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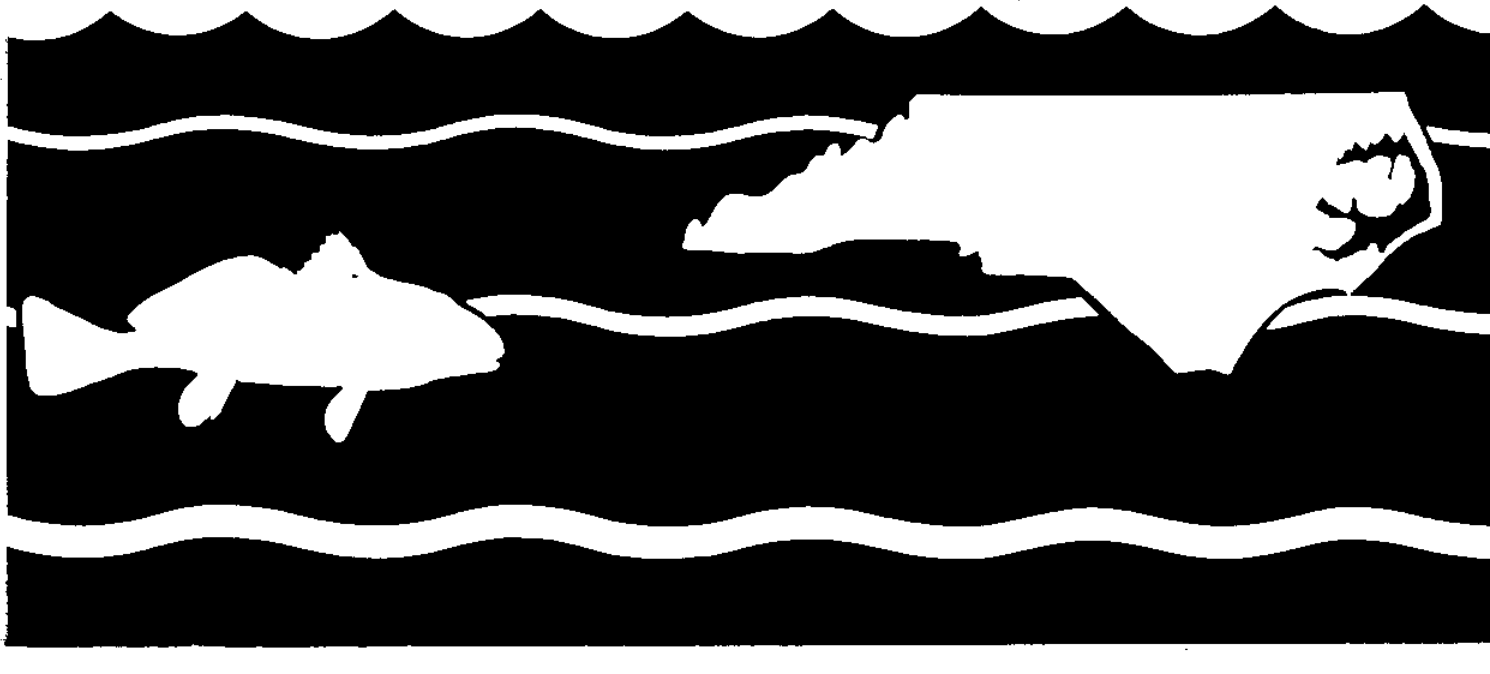

FIELD AND EXPERIMENTAL STUDIES OF THE SYSTEMATICS AND ECOLOGY OF *Ulva curvata* AND *Ulva rotundata*

by
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CHARLES FREDERICK RHYNE. Field and Experimental Studies on the Systematics and Ecology of *Ulva curvata* and *Ulva rotundata*. (Under the direction of MAX H. HOMMERSAND.)

An investigation of the biology of two species of *Ulva* found along the east coast of the United States was undertaken in order to gain a better understanding of their taxonomy and ecology. *U. curvata* was the only *Ulva* entity found in marsh-like habitats under lowered salinity during the winter-spring season between Georgia and New Jersey. *U. rotundata* appeared to be the dominant *Ulva* species in more saline habitats. Both species are new records for the Western Hemisphere.

In order to establish the range of distribution of *Ulva curvata* mating studies were carried out with plants from the principal research site in Calico Creek near Morehead City, North Carolina and material from several localities extending from the Jekyll River, Brunswick, Georgia to the Navesink River, New Jersey. Male and female gametes released from thalli obtained at each locality mated with gametes from Calico Creek material and produced viable zygotes which germinated normally.

The life cycle of *Ulva curvata* was demonstrated in field and laboratory studies to involve an alternation of isomorphic gametophyte and sporophyte generations. Thalloid forms of both generations appear between November and May and alternate with a microscopic filamentous stage present on oyster shells and other solid substrates from May to November. Gametophytes predominate in the field during the early growing season in Fall and early Winter and are gradually replaced by sporophytes in late Winter and early Spring. At the research site in Calico Creek female gametophytes were much more prevalent than male gametophytes and sporophytes released zoospores which developed more commonly into female plants. While all nuclear phases and growth forms appear to be connected to one another by meiosis and syngamy, a high frequency of somatic diploidization of female plants leading to parthenosporophytes that carry a potential for regenerating only female thalli has greatly biased the life cycle in Calico Creek.

Field studies showed that populations of *Ulva curvata* in Calico Creek release swarmers in strictly cyclical patterns with peaks of reproductive activity every 14-15 days between January and April. Release occurred 3-5 days before spring tide in 1970 and 1 day before to 1 day after spring tide in 1971. Germination, early development, thallus growth, reproduction and senescence were investigated experimentally under controlled conditions in the laboratory in order to account for the life history and ecological adaptations of *Ulva curvata* as seen in the field. A temperature greater than 15°C and light intensities of 250 ft-c stimulated germination of all swarmer types. Further development into minute tubular plantlets occurred only if the temperature was reduced below 18-20°C in the laboratory. Morphogenesis of the small tubular thallus into an expanded blade characteristic of *Ulva* was found to occur with regularity only if cultures received mud-water extract and agitation. Experiments on growth and reproduction showed that agitation, high NH_4-N levels and daily cycles of high (20°C) and low (9°C) temperatures and high (800 ft-c) and low (250 ft-c) light intensities favored high growth rates of the vegetative thallus while stationary cultures, low NH_4-N levels and constant high light intensities promoted spontaneous reproduction.

Mitosis of vegetative cells of *Ulva curvata* were essentially synchronous in the field and laboratory and occurred between 9:30 p.m. and 11:45 p.m. The nucleus migrates from a position near the upper cell membrane to the lower cell membrane in the early afternoon while the chloroplast moves across the face of the cell. In contrast, mitosis in *U. rotundata* appeared to occur between 9:30 a.m. and 11:15 a.m. with no shift in position of the nucleus or chloroplast.

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INTRODUCTION

The marine alga *Ulva* is wide ranging in its distribution having been observed in both estuarine and marine environments extending from the poles to the equator. Originally most entities were referred to *Ulva lactuca* Linnaeus; however, the number of species has grown steadily during the past century. De Toni (1889) listed 22 species of *Ulva*. Dawson (1962) included 17 species as newly described since De Toni with approximately 5-6 new species having been added since then. Recent taxonomic revisions of *Ulva* have been based on genetic, physiological and experimental studies carried out in different parts of the world. Even with the rather vast amount of information available it appears that the typification of the genus *Ulva*, the phylogenetic affinities of the family Ulvaceae and the taxonomic status of the order Ulvales itself are still in question. As Papenfuss (1960) has aptly stated: "It is hardly believable that so much should be wrong with the nomenclature of such a classical and ubiquitous genus as *Ulva*."

The taxonomic and nomenclatural status of the genus *Ulva* is discussed by Silva (1952) and later by Papenfuss (1960). Historically, *Ulva* is one of the four oldest genera of algae, having been included along with *Fucus*, *Conferva* and *Chara* in Linnaeus' Species Plantarum (1753), the starting point of botanical nomenclature. The genus *Ulva* was assigned nine species, of which six were later removed to other genera. Of the three remaining species: *Ulva linza*, *U. latissima* and *U. lactuca*, the first is thought by most workers to be a species of *Enteromorpha*, while the type specimen of *U. latissima* appears to both Silva and Papenfuss to be representative of *Laminaria saccharina*, a phaeophycean alga. *Ulva lactuca* has remained the only Linnaean species representative of *Ulva* as currently circumscribed. As discussed by Papenfuss (1960) this typification is entirely contrary to

Linnaeus' diagnosis of *Ulva* in that Linnaeus stated that he regarded *Ulva* to be plants that were hollow. Consequently, *Ulva* should be typified by one of the hollow species and not by *U. lactuca* or *U. latissima*. The first species listed by Linnaeus in Species Plantarum was *Ulva intestinalis*, and it is highly probable that it was from this entity that he drew his diagnosis and that it should serve as the lectotype of *Ulva*. On this basis *Ulva* becomes a synonym of the conserved name *Enteromorpha* which also has *U. intestinalis* as lectotype (Silva, 1952). In order to retain the current concept of *Ulva* L. (a genus having a distromatic thallus with the two cell layers adhering to each other) it is necessary that the name be conserved as of a later author. Papenfuss (1960) proposes that the name *Ulva* be conserved in the sense of Thuret 1854 who excluded from the genus both *U. lactuca* L. and several monostromatic species for which Thuret had established the genus *Monostroma*. Papenfuss suggests that *U. rigida* (C. Agardh) Thuret 1854 best lectotypifies *Ulva* Thuret in view of the great uncertainty concerning the species represented by the type of *U. lactuca*.

The identification of *Ulva lactuca* L. has been a subject of controversy. Several morphological characteristics of the authentic specimens in the Linnaean Herbarium led Papenfuss (1960) to question strongly the name *U. lactuca* as it was applied to Linnaeus' lectotype. The occurrence of hyaline rhizoids between the cell layers in some areas suggested a feature of the genus *Letterstedtia*. The Linnaean description of the type as being palmate is not a feature of the plant that is currently known by this name. It was evident to Papenfuss that after the plant had been soaked out it did not appear typical. Cross sections of the thallus did show, however, that the blade was distromatic. He concluded that *U. lactuca* is a species of *Ulva* in the current sense, but that it was not certain that the type specimen in the Linnaean Herbarium was an example of the taxon currently known by the name *U. lactuca*. As Silva (1952) has pointed out, early post-Linnaean authors included in *Ulva* algae with expanded, or at times, gelatinous thalli of any color. Lamouroux (1813) removed membranous red algae to his Floridées and brown algae to his Dictyotées. The present circumscription of *Ulva* limiting it to membranous green algae having a distromatic thallus came about as a result of the removal of

tubular forms to *Enteromorpha* by Greville (1830), and the splitting off of *Porphyra* by C. Agardh (1824) and *Monostroma* by Thuret (1854). Dumortier (1822) latinized the spelling of Lamouroux's Ulvacées to Ulvaceae.

Ulva was at first placed by C. Agardh in his order Confervoideae in 1817. Subsequently he transferred *Ulva* to his order Ulvoideae in 1822 which he renamed the Ulvaceae in 1824. The Ulvaceae was one of six orders recognized by Agardh and was comprised of some 11 genera of membranous, tubular or spongy green, brown and red algae. When Harvey (1836) established 4 divisions based on spore color the Ulvaceae was included as one of 8 tribes under the Chlorospermae. Interestingly, two red algal genera, *Bangia* and *Porphyra*, remained in Ulvaceae until they were removed by Thuret (in Le Jolis, 1963).

Stizenberger (1860) and Rabenhorst (1868) separated the freshwater green algae into 4 orders with the Ulvaceae placed in their Nemastophyceae. Later De Toni (1889), Wille (1890) and Blackman (1900) substituted C. Agardh's Confervoideae as the ordinal name for the taxon which includes the family Ulvaceae in place of Nemastophyceae.

Borzi (1895) had placed the Ulvaceae in his new order Ulotrichales. Similarly, Blackman and Tansley (1902) broke up the largely artificial order Confervoideae of De Toni and Wille and removed the Ulvaceae to a new order Ulvales under the Chlorophyceae (Isokontae). They contended that the green parenchymatous algae were distinct in their morphology from the filamentous habit of typical Ulotrichales. Wille (1909) later elaborated on his earlier classification of the green algae placing the Ulvaceae in his order Chaetophorales (= Confervales).

In present day treatments Ulvaceae is now placed in Ulvales as first proposed by Blackman and Tansley (1902) or in Ulotrichales as first proposed by Borzi (1895). The recognition of the Ulvales as a separate order was strongly influenced by the discovery that the life cycles of some species of *Ulva* or *Enteromorpha* include an alternation of gametophyte and sporophyte generations unlike members of the Ulotrichales in which the vegetative thallus is haploid and only the zygospore stage is diploid (Hartmann, 1929; Kylin, 1931; Föyn, 1934).

Blackman and Tansley were followed by such later workers as West (1916), Setchell and Gardner (1920), Hamel (1931), Newton (1931), Feldman (1937), Doty (1947), Kylin (1949) and Smith (1950).

The placement of Ulvaceae in the order Ulotrichales was backed by the observation that the filamentous germling stages of *Ulva* and *Enteromorpha* show a striking resemblance to filaments of *Ulothrix* (Fritsch, 1935). It was also established that some Ulotrichales, such as *Fritschiella* and *Draparnaldiopsis*, show an alternation of isomorphic generations (Singh, 1945, 1947) and that some are partly parenchymatous (*Fritschiella*) or show a tendency in that direction (*Stigeoclonium* and *Draparnaldia*). Authorities who have supported this second alternative include Oltmanns (1922), Collins (1909), Børgesen (1913), Chapman (1952b) and Papenfuss (1960). De Toni (1889) and Knight and Park (1931) recognized the Confervoideae rather than the Ulotrichales as the all embracing order. Heering in Pascher (1914) followed by Fritsch (1935) modified the arrangement somewhat by establishing two suborders, the Ulotrichineae and the Prasiolineae under the Ulotrichales.

The family Ulvaceae has in the past included the genera *Ulva* Linnaeus (1753), *Enteromorpha* Link (1820), *Percusaria* Bory (1823), *Letterstedtia* Areschoug (1851), *Ulvaria* Ruprecht (1851), *Monostroma* Thuret (1854), *Capsosiphon* Gobi (1879), *Blidingia* Kylin (1947), *Lobata* and *Gemina* Chapman (1952a), *Rhizenteron* Dangeard (1952), *Feldmannodora* Chadefaud (1957) and *Ulvopsis* Gayral (1964). *Monostroma* was placed in its own family Monostromataceae by Suneson (1947) based on the developmental and life history studies of Kunieda (1934). *Capsosiphon* was removed to the Capsosiphonaceae by Chapman (1952a) and *Percusaria* to the Percusariaceae by Bliding (1968). *Blidingia*, which has an ontogeny similar to *Monostroma*, was placed in the Monostromataceae by Bliding (1968). *Letterstedtia* and *Lobata* were synonymized under *Ulva* by Papenfuss (1960), while species of *Gemina* were transferred either to *Ulva* or *Enteromorpha* by Papenfuss (1960). According to Bliding (1968) *Ulvopsis* is to be included under *Monostroma*.

Gayral (1964), Bliding (1968) and Vinogradova (1969) have all proposed major changes in the Ulvaceae. Gayral includes the genera *Percusaria, Enteromorpha, Ulvaria, Ulvopsis, Blidingia, Ulva* and

Kornmannia in Ulvaceae. Bliding includes only *Ulva*, *Enteromorpha* and *Ulvaria*, while Vinogradova places *Enteromorpha*, *Ulva*, *Percusaria* and *Ulvaria* in Ulvaceae.

A serious taxonomic revision of Ulvaceae was carried out by Bliding commencing with work on *Enteromorpha* in 1938 and culminating in two monographic studies dealing primarily with *Enteromorpha* (1963) and *Ulva* (1968) found in Europe. In his early papers Bliding drew attention to the impossible task of delimiting specific entities within *Ulva* and *Enteromorpha* based solely on anatomical and morphological characters.

Extensive experiments based on intraspecific and interspecific crosses as well as intergeneric crosses in *Ulva* and *Enteromorpha* convinced Bliding (1955,1963,1968) that strict intersterility barriers do exist between species of the Ulvaceae. Föyn (1955,1958) also showed that the plant known as *U. lactuca* in Europe can be separated into three species, all sexually isolated from one another. The young plantlets showed certain differences, but the mature plants were virtually identical. Kapraun (1970) confirmed Bliding's work with hybridization experiments which showed that interspecific crosses failed to give viable zygotes capable of subsequent development in *Enteromorpha* and *Ulva*.

From the results of his breeding experiments Bliding was able to establish that certain cytological and anatomical characters are relatively stable and therefore useful for the identification and characterization of species. These include: (1) number of pyrenoids in a vegetative cell; (2) form and arrangement of cells in surface view; (3) size of cells in surface and cross sectional view; (4) diameter of the thallus in cross sectional view at different levels; and (5) morphology of the young germlings.

Early studies on reproduction in the genus *Ulva* began with Thuret (1850) who found that all cells at the margin of the thallus in *Ulva lactuca* became fertile and released biflagellated and quadriflagellated swarmers. He called both types zoospores. In 1907 Schiller demonstrated that the biflagellated bodies were gametes. Föyn (1929,1934) was the first to establish that the life cycle of *U. lactuca* consisted of an alternation of isomorphic generations.

Cytological studies by Föyn showed that the chromosome number was doubled at the time of gametic union and that nuclear division prior to zoospore formation was meiotic. He also showed that parthenogenesis may occur with the resultant thallus possessing occasional groups of diploid cells scattered among the smaller haploid cells. At the time of swarmer release both bi- and quadriflagellated swarmers are seen coming from the same thallus. Moewus (1938) confirmed these results in culture studies.

Föyn (1958) established a new species of *Ulva* from Portugal (*U. mutabilis*) and investigated its life cycle in detail in culture. Although *U. mutabilis* is invalidly described for a lack of a Latin diagnosis a great deal of valuable information has come forth. In 1959 Föyn demonstrated an unusual variation in the life cycle for this species. It was found that gametes of both mating types are capable of parthenogenetic development with a doubling of the chromosome number at a certain stage (diploidization) leading to formation of a sporophyte that is homozygous for mating type. Föyn has called such plants diploid parthenosporophytes. Unfertilized gametes can also develop into haploid gametophytic plants as well.

Subsequent experimental studies of mutants of *Ulva mutabilis* by associates of Föyn at the University of Oslo have brought forth relevant data in the fields of morphogenesis, genetics, cytology and basic physiology of this species (Bråten, 1971; Bråten and Lovlie, 1968; Fjeld, 1970,1972; Løvlie, 1964,1968,1969; Løvlie and Bråten, 1968, 1970; Thiadens and Zeuthen, 1967).

Løvlie and Bråten (1970) have studied the mitotic sequence in the vegetative cell of the laboratory-reared diploid sporophyte of the mutant "slender" of *Ulva mutabilis*. Cell division was synchronized by light:dark cycles, whereby maximum mitotic activity occurred one hour after the lights were switched off. Mitosis is preceded by rearrangements of the nucleus and Golgi apparatus in which both migrate from a position near the inner cell membrane. Mitosis then occurs as in more classical mitotic schemes except that the nuclear membrane disappears at the poles and persists as a boundary around the mitotic apparatus. The authors suggest that the synthesis of new membrane material starts at telophase and preferentially takes place in

the region of the Golgi complex and associated nucleus. They base their assumption on the common observations of others that Golgi vesicles open and release their contents onto the cell surface. The formation of wall material starts at late telophase as the two nuclei approach each other with further synthesis being accomplished by the migration of the nucleus and Golgi complex from the lower cell membrane up through the chloroplast to the outer cell membrane as observed in interphase.

Chromosome counts in the genus *Ulva* have established a range n= 10-13, 2n=20-26 for the species studied. *U. lactuca*: Carter (1926) n=10; Föyn (1934) n=13, 2n=26; Sarma (1958) n=10, 2n=20; Kapraun (1970) n=10, 2n=20. *U. fasciata*: Kapraun (1970) n=10, 2n=20. *U. linza*: (=*Enteromorpha linza*) Levan and Levring (1942) 2n=24-25.

Thiadens and Zeuthen (1967) demonstrated experimentally that sporulation in the mutant "slender" of *Ulva mutabilis* could be controlled by replacing the old culture medium with new medium or by adding a mixture of seven vitamins to the old medium. Replacement of the old culture medium has been used to induce reproductive activity in many studies on a wide variety of algae with varying degrees of success. This shocklike treatment appears to stimulate reproduction in both *Ulva* and *Enteromorpha*. Fjeld (1972) has been able to induce sporulation in *U. mutabilis* by storing plants at 5°C in continuous light at 60 ft-c. When zoospores were needed pieces of sporophytic tissue were transferred to fresh medium in the middle of a light cycle and given high intensity light. Zoospores were cleaved out during the third dark period after transfer and were released in the following light period after a second transfer to fresh medium.

Studies on techniques for growing members of the Ulvaceae in culture are numerous (Kylin, 1941,1942; Levring, 1946; Provasoli, 1958, 1964; Föyn, 1960; Gayral, 1967; Baudrimont, 1961; Nasr and Bekheet, 1970; Nasr et al., 1968; Berglund, 1969 and Kapraun, 1970). It is interesting to note that no artificial seawater medium has been reported that is adequate to support normal growth of the ubiquitous seaweed *Ulva lactuca* (Levring, 1946; Föyn, 1960 and Provasoli, 1964).

Provasoli (1958) working with *Ulva lactuca* found that natural

seawater had to be enriched with nitrogen, phosphorous, trace metals and vitamins along with adenine and kinetin to produce a near normal thallus in culture; otherwise, slow growing callus-like clumps would appear. Provasoli (1969) and Provasoli and Pinter (1964,1966) have demonstrated that chemical symbiosis is indeed a very important factor in the growth and normal morphology of several benthic marine algae. When both *Ulva lactuca* and *Monostroma oxyspermum* (=*Ulvaria oxysperma*) were deprived of their usual microflora in culture they completely lost the thalloid morphology and became respectively, a colony of uniseriate branching filaments or an assemblage of loose cells attached to a large rhizoidal cell. Several phenolic compounds such as ferulic and homovanillic acids restored normal morphology to *Ulva* (Provasoli and Pinter, 1966) while the addition of a supernatent of *Sphacelaria* (Phaeophyceae) or the brown exudate of *Fucus* restored normal morphology in *Monostroma* (Provasoli and Pinter, 1964). Interestingly, similar compounds are found in sea muds (Degens et al., 1963) and in degraded humic acid (Mathur and Paul, 1966).

Mud and soil water extracts have been continually used as supplements to culture media for solving growth and morphology problems. Vitamins, such as B_{12}, and chelated trace metals are undoubtedly important factors found in these extracts, as many workers interested in marine culture media for seaweeds have suggested. It was this medium (seawater enriched with N, P and soil extract) that Föyn (1934) formulated and used in his classical work on the life cycle of *Ulva lactuca* and later *U. mutabilis*. Gayral (1967) reported elongate tubular growth for *U. lactuca* in culture in supplemented seawater medium, while *U. curvata* attained a near normal morphology with similar treatment, but only after nine months. Kapraun (1970) also had difficulty in growing young plants of *U. lactuca* in culture. Plants grew to a length of 4 cm and had a tendency for the distromatic blade to separate near the base forming an intestiform stalk; nevertheless, cytological characters and thallus thickness of the cultured plants were similar to material collected in the field.

Germling development has been studied by several workers and more than one developmental scheme devised. Arasaki (1946) employed the term "*Ulva*-type" for the germination pattern found in *Ulva* and

Enteromorpha in which swarmers developed directly into uniseriate filaments with one or more rhizoidal cells at the base. Bliding (1963) found this type of germination pattern to occur in *Ulva, Ulvaria* and in most species of *Enteromorpha*, the three genera comprising Ulvaceae according to Bliding. *Capsosiphon* of the Capsosiphonaceae demonstrates this pattern in a modified way. A second group in which a single layered disc of holdfast cells appears before the erect filament arises is seen in *Enteromorpha hendayensis*; while in certain varieties of *E. flexuosa* the young filaments (3-5 cells long) become hook-shaped. The remaining genera comprising the Percusariaceae and Monostromataceae of the Ulvales have distinctly different germination patterns.

Chihara (1968) studying *Ulva scagelii* found that the germination pattern was like that of *Enteromorpha hendayensis* as well as *U. linearis, U. gayralii* and *E. flabellata*. Chihara calls this germination pattern the "*Ulva scagelii*-type" and the germination pattern in the other species the "*Ulva lactuca*-type."

Kapraun (1970) showed that germling development in *Ulva lactuca, U. fasciata, Enteromorpha clathrata, E. ramulosa* and *E. lingulata* all show direct development of a tubular thallus of the "*Ulva lactuca*-type." In *E. prolifera, E. flexuosa* and *E. salina*, initial rhizoidal development corresponded to the "*Ulva scagelii*-type" of Chihara. Kapraun showed that the germination pattern was characteristic of both gametophyte and sporophyte generations of a species. Gayral (1967) also found a similar species-dependent developmental sequence for germlings in the Ulvaceae.

Ecological studies of the reproduction of marine algae in the field are many; however, studies focusing on the cyclic phenomenon of swarmer release are few. Lunar cycles are known for several marine organisms and reproduction of some of them are restricted to the period of spring or neap tides or to days having some fixed relationship to these times. Reproductive rhythms that are known to occur correspond either to the lunar cycle of 29.5 days or to 1/2 this cycle or 14.7 days (Hauenschild, 1961).

Williams (1905) and Hoyt (1907) were the first to describe in detail the lunar and semi-lunar fruiting period in *Dictyota dichotoma* (Phaeophyceae). Smith (1947) has shown that *Ulva lobata* fruits at

fortnightly intervals with gametophytes releasing early and sporophytes later during the reproductive period. Christie and Evans (1962) found that release of spores occurred 3-5 days before the highest tides of each month in *Enteromorpha intestinalis* in England. Townsend and Lawson (1972) described a semi-lunar rhythm for *E. flexuosa* in Ghana in which maximum release occurred two days before to two days after the highest spring tides.

Brown et al. (1953) and Naylor (1958) have shown that in many marine invertebrates there is a coexistence of diurnal (24 hour) and tidal (12.4 hour) periods, with the two overlapping cycles generating beats at 14.7 day intervals. Müller (1962) subjected *Dictyota dichotoma* to light and dark regimes in the laboratory with varying intervals of light at intensities as low as 3 lux. A burst of reproductive bodies was released 10 days later with subsequent fruiting and release every 16-18 days for 5 cycles much as in nature. The results of these experiments suggested to Müller that the endogenous rhythm operating in *Dictyota* must then be integrated from both diurnal and tidal components with moonlight acting as a phase-setting mechanism. This aspect of marine algal ecology is quite important as Castenholz (1967) has pointed out in that reproduction and colonization by many algae are narrowly seasonal and often display lunar or semi-lunar patterns. The environmental conditions during a period of a few days can mean the difference between establishment or failure for the next season.

The current classification of species of *Ulva* on the east coast of the United States is based largely on morphological characters that show great phenotypic variability and has not changed substantially since the work of Collins (1909). A broad investigation of the biology of *Ulva* taxa is needed before proper identification and systematic revision of the Atlantic North American species will be possible. In this study two species of *Ulva* which are newly recorded from North America, *U. curvata* and *U. rotundata*, are investigated with the purpose of gaining a better understanding of their systematics and ecology.

The approach taken in this research has been to investigate developmental stages in vegetative growth and reproduction in *Ulva curvata* in the field at a site in Calico Creek near Morehead City,

North Carolina, and in a controlled environment in the laboratory. The identity of specimens occurring outside the Morehead City area is confirmed through breeding experiments. The response of different stages of the life cycle to a range of parameters, such as light intensity, photoperiod, temperature and nutrients is followed experimentally. Combining the results of field and laboratory studies has led to a species concept for *Ulva curvata* based on an analysis of its developmental morphology, cytology and life history and its range of ecological adaptation.

MATERIALS AND METHODS

Field Methods

Observations upon an algal community comprised mainly of *Ulva* and *Enteromorpha* were begun in November, 1968, at Morehead City, North Carolina. The research site is located at the end of 13th Street near the mouth of Calico Creek in the Newport River basin (fig. 1). Calico Creek is a eutrophicated estuarine tidal creek which drains both residential and limited agricultural areas and receives both raw and treated sewage through the town's sewage treatment plant. The shore line along Calico Creek is representative of the tidal creeks and marshes in the Beaufort-Morehead City area. The physiography is also representative of many of the estuarine marshes along the central and southern Atlantic coast. Extensive areas of the intertidal zone are covered by oyster reefs set in a fine mud substrate. Higher in the intertidal zone a more sandy substrate is seen which gives way to extensive stands of *Spartina alterniflora* in the upper and supralittoral zones.

During the period from November to May of the years 1968 to 1971, large populations of *Enteromorpha intestinalis, E. prolifera, E. linza* and *Ulva curvata* were found growing on all available hard substrates in Calico Creek (fig. 2A-C). Monthly from December to April, sizable populations of *Porphyra umbilicalis* were seen growing on the exposed oyster shell reef, while numerous tufts of *Ectocarpus siliculosus* were observed growing from both oyster shells and *Spartina* culms. A band of *Ulvaria oxyspermum* was also observed in the upper littoral zone growing well where the *Spartina* was most sparse. Clumps of both *Gracilaria* sp. and *Gelidium crinale* were observed occasionally in a dwarfed state during the spring and summer periods. It should be noted that from May to early November no conspicuous benthic algae are present in this environment (fig. 2D-E). Attached diatoms, bluegreen

algae and the minute filaments of *Enteromorpha* and *Ulva* are the algal vegetation at that time.

During the active growing season of November to May the principal research area was visited every one to three weeks and studied daily from January to April in 1971. Additional collection sites were in Bogue Sound at the Institute of Marine Sciences (I.M.S.), at Fort Macon, in Southport, N. C., and at Little Creek, Virginia. In April, 1971, collections were extended to Charleston, South Carolina and Brunswick, Georgia. During April, 1972, sites in New Jersey, Rhode Island and Massachusetts were visited and plants collected.

At the Calico Creek research site various environmental parameters were measured at the time of collections or recorded over a period of time:

Salinity - Samples were taken periodically and measured in the field with an A.O. Goldberg refractometer.

Temperature - Single measurements were obtained with a standard centigrade thermometer. Long term readings were gathered by a single channel Rustrak recorder. Annual temperature data could not be secured at the research site due to the lack of sufficient protection for recording equipment. Water temperature readings are therefore represented by recorded data at the I.M.S. in Bogue Sound during the years 1970, 1971 and in the pollution ponds at the Calico Creek sewage treatment plant in 1969. Air temperature was measured by a Taylor chart recorder located at the I.M.S.

Tide Factors - Lengths of inundation and exposure at different points along the intertidal zone were estimated from direct observation of stationary marks through several tidal cycles.

Nutrients - Nitrogen as NH_4-N was analyzed using the method of Solorzano (1969). Samples were taken periodically at all stages of tide height particularly during the winter-spring months of 1971. Determinations were made on a Beckman DU spectrophotometer at the I.M.S. or a Cary-14 recording spectrophotometer at the campus.

Light - Illumination was measured by both a Weston model 756 photometer and a Whitney LMD-8A submarine photometer. Solar radiation was measured on a diurnal basis with a Belmont Pyroheliograph using

weekly chart strips. Insolation was also collected using an O.T.I. Mark IV Solameter integrating pyroheliometer. Both instruments were located on the roof of the I.M.S.

Rainfall - Rainfall data was collected from a standard rain gauge located at the I.M.S.

Laboratory Methods

Material collected in the field was brought back to the laboratory either in Chapel Hill or at Morehead City, generally on the day of collection. Thalli maintained as stock material were placed in plastic trays with unfiltered Calico Creek water or water from the collection site. Thalli used as experimental material were washed in sterile Calico Creek water and introduced into various culture media.

Two walk-in constant temperature rooms set at 9°C $\pm$ 1°C and 18°C $\pm$ 1°C and a table mounted metal culture box at a temperature of 24°C $\pm$ 2°C were used. All rooms were equipped with 20 or 40 watt cool white fluorescent bulbs in conjunction with 75 watt incandescent spot lights. Illumination was measured with a Weston model 756 quartz-fitted photometer. Light intensity, controlled by filters and by distance from the lamps, ranged from 50 to 1000 ft-c. Photoperiod was regulated by time clocks.

Calico Creek water brought in from the field was generally left in a dark room at 3-4°C to age for two to five weeks. Before use the water was filtered through Millipore HA 0.45 μ filters. Best control over germination and growth was attained with von Stosch's medium, as cited by Ott (1966), although other supplemental and artificial sea water media were tested. A modification was made by substituting NH_4Cl for the $NaNO_3$ which increased the nitrogen level to 10 mg/l or 0.187 μM NH_4-N. The microelement stock solutions were prepared by dissolving the salts listed below in separate aliquots of 200 ml distilled water:

NH_4Cl	2.0 g
$Na_2HPO_4 \cdot 12H_2O$	2.15 g
$FeSO_4 \cdot 7H_2O$	55.6 mg
$MnCl_2 \cdot 4H_2O$	3.9 mg
$Na_2EDTA \cdot 2H_2O$	0.74 g

The vitamins below were dissolved in a single aliquot of 200 ml distilled water:

biotin	0.2 mg
thiamin	40.0 mg
B_{12} solution (1 mg/ml)	0.2 ml

One ml of each of the six stock solutions was added to 1 liter of filtered sea water. GeO_2 was added as 1.0 ml/l from a 1% solution as described in Lewin (1966). This was highly effective against diatom contamination when cultures were subjected to high light intensity. Germination and early germling growth was greatly enhanced by the addition of a mud-water extract. This supplement similar to Erd-Schrieber solution (Föyn, 1934) consisted of mud collected at the base of the *Spartina* in Calico Creek. Approximately 300 ml of mud was added to 1 liter of Calico Creek water and heated to 85°C. This solution was left standing overnight to be filtered through a Whatman #1 filter paper the next day. The filtrate was then autoclaved at 25 psi for 20 minutes and frozen until used. The mud-water extract was added to the medium at a concentration of 25 ml/l.

Experimental material was kept unialgal; however, occasional outbreaks of coccoid and filamentous blue-green algae occurred. *Tetraselmis maculata* (Prasinophyceae) was found to be a common contaminant. Germlings were generally dragged through sterile seawater agar several times to remove epiphytic organisms. No attempt was made to grow plants in axenic culture.

Containers used in most experiments were 300 ml Pyrex culture dishes (80 mm X 100 mm) or 250 and 500 ml Erlenmeyer flasks when used in shaking experiments.

Swarmers (gametes, zoospores) were collected by pipette at the meniscus of the culture jar shortly after their release from the parent material. When a sufficient amount of swarmers was available it was put through several (5-10) sterile seawater washes and collected by phototaxis. The reproductive bodies were then placed on coverslips, allowed to attach in 3 to 12 hours and flooded with media to start new cultures. Gametes used for mating purposes were collected again at the meniscus and both mating types introduced into a small vial. Shortly after fusion zygotes became negatively phototactic and were

drawn from the shaded side of the vial, while unfertilized gametes were positively phototactic and could be taken off the illuminated side.

For experiments on growth of thalli a disc of tissue having an area of either 0.45 cm^2 or 1.80 cm^2 was removed by means of a cork boring tube. Discs were regularly taken from the central area of the thallus tissue to normalize for variations in the growth potential of different parts of the thallus. Discs were measured individually at the beginning of each experiment and generally there was never more than 4-5% variation in size. The areas of each disc was measured on a grid having 100 squares/cm^2. The use of discs greatly facilitated measurement in growth and reproduction experiments. Successive removal of discs from previously growing discs or thalli perpetuated clones of reproductive isolates over long periods of time in the laboratory.

The use of a crossed-gradient apparatus allowed for controlled experiments on germination, growth and reproduction, designed with variables of light intensity, temperature, photoperiod and nutrient factors. The apparatus consisted of a plywood tray 86 cm long, 44.5 cm wide and 8.5 cm deep, a small refrigeration unit and heat exchange unit placed at one end, and a thermostated hot water bath connected by tubing to a second heat exchange unit placed at the other end of the tray. Both heat exchange units were buried in medium-grain construction sand allowing a stable gradient in temperature to develop from one end of the tray to the other. A gradient was established from 5°C $\pm$ 1.5°C to 25°C $\pm$ 2.5°C in an ambient temperature of 10°C. Intervals of 5°C were chosen for the experiments. A light intensity gradient ranging from 100 ft-c $\pm$ 20 ft-c to 1000 ft-c $\pm$ 55 ft-c was crossed at right angles to the temperature axis by utilizing one 30 watt cool white and two high intensity cool white "Power Groove" fluorescent bulbs. Intervals in the light gradient were obtained by adhering Zip-a-Tone stipple paper as density filters to glass plates covering each 60 ml culture jar.

The apparatus just described is identical in concept to that designed and used by Halldal and French (1956,1958. Edwards and van Baalen (1970) followed the same principles in constructing a

crossed-gradient system in order to study growth of species of benthic marine algae in experimental culture.

Investigations into the nuclear cytology of *Ulva* were carried out to determine the ploidy levels of thalli and mitotic phases in cell division. Chromosome counts were determined using Bouin-Debosque fixing fluid and Gomori's haematoxylin stain following the procedure of Løvlie (1964). It should be pointed out that chromosomes in the Ulvaceae are exceedingly small, while chromatin material also tended to confuse counts. It was necessary to use only those nuclei in late prophase just as they were entering metaphase in order to distinguish between chromosome "dots" and chromatin.

RESULTS OF FIELD AND EXPERIMENTAL STUDIES

Identification of *Ulva curvata* (Kützing) De Toni

Ulva curvata was first described by Kützing in 1845 as the entity *Phycoseris curvata*, collected in the Baltic Sea on the island of Rügen. De Toni (1889) later transferred it to the genus *Ulva* and, according to Bliding (1968), the species was overlooked or included in the complex "*lactuca*." Schiller (1928) appears to be the first person to have described it thoroughly with material from Helgoland, Germany. Van den Hoek (1963) found *U. dangeardii* Gayral and Mazencourt (1958) from Morocco and *U. incurvata* Parriaud (1958) from southern France to be conspecific with the earlier described *U. curvata* (Kützing) De Toni (1889). Bliding investigated the type specimen of *Phycoseris cornucopiae* Kützing and lists it also as a synonym of *U. curvata*.

Ulva curvata, as described by Bliding (1968), is a plant with a small asymmetric stipe at the base reflecting a curved growth pattern and morphology. The thallus lacks conspicuous fenestrations and microscopic teeth on the margins. Rectangular and more or less polygonal cells in rows alternate with cells in an unordered fashion. Cells average 11.5 to 17.0 μ long and 11.5 μ wide, while cells from cultured material seven months old were 20 μ long and 15 μ wide. The thickness of the thallus measures 34-39 μ at the upper margin of the thallus, 44 μ in the upper central area, 75 μ toward the base and 85 μ in the rhizoidal region. The chloroplast is parietal with one pyrenoid.

In Europe *Ulva curvata* has been demonstrated to possess an isomorphic alternation of generations (Bliding, 1968). Male gametes measure 6.2 X 2.0 μ, female gametes 7.3 X 3.3 μ and zoospores 11.2 X 5.4 μ in size. Early development is characterized by an unusually long time spent in the "*Enteromorpha*-like" tubular stage with a single top cell. The young thallus is also characteristically curved from the basal holdfast. While *U. curvata* is now known from Morocco to

Sweden, Bliding's observations were based upon material from Mediterranean France, Brittany, Bay of Biscay and the west coast of Sweden.

A herbarium specimen of the isotype of *Ulva dangeardii* Gayral and Mazencourt was kindly sent to the author by F. Ardré of the Museum national d'Histoire naturelle. The specimen (fig. 4F), collected from an estuary near Rabat in Morocco on April 21, 1958, shows an asymmetric base with a decidedly recurved growth pattern in relation to the holdfast. Cellular features as described in Gayral and Mazencourt (1958) show cells 6-12 μ long and 4-8 μ wide in a random arrangement (fig. 5A-B). The thickness of the thallus in the upper region was found to be about 40 μ. One pyrenoid was faintly discernible after I_2KI staining. Although cell size in the specimens of *U. dangeardii* average somewhat smaller than that in plants from Calico Creek, the external appearance of the thallus is quite similar. As was mentioned earlier, van den Hoek (1963) placed both *U. dangeardii* and *U. incurvata* in synonomy under *U. curvata* based on morphological and anatomical characters. Additional representative European specimens of *U. lactuca* and *U. rigida* sent by F. Ardré demonstrate the distinctiveness of *U. dangeardii* with its striking asymmetrical morphology.

Collections of *Ulva* taken over a three year period from Georgia to Massachusetts have provided a large number of specimens that have been examined morphologically and cytologically and used in breeding experiments. The Calico Creek material was subjected to Bliding's systematic approach and criteria and was found to be identifiable with *U. curvata* (Kützing) De Toni (Table 1). Observations of Calico Creek plants were made during early, mid and late growing seasons (November to May) of 1969-1971. Cell size was measured in 40-60 plants during each growing season. A random selection of 10-20 cells per 2-3 fields (175 μ^2) per plant was measured in surface view for cell dimensions. Thallus thickness was measured in a total of 60-75 plants during the same period. Dimensions of 25 I_2KI-fixed reproductive bodies were determined for each of 5-10 plants of each genetic phase, mostly during the mid and late reproductive periods of 1970-1972.

The normal morphology characteristic of both gametophyte and sporophyte plants is seen in figures 3A-C. Thalli are generally 10-35 cm in length but can reach one meter, becoming irregular in shape.

Specimens collected in Georgia in the Jekyll River estuary (fig. 3D) and in northern New Jersey in the Navesink River (fig. 3E) look similar to Calico Creek material.

TABLE 1

A comparison of morphological and cytological observations of plants from Calico Creek with data published by Bliding (1968) for *Ulva lactuca* and *Ulva curvata* from Atlantic Europe.

CHARACTER	*U. lactuca*	*U. curvata*	C.C. PLANTS
External morphology	Symmetric base	Asymmetric base	Asymmetric base
Cell measurements in surface view	18 μ X 13.5 μ	11-17 μ X 11.5 μ	10-18 μ X 7-11 μ
Thallus thickness			
Upper margin	48 μ	34-39 μ	33-40 μ
Central portion	60 μ	44 μ	——
Toward base	80-90 μ	75 μ	60-70 μ
Rhizoidal area	100 μ	85 μ	70-80 μ
Chloroplast position	Across outer face of cell	Lateral	Lateral
Number of pyrenoids	One	One	One
Male gametes	6.8 μ X 2.1 μ	6.2 μ X 2.0 μ	5.4-6.4 μ X 2.0 μ
Female gametes	7.6 μ X 3.6 μ	7.3 μ X 3.3 μ	6.4-7.4 μ X 3.5 μ
Zoospores	9.7-12.2 μ X 5.9-6.8 μ	11.2 μ X 5.4	8-11.5 μ X 4.0-5.5 μ

Light microscope surface views of the thallus show cells with parietal chloroplasts along the side walls with one pyrenoid in 95% of the cases (fig. 4A-B). Approximately 5% of the cells observed had two pyrenoids. Cells measured 7-11 μ X 10-18 μ arranged in longitudinal rows or commonly oriented randomly. The thickness of the thallus in the upper portion is 33-40 μ ranging to 60-70 μ toward the base. Male gametes measure 5.4-6.4 μ X 2.0 μ, female gametes 6.4-7.4 μ X 3.5 μ and zoospores 8-11 μ X 4-5.5 μ (fig. 4C-E). The zoospore from Calico Creek material was found in most cases to be smaller than the average reported by Bliding and to be positively phototactic throughout

motility. The plant demonstrates isomorphic alternation of generations with both sporophyte and gametophyte present during the growing period. Both male and female gametes are capable of parthenogenetic development.

Mating studies were carried out with plants from Calico Creek and material from other localities along the Atlantic coast. Collection sites include: Jekyll River, Brunswick, Georgia; Folly River, Charleston, South Carolina; Southport and Shallotte, North Carolina; Little Creek and Lynhaven Inlet, Virginia; Cold Spring Inlet, Cape May, New Jersey; Point Judith Pond, Rhode Island; and Acoaxet, Onset and Woods Hole, Massachusetts. Gametophytes from these localities were induced to release gametes and matings were attempted with Calico Creek gametes. Results showed copulatory activity and zygote formation between Calico Creek plants and plants from Georgia, South Carolina, North Carolina, Virginia, and New Jersey. Although the plants collected in Rhode Island, Acoaxet and Onset, Massachusetts looked cytologically and morphologically similar to Calico Creek material, gametes could not be induced into release.

Plants collected at Woods Hole harbor on rocks released biflagellated swarmers (6.4-8.0 μ long) that did not mate with either mating type from the other localities. These plants were morphologically similar to *Ulva rigida*, a common entity in the locality (fig. 5F). It should be stressed that all collection sites except Woods Hole, Massachusetts, consisted of *Spartina* marsh areas, generally with oyster shells serving as the substrate for attachment of *Ulva*.

An isolate of *Ulva lactuca*, identified and distributed by the Carolina Biological Supply Company, Burlington, N. C., and reported to have been collected originally in Massachusetts, was given to the writer. The material kept in culture later released small (5.0-6.0 μ long) biflagellated swarmers that did not mate with material of either mating type from Calico Creek or Lynhaven Inlet, Virginia.

Identification of *Ulva rotundata* Bliding

In the course of making observations and collections in the

Morehead City-Beaufort area another entity was found growing from late October to early June. It was relatively common on hard substrates in Bogue Sound, in areas of Bogue Inlet and at Atlantic Beach facing the open ocean with salinities ranging from 31 to 35°/oo. After comparison with known species of *Ulva* along this coast and in Europe it was decided that these specimens were comparable to *Ulva rotundata* Bliding 1968.

Small rather stunted looking plants were observed in Bogue Sound from October to December (fig. 5C). Plants collected in April and May were considerably larger, appearing lobate to somewhat rotundate (fig. 5D). Except for cell size and the number of pyrenoids these plants could easily be mistaken for *Ulva lactuca*.

Ulva rotundata as described by Bliding has been found from the Canary Islands to northern Norway. Plants from the Canary Islands were a few cm high in the upper littoral zone while plants from Norway were reported up to 2 dm tall in the sublittoral zone. According to Bliding cell dimensions are 26 x 20 μ while some cells reach 30-36 μ in length. The thickness of the thallus is 56 μ in the upper portion and ranges to 75 μ in the lower parts. A parietal chloroplast with 1-3 pyrenoids lies across the outer face of the cell or sometimes along the side wall. Male gametes measure 5.1 X 2.5 μ, female gametes, 6.7 X 3.6 μ and zoospores, 10.1 X 5.7 μ. Bliding reports that this species has an isomorphic alternation of generations.

Cells measure 20-30 μ long by 12-20 μ broad with the long axis oriented randomly in plants collected in Bogue Sound. The chloroplast lies across the outer face of the cell and contains (1)2-4(5) pyrenoids (fig. 5E). Thalli are 50-60 μ thick in the upper portions and 75-80 μ in the lower parts. To date only large biflagellated swarmers (7.0-9.0 μ long) have been observed that do not mate with either of the gametes of *Ulva curvata*. The biflagellated swarmers are capable of direct germination without sexual fusion. Morphological and cytological observations of plants collected by the author in Bogue Sound are contrasted with data published by Bliding (1968) for *U. rotundata* in Table 2. Measurements were made on 45 plants taken during the growing season from November to April, 1971-1972 and in December, 1972. Dimensions of 25 I_2KI and fixed reproductive bodies were determined

for 18 plants during the reproductive months from December to February in 1971-1972 and again in December, 1972.

TABLE 2

A comparison of morphological and cytological observations of plants collected in Bogue Sound with data published by Bliding (1968) for *Ulva rotundata*.

CHARACTERS	*U. rotundata* Bliding	BOGUE SOUND PLANTS
External morphology	Lobate	Lobate
Cell measurements in surface view	26-36 μ X 20 μ	20-30 μ X 12-20 μ
Thallus thickness		
Upper portion	56 μ	50-60 μ
Lower portion	75 μ	75-80 μ
Chloroplast position	across outer face of cell or along side wall	across outer face of cell
Number of pyrenoids	1-3(5)	(1)2-4(5)
Male gametes	5.1 μ X 2.5 μ	
Female gametes	6.7 μ X 3.6 μ	one size of swarmer observed
Zoospores	10.1 μ X 5.7 μ	7.0-9.0 μ X 4.0 μ

Germination and Development of the Early Tubular Thallus

During the months of late May to early July oyster shells (*Crassostrea virginica*) in Calico Creek are relatively clean with a minimum of epizoic growth (fig. 6A), except for what appears to be germination products of *Enteromorpha* and *Ulva*. These are seen as minute uniseriate to slight pluriseriate filaments. Cellular masses and very short ($\leq$ 100 μ) filaments are observed (fig. 6B-C). Toward the end of the summer and early fall the shells become increasingly darker green with an evident scruffy mat of algal filaments (fig. 6D). Microscopic observations showed tubular *Enteromorpha*-like germlings up to 500 μ

long on or just beneath the surface of the oyster shells. *Spartina* culms, root stocks and other solid material can also serve as substrates. During the second to third week in November small, somewhat expanded thalli were observed in the *Spartina* zone in 1969-70. Thalli were not seen in this zone during the 1971-72 germination period until late January, 1972. Thalli appeared on the more exposed intertidal oyster shell reef in late November to early December during the 1969 season. However, their appearance was not observed until early March in 1970, mid-March in 1971, and early April in 1972.

The differences in the time of germination for *Ulva curvata* between the *Spartina* and oyster reef zones suggested that, perhaps, two different *Ulva* species were represented, one in each area; or, alternatively, that different genetic phases (gametophyte or sporophyte) inhabited one or the other zones. Both biflagellated and quadriflagellated swarmer types were released by thalli from the two zones and chromosome counts of vegetative cells confirmed that both haploid and diploid thalli were represented. Mating experiments were performed using gametophytes from both areas, whereupon zygotes resulted when gametes of opposite mating type were brought together. It thus appeared that a single species occupies the two ecological zones. As will be brought out later, a greater proportion of the plants in the *Spartina* zone was found to be gametophytes and primarily female plants during the early months of November, December and January.

Germination studies were initiated under controlled environments in order to investigate the possible causative factors responsible for the sporadic appearance of the small germlings (1-5 mm) in the oyster reef zone, and the irregular appearance each year in the *Spartina* zone in the early part of the season. Oyster shells were collected in the intertidal zone during the months from June through October and brought back into the laboratory. Shells broken up to a convenient handling size were subjected to experimental parameters of varying light intensity, temperature, photoperiod and nutrients. Figures 7A-B show the germination potential and growth of tubular thalli on shells in the laboratory that were collected in the *Spartina* zone during August, 1971. A single figure is used for each experimental photoperiod as the results from triplicate experiments were virtually identical. The

10.5:$\overline{13.5}$ photoperiod (fig. 7A) represents the day length at the time plantlets first appear in nature. When the day length was increased to 14.5:$\overline{9.5}$ (fig. 7B) comparable to the photoperiod in June, germling development was unchanged. Levels of 0.5, 1.0 and 10.0 mg/l NH_4-N added in separate experiments were found only to increase thallus growth over that of the controls with no evidence of any effect on induction of germination.

When shells containing both *Enteromorpha* and *Ulva* were collected from July through September and held constant at 25°C and 400 ft-c as stock material no signs of germling development were observed; however, when these shells were transferred to 18°C at 400 ft-c, plantlets developed in 10-25 days. It should be noted that shells that appeared to be void of any discoloration or mat-like surface did not show signs of thalli even after 40 days in culture. Shells collected in the oyster reef zone showed comparable germination and germling appearance in all temperature and light intensity regimes as shells collected in the *Spartina* zone. Experimentally the only difference observed between the germination patterns in these two zones was the greater incidence of *Enteromorpha* thalli on the oyster zone shells.

Figure 7C illustrates the germination potential of shells collected from mid-May to early June, 1971. As mentioned earlier, shells collected during this period show only minute filaments up to 100 μ in the field. In the crossed-gradient apparatus the filaments increase in length to about 500 μ in high temperatures, especially at high light intensities.

Germinations were carried out using the different reproductive bodies (male and female gametes, zoospores and zygotes) obtained from *Ulva curvata* thalli in culture. Results of germination experiments under crossed-gradients are seen in figure 8. Germination is said to have occurred if at least one cross wall is formed. The average length of at least 25 germlings per coverslip was measured. Gametes of both mating types are capable of parthenogenetic development; however, there is a difference in germination potential between the male and female gamete and the other swarmer types. The male gamete appears to be capable of germination only under comparatively high temperatures and light intensities in the laboratory (fig. 8).

Germination of all the reproductive bodies appears to be strongly influenced by temperature and light intensity (fig. 8). A lower limit of 15°C and in some cases 20°C is needed before germination will occur and early growth continues. One week in the apparatus was sufficient time to determine germination potential. Germination potential was similar with different culture media and photoperiods; however, mud-water extract was an essential ingredient in the medium in order to obtain normal development.

When reproductive bodies were placed in high temperature (25-28°C) and high light intensity (500-800 ft-c) germination proceeded quickly, giving rise to a tubular stage 1.0-2.0 mm long in 10-20 days. These plants develop further only at an exceedingly slow rate.

From these experimental results it appears that temperature may well be a dominant controlling factor in initiating germination and in the subsequent appearance of the tubular thallus. Water and air temperature, insolation and rainfall were followed and analyzed during the early germination phase in the field. As mentioned earlier reproductive bodies have germinated by early to mid-summer but do not appear as macroscopic entities until early November. Temperature data, although collected on an annual basis, are presented only from September through December, the period in which both water and air temperatures appear to be significant for germling development. Minimum and maximum daily air and water temperatures are seen in figure 10. The ranges correspond to the temperatures recorded during each 24 hour period under both exposure and inundation conditions.

Macroscopic thalli of *Ulva curvata* first appeared during the first to third week in November, 1969-1971 and seemingly can be correlated with the observed decrease in air and water temperatures as seen in figure 10. A precipitous drop in temperature was recorded in the years 1969 and 1971, while a more gradual decline appears to have occurred in 1970.

Insolation measured as cal/cm^2/month was calculated and is shown in figure 9 for the months of September through December. The data are quite similar from year to year except, perhaps, for a low value recorded for September, 1969. Thalli on the open oyster shell reef are subject to 100% of the measured insolation at low tide.

Measurements made during various phases of the total cycle revealed a reduction of 55-75% of the incident light below 1 meter of clear water. The drop in light intensity was 80-95% when 1 meter of turbid water lay over the oyster zone. Measurements of light attenuation at the base of *Spartina* plants showed a fall off of 60-70% of the light at low tide and a loss of 85-95% under 0.5 meters of turbid water. It is seen that light intensity differs widely over available substrates between the *Spartina* zone and the open oyster reef area at low tide. The height of the tide and turbidity of the water attenuated the light to a large degree.

Salinity measurements were not taken on a regular basis but were found to range on an average from 24-29°/oo. Wider fluctuations were observed from 18-31°/oo after abnormal tides due to storms and strong winds during the September-December period. Laughinghouse et al. (1971) found salinities in Calico Creek measured at the experimental pollution ponds located at the Morehead City sewage treatment plant to range from 17-33°/oo from April to August, 1970. During low tide and significant rainfall salinities dropped below 20°/oo. Rainfall which plays an important part in seawater dilution, particularly at low tide, was measured on both an annual and monthly basis, with attention being paid to the August-November period (table 3).

Some interesting observations were made in the laboratory regarding the very early stages of germling morphology. At first the germlings appeared to be of two types. Zygotes and parthenogenetic gametes produced an early upright uniseriate filament well before the differentiated basal cell develops a sparse rhizoidal system (fig. 11A). Quadriflagellate zoospores, on the other hand, quickly produced a large irregular rhizoidal basal system before any significant growth of the erect filament (fig. 11B). Both germling types, however, converged becoming morphologically similar in 10-20 days. These observations are much like the results obtained by Kapraun (1970) for several species of *Ulva* and *Enteromorpha*. In contrast, the basal portion of gametes and zygotes grown in an agitated culture medium forms a tight disc of rhizoidal filaments that were closely appressed (fig. 11C). When zoospores were grown in mud-water extract medium, the rhizoidal portion was normally large, but when subjected to high

NH_4-N levels the development of the rhizoidal portion was much reduced (fig. 11E). It appears that early germling development and differentiation is controlled to a certain degree by exogenous factors at least in experimental culture.

TABLE 3

Monthly and annual rainfall in inches over a four year period at I.M.S. Totals for the months August-November are given in brackets.

	1968		1969		1970		1971	
Jan.	4.85		3.08		2.66		6.82	
Feb.	0.99		3.12		5.22		3.91	
March	2.65		4.77		5.40		4.24	
April	3.70		1.84		2.18		3.11	
May	2.79		2.05		3.02		2.96	
June	3.09		5.53		3.79		3.20	
July	12.66		9.28		8.98		5.49	
August	3.91		3.80		4.57		8.06	
Sept.	10.76		2.55		2.82		8.71	
Oct.	11.93	[31.43]	1.65	[11.16]	4.15	[14.45]	10.66	[29.48]
Nov.	4.83		3.16		2.91		2.05	
Dec.	1.89		5.77		2.74		1.98	
Total	64.00		46.60		48.44		61.19	

All germling types except that produced by the male gamete later formed secondary erect thalli adventitiously from the rhizoidal filaments (fig. 12) in the same manner as has been described by Gayral and Mazencourt (1958) for *Ulva dangeardii* (=*U. curvata*) from Morocco.

Development of the Juvenile Thallus

In the field *Ulva* plantlets can be distinguished from juvenile *Enteromorpha* by their expanded thalli. It was possible because of

differences in width to recognize the first appearance of *Ulva* among the complex of *Enteromorpha* species. It could not be determined precisely how long a period elapsed between the first appearance of macroscopic tubular thalli and expanded thalli for *U. curvata* in the field. The time was estimated, however, to be about 1-3 weeks.

Laboratory conditions greatly affected the primary morphological change from the tubular to the flattened expanded form of the thallus. As several workers have pointed out, normal morphology is extremely difficult to obtain in culture with species of *Ulva*. This was borne out for the most part in the present research. Figures 13A-E show a common developmental series obtained in the laboratory. Under static culture conditions with a variety of photoperiods, temperatures, types of media and rates of nutrient replenishment, a standard growth pattern is realized with all four types of reproductive bodies. Figure 13D shows results of prolonged growth of germlings after several months in culture. An intestiform-like blade was usual in all culture conditions. A larger somewhat more expanded thallus was realized if the culture medium was changed at least once every two days (fig. 13E). Similar results were reported for *U. lactuca* by Kapraun (1970), Gayral (1967) and Provasoli (1958,1964) and for *U. fasciata* in axenic culture by Kapraun (1970).

Frequent changes of media that contained mud-water extract and high levels of NH_4-N were factors that helped produce a near normal thallus in static culture. When tubular germlings 1-3 mm in length were subjected to various modes of agitation, development in most cases changed markedly. Tubular germlings attached to coverslips in Erlenmeyer flasks began expanding into blades after 5-10 days on both rotary and wrist action mechanical shakers. Figures 14A-B compare the size and thalloid morphology of plantlets grown in static and in agitated cultures. In figure 15 the ratio of length to width is plotted for germlings grown under identical conditions except that part were maintained under static conditions while the rest were mechanically agitated. The length to width ratio is clearly an important factor as it applies to morphogenesis and differentiation. When germlings were grown with agitation but without the addition of mud-water

extract growth in the transverse direction was slow but not so slow as in static culture. Figures 14C-D show thalli that were grown in agitated cultures from zoospores and female gametes respectively during a period of three months. Each plant measured approximately 20-22 cm in length. Growth was still continuing but the experiment was terminated because large containers needed for optimal shaking conditions were not available.

Thallus Growth and Reproduction

The rapid growth of mature thalli and a strictly cyclical formation and release of reproductive bodies in the field suggested to the writer that these two phenological events should be studied together. Experimental control over both growth and reproduction was essential and the two activities are discussed in conjunction with each other.

In both the *Spartina* and oyster reef zones thalli grew quickly until they reached a mature size of 20-35 cm in 4-8 weeks. It proved to be difficult to measure entire attached plants in the field, due to periodic detachment and fragmentation, so discs were cut from thalli and used as indicators of growth. The discs were placed in plexiglass cylinders (4 X 6 cm) and secured at different levels in the intertidal zone. Growth rates were recorded only when reproduction had not occurred or silting had not buried the chambers. Growth rates ranged from 5% to 66% over three day intervals and from 12% to 157% over seven day intervals. During the months March-April 1971, 37 discs were measured over a 3 day period, while 20 discs were measured over a 7 day period. These rates of growth are considerably lower than rates observed in the laboratory and appear to be lower than for intact thalli.

Thalli were found growing at the lowest limit of the oyster shell reef in the intertidal zone. Transplant experiments showed that when oyster clutches and their associated *Ulva* thalli were moved further down in the lower intertidal zone past the level of established oyster shells, growth of the plants ensued as long as silting did not cover the shell substrate. When fourteen oyster clutch transplants were

moved distances up to 2.5 meters beyond the established lower limit of oyster growth, thalli on these shells grew and reproduced through the remainder of the season.

Records kept on the types of swarmers released from thalli collected in the field revealed a trend in which gametophytes were more prevalent early in the season from December to February, while sporophytes predominated later in the season from February to April (table 4). It should be noted that diploid sporophytic thalli releasing smaller positively phototactic zoospores (8-9.5 μ X 4.0-4.5 μ) were more common than those releasing larger negatively phototactic zoospores. The same trends were observed in both the *Spartina* and oyster reef populations.

It was not realized that a one-sided ratio of female to male gametophytes existed until extensive cross-breeding experiments were attempted between Calico Creek material and plants from other areas. This imbalance so strongly in favor of female gametophytes was not suspected because the only available biflagellated swarmer (female gamete) was thought to be an asexual reproductive body, corresponding to the biflagellated zoosporid described by Bliding (1963). Plants collected from other areas along the Atlantic coast showed greater percentages of male gametophytes. These plants released male gametes that were capable of mating with female gametes produced in Calico Creek thalli. Table 4 shows the number of male and female gametophytes collected in Calico Creek on several dates, indicating a much higher incidence of female plants.

Thalli reproduce periodically by cleavage of vegetative cells into 8 to 16 motile bodies. The reproductive areas are located along the margin in a band 5 to 20 mm wide. These marginal areas appear slightly greener at the time swarmer bodies are formed in the female gametophytes and less green in the male gametophytes. This is probably due to the small amount of chlorophyll pigment present in the male gamete as was found by Levring (1955) in *U. lactuca*. Evidence of prior swarmer release is seen as a white marginal strip around the upper portion of the thallus. This strip will slough off shortly under field conditions.

TABLE 4

Ratio of gametophyte to sporophyte plants during reproductive periods (1970-1972) and ratio of female to male gametophytes

Date of Collection	Number of Plants Releasing	Number of thalli producing biflag. swarmers (Gametophytes)	Number of thalli producing quadriflag. swarmers (Sporophytes)	Gametophytes Male	Gametophytes Female
13 Dec. 1970	14	10	4		
11 Jan. 1971	17	13	4		
27 Jan. "	11	9	2		
24 Feb. "	10	7	3		
25 Feb. "	9	6	3		
10 March "	19	7	12		
11 March "	8	4	4		
25 March "	12	5	7		
26 March "	15	4	11		
10 April "	20	5	15	0	5
24 April "	11	4	7	1	3
25 April "	13	3	10	0	3
14 Dec. "	12	10	2	1	9
30 Dec. "	16	11	5	2	9
30 Jan. 1972	10	6	4	2	4
31 Jan. "	9	6	3	1	5
14 Feb. "	6	3	3	0	3
23 March "	4	1	3	0	1

During the months January-April, 1969-1971, swarmers were released at regular periodic intervals. The percentage of thalli that were reproductive are graphed in figure 16, based on counts made each day over a 10 day period in February, 1970 and in March, 1971. Time periods in the graph correspond to the number of days before and after a new or full moon of each month. The total number of plants collected ranged from 35-45 per day during the February, 1970 study and from 35-50 plants per day in March, 1971 study.

In 1970 swarmers were released in nature 3 to 5 days before each maximum spring tide correlating with new and full moons of the month. Release was maximal for both gametes and zoospores during this period. On days prior to and after this period thalli showed little or no tendency to release. During 1971 the release of swarmers shifted from 3-5 days before spring high tide to one day before to one day after spring high tide. The semi-lunar reproductive rhythm was not analyzed in 1969. This cyclic phenomenon carries with it an inherent problem for the gathering of reproductive bodies in the field. Only twice a month for 3-4 days at a time can large quantities of swarmers be collected. Unusually low temperature or high tides due to winds appeared to affect the 3-4 day reproductive period by decreasing the intensity of reproductive activity.

Environmental data including temperature, insolation, photoperiod, rainfall, and NH_4-N levels were collected during the four month period of reproductive activity in hopes of detecting cyclic patterns. Close examinations of environmental factors taken singly showed that large excursions of parameter intensity occurred within minutes, hours or days without any hint of a possible rhythm-setting factor. The exogenous parameter that appeared most important for entraining a semilunar rhythm was the tidal cycles generated by the changing phases of the moon. The periods of exposure and inundation in the intertidal zone during three successive spring tide cycles in March and April are seen in figure 17 in relation to photoperiod. Lengths of inundation and exposure at different points along the intertidal zone were estimated from stationary marks during the different phases of the tidal cycle. The semi-diurnal tide along this section of the coast dictates

that low and high spring tides occur at the same times of the day at two week intervals. The lowest spring tides of the month occur at 2:00 to 3:00 p.m. and the highest tides are at 8:30 to 9:30 a.m. during daytime. Periodic storms and wind driven tides alter the occurrence and length of inundation and exposure over the intertidal zone as would be expected; however, it is this ever present factor that appears to express the greatest cyclic behavior at approximately 12.4 hour intervals.

Experiments were designed to simulate the intertidal environment in hopes of elucidating factors involved in the semi-lunar reproductive rhythm. Dessication and inundation experiments programmed for 50 minute tidal shifts daily in relation to photoperiod were attempted. Random release ensued without any hint of a cyclic pattern developing in the laboratory. The semi-lunar rhythm was either dampened or non-existent after plants had been in culture for a short period.

Certain stresses or their combinations tended to promote reproduction. A single stimulus was usually not sufficient to induce reproductive activity. However, when plants were placed in low light (25-75 ft-c) and low temperature (7-9°C) for 3 to 10 days and then removed to a higher light intensity (250-450 ft-c) and temperature (17-20°C) induction and subsequent release of reproductive bodies was enhanced. It is interesting to note that in 90% of the cases when thalli were transferred from low temperature, low light and high NH_4-N to higher temperatures and light intensities and a low NH_4-N medium, swarmers were released 72 hours later. The 72 hour lag is identical to that found by Fjeld (1972) for *Ulva mutabilis*. The margins of discs were always observed to become reproductive no matter from what part of the parent thallus the disc was cut.

A common behavior toward induction of reproductive activity in relation to NH_4-N concentrations and light intensity levels is seen in figure 18. Experiments were done in triplicate with Calico Creek water containing 60 µg/l NH_4-N and 260 µ g/l NH_4-N. Culture conditions were static, maintained at a temperature of 14°C, but the medium was changed daily. Only the highest and lowest growth rates of each set of three discs are plotted. The data show the following: (1) low

and high NH_4-N levels give nearly the same growth rates in low intensity light; (2) a higher growth rate is obtained at higher light intensities in the high NH_4-N medium; (3) reproduction takes place in all discs 3-4 days after transfer from low light to high light in the low NH_4-N; (4) vegetative growth continues in those discs grown in high NH_4-N after transferring from low to high light.

In figure 19 the effects of several experimental parameters on growth and reproduction are compared. The number of discs becoming reproductive by the seventh day is registered at the top of each series as the percentage of the total number of discs tested. Each experimental series represents 5-7 experiments using 10-15 discs per experiment. The incidence of reproduction is high in low NH_4-N concentrations, high light, high temperature and static conditions and decreases progressively with a shift to the opposite conditions. Maintenance of vegetative growth was best achieved by shaking the cultures and by frequent changes of the culture medium with high levels of NH_4-N. A comparison of disc growth in static, aerated and mechanically agitated cultures is seen in figure 20. The data in several experiments show that air agitation allowed even less growth than static conditions.

Cytology of the Dividing Vegetative Cell

Under certain conditions in the laboratory, tissue growth was extremely rapid with growth rates as high as 140%/24 hours having been recorded. Time of division, percentage of cells undergoing division, and cellular morphologies during division were observed for both *Ulva curvata* and *U. rotundata*.

After random sampling of thalli in Calico Creek it was suspected that mitotic division occurred primarily in the evening. Observations based on thalli collected and fixed every hour between 7:00 a.m. and 1:00 a.m. indicated that material from the field was undergoing mitosis between 9:30 p.m. and 11:45 p.m.

Before mitotic activity could be analyzed, several characteristics of cell division had to be evaluated. Just prior to division in *Ulva curvata* the chloroplast in its normal position along the side

wall (fig. 21A) comes to lie beneath the outer face of the cell (fig. 21B). The once distinct pyrenoid disappears and the cell remains in this state for 45-60 minutes (fig. 21C) prior to nuclear division. Cells stained with I_2KI or Gomori's haematoxylin showed that mitosis is completed during a 30 to 40 minute period. Observations on the mitotic sequence are based on 45 separate tissue preparations of both haploid and diploid plants. The entire sequence from vegetative cell to newly formed daughter cells takes place in about 1.25 to 1.75 hours. Cells undergoing a chloroplast shift are destined to divide, whereas those cells in which the chloroplast remains along the side wall do not divide during that mitotic period (fig. 21D).

The nucleus was observed to migrate from its position near the outer membrane through the center of the cell to a position near the inner membrane by early afternoon. Prior to division the nucleus is visible only when the cells are viewed from the side of the inner cell membrane (fig. 21E). The tissue must be macerated after staining in order to split the distromatic thallus exposing the nucleus at the surface of the inner membrane. A cross sectional view of a thallus with a nucleus adjacent to the inner membrane beneath the chloroplast is seen in figures 22A-B.

Observations showed that the vegetative nucleus entered prophase along side the inner membrane around 9:45 to 10:00 p.m. The first signs of daughter cell formation occurred around 10:45 to 11:00 p.m. Figures 22C-H show the sequence of nuclear division in a gametophyte thallus as viewed from the side of the inner membrane. Chloroplast division appears to occur at the same time cell division is taking place. Mitosis and plastid division as well as the entire sequence of cytokinesis appears to be identical to that observed in *Ulva mutabilis* by Løvlie and Bråten (1968,1970).

The mitotic index and phasing of cell division in discs of *Ulva curvata* parthenosporophytes, male and female gametophytes growing at rates of 100%/day or greater are seen in figure 23. Calculations are based on the percentage of cells dividing between the hours of 6:00 p.m. and 3:00 a.m. observed in a 175 μ^2 ocular rectangle encompassing 130 $\pm$ 6 cells. An average of six fields per disc were counted using

three discs for each of the three different thalli observed. As can be seen in figure 23 those cells that were to divide did so with a peak around 11:00 p.m. The number of cells dividing at this time ranged from 48 to 59% of the total.

When the same behavior was analyzed in *Ulva rotundata* the time of division occurred between 9:30 p.m. and 11:15 p.m., with as high a degree of synchrony as that observed in *U. curvata*. The nucleus remained in position next to the upper membrane during division and did not migrate to the side of the lower membrane as in *U. curvata* (fig. 22I). The position of the chloroplast in *U. rotundata* is normally across the face of the outer membrane and it remains *in situ* during the entire division period. Chloroplast division appears to occur at the same time as cell division with each plastid migrating down the side walls as in *U. curvata*. Chloroplasts of the two new daughter cells contain 1-2 pyrenoids that appear visually to have persisted throughout cell division (fig. 22J).

Reproductive Races

Laboratory studies show that *Ulva curvata* possesses a life cycle in which a haploid thalloid gametophyte that releases biflagellated gametes alternates with an isomorphic diploid sporophyte that produces quadriflagellated zoospores (fig. 24-I). Chromosome counts of vegetative thalli fixed at a late prophase stage around 11:00 p.m. showed what appeared to be 10 to 12 chromosomes for plants releasing gametes (fig. 25A-B) and 20 to 24 chromosomes for plants releasing zoospores (fig. 25C). Meiosis was not observed. The quadriflagellated zoospores were 10-11 μ long and negatively phototactic only if the parent sporophyte was the result of male and female gamete fusions. Both male and female gametophytes resulted from germinations of the zoospores but determinations of the ratios of male to female progeny were erratic due to germination difficulties, mortality rates of germlings and the large number of plantlets needed for sufficient counts. The gametes of both mating types are capable of parthenogenetic development back into gametophyte plants.

In the course of laboratory investigations anomalies were observed regarding the type of reproductive body produced and the pattern of release. On occasion a plant newly collected from the field and induced to release swarmers would produce both bi- and quadriflagellated bodies at the same time or possibly biflagellated swarmers first and then quadriflagellated swarmers later after several release periods. These thalli were maintained and used in further experiments.

At first there appeared to be two separate races, one being haploid gametophytic plants that released biflagellate gametes capable of mating with gametes of the opposite mating type (fig. 24-IIa), and the other sporophyte plants that released quadriflagellated zoospores (fig. 24-IIb). It was observed that parthenogenetic development of gametes sometimes produced a thallus which released first biflagellated gametes that were capable of mating and later after several further release periods the same thallus produced quadriflagellated zoospores 8-9 μ long that were positively phototactic. The zoospores germinated and produced normal thalli. At times during the release of the quadriflagellated zoospores another swarmer type was noticed in which the motile bodies appeared as the partial fusion of two isogamous gametes with two eyespots and four flagella. Figures 25D-E show the swarmer compared to normal anisogamous fused gametes. These fused swarmers were comparable in size to either male or female gametes depending upon the sex of the original thallus. The subsequent development of the fused swarmers could not be followed as isolation was difficult because both swarmer types released were positively phototactic.

A second reproductive race was observed beginning with what was thought to be a diploid thallus (fig. 24-IIb). These plants released quadriflagellated zoospores 8-9 μ long and positively phototactic identical to those released by plants mentioned above. These swarmers developed into thalli which released the same three types of reproductive bodies seen in figure 24-IIa; however, the biflagellated gametes and quadriflagellated fused bodies were the size of female gametes in all cases. The biflagellated gametes did not mate with either male or female gametes, but developed parthenogenetically.

Microscopic investigations of young male and female gametophytic thalli resulting from parthenogenetic germination of gametes contained cellular areas that appeared to be considerably larger than typical haploid cells (fig. 25F-G). These areas appeared to release swarmers, but unfortunately they could not be isolated and chromosome numbers were not determined. These areas were particularly evident at the time both biflagellate and quadriflagellate swarmers were being released. It is assumed that these mosaic-like areas could be diploid and the thallus contains a mixture of haploid and diploid matrices (n + 2n).

Decline and Senescence of *Ulva* Populations

The *Ulva* population declines in the field in late spring when rates of reproduction begin to outstrip vegetative growth or when thalli become detached from their substrates. Under some conditions thalli will turn dark green, become thickened, contorted and often fenestrate or lacerate. *Ulva* biomass declines first in the oyster reef population in mid to late April. The population disappeared completely by late May in 1969, early May in 1970, early June in 1971 and late May in 1972. The *Ulva* population in the *Spartina* zone declined shortly afterwards; however, thalli could still be seen four to six weeks after *Ulva* thalli had completely disappeared in the oyster zone.

No one environmental factor or combination of factors were uniquely responsible for senescence in the *Ulva* population. Temperature and insolation, followed as mean daily values, increased sporadically but slowly from March 15 to May 31 in all four years from 1969 to 1972. Along with rising temperature and light intensity, fortnightly reproductive activity was observed to increase at the expense of vegetative growth.

In 1970 and 1971 thalli that were thick and dark green developed late in the season among the oyster reef population. The number of pyrenoids increased from one to two or three and minute protuberances were noticed along the thallus margins. These plants resembled closely *Ulva rigida*, a species common along the coast of New England.

During this period from May to late June certain populations with characteristics of *U. lactuca* var. *latissima* (=*U. gigantea*) were occasionally found unattached, particularly in Bogue Sound, North Carolina and Lynhaven Inlet, Virginia. The thalli can reach 1.5 meters in length becoming rather irregular in form and fenestrate (fig. 25H).

Morphological changes associated with senescence in the field could not be studied effectively in the laboratory. Experimental conditions can easily alter the morphological and physiological attributes of an organism. Material placed in static culture with infrequent changes of the medium and relatively high light intensities became moribund in 7-15 days. Intensities above 250 ft-c appear to be detrimental to normal growth and development under static culture conditions in *Ulva curvata* as in other species of *Ulva* and *Enteromorpha*. Temperatures above 22-23°C also appear to be harmful. Thalli or discs became pale green to yellow under adverse laboratory conditions. Microscopic examination showed that cells accumulated starch grains which completely filled the cell. If caught in time the process could be reversed by transferring discs or thalli to a fresh medium containing high NH_4-N levels with agitation and light intensities less than 200 ft-c.

DISCUSSION

Identification and Distribution of *Ulva curvata* and *Ulva rotundata*

Phycologists have long recognized the great variability in the phenotypic expression of morphological features in *Ulva* and the inherent difficulty of classifying this genus into distinct and easily recognized species. A flat membranous distromatic thallus with little or no branching presents a minimum number of morphological and anatomical characters. Bliding's monographic studies of the European taxa in Ulvales (1963,1968) is a carefully synthesized treatment that critically examines all European species from both a classical and an experimental viewpoint. No comparable study has been carried out, so far, with the species of *Ulva* or *Enteromorpha* occurring in Atlantic North America.

Taylor (1957,1960) recognizes a single broadly membranous species of *Ulva*, *U. lactuca* L. including four varieties, var. *latissima* (L.) De Candolle, var. *rigida* (C.Ag.) Le Jolis, var. *lacinulata* (Kütz.) Taylor and var. *mesenteriformis* (Roth) Collins extending from Maine to Florida and along the Gulf coast of the United States. A second species, *U. fasciata*, which is normally divided into narrow segments is found from North Carolina southward into tropical waters. A third species, *U. profunda*, which was described by Taylor in 1928 from material dredged in 15-67 meters off the Florida coast has not been reported since. Specimens of *Ulva* occurring in Florida and in Texas have been referred to *U. lactuca* in recent reports (Humm and Taylor, 1961; Kapraun, 1970).

The variety *rigida* has been recognized as a distinct species of exposed marine habitats in many parts of the world (C. Agardh, 1822; Thuret, 1854; Feldmann, 1937; Papenfuss, 1960 and Bliding, 1968).

According to Bliding (1968) the thallus is typically lobed and dark green, often with minute marginal teeth or spines. Cells measure 15-22 μ X 11-17 μ averaging 18 X 14 μ in mature plants and 29 X 16 μ in younger plants. Cells in the upper portion of the thallus appear quite rounded or ball-shaped, while cells in the lower area of the blade are much taller than broad. Mature cells generally have two pyrenoids. Southern forms of this species can have as many as eight pyrenoids. *Ulva rigida* includes the species *U. lacinulata* Kützing according to Bliding.

Variety *latissima* L., sensu J. Agardh, has been included under *Ulva gigantea* (Kützing) Bliding by Bliding (1968). Except for the basal portion of the plant, the thallus is very thin (<35 μ) with small to medium size quadrangular cells and typically one pyrenoid. The description given by Bliding leads one to believe that this plant, first described by Kützing as *Phycoseris gigantea*, could possibly be *U. lactuca* var. *latissima*, so common in quiet shallow estuarine waters along the eastern coast of the United States (Taylor, 1957,1960). Recently Webber and Wilce (1971), following Bliding, have recognized *U. gigantea* from a salt marsh at Ipswich in Massachusetts.

The fourth variety, var. *mesenteriformis*, originally described as *Ulva mesenteriformis* by Roth from the Baltic Sea, was later placed under *U. lactuca* by Collins in 1900. This variety has been reported only once by Collins from marsh pools in Connecticut. J. Agardh (1883) indicates that *U. mesenteriformis* may be a juvenile form of the later entity, *U. cornucopiae* (Kützing) J. Agardh, which Bliding (1968) lists as a synonym under *U. curvata*.

According to Bliding, typical *Ulva lactuca* is a northern species occurring primarily in the littoral zone in both marine and estuarine waters. The thallus is quite variable in shape, 45-60 μ thick except at the base, lacks microscopic teeth, and typically possesses one pyrenoid that is centrally located in a surface oriented chloroplast. The presence of *U. lactuca* L., in the sense of Bliding's circumscription of that species, has not been reconfirmed for plants from North America.

Ulva occurring in the Morehead City-Beaufort area of North Carolina that was investigated in this research at first appeared to be a

variety of *Ulva lactuca*. Analysis of morphological, anatomical and cytological features according to Bliding's methods, however, indicated that it compared more favorably with Bliding's (1968) circumscription of *U. curvata* than with any other North Atlantic species of *Ulva*. Examination of the isotype of *Ulva dangeardii* Gayral and Mazencourt, a taxonomic synonym of *U. curvata* (van den Hoek, 1963; Bliding, 1968), with its distinctive recurved growth pattern greatly helped in the identification of these plants.

The entities *Ulva curvata* and *U. lactuca* are strikingly similar looking plants. Although the asymmetric base is observed in at least 80% of the thalli collected in Calico Creek, it is not a strong enough character to be used alone in separating this species from *U. lactuca*. It appears that dimensions for both cell size and thallus thickness place the Calico Creek plants and other specimens distributed from Georgia to New Jersey within the range of *U. curvata*. The clearest diagnostic feature of *U. curvata*, sensu Bliding, that also occurs in the North Carolina plants is the presence of a comparatively small chromatophore that tends to lie to one side of the cell.

Breeding experiments indicate that *Ulva curvata* is the only *Ulva* entity occurring in marsh-like habitats on a mud-shell substrate under lowered salinity during the winter-spring season between Georgia and New Jersey. Unfortunately, material collected in Rhode Island and Massachusetts could not be induced into reproductive activity, although their morphology, cytology and ecology were similar to plants found in the southern localities. The plants collected at Woods Hole, Massachusetts, resembling *U. rigida* as well as specimens from Massachusetts labeled *U. lactuca* obtained from the Carolina Biological Supply Co. were found not to mate with plants of *U. curvata* from Calico Creek and other areas along the Eastern Coast.

Late in the growing season large unattached thalli were observed in Calico Creek. Thalli could be found floating in both the lower North and Newport Rivers as well as in Bogue Inlet and Bogue Sound. The general appearance of these plants fit the circumscription of *Ulva lactuca* var. *latissima* with their somewhat lighter thallus coloration and smaller membrane thickness while also being fenestrate to lacerate

at times.

Toward the latter part of the growing season, particularly at the Calico Creek research site in 1970 and 1971, many attached plants took on the appearance of *Ulva lactuca* var. *rigida* when thalli became darker green and increased in thickness from 60–70 μ to 80–90 μ while the pyrenoid number increased from one to two or three. The margins of thalli were found to possess minute protuberances. This last character, so evident in *U. lactuca* var. *rigida* developed concomitantly with detachment of thalli during senescence.

During the period from November to May a second broadly membranous entity that also resembles the classical characterization of *Ulva lactuca* was found commonly in Bogue Sound and Atlantic Beach. These plants resemble *Ulva rotundata* Bliding, particularly with respect to over-all thallus form and thickness, cell size and pyrenoid number. Breeding studies with biflagellated swarmers of this species gave negative results in mating attempts with gametes of *U. curvata*.

It now appears from the available data that the common *Ulva* species present in estuarine habitats during the late fall to late spring throughout the central and southern Atlantic Coast is not *U. lactuca* but *U. curvata* with a strong possibility that *U. rotundata* is the prevailing species in more saline habitats. The identification of *Ulva curvata* and *Ulva rotundata* mark these two species as new records for the Western Hemisphere.

Föyn collected material of *Ulva* in southern Portugal in 1952 which did not mate with either *U. lactuca* or *U. thuretii* (=*U. rigida*, sensu Bliding, 1968). Föyn (1958) referred to these plants as *U. mutabilis*. Although the writer has not seen authentic material of the species, several morphological and cytological attributes appear to be common to plants of *U. curvata* from this coast. Föyn found the mature thallus of *U. mutabilis* differs very little morphologically from *U. lactuca* or *U. thuretii*; however, young plantlets were decidedly different in having a tubular morphology. A compact dense felt of rhizoids develops at the basal attachment disc from which new thalli soon arise in projecting tufts. These characters were not seen in either *U. lactuca* or *U. thuretii* according to Föyn, but have been observed on occasion in

U. curvata from North Carolina by the writer.

Cells of *Ulva mutabilis* normally have one pyrenoid with a parietal chloroplast much like *U. curvata*. The description of the migration of the nucleus prior to cell division in *U. mutabilis* (Løvlie and Bråten, 1968,1970) is identical with that observed in *U. curvata* from Calico Creek. The phenomenon has not been reported in either *U. lactuca* or *U. thuretii* (=*U. rigida*) and was not seen in *U. rotundata* by the writer. The thallus of *U. mutabilis* was described by Föyn as being 53-68 μ thick in the upper portion, this being closer in size to both *U. lactuca* and *U. thuretii* than to *U. curvata*. The few figures of *U. mutabilis* show the basal portion of the thallus to possess both asymmetrical and symmetrical patterns. The life cycle of *U. curvata* proposed by the writer is very similar to that of *U. mutabilis* (Föyn, 1959) in such aspects as parthenogenetic development of both male and female gametes with subsequent diploidization. Föyn (1934,1955) did observe these same phenomena in both *U. lactuca* and *U. thuretii*.

Discussion of the Life History of *Ulva curvata*

Field and laboratory studies revealed that identical gametophytic and sporophytic winter-spring thalli alternate with microscopic summer-fall stages. *Ulva curvata*, therefore, possesses an alternation of isomorphic generations that is typical for Ulvaceae (fig. 24-I). Plants collected in Calico Creek from several populations showed haploid male gametophytes releasing positively phototactic biflagellated gametes (5.4-6.4 μ X 2.0 μ) which showed short quick movements, were pale green in color and were as much as 37% smaller than female gametes (fig. 26A). Female gametophytes released biflagellated gametes (6.4-7.4 μ X 3.5 μ) that were bright green and moved much more slowly (fig. 26A). Gamete matings resulted in negatively phototactic quadriflagellated zygotes (fig. 26B) which developed into diploid thalli. Diploid plants released negatively phototactic quadriflagellated zoospores (fig. 26C) (10-11.5 μ X 5.0-5.5 μ). Direct development of these swarmer bodies gave rise to both male and female haploid gametophyte

plants, although a 1:1 meiotic segregation for mating type could not be proved. Both male and female gametes commonly developed directly back into haploid gametophyte plants in the absence of sexual fusion (fig. 26D).

Figure 26E depicts what appears to take place in culture when parthenogenetic gametophytes are induced to release reproductive bodies. Young thalli released biflagellated gametes (fig. 26F) during the first or first few release periods. It was not until later that more than one type of swarmer was released from the same thallus. The later-formed reproductive bodies were quadriflagellated, positively phototactic zoospores measuring 8-9.5 μ X 4.0 μ (fig. 26H). Their germination gave rise to haploid plants with the same mating type as the original gametophyte. On occasion, a third reproductive body was observed which was positively phototactic and possessed the characteristics of zygotes (two eyespots, four flagella and a fused body morphology). These motile swarmers (fig. 26G) resembled fused isogamous gametes measuring the same size as male or female gametes depending on the mating type of the original gametophyte. They were seen at the time zoospores or zoospores mixed with gametes were being released, but not with gametes alone. It was not possible to follow the germination history of this swarmer type. It should be noted that the occurrence of both the zoospores and the zygote-like swarmers was observed only rarely with parthenogenetically produced male gametophytes, while this mode of reproduction was common in parthenogenetically produced female gametophytes.

Microscopic analysis of young parthenogenetically developed gametophytes revealed mosaic-like areas of increased cell size among the more common and smaller haploid vegetative cells of the thallus. At the time quadriflagellated zoospores (8-9.5 μ long) were released both the mosaic-like areas of large cells and the more frequent smaller cells contained reproductive bodies. The ploidy level of the mosaic-like areas were never determined nor was the type of swarmer released from these areas characterized.

Certain field and laboratory observations suggest strongly that diploidization may be occurring within *Ulva curvata*. The finding of

mature diploid thalli in the field releasing identical quadriflagellated positively phototactic zoospores (8-9.5 μ long) which later germinated into haploid gametophytes with the same mating type as the original parent strengthens the case for diploidization. These particular thalli as mentioned earlier were found to be more common later in the growing season. The seasonality of sporophytes in the field suggests that parthenogenetically developing gametophytes become totally diploid later in the season. Mosaic-like areas found in young haploid plantlets grown in the laboratory may well be the beginnings of diploidization that never reached their full potential under culture conditions. These observations support the model first proposed by Föyn that a somatic doubling of the chromosome complement (diploidization) is achieved in certain areas of the thallus in *Ulva lactuca* (Föyn, 1955) or in the entire young gametophyte as in *U. mutabilis* (Föyn, 1958). Föyn (1958,1959) has shown that complete diploidization can occur at the 100 cell stage in either the male or female gametophyte. Such plants, designated as parthenosporophytes, are capable of releasing quadriflagellated zoospores (in this case the same size as those released by normal diploid sporophytes) after meiotic division. Upon germination they give rise to gametophytes of the same sex as that from which they were derived. If areas of increased cell size are diploid having resulted from a somatic doubling of the chromosome number, then the presence of fused isogamous gamete-like swarmers may indicate an incomplete transition from mitotically derived gametes to meiotically derived zoospores in mosaic-like areas within the thallus. Mosaics were not observed in the early release periods, but their later appearance both within and outside reproductive areas could account for the occurrence of both gametes and zoospores at certain times.

The life cycle just described for *Ulva curvata* replete with six different kinds of motile reproductive bodies was also observed in nature, emphasizing that culture conditions and manipulations were not dictating abnormal patterns. Completely diploid thalli which released small positively phototactic zoospores were not obtained in culture although they were seen in nature. All observed nuclear phases and

growth forms appear to be connected to one another by meiosis, syngamy and what appears to be somatic diploidization.

While the life cycle of *Ulva curvata* is similar to that of *U. mutabilis* (Föyn, 1958,1959) the two species differ in two respects. First, a much higher incidence of female to male gametophytes was found in Calico Creek material of *U. curvata* and, more important, the male and female reproductive bodies were found to have different requirements for germination. Secondly, while the zoospores released from Föyn's parthenosporophytes are described as being similar to those released from normal sporophytes, they were found to be quite different in *Ulva curvata*. Zoospores produced by normal diploid sporophytes were negatively phototactic, 10-11.5 μ long and gave rise to both male and female gametophytes, while zoospores from other diploid sporophytes were positively phototactic, 8-9.5 μ long and gave rise to only one or the other mating type gametophyte in *U. curvata*.

The reproductive histories as observed and discussed by Kapraun (1970) for *Enteromorpha prolifera* show striking similarities to *Ulva curvata*. Kapraun found that *E. prolifera* included four different races. The first demonstrated a normal alternation of isomorphic generations. The second possessed both male and female gametophytes releasing quadriflagellated swarmers of the same size and having the same phototactic responses as *U. curvata* parthenosporophytes. The third produced bi- and quadriflagellated swarmers that were released alternately with no sign of a sexual cycle. The fourth released only biflagellated swarmers which were the same size as gametes but were unable to mate. Kapraun could not ascertain whether this latter race was reproducing by biflagellated zoospores or by parthenogenetic development of gametes. Kapraun questioned whether the sequential alternation of bi- and quadriflagellated swarmers from the same thallus was unique to *Enteromorpha prolifera* from the Texas coast or was more widespread in occurrence, but unreported due to lack of studies of material in culture.

It appears that *Ulva curvata*, like *Enteromorpha prolifera*, releases both flagellated types of swarmers with the biflagellated bodies appearing first and the quadriflagellated bodies coming at a

later period, or with both occurring together. The biflagellated swarmers act as gametes in most cases, while the quadriflagellated swarmers act as zoospores giving rise to gametophytes of only one mating type.

The potential for parthenogenetic development of gametes is particularly strong for this species in Calico Creek and, seemingly, favors the female gametophyte, as seen in germination studies involving the different swarmer types in the laboratory. The partial and later total diploidization of the female gametophyte thallus could possibly account for the seasonal pattern leading from gamete to zoospore release. The concept of independent speciation of a certain phase in the life cycle which has been discussed by Bernatowicz (1958) could conceivably be applied here. A shift in the life cycle from a mode of sexual reproduction involving haploid gametophytes and diploid sporophytes to one of parthenogenetic diploidization could possibly lead to clones that are homozygous for mating type and to an asexual life cycle. The possibility exists that *Ulva curvata* in Calico Creek may well be expressing a gradual shift from a facultative alternation of generations to an obligate asexual cycle involving only female plants. Several species of both *Ulva* and *Enteromorpha* as well as other genera in the Ulvales show an asexual life cycle in which reproduction involves only biflagellated and/or quadriflagellated zoosporids (Bliding, 1963) that are morphologically similar to swarmers found in *U. curvata*.

The Semi-Lunar Rhythm in the Reproduction of *Ulva curvata*

The rhythmic release of swarmers in *Ulva curvata* is but one of many physiological functions displayed by both plants and animals that are timed to either circadian (24 hours), tidal (12.4 hours), semi-lunar (14.8 days), lunar (29.5 days) or annual (365 days) rhythms. The fundamental nature of these biological clocks remains enigmatic.

It is a well known fact that activities within many invertebrate organisms display regular cyclic patterns related to tides and lunar

phases both in the field and in the laboratory. The mussel *Mytilus* shows pumping rates coupled to the rise and fall of the tides (Rao, 1954), while the amphipod *Synchelidium* shows migration patterns regulated to tidal fluctuation, (Enright, 1963). Examples within the algae are the vertical migration of *Euglena obtusa*, (Palmer and Round, 1965), the diatom *Hantzschia amphioxys* (Palmer and Round, 1967) and the chrysomonad *Chromulina psammobia* (Fauré-Fremiet, 1950), all coinciding with tidal rhythms. It is interesting to note that in each of these cases the rhythm continues in the laboratory without obvious tidal influence. Semi-lunar and lunar rhythms also appear to influence the reproductive activity of several groups of intertidal algae. The green algal genera *Enteromorpha* (Christie and Evans, 1962; Townsend and Lawson, 1972) and *Ulva* (Smith, 1947) are commonly found possessing a fortnightly reproductive rhythm. The green alga *Halicystis* (Hollenberg, 1936; Page and Kingsbury, 1968; Page and Sweeney, 1968) and particularly the brown alga *Dictyota* (Williams, 1905; Hoyt, 1927; Bünner and Müller, 1961; Müller, 1962 and Vielhaben, 1963) have been investigated in the laboratory in some detail.

In *Ulva curvata* in Calico Creek the release of swarmers conforms to a strictly cyclical pattern with intervals of 14-15 days between peaks of maximum reproductive activity. During the winter and spring of 1970 swarmers were consistently shed every three to five days before a new or full moon period, with the percentage of release per day forming a bell-shaped curve. In between release periods thalli were in a vegetative state and showed no sign of pending reproductive activity. In 1971 the peak of maximum release shifted to a span from one day before to one day after spring tides, but the period was still semi-lunar. In both 1970 and 1971 there were occasions when the release period was diminished and thalli showed little sign of reproductive activity. These occurrences were generally preceded by inclement weather with overcast skies and wind-driven tides creating lows and highs of abnormal duration. It should be noted that the semi-lunar rhythm was equally evident during periods in which the air temperature fell below 0°C as at temperatures of 18-25°C.

Plants brought into the laboratory generally demonstrated only

one rhythmic cycle in the release of reproductive bodies if collected several days prior to their scheduled release in nature. When plants were collected and returned more than a week before release the chances that release would occur in the laboratory at the same time as in the field were remote. The semi-lunar rhythm is quickly dampened out of existence under laboratory conditions.

Air and water temperature and insolation may fluctuate widely within a matter of minutes or hours in the intertidal zone in Calico Creek. It is apparent that these factors do not demonstrate any short or long term cyclic tendencies that might entrain a semi-lunar rhythm. Two environmental parameters, however, do generate regular cycles, these being the daily periods of light and darkness (24 hours) and the moon-driven tidal cycles (12.4 hours). These two rhythms, diurnal and tidal, were demonstrated mathematically by Brown et al. (1953) to interact in such a way as to generate beats every 14.7 days corresponding to the semi-lunar period.

The existence of a reproductive rhythm in *Ulva curvata* with a semi-lunar period showing peaks of activity around full moon and new moon (spring tides) suggests a strong connection between the phases of the moon and reproductive activity in *Ulva*. Along the North Carolina coast the tides are semi-diurnal generating two highs and two lows of about the same magnitude every 24.8 hours corresponding to the lunar day. Spring tides always occur between 8:30 and 9:30 a.m. and spring lows between 2:00 and 3:00 p.m. throughout the year.

Bünning and Müller (1961), Müller (1962) and Vielhaben (1963) have carried out experiments with the brown alga *Dictyota dichotoma*. Bünning and Müller's material collected at Helgoland, Germany shows some similarities to *Ulva curvata* in that both plants observe a strict semi-lunar reproductive rhythm in the field, but synchrony is lost under artificial photoperiods in the laboratory. It was found that when *Dictyota* was placed in culture under natural light, oogonia were released every 14-15 days. Since Hauenschild (1961) had demonstrated that light at night, such as moonlight, may be necessary for the entrainment of a lunar reproductive rhythm in the polychaetous marine worm *Platynereis*, Müller (1962) tested this possibility in

Dictyota by leaving a light on during the night at 28 day intervals, thus exposing the plants to a simulated full moon. Release was obtained under the artificial photoperiod every 16-18 days for at least five semi-lunar cycles. It was found that a light as low in intensity as three lux was sufficient to cause entrainment. Although the period was not a perfect 14.7 day rhythm it was sufficiently close to implicate moonlight as the controlling factor in the semi-lunar rhythm of *Dictyota*.

Müller further suggested that tidal and diurnal rhythms were operating endogenously in *Dictyota* giving beats at 14.7 days. This hypothesis could be tested by changing the frequency of one of the rhythms thus changing the position of the beat. The way in which tidal rhythms are reset is not known, but circadian rhythms can easily be entrained to different photoperiods.

An endogenous semi-lunar rhythm may well be operating in *Ulva curvata* in response to both tidal and diurnal cycles, perhaps, with full moon acting as a phase setting mechanism. Since reproductive rhythms that are observed in the field damp rapidly and disappear within one cycle in the laboratory, it is unlikely that simple experiments employing simulated full moons at 28 day intervals will work with *Ulva*. Very likely, the provision of a complex set of parameters involving light intensity, temperature and exposure to dessication with control of both diurnal, tidal and lunar cycles will be needed in order to achieve sufficient control in the laboratory to obtain fortnightly rhythms of swarmer production and release. Some preliminary experiments were attempted by the author, but so far, these have not been successful.

It has not proved possible at the present time to account for the shift in the period of maximum swarmer release between the years 1970 and 1971. An attempt to correlate the observed phase shift with possible differences between the time of moon rise and set for the two years proved negative. A check showed that moon rises and sets were almost identical for any phase of the moon in both 1970 and 1971.

Even though no plausible theory is suggested by the data to account for the vagaries of reproduction and swarmer release in *Ulva*

continued research along this line may well lead to a better understanding of semi-lunar cycles based on field observations coupled with carefully designed laboratory experiments.

Ecological and Experimental Control over Stages in the Life Cycle of *Ulva curvata*

The relationship between field observations of growth and reproduction and laboratory studies of environmental parameters which may exert a controlling influence on morphogenesis is summarized in table 5. Investigations in the field showed that minute (<100 μ long) filaments representing the germlings of both *Ulva* and *Enteromorpha* species were present by late June on oyster shells in Calico Creek. When these shells were subjected to temperatures of 20-30°C in the laboratory the filaments grew at least 500 μ in length and later developed into tubular plantlets. Lower temperatures appeared to keep the filaments in check blocking further growth. When filaments 200-500 μ long were collected on shells in mid to late summer they developed into plantlets only at temperatures below 20°C, comparable to water temperatures measured in late October or early November in Calico Creek.

Photoperiod was found not to be a controlling factor in germination or in the development of a tubular thallus as seen in figures 7A-B. Experiments using crossed gradients of temperature and light intensity confirmed other field and laboratory observations. Germination occurred only between 15-25°C, comparable to late spring or early summer temperatures with further development occurring between 10-18°C, comparable to late fall temperatures. The sudden drop in temperature very early in November in the years 1969 and 1971 and to a lesser extent in 1970 and 1972 may indeed be significant in germling development, as macroscopic plantlets were observed shortly afterward. While the first appearance of *U. curvata* thalli the second and third week in November in the *Spartina* zone seems to be related to a sharp decline in temperature the variation observed in the time and season when plantlets appeared in the oyster reef zone seems to be due to

TABLE 5

OBSERVATIONS ON PARAMETER CONTROL OVER STAGES IN THE LIFE CYCLE IN *ULVA CURVATA*

STAGES	FIELD OBSERVATIONS	EXPERIMENTAL OBSERVATIONS
Germination	Filamentous germination products observed on oyster shells by late June.	Female gametes and zoospores germinate at minimum temperatures of 15-20°C, 100 ft-c. Male gametes germinate at minimum temperature of 20-25°C, 500-1000 ft-c. Adventitious plantlets produced by rhizoids from all germling types except male gametophytes.
First appearance of tubular thallus	Observed by mid-November, 1969-1972 in *Spartina* zone; on oyster shell reef by late November, 1969, early March, 1970, mid-March, 1971, early April, 1972 and mid-November, 1972. Appearance in the *Spartina* zone appears to correspond to recorded temperature drops in early November.	Tubular germlings develop in 10-20 days at temperatures of 18-25°C, 500-800 ft-c. Basal morphology of germlings variable according to culture conditions.
Formation of expanded blade	Expanded blade observed 1-3 weeks after first sign of tubular thallus.	Normal morphology occurs only upon addition of mud-water extract with agitation.

TABLE 5

(continued)

STAGES	FIELD OBSERVATIONS	EXPERIMENTAL OBSERVATIONS
Growth of the mature thallus	November-May, 0-25°C; measured growth rates up to 23%/day.	Measured increase in disc area up to 143%/day with cyclic temperature and light intensities, high levels of NH_4-N and agitated culture conditions.
Formation and release of reproductive bodies	December to April, 0-25°C, 9.75-12.5 hour day length; formed and released on 14.7 day intervals corresponding to spring tides. High ratio of female to male gametophytes. Gametophytes more prevalent from November to February, sporophytes more common from February to April. Both phases present during entire season.	8-25°C, 9.5-14.5 hour day length; formed and released upon transfer to high temperature, high light intensities and low NH_4-N levels in medium.
Decline and Senescence	Reduction in biomass due to reproduction overtaking vegetative growth and to detachment of thalli. Population declining from early May to early June on oyster reef, 4-6 weeks later in *Spartina* zone. Polymorphism in *Ulva curvata* populations in April to May in which plants may resemble *U. lactuca* var. *rigida* or *U. lactuca* var. *latissima*.	

other causes. Marked differences in environment occur between the two zones, particularly in relation to total insolation, tidal inundation and exposure, and to a lesser extent temperature. It is, however, very difficult to point to a factor or factors that exert a controlling influence over the variable emergence time for plantlets in the oyster reef zone.

Gayral (1967) and Kapraun (1970) have demonstrated that the developmental pattern in germlings of some members of the Ulvaceae are species dependent. Kapraun (1970) found that an erect tubular thallus arises initially from gametes and zoospores in species having an alternation of generations, while in those species with only an asexual life cycle the germlings first produce a rhizoidal holdfast. Observations made by the writer indicate that there is a strong tendency for zoospores to produce an abundance of basal rhizoids first, while gametes and zygotes germinate initially into an erect thallus in *Ulva curvata*. Great variability was evidenced in the pattern of early development when swarmers were subjected to different kinds of media or when they were placed either in static or agitated culture conditions. Most differences were lost as the germlings reached the age of 10-20 days.

The work of Provasoli (1958,1964) has indicated that certain factors in the medium such as vitamins, chelated metals and growth promoters must be present before normal morphogenesis will occur in *Ulva lactuca* grown in culture. The results of laboratory experiments showed that tubular plantlets of *U. curvata* seldom developed into flattened thalli under standard culture conditions. When mud-water extract was added to the medium and replenished every two days, larger and somewhat more normal looking thalli resulted; however, when germlings of all types of swarmers were mechanically agitated, expanded blades were soon formed and subsequently developed into normal looking plants.

The 2-4 cm long tubular plantlets so commonly observed in culture were never seen in the field. When cultures were shifted from static to agitated conditions a morphology like that of natural plants was obtained in the presence of soil-water additives.

Vitamins, chelated trace metals and other complex growth factors formed by bacterial action on various substrates are undoubtedly present in mud-water extract and most likely play an important role in morphogenesis. The factors involved must also be active after autoclaving, as similar results are obtained when the mud-water extract is centrifuged and millipore filtered without autoclaving. The work by Provasoli and Pinter (1966) showing that certain phenolic compounds found in estuarine and marine substrates restored a normal morphology to *Ulva*, may also be important here. Further observations of Provasoli and Pinter (1964,1966) and Provasoli (1969) which strongly suggest that the microflora growing epiphytically on certain seaweeds is important for a normal development probably applies to *U. curvata*. The mucilage coating of many seaweeds harbors large colonies of bacteria and may well conceal a chemical symbiotic relationship. Sieburth (1968) and others have shown that biologically active excretory products from various seaweeds can have an opposite effect upon bacterial populations, controlling or inhibiting their growth by means of chemical antibiosis. Though there have been many convincing demonstrations of the virtues of soil-water extract and its panacean effects upon morphogenetic processes in the algae, it should be stressed that while mud-water extract was indispensable for normal germination and development of young plantlets in static culture, it did not approach the effectiveness of agitation, even in the absence of mud-water extract.

There appears to be a strong tendency in statically grown plants for longitudinal cell division to predominate forming long narrow thalli 8 to 13.5 times longer than broad. Thalli were more normal with agitation, growth was more rapid and transverse divisions were stimulated resulting in thalli that were only 1.5 to 4.5 times longer than broad. Plants found in the intertidal zone in Calico Creek receive not only an influx of fresh nutrients twice each day, but also environments ranging from static conditions at low tide to mildly or highly agitated conditions during periods of tidal flux. Natural agitation in the intertidal zone may well facilitate nutrient transport by sweeping away diffusion barriers at the interface of the thallus allowing a greater uptake of nutrients to occur, and in some

way may also stimulate longitudinal cell divisions resulting in increased transverse growth.

Control over growth and reproduction of the mature thallus or of a disc cut from a thallus was found to be dependent upon strongly interacting chemical and physical parameters. Ammonia supported high growth rates better than nitrate and strongly inhibited reproduction. The growth rate of excised discs ranged from 200 to 1000% per week in a continuously agitated culture medium containing mud-water extract, 10 mg/l NH_4-N, a 12:$\overline{12}$ photoperiod, 100 ft-c and 17-18°C during the first 6 hours of the light period and 250 ft-c and 9-10°C during the last 6 hours of the light period. Reproductive activity was zero under these conditions. Low NH_4-N levels and static conditions increased the incidence of reproduction and decreased the growth rate.

Increased light intensities up to 1000 ft-c stimulate both growth and reproduction. As seen in figure 21, growth is much more rapid at 750 ft-c (series 2) than at 250 ft-c (series 3), even though NH_4-N was added. Reproductive activity is enhanced at the higher light intensity in the absence of added NH_4-N and is depressed with its addition (series 4). When light intensity and temperature are cycled during the light period (series 5) the addition of NH_4-N appreciably stimulated growth rates and depressed reproductive activity.

In all of these experiments the thalli were maintained under static conditions. Agitation markedly stimulated the growth rate of excised discs (series 6-8) and further limited the incidence of reproduction. The combination of agitation and added NH_4-N gave the highest mean growth rate observed (approximately 585%/week), and a reproductive incidence of 0-8%. A comparison of series 6 and 2, both lacking added NH_4-N, indicates the importance of agitation in reducing the incidence of reproduction. Similarly series 7 and 4 which contain added NH_4-N clearly show the importance of agitation to vegetative growth. The effect of agitation could not be accounted for as having resulted from increased O_2 or CO_2 tension. Bubbling air through the medium gave lower growth rates than mechanical agitation.

It should be noted that the growth rates in media bubbled with air were even less than in static cultures. Reasons for this apparent discrepancy are not clear at this time. The possibility exists that supersaturated O_2 tensions in the medium generate conditions that are detrimental to growth.

In another experiment designed to test two parameters suspected as being important in the experimental control over growth and reproduction series of discs were subjected to both low (60 µg/l) and high (260 µg/l) concentrations of NH_4-N and low light intensities (200 ft-c) for four days, whereupon the light intensity was increased to 900 ft-c. Four days after the change in light intensity discs growing in low NH_4-N reproduced in total. Discs grown in high NH_4-N did not show signs of reproductive activity but continued vegetative growth at a rate of 51%/day. It is clear from these data that light intensity and NH_4-N levels interact under experimental conditions in exerting control over growth and reproduction in *Ulva curvata*.

Studies on growth and growth rates in species of *Ulva* are few. Nasr et al. (1968) reported that urea appeared to be a better source of nitrogen than either nitrate or ammonia, particularly in the synthesis of several amino acids in *U. lactuca*. Kapraun (1970) attempted to enhance the growth of *Ulva lactuca* using 0.25 g/l urea and found that the fronds became intestiform and contorted within three weeks. Waite and Gregory (1969) reported obtaining maximum growth rates for discs cut from *Ulva latissima* (=*U. gigantea*?) at 0.7 mg/l NH_4-N in experiments in which the levels ranged from 0.4 to 1.2 mg/l. Observations on growth were not possible at temperatures above 15°C in *U. curvata* as discs became reproductive at these temperatures. Burrows (1971) found disc growth rates in *U. lactuca* to be 57%/week when grown in a mud-water extract medium. Kale and Krisnamunthy (1967) have shown that the growth rate of excised strips from different areas of the thallus in *Ulva lactuca* was not uniform but followed definite patterns in which the apical region was a region of predominant elongation, while the middle, midbasal and marginal regions showed diffuse growth. In later experiments these workers (1969) reported that IAA is distributed in a distinct pattern

in the thallus. The hypothesis was suggested that maximum growth is obtained with optimum levels of IAA and that excess exogenously supplied IAA actually retards growth.

Growth rates of *Ulva curvata* could not be measured satisfactorily in the field. Qualitative observations, however, showed that growth took place from the small expanded plantlet stage (0.5-2.0 cm long) to plants 10-20 cm long in just two to three weeks. These growth rates indicate that prevailing conditions were optimal in the field during this period. The high levels of NH_4-N in Calico Creek, the presence of currents and tidal flux along with cycling temperatures and light intensities may well be factors stimulating the rapid vegetative growth rates seen in the field.

Experimental stimulation of vegetative growth by simulating these parameters in the laboratory was measured directly as a high mitotic index and as growth rates exceeding 100% per day. Approximately 50% of the cells were found to be dividing each day with the peak division period occurring at about 11:00 p.m. Cell division in *Ulva curvata* appears to be of the phased type, as discussed by Sweeney and Hastings (1958) and Hastings and Sweeney (1964) for the dinoflagellate *Gonyaulax*. The authors have shown that cell division occurs synchronously at 24 hour intervals while the generation times are multiples of this value. Løvlie (1964) has reported that distal portions of the blade of *U. mutabilis* doubles every 36 hours suggesting that certain cells may divide every 24 hours while others divide every 48 hours. Løvlie also demonstrated that the mutant "slender" doubles its mass every 24 hours with the consequences that every cell must be dividing during each 24 hour mitotic period. The degree of synchrony in the mutant seems to be as high as that which can be induced in mass cultures of *Euglena* (Cook, 1961).

The cytological studies revealed differences between *Ulva curvata* and *U. rotundata* in the time of cell division and cellular morphologies during mitosis. *U. rotundata* also possesses a phased type of cell division, but with peak mitotic activity occurring between 9:30 and 11:15 a.m. The nucleus in *U. rotundata* remains *in situ* near the upper membrane and is always observable in both

interphase and during division by the refractive staining of I_2KI. In complete opposition was the finding that the nucleus in *U. curvata* migrates from a lateral position near the outer cell membrane to a central position near the lower cell membrane by early afternoon. Nuclear division then occurs with the nucleus adjacent to the inner membrane between 9:30 and 11:45 p.m. The stages of mitosis and synchrony of cell division found in *U. curvata* are virtually identical to observations reported for *U. mutabilis* by Løvlie and Bråten (1970). The migration of the nucleus and Golgi apparatus from a position near the lower membrane appears to signal the onset of a true division furrow which begins at the lower membrane and extends through the center of the cell to the upper membrane. The furrow bisects the vacuole and chloroplast. Daughter chloroplasts then migrate to their normal position along the side walls (Løvlie and Bråten, 1968,1970). Experimental manipulation of parameters such as temperature, light intensity, nutrients and photoperiod does not change the mitotic process in either species. The finding of two strikingly different mitotic systems with regard to nuclear movement and time of division is indeed puzzling at present.

The Genus *Ulva* as a Biological Indicator of Eutrophication

The eutrophication of marine systems either due to natural causes or to man's activities has been investigated extensively in recent years, particularly as it affects phytoplankton communities (Provasoli, 1969; Ryther and Dunstan, 1971; Ryther et al., 1972). Pollution of estuarine and marine environments by sewage discharge is now being earnestly investigated (Olson and Burgess, 1967; Robbins and Colwell, 1972) due to the fact that approximately one-third of the total population in the United States is located on estuaries which are being fouled with domestic and industrial waste (De Falco, 1967; Ketchum, 1969). O'Sullivan (1971) has summarized the ecological effects of sewage discharge in the marine and estuarine environments. Extensive research has been carried out at Morehead City, North Carolina with emphasis on estuarine ecosystems exposed to treated sewage wastes (Odum and Chestnut, 1970; Kuenzler and Chestnut,

1971).

It has been found that nutrient buildup from sewage effluent greatly increases the biomass of certain benthic marine algae particularly the genera *Ulva* and *Enteromorpha* (Cotton, 1911; Letts and Richards, 1911; Wilkinson, 1964; Sawyer, 1965). Sawyer (1965) observed enormous growths of *Ulva* reported as "sea lettuce" during the summer months in Boston Harbor due to municipal sewage discharge. Hanks (1966) described large detached masses of *Ulva* reported as *U. lactuca* in the Chesapeake Bay. Newspaper accounts have described excessive growths of *U. lactuca* in and around Norfolk, Virginia. In each of these cases the results have been fish and invertebrate kills, noxious odors, house and boat paint peeling and the loss of revenue at seashore recreational areas.

In light of the many incidences of eutrophication along urban coastal areas a demand has recently arisen for improved assessment of polluted areas. Stein and Denison (1967) remark that indicator organisms by themselves reveal more about water quality than chemical indicators can provide when used alone. They also point out that an ideal indicator is an organism that is sessile and unable to move away from the pollutant. An organism is of little value as a measure of pollution unless it spends the majority of its life cycle in the areas under investigation. Attached marine algae appear to have a number of advantages as possible indicator organisms. This is particularly true in the case of *Ulva curvata* since its specific growing and dormant periods both occur in a highly eutrophicated body of water (Calico Creek) and in a relatively clean body of water (Dill Creek). Both creeks flow eastward and empty into the lower Newport River which connects with Bogue Sound and the Atlantic Ocean near Beaufort, North Carolina.

Marshall (1970) has shown that *Spartina* growth is greater in Calico Creek compared to Dill Creek with its considerably lower nitrogen and phosphate levels. Determinations made by Woods (1970) on N and P components in experimental ponds receiving treated sewage indicate that high levels of both nutrients are being released into Calico Creek. Although biomass and productivity studies were not

carried out by the writer, direct observations did indicate heavier growths of *Ulva* and *Enteromorpha* in Calico Creek compared with Dill Creek. Edwards (1972) assayed the benthic algal flora of three estuaries in England correlating algal abundance and distribution with the properties of the different types of pollutants.

Oglesby (1967) has suggested that measuring the size of an organism could serve as a relatively simple method for evaluating nutrient pollution. Size variations in relation to the extent of pollution would obviously be a better indicator than the mere presence or absence of a species. Burrows (1971) remarked on the potential suitability of using a marine alga such as *Ulva* as a biological indicator of pollution. She found that discs cut from thalli of *Ulva* responded favorably to media from various pollution sources. Photosynthesis was stimulated in *Ulva* when ^{14}C-labelled sodium acetate was added to the culture medium, suggesting that thalli are utilizing this organic carbon source.

Letts and Richard (1911) and Wilkinson (1964) have shown that *Ulva* can make use of nitrogen in the form of ammonia. Under pollution conditions plants have a high nitrogen and organic sulfur content compared to thalli taken from unpolluted waters. Laboratory studies carried out by the writer with *U. curvata* demonstrate a clear relationship between the levels of ammonium in the medium and the rate of vegetative growth as well as the inhibition of reproduction in this species. Nitrogen levels in the estuarine environment may well be the critical limiting factor that determines the growth rate and the size of the standing crop in most situations. Because of its sessile habit, simple morphology and ease of handling in culture, *Ulva* and similar macrobenthic algae may prove to be especially useful as indicators of nutrient pollution in future studies on eutrophication of marine and estuarine environments.

SUMMARY

An investigation of the biology of two species of *Ulva* found along the east coast of the United States was undertaken in order to gain a better understanding of their taxonomy and ecology. *U. curvata* was the only *Ulva* entity found in marsh-like habitats under lowered salinity during the winter-spring season between Georgia and New Jersey. *U. rotundata* appeared to be the dominant *Ulva* species in more saline habitats. *U. curvata* and *U. rotundata* possess morphological attributes in common with *U. lactuca* or its varieties, var. *latissima* and var. *rigida*. Both species are new records for the Western Hemisphere.

In order to establish the range of distribution of *Ulva curvata* mating studies were carried out with plants from the principal research site in Calico Creek near Morehead City, North Carolina and material from several localities extending from the Jekyll River, Brunswick, Georgia to the Navesink River, New Jersey. Male and female gametes released from thalli obtained at each locality mated with gametes from Calico Creek material and produced viable zygotes which germinated normally. Although plants were collected in Rhode Island and Massachusetts which looked cytologically and morphologically similar to Calico Creek plants, gametes could not be induced into release and breeding tests were not performed.

The life cycle of *Ulva curvata* was demonstrated in field and laboratory studies to involve an alternation of isomorphic gametophyte and sporophyte generations. Thalloid forms of both generations appear between November and May and alternate with a microscopic filamentous stage present on oyster shells and other solid substrates from May to November. Gametophytes predominate in the field during the early growing season in Fall and early Winter and are gradually replaced by sporophytes in late Winter and early

Spring. At the research site in Calico Creek female gametophytes were much more prevalent than male gametophytes and sporophytes released zoospores which developed more commonly into female plants. Asexual propagation by plantlets produced adventitiously from the rhizoids of germlings was seen commonly in culture in female gametophytes and in sporophytes, but was absent in male gametophytes.

While all nuclear phases and growth forms appear to be connected to one another by meiosis and syngamy, a high frequency of somatic diploidization of female plants leading to parthenosporophytes that carry a potential for regenerating only female thalli has greatly biased the life cycle in Calico Creek. The possibility exists that *U. curvata* may well be expressing a gradual shift from a facultative alternation of generations to an obligate asexual life cycle involving only female plants.

Field studies showed that populations of *Ulva curvata* in Calico Creek release swarmers in strictly cyclical patterns with peaks of reproductive activity every 14-15 days between January and April. Release occurred 3-5 days before spring tide in 1970 and 1 day before to 1 day after spring tide in 1971. Experiments carried out in the laboratory to determine mechanisms involved in the semi-lunar cycle were unsuccessful.

Germination, early development, thallus growth, reproduction and senescence were investigated experimentally under controlled conditions in the laboratory in order to account for the life history and ecological adaptations of *Ulva curvata* as seen in the field. A temperature greater than 15°C and light intensities of 250 ft-c stimulated germination of all swarmer types. The male gamete was found to need still higher temperatures and light intensities for germination. Further development into minute tubular plantlets occurred only if the temperature was reduced below 18-20°C in the laboratory. Morphogenesis of the small tubular thallus into an expanded blade characteristic of *Ulva* was found to occur with regularity only if cultures received mud-water extract and agitation. Experiments on growth and reproduction showed that agitation, high NH_4-N levels and daily cycles of high (20°C) and low (9°C) temperatures and high (800 ft-c) and low

(250 ft-c) light intensities favored high growth rates of the vegetative thallus while stationary cultures, low NH_4-N levels and constant high light intensities promoted spontaneous reproduction. These latter parameters also contribute to early senescence of plants in culture.

Mitosis of vegetative cells of *Ulva curvata* were essentially synchronous in the field and laboratory and occurred between 9:30 p.m. and 11:45 p.m. A shift in the position of the nucleus and chloroplast preceded mitosis. The nucleus migrates from a position near the upper cell membrane to the lower cell membrane in the early afternoon while the chloroplast moves across the face of the cell. In contrast, mitosis in *U. rotundata* appeared to occur between 9:30 a.m. and 11:15 a.m. with no shift in position of the nucleus or chloroplast.

These studies demonstrate that the genus *Ulva* can serve as an important indicator organism of eutrophication. Because of its simple morphology and ease of handling in culture, *Ulva* should prove to be a valuable tool in the analysis of estuarine environments subject to pollution.

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FIGURE 1

Map of Morehead City area showing Calico Creek and principal research site.

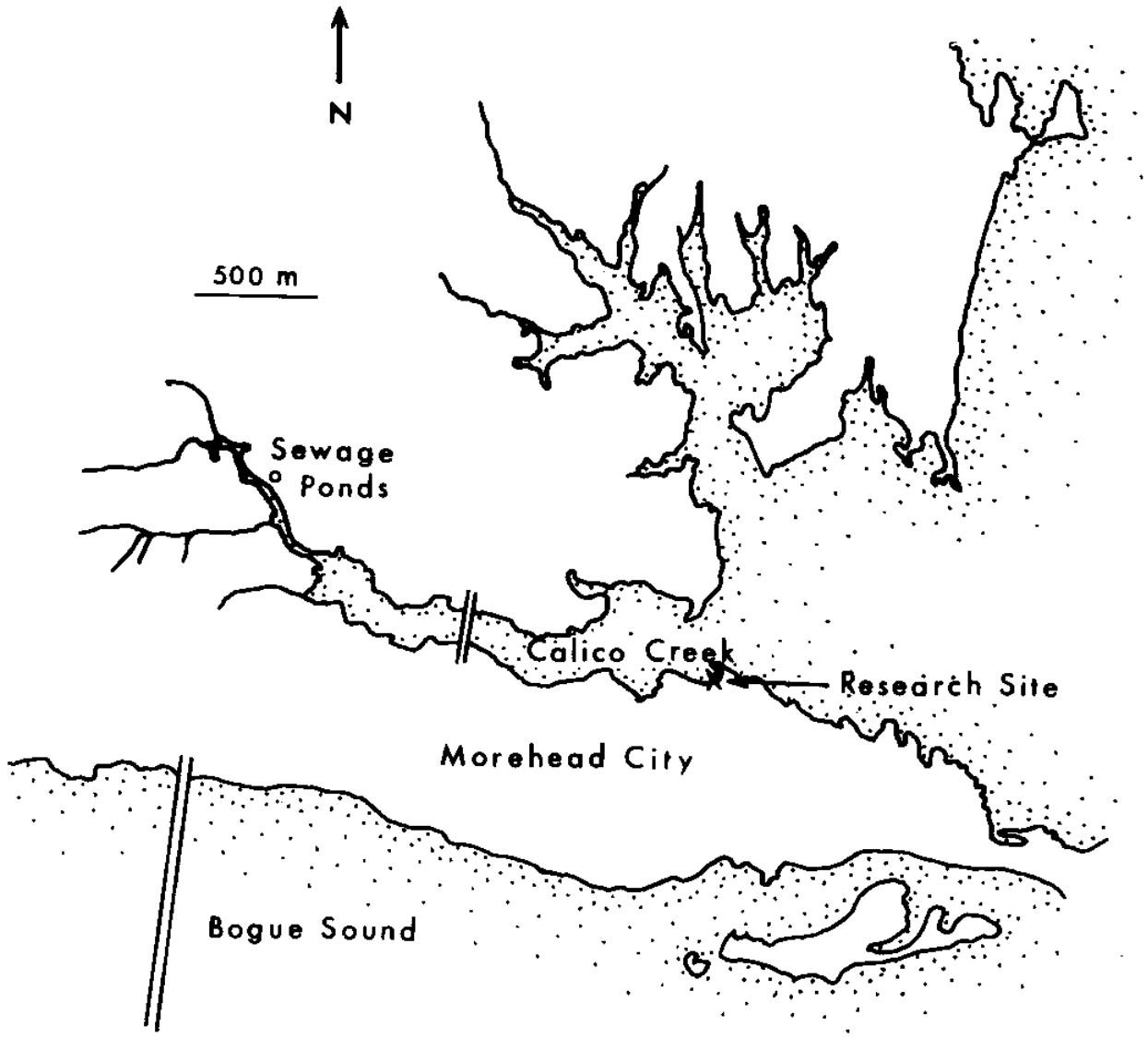

N
500 m
Sewage
Ponds
Calico Creek
Research Site
Morehead City
Bogue Sound

FIGURE 2

Intertidal zone at Calico Creek research site as it appears in March (A-C) and October (D-E)

A: Lower intertidal zone of oyster reef area showing large biomass of *Ulva curvata* thalli attached to shells

B: Upper intertidal zone and edge of *Spartina* area

C: Close up of oyster reef showing cover of *Ulva curvata* thalli

D: Upper intertidal zone of oyster reef

E: Close up of upper oyster reef zone showing bare shells

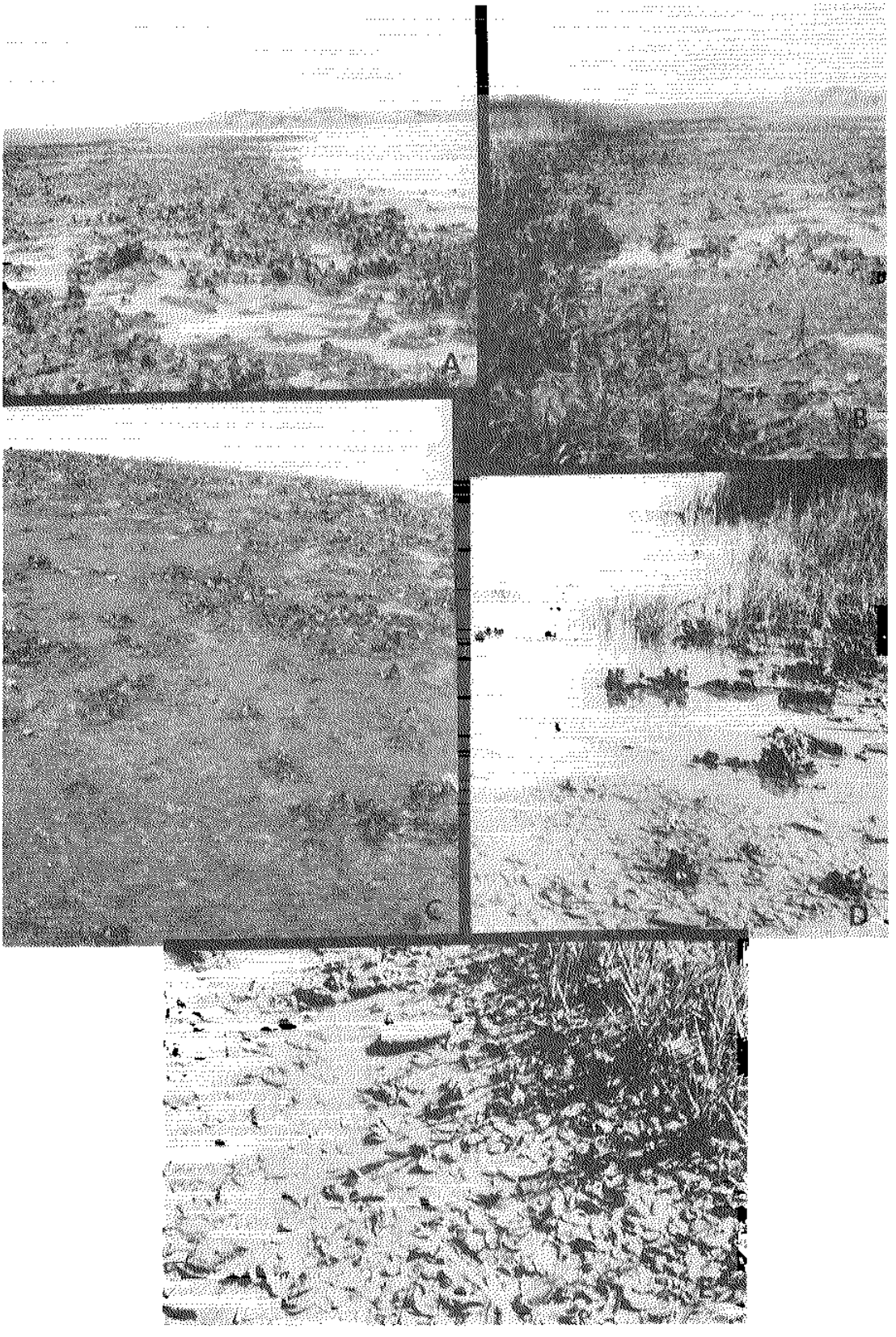

A
B
C
D

FIGURE 3

Habit of plants of *Ulva curvata* from the field

A: Young plants from Calico Creek

B: Mature gametophyte from Calico Creek

C: Mature sporophyte from Calico Creek

D: Plant from Jekyll River, Georgia

E: Plant from Navesink River, New Jersey

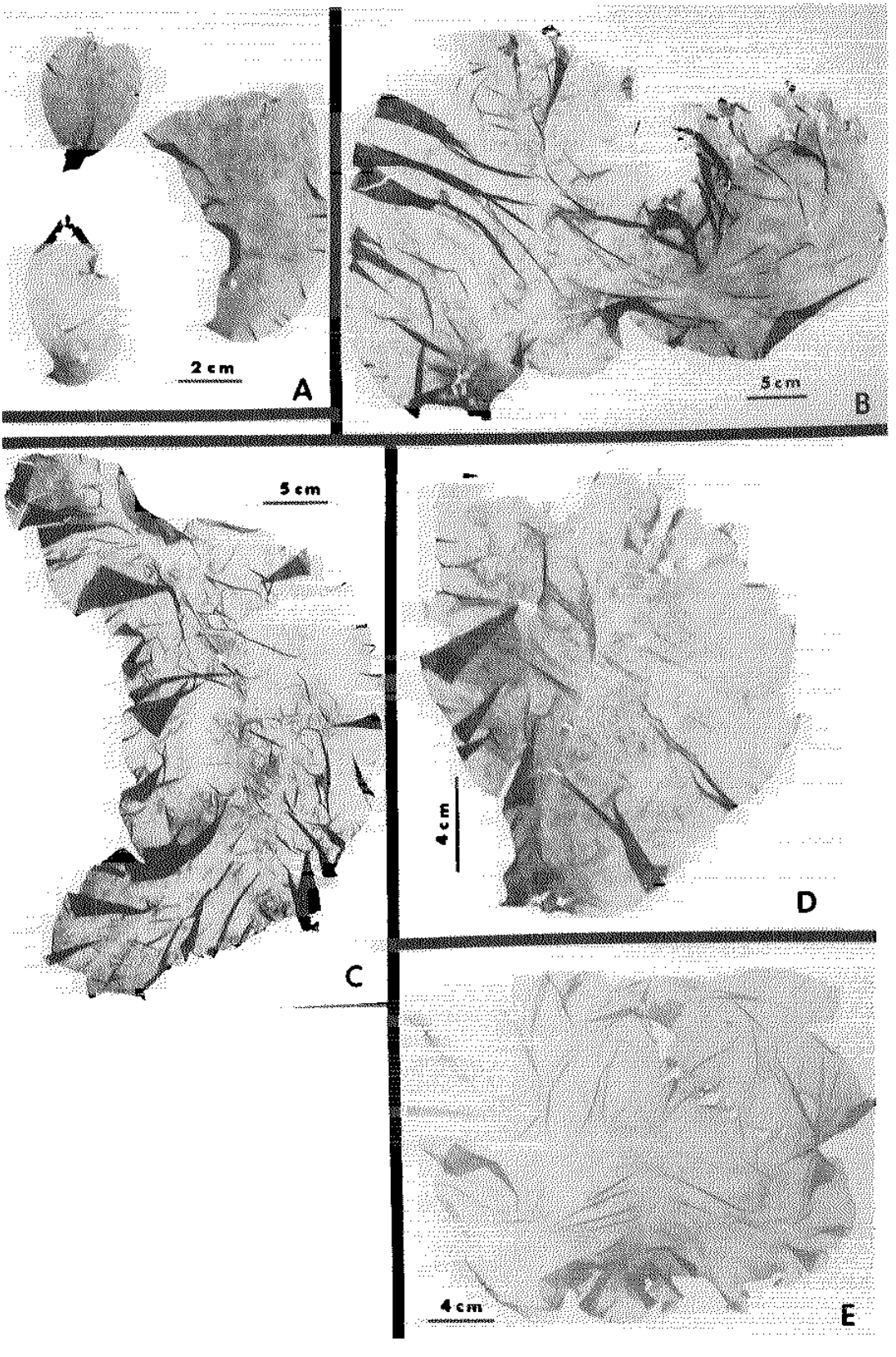

2 cm
A
5 cm
B
5 cm
C
4 cm
D
4 cm
E

FIGURE 4

A-B: Surface view of cells in *Ulva curvata* showing lateral disposition of chloroplast and single pyrenoid

C: Male gamete

D: Female gamete

E: Zoospore

F: Habit of an isotype of *Ulva dangeardii* (=*U. curvata*)

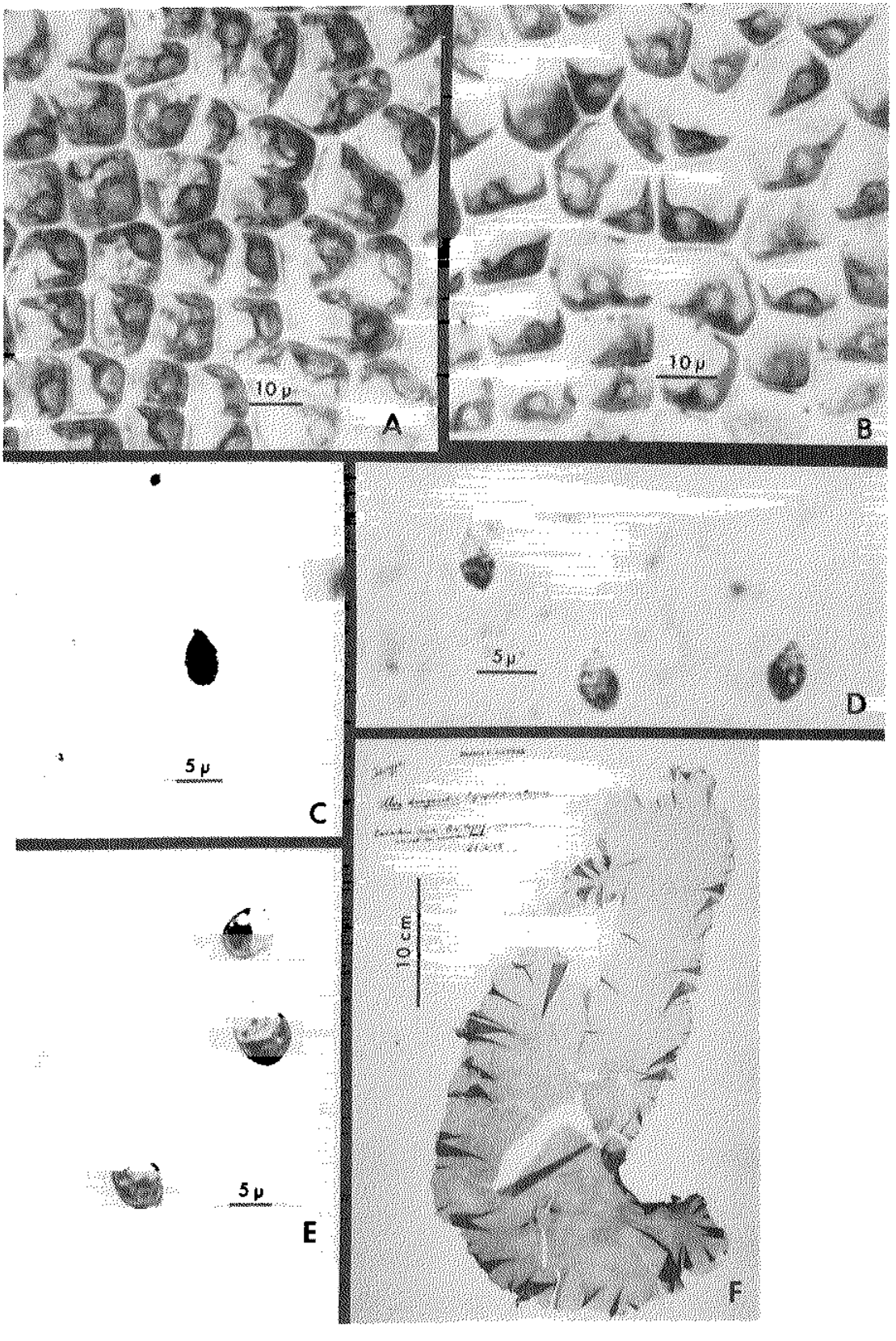

10 µ
A
10 µ
B
5 µ
C
5 µ
D
5 µ
E
10 cm
F

FIGURE 5

A-B: Surface view of cells in *Ulva dangeardii*

C-E: *Ulva rotundata*

C: Young plants from Bogue Sound

D: Mature plant from Bogue Sound

E: Surface view of cells showing 1-3 pyrenoids, nucleus and chloroplast across face of cell

F: *Ulva rigida* collected at Woods Hole, Massachusetts

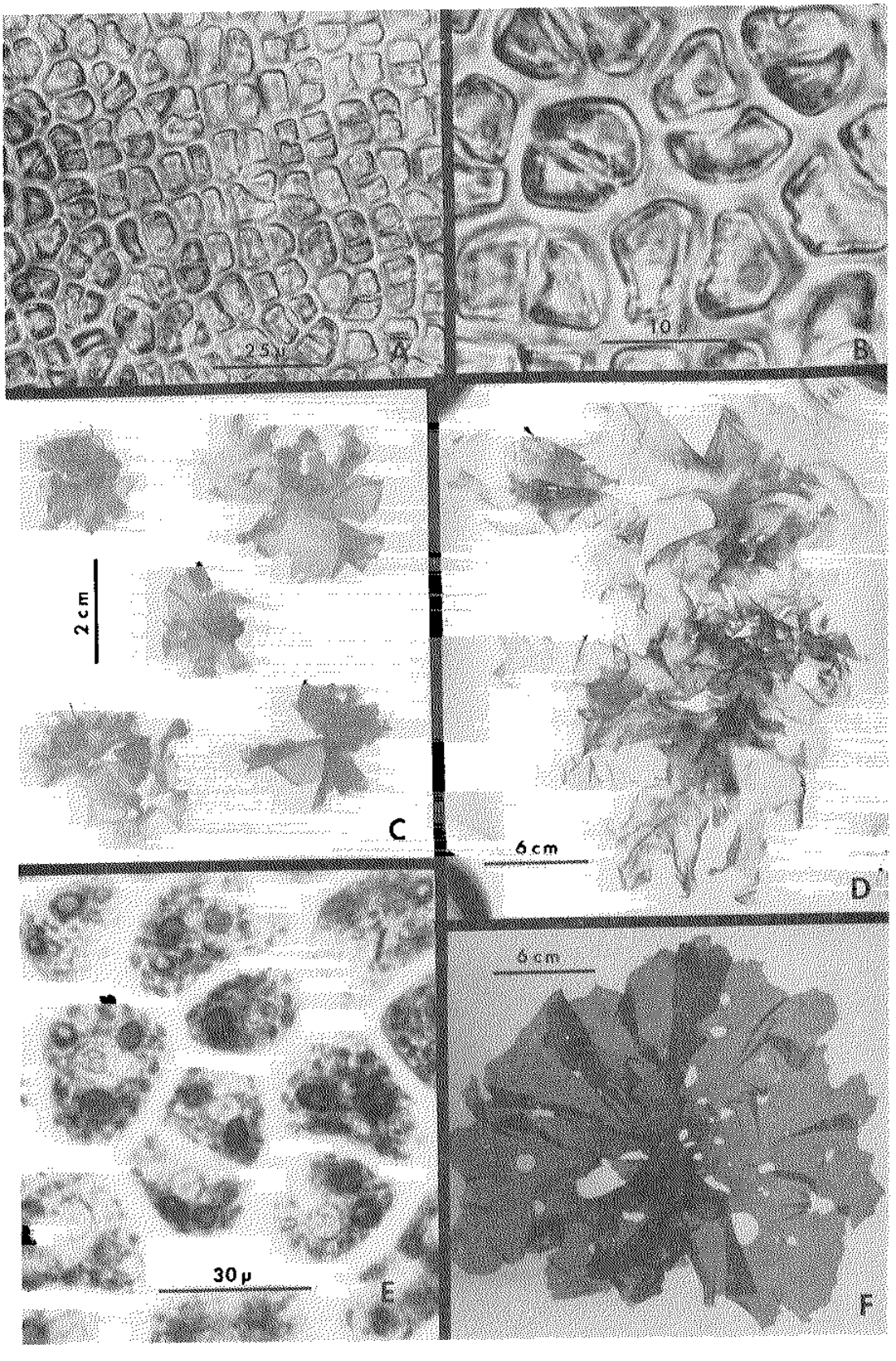

2.5 µ
A
10 µ
B
2 cm
C
6 cm
D
30 µ
E
6 cm
F

FIGURE 6

A: Oyster shells collected in Calico Creek during mid-summer

B-C: Germlings found on surface of shells during mid-summer

D: Oyster shell from Calico Creek in mid-November showing plantlets up to 0.5 cm long

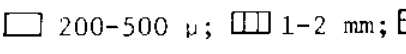
5 cm
A
10 μ
B
20 μ
C
2 cm
D

FIGURE 7

Growth rates of filaments and plantlets on oyster shells in crossed gradient apparatus in response to varying light intensities, temperatures, photoperiods and collection dates

Duration of experiments: 14 days

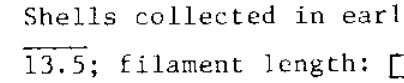
A-B: Shells collected in August, 1971; filament length:



A: Photoperiod 10.5:$\overline{13.5}$

B: Photoperiod 14.5:$\overline{9.5}$

C: Shells collected in early June, 1971, Photoperiod 10.5:



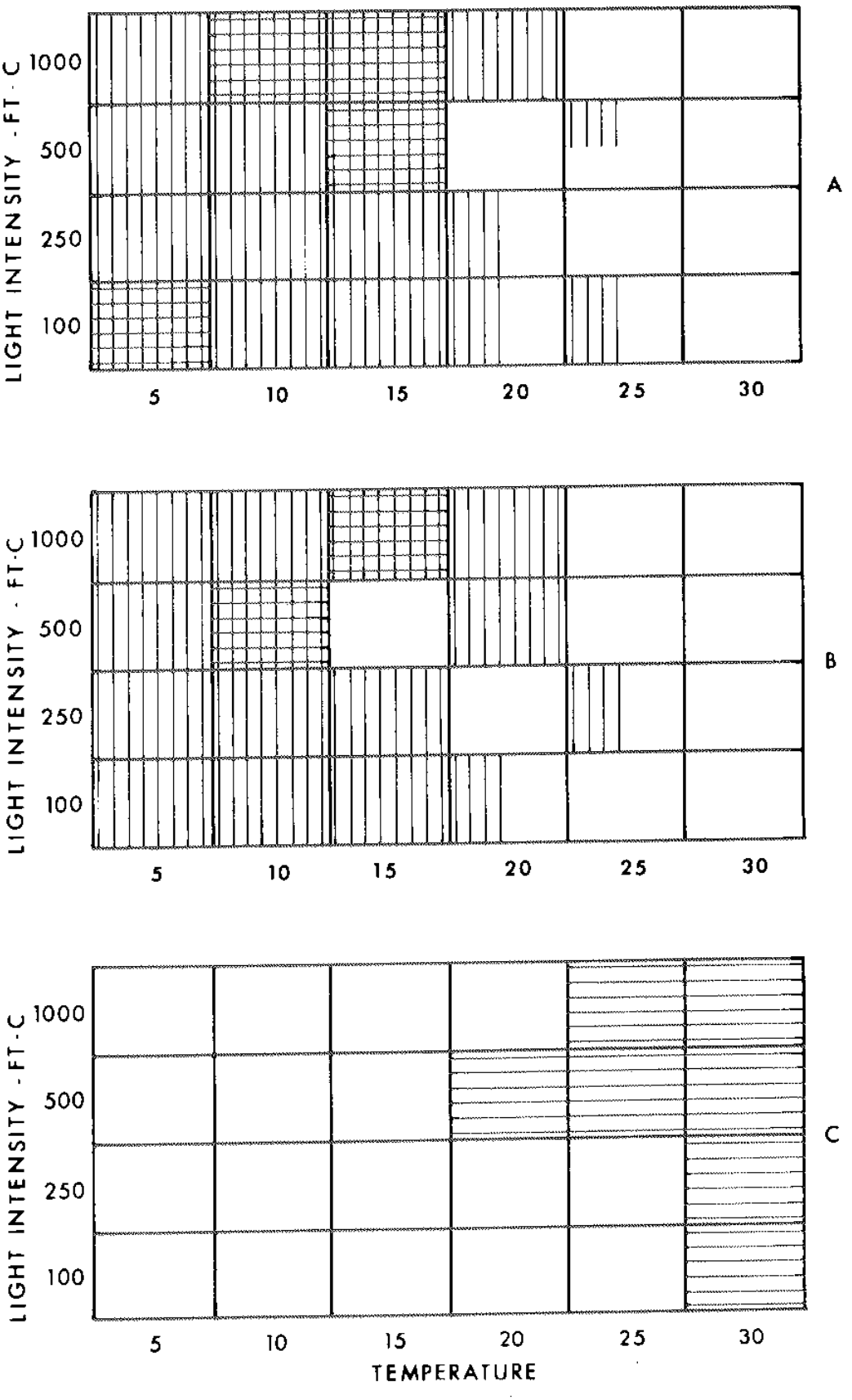
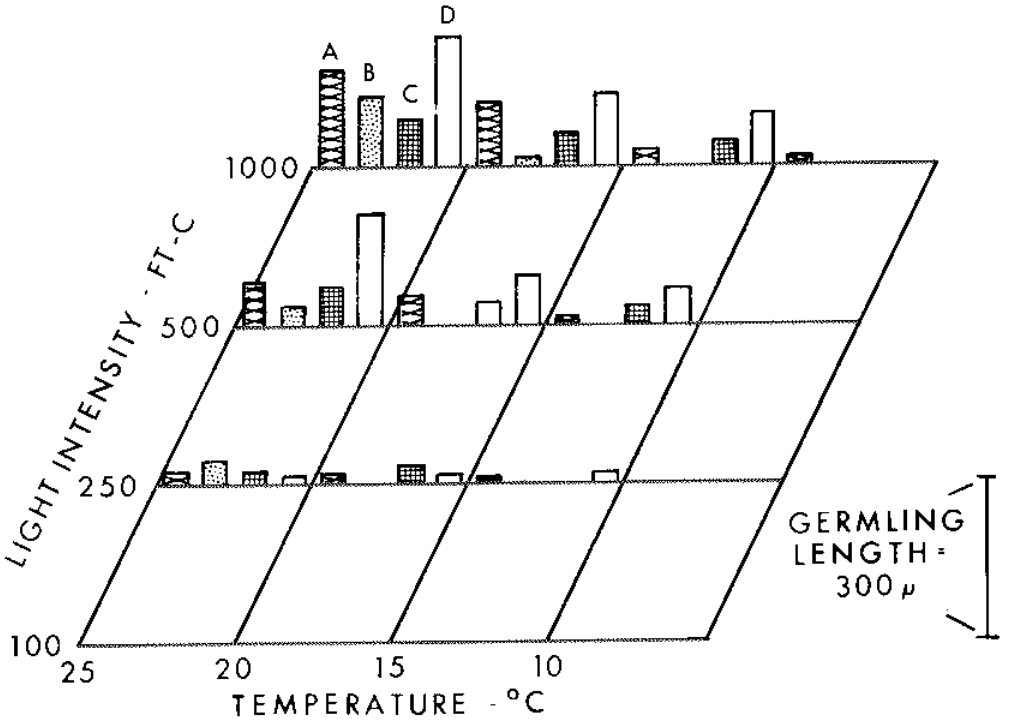

LIGHT INTENSITY - FT - C
1000
500
250
100
5
10
15
20
25
30
A
LIGHT INTENSITY - FT-C
1000
500
250
100
5
10
15
20
25
30
B
LIGHT INTENSITY - FT-C
1000
500
250
100
5
10
15
20
25
30
TEMPERATURE
C

FIGURE 8

Germination and early growth of the different reproductive bodies of *Ulva curvata* as a function of light intensity and temperature. Photoperiod: 12:$\overline{12}$; Medium: modified von Stosch with 10 mg/l NH_4-N and mud-water extract; Duration: 7 days
A: Female gametes, B: Male gametes, C: Zoospores, D: Zygotes

FIGURE 9

Insolation during the months September-December in $cal/cm^2/month \times 10^3$


A
B
C
D
1000
500
250
100
LIGHT INTENSITY - FT-C
25
20
15
10
TEMPERATURE - °C
GERMLING LENGTH = 300 μ

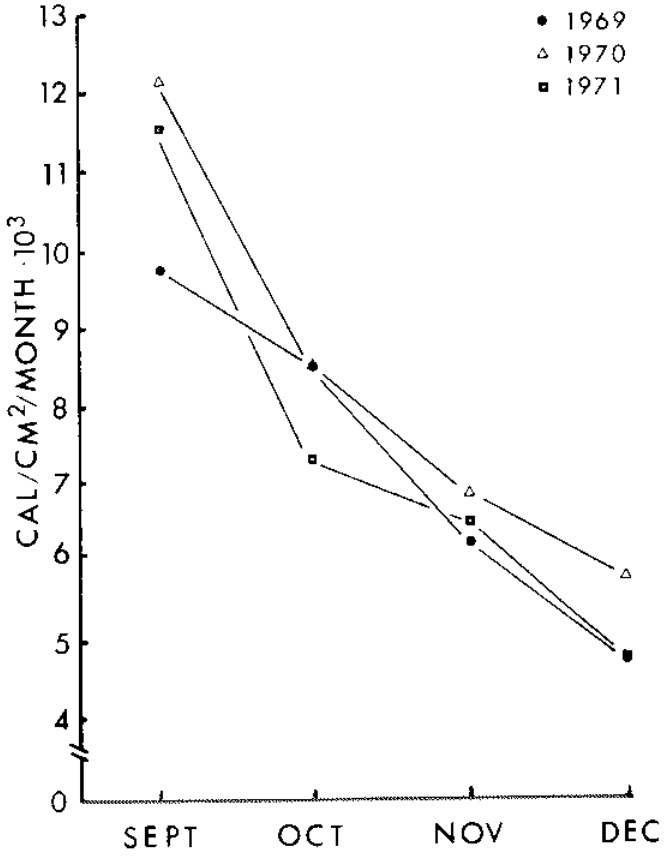

1969
1970
1971
13
12
11
10
9
8
7
6
5
4
0
CAL/CM²/MONTH ·10³
SEPT
OCT
NOV
DEC

FIGURE 10

Minimum and maximum daily water and air temperatures at Morehead City; vertical bars depict air temperature ranges; horizontal bars show water temperature ranges.

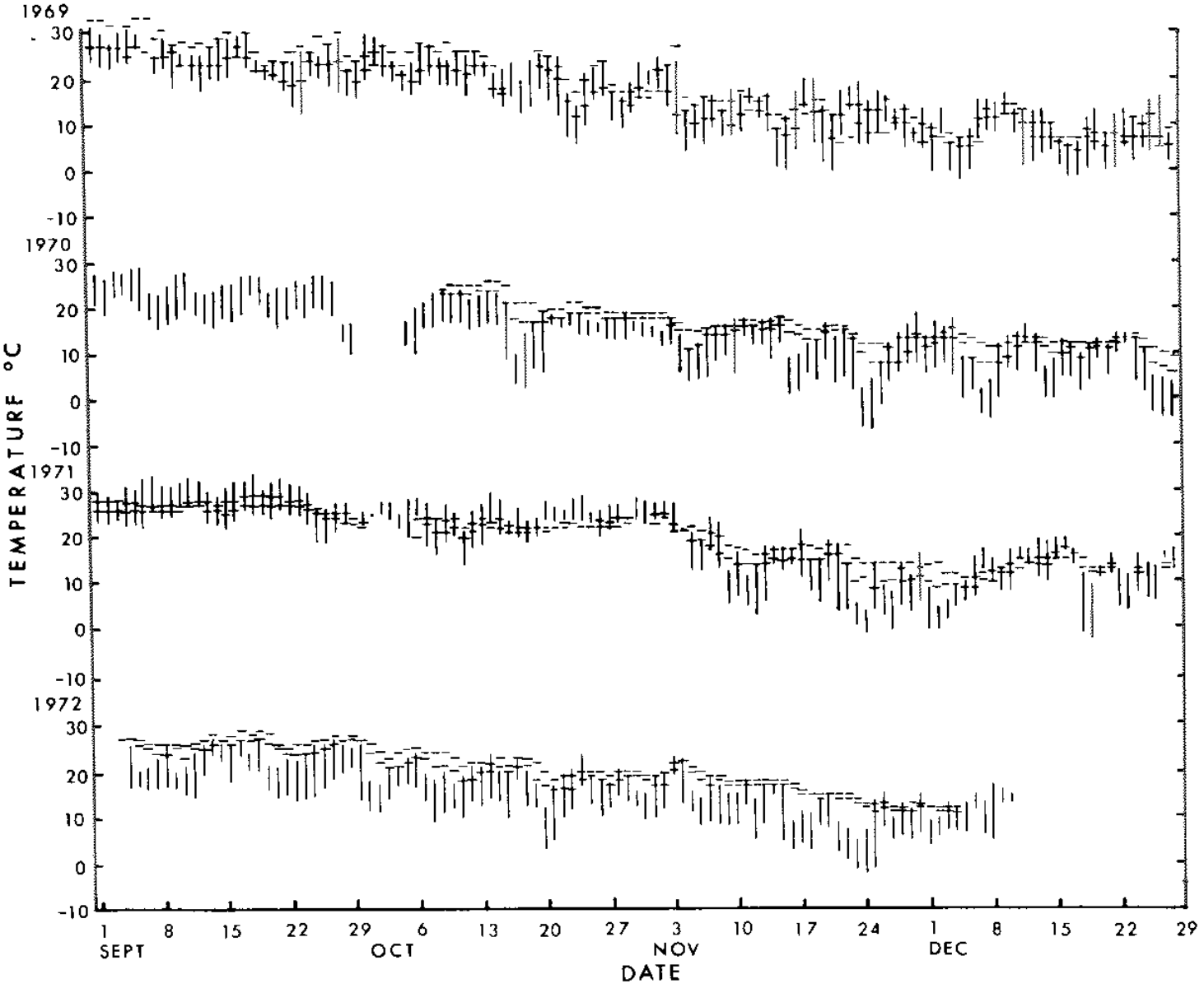

1969
1970
1971
1972
30
20
10
0
-10
TEMPERATURE °C
1 8 15 22 29 6 13 20 27 3 10 17 24 1 8 15 22 29
SEPT
OCT
NOV
DEC
DATE

FIGURE 11

Germling morphology in *Ulva curvata*

A: From zygotes and unfertilized gametes in static culture

B: From zoospores in static culture

C: From zygotes and unfertilized gametes in agitated culture

D: From zygotes and unfertilized gametes in experimental pond water

E: From zoospores in high concentrations of NH_4-N

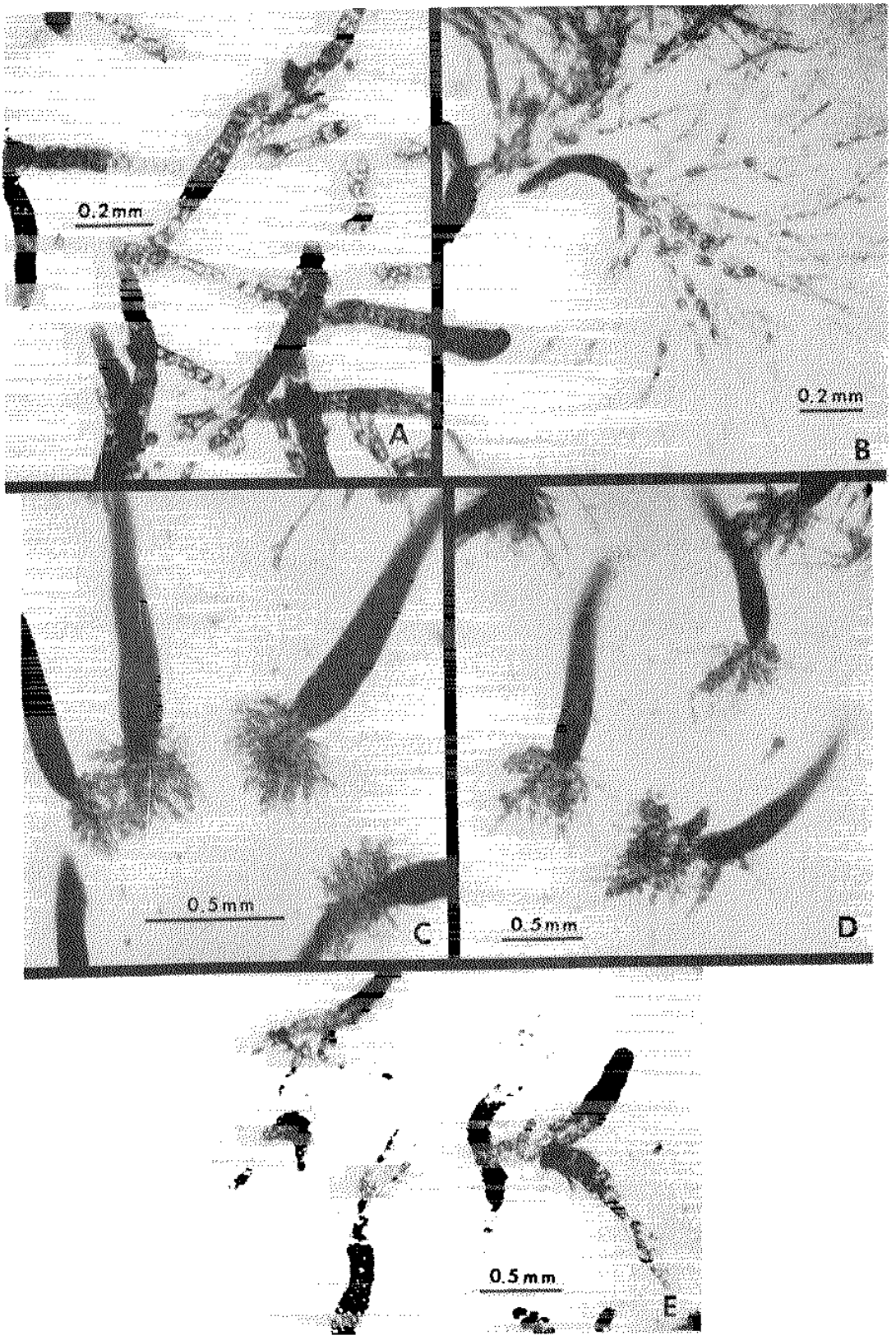

0.2mm
A
0.2mm
B
0.5mm
C
0.5mm
D
0.5mm
E

FIGURE 12

Germlings developing adventitiously from the basal filaments of female gametophytes of *Ulva curvata*

A-B: Early stages in development

C-D: Later stages prior to detachment

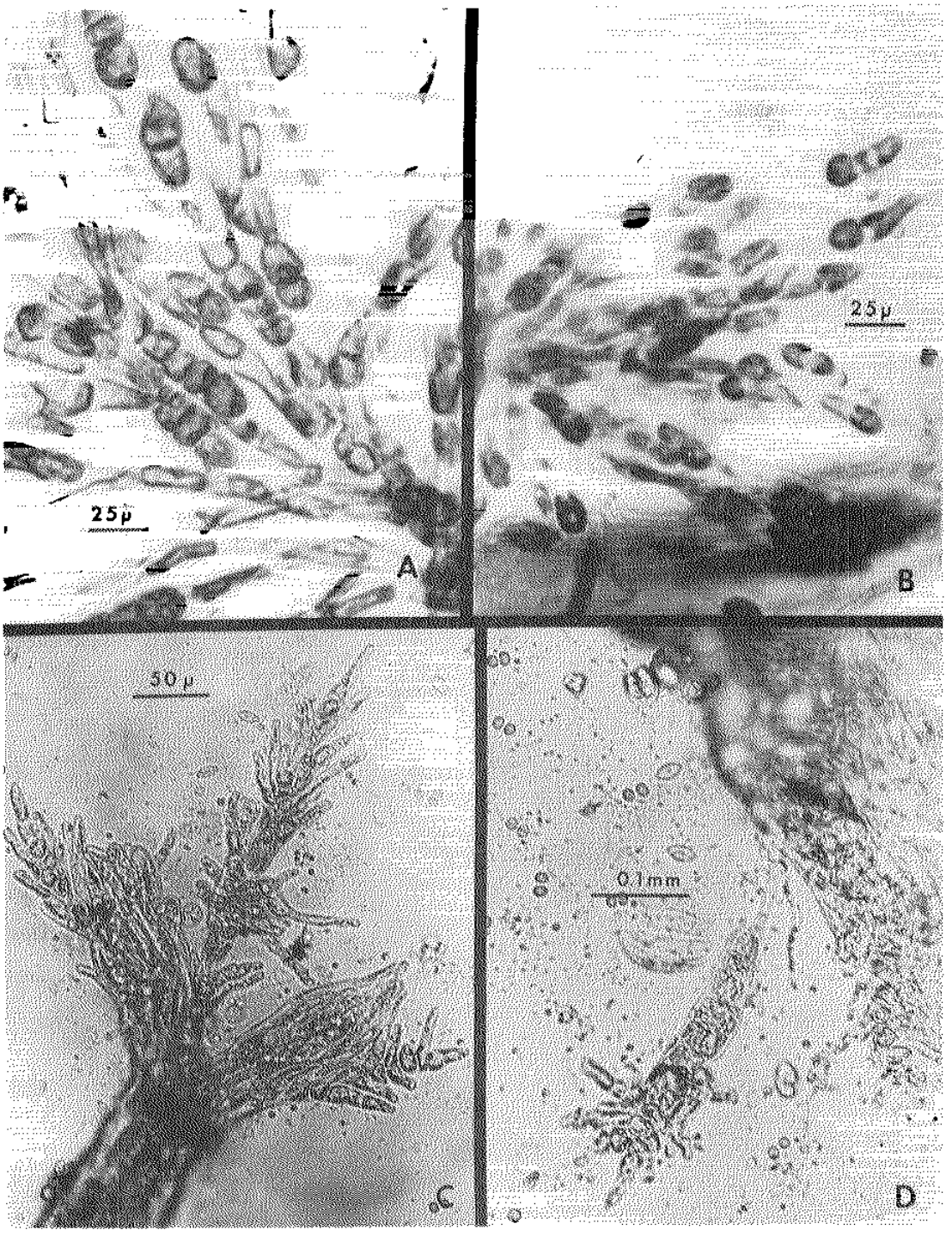

25μ
A
25μ
B
50μ
C
0.1mm
D

FIGURE 13

Growth of sporophyte of *Ulva curvata* in still culture

A: One week

B: Two weeks

C: Two months

D: Five months

E: Appearance of thallus after five months with frequent changes of culture medium

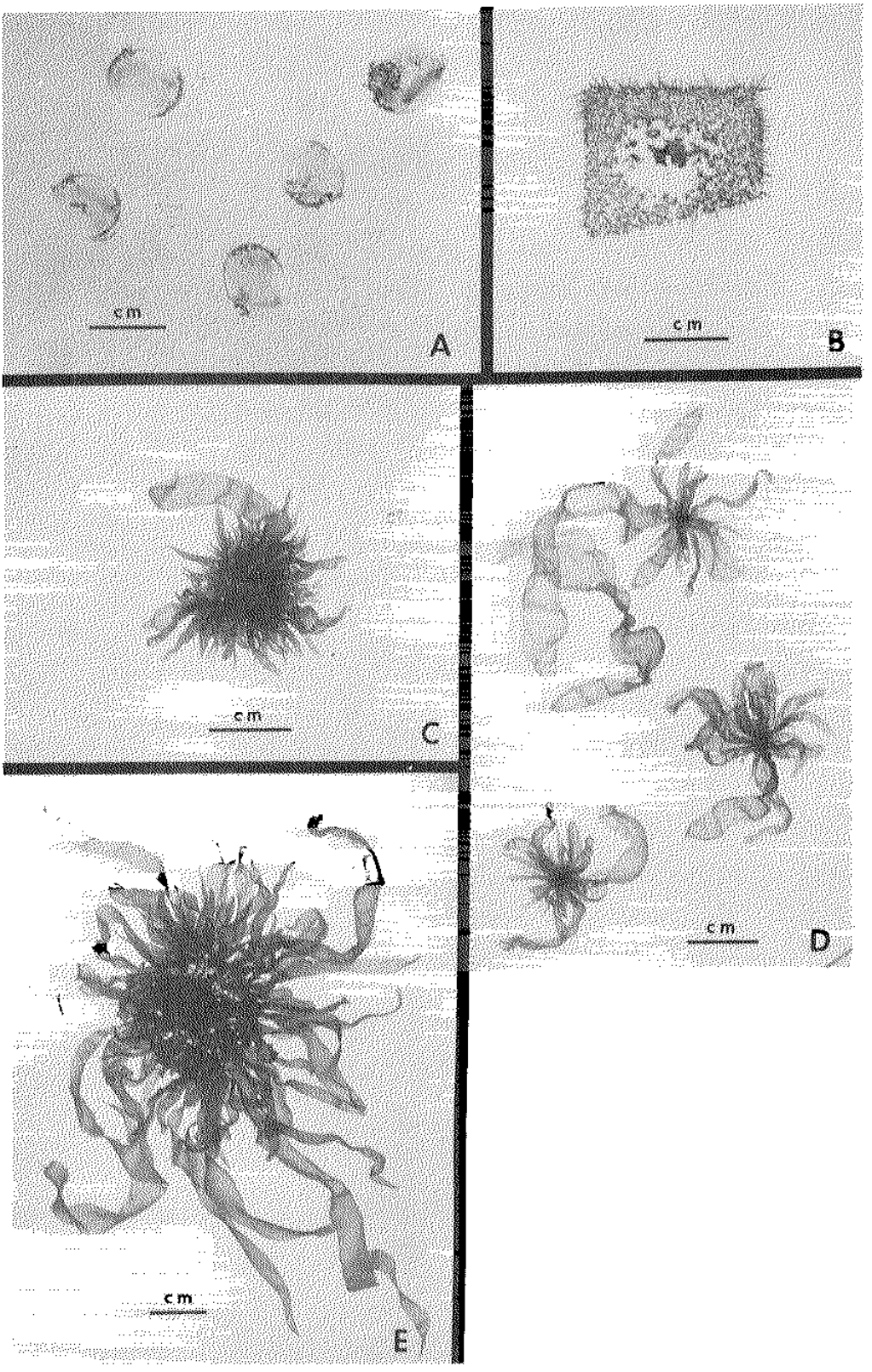

c m
A
c m
B
c m
C
c m
D
c m
E

FIGURE 14

A-B: Comparison of size of plantlets grown in static culture (bottom) with plantlets grown in agitated culture (top)

A: Two week old cultures

B: Three week old cultures

C-D: Three month old plants grown from germlings on shaker

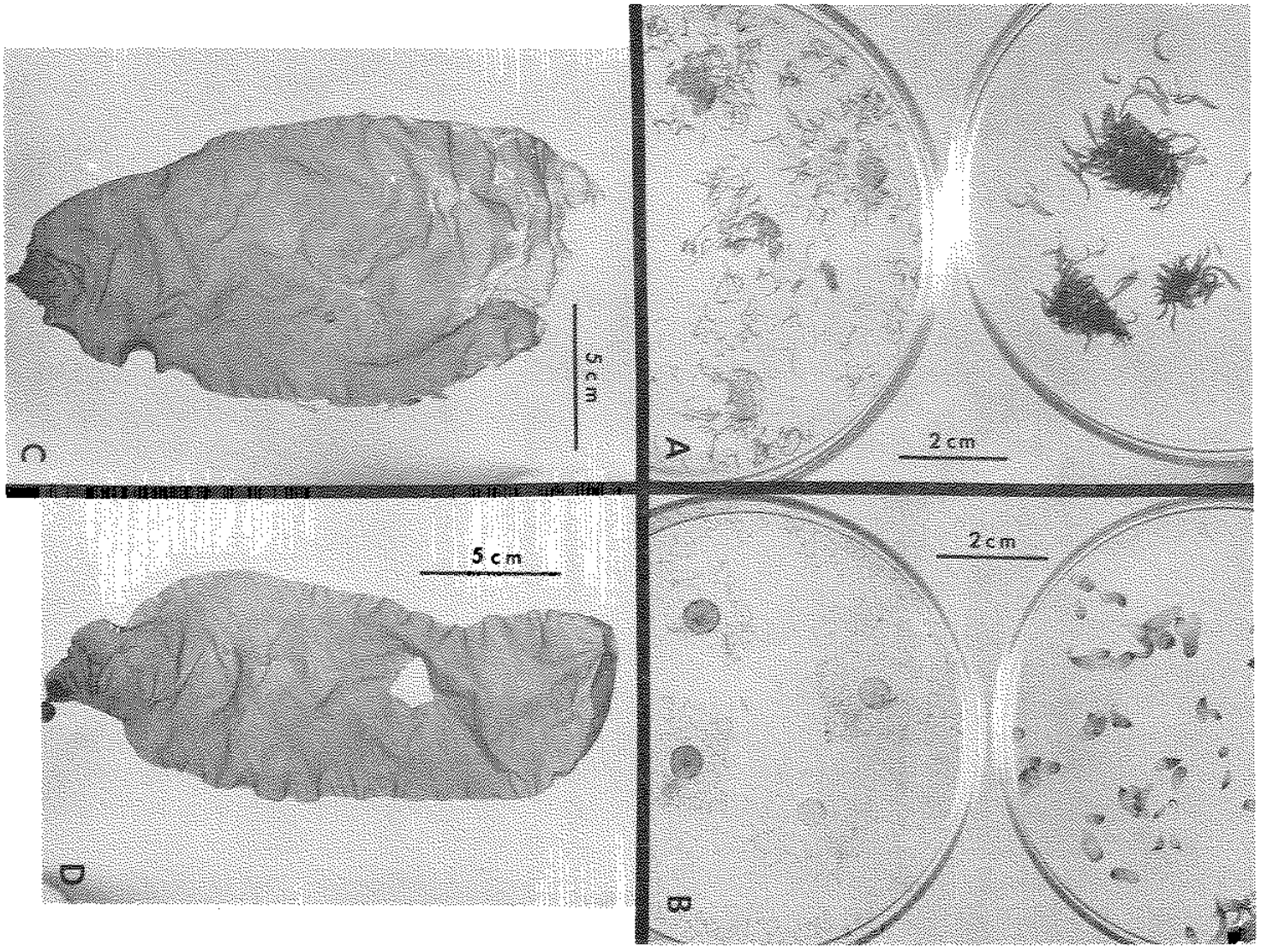
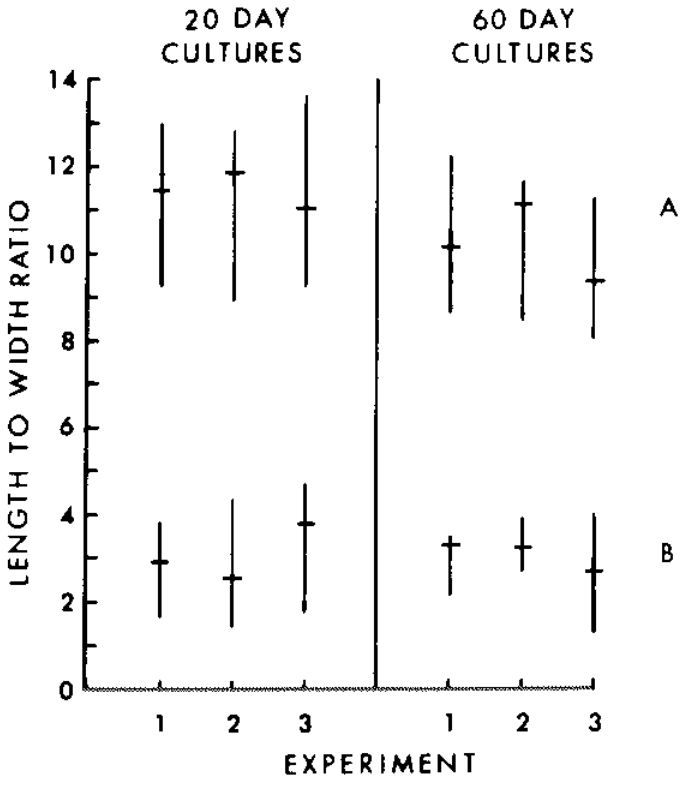

A
2 cm
B
2 cm
C
5 cm
D
5 cm

FIGURE 15

Ratio of length to width for plants grown under static or agitated culture conditions. Vertical bars indicate the length/width ratio; horizontal bars give the mean values
Temperature: 18°C; Photoperiod: 12:$\overline{12}$
Each experiment contained 25-32 plants
A: Static conditions
B: Agitated conditions

FIGURE 16

Percentage of thalli found to be reproductive before and after spring tides at Calico Creek research site in February, 1970 and March, 1971
The day of maximum spring tide is indicated by "0"


20 DAY CULTURES
60 DAY CULTURES
LENGTH TO WIDTH RATIO
14
12
10
8
6
4
2
0
A
B
1
2
3
1
2
3
EXPERIMENT

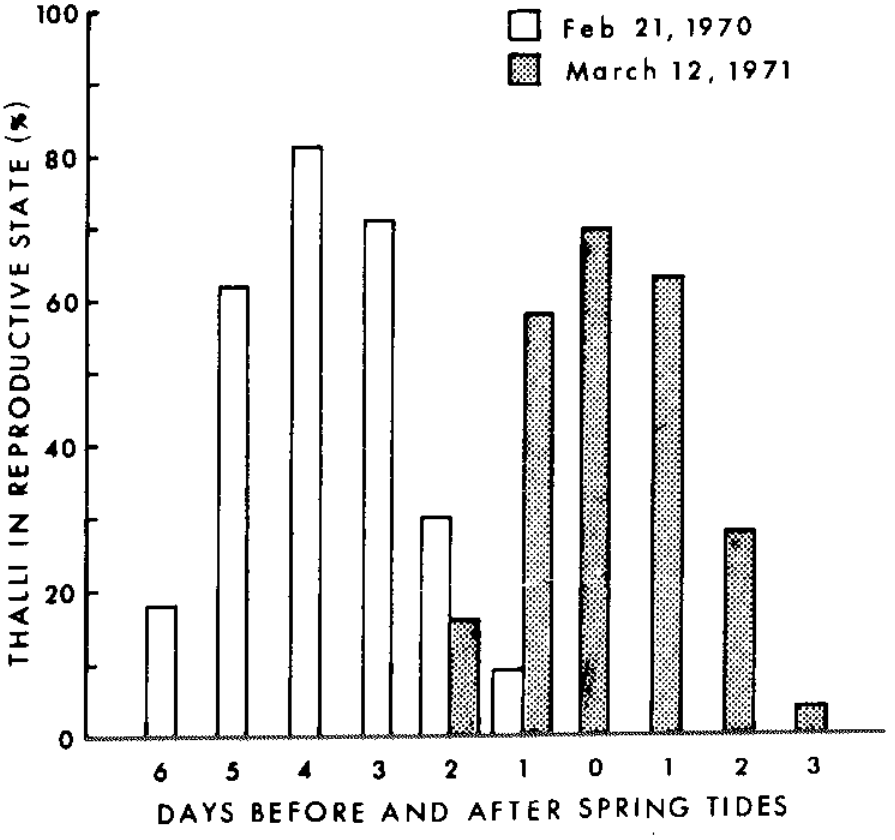

Feb 21, 1970
March 12, 1971
THALLI IN REPRODUCTIVE STATE (%)
100
80
60
40
20
0
6
5
4
3
2
1
0
1
2
3
DAYS BEFORE AND AFTER SPRING TIDES

FIGURE 17

Tidal cycle at Calico Creek, March 1-April 11, covering three spring tide periods (s). Vertical lines show time of exposure of oyster reef, horizontal lines indicate the length of the daylight period.

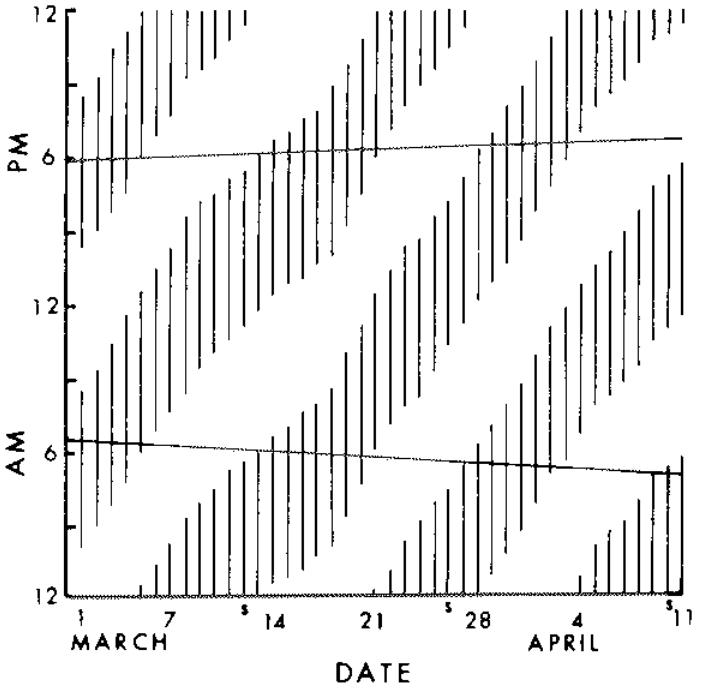
FIGURE 18

Growth rates and reproductive potential in discs cut from *Ulva curvata* grown under 200 ft-c for 4 days and transferred to high light for six more days under static culture conditions; temperature 14°C; photoperiod 12:$\overline{12}$

A: 260 µg/l NH_4-N in medium

B: 60 µg/l NH_4-N in medium

Three discs were used in each experimental series


12
PM
6
12
AM
6
12
1
7
14
21
28
4
11
MARCH
APRIL
DATE

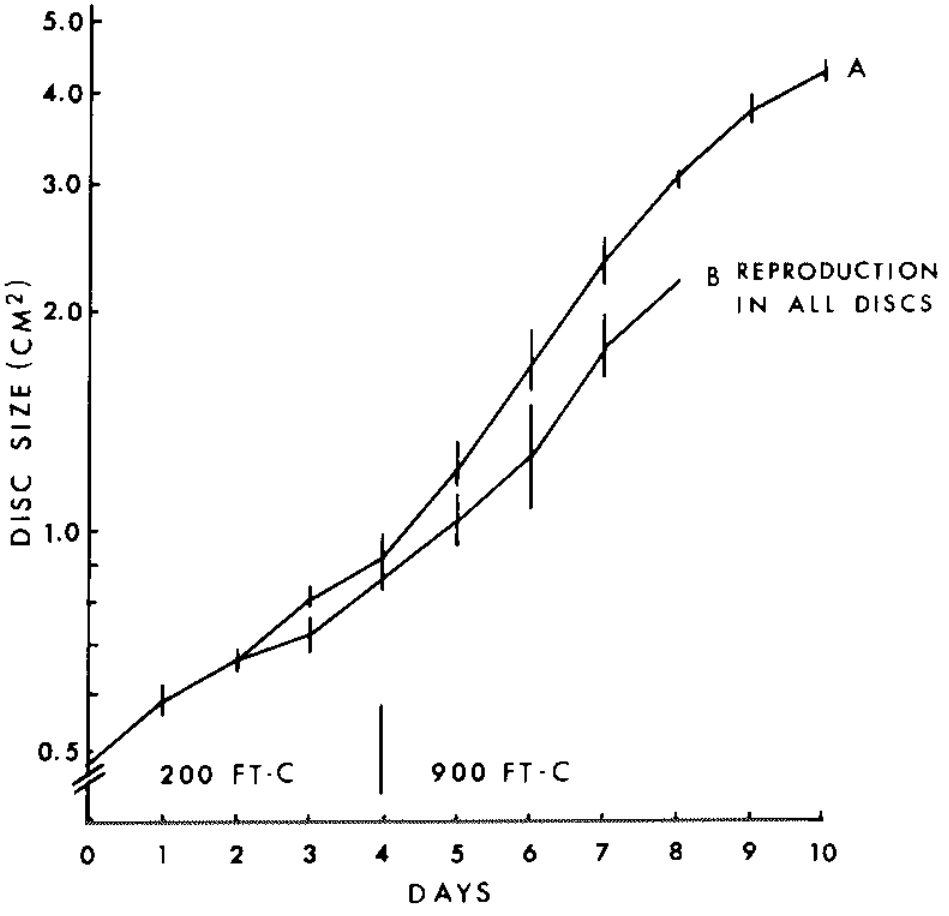

5.0
4.0
3.0
2.0
1.0
0.5
DISC SIZE (CM²)
A
B REPRODUCTION
IN ALL DISCS
200 FT-C
900 FT-C
0
1
2
3
4
5
6
7
8
9
10
DAYS

FIGURE 19

Percent increase in growth of discs for 7 days under a 12:$\overline{12}$ photoperiod

Experimental series:

1. still cultures, no added NH_4-N, 250 ft-c
   a. 9-10°C
   b. 17-18°C
2. still cultures, no added NH_4-N, 750 ft-c
   a. 9-10°C
   b. 17-18°C
3. still cultures, 10 mg/l NH_4-N, 250 ft-c
   a. 9-10°C
   b. 17-18°C
4. still cultures, 10 mg/l NH_4-N, 250 ft-c, 17-18°C
5. still cultures, 10 mg/l NH_4-N
   17-18°C during first 6 hours of light period at 800 ft-c
   9-10°C during second 6 hours of light period at 250 ft-c
   9-10°C during dark period
6. agitated cultures, no added NH_4-N, 800 ft-c, 17-18°C
7. agitated cultures, 10 mg/l NH_4-N, 800 ft-c, 17-18°C
8. agitated cultures, other conditions as in 5

The percentage of discs found to be reproductive by the 7th day is indicated for each series

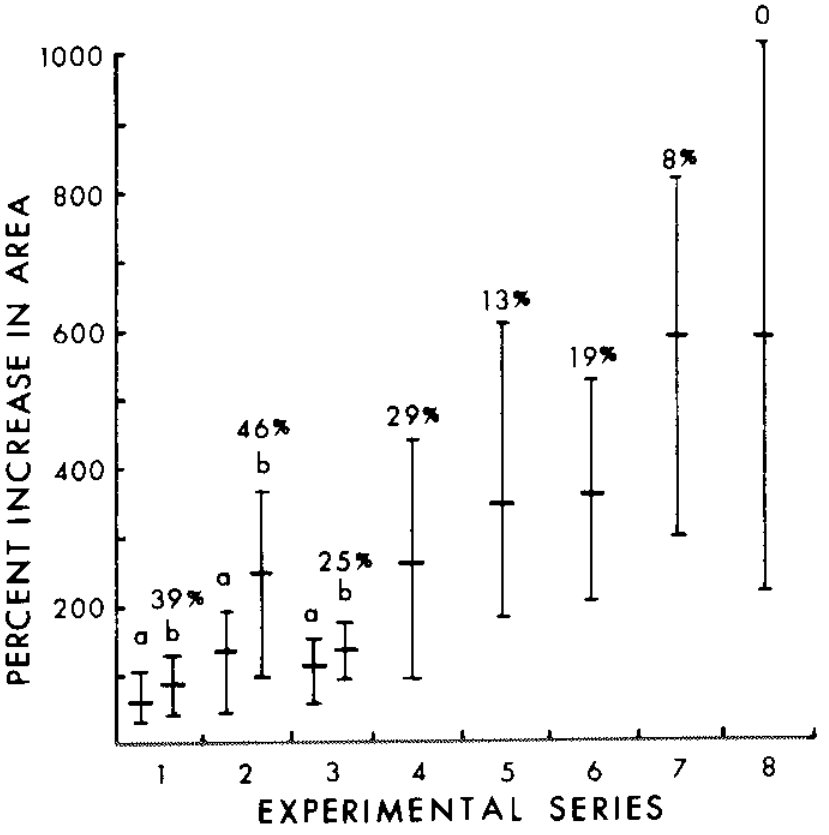
FIGURE 20

Percent increase in growth of discs for 4 days under a 12:$\overline{12}$ photoperiod, 10 mg/l NH_4-N

Temperatures: a. 9-10°C, b. 17-18°C

Experimental series:

1. still cultures
2. air agitated cultures
3. mechanically agitated cultures


PERCENT INCREASE IN AREA
1000
800
600
400
200
39%
46%
25%
29%
13%
19%
8%
0
a
b
a
b
a
b
1
2
3
4
5
6
7
8
EXPERIMENTAL SERIES

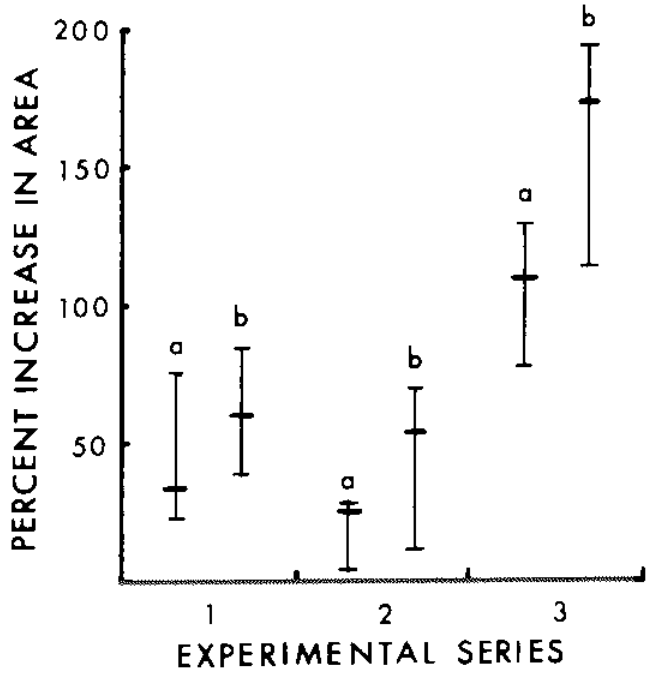

PERCENT INCREASE IN AREA
200
150
100
50
a
b
a
b
a
b
1
2
3
EXPERIMENTAL SERIES

FIGURE 21

Surface view of cells of *Ulva curvata* during mitosis

A: Position of nucleus near the outer cell membrane in interphase, mid-morning

B: Lateral position of chloroplast in non-dividing cells, contrasted with chloroplast position across face of cell in cells destined to divide that day, early afternoon

C: Loss of pyrenoid, late afternoon

D: Surface view showing a few cells with chloroplast across face of cell prior to cell division

E: Nuclei in prophase of mitosis as viewed from inner surface of cells prior to division, ca. 9:00 p.m.

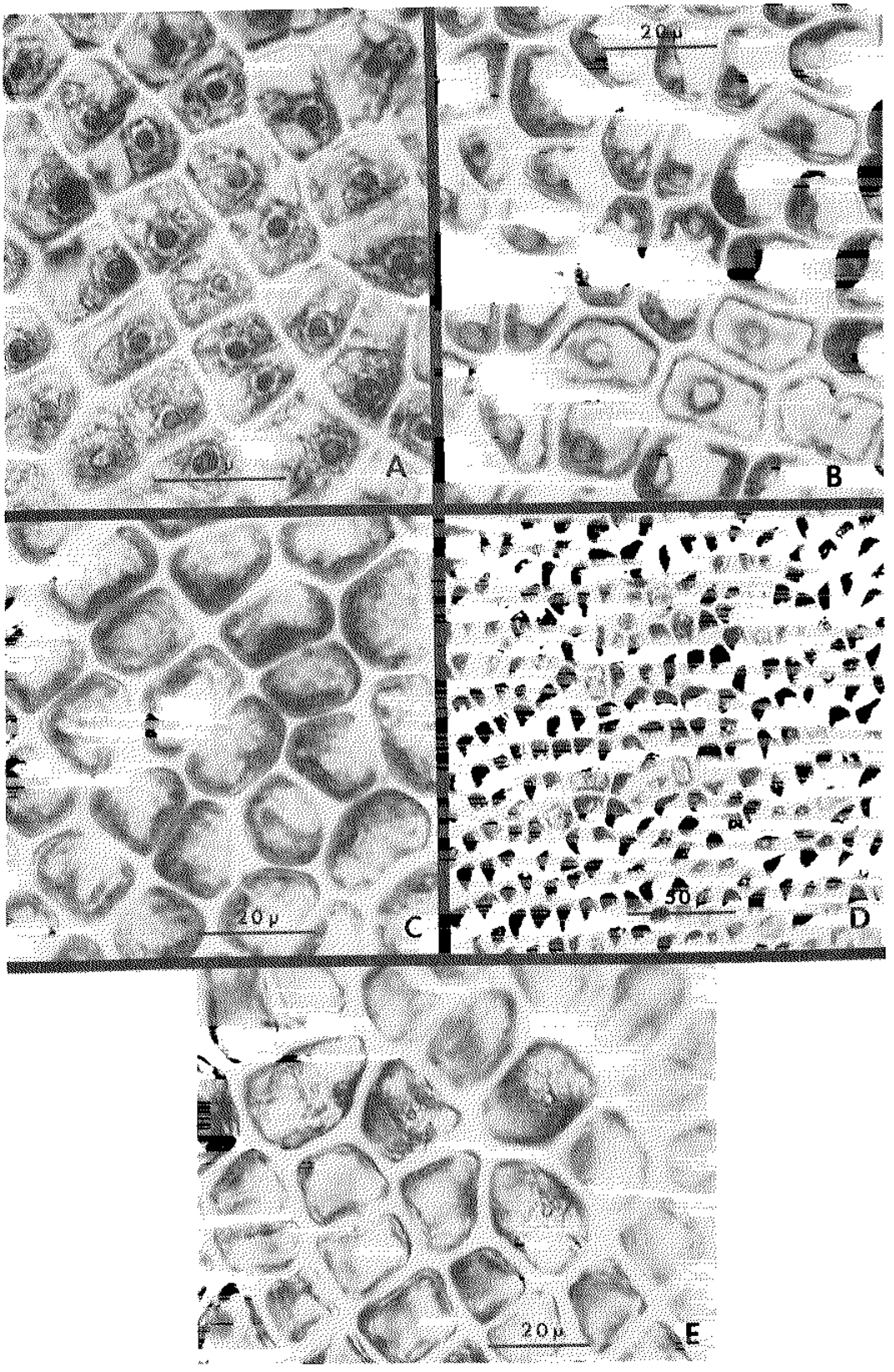

20 µ
A
20 µ
B
20 µ
C
50 µ
D
20 µ
E

FIGURE 22

A-H: Nuclear position and mitosis in *Ulva curvata*

A-B: Cross section showing nucleus after it has migrated to a position adjacent to the inner cell membrane

C: Interphase

D: Prophase

E: Metaphase

F: Late Anaphase

G: Telophase

H: Daughter nuclei

I-J: Nuclear division in *Ulva rotundata*

I: Nucleus in late prophase at the upper cell membrane

J: Daughter nuclei

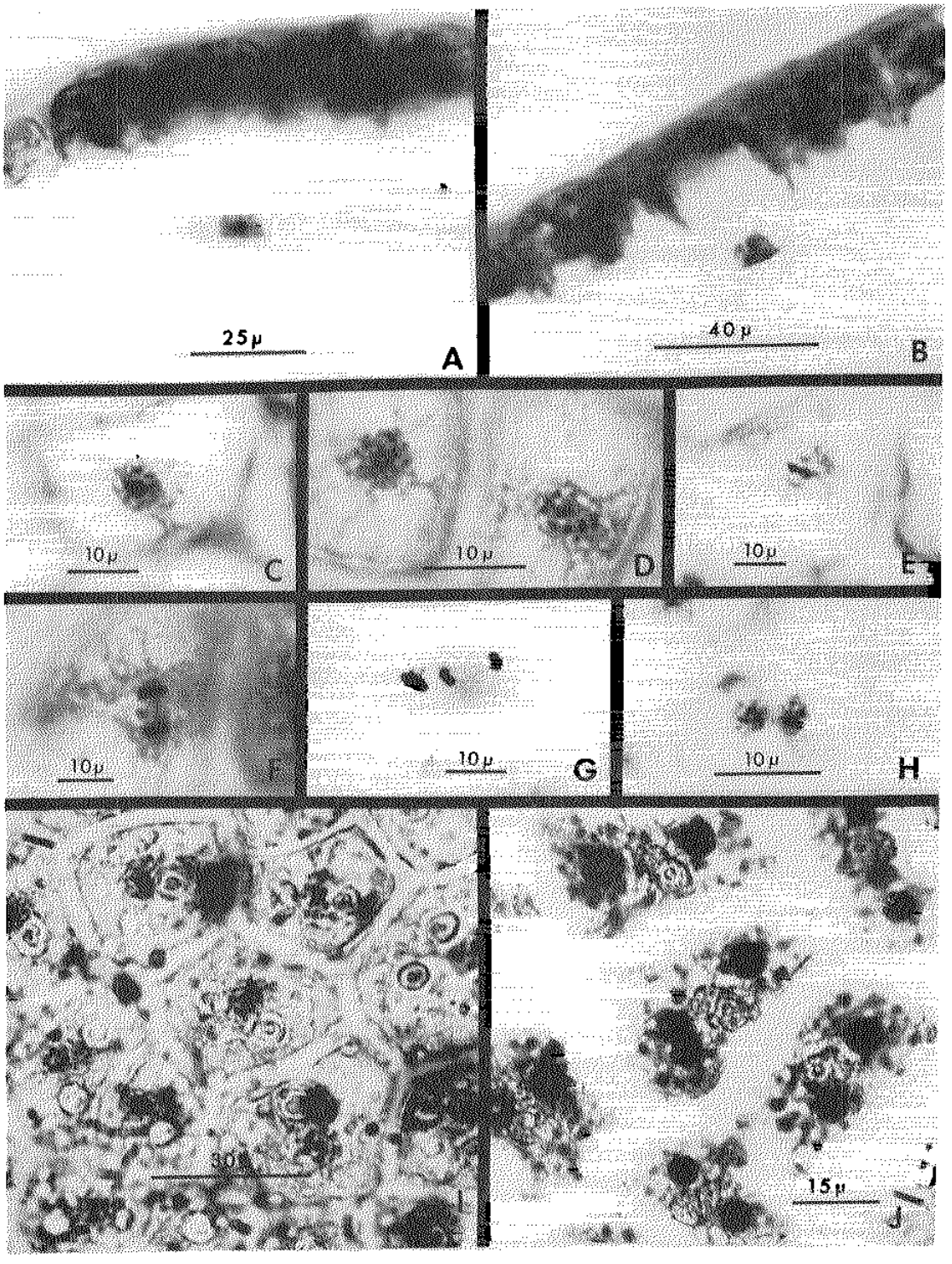

25 µ
A
40 µ
B
10 µ
C
10 µ
D
10 µ
E
10 µ
F
10 µ
G
10 µ
H
30 µ
I
15 µ
J

FIGURE 23

Mitotic index in cells of *Ulva curvata*

Female gametophyte . . . . ●

Male gametophyte . . . . . △

Parthenosporophyte . . . . ▫

Indices are based on measurements of six fields (130 ± 6 cells/field) for three discs of each thallus type

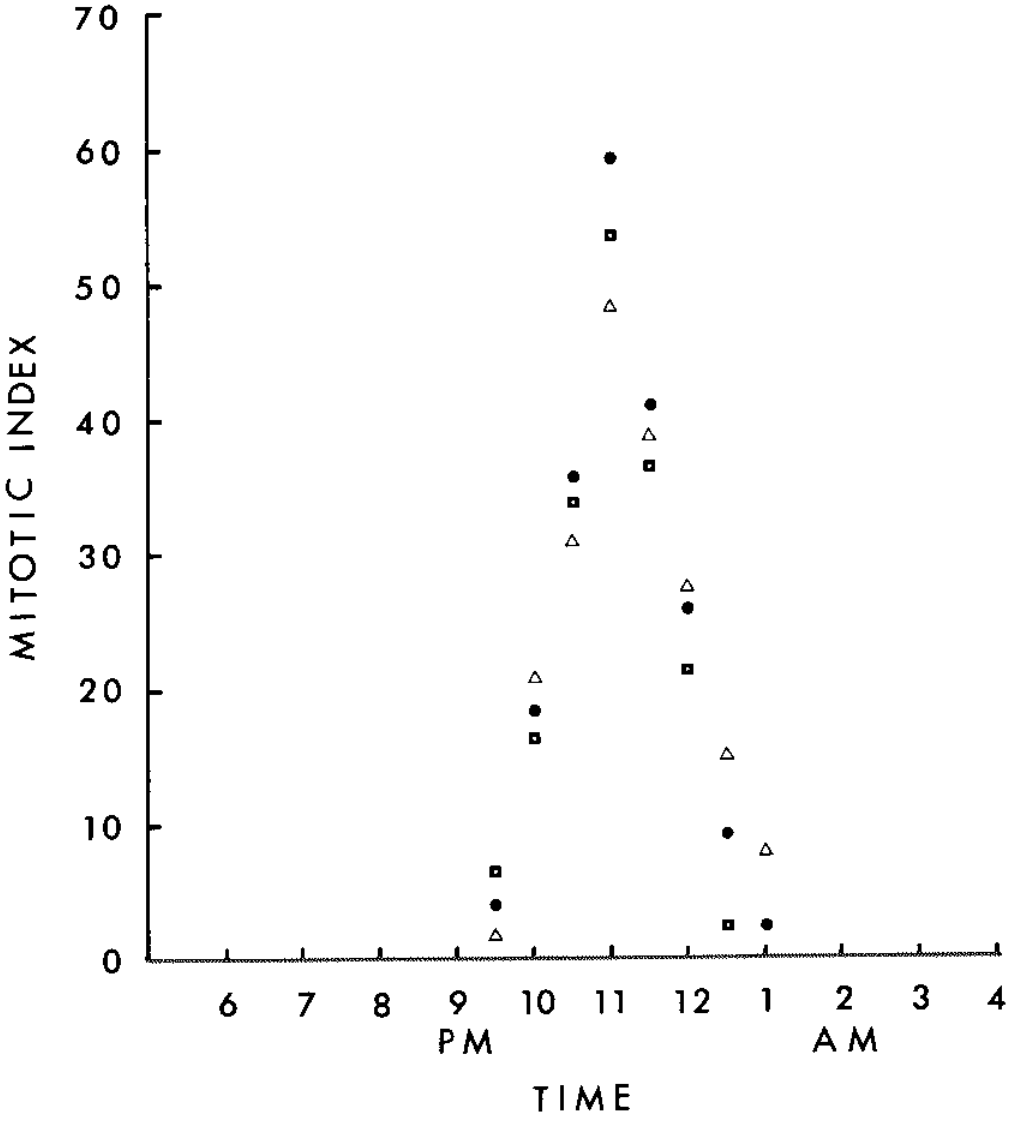

70
60
50
40
30
20
10
0
MITOTIC INDEX
6
7
8
9
10
11
12
1
2
3
4
PM
AM
TIME

FIGURE 24

Laboratory observations on isolated reproductive races in *Ulva curvata*

I. Isomorphic alternation of generations

IIa. Isolate originating as a parthenogenetically developed haploid gametophyte later giving rise to biflagellated gametes, quadriflagellated zoospores and quadriflagellated fused swarmers from the same thallus

IIb. Isolate presumed to have originated as a diploid sporophyte later giving rise to the same three types of swarmers as in IIa

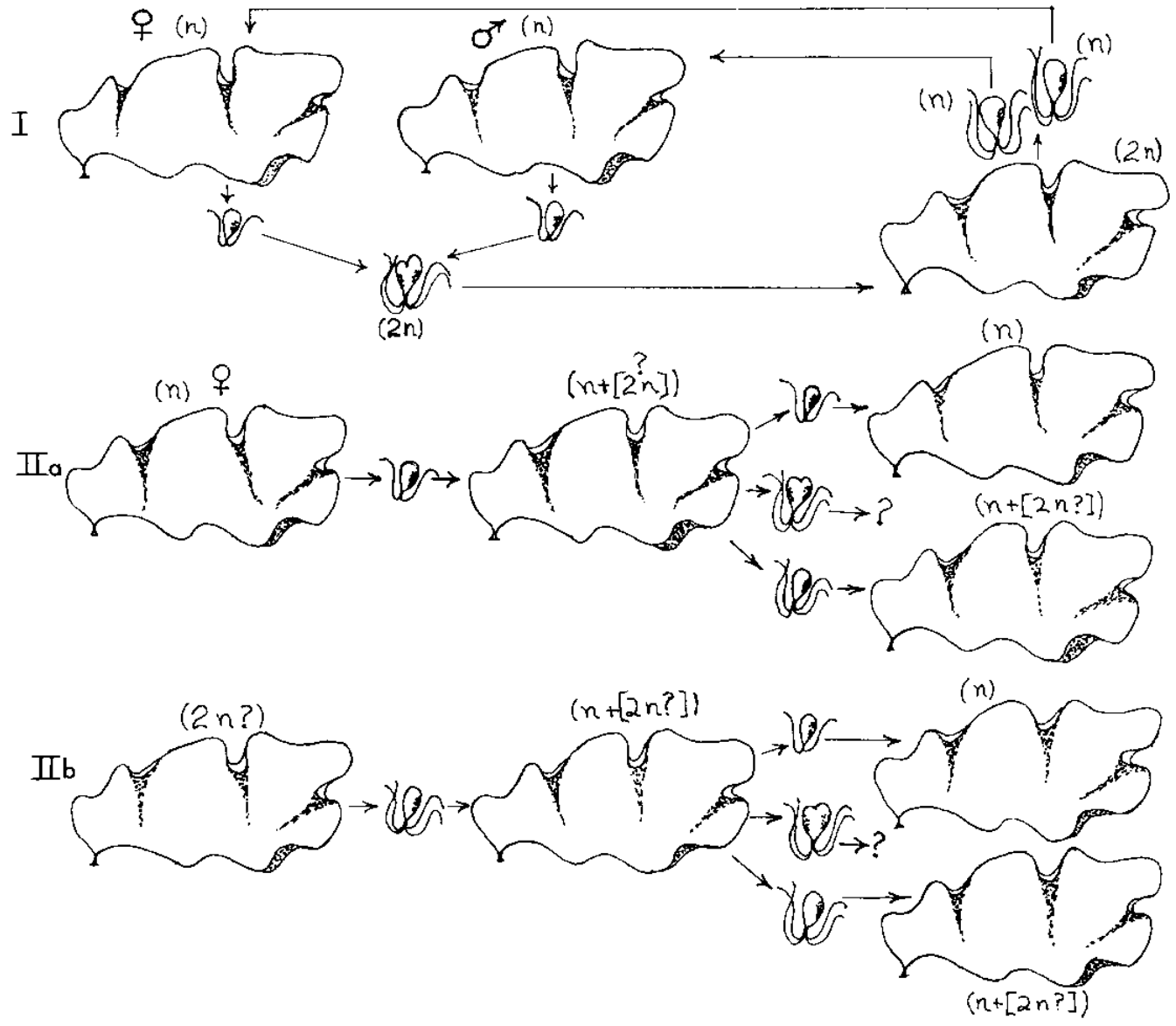

I
♀ (n)
♂ (n)
(n)
(n)
(2n)
(2n)
IIa
(n) ♀
(n+[2?n])
(n)
?
(n+[2n?])
IIb
(2n?)
(n+[2n?])
(n)
?
(n+[2n?])

FIGURE 25

A-C: Chromosome figures in *Ulva curvata*

A-B: Haploid number of 10-12 observed in all thalli releasing biflagellated gametes

C: Diploid number of 20-24 observed in all thalli releasing quadriflagellated zoospores

D: Quadriflagellated isogamously fused swarmer

E: Normal anisogamously fused male and female gametes

F-G: Areas of large, presumedly diploid cells observed among smaller haploid cells in parthenogenetically developed gametophyte plantlets

H: Habit of plant of *Ulva curvata* collected late in the spring, 1971, that resembles *Ulva rigida*

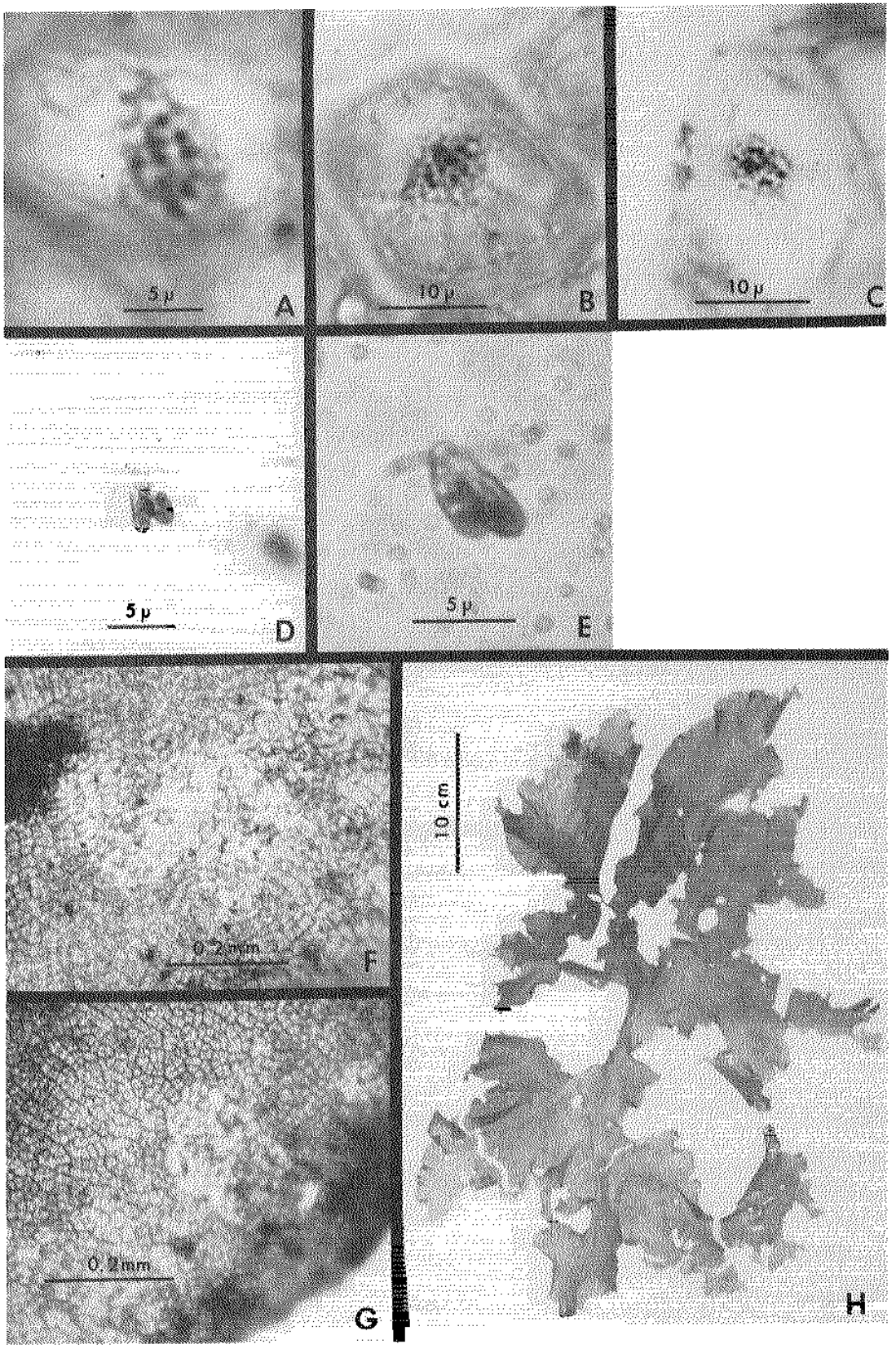

5 μ
A
10 μ
B
10 μ
C
5 μ
D
5 μ
E
0.2mm
F
0.2mm
G
10 cm
H

FIGURE 26

Proposed life cycle for *Ulva curvata* based on both field and laboratory observations

A: Biflagellated male and female gametes released from haploid gametophytes

B: Anisogamous gamete fusions producing diploid sporophytes

C: Negatively phototactic quadriflagellated zoospores producing haploid male and female gametophytes

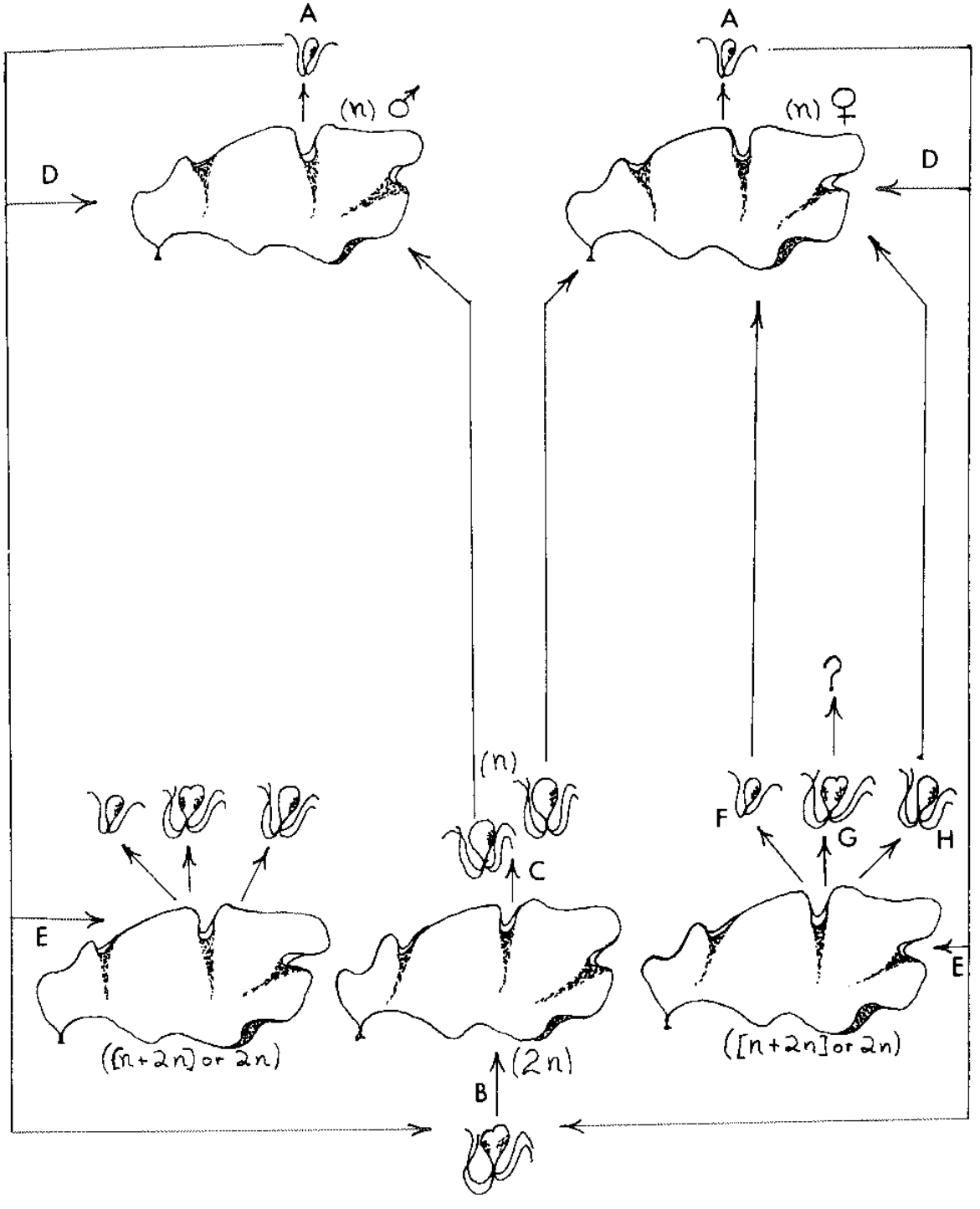
D: Parthenogenetic development of male and female gametes into haploid gametophytes

E: Development of parthenosporophytes either with mosaics of diploid and haploid tissue or entirely diploid. (Partheno-sporophytes developed from both male and female gametes released three types of swarmers designated F, G and H. Swarmers released from male parthenosporophytes were not observed.

F: Biflagellated gametes identical to those in A

G: Isogamously fused zygote-like bodies the size of male or female gametes

H: Quadriflagellated zoospores smaller than those in C and positively phototactic giving rise to haploid gametophytes


A
(n) ♂
D
A
(n) ♀
D
?
(n)
C
F
G
H
E
E
([n+2n] or 2n)
([n+2n] or 2n)
(2n)
B

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UNC-SG-73-09. Rhyne, C. F. Field and ecological studies of the systematics and ecology of Ulva curvata and Ulva rotundata.

* Available from: Sea Grant Program, School of Public Health, University of North Carolina, Chapel Hill, N. C. 27514