A SURVEY OF ZOOPORE AND SPERM ULTRASTRUCTURE
IN THE LAMINARIALES (PHAEOPHYCEAE)
by
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Date **11 August 1980**
Zoospores of 17 species in 14 genera and sperm of 13 species in 11 genera of Laminariales were studied by electron microscopy. The zoospores are unique in the brown algae in lacking both an eyespot in the single chloroplast and an associated swelling at the base of the shorter, posterior flagellum. Spores of all species possess a distal whiplash on the longer, mastigoneme-bearing anterior flagellum; although it is only seldom preserved for electron microscopy, this appendage may sometimes be as long as the mastigoneme-bearing portion of the flagellum. A microtubular cytoskeleton with connections to the flagellar basal bodies is responsible for maintaining the shape of the zoospore.

The sperm are also unique in the brown algae. They are elongate and possess two to three plastids and several mitochondria, but lack an eyespot. Their most distinctive feature is the long posterior flagellum which tapers distally as the doublet microtubules of the axoneme are transformed into singlets and then decrease in number. The sperm also bear a distal whiplash on the mastigoneme-bearing anterior flagellum.

These laminarialean zoospores and sperm are ultrastructurally distinct from those known in other orders of brown algae, and from Chorda of the Laminariales, which confirms that Chorda is primitive. The longer posterior flagellum of the sperm recalls similar modifications in sperm of other oogamous brown algae, and suggests a common
functional significance.
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CHAPTER 1. INTRODUCTION

I. Importance Of Cytological Characteristics In Algal Taxonomy

In the many divisions and classes of algae recognized in various classification schemes, the nature of the flagellar apparatus is of primary importance. The Rhodophyta are characterized by complete absence of flagella or related structures such as centrioles. Pyrrophyta and Euglenophyta possess many unique cytological features in their nuclear organization and cell armor but can be just as easily separated by their distinctive flagellar arrangements. Among the Chlorophyta, the Prasinophyceae and Charophyceae are readily separated from each other and other Chlorophyta by peculiar features of their flagella. The relationship of the classes comprising the Chromophyta of Christensen (1962, cited in Manton 1965) is based on flagella as much as pigmentation, and the recent recognition of the classes Eustigmatophyceae (Hibberd and Leedale 1970) and Prymnesiophyceae (Hibberd 1976) are based largely on flagellar characteristics. It is often suggested that the Oomycetes are descended from a heterokont algal ancestor (Taylor 1978); and recent work (Barr and Hadland-Hartmann 1978) attempts to construct a taxonomy and phylogeny of Chytridiomycetes on the basis of zoospore ultrastructure, including consideration of the flagellar apparatus. In a recent text (van den Hoek 1978) the Chrysophyceae, Xanthophyceae, Bacillariophyceae,
Chloromonadophyceae, and Phaeophyceae are grouped into the Heterokontophyta, based on the possession of heterokont flagella (one with mastigonemes, one without) or an obvious modification of the heterokont plan (e.g., uniflagellate sperm of centric diatoms).

II. Motile Cells Of Brown Algae

Within the Phaeophyceae the only flagellated cells are zoospores and gametes (collectively termed "swarmers"), and they have received relatively little attention from taxonomists; classification has been based largely on vegetative construction and life history. Before the advent of electron microscopy it was known (Fritsch 1945) that swarmers of brown algae (with the exception of the uniflagellate sperm of at least some Dictyotales) possess two laterally attached flagella unequal in length, the longer directed forward and bearing mastigonemes (Longest 1946), the shorter directed backward and bearing only a terminal "whiplash" (except in some Fucales the posterior flagellum is the longer). An eyespot was known to be located near the attachment of the flagella, associated with a plastid; one or more plastids and one nucleus were known to be present. The size of the nucleus was a subject of controversy among earlier light microscopists (Kylin 1920).

To date, approximately thirty-five publications have reported on electron microscopical studies of phaeophycean reproductive cells, confined to observations of the orders Ectocarpales, Chordariales, Cutleriales, Dictyotales,
Laminariales and Fucales. Some examine only one species but in
detail (Ectocarpus: Baker and Evans 1973a, 1973b; Lofthouse and
Capon 1975; Pilayella: Markey and Wilce 1976a, 1976b; Cutleria:
Caram 1975, La Claire and West 1978, 1979; Chorda: Toth 1974,
1976a; Fucus: Manton and Clarke 1951a, 1951b, 1956, Berkaloff
and Rousseau 1979). Others treat only one aspect in several
species (Loiseaux 1973: unilocular sporangia of Pilayella,
Elachista, Hecatonema; Manton et al. 1953: flagella of sperm
of Dictyota, Himanthalia, Pelvetia, Ascophyllum, Fucus;
Loiseaux and West 1970: mastigonemes of Compsonea, Giffordia,
Hecatonema, Leptonematella, Myrionema, Ralfsia). Many studies
are only concerned with one aspect of one species (e.g., Bouck
1970, on eyespots of Fucus sperm; Cassell and Pollock 1978 on
Fucus sperm mitochondria) or only superficially examine several
aspects. Several of the earlier papers used techniques that
have become so outmoded that the work needs to be repeated with
modern methods, as the results cannot be compared usefully with
recent findings.

There has been no attempt at a comprehensive survey of
phaeophycean motile cells since Manton's (1964) comparison of
the Scytosiphon zoospore with the sperm of Fucales and
Dictyota, but it is now possible to make some further
generalizations based on additional available information. It
appears that the primitive phaeophycean motile cell always
lacks a cell wall, but possesses a nucleus with a closely
associated dictyosome, several mitochondria, one or more
chloroplasts, and two flagella inserted laterally. The longer,
anteriorly directed flagellum bears mastigonemes, whereas the
shorter, trailing flagellum has no lateral appendages but possesses a basal swelling intimately appressed to the plasmalemma directly overlying an eyespot contained within a chloroplast. The basal bodies of the flagella are associated with a group of microtubules that appears to serve as a cytoskeleton, maintaining the shape of the swarmer.

III. Significance Of Motile Cell Specialization

A. The "Primitive Fucoid" Of Manton

Manton (1964) proposed that the modifications she observed in the sperm of Fucus were evidence that Fucus was less primitive than Cystoseira, whose sperm resembles the zoospores of Scytosiphon. Formerly, great emphasis had been given to the reduction of the number of eggs produced in the oogonium of these genera, eight in Fucus considered more primitive than the one (with seven aborted nuclei) in Cystoseira (Fritsch 1945). Before Manton, Kylin (1940) had stressed the difference between the sperm of Fucus (with its longer posterior flagellum) and zoospores of non-fucalean brown algae (with their shorter posterior flagella). He proposed the segregation of the Order Fucales into a separate Class Cyclosporeae, which he considered to have originated from a phyletic line of diploid ancestors entirely removed from the other brown algae that show alternation of haploid and diploid generations. He failed to note that only sperm of the family Fucaceae (indeed only those of the Northern Hemisphere) have this flagellar pattern.
Although Fritsch (1945) stated with regard to the relative lengths of the two flagella in the Fucales "The evidence...is conflicting," many subsequent authors (Papenfuss 1951, 1955; Smith 1955; Scagel et al. 1965; Scagel 1966; Dawson 1966; Bold and Wynne 1978; Boney 1978) have accepted Kylin's contention. However Manton, who did not seem to be aware of the controversy, had pointed out that in several British Cystoseiraceae the sperm "In shape...are all exactly comparable to the average brown algal zoospore...As in the zoospore...the short hind flagellum being apparently stuck to the surface of the eyespot and the longer front flagellum being a 'Flimmergeissel' of the ordinary kind" (Manton 1964, p. 247).

Other significant modifications of brown algal sperm are the "proboscis" of the Fucaceae with the longer hind flagellum, spiny flagella, and the lack of eyespots. All these specializations are confined to gametes of oogamous taxa, where the radical transformation of the female gamete from a swimming isogamete to a non-motile egg as much as 20,000 times the mass of the sperm (e.g., Fucus, Jaenicke 1977) has proceeded hand in hand with modification of the male gamete.

The taxonomy of the brown algae has traditionally been based on consideration of both vegetative structure and reproductive characteristics, and among the latter the type of life history and the form of the sporangia are paramount. It is obvious that life history and reproductive structures are functionally related, so one would anticipate that those orders possessing the more specialized, oogamous life histories (Fucales, Dictyotales, Laminariales, Desmarestiales,
Sporochnales) would have modified sperm. If one wished to determine the variation in sperm structure throughout the brown algae, the first place to look would be at the oogamous taxa that are still poorly known. The means of investigation must be electron microscopy, since contradictory descriptions have resulted from light microscopy of these small cells. The variation in sperm structure among the families of the Fucales suggests that sperm ultrastructure may be useful for distinguishing taxa at the level of family as well as order. Variation in the occurrence of eyespots (Sauvageau 1911, Roberts 1978) might be a good indicator of phyletic series within families, as loss is presumably an advanced characteristic.

Speculation has been made (Manton 1964) about the possible functional significance of sperm modifications. While only experimental studies (cf. Jaenicke 1977) can reveal the true importance of these structural alterations, a more complete knowledge of the variations among the different phyletic lines with oogamous reproduction may facilitate our comprehension by discovering universal changes relating to oogamy per se.

IV. Choice Of Laminariales As Subject Of Investigation

I chose to survey the ultrastructure of zoospores and sperm of Laminariales for several reasons. The Laminariales are not well known ultrastructurally, and the great difference between the sporophyte and gametophyte in size and construction suggests that the life history is highly derived (Fritsch 1945). All Laminariales are oogamous, so a specialized sperm
may be expected, especially as many reports (Sauvageau 1918, Kanda 1936, 1938, Fritsch 1945, Kemp and Cole 1961) suggest that the zoospores of some species lack eyespots and so are unique in the brown algae.

The Laminariales are also very diverse, with 16 genera and perhaps 30 species in the British Columbia flora. Nine of these genera are monotypic, but two, Laminaria (9 species) and Alaria (5 species) have enough representatives in our waters to permit the study of both inter- and intra-generic variation. Because zoosporangia in Laminariales are borne in sori whose presence and degree of maturity are evident in the field, and culture methods for growing the gametophytes are well established, this order is especially suitable for an ultrastructural study of its reproduction.

Furthermore, there is an extensive literature covering reproduction in the Laminariales (Fritsch 1945). Since Fritsch published his review of algal structure and reproduction, there have been numerous further light-microscope studies (especially those of Cole et al. and various Japanese and European workers). Ultrastructural studies have been limited to those on Laminaria (Manton and Clarke 1951, whole mounts of spores only; Bisalputra et al. 1971, oogenesis only), Chorda sporogenesis, release and germination (Toth 1974, 1976a), Macrocystis sporogenesis (Chi and Neushul 1972, Gherardini and North 1972, Chi 1973) and a very incomplete account of Macrocystis spermatogenesis (Gherardini and North 1972).

This body of literature shows that certain issues should be addressed, such as presence or absence of eyespot, number of
plastids, and presence or absence of the anterior "whiplash" in both zoospores and sperm. The diversity of species ensured that a good account of variation within the order could be made from the information gathered, and common features would be seen often enough so that generalizations about the whole order would be justified, such as mechanisms of sporogenesis and spermatogenesis. With the results of such a survey in hand it would be possible to make more informed approaches to investigation of other brown algae, as well as to gain a better evaluation of relationships between the Laminariales and the rest of the Phaeophyta.
CHAPTER 2. MATERIALS AND METHODS

I. Collections

Most sporophytes were collected in the environs of the Bamfield Marine Station, on Barkley Sound, Vancouver Island. Additional Vancouver Island collections were made at Port Hardy, Botanical Beach (near Pt. Renfrew), Sombrio River, Sooke and Victoria. Other collections were made at Bath Island, off Gabriola Island in the Strait of Georgia; Stanley Park, Vancouver; Whidbey Island, Washington; and Marine Gardens and Boiler Bay, Oregon.

Table I lists the taxa collected, and shows whether zoospores, sperm, or both were studied, and by which techniques.
<table>
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<th>Species</th>
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<th>Sperm</th>
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<td>Whole mount</td>
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<td>Bory</td>
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<tr>
<td>2. <em>Alaria marginata</em></td>
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<tr>
<td>Postels et Ruprecht</td>
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<td>3. <em>A. nana</em></td>
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<td>Schrader</td>
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<tr>
<td>4. <em>A. taeniata</em></td>
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<tr>
<td>Kjellman</td>
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<td>5. <em>A. tenuifolia</em></td>
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<td>Setchell</td>
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<tr>
<td>6. <em>Costaria costata</em></td>
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<tr>
<td>(Turner) Saunders</td>
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<tr>
<td>7. <em>Cymathere triplicata</em></td>
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<td>(P. et R.) J. Agardh</td>
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<tr>
<td>8. <em>Dictyoneurum californicum</em></td>
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<tr>
<td>Ruprecht</td>
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<tr>
<td>9. <em>Eisenia arborea</em></td>
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<td>Areschoug</td>
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<td>10. <em>Hedophyllum sessile</em></td>
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<tr>
<td>(C. Ag.) Stechell</td>
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<td>11. <em>Laminaria groenlandica</em></td>
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<td>Rosenvinge</td>
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<td>12. <em>L. saccharina</em></td>
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<td>(L.) Lamouroux</td>
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<td>13. <em>Lessoniopsis littoralis</em></td>
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<td>(Tilden) Reinke</td>
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<td>14. <em>Macrocystis integrifolia</em></td>
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<td>Bory</td>
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<tr>
<td>15. <em>Nereocystis luetkeana</em></td>
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<td>(Mert.) P. et R.</td>
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<td>16. <em>Pleurophycus gardneri</em></td>
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<td>Setchell et Saunders</td>
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<tr>
<td>17. <em>Postelsia palmaeformis</em></td>
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<tr>
<td>Ruprecht</td>
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<tr>
<td>18. <em>Pterygophora californica</em></td>
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II. Gametophyte Cultures

Gametophyte cultures were established from field-collected sporophytes by cutting pieces of fertile sori from plants kept under refrigeration for one to four days after collection, washing the pieces in cold running tap water to remove contaminating organisms, and leaving the pieces in 0.4 um-filtered seawater in petri dishes at 10 C until spore release occurred (within a few minutes to several days). The sorus pieces were then removed, and after spore settlement the seawater was replaced with culture medium.

All cultures were maintained in 15 x 90 mm plastic petri dishes, in SWM-3 medium (Chen et al. 1969), without soil extract, liver extract, or TRIS buffer, at 5, 10, or 15 C. Light was provided by cool-white fluorescent tubes at intensities of 7.7-45 uE/sq. m/sec with a 12:12 or 18:6 -hour light:dark photoperiod.

Stock cultures were maintained with changes of medium at one- to three-month intervals; these cultures generally remained vegetative. Subcultures were started by gently separating the entangled gametophytes by hand with a tissue homogenizer, and growing these gametophytes at a low density (1-5/sq. mm) under red light ("cinemoid" No. 14 "ruby" filter) at a light intensity of 1.1-5.5 uE/sq. m/sec or elevated temperature (15-19 C) under white light for one to four months. When gametophytes were clearly differentiated as to sex, and were large enough to be individually picked up by pipette or forceps, separate male and female cultures were started and grown under red light at 10 C for one to four months or under
white light at 10°C for 12 months but without change of medium.
When brought into white light and fresh medium, these cultures
became fertile.

Sperm release was obtained by placing the fertile
gametophytes in drops of filtered seawater in individual
depressions of Terasaki dishes (Falcon Plastics). The sperm
were thus released into a small volume of water so that high
concentrations were available for electron microscopy.

III. Light Microscopy

An Olympus CK inverted microscope providing magnifications
up to 400x was used to monitor the condition of cultures and to
observe spore and sperm release. A Leitz cooling stage was
fitted to a Wild M20 microscope for long-term (several hours)
observations and photography of spores and sperm at higher
magnification. This microscope was fitted with Wild phase-
contrast optics; additionally, an Olympus "Hoffman modulation
contrast" condenser and objectives were adapted to this
instrument to take advantage of the cold stage. During
prolonged observation, spores and sperm were maintained in an
observation cell made by removing a rectangle from the central
area of a piece of acetate adhesive tape stuck to a microscope
slide. A drop of cell suspension was placed in this rectangle
and covered with a coverslip, forming a well of reasonably
large volume, shallow enough that its full depth could be
focused under oil immersion, and that could be easily sealed to
prevent evaporation.

Photographs were taken with a Leica M1 or Wild
Microphotoautomat 35 mm camera using Kodak PAN-X, TRI-X, Ektachrome or Kodachrome films.

IV. Electron Microscopy

For electron microscopy of zoospores before release, pieces of mature sori were cut up and placed in fixative. For pre-release sperm, gametophytes were transferred from culture medium to fixative. Swimming sperm and spores were collected on Millipore filters (Bisalputra et al.; 1973); 3 um pore size was used for very dense spore suspensions, 0.45 um for sparse spore and sperm suspensions. After fixative was passed through the filter, the membrane with adherent cells was removed from the filtration apparatus and processed as if it were a thin piece of tissue. Filters with very sparse cell aggregations were cut into convenient sized pieces and embedded in agar after osmication to prevent loss of cells.

Several fixative recipes were employed. Initially, the fixative mixture contained 0.1M cacodylate buffer diluted 1:1 with filtered seawater, 2.5% glutaraldehyde and 5% sucrose, at pH 7.4. Other combinations tried were 2% glutaraldehyde in 0.1M cacodylate with 10% sucrose; and 2% glutaraldehyde and 2% paraformaldehyde in 0.1M cacodylate buffer with 6.8% sucrose. The best results were obtained with the use of 0.3M PIPES buffer (Salema and Brandao 1973), usually with 2.5% glutaraldehyde and 6.6% sucrose (PIPES buffer is apparently non-toxic and is therefore safer to handle than cacodylate).

Other additives used from time to time were 0.1% osmium tetroxide (Barr and Hadland-Hartmann 1978) or 2%
paraformaldehyde.

After aldehyde fixation, a graded series of rinses served to gradually lower the osmolarity of the specimen environment prior to postfixation with 1% osmium tetroxide: first rinse in 0.3M PIPES with 6.6% sucrose; second rinse in 0.15M PIPES in 5% sucrose; third rinse in 0.15M PIPES without sucrose, then osmication in 0.07M PIPES. Osmication proceeded at room temperature for 2-3 h or overnight under refrigeration followed by 1-2 h at room temperature. Occasionally 0.1% ruthenium red was added to the postfix to enhance polysaccharide staining, or thiocarbohydrazide treatment (OTOTO of Postek and Tucker 1977) to enhance lipid retention. Rinsing in distilled water was followed by dehydration in a methanol series of 10%, 25%, 50%, 70%, 90% and 100% methanol with 0.5 NaCl added (Millonig 1966), several changes in 100% methanol without NaCl, 1:1 methanol:propylene oxide, and 100% propylene oxide, followed by gradual infiltration with Epon, Spurr's resin or the ultra low viscosity resin of Mascorro et al. (1976). Resin was polymerized at 70 C (or 85 C for Mascorro resin), for 8-24 h. Sections were cut with glass or diamond knives on a Reichert OM-3 ultramicrotome, mounted on carbon-stabilized, colloidin-coated grids, stained routinely in uranyl acetate and lead citrate, and viewed in a Zeiss EM-10 electron microscope.

For whole-mounts of spores and sperm, the swimming cells were fixed by addition of glutaraldehyde to a 1% concentration, or by osmium tetroxide to a 0.1% concentration, or both fixatives together. The suspension of fixed cells was centrifuged at 1000 X G for five minutes, the cell pellet
rinsed in distilled water, centrifuged and resuspended, then drops of cells were placed on colloidal-carbon coated grids and allowed to air dry. Alternatively, especially when only small numbers of cells were available, individual drops of cell suspension were placed on coverslips, fixed in vapor from 2% osmium tetroxide for one minute and transferred to grids after 15 min, or placed directly on grids and vapor fixed. To observe spore settlement, drops of spore suspension were placed on gold grids (to avoid copper toxicity that might affect spore behavior) coated with colloidal-carbon. These were stuck at a tiny portion of the margin to the edge of masking tape glued sticky-side up on a microscope slide, with the grid film facing upward. The slide was inverted and put on the cooling stage over a water chamber to prevent evaporation, and observed until spores could be seen settling, whereupon the inverted slide was placed over an osmium vapor chamber to fix the spores in the act of settling. After vapor fixation, the seawater was allowed to evaporate dry and the salts were rinsed away by application and removal of individual drops of distilled water. After final drying, the cells were observed in the electron microscope, or were first shadowed with platinum-palladium or negative-stained with phosphotungstic acid.
CHAPTER 3. RESULTS

These results represent a composite of all data derived from the study of zoospores of 14 genera and sperm of 11 genera of Laminariales.

I. Zoospores

A. Sporangia

All Laminariales produce their sporangia in aggregations termed sori. The sorus is distinguishable macroscopically as a raised region, usually darker than or a different color from the neighboring vegetative tissue. It is comprised of sporangia and accompanying sterile cells, the paraphyses, which are longer than the sporangia (Figs. 1, 2). The abutting distal tips of paraphyses provide a protective cover for sporangia. Sporangia and paraphyses are produced (at least initially) in a 1:1 ratio, as each paraphysis is produced by a meristoderm cell which next produces a sporangium.

Paraphyses contain large osmiophilic globules, which are absent in the sporangium (Figs. 3, 4) and may represent tannic substances that serve to deter grazing (Conover and Sieburth 1965). Often an outer layer, which has been termed a "cuticle" (Hanic and Craigie 1969), is produced at the tips of the paraphyses (Figs. 1, 2, 3). The ultrastructure of this layer is similar to that of the underlying paraphysis wall. It could be
an artifact of differential shrinkage during preparation for microscopy, which results in a pulling away of the oldest layer of wall material. In preparations made from sori that have already released most of their sporangial contents, the tips of paraphyses are often swollen and no longer confluent (Fig. 6), implying either that pre-existing lines of weakness allow tips to separate, or that an enzyme dissolves the substance holding the tips together. Dissolution of hydrophilic polysaccharides in the wall would result in swelling of tips of paraphyses.

Sporangia are clavate and very elongate, typically 100 μm long and 15 μm in diameter. The walls are thinner than the walls of paraphyses (Fig. 7), and the distal tips are lined with a "cap" of material that appears granular in the electron microscope, in contrast to the fibrillar appearance of the wall proper (Figs. 4, 5). This cap underlies the site of dehiscence of the sporangium, and so presumably serves some function relating to bursting of the cell wall. Sporangia often develop with varying degrees of synchrony (cf., Angst 1929), for uncleaved sporangia are found next to apparently mature ones (Fig. 7). Even the uncleaved sporangia show considerable organization in the association of organelles; a given plane of section frequently shows many basal bodies, nuclei or other organelles in similar configurations in different areas of the section (Fig. 8). This indicates that the development of spores within a sporangium is very tightly coordinated.
B. Zoospore Structure

As cleavage of the cytoplasmic mass of the sporangium occurs, the spores begin to take shape. Their pre-release form is largely determined by the close packing that prevails before the sporangium is mature (Figs. 9-11), although in later stages some longitudinal extension is evident (Figs. 12, 13). They measure from 4 x 4 um to 4 x 8 um, depending on the orientation of the section through the spore. After release, the spores are free to adopt a more elongate form and measure about 5 x 7 um in sections. Whole mounts that retain their shape, and (presumably) are shrunken minimally during air-drying, measure about 4 x 8 um. In some specimens the anterior "nose" of the spore is still apparent (Fig. 15). It is possible for the mass of the uncleaved spores to be prematurely released from the sporangium, presumably with the protruding flagella functional (Fig. 15); the fate of such a spore mass is unknown.

Cytological Components Of The Zoospore

The microtubular apparatus consists of the cytoskeleton and the flagellar axonemes. The flagella are produced prior to cleavage of the sporangial cytoplasm; they all protrude from the cytoplasm of the sporangium, the basal body of each associated with a chloroplast (Figs. 8, 9). After cleavage, elements of the cytoskeleton occasionally become visible. "Bands" of parallel microtubules (Figs. 14, 17), as well as apparent single tubules (Fig. 13), are present. It is the cytoskeleton which permits the zoospore to have a form other
than the lowest-energy shape of a sphere.

There is a definite association, and perhaps connection, between the cytoskeleton and the basal bodies of the flagella. Clearest is the association of a band of 7-9 microtubules with the anterior basal body (Figs. 17, 20-24). Immediately adjacent to longitudinal sections of basal bodies a dense granularity can be seen (Figs. 18, 19, 21, 23C); this may represent the termination of a tubular band (Fig. 24), although it is difficult to determine whether or not it is tubular. It is seen only in certain sections, so it does not surround the basal body. In sections where the tubular construction of the bands is clear (Figs. 17, 22, 23B), the tubules can sometimes form an arc, which explains why a band is rarely caught entirely in the plane of a longitudinal section. A short distance from the basal bodies some of the microtubules spread out into the cytoplasm and are less conspicuous, especially in oblique section. The anterior band (or at least some tubules, perhaps separated) arcs around the anterior periphery of the cell (Figs. 13, 25). This provides the necessary support for the prominent "nose" of the spore. Other tubules converge at the point of the nose (Fig. 26); their relationship with the microtubules of the bands or the basal bodies is not clear. Occasionally, microtubules can be seen in the posterior portion of the spore (Fig. 26), but the presence of large organelles such as the nucleus and plastid probably provides most of the rigidity for this part of the cell.

The cytoskeletal configuration is best visualized with the help of "exploded" cells, in which only the microtubular
apparatus is preserved, the rest of the spore having been removed by osmotic and surface tension shocks during preparation for electron microscopy (Figs. 27-30; all these specimens are of *Alaria tenuifolia*). Eight microtubules can be counted in the anterior band (Fig. 28); in some preparations the anterior and posterior basal bodies were connected by a short band (Figs. 27, 29, 39, 43) of about 10 microtubules (Fig. 30). In some specimens (Figs. 28, 29) the anterior band extends at least 8.3 μm; it may loop around the cell periphery to connect with the posterior basal body (Figs. 24, 32), forming a continuous strand (Fig. 27).

The flagella constitute the remainder of the microtubular apparatus. They are similar to those that have been described for zoospores of other brown algae by some workers, although they are not formed within cytoplasmic vesicles as is the case in many species. They are inserted laterally (Fig. 31), and it is convenient to designate the side of the spore bearing the flagella as the ventral surface. Before release the basal bodies viewed in section were sometimes parallel to each other (Figs. 33, 34), but after release they are inserted perpendicular to each other (Fig. 32), so reorientation must occur as the released spore changes to a swimming posture. In this configuration the spore becomes almost bullet-shaped and the anterior flagellum is directed forward, while the posterior flagellum trails to the rear (Fig. 37).

The anterior flagellum consists of two parts (Fig. 37). The proximal portion is up to 22 μm long, bears mastigonemes, and in cross-section shows the usual "9+2" microtubule
arrangement of the axoneme. The distal portion, which may be as much as 17.5 μm long, bears no appendages and is merely an extension of the two central microtubules of the axoneme (Fig. 38). Although this distal "whiplash" was rarely preserved, its presence was evident in all species examined. It may be transitory or very labile, which would account for its rarity.

Before release of the spore the mastigonemes are confined to 1/2 to 1/3 of the circumference of the flagellum (Figs. 34-36). In the swimming spore they are probably arranged in two rows on opposite sides of the flagellum, because whole mounts invariably show uniform distribution of the mastigonemes on both sides (Figs. 37, 42, 45). In disrupted flagella where the mastigonemes remain attached, this same arrangement is found (Figs. 39, 42, 43). The most plausible explanation for this is that the rows are attached to opposite microtubules of the axoneme, rather than to the flagellar membrane. In intact mastigonemes that have been removed from the flagellum, a basal piece may be preserved that is spherical (Fig. 41), presumably the remnant of the attachment to the flagellum. The proximal portion of such detached mastigonemes is 1.4 μm long and in negative-stained preparations (Figs. 40, 91) and cross-sections (Fig. 75) shows a tubular construction with an electron-dense core. The distal portion is a fine filament up to 0.8 μm long (Fig. 44). The mastigonemes usually appear straight in whole mounts, evidence of an inherent stiffness that should be functionally significant.

The posterior flagellum is up to 12 μm long and bears no
mastigonemes (Figs. 15, 47). The terminal attenuated tip usually is well preserved (Fig. 46) and represents a continuation of the two central microtubules as in the anterior flagellum. However, the tip has a swelling which is not present on the anterior whiplash. The usual "9+2" axoneme is present, despite the fact that this flagellum does not appear to beat during locomotion.

"Exploded" flagella show clearly the structure of the axonemes, including the independence of the two central microtubules, in contrast to the integral structure of the doublets (Fig. 27). The coherence of the two singlets in the tip of the posterior flagellum (at the very end of the "9+2" region) is striking (Figs. 27, 28). Similarly, in the anterior flagellum the terminal filament is often not preserved except at its very base (Figs. 38, 42, 99, 103). The anterior flagella were found disrupted much more often than the posterior, and they were always fragmented starting at the distal end, never at the basal end or points in between (Figs. 42, 45). This, combined with the tendency for the distal filament or "whiplash" to be lost, suggests that the end of the anterior flagellum is fundamentally different from the posterior flagellum. Occasionally connections between adjacent microtubule doublets were preserved in the anterior flagellum, but they were not evident in the the posterior flagellum (Fig. 27). If these connections represent dynein arms, their absence would be consistent with the apparent absence of beating in the posterior flagellum.
Plastids

At the time of release, there is one plastid per spore, appressed to the posterior side of the nucleus and folded around it, giving the appearance of two plastids in many sections (Fig. 48). The plastid typically measures 3.5 x 1.25 μm, although when wrapped around the nucleus it may be 8.3 μm long and only 0.75 μm thick. This is smaller than plastids in paraphyses, which measure 7.75 x 2 μm. Plastids possess the usual 3-thylakoid bands with connections between adjacent bands. Occasionally membrane-bound inclusions, presumably thylakoid precursors, were seen in the stroma (Figs. 49, 50, 51). The ring genophore is evident in sections that are cut in a plane perpendicular to the plane of flattening of the "disk" of the plastids (Fig. 49). No pyrenoids were seen.

In the swimming zoospore, part of the plastid is adjacent to the plasmalemma in the region where the posterior flagellum passes posteriorly after emerging from the spore midway along the "ventral" surface (Fig. 31). This is the location of the eyespot in the plastid in all species of brown algae and Chrysophyceae in which this structure is known. However, there was no trace in any of the species examined of an eyespot in the plastids and no pronounced swelling of the posterior flagellum in this region. Plastoglobuli, when present, were scattered and never aggregated into clumps that resembled eyespots (Figs. 49, 50).

The plastid begins to divide after release (Figs. 25A, 52), and by the time wall deposition commences in
the settled spore, the two resulting plastids are separated from each other (Fig. 53).

Mitochondria

The mitochondria are typical of brown algae: ovoid, lobed or cupped at times (Fig. 54) (probably when dividing), commonly measuring 1.7 x 0.4 μm. There are several mitochondria per spore, not precisely located in relation to other organelles, but generally confined to the anterior of the spore, especially in the ventral portion. The tubular cristae possess tubular inclusions lacking in mitochondria of vegetative cells (Figs. 55, 56). These inclusions run parallel to the axis of the crista, number as many as four per crista, and have a diameter of about 12 nm. They may extend nearly the entire length of the crista (Fig. 56). They appear in the cristae before cleavage of the sporangial cytoplasm and persist at least until germination of the settled spore.

Nucleus

The multinucleate sporangium cleaves to form uninucleate spores. The spore nuclei are roughly spherical and measure about 3 μm in diameter. Nucleoli are rarely seen (Fig. 57). Heterochromatin is conspicuous in pre-cleavage nuclei (Figs. 58, 59), and it disappears after cleavage only to reappear before release of the spores, remaining prominent until after spore settlement (Figs. 25, 52, 53). Nuclear pores and extensions of the nuclear envelope are evident (Figs. 60-
The nucleus and plastid are closely associated, the outer membrane of the nuclear envelope being continuous with the chloroplast endoplasmic reticulum (Fig. 62). The nucleus typically is appressed to the plastid on its posterior side, closely associated with both the golgi apparatus and a system of ER stacks toward the anterior (Figs. 64, 65).

Endomembrane System

The perinuclear golgi apparatus is prominent in all stages of spore development from pre-cleavage to the settled spore. It may be nestled in a concavity of the nucleus at times (Fig. 66). Similar stacks of rough ER are frequently present, and in precleavage nuclei these stacks occasionally surround the nucleus completely in one plane (Fig. 67). Sometimes arrays of ribosomes are conspicuous in these stacks (Figs. 68, 69).

The products of both golgi vesicles and ER arrays are not often obvious, despite the presence of numerous membrane-bound inclusions in zoospores. These vesiculate inclusions are of several types. Electron-dense vesicles, presumably osmiophilic although not notably intensified by thiocarbohydrazide treatment, are present from the pre-cleavage stage to germination of the spore. They resemble the larger globules found in the paraphyses, and only occasionally is their bounding membrane discernible (Fig. 70). In the spore they measure up to 0.6 um in diameter, and are usually scattered around the cell, although occasional aggregations may occur (Figs. 14, 71). They most closely resemble the "type A vacuole"
of Rawlence (1973).

Another conspicuous vesicle type, similar to the "type B vacuole" of Rawlence (1973) contains a homogeneous, finely granular material (Figs. 9, 10) that may be partly or wholly leached by some fixations (Figs. 5, 27, 48, 59, 99). These inclusions are often large (to 1.3 um), filling as much as 1/3 of the area of certain spore sections (Figs. 10, 73). They are confluent in many specimens (Figs. 9, 25C, 109), evidently having been squeezed together. Often the bounding membrane is indistinct, and in released spores the contents may break through the plasmalemma (Figs. 25C, 25F). They probably constitute a point of weakness in the structure of the spore where deformation or disruption of membranes is likely to occur. Their contents are retained and enhanced in electron density by thiocarbohydrazide treatment (Figs. 73, 74), supporting Rawlence's (1973) contention that they contain lipid or polyphenolic material, both of which should be osmiophilic.

These vesicles were also present from pre-cleavage through spore germination, and at times the perinuclear rough endoplasmic reticulum (ER) stacks of the pre-release spores appeared to be producing these vesicles (Fig. 72).

A third kind of vesicle, corresponding to the "type C vacuole" of Rawlence (1973), has an electron-transparent matrix with scattered fibrillar or granular material. In early stages these vesicles produce the extracytoplasmic matrix that surrounds the cytoplasm in the sporangium, presumably consisting of alginic acid and fucoidan (cf. McCully 1968a).

Indistinct vesicles such as these were occasionally seen
at the plasmalemma of pre-cleavage sporangia in association with mastigonemes emerging from the cytoplasmic mass (Figs. 75, 76). This was the only time that structured contents were seen in such vesicles, which have largely disappeared by the time the spores are mature. When the released spores settle and begin to produce a wall, vesicles containing tubular membranes appear, perhaps comparable to the "type D vacuole" of Rawlence (1973).

Membrane stacks, with only limited or in some cases no association with the cytoplasm, were occasionally seen in sporangia (Figs. 77-80). Sometimes they appeared to be involved with formation of the flagella (Figs. 79, 80). They were often accompanied by membrane-bounded vesicles containing dense granular material similar to neighboring cytoplasm, but free in the extracytoplasmic space (Figs. 79, 81).

No consistent differences among species were observed in any of the preceding cellular contents. However, it is striking that differences were observed in a vesicle type that is unique to the mature zoospore: these are the presumptive adhesion vesicles. Their contents are extruded from the cytoplasm when the swimming zoospore settles, forming a layer of material that provides for adhesion of the settled spore to the substrate.

These vesicles are formed after cleavage of the sporangial protoplast, but well before spore release. The contents are usually differentiated into a core and periphery of differing electron densities. One type of adhesion vesicle, observed in Pterygophora (Fig. 66), Lessoniopsis (Fig. 14), Alaria
(Fig. 25C), and *Hedophyllum* (Fig. 82), is spherical with a fibrous core and a granular, more electron-dense periphery. A second type, observed in *Macrocrystis integrifolia* (Fig. 83) and *Laminaria groenlandica* (Fig. 84), has a light, granular core and a much darker, granular periphery; these vesicles are markedly elongate. A third kind is also elongate, and often curved, with a core and periphery of a uniformly granular, electron-dense material, separated by a lighter, very fine line that could not be resolved as a membrane. This kind was observed only in *Laminaria saccharina* (Fig. 85) fixed in chromate buffer. A fourth kind of adhesion vesicle was also elongated and curved, with a granular, electron-dense core and reticulate periphery, observed in *Agarum* (Fig. 86), *Postelsia* (Fig. 87), and *Eisenia* (Fig. 13).

Perhaps the most distinctive type of adhesion vesicle was observed in *Cymathere tripliaca* (Fig. 10, 52, 61, 65, 73, 74). This form was consistently present in material collected from widely separated populations fixed in both cacodylate and PIPES buffers. These vesicles have an elongated outline, an electron-dense periphery, and a core of electron-dense material forming striae in a lighter, fibrous matrix (Fig. 89). Cross-sections of these vesicles show that the dense core material may be continuous within the striation, or may possess a lighter inner core (Fig. 88).

Fig. 126A-B shows a generalized zoospore model based on my observations.
C. Zoospore Function

The structure and activity of the flagella are related to the two main functions of the released zoospore, dispersal and settlement. The anterior flagellum is held in front of the swimming spore and beats sinusoidally. The posterior flagellum does not appear to beat, but presumably functions as a rudder. There is no sign of phototaxis, such as aggregation of swimming spores or selection of site of settlement in relation to light. Nor do the spores seem to prefer the bottom of a container to the sides when settling, and so show no geotaxis. In some species the spores will swim for at least 24 hours if suitable conditions are provided (crowding in the presence of mucilages from the sorus), while they will settle within minutes if not crowded and given access to substrate.

The spore attaches to the substrate by the tip of the anterior flagellum, and may remain attached in this way and continue beating the flagellum for as long as 20 minutes without settling. Resorption of the flagella and attachment of the spore to the substrate were not observed with the light microscope, but spores fixed for electron microscopy have been found to exhibit all the stages from swimming to germination.

There is good agreement in the literature that the anterior flagellum makes the first contact with the substrate. Although it is rarely preserved in electron microscope preparations (Figs. 92-103), the long anterior whiplash is implicated in this action, and that it has adhesive properties is indicated by occasional preservation of clumps of mastigonemes on its tip (Figs. 90, 91, 92).
mastigonemes are not adhesive is suggested by the fact that they rarely appear clumped together along the anterior flagellum, and clumps of mastigonemes have not been observed sticking to the substrate. If adhesion is accompanied by increased fluidity of the membrane, this might account for the lability of the whiplash. It is important to note that sections of flagella that show only the two central microtubules are very rare within the sporangium, and could be attributed to the whiplash of the posterior flagellum (Figs. 104-106). There is no sign that the whiplash is removed from the anterior flagellum, for I have never found free whiplashes in whole mount preparations, even though free mastigonemes can be seen. This could be because the whiplash is so labile that it disintegrates completely when it is removed.

After adhesion of the anterior flagellum, the zoospore is drawn to the substrate by withdrawal of the axoneme into the spore. In some sections flagella are appressed to (Fig. 65), or fused with the plasmalemma (Figs. 107, 108). The axoneme may be drawn in either straight or by fusion of the flagellar membrane with the plasmalemma (Fig. 109). This probably depends on the orientation of the spore body with respect to the flagellum, which would vary under circumstances of water motion.

The axonemes preserve their organization long after withdrawal. For a time the two basal bodies may retain their association following withdrawal of at least part of one axoneme (Fig. 110), but eventually they move away from the site
of insertion (Fig. 115). Similarly, the axonemes will be found later removed from the plasmalemma and deeper inside the cell (Figs. 111-120). Once the spore has rounded up at about the time of axoneme resorption, the cytoskeleton is not detectable and has probably been disassembled. Presumably at least partial disassembly would be required for movement of the basal bodies during resorption of the axonemes. The adhesion vesicles are not extruded (Fig. 112) until after axonemes are inside the spore. The vesicles remain stuck to the exterior of the plasmalemma after exocytosis (Figs. 112, 119), and often open (Figs. 113, 115, 116, 118), releasing their contents which glue the spore to the substrate.

As the spore begins to form a wall, vesicles deposit material outside the plasmalemma, intercalating between the plasmalemma and any attached remnants of adhesion vesicles (Fig. 117). The axonemes are still evident as more wall material is laid down (Figs. 118, 119), but they are scattered through the cytoplasm. They can still be seen after formation of the germination tube commences (Fig. 120). In whole mount preparations, the diameter of the settled spores is larger than that of spores still in the swimming state (121). This increase in diameter, and the appearance of other settled spores as "double blob" figures (Figs. 122, 123) can be attributed to the presence of the withdrawn axonemes inside the cells. The mastigonemes may be cast off the flagella during withdrawal, because they are seen scattered about (Fig. 124), or forming a trail that leads to the settled spore (Fig. 125).
II. Sperm

A. Antheridia

Antheridia develop on male gametophytes, usually in a terminal or lateral position (Fig. 127); if intercalary, at least one of the neighboring cells is another antheridium (Fig. 129). They may be formed singly or in clusters that are essentially rudimentary plurilocular structures (pluriloccs) (Figs. 130-133), for a confluent outer wall is often detectable around adjacent antheridia (Figs. 131, 132). They are more or less conical in shape, measuring 5 to 9 um in longest dimension. The apex of the cone is the site of dehiscence, and it is lined with a "cap" of differentially staining material that is granular in the electron microscope (Figs. 133-136). The walls are surprisingly effective barriers to penetration of fixatives, and often nearby vegetative cells show superior preservation.

B. Sperm Structure

One sperm is formed per antheridium. Within the antheridium, sperm measure from 1.7 x 7.2 um to 2.5 x 5 um. They appear to be folded before release (Figs. 137-139), like Fucus sperm. Early in the course of differentiation of the antheridial cytoplasm the future basal bodies can be seen (Figs. 140-141); the microtubular assemblages are not positioned perpendicular to each other as centrioles would be.
The sperm possesses a band of microtubules in the anteriormost portion of the cell which runs parallel to the basal bodies in the fold of the cell that encloses the flagella (Figs. 137, 143-147). At this time a part of the anterior microtubular band is associated with the basal bodies, which are parallel to each other, similar to the situation in the zoospores (Figs. 137, 142B-D, 145). After release, the sperm unfolds so that the angle between the basal bodies increases, possibly to as much as 180 degrees, as in the Fucus sperm, and the anterior band of the cytoskeleton loops around the anterior periphery of the cell, as in the zoospore (Figs. 148, 149).

The anterior flagellum of the sperm is essentially the same as that of the zoospore, for it has a basal portion 16-18 um long that bears mastigonemes (Figs. 152, 155, 157, 158), and a distal portion at least 10 um long that is a continuation of the two central microtubules (Figs. 150, 153, 154). Fewer sperm were obtainable than zoospores; consequently these measurements probably do not represent the dimensions of the fully intact anterior flagellum, because here again the distal whiplash is apparently labile and difficult to preserve.

The most remarkable feature of the sperm is the posterior flagellum, which is up to 38 um long and tapers distally (Figs. 150-152, 154-160). Observations of unshadowed and unstained whole mounts (Figs. 151, 155, 157, 158) and cross-sections of flagella within antheridia (Figs. 161-163) both show that this taper is the result of a decrease in the number of microtubules in the axoneme, with a change of the doublets into singlets. The termination point of microtubules can be
detected when a fraying of the posterior flagellum occurs (Figs. 164, 165); a microtubule not anchored in place at its distal end may tear loose from the axoneme short of the end of the flagellum, a phenomenon that virtually never occurs in a flagellum in which all the axonemal microtubules end at a common terminus.

Plastids

The plastids of the sperm are smaller than those of the zoospores or vegetative cells of the gametophytes, measuring from 0.7 x 1.5 µm to 0.33 x 2.0 µm in section. They are oval in virtually all section planes, rarely bent or folded. The usual 3-thylakoid stacks are present, but only 3-4 per plastid. In contrast to the spores, there are 2-3 plastids in each sperm, usually next to the nucleus (Figs. 166-168, 173, 175). As in the zoospores, there is no eyespot.

Mitochondria

As many as seven mitochondrial sections, measuring up to 0.54 x 1.65 µm, can be seen in one section of a sperm. They are usually positioned at the anterior and ventral surfaces (Figs. 137, 142A). The tubular cristae possess the same tubular inclusions found in the mitochondria of zoospores (Fig. 169).
**Nucleus**

The nucleus is globular to irregular, reflecting a closer packing of the membrane-bound organelles than in zoospores (Figs. 137-139, 172, 177). It is usually elongated along the anterior-posterior axis, from 2.2 x 2.8 um to 1.6 x 3.5 um in dimensions. It is positioned between the dorsal, posterior plastids and the ventral, anterior mitochondria. Heterochromatin is rarely evident, in contrast to the zoospore nucleus. No nucleoli were seen.

**Endomembrane System**

The golgi apparatus is active in early stages of differentiation of antheridia (Fig. 170), cisternae forming vesicles that empty into the channel within which the flagella appear (Figs. 143, 171). In more mature sperm the golgi apparatus is much less apparent than in the zoospores. Stacks of ER sometimes occur on the dorsal side of the nucleus (Fig. 172), and electron-transparent vesicles, similar to those in the developing zoospores, produce the material that fills the extracytoplasmic space which surrounds the mature sperm (Figs. 142B-D, 175). Vesicles producing membranous material are apparently involved in formation of the flagella (Fig. 143), and free membrane aggregations (Figs. 173, 177), often associated with the flagella, may occur outside the cytoplasm. Four to five granular vesicles up to 0.55 um in diameter, similar to the "type B vacuoles" of the zoospores, are positioned characteristically in the anterior of the sperm.
Electron-dense globules may be the counterparts of similar bodies seen in zoospores (Figs. 137, 142, 171, 176, 178). However, because a bounding membrane was never visible, and they were found in anomalous positions at times, such as the flagella and outside the cytoplasm, these globules may represent an artifact of osmium precipitation.

Fig. 183 is a generalized sperm model based on my observations.

C. Sperm Function

Upon release, sperm are active swimmers, propelled by beating of the anterior flagellum. According to light microscope observations, the posterior flagellum does not beat, but it may show a bend when the sperm is not moving rapidly (Figs. 180-182). A sinuous waveform demonstrates the greater activity and flexibility of the anterior flagellum. Shortly after release, masses of sperm gather around eggs, demonstrating that the sperm are chemotactic.
CHAPTER 4. DISCUSSION

I will discuss my results with the Laminariales in light of what is known of other brown algal reproductive structures so that the significance of my findings is made clear.

I. Sporangia

A. Unilocular Sporangia

Unilocular sporangia may be defined as sporangia that at some stage in their development contain more than two nuclei (this excludes both conventional plurilocular structures and one-chambered, mitotically-derived structures). This definition emphasizes their long-recognized role as the only known site of meiosis in brown algae (Fritsch 1945), although in many life histories it has been shown that meiosis fails to occur during unispore formation (Bold and Wynne 1978). Unilocals may be spherical or ovoid to clavate, pedicellate, sessile or immersed in the thallus. The most primitive unilocular sporangia may be considered to be those that are solitary and globular in form. Unilocals that are tightly packed in a sorus may be clavate (e.g., Laminariales).

Unilocals may be solitary or clustered, occasionally in catenate series (e.g., Arthrocladia, Ascoseira, Pilayella) or may occur in distinctive aggregations termed sori, which may include specialized non-sporangial cells. When sori are
immersed in the thallus the resulting structure is called a conceptacle (e.g., Splachnidium, Notheia, Ascoseira, "Fucales" sensu lato). Sori vary from irregular, scattered groups of sporangia too small to be seen by the unaided eye (e.g., Punctaria), to larger aggregations with a distinct organization (e.g., Soranthera, with specialized multicellular "paraphyses" and a central tuft of trichothallic hairs), to very large continuous expanses (e.g., Himantothallus (Moe and Silva 1977), all Laminariales) that may be confined to specialized laterals (e.g., Alaria, Lessoniopsis).

The elongated sporangia of Laminariales, resulting from the close packing of sporangia and paraphyses in the sorus, clearly demonstrate the high degree of organization which must exist within any such structure. This organization is evident in sections taken through the sporangium; the cytoplasm is distributed along an axis in a way that cannot be readily seen in globose sporangia of other groups such as Ectocarpales (cf. Loiseaux 1973, Baker and Evans 1973a, Markey and Wilce 1976a). Each nucleus is located close to the periphery of the cytoplasm and the flagella all protrude radially, neither of which is possible in a spherical sporangium. This accounts for the lack of flagellar vesicles within the cytoplasm and suggests that there are no fundamental differences in the way flagella are formed among brown algae (cf. Markey and Wilce 1976a p. 168). The most highly modified unilocular sporangia are found in Notheia with its micro- and macrosporangia (Nizamuddin and Womersley 1960) or Syringoderma abyssicola, which produces cross-walls within the sporangium (Walker and Henry 1978).
B. **Plurilocular Sporangia**

In plurilocular sporangia cross-walls are always formed after each mitotic division [the lack of cross-walls reported by Knight (1923) in *Pilayella* has been disproved by Harkey and Wilce (1976a)]. Plurilocular sporangia occur on diploid sporophytes of only the vegetatively less advanced brown algae [they are not known in Laminariales, Desmarestiales, Sporochnales, Cutleriales, Dictyotales, or "Fucales" sensu lato (Fritsch 1945)], where they serve to recycle the sporophyte. Similar plurilocular structures that occur on gametophytes are considered gametangia, even if the "gametes" may develop parthenogenetically (Fritsch 1945, Bold and Wynne 1978). These sporangia may also be solitary, clustered, or grouped into sori.

II. **Spores**

A. **Variability Among Spores**

Few previous studies have examined released spores or gametes except as whole mounts, for once free of the protection of the sporangium or gametangium, the swarmer appears to be very fragile. For example, mastigonemes of actively swimming cells are usually not preserved unless an initial osmium fixation is used, yet this results in very poor preservation of the cell contents (cf., Cheignon 1964). Swarmers are very likely to be fixed before or after their period of optimum
vitality if attention is not paid to conditions of release (Chi 1973) or timing of fixation (Toth 1976a).

The primitive zoospore has already been described in the Introduction; very little variation among brown algal zoospores has been reported to date. The large (to 12 μm in length) spores from the unilocular sporangia of *Ectocarpus* were found to contain up to six dictyosomes rather than the one of most other (smaller) spores, and the plastid was not appressed to the nucleus (Baker and Evans 1973b); both these modifications can be attributed to the large size of the cell.

B. The Missing Eyespot

In most respects laminarialean zoospores resemble those of other brown algae. The most significant difference from other orders is the lack of an eyespot. This feature had been reported by some previous workers [Sauvageau 1918, Kanda 1936, 1938, Papenfuss 1942, Bold and Wynne 1978 (no authority cited)], but not by others (Meyers 1928, Angst 1929, Hollenberg 1939); all (Sauvageau 1918, Kylin 1918, Kanda 1938, Toth 1974, Bold and Wynne 1978) agree, however, that *Chorda* possesses an eyespot [and also perhaps, *Saccorhiza* (Sauvageau 1918, Kain 1969, Norton and Burrows 1969)]. The supposition of Chi (1973) and Walker (1980) that osmiophilic globules in the cytoplasm of the zoospore of *Macrocystis* and *Nereocystis* are extraplastidic eyespot globules can be discounted for several reasons: 1) Although the globules may be aggregated at the posterior of the cell in association with a band of microtubules, in other parts of the cell apparently identical globules can be found.
2) The mass of globules is not bounded by a membrane; although eyespots of some algal groups may lack a membrane when located outside a plastid (Dodge 1973), this is never the case in the flagellate groups thought to be most closely related to brown algae (Chrysophyceae, Xanthophyceae). 3) Although the globules are osmiophilic, they are not necessarily lipid; in fact, they are much more osmiophilic than known phaeophycean eyespots and could be composed of some other osmiophilic substance. They resemble vesicles in the paraphyses of the sorus similar to those in *Fucus* epidermal cells thought to contain phenolic material (McCully 1968b). 4) Light microscopy fails to reveal the presence of any pigmented bodies in the live zoospore other than the plastid; all other algal eyespots are visible in the light microscope. 5) There is no association of the globules with the flagellar apparatus; such an association is known in all brown algae and related groups in which any eyespot is found. 6) Numerous observers (Hollenberg 1939, Papenfuss 1942, Suto 1950, Saito 1975, my own observations) have noted the lack of phototaxis in the behavior of zoospores of many Laminariales; by contrast, in *Chorda*, shown by electron microscopy (Toth 1974) to possess an eyespot, reports of phototaxis appear to be universal (Kylin 1918, Sauvageau 1918, Toth 1976a).

I propose that this loss of the eyespot can be accounted for by a consideration of the the reproductive ecology of Laminariales. All Laminariales except *Chorda* produce sporangia in sori that cover an expanse of blade much larger than the sporangial surface of most other algae, and most species occur
in dense stands, even forming "forest"-like kelp beds (e.g., *Macrocystis*). Spore release by such close aggregations of plants can result in dense settlements of spores near the parent sporophytes. Such dense settlements may result in poor growth because of shading by the parents (Anderson and North 1965). Under these conditions there may be selection for a mechanism to prevent settling of the spores too soon. A loss of the eyespot and associated phototaxis would ensure that spores would find a substrate at random, rather than seeking the sea bottom by negative phototaxis (cf., Kylin 1918). By staying motile longer, spores would be more likely to be carried further by water currents.

C. How Many Plastids?

In various other orders of brown algae there may be from one to several plastids per swarmer. Several authors (Chi and Neushul 1972, Collins and Kugrens 1975) have reported the occurrence of more than one plastid in zoospores of Laminariales. This conclusion has most likely been based on sections which show two lobes of the plastid as it lies folded around the nucleus (cf., Sauvageau 1918). My observations agree with those of Toth (1976a) that there is only one plastid per spore, which begins to divide after the spore is released from the sporangium. Because I observed no pyrenoids, I consider reports of pyrenoids (Chi 1973, Walker 1980) in pre-release spores questionable; the matrix of these "pyrenoids" does not appear differentiated from the stroma. These appendages of the plastid may be lobes formed at plastid
division.

D. Mitochondria

The tubular intracristal inclusions found in mitochondria in both zoospores and sperm of Laminariales appear identical to those in five other orders of brown algae (Markey and Wilce 1976a, Cassell and Pollock 1979, Henry 1979). Because they are not evident in vegetative cells, or the sporangia before flagella differentiate, it seems likely that their presence is related to the respiratory demands of motility.

E. The Cytoskeleton

The cytoskeleton of swarmers has received little attention since Manton (1964) compared the fucacean "proboscis" to the cytoskeletal microtubular bands in Cystoseira sperm and Scytosiphon spores. Toth (1976a) failed to observe the cytoskeleton in zoospores of Chorda, but he apparently was looking at spores that were rounded up, and in such spores the cytoskeleton of necessity has been disassembled. Although Manton (1964) discounted the skeletal function of cytoplasmic microtubules, their appearance as the zoospore becomes elongated and disappearance as it rounds up in settling constitute strong circumstantial evidence of their skeletal role. A close association with the basal bodies of the flagella is also consistent with mechanical support, because the forces generated by beating of the flagella must be transmitted to the body of the spore through a structure
capable of withstanding such stresses without permitting damage or deformation of the cell. The microtubules extending from the region of the basal bodies could distribute these forces within the cytoplasm so that no one component would be overly stressed. This rationalization accounts for the wide, if not universal, occurrence of flagellar roots of various forms among flagellates and ciliates (Pitelka 1963, Hibberd 1976).

F. The Flagella

Most of the characteristics of laminarialean zoospore flagella demonstrated by this study have been previously encountered by other workers, but some aspects are obscure and others are subject to controversy. Several authors (Bouck 1969, Loiseaux and West 1970, Markey and Wilce 1976a, 1976b, LaClaire and West 1978, Toth 1974) have noted that brown algal mastigonemes are found in pre-release swarvers only on one side of the flagellum. Some (Loiseaux and West 1970, Toth 1976a, LaClaire and West 1978) believe that mastigonemes in the swimming spore are arranged unilaterally or helically, and that the bilateral appearance in whole-mount preparations is an artifact. However, sections of free-swimming Fucus sperm (Cheignon 1964), and whole mounts of "exploded" flagella show that the attachment of the mastigonemes is bilateral in the active swarmer. Indeed, a helical arrangement can be ruled out because in that case, the mastigonemes should show a periodic "wavy" pattern in whole mounts. Similarly, a unilateral distribution of mastigonemes should yield many flagella with asymmetric distributions of mastigonemes to one side or the
other in many specimens. A uniform distribution around the flagellar circumference might show a symmetrical array, but this is incompatible with the rows of mastigonemes associated with axonemal microtubules that can be seen in "exploded" flagella. Although only limited attempts have been made to analyse the hydromechanical function of flagella, some (Holwill 1974) suggest that a bilateral arrangement of mastigonemes is necessary for the reversal of thrust evident in all flagella with stiff, rigidly anchored mastigonemes. All anteriorly directed flagella that beat sinusoidally seem to share this construction. Therefore, it is possible to reject on hydromechanical grounds Caram's (1975) contention that in the sperm of *Cutleria* the anterior flagellum is short and simple and the posterior is long and adorned with mastigonemes.

The whiplash was found on the anterior flagellum of both zoospores and sperm in all species of Laminariales studied. Numerous references to the adhesive function of the anterior flagellum are cited by Fritsch (1945) and Friedmann (1961). This whiplash has been shown to be as long as the mastigoneme bearing portion in many specimens (Petersen et al., 1958), Muller and Falk 1973, Toth 1976a). Its presence has been reported in *Chordaria* (Petersen et al., 1958), *Ectocarpus* and *Sorocarpus* (Muller 1965), *Chorda* (Toth 1976a), *Desmarestia*, *Soranthera*, *Coilodesme*, *Analipus*, *Syringoderma*, and *Fucus* (unpub. obs.). It is visible under phase-contrast in the light microscope (Muller 1965), appearing as a loop or loosely wound helix. But it is not preserved in most electron microscope preparations after standard aldehyde or osmium fixation (Muller
and Falk 1973). Because the tip of the anterior flagellum is the site of first substrate contact by settling swarmers (Fritsch 1945), the existence of a specialized structure facilitating attachment should not come as a surprise. The coiling of the whiplash observed in the light microscope is sometimes preserved in electron microscope preparations (Muller and Falk 1973, Toth 1976a), but since other configurations have been found (Petersen et al. 1958), it may be that its form is altered by the preparatory methods, or just that it is not readily visible by light microscopy if it is not coiled. The rarity of preservation of the whiplash in preparations for the electron microscope has been attributed to failure of fixation (Muller and Falk 1973), but a second possibility remains, that the whiplash is not formed until the swarmer is ready to settle. This would account for so few whiplashes appearing in sections of swarmers before release; those that are seen are attached to the posterior flagellum. Nevertheless, the hypothesis of extreme lability is consistent with the fact that the anterior flagellum is disrupted much more frequently than the posterior, and always starting from the distal end. Adhesive function might be tied to an intrinsic fluidity of the whiplash membrane, making fixation difficult. The loss of mastigonemes after aldehyde fixation demonstrates that even the "best" fixatives may fail to preserve certain components. It would be interesting to look for a similar structure in fungal zoospores of similar design (e.g., Oomycetes) and Chrysophyta known to have settled stages in their life cycles.

The posterior flagellum of zoospores of Laminariales
differs from those of most brown algal swarmers in lacking a basal swelling, since there is no eyespot. It should be noted that the short whiplash of this flagellum is usually well preserved. Although the construction seems to be the same as the long anterior whiplash (2 central single microtubules covered by membrane), its ease of preservation may be associated with its lack of involvement in settling, and consequent lack of fluidity. This flagellum is rigid (Friedmann 1961, Toth 1976a) but may execute beating motions (Couch 1941, Muller and Falk 1973). That it is not necessary for motility is suggested by the fact that in the sperm of Dictyota it is missing. No mastigonemes have been seen on this flagellum [I have concluded that the report by Loiseaux and West (1970) of short tufted mastigonemes on the posterior flagellum of Giffordia oviger can be dismissed as contamination of their cultures by a colorless flagellate, probably a bodonid].

G. The Adhesion Vesicles

All cytoplasmic vesicles seen in this study of laminarialean zoospores are comparable to those seen in other brown algal zoospores by previous authors. However, the great variation in the appearance of the contents of the adhesion vesicles ["lamellate vesicles" of Chi (1973), "plaques" of Walker (1980)] among the species of Laminariales examined was unexpected. Their derivation from the golgi apparatus has been established (Baker and Evans 1973a, 1973b, Chi 1973, Walker 1980). Some of the variation may be attributable to different
stages of formation, and this makes establishment of structural types dubious at present. However, it is probably safe to make a few generalizations. Most of the adhesion vesicles in Laminariales show more structural complexity than those of "lower" brown algae (cf. Baker and Evans 1973a, 1973b, Loiseaux 1973, my unpub. obs.). Internal membranous components have been shown in fixations with added ruthenium red (Chi 1973) or chromate (Fig. 85). The striations formed by the granular contents in Cymathere were observed consistently in several different collections and fixations. Although vesicle types encountered in this study could not be correlated with family concepts (Fritsch 1945) in the Laminariales, it is noteworthy that the vesicles described by Chi (1973) in Macrocystis pyrifera appear identical to those found in M. integrifolia.

H. Other Modified Spores

Although lack of an eyespot sets most Laminarialean zoospores apart from those of more primitive brown algae, spores are modified even more dramatically in some other orders. Only one instance of heterospory has been reliably recorded in the brown algae, in the reproduction of Notheia, where two sizes of sporangia produce macrospores and microspores (Nizamuddin and Womersley 1960).

In Dictyotales and Tilopteridales, large, non-motile spores are produced in the unilocular sporangium. The tetra- or octospires of Dictyotales are uninucleate products of meiosis. It is significant that in the production of the
tetraspores only four nuclei are produced (Fritsch 1945), whereas in oogonia of Fucales eight nuclei result, no matter how many eggs are eventually produced (from one to eight). In Tilopteridales only one large, non-motile monospore is produced which is quadrinucleate, at least initially (Fritsch 1945). In both Dictyotales and Tilopteridales the lack of flagella in these spores can be attributed to their large size; a flagellum would be useless to propel such large cells (the same reasoning can be applied to the eggs of oogamous forms).

III. Gametangia

A. Plurilocular Gametangia

The most primitive gametangia are obviously plurilocular. The chambers of the gametangia may form a simple filament (e.g., in Scytosiphon), or a hollow tube (e.g., Tilopteris) (Fritsch 1945). In many cases a parenchyma is formed, even in species showing no other parenchymatous structures (e.g., in Ectocarpales, Chordariales). Frequently gametangia are grouped in sori.

Specialized plurilocular gametangia occur in anisogamous and oogamous species. Anisogamy involves fusion of gametes produced in distinctly larger-chambered (female) and smaller-chambered (male) gametangia (e.g., Cutleria), although rarely the two sizes of chamber may occur in the same gametangium (e.g., Giffordia mitchelliae (Muller 1969)). Even when a gametangium produces a very specialized gamete (i.e., in
oogamy), the gametangium may retain an obvious plurilocular character [e.g., antheridia of Dictyotales (Fritsch 1945)].

In many groups, however, the gametangia have been reduced to solitary or clustered single locules, so that their homology with pluriloccs is obscured, as with oogonia of Dictyotales, and both types of gametangia of Laminariales, Desmarestiales and Sporochnales. Note, however, that antheridia of Dictyotales are unquestionably plurilocular and that the antheridium of Nereia (Sporochnales) is reported to be two-chambered (Fritsch 1945). Therefore, it is inappropriate to refer to such one-chambered gametangia as "unilocular" (cf. Setchell and Gardner 1925, p. 590; Kanda 1936, p. 256), as such terminology obscures the unmistakable homologies among different orders.

B. Antheridia Of Laminariales

Although in Laminariales most antheridia are borne singly on the male gametophytes, occasional clusters of gametangia show that these structures may be regarded as reduced pluriloccs. The confluence of the outer walls indicates that the adjacent antheridia are formed by cell division within a common wall, as in the formation of more conventional plurilocular structures (cf. Markey and Wilce 1976b).

The "cap" of material lining the apex of the antheridium is undoubtedly involved in the triggering of sperm release. It has been shown (Luning and Muller 1978, Muller et al. 1979) that release occurs within 12 seconds of contact with a substance produced by fertile female gametophytes in several Laminariales. Such a rapid response implies a specialized
mechanism for triggering dehiscence of the antheridium; the differential staining of the antheridial cap shows that it is chemically differentiated from the other antheridial contents and wall. Its superficial resemblance to the cap found in the apex of Laminariales zoosporangia may indicate that sporangial release in this order is induced by a more specialized mechanism (e.g., an enzymatic breakdown of the tip of the sporangium) than is the case in "lower" brown algae in which no predetermined site of dehiscence is discernible, release being triggered by osmotic forces.

IV. Gametes

A. Variation Among Gametes

Three different patterns of sexual reproduction in brown algae have been recognized traditionally: isogamy, anisogamy and oogamy (Fritsch 1945). "Isogametes" are most commonly produced among the Ectocarpales (sensu Fritsch 1945). The few that have been studied ultrastructurally show little to distinguish them from zoospores, or the males from the females (Markey and Wilce 1976a, 1976b, Muller and Falk 1973). Ectocarpus has been the most carefully studied (Papenfuss 1935, Fritsch 1945, Muller 1967, 1972a, 1972b, 1975, Muller and Falk 1973), and as in all other "isogamous" species that have been examined rigorously, the male and female swarmers are differentiated behaviorally, and so should not be termed isogamous in the strict sense of the word. The female swims
only for a short time, perhaps 15 minutes, while the male may remain motile for eight hours or more. Upon settling, the female begins to secrete a pheromone that attracts the males. The males make contact with the settled female with the tip of the anterior flagellum, and when one succeeds in fusing with the female, pheromone production evidently stops, because the other male gametes swim away. Gametes may also settle without fusing and regenerate the haploid gametophyte. Although there are reports of fusions of swimming gametes in isogamous species (Papenfuss 1934, Loiseaux 1967), these observations have never been confirmed by cytological evidence and should be regarded with suspicion.

There are few well-documented examples of what has been classically recognized as anisogamy, in which both gametes are motile but the female is larger than the male. The best known is *Cutleria*, in which the female gametes have about 30 times the volume of the male gametes (Jaenicke 1977) and possess several plastids (LaClaire and West 1978), while the male has but one plastid (Caram 1975), although both have eyespots. Here again the female gamete settles promptly, secretes a sex pheromone (different from the one produced by *Ectocarpus*) and males are attracted as in *Ectocarpus* (Muller 1974). In *Colpomenia peregrina* (Clayton 1979) and *Giffordia mitchelliae* (Muller 1969) the size difference between the two gametes is not as great as in *Cutleria*, but the male gametes are noticeably paler. Essentially the same mating reaction occurs as was observed in *Cutleria*, but in *Colpomenia* both gamete types could develop parthenogenetically.
In oogamy, a motile sperm fuses with a large, unflagellated egg. In the sperm the complement of organelles is often reduced or modified from that found in zoospores or isogametes. In *Dictyota* only the anterior flagellum protrudes from the sperm, decorated with a row of small spines along with the usual mastigonemes, and the plastid is vestigial. Sperm of *Himanthalia*, *Xiphophora* (Manton 1956), and *Hormosira* (Forbes and Hallam 1978) in the Fucales all possess a larger spine near the terminus of the mastigoneme-bearing flagellum. In the Fucaceae of the Northern Hemisphere the sperm bear a "proboscis" of membrane-bound microtubules, and the posterior flagellum is longer than the anterior (Manton 1964, my unpub. obs.). *Pelvetia* (Oltmanns 1922, my unpub. obs.), *Pelvetiopsis*, and *Hesperophycus* (my unpub. obs.) sperm all lack eyespots in contrast to *Fucus* and *Ascophyllum*. Some species of *Sargassum* (Fletcher and Fletcher 1975) and *Cystoseira* (Sauvageau 1911, Roberts 1978) lack eyespots, even though their sperm are otherwise similar to more primitive gametes of isogamous species, since their flagella are unmodified (Manton 1964, my unpub. obs.).

Less is known of the sperm of oogamous *Sphacelariales* (Moore 1951), *Desmarestiales* (Fritsch 1945) or *Sporochnales* (Caram 1965), as no electron microscopy and little light microscopy has been done with them. It does appear that eyespots may be absent in all. *Laminariales*, *Desmarestiales* and *Sporochnales* all share a tendency to retain their eggs on the oogonia of their diminutive gametophytes; this common aspect of their reproduction might be expected to have some
bearing on their fertilization biology and thus the structure of their sperm.

The eggs of *Fucus* have been shown (Muller and Jaenicke 1973) to produce a sperm-attracting pheromone, and as already mentioned, fertile female gametophytes of several Laminariales produce a substance that not only attracts sperm but is also responsible for their release from the antheridium (Luning and Muller 1978, Muller et al. 1979).

### B. Sperm of Laminariales

Descriptions of sperm of Laminariales have shown considerable disagreement (Fritsch 1945). They have reported presence (McKay 1933, Hollenberg 1939, Kemp and Cole 1961) and absence (Gherardini and North 1972) of plastids, presence (Sauvageau 1918, McKay 1933, Hollenberg 1939) and absence (Kanda 1936, 1938, Kemp and Cole 1961) of eyespots; a longer anterior and shorter posterior flagellum (McKay 1933), "the usual two laterally attached flagella" (Fritsch 1945), "flagella of different lengths... size difference... not as sharply pronounced as in the zoospore" (Kemp and Cole 1961) or a posterior flagellum as long as the anterior (Kanda 1936, 1938).

I have found that the general architecture of the sperm cell of Laminariales shows resemblance to both Laminariales zoospores and fucacean sperm. The microtubular bands of the cytoskeleton in the anterior of the Laminariales sperm are undoubtedly homologous with those of the zoospore, but within the antheridium the sperm is folded, similar to the pre-release
Fucus sperm (Manton and Clarke 1956, Berkaloff and Rousseau 1979). The flagella of the sperm are likewise formed within a channel or pocket that in the later stages of spermatogenesis persists as the anterior fold. This fold, with its cytoskeletal microtubular band, is reorganized upon release so that it points forward. This change in the orientation of the band is accompanied by a change in the angle between the basal bodies from zero to 180 degrees, as in the Fucus sperm, in contrast to the 90 degree angle found in the zoospores.

The very long and tapering posterior flagellum of the sperm of Laminariales has no known counterpart in other brown algae. The construction of this flagellum resembles that reported in the hypermastiginid flagellate Trichonympha (Gibbons and Grimstone 1960). In both the laminarialean sperm and Trichonympha, tapering of the flagellum is the result of a reduction in the number of microtubular doublets to singlets, then reduction in the number of singlets. The outline of these singlet microtubules becomes somewhat rectilinear towards the tip, indicating an unusual substructure.

Although more than one plastid is present in the sperm, they are reduced in size from those of the zoospore. Thylakoid morphology is normal, so they may be assumed to be functional. Having several small plastids may facilitate formation of the elongated shape of the sperm, since a large plastid would tend to dominate the volume available, resulting in a more globose, less hydrodynamically efficient shape. The lack of eyespots in the sperm is consistent with the importance of chemotaxis in sperm function. Lack of adhesion vesicles is consistent with
the goal of fusion with the egg rather than adhesion to an inert substrate.

C. Comparison With Other Brown Algal Sperm

There are several points of similarity between laminarialean sperm and those of other oogamous orders, as is illustrated diagrammatically in Fig. 184. The various specialized sperm types can be derived from the primitive, zoospore-like sperm found in isogamous species and (apparently) even some Fucales. The reported lack of eyespots in some Fucales, Sporochnales, Dictyotales and Sphacelariales has already been mentioned. Desmarestia viridis sperm also lack eyespots and have a prominent anterior cytoskeletal loop and a longer hind flagellum, but this flagellum is constructed differently (see Appendix). A long posterior flagellum (which retains the "9 + 2" construction throughout its length) is found in specialized fucacean sperm that lack spines on the anterior flagellum (Fucaceae of Northern Hemisphere). Elongated sperm bodies also occur in Fucaceae and Durvillaea, as well as Desmarestia viridis. This presumably results in greater hydrodynamic efficiency. The longer posterior flagellum may serve as a stabilizing rudder, necessary to ensure stability in the presence of a very rapidly beating anterior flagellum. The longer flagellum, as well as the elongated cell shape and the "proboscis" of some Fucaceae, all contribute to a larger surface area, important in the reception of chemotactic signals. It has been shown in Ectocarpus (Jaenicke 1977) that the sex pheromone is first taken up by the
anterior flagellum of the male gamete. An elongated receptor mechanism should allow more sensitive detection of concentration gradients of pheromones, facilitating location of the source of emission (the female gamete). While *Dictyota* sperm have no posterior flagellum, they have spines on the anterior flagellum, recalling the several Fucales (*Himanthalia, Xiphophora, Hormosira*) with short posterior flagella and an anterior flagellar spine. I have indicated with question marks in Fig. 184 the possibility that these spiny flagella might lack whiplashes (they have not been reported); it is conceivable that in these instances the failure to observe this structure reflects its absence, the spines substituting in some way for the whiplashes in sperm-egg contact. The significance of these various sperm morphologies for swimming behavior or gamete interaction has not been established. Some of these questions might be answered by motion studies of swimming, or ultrastructural examination of gamete contact.

An understanding of the functional significance of sperm morphologies will be required to make the best use of these characteristics in taxonomy. Fortunately, oogamy is widespread among the orders of the brown algae, and the evolution of certain modifications (e.g., longer posterior flagellum, spines) in different phyletic lines appears to have been accomplished by different mechanisms, so merely analogous structures are readily distinguished from true homologies. I have therefore indicated in Fig. 184 that sperm modifications found among oogamous orders of brown algae have occurred as a radiation from a primitive ancestral form, rather than as a
hierarchical series of changes; the laminarialean, Desmarestia, fucacean, spiny fucalean and Dictyota sperm types can be considered to be at comparable levels of advancement. Where some modifications have occurred less widely (e.g., loss of eyespots in Fucales) phyletic series within families may be made evident. Seemingly anomalous features may point to taxa that have been neglected or misinterpreted in morphological studies (e.g., Durvillaea).

The "isogamous" and anisogamous brown algae may yet show interesting variations in swarmer structure. One might expect to find adhesion vesicles in gametes capable of parthenogenesis, or the timing of adhesion vesicle formation might be delayed until after release. Better understanding of the cytological mechanisms involved in gamete fusion might prevent the misinterpretation of life cycles that has plagued studies of lower (and some higher) brown algae. Especially in the Southern Hemisphere, where the Sporochnales, Desmarestiales, Dictyotales, Sphacelariales and "Fucales" all attain their greatest diversity, ultrastructural investigation of swarmer can be expected to provide important information for a better understanding of the brown algae.

V. Implications For Phyletics Of Laminariales

The unique zoospores and sperm of Laminariales set this order distinctly apart from other brown algae, corroborating taxonomy based on vegetative features. Chorda is marked as primitive, in agreement with previous judgements (Fritsch 1945). Kanda's (1938) reports of a short hind flagellum and an
eyespot in *Chorda* sperm are probably correct, judging from the fact that he noted the longer posterior flagellum and lack of eyespots in other species.

If *Saccorhiza* does indeed have an eyespot in its zoospore, it is likely the most primitive member of the Laminariales, excluding *Chorda*. It would be most interesting then to know what the sperm of *Saccorhiza* look like. A primitive zoospore is consistent with Sauvageau's (1918) report of a few sporophytic embryos in his *Saccorhiza* cultures showing an apical hair early in development (cf. *Chorda*). The retention of hair-pits on the adult thallus (Norton and Burrows 1969) recalls *C. tormentosa*, and the lack of sieve cells and occurrence of sorus on the stipe and basal "bulb", as well as the blade (Norton and Burrows 1969), contrasts with all other Laminariales except *Chorda*, in which there is no differentiated stipe and a very simple internal anatomy.

**VI. Implications For Swarmer Function And Life Histories**

Some of my findings, combined with consideration of information available from current literature, suggest several generalizations that could be made concerning reproduction in brown algae.

The structure of flagella must be subject to hydromechanical constraints. Since mastigonemes are apparently crucial for generation of a pulling force in anteriorly directed, sinusoidally beating flagella, we may safely predict that all brown algal swarmers will eventually be found to possess anterior flagella of the kind I have described. If the
posterior flagellum is not essential for propulsion, (cf. Dictyota sperm), then its form may be freely altered to perform other functions, such as chemotaxis (cf. Laminariales and Desmarestia sperm).

The wide (and probably universal) occurrence of the anterior whiplash throughout the brown algae has not been generally recognized. Since it appears to play a key role in zoospore settling and gamete interaction, we may be very skeptical of reports of gametes fusing while swimming (Papenfuss 1934, Caram 1964), and doubly suspicious of reports of fusions of spores from unilocular sporangia.

The release of uncleaved cytoplasmic masses from sporangia indicates that observation of "fusion products" cannot be assumed to provide evidence of sexuality. "Fused" zoospores were reported in Laminariales several times (Kain 1979) before the life history was first described by Sauvageau (1915).

There are numerous reports of fusions of spores from unilocular sporangia (Fritsch 1945, Caram 1972), supposedly resulting in the formation of diploid zygotes. But more recently exhaustive experimental evidence has been presented (Muller 1975) which casts doubt on the likelihood of this occurring. Therefore there is good reason to believe that fundamental differences exist between spores and gametes, though both are haploid. Better understanding of sporangium dehiscence (Toth 1976b) and sexual interactions (Luning and Muller 1978) in taxa such as the Laminariales, which are considered to be well understood, might improve the standards used by observers describing life histories of more enigmatic
The distinction between spores and gametes may arise from the fundamental properties of the unilocular sporangium, with its unique nuclear behavior (consider that sexuality is basically a nuclear phenomenon). It appears that the occurrence of free nuclear divisions (during meiosis) is sufficient to unmistakably identify a sporangium as homologous with such well known unilocular sporangia as those in Laminariales. By this reasoning, the four-nucleate "monosporangia" of Tilopteridales must be unilocular sporangia, while (at least some of) the uninucleate "monospores" are eggs that can develop parthenogenetically, but which have nevertheless shown evidence of sexual function (Sundene 1966).

This approach also enables us to interpret the life history of the "Fucales" (sensu lato) as homologous with other brown algae (a vexed question indeed) (Smith 1938, Kylin 1940, Fritsch 1945, Caplin 1968, Jensen 1974).

In "Fucales", the unilocular reproductive structures which produce cells that fuse sexually upon release have been considered unilocular gametangia. As with unilocular sporangia, these gametangia are the site of meiosis and thus occur on diploid plants. Traditionally all brown algae that possess this "animal-like" life history have been placed in the Fucales (they share a second characteristic feature in bearing these gametangia in conceptacles). This is true among all the conventionally recognized Fucales, but there is nevertheless significant variation in the construction of the gametangia in this group. In some, the oogonia (e.g., in Fucus) produce
several eggs, or the antheridia (e.g., in *Cystoseira*), produce many sperm in a series of events that shows no essential difference from what is found in a unilocular sporangium; meiosis occurs, mitosis follows to form 8-64 nuclei, and the cytoplasm cleaves, the products being released without further complication. In other cases, after meiosis in the oogonium, walls may be formed around the four division products either before [*Bifurcariopsis* (Jensen 1974)] or after [*Xiphophora* (Naylor 1954)] subsequent mitosis. Similarly, in antheridia a wall (called an "endochiton") may be formed inside the original gametangial wall [*Himanthalia* (Manton 1964), *Fucus* (Berkaloff and Rousseau 1979), *Xiphophora*, *Hormosira*, *Durvillaea* (Moestrup 1977)]; the wall develops some time between meiosis and cleavage of the cytoplasm.

Although complete knowledge of the life history is not yet available, analogous internal walls in the unilocular sporangia of *Ascoseira* (Skottsberg 1921, Moe and Henry unpub. obs.) and *Syringodermia* (Walker and Henry 1978, and unpub. obs.) likely signify that these species produce gametes from their unilocular reproductive structures, in exact analogy with the Fucales. This widespread evidence of walls or wall remnants within "unilocular gametangia" suggests that in these instances, if not in all "Fucales", the "gametes" are exactly that, not spores acting as gametes as proposed by Smith (1938) and Caplin (1968). The evident homologies with plurilocular gametangia also argue against Kylin's (1940) contention that the fucalean life history has no counterpart in the other brown algae, the primary justification for his segregation of the
Fucales in the Class Cyclosporeae. The variation in form and appearance of these internal walls, as well as parallel developments in the obviously unrelated genera Ascoseira and Syringoderma, suggest that the Fucales as conceived by Kylin are polyphyletic, this "animal-like" life history having evolved more than once in the brown algae.

Further ultrastructural studies are required to confirm these homologies. The more obvious correspondence between laminarialean gametangia and plurilocular gametangia of "lower" brown algae might be more widely recognized if the terms "uniloc" and "pluriloc" were redefined or abandoned in favor of terms which explicitly denote the differences in cytology of development.

The terms "mitosporangium", "meiosporangium", "mitogametangium", and "meiogametangium (von Denfer 1972) are admirably expressive, but their adoption fails to solve certain terminological problems. Unfortunately, the term "meiosporangium" has been used to refer to certain plurilocular structures (Fritsch 1945). If this archaic usage be overlooked, there still might be argument that "meiosporangium" is not a valid term in taxa shown to lack meiosis in the life history, even though they possess structures obviously homologous with normal, meiotic "unilocs". On the other hand, although it may be known that a taxon has a sexual life history, in a given specimen the ploidy level may not be known, so the role of the products of a mitotically produced reproductive structure is unknown (is it a mitosporangium or a mitogametangium?). Perhaps a term such as "zoidangium" would
be useful, because it does not denote the role of the "zoid". But "zoidangium" cannot be used to refer to reproductive structures with non-motile products (e.g., "monosporangia" of Tilopteridales). Since we have no single word for "reproductive structure" (i.e., "sporangium-or-gametangium"), we cannot easily coin a non-committal term that is both etymologically elegant and expressive. Perhaps "coenangium" or "meiotangium" might be used for meiosporangium/meiogametangium, and "mitotangium" for mitosporangium/mitogametangium.

Since differences in cytology of development are intimately related to the roles of the products of reproductive structures in life histories, a more illuminating terminology would assist those attempting to analyse enigmatic life histories, such as Tilopteridales, Fucales, Syringoderma and Ascoseira. We must not be surprised to encounter taxa that defy our efforts at typification. When the fundamental properties of "typical" reproduction are understood, a grasp of the origins and functioning of exceptions to the rule will be within reach.
Fig. 1. *Macrocystis pyrifera*. Blade cross-section in sorus region. The blade surface curls inward where it has been cut (arrow), causing the "cuticle" (C) to separate from the paraphyses. 1 um thick section, stained osmium tetroxide, methylene blue-azure II.

Fig. 2. *Laminaria saccharina*. Cross-section through blade showing mucilage ducts (arrow) and sorus. 1 um thick, osmium, methylene blue-azure II.

Fig. 3. *L. saccharina*. Section through sorus. Note abutting tips of paraphyses (Pa) forming protective layer over sporangia (Sp), osmiophilic globules (O) in paraphysis tips, "cuticle" lifted up.

Fig. 4. *Dictyoneurum californicum*. Longitudinal section of paraphyses and sporangia showing swollen paraphysis tips, osmiophilic material in paraphyses (arrow), granular material (gr) in apex of sporangia.

Fig. 5. *Alaria tenuifolia*. Postcleavage zoosporangium in longisection showing partly leached "type b" vesicles (B) and granular material at sporangium apex.

Fig. 6. *Lessonoipsis littoralis*. Cross-section of blade with sporangia largely released from sorus. Note swollen tips of paraphyses (arrow). 5 um-thick section, stained with toluidine blue.
Fig. 7. *Dictyoneurum*. Cross-section of paraphyses and sporangia. Walls of paraphyses are thicker than those of sporangia; top sporangium is cleaved, the bottom is not. Note osmiophilic globules in paraphyses.

Fig. 8. *Dictyoneurum*. Oblique section through sporangium showing several emergent flagella (F), each associated at basal body with a plastid.

Fig. 9. *Dictyoneurum*. Longitudinal section through sporangium showing close-packing of spores, conspicuous "type b" vesicles.

Fig. 10. *Cymathere triplicata*. Cross-section of sporangium showing packing of spores, prominent "type b" vesicles.

Fig. 11. *Pterygophora californica*. Longissection of sporangium showing close-packing of spores.
PLATE 3

Fig. 12. Eisenia arborea. Longissection of sporangium; one spore shows considerable elongation (arrow).

Fig. 13. Higher magnification view of spore indicated in Fig. 12. Note cytoskeletal microtubule (arrow).

Fig. 14. Lessoniopsis. Spore within sporangium showing four parallel cytoskeletal microtubules (arrow). O=osmiophilic vesicle, AV=adhesion vesicle.

Fig. 15. Pterygophora. Whole-mount of spores showing well-preserved "nose" (arrow). AF=anterior flagellum, PF=posterior flagellum.

Fig. 16. Alaria tenuifolia. Whole-mount, shadowcast zoospores and undivided sporangial mass (U).

Fig. 17. Cymathere. Spore within sporangium, showing flagellum in cross-section at emergence from spore, with associated band of 9 microtubules (arrow).
Fig. 18. *Macrocystis integrifolia*. Section through flagellum of pre-release spore showing dense material (arrow) arrayed parallel to basal body. Note association with plastid (P).

Fig. 19. *Dictyoneurum*. (Same sporangium as Fig. 9) Section through anterior flagellum showing unilateral attachment of mastigonemes (Ma), dense material (arrow) parallel to basal body, association of basal body with plastid.

Fig. 20. *Hedophyllum sessile*. Section through released zoospore in plane of flagellar bases. Note microtubular band (arrow) near anterior basal body.

Fig. 21. *A. tenuifolia*. Released zoospore sectioned through attachment of one flagellum, showing parallel, possibly tubular material (arrows) next to basal body.

Fig. 22. *A. tenuifolia*. Released zoospore showing posterior flagellum attachment, anterior flagellum's basal body perpendicular (straight arrow), curved band of microtubules (curved arrow). Note dividing plastid and lack of eyespot, also leached "type b" vesicle.
Figs. 23A-23E. *A. tenuifolia*. Released zoospore. Serial sections through (probably posterior) flagellum, showing parallel tubular band of microtubules (arrow), and band (curved arrow) associated with other, perpendicular basal body (bb).

Fig. 24. *A. tenuifolia*. Section through released zoospore showing flagellar attachment, associated microtubular bands (arrows) and other cytoskeletal microtubules (mt).
PLATE 6

Figs. 25A-25G. A. tenuifolia. Serial sections through released zoospore showing cytoskeletal microtubules (arrows) at "nose" of spore. Fig. 25B: detail of microtubules (arrow) in Fig. 25A.

Fig. 26. A. tenuifolia. Released zoospore showing convergence of cytoskeletal microtubules in "nose" (arrow).
Fig. 27. *A. tenuifolia*. "Exploded" zoospore, showing microtubular skeletons of anterior flagellum, posterior flagellum, and cytoskeleton (Cy). Note persistence of tip of posterior flagellum, connection between basal bodies (large arrow), connecting material between adjacent doublets (thin arrow). Two central singlet microtubules have been torn away from rest of axoneme (curved arrow), but 2 microtubules are detectable distally (short arrow).
Fig. 28. *A. tenuifolia*. "Exploded" zoospore showing partially disrupted anterior cytoskeletal band of microtubules (Cy), and posterior band. Note preservation of tip of posterior flagellum.

Fig. 29. *A. tenuifolia*. "Exploded" spore showing partially disrupted anterior cytoskeletal band.

Fig. 30. *A. tenuifolia*. "Exploded" spore showing intact anterior cytoskeletal band and connecting microtubules (arrow) between basal bodies.
Fig. 31. A. tenuifolia. Released zoospore showing lateral insertion of the two flagella. Note lack of eyespot in plastid near posterior flagellum.

Fig. 32. Detail of basal bodies (Fig. 31) and posterior band of cytoskeletal microtubules (arrow).

Fig. 33. Cymathere. Pre-release zoospore showing parallel basal bodies.

Fig. 34. Dictyoneurum. Pre-release zoospore with parallel basal bodies. Note association with plastid, unilateral attachment of mastigonemes (Ma).

Figs. 35-36. Dictyoneurum. Pre-release flagella showing unilateral mastigoneme attachment.
**PLATE 10**

Fig. 37. *Alaria nana*. Whole mount, unshadowed, unstained zoospore showing long anterior whiplash (W), bilateral arrangement of mastigonemes.

Fig. 38. *A. tenuifolia*. Detail of anterior whiplash (largely removed) Membrane has collapsed around axoneme showing presence of two singlet microtubules.

Fig. 39. *A. tenuifolia*. "Exploded" zoospore showing attachment of mastigonemes in two rows on opposite sides of flagellum (arrows).
Fig. 40. *A. nana.* Negative-stained mastigonomes showing dense core (arrow) and terminal filaments (TF).

Fig. 41. *A. tenuifolia.* Shadowcast, detached mastigonomes, some with spherical basal piece (arrow).

Fig. 42. Shadowcast, partly disrupted flagellum showing persistence of distal whiplash base (arrow), bilateral mastigomene arrangement.

Fig. 43. *A. tenuifolia.* "Exploded" spore showing connections between anterior and posterior basal bodies (arrow), bilateral arrangement of mastigonomes.
Fig. 44. A. *nana*. Negative-stained mastigonemes showing distal terminal filaments.

Fig. 45. A. *tenuifolia*. Shadowcast spore showing partly disrupted anterior flagellum with bilateral mastigoneme attachment, intact posterior flagellum.

Fig. 46. A. *tenuifolia*. Detail of posterior flagellum tip with terminal swelling.

Fig. 47. A. *tenuifolia*. 2 Shadowcast zoospores, with prominent swellings of the tips of posterior flagella.
Fig. 48. **Pterygophora.** Zoosporangium showing various sections through plastids as they enfold nucleus. Note large "type b" vesicles.

Fig. 49. **Costaria costata.** Zoospore within sporangium. Plastid showing plastoglobuli (pg) and membrane-bound inclusions (straight arrow) and ring genophore (curved arrow).

Figs. 50-51. **Postelsia palmeformis.** Zoospores within sporangium, showing plastoglobuli and membrane-bound inclusion (arrow).

Fig. 52. **Cymathere.** Released zoospore showing dividing plastid, adhesion vesicles (arrow).

Fig. 53. **Cymathere.** Settled zoospore showing divided plastid, released adhesion vesicles (AV), cell wall (arrow).
PLATE 14

Fig. 54. *Macrocystis*. Mitochondria (M) in pre-cleavage sporangium showing lobing.

Figs. 55-56. *Pterygophora*. Mitochondria showing intracristal tubules (T, arrows).

Fig. 57. *Costaria*. Zoospore within sporangium, nucleoli evident (arrows).

Fig. 58. *Cymathere*. Pre-cleavage sporangium nuclei with heterochromatin.

Fig. 59. *Dictyoneurum* Pre-cleavage sporangium nuclei with heterochromatin.
Fig. 60. *Postelsia*. Pre-release zoospore, nucleus with pores (arrow) associated plastid and golgi (G).

Figs. 61-63. *Cymathere*. Pre-release zoospores, nucleus with enfolding plastid, nuclear pores (short arrows). Note extensions of nuclear envelope around plastid (curved arrows) and into cytoplasm (long arrows).
Fig. 64. *Hedophyllum*. Pre-release zoospore showing nucleus enfolded by plastid, associated golgi and endoplasmic reticulum (er) stacks.

Fig. 65. *Cymathere*. Released zoospore. Note nucleus with cytoplasmic extension (arrow), golgi opposite endoplasmic reticulum stacks (curved arrow), appressed flagellum (F).

Fig. 66. *Pterygophora*. Pre-release zoospore, nucleus with golgi in invagination.

Fig. 67. *Lessoniopsis*. Pre-cleavage sporangium with endoplasmic reticulum stacks surrounding nuclei.

Fig. 68. *Hedophyllum*. Pre-cleavage sporangium nucleus showing endoplasmic reticulum stacks with ribosome arrays.

Fig. 69. *Hedophyllum*. Detail of spore from Fig. 64. Endoplasmic reticulum stacks with ribosome arrays, adjacent golgi.
**PLATE 17**

Fig. 70. *Pterygophora*. Released zoospore showing osmiophilic vesicles with distinct bounding membrane (arrows).

Fig. 71. *A. tenuifolia*. Released zoospore with aggregation of osmiophilic globules superficially resembling an eyespot (arrow).

Fig. 72. *Postelsia*. Pre-release zoospore nucleus with rough endoplasmic reticulum stacks forming contents of "type b" vesicle.

Figs. 73-74. *Cymathere*. Pre-release zoospores treated with thiocarbohydrazide-osmium. Note retention of "type b" vesicle contents.
Figs. 75-76. *Dictyoneurum*. Pre-cleavage sporangium with mastigonomes in close association with precursors in cytoplasm (curved arrow). Electron-dense core of mastigonomes is evident (thin arrow).

Figs. 77-78. *Dictyoneurum*. Pre-cleavage sporangia with extracytoplasmic membrane stacks (arrow).
**PLATE 19**

Figs. 79-80. *Dictyoneurum*. Pre-cleavage sporangia with extracytoplasmic stacks (E) enclosing flagella.

Fig. 81. *Dictyoneurum*. Pre-cleavage sporangium with extracytoplasmic vesicles (V) among flagella. Note cross-section of flagellum with only 2 singlet microtubules (arrow).

Fig. 82. *Hedophyllum*. Pre-release zoospore with adhesion vesicles (AV).

Fig. 83. *Macrocystis*. Pre-release spore with adhesion vesicles (arrow).

Fig. 84. *Laminaria groenlandica*. Pre-release spore with adhesion vesicles (arrow).

Fig. 85. *L. saccharina*. Pre-release spore with adhesion vesicles. Note apparent membrane in negative image (arrow) (chromate fix).
PLATE 20

Fig. 86. Agarum cribosum. Pre-release zoospore with adhesion vesicles (arrow).

Fig. 87. Postelsia. Pre-release spore with adhesion vesicles (arrow).

Fig. 88. Cymathere. Pre-release zoospore with adhesion vesicles. One vesicle shows electron-transparent central core (arrow).

Fig. 89. Cymathere. Adhesion vesicle in longissection.

Fig. 90. A. nana. Zoospore with mastigoneme clump at tip of anterior whiplash.

Fig. 91. Detail of (Fig. 90) mastigoneme clump.
PLATE 21

Fig. 92. *Hedophyllum*. Zoospore with mastigonemes adhering to anterior whiplash (arrow).

Fig. 93. *Agarum*. Zoospore with anterior whiplash.

Fig. 94. *A. tenuifolia*. Zoospore with anterior whiplash.

Fig. 95. *Cymathere*. Zoospores, anterior whiplash not preserved.

Fig. 96. *Eisenia*. Zoospore with anterior whiplash.

Fig. 97. *Costaria*. Zoospore with anterior whiplash.
PLATE 22

Fig. 98. Lessoniopsis. Zoospore, anterior whiplash not preserved.

Fig. 99. Macrocystis. Zoospore, only basal remnant of anterior whiplash preserved.

Fig. 100. Nereocystis leutkeana. Zoospore with anterior whiplash.

Fig. 101. Pleurophycus gardneri. Zoospore with anterior whiplash.

Fig. 102. Postelsia. Zoospore, anterior whiplash and mastigonemes not preserved.

Fig. 103. Pterygophora. Zoospores, only basal remnant of anterior flagellum preserved; full extent of posterior flagellum visible.
Fig. 104. Cymathere. Section through pre-release flagella, one through tip with only 2 singlet microtubules (arrow).

Fig. 105. Cymathere. Section through pre-release flagella, one showing region near tip; 9+2 pattern lost, three doublets and two singlets (arrow) evident.

Fig. 106. Dictyoneurum. Pre-release flagellum in longisection, with whiplash.

Figs. 107-109. A. tenuifolia. Settling zoospores showing resorption of axonemes (arrows).

Fig. 110. A. tenuifolia. Grazing section through released zoospore near basal bodies; one axoneme is withdrawn (note basal plates, [arrows]), one is still emergent.
PLATE 24

Fig. 111. A. _tenuifolia_. Released zoospore showing peripheral, withdrawn axoneme (arrow).

Fig. 112. A. _tenuifolia_. Settled zoospore extruding adhesion vesicles (arrow). Note withdrawn axonemes (curved arrow).

Fig. 113. _Cymathere_. Settled zoospore showing released, opened adhesion vesicles (arrow).

Fig. 114. _Cymathere_. Settled zoospore with extruded adhesion vesicles, withdrawn axonemes (arrow).

Fig. 115. _Macrocystis_. Settled zoospore showing withdrawn basal body (arrow), extruded adhesion vesicles.

Fig. 116. _Macrocystis_. Settled zoospore showing extruded, opened adhesion vesicles.
PLATE 25

Figs. 117-120. *Alaria tenuifolia*. Settled zoospores showing extruded adhesion vesicles and wall development (arrows), Fig. 120 shows formation of germination tube.
Fig. 121. A. tenuifolia. Shadowcast zoospores, one settled (Se), one still active (Ac). Note scattered mastigonemes.

Figs. 122-123. A. tenuifolia. Settled zoospore forming "double-blob" figures.

Fig. 124. A. tenuifolia. Settled zoospores with scattered mastigonemes.

Fig. 125. A. tenuifolia. Settled zoospore showing trail of mastigonemes, possibly indicating path of flagellum withdrawal.
Fig. 126A. Diagrammatic representation of a generalized Laminarialean zoospore, showing probable arrangement of cytoskeletal microtubules and major organelles. AF = anterior flagellum, B = "type b" vesicle, M = mitochondrion, Ma = mastigonemes, N = nucleus, P = plastid.

Fig. 126B. Diagrammatic representation of a "dorsal view" of a zoospore, depicting configuration of organelles and vesicles. Av = adhesion vesicle, er = endoplasmic reticulum, G = golgi apparatus, O = osmiophilic vesicle.
Fig. 127. *Macrocystis*. Male gametophyte. 1 μm-thick section stained with methylene blue-azure II. Antheridia are commonest at periphery of sphere of filaments.

Fig. 128. *Alaria taeniata*. Gametophyte filament showing lateral position of antheridia (A).

Fig. 129. *Macrocystis*. Gametophyte with intercalary antheridium. Note granular material (gr) at apex of terminal antheridium.

Fig. 130. *A. nana*. Gametophyte. Note size difference between plastids of developing sperm (arrows) and vegetative cells.

Figs. 131-132. *Macrocystis*. Antheridal clusters showing plurilocular nature of clusters, confluent outer walls (arrows).
PLATE 29

Fig. 133. *Macrocystis*. Antheridia. Note confluence of outer walls of cluster (arrow).

Fig. 134. *Macrocystis*. 1 um-thick section showing differential staining of material at apex of antheridium (arrow). Methylene blue-azure II.

Fig. 135. *A. nama*. Same antheridium as Fig. 130. Note granular material at apex of antheridium.

Fig. 136. *A. taeniata*. Note granular material at apex of antheridium.
Fig. 137. *A. taeniata*. Longissection through developing sperm showing fold enclosing flagella, association of parallel basal bodies with cytoskeletal microtubules in fold (arrow). Note "type b" vesicles (B).

Fig. 138. *A. tenuifolia*. Developing sperm showing fold enclosing flagellum, extracytoplasmic membranes (E).

Fig. 139. *Macrocystis*. Developing sperm showing parallel basal bodies enclosed by fold of cytoplasm.

Fig. 140. *Pterygophora*. Early antheridium with evident future basal bodies (arrows). Note they are not perpendicular to each other.

Fig. 141. Detail of basal bodies (Fig 140).
Figs. 142A-D. *A. taeniata*. Serial sections through developing sperm showing insertion of basal bodies in fold of cytoplasm, band of microtubules in fold and associated with basal bodies (between arrows). Note "type b" vesicles.
Fig. 143. *Alaria taeniata*. Early antheridium with active golgi (G), membraneous vesicles associated with flagella (curved arrow). Note band of microtubules (straight arrow).

Fig. 144. *Macrocystis*. Developing sperm showing association of microtubular bands (short arrow) and similar electron-dense material with basal bodies (long arrow).

Fig. 145. *A. taeniata*. Developing sperm showing cytoskeletal microtubules in fold enclosing flagella (arrows). Note vesicles (V) forming extracytoplasmic matrix.

Fig. 146. *A. taeniata*. Developing sperm. Note microtubular band parallel to flagella (arrow).
Fig. 147. *Macroystis*. Developing sperm. Note electron-dense (microtubular?) material surrounding basal body of flagellum (arrow).

Figs. 148-149. *Pterygophora*. Released sperm, whole mount, shadowcast. Note 180 degree orientation of anterior and posterior flagella, and loop of cytoskeletal microtubules in "nose" (arrow).

Fig. 150. *A. taeniata*. Whole mount of sperm, unshadowed, unstained, showing tapering posterior flagellum, whiplash (W) on anterior flagellum.

Fig. 151. *Costaria*. Whole mount sperm, unstained, unshadowed, showing taper of axoneme of posterior flagellum, basal remnant of anterior whiplash.

Fig. 152. *Dictyteuremum*. Whole mount sperm, unstained, unshadowed, with well-preserved anterior whiplash. Many bacteria also present.
Fig. 153. Detail of whiplash (Fig. 152).

Fig. 154. Lessoniopsis. Negative-stained sperm showing anterior whiplash, mastigonemes.

Fig. 155. Hedophyllum. Unstained, unshadowed sperm with "exploded" anterior flagellum. Note association of mastigonemes with axonemal microtubules.

Fig. 156. Pleurophycus. Shadowcast sperm, showing remnant of anterior whiplash.
Plate 35

Figs. 157-158. *Macrocystis*. Unshadowed, unstained sperm clearly showing tapering of axoneme of posterior flagellum, mastigonemes, and basal remnant of anterior whiplash.

Figs. 159-160. *Macrocystis*. Sperm on EM grid film, photographed with 40x phase-contrast objective. Note taper of posterior flagellum, anterior whiplash (arrow).

Fig. 161. *Macrocystis*. Cross-section of tapering posterior flagellum within antheridium. Note doublets have become singlets.

Fig. 162. *Pterygophora*. Sections of tapering posterior flagellum.

Fig. 163. *A. taeniata*. Sections of tapering posterior flagellum.
PLATE 36

Fig. 164. *Pterygophora*. Shadowcast sperm with microtubules torn free of axoneme (arrow).

Fig. 165. Detail of free microtubules (Fig. 164). They appear to be doublets or closely adherent singlets.

Fig. 166. *Macrocystis*. Developing sperm showing at least three plastids in section.

Fig. 167. *A. taeniata*. Developing sperm showing two elongate plastids.

Fig. 168. *A. nana*. Developing sperm with at least 2 plastids; note few thylakoids.

Fig. 169. *A. taeniata*. Mitochondria of developing sperm. Note intracristal tubules (arrow).
Fig. 170. *A. nana*. Early antheridium showing well-developed golgi.

Fig. 171. *Macrocystis*. Developing sperm showing endoplasmic reticulum arrays associated with flagellar cavity (between arrows). Note "type b" vesicles.

Fig. 172. *A. nana*. Developing sperm showing endoplasmic reticulum arrays parallel to nuclear membrane (arrow).

Fig. 173. *A. taeniata*. Developing sperm. Note extracytoplasmic membranes and vesicles producing extracytoplasmic matrix.
PLATE 38

Fig. 174. *A. taeniata*. Developing sperm with active Golgi producing vesicles emptying into cavity.

Figs. 175-177. *A. taeniata*. Developing sperm showing extracytoplasmic membranes, many associated with flagella. Note "type b" vesicles.
PLATE 39

Figs. 178-179. A. taeniata. Developing sperm with extracytoplasmic membranes.

Fig. 183. Diagrammatic representation of generalized Laminarialean sperm, showing configuration of major organelles and tapering of posterior flagellum. AF = anterior flagellum, B = "type b" vesicle, M = mitochondrion, Na = mastigonemes, N = nucleus, P = plastid, PF = posterior flagellum.
PLATE 41

Fig. 184. Diagrammatic representation of the better-known brown-algal sperm types. The advanced forms may be derived by radiation from a primitive form (such as that of Ectocarpus) which resembles a zoospore. The most significant modifications are the loss of the eyespot, elongation of the body of the sperm, prolongation of the anterior of the sperm, and elongation of the posterior flagellum or presence of spines on the anterior flagellum.
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Ultrastructure Of Sperm Of Desmarestia viridis

Desmarestia viridis was studied by the same techniques used with Laminariales. The zoospores (not illustrated) are similar to those of most brown algae; they possess an eyespot, and the longer, anterior, mastigoneme-bearing flagellum bears a distal whiplash.

The sperm are elongate (Figs. 1, 2) and lack an eyespot, but they contain a nucleus, several mitochondria, "type b" and osmiophilic vesicles (Figs. 6-8). The anterior flagellum is similar to that of other brown algal swarmers. The mastigoneme-bearing portion is about 20 um long; occasionally a distal whiplash is preserved (not illustrated). The posterior flagellum bears a distal whiplash (Figs. 1-5) that may extend this flagellum to as much as 35 um in length. In well-preserved specimens (Figs. 1, 2) an anterior rostrum, similar to the "proboscis" of the sperm of Vaucheria (Moestrup 1970) is evident. Fig. 2 indicates that a cytoskeletal loop supports the rostrum.

The posterior whiplash, like the anterior whiplash, is an extension of the two central microtubules of the flagellar axoneme. In contrast to the anterior whiplash, it is often well preserved by osmium vapor fixation, and it frequently is apparent in sections of pre-release sperm.

The elongated shape, lack of eyespot, and long posterior
flagellum of the Desmarestia viridis sperm recall the sperm of Laminariales, but it is significant that in D. viridis flagellar extension has occurred by a different structural modification (prolongation of the two central anomemal microtubules only) from those found in other brown algal sperm: in the Laminariales the posterior flagellum tapers distally, while in the Fucaceae it retains the "9 + 2" construction throughout its entire length. This difference indicates that despite the similarities of the desmarestialean and laminarialean life histories and gametophyte morphologies, these two orders are not closely related.
Fig. 1. *Desmarestia viridis* sperm. Whole mount, unstained, unshadowed.

Fig. 2. Sperm showing some disruption of the anterior part of the cell; the cytoskeleton of the rostrum has resisted disruption. Posterior whiplash only partially preserved.

Fig. 3. Sperm with mastigonemes retained on anterior flagellum. Cell shape is distorted.

Fig. 4. Cell fixed with 1% tannic acid added before osmium vapor. Mastigonemes have been preserved, but are shrivelled, as is posterior whiplash. Cell shape is distorted.
Fig. 5. Sperm with well preserved mastigonemes and posterior whiplash. Cell shape is distorted.

Fig. 6. Section through sperm within antheridium. Note cross-section through posterior whiplash (arrows), nucleus (N), mitochondria (M).

Fig. 7. Sperm within antheridium. Note the flagellum (F) lying within membrane-enclosed region.

Fig. 8. Sperm within antheridium. Note longisection of posterior whiplash (arrow), "type b" vesicles (B), osmiophilic vesicles (O).