LIFE HISTORY STUDIES IN MONOSTROMA FUSCUM (POST. AND RUPR.) WITTR.

by

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INTRODUCTION

The author's interest in the genus Monostroma was aroused while engaged in cultural studies of marine algae with Dr. R. F. Scagel at Friday Harbor, Washington during the summer of 1959. At that time, Dr. Scagel and Dr. Yamada of Hokkaido University suggested a study to determine the relationship between the entities bearing the name M. zostericola in Japan and in North America. Other native species of Monostroma were collected also and considerable difficulty was experienced in assigning these entities to described species of Monostroma. Cultures of these entities were started in an attempt to establishing their taxonomic status.

Five entities were recognized among those studied, one of which was considered to be M. fuscum. This form first attracted the author's attention because the thickness of the thalli varied considerably from the published dimensions and the organization of its chloroplasts differed from that of other species studied. Later, fertile plants of this species, collected along the Oregon Coast in June 1960, were observed to produce in some cases biflagellate and, in others, quadriflagellate swarmers. As there was at that time no record of any species of Monostroma with an isomorphic alternation of gametophytic and sporophytic generations, the decision was made by the

author and his major professor, Professor H. K. Phinney, to concentrate on a study of the life history of this entity. The study has been made at Friday Harbor Laboratories, San Juan Island, Washington and Oregon State University, Corvallis, Oregon and along the coastal areas near these facilities.

While the objective of this study was to elucidate the essential features of the life history of this species, from observations of collections and from cultures, it must be recorded that apparent differences were seen between the plants growing in the two areas. The means of thallus thicknesses of the two populations lie within the limits often given for two different varieties of M. fuscum. The anatomical features of the plant were studied at both localities and are here reported together. The life history information was obtained primarily from observations of plants growing at San Juan Island with some corroborating evidence from germling cultures from plants collected in Oregon. Description of the cytology was derived almost entirely from plants collected along the Oregon coast.

The two varieties of M. fuscum considered in this study are widely distributed in the colder waters of the northern hemisphere. M. fuscum, var. splendens (Rupr.) Rosenv. has been collected from the Kurile Islands by Nagi (20, p. 21); from the Aleutian Islands, the Arctic Ocean, and the Bering Sea by Ruprecht, according to DeToni (10, p. 107); from Alaska by Saunders (29, p. 409); from the west

coast of North America by Setchell and Gardner (33, p. 242); from Greenland by J. Agardh, according to Rosenvinge (26, p. 940) and also by Rosenvinge (26, p. 940); and on the coast of Norway and Greenland by J. Agardh, according to DeToni (10, p. 108).

M. fuscum var. Blyttii (Areschoug) Collins has been reported from the northwest coast of Norway by Blytt, according to DeToni (10, p. 108); along the northeast coast of North America by Farlow, according to DeToni (10, p. 108); and along the northwest coast of North America by Setchell and Gardner (33, p. 243).

REVIEW OF LITERATURE

The Monostroma fuscum Complex of the Pacific Northwest

None of the Ulvaceae described by Linneaus have been found to belong in the modern genus Monostroma. However, plants possessing the monostromatic character were known to early post Linnean authors who either placed them under the Linnean binomial Ulva lactuca that many early workers believed was based on a monostromatic plant or they described them as new species of Ulva as in the case of U. bullosa Roth 1806. The type of U. lactuca was examined in 1954 in the Linnean Herbarium by Dr. G. F. Papenfuss who found it to be distromatic, thus ending two centuries of uncertainty as to the nature of this species.

The next species to be described in the genus Monostroma were published by Postels and Ruprecht in 1850 (23, p. 21) as U. fenestrata and U. fusca. Kutzing in Phycologia Generalis, 1843 (17, p. 294), retained the name Ulva for monostromatic species based upon U. lactuca, and described the genus Phycoseris for the distromatic species. Ulva Blyttii was described by Areschoug in 1850 according to Wittrock (38, p. 49) and in 1851 Ruprecht (28, p. 409) proposed the genus Ulvaria based on Ulva fusca Post. and Rupr. and Ulva splendens Rupr. This new genus was distinguished from Ulva on the basis of

fruiting structures. According to Papenfuss (22, p. 309) Thuret (36, p. 29) proposed the Genus Monostroma in his Note sur la synonymie des Ulva Lactuca et latissima, L., suivie de quelques remarques sur la tribu des Ulvacées. The sections Ulvaria and Eumonostroma were proposed within the genus Monostroma by DeToni in 1889 (10, p. 108). These sections were distinguished by anatomical characters, Ulvaria having parenchymatous thalli and Eumonostroma being more or less gelatinous.

A monograph of the genus Monostroma prepared by Wittrock in 1866 (38, p. 24) reviewed descriptive information available at that time and included some of the author's own observations on the species M. fuscum, M. splendens, and M. Blyttii. These same taxa were treated as forms or varieties of M. fuscum by Collins in 1903 (8, p. 213) and in 1909 (9, p. 10) and by Setchell and Gardner in 1920 (33, p. 242) in taxonomic descriptive works on the Chlorophyta of North America.

Considering the differences of life cycles seen in some Monostroma and those seen in Enteromorpha and Ulva, Kuneida in 1934 (16) expressed the opinion that Monostroma should be placed under a family, Monostromaceae. This view was supported by Suneson in 1947 (35, p. 240) and has been followed in more recent works by Papenfuss (22, p. 315), Arasaki (1) and Scagel (30, p. 34).

In 1960 Papenfuss (22, p. 315) proposed the name Monostroma for conservation against Ulvaria in view of the "world-wide distribution of Monostroma and the frequent reference to it in floristic and morphological publications". In the same year a proposal was made by Bliding (4, p. 172) to retain the name Ulvaria for those species of Monostroma in which reproduction is by asexual biflagellate swarmers.

Life History Studies in the Genus Monostroma

Details of swarmer production, fertilization, and development from the zygote have become known in a number of species of Monostroma, but in no species has there been an elucidation of the complete life history including the point of meiosis. However, it has become apparent that this taxon, as presently conceived, exhibits a variety of life histories. Few genera within the Chlorophta possess a comparable diversity of life histories.

Wittrock's extensive monographic treatment of this genus (38, p. 24) included a brief section on reproduction. Wittrock expressed indebtedness to Areschoug for much information, particularly for observations on swarmers of M. Grevillei and he also cited the published works of G. Thuret (36, p. 29). He stated that the observed number of flagella in M. Grevillei was two and in M. bullosum four, and suggested that in every species there were two types of zoospores;

one with four, the other with two flagella. Wittrock indicated that the former germinate immediately while the latter became resting spores.

The original report containing studies of cultures of Monostroma bullosum by Reinke in 1878 (25) was unavailable but Carter, in 1926 (6, p. 680) and Moewus, in 1938 (19, p. 364) reviewed Reinke's work and recounted his description of the enlargement of the zygote and its division after five months into eight cells. Carter relates that, "cell division proceeds in such a way as to preserve the structure of a hollow sphere which is the form of the young Monostroma plant." Chodat, in 1894 (7) studied the same plant, and reported that zygotes or zoospores divided into two, then four cells. The four cells were arranged in either a linear series or in the form of a "T". These four-celled structures, after further division, developed into juvenile plants. Chodat implied that division of the zygote proceeded without enlargement or dormancy.

Some aspects of the life history of the fresh water species M. membranacea were added by West in 1903 (37). According to this author, zygotes in this alga enlarged and germinated after several weeks, dividing into four cells. The author described the young plants, as developing through irregularly oriented divisions of the four cells.

Carter in 1926 (6, p. 680) undertook to discover cytological affinities or contrasts that might exist between the Ulvaceae and

members of the order Ulotrichales. This objective was not attained because of the small size of the nucleus, but the work was a valuable introduction to cytological study in the family, and included details for two species, M. latissimum and M. Grevillei var. Vahlii. Behavior of the pyrenoid during vegetative cell division and certain nuclear details were given for these two species. Cultural study was made only of M. latissimum. In this species, the zygote and the gametes of either sex following attachment enlarged to about 50μ in 38 days at which time the cultures were terminated.

In 1931 Miyake and Kuneida (18, p. 348) studied an unidentified Japanese species of Monostroma and carefully described the morphology and behavior of gametes. The zygote wall in this species thickened and the zygote enlarged to a diameter of approximately 15-20μ in 4-5 weeks. Cell division occurred in some zygotes after 40 days. A three-celled filament was the most advanced stage noted when the culture was terminated after 60 days.

An important contribution to the knowledge of life histories was made by Kunieda in 1934 (16) in his study of an unidentified species of Monostroma from the region of Misaki. Zygotes in this plant enlarged and after four to five months released 32 zoospores. The zoospores germinated immediately, developing into sexual plants. Yamada and Tatewaki later implied (41) that the species with which Kuneida was working was M. nitidum.

Bliding, in 1935 (3, p. 66), reported that reproduction was entirely asexual in M. Wittrockii. The biflagellate swarmers, after attaching and developing a cell wall, grew into filaments. From Bliding's illustrations it appears that the vesicular form was present at about the ten-celled stage. A rhizoidal system was not present in the plants he illustrated. Bliding stated that his findings were in agreement with those reported by Bornet in 1880 (5, p. 176), who, according to Bliding, also found that M. Wittrockii reproduced only asexually.

Yamada and Saito (40, p. 43), working with M. angicava, reported in 1938 that gametes fused to form zygotes which enlarged to 55-80μ. The cysts germinated after five months liberating zoospores. The zoospores germinated and produced branching filaments, which by further growth, formed a dense disk. A monostromatic thallus developed from a layer of cells which uplifted in the center of the disk. This life history was essentially the same as in the Misake species reported by Kunieda.

A unique life history was reported by Yamada and Saito in 1938. (40, p. 47), for M. pulcrum. Fertile thalli produced quadriflagellate swarmers and these developed into spherical structures 50-80μ in diameter. These spherical structures resembled the zygotes of M. angicava illustrated by Yamada not only in appearance but in the fact that they also produced quadriflagellate swarmers. These

quadriflagellate swarmers, unlike those produced by the thalli, when released began to undergo division. Their development was not followed beyond the two-cell stage.

Moewus in 1938 (19, p. 364), demonstrated sexuality and the occurrence of an enlarged zygote in M. Wittrockii. Moewus verified statements made by Kunieda, that the enlarged zygote represented the sporophyte generation and formed 32 zoospores. He also deduced evidence for genetypic sex determination in this alga.

Yamada and Kanda in 1941 (39, p. 218), reported that in M. zostericola as in M. pulcrum, fertile thalli released quadriflagellate swarmers. The quadriflagellate swarmers in this plant, however, developed into thalli similar to those found in the field. The existence of the sexual phase in this alga was reported by Yamada and Tatewaki in an abstract published in 1959 (41).

A brief description of zygote formation and enlargement in M. Grevillei was given by Schreiber in 1941 (32). Details of germination were not given.

Descriptions of the life histories of M. nitidum (?) and M. latissimum were given in a study of eleven species of the Ulvales by Arasake in 1946 (1). In these species, both gametes and zygotes germinated to form germlings that developed into tubiform thalli at about the 10-celled stage. These thalli eventually opened to the form typical of these species. A basal rhizoid system was not indicated by

his illustrations.

Enlargement of the zygote of M. Grevillei was reported by Suneson in 1947 (35, p. 240), but germination was not observed.

A study of four Japanese species of Monostroma reported by Iwamoto in 1960 (14, p. 93) included descriptions of two new species. In one, M. tubiforme, biflagellate swarmers developed into trumpet-shaped thalli without development of a juvenile basal disk. Swarmers were not observed in the second of the two new species, and propagation by gemmation was proposed.

The first report of the existance of both gamete-producing and zoospore-producing thalli in a species of Monostroma was made by Gayral in 1961 (13) in a brief description of the thallus structure and morphology of swarmers of M. obscurum.

METHODS AND MATERIALS

Collecting Sites

Collecting areas were selected with consideration for ease of collecting and accessability to the laboratory facility. Populations of M. fuscum were chosen at two locations near Friday Harbor Laboratories and at one location near Oregon State University. An abundance of M. fuscum was found attached to rocks in the sub-tidal and very low intertidal zone in many places along the accessible west shore of San Juan Island. Two areas that differed in type of habitat were selected along this west shore; these were the beach at Mount Dallas (Lat. 48°30'21"N., Long. 123°08'23"W.) and the bay at Edwards Reef (Lat. 48°29'56"N., Long. 123°07'37"W.) M. fuscum grew abundantly in tide pools of the mid-intertidal zone at Marine Gardens, Otter Rock, Oregon. The densest populations were located just south of the large rock outcropping in the center of the Marine Gardens area.

Collecting Procedures

At San Juan Island collections were made by wading at all locations during periods of low tide. During periods of high tide plants were collected at the Mount Dallas beach by diving. Diving equipment included snorkel and wet suit. Plants were placed in

11 x 14 inch bags made of plastic screen material. The top of each bag was equipped with a steel spring closure that could be opened easily while wearing gloves and remained closed at other times. The area at Edwards Reef was found suitable for collecting by casting a hook since this area was more protected from tidal currents than the beach at Mount Dallas and kelp was less abundant.

At the Marine Gardens area, plants were collected from shallow tide pools of the mid-intertidal zone by wading. The tide pools were accessible every day during low tides.

Selection of Material for Study

The visual selection of plants at or near a desired stage of swarmer development was possible because of the progressive change in color of the thallus margin occurring with the differentiation of fertile cells. Often the stage of reproductive development of a plant could be estimated to within a day by comparing the lighter green of the fertile area with the darker green vegetative portion of the thallus. This difference in color could be detected best in submerged plants and most ideally while diving, since the refractions at the air-water interface were absent and a closer view of the material was possible. At night the plants were examined against a dark background while illuminated by an electric lamp attached to the collectors hat.

Transportation and Storage of Living Materials

Material at a stage just prior to normal release of swarmers was placed between folds of newspaper and kept in a plastic bag surrounded by crushed ice. The material was retained in these packages during refrigerated storage in the laboratory. The release of most swarmers was delayed in this manner as much as two days beyond the normal time of release.

Materials at earlier stages of swarmer formation were placed in a styrofoam bucket containing sea water chilled by ice contained in a plastic bag. The plant material was stored in the laboratory in a plastic bucket that was provided with a continuous change of water. At the collecting site material was also placed directly into ice cube trays containing sea water and held at 12° C. until the time of study. Plants containing cells in which the nuclei had migrated to an equatorial position differentiated normally both in light and dark storage.

Swarming

As they matured, swarmers were discharged from material stored in the light, but in the dark, discharge of swarmers was delayed for periods of as much as two days. Uninterrupted darkness was necessary to effectively prevent discharge since brief exposure to light occasioned by removing material from the bucket or ice cube

tray caused discharge of all fertile material.

To obtain swarmers for study, portions of fertile plants were taken from newspaper or dark storage in sea water and transfered to plastic ice cube trays containing sea water. The temperature of the trays was maintained at 12° C. on a circulating sea water table or in a refrigerated culture box. The trays were illuminated from above by fluorescent lights. Swarmers were observed at the surface of the water within 30 minutes following the termination of darkness. Swarmers responded phototactically and it was possible to obtain swarmers quite free of diatoms by making successive transfers into purified sea water. Plastic ice cube trays illuminated unilaterally from above were found very suitable for this purpose.

Attachment of Zoospores and Unfertilized Gametes

Early ontogeny of germlings from unfertilized gametes, zygotes, and zoospores was followed by studying them attached to glass microscope slides. The juvenile vesicular stages were grown on unglazed, white tile and observed macroscopically with vertical or lateral illumination. The tile provided a firm substrate for the holdfast and presented a white background which contrasted with the alga.

The slides (or tiles) on which zoospore or unfertilized gamete germlings were to be established were placed on a porcelin tray under which sea water was circulated at 12° C. A single drop of water

containing swarmers was transfered from the ice cube tray to a position approximately 1/2 inch from one end of each microscope slide. Evaporation of the drop of water was retarded by maintaining a high relative humidity in the surrounding air. The slides remained on the tray for 1-12 hours while part of the swarmers became attached to the slides. The slides were then rinsed and transfered to culture dishes.

Zygote Formation and Attachment

Gametes of opposite mating strains were identified by trial. Drops of water containing gametes were mixed and presence of the two sexes could be determined macroscopically by clumping activity and microscopically by observing zygotes. Zygotes for culture were obtained by mixing 2 cc of water containing a high concentration of gametes of each sex in a syracuse watch glass half-full of sea water. Soon after plasmogamy, the zygotes became attached to the bottom of the dish. After a period of from 5-10 minutes the dish was vigorously rinsed with a stream of sea water that removed all but the attached zygotes. The zygotes were then transfered to tiles or slides with a camels hair brush and placed in culture.

Zygotes were also produced directly on the microscope slides by placing drops containing freshly released gametes of the two sexes on a chilled slide. Fusion cells were concentrated by illuminating

from the side. The unmated gametes were removed by vigorous rinsing in sea water after the fusion cells had become attached.

Purification of Sea Water Medium

A number of procedures were tried to remove contaminating organisms from water used in cultures. Centrifugation with a Foerst plankton centrifuge, filtration through shark skin filter paper, sand, and glass wool proved unsuccessful in removing diatoms. Diatoms were successfully removed only by micropore filtration and by autoclaving the sea water.

Use of water that contained diatom contaminants necessitated constant care to prevent the diatoms and bacteria from overgrowing the developing germlings. Young cultures were brushed every two weeks with a camels hair brush. The more mature cultures were brushed with an ordinary 1/2" wide paint brush with bristles shortened to about 3/4 inch.

Composition of the Culture Solution and the Conditions of Culture

Germlings were cultured on slides or tile submerged in 125 ml of enriched, filtered sea water contained in a pint wide mouth jar covered with a petri plate. The culture solution consisted of 125 ml of sea water to which was added approximately 0.75 ml of a solution containing 300 gm of $NaNO_3$/liter of distilled water and 0.75 ml of a

solution containing 22 gm of NaH_2PO_4/liter of distilled water. The culture solution was changed every two weeks. A culture temperature of approximately 12° C. was maintained by circulating sea water at Friday Harbor Laboratories and at Oregon State University in a culture chamber improvised from a soft drink cooler. Continuous overhead illumination was provided at Friday Harbor Laboratories by warm white fluorescent tubes yielding 100-300 f. c. At Oregon State University 3 delux white and one blue tube provided 200-300 f. c. of illumination for fourteen hours out of twenty four. At Oregon State University the cultures rested upon a shaker platform that oscillated 120 cycles per minute.

Methods of Study of Germling Development

Study of details of germination and development of zoospores, zygotes, and of unfertilized gametes developing on glass slides was made by covering the growth with a coverslide and observing microscopically. Slides were not returned to culture except in the studies of germlings on mapped slides. The cultures on tiles were observed with a stereo dissecting microscope.

After plants had grown in culture for 11 months they were provided with natural growing conditions. The tiles on which the plants were growing were attached to a submerged platform in the shelter of Friday Harbor. The platform was weighted and suspended from a

float and maintained one meter below the surface.

Cytological Technique

Material for study of differentiation of zoosporangia and gametangia was obtained from plants exhibiting color changes in the marginal fringe of the thallus characteristic of plants entering the fertile condition. Portions of these plants, 3-4 cm square, were placed in sea water in numbered compartments of plastic ice cube trays and transported to the laboratory where the type of swarmer produced was determined. Portions 5 mm square of the same thalli were killed and fixed in the field by plunging them into numbered two dram vials half-filled with a solution of one part glacial acetic acid and three parts 95% ethyl alcohol. Following fixation for from 3-12 hours, the fixed material was rinsed twice in 70% alcohol and stored in 70% alcohol at 8^{o} C. until it was stained by the Feulgen technique.

While fixed material was being processed, living replicates were maintained in the culture cabinet. As swarmers were liberated, a record was made of each numbered specimen as being sporophyte or gametophyte. The fixed materials were then transfered to two vials, one containing gametophyte material and the other containing sporophyte material. The material was processed in carriers made of 4 cm long sections of half-inch polyethylene tubing covered at one end by #20 mesh bolting cloth.

Material to be stained was brought down to water through 50, 30, 15 and 5% ethyl alcohol with 5 minutes in each grade. After 30 minutes in 1% chromic acid the material was washed in running water for thirty minutes. The staining schedule was modified from Johansen (15, p. 95). Hydrolysis at 60° C. in HCl for 10 minutes was followed by 3 hours in Schiffs reagent. Material was then passed through three changes of the differentiating solution described by Johansen, with 5 minutes in each change. Dehydration through 5, 15, 30, 50, 70, 95, and 100% ethyl alcohol with a period of 5 minutes in each grade followed. The material then passed through 1:1 ethyl alcohol and xylol and finally into xylol. Whole mounts were made in Canada balsam. Sections were made by hand following removal of material from the final differentiating solution. A thin layer of albumin was applied to a slide and heated over a flame. The sections were applied to the slide in a drop of water and pressed onto the albumin under a cover slip. The sectioned material was dehydrated through the alcohol series listed above and mounted in Canada balsam.

Photography

Photomacrographs were made with 35 mm single lens reflex camera equipped with a lens of 40 cm focal length. In some cases extension tubes were employed. Photomicrographs also were made with a 35 mm reflex camera. Flash photomicrographs were made

using a standard electronic flash manufactured for photomacrography. Light from the flash tube was collimated and directed into the optical axis of the microscope by means of a beam splitter located between the microscope and the microscope illuminator. Kodak High Contrast Copy film was used to record gametes and early germling stages photographed through 4 mm and 2 mm F. L. microscope objectives. The film was developed 8 minutes in Kodak DK 50 at 20° C. Photomacrographs and photomicrographs recorded through the 16 mm F. L. objective and the photomicrographs of materials of higher contrast photographed through the 4 mm F. L. microscope objective were made using Kodak Panatomic-X film, developed 3 1/2 minutes in Kodak DK 50 at 20° C.

RESULTS

Description of Monostroma fuscum

Proposed Life Cycle

Although it was not possible to delineate the entire life history in culture, indirect evidence was obtained for a cycle consisting of an alternation of isomorphic, heterothallic haploid gametophyte and a diploid sporophyte plant. Meiosis has been shown to occur in the initial divisions within the zoosporangia. Additional evidence supporting the existence of this type of life history consisted of the complete morphological similarity of the vegetative sporophytic and gametophytic plants and the similarity of ontogenetic development of germlings from zygotes and zoospores.

Details of this evidence are presented below.

Ecology

At the beach at Mount Dallas the plants formed a more or less continuous population extending for a distance of over 300 yards along open, exposed coast. The intertidal zone here was characterized by a precipitous rock slope which gave way abruptly to a subtidal shelf of broken rock. The shelf sloped gently from 0.0 MLT* to -1 or -3

*Mean low tide datum.

meters MLT and then fell off rapidly. Current and wave action was as severe as on any part of the island. At the upper limit of growth of M. fuscum (+1 meter MLT) a few dwarfed plants were found crowded among other species of Ulvales. The zone of dense growth of the alga extended from lowest tide line to -2 or -3 meters MLT. This lower limit of dense growth corresponded closely with the upper limit of the densest sea urchin population. The maximum depth for the alga was not determined but plants were found sparsely at -8 meters MLT.

The bay at Edwards Reef was protected from wave action and current by a reef that partially obstructed the mouth of the bay along the north side. The alga grew thickly in the north part of the bay where the depth was less than 1 1/2 meters MLT. Growth of M. fuscum here extended up to a level of about -1/2 meter MLT where it was replaced by Ulva species.

The area at Marine Gardens, Otter Rock, Oregon was characterized by tide channels and flat, intertidal reef. M. fuscum was found scattered among other species of the Ulvales. The plants were exposed to severe wave action and during summer periods of low tide they were exposed to high day time temperatures.

Vegetative Morphology

Some difficulty was experienced in distinguishing macroscopi-

cally, living plants of M. fuscum from species of Ulva. Distinction on the basis of color was possible only with some species of Ulva. The presence of the funnel shaped holdfast in M. fuscum served to distinguish it from species of Ulva when plants were not too greatly torn by wave action.

The thalli of M. fuscum attached poorly when dried on herbarium paper and were distinctly fragile. A valuable taxonomic characteristic was the dark brownish-green color assumed by plants on drying. This darkening was seen in plants washed up on beaches and was present in varying degrees in herbarium material. When an explanation for the darkening was sought, Dr. Meeuse of the University of Washington suggested the gum gaicum test for polyphenyl oxidase. The results of this test were positive. Further tests conducted by Dr. Meeuse in the laboratory at the University of Washington showed that polyphenyl oxidase activity in this alga was significantly higher than in one other species of Monostroma and in one species of Ulva tested.

Plants identified as M. fuscum were collected in various stages of development. Plants 2-4 mm in length were obpyriform vesicles, tapering downward into a stipe and holdfast. Vesicles approximately 5 mm in length were observed to be somewhat more pale in a region distal to the maximum diameter of the vesicle. Cells of this distal region were observed to be enlarged with respect to cells of the

proximal portion of the vesicle. Some vesicles were found with this distal cap region ruptured and others were observed to have entirely lost the distal cap of enlarged cells. Following the loss of the cap the plants were funnel-shaped above the short, cylindrical stipe. In regions subjected to wave action the enlarging plants became perforated and more or less torn, sometimes to the extent that segments of the blade became detached.

The average length of the blades, measured from stipe to tip, varied seasonally. Plants examined in all seasons at Marine Gardens had blades varying in length from 5 cm in December to 20 cm in July. When observed in August, plants at San Juan Island had an average blade length of 35 cm.

The thickness of blade was fairly uniform except below a point 1 1/2 cm above the holdfast where the thickness was increased by intramatrical rhizoids. The thickness immediately above the stipe was 200-400μ .

Thickness of blade in plants collected at San Juan Island and at Marine Gardens is shown in Table I. The mean thickness of 89 plants collected at the two San Juan Island sites was 61.2μ. The mean blade thickness of 47 plants collected at Marine Gardens was 49.4μ. A greater variation was seen in the thickness of plants collected at Marine Gardens in the winter than in the summer but the mean thickness of the winter and summer collections compared closely. No

Table I. Blade thickness in Monostroma fuscum collected at the bay at Edwards Reef, the beach at Mount Dallas, and at Marine Gardens.

Collection site	Date	Type of plant (gametophyte or sporophyte)	Depth of attachment referred to mean low tide (meters)	Number of plants sampled	Range of thickness (μ)	Mean thickness (μ)
Bay at Edwards Reef	8-25-61	gametophyte	0.6	31	53-70.5	61.6
		sporophyte	0.6	30	50-73	63.2
	9-6-61	gametophyte	0.6	9	55-65	61.3
		sporophyte	0.6	9	55-66	60.4
	Mean blade thickness of 89 plants					61.2
Beach at Mount Dallas	9-2-61	mixed gametophyte and sporophyte	- 2.0	11	53-63	56.9
Marine Gardens	10-7-61	mixed gametophyte and sporophyte	+2.0	17	38-64	50.9
	6-21-62	mixed gametophyte and sporophyte	- 0.3	6	42-52	48.2
		mixed gametophyte and sporophyte	+0.3	13	40-53	47.5
		mixed gametophyte and sporophyte	+2.0	11	40-54	50.0
	Mean blade thickness of 47 plants					49.4

significant difference was seen between the blade thickness of gametophyte and sporophyte plants. The difference in blade thickness observed between plants growing at +1.6 meters MLT and plants growing at -0.3 meters MLT at Marine Gardens was small.

Not shown in this table are the measurements recorded for portions of two plants collected from deeper water. A segment of a plant collected by dredge in Roach Harbor, Washington at a depth of 25 feet measured 78μ. A segment of M. fuscum was found in the spiral valve of a rat fish, Hydrolagus colliei, which was taken by net at a depth of 30 fathoms in Lopez sound, Washington by John S. Laurie. This segment was 82μ thick. Holdfasts were not present on either specimen. It could not be determined if these plants grew attached at these depths or if they had become detached and drifted to deeper areas.

More distal portions of the blades were monostromatic (Plate I, Figure 3) and uniformly thick except for a narrow band of cells which decreased in thickness out to the margin of the blade (Plate I, Figure 5). The cells of the monostromatic region seen in surface view were closely set and had a diameter of 10-20μ (Plate I, Figures 1 and 2). In monostromatic portions, the cell walls at the surface of the thallus were 36μ thick and thickened toward the holdfast.

The basal 1 1/2-2cm of the blade was thickened by the presence of intramatrical rhizoids arising from cells of this region (Plate I,

Figure 4). These cells were somewhat larger than cells of the more distal, monostromatic portion of the blade. The rhizoids were directed toward the stipe and were found only within the wall which formed ontogenetically the inner surface of the vesicle. The stipe was 1/2 - 1 1/2 mm in length, hollow at the top, becoming solid below. It was approximately 1 mm wide and consisted of a single layer of cells surrounding a central core of rhizoids.

Vegetative Cytology

An enlarged central vacuole occupied more than two-thirds of the cell lumen. The chloroplast was concentrated at both ends of the cell. These polar masses were connected by thin parietal strands that passed between the vacuole and the lateral cell walls. The chloroplast was approximately ring-shaped when viewed from the end of the cell that was ontogenetically the inner surface of the vesicle. The nucleus was located within this ring. In recently divided cells the chloroplast was open along the most recently formed lateral wall. The chloroplast was thin, but continuous over the opposite end of the cell. Here, there were usually one or two large pyrenoids. Somewhat smaller pyrenoids were located in the denser regions of the chloroplast.

The nucleus was round to slightly irregular in shape, 3 1/2-5 1/2 μ in diameter. It was located adjacent to the end of the cell that

was ontogenetically the inner surface of the vesicle and near one of the lateral walls of the cell (Plate I, Figure 2; Plate II, Figures 10 and 11). One to three heteropycnotic bodies staining with Schiffs reagent were present in the interphase nuclei of both haploid and diploid vegetative cells. Nucleoli were observed in some living vegetative cells (Plate I, Figure 2).

During vegetative cell division, the nucleus remained at the end of the cell nearest the wall which was ontogenetically the inner surface of the vesicle. The spindle axes of these mitotic figures were oriented randomly in a plane parallel to the plane of the thallus. The chromosomes at this time remained closely associated and could rarely be seen as discrete structures.

Reproduction

Gross Aspects of the Formation and Discharge of Zoospores and Gametes

The main extra-nuclear events during the formation of zoospores and gametes were similar with respect to time. Macroscopically the first indication of approaching fertility was the appearance of a lighter shade of green in the marginal 0.2-10 cm of the blade. This lighter green color resulted from changes in chloroplast organization occurring at this time (Plate I, Figure 6). This differentiation occurred in nearly all cells of the fringe except for a marginal band 2-6 cells

wide. The next distinct cytological event was the migration of the nucleus to an equatorial position at one side of the vacuole (Plate I, Figure 7). This was observed to occur in most material within approximately one day after granulation began.

Pore development occurred on the ontogenetically outer surface of the vesicle and was initiated at about the time of nuclear migration. Pore formation was normally accompanied by a curling or rolling of the fertile area of the blade, the pores being located on the convex surface. After migration to the center of the cell, the nucleus became noticably enlarged (Plate II, Figure 14). Staining revealed the nuclei of the gametangia and zoosporangia to be at prophase at this time. After the two-cell stage (Plate I, Figure 8) and during completion of the divisions leading to the formation of zoospores or gametes (Plate I, Figure 9), a change again began to occur in the color of the fringe area. A dark yellow-green appeared in sporophyte plants and a somewhat lighter green to greenish-tan color in the gametophyte plants. After developing stigmas, flagella and becoming pyriform, zoospores or gametes were discharged from the cells of the fertile fringe. Following discharge, the marginal area consisted primarily of empty cell walls and appeared as a translucent fringe on the blade. In nature, the fringe became detached from the blade after one or two days.

At San Juan Island the discharge of zoospores and gametes was periodic. Both were discharged simultaneously from separate plants during a 4-5 day swarming period. Collections at San Juan Island were made primarily during the swarming periods as interest was centered around the study of the zoospores and gametes and of germling development. Marine Gardens was visited for the purpose of obtaining material prior to the discharge of zoospores and gametes when zoosporangia and gametangia were at first division prophase. The condition of plants in the two areas at the times of collection is recorded in Table II.

Zoospores and gametes were frequently discharged in such numbers that they appeared as green clouds in still water. The densest concentrations occurred in the mornings following the first exposure to light. A minor release of zoospores and gametes also occurred during the afternoons. Plants from the two San Juan Island areas normally released the greatest number of zoospores and gametes on one or two days midway in the 4-5 day swarming period. Variation in length of the period of release of zoospores and gametes resulted from variation in the stage of development of various portions of the same plant and especially from variation among different plants in the same population. Some variation in the stage of development among different plants was correlated with type of habitat. For example, at Mount Dallas beach the plants in a cluster as large as 3

Table II. Summary of data concerning cyclic maturation and discharge of zoospores and gametes by M. fuscum.

Collection site	Dates at which the majority of plants in the population were observed in the following condition with respect to fertility				Interval between consecutive equivalent points in the cycle of maturation and discharge of zoospores and gametes
	No noticable differentiation of zoosporangia and gametangia*	Zoosporangia and gametangia at first division prophase	Zoosporangia and gametangia at the 2-cell stage	Major discharge of zoospores and gametes	
Mount Dallas beach and at Edwards Reef. 1961	July 8			July 11	
				July 20	9 days
	July 25			July 29	10 days
			Aug. 4	Aug. 6	8 days
			Aug. 14	Aug. 16	10 days
		Aug. 22		Aug. 26	10 days
		Sept. 1		Sept. 5	10 days
	Sept. 7				
Marine Gardens. 1961-1962				Oct. 7	
			Oct. 14		
		Dec. 25			
	Jan. 6	Jan. 9			15 days
		Jan. 25			16 days
		Feb. 8			14 days
		Feb. 19			11 days

* Nuclei in cells of the fringe were not observed to have migrated to the center of the cell or chloroplasts showed no granulation macroscopically.

meters in diameter, all at approximately the same stage of differentiation frequently would be approximately two days out of phase with other nearby groups of plants. Environmental factors such as density of growth or amount of cover did not seem characteristically associated with these differences between populations. It was also observed that the last few plants to release swarmers in a period were found growing at -3 meters MLT. It did not appear from the limited number of observations made at this depth, however, that the period of maximum release of swarmers from these plants was later than the average of the whole population. However, the average condition of widely separated populations appeared to be much the same. The plants in early August growing in the sheltered bay at Edward's Reef appeared to be within one day of the condition of plants one mile distant along the exposed shore at Mount Dallas Beach and at Pearl Point, a similar type of location on San Juan Island, approximately 15 miles distant.

Periodicity of Formation and Discharge of Zoospores and Gametes

At San Juan Island the period of discharge was followed by a period of from 4-6 days when all cells of the blade in the majority of plants were either at interphase or dividing vegetatively. The most consistently observed point in the cycle at San Juan Island was the time of major release of zoospores and gametes. The interval

between consecutive equivalent points in the period apparently was not constant. The average interval for July through August 6th was 9 days. The interval observed during August and September was 10 days.

In Oregon from late December 1961 through February 1962, effort was directed primarily to obtain zoosporangia and gametangia at prophase of the first division for chromosome studies. Marine Gardens was visited on December 25th to determine the approximate condition of the plants. They were found to range from plants with fringe cells at interphase to plants with swarmers ready for release. The fringe cells of the majority of plants were at prophase of the first division. The area was visited on January 6th when the chloroplasts of fringe cells showed no granulation. On January 9th a light green fringe associated with differentiation was present in many plants and the average condition of the nuclei of zoosporangia and gametangia was prophase of the first division. Material at prophase of the first division was collected at intervals of 16 days in January, 14 days in January and February and 11 days in February.

During the winter months, visits were made to the Marine Gardens only at times prior to swarmer release. However, when segments of the plants were maintained in the laboratory they liberated swarmers in 1-5 days whether cultured in the light or in the dark, indicating that the plants are not entirely vegetative or in some

suspended nuclear phase during the winter months and that they do periodically produce swarmers.

The periods of fertility in M. fuscum did not coincide with the lunar period as reported by Smith (34) in Ulva lobata. Moreover, the interval between periods of fertility did not appear to be constant through the seasons. Longer intervals occurred between equivalent stages in the fertility cycle in San Juan Island plants in late August and September as compared to the interval in July and early August. Also a longer interval appears to have prevailed during the winter months from the data available from Marine Gardens. This suggests that the intervals between successive stages in fertility are influenced by some factor or factors which vary seasonally, such as the total solar energy received.

Cytology of Zoospore Development

The following discussion of cytological details relates primarily to observations on material collected from Marine Gardens, Otter Rock, Oregon. There are included some less detailed observations on plants from San Juan Island.

The onset of differentiation of the zoosporangia was usually marked by granulation of the chloroplast associated with the detachment and fragmentation of the starch sheath and was followed by the gradual disappearance of the pyrenoids. Following the initiation of

structural changes in the chloroplast the nucleus migrated to the center of the cell. The extent of granulation of the chloroplast and disorganization of the pyrenoid during the early stage of differentiation was variable and therefore not a reliable index of the exact progress of nuclear events. Pore formation usually occurred at approximately the time the nucleus migrated to the center of the cell, but examples were seen in which pore formation occurred after migration of the nucleus. The pore was first distinguishable as an area of a different optical property in the cell wall surrounded by an upraised border (Plate I, Figure 7).

After the nucleus had migrated to the center of the cell it became suspended by cytoplasmic strands (Plate II, Figure 13). At this time the nucleus exhibited features characteristic of early prophase such as increased nuclear volume and increased number of heterochromatic structures (Plate II, Figure 13). Later discrete chromosomal fibers became visible and the nucleolus disappeared (Plate II, Figure 14). In late prophase, diakinesis figures were clearly discernible. In many diploid nuclei one "O" shaped bivalent (Plate II, Figures 18 and 19) was seen with 8 dense chromosome bodies some of which gave evidence of being bivalents by their biparted ends. In some nuclei, two "O" figures evidencing two chiasmata were seen. A chromosome number of $1n = 9$ was the best count from sporophytic material (Plate II, Figures 17, 18 and 19).

A count of 9 chromosomes was made in a number of nuclei which were at metaphase of meiosis II. Chromosome numbers were not determined in material collected at San Juan Island.

The plane of the first meiotic division in zoosporangia was parallel to the plane of the thallus (Plate I, Figure 8; Plate II, Figure 15). In the second meiotic division, the plane of division was perpendicular to the plane of the thallus (Plate II, Figure 16). Orientation of the next few divisions was not determined but it was noted that in later nuclear divisions orientation of the planes of division was random. In one preparation studied of the thicker material from San Juan Island the plane in the second nuclear division in the zoosporangium was parallel to the plane of the thallus.

Divisions taking place within a single zoosporangium occurred nearly synchronously and all nuclei in a zoosporangium were commonly at one nuclear phase or occasionally at two consecutive phases. Variation in phase from cell to cell was typical (Plate II, Figures 12 and 16) and phases ranging from mid-prophase to the 4-celled stage were seen in a single preparation. It was difficult to count living zoospores in mature zoosporangia, (Plate I, Figure 9). The number of zoospores was more easily estimated by counting the nuclei present in stained material. The number present appeared to be related to the size of the zoosporangia. In sporophytic material collected in February at Marine Gardens the nuclei counted

approximated 64 in some and 32 in others. The number of swarmers was not always a figure in the geometric progression 16, 32, 64, 128, ... as some zoosporangia contained 9 and some 10 zoospores.

Discharge of Zoospores

Zoospores were ordinarily motionless prior to release. They were either arranged with the rostra directed toward the center of the cell or more or less at random (Plate I, Figure 10). Zoospores were ordinarily discharged too rapidly to be counted. When discharged from zoosporangia having small pores however, the process occurred more slowly. When passing through the pore, the zoospores assumed a dumbell shape while cell organalles moved from the posterior to the enlarging anterior. The exit of one zoospore was followed quickly by the movement of another, flagellar end first, to the pore opening as though carried passively by a current. The zoospores were motile immediately, or within a few seconds after discharge.

Description of Motile Zoospores

Zoospores were quadriflagellate and pyriform in shape (Plate I, Figure 11). The posterior end was usually rounded but at times pointed. Zoospores from material collected at San Juan Island were 7.7-10.6 μ long and 3.1-4.3 μ wide. Zoospores from Marine

Gardens material measured 7.6-11.5 μ long and 2.5-5.5 μ wide. The stigma was usually submedian in position. Both in summer and in winter greater variation in size was observed among zoospores from Marine Gardens than from San Juan Island. In plants collected at Marine Gardens, numerous, abnormally large, spherical swarmers were seen among discharged zoospores and in zoosporangia prior to discharge. In some cases the abnormal zoospores bore the proper number of flagella and a single stigma, but in other cases more than one stigma was observed.

Immediately following liberation, a variable proportion of zoospores were positively phototactic and formed dense swarms on the bright side of a unilaterally illuminated tray. These positively phototactic zoospores exhibited a change in reaction later in the swarming period as indicated by the decrease in numbers of zoospores congregated at the surface under the culture lights.

Swarming terminated with the disappearance of the flagella and by attachment and rounding up of the zoospore. The duration of the swarming period rarely exceeded 6 hours and many zoospores lost motility within one hour.

Development of Germlings from Zoospores

Zoospores from plants collected at Mount Dallas were cultered until they had grown to plants with blades 2-3 cm in diameter. The

zoospores from plants obtained from Marine Gardens were cultured to the 15-celled stage. The development of germlings from these two sources were entirely comparable through the 15-celled stage.

Although often still approximately pyriform at the end of the swarming period, all zoospores became spheroidal following attachment.(Plate I, Figure 15). Following 2-4 days in culture pyrenoids were distinguishable. At this time the growing cell developed a somewhat pyriform shape as a result of elongation. Initial elongation of the germinating zoospore resulted in the formation of a basal cell at the first division (Plate I, Figure 16). This elongation usually occurred on the side of the cell away from the source of light, but some lack of conformity in this tropistic elongation occurred among closely crowded zoospores. As the eyespot was located randomly on the elongating germling, growth did not appear to be polar.

The first division was transverse and occurred 4-8 days following attachment (Plate I, Figure 17). Subsequent to the first division, elongation continued at both poles of the germling but was most rapid at the basal end. Normally both daughter cells of the first division divided transversely giving a 4-celled uniseriate germling. The multiseriate condition was introduced at the 3-8 celled stage by cell divisions in planes more or less parallel to the longitudinal axis of the germling. Once begun, the biseriate

condition became established at random locations along the filament. Cells in biseriate segments also divided in planes radial to the longitudinal axis of the filament. By the 15-celled stage the germling was almost entirely multiseriate. This stage usually bore rhizoids which developed from basal cells of the germling (Plate II, Figures 3 and 4).

Further divisions of the multiseriate germling resulted in a slender tubular plant that attained a length of 2 mm in approximately 60 days. The growth of the germling of this stage was most rapid along the longitudinal axis. The germling then began to increase rapidly in diameter at the distal end resulting in the typical vesicle (Plate II, Figure 5).

By the end of 150 days, the vesicle opened by a rupture in a region distal to the greatest diameter of the vesicle. Enlargement of the cells in this cap region preceded rupture of the distal cap. A more complete description of the vesicular stage is presented in the discussion of germlings developing from zygotes.

When plants had reached a size of 1 cm in diameter at 11 months they were placed on a submerged platform maintained at 1 meter below the surface of the water in the shelter of Friday Harbor. The plants grew to a diameter of 2-3 cm in one month under these conditions. The thalli did not become fertile. Observations were terminated when germlings had reached an age of one year.

Cytology of Gamete Formation

The following discussion of cytological details was primarily compiled from observations made on material growing at Marine Gardens. A single observation on a specimen collected at San Juan Island is given at the close of this section.

Early differentiation in gametangia was not distinguishable from that in zoosporangia with respect to structural changes that occurred in the chloroplast, the migration of the nucleus, and the formation of pores. The time schedule of these events did not differ from that seen in diploid plants. At the first division in the gametangia, the nuclei became distinguishable from diploid nuclei at late prophase by their thinner, less dense chromosomes. Chromosome counts and study of chromosome morphology in gametophyte material was possible at metaphase of the first mitotic division leading to gamete formation. The chromosome number in haploid material was 9 (Plate II, Figure 20, 21 and 22). The chromosomes fell into four general size groups. Lengths measured at a typical metaphase plate of the first division of the gametangium were: one chromosome 1.4μ, three chromosomes 0.8-1.0μ, four chromosomes 0.5-0.7μ, and one chromosome 0.4μ.

The orientations of the divisions within the gametangia did not differ from those described in zoosporangia. The number of gametes

per gametangium was approximated by estimating the number of nuclei within gametangia in which nuclear divisions were complete. In material from Marine Gardens collected in February, the number of nuclei in some gametangia approximated 64 and in others 128. In one collection of gametophyte material from San Juan Island the number of gametes in some gametangia approximated 128 and in others 256.

Description of Gametes

Liberated gametes were distinguishable from zoospores by their lighter, green color when in a group of a single type of swarmer. The color of the gametes varied from a shade of green slightly lighter than that of the zoospores to a pale brownish green.

Gametes were biflagellate and pyriform to elliptical (Plate I, Figure 12 and 13). The anterior end was pointed or somewhat round. The posterior tended to be more pointed than in the zoospore. Gametes in the San Juan Island material were 5.0-9.2 μ long and 3.2-5.6 μ wide. Gametes from plants collected at Marine Gardens measured 5.2-7.4 μ long and 2.2-4.7 μ wide. The stigma was equatorial or supra median.

As in the zoosporangia, occasional abnormally large motile cells appeared in the gametangia in plants from Marine Gardens. Some abnormal gametes bore the proper number of flagella and a

single stigma, others had as many as 5 stigma.

Activity of Gametes and Zygote Formation

The gametophytes were heterothallic and zygotes resulted only from combination of gametes produced by plants representing two mating types. Zygotes were not formed among gametes produced by the same plant or among gametes produced by plants of the same mating type. Table III shows the results of all possible combinations of gametes from 6 plants collected at Marine Gardens during June of 1960. This mating pattern was replicated many times in the laboratory with plants both from Marine Gardens and San Juan Island.

Table III. Results of combinations of gametes of 6 plants of Monostroma fuscum.

	1	2	3	4	5	6
1	-	-	-	-	Z*	Z
2	-	-	-	-	Z	Z
3	-	-	-	-	Z	Z
4	-	-	-	-	Z	Z
5	Z	Z	Z	Z	-	-
6	Z	Z	Z	Z	-	-

* Z = Zygotes produced.

A macroscopic difference in the shade of green of the two strains of gametes could usually be detected during swarming. A difference in average size of gametes from plants representing the two sexes was suspected in several cases in material from San Juan Island and Marine Gardens. Direct proof of anisogamy by a comparison of average sizes of gametes of a number of plants which have been compared with regard to mating type remains to be obtained.

The association of gametes in pairs prior to fusion was observed microscopically in very dilute suspensions of the two mating types and in preparations in which the distance between the slide and cover slip restricted gamete movement more or less to one plane. However, when swarmer concentrations were high enough for the suspension to be noticeably colored, zygote formation was preceded by clumping (Plate I, Figure 14) that could be easily observed macroscopically. The clumps were congregations of from three to hundreds of extremely active gametes in close lateral association but only rarely in contact. The gametes appeared to leave and reenter the clumps frequently. Motile zygotes were occasionally seen in the vicinity of the clump (Plate I, Figure 14) but were not seen to enter the clumps. A single clump at times divided into two and at times two clumps would coalesce. During the more active, early period of clumping the diameter of the clump was consistant in size with an association of gametes by flagella tips. In swarms of

recently released gametes, the number of clumps rapidly decreased concurrent with diminishing numbers of unmated gametes. Zygotes were formed more slowly by gametes which had been combined after they had been swarming for some time, and zygotes were not formed in mixtures of gametes that had been swarming separately for more than 24 hours. However, abundant clumping was observed to occur among gametes that had been combined 36 hours after release. Gametes that persisted in clumps, unable to form zygotes, finally became quiescent and remained attached by flagella tips after flagellar activity ceased. It would appear that a reaction involving association of gametes of both sexes results in clumping and is followed by a second reaction responsible for fusion of the protoplasts. With passage of time the former reaction appears to diminish in strength less rapidly than the later.

For a time following plasmogamy, the fused gametes were motile and negatively phototactic. Swarming terminated in less than 30 minutes with the disappearance of flagella. The fusion cell at this time began rounding up and became attached to the substrate. Mating material from San Juan Island killed periodically and stained showed karyogamy complete in 50 percent of fused gametes in 12 hours. After 49 hours, very few cells with unfused nuclii remained.

Development of Germlings from Zygotes

Zygotes produced from gametes obtained from plants at Mount Dallas were cultured and grown to adult plants 2-3 cm in diameter. Zygotes from material collected at Marine Gardens were cultured through the 10-celled stage. Details were comparable throughout the equivalent stages.

The development of germlings from zygotes was slower than from zoospores. The first division parallel to the germling axis occurred by the 9th day in zoospore germlings but not until the 12th day among zygote germlings. Aside from this difference in rate of growth in the initial stages, the ontogeny of plants developing from zygotes and zoospores was essentially the same. Ontogeny included progression from uniseriate through multiseriate to tubular juvenile stages. The tubular germlings enlarged distally forming a vesicle. As in zoospore germlings, in the late vesicular stage cells became abnormally large in a region distal to the greatest diameter of the vesicle (Plate II, Figure 9). The differentiation was first detected as a lighter green color in this region and later by a translucent appearance. The distal cap ruptured and became detached when the thallus had reached a length of from 4-10 mm. The distal margin of the resulting trumpet shaped plants continued to increase in diameter producing a more or less centrally attached blade.

Observations were discontinued after one year when the thallus reached a blade diameter of 2-3 cm. As with the germlings produced from zoospores, the last month of growth was under natural conditions. Improved growth was observed under these natural conditions but the thalli did not produce reproductive cells.

Development of Germlings from Unfertilized Gametes

Germlings were cultured through the 15-celled stage from unfertilized gametes from plants collected from San Juan Island and to the 6-celled stage from unfertilized gametes from plants collected at Marine Gardens. Growth of these germlings was slower than in zoospore or zygote germlings. The first division parallel to the germling axis occurred after 9 days in zoospore germlings, after 12 days in zygote germlings and in germlings from unfertilized gametes only after 14 days. Mortality and abnormality among germlings was highest in those developing from unfertilized gametes. The rate of occurrence of abnormality was particularly high in those that appeared very pale green while swarming. The form and the rate of growth of well established germlings from unfertilized gametes at the 8-15 celled stages was equivalent to those at comparable stages of germlings from zygotes and zoospores. It was not determined whether germlings from gametes resulted in a haploid gametophyte, a development which Drew (11, p. 352) chose to call

apomictosis or whether there was doubling of the chromosome number giving a diploid plant by true parthenogenesis.

Regeneration by Proliferation

Eleven months-old, cultured plants developed from zoospores and zygotes were suspended at a depth of one meter on a submerged platform in Friday Harbor. In addition to an increased rate of growth of some of the plants it was noted that plantules proliferated both from stunted thalli and from the holdfasts of plants that had remained after accidental destruction of the stipe and blade while removing growths of diatoms by brushing. During the growth of these plantules, the various stages resembled comparable stages of germlings developed from zoospores and zygotes. Observations, however, had to be discontinued after the plants reached 3 mm in length.

PLATE 1

Figures 1-3, 6, 8-10. Living material collected at Marine Gardens, Otter Rock, Oregon.

Figures 4, 5, 7, 11-18. Living material collected at San Juan Island, Washington.

Figures 1-3. Cells of the monostromatic part of the blade at interphase.

Figure 1. Thallus viewed from the surface that is ontogentically the outside of the vesicle. 1000X.

Figure 2. Thallus viewed from the surface that is ontogenetically the inside of the vesicle. 1000X.

Figure 3. Cross section of the blade in vegetative condition. 500X.

Figure 4. Cross section of the blade 0.5 mm above the stipe showing the downward directing rhizoids. 85X.

Figure 5. Cross section at the margin of the blade. 500X.

Figures 6-9. Cross section of the fertile fringe showing various stages of development.

Figure 6. Cells with granular chloroplast. 500X.

Figure 7. Centrally located nuclei that have recently migrated from the end of the cell. 500X.

Figure 8. Zoosporangia or gametangia at the two celled stage. 500X.

Figure 9. Zoosporangia. Some empty, others containing an estimated 32 or 64 zoospores. 500X.

Figure 10. Surface view of zoosporangia showing some with zoospores oriented toward the center of the zoosporangia. 500X.

Figure 11. Zoospores recently released. 1000X.

Figure 12. Gametes recently released. 1000X.

Figure 13. Gametes recently released. 1000X.

Figure 14. Electronic flash photomicrograph of clumping and zygote formation. The apparent adherence of gametes by flagella tips is characteristic in matings of gametes that have been held separately for 6-36 hours before mating. 750X.

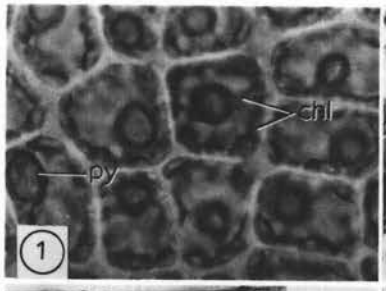
Figure 15. Zygotes at 2 hours. 1000X.

Figure 16. Zygotes at 4 days. 1000X.

Figure 17. A two-cell diploid germling left, and a one-cell germling right. At 9 days. 500X.

Figure 18. Five-celled diploid germling at approximately 13 days. 500X.

Chl, chloroplast; cl, clump; f, flagella; N, nucleus; n, nucleolus po, pore; py, pyrenoid; rh, rhizoids; s, stigma; w, wall; z, zoospore zy, zygote.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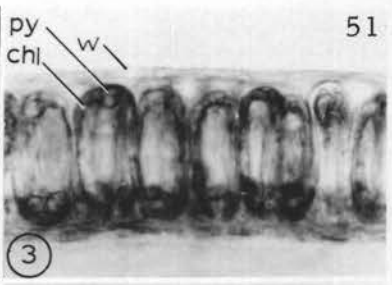
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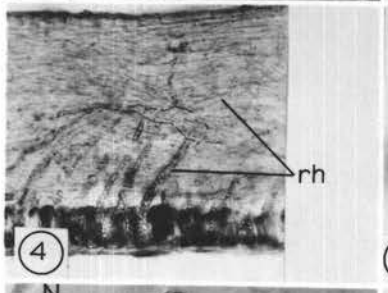
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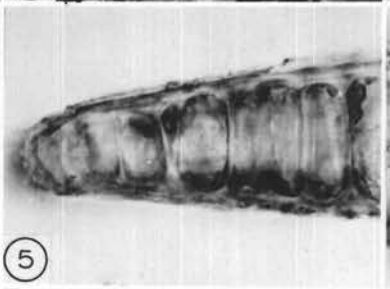
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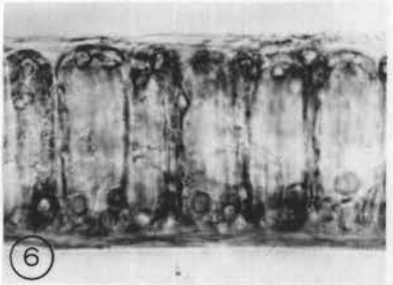
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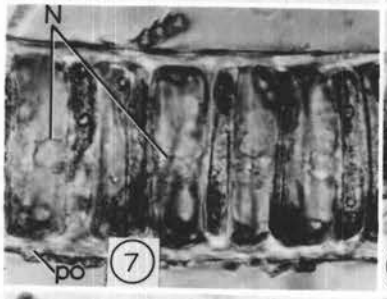
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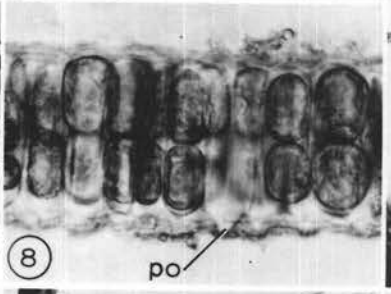
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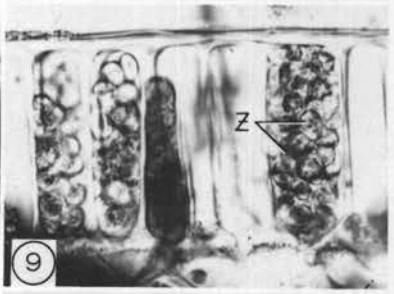
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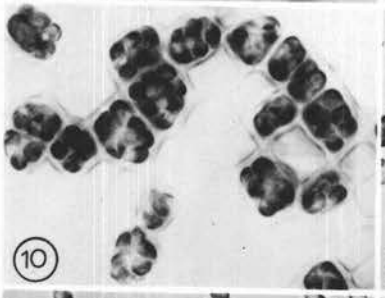
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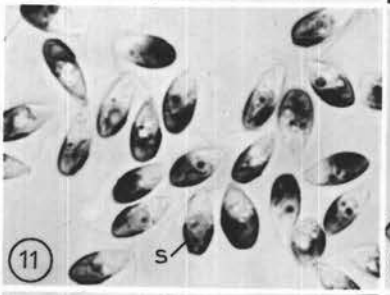
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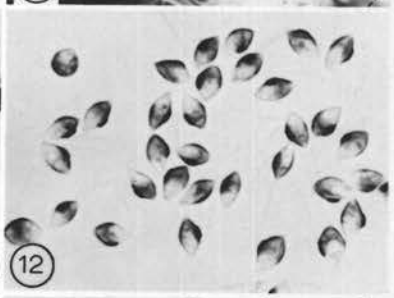
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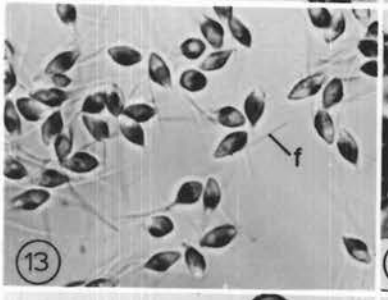
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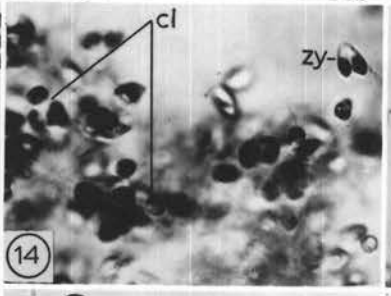
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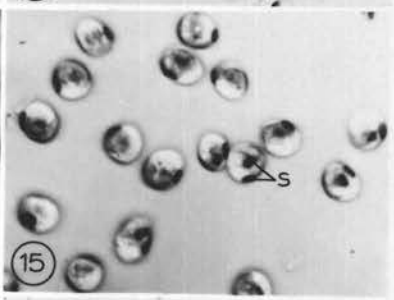
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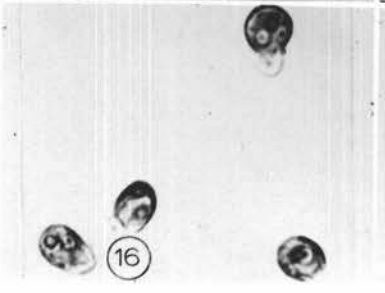
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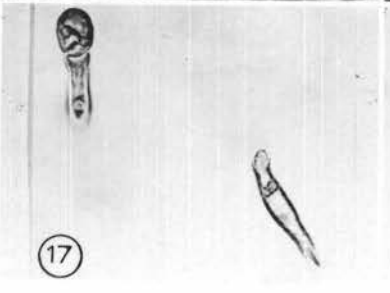
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PLATE II

Figure 1-9. Living material collected at San Juan Island, Washington.

Figure 10-22. Fixed and stained material collected at Marine Gardens, Otter Rock, Oregon.

Figure 1. Germling from an unfertilized gamete showing the first cell division with the axis parallel to the longitudinal axis of the germling. At 15 days. 500X.

Figure 2. Germlings developed from zoospores at 9 days showing oblique cell wall. 500X.

Figure 3. Multiseriate germling developed from a zoospore. 29 days. Grown in unenriched sea water. 150X.

Figure 4. Germling developed from zoospore. Rhizoids are present and a small germling may be seen propagating from the base. 30 days. 100X.

Figure 5. Vesicular stage in germlings developed from zoospore. 3 months. 18X.

Figure 6. Trumpet-shaped plant and germlings at the vesicular stage. All grown from zoospores. 5 months. 2 1/2X.

Figure 7. Vesicular stage in germlings developed from zygotes. Distal cap of enlarged cells in (a) is intact. Cap is partly detached from the vesicle in (b). 5 months. 2 1/2X.

Figure 8. Trumpet-shaped plant grown from a zygote. 5 months. 2 1/2X.

Figure 9. Surface view of cells at the point of transition between the typical thallus cells below and the enlarged cells of the cap above. 5 month old vesicle grown from a zygote. 500X.

Figure 10. Cross section of diploid thallus with cells at interphase. 1000X.

Figure 11. Surface view of the diploid thallus with cells at interphase, nucleus is located at the end of the cell. 1000X.

Figure 12. Surface view of the haploid thallus with cells at prophase and metaphase, nuclei are located equidistant from ends of the cell. 1000X.

Figure 13, 14. Cross section of the diploid thallus with cells at prophase. 1000X.

Figure 15. Cross section of a diploid cell with a nucleus at anaphase. 1000X.

Figure 16. Surface view of the thallus showing the second nuclear division in a gametangum with one pair of chromosomes trailing in anaphase. Other prophase nuclei may be seen. 1000X.

Figures 17-19. Chromosomes at late prophase of meiosis I. The first nuclear division in zoosporangia. 3000X.

Figures 20-22. Chromosomes at mitotic metaphase of the first nuclear division in the gametangia. 3000X.

g, proliferating germling; rh, rhizoids; N, Nucleus; n, nucleolus.

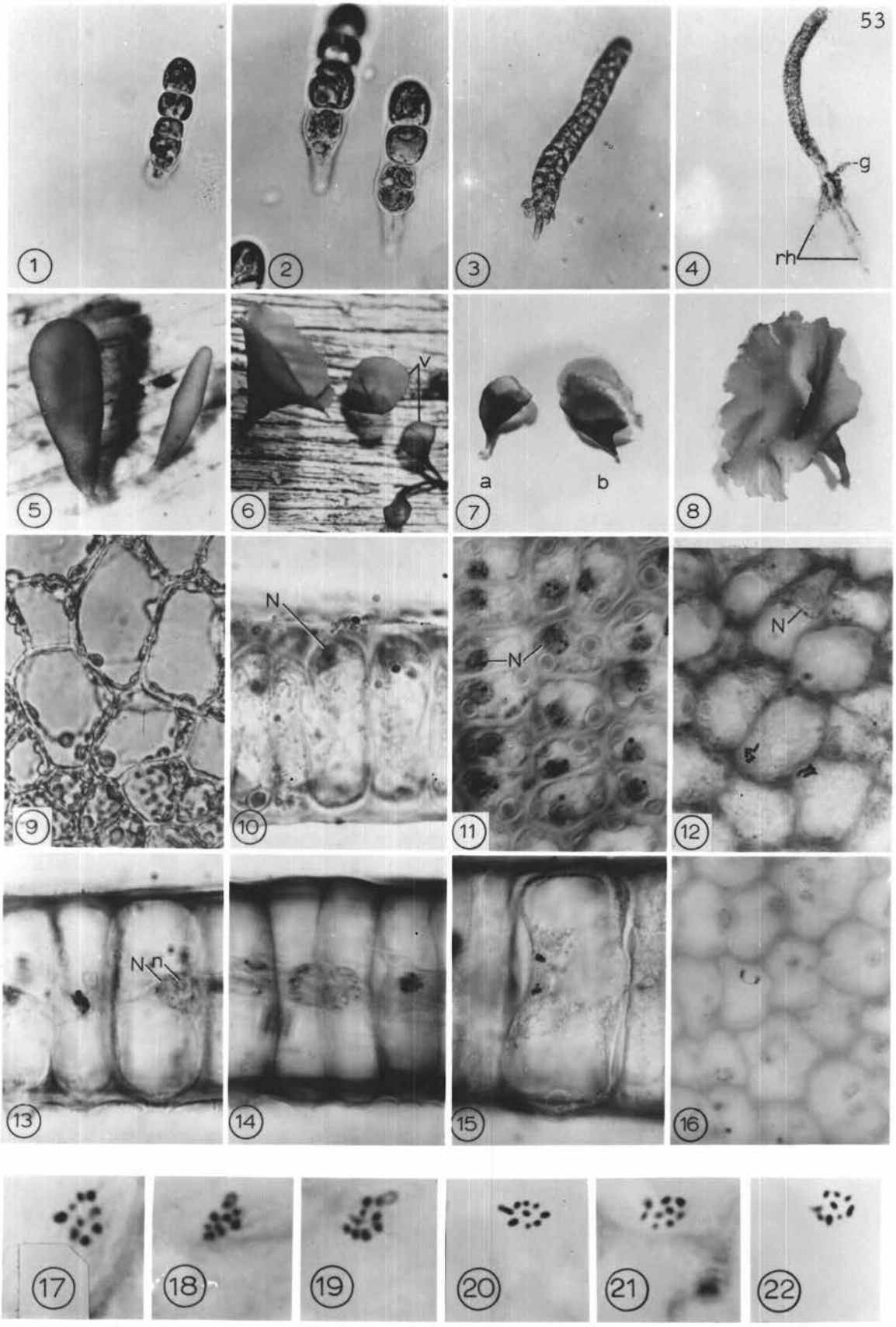
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PLATE III.

Diagram of the generalized life cycle of haplobiontic species of Monostroma.

(a and b) Adult gametophyte plants

(c and d) Gametes

(e) Recently formed zygote

(f) Recently attached zygote

(g) Enlarged, germinating zygote

(h) Zoospore

(i) Branched uniseriate germling

(j) Disk of densely compacted filaments

(k) Juvenile gametophytic vesicle

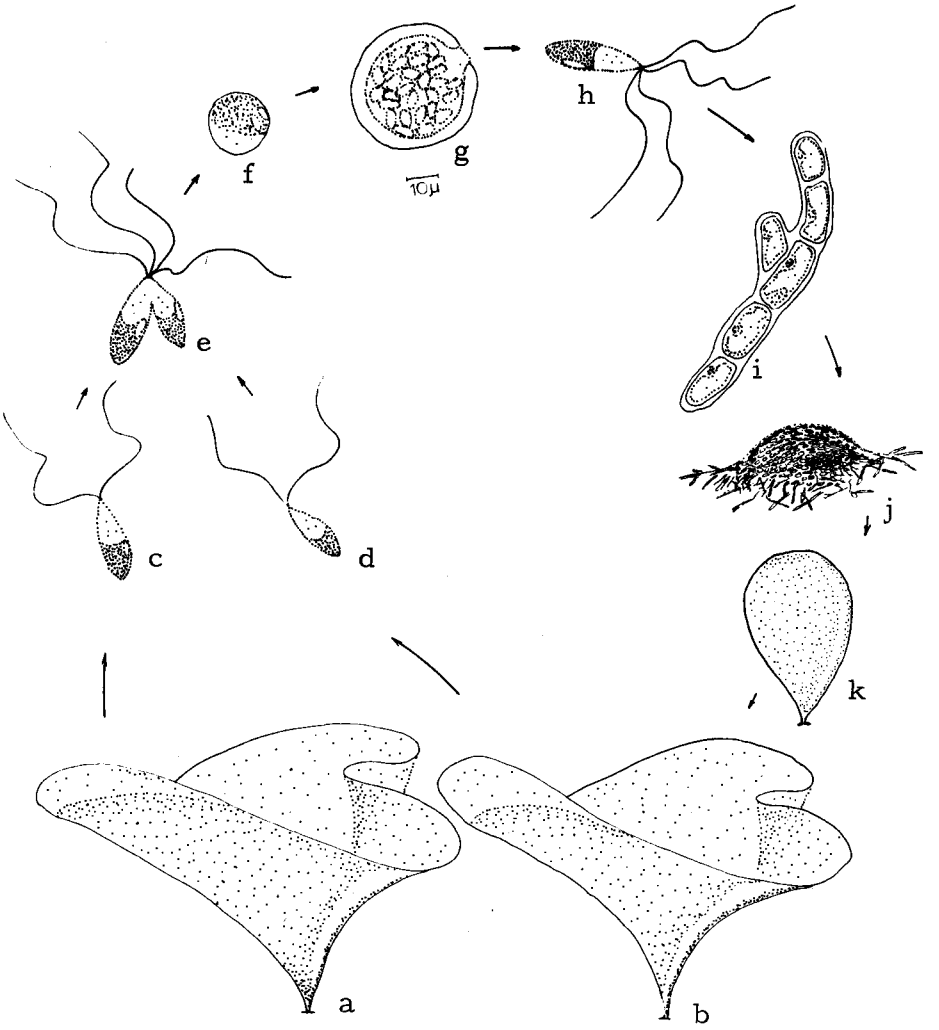
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PLATE IV

Diagram of the tentative life cycle of Monostroma fuscum.

(a) Zoospore

(b) One-celled germling developed from a zoospore at two days

(c) Two-celled germling developed at approximately one week

(d) Germling developed from a zoospore becoming multiseriate at approximately two weeks.

(e) Elongating germling with rhizoids, developed from a zoospore

(f) Vesicular, obpyriform plant developed from a zoospore becoming chlorotic above at approximately 4 months

(g) Trumpet shaped thallus after loss of the distal cap of chlorotic cells at 6 months developed from a zoospore

(h and i) Heterothallic gametophyte plants

(j and k) Gametes from the two sexual strains

(l) Clumping of gametes

(m) Zygote formed in the clump

(n) Zygote formed by direct fusion of gametes outside the clump

(o, p, q, r, s, and t) Ontogeny of the germling developed from a zygote shown at the same magnifications as the corresponding morphologically similar germlings developed from zoospores

(u) Sporophyte plant

(v) Vegetative cell seen in a cross section of the blade (typical of both diploid and haploid plants)

(w) Meiosis I prophase of the zoosporangium

(x) Zoosporangium at the time of discharge

(y) Two day old germling developed from a gamete

(z) Germling developed from an unfertilized gamete at the two-cell stage at approximately two weeks

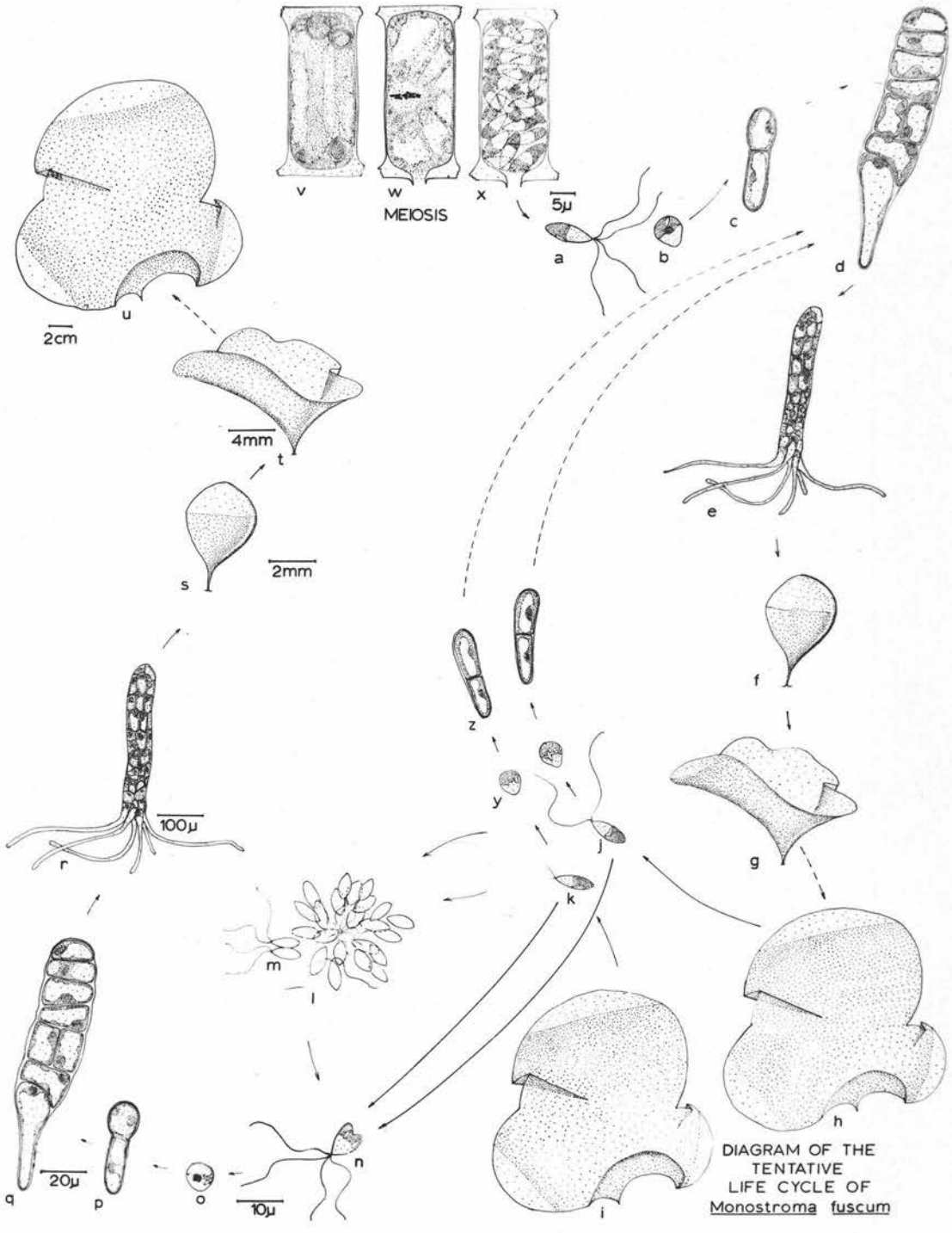

DIAGRAM OF THE TENTATIVE LIFE CYCLE OF Monostroma fuscum

DISCUSSION

Taxonomy Below the Species Level

Wittrock in his monograph of the genus Monostroma (38, p. 49) distinguished the species M. fuscum, M. Blyttii, and M. splendens, in part on the relative shape of cells, relative amount of gloss and thickness of the blade. The thickness of M. fuscum was 20-25 μ; M. splendens, 49-53 μ; and M. Blyttii, 65-72 μ. M. Blyttii was described as less glossy than M. splendens. Rosenvinge reported in 1893 (26, p. 941) that M. fuscum collected in Greenland had a continuous range in shape of cells and blade thickness from individuals 50 μ thick answering the description of M. splendens with its thicker walls to individuals 25 μ thick with the thinner external wall and prismatic cuboidal cells as described in M. fuscum. The plants collected by deA. Saunders in 1901 (29, p. 409) on the Harriman Alaska Expedition ranged from 40-60 μ. Later Collins (8, p. 213) indicated three forms under M. fuscum; Forma typica, 20-35 μ; forma splendens, 50-55 μ; and forma Blyttii, 60-70 μ. It is not always clear in these reports whether the measurements given were made on soaked herbarium specimens or on living material, nor do these writers indicate the number of plants represented in the sample

Eighty-nine plants collected at San Juan Island ranged in thickness from 50 to 74 μ. The range in thickness in a group of 47 plants

collected from Marine Gardens was 38 to 64 μ. The means of the thicknesses of the two populations differ by 12.5 μ and lie within the limits of the two varieties, fuscum and splendens. M. fuscum grew primarily subtidally and in the low intertidal zone at San Juan Island while at Marine Gardens the plants grew most abundantly in the mid-intertidal zone and only rarely in the low intertidal zone. It was not determined whether the plant grew subtidally at Marine Gardens. Differences in blade thickness and the ecological niche occupied by plants growing in these two areas may result directly from physical factors and plant population pressures. It is also possible that blade thickness and the intertidal zone occupied are indicative of genotypic differences between the Oregon and Washington plants. Data on thickness of blade of plants collected at Marine Gardens, makes it appear unlikely that this dimension is directly affected by differences in depth of the water in which the plants were attached. From this one consideration it would appear that variation in blade thickness observed in the population at San Juan Island and at Marine Gardens is genotypic and that these plants represent two taxa of subspecific rank.

Taxonomy Above the Species Level

Recently, G. F. Papenfuss (22, p. 315) characterized the genus Monostroma as consisting of species in which the macroscopic plant is a sporophyte and no sexual generation is present and species in which the only macroscopic plant is the gametophyte generation. The latter group comprises the largest number of species for which life history information is available. In these sexually reproducing entities, the zygote undergoes enlargement as a single celled microscopic structure and later discharges quadriflagellate swarmers that are presumed to be zoospores. This type of life history has been demonstrated in M. angicava (40, p. 43), M. nitidum (1), (16), M. Wittrockii (19, p. 364), and M. latissimum (1). Germination of the zoospore follows one of two patterns. In one pattern (Plate III occurring in some species such as M. angicava the germinating zoospore produces a branching filament that develops into a dense disk. A single layer of cells in the center of the disc uplifts into a dome. This dome enlarges into a vessicle that later opens distally into the typical adult vegetative thallus. In the other type, characteristic of M. latissimum, the germling arising from the zoospore develops by randomly oriented divisions into a vesicle at approximately the

10-celled stage. In both sexual life cycle patterns the sporophytic generation is microscopic.

A much different type of life history was exhibited in plants of M. fuscum from Washington and Oregon (Plate IV). The life cycle of M. fuscum appeared to be monomorphic diplohaplontic. This characterization however remains tentative because production of gametes by plants grown in culture from zoospores, or production of zoospores by plants grown in culture from zygotes was not obtained. Support for the existence of this life cycle in this alga was derived from, (1) the presence of morphologically identical haploid, gamete-producing and diploid, zoospore-producing thalli in the natural population and (2) the similarity of ontogeny in cultured plants developed from zoospore and zygotes. Evidence that the cycle is truly sexual is based on the observations of meiosis in the first divisions of zoosporangia and karyogamy following gamete fusion.

With an assumed monomorphic diplohaplontic life cycle, M. fuscum sould appear to more resemble Enteromorpha than most sexual species of Monostroma. The development of germlings from zoospores and zygotes in M.fuscum also differs from patterns of germling development described in haplontic species. In M. fuscum, germlings developed as uniseriate filaments to the 3-8 celled stage before becoming multiseriate. The development at this period recalls that illustrated for U. lactuca by Schiller (31, Plate 1) and for E. intestinalis by

Bliding (2, p. 235).

There is evidence that these life history features of a monomorphic diplohaplontic life cycle and an Ulva-Enteromorpha like germling development are characteristic of a complex of species. The genus Monostroma was divided by DeToni (10, p. 108) into two sections on the basis of a difference in thallus texture. The section Eumonostroma comprised species with soft gelatinous thalli. The section Ulvaria contained six species; M. crispatum, M. leptodernum, M. obscurum, M. fuscum, M. splendens, and M. Blyttii. The latter three entities are now recognized as varieties of M. fuscum. The species M. obscurum is known to resemble M. fuscum in features other than thallus texture. The presence of a bilobed chloroplast in M. fuscum and M. obscurum was reported by Rosenvinge in 1932 (27). More recently, in 1961, morphological similarity of sporophyte and gametophyte plants of M. obscurum was reported by Gayral (13). Considering all available information concerning M. fuscum and M. obscurum, it is now possible to make a more detailed characterization of the group Ulvaria.

The taxon Ulvaria includes monostromatic members of the Ulvaceae which have the following characteristics; (1) a monomorphic, diplohaplontic life cycle, (2) a simple, uniseriate filamentous, early germling that becomes multiseriate at the 3-8 celled stage, (3) a juvenile vesicle that at a size of less than 1.0 cm opens by dehiscence

of a distal cap of enlarged cells, (4) a blade that is relatively inelastic and non-gelatinous, and (5) a chloroplast concentrated in two polar masses. It remains to be seen if all of these characteristics are true of M. obscurum and the other entities in DeToni's Ulvaria.

Ulvaria would appear to occupy a phylogenetic position closer to Ulva and Enteromorpha than to Monostroma on the basis of characters 1, 2, and 4 above. On the basis of chromosome number M. fuscum with n=9 appears to be more closely related to Enteromorpha than Ulva. Fǿyn (12, p. 160) has reported n=13 for Ulva lactuca while Niizeki (21) and Ramamathan (24, p. 381) have reported n=12 and n=10 for Enteromorpha linza and E. compressa respectively. An additional resemblance to Enteromorpha is apparent in the monostromatic blade. However, the random pattern of cell divisions resulting in growth of the broad blade of M. fuscum is more akin to the pattern of development of the blade in Ulva.

The genera Ulva and Enteromorpha have usually been distinguished taxonomically on the basis of separation or non-separation of cell layers in the blade. It would appear that the difference in chloroplast organization that distinguishes Ulvaria from Ulva and Enteromorpha and species in DeToni's Eumonostroma is an equally distinct and important taxonomic character. It would appear that Ulvaria could be properly regarded as a genus related to the Ulva-Enteromorpha complex.

SUMMARY

A study of the life history of Monostroma fuscum was made using plants growing at Marine Gardens, Oregon and at San Juan Island, Washington. Plants from these two localities were sufficiently different in thallus thickness that some would designate the San Juan Island plants as the variety Blyttii and the Marine Gardens plants as the variety splendens.

Juvenile plants growing in nature were vesicular above a short stipe and holdfast. Eventually the vesicle opened distally into a flat blade which in the adult plant was centrally attached and more or less divided into lacineae. The blade was uniformly thick and monostromatic above and thickened below by the presence of intramatrical rhizoids extending downward within what was ontogenetically the inner wall of the vesicle.

The chloroplast was concentrated at both ends of the cell. These polar masses were connected by parietal strands which ran between the vacuole and the lateral walls of the cells. The nucleus of the vegetative cell occupied a position adjacent to the, ontogenetically, inner wall of the vesicle.

Populations of M. fuscum included morphologically identical diploid zoospore producing and haploid, gamete producing plants. In haploid plants gamete formation occurred in cells of the marginal

0.2-10 cm of the blade. Differentiation of the gametangia was grossly evident as a slight change in color in the margin of the thallus caused by changes in plastid structure occurring 4-5 days preceding discharge of gametes. Following the initiation of changes in chloroplast structure, the nucleus migrated to as equatorial position and pore formation began. The first division of the gametangium was mitotic. A chromosome number of ln=9 was observed at metaphase of this division. Discharge of gametes at San Juan Island was periodic, occurring in the mornings during a 4-5 day swarming period. The average interval between periods of swarming at San Juan Islands was 9-10 days during July and August and 10 days during late August and September. The periodic discharge of gametes during the winter months at Marine Gardens was inferred to occur at intervals longer than that observed at San Juan Island. Gametophytic plants were heterothallic. Some evidence exists for a slight degree of anisogamy. Zygote formation appeared to involve two reactions: one involving association of gametes by flagella resulting in clumping, the other involving fusion of the protoplasts. The former reaction decreased in activity more rapidly than the latter. In one mating of plants from San Juan Island karyogamy followed plasmogamy by 12 hours in 50 percent of the fused gametes.

Early ontogeny of germlings developed from zygotes involved a progression from uniseriate through multiseriate to tubular stages

and resembled somewhat the development described for species of Enteromorpha and Ulva. In more advanced cultures developed from zygotes obtained at San Juan Island the tubular germlings enlarged distally into obpyriform vesicles which opened by rupture and dehiscence of a cap comprised of the portion distal to the maximum diameter of the vesicle. The resulting trumpet shaped thalli enlarged at the distal margin into typical adult thalli.

Exclusive of details of nuclear events the cytological details associated with the formation and discharge of zoospores in diploid thalli were similar to, and occurred simultaneous with, the corresponding events in gametophyte thalli. Meiosis occurred in the first division of the zoosporangia. Nine bivalents were present in this division. The ontogeny of germlings developing from zoospores was followed to adult thalli and was found to be similar to that of germlings developing from zygotes. Germlings produced from unmated gametes were identical through the tubular stage to germlings from zygotes and zoospores.

The existence in M. fuscum of diploid, zoospore producing and haploid, gamete producing thalli, the determination of the points of karyogamy and meiosis, and the similarity in ontogeny and form of the adult plants developing from zoospores and zygotes provided evidence of a monomorphic, diplohaplontic life cycle in this alga. The occurrence of meiosis in the first division of the zoosporangia was

consistent with the sporic meiosis reported for other members of the Ulvaceae.

The existence of morphologically similar sporophyte and gametophyte plants in M. obscurum has recently been reported by Gayral. The similarity of M. fuscum and M. obscurum as regards thallus texture was previously recognized by DeToni who assigned them along with other plants to the section Ulvaria of the genus Monostroma. This study has provided evidence that Ulvaria is a valid taxon and that it includes monostromatic Ulvaceae with the following characteristics: (1) a monomorphic, diplohaplontic life cycle, (2) a simple, uniseriate, filamentous, early germling that becomes multiseriate at the 3-8 celled stage, (3) a juvenile vesicle that at a size of less than 1.0 cm opens by rupture and dehiscence of the portion distal to the maximum diameter of the vesicle, (4) a blade that is relatively inelastic and non-gelatinous, and (5) a chloroplast concentrated in two polar masses.

Thus characterized, the group Ulvaria appears to occupy a phylogenetic position closer to Enteromorpha and Ulva than to Monostroma. Ulvaria is obviously as distinct from the genera Monostroma, Ulva, and Enteromorpha as Enteromorpha is from Ulva. Chromosome numbers suggest a greater affinity between Ulvaria and Enteromorpha than between Ulvaria and Ulva.

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