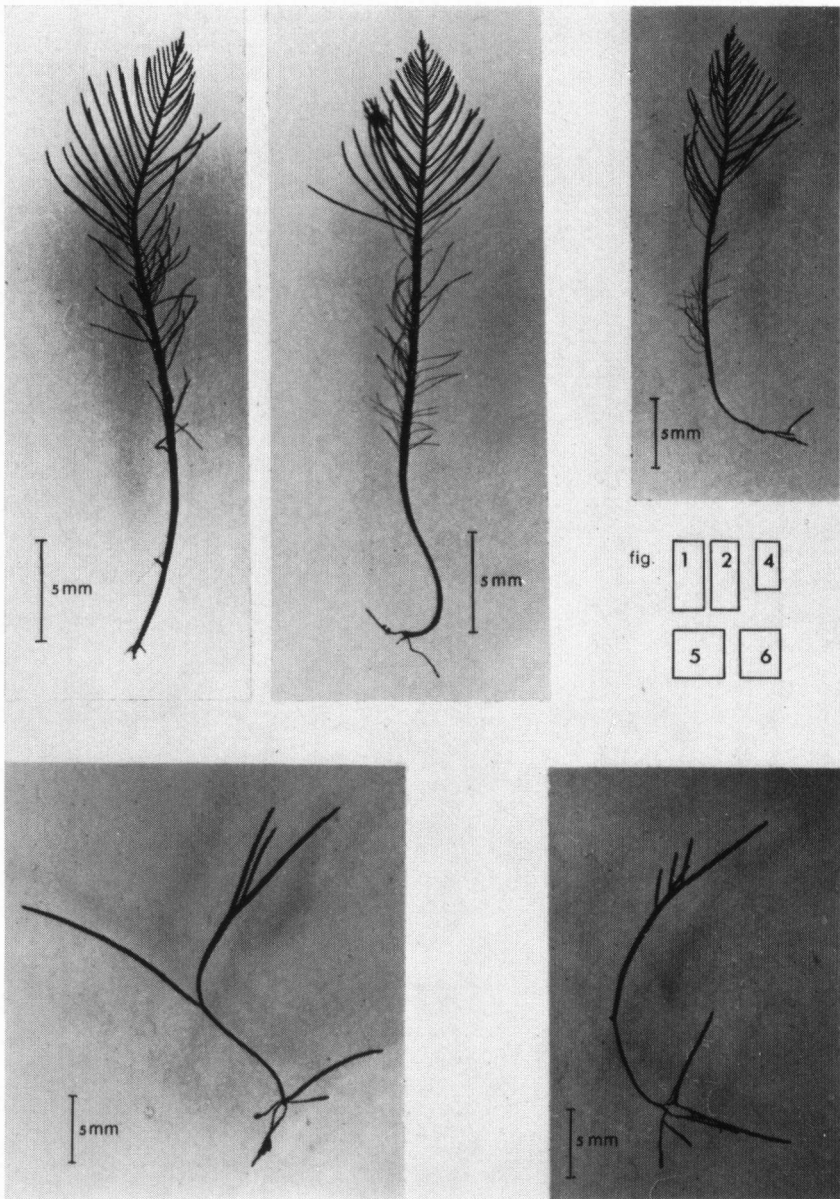


Fig. 1. Two weeks old male plant of *Bryopsis plumosa*, Naples material (grown in culture from an isolated determinate lateral).  
 Fig. 2. Two weeks old female plant of *Bryopsis plumosa*, Naples material (grown in culture from an isolated determinate lateral).  
 Fig. 4, 5, 6. Morphologically diverging plants of *Bryopsis plumosa* grown in culture from stephanokontic zoids produced by one and the same filamentous germling in the offspring of Naples material.

gametes, respectively. These plants were morphologically similar to the original plants from nature. Mixtures of male and female gametes produced a large number of filamentous creeping germlings. Twenty five two to three months old germlings were observed and none of these had grown either directly, or indirectly via the production of stephanokontic zoids, into new *Bryopsis* plants within seven weeks after transfer into fresh medium. On the other hand thirty germlings about ten months old were observed, and of these four grew directly, and five indirectly via stephanokontic zoids into new *Bryopsis* plants within 7 weeks after transfer of the germlings into fresh medium. A diagram of the life-history of *Bryopsis plumosa* from Naples is given in *fig. 3*. The stephanokontic zoospores from one germling produced female, male, and some monoecious plants (for example in 30 offspring of one germling there were 15 female, 11 male, and 3 monoecious plants; in 30 of another there were 7 female, 13 male, and 2 monoecious plants). In some plants it was impossible to determine the sex. The morphology of some plants produced by one germling via the production of stephanokontic zoids is depicted in the *figs. 4, 5* and *6*. These figures illustrate the fact that the offspring of one germling contained morphologically diverging *Bryopsis* plants. In vegetative subcultures these different morphological types remained constant.

### 3.2. Crosses between Naples material and Roscoff material

It proved possible to fertilize female gametes produced by Naples material with male gametes produced by Roscoff material and *vice versa*. Thus hundreds of germlings were obtained. Germlings about 10 months old all produced stephanokontic zoids within seven weeks of observation after transfer into fresh medium. The stephanokontic zoids grew into new fertile *Bryopsis* plants. None of the 30 germlings observed either grew out directly or remained permanently in the germling stage.

### 3.3. Crosses between Naples material and Zeeland material

It also proved possible to fertilize female gametes from Zeeland material with male gametes from Naples material. Hundreds of filamentous germlings were obtained. Germlings about 10 months old, after transfer into fresh medium, only grew directly into new *Bryopsis* plants. During the 7 weeks of observation none of the total number of 30 germlings observed produced stephanokontic zoids or remained permanently in the germling stage.

### 3.4. *Bryopsis plumosa* from Banyuls

Germlings about two months old (30 observed) derived from material collected at Banyuls grew neither directly nor indirectly, via production of stephanokontic zoids, into new *Bryopsis* plants within seven weeks after transfer into fresh medium. Seven out of thirty 5 months old germlings produced stephanokontic zoids within seven weeks of being transferred into fresh medium. Only one germling grew directly into a new *Bryopsis* plant.

### 3.5. *Bryopsis plumosa* from Biarritz

Germlings about two months old (eight germlings observed) did not grow directly, nor indirectly via the production of stephanokontic zoids, into new *Bryopsis* plants during the seven weeks after transfer into fresh medium. Within four months two germlings grew out indirectly via the production of stephanokontic zoids.

Four out of twelve four months old germlings produced stephanokontic zoids during seven weeks after transfer into fresh medium.

### 3.6. 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 *Bryopsis* thalli derived from material collected in Naples, Banyuls, Biarritz, Roscoff, and Zeeland, were stained red by Congo red. The cell walls of the germlings derived from material collected in the same localities did not stain at all in the same period of observation. With a zinc-chlor-iodide solution the cell walls of the *Bryopsis* thalli immediately stained violet whereas those of the germlings remained unstained. In germlings directly developing into new *Bryopsis* plants only the inner wall layers stained with zinc-chlor-iodide. This phenomenon was particularly clearly observed in germlings obtained from Zeeland material and crosses between Naples and Zeeland material.

## 4. DISCUSSION

The first description of a life-history in *Bryopsis plumosa* was based on observations made on *Bryopsis plumosa* collected at Porto Santa Lucia in Naples (ZINNECKER 1935). These observations gave evidence of a monophasic, diplontic life-history, in which meiosis took place preceding the formation of anisogametes in the determinate laterals. Until recently this type of life-history was widely accepted as characteristic for the whole genus *Bryopsis* (FRITSCH 1945; IYENGAR 1951; SMITH 1955; BOLD 1957; CHADEFAUD 1966). The present observations were made on material collected in exactly the same locality where Zinnecker collected her *Bryopsis plumosa* plants. In contrast to her observations the present author's results show that both a monophasic life-history and a heteromorphic biphasic life-history can be realised within one and the same *Bryopsis plumosa* population from Naples. The filamentous germlings into which the zygotes developed grew partly directly, partly indirectly via the production of stephanokontic zoids, into new *Bryopsis* plants. Germlings derived from *Bryopsis plumosa* collected at Banyuls behaved more or less the same as the germlings from Naples material.

In comparing the life-histories of *Bryopsis plumosa* from Naples and Banyuls with that of *Bryopsis plumosa* from Roscoff (see RIETEMA 1969) it was striking that Roscoff germlings always divided into stephanokontic zoids (and never grew directly into *Bryopsis* plants) and further could much more easily be induced to "break" their "dormancy" than the Banyuls and Naples germlings. As in the Naples and Banyuls germlings it was not easy to break the "dormancy"

of the germlings derived from *Bryopsis plumosa* collected in Biarritz. These germlings also grew indirectly via the production of stephanokontic zoids into new *Bryopsis* plants. All above-mentioned observations suggest that *Bryopsis plumosa* populations from more southern shores of Europe have a filamentous germling stage in their life-history that is capable of producing stephanokontic zoids.

*Bryopsis plumosa* from Zeeland (a more northern shore in Europe) lacks this capacity completely. Observations made on germlings from *Bryopsis hypnoides* collected on Helgoland (NEUMANN 1969a) suggest that this *Bryopsis* species in northern regions also lacks this capacity. The disappearance of a stage (the formation of stephanokontic zoids) in the life-history of a northern population of *Bryopsis plumosa* (Zeeland) and the rapid "break" of the "dormancy" of the filamentous germling stage of Roscoff and Zeeland populations of this species tend to "accelerate" the life-histories of the more northern populations as compared with more southern populations.

Successful interbreeding between Naples, Roscoff, and Zeeland material suggest that there are no genetic barriers between *Bryopsis plumosa* populations from these three regions.

The fact that the germlings obtained from crosses between Naples and Roscoff material under the applied cultural conditions always produced stephanokontic zoids, while the germlings obtained from crosses between Naples and Zeeland material and crosses between Roscoff and Zeeland material (RIETEMA 1969) always grew out directly, indicates that the capacity of germlings to produce, or not to produce, motile stephanokontic zoids is genetically determined. The occurrence of both possibilities in germlings derived from *Bryopsis plumosa* from Naples and Banyuls suggests, however, that differences in the mode of development of the filamentous germling phase, and hence the occurrence of a monophasic or biphasic life-history, are without fundamental taxonomic importance.

The fundamental similarity of germlings producing stephanokontic zoids and germlings developing directly into new *Bryopsis* plants is confirmed by their identical negative reaction to the zinc-chlor-iodide and Congo red stain. The walls of the *Bryopsis* phase always give a positive reaction to both stains. Apparently the chemical composition of the wall of the germling phase differs considerably from that of the *Bryopsis* phase. In this respect it is interesting that germlings developing directly into new *Bryopsis* plants had an outer wall-layer (the original wall of the germling phase) that did not stain and an inner wall-layer (continuous with the wall of the *Bryopsis* phase) that stained with zinc-chlor-iodide. The terms monophasic and diplontic as applied to the life-history of *Bryopsis plumosa* are therefore incorrect and in any case premature.

As mentioned above, ZINNECKER (1935) produced caryological evidence for the occurrence of meiosis previous to the formation of the gametes in the determinate laterals. The fact that some of the stephanokontic zoids produced by one germling (Naples material) grew into male *Bryopsis* plants, some into female *Bryopsis* plants, and only a few into monoecious *Bryopsis* plants, and the fact

that the offspring from one germling may contain morphologically diverging plants (the characters of which remain constant after further vegetative subculturing) rather suggest that meiosis takes place somewhere in the germling phase. Therefore, a caryological reinvestigation on the site of meiosis in the life-histories of *Bryopsis plumosa* is required. If meiosis proved to take place previous to the formation of stephanokontic zooids the lifehistory of *Bryopsis* would resemble the heteromorphic biphasic life-history of *Halicystis ovalis* which has *Derbesia marina* as a sporophytic phase (NEUMANN 1969b).

Possibly *Bryopsis halymeniae* which has *Derbesia neglecta* as a sporophytic phase in its life-history (HUSTEDE 1964) could serve as an intermediate between *Halicystis* and *Bryopsis* species which have in their life-histories only a reduced sporophytic phase (*viz.* a creeping filamentous phase producing stephanokontic zooids).

*Bryopsis monoica* and southern populations of *Bryopsis hypnoides* also have in their life-histories a filamentous, creeping germling phase which produces stephanokontic zooids (Rietema, unpublished).

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